

Synthesis, Biological Evaluation and Molecular Modeling Studies of 5-[4-(substituted) benzylidene or benzyl] thiazolidine-2,4-dione with Oral Antihyperglycemic Activity

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Abstract: A new series of 5-[4-(substituted) benzylidene or benzyl] thiazolidine-2,4-dione have been synthesized using economical synthetic routes. The synthesized compounds **4a-4c**, **7a-7c** and **14a-14c** were evaluated for their oral antihyperglycemic activity by fructose induced hyperglycemia in Wistar rats. From the results, compounds **7c** and **14b** have appreciable blood glucose-lowering effect compared to that of the reference drug, pioglitazone. Hypoglycemic activity of all compounds was compared with the results of their docking after removal of the co-crystallized ligand present in the 2PRG structure. Out of these compounds, compound **14b** shows better interaction with amino acid residues of PPAR- and also shows better oral antihyperglycemic activity in this series.

Key Words: Thiazolidinediones (TZD's), Glitazone, Blood glucose-lowering effect, Docking.

Introduction

Diabetes mellitus is a chronic metabolic disorder characterized by high levels of glucose in blood due to non-secretion of insulin or insulin insensitivity. Either of the factors causes disturbances in carbohydrate, lipid and protein metabolism.¹ Type 1 diabetes mellitus (Insulin dependent diabetes mellitus, IDDM) and Type 2 diabetes mellitus (Non insulin dependent diabetes mellitus, NIDDM) are now recognized as serious global health problem, growing rapidly worldwide² and taking its place as one of the main threats to human health in the 21st century.³

More than 90% of diabetic patients suffer from type 2 diabetes, which is characterized by insulin resistance and hyperglycemia. It has been estimated that a large number of type 2 diabetics remain undiagnosed. Several epidemiological and clinical studies indicate a direct relationship between hyperglycemia and long term microvascular and macrovascular complications such as neuropathy, retinopathy, nephropathy, myocardial infarction, atherosclerosis, coronary artery disease, stroke, and lower limb amputation, which develop as the disease progresses, gradually decrease quality of life of diabetic patients. Therefore, it is essential to control blood glucose levels during the early stages of the disease.^{4,5} This metabolic disease is also linked to a wide spectrum of other pathophysiologic conditions including dyslipidemia (hypertriglyceridemia, decreased serum HDL cholesterol, and increased small dense LDL particles), hypertension, hyperuricemia, increased plasminogen activator inhibitor-1 (PAI-1), abnormal fibrinolytic system, and abdominal obesity.⁶

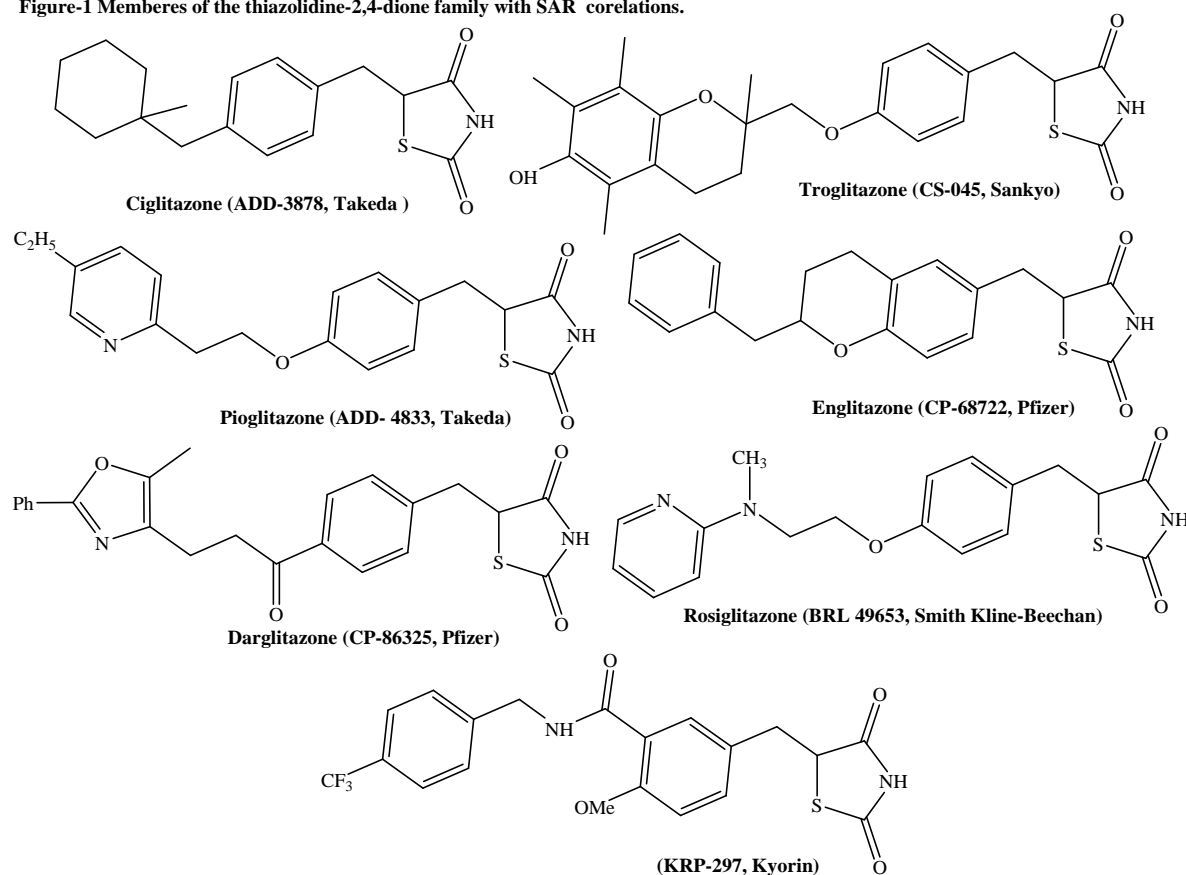
Therapy for type 2 diabetes primarily has been aimed at improving glycemic control via a combination of diet, exercise,⁷ and current therapeutic agents such as insulin formulations, sulfonylureas, metformin, acarbose, thiazolidinediones (TZDs), dipeptidyl peptidase IV inhibitors, Glucagon-like peptide (GLP)-1 analogs.⁸ 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. TZDs are known to be selective agonists of peroxisome proliferator-activated receptor- (PPAR-), thereby increasing insulin sensitivity at adipose, muscle and hepatic tissues.⁹

Of the thiazolidine-2,4-dione compounds, ciglitazone, troglitazone, pioglitazone, englitazone, darglitazone, rosiglitazone and KRP-297 have been clinically examined. Unfortunately, ciglitazone, englitazone, darglitazone and KRP-297 were discontinued in clinical development (Figure-1). Troglitazone, after launching in 1997 was subsequently withdrawn from the market due to its hepatotoxicity.^{10,11} Between 1997 and 1999, rosiglitazone and pioglitazone was approved by the FDA for the treatment of type 2 diabetes.¹² Rosiglitazone is more potent ligand of PPAR- and shows efficient insulin-sensitization in type 2 diabetes patients.¹³ It was also associated with a significantly increased the risk of myocardial infarction, heart failure and death as a result of cardiovascular complication.¹⁴

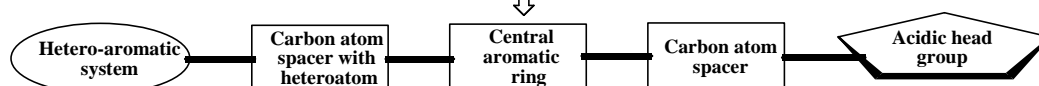
In contrast to rosiglitazone however, pioglitazone is associated with significantly lower risk of death and myocardial infarction. Serious heart failure is increased by pioglitazone, although without an associated increase in mortality.¹⁵ It also showed favorable reductions in serum total cholesterol, triglycerides, LDL cholesterol, VLDL cholesterol and increase in HDL cholesterol as compared to rosiglitazone. In addition to this, pioglitazone has been shown to possess partial PPAR- agonistic activity that may be responsible for its differential effects seen, as compared to rosiglitazone.¹⁶

Typical structure-activity-relationships (SAR) study of most of the potent antihyperglycemic TZD compounds containing acidic head group, carbon atom spacer, central aromatic ring, carbon atom spacer with heteroatom and substituted hetero-aromatic group (Figure-1).

Figure-1 Members of the thiazolidine-2,4-dione family with SAR correlations.



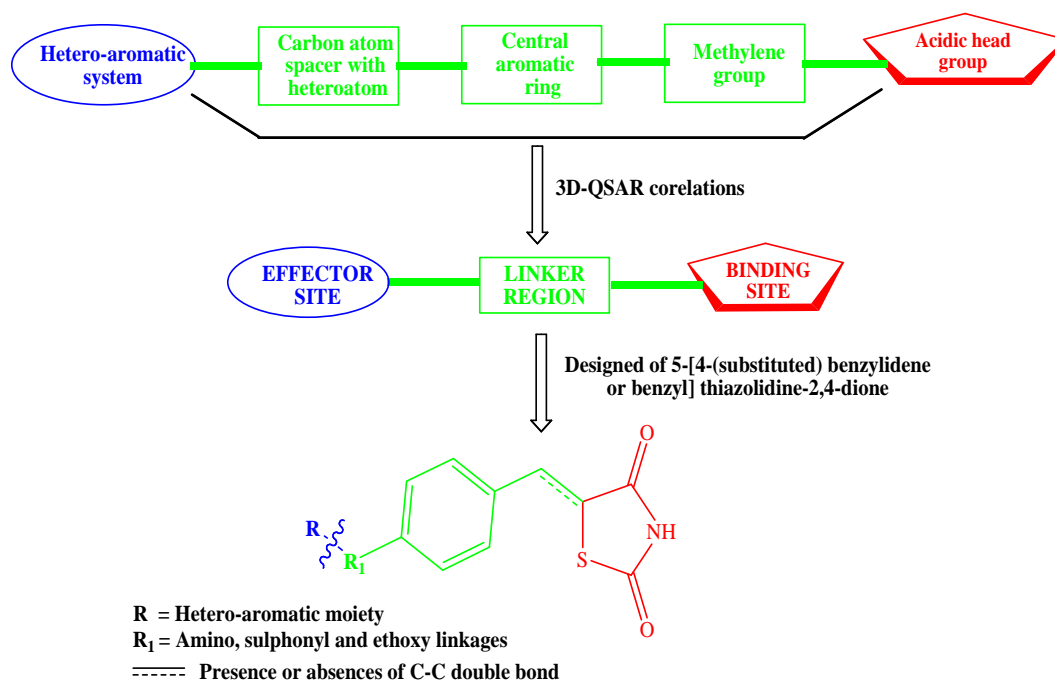
SAR correlations



iA three-dimensional quantitative structure-activity-relationships (3D-QSAR) study of thiazolidine-2,4-dione antihyperglycemic agents bind with PPAR- receptor reveal that, the binding site is essential in all TZDs. A large number of variations to this ring were reported but no correlation was observed with the acidic strength and activity. Therefore, structural factors override the acid strength in this region. The effector site is a secondary region that modifies the pharmacokinetic and toxicity profiles. This is an important region of the molecule where wide choice of lipophilic substituents can be made to design better antihyperglycemic agents. And there is linker region present between the binding and the effector sites. This region containing central aromatic ring is essential for activity. Because this region has a limited space and hence large substituted fragments in the central aromatic region leads to reduced activity (Figure-2).¹⁷ Based on this conclusion, the present invention was aimed at the modification of effector site and linker region.

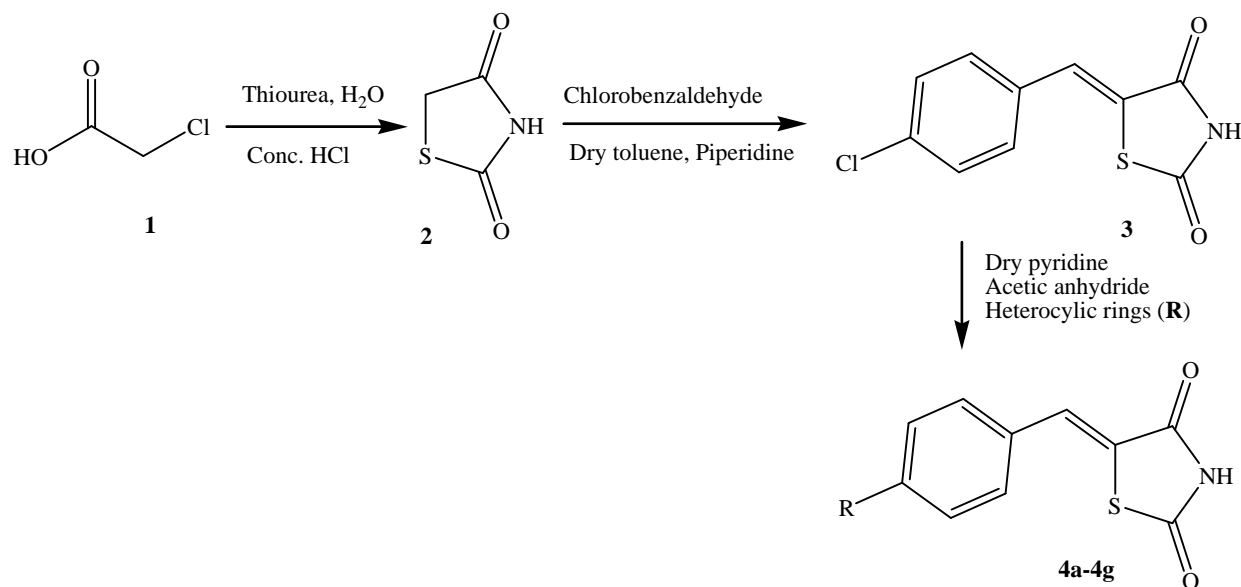
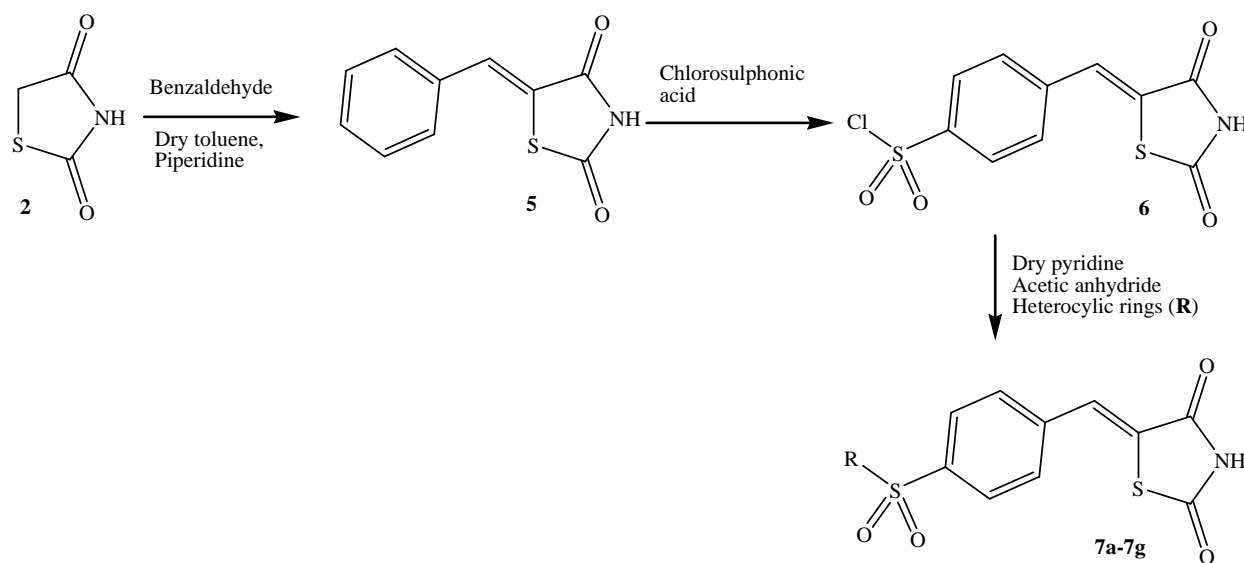
In the present work, thiazolidine-2,4-dione moiety serves as the acidic head end and the hetero-aromatic moiety (**R**) serves as the tail end of the molecules. Both ends are linked through the 5-position of thiazolidine-2,4-dione moiety via benzylidene, para sulphonyl benzylidene and ethoxybenzyl linkages towards development of better and safer glitazones than the available ones (Figure-2).

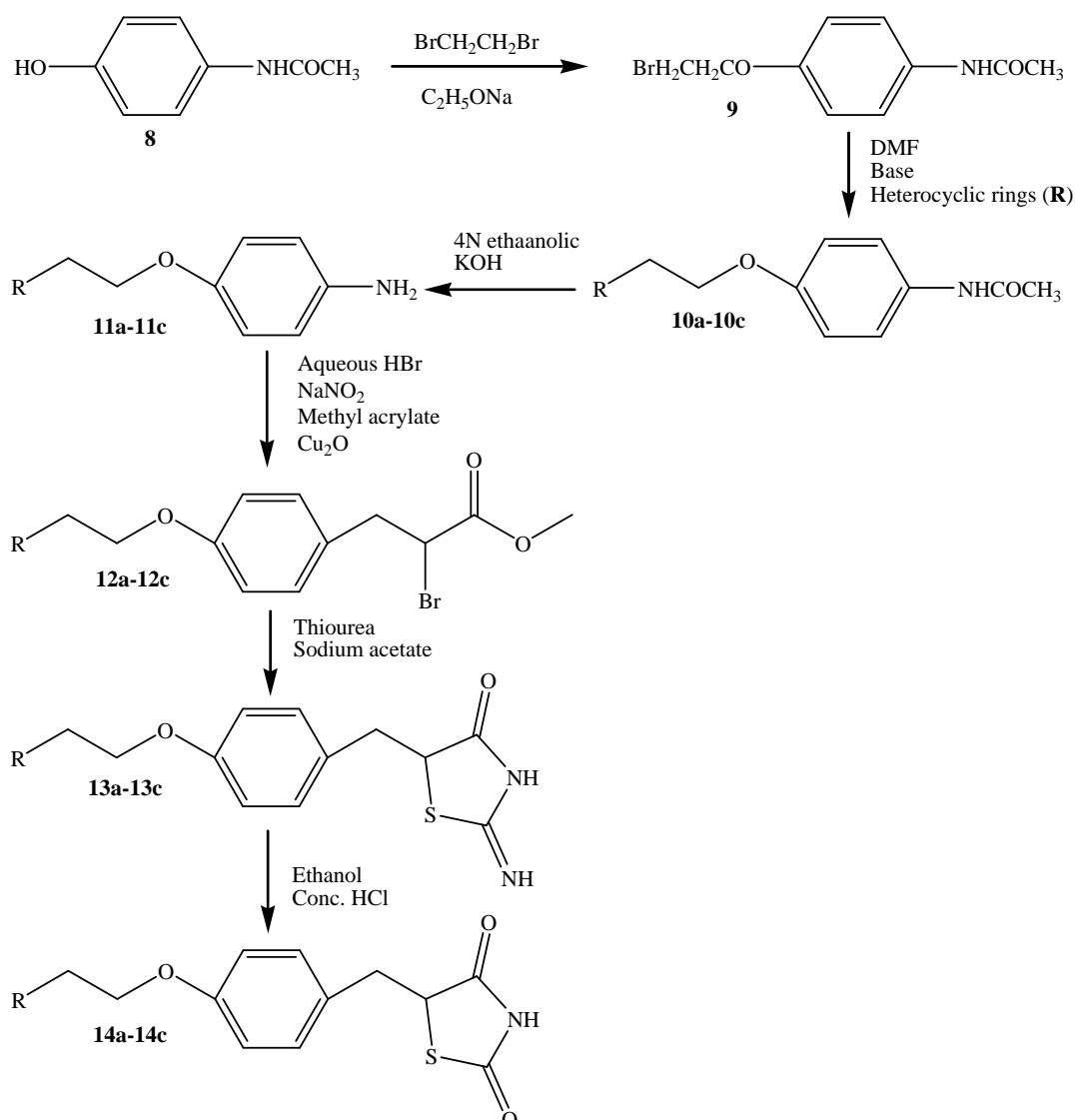
Figure-2 SAR and 3DQSAR correlations. Based on correlation, designed of 5-[4-(substituted) benzylidene or benzyl] thiazolidine-2,4-dione



Experimental

The general strategies for synthesis of some new 5-[4-(substituted) benzylidene or benzyl] thiazolidine-2,4-dione derivatives were adopted from literature reports with minor modifications wherever necessary as illustrated in schemes 1, 2 and 3.

Scheme-1. Synthesis of 5-(4-(substituted)benzylidene)thiazolidine-2,4-diones.**Scheme-2. Synthesis of 5-[4-(substituted)sulfonylbenzylidene]thiazolidine-2,4-dione.**

Scheme-3. Synthesis of 5-[4-(substituted)ethoxybenzyl]thiazolidine-2,4-dione

All the solvents used were of analytical grade. Reactions were monitored with the help of TLC using precoated silica gel as stationary phase, using appropriate solvent system as mobile phase and iodine vapors as visualizing agent. Melting points were determined in Elico melting point apparatus and are uncorrected. IR spectrum of all compounds was recorded on Perkin-Elmer AC-1 Spectrophotometer (Glenmark Pharmaceutical Ltd, Nashik) by using KBr pellet and values are expressed in cm^{-1} . NMR spectra were recorded on BROOKER spectrophotometer (Analytical center, University of Pune, Pune.) by using varian 800 MHz in DMSO-d_6 and CDCl_3 as solvent and chemical shift are reported in ppm. Mass spectrum were recorded on Shimadzu QP2010 (MGV college of Pharmacy, Nashik.) and mass values were reported in m/z.

Synthesis of Thiazolidine-2,4-dione [2].¹⁸

In a 250ml three-necked flask was placed, a solution containing chloroacetic acid (56.6g, 0.6mol) in water (60ml) and thiourea (45.6g, 0.6mol) dissolved in water (60ml). The mixture was stirred for 15min to obtain a white precipitate, accompanied by considerable cooling. To the contents of the flask was then added slowly concentrated hydrochloric acid (60ml) from a dropping funnel. The flask was then connected with a reflux condenser and gentle heat applied to effect complete dissolution, after which the reaction mixture was stirred and refluxed at $100\text{-}110^\circ\text{C}$ for 8-10hrs. On cooling, the content of the flask solidifies into cluster of white needles. The product was filtered and washed with water to remove traces of hydrochloric acid and dried. It was purified by recrystallization from ethanol. Yield 85%, mp $123\text{-}125^\circ\text{C}$.

Synthesis of 5-(4-chlorobenzylidene)thiazolidine-2,4-dione [3].

In a 250ml three-necked flask provided with a Dean-Stark apparatus, chlorobenzaldehyde (26g, 0.188mol) and thiazolidine-2,4-dione (22g, 0.188mol) were together suspended in dry toluene. To this catalytic amount of piperidine (1ml) was added. The mixture was refluxed with stirring. After complete removal of water and when temperature crossed 110°C the reaction was stirred for a further 30min. On cooling, the product precipitated out from toluene. The compound was filtered and washed with cold, dry toluene and dry ethanol. Yield 93%, mp 240-242°C.

General procedure for synthesis of 5-[4-(substituted)benzylidene]thiazolidine-2, 4-dione [4a-4c].

Heterocyclic rings (**R**) (0.1mol) treated with 5-(4-chlorobenzylidene)thiazolidine-2,4-dione (0.1mol) in the presence of dry pyridine (4ml) and acetic anhydride (20ml) mixture. The reaction mixture was refluxed for 2-3hrs (monitored by TLC), after cooling the reaction mixture was poured on crushed ice with stirring. Thus separated solid was then filtered. The resulting crude compound was dried and recrystallized by using ethanol as recrystallization solvent.

Synthesis of 5-benzylidene-thiazolidine-2,4-dione [5].¹⁸

In a 250ml three-necked flask provided with a Dean-Stark apparatus, benzaldehyde (20g, 0.188mol) and thiazolidine-2,4-dione (22g, 0.188mol) were together suspended in dry toluene. To this catalytic amount of piperidine (1ml) was added. The mixture was refluxed with stirring. After complete removal of water and when temperature crossed 110°C the reaction was stirred for a further 1hr. On cooling, the product precipitated out from toluene. The compound was filtered and washed with cold, dry toluene and dry ethanol. Yield 89-93%, mp240-242°C

Synthesis of 5-(4-chlorosulphonylbenzylidene)thiazolidine-2,4-dione [6].¹⁸

5-Benzylidene thiazolidine-2,4-dione (8g, 0.0388mol) was placed in a 100ml round-bottom flask equipped with a condenser and a dropping funnel. Chlorosulphonic acid (18.08g, 0.155mol) was placed at room temperature using the dropping funnel. The reaction was found to be exothermic, after addition of chlorosulphonic acid was over the reaction mixture was refluxed for 1hr on a water bath. The reaction mass was cooled and poured in a thin stream with stirring into crushed ice contained in a 1L beaker. It was filtered and dried and purified by recrystallization from ethanol. Yield 68%, mp 180-181°C.

General procedure for synthesis of 5-[4-(substituted)sulfonyl benzylidene] thiazolidine-2,4-dione [7a-7c].¹⁸

Heterocyclic rings (**R**) (0.1mol) treated with 5-(4-chlorosulphonylbenzylidene) thiazolidine-2, 4-dione (0.1mol) in the presence of dry pyridine (4ml) and acetic anhydride (20ml) mixture. The reaction mixture was refluxed for 2-3hrs (monitored by TLC), after cooling the reaction mixture was poured on crushed ice with stirring. Thus separated solid was then filtered. The resulting crude compound was dried and recrystallized by using ethanol as recrystallization solvent.

Synthesis of 4-[2-(bromo ethoxy)] acetanilide [9].

To dry ethanol (533ml), sodium metal (20.4g, 0.88mol) was added slowly in small pieces. Then the resulting hot reaction mixture was cooled on a water-bath. Above cooled mixture, paracetamol (120g, 0.88mol) was added followed by slow addition of ethylene dibromide (153g, 0.88mol). Then the reaction mixture was refluxed for 3-4hrs. After completion of the reaction, the reaction mixture was dumped into ice cold water, and the precipitated solid was collected by filtration under suction. Yield 65%.

General procedure for synthesis of 4-[2-(substituted) ethoxy] acetanilide [10a-10c].

To a solution of an appropriate substituted heterocyclic rings (**R**) (0.025mol) in dry Dimethyl Formamide (37.5ml) at 25°C was added an appropriate base (0.027mol), and the reaction mixture stirred for 0.25-1 hr. A solution of 4-[2-(bromoethoxy)] acetanilide (0.025mol) in dry Dimethyl Formamide (12.5ml) was added and stirred further 0.25-6 hrs. The reaction mixture was dumped into ice cold water and precipitated solid was filtered under suction and dried in oven. Yield 67 to 75%.

Synthesis of 4-[2-(substituted) ethoxy] aniline [11a-11c].

A mixture of 4-(2-(substituted) ethoxy) acetanilide (0.016 mol), 4N potassium hydroxide (75ml) and ethanol (75ml) was refluxed for 24hrs and dumped in ice cold water and precipitated solid were filtered and dried in oven. Yield 35 to 40%.

General procedure for synthesis of Methyl-2-bromo-3-[4-(substituted)ethoxybenzyl] propionate [12a-12c].¹⁹

A solution of sodium nitrite (0.24mol) in water (30ml) was added drop wise to a stirred and ice cold mixture of appropriate 4-[2-(substituted) ethoxy] aniline (0.22mol), aqueous hydrobromic acid (47 %, 0.88mol), methanol (200ml) and acetone (500ml) below 5°C. The whole reaction mixture was stirred at 5°C for 30 min and methyl acrylate (1.32 mol) was added and the temperature was raised to 38°C. Powdered cuprous oxide (0.014mol) was added in small portions to vigorously stirred mixture. After a nitrogen gas evolution had ceased, the reaction mixture was concentrated in vacuo. The residue was diluted with water, made alkaline with concentrated ammonia hydroxide and extracted with ethyl acetate. The ethyl acetate extract was washed with brine, dried magnesium sulphate and concentrated in vacuo to give crude oil. Yield 60 to 70%.

General procedure for synthesis of 2-imino-5-[4-(substituted)ethoxybenzyl]-4-thiazolidinone [13a-13c].¹⁹

A mixture of methyl-2-bromo-3-[4-(substituted)ethoxybenzyl] propionate (crude oil, 0.087 mol), thiourea (0.087mol), sodium acetate (0.087mol) and ethanol (350ml) was stirred under reflux for 3hrs, and concentrated in vacuo. The residue was neutralized with aqueous sodium bicarbonate and Et₂O (150ml) – hexane (150ml) was added. The whole was stirred at room temperature for 15min, and the crystals were collected by filtration to imino compound. Yield 57 to 65%.

General procedure for synthesis of 5-[4-(substituted)ethoxybenzyl]thiazolidine-2,4-diones [14a-14c].¹⁹

A mixture of 2-imino-5-[4-(substituted)ethoxybenzyl]4-thiazolidinone (0.046mol), 2N HCl (200ml) and ethanol (200 ml) was stirred under reflux for 12hrs. The reaction mixture was concentrated in vacuo. The residue was diluted with water, neutralized with saturated aqueous sodium bicarbonate and extracted with chloroform. The chloroform extract was washed with brine, dried with anhydrous magnesium sulphate and concentrated in vacuo to give the title compound.

5-[4-(2-amino-3-carbethoxy-4,5,6,7-tetrahydrobenzothiophene)benzylidene]thiazolidine-2,4-dione

[4a]: Yellow powder; m.p.195-197°C; % yield: 72%; Rf: 0.67 (Chloroform: Acetone::8:2); IR(KBr) 3428(NHstr.sec.amine), 3287(NHstr.TZD), 3032(CHstr.Ar.), 1750 (C=Ostr.TZD), 1648(C=Cstr.Ar.), 1557(C=Cstr.heterocyclic), 1215(C-Nstr.), 759(C-S-Cstr. TZD), 703(C-S-Cstr.thiophene).

5-[4-(2-Amino-3-carbethoxy-4,5-dimethoxythiophene)benzylidene]thiazolidine-2,4-dione[4b]:

Brown powder; m.p.186-188°C; % yield: 72%; Rf: 0.65(Chloroform:Acetone:: 8:2); IR (KBr) 3422(NHstr.sec.amine), 3233(NHstr.TZD), 3076(CHstr.Ar.), 1738(C=Ostr.), 1665 (C=Cstr.Ar), 1557(C=Cstr.heterocyclic), 1254(C-Nstr.), 723(C-S-Cstr.TZD), 705(C-S-Cstr. thiophene). ¹HNMR(800MHz,DMSO-d₆) 12.62(1H,NH,TZD), 10.04(1H,NH,sec. amine), 7.80(1H,Arylidene), 7.45(2H,Ar.),7.27(2H,Ar.), 4.30(2H,CH₂,ester), 2.70(3H,CH₃), 1.38(3H, CH₃,ester). MS *m/z* 402 (M)⁺.

5-[4-(2-amino-3,5-dicarbethoxy-4-methylthiophene)benzylidene]thiazolidine-2,4-dione[4c]:

Dark brown powder; m.p.207-209°C; %yield:78%; Rf:0.73 (Chloroform: Acetone ::9:1); IR (KBr) 3434(NHstr.sec.amine), 3251(NHstr.TZD), 3071(CHstr.Ar.), 1733(C=O str.), 1640(C=Cstr.Ar.), 1587(C=Cstr.heterocyclic), 1254(C-Nstr.), 759(C-S-Cstr.TZD), 692 (C-S-Cstr.thiophene).

5-[4-(2-Amino-3-carbethoxy-4,5,6,7-tetrahydrobenzothiophene)sulfonylbenzylidene]thiazolidine-2,4-dione

[7a]: Dark yellow crystal; m.p.268-270°C; % yield: 51%; Rf: 0.95 (Chloroform: Acetone:: 9:1); IR (KBr) 3405(NHstr.TZD), 3299(NHstr. sulfonamide), 3025 (CHstr.Ar.), 1742(C=Ostr.), 1648(C=Cstr.Ar.), 1562(C=Cstr.heterocyclic), 1367(S=Ostr.), 1215(C-Nstr.), 742(C-S-Cstr.thiophene), 706(C-S-Cstr.TZD). ¹H NMR (800MHz,DMSO-d₆) 10.56(1H,NH,TZD), 8.18(1H,NH,sulfonamide), 7.32(1H,arylidene), 6.18(2H,Ar.), 5.92(2H, Ar.), 4.15(2H,CH₂,ester), 2.84(2H,CH₂,cyclohexene), 2.65(2H,CH₂,cyclohexene), 2.32(2H, CH₂,cyclohexene), 1.84(2H,CH₂,cyclohexene), 1.30(3H,CH₃,ester). MS *m/z* 492 (M)⁺.

5-[4-(2-amino-3-carbethoxy-4,5-dimethoxythiophene)sulfonylbenzylidene]thiazolidine-2,4-dione[7b]:

Faint brown powder; m.p.213-215⁰C; % yield: 65%; Rf: 0.67 (Chloroform: Acetone::8:2); IR (KBr) 3422 (NHstr.TZD), 3248(NHstr.sulfonamide), 3030(CHstr.Ar), 1745(C=Ostr.), 1655(C=Cstr.Ar.), 1560(C=Cstr.heterocyclic), 1340(S=Ostr.), 754(C-S-Cstr. TZD), 696(C-S-Cstr.thiophene).

5-[4-(2-Amino-3,5-dicarbethoxy-4-methyl thiophene) sulfonyl benzylidene] thiazolidine-2,4-dione[7c]:

Brown needle crystal; m.p.226-228⁰C; % yield: 78%; Rf: 0.87 (Chloroform: Acetone:: 8:2); IR (KBr) 3383(NHstr.TZD), 3285(NHstr.sulfonamide), 3030(CHstr.Ar.), 1767(C=Ostr.), 1629(C=Cstr.Ar), 1570(C=Cstr.heterocyclic), 1374(S=Ostr.), 761(C-S-Cstr. TZD), 684(C-S-Cstr.thiophene). ¹H NMR(800MHz,DMSO-d₆) 10.60(1H,NH,TZD), 8.20 (1H,NH,sulfonamide), 7.80(1H,arylidene), 6.78(2H,Ar.), 6.57(2H,Ar.), 4.27(2H,CH₂,ester), 1.36(3H,CH₃,ester), 0.86(3H,CH₃). MS *m/z* 524 (M)⁺.

5-[4-(2-Amino-3-carbethoxy-4,5,6,7-tetrahydrobenzo thiophene) ethoxy benzyl] thiazolidine-2,4-dione [14a]:

Faint brownish amorphous powder; m.p.246-248⁰C; % yield: 62%; Rf:0.84 (Chloroform: Acetone::8:2); IR (KBr) 3432(NHstr.TZD), 3423(NHstr.sec. amine), 3035(CHstr.Ar.), 2943(CHstr.alipatic), 2885(CH₂str.), 1742(C=Ostr.TZD), 1650 (C=Cstr.Ar.), 1562 (C=Cstr.heterocyclic), 1209 (C-Nstr.secondary), 1147 (C-Ostr.ester), 730 (C-S-Cstr.TZD). ¹H NMR(800MHz,CDCl₃) 7.24(1H,NH,TZD), 6.12(2H, 3 and 5 position, Ar.), 5.85(2H, 2 and 6 position,Ar), 4.32(2H,CH₂,ester), 4.24(2H, H to oxo, ethoxy), 4.12 (1H,CH,methine proton,TZD-5-H), 3.55(2H,CH₂), 2.75(2H, H to amino nitrogen, ethoxy), 2.04(4H,CH₂,cyclohexane), 1.54(4H,CH₂,cyclohexane), 1.32(3H,CH₃,ester) 0.82(1H,NH, sec.amine). MS *m/z* 474 (M)⁺.

5-[4-(2-Amino-3-carbethoxy-4,5-dimethylthiophene) ethoxy benzyl] thiazolidine-2,4-dione [14b]:

Brown amorphous powder; m.p.220-223⁰C; % yield: 60%; Rf:0.76 (Chloroform: Acetone::8:2); IR (KBr) 3412(NHstr.sec.amine), 3323(NHstr.TZD), 3085(CHstr.Ar.), 2943(CHstr.alipatic), 2885(CH₂str.), 1742(C=Ostr.TZD), 1663(C=Cstr.Ar.), 1554(C=Cstr. heterocyclic), 1214(C-Nstr.), 1180(C-Ostr.ester), 754(C-S-Cstr.TZD). ¹H NMR(800MHz, CDCl₃) 7.45(1H,NH,TZD), 6.97(2H, 3 and 5 position,Ar.), 6.82(2H, 2 and 6 position,Ar.), 4.20(1H,CH,methine proton,TZD-5-H), 4.34(2H,CH₂,ester), 4.15(2H, H to oxo, ethoxy), 3.45(2H,CH₂), 3.25(2H, H to amino nitrogen, ethoxy), 2.10(3H,CH₃), 2.30 (3H,CH₃), 1.30 (6H,CH₃,ester) 0.86 (1H,NH,sec.amine). MS *m/z* 448 (M)⁺.

5-[4-(2-Amino-3, 5-dicarbethoxy-4-methyl thiophene) ethoxy benzyl] thiazolidine-2,4-dione [14c]:

Blackish brown amorphous powder; m.p.238-240⁰C; % yield:62%; Rf:0.86 (Chloroform: Acetone:: 2:8); IR (KBr) 3487(NHstr.sec.amine), 3254(NHstr.TZD), 3045 (CHstr.Ar.), 2954(CHstr.aliphatic), 2885(CH₂str.), 1740(C=Ostr.TZD), 1668 (C=Cstr.Ar.), 1550(C=Cstr.heterocyclic), 1208(C-Nstr.), 1181(C-Ostr.ester), 735(C-S-Cstr. TZD). ¹H NMR (800MHz,CDCl₃) 7.39(1H,NH,TZD), 6.87(2H, 3 and 5 position Ar.), 6.52 (2H, 2 and 6 position,Ar.), 4.62(1H,CH, methine proton, TZD-5-H), 4.24(2H,CH₂,ester), 4.02 (2H, H to oxo,ethoxy), 3.69(2H,CH₂), 3.12(2H, H to amino nitrogen, ethoxy), 2.20(3H,CH₃), 1.21(6H,CH₃, ester) 0.85 (1H,NH,sec.amine). MS *m/z* 506 (M)⁺.

Anti-hyperglycemic activity evaluation

The compounds **4a-4c**, **7a-7c** and **14a-14b** were evaluated for their antihyperglycemic activity in fructose-induced hyperglycemia Male albino rats of Wistar strain. The rats weighing were obtained at 6–8 wk of age (130-180g) from National Institute of Biosciences, Pune and maintained at 25 ± 2 °C and also its relative humidity (50 ± 5%) with 12 h light/ 12 h dark cycles. The rats were given standard laboratory chow and water ad libitum. The Institutional Animal Ethical Committee of MVPS's College of Pharmacy approved the proposal.

Fructose was obtained from Qualigens. Pioglitazone was obtained as a gift sample from Glenmark Pharmaceuticals, Nashik, India. Glucose estimation kits were from Accusure, TaiDoc Technology Corporation, Taiwan. Reagents and chemicals used in the study were of analytical grade. Test agents and standard drug were formulated in suspension by using 2% w/v sodium carboxy methyl cellulose (CMC) and distilled water before dosing.

Fructose-induced hyperglycemia in rats²⁰

Animals were divided into four groups, a normal control, positive control, standard drug and test compounds **4a-4c**, **7a-7c** and **14a-14b**. Each groups (i.e. normal control, positive control, and standard drug)

and sub-group of test compounds **4a-4c**, **7a-7c** and **14a-14b** were consisting of three rats per group. With economic use of animals in this preliminary test designed, for selection of more active compounds among the test compounds.

The normal control group fed with normal diet without fructose, it was also given sodium carboxy methyl cellulose (2% w/v) in distilled water through oral gavage. This dose was well-tolerated by the animals with no evidence of diarrhea as reported in the literature.²¹ Whereas, all remaining groups fed with normal diet 40% w/v aq. fructose solution (4g/kg/day), by oral gavage for 0-18 days. The induction of hyperglycemia in all high fructose-fed groups was confirmed by measuring the blood glucose level on 18 day of the experiment. On 18 day, all groups of rats were fasted for 8 hrs. After which blood glucose level of each animal was measured (taken as 0 hr) with the help of glucometer by removing blood drop from the tail vein of the fasted rats and immediately thereafter administered the suspension of the test compounds orally at desired dose levels, that is, 100mg/kg body weight and 20mg/kg body weight of standard drug, after 2 hrs monitoring of blood glucose level.

The selection of more potent compounds based on, percentage changes in blood glucose levels for each group (**Table-1**). The selected active compounds **7c** and **14b** were further tested for their antihyperglycemic activity against standard drug, pioglitazone by using the same animal with minor modification, five animals per group (i.e. normal control, positive control, standard drug and test compounds **7c** and **14b**).

Except normal control group, all groups of rats were fed with normal diet 40% w/v aq. fructose solution (4g/kg/day), by oral gavage for 0-21 days. The induction of hyperglycemia was confirmed by measuring the blood glucose level on 0, 7, 14 and 21 day of the experiment. During this period 0-21day the animals were simultaneously checked in respect of different parameters having co-relation with diabetes and hyperglycemia such as body weight, blood glucose levels, food intake per group and water intake per group (**Table-2**).

On 21st day, all groups of rats were fasted for 8 hrs at night. After which blood glucose levels of all animals were measured (taken as 0 hr) and immediately thereafter drug treatment was given orally at earlier desired dose levels and followed by monitoring of blood glucose level at different time interval (**Table-3**).

Molecular docking studies

The docking analysis was carried out on the PPAR- binding site in the 2PRG obtained from the RCSB Protein Data Bank, <http://www.rcsb.org/pdb>, where the residues were bonded more closely to the rosiglitazone agonist, co-crystallized with PPAR-. In this crystal structure, the LBD forms a homodimer in which both monomers have nearly identical C conformations. The structure of the "A" monomer of the LBD homodimer was chosen as the target for docking studies. The rosiglitazone agonist was extracted from the complex. During the calculations, the active site was determined within a grid box centered on the co-crystallized ligand (X=49.19, Y= -37.083, Z=19.009) and ensuring that the active site region was covered²². The docking analyses of compounds **4a-4c**, **7a-7c** and **14a-14b**, pioglitazone and rosiglitazone were carried out on docking software of Schrodinger Maestro Module Glide Version 9.2.

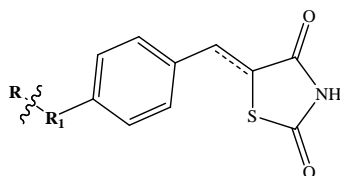
Results and Discussion

The selection of effector site of newly synthesized TZD's compounds is based on effector site of rosiglitazone. Pyridine ring of rosiglitazone is replaced by thiophene ring which is isosteric to pyridine. The linker moiety is also changed; benzylidene is replaced in some compounds by para sulphonyl benzylidene and ethoxybenzyl linkage, to study its effect on SAR. Because, the nitrogen of amino group of various links is attached to various substituted thiophene moieties so that the acidity of the hydrogen of amino group and TZD moiety changes marginally that may have effect in binding of molecule with the receptor.

The results of Table-1 indicate that, direct linkage of substituted **R** moiety to phenyl ring of 5-benzylidenethiazolidine-2,4-dione gave **4a-4c**, shows poor oral hypoglycemic activity. While compounds containing sulphonyl and ethoxy linkages help in showing activity possibly when suitable **R** moiety is present. This result helps in selecting of active compounds **7c** and **14b**, for further testing by using five animals per group for 21 days. Because, both promising active compounds **7c** and **14b** containing geometrical shape and

size similar with rosiglitazone drug. It is clearly indicated that, linkage between central aromatic region and lipophilic fragment is also important for activity. Because, this spacer has to present the lipophilic fragment in the proper spatial orientation to exhibit high activity.¹⁷

Table-1: Final structures, preliminary anti-hyperglycemic activity and docking studies of all synthesized TZDs compounds



Comp code	Heterocyclic ring (R)	Linkages (R ₁)	Double bond (-----)	Dose (mg/kg)	Activity (%)	Docking score
4a		No	Yes	100	3.64	-5.409
4b		No	Yes	100	4.31	-5.685
4c		No	Yes	100	6.15	-6.721
7a		Sulphonyl	Yes	100	7.29	-6.247
7b		Sulphonyl	Yes	100	5.71	-7.640
7c		Sulphonyl	Yes	100	33.33	-9.22
14a		Ethoxy	No	100	5.38	-8.368
14b		Ethoxy	No	100	33.64	-9.510
14c		Ethoxy	No	100	5.30	-8.297
Std.	Pioglitazone			20	41.83	-10.338

Table-2: Effects of fructose feeding on food intake, water intake, body weight and blood glucose levels of rats on day zero (Initial) and day 21st (Final) before oral administration of drugs on day 21st.

Groups	Food Intake (g)	Water intake (ml)	Body weight (g)		Blood glucose (mg/dl)	
			Initial	Final	Initial	Final
Normal control	50.27 ± 0.66	55.68 ± 0.75	132.20 ± 2.17	164.60 ± 3.36	95.40 ± 1.96	97.80 ± 1.68
Positive control	36.36 ± 0.64 ^a	79.59 ± 0.64 ^a	134.60 ± 2.94	181.20 ± 4.59 ^a	93.80 ± 1.39	156.00 ± 3.04 ^a
Pioglitazone	35.18 ± 0.59 ^a	81.18 ± 0.80 ^a	131.40 ± 2.76	179.40 ± 4.20 ^a	91.60 ± 1.20	152.60 ± 3.98 ^a
Test compound 7c	36.09 ± 1.00 ^a	81.00 ± 1.29 ^a	134.80 ± 3.26	180.20 ± 2.41 ^a	92.20 ± 1.01	146.40 ± 2.71 ^a
Test compound 14b	35.61 ± 2.30 ^a	79.81 ± 1.57 ^a	138.60 ± 2.92	179.20 ± 4.43 ^a	93.08 ± 1.00	148.80 ± 3.92 ^a

The results are expressed as mean ± SEM. The data is analyzed using One-way Analysis of Variance (ANOVA) followed by Dunnett's test.

(n = 5), ^apositive control, pioglitazone and test compounds compared to normal control (P < 0.05).

Table 3: Antihyperglycemic effect of test compounds and the standard drug pioglitazone in fructose loaded hyperglycemic rats.

Groups	Blood glucose level (mg/dl)			
	0hr	2hrs	5hrs	24hrs
Normal control	97.80 ± 1.68	96.20 ± 2.08	96.00 ± 1.22	89.40 ± 1.63
Positive control	156.00 ± 3.04	146.00 ± 2.32	143.20 ± 2.05	139.40 ± 2.48
Pioglitazone	152.60 ± 3.98	112.20 ± 1.31 ^a	92.20 ± 1.35 ^a	120.40 ± 1.63 ^a
Test compound 7c	146.40 ± 2.71	125.60 ± 1.13 ^a	100.80 ± 2.41 ^a	128.20 ± 2.08 ^a
Test compound 14b	148.80 ± 3.92	122.20 ± 2.57 ^a	99.80 ± 1.49 ^a	120.20 ± 0.58 ^a

Test compound = 100mg/kg,p.o.

Reference standard, Pioglitazone = 20mg/kg,p.o.

The results are expressed as mean ± SEM. The data is analyzed using One-way Analysis of Variance (ANOVA) followed by Dunnett's test. (n = 5), ^a Pioglitazone and test compounds compared to positive control (P < 0.05).

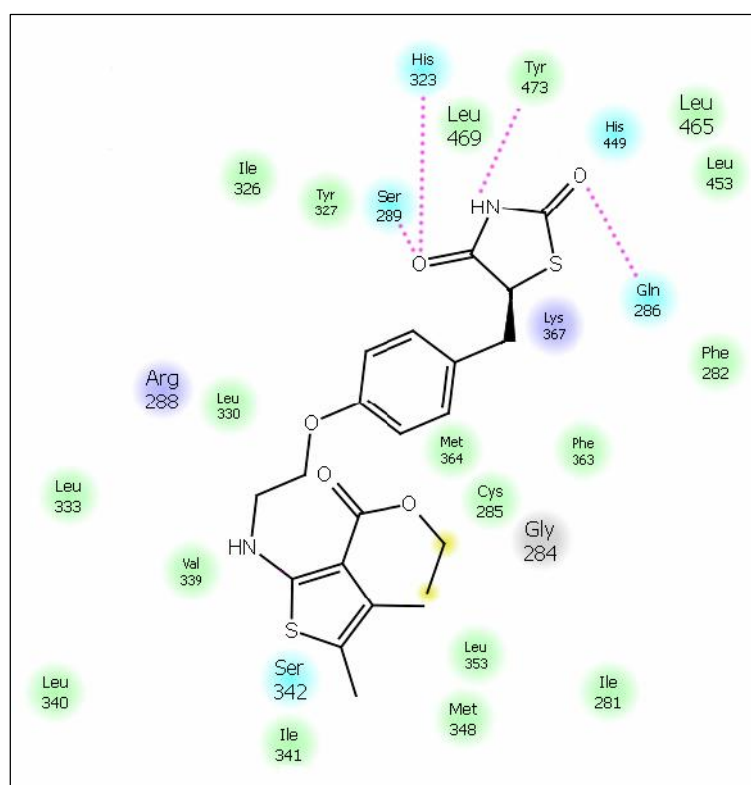
The different parameters studies of promising active compounds **7c** and **14b** during before administration of drugs in fructose-fed rats and finally results was compare with normal control group as reported in Table-2. There is significantly (p<0.05) decrease the food intake capacity of the fructose-fed rats. Feeding of fructose also shows the significantly (p<0.05) increases the fluid intake, body weight and blood glucose levels of the animals. Because, oral feeding of fructose which will convert into glucose in absorption process. The significantly increase the fluid intake; it is sign of development of diabetes.

The results of Table-3 indicate that, blood glucose profile of the selected compounds **7c** and **14b** at various time intervals. Both compounds were significantly (p<0.05) reduce the rise in blood glucose levels of fructose induced hyperglycemia in comparison with positive control group. The standard drug pioglitazone caused nearly reduced blood glucose level at 20mg/kg dose in fructose induced hyperglycemia. After two hour, pioglitazone shows onset of activity is rapid. It is maintain up to five hour and may be more than that, because pioglitazone bind with protein and its metabolite (M₁, M₂ and M₃) are also active.²³

The active compounds **7c** and **14b** shows, closely similar pattern of activity in comparison with the activity of standard drug of pioglitazone at various time intervals but at considerably higher doses. Like many other TZDs reported in literature, the activity of TZDs compounds **4a-4c**, **7a-7c** and **14a-14b** probably depend on TZD acidic head group allowing several specific hydrogen-bonding interactions with His449, Tyr473, His323, Ser289 and Gln286 residues²⁴ and producing quite significant activity. These specific hydrogen-bonding interactions were confirmed with the help of docking studies.

The most stable docking score of TZD's **4a-4c**, **7a-7c** and **14a-14b** complexed with the PPAR- LBD are listed in Table-1. The binding profile of the 5-[4-(substituted) benzylidene or benzyl] thiazolidine-2,4-dione was compared with the profile of the rosiglitazone molecule. The ligand 5-[4-(2-Amino-3-carethoxy-4,5-dimethylthiophene) ethoxy benzyl] thiazolidine-2,4-dione **14b**, interact mainly with PPAR- through the formation of four hydrogen bonds between the TZD acidic group of the benzyl ring and the Gln286, Tyr473, His323 and Ser289 residues, respectively (Figure-3). Actually, the His449 residue also interacts with rosiglitazone in the crystal structure through hydrogen bonding.

Figure 3: Main hydrogen bonds between (R) 5-[4-(2-Amino-3-carethoxy-4,5-dimethyl thiophene) ethoxy benzyl] thiazolidine-2,4-dione and PPAR-



Conclusion

On comparison of experimental data of activity and their docking scores of the present set of compounds **4a-4c**, **7a-7c** and **14a-14b**, it may be concluded that, direct linkage of substituent **R** moiety to 4-position of phenyl ring of 5-benzylidene thiazolidine-2,4-dione compounds **4a-4c** gave poor oral antihyperglycemic activity experimentally and also showed low docking scores compared to other compounds **7a-7c** and **14a-14b**. Whereas compounds **7c** and **14b** containing sulphonyl or ethoxy linkage respectively. Both compounds gave promising activity and also showed comparatively higher docking scores. This result is possible due to linker moiety (sulphonyl or ethoxy) present in compounds **7c** and **14b** favouring binding with receptor and also in docking.

From the present studies, it is not yet clear whether presence of carbon-carbon double bond favours or disfavours activity and therefore required further studies on synthesis of newer compounds and their experimental activity and docking studies.

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