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Original article

Synthesis, glucose uptake activity and structure–activity relationships of some novel glitazones incorporated with glycine, aromatic and alicyclic amine moieties via two carbon acyl linker

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ABSTRACT

Three series of novel glitazones were designed and prepared by using appropriate synthetic schemes to incorporate glycine, aromatic and alicyclic amines via two carbon linker. Compounds were synthesized both under conventional and microwave methods. Nineteen out of twenty four synthesized compounds were evaluated for their *in vitro* glucose uptake activity using isolated rat hemi-diaphragm. Compounds, **6**, **9a**, **13a**, **13b**, **13c**, **13f** and **13h** exhibited significant glucose uptake activity. Illustration about their synthesis and *in vitro* glucose uptake activity is described along with the structure–activity relationships.

1. Introduction

Type 2 diabetes is a non-insulin dependent type of diabetes mellitus (NIDDM) characterized by high blood glucose levels due to impaired insulin action [1]. Insulin resistance in skeletal muscle, adipose tissue and liver is an important distinctive feature of NIDDM and is also a contributing factor in atherosclerosis, hypertension, lipid disorders and polycystic ovarian syndrome [2]. Increasingly, there is an evidence to suggest that the presence of insulin resistance increases cardiovascular risk in patients with type 2 diabetes [3,4] and this metabolic syndrome is now recognized as a major risk factor for coronary heart diseases [5]. Several studies have shown that decreasing the insulin resistance is an ultimate need for the NIDDM [6–8]. Among the oral hypoglycemics, sulfonylureas are the most commonly used but they have a tendency to induce serious hypoglycemia [9]. However, the non-sulfonylureas do not increase insulin secretion or cause serious hypoglycemia rather they enhance the insulin action which is much required and this has led to the evolution of glitazones as insulin sensitizers [10].

Thiazolidine-2,4-diones (TZD's) have become a pharmacologically important class of heterocyclic compounds since their introduction in the form of glitazones into clinical use for the treatment of type 2 diabetes [11,12]. TZDs are known to be selective agonists of peroxisome proliferator-activated receptor-γ (PPAR-γ), thereby increasing insulin sensitivity at adipose, muscle and hepatic tissues [13,14]. Reports also claim that, TZDs are known to normalize elevated blood glucose, lipid, and insulin levels in rodent models. Some studies have indicated that the TZDs have an impact on different elements of carbohydrate metabolism, thereby may delay or prevent the progression of atherosclerotic disease in patients with type 2 diabetes [13,15–17].

The TZDs are known to exert beneficial effects on lipid abnormalities, endothelial function, hemostasis and inflammation [18]. TZDs are also known to lower blood pressure thus reduce chances of heart failure and microalbuminuria in patients with type 2 diabetes [19]. TZDs are known to cause weight gain, hepatotoxicity and fluid retention [20], these side effects are more common during combination therapy with sulfonylureas or insulin [20,21]. Fluid retention associated with TZDs may result in mild to moderate dose-related edema and the incidence of edema increases in combination with insulin therapy [22,23]. Therefore, TZDs are not recommended for use in class III or IV heart failure patients at New

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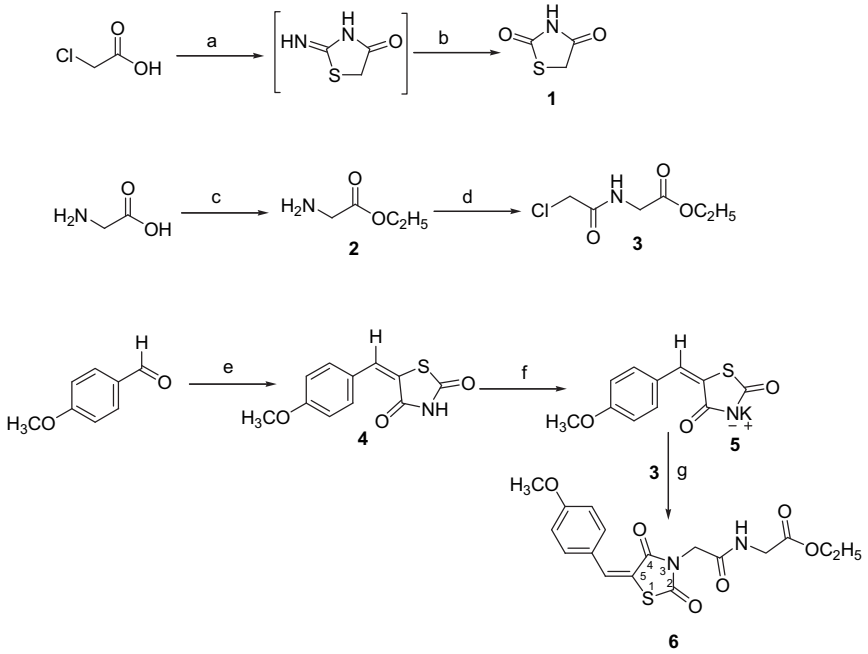
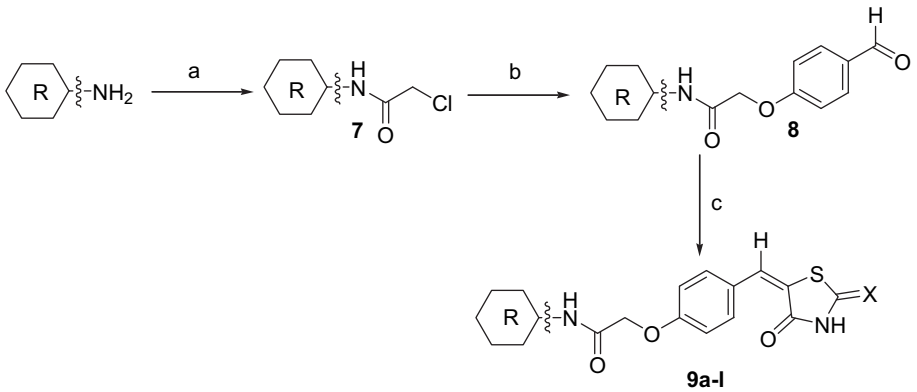


Scheme 1. Reaction protocol for the synthesis of glycine incorporated glitazone (**6**). Reagents and conditions: (a) Water, thiourea, stirred at 0–5 °C, 20 min (b) Conc. HCl, reflux, 10–12 h. (c) Ethanol, $SOCl_2$, stirred at 0–5 °C for 5–6 h and reflux for 1 h; (d) $CHCl_3$, triethyl amine, chloroacetyl chloride, 0–5 °C, stirred, 12–15 h; (e) Dry toluene, TZD **1**, piperidine, acetic acid, reflux at 110 °C, 10–14 h (MW at 700 W for 20–30 min); (f) C_2H_5OH, KOH, stirred, 1 h; (g) DMF, **3**, stirred with reflux, 96 h.

York Heart Association, USA [22,23]. Troglitazone, the first marketed TZD was recalled from the market because of its increased risk of hepatotoxicity [24]. However, drugs such as rosiglitazone and pioglitazone are still in the market with a regular monitoring [25,26]. In light of these beneficial and risk factors associated with the glitazones, herein, we have made an attempt to design, synthesize and evaluate some novel glitazones with structural diversity for their possible antidiabetic activity.

2. Chemistry

Glitazones are known to have adverse side effects by producing toxic metabolites in the body after their metabolism [27]. As there are no reports so far about glitazones which are incorporated with natural substrates like amino acids, therefore, we have made an attempt to incorporate glycine (a simple, natural and polar amino acid) in glitazone structure according to Scheme 1. First, the basic nucleus thiazolidinedione **1** was obtained by reacting equimolar amounts of thiourea and chloroacetic acid. The *C*-terminal of glycine was blocked by converting it to the ethyl ester **2** rather the methyl ester. As the ethyl ester will release a relatively safer ethyl alcohol after its hydrolysis during first pass metabolism in the body. After that, *N*-terminal was extended by acylation reaction with chloroacetyl chloride (a two carbon acyl linker) to obtain **3** [28]. Simultaneously, the TZD was subjected to the Knoevenagel condensation with an anisaldehyde to obtain corresponding 5-benzylidene thiazolidine-2,4-dione **4**. The acidic NH group of TZD ring of compound **4** was deprotonated by transforming it to the potassium salt **5**. Later, the chloride terminal of **3** was coupled to the compound **5** to get the final compound **6** with a poor yield of 17%. Due to the poor yield, this synthetic route lacks its scope to generate diverse glitazones containing natural substrates like amino acids and thus needs an alternative strategy.



Scheme 2. Reaction protocol for the synthesis of compounds **9a–l**. Reagents and conditions: (a) $CHCl_3$, triethyl amine, chloroacetyl chloride, stirred at 0–5 °C, 2 h; (b) Dry acetone, 4-hydroxybenzalaldehyde, anhydrous K_2CO_3, stirred at rt, 72 h; (c) Dry toluene, thiazolidinedione or rhodanine, piperidine, acetic acid, reflux at 110 °C for 10–14 h or MW at 700 W for 20–30 min.

From our previous studies, we have learned that glitazones normally need to possess a polar thiazolidinedione ring system as their head followed by benzyloxy moiety as their trunk which in turn connected to the hydrophobic tail via a two carbon acyl linker for exhibiting better antidiabetic activity [28]. Therefore, we adopted Scheme 2 to synthesize twelve new compounds (**9a**–**l**). The tail part was first designed and synthesized by connecting various aromatic/alicyclic amines to the two carbon acyl linker. The acylated amines **7** were then connected to the hydroxyl group of *p*-hydroxy benzaldehyde to obtain **8**. The motive for us to select *p*-hydroxy benzaldehyde here was, in the past we had found some similar kind of glitazones containing vanillin as part of their trunk with potential antidiabetic activity [29]. The hydrophobic trunk was connected to the hydrophobic tail via two carbon acyl linker and later aldehyde functional was condensed on to the head group which may be a thiazolidinedione **1** or its bioisostere rhodanine (Fig. 1) to obtain compounds **9a**–**l** by Knoevenagel condensation reaction.

Compounds **13a**–**k** were synthesized according to Scheme 3. These glitazones are structurally similar to compound **6**, however, polar glycine part of the structure is replaced with hydrophobic aniline. Aniline was acylated with two acyl carbon linker to form 2-chloro-*N*-phenylacetamide **10** [28,29]. The potassium salt of thiazolidinedione **11** was coupled to the halide terminal of **10** to get 2-(2,4-dioxothiazolidin-3-yl)-*N*-phenylacetamide **12**. In the final step, compound **12** was subjected to the Knoevenagel condensation with various aryl aldehydes to get compounds **13a**–**k**.

Knoevenagel condensation reactions were performed both under microwave and conventional methods using toluene as the hydrophobic solvent. Microwave method showed better results in terms of reaction time and yields when compared to the conventional method (Table 1).

Structures of all the synthesized compounds were confirmed by IR, ^{1}H NMR, ^{13}C NMR and Mass spectral analyses. IR spectrum of all the final compounds, showed characteristic peaks for N–H stretching in the range of 3000–3400 cm^{-1} and C=O stretching in the range of 1685–1740 cm^{-1} to confirm the presence of thiazolidinedione or rhodanine ring system. ^{1}H NMR showed a characteristic broad singlet peak in the range of 12.5–13.9 δ ppm to confirm the presence of NH proton of thiazolidinedione or rhodanine scaffolds in compounds **9a**–**l**. This large deshielding effect on NH proton is attributed to the presence of electron withdrawing carbonyl groups. The compounds obtained according to Schemes 2 and 3 (**13a**–**k**), showed a characteristic peak for CH_2 between 4.5 and 4.8 δ ppm. In principle two geometrical isomers, namely, *E* and *Z* are possible for all the Knoevenagel condensed products. However, all the compounds exhibit only the *Z* configuration as expected from our previous studies because in ^{1}H NMR spectra, peak for =CH was found unusually between 7.7 and 8.2 δ ppm. ^{13}C NMR spectra showed signal for =CH in Knoevenagel condensed products at about 132 δ ppm. The reason for this deshielding is attributed to the *cis* position of the carbonyl function of highly electronegative TZD or 2-thioxo-thiazolidine-4-one ring to the =CH and hence the *Z* configuration. *Cis* positioning is due to the high degree of thermodynamic stability of these compounds because of the intramolecular hydrogen bond that can be formed between the hydrogen atom of =CH and the oxygen atom in TZD or 2-thioxo-thiazolidine-4-one [29].

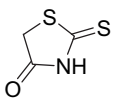


Fig. 1. Structure of 2-thioxo-thiazolidine-4-one or rhodanine.

3. Pharmacology

3.1. Glucose uptake activity

Antidiabetic activity of the compounds **6**, **9a**, **9b**, **9g**–**l**, **13a**–**i** and **13k** were evaluated by measuring glucose uptake using *in vitro* rat hemi-diaphragm model according to the standard procedure reported by Walaas and Chattopadhyay [30,31]. The institutional animal ethics committee (IAEC) of JSS College of Pharmacy approved the proposal. Rat diaphragm was selected because striated muscle is quantitatively the most important tissue for glucose disposal in the body [32–34]. Insulin stimulates glucose uptake by causing translocation of the transporters from an intracellular site to the plasma membrane. In muscles, two types of glucose transporter iso-forms are expressed, namely, GLUT1 and GLUT4. The latter is quantitatively more abundant in adult rat muscle and is distributed in intracellular compartments of basal state, from here it will be rapidly translocated to the plasma membrane in response to the insulin. Glucose uptake was measured in mg/dl/45 min at the dose of 1 mg and 2 mg both in the absence and presence of insulin in triplicates. The results are expressed as mean ± standard error of mean (SEM) and are shown in Table 2.

3.2. Statistics

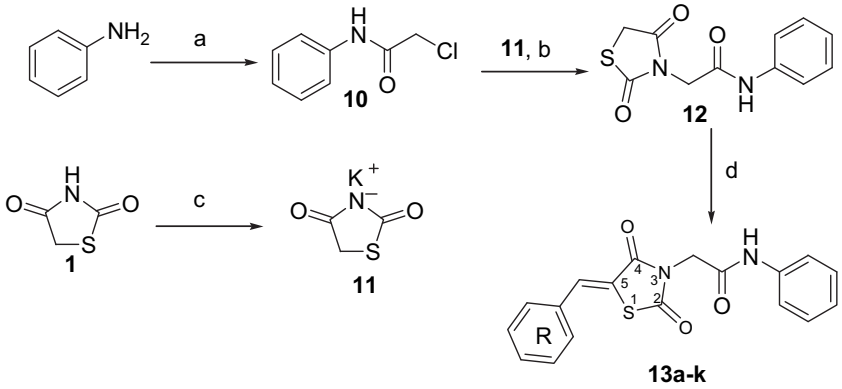
Statistical comparisons between the groups were performed on all the four sets using one-way ANOVA and Dunnet's multiple comparison post-test on graphPad Prism 4.0 software for Windows (San Diego, California, USA).

4. Results and discussion

In continuation with our earlier work on glitazones and with certain rationale some novel glitazones have been designed manually, synthesized, analyzed and evaluated for their glucose uptake activity. The results in Table 2 indicate that, most of the compounds exhibit glucose uptake activity ranging from good to moderate and from moderate to weak. The results also indicate that increasing concentration of test compound beyond 1 mg did not produce any significant increase in the glucose uptake by the diaphragm both in presence and absence of insulin.

Comparatively, compounds exhibited good glucose uptake activity in presence of insulin proving that they are insulin sensitizers. It is quite evident, therefore, that this class of compounds tend to sensitize the tissue cells to take up the insulin which later leads to the glucose utilization by the cells [35–37]. Compounds **13a**, **13b**, **13c**, **6**, **9a**, **13f** and **13h** exhibited significant glucose uptake activity amongst the compounds screened. However, rosiglitazone, a standard drug exhibited highest glucose uptake activity indicating its supremacy over the title compounds. Compounds **13d**, **13g**, **9j**, **9b** and **9i** have exhibited moderate glucose uptake activity. Whereas, rest of the compounds exhibited weak glucose uptake activity. Compound **13e** alone failed to show any glucose uptake activity both in presence and absence of insulin.

The structure–activity relationships for the screened compounds are as follows. 5-Benzylidene-1,3-thiazolidine ring system is found to be the pharmacophore structural part as all most all the compounds possessed this substructure. Figs. 2 and 3 represent energy minimized and aligned structures of glitazones from the present and previous study, respectively [29]. Glitazones with polar thiazolidine-2,4-dione as their head group produced good activity when compared to their corresponding bioisoteres, 2-thioxo-thiazolidine-4-ones **9b**, **9d**, **9f**, **9h**, **9j** and **9k**. Compound **13a**, structurally containing 4-methoxy benzylidene ring connected to **12** showed maximum glucose uptake activity amongst all the compounds



Scheme 3. Reaction protocol for the synthesis of compounds **13a–k**. Reagents and conditions: (a) $CHCl_3$, triethyl amine, chloroacetyl chloride, 0–5 °C, stirred for 2 h; (b) DMF, **11**, stirred with reflux, 90 h; (c) C_2H_5OH, KOH, stirred, 1 h; (d) Dry toluene, substituted aldehydes, piperidine, acetic acid, reflux at 110 °C for 10–14 h or MW at 700 W for 20–30 min.

reported. Compound **6**, with similar substitution at the 5th position of TZD ring and ethyl ester of glycine connected to the 3rd position of TZD exhibits glucose uptake activity closer to that of **13a**. Compounds belonging to the same series **13b**, **13c**, **13f** and **13h** with other hydrophobic groups as their tail also produced significant glucose uptake activity. Decreasing ring size from six membered benzene to the five membered furan (**13i**) found to reduce the activity. Electron donating hydroxyl group at *ortho* position over the benzylidene ring in compound **13c** showed good activity. However, surprisingly, combination of hydroxyl group and methoxy groups at *meta* and *para* positions in compound **13k** reduced glucose uptake activity. Compound **13e**, alone from this series failed to produce any glucose uptake activity with *o*-chloro benzene as its hydrophobic tail.

Compound **9a**, with aniline as its tail alone produced significant glucose uptake activity from its series of compounds having hydrophobic tails connected to the 5-benzylidene structure via a two carbon acyl linker. Rest of the compounds from this series produced moderate glucose uptake activity (**9b**, **9g–l**).

5. Conclusion

New glitazones have been manually designed, synthesized and evaluated for their glucose uptake activity using rat hemi-diaphragm. Among the glitazones investigated, compounds **13a**, **13b**, **13c**, **6**, **13f** and **13h** have exhibited significant glucose uptake activity. Compound **13a** happens to be the most potent of all the glitazones reported here. These results indicate a need for the agonistic study of these compounds toward PPAR-γ and PPAR-α receptors followed by *in vivo* studies to prove their potency and efficacy.

6. Experimental protocols

All the synthetic work was done by procuring required laboratory and analytical grade chemicals, reagents and solvents. Solvents were purified and dried according to the standard procedures. Microwave assisted synthesis was performed using scientific microwave system CATA R from Catalyst Systems (Pune, India). The melting points of the synthesized compounds were determined using VeegoVMP-1 apparatus and are uncorrected. The IR spectra were recorded on Shimadzu FT-IR spectrometer using KBR pellet technique and are expressed in cm^{-1}. ^1H NMR and ^{13}C NMR spectra were recorded on AV-III 400 and 500 MHz spectrometer using DMSO-d_6 as solvent and TMS as internal standard. Mass spectra were recorded using Perkin–Elmer-high resolution mass spectrometer (HRMS) under electro spray ionization technique using time of flight mass analyzer. Auto analyzer (Merck-Microlab 200) was used for the estimation of glucose.

6.1. Chemistry

6.1.1. Synthesis of thiazolidine-2,4-dione (*1*)

Equimolar amounts of chloroacetic acid (0.6 M) and thiourea (0.6 M) were dissolved each in 60 ml of water separately and mixed slowly at 0–5 °C and stirred for 20 min to form a white precipitate of 2-imino-thiazolidine-4-one. Conc. HCl (60 ml) was added and refluxed for about 10–12 h. Reaction was monitored through TLC (Chloroform: Methanol, 9:1). Reaction mixture was allowed to cool to form white solid crystals, washed with water and dried. White crystals, yield 79%, m.p. 123–125 °C; IR (KBr) ν_{max} in cm^{-1}: 3132 (N–H), 1739 (C=O), 1734 (C=O); ^1H NMR (DMSO-d_6, 400 MHz) δ ppm: 4.42 (s, 2H, CH_2), 12.54 (bs, 1H, NH).

6.1.2. Synthesis of ethyl-2-aminoacetate (*2*)

To a suspension of glycine (0.04 M) in 40 ml of ethanol on an ice bath, thionyl chloride (0.16 M) was added drop wise for about 45 min at 0–5 °C and later refluxed for 1 h. Reaction was monitored through TLC by staining the plates with Ninhydrin reagent. The excess of ethanol was distilled off by adding 50 ml of toluene and the residue obtained was dried to get white crystals of **2**.

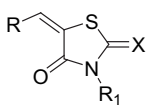
6.1.3. Synthesis of ethyl 2-(2-chloroacetamido) acetate (*3*)

An equimolar solution of ethyl-2-aminoacetate **2** (0.2 M) and triethyl amine (0.2 M) in dry chloroform (50 ml) was stirred at 0–5 °C. Chloroacetyl chloride (0.21 M) was added drop wise for a period of about 30–45 min and later stirred for 24–30 h. The reaction was monitored through TLC by staining the plates with Ninhydrin reagent. After completion of the reaction, solvent was distilled off. The solid obtained was washed with $NaHCO_3$, later with water and dried over anhydrous Na_2SO_4. White crystalline solid, yield 35%; m.p. 45–50 °C; IR (KBr) ν_{max} in cm^{-1}: 3269 (N–H), 1739 (C=O), 1649 (C=O), 717 (C–Cl); ^1H NMR (DMSO-d_6, 500 MHz) δ ppm: 1.30 (t, 3H, CH_3), 3.85 (s, 2H, CH_2), 4.10 (q, 2H, CH_2), 4.19 (s, 2H, CH_2), 8.70 (bs, 1H, NH).

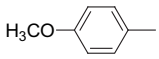
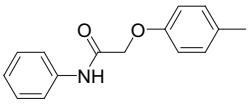
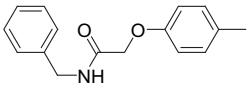
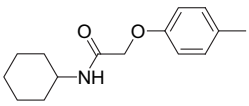
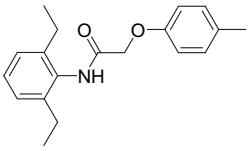
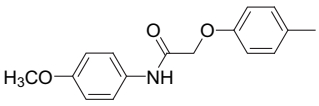
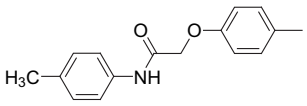
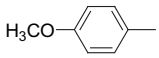
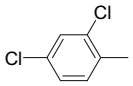
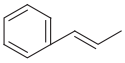
6.1.4. Procedure for the Knoevenagel condensation reaction *4*

To a suspension of thiazolidinedione **1** (0.01 M) in dry toluene, anisaldehyde (0.01 M), catalytic amounts of piperidine (0.0005 M), acetic acid (0.0005 M) and 4–5 dried molecular sieves were added. Under conventional method, the reaction mixture was stirred for about 5 min and refluxed at 110 °C with occasional stirring for 10–14 h. Under microwave method, reaction mixture was stirred for about 5 min and then irradiated with microwaves at 700 W power for about 20–30 min. The reaction was monitored through TLC. The reaction mixture was allowed to cool, precipitate obtained was filtered and recrystallized with aqueous ethanol.

Table 1
Structures of the synthesized glitazones and comparative data for the Knoevenagel condensation reaction under microwave and conventional methods.



Comp	R_1
4	Ethyl 2-acetamidoacetate
9a-l	H
13a-k	*N*-Phenylacetamide

Comp	R	X	Reaction Time		Yield (%)	
			MW(min)	Conv(h)	MW	Conv
6		O	20	10	19	17
9a		O	25	10	85	70
9b		S	20	10	88	72
9c		O	25	12	80	68
9d		S	20	10	82	69
9e		O	30	13	65	50
9f		S	30	13	60	50
9g		O	32	14	55	52
9h		S	30	14	53	50
9i		O	30	10	75	70
9j		S	30	10	78	71
9k		O	30	11	65	50
9l		S	30	13	68	50
13a		O	28	10	79	70
13b		O	30	14	80	75
13c		O	30	10	78	73
13d		O	35	13	68	55

(*continued on next page*)

Table 1 (*continued*)

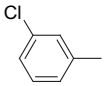
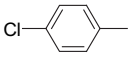
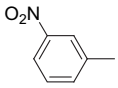
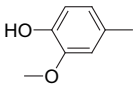
Comp	R	X	Reaction Time		Yield (%)	
			MW(min)	Conv(h)	MW	Conv
13e		O	25	14	65	58
13f		O	25	13	68	60
13g		O	25	12	65	55
13h		O	20	10	78	75
13i		O	30	10	80	72
13j		O	31	12	40	35
13k		O	30	10	74	68

Table 2
Glucose uptake activity of the synthesized glitazones at two different dose levels both in absence and presence of insulin.

S. No.	Comp	Glucose uptake (mg/dl/45 min)			
		No insulin		With insulin	
		1 mg	2 mg	1 mg	2 mg
1	**6**	18.28 ± 1.30**	18.79 ± 0.42**	41.50 ± 4.42**	43.07 ± 2.36**
2	**9a**	18.46 ± 2.16**	18.50 ± 2.22**	41.16 ± 1.30**	42.69 ± 1.25**
3	**9b**	16.16 ± 1.47**	16.33 ± 1.66**	34.66 ± 1.66**	35.33 ± 2.49**
4	**9g**	15.82 ± 3.60*	15.00 ± 3.36	29.00 ± 1.36**	29.80 ± 1.30**
5	**9h**	14.50 ± 1.42	15.66 ± 1.33	28.83 ± 0.30*	28.50 ± 1.52*
6	**9i**	16.66 ± 1.55**	16.83 ± 2.60**	34.16 ± 1.47**	35.66 ± 2.42**
7	**9j**	16.91 ± 1.67**	17.01 ± 0.90**	35.00 ± 1.77**	37.09 ± 0.36**
8	**9k**	16.00 ± 1.73**	16.16 ± 1.30**	33.83 ± 2.47**	33.50 ± 1.50**
9	**9l**	15.33 ± 0.49	16.56 ± 1.30**	33.79 ± 1.30**	34.16 ± 0.47**
10	**13a**	18.71 ± 2.36**	19.66 ± 1.42**	42.16 ± 1.40**	43.90 ± 0.42**
11	**13b**	18.33 ± 1.33**	18.50 ± 2.42**	41.93 ± 2.49**	42.44 ± 1.30**
12	**13c**	18.00 ± 0.36**	18.33 ± 1.21**	41.85 ± 0.40**	42.26 ± 1.33**
13	**13d**	15.00 ± 1.36	16.16 ± 1.30**	36.16 ± 1.47**	36.66 ± 1.33**
14	**13e**	13.81 ± 0.30	14.03 ± 1.60	26.03 ± 1.49	27.16 ± 0.47
15	**13f**	17.83 ± 0.30**	17.63 ± 4.30**	39.00 ± 1.36**	40.500 ± 0.42**
16	**13g**	16.83 ± 1.24**	16.90 ± 1.36**	36.03 ± 1.47**	36.66 ± 1.61**
17	**13h**	17.72 ± 2.30**	18.00 ± 1.25**	38.30 ± 3.49**	39.00 ± 0.25**
18	**13i**	15.66 ± 1.55	15.86 ± 1.30*	32.16 ± 0.70**	32.29 ± 3.55**
19	**13k**	15.50 ± 1.50	16.96 ± 1.47**	28.50 ± 1.34*	30.83 ± 1.65**
20	Std	19.00 ± 2.73**	20.36 ± 1.70**	48.34 ± 4.49**	50.33 ± 5.29**

Group 1(Tyrode): 14.166 ± 0.477; Group 2 (Insulin): 26.51 ± 1.522*; **$p < 0.01$, *$p < 0.05$, Std: Rosiglitazone; Values are mean ± SEM; $n = 3$.

*6.1.5. Procedure for the synthesis of the potassium salt of 5-arylidene-thiazolidine-2,4-diones **5***

To a suspension of 5-arylidene-2,4-thiazolidinedione **4** (0.25 M) in 50 ml of ethanol, potassium hydroxide (15.4 g, 0.275 M) was added and stirred at room temperature for about 1 h and warmed on steam water bath for 10 min. The solid potassium salt of TZD was collected by filtration and dried under vacuum.

*6.1.6. Procedure for the synthesis of (Z)-ethyl-2-(2-(5(4-methoxybenzylidene)-2,4-dioxothiazolidin-3-yl)acetamido)acetate (**6**)*

To a suspension of the potassium salt of 5-arylidene-2,4-thiazolidinediones (0.02 M) in 50 ml of DMF, compound **3** was added slowly with stirring. Reaction mixture was refluxed with continued stirring for about 96 h. Reaction was monitored through TLC. After completion, reaction mixture was poured into ice cold water (500 ml), formed solid product was collected after filtration. The resultant solid **6** was recrystallized using methanol. White amorphous solid, yield 17%; m.p. 238–240 °C; IR (KBr) ν_{max} in cm^{-1}: 3296 (N–H), 3061 (ArC–H), 2953 (AliC–H), 1739 (C=O), 1681 (C=O), 1180 (ester C–O); ^{1}H NMR (DMSO-d_6, 500 MHz) δ ppm: 1.21 (t, 3H, CH_3), 3.83 (s, 3H, CH_3), 3.88 (q, 2H, CH_2), 4.13 (d, 2H, CH_2), 4.32 (s, 2H, CH_2), 7.11 (d, 2H, Ar–H), 7.60 (d, 2H, Ar–H), 7.95 (s, 1H, =CH), 8.70 (bs, 1H, NH); HRMS (ES-TOF) *m/z* found 411.1079 (M + Na), calculated 411.0895 (M + Na).

*6.1.7. Procedure for the acylation of substituted amines (**7**)*

Acylated amines **7** were prepared by following the procedure described for the preparation of compound **3** by taking corresponding substituted amine as a substrate.

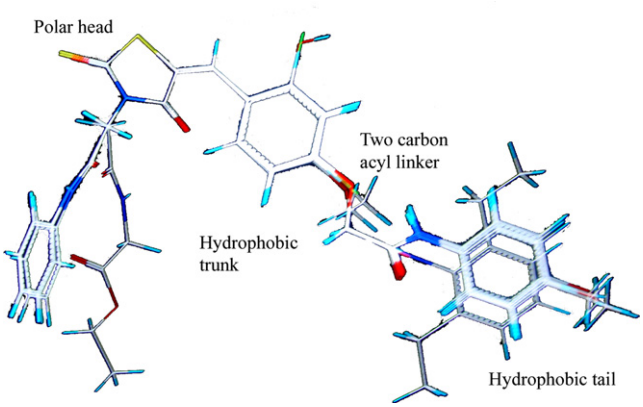


Fig. 2. Glitazones of the present study after energy minimization and alignment using Sybyl 6.7.

*6.1.8. General procedure for connecting various acylated amines to the p-hydroxy benzaldehyde (**8**)*

To a solution of acylated amine **7** (0.02 M) in dry acetone, 4-hydroxy benzaldehyde (0.022 M), and triturated anhydrous K_2CO_3 (0.021 M) were added and stirred at 30–34 °C for about 40–48 h. The reaction was monitored by TLC (pet ether:ethyl acetate:methanol, 7:2:1). Subsequently the reaction mixture was poured into water, extracted with 3 × 20 ml of ethyl acetate, washed with 5% aqueous NaOH and saturated NaCl solutions. The organic layer was evaporated and residue collected was purified from column chromatography using 5% ethyl acetate in pet ether as solvent over silica gel.

*6.1.9. General procedure for the Knoevenagel condensation reactions (**9a**–**l**)*

Compounds **9a**–**l** were prepared by following the procedure for Knoevenagel condensation reaction as described for the synthesis of compound **4**.

*6.1.9.1. (Z)-2-(4-((2,4-Dioxothiazolidin-5-ylidine)methyl)phenoxy)-N-phenylacetamide (**9a**).* Pale brown amorphous solid, yield 70%; m.p. 218–221 °C; IR (KBr) ν_{max} in cm^{-1}: 3387 (N–H), 3144 (N–H), 1739 (C=O), 1685 (C=O), 1176 (ester C–O); ^{1}H NMR (DMSO-d_6, 500 MHz) δ ppm: 4.82 (s, 2H, CH_2), 7.08 (t, 1H, Ar–H), 7.17 (m, 2H, Ar–H), 7.33 (t, 2H, Ar–H), 7.58 (d, 2H, Ar–H), 7.58 (d, 2H, Ar–H), 7.71 (s, 1H, =CH), 10.54 (s, 1H, NH), 12.57 (bs, 1H, thiazolidinedione NH); ^{13}C NMR (DMSO-d_6, 125 MHz) δ ppm: 67.52 (CH_2), 115.65 (=C), 116.05, 120.16, 121.22, 124.22, 126.56, 129.22, 130.58, 130.60, 132.21, 132.47, 138.78, 160.00 (aromatic carbons), 132.12 (=CH), 166.48 (C=O), 167.91 (C=O), 168.43 (C=O); HRMS (ES-TOF) *m/z* found 377.0981 (M + Na), calculated 377.0586 (M + Na).

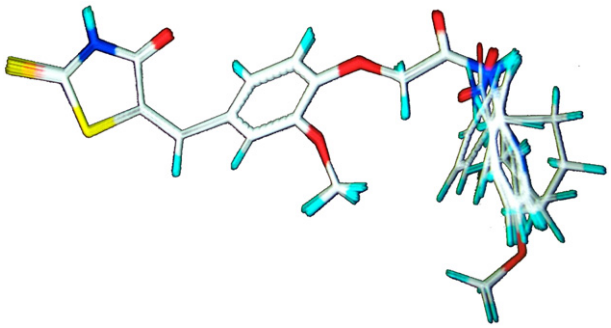


Fig. 3. Glitazones of our previous study after energy minimization and alignment using Sybyl 6.7 [29].

*6.1.9.2. (Z)-2-(4-((4-Oxo-2-thioxothiazolidin-5-ylidine)methyl)phenoxy)-N-phenylacetamide (**9b**).* Yellowish amorphous solid, yield 72%; m.p. 225–228 °C; IR (KBr) ν_{max} in cm^{-1}: 3365 (N–H), 3053 (N–H), 1712 (C=O), 1651 (C=O), 1197 (C=S), 1170 (ester C–O). ^{1}H NMR (DMSO-d_6, 500 MHz) δ ppm: 4.86 (s, 2H, CH_2), 7.08 (t, 1H, Ar–H), 7.17 (d, 2H, Ar–H), 7.33 (t, 2H, Ar–H), 7.62 (m, 4H, Ar–H), 7.60 (s, 1H, C=CH), 10.52 (bs, 1H, NH), 13.70 (bs, 1H, rhodanine NH). HRMS (ES-TOF) *m/z* found 393.0369 (M + Na), calculated 393.0401 (M + Na).

*6.1.9.3. (Z)-N-Benzyl-2-(4-((2,4-dioxothiazolidin-5-ylidine)methyl) phenoxy)acetamide (**9c**).* White amorphous solid, yield 68%; m.p. 230–233 °C; IR (KBr) ν_{max} in cm^{-1}: 3342 (N–H), 3111 (N–H), 1739 (C=O), 1689 (C=O), 1182 (ester C–O). ^{1}H NMR (DMSO-d_6, 500 MHz) δ ppm: 4.43 (d, 2H, CH_2), 4.57 (s, 2H, CH_2), 7.1–7.4 (m, 5H, Ar–H), 7.60 (d, 2H, Ar–H), 7.87 (d, 2H, Ar–H), 7.95 (bt, 1H, NH), 8.74 (s, 1H, =CH), 12.50 (bs, 1H, thiazolidinedione NH); HRMS (ES-TOF) *m/z* found 391.08101 (M + Na), calculated 391.08216 (M + Na).

*6.1.9.4. (Z)-N-Benzyl-2-(4-((4-oxo-2-thioxothiazolidin-5-ylidine) methyl)phenoxy)acetamide (**9d**).* Yellowish amorphous solid, yield 69%; m.p. 235–238 °C; IR (KBr) ν_{max} in cm^{-1}: 3385 (N–H), 3061 (N–H), 1724 (C=O), 1662 (C=O), 1195 (C=S), 1165 (ester C–O); ^{1}H NMR (DMSO-d_6, 500 MHz) δ ppm: 3.64 (d, 2H, CH_2), 4.55 (s, 2H, CH_2), 7.10–7.90 (m, 9H, Ar–H), 7.70 (s, 1H, =CH), 7.95 (d, 1H, NH), 13.70 (bs, 1H, rhodanine NH); HRMS (ES-TOF) *m/z* found 407.0633 (M + Na), calculated 407.0582 (M + Na).

*6.1.9.5. (Z)-N-Cyclohexyl-2-(4-((2,4-dioxothiazolidin-5-ylidine) methyl)phenoxy)acetamide (**9e**).* Pale greenish amorphous solid, m.p. 190–193 °C; IR (KBr) ν_{max} in cm^{-1}: 3294 (N–H), 3061 (N–H), 1737 (C=O), 1685 (C=O), 1178 (ester C–O); ^{1}H NMR (DMSO-d_6, 500 MHz) δ ppm: 1.25 (m, 4H, $2CH_2$), 1.57 (m, 2H, CH_2), 1.71 (m, 4H, $2CH_2$), 3.34 (m, 1H, CH), 4.53 (s, 2H, CH_2), 7.09 (d, 2H, Ar–H), 7.56 (d, 2H, Ar–H), 7.74(s, 1H, =CH), 7.95(d, 1H, NH), 12.47(bs, 1H, thiazolidinedione NH); HRMS (ES-TOF) *m/z* found 383.1325 (M + Na), calculated 383.1204(M + Na).

*6.1.9.6. (Z)-N-Cyclohexyl-2-(4-((4-oxo-2-thioxothiazolidin-5-ylidine) methyl)phenoxy)acetamide (**9f**).* Yellowish amorphous solid, yield 50%; m.p. 205–209 °C; IR (KBr) ν_{max} in cm^{-1}: 3402 (N–H), 3034 (N–H), 1707(C=O), 1654 (C=O), 1195 (C=S), 1172 (ester C–O); ^{1}H NMR (DMSO-d_6, 500 MHz) δ ppm: 1.21 (m, 4H, $2CH_2$), 1.57 (m, 2H, CH_2), 1.70 (m, 4H, $2CH_2$), 3.34 (m, 1H, CH), 4.53 (s, 2H, CH_2), 7.06–7.11 (m, 2H, Ar–H), 7.56–7.61(m, 2H, Ar–H), 7.91 (s, 1H, =CH), 13.7 (bs, 1H, rhodanine NH); HRMS (ES-TOF) *m/z* found 399.0986 (M + Na), calculated 399.1077 (M + Na).

*6.1.9.7. (Z)-N-(2,6-Diethylphenyl)-2-(4-((2,4-dioxothiazolidin-5-ylidine)methyl)phenoxy)acetamide (**9g**).* White amorphous solid, yield 50%; m.p. 229–233 °C; IR (KBr) ν_{max} in cm^{-1}: 3201 (N–H), 3037 (N–H), 1741 (C=O), 1691 (C=O), 1178 (ester C–O); ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 1.22 (m, 6H, $2CH_3$), 2.50 (m, 4H, $2CH_2$), 4.53 (s, 2H, CH_2), 7.09 (d, 2H, Ar–H), 7.61 (d, 2H, Ar–H), 7.69 (d, 2H, Ar–H), 7.78 (s, 1H, =CH), 7.99 (t, 1H, Ar–H), 12.53 (bs, 1H, thiazolidinedione NH); HRMS (ES-TOF) *m/z* found 433.1287 (M + Na), calculated 433.1308 (M + Na).

*6.1.9.8. (Z)-N-(2,6-Diethylphenyl)-2-(4-((4-oxo-2-thioxothiazolidin-5-ylidine)methyl)phenoxy)-acetamide (**9h**).* Yellowish amorphous solid, yield 52%; m.p. 242–245 °C; IR (KBr) ν_{max} in cm^{-1}: 3225 (N–H), 3037 (N–H), 1714 (C=O), 1651 (C=O), 1197 (C=S), 1176 (ester C–O); ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 1.22 (m, 6H, $2CH_3$), 2.53 (m, 4H, $2CH_2$), 4.81 (s, 2H, CH_2), 7.09 (d, 2H, Ar–H), 7.61 (d, 2H, Ar–H), 7.69(d, 2H, Ar–H), 7.78(s, 1H, =CH), 7.99 (t, 1H,

Ar–H), 9.55 (s, 1H, NH), 13.68 (bs, 1H, rhodanine NH). HRMS (ES-TOF) *m*/*z* found 449.1009 (M + Na), calculated 449.1088 (M + Na).

*6.1.9.9. (Z)-2-(4-((4-Oxo-2-thioxothiazolidin-5-ylidine)methyl)phenoxy)-N-(4-methoxyphenyl)-acetamide (**9i**).* Pale brownish amorphous solid, yield 50%; m.p. 245–247 °C; IR (KBr) ν_{max} in cm^{-1}: 3389 (N–H), 3126 (N–H), 3026 (Methoxy OCH_3), 1737 (C=O), 1683 (C=O), 1186 (ester C–O); ^1H NMR (DMSO-d_6, 400 MHz) δ ppm: 3.70 (s, 3H, CH_3), 4.75 (s, 2H, CH_2), 6.90 (d, 2H, Ar–H), 7.25 (d, 1H, Ar–H), 7.05 (d, 1H, Ar–H), 7.15 (d, 2H, Ar–H), 7.51 (d, 1H, Ar–H), 7.19 (d, 1H, Ar–H), 7.75 (s,1H, =CH), 10.0 (s, 1H, NH), 12.5 (bs, 1H, thiazolidinedione NH); HRMS (ES-TOF) *m*/*z* found 407.0401 (M + Na), calculated 407.0518 (M + Na).

*6.1.9.10. (Z)-N-(4-Methoxyphenyl)-2-(4-((4-oxo-2-thioxothiazolidin-5-ylidine)methyl)phenoxy)-acetamide (**9j**).* Yellowish amorphous solid, yield 71%; m.p. 250–253 °C; IR (KBr) ν_{max} in cm^{-1}: 3398 (N–H), 3134 (N–H), 2999 (Methoxy OC–H), 1734 (C=O), 1687 (C=O), 1182 (C=S), 1172 (ester C–O); ^1H NMR (DMSO-d_6, 400 MHz) δ ppm: 3.75 (s, 3H, CH_3), 4.82 (s, 2H, CH_2), 6.91 (d, 2H, Ar–H), 7.25 (d, 2H, Ar–H), 7.05 (d, 2H, Ar–H), 7.15 (d, 2H, Ar–H), 7.21 (s,1H, =CH) 10.0 (s, 1H, NH), 13.7 (bs, 1H, rhodanine NH). HRMS (ES-TOF) *m*/*z* found 423.0486 (M + Na), calculated 423.0459 (M + Na).

*6.1.9.11. (Z)-2-(4-((2,4-Dioxothiazolidin-5-ylidine)methyl)phenoxy)-N-p-tolylacetamide (**9k**).* Pale yellowish amorphous solid, yield 50%; m.p. 235–238 °C; IR (KBr) ν_{max} in cm^{-1}: 3350 (N–H), 3064 (N–H), 1714 (C=O), 1697 (C=O), 1182 (ester C–O); ^1H NMR (DMSO-d_6, 400 MHz) δ ppm: 2.54 (s, 3H, CH_3), 4.50 (s, 2H, CH_2), 7.04–7.15 (m, 4H, Ar–H), 7.39 (s, 1H, =CH), 7.41–7.52 (m,4H, Ar–H), 9.90 (s,1H, NH), 12.52 (bs, 1H, thiazolidinedione NH); HRMS (ES-TOF) *m*/*z* found 391.0699 (M + Na), calculated 391.0715 (M + Na).

*6.1.9.12. (Z)-2-(4-((4-Oxo-2-thioxothiazolidin-5-ylidine)methyl)phenoxy)-N-p-tolylacetamide (**9l**).* Pale brownish amorphous solid, yield 50%; m.p. 239–242 °C; IR (KBr) ν_{max} in cm^{-1}: 3284 (N–H), 3036 (N–H), 1685 (C=O), 1664 (C=O), 1199 (C=S), 1176 (ester C–O). ^1H NMR (DMSO-d_6, 400 MHz) δ ppm: 2.50 (s, 3H, CH_3), 4.51 (s, 2H, CH_2), 7.04–7.20 (m, 4H, Ar–H) 7.39 (s, 1H, =CH), 7.42–7.68 (m, 4H, Ar–H), 9.91 (s, 1H, NH), 13.70 (bs, 1H, rhodanine NH). HRMS (ES-TOF) *m*/*z* found 407.0691 (M + Na), calculated 407.0714 (M + Na).

*6.1.10. Synthesis of 2-chloro-N-phenylacetamide (**10**)*

The compound **10** was prepared following the procedure described for the preparation of compound **3** by taking aniline.

*6.1.11. Synthesis of potassium salt of thiazolidine-2,4-dione (**11**)*

Compound **11** was prepared following the procedure for the preparation of **5** by taking thiazolidine-2,4-dione.

*6.1.12. 2-(2,4-Dioxothiazolidin-4-yl)-N-phenylacetamide (**12**)*

Compound **12** was prepared as per the procedure followed for synthesis of compound **6** by taking **11** in place of potassium salt of 5-arylidene-2,4-thiazolidinediones and **8** in place of **3**. White amorphous solid, yield 55%; IR (KBr) ν_{max} in cm^{-1}: 3259 (Amide NH), 1763 (ring C=O), 1685 (Amide C=O); ^1H NMR (DMSO-d_6, 500 MHz) δ ppm: 4.33 (s, 2H, CH_2), 4.34 (s, 2H, CH_2), 7.07 (t, 1H, Ar–H), 7.32 (t, 2H, Ar–H), 7.54 (d, 2H, Ar–H), 10.31 (s, 1H, NH).

*6.1.13. General procedure for the Knoevenagel condensation of 2-(2,4-dioxothiazolidin-4-yl)-N-phenylacetamide **12** with various aldehydes (**13a–k**)*

Compounds **13a–k** were prepared by following the procedure for Knoevenagel condensation reaction described above for the synthesis of compound **6**.

*6.1.13.1. (Z)-2-(5-(4-Methoxybenzylidine)-2,4-dioxothiazolidin-3-yl)-N-phenylacetamide (**13a**).* Pale brown amorphous solid, yield 70%; m.p. 201–204 °C; IR (KBr) ν_{max} in cm^{-1}: 3300 (N–H), 2929 (C–H), 1741 (C=O), 1685 (C=O), 1180 (ether C–O); ^1H NMR (DMSO-d_6, 500 MHz) δ ppm: 3.84 (s, 3H, CH_3), 4.51 (s, 2H, CH_2), 7.08 (t, 1H, Ar–H), 7.14 (m, 4H, Ar–H), 7.33 (t, 2H, Ar–H), 7.64 (d, 2H, Ar–H), 7.95 (s, 1H, =CH), 10.39 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 125 MHz) δ ppm: 44.45 (CH_2), 56.02 (OCH_3), 118.16 (=C), 124.16 (=CH), 115.51, 115.53, 119.63, 119.65, 125.81, 129.34, 129.36, 132.85, 132.87, 134.07, 138.87, 161.79 (aromatic carbons), 164.31 (C=O), 165.89 (C=O), 167.68 (C=O); HRMS (ES-TOF) *m*/*z* found 391.0694 (M + Na), calculated 391.0797 (M + Na).

*6.1.13.2. (Z)-2-(5-(2,4-Dichlorobenzylidine)-2,4-dioxothiazolidin-3-yl)-N-phenylacetamide (**13b**).* Pale yellow amorphous solid, yield 75%; m.p. 211–213 °C; IR (KBr) ν_{max} in cm^{-1}: 3261 (N–H), 1743 (C=O), 1701 (C=O), 1656 (C=O); ^1H NMR (DMSO-d_6, 400 MHz) δ ppm: 4.50 (s, 2H, CH_2), 7.07 (t, 1H, Ar–H), 7.33 (t, 1H, Ar–H), 7.55 (d, 2H, Ar–H), 7.65 (d, 2H, Ar–H), 7.81 (s, 1H, Ar–H), 8.10 (s, 1H, =CH), 10.42 (s, 1H, NH). HRMS (ES-TOF) *m*/*z* found 428.0018 (M + Na), calculated 427.9991 (M + Na).

*6.1.13.3. (Z)-2-(5-(2-Hydroxybenzylidine)-2,4-dioxothiazolidin-3-yl)-N-phenylacetamide (**13c**).* Yellow amorphous solid, yield 73%; m.p. 235–238 °C; IR (KBr) ν_{max} in cm^{-1}: 3292 (Phenol O–H), 3055 (N–H), 1732 (C=O), 1676 (C=O), 1662 (C=O); ^1H NMR (DMSO-d_6, 400 MHz) δ ppm: 2.9 (s, 1H, OH), 4.5 (s, 2H, CH_2), 6.97 (m, 2H, Ar–H), 7.06 (m, 1H, Ar–H), 7.31 (m, 2H, Ar–H), 7.39 (m, 1H, Ar–H), 7.54 (t, 2H, Ar–H), 7.88 (s, 1H, Ar–H), 8.25 (s, 1H, =CH), 10.4 (s, 1H, NH); HRMS (ES-TOF) *m*/*z* found 377.0583 (M + Na), calculated 377.0679 (M + Na).

*6.1.13.4. (Z)-2(2,4-Dioxo-5-(3-phenylallylidene)thiazolidin-3-yl)-N-phenylacetamide (**13d**).* Yellow amorphous solid, yield 55%; m.p. 197–200 °C; IR (KBr) ν_{max} in cm^{-1}: 3325 (NH), 1741 (C=O), 1689 (C=O), 1666 (C=O); ^1H NMR (DMSO-d_6, 400 MHz) δ ppm: 4.55 (s, 2H, CH_2), 7.15 (d, 1H, =CH), 7.21 (d, 1H, =CH), 7.61 (s, 1H, =CH), 7.65–7.65 (m, 10H, Ar–H), 10.50 (s, 1H, NH). HRMS (ES-TOF) *m*/*z* found 387.0754 (M + Na), calculated 387.0807 (M + Na).

*6.1.13.5. (Z)-2-(5-(2-Chlorobenzylidine)-2,4-dioxothiazolidin-3-yl)-N-phenylacetamide (**13e**).* Pale yellow amorphous solid, yield 58%; m.p. 202–205 °C; IR (KBr) ν_{max} in cm^{-1}: 3273 (N–H), 1753 (C=O), 1703 (C=O), 1666 (C=O); ^1H NMR (DMSO-d_6, 400 MHz) δ ppm: 4.54 (s, 2H, CH_2), 7.07 (t, 1H, Ar–H), 7.33 (t, 2H, Ar–H), 7.55 (d, 2H, Ar–H), 7.65 (d, 2H, Ar–H), 7.88 (t, 2H, Ar–H), 8.11 (s, 1H, =CH), 10.44 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 100 MHz) δ ppm: 44.01 (CH_2), 124.78 (=C), 128.67 (=CH), 119.12, 119.14, 123.67, 128.14, 128.94, 128.99, 130.35, 130.83, 132.14, 132.16, 134.45, 138.31 (ArC), 163.60 (C=O), 164.85 (C=O), 166.79 (C=O). HRMS (ES-TOF) *m*/*z* found 395.0321 (M + Na), calculated 395.0298 (M + Na).

*6.1.13.6. (Z)-2-(5-(3-Chlorobenzylidine)-2,4-dioxothiazolidin-3-yl)-N-phenylacetamide (**13f**).* White amorphous solid, yield 60%; m.p. 207–210 °C; IR (KBr) ν_{max} in cm^{-1}: 3265 (N–H), 1753 (C=O), 1697 (C=O), 1670 (C=O); ^1H NMR (DMSO-d_6, 400 MHz) δ ppm: 4.50 (s, 2H, CH_2), 7.12 (t, 1H, Ar–H), 7.32 (t, 2H, Ar–H), 7.54 (d, 2H, Ar–H), 7.50–7.59 (m, 3H, Ar–H), 7.75 (s, 1H, Ar–H), 7.90 (s, 1H, =CH), 10.41 (s, 1H, NH); HRMS (ES-TOF) *m*/*z* found 395.0330 (M + Na), calculated 395.0298 (M + Na).

*6.1.13.7. (Z)-2-(5-(4-Chlorobenzylidine)-2,4-dioxothiazolidin-3-yl)-N-phenylacetamide (**13g**).* Brown amorphous solid, yield 55%; m.p. 206–208 °C; IR (KBr) ν_{max} in cm^{-1}: 3279 (N–H), 1745 (C=O), 1697 (C=O), 1666 (C=O); ^1H NMR (DMSO-d_6, 400 MHz) δ ppm: 4.52 (s, 2H, CH_2), 7.07 (t, 1H, Ar–H), 7.33 (t, 2H, Ar–H), 7.55 (d, 2H, Ar–H),

7.65 (d, 2H, Ar–H), 7.88 (d, 2H, Ar–H), 7.88 (s, 1H, =CH), 10.4 (s, 1H, NH). HRMS (ES-TOF) *m/z* found 395.0326 (M + Na), calculated 395.0298 (M + Na).

*6.1.13.8. (Z)-2-(5-(Benzylidine)-2,4-dioxothiazolidin-3-yl)-N-phenylacetamide (**13h**).* White amorphous solid, yield 75%; m.p. 202–205 °C; IR (KBr) ν_{max} in cm^{-1}: 3275 (N–H), 1749 (C=O), 1697 (C=O), 1662 (C=O); ^1H NMR (DMSO-d_6, 400 MHz) δ ppm: 4.52 (s, 2H, CH_2), 7.07 (t, 1H, Ar–H), 7.32 (t, 2H, Ar–H), 7.54 (m, 5H, Ar–H), 7.67 (d, 2H, Ar–H), 7.90 (s, 1H, =CH), 10.4 (s, 1H, NH); HRMS (ES-TOF) *m/z* found 361.0561 (M + Na), calculated 361.0759 (M + Na).

*6.1.13.9. (Z)-2-(5-(Furan-2-ylmethylene)-2,4-dioxothiazolidin-3-yl)-N-phenylacetamide (**13i**).* Pale orange amorphous solid, yield 72%; m.p. 213–216 °C; IR (KBr) ν_{max} in cm^{-1}: 3275 (N–H), 1741 (C=O), 1693 (C=O), 1664 (C=O); ^1H NMR (DMSO-d_6, 400 MHz) δ ppm: 4.51 (s, 2H, CH_2), 6.79 (d, 1H, Ar–H), 7.04 (t, 1H, Ar–H), 7.18 (d, 2H, Ar–H), 7.32 (t, 1H, Ar–H), 7.56 (t, 2H, Ar–H), 7.82 (s, 1H, =CH), 8.14 (d,1H, Ar–H), 10.4 (s, 1H, NH); HRMS (ES-TOF) *m/z* found 351.0491 (M + Na), calculated 351.0380 (M + Na).

*6.1.13.10. (Z)-2-(5-(3-Nitrobenzylidene)-2,4-dioxothiazolidin-3-yl)-N-phenylacetamide (**13j**).* Yellow amorphous solid, yield 35%; m.p. >300 °C; IR (KBr) ν_{max} in cm^{-1}: 3271 (N–H), 1763 (C=O), 1696 (C=O), 1682 (C=O); ^1H NMR (DMSO-d_6, 400 MHz) δ ppm: 4.62 (s, 2H, CH_2), 7.24 (t, 1H, Ar–H), 7.43 (t, 2H, Ar–H), 7.61 (d, 2H, Ar–H), 7.69–7.78 (m, 3H, Ar–H), 7.90 (s, 1H, =CH), 7.80 (s, 1H, Ar–H), 10.52 (s, 1H, NH); HRMS (ES-TOF) *m/z* found 406.0614 (M + Na), calculated 406.0576(M + Na).

*6.1.13.11. (Z)-2-(5-(3-Hydroxy-4-methoxybenzylidine)-2,4-dioxothiazolidin-3-yl)-N-phenylacetamide (**13k**).* Yellow amorphous solid, yield 68%; m.p. 232–235 °C; IR (KBr) ν_{max} in cm^{-1}: 3433 (Phenol O–H), 3246 (N–H), 1734 (C=O), 1674(C=O); ^1H NMR (DMSO-d_6, 400 MHz) δ ppm: 3.82 (s, 3H, CH_3), 4.55 (s, 2H, CH_2), 6.95 (d, 2H, Ar–H), 7.07 (t, 1H, Ar–H), 7.15 (t, 1H, Ar–H), 7.26 (s, 1H, Ar–H), 7.31 (t, 1H, Ar–H), 7.54 (d, 2H, Ar–H), 7.90(s, 1H, =CH), 9.9 (bs, 1H, vannilin OH), 10.45 (s, 1H, NH); HRMS (ES-TOF) *m/z* found 407.0742 (M + Na), calculated 407.0795 (M + Na).

6.2. Determination of glucose uptake activity

Glucose uptake measurement: Six well microtitre plates were selected for the study with each well capacity of 5 ml ($n = 3$). Plates were divided into following groups; Group 1: 2 ml of Tyrode solution with 2000 mg/l glucose. Group 2: 2 ml of Tyrode solution with 2000 mg/l glucose and regular insulin (Nova Nardisk, 40 IU/ml) 5 μl containing 0.2 units of insulin. Groups 3–21: 2 ml of Tyrode solution with 2000 mg/l glucose and 1 mg of the test compound **6**, **9a**, **9b**, **9g**–**l**, **13a**–**i** and **13k**. Group 22: 2 ml of Tyrode solution with 2000 mg/l glucose and 1 mg of rosiglitazone (standard). Groups 23–41: 2 ml of Tyrode solution with 2000 mg/l glucose, regular insulin 5 μl containing 0.2 units of insulin and 1 mg of the test compound **6**, **9a**, **9b**, **9g**–**l**, **13a**–**i** and **13k**. Group 42: 2 ml of Tyrode solution with 2000 mg/l glucose, regular insulin 5 μl containing 0.2 units of insulin and 1 mg of rosiglitazone (standard). Groups 43–61: 2 ml of Tyrode solution with 2000 mg/l glucose and 2 mg of the test compound **6**, **9a**, **9b**, **9g**–**l**, **13a**–**i** and **13k**. Group 62: 2 ml of Tyrode solution with 2000 mg/l glucose and 2 mg of rosiglitazone (standard). Groups 63–81: 2 ml of Tyrode solution with 2000 mg/l glucose, regular insulin 5 μl containing 0.2 units of insulin and 2 mg of the test compound **6**, **9a**, **9b**, **9g**–**l**, **13a**–**i** and **13k**. Group 82: 2 ml of Tyrode solution with 2000 mg/l glucose, regular insulin 5 μl containing 0.2 units of insulin and 2 mg of rosiglitazone (standard). Wistar rats of either sex were maintained on a standard pellet diet, water ad libitum, and fasted overnight. The animals were killed by decapitation under ether anaesthesia and diaphragms were taken out swiftly avoiding trauma and divided into two halves and weight was noted. The hemi-diaphragms were then rinsed in cold Tyrode solution (without glucose) to remove any blood clots and quickly transferred to the respective wells. The plates were closed with the lids and incubated for 45 min at 21 °C with shaking at 60 cycles per min. Following the incubation, the glucose content of the incubated wells was measured by GOD/POD enzymatic method using Merckotest glucose kit.

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