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CONTENTS

Serum glucose and triglyceride lowering activity of some novel glitazones against dexamethasone-induced hyperlipidemia and insulin resistance

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ABSTRACT

Objectives: To study the serum glucose and triglyceride lowering activity of some novel glitazones against dexamethasone-induced hyperlipidemia and insulin resistance in rats.

Materials and Methods: Serum glucose and triglyceride lowering activity of the test compounds and a standard, rosiglitazone, was tested at a dose of 10 mg/kg p.o. against dexamethasone-induced hyperlipidemia and insulin resistance in Sprague-Dawley (SD) rats. On day 11 of treatment, blood was collected for the estimation of serum glucose and triglyceride levels.

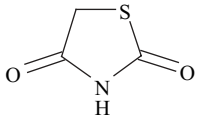
Results: All the 14 compounds and rosiglitazone significantly ($P < 0.05$) decreased dexamethasone-induced elevation of serum glucose when compared to dexamethasone-alone-treated group. Among these compounds, compound 10 showed better antihyperglycemic activity than rosiglitazone. Compounds 7, 8 and 9 have shown significant ($P < 0.05$) serum triglyceride lowering activity than did rosiglitazone. Out of the 14 compounds tested, only compounds 7, 8 and 9 have shown both serum glucose and triglyceride lowering activity.

Conclusion: The present study indicates that some of the newer glitazones show significant glucose and triglyceride lowering activity. In comparison to rosiglitazone, these glitazones show comparable serum glucose lowering and better triglyceride lowering activity.

KEY WORDS: Dexamethasone, glitazones, hyperlipidemia, insulin resistance, peroxisome proliferator-activated receptor

Diabetes is a global disease with a huge adverse impact on health and mortality, particularly from cardiovascular disorders. It occurs at any time of life from infancy to old age. Type-2 diabetes is primarily a lifestyle disorder, which accounts for around 90% of diabetes cases and increasing at an astonishing rate, particularly in developing countries like India. In 1995, it has been estimated that around 135 million people had this condition, and this may increase to as many as 300 million by the year 2025.[1]

Most commonly employed oral hypoglycemic agents are sulfonylureas and biguanides. These drugs, however, have disadvantages such as primary and secondary failure of efficacy as well as the potential for induction of severe hypoglycemia.[2] There is a need, therefore, for new candidate molecules that may effectively reduce insulin resistance or potentiate insulin action in genetically diabetic or obese individuals. New drugs that reverse insulin resistance without stimulating insulin release from β-cells also fulfill a major medical need in the treatment of non-insulin-dependent diabetes mellitus (NIDDM). The search for such drugs with a potential to reduce long-term complications of NIDDM is, therefore, of current interest. Between 1997 and 1999, a new class of drugs called 'glitazones' was approved by the FDA for the treatment of type-2 diabetes.[3] These agents share a common chemical structure, namely thiazolidine-2,4-dione (TZD).



Glitazones correct hyperglycemia by enhancing tissue sensitivity to insulin,[4-6] as a result of which glitazone treatment is not associated with dangerous hypoglycemic incidents that are normally observed with conventional sulfonylurea agents and insulin therapy.[7] In the mid-1990s, the molecular target of glitazones was reported to be peroxisome proliferator-activated receptor gamma (PPAR-γ).[8-11] Undesirable side-effects associated with existing glitazones, however, have been observed in animal and human studies, including cardiac hypertrophy, haemodilution and severe liver toxicity.[12-17]

Furthermore, it is known that PPAR-α, another nuclear receptor belonging to PPAR family, regulates genes that are important for lipid metabolism. As people with diabetes often have adverse changes to their plasma lipid profile,[18] it would be worthwhile to find an agent that could activate PPAR-α. An existing class of medicines that act on PPAR-α and being prescribed to control dyslipidaemia are fibrates. A single agent that could stimulate both PPAR-α and PPAR-γ, however, would be more valuable.[19] The focus today is, therefore, on the development of newer glitazone PPAR agonists, which might surmount the problem associated with the known TZDs, and thus offer advantage as anti-diabetic agents. The objective of the present study was, therefore, to evaluate some newer glitazone molecules for their serum glucose and triglyceride lowering potential.

Materials and Methods

Animals

Male Sprague-Dawley rats weighing between 200 and 250 g were procured from in-house animal facility of J.S.S. College of Pharmacy, Ootacamund. The animals were housed under standard conditions of temperature (22 ± 3°C) and relative humidity (30-70%) with a 12:12 light-dark cycle. The animals were fed with standard pellet diet (Amrit Feeds Ltd., Bangalore) and water *ad libitum*. The Institutional Animal Ethics Committee (IAEC) of J.S.S. College of Pharmacy approved the proposal.

Chemicals

Dexamethasone sodium phosphate was obtained as a gift sample from M/s. Strides Arcolabs, Bangalore, India. Triglyceride and glucose estimation kits were from Ecoline, Merck Ltd., Mumbai, India. All the other reagents and chemicals used in the study were of analytical grade.

Synthesis of newer glitazones

Novel glitazones were synthesized with an appropriate synthetic scheme using both conventional and microwave methods of synthesis. The synthesized glitazones were characterized for their structures by IR, NMR, mass and elemental analysis. The details of synthesis and characterization of these glitazones are reported elsewhere.[20] The structures of synthesized glitazones, which are selected for pharmacological screening based on quantitative structure activity relationship (QSAR) studies, are given in Figure 1.

Preparation of the test item for oral gavage

All the 14 compounds and rosiglitazone were suspended in 0.5% w/v sodium carboxymethyl cellulose (CMC) at the concentration of 1 mg/ml. These suspensions were administered at a dose volume of 10 ml/kg body weight.

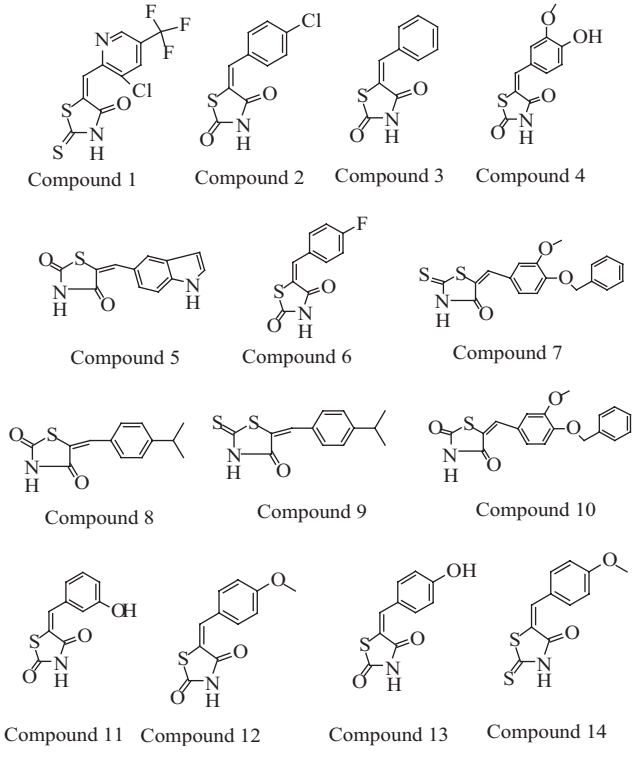
Dose selection

All the newly synthesized glitazones were structural analogs of rosiglitazone. All these compounds were, therefore, tested at an effective anti-diabetic dose of rosiglitazone in rats, i.e., 10 mg/kg, p.o. As a preliminary study, all these synthesized compounds and rosiglitazone were tested at a single dose level.

Dexamethasone-induced insulin resistance and hyperlipidemia[21]

Animals were divided into 17 groups, each consisting of six rats. Rats in the first group received vehicle (10 ml/kg p.o.) and served as control group, while the second group of rats received vehicle (10 ml/kg p.o.) plus dexamethasone (10 mg/kg s.c.) and served as positive control group. Rats in experimental groups 3-16 were treated with compounds 1-14 (10 mg/kg p.o.) plus dexamethasone (10 mg/kg s.c.), whereas rats in the 17th group were treated with rosiglitazone (10 mg/kg p.o.) plus dexamethasone (10 mg/kg s.c.). All the animals received their respective assigned treatment daily for a period of 10 days. Rats of group 2-17 were daily fasted overnight before dexamethasone treatment.[21] On day 11, the animals were anesthetized with ether, and blood was collected from retero-orbital plexus. Serum was then separated for the estimation of glucose and triglyceride using Ecoline triglyceride and glucose estimation kits.

Figure 1: Structures of some novel glitazones synthesized and screened



Statistics

All the results were expressed as mean ± SEM, and the data were analyzed using one-way ANOVA followed by Dunnett's multiple comparison post-test using GraphPad Prism 4 software. *P*-values <0.05 were considered significant.

Results

Effect on serum glucose level

All the 14 compounds and the standard, rosiglitazone, at a dose of 10 mg/kg p.o. significantly ($P < 0.05$) decreased dexamethasone-induced elevation of serum glucose. Among the 14 compounds tested, compound 10 (group 12) showed maximum anti-hyperglycemic activity that was superior to rosiglitazone (group 17) [Table 1].

Table 1

Effect of glitazones on dexamethasone-induced elevation in serum glucose levels

Group	*Treatment*	*Serum glucose (mg/dl)*	*Serum triglyceride (mg/dl)*
1 (Normal)	Vehicle (10 ml/kg, p.o.)	61.5 ± 1.5	58.3 ± 1.9
2 (Positive control)	Vehicle (10 ml/kg, p.o.)	377.5 ± 39.5*	146.3 ± 12.3*
3	Compound-1 (10 mg/kg, p.o.)	140.3 ± 19.2*	119.8 ± 18.9
4	Compound-2 (10 mg/kg, p.o.)	102.0 ± 2.4*	219.0 ± 14.6
5	Compound-3 (10 mg/kg, p.o.)	101.0 ± 10.8*	229.3 ± 16.5
6	Compound-4 (10 mg/kg, p.o.)	87.0 ± 5.05*	139.3 ± 14.4
7	Compound-5 (10 mg/kg, p.o.)	102.5 ± 10.2*	121.3 ± 13.8
8	Compound-6 (10 mg/kg, p.o.)	118.3 ± 16.1*	120.0 ± 10.7
9	Compound-7 (10 mg/kg, p.o.)	81.5 ± 10.6*	86.8 ± 15.8*
10	Compound-8 (10 mg/kg, p.o.)	88.0 ± 4.9*	96.8 ± 13.3*
11	Compound-9 (10 mg/kg, p.o.)	83.2 ± 11.2*	99.5 ± 11.6*
12	Compound-10 (10 mg/kg, p.o.)	61.5 ± 10.3*	125.0 ± 13.8
13	Compound-11 (10 mg/kg, p.o.)	77.7 ± 12.7*	120.2 ± 13.5
14	Compound-12 (10 mg/kg, p.o.)	72.5 ± 11.3*	144.5 ± 13.9
15	Compound-13 (10 mg/kg, p.o.)	67.7 ± 8.5*	123.5 ± 14.9
16	Compound-14 (10 mg/kg, p.o.)	79.2 ± 5.2*	126.3 ± 19.6
17	Rosiglitazone (10 mg/kg, p.o.)	69.2 ± 2.1*	106.8 ± 8.2*

Values mean ± SEM, $n = 6$, *$P < 0.05$. Animals of groups 2 to 17 also received dexamethasone (10 mg/kg, s.c.)

Effect on serum triglyceride level

Out of the 14 compounds, compounds 7 (group 9), 8 (group 10) and 9 (group 11) showed significant triglyceride lowering activity as compared to dexamethasone-alone-treated group. Among these, compound 7 showed better activity when compared to the other two. Compounds 7, 8 and 9, however, showed better activity when compared to the standard, rosiglitazone [Table 1].

Discussion

Insulin resistance in type-2 diabetes is not only associated with hyperglycemia but also with hyperlipidemia and atherosclerosis.[18,22] A drug that simultaneously ameliorates insulin resistance and hyperlipidemia, therefore, facilitates better management of type-2 diabetes. The present study aims towards the development of such a drug. Out of the 14 compounds screened, only three compounds, namely compounds 7, 8 and 9, showed both serum glucose and triglyceride lowering activity. Compound 10, which was similar in structure to compound 7 except an oxo (=O) substitution instead of thione (=S) at position 2 in the thiazolidine ring, however, showed only glucose lowering effect, which was better than rosiglitazone. The absence of triglyceride lowering effect of this compound may be attributed to the presence of oxo substitution in the thiazolidine ring instead of thio. The same, however, was not observed with compounds 8 and 9, where both share similar structure except oxo and thione substitutions at position 2 of the thiazolidine ring, respectively. Compound 8, unlike compound 10, showed a significant triglyceride lowering potential; this may be attributed to the presence of an entirely different side chain at position 5 in the thiazolidine ring when compared to compound 10.

Compounds 7, 8 and 9 significantly lowered dexamethasone-induced serum glucose and triglyceride levels. In comparison to rosiglitazone, all these compounds have shown relatively less anti-diabetic activity, but better anti-hyperlipidemic activity. Among compounds 7, 8 and 9, compound 7 exhibited better anti-hyperlipidemic activity.

The better anti-hyperlipidemic activity of these three compounds when compared to rosiglitazone suggests that these compounds, in addition to PPAR-γ, may activate PPAR-α receptors, thereby resulting in better anti-hyperlipidemic activity. Further studies, however, are needed to confirm the same.

Conclusion

The results of the present study indicate that some of the newer glitazones synthesized show significant glucose and triglyceride lowering activities. In comparison to rosiglitazone, all these compounds show comparable serum glucose lowering and better triglyceride lowering activity.

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