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Novel glitazones: Design, synthesis, glucose uptake and structure–activity relationships

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ABSTRACT

Glitazones are known to exhibit antihyperglycemic activity by decreasing peripheral insulin resistance. In the present study, we have designed some novel glitazones based on the structure–activity relationships as possible PPAR-γ agonists. The manually designed glitazones were synthesized by using the appropriate synthetic schemes and screened for their in vitro antihyperglycemic activity by estimating glucose uptake by rat hemi-diaphragm, both in the absence and in the presence of external insulin. Some of the glitazones exhibited good antihyperglycemic activity in presence of insulin. Illustration about their design, synthesis, evaluation, and structure–activity relationships is described.

Type-2 diabetes mellitus is a chronic metabolic disorder that results from defects in both insulin secretion and insulin action. Although it has been primarily regarded as a disorder of glucose metabolism and homeostasis, more recently it has been viewed as a constellation of metabolic disturbances.[1] Diabetes is a global burden and is rising all over the world due to various reasons.[2]

Thiazolidinediones or glitazones, specifically targeted to combat insulin resistance, offered a new approach to the treatment of type-2 diabetes as 'insulin sensitizers'.[3] In December 1997, the first drug from this class, troglitazone[4] was suspended from marketing in the UK due to concerns of drug-induced hepatotoxicity. In June 1998, the National Institute of Health terminated a study investigating troglitazone's potential for preventing type-2 diabetes due to documented cases of fatal hepatotoxicity.

In 1999, two new thiazolidinediones, pioglitazone and rosiglitazone, were approved by the US FDA for the treatment of type-2 diabetes. To date, the incidence of hepatotoxicity appears to be minor with both pioglitazone and rosiglitazone. There are, however, other factors that limit the use of these drugs such as weight gain, congestive heart failure etc. There is, therefore, a need for the development of newer and safer drugs from this class.[5]

Glitazones are agonists for the peroxisome proliferator-activated receptor, PPAR-γ, which regulates the transcription of insulin-responsive genes involved in the control of glucose production, transport, and utilization.[6] Several new insulin sensitisers are currently under investigation. Some of these belong to the glitazone class, but others have different chemical structures.[7,8]

In recent years, we have been engaged in a research program directed towards the development of novel glitazones. We have made efforts to elucidate quantitative structure–activity relationships,[9,10] developed methods to synthesize glitazones efficiently[11] and screened them for their antihyperglycemic activity.[12] In the present communication we describe results on designing some novel glitazones, their synthesis, glucose uptake activity, and their structure–activity relationships.

From our previous studies we have learnt that glitazones normally need to possess a polar thiazolidinedione ring system as a head followed by hydrophobic benzyloxy moiety as trunk linked by a two carbon atom linker and a hydrophobic ring as a tail for better antihyperglycemic activity (Fig. 1).[13]

Keeping these structural features in mind, we have designed newer glitazones having structures similar to this template pharmacophore structure. The synthetic scheme adopted is shown in Scheme 1. The tail part was first designed and synthesized, by con-

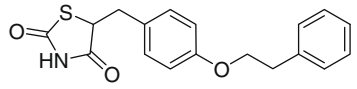

Figure 1. Simplified, typical pharmacophore structure of PPAR agonists.

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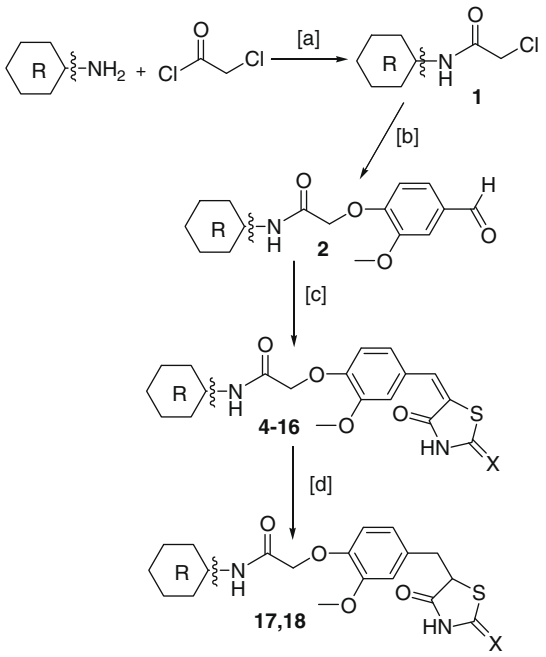


Scheme 1. Synthesis of glitazones. Reagents and conditions: (a) $CHCl_3$, triethylamine, 0–5 °C to rt, stirred for 6 h, 90–99%. (b) Acetone, vanillin, K_2CO_3, stirred at rt for 36 h, 65–80%. (c) Toluene, **3** or rhodanine, piperidine, acetic acid, molecular sieves, reflux, 110 °C for 15 h or MW irradiation 700 W, 30 min, 70–81%. (d) Acetic acid, 5% Pd on charcoal, H_2 at 50 Psi, 14 h, 85–87%.

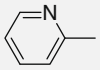
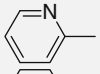
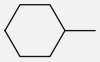
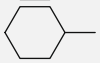
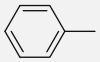
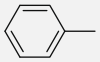
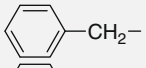
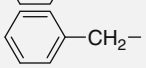
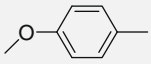
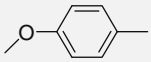
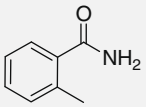
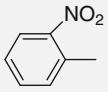
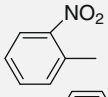
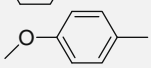
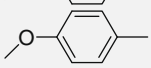
necting various aromatic/alicyclic amines with the two carbon linker. The acylated amines **1** were then connected to the *p*-hydroxy group of vanillin to obtain **2**. Vanillin, instead of *p*-hydroxy benzaldehyde, was used because vanillin is a natural product. There are no reports so far about glitazones which are incorporated with a natural substrate like vanillin as part of their trunk. The trunk which is connected with the hydrophobic tail via the two carbon linker was then connected to the head group thiazolidinedione **3** or its bioisoster, that is, 2-thiaxo-thiazolidine-4-one or rhodanine ring system to obtain **4–16**. Compounds **12** and **13** were further subjected to hydrogenation to reduce C=C using 5% Pd on charcoal to get the compounds **17** and **18** (Table 1).

Thiazolidinedione **3** was prepared by reacting equimolar amounts of chloroacetic acid and thiourea under ice cold conditions. The white precipitate of 2-imino thiazolidine-4-one obtained was then acidified and refluxed with HCl for 12 h to get white crystals of thiazolidinedione (Scheme 2).[11] All the compounds were analyzed using IR, ^{1}H NMR, ^{13}C NMR, and HRMS analytical methods.[14]

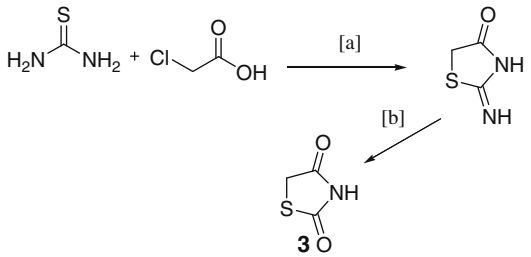
The antidiabetic activities of compounds **4–18** were measured using glucose uptake by rat hemi-diaphragm according to the methods described by Walaas[15] and Chattopadhyay.[16] The institutional animal Ethics Committee (IAEC) of JSS College of Pharmacy approved the proposal. Rat diaphragm was selected because striated muscle is quantitatively the most important tissue for glucose disposal in the animal body. The glucose content was measured[17] and the glucose uptake was calculated as the difference between the initial and final glucose content. Data were expressed as mean ± standard error of mean (SEM) and shown in Table 1.[18]

Statistical comparisons between the groups were performed in two sets using one-way ANOVA followed by Dunnet's multiple comparison post-test using graphPad Prism 4.0 software for Windows (San Diego, California, USA). In the first set, group 1 that served as a control was compared with groups 3–18 including the analogous standard drug (rosiglitazone). In the second set group 2 that served as control was compared with groups 19–34.

Table 1
List of glitazones synthesized and their effect on glucose uptake by the isolated rat hemi-diaphragm

Compd	R	X	Glucose uptake (mg/g/45 min)	
			No insulin	With insulin
4		O	12.25 ± 1.37	32.00 ± 0.91**
5		S	9.75 ± 1.25	30.00 ± 0.81*
6		O	9.50 ± 1.32	27.25 ± 1.10
7		S	11.75 ± 1.03	30.00 ± 0.91*
8		O	12.00 ± 1.29	28.50 ± 1.19
9		S	8.0 ± 1.08	24.75 ± 0.85
10		O	10.50 ± 0.64	31.75 ± 1.31**
11		S	10.25 ± 1.10	26.75 ± 0.85
12		O	10.75 ± 0.85	36.25 ± 1.10**
13		S	9.75 ± 0.47	33.50 ± 1.19**
14		O	10.00 ± 0.70	29.00 ± 0.91
15		O	9.75 ± 0.85	26.25 ± 1.03
16		S	9.00 ± 0.70	25.50 ± 0.64
17		O	11.25 ± 0.85	30.25 ± 0.85*
18		S	9.50 ± 0.64	28.25 ± 0.85
Std	Rosiglitazone		11.25 ± 0.47	35.25 ± 1.25**

Group 1: 8.75 ± 0.75, Group 2: 25.25 ± 1.1. *P <0.05, **P <0.01, Std: standard drug.



Scheme 2. Preparation of thiazolidine-2,4-dione. Reagents and conditions: (a) 0–5 °C, water, stirring for 15 min. (b) HCl, reflux 12 h, 89%.

We neither compared set one versus set two groups nor all the groups with each other, as it is well known biologically that insulin enhances the glucose uptake. The focus was, therefore, turned to comparing the differences made by the presence of compounds.

There is a reasonable correlation between the groups 3–18 versus groups 19–34 with a correlation coefficient value of 0.2982

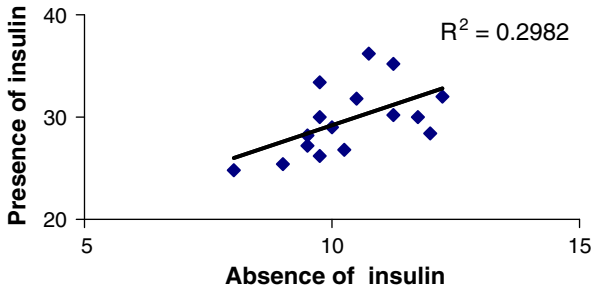


Figure 2. Correlation between the groups 3–18 versus groups 19–34.

(Fig. 2). This indicates some degree of correlation for the compounds with respect to their glucose uptake enhancement in the absence and the presence of insulin.

The results of the in vitro glucose uptake study indicate that all the compounds enhance the glucose uptake by the tissue except compound **9** (groups 8 and 24). The compounds exhibit from weak to moderate and from moderate to significant glucose uptake. The results also reveal one key aspect, namely, the compounds significantly enhance the glucose uptake in the presence of the insulin rather in the absence of external insulin. It is quite evident, therefore, that this class of compounds tends to sensitize the tissue to take up insulin which later enhances the glucose utilization by the tissue cells.[19] Out of the 15 compounds, compounds **12** (group 27), **13** (group 28), **4** (group 19), and **10** (group 25) including rosiglitazone significantly enhance the glucose uptake in the presence of insulin. Compound **12** is seen to be the most active compound even when compared to rosiglitazone. It is, therefore, a candidate compound to investigate further. Compounds **17** (group 32), **5** (group 20), and **7** (group 22) exhibit reasonably good glucose uptake in the presence of insulin.

We have also investigated the structure–activity relationships based on the results obtained. In doing so, we defined the pharmacophore part of the structure by aligning energy minimized and conformationally analyzed structures against common substructure and the most active compound **12** by atom fit method using Sybyl 6.7 software (Tripos, USA).[20] The superimposed common part of the structure, namely, polar thiazolidinedione or its bioisosteric rhodanine head followed by hydrophobic benzyloxy trunk which in turn is connected with two carbon linker in association with the amide bond, seems to be the pharmacophore (Fig. 3). If we disconnect and analyze individually the different parts of the structures, it seems that compounds with thiazolidinedione moiety as a head group shows better activity when compared to its bioisostere rhodanine. Compound **7** alone is an exception to this statement. This indicates the necessity of a relatively more polar head. Regarding the hydrophobic trunk part, it appears vanillin is doing extremely good as many of the compounds in the series contain the same. Two carbon linker in the form of amide (CH_2CONH) which is the common structural moiety in all the compounds appears to be a requirement for the activity.

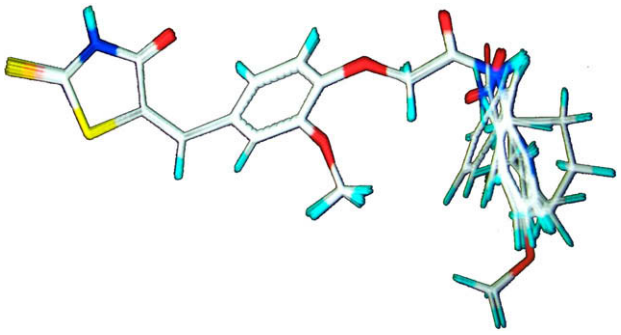


Figure 3. Energy minimized, aligned image of all the synthesized and screened glitazones.

The hydrophobic tail part which differs in most of the compounds is of major interest. Compound **12** with anisidine moiety is the most active compound from this series, even when compared with the standard drug (rosiglitazone). Compound **13** with a similar tail and rhodanine as a head also show equally good activity. This prompted us to go one step ahead with these compounds. Hence, we reduced the C=C of compounds **12** and **13** to yield compounds **17** and **18**. Compounds **17** and **18**, however, failed to meet the expectations.

In conclusion, we have found a new series of glitazones with different body make up having promising levels of glucose uptake activity. Compound **12** seems to be the candidate compound to investigate further for its safety and efficacy. The in vitro and in vivo studies on the title compounds will be reported in due course.

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14. *Structural data for some representative molecules* (**12**): IR (KBr) 3369, 3200, 3072, 3005, 1697, 1676, 1514, 1271, 1151, 1037, 829; ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.70 (s, 3H, CH_3), 3.85 (s, 3H, CH_3), 4.74 (s, 2H, CH_2), 6.88 (d, J = 9.02 Hz, 2H, ArH), 7.06–7.25 (m, 3H, ArH), 7.50(d, J = 9.02 Hz, 2H, ArH), 7.74 (s, 1H, =CH), 9.93 (s, 1H, NH), 12.50 (bs, 1H, NH); ^{13}C NMR (400 MHz, DMSO-d_6) δ 55.12, 55.64, 67.90, 113.87, 121.02, 123.35, 126.62, 131.42, 131.93, 149.17, 149.38, 155.51, 165.53, 167.33; HRMS (ES-TOF) m/z found 437.0013 $(M+Na)^+$, calcd 437.0 $(M+Na)^+$. (**13**) IR (KBr) 3367, 3198, 3072, 3001, 1708, 1678, 1512, 1442, 1209, 1037, 831; ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.71 (s, 3H, CH_3), 3.86 (s, 3H, CH_3), 4.75 (s, 2H, CH_2), 6.87 (d, J = 9.04 Hz, 2H, ArH), 6.89–7.22 (m, 3H, ArH), 7.51(d, J = 9.04 Hz, 2H, ArH), 7.59 (s, 1H, =CH), 9.97 (s, 1H, NH), 13.73 (bs, 1H, NH); ^{13}C NMR (400 MHz, DMSO-d_6) 55.12, 55.66, 67.79,

113.86, 120.97, 123.24, 124.09, 126.58, 131.40, 131.70, 149.23, 149.72, 155.46, 165.41, 195.69; HRMS (ES-TOF) m/z found 453.0118 $(M+Na)^+$, calcd 453.0 $(M+Na)^+$. (**17**) ^1H NMR (500 MHz, DMSO-d_6) δ 3.03 (dd, J = 14.5, 5.0 Hz, 1H, CH_2), 3.35 (dd, J = 14.5, 5.0 Hz, 1H, CH_2), 3.73 (s, 3H, CH_3), 3.79 (s, 3H, CH_3), 4.61 (s, 2H, CH_2), 4.88 (dd, J = 9.5, 4.0 Hz, 1H, CH), 6.75 (d, J = 8.5 Hz, 1H, ArH), 6.89 (d, J = 9.0 Hz, 2H, ArH), 6.90 (s, 1H, ArH), 6.93 (d, J = 8.5 Hz, 1H, ArH), 7.52 (d, J = 9.0 Hz, 2H, ArH), 9.86 (s, 1H, NH), 12.04 (bs, 1H, NH); ^{13}C NMR (400 MHz, DMSO-d_6) 36.97, 52.92, 55.11, 55.59, 68.67, 113.36, 113.82, 114.49, 121.11, 130.76, 131.37, 146.46, 148.99, 155.45, 166.08, 171.69, 175.82; HRMS (ES-TOF) m/z found 439.0026 $(M+Na)^+$, calcd 439.0 $(M+Na)^+$.

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18. *(a) Glucose uptake measurement*: Six well microtitre plates were selected for the study with each well capacity of 5 ml (n = 4). 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 3–17: 2 ml of Tyrode solution with 2000 mg/l glucose and 2 mg of the test compound **4**–**18**. Group 18: 2 ml of Tyrode solution with 2000 mg/l glucose and 2 mg of rosiglitazone (standard). Groups 19–33: 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 **4**–**18**. Group 34: 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). 68 Wistar rats of either sex were maintained on a standard pellet diet, water ad libitum, and fasted overnight. The animals were killed by decapitation and diaphragms were taken out swiftly avoiding trauma and divided into two halves. The hemi-diaphragms 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 kit and Merck-Microlab 200 analyser.
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