Bioorganic Chemistry 56 (2014) 27–33

Contents lists available at ScienceDirect

Bioorganic Chemistry

journal homepage: www.elsevier.com/locate/bioorg

Design, synthesis and glucose uptake activity of some novel glitazones

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ARTICLE INFO

Article history:
Received 23 September 2013
Available online 24 May 2014

Keywords:
Thiazolidinediones
Glitazones
Glucose uptake activity
Rat hemi-diaphragm

ABSTRACT

Herein, we report a library consisting of some novel glitazones containing thiazolidinedione and its bio-isosteres, rhodanine and oxadiazolidine ring structures as their basic scaffold for their antidiabetic activity. Twelve novel glitazones with diverse chemical structures were designed and synthesized by adopting appropriate synthetic schemes and analyzed. Later, subjected to *in vitro* glucose uptake assay in the absence and presence of insulin to confirm their antidiabetic activity using rat hemi-diaphragm. The titled compounds exhibited glucose uptake activity ranging weak to significant activity. Compounds **4**, **5**, **9**, **11**, **15**, **16**, **19** and **20** showed considerable glucose uptake activity apart from rosiglitazone, a standard drug. Compound **16** happens to be the candidate compound from this study to investigate further. The illustration about their design, synthesis, analysis and glucose uptake activity is reported here along with the *in vitro* and *in silico* study based structure–activity relationships.

1. Introduction

Diabetes mellitus is a metabolic disease characterized by hyperglycemia arises as a consequence of relative or absolute deficiency of insulin secretion or resistance to insulin action or in combination of many factors [1]. Insulin is a hormone necessary for normal carbohydrate, protein and fat metabolism in mammals. Insulin resistance is one of key characteristic features of non-insulin dependent diabetes mellitus (NIDDM) [2]. Patients with NIDDM often suffer from dyslipidemia in the form of high plasma triglycerides and low HDL cholesterol levels these factors are considered as major risk factors for coronary heart diseases [3]. The primary therapy for NIDDM is caloric restriction of diet and regulated aerobic exercises. When lifestyle modifications do not result in normalization or near normalization of metabolic abnormalities, pharmacologic therapy is unavoidable [4]. Before 1990, sulfonylureas and biguanides were the oral antidiabetic agents available for the treatment of type 2 diabetes to enhance the insulin secretion and action [5,6]. Many drawbacks associated with these drugs restrict their use and creates opportunity to develop some novel insulin sensitizers to reduce insulin resistance [7,8]. Fortunately, since from 1990, there is an explosion with the introduction of new classes of antidiabetic drugs to the market especially glitazones [9]. The pioneering discovery of ciglitazone by the group of scientists at Takeda Co., Japan, for insulin resistance by potentiating insulin action in genetically diabetic and or, obese animals lead to the development of new glitazones or thiazolidine-2,4-diones (TZDs) [10–12]. Among these TZDs, troglitazone was the first approved by USFDA (United States Food and Drug Administration) in late 1990s for the treatment of NIDDM followed by pioglitazone and rosiglitazone [13–15]. These agents share a common partial chemical structure, TZD, and are commonly called as glitazones. These glitazones correct hyperglycemia by enhancing insulin sensitivity at adipose, hepatic and skeletal muscle tissues. Recently, rosiglitazone has been recalled from the market due to its cardiovascular concerns and on the other hand this has created an opportunity to develop some newer glitazones. The major molecular target for these glitazones happens to be the peroxisome proliferator activated receptor-gamma (PPAR-γ) which regulates the gene expression mainly in the adipose tissues [16].

PPAR-γ and its modulators are well known to play key role in treating NIDDM, gastrointestinal diseases, and genetic disorders associated with glucose homeostasis and lipid uptake [17–20].

In the past we have reported the design, synthesis and evaluation of some novel TZDs [21–23]. In continuation, the present interest on TZDs is mainly due to their structural diversity based on structure–activity relationships learnt in the past.

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http://dx.doi.org/10.1016/j.bioorg.2014.05.006
0045-2068/

2. Results and discussion

2.1. Chemistry

Relationship between the structure and antihyperglycemic activity of the substituted TZDs and related compounds has been a subject of intensive investigation. TZDs with significant antihyperglycemic activity are known for their common structural features, namely, acidic head group connected to a lipophilic tail by a phenoxyalkyl linker (Fig. 1).

The structure–activity relationships drawn from our previously generated CoMSIA model are [21], the superimposed common part of the structures, namely, polar rhodanine head followed by polar tyrosine or phenylalanine moiety at the third position and benzylidene moiety at the fifth position of the rhodanine ring seems to be the pharmacophore, as most of the compounds possessed this common substructure. This indicated the necessity of relatively more polar acidic groups nearer the thiazolidine head. The hydrophobic benzyloxy trunk, which in turn connected to the two carbon acyl linker in association with the amide bond, also identified as the important structural components for the compounds to exhibit significant antidiabetic activity. Regarding the hydrophobic trunk part, vanillin was found to be extremely good as many of the active compounds in the series contain the same.

In light of this back ground and considering all these pharmacophore features we have used three synthetic schemes to design and synthesize some novel glitazones. First, following Scheme 1, we have synthesized the basic nucleus TZD **1** which happens to be the head part of glitazones by reacting chloroacetic acid and thiourea. Vanillin, which happens to be the trunk portion of glitazones, is connected to a two carbon linker to get **2**. Further, it is connected to the hydrophobic morpholine, which happens to be the hydrophobic tail part of glitazones to yield **3**. At last, **1** is subjected to the Knoevenagel condensation reaction with **3** to get glitazone **4**. Similarly, we used rhodanine which happens to be the one of the bioisosteres of TZD to get **5**.

Para amino benzoic acid (PABA) substituted TZDs were designed to enhance the acidity near the TZD ring system was prepared by adopting Scheme 2. First, PABA is connected to the two carbon linker using chloroacetyl chloride to form **6** and later reacted with the prepared potassium salt of TZD **7** using DMF as solvent to obtain **8** in moderate yields. Later, compound **8** was condensed with various substituted aldehydes using Knoevenagel condensation reaction to get **9**–**16**. Since, oxadiazole ring system also happens to be bioisosteric with the TZD ring system, attempt has been made to synthesise some novel oxadiazole based glitazones by adopting Scheme 3. Here, we first reacted ethylacetoacetate and hydrazine hydrate under neat condition to form hydrazide **17**, instantly at 100% yields. Later, we converted **17** to the substituted oxadiazole **18** by reacting with carbon disulfide under basic conditions. The NH group of **18** is subjected to the Mannich reaction with aniline and benzyl amine to get **23** and **24**, respectively, having quite similar structural frame as that of a usual glitazones.

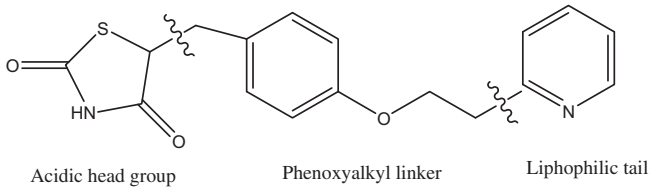


Fig. 1. Pharmacophore structural components of glitazones.

Knoevenagel condensation reactions were performed both under microwave and conventional methods. However, microwave method happens to be the easy and efficient method over the conventional method as it showed better results in terms of time and yields when compared to the conventional method. All the synthesized compounds were analyzed by IR, NMR and Mass spectral studies to confirm their structures. 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 at about 7.7 δ ppm. ^{13}C NMR spectra showed signal for =CH in Knoevenagel condensed products at about 133 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 [19,20].

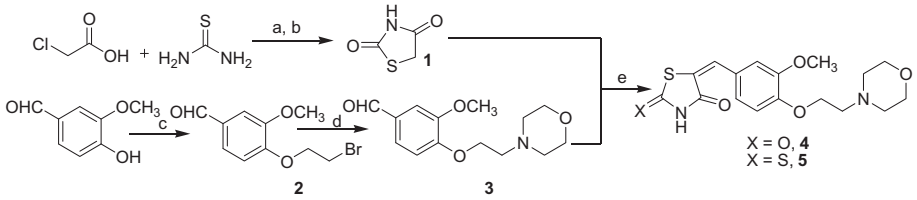
2.2. Glucose uptake by rat hemi diaphragm

Antidiabetic activities for the twelve compounds were measured using glucose uptake by rat hemi-diaphragm method according to the previously reported protocols and the procedure that we had reported earlier [19–22]. Rat diaphragm was selected because the striated muscle is quantitatively the most important tissue for glucose disposal in the animal body. The glucose content was measured and the glucose uptake was calculated as the difference between the initial and final glucose content at 2 mg of optimized drug concentration [21–23]. The glucose uptake by rat hemi-diaphragm was measured in mg/dl. Data was expressed as mean ± standard error of mean (SEM) and are shown in Table 1. Statistical comparisons between the groups were performed in two sets against the respective control in one-way ANOVA followed by Dunnet's multiple comparison post-test using graphPad Prism 4.0 software for Windows (San Diego, California, USA).

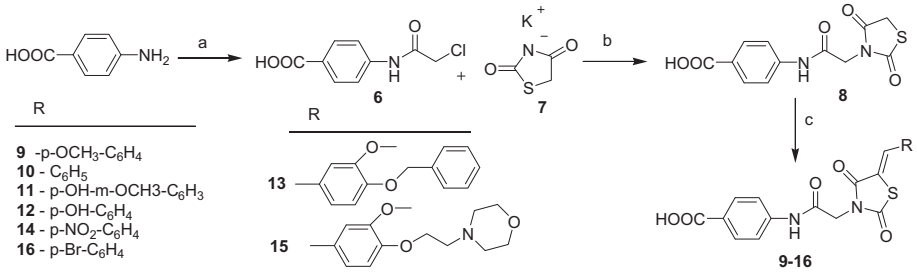
The results of the *in vitro* glucose uptake study indicate that the compounds **4**, **5**, **9**, **11**, **15**, **19** and **20** ($p < 0.05$) enhance the glucose uptake significantly by the tissue cells. Compound **16** happens to be the most potent compound amongst all the glitazones studied here by enhancing the glucose uptake significantly ($p < 0.01$). However, rosiglitazone, being a standard drug demonstrated its superiority over all the glitazones studied here. Rest of the compounds exhibit weak to moderate glucose uptake activity. However, glitazones **13** and **14** failed to produce the glucose uptake activity especially in the presence of insulin.

We have investigated the structure–activity relationships based on the results obtained. To define the pharmacophore we aligned energy minimized and conformationally analyzed structures of **4**, **5**, **9**–**16** against common atoms by atom fit method using Schrodinger 9.2 software (Fig. 2). The superimposed common substructure that contains polar thiazolidinedione or its bioisosteres, rhodanine or oxadiazole-2-thione ring as head group followed by hydrophobic benzyloxy trunk happens to be the pharmacophore. The acidic TZD ring is extended with PABA via two carbon acyl linker and perhaps this could also be the one of the important reasons for compounds **9**, **11**, **15** and **16** to exhibit significant glucose uptake activity. Compound **16**, the most active compound contains para bromo benzylidene moiety at the fifth position of TZD scaffold. Surprisingly, compounds **19** and **20** with oxadiazolidine-2-thione scaffold also exhibited good glucose uptake activity.

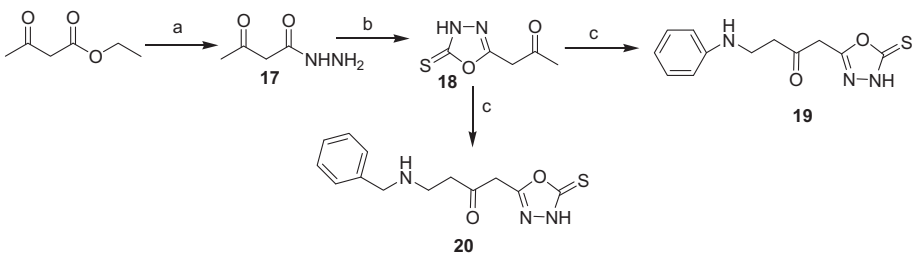
Furthermore, we have docked all the reported glitazones here against PPAR-γ protein (PDB ID: 2PRG) [23] using regular protocol using the Glide molecular docking tool implemented in the Schrodinger software (Schrödinger, L.L.C.) [24]. The binding conformations of the standard drug rosiglitazone and compound **16** at the active site of PPAR-γ is as shown in Fig. 3. Key aspect here is that, carboxyl group of PABA is superimposed over the TZD ring and this indicates that they are bioisosteric with one another. However, the binding mode of tail part of the structures differs considerably. The



Scheme 1. Reagents and conditions: (a) water, 0–5 °C, stir for 30 min, (b) HCl, reflux for 12 h, (c) dibromoethane, dry acetone, anhydrous K_2CO_3, stir at 50 °C, 48 h, (d) morpholine, dry acetone, anhydrous K_2CO_3, stir under at rt for 48 h and (e) thiazolidine-2,4-dione or rhodanine, dry toluene, piperidine, acetic acid, molecular sieves, MW irradiation; 420 W, 30–45 min, conventional; reflux at 110 °C for 12–15 h with stirring.



Scheme 2. Reagents and conditions: (a) triethylamine, chloroacetylchloride, dry chloroform, 0–5 °C, stir for 24–30 h, (b) DMF, stir at 50–70 °C for 48 h and (c) aryl aldehyde, dry toluene, piperidine, glacial acetic acid, MW irradiation; 420 W, 30–45 min, conventional; reflux at 110 °C for 12–15 h with stirring.



Scheme 3. Reagents and conditions: (a) hydrazine hydrate, neat, stir at 0–5 °C for 3 min, (b) ethanol (50 ml), CS_2, 0–5 °C, stir for 30 min and reflux for 6 h and (c) 37% formaldehyde, aniline/benzylamine, ethanol, stir at rt for 2 h.

Table 1
In vitro glucose uptake study for the glitazones by isolated rat hemi-diaphragm.

Compound/ group	In absence of insulin (mg/dl/ g/45 min) Mean ± SEM	In presence of insulin (mg/dl/ g/45 min) Mean ± SEM
Control	20.89 ± 1.41	33.55 ± 1.00
4	29.31 ± 2.36*	40.36 ± 2.40*
5	29.06 ± 0.44*	38.29 ± 0.71
9	29.05 ± 1.64*	39.15 ± 2.57
10	18.90 ± 1.40	35.33 ± 1.33
11	28.79 ± 2.21	42.02 ± 3.53*
12	19.48 ± 1.40	34.05 ± 1.55
13	23.93 ± 1.07	33.88 ± 1.67
14	15.27 ± 1.23	34.00 ± 1.00
15	25.41 ± 1.41	40.33 ± 2.33*
16	34.71 ± 3.29**	47.65 ± 2.87**
19	28.50 ± 1.50	41.44 ± 1.56*
20	30.40 ± 1.62*	40.36 ± 1.49*
Rosiglitazone	36.00 ± 1.00**	50.50 ± 1.50**

Statistics: Values are mean of triplicate ± SEM.
* $p < 0.05$ compared to the respective control, one way-ANOVA followed by Dunnett's post-test.
** $p < 0.01$ compared to the respective control, one way-ANOVA followed by Dunnett's post-test.

carboxyl group of PABA has made hydrogen bonding interactions at a distance of 1.503, 1.617 and 2.402 Å with residues His323, His449 and Tyr473, respectively (Fig. 4), whereas TZD ring of rosiglitazone also makes the same interactions at almost same distances [25–27].

3. Experimental methods

The melting points of the synthesized compounds were determined in open capillaries using Veego VMP-1 apparatus and are expressed in °C and are uncorrected. The IR-spectra of compounds were recorded on Shimadzu FT-IR spectrometer using KBr pellets technique and are expressed in cm^{-1}. ^1H NMR, ^{13}C NMR spectra was recorded on AV-III 400 MHz spectrometer using DMSO-d_6 as solvent and TMS as internal standard. The chemical shifts were reported in δ ppm. Mass spectra were obtained using JEOL GC mate mass spectrometer under EI^+ ionization technique/mode. Microwave assisted synthesis was performed using microwave system CATA R from Catalyst system (Pune, India). Auto analyzer (Merck) was used for the glucose estimation during *in vitro* glucose uptake study by rat hemi-diaphragm method. TLC were performed to monitor the reactions and to determine purity of the products. Compounds were purified by re-crystallization using suitable solvents.

3.1. Procedure for the preparation of thiazolidine-2,4-dione [*1*]

Thiazolidine-2,4-dione [3] was prepared according to the Scheme 1. Equimolar amount of thiourea (0.3 M) and chloroacetic

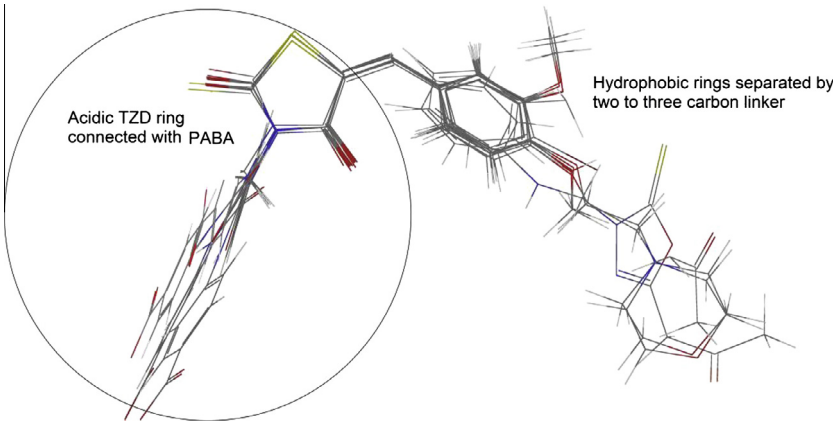


Fig. 2. Aligned glitazones by the atom fit method depicting pharmacophore.

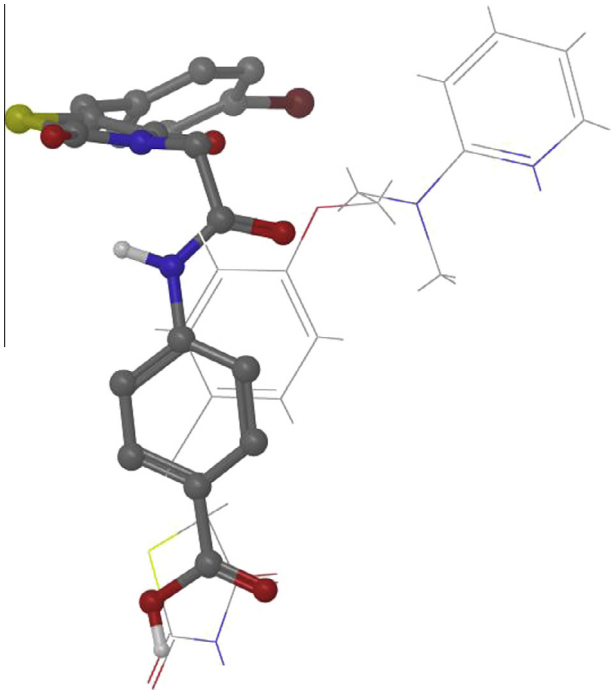


Fig. 3. The binding conformations of rosiglitazone (antialias representation) and compound **16** (ball and stick representation).

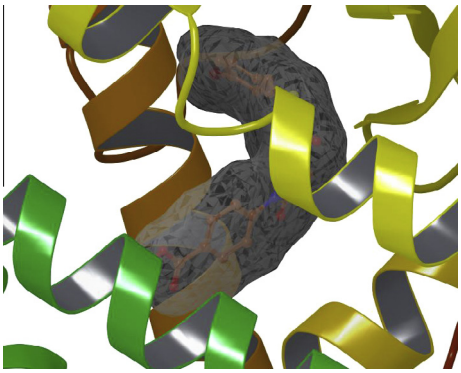


Fig. 4. Binding mode of compound **16** at the active site of the PPAR-γ.

acid (0.3 M) was taken separately in 30 ml of water and mixed under ice cold condition (0–5 °C) with stirring for 20 min to form white precipitate of 2-imino-thiazolidine-4-one. Concentrated HCl (30 ml) was added and refluxed for 12 h. Reaction was monitored through TLC (ethyl acetate:pet ether, 2:3). The white crystalline solid obtained after cooling was filtered, washed with water and dried. The yield was found to be 80%.

*3.2. Thiazolidine-2,4-dione [**1**]*

White crystalline solid, Yield 80%, M.P. 123–125 °C, IR (KBr, cm^{-1}): 3126.7 (N—H, broad peak, stretch), 1735.9 (C=O, stretch), 1849.8 (C=O, stretch), 1163.1 (C=S, stretch).

^{1}H NMR (δ ppm, DMSO-d_6) δ: 4.4 (s, 2H, CH_2), 12.5 (s, 1H, NH).

*3.3. Procedure for the preparation of 4-(2-bromoethoxy)-3-methoxybenzaldehyde [**2**]*

One equivalent of vanillin, 2.5 equivalent of dibromoethane along with 1.2 equivalent of anhydrous K_2CO_3 and 70 ml of dry acetone were transferred to a flask. Then it was refluxed at 50 °C for 48 h. Reaction was monitored through TLC (ethyl acetate:pet ether, 2:3). After completion of the reaction, solvent was evaporated and the reaction mixture was transferred to the beaker containing 200–300 ml of water and stirred for 20–30 min. Resulting suspension was filtered. The solid obtained was then dissolved in 10% NaOH solution, stirred for 10 min and then filtered. The solid obtained was washed with plain water and dried. The white crystals of the pure product was taken forward for connecting it to the morpholine.

*3.4. Procedure for connecting **2** to morpholine [**3**]*

4-(2-Bromoethoxy) benzaldehyde (1 eq) was connected to morpholine (1.5 eq) by stirring with anhydrous K_2CO_3 (1.1 eq) in dry acetone for 48 h. Reaction was monitored through TLC (ethyl acetate:pet ether, 2:3). After completion of the reaction the solvent was evaporated and the reaction mixture was transferred to the beaker containing 200–300 ml of water and stirred for 20–30 min. Resulting suspension was filtered. The solid obtained was then dissolved in 10% NaOH solution, stirred for 10 min and then filtered. The solid obtained was washed with plain water and dried. Later, it was subjected to the Knoevenagel condensation to get the final compounds.

3.5. General procedure for Knovenagel condensation

The morpholine incorporated vanillin was subjected to the Knoevenagel condensation reaction to get the final compounds [**4** and **5**].

Conventional method: Rhodanine (0.01 M) and thiazolidin-2,4-dione (0.01 M) in dry toluene was transferred to flask. Then morpholine incorporated vanillin (0.012 M), piperidine (3 drops), glacial acetic acid (2–3 drops) and molecular sieves were added. The reaction mixture was refluxed at 110 °C with occasional stirring for 12–15 h. The reaction was monitored through TLC. Upon completion of the reaction, reaction mixture was allowed to cool and precipitated solid was filtered off. The corresponding product was re-crystallized from ethyl acetate.

Microwave method: Rhodanine (0.01 M) and thiazolidin-2,4-dione (0.01 M) in dry toluene was transferred to flask. Then morpholine incorporated vanillin (0.012 M), piperidine (3 drops), glacial acetic acid (2–3 drops) and molecular sieves were added. The reaction mixture was irradiated with microwaves at 700 W for 30–45 min. The reaction was monitored through TLC. Upon completion of the reaction, reaction mixture was allowed to cool and precipitated solid was filtered off and washed with cold chloroform. The corresponding product was re-crystallized with ethyl acetate.

3.6. (Z)-5-(4-(2-morpholinoethoxy)-benzylidene)-thiazolidine-2, 4-dione [4]

Yellow amorphous solid, Yield 85%, M.P. 280–285 °C, IR (KBr, cm^{-1}): 3236.6 (N—H, broad peak, stretch), 2877.8 (Aliphatic C—H, stretch), 1697.4 (C=O, stretch), 1589.4 (Aromatic C=C, stretch), 1396.5 (C—O, stretch), 1265.3 (C=S, stretch), 1138.0 (C—N, stretch). ^{1}H NMR (δ ppm, DMSO-d_6) δ: 2.05 (t, 4H, $2CH_2$), 3.20 (t, 2H, CH_2), 3.80 (s, 3H, OCH_3), 3.85 (t, 4H, $2CH_2$), 4.41 (t, 2H, CH_2), 7.20 (d, 1H, ArH), 7.41 (d, 1H, ArH), 7.70 (s, 1H, =CH), 12.51 (bs, 1H, NH). Mass (*m*/*z*): Molecular ion peak found, 364.4198 (peak calculated, 364.4201 for $C_{17}H_{20}N_2O_5S$), 52.34 (base peak). Mass fragment (*m*/*z*): 321.25, 278.97 and 219.65.

3.7. (Z)-5-(4-(2-morpholinoethoxy)-3-methoxybenzylidene)-2-thioxothiazolidin-4-one [5]

Yellow amorphous solid, Yield 90%, M.P. 300–305 °C IR (KBr, cm^{-1}): 3140.2 (N—H, broad peak, stretch), 1685.8 (C=O, stretch), 1579.7 (Aromatic C=C, stretch), 1246.0 (C—O, stretch), 1271.1 (C=S, stretch), 1168.9 (C—N, stretch). ^{1}H NMR (δ ppm, DMSO-d_6) δ: 1.80 (t, 4H, $2CH_2$), 3.02(t, 2H, CH_2), 3.79 (s, 3H, OCH_3), 3.82 (t, 4H, $2CH_2$), 4.44 (t, 2H, CH_2), 7.20 (d, 1H, ArH), 7.41 (d, 1H, ArH), 7.69 (s, 1H, =CH), 13.78 (bs, 1H, NH). ^{13}C NMR (δ ppm, DMSO-d_6) δ: 43.63 (2C, CH_2), 43.64 (1C, CH_2), 55.48 (2C, CH2), 55.49 (1C, CH_3), 113.76 (1C, =C), 122.59 (2C, ArC), 125.85, 129.91 (2C, ArC), 132.13 (2C, ArC), 133.12 (1C, =CH), 169.32 (1C, C=O), 191.45 (1C, C=S). Mass (*m*/*z*): peak found, 380.4789 (peak calculated, 380.4796 for $C_{17}H_{20}N_2O_4S_2$), 68.58 (base peak). Mass fragment (*m*/*z*): 337.19, 294.60, 250.85, and 235.82.

3.8. Synthesis of 4-(2-chloroacetamido) benzoic acid [6]

In Scheme 2, PABA (1 eq) and triethylamine (1.7 eq) along with dry chloroform (50 ml) was transferred to flask fitted with guard tube. The mixture was allowed to stir under room temperature to form a uniform mixture. Chloroacetyl chloride (1.5 eq) was added drop wise under ice cold condition with continuous stirring. The reaction mixture was allowed to stir for about 24–30 h. Reaction was monitored through TLC (ethyl acetate:pet ether, 2:3). After the reaction was completed the solvent was evaporated. The crude product obtained was washed with water and dried to get the 4-(2-chloroacetamido) benzoic acid. It was taken directly for the next step without any further purification. The yield was found to be 85%.

3.9. Synthesis of potassium salt of thiazolidine-2,4-dione [7]

Thiazolidine-2,4-dione (8 g) was dissolved in 12.5 ml of ethanol. To this solution, KOH (4.2 g) in ethanol (10 ml) was added. The mixture was stirred for 2 h. The white crystalline solid was collected after filtration, washed with ethanol and dried under vacuum to get potassium salt of thiazolidinedione.

3.10. Synthesis of 4-(2-(2,4-dioxothiazolidin-3-yl)acetamido)benzoic acid [8]

Potassium salt of 2,4-thiazolidinediones (2 eq) in DMF was taken into flask. Then 4-(2-chloroacetamido) benzoic acid was added slowly with stirring. After the addition was complete, the mixture was allowed to stir and heated at 50–60 °C under reflux for 24 h. Reaction was monitored through TLC. After completion of the reaction, the solvent was evaporated and the mixture was poured in water (500 ml) and product precipitated as a solid collected on a filter and dried. The yield was found to be 60%.

3.11. 4-(2-(2,4-Dioxothiazolidin-3-yl)acetamido)benzoic acid [8]

Cream amorphous solid, Yield 89%, M.P. 240–265 °C IUPAC: 4-(2-(2,5-dioxothiazolidin-3-yl)acetamido)benzoic acid. IR (KBr, cm^{-1}): 3353.0 (N—H, broad peak, stretch), 3012.9 (Aromatic C—H, stretch), 2941.5 (Aliphatic C—H, stretch), 1674.2 (C=O, stretch), 1593.2 (Aromatic C=C, stretch), 1271.1 (C=S, stretch), 1246.0 (C—O, stretch), 1182.4 (C—N, stretch).

3.12. General procedure for Knoevenagel condensation [9–16]

The resultant solid was subjected to the Knoevenagel condensation reaction to get the final compounds **(9–16)**.

Conventional method: 4-(2-(2,4-Dioxothiazolidin-3-yl)acetamido)benzoic acid (0.01 M) in dry toluene was transferred in flask. Then aldehydes (0.01 M), piperidine (3 drops), acetic acid (2–3 drops) and molecular sieves were added. The reaction mixture was stirred for 5 min and then refluxed at 110 °C with occasional stirring for 2 h. The reaction was monitored through TLC. Upon completion of the reaction, reaction mixture was allowed to cool and precipitated solid was filtered off. The resultant solid was re-crystallized with methanol.

Microwave method: 4-(2-(2,4-Dioxothiazolidin-3-yl)acetamido)benzoic acid (0.01 M) in dry toluene was transferred in flask. Then aldehydes (0.01 M), piperidine (3 drops), acetic acid (2–3 drops) and molecular sieves were added. The reaction mixture was stirred for 5 min and then heated under microwave irradiation (420 W) for 20–40 min. The reaction was monitored through TLC. Upon completion of the reaction, reaction mixture was allowed to cool and precipitated solid was filtered off. The resultant solid was re-crystallized with methanol.

3.13. 4-(2-((E)-5-(4-Methoxy benzylidene)-2,4-dioxothiazolidin-3-yl)acetamido)benzoic acid [9]

Light yellow amorphous solid, Yield 85%, M.P. 220–228 °C, IR (KBr, cm^{-1}): 3319.6 (N—H, broad peak, stretch), 3061.1 (Aromatic C—H, stretch), 2951.1 (Aliphatic C—H, stretch), 1680.0 (C=O, stretch), 1539.2 (Aromatic C=C, stretch), 1413.8 (C—N, stretch), 1386.8 (C—O, stretch), 1176.6 (C=S, stretch). ^{1}H NMR (δ ppm, DMSO-d_6) δ: 4.04 (s, 3H, OCH_3), 4.91 (s, 2H, CH_2), 7.59 (d, 2H,

ArH), 7.62 (d, 2H, ArH), 7.80 (s, 1H, =CH), 7.88 (d, J = 7.2 Hz, 2H, ArH), 7.92 (d, J = 7.6 Hz, 2H, ArH), 12.30 (bs, 1H, NH). ^{13}C NMR (δ ppm, DMSO-d_6) δ: 43.54 (1C, CH_2), 63.03 (1C, CH_3), 118.43 (2C, ArC), 118.68 (1C,=C), 123.72 (2C, ArC), 130.70 (2C, ArC), 134.02 (1C,=CH), 141.90 (3C, ArC), 143.16 (3C, ArC), 164.94 (1C, C=O), 165.88 (1C, C=O), 171.59 (1C, C=O), 171.96 (1C, C=O). MS (*m*/*z*): peak found, 412.4209 (peak calculated, 412.4215 for $C_{20}H_{16}N_2O_6S$). Mass fragments (*m*/*z*): 397.63, 387.35, 332.80, 293.48, 178.12, 164.16, 150.25, 137.29, 120.36 (base peak).

3.14. 4-(2-((E)-5-Benzylidene-2,4-dioxothiazolidin-3-yl) acetamido)benzoic acid [**10**]

Yellow amorphous solid, Yield 80%, M.P. 201–205 °C, IR (KBr, cm^{-1}): 3140.2 (N—H, broad peak, stretch), 3003.2 (Aromatic C—H, stretch), 2847.0 (Aliphatic C—H, stretch), 1685.8 (C=O, stretch), 1467.8 (Aromatic C=C, stretch), 1423.5 (C—N, stretch), 1354.0 (C—O, stretch), 1168.9 (C=S, stretch). ^{1}H NMR (δ ppm, DMSO-d_6) δ: 4.90 (s, 2H, CH_2), 7.07–7.55 (m, 5H, ArH), 7.78 (s, 1H, =CH), 7.80 (d, J = 7.6 Hz, 2H, ArH), 7.88 (d, J = 7.1 Hz, 2H, ArH), 10.4 (bs, 1H, NH). MS (*m*/*z*): peak found, 382.3979 (peak calculated, 382.3986 for $C_{19}H_{14}N_2O_5S$). Mass fragments (*m*/*z*): 350.12, 287.39, 167.47 and 120.36 (base peak).

3.15. 4-(2-((E)-5-(4-hydroxy-3-methoxybenzylidene)-2, 4-dioxothiazolidin-3-yl)acetamido)benzoic acid [**11**]

Brown amorphous solid, Yield 88%, M.P. 235–240 °C. ^{1}H NMR (δ ppm, DMSO-d_6) δ: 4.09 (s, 3H, OCH_3), 4.93 (s, 2H, CH_2), 7.61 (m, 2H, ArH), 7.63 (d, J = 7.4 Hz, 2H, ArH), 7.74 (d, J = 7.7 Hz, 2H, ArH), 7.79 (s, 1H, =CH), 10.5 (bs, 1H, NH). ^{13}C NMR (δ ppm, DMSO-d_6) δ: 43.47 (1C, CH_2), 63.01 (1C, CH_3), 118.66 (1C,=C), 118.85 (2C, ArC), 123.84 (2C, ArC), 130.67 (2C, ArC), 132.98 (1C, =CH), 143.02 (3C, ArC), 143.13 (3C, ArC), 164.56 (1C, C=O), 165.79 (1C, C=O), 171.54 (1C, C=O), 171.91 (1C, C=O). MS (*m*/*z*): peak found, 428.4211 (peak calculated, 428.4206 for $C_{20}H_{16}N_2O_7S$). Mass fragments (*m*/*z*): 414.04, 334.96, 294.32, 178.92, 163.98, 121.19 (base peak), 93.32.

3.16. 4-(2-((E)-5-(4-hydroxybenzylidene)-2,4-dioxothiazolidin-3-yl) acetamido benzoic acid [**12**]

Deep brown amorphous solid, Yield 90%, M.P. 195–202 °C. ^{1}H NMR (δ ppm, DMSO-d_6) δ: 4.91 (s, 2H, CH_2), 7.59 (d, 2H, ArH), 7.61 (d, J = 7.5 Hz, 2H, ArH), 7.74 (d, J = 7.2 Hz, 2H, ArH), 7.78 (s, 1H, =CH), 7.89 (d, J = 8.1 Hz, 2H, ArH), 10.90 (bs, 1H, OH). MS (*m*/*z*): peak found, 398.3918 (peak calculated, 398.3924 for $C_{19}H_{14}N_2O_6S$). Mass fragments (*m*/*z*): 377.64, 343.69, 276.50, 187.36, 120.25 (base peak).

3.17. 4-(2-((E)-5-(4-benzyloxy)-3-methoxybenzylidene)-2, 4-dioxothiazolidin-3-yl)acetamido)benzoic acid [**13**]

Deep brown amorphous solid, Yield 86%, M.P. 250–255 °C. ^{1}H NMR (δ ppm, DMSO-d_6) δ: 4.08 (t, 3H, OCH_3), 4.92 (t, 2H, CH_2), 7.60 (d, J = 7.2 Hz, 2H, ArH), 7.67 (d, J = 7.6 Hz, 2H, ArH), 7.75 (s, 1H, =CH), 7.88 (d, J = 8.0 Hz, 2H, ArH), 7.90 (d, J = 7.9 Hz, 2H, ArH), 7.94 (d, 2H, ArH), 7.99 (d, 2H, ArH), 10.5 (bs, 1H, NH). MS (*m*/*z*): peak found, 518.5379 (peak calculated, 518.5398 for $C_{27}H_{22}N_2O_7S$). Mass fragments (*m*/*z*): 503.34, 478.54, 436.32, 357.40, 276.50, 185.44 (base peak).

3.18. 4-(2-((E)-5-(4-nitrobenzylidene)-2,4-dioxothiazolidin-3-yl) acetamido)benzoic acid [**14**]

Light brown amorphous solid, Yield 80%, M.P. 225–230 °C. ^{1}H NMR (δ ppm, DMSO-d_6) δ: 4.92 (s, 2H, CH_2), 7.59 (d, J = 7.0 Hz, 2H, ArH), 7.61 (d, J = 7.5 Hz, 2H, ArH), 7.68 (d, 2H, ArH), 7.93 (s, 1H, =CH), 7.94 (d, 2H, ArH), 8.08 (bs, 1H, NH). ^{13}C NMR (δ ppm, DMSO-d_6) δ: 45.23 (1C, CH_2), 120.53 (2C, ArC), 120.57 (2C, ArC), 120.69 (1C, =C), 130.58 (2C, ArC), 132.80 (1C, =CH), 143.29 (3C, ArC), 144.76 (3C, ArC), 167.08 (1C, C=O), 168.83 (1C, C=O), 173.71 (1C, C=O). MS (*m*/*z*): peak found, 427.3908 (peak calculated, 427.3914 for $C_{19}H_{13}N_3O_7S$). Mass fragments (*m*/*z*): 412.45, 398.36, 351.36, 298.39, 187.07, 120.25 (base peak).

3.19. 4-(2-((E)-5-(3-(2-morpholinoethoxy)-4-methoxybenzylidene)-2,4-dioxothiazolidin-3-yl)acetamido)benzoic acid [**15**]

Yellow amorphous solid, Yield 85%, M.P. 214–220 °C. ^{1}H NMR (δ ppm, DMSO-d_6) δ: 4.43 (t, 3H, OCH_3), 4.92 (s, 2H, CH_2), 7.61 (d, J = 7.3 Hz, 2H, ArH), 7.63 (d, J = 7.7 Hz, 2H, ArH), 7.65 (d, 2H, =CH), 7.68 (d, J = 8.2 Hz, 2H, ArH), 7.71 (d, J = 8.0 Hz, 2H, ArH), 7.93 (d, 2H, ArH), 7.96 (d, 2H, ArH), 8.08 (bs, 1H, NH). MS (*m*/*z*): peak found, 541.5721 (peak calculated, 541.5723 for $C_{26}H_{27}N_3O_8S$). Mass fragments (*m*/*z*): 523.34, 480.34, 445.36, 436.32, 267.89, 265.43, 120.25 (base peak).

3.20. 4-(2-((E)-5-(4-bromobenzylidene)-2,4-dioxothiazolidin-3-yl) acetamido)benzoic acid [**16**]

Cream amorphous solid, Yield 84%, M.P. 260–265 °C. ^{1}H NMR (δ ppm, DMSO-d_6) δ: 4.91 (s, 2H, CH_2), 7.57 (d, J = 7.1 Hz, 2H, ArH), 7.60 (d, J = 7.2 Hz, 2H, ArH), 7.64 (d, J = 7.5 Hz, 2H, ArH), 7.77 (s, 1H, =CH), 7.86 (d, J = 7.8 Hz, 2H, ArH), 10.6 (bs, 1H, NH). ^{13}C NMR (δ ppm, DMSO-d_6) δ: 43.82 (1C, CH_2), 118.20 (2C, ArC), 118.59 (1C,=C), 123.72 (2C, ArC), 130.15 (2C, ArC), 132.85 (1C,=CH), 141.46 (3C, ArC), 143.04 (3C, ArC), 165.76 (1C, C=O), 167.59 (1C, C=O), 171.55 (1C, C=O), 173.62 (1C, C=O). MS (*m*/*z*): peak found, 461.2926 (peak calculated, 461.2934 for $C_{19}H_{13}BrN_2O_5S$). Mass fragments (*m*/*z*): 445.69, 412.11, 398.34, 299.69, 211.46, 120.25 (base peak).

3.21. Synthesis of 3-oxobutanehydrazide [**17**]

According to Scheme 3, ethylacetoacetate (0.002 M) was taken in flask. Hydrazine hydrate (0.002 M) was added drop wise under ice cold condition with constant stirring for 5 min. The reaction completes quickly and the resultant solid was re-crystallized with alcohol to get white crystals of corresponding hydrazide.

3.22. 3-Oxobutanehydrazide [**17**]

White crystalline solid, Yield 90%, M.P. 267–273 °C.

IR (KBr, cm^{-1}): 3128.6 (N—H, broad peak, stretch), 2885.5 (Aliphatic C—H, stretch), 1776.5 (C=O, stretch).

3.23. Synthesis of 1-(4,5-dihydro-5-thioxo-1,3,4-oxadiazol-2-yl) propan-2-one [**18**]

3-Oxobutanehydrazide (0.01 M) was taken in flask. Ethanol of 50 ml was added along with KOH (0.015 M) and CS_2 (0.015 M). The reaction mixture was refluxed for 6 h. The reaction mixture was monitored through TLC (ethyl acetate:pet ether, 10:1). Upon completion of the reaction, the reaction mixture was allowed to cool and ethanol was distilled off. The residue left was acidified with dil.HCl. Then it was kept in the refrigerator for one day. The precipitated solid was filtered off to get red crystals.

3.24. 1-(4,5-Dihydro-5-thioxo-1,3,4-oxadiazol-2-yl)propan-2-one [18]

Red amorphous solid, Yield 80%, M.P. 280–285 °C. ^{1}H NMR (δ ppm, $CDCl_3$) δ: 2.27 (s, 3H, CH_3), 2.58 (s, 2H, CH_2), 7.36 (bs, 1H, NH). MS (*m*/*z*): Molecular ion peak found, 158.1810 (peak calculated, 158.1804 for $C_5H_6N_2O_2S$), 75.19 (base peak). Mass fragments (*m*/*z*): 132.23, 104.26, 72.29.

3.25. General procedure for Mannich reaction [19,20]

1-(4,5-Dihydro-5-thioxo-1,3,4-oxadiazol-2-yl)propan-2-one (**18**) was taken in flask. To this solution, primary aromatic amine (benzylamine/aniline) and 37% formaldehyde solution were added. Then the reaction mixture was stirred at room temperature for 2 h. and allowed to stand overnight. The reaction mixture was monitored through TLC (ethyl acetate:pet ether, 10:1). Upon completion of the reaction, the separated solid was filtered, washed with cold ethanol, dried and recrystallized from aqueous ethanol to get the product (**19**, **20**).

3.26. 1-(4-((Anilino)methyl)-4,5-dihydro-5-thioxo-1,3,4-oxadiazol-2-yl)propan-2-one [19]

Orange amorphous solid, Yield 85%, M.P. 270–275 °C. ^{1}H NMR (δ ppm, $CDCl_3$) δ: 2.27 (s, 2H, CH_2), 2.95 (t, 2H, CH_2), 4.48 (t, 2H, CH_2), 4.93 (bs, 1H, NH), 6.60–7.10 (m, 5H, ArH), 7.90 (bs, 1H, NH/SH). MS (*m*/*z*): Molecular ion peak found, 263.0703 (peak calculated, 263.0714 for $C_{12}H_{13}N_3O_2S$), 75.89 (base peak). Mass fragments (*m*/*z*): 244.25, 132, 23121.21, 78.33.

3.27. 1-(4-((Benzylamino)methyl)-4,5-dihydro-5-thioxo-1,3, 4-oxadiazol-2-yl)propan-2-one [20]

Yellow amorphous solid, Yield 85%, M.P. 200–205 °C. ^{1}H NMR (δ ppm, $CDCl_3$) δ: 2.32 (m, 2H, CH_2), 2.45 (s, 2H, CH_2), 3.23 (m, 2H, CH_2), 3.81 (m, 2H, CH_2), 4.61 (m, 1H, NH), 7.18–7.35 (m, 5H, ArH), 8.01 (bs, 1H, NH/SH) MS (*m*/*z*): peak found, 277.24 (peak calculated, 277.34). Mass fragments (*m*/*z*): 187.53, 89.78 (base peak). MS (*m*/*z*): Molecular ion peak found, 277.0989 (peak calculated, 277.0980 for $C_{13}H_{15}N_3O_2S$), 89.78 (base peak). Mass fragments (*m*/*z*): 155.70, 109.79, 95.8, 74.78.

3.28. Determination of glucose uptake activity

Glucose uptake measurement: Six-well microtitre plates were selected for study with each well capacity of 5 ml ($n = 3$). Plates were divided into following groups: Group 1: 2 ml of Tyrode solution with 20,000 mg/l glucose; Group 2: 2 ml of Tyrode solution with 20,000 mg/l glucose and regular insulin (Nova Nardisk, 40 IU/ml), 5 μl containing 0.2 units of insulin; Groups 3–14: 2 ml of Tyrode solution with 20,000 mg/l glucose and 2 mg of the test compounds; Group 15: 2 ml of Tyrode solution with 20,000 mg/l glucose and 2 mg of rosiglitazone (standard); Groups 16–27: 2 ml of Tyrode solution with 20,000 mg/l glucose, regular insulin 5 μl containing 0.2 units of insulin and 2 mg of the test compounds; and Group 28: 2 ml of Tyrode solution with 20,000 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 euthanized by decapitation, and diaphragms were taken out swiftly avoiding trauma and divided into two halves. The hemidiaphragms were then rinsed in cold Tyrode solution (without glucose) to remove any blood clots and 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.

4. Conclusion

We have designed and synthesized some novel glitazones by adopting three synthetic schemes and confirmed their structures. Compound **16** was found to be the candidate compound to investigate further for its antidiabetic activity. For the first time we are reporting some novel glitazones kind of structures containing oxadiazolidine ring instead of TZD.

Acknowledgment

We acknowledge AICTE, New Delhi, India for funding this project and for providing Schrodinger molecular modeling tools under the RPS Scheme during the year 2011.

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