NJC

PAPER

View Article Online
View Journal

Cite this: DOI: 10.1039/c5nj03589a

Novel benzothiazole based sulfonylureas/sulfonylthioureas: design, synthesis and evaluation of their antidiabetic potential†

Chetna Kharbanda,[a] Mohammad Sarwar Alam,*[a] Hinna Hamid,*[a] Kalim Javed,[a] Sameena Bano,[a] Yakub Ali,[a] Abhijeet Dhulap,[b] Parwez Alam[c] and M. A. Q. Pasha[c]

Received (in Montpellier, France) 17th December 2015,
Accepted 17th May 2016

DOI: 10.1039/c5nj03589a

www.rsc.org/njc

In the present study, twenty-eight benzothiazole based sulfonylureas/sulfonylthioureas were synthesized and were assessed for their antidiabetic effect in a normoglycemic rat model by the *in vivo* oral glucose tolerance test (OGTT). All the synthesized compounds were studied for their interactions inside the PPAR-γ receptor site through a docking study. Subsequently, *in vitro* PPAR-γ transactivation assay was performed on ten active compounds **7c**, **7d**, **7i–l**, **8c**, **8d**, **8g**, and **8h** which showed potent antidiabetic activity in the OGTT (better than standard drugs) and also showed a good dock score with the PPAR-γ receptor site. These active ten compounds were also found to transactivate PPAR and therefore were assessed for their antidiabetic potential on the STZ induced diabetic model. Effects of these compounds on body weight were also monitored during the course of study. Furthermore, the most effective compound **7j** was evaluated for its effect on PPAR-γ gene expression.

Introduction

The peroxisome proliferator-activated receptor-γ (PPAR-γ) is abundantly found in adipose tissue, macrophages, skeletal muscles and intestinal cells. Important functions of PPAR-γ include regulation of glucose homeostasis, adipogenesis and insulin sensitivity.[1] Activation of PPAR-γ stimulates the insulin dependent glucose transporter GLUT4 protein which accelerates the transportation of fatty acids synthesized from glucose. This process increases both the storage capacity and fatty acid flux in the adipocyte which in turn helps in glucose homeostasis. In addition, the ligand-binding domain of PPAR-γ contains a large binding pocket that allows the interaction of diverse types of ligands to form ligand–receptor complexes.[2]

Despite being primitive, sulfonylureas are still being used as oral antidiabetic agents either alone or in combination with other classes of oral antidiabetic drugs. Their intriguing antidiabetic property is still provoking scientists to synthesize new sulfonylurea derivatives with lesser side effects. Sulfonylureas mainly act by stimulating insulin secretion by binding to sulfonylurea receptors (SURs) of ATP sensitive potassium ion channels present in pancreatic β-cells.[3] Though thiazolidinediones represent a class of antidiabetic compounds generally known as PPAR-γ agonists, several marketed sulfonylureas like glibenclamide, glimepiride, gliquidone and glipizide have recently been proven to improve insulin sensitization by acting as PPAR-γ agonists in addition to binding with SU-receptors. It has been studied that glibenclamide competitively binds to the PPAR-γ receptor site with respect to marketed PPAR-γ agonists like pioglitazone and rosiglitazone.[4,5] Benzothiazole derivatives encompass multiple applications in bioorganic and medicinal chemistry due to a unique bicyclic ring system of benzothiazole. The pharmacological profile of compounds containing the benzothiazole moiety comprises antimicrobial,[6–10] anticancer,[11] anthelmintic[12] and anti-diabetic[13] activities.

Therefore, the present work emphasizes the synthesis of benzothiazole based sulfonylureas as an approach to design new antidiabetic agents along with subsequent control over weight gain. The effect of these synthesized compounds as PPAR-γ agonists was also studied.

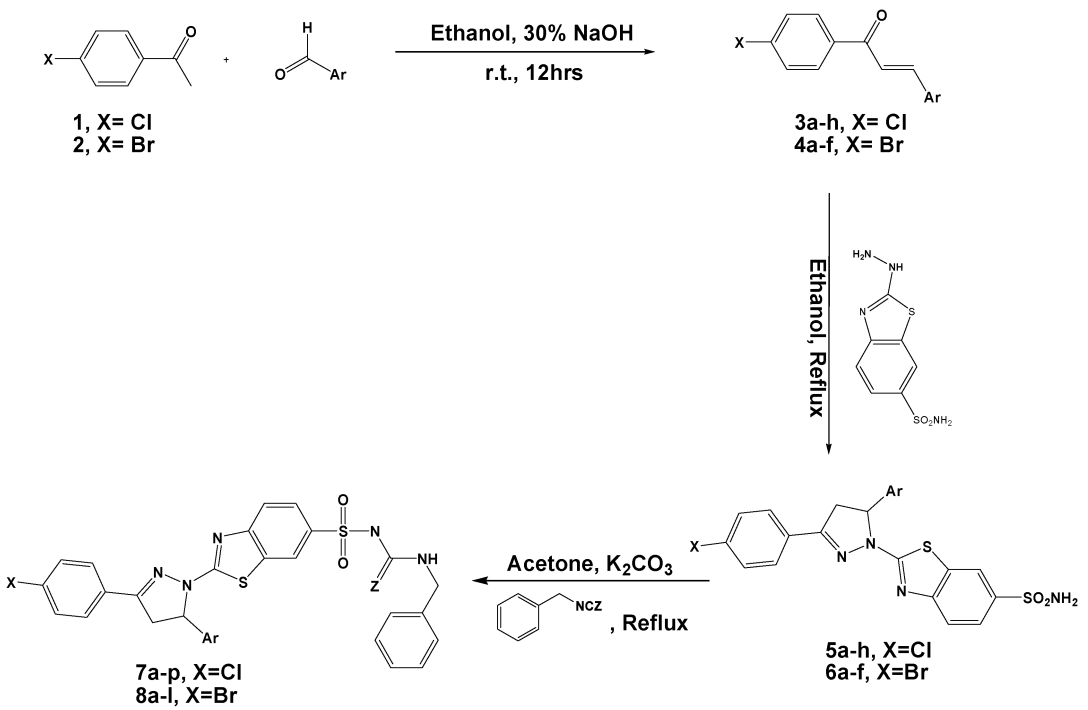
Results and discussion

Chemistry

The synthetic route used to synthesize benzothiazole based sulfonylurea derivatives is outlined in Scheme 1. The structural

[a] Department of Chemistry, Faculty of Science, Hamdard University, New Delhi-110 062, India. E-mail: msalam@jamiahamdard.ac.in, msalam5555@gmail.com, hhmaid@jamiahamdard.ac.in; Fax: +91 11 26059663; Tel: +91 11 26059688 (5555), (5559)
[b] CSIR Unit for Research and Development of Information Products, Pune-411038, India
[c] Functional Genomics Unit, CSIR-Institute of Genomics & Integrative Biology, Delhi, India

† Electronic supplementary information (ESI) available. See DOI: 10.1039/c5nj03589a



Scheme 1 Synthesis of benzothiazole based sulfonylurea derivatives.

elucidation was based on ^{1}H NMR, ^{13}C NMR, IR, mass spectra and CHNS data of synthesized compounds. ^{1}H NMR spectra of all the synthesized compounds were recorded at 300–500 MHz frequency in parts per million (ppm) using TMS as the standard. The appearance of three double doublets in the range of δ 3.00–6.00 ppm confirmed the formation of the pyrazoline ring. Coupling with benzoisocyanate/benzoisothiocyanate was verified by increase in signals due to aromatic protons depending on the substituent and from the presence of the singlet of CH_2 at around δ 4.65 ppm. Mass spectra showing specific peaks at $[M + 1]^+$ further confirmed the syntheses of desired products. The structure of each compound indicating different substitutions has been provided in Fig. S1 (ESI†).

Oral glucose tolerance test (OGTT)

Primarily, all the synthesized compounds were scrutinized by loading glucose in normal rats to assess their antidiabetic potential. From the results of the oral glucose tolerance test, it was observed that ten compounds namely **7c**, **7d**, **7i–l**, **8c**, **8d**, **8g** and **8h** significantly lowered the plasma glucose level as compared to the standard drug glibenclamide. Moreover, the alleviation in the plasma glucose level that was observed for a PPAR-γ agonist, rosiglitazone, after 90 min was less than twelve compounds. The results of the OGT test are illustrated in Fig. 1. It was seen that the plasma glucose concentration of animals in the control group suddenly increased after glucose load while the groups administered with synthesized compounds showed a gradual increase in the plasma glucose level. The increase in the plasma glucose level of groups administered with synthesized compounds started reducing with the passage of time and came near to normal after 90 min for ten compounds **7c**, **7d**, **7i–l**, **8c**, **8d**, **8g** and **8h**.

Docking studies

As mentioned earlier, sulfonylureas can act through the activation of PPAR-γ in addition to their action on K^{+}_{ATP} channels. Therefore, before proceeding further we first carried out molecular docking studies of synthesized compounds against the PPAR-γ target. Dock scores of all the synthesized compounds were compared with the standard drug, rosiglitazone, which is a PPAR-γ agonist. Interestingly, eighteen (**7a–d**, **7f**, **7i–o**, **8c**, **8d**, **8f–h**, and **8j**) out of twenty eight synthesized compounds showed a higher dock score than rosiglitazone (−5.72). It should be noted that a negative value indicates low energy which in turn indicates likely binding interactions to make a stable system *i.e.* the more negative the value, the more stable the system will be. Careful examination of docking images showed that most of the compounds were either deeply buried inside the receptor site or formed H-bonds with amino acid residues of the receptor site. Some of the compounds displayed more than one H-bonding interactions. The images of the compounds displaying a high docking score are shown in Fig. 2 and dock scores are given in Table S1 (ESI†). The ligand interaction diagram of compound **7k** with the highest dock score of −10.06 showed the presence of π–π stacking as well as H-bonding with LYS-261 and ARG-280 and compound **8g** (−10.03) formed multiple H-bonds with LYS-261 present in the receptor site while the dock score of rosiglitazone was only −5.72 though it showed π–π stacking with the GLU-272 amino acid residue of the receptor site.

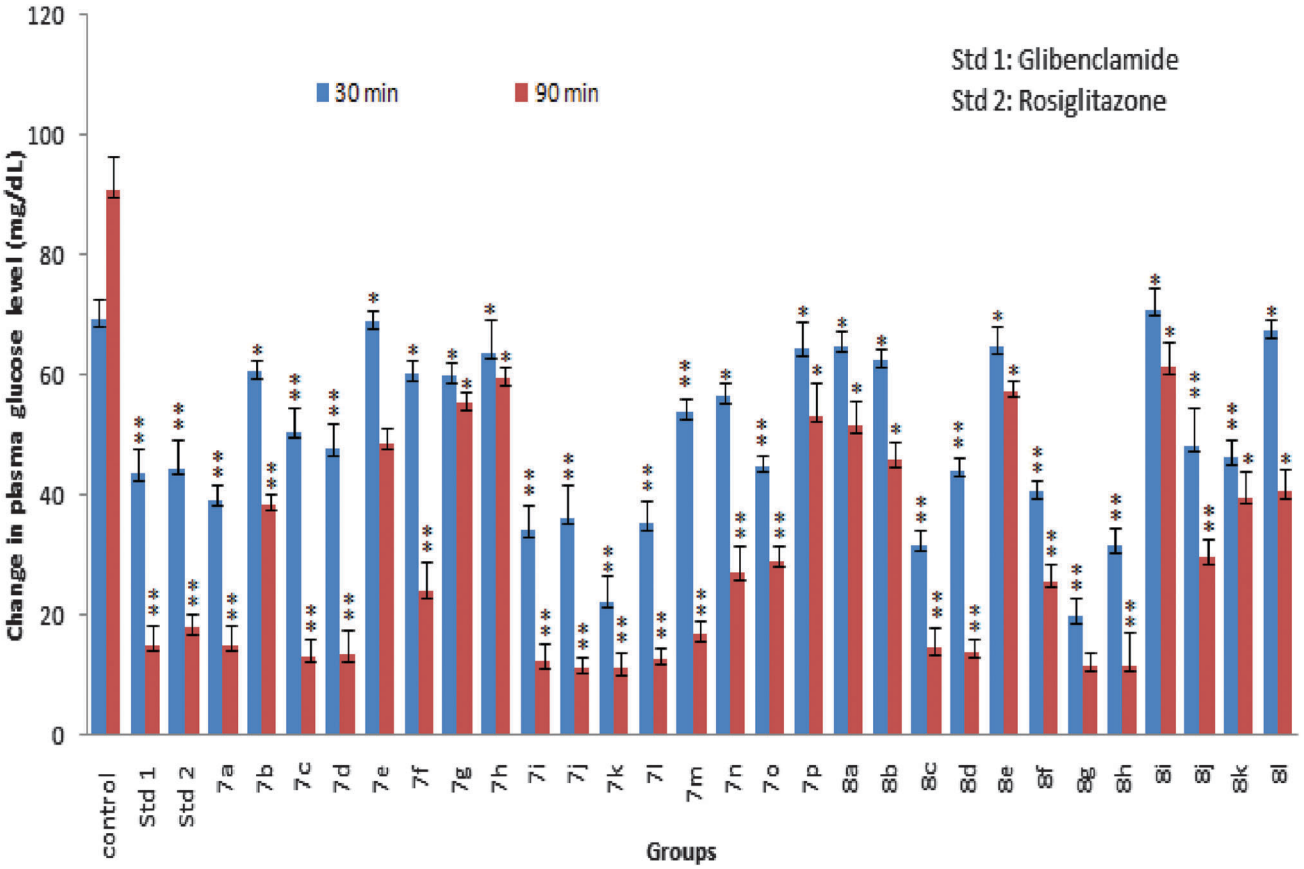


Fig. 1 Antidiabetic effect of treating wistar rats with synthesized compounds by the Oral Glucose Tolerance Test. Data are analyzed by one way ANOVA followed by the Dunnett test and expressed as mean ± SEM from six observations; * represents a change as compared to control; ** indicates $p < 0.01$ & * indicates $p < 0.05$.

***In vitro* PPAR transactivation assay.** To confirm that the synthesized compounds are acting through transactivation of PPAR-γ, ten compounds **7c**, **7d**, **7i–l**, **8c**, **8d**, **8g** and **8h** (that showed good oral glucose tolerance and have a high dock score) were scrutinized by *in vitro* PPAR transactivation assay (Table 1). All the active compounds showed moderate alleviation of PPAR in transactivation assay. Captivatingly, compound **7k** which exhibited highest dock score transactivated PPAR by only 54.93% as compared to rosiglitazone which showed 81.68% elevation in PPAR transactivation. Another two compounds **7j** and **8h** showed PPAR transactivation as much as compound **7k** *i.e.* 54.01 and 54.29%, respectively. The synthesized compounds showed high *in vivo* antidiabetic activity but less PPAR transactivation than rosiglitazone which inferred that PPAR transactivation is one of the targets aimed by these synthesized compounds in order to exhibit antidiabetic activity in a more complex *in vivo* system.

Antidiabetic activity on STZ-induced diabetic rats

Ten compounds (**7c**, **7d**, **7i–l**, **8c**, **8d**, **8g**, and **8h**) were further evaluated for their effect in the STZ induced diabetic model. It was found that the supplementation of diabetic rats with compounds **7i–l**, **8g** and **8h** caused significant lowering of the plasma glucose level on 15th day of the study (Fig. 3). These six compounds restored the plasma glucose level almost close to normal. Remaining four compounds (**7c**, **7d**, **8c** and **8d**) assuaged the plasma glucose level almost as appreciably as standard drugs, glibenclamide and rosiglitazone.

Structure–activity relationship

To understand the effect of different substitutions on *in vivo* antidiabetic activity, correlations have been drawn as follows (Fig. 4):

- Sulfonylureas derived from *p*-chloroacetophenone are in general more biologically active than *p*-bromoacetophenone derivatives.
- Fluoro substitution on the aryl ring exhibited higher antidiabetic activity than other substitutions while chloro substitution on the aryl group significantly lowered *in vivo* activity.
- Replacing the aryl ring with heterocycle like thiophene also displayed significant biological activity.
- Substitution of methyl and methoxy on the aryl ring lowered biological activity.
- In the case of *p*-chloro derivatives, sulfonylureas are more active than sulfonylthioureas while the trend is reverse in *p*-bromo derivatives with few exceptions.

Body weight

Increase in body weight is one of the closely associated concerns of treatment with sulfonylureas. Therefore, the change in

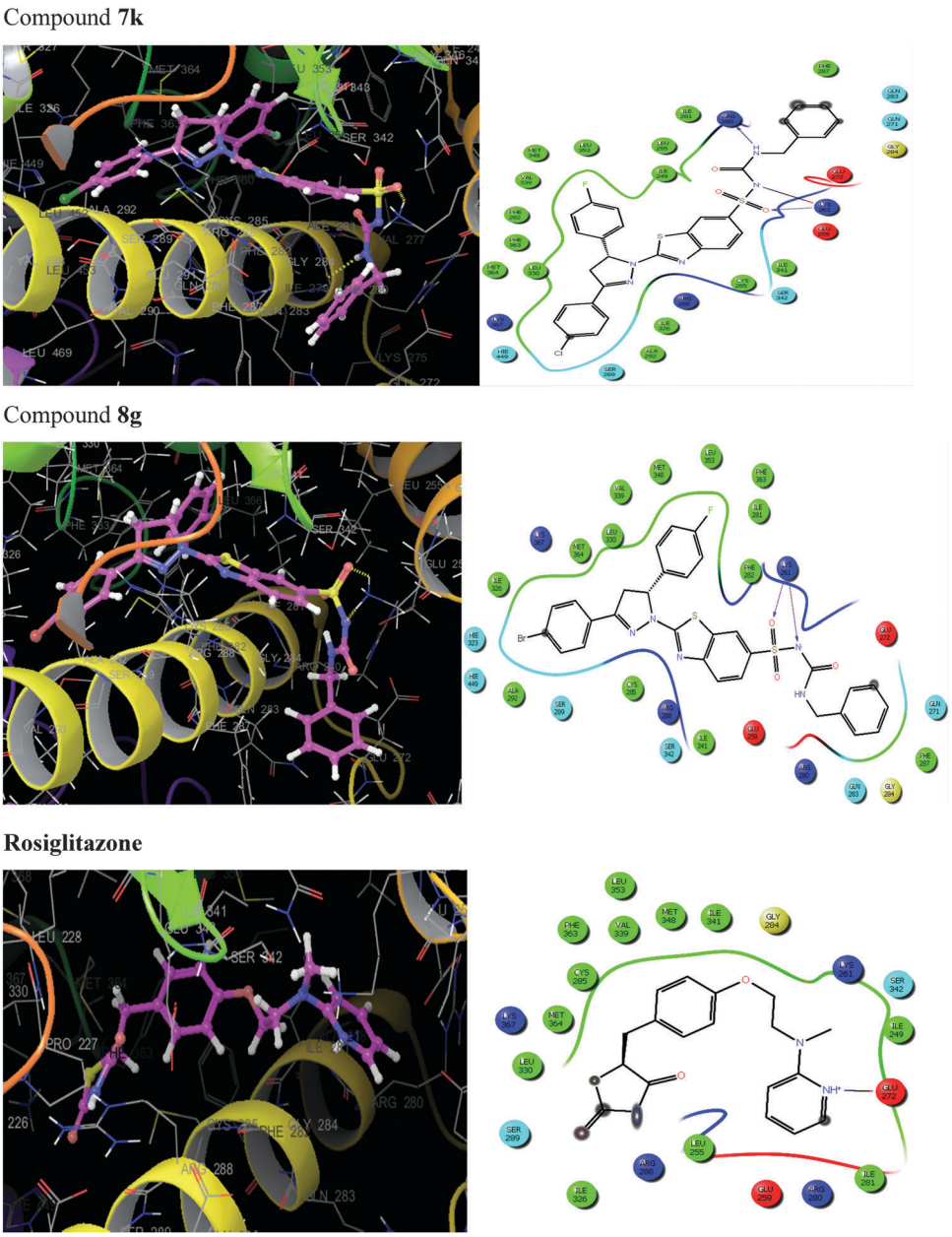


Fig. 2 Docking images of compounds **7k**, **8g** and rosiglitazone.

Table 1 *In vitro* PPAR-γ transactivation assay on the 3T3-L1 cell line

Compounds	% PPAR transactivation (mean ± SEM)
Control	0.15 ± 0.001
Rosiglitazone	81.68 ± 0.001
7c	43.48 ± 0.001
7d	43.69 ± 0.002
7i	45.85 ± 0.001
7j	54.01 ± 0.001
7k	54.93 ± 0.001
7l	49.58 ± 0.001
8c	43.11 ± 0.001
8d	41.49 ± 0.001
8g	53.21 ± 0.001
8h	54.29 ± 0.002

body weight post treatment with active compounds (**7i–l**, **8g** and **8h**) was carefully observed over the period of 15 days of study. In contrast to STZ induced control where significant weight loss was observed, the animals treated with glibenclamide and rosiglitazone exhibited increase in body weight. However, the treatment with active compounds **7i**, **7j**, **7l** and **8g** maintained the body weight and did not allow much increase up to 15 days of study (Fig. 5).

PPAR-γ gene expression

The most potent compound **7j** was evaluated for its effect on the expression of PPAR-γ target genes. The expression of PPAR-γ genes was measured in 3T3-L1 fibroblasts in comparison with

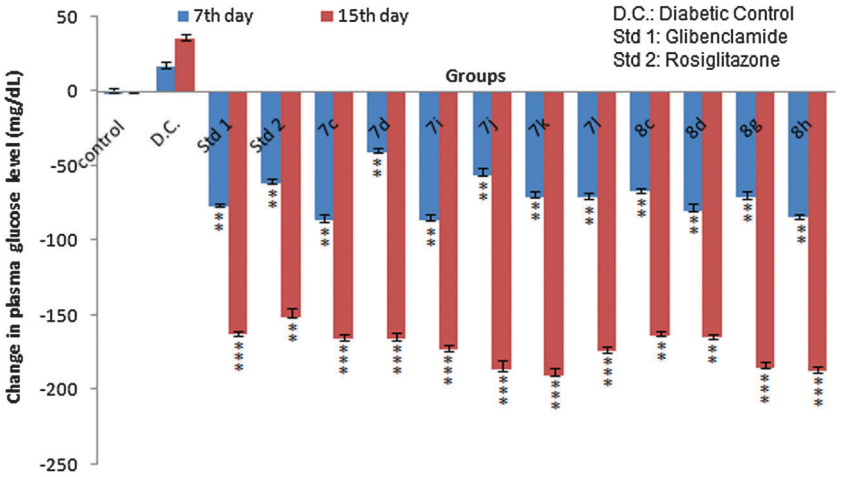


Fig. 3 Antidiabetic effect of synthesized compounds on STZ induced diabetic animals. Data are analyzed by one way ANOVA followed by the Dunnett test and expressed as mean ± SEM from six observations; * represents a change as compared to diabetic control; ** indicates $p < 0.01$ & *** indicates $p < 0.001$.

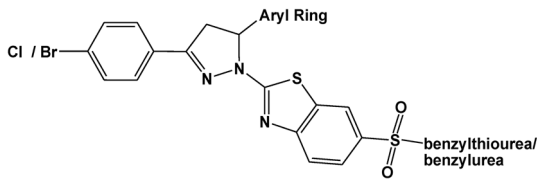


Fig. 4 General structure of synthesized benzothiazole based sulfonylurea.

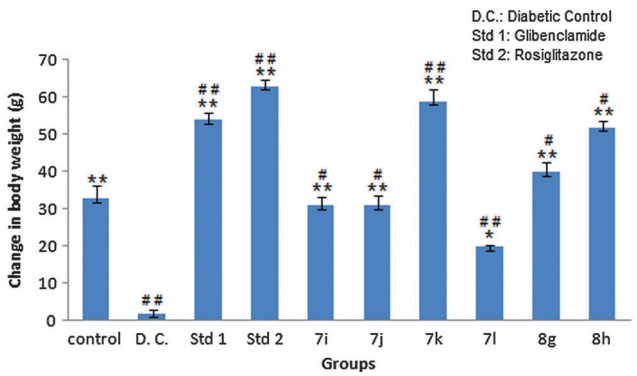


Fig. 5 Change in body weight after a 15 day study on STZ-induced diabetic rats. Data are analyzed by one way ANOVA followed by the Bonferroni 't' test and expressed as mean ± SEM from six observations; * represents a significant change as compared to diabetic control; # represents a significant change as compared to normal control. ** Indicates $p < 0.01$ & * indicates $p < 0.05$; ## indicates $p < 0.05$ & # indicates $p < 0.01$.

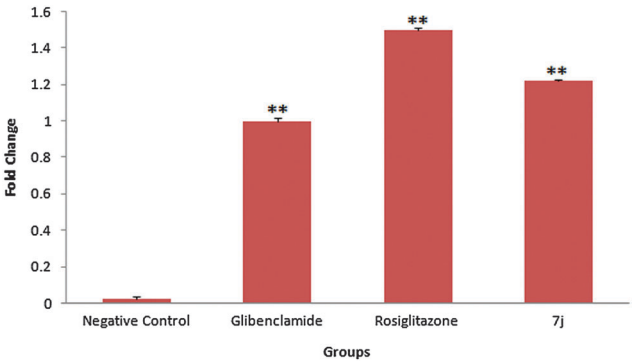


Fig. 6 PPAR-γ gene expression evaluation of compound **7j** in comparison with standards, glibenclamide and rosiglitazone. Data are analyzed by one way ANOVA followed by the Dunnett test and expressed as mean ± SEM from three observations; * represents change as compared to control; ** indicates $p < 0.01$. 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.

standard drugs, rosiglitazone and glibenclamide (Fig. 6). The standard drug, glibenclamide, amplified the expression of PPAR-γ genes to lesser extent than rosiglitazone despite having strong *in vivo* antidiabetic activity. Similarly, compound **7j** significantly enhanced the expression of PPAR-γ genes more than glibenclamide but less than rosiglitazone. It was found that rosiglitazone increased gene expression by 1.5 fold while the treatment with compound **7j** caused 1.22 fold elevation. Glibenclamide, on the other hand, showed less significant elevation in gene expression as compared to compound **7j** and rosiglitazone.

Conclusion

It can be concluded that four compounds **7i**, **7j**, **7l** and **8g** exhibited significant antidiabetic activity as compared to standard drugs without showing a much increase in body weight. The most active compound **7j** also showed an increase in PPAR-γ gene expression. Therefore, the present study demonstrated that the antidiabetic effect of these compounds might partially be due to the activation of PPAR-γ. Furthermore, it can be concluded that the combination of benzothiazole with sulfonylurea allows us to design more of the biologically

active molecules that can lead to discovery of potential drug candidates.

Experimental data

General synthesis of final products (7a–p and 8a–l)

p-Chloroacetophenone (**1**) and *p*-bromoacetophenone (**2**) were made to react with appropriate aldehyde to give corresponding chalcones, **3a–h** and **4a–f**. The synthesis of intermediate pyrazolines (**5a–h** and **6a–f**) was carried out by reacting synthesized chalcones (1.0 mmol) with 1.2 mmol of 2-hydrazinobenthothiazole-6-sulfonic acid amide.[14] The pyrazolines were reacted with appropriate benzylisocyanate or benzylisothiocyanate in the presence of anhydrous potassium carbonate and acetone by conventional reactions.[15] After reaction completion, the reaction mixture was carefully concentrated and filtered. The solid so obtained was washed with water, dried, and crystallized from acetone (Scheme 1). The compounds were finally recrystallized from ethanol to give pure desired products (**7a–p** and **8a–l**).

{2-[3-(4-Chloro-phenyl)-5-(4-methoxy-phenyl)-4,5-dihydro-pyrazol-1-yl]-benzothiazole-6-sulfonyl}*N*′-benzylurea (7a). Yield = 64.2%, m.p. 148–149 °C, R_f = 0.57, toluene : ethyl acetate : formic acid, 5 : 4 : 1. IR ν_{max} (KBr): 3363, 3242 & 1541 (N–H), 1595 (C=N), 1321 & 1151 cm^{-1} ($SO_2N<$). ^1H NMR (400 MHz, DMSO, δ_{ppm}): 3.55 (1H, dd, J = 5.2 Hz, 18.0 Hz), 3.71 (3H, s, OCH_3), 4.25 (2H, s, CH_2), 4.63 (1H, dd, J = 12.0 Hz, 17.6 Hz), 5.79 (1H, dd, J = 6.0 Hz, 12.0 Hz), 6.25 (2H, s, NH), 7.20–7.38 (16H, m, Ar). ^{13}C NMR (125 MHz, DMSO, δ_{ppm}): 43.41 (C_4), 53.57 ($\underline{C}H_2$), 57.16 (C_5), 63.47 (OCH_3), 113.95–143.76 (C aromatic), 154.80 (C=N), 155.23 (N_1–$\underline{C}$ benzothiazolyl), 159.56 (C=O). FAB-MS m/z: 632 $[M + 1]^+$. Elemental analysis: calculated for $C_{31}H_{26}ClN_5O_4S_2$; C = 58.90, H = 4.15, N = 11.08, S = 10.14. Found: C = 58.92, H = 4.18, N = 11.11, S = 10.17.

{2-[3-(4-Chloro-phenyl)-5-(4-methoxy-phenyl)-4,5-dihydro-pyrazol-1-yl]-benzothiazole-6-sulfonyl}*N*′-benzylthiourea (7b). Yield = 61.7%, m.p. 149–150 °C, R_f = 0.57, toluene : ethyl acetate : formic acid, 5 : 4 : 1. IR ν_{max} (KBr): 3363, 3241 & 1539 (N–H), 1592 (C=N), 1318 & 1148 cm^{-1} ($SO_2N<$). ^1H NMR (400 MHz, DMSO, δ_{ppm}): 3.58 (1H, dd, J = 5.6 Hz, 17.4 Hz), 3.72 (3H, s, OCH_3), 4.26 (2H, s, CH_2), 4.53 (1H, dd, J = 11.2 Hz, 17.6 Hz), 5.68 (1H, dd, J = 6.0 Hz, 12.2 Hz), 5.89 (2H, s, NH), 7.12–8.04 (16H, m, Ar). ^{13}C NMR (125 MHz, DMSO, δ_{ppm}): 43.56 (C_4), 57.68 (C_5), 63.28 (CH_2), 54.31 (OCH_3), 110.41–129.32 (C aromatic), 154.81 (C=N), 155.64 (N_1–$\underline{C}$ benzothiazolyl), 158.60 (C=S). FAB-MS m/z: 649 $[M + 1]^+$. Elemental analysis: calculated for $C_{31}H_{26}ClN_5O_3S_3$; C = 57.44, H = 4.04, N = 10.80, S = 14.84. Found: C = 57.45, H = 4.06, N = 10.85, S = 14.91.

{2-[3-(4-Chloro-phenyl)-5-thiophen-2-yl-4,5-dihydro-pyrazol-1-yl]-benzothiazole-6-sulfonyl}*N*′-benzylurea (7c). Yield = 64.4%, m.p. 236–237 °C, R_f = 0.55, toluene : ethyl acetate : formic acid, 5 : 4 : 1. IR ν_{max} (KBr): 3361, 3244 & 1541 (N–H), 1591 (C=N), 1318 & 1148 cm^{-1} ($SO_2N<$). ^1H NMR (400 MHz, DMSO, δ_{ppm}): 3.58 (1H, dd, J = 5.5 Hz, 17.8 Hz), 4.15 (1H, dd, J = 11.2 Hz, 17.6 Hz), 4.71 (2H, s, CH_2), 5.88 (1H, dd, J = 5.6 Hz, 11.5 Hz), 6.09 (2H, s, NH), 6.98–8.01 (15H, m, Ar). ^{13}C NMR (125 MHz, DMSO, δ_{ppm}): 43.33 (C_4), 53.86 ($\underline{C}H_2$), 63.73 (C_5), 104.22–131.37 (C aromatic), 155.50 (C=N), 159.85 (N_1–$\underline{C}$ benzothiazolyl), 170.30 (C=O). FAB-MS m/z: 608 $[M + 1]^+$. Elemental analysis: calculated for $C_{28}H_{22}ClN_5O_3S_3$; C = 55.30, H = 3.65, N = 11.52, S = 15.82. Found: C = 55.35, H = 3.71, N = 11.56, S = 15.87.

{2-[3-(4-Chloro-phenyl)-5-thiophen-2-yl-4,5-dihydro-pyrazol-1-yl]-benzothiazole-6-sulfonyl}*N*′-benzylthiourea (7d). Yield = 59.2%, m.p. 181–182 °C, R_f = 0.56, toluene : ethyl acetate : formic acid, 5 : 4 : 1. IR ν_{max} (KBr): 3361, 3239 & 1551 (N–H), 1594 (C=N), 1321 & 1148 cm^{-1} ($SO_2N<$). ^1H NMR (400 MHz, DMSO, δ_{ppm}): 3.57 (1H, dd, J = 5.6 Hz, 18.0 Hz), 4.12 (1H, dd, J = 11.5 Hz, 17.2 Hz), 4.63 (2H, s, CH_2), 6.20 (1H, dd, J = 6.0 Hz, 12.0 Hz), 6.48 (2H, s, NH), 6.97–8.43 (15H, m, Ar). ^{13}C NMR (125 MHz, DMSO, δ_{ppm}): 42.79 (C_4), 57.41 (C_5), 63.18 (CH_2), 112.01–129.56 (C aromatic), 155.58 (C=N), 156.49 (N_1–$\underline{C}$ benzothiazolyl), 158.44 (C=S). FAB-MS m/z: 624 $[M + 1]^+$. Elemental analysis: calculated for $C_{28}H_{22}ClN_5O_2S_4$; C = 53.88, H = 3.55, N = 11.22, S = 20.55. Found: C = 53.95, H = 3.61, N = 11.27, S = 20.62.

{2-[3-(4-Chloro-phenyl)-5-(2-chloro-phenyl)-4,5-dihydro-pyrazol-1-yl]-benzothiazole-6-sulfonyl}*N*′-benzylurea (7e). Yield = 64.2%, m.p. 198–199 °C, R_f = 0.55, toluene : ethyl acetate : formic acid, 5 : 4 : 1. IR ν_{max} (KBr): 3362, 3237 & 1541 (N–H), 1595 (C=N), 1318 & 1147 cm^{-1} ($SO_2N<$). ^1H NMR (400 MHz, DMSO, δ_{ppm}): 3.61 (1H, dd, J = 5.6 Hz, 17.2 Hz), 4.26 (1H, dd, J = 11.6 Hz, 17.2 Hz), 4.66 (2H, s, CH_2), 6.18 (1H, dd, J = 6.0 Hz, 12.0 Hz), 6.49 (2H, s, NH), 7.11–7.92 (16H, m, Ar). ^{13}C NMR (125 MHz, DMSO, δ_{ppm}): 46.25 (C_4), 53.87 ($\underline{C}H_2$), 63.96 (C_5), 100.18–129.47 (C aromatic), 153.58 (C=N), 158.41 (N_1–$\underline{C}$ benzothiazolyl), 171.90 (C=O). FAB-MS m/z: 636 $[M + 1]^+$. Elemental analysis: calculated for $C_{30}H_{23}Cl_2N_5O_2S_3$; C = 56.60, H = 3.64, N = 11.00, S = 10.07. Found: C = 56.66, H = 3.68, N = 11.07, S = 10.09.

{2-[3-(4-Chloro-phenyl)-5-(2-chloro-phenyl)-4,5-dihydro-pyrazol-1-yl]-benzothiazole-6-sulfonyl}*N*′-benzylthiourea (7f). Yield = 59.7%, m.p. 183–184 °C, R_f = 0.55, toluene : ethyl acetate : formic acid, 5 : 4 : 1. IR ν_{max} (KBr): 3361, 3233 & 1539 (N–H), 1590 (C=N), 1314 & 1141 cm^{-1} ($SO_2N<$). ^1H NMR (400 MHz, DMSO, δ_{ppm}): 3.59 (1H, dd, J = 5.6 Hz, 17.4 Hz), 4.23 (1H, dd, J = 11.5 Hz, 17.6 Hz), 4.62 (2H, s, CH_2), 6.08 (1H, dd, J = 6.0 Hz, 12.0 Hz), 6.36 (2H, s, NH), 7.16–8.26 (16H, m, Ar). ^{13}C NMR (125 MHz, DMSO, δ_{ppm}): 43.47(C_4), 57.61 (C_5), 63.69 (CH_2), 112.00–132.14 (C aromatic), 154.61 (C=N), 155.48 (N_1–$\underline{C}$ benzothiazolyl), 158.98 (C=S). FAB-MS m/z: 652 $[M + 1]^+$. Elemental analysis: calculated for $C_{30}H_{23}Cl_2N_5O_2S_3$; C = 55.21, H = 3.55, N = 10.73, S = 14.74. Found: C = 55.24, H = 3.58, N = 10.78, S = 14.77.

{2-[3-(4-Chloro-phenyl)-5-(4-chloro-phenyl)-4,5-dihydro-pyrazol-1-yl]-benzothiazole-6-sulfonyl}*N*′-benzylurea (7g). Yield = 65.3%, m.p. 234–235 °C, R_f = 0.55, toluene : ethyl acetate : formic acid, 5 : 4 : 1. IR ν_{max} (KBr): 3362, 3240 & 1545 (N–H), 1601 (C=N), 1320 & 1149 cm^{-1} ($SO_2N<$). ^1H NMR (400 MHz, DMSO, δ_{ppm}): 3.68 (1H, dd, J = 6.0 Hz, 18.0 Hz), 4.15 (1H, dd, J = 12.2 Hz, 18.0 Hz), 4.67 (2H, s, CH_2), 5.87 (1H, dd, J = 5.6 Hz, 12.0 Hz), 6.09 (2H, s, NH), 7.02–8.01 (16H, m, Ar). ^{13}C NMR (125 MHz, DMSO, δ_{ppm}): 44.12 (C_4), 54.18 ($\underline{C}H_2$), 63.25 (C_5), 110.50–125.98 (C aromatic), 153.48 (C=N), 155.74 (N_1–$\underline{C}$ benzothiazolyl), 158.16 (C=O). FAB-MS m/z: 636 $[M + 1]^+$. Elemental analysis: calculated for $C_{30}H_{23}Cl_2N_5O_3S_2$; C = 56.60, H = 3.64, N = 11.00, S = 10.07. Found: C = 56.62, H = 3.69, N = 11.11, S = 10.08.

{2-[3-(4-Chloro-phenyl)-5-(4-chloro-phenyl)-4,5-dihydro-pyrazol-1-yl]-benzothiazole-6-sulfonyl}*N*′-benzylthiourea (7h). Yield = 61.3%, m.p. 168–169 °C, R_f = 0.57, toluene : ethyl acetate : formic acid, 5 : 4 : 1. IR ν_{max} (KBr): 3362, 3241 & 1545 (N–H), 1595 (C═N), 1318 & 1151 cm^{-1} ($SO_2N<$). ^1H NMR (400 MHz, DMSO, δ_{ppm}): 3.69 (1H, dd, J = 5.6 Hz, 18.0 Hz), 4.14 (1H, dd, J = 12.0 Hz, 17.4 Hz), 4.61 (2H, s, CH_2), 5.89 (1H, dd, J = 6.0 Hz, 12.0 Hz), 6.93 (2H, s, NH), 6.94–8.16 (16H, m, Ar). ^{13}C NMR (125 MHz, DMSO, δ_{ppm}): 43.59 (C_4), 57.36 (C_5), 63.42 (CH_2), 112.14–132.56 (C aromatic), 155.72 (C═N), 156.39 (N_1–C benzothiazolyl), 158.64 (C═S). FAB-MS m/z: 652 $[M + 1]^+$. Elemental analysis: calculated for $C_{30}H_{23}Cl_2N_5O_2S_3$; C = 55.21, H = 3.55, N = 10.73, S = 14.74. Found: C = 55.27, H = 3.61, N = 10.75, S = 14.79.

{2-[3-(4-Chloro-phenyl)-5-phenyl-4,5-dihydro-pyrazol-1-yl]-benzothiazole-6-sulfonyl}*N*′-benzylurea (7i). Yield = 67.0%, m.p. 161–162 °C, R_f = 0.54, toluene : ethyl acetate : formic acid, 5 : 4 : 1. IR ν_{max} (KBr): 3367, 3245 & 1541 (N–H), 1596 (C═N), 1323 & 1151 cm^{-1} ($SO_2N<$). ^1H NMR (500 MHz, DMSO, δ_{ppm}): 3.48 (1H, dd, J = 6.5 Hz, 17.0 Hz), 3.95 (1H, dd, J = 11.5 Hz, 17.5 Hz), 4.62 (2H, s, CH_2), 5.87 (2H, s, NH), 6.40 (1H, dd, J = 5.5 Hz, 12.5 Hz), 6.94–8.16 (17H, m, Ar). ^{13}C NMR (125 MHz, DMSO, δ_{ppm}): 43.42 (C_4), 43.98 ($\underline{C}H_2$), 63.94 (C_5), 100.00–135.37 (C aromatic), 153.50 (C═N), 155.85 (N_1–C benzothiazolyl), 158.55 (C═O). FAB-MS m/z: 602 $[M + 1]^+$. Elemental analysis: calculated for $C_{30}H_{24}ClN_5O_3S_2$; C = 59.84, H = 4.02, N = 11.63, S = 10.65. Found: C = 59.88, H = 4.10, N = 11.68, S = 10.67.

{2-[3-(4-Chloro-phenyl)-5-phenyl-4,5-dihydro-pyrazol-1-yl]-benzothiazole-6-sulfonyl}*N*′-benzylthiourea (7j). Yield = 63.7%, m.p. 204–205 °C, R_f = 0.55, toluene : ethyl acetate : formic acid, 5 : 4 : 1. IR ν_{max} (KBr): 3368, 3244 & 1547 (N–H), 1598 (C═N), 1322 & 1152 cm^{-1} ($SO_2N<$). ^1H NMR (400 MHz, DMSO, δ_{ppm}): 3.84 (1H, dd, J = 6.0 Hz, 17.8 Hz), 4.18 (1H, dd, J = 11.2 Hz, 17.4 Hz), 4.66 (2H, s, CH_2), 5.89 (1H, dd, J = 6.0 Hz, 11.6 Hz), 6.18 (2H, s, NH), 7.04–8.01 (17H, m, Ar). ^{13}C NMR (125 MHz, DMSO, δ_{ppm}): 43.59 (C_4), 57.29 (C_5), 63.52 (CH_2), 100.20–132.41 (C aromatic), 154.58 (C═N), 155.45 (N_1–C benzothiazolyl), 158.35 (C═S). FAB-MS m/z: 618 $[M + 1]^+$. Elemental analysis: calculated for $C_{30}H_{24}ClN_5O_2S_3$; C = 58.29, H = 3.91, N = 11.33, S = 15.56. Found: C = 58.35, H = 3.96, N = 11.41, S = 15.62.

{2-[3-(4-Chloro-phenyl)-5-(4-fluoro-phenyl)-4,5-dihydro-pyrazol-1-yl]-benzothiazole-6-sulfonyl}*N*′-benzylurea (7k). Yield = 55.7%, m.p. 158–159 °C, R_f = 0.56, toluene : ethyl acetate : formic acid, 5 : 4 : 1. IR ν_{max} (KBr): 3365, 3242 & 1542 (N–H), 1592 (C═N), 1321 & 1151 cm^{-1} ($SO_2N<$). ^1H NMR (500 MHz, DMSO, δ_{ppm}): 3.60 (1H, dd, J = 5.6 Hz, 17.8 Hz), 4.27 (1H, dd, J = 11.5 Hz, 17.6 Hz), 4.41 (2H, s, CH_2), 5.86 (1H, dd, J = 5.6 Hz, 11.5 Hz), 6.21 (2H, s, NH), 6.94–7.85 (16H, m, Ar). ^{13}C NMR (125 MHz, DMSO, δ_{ppm}): 43.67 (C_4), 59.79 ($\underline{C}H_2$), 63.72 (C_5), 100.00–137.87 (C aromatic), 143.76 (C═N), 155.23 (N_1–C benzothiazolyl), 158.47 (C═O). FAB-MS m/z: 620 $[M + 1]^+$. Elemental analysis: calculated for $C_{30}H_{23}ClFN_5O_3S_2$; C = 58.11, H = 3.74, N = 11.29, S = 10.34. Found: C = 58.20, H = 3.81, N = 11.35, S = 10.38.

{2-[3-(4-Chloro-phenyl)-5-(4-fluoro-phenyl)-4,5-dihydro-pyrazol-1-yl]-benzothiazole-6-sulfonyl}*N*′-benzylthiourea (7l). Yield = 59.4%, m.p. 220–221 °C, R_f = 0.57, toluene : ethyl acetate : formic acid, 5 : 4 : 1. IR ν_{max} (KBr): 3364, 3244 & 1541 (N–H), 1600 (C═N), 1319 & 1151 cm^{-1} ($SO_2N<$). ^1H NMR (400 MHz, DMSO, δ_{ppm}): 3.82 (1H, dd, J = 6.0 Hz, 17.8 Hz), 4.22 (1H, dd, J = 11.6 Hz, 17.2 Hz), 4.67 (2H, s, CH_2), 5.86 (1H, dd, J = 6.0 Hz, 12.0 Hz), 6.17 (2H, s, NH), 7.12–8.94 (16H, m, Ar). ^{13}C NMR (125 MHz, DMSO, δ_{ppm}): 43.95 (C_4), 57.68 (C_5), 63.49 (CH_2), 110.46–132.53 (C aromatic), 154.31 (C═N), 156.40 (N_1–C benzothiazolyl), 158.24 (C═S). FAB-MS m/z: 637 $[M + 1]^+$. Elemental analysis: calculated for $C_{30}H_{23}ClFN_5O_2S_3$; C = 56.64, H = 3.64, N = 11.01, S = 15.12. Found: C = 56.67, H = 3.68, N = 11.04, S = 15.17.

{2-[3-(4-Chloro-phenyl)-5-(4-dimethylamino-phenyl)-4,5-dihydro-pyrazol-1-yl]-benzothiazole-6-sulfonyl}*N*′-benzylurea (7m). Yield = 67.2%, m.p. 268–269 °C, R_f = 0.54, toluene : ethyl acetate : formic acid, 5 : 4 : 1. IR ν_{max} (KBr): 3361, 3238 & 1539 (N–H), 1588 (C═N), 1318 & 1147 cm^{-1} ($SO_2N<$). ^1H NMR (400 MHz, DMSO, δ_{ppm}): 2.87 (6H, s, $N(CH_3)_2$), 3.59 (1H, dd, J = 5.6 Hz, 18.0 Hz), 4.03 (1H, dd, J = 12.2 Hz, 17.6 Hz), 4.68 (2H, s, CH_2), 5.76 (1H, dd, J = 6.0 Hz, 12.0 Hz), 6.23 (2H, s, NH), 7.03–8.52 (16H, m, Ar). ^{13}C NMR (125 MHz, DMSO, δ_{ppm}): 43.26 (C_4), 54.10 ($N(\underline{C}H_3)_2$), 55.36 ($\underline{C}H_2$), 63.40 (C_5), 112.96–130.19 (C aromatic), 150.34 (C═N), 155.24 (N_1–C benzothiazolyl), 159.27 (C═O). FAB-MS m/z: 646 $[M + 1]^+$. Elemental analysis: calculated for $C_{32}H_{29}ClN_6O_3S_2$; C = 59.57, H = 4.53, N = 13.03, S = 9.94. Found: C = 59.61, H = 4.56, N = 13.08, S = 10.02.

{2-[3-(4-Chloro-phenyl)-5-(dimethylamino-phenyl)-4,5-dihydro-pyrazol-1-yl]-benzothiazole-6-sulfonyl}*N*′-benzylthiourea (7n). Yield = 62.3%, m.p. 190–191 °C, R_f = 0.55, toluene : ethyl acetate : formic acid, 5 : 4 : 1. IR ν_{max} (KBr): 3365, 3239 & 1541 (N–H), 1597 (C═N), 1321 & 1150 cm^{-1} ($SO_2N<$). ^1H NMR (400 MHz, DMSO, δ_{ppm}): 2.89 (6H, s, $N(CH_3)_2$), 3.61 (1H, dd, J = 5.2 Hz, 17.0 Hz), 4.12 (1H, dd, J = 12.0 Hz, 17.6 Hz), 5.87 (1H, dd, J = 6.0 Hz, 12.0 Hz), 6.11 (2H, s, NH), 7.08–7.94 (16H, m, Ar). ^{13}C NMR (125 MHz, DMSO, δ_{ppm}): 43.53 (C_4), 54.68 ($N(\underline{C}H_3)_2$), 57.43 (C_5), 63.47 (CH_2), 112.81–134.32 (C aromatic), 154.32 (C═N), 155.76 (N_1–C benzothiazolyl), 158.55 (C═S). FAB-MS m/z: 661 $[M + 1]^+$. Elemental analysis: calculated for $C_{32}H_{29}ClN_6O_2S_3$; C = 58.12, H = 4.42, N = 12.71, S = 14.55. Found: C = 58.16, H = 4.44, N = 12.75, S = 14.60.

{2-[3-(4-Chloro-phenyl)-5-(4-methyl-phenyl)-4,5-dihydro-pyrazol-1-yl]-benzothiazole-6-sulfonyl}*N*′-benzylurea (7o). Yield = 54.2%, m.p. 144–145 °C, R_f = 0.55, toluene : ethyl acetate : formic acid, 5 : 4 : 1. IR ν_{max} (KBr): 3363, 3242 & 1541 (N–H), 1592 (C═N), 1319 & 1154 cm^{-1} ($SO_2N<$). ^1H NMR (400 MHz, DMSO, δ_{ppm}): 3.10 (3H, s, CH_3), 3.41 (1H, dd, J = 6.0 Hz, 17.4 Hz), 3.96 (1H, dd, J = 11.6 Hz, 17.4 Hz), 4.69 (2H, s, CH_2), 5.81 (1H, dd, J = 6.0 Hz, 12.0 Hz), 6.09 (2H, s, NH), 7.04–8.03 (16H, m, Ar). ^{13}C NMR (125 MHz, DMSO, δ_{ppm}): 20.12 (CH_3), 43.61 (C_4), 57.56 (C_5), 64.02 (CH_2), 112.01–132.17 (C aromatic), 154.90 (C═N), 155.95 (N_1–C benzothiazolyl), 159.61 (C═S). FAB-MS m/z: 616 $[M + 1]^+$. Elemental analysis: calculated for $C_{31}H_{26}ClN_5O_3S_2$; C = 60.43, H = 4.25, N = 11.37, S = 10.41. Found: C = 60.49, H = 4.28, N = 11.42, S = 10.45.

{2-[3-(4-Chloro-phenyl)-5-(4-methyl-phenyl)-4,5-dihydro-pyrazol-1-yl]-benzothiazole-6-sulfonyl}*N*′-benzylthiourea (7p). Yield = 57.5%, m.p. 198–199 °C, R_f = 0.55, toluene : ethyl acetate : formic acid, 5 : 4 : 1. IR ν_{max} (KBr): 3364, 3241 & 1542 (N–H), 1592 (C═N), 1318 & 1154 cm^{-1} ($SO_2N<$). ^1H NMR (400 MHz, DMSO, δ_{ppm}): 3.19 (3H, s, CH_3), 3.39 (1H, dd, J = 6.0 Hz, 18.0 Hz),

3.95 (1H, dd, J = 11.6 Hz, 17.6 Hz), 4.75 (2H, s, CH_2), 5.85 (1H, dd, J = 5.6 Hz, 12.0 Hz), 6.25 (2H, s, NH), 7.04–8.05 (16H, m, Ar). ^{13}C NMR (125 MHz, DMSO, δ_{ppm}): 20.32 (CH_3), 43.69 (C_4), 57.41 (C_5), 63.52 (CH_2), 112.21–132.41 (C aromatic), 154.92 (C═N), 155.63 (N_1–C benzothiazolyl), 161.21 (C═S). FAB-MS m/z: 632 $[M + 1]^+$. Elemental analysis: calculated for $C_{31}H_{26}ClN_5O_2S_3$; C = 58.89, H = 4.15, N = 11.08, S = 15.22. Found: C = 58.92, H = 4.22, N = 11.12, S = 15.25.

{2-[3-(4-Bromo-phenyl)-5-(4-methoxy-phenyl)-4,5-dihydro-pyrazol-1-yl]-benzothiazole-6-sulfonyl}*N′*-benzylurea (8a). Yield = 59.8%, m.p. 139–140 °C, R_f = 0.57, toluene : ethyl acetate : formic acid, 5 : 4 : 1. IR ν_{max} (KBr): 3365, 3241 & 1542 (N–H), 1594 (C═N), 1318 & 1149 cm^{-1} ($SO_2N<$). ^{1}H NMR (400 MHz, DMSO, δ_{ppm}): 3.65 (1H, dd, J = 6.0 Hz, 17.4 Hz), 3.83 (3H, s, OCH_3), 4.43 (1H, dd, J = 11.6 Hz, 17.6 Hz), 4.67 (2H, s, CH_2), 5.88 (1H, dd, J = 6.0 Hz, 12.0 Hz), 6.12 (2H, s, NH), 6.99–8.04 (16H, m, Ar). ^{13}C NMR (125 MHz, DMSO, δ_{ppm}): 43.57 (C_4), 57.38 ($\underline{C}H_2$), 63.88 (C_5), 64.53 (OCH_3), 109.36–128.97 (C aromatic), 155.90 (C═N), 158.05 (N_1–C benzothiazolyl), 171.19 (C═O). FAB-MS m/z: 676 $[M + 1]^+$. Elemental analysis: calculated for $C_{31}H_{26}BrN_5O_4S_2$; C = 55.03, H = 3.87, N = 10.35, S = 9.48. Found: C = 55.07, H = 3.90, N = 10.38, S = 9.52.

{2-[3-(4-Bromo-phenyl)-5-(4-methoxy-phenyl)-4,5-dihydro-pyrazol-1-yl]-benzothiazole-6-sulfonyl}*N′*-benzylthiourea (8b). Yield = 67.0%, m.p. 269–270 °C, R_f = 0.57, toluene : ethyl acetate : formic acid, 5 : 4 : 1. IR ν_{max} (KBr): 3363, 3241 & 1542 (N–H), 1594 (C═N), 1320 & 1151 cm^{-1} ($SO_2N<$). ^{1}H NMR (400 MHz, DMSO, δ_{ppm}): 3.67 (1H, dd, J = 5.4 Hz, 17.2 Hz), 4.00 (3H, s, OCH_3), 4.79 (1H, dd, J = 12.0 Hz, 17.6 Hz), 5.98 (1H, dd, J = 6.0 Hz, 12.0 Hz), 6.23 (2H, s, NH), 7.01–8.07 (16H, m, Ar). ^{13}C NMR (125 MHz, DMSO, δ_{ppm}): 43.26 (C_4), 54.64 ($\underline{C}H_2$), 58.15 (C_5), 64.36 (OCH_3), 110.89–132.02 (C aromatic), 155.49 (C═N), 158.37 (N_1–C benzothiazolyl), 159.14 (C═S). FAB-MS m/z: 692 $[M + 1]^+$. Elemental analysis: calculated for $C_{31}H_{26}BrN_5O_3S_3$; C = 53.75, H = 3.78, N = 10.11, S = 13.89. Found: C = 53.77, H = 3.81, N = 10.16, S = 13.92.

{2-[3-(4-Bromo-phenyl)-5-thiophen-2-yl-4,5-dihydro-pyrazol-1-yl]-benzothiazole-6-sulfonyl}*N′*-benzylurea (8c). Yield = 62.3%, m.p. 207–208 °C, R_f = 0.57, toluene : ethyl acetate : formic acid, 5 : 4 : 1. IR ν_{max} (KBr): 3367, 3245 & 1541 (N–H), 1591 (C═N), 1321 & 1151 cm^{-1} ($SO_2N<$). ^{1}H NMR (400 MHz, DMSO, δ_{ppm}): 3.68 (1H, dd, J = 5.6 Hz, 17.4 Hz), 4.22 (1H, dd, J = 11.8 Hz, 17.6 Hz), 4.67 (2H, s, CH_2), 5.87 (1H, dd, J = 5.6 Hz, 12.0 Hz), 6.22 (2H, s, NH), 7.06–8.19 (15H, m, Ar). ^{13}C NMR (125 MHz, DMSO, δ_{ppm}): 43.22 (C_4), 48.58 ($\underline{C}H_2$), 63.87 (C_5), 100.09–134.27 (C aromatic), 153.61 (C═N), 155.85 (N_1–C benzothiazolyl), 158.29 (C═O). FAB-MS m/z: 652 $[M + 1]^+$. Elemental analysis: calculated for $C_{28}H_{22}BrN_5O_3S_3$; C = 51.53, H = 3.40, N = 10.73, S = 14.74. Found: C = 51.57, H = 3.46, N = 10.75, S = 14.79.

{2-[3-(4-Bromo-phenyl)-5-thiophen-2-yl-4,5-dihydro-pyrazol-1-yl]-benzothiazole-6-sulfonyl}*N′*-benzylthiourea (8d). Yield = 63.2%, m.p. 190–191 °C, R_f = 0.56, toluene : ethyl acetate : formic acid, 5 : 4 : 1. IR ν_{max} (KBr): 3361, 3239 & 1539 (N–H), 1589 (C═N), 1320 & 1148 cm^{-1} ($SO_2N<$). ^{1}H NMR (500 MHz, DMSO, δ_{ppm}): 3.62 (1H, dd, J = 5.5 Hz, 17.0 Hz), 4.29 (1H, dd, J = 11.5 Hz, 17.0 Hz), 4.67 (2H, s, CH_2), 6.19 (1H, dd, J = 6.0 Hz, 11.5 Hz), 6.47 (2H, s, NH), 6.94–8.37 (15H, m, Ar). ^{13}C NMR (125 MHz, DMSO, δ_{ppm}): 43.67 (C_4), 59.79 (C_5), 63.47 (CH_2), 100.00–137.87 (C aromatic), 154.80 (C═N), 155.23 (N_1–C benzothiazolyl), 178.94 (C═S). FAB-MS m/z: 667 $[M + 1]^+$. Elemental analysis: calculated for $C_{28}H_{22}BrN_5O_2S_4$; C = 50.29, H = 3.32, N = 10.47, S = 19.18. Found: C = 50.25, H = 3.31, N = 10.41, S = 19.22.

{2-[3-(4-Bromo-phenyl)-5-(4-chloro-phenyl)-4,5-dihydro-pyrazol-1-yl]-benzothiazole-6-sulfonyl}*N′*-benzylurea (8e). Yield = 65.3%, m.p. 217–218 °C, R_f = 0.55, toluene : ethyl acetate : formic acid, 5 : 4 : 1. IR ν_{max} (KBr): 3365, 3241 & 1542 (N–H), 1594 (C═N), 1318 & 1149 cm^{-1} ($SO_2N<$). ^{1}H NMR (400 MHz, DMSO, δ_{ppm}): 3.40 (1H, dd, J = 4.8 Hz, 18.0 Hz), 4.13 (1H, dd, J = 10.4 Hz, 17.6 Hz), 4.31 (2H, s, CH_2), 5.91 (1H, dd, J = 6.8 Hz, 11.6 Hz), 6.23 (2H, s, NH), 7.00–7.82 (16H, m, Ar). ^{13}C NMR (125 MHz, DMSO, δ_{ppm}): 43.67 (C_4), 53.99 ($\underline{C}H_2$), 63.58 (C_5), 110.42–128.37 (C aromatic), 153.86 (C═N), 163.17 (N_1–C benzothiazolyl), 168.52 (C═O). FAB-MS m/z: 680 $[M + 1]^+$. Elemental analysis: calculated for $C_{30}H_{23}BrClN_5O_3S_2$; C = 52.91, H = 3.40, N = 10.28, S = 9.42. Found: C = 52.94, H = 3.41, N = 10.31, S = 9.47.

{2-[3-(4-Bromo-phenyl)-5-(4-chloro-phenyl)-4,5-dihydro-pyrazol-1-yl]-benzothiazole-6-sulfonyl}*N′*-benzylthiourea (8f). Yield = 59.8%, m.p. 212–213 °C, R_f = 0.57, toluene : ethyl acetate : formic acid, 5 : 4 : 1. IR ν_{max} (KBr): 3365, 3242 & 1539 (N–H), 1589 (C═N), 1321 & 1152 cm^{-1} ($SO_2N<$). ^{1}H NMR (300 MHz, DMSO, δ_{ppm}): 3.68 (1H, dd, J = 5.1 Hz, 17.1 Hz), 4.16 (1H, dd, J = 11.7 Hz, 17.1 Hz), 4.66 (2H, s, CH_2), 5.85 (1H, dd, J = 4.8 Hz, 12.0 Hz), 6.27 (2H, s, NH), 7.06–8.85 (16H, m, Ar). ^{13}C NMR (125 MHz, DMSO, δ_{ppm}): 43.49 (C_4), 54.72 (C_5), 63.44 (CH_2), 100.01–134.08 (C aromatic), 154.84 (C═N), 155.54 (N_1–C benzothiazolyl), 168.14 (C═S). FAB-MS m/z: 695 $[M + 1]^+$. Elemental analysis: calculated for $C_{30}H_{23}BrClN_5O_2S_3$; C = 51.69, H = 3.33, N = 10.05, S = 13.80. Found: C = 51.72, H = 3.36, N = 10.06, S = 13.83.

{2-[3-(4-Bromo-phenyl)-5-(4-fluoro-phenyl)-4,5-dihydro-pyrazol-1-yl]-benzothiazole-6-sulfonyl}*N′*-benzylurea (8g). Yield = 65.9%, m.p. 163–164 °C, R_f = 0.55, toluene : ethyl acetate : formic acid, 5 : 4 : 1. IR ν_{max} (KBr): 3362, 3239 & 1541 (N–H), 1595 (C═N), 1321 & 1149 cm^{-1} ($SO_2N<$). ^{1}H NMR (300 MHz, DMSO, δ_{ppm}): 3.42 (1H, dd, J = 4.8 Hz, 17.1 Hz), 4.12 (1H, dd, J = 11.7 Hz, 17.6 Hz), 4.66 (2H, s, CH_2), 5.89 (1H, dd, J = 4.8 Hz, 11.7 Hz), 6.04 (2H, s, NH), 7.06–8.88 (16H, m, Ar). ^{13}C NMR (125 MHz, DMSO, δ_{ppm}): 43.56 (C_4), 53.72 ($\underline{C}H_2$), 63.76 (C_5), 112.50–128.42 (C aromatic), 153.65 (C═N), 157.03 (N_1–C benzothiazolyl), 158.95 (C═O). FAB-MS m/z: 664 $[M + 1]^+$. Elemental analysis: calculated for $C_{30}H_{23}BrFN_5O_3S_2$; C = 54.22, H = 3.49, N = 10.54, S = 9.65. Found: C = 54.25, H = 3.47, N = 10.61, S = 9.67.

{2-[3-(4-Bromo-phenyl)-5-(4-fluoro-phenyl)-4,5-dihydro-pyrazol-1-yl]-benzothiazole-6-sulfonyl}*N′*-benzylthiourea (8h). Yield = 65.5%, m.p. 157–158 °C, R_f = 0.57, toluene : ethyl acetate : formic acid, 5 : 4 : 1. IR ν_{max} (KBr): 3365, 3241 & 1542 (N–H), 1594 (C═N), 1318 & 1149 cm^{-1} ($SO_2N<$). ^{1}H NMR (400 MHz, DMSO, δ_{ppm}): 3.58 (1H, dd, J = 5.6 Hz, 18.0 Hz), 4.22 (1H, dd, J = 5.6 Hz, 17.4 Hz), 4.69 (2H, s, CH_2), 5.87 (2H, s, NH), 6.18 (1H, dd, J = 6.4 Hz, 12.0 Hz), 6.98–8.94 (16H, m, Ar). ^{13}C NMR (125 MHz, DMSO, δ_{ppm}): 43.62 (C_4), 57.79 (C_5), 63.49 (CH_2), 112.15–132.43 (C aromatic), 154.81 (C═N), 155.40 (N_1–C benzothiazolyl), 158.72 (C═S). FAB-MS m/z: 680 $[M + 1]^+$. Elemental analysis: calculated for $C_{30}H_{23}BrFN_5O_2S_3$; C = 52.94, H = 3.41, N = 10.29, S = 14.13. Found: C = 52.95, H = 3.46, N = 10.31, S = 14.15.

{2-[3-(4-Bromo-phenyl)-5-(4-dimethylamino-phenyl)-4,5-dihydro-pyrazol-1-yl]-benzothiazole-6-sulfonyl}*N*′-benzylurea (8i). Yield = 62.1%, m.p. 164–165 °C, R_f = 0.56, toluene : ethyl acetate : formic acid, 5 : 4 : 1. IR ν_{max} (KBr): 3362, 3244 & 1541 (N–H), 1592 (C═N), 1319 & 1151 cm^{-1} ($SO_2N<$). ^1H NMR (400 MHz, DMSO, δ_{ppm}): 2.86 (6H, s, $N(CH_3)_2$), 3.61 (1H, dd, J = 5.6 Hz, 17.2 Hz), 4.21 (1H, dd, J = 11.6 Hz, 17.8 Hz), 4.67 (2H, s, CH_2), 5.76 (1H, dd, J = 6.0 Hz, 11.5 Hz), 6.23 (2H, s, NH), 7.03–8.51 (16H, m, Ar). ^{13}C NMR (125 MHz, DMSO, δ_{ppm}): 43.38 (C_4), 54.09 ($\underline{C}H_2$), 56.24 ($N(CH_3)_2$), 63.57 (C_5), 112.30–131.62 (C aromatic), 153.42 (C═N), 155.61 (N_1–$\underline{C}$ benzothiazolyl), 164.11 (C═O). FAB-MS m/z: 689 $[M + 1]^+$. Elemental analysis: calculated for $C_{32}H_{29}BrN_6O_3S_2$; C = 55.73, H = 4.24, N = 12.19, S = 9.30. Found: C = 55.76, H = 4.29, N = 12.21, S = 9.32.

{2-[3-(4-Bromo-phenyl)-5-(4-dimethylamino-phenyl)-4,5-dihydro-pyrazol-1-yl]-benzothiazole-6-sulfonyl}*N*′-benzylthiourea (8j). Yield = 62.4%, m.p. 211–212 °C, R_f = 0.56, toluene : ethyl acetate : formic acid, 5 : 4 : 1. IR ν_{max} (KBr): 3365, 3241 & 1542 (N–H), 1594 (C═N), 1318 & 1149 cm^{-1} ($SO_2N<$). ^1H NMR (400 MHz, DMSO, δ_{ppm}): 2.87 (6H, s, $N(CH_3)_2$), 3.61 (1H, dd, J = 5.5 Hz, 18.0 Hz), 4.23 (1H, dd, J = 12.0 Hz, 17.2 Hz), 4.66 (2H, s, CH_2), 5.76 (1H, dd, J = 5.6 Hz, 11.8 Hz), 6.29 (2H, s, NH), 7.01–8.05 (16H, m, Ar). ^{13}C NMR (125 MHz, DMSO, δ_{ppm}): 43.62 (C_4), 54.31 ($N(\underline{C}H_3)_2$), 57.79 (C_5), 63.49 (CH_2), 112.21–132.43 (C aromatic), 154.81 (C═N), 155.40 (N_1–$\underline{C}$ benzothiazolyl), 158.99 (C═S). FAB-MS m/z: 705 $[M + 1]^+$. Elemental analysis: calculated for $C_{32}H_{29}BrN_6O_2S_3$; C = 54.46, H = 4.14, N = 11.91, S = 13.63. Found: C = 54.50, H = 4.16, N = 11.94, S = 13.68.

{2-[3-(4-Bromo-phenyl)-5-(4-methyl-phenyl)-4,5-dihydro-pyrazol-1-yl]-benzothiazole-6-sulfonyl}*N*′-benzylurea (8k). Yield = 61.0%, m.p. 225–226 °C, R_f = 0.55, toluene : ethyl acetate : formic acid, 5 : 4 : 1. IR ν_{max} (KBr): 3362, 3242 & 1540 (N–H), 1591 (C═N), 1319 & 1147 cm^{-1} ($SO_2N<$). ^1H NMR (400 MHz, DMSO, δ_{ppm}): 2.81 (3H, s, CH_3), 3.40 (1H, dd, J = 6.0 Hz, 17.2 Hz), 4.11 (1H, dd, J = 11.6 Hz, 18.0 Hz), 4.67 (2H, s, CH_2), 5.90 (1H, dd, J = 6.0 Hz, 12.4 Hz), 6.19 (2H, s, NH), 7.07–8.43 (16H, m, Ar). ^{13}C NMR (125 MHz, DMSO, δ_{ppm}): 21.11 (CH_3), 43.56 (C_4), 58.16 ($\underline{C}H_2$), 63.49 (C_5), 121.05–138.40 (C aromatic), 155.71 (C═N), 158.45 (N_1–$\underline{C}$ benzothiazolyl), 164.41 (C═O). FAB-MS m/z: 660 $[M + 1]^+$. Elemental analysis: calculated for $C_{31}H_{26}BrN_5O_3S_2$; C = 56.36, H = 3.97, N = 10.60, S = 9.71. Found: C = 56.38, H = 3.99, N = 10.61, S = 9.76.

{2-[3-(4-Bromo-phenyl)-5-(4-methyl-phenyl)-4,5-dihydro-pyrazol-1-yl]-benzothiazole-6-sulfonyl}*N*′-benzylthiourea (8l). Yield = 59.8%, m.p. 221–222 °C, R_f = 0.56, toluene : ethyl acetate : formic acid, 5 : 4 : 1. IR ν_{max} (KBr): 3365, 3240 & 1542 (N–H), 1591 (C═N), 1321 & 1149 cm^{-1} ($SO_2N<$). ^1H NMR (400 MHz, DMSO, δ_{ppm}): 3.11 (3H, s, CH_3), 3.86 (1H, dd, J = 6.4 Hz, 17.6 Hz), 4.62 (1H, dd, J = 11.5 Hz, 17.2 Hz), 4.71 (2H, s, CH_2), 5.86 (1H, dd, J = 5.5 Hz, 12.0 Hz), 6.28 (2H, s, NH), 7.02–8.01 (16H, m, Ar). ^{13}C NMR (75 MHz, DMSO, δ_{ppm}): 19.08 (CH_3), 43.65 (C_4), 47.95 (C_5), 63.21 (CH_2), 119.50–137.75 (C aromatic), 156.12 (C═N), 165.81 (N_1–$\underline{C}$ benzothiazolyl), 178.94 (C═S). FAB-MS m/z: 676 $[M + 1]^+$. Elemental analysis: calculated for $C_{31}H_{26}BrN_5O_2S_3$; C = 55.02, H = 3.87, N = 10.35, S = 14.22. Found: C = 55.05, H = 3.91, N = 10.41, S = 14.27.

Biological experiment

Animals

Male rats of Wistar strain[16] were obtained from the Central Animal house at Hamdard University, New Delhi. Animals were housed at an ambient temperature (25 ± 2 °C) with *ad libitum* access to water and food. The experiments were performed in compliance with Institutional Animals Ethics Committee guidelines, approved by the Institutional Animal Ethics Committee of the University, Jamia Hamdard, New Delhi, India (Registration No. 757-CPSCEA).

In vivo antidiabetic activity by the OGTT

All the synthesized compounds (**7a–p** and **8a–l**) were evaluated for oral glucose tolerance by the earlier reported method.[17] Overnight fasted animals (130–180 g) were randomly divided into thirty one groups of five rats. The control group comprising healthy rats was administered with the vehicle (10% carboxymethylcellulose) only. Two groups of animals were administered with standard drugs glibenclamide (30 mg kg^{-1} b.w.) and rosiglitazone (36 mg kg^{-1} b.w.). An oral dose of 30 mg kg^{-1} b.w. synthesized compounds suspended in 10% CMC was given to the remaining animal groups. Post 30 minutes of dosing, 3 g kg^{-1} b.w. glucose solution was given to all animals. Blood samples were withdrawn from retro-orbital plexus just prior to glucose load and after 30 and 90 min. The working solution of each sample was prepared and quantified according to the manufacturer's instructions in the GOD-POD kit.[18]

Docking studies

The crystal structure of PPAR-γ protein (accession number 3CS8) was downloaded from the protein data bank.[19] The structure of the PPAR-γ receptor contains two identical monomeric chains (A & B). For the ease of understanding, one of the chains (chain B) was eliminated while the water molecules in close proximity of ligands were retained. 3D-structures of proteins and ligands were prepared using the LigPrep module and energy minimization was carried out using the OPLS 2005 force field in Schrondinger software. The chiralities of ligands were maintained during this step. The minimized reference protein was used to generate a grid which was used to dock new ligands. Docking studies on the low energy state of ligands were performed using Glide software with extra precision. The glide score and ligand properties of all the ligands were inscribed by XP descriptor information.

In vitro PPAR transactivation assay

Human embryonic kidney (HEK 293) cells were cultured in DMEM containing 10% FBS at 37 °C in a 5% CO_2 humidified atmosphere.[20] When 70–80% cell confluency was obtained, cells were inoculated in a 96 well plate containing approximately 70 000 cells per well. Transfection of cells was done with 2.5 μL of PPRE-Luc, 6.67 μL of PPAR-γ, 1.0 μL of renilla and 20 μL of lipofectamine. Test compounds (10 μM) were added to cells for 24 h after 5 h of transfection. Cells were collected with the help of lysis buffer and luciferase activity was monitored on the luminometer by using a luciferase assay kit. The manufacturer's instructions were followed while using the luciferase kit. Glibenclamide and rosiglitazone were taken as reference drugs.

In vivo antidiabetic activity on the diabetic model

A fresh solution of streptozotocin (45 mg kg^{-1} b.w.) in citrate buffer (pH 4.5, 0.1 M) was administered intraperitoneally to prepare a diabetic model. The blood glucose was monitored daily to ensure the progression of diabetes. Animals having blood glucose levels more than 250 mg dL^{-1} were divided into thirty two groups. Apart from these animals, a group of healthy rats comprised a control group and was administered with the vehicle only. The diabetic control group was also given 10% CMC only. Two groups of animals marked as standard groups were treated with 30 mg kg^{-1} b.w. glibenclamide and 36 mg kg^{-1} b.w. rosiglitazone. Remaining groups were orally administered with 30 mg kg^{-1} b.w. of synthesized compounds. The same dosing pattern was followed for 15 days and plasma glucose concentration of blood samples was estimated on 0, 7, and 15th day post-dose administration by the GOD-POD method.

Body weight

Once diabetes was established in animals after induction of STZ, body weights of all the animals were recorded. Then on the final day of study *i.e.*, on 15th day the body weight of groups treated with standard drugs and active compounds was measured. The change in body weight was studied against the control group to understand the effect of treatment on weight.

Gene expression

3T3-L1 cells (American Type Culture Collection) were seeded in a 24 well plate using DMEM containing 10% calf serum (Invitrogen). The mixture was incubated for 24 h to attain 70% confluency. Cells were treated with 10 μM of compound **7j**, 10 μM standards (glibenclamide and pioglitazone) as positive control and DMSO as negative control and were again incubated for 24 h at 37 °C and 5% CO_2. Cells were transferred to 1.5 ml micro centrifuge tubes after 24 h and the total RNA was isolated by TRI Reagent® (Molecular Research Centre). The quantity and quality of RNA were determined on a NanoDrop ND-2000c spectrophotometer and integrity was verified on a 1.5% agarose gel. The total RNA (1 μg) so obtained was used to generate cDNA using an EZ-first strand cDNA synthesis kit for RT (reverse transcription)-PCR (Biological Industries). Pearl Primer software was used to design primers of PPAR-γ and β-actin for real-time PCR. 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.[21] Real-time PCR was performed in triplicate on an ABI Prism 7300 Sequence Detection System (Applied Biosystems) using the SYBR Green PCR Master Mix (Applied Biosystems). PCR 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.

Conflict of interest

The authors have no conflict of interest.

Acknowledgements

The authors wish to express their thanks to Dr G. N. Qazi, Vice Chancellor, Jamia Hamdard for providing necessary research facilities The authors are thankful to Dr Javed Iqbal, former Director, Institute of Life Sciences (ILS), Hyderabad, for providing necessary *in vitro* facilities and Dr Parimal Misra for his help in performing *in vitro* studies. CK is also thankful to University Grant Commission for providing financial assistance.

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