Scholars Academic Journal of Pharmacy (SAJP) **ISSN 2320-4206 (Online)**
Sch. Acad. J. Pharm., 2014; 3(1):26-37 **ISSN 2347-9531 (Print)**

(An International Publisher for Academic and Scientific Resources)
www.saspublisher.com

Research Article

Benzisoxazole containing Thiazolidinediones as Peroxisome Proliferator Activated Receptor-γ Agonists: Design, Molecular Docking, Synthesis & anti-diabetic studies

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Abstract: A series of novel benzisoxazole containing thiazolidinediones were designed, docked with PPAR-γ protein leading to identification of a highly potent PPAR-γ agonist, compound S7. Based on molecular docking studies and lipinski's rule of five, nine analogues out of 12 were synthesized and characterized by FT-IR, 1H-NMR and Mass spectra. Anti-diabetic activity of nine analogues was evaluated in alloxan (70 mg/kg, i.v.)-induced diabetes in mice [single-dose one day study]. The molecular docking and the pharmacological studies revealed that the distances between the acidic group and the linker, when a ligand was complexed with PPAR-γ protein, are important for the potent activity. The acidic head part of S7 makes intensive hydrophobic interaction with the PPAR-γ protein resulting in potent activity.
Keywords: Design, Molecular docking, PPAR-γ agonists, anti-diabetic activity.

INTRODUCTION

The prevalence of type 2 diabetes mellitus, a multifactorial heterogeneous group of disorders resulting from defects in insulin secretion, insulin action, or both, has increased dramatically over the past several decades. Major influencing factors are change in human environment, behavior, and lifestyle. The metabolic syndrome, a deadly quartet of insulin resistance, central obesity, dyslipidaemia, and hypertension, is associated with increased risk of cardiovascular diseases [1].

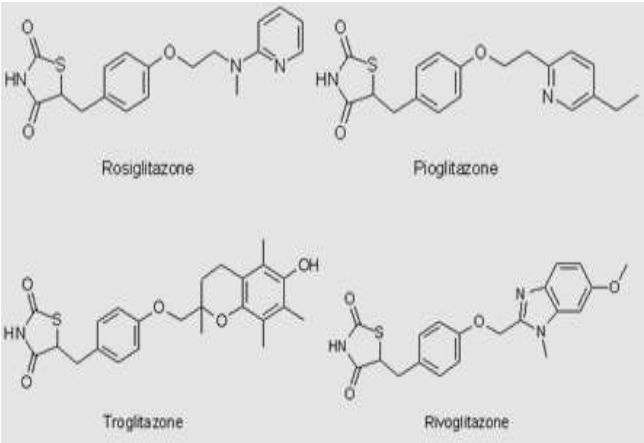


Fig. 1: Different PPAR--γ agonists

The peroxisome proliferator-activated receptors (PPARs) are lipid-activated transcription factors exerting several functions in development and metabolism [2]. They serve as major targets of drugs effective in treatment of metabolic disorders. The modulation of PPAR activity might be an effective therapy for metabolic syndrome including obesity. The three PPAR subtypes, PPAR-α, PPAR-β, and PPAR-γ, have been the focus of extensive research during the past decade [3]. The currently marketed PPAR-α agonists have only modest net efficacy and have the potential for several undesired side effects. Novel PPAR ligands are now being developed that possess broader efficacies and improved tolerability compared with currently available therapeutic agents3 (Fig. 1). The lipid-lowering and cardioprotective effect of PPAR-β agonists, insulin-sensitizing effect of PPAR-α agonists, and fatty acid catabolism by PPAR-γ agonists are well documented [3-6]. We are constantly designing and synthesizing several novel heterocyclic ring systems for different biological activities including diabetes. In continuation of these works, we explored new hydrophobic building block as the tail part of the benzisoxazole based PPAR-γ agonists. This article describes the design, molecular docking and synthesis of novel PPAR-γ agonists with hydrophobic tail selected from commercially available building blocks, which were further modified to improve the activity. In addition, anti-diabetic activity of active analogs in alloxan-induced diabetic mice model was carried out.

EXPERIMENTAL SECTION

Chemicals

Chemicals used in the synthesis of the titled compounds were purchased from, Sigma-Aldrich Pvt. Ltd, S.D. Fine Chem Pvt. Ltd. and Spectrochem Pvt. Ltd. They were Thiourea, Chloroacetic acid, conc.HCl, substituted aromatic aldehydes, Piperidine, methylene chloride, benzene, toluene, triethylamine, pyridine, dimethyl formamide, 5-Chloro-3-phenyl-2,1-benzisoxazole,2-methyl amino ethanol,2-ethyl amino ethanol,2-propyl amino ethanol, tetrahydrofuran, anhydrous magnesium sulfate, Tributylphosphine, azodicarbonyl dipiperidine.

Instruments and software

Melting points of synthesized compounds were determined on SHITAL-Digital Programmable melting point apparatus and are uncorrected; FT-IR spectra were recorded on Bruker spectrophotometer by using KBr pellets. The ^{1}H-NMR was recorded on Bruker Avance III NMR 500 MHz instruments using DMSO as solvent and TMS as internal standard, chemical shifts are expressed as δ values (ppm).

Design of PPAR-γ agonists

The review of the literature [5, 6] shows that a typical PPAR agonist consists of an acidic head attached to an aromatic scaffold, a linker, and a hydrophobic tail. In continuation of our studies on benzisoxazole based PPAR-γ agonists, the Thiazolidinedione was retained as a core skeleton for the acidic head [7], the aliphatic linker length was fixed at three carbons on the basis of our previous observations and literature reports [7-9]. Sahoo *et al.* [8, 9] have reported improvement in PPAR-γ agonist activities upon introduction of a propyl group in the hydrophobic tail. Therefore, the design was carried out in the following manner. There are mainly three parts in the pharmacophore. Thiazolidinedione moiety (Head part), which is already a proven anti-diabetic agent. The thiazolidinedione moiety is attached to a hydrophobic tail, i.e. phenyl benzisoxazole through an aliphatic linker. The common structural features of PPAR-γ agonist & its resemblance to the currently designed molecule is shown in the Fig. 2 & 3.

The design of PPAR-γ agonist is adopted on the basis of the standard anti-diabetic drug Rosiglitazone which is a well known PPAR-γ agonist.

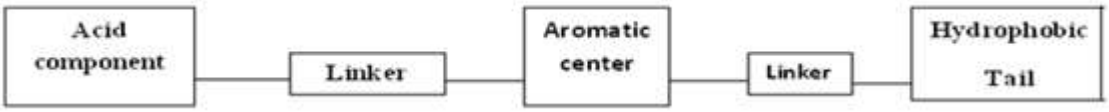


Fig. 2: Common structural features of PPAR agonists

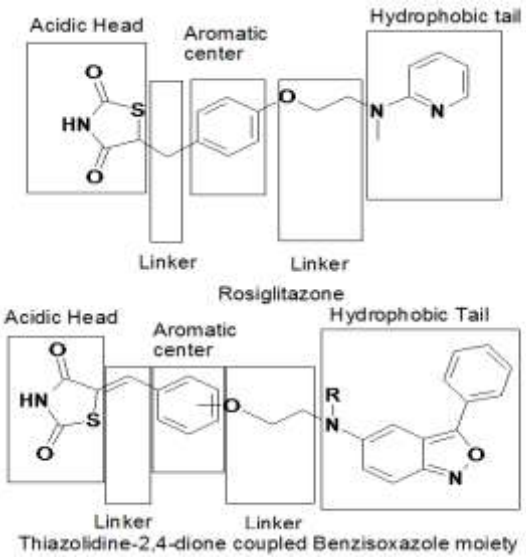
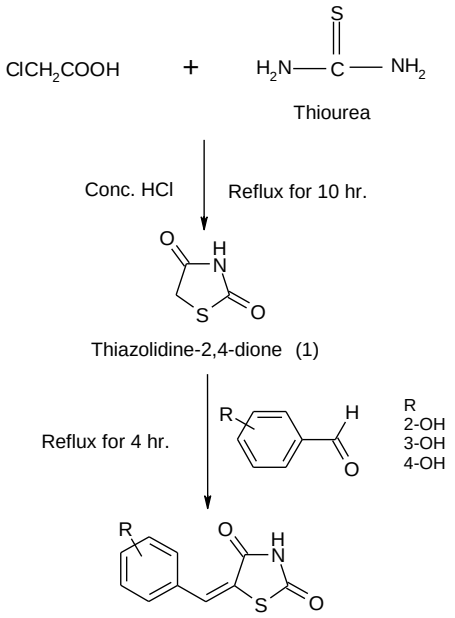


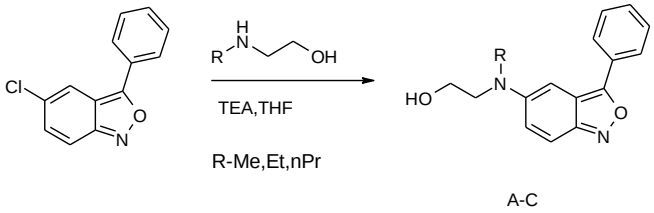
Figure 3: Structure showing the similarity between a PPAR-γ agonist & the proposed Ligand

Scheme of Synthesis

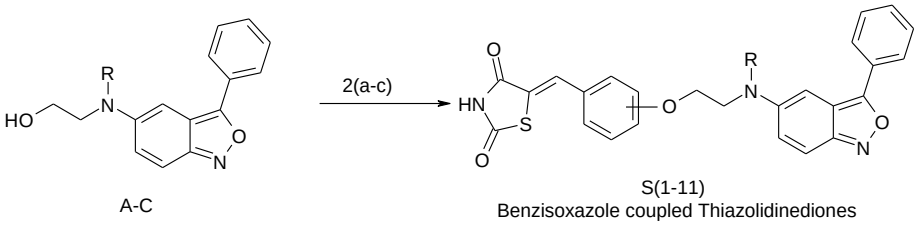
a) Synthesis of 5-arylidene thiazolidine-2,4-diones



b) Synthesis of 2-(Benzoxazol-2-yl-substituted amino)ethanol



c) Synthesis of benzisoxazole coupled Thiazolidine-2,4-diones



Compd. Code	S1	S2	S3	S5	S6	S7	S9	S10	S11
R	Me	Et	*n*-Pr	Me	Et	*n*-Pr	Me	Et	*n*-Pr
-OH Substituent	4-OH			3-OH			2-OH		

Molecular docking studies [10]

For the present study bioinformatics tools, biological databases like PubMed, Drug Bank, PDB (ProteinDataBank) and software's like Molegro Virtual Docker version 5.5, ACD Chem Sketch, Corina software, UCSF Chimera1.6.2 were used. The structure of Ligand binding domain was retrieved from Protein databank (PDB ID: 2PRG, Ligand-Binding Domain of the Human Peroxisome Proliferator Activated Receptor Gamma; Fig. 4).

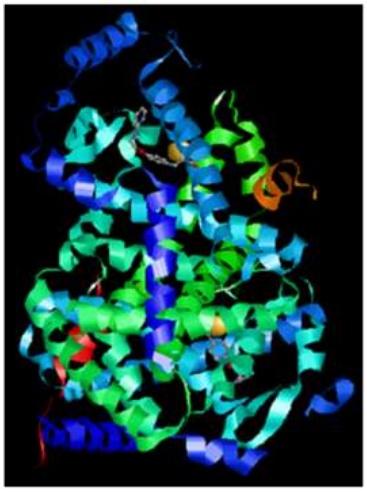


Fig. 4: Ligand-binding domain of the human peroxisome proliferator activated receptor –γ (PDB ID: 2PRG)

The structures of the protein along with a PPAR-γ agonist, Rosiglitazone was obtained from Protein databank (www.rcsb.com). Using ACDLABS/ChemSketch (www.acdlabs.com) the 2D structures of the analogue, the benzisoxazole coupled Thiazolidinedione ligands were sketched.The 2D structures were converted to the respective 3D-mol 2 files using Chimera software (UCSF-Chimera). Out of 12 analogues, 9 followed Lipinski's rule of Five for drug-likeness & were calculated using Molinspiration online server & the results are depicted in Table 1. These analogues were then searched against various chemical structure databases for similarity with an existing structure. The databases taken in this step are: Pub Chem (pubchem.ncbi.nlm.nih.gov), KEGG, Molsoft (Mol Cart) (www.molsoft.com/molcart.html), Hic-Up (xray.bmc.uu.se/hicup) and Chem Bank (chembank. broadinstitute.org/). The similarity of structures were observed with the PPAR-γ agonist, Rosiglitazone & further subjected for docking studies. The Molecular Docking is performed in Molegro Virtual Docker (MVD) version 5.5 (http://www.molegro.com). The possible active sites and cavities were detected for Chain A of 2PRG using Molegro Virtual Docker. The following Parameters were used for Cavity Detection as shown in Fig. 5. The Surface plot of the Protein along with a PPAR-γ agonist, Rosiglitazone in Fig. 6 & 7 shows the position of the active site.

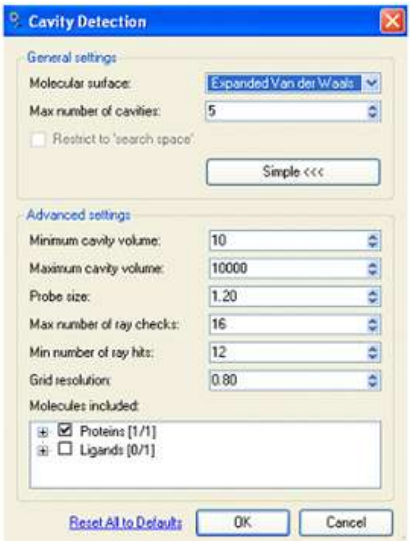


Fig. 5: Parameters of Molegro Virtual Docker

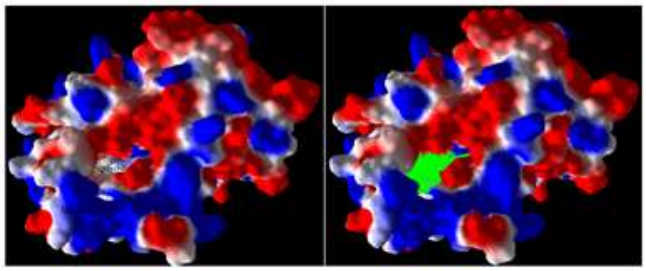


Fig. 6: Surface plot of the Protein with a PPAR–γ agonist, Rosiglitazone

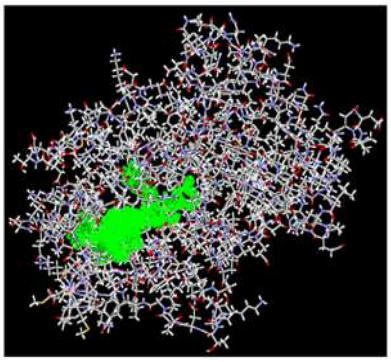


Fig. 7: Position of the active site shown in Molegro Virtual Docker (MVD)

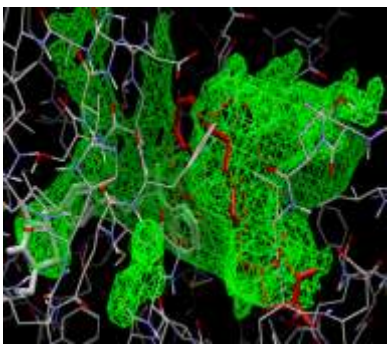


Fig. 8: Result of Active Site Prediction of the detected cavity with the Ligand (S7 in Red) &Rosiglitazone (In white). (Chain-A, Vol+185.856)

The Molecular Docking is performed in Molegro Virtual Docker (MVD). The following Parameters were used for Docking using Molegro Virtual Docker. Docker uses the Mol Dock docking engine to predict ligand- protein interactions. Mol Dock is based on a new hybrid search algorithm called guided differential evolution.

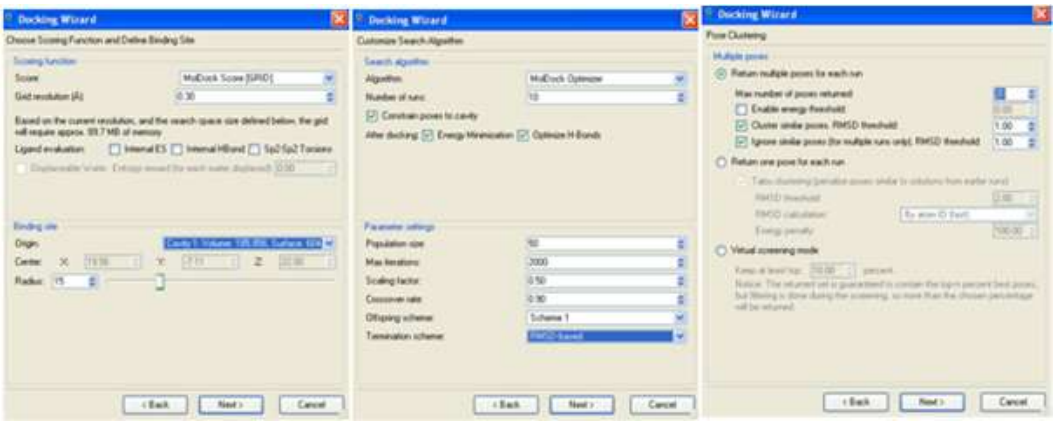


Fig. 9: Docking parameters shown in Molegro Virtual Docker

The ligands were manually checked before docking and corrected in those cases were it had failed. Water molecules with the protein structures were excluded from the docking experiments; the docking is then allowed to run for some time for Zopolrestat & prepared ligands with the target protein. The poses having the good Mol Dock and Docking score are selected for the further analysis.

Docking studies of S (1-12) for PPAR- γ agonist activity PDB ID: 2PRG

2PRG is a ligand-binding domain of the human peroxisome proliferator activated receptor gamma. The interactions of 5-substituted thiazolidine-2, 4-dione as PPAR-**γ** agonists with the amino acid residues were carried out using Molegro Virtual Docker 5.5. & these interactions were compared with the standard PPAR-**γ** agonists. Docking results tabulated between Human Peroxisome Proliferator Activated Receptor-Gamma along with the proposed ligands & an active PPAR-γ agonist, Rosiglitazone & are depicted in Table 1.

Table 1: Molecular docking results of 5-[(3-{2-[(3-phenyl-2, 1-benzoxazol-5-yl) (alkyl) amino]ethoxy}phenyl) methylidene]-1,3-thiazolidine-2,4-diones (Benzisoxazole coupled Thiazolidine-2, 4-dione analogues

Compound Code	H-Bond	MolDock Score	Interaction	MW	Docking score
S1	-0.840	-176.65	-185.91	471.52	-140.54
S2	-3.466	-178.33	-184.43	485.55	-141.24
S3	0	-192.77	-200.99	499.58	-147.90
S4	-1.900	-190.44	-186.63	513.60	-149.53
S5	0	-171.22	-174.21	471.52	-140.89
S6	0	-166.39	-173.67	485.55	125.06
S7	**-3.738**	**-178.50**	**-187.27**	**499.58**	**-131.92**
S8	-0.572	-183.22	-192.73	513.60	-149.35
S9	0	-176.51	-171.92	471.52	-159.03
S10	-2.756	-185.45	-188.69	485.55	-150.69
S11	0	-188.85	-167.13	499.58	-139.19
S12	-0.4888	-184.99	-194.77	513.60	-155.17
Rosiglitazone	**-3.474**	**-135.70**	**-133.25**	**357.43**	**-124.78**

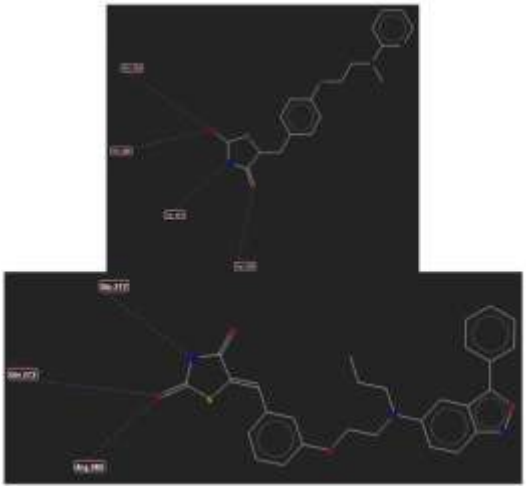


Fig. 10: The results of docking shown with contacts Rosiglitazone (Above) & Proposed Ligand (Below)

Lipinski's Rule of Five for drug likeness to check oral bioavailability

Lipinski's Rule of Five is a rule of thumb to evaluate drug likeness or determine if a chemical compound with a certain pharmacological or biological activity has properties that would make it a likely orally active drug in humans. The rule was formulated by Christopher A. Lipinski in 1997, based on the observation that most medication drugs are relatively small and lipophillic molecules.

The rule describes molecular properties important for a drug's pharmacokinetics in the human body, including their absorption, distribution, metabolism, and excretion. However, the rule does not predict if a compound is pharmacologically active.

The rule is important for drug development where a pharmacologically active lead structure is optimized step-wise for increased activity and selectivity, as well as drug-like properties as described by Lipinski's rule. Lipinski's rule states that, in general, an orally active drug has no more than one violation of the following criteria:

- Not more than 5 hydrogen bond donors (nitrogen or oxygen atoms with one or more hydrogen atoms).
- Not more than 10 hydrogen bond acceptors (nitrogen or oxygen atoms).
- A molecular mass less than 500 daltons.
- An octanol-water partition coefficient log *P* in -0.4 to +5.6 range

All the docked analogues were checked for this rule using online version of Molinspiration software & the results are depicted in Table 2.

Table 2: Property prediction for 5-[(3-{2-[(3-phenyl-2,1-benzoxazol-5-yl) (alkyl) amino]ethoxy}phenyl) methylidene]-1,3-thiazolidine-2,4-diones (Benzisoxazole coupled Thiazolidinediones) by Lipinski's rule of five using Molinspiration software

Comp. Code	R	-OH Substituent	Molecular mass (Dalton)	Hydrogen bond donors	Hydrogen bond acceptors	Partition coefficient	No. of violations
S1	Me	4-OH	471.538	1	7	4.36+/- 0.88	0
S2	Et		485.565	1	7	4.89+/- 0.88	0
S3	*n*-Pr		499.592	1	7	5.42+/- 0.88	0
S4	*n*-Bu		513.619	1	7	5.95+/- 0.88	2
S5	Me	3-OH	471.538	1	7	4.31+/- 0.75	0
S6	Et		485.565	1	7	4.84+/- 0.75	0
S7	*n*-Pr		499.592	1	7	5.37+/- 0.75	0
S8	*n*-Bu		513.619	1	7	5.90+/- 0.75	2
S9	Me	2-OH	471.538	1	7	4.42+/- 0.88	0
S10	Et		485.565	1	7	4.95+/- 0.88	0
S11	*n*-Pr		499.592	1	7	5.48+/- 0.88	0
S12	*n*-Bu		513.619	1	7	6.01+/- 0.88	2
Rosiglitazone			357.43	1	6	2.56+/- 0.79	0
Normal range			<500	<5	<10	-0.4 to +5.6	

Synthesis of 5-arylidene-2, 4-thiazolidinediones [11-13] 2(a-c)

2, 4- thiazolidinediones (1) were synthesized by condensation of equimolar amounts of Thiourea and Chloroacetic acid in presence of Conc. Hydrochloric acid. Knoevenagel condensation between the aromatic aldehyde and 2, 4-thiazolidinedione in refluxing ethanol, containing a catalytic amount of piperidine to gave substituted 5-arylidene-2, 4-thiazolidinedione.

Synthesis of 2-(Benzisoxazol-2-yl-substituted amino) ethanol compounds [14] (A-C)

A solution of 5-Chloro-3-phenyl-2,1-benzisoxazole (2.00 g, 13.02 mmol), 2-substituted aminoethanol (1.47 g, 19.57 mmol) and triethylamine (1.98 g, 19.57 mmol) in tetrahydrofuran (30 mL) was stirred at 70°C for 2 h. The reaction mixture was then extracted with ethyl acetate. The combined organic extracts were washed with water, dried over anhydrous magnesium sulfate, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography (eluant, *n*-hexane/ethyl acetate = 1:1 v/v) to afford 2.24 g (89%) of the title compound (A-C) as a yellow powder:

Synthesis of 5-[(3-{2-[(3-phenyl-2, 1-benzoxazol-5-yl) (alkyl) amino]ethoxy}phenyl) methylidene]-1,3-thiazolidine-2,4-diones [14] (Benzisoxazole coupled Thiazolidinediones)

Tributylphosphine (1.2 mL, 0.6 M in dry toluene, 0.60 mmol) in anhydrous toluene was added dropwise to a mixture of the alcohol (A-C) (57.7 mg, 0.30 mmol), 5-(4-hydroxybenzyl)-3-triphenylmethyl thiazolidine-2, 4-dione 2(a-c) (93.1 mg, 0.20 mmol), azodicarbonyl dipiperidine (151.4 mg, 0.60 mmol) and anhydrous toluene (2 mL). The resulting mixture was then stirred at room temperature for 6 h. Insoluble materials were filtered away and the filtrate was concentrated under reduced pressure.

Anti-diabetic activity of synthesized compounds

Animals

Swiss Albino mice of either sex weighing between 25-30 g were used in antidiabetic screening. Animals were housed under standard condition of temperature of the experimental room was maintained constant at 25°C and lightening was kept artificial. The sequence was 12 h light and 12 h dark. Conventional laboratory diets and water were provided *ad-libitum.* Studies were carried out at S.E.T's College of Pharmacy,Dharwad, College animal House used for housing of animals. Approval was taken from committee for the purpose of control and supervision of experiments on animals (CPCSEA) and Institutional animal ethical committee (IAEC).

Induction of diabetes

The initial fasting serum glucose (SG) was estimated by glucose oxidase peroxidase (GOD/POD) method. The animals showing SG 80-120 mg/dl were selected for study and injected with alloxan (70 mg/kg, i.v.) alloxan monohydrate was purchased from spectrochem, India. After 48 h of alloxan injection the blood was removed by retro orbital plexus technique (ROP) and SG was estimated. The animals showing SG levels above 200 mg/dl were consider diabetic and selected for study [15, 16]. Rosiglitazone was used as standard drug.

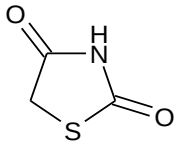
Experimental design single-dose one-day study

The experimental rats were divided into 12 groups of five mice each treated as Group 1: Normal control received 1% CMC; Group 2: Diabetic control (DC) received 1% CMC; Group 3 to 11: DC mice treated with S1, S2, S3, S5, S6, S7, S9, S10 and S11 (30 mg/kg, p.o) respectively; Group 12: DC mice treated with Rosiglitazone (30 mg/kg, p.o).

Blood samples were collected at 0, 2, 4, 6 and 24 h after extract/ GLB administration. SG was estimated by the enzymatic glucose oxidase method. Percentage reduction in glycemia was calculated with respect to the initial (0 h) level according to: Percentage reduction in glycemia ¼ [(Gi_Gt) X Gi] 100; Where Gi is initial glycemia and Gt is glycemia at 2, 4, 6 and 24 h. The data obtained were analyzed by one-way ANOVA followed by Dunnet test [17] and have been depicted in Table 11.

Physical and Spectral data

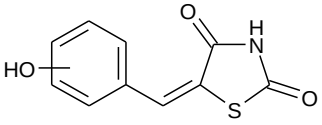
Table 3: Physical data of 2, 4-thiazolidinedione (1)



Sl. No.	Molecular Formula	Molecular Weight	mp (°C)	Yield (%)
1	$C_3H_3NO_2S$	117	123-125	80.16

Recrystallization solvent: ethanol

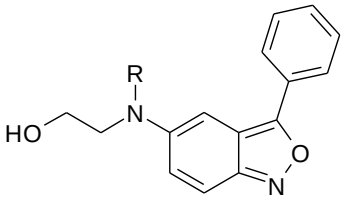
Table 4: Physical data of Substituted 5-arylidene-2, 4-thiazolidinedione 2(a- c)



Sl. No.	-OH Substituent	Molecular Formula	Molecular Weight	mp (^oC)	Yield (%)
2a	4-OH	$C_{10}H_7NO_2S$	221	278-280	73.30
2b	3-OH	$C_{10}H_7NO_2S$	221	240-242	71.65
2c	2-OH	$C_{10}H_7NO_2S$	221	282-285	71.36

Recrystallization solvent: ethanol

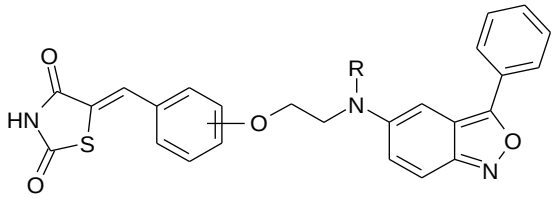
Table 5: Physical data of 2-(Benzisoxazol-2-yl-substituted amino) ethanol compounds (A-C)



Sl. No.	R	Molecular Formula	Molecular Weight	mp (^oC)	Yield (%)
A	Me	$C_{17}H_{13}NO_3S$	311	240-243	67.68
B	Et	$C_{17}H_{12}NO_3S$ Cl	345	245-246	70.30
C	*n*-Pr	$C_{17}H_{12}NO_3S$ Cl	345	245-247	72.27

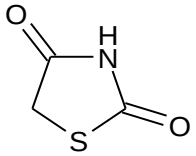
Recrystallization solvent: ethanol

Table 6: Physical data of 5-[(3-{2-[(3-phenyl-2,1-benzisoxazol-5-yl) (alkyl) amino]ethoxy}phenyl) methylidene]-1,3-thiazolidine-2,4-diones (Benzisoxazole coupled Thiazolidinediones)



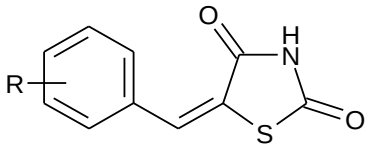
Sl. No.	R	-OH Substituent	Molecular Formula	Molecular Weight	mp (^oC)	Yield (%)
S1	Me	4-OH	$C_{17}H_{13}NO_3S$	471.5	250-52	74.45
S2	Et		$C_{17}H_{12}NO_3S$ Cl	485.5	245-48	68.77
S3	*n*-Pr		$C_{17}H_{12}NO_3S$ Cl	499.6	255-57	67.03
S5	Me	3-OH	$C_{17}H_{13}NO_3S$	471.5	221-23	65.00
S6	Et		$C_{17}H_{12}NO_3S$ Cl	485.5	239-43	71.50
S7	*n*-Pr		$C_{17}H_{12}NO_3S$ Cl	499.6	215-18	75.00
S9	Me	2-OH	$C_{17}H_{13}NO_3S$	471.5	240-43	67.68
S10	Et		$C_{17}H_{12}NO_3S$ Cl	485.5	245-46	70.30
S11	*n*-Pr		$C_{17}H_{12}NO_3S$ Cl	499.6	245-47	72.27

Table 7: Spectral data of 2, 4-thiazolidinedione (1)



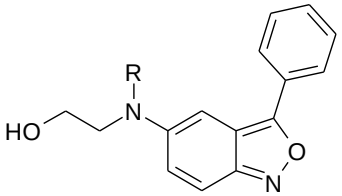
Sl. No.	IR spectra (KBr cm^{-1})	^{1}H-NMR spectra (δ, ppm)	Mass Spectra (m/z value)
1	1673.8, 1739.9(C=O), 3134.0(N-H)	8.75 (1H, s, NH), 3.72,3.75 (2H, d, CH_2)	------

Table 8: Spectral data of 5-arylidene-2, 4-thiazolidinediones 2(a - c)



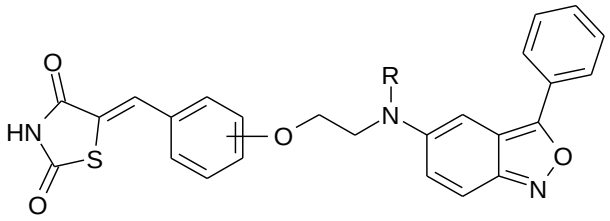
Sl. No.	R	IR spectra (KBr cm^{-1})	^{1}H-NMR spectra (δ, ppm)	Mass Spectra (m/z value)
2a	4-OH	1678.3, 1729.0(C=O), 3137.3(N-H), 3024.0(C-H; aromatic), 3421.9(O-H)	8.59 (s, 1H), 7.81 – 7.75 (m, 2H), 7.40 (td, *J* = 7.5, 1.5 Hz, 1H), 7.11 (td, *J* = 7.5, 1.6 Hz, 1H), 7.00 (dd, *J* = 7.5, 1.5 Hz, 1H), 5.43 (s, 1H).	205
2b	3-OH	1691.0, 1738.3(C=O), 3141.6(N-H), 3034.8(C-H; aromatic)	8.60 (s, 1H), 7.97 (s, 1H), 7.52 (t, *J* = 7.5 Hz, 1H), 7.29 – 7.23 (m, 1H), 7.01 – 6.94 (m, 2H), 4.72 (s, 1H).	
2 c	2-OH	1678.6, 1723.0(C=O), 3125.2(N-H), 3003.2(C-H; aromatic), 3404.8(O-H)	8.60 (s, 1H), 7.97 (s, 1H), 7.77 – 7.71 (m, 2H), 6.83 – 6.77 (m, 2H), 4.82 (s, 1H).	

Table 9: Spectral data of Substituted 2-(Benzisoxazol-2-yl-substituted amino) ethanol compounds (A-C)



Sl No.	R	IR spectra (KBr cm^{-1})	^{1}H-NMR spectra (δ, ppm)	Mass Spectra (m/z value)
A	Me	1679.2, 1729.7(C=O), 3038.1(C-H; aromatic), 1592.8(C-N), 3420.8(O-H)	8.11 – 8.03 (m, 2H), 7.65 – 7.55 (m, 4H), 7.16 (dd, *J* = 7.5, 1.5 Hz, 1H), 7.09 (d, *J* = 1.5 Hz, 1H), 3.78 – 3.65 (m, 4H), 0.90 (s, 1H).	268
B	Et	1679.7, 1730.1(C=O), 3036.0(C-H; aromatic), 1592.7(C-N), 3421.0(O-H)	8.11 – 8.03 (m, 2H), 7.65 – 7.55 (m, 4H), 7.16 (dd, *J* = 7.5, 1.5 Hz,1H), 6.61 (d, *J* = 1.7 Hz, 1H), 3.98 – 3.91 (m, 1H), 3.76 – 3.69 (m, 3H), 3.40 (q, *J* = 6.3 Hz, 2H), 1.12 (t, *J*= 6.3 Hz, 3H).	282
C	*n*-Pr	1665.7, 1731.3(C=O), 3053.7(C-H; aromatic), 1594.7(C-N), 3420.0(O-H)	8.11 – 8.03 (m, 2H), 7.65 – 7.56 (m, 4H), 7.19 (dd, *J* = 7.4, 1.6 Hz, 1H), 7.00 (d, *J* = 1.6 Hz, 1H), 3.99 (t, *J* = 3.7 Hz, 1H), 3.73 (t, *J* = 3.7 Hz, 2H), 3.68 – 3.62 (m, 2H), 3.50 t, *J* = 5.1 Hz, 1H), 1.70 (d td, *J* = 11.8, 6.7, 5.1 Hz, 2H), 1.36 (s, 1H), 0.87 (t, *J* = 6.7 Hz, 3H).	296

Table 10: Spectral data of 5-[(3-{2-[(3-phenyl-2,1-benzisoxazol-5-yl) (alkyl)amino] ethoxy}phenyl) methylidene]-1,3-thiazolidine-2,4-diones (Benzisoxazole coupled Thiazolidinediones)



Sl No.	R	IR spectra (KBr cm^{-1})	^{1}H-NMR spectra (δ, ppm)	Mass Spectra (m/z value)
S1	Me	1679.2, 1729.7(C=O), 3038.1(C-H; aromatic), 1592.8(C-N), 3420.8(O-H)	8.59 (s,NH, 1H), 8.11 – 8.03 (m, 2H), 7.99 – 7.89 (m, 3H), 7.65 – 7.56 (m, 4H), 7.25 – 7.18 (m,1H benzisoxazole & Ar 4-OH 2H 3H), 6.78 (d, *J* = 1.5 Hz, 1H Ar of benzisoxazole), 4.37 (t, CH_2,*J* = 7.1 Hz, 2H), 4.25 – 4.18 (m,CH_2, 1H), 3.88 (t,CH_2, *J* = 7.1 Hz, 1H).	471
S2	Et	1679.7, 1730.1(C=O), 3036.0(C-H; aromatic), 1592.7(C-N), 3421.0(O-H)	8.60 (s,NH, 1H), 8.11 – 8.03 (m, 2H), 7.99 – 7.89 (m, 3H), 7.67 – 7.55 (m, 4H), 7.25 – 7.17 (m, 2H), 6.59 (d, *J* = 1.5 Hz, 1H), 4.44 (t, CH_2,*J* = 7.2 Hz, 2H), 4.14 (t, CH_2,*J* = 7.1 Hz, 1H), 3.93 (t, CH_2,,*J* = 7.1 Hz, 1H), 3.40 (q,CH_2 *J* = 6.3 Hz, 2H), 1.12 (t,CH_3 *J* = 6.3 Hz, 3H).	485
S3	*n*-Pr	1665.7, 1731.3(C=O), 3053.7(C-H; aromatic), 1594.7(C-N), 3420.0(O-H)	8.61 (s, NH,1H), 8.11 – 8.03 (m, 2H), 7.99 – 7.89 (m, 3H), 7.65 – 7.55 (m, 4H), 7.25 – 7.17 (m, 2H), 6.72 (d, *J* = 1.7 Hz, 1H), 4.44 (t,CH_2, *J* = 3.9 Hz, 2H), 4.17 (t,CH_2, *J* = 3.9 Hz, 1H), 3.87 (t,CH_2, *J* = 3.9 Hz, 1H), 3.65 (t,CH_2, *J* = 7.5 Hz, 1H), 3.55 (t,CH_2, *J* = 7.5 Hz, 1H), 1.72 (td,CH_2, *J* = 7.3, 6.4 Hz, 2H), 0.87 (t,CH_3, *J* = 6.7 Hz, 3H).	499
S5	Me	1690.2, 1822.6(C=O), 3089.1(C-H; aromatic), 1585(C-N), 3395(O-H)	8.59 (s, NH,1H), 8.11 – 8.03 (m, 2H), 7.97 (s, 1H), 7.73 – 7.66 (m, 3H), 7.65 – 7.55 (m, 4H), 7.41 – 7.31 (m, 2H), 7.03 (dt, *J* = 7.5, 1.5 Hz, 1H), 4.07 (t, CH_2,*J* = 3.8 Hz, 2H), 4.01 (s, CH_2,1H), 3.68 (s, CH_2,1H), 3.04 (t,CH_2, *J* = 3.8 Hz, 1H), 2.72 (t, CH_2,*J* = 3.8 Hz, 1H),2.43(s,CH_3)	471
S6	Et	1710.7, 1710.1(C=O), 3020.0(C-H; aromatic), 1575.7(C-N), 3405.0(O-H)	8.61 (s, NH,1H), 8.11 – 8.03 (m, 2H), 7.97 (s, 1H), 7.73 – 7.66 (m, 2H), 7.65 – 7.52 (m, 5H), 7.41 – 7.31 (m, 2H), 7.03 (dt, *J* = 7.5, 1.5 Hz, 1H), 4.07 (t,CH_2, *J* = 6.9 Hz, 2H), 4.01 (s,CH_2, 1H), 3.64 (s, CH_2,1H), 3.01 (t,CH_2, *J* = 6.9 Hz, 1H), 2.85 (q, CH_2,*J* = 6.3 Hz, 2H), 2.72 (t,CH_2, *J* = 7.0 Hz, 1H), 1.17 (t,CH_3, *J* = 6.3 Hz, 3H).	485
S7	*n*-Pr	1615.7, 1721.3(C=O), 3015.7(C-H; aromatic), 1510.7(C-N), 3320.0(O-H)	8.61 (s, NH,1H), 8.11 – 8.03 (m, 2H), 7.97 (s, 1H), 7.73 – 7.55 (m, 7H), 7.41 – 7.31 (m, 2H), 7.03 (dt, *J* = 7.5, 1.5 Hz, 1H), 4.10 – 4.04 (m, CH_{2+1},3H), 3.67 (s,CH_2, 1H), 3.04 (t, CH_2,*J* = 3.9 Hz, 1H), 2.90 (t,CH_2, *J* = 7.5 Hz, 1H), 2.75 (t,CH_2, *J* = 3.9 Hz, 1H), 2.45 (t,CH_2, *J* = 7.5 Hz, 1H), 1.66 – 1.56 (m,CH_2, 2H), 0.87 (t,CH_3, *J* = 6.6 Hz, 3H).	499
S9	Me	1652.4, 1715.6(C=O), 3027.2(C-H; aromatic), 1521.0(C-N), 3205.0(O-H)	8.65 (s, NH,1H), 8.11 – 8.03 (m, 2H), 7.95 (dd, *J* = 7.5, 1.5 Hz, 1H), 7.78 (s,CH, 1H), 7.66 – 7.55 (m, 4H), 7.40 (td, *J* = 7.5, 1.7 Hz, 1H), 7.27 – 7.16 (m, 2H), 6.99 (dd, *J* = 7.5, 1.5 Hz, 1H), 6.66 (d, *J* = 1.5 Hz, 1H), 4.33 (t,CH_2, *J* =	471

			7.1 Hz, 2H), 4.21 – 4.14 (m, CH_2,1H), 3.77 (t,CH_2, *J* = 7.1 Hz, 1H),2.75 (s,CH_3)	
S10	Et	1634.9, 1714.6(C=O), 3028.8(C-H; aromatic), 1553.1(C-N), 3425.5(O-H)	8.11 – 8.03 (m, 2H), 7.95 (dd, *J* = 7.5, 1.5 Hz, 1H), 7.81 (s,CH, 1H), 7.69 (s,NH, 1H), 7.65 – 7.54 (m, 4H), 7.40 (td, *J* = 7.5, 1.7 Hz, 1H), 7.27 – 7.14 (m, 2H), 7.02 – 6.92 (m, 2H), 4.32 (t,CH_2, *J* = 4.0 Hz, 2H), 4.13 (t,CH_2, *J* = 4.0 Hz, 1H), 4.03 (t,CH_2, *J* = 3.9 Hz, 1H), 3.40 (q,CH_2, *J* = 6.3 Hz, 2H), 1.12 (t,CH_3, *J* = 6.3 Hz, 3H).	485
S11	*n*-Pr	1620.1, 1742.1(C=O), 3049.1(C-H; aromatic), 1522.1(C-N), 3415.0(O-H)	8.67 (s, NH,1H), 8.11 – 8.03 (m, 2H), 7.95 (dd, *J* = 7.5, 1.5 Hz, 1H), 7.79 (s, CH,1H), 7.65 – 7.55 (m, 4H), 7.40 (td, *J* = 7.5, 1.7 Hz, 1H), 7.27 – 7.16 (m, 2H), 6.99 (dd, *J* = 7.5, 1.5 Hz, 1H), 6.73 (d, *J* = 1.7 Hz, 1H), 4.37 (t, CH_2,*J* = 7.0 Hz, 2H), 4.08 (t, CH_2,*J* = 7.0 Hz, 1H), 3.95 (t, CH_2,*J* = 7.0 Hz, 1H), 3.67 (t,CH_2,, *J* = 5.1 Hz, 1H), 3.50 (t,CH_2, *J* = 5.2 Hz, 1H), 1.60 – 1.50 (m,CH_2, 2H), 0.87 (t,CH_3, *J* = 6.7 Hz, 3H).	499

Table 11: Effect of different 5-[(3-{2-[(3-phenyl-2,1-benzisoxazol-5-yl) (alkyl)amino]ethoxy}phenyl) methylidene]-1,3-thiazolidine-2,4-diones (Benzisoxazole coupled thiazolidinedione analogues) on alloxan induced hyperglycemia [Single-dose one-day study]

Treatment groups	% change in serum glucose			
	After 2 h	**After 4 h**	**After 6 h**	**After 24 h**
Normal Control	0.98±0.08	1.16±0.21	1.92±0.43	1.18±0.65
Diabetic control (DC) [Alloxan 70 mg/kg, i.v]	1.71±0.19	2.16±1.79	2.81±1.59	1.46±1.25
DC + S1 [30 mg/kg, p.o]	-21.67±4.52[b]	-18.54±6.48[b]	-17.65±5.22[b]	-14.01±5.38[b]
DC + S2 [30 mg/kg, p.o]	-43.37±8.13[b]	-41.71±9.91[b]	-42.17±5.27[b]	-28.21±8.01[b]
DC + S3 [30 mg/kg, p.o]	-20.13±3.68[b]	-17.81±5.21[b]	- 15.08±6.31[b]	-12.34±4.81[b]
DC + S5 [30 mg/kg, p.o]	-38.65±4.52[b]	-35.76±4.31[b]	-32.76±2.71[b]	-26.51±3.91[b]
DC + S6 [30 mg/kg, p.o]	-4.27±1.94	-6.93±2.41	-9.16±2.58	-3.98 ±1.83
DC + S7 [30 mg/kg, p.o]	**-46.82±5.33[b]**	**-43.28±5.03[b]**	**-40.61±4.81[b]**	**-30.62±6.24[b]**
DC + S9 [30 mg/kg, p.o]	-15.67±4.61	-13.14±5.13	-12.54±6.41	-10.98±6.12
DC + S10 [30 mg/kg, p.o]	-40.31±6.12[b]	-38.14±7.12[b]	-36.78±8.02 [b]	-29.61±7.19 [b]
DC + S11[30 mg/kg, p.o]	-9.56±3.27	-7.02±2.43	-5.87±3.21	-4.98±1.96
DC + Rosiglitazone [30 mg/kg, p.o]	**-26.16±5.28[b]**	**-28.70±4.49[b]**	**-20.84±3.98 [b]**	**-17.24±4.37[b]**

All values are expressed as mean ±SEM, n=5

\+ indicates increase in blood glucose, - indicates decrease in blood glucose, [b]P<0.01 comparable to DC group

RESULTS & DISCUSSION

All the results obtained during the characterization and pharmacological activities of the synthesized compounds are included in the Tables from 2-11. The PPAR-γ agonists, Benzisoxazole coupled Thiazolidinediones were designed and subjected for docking studies. All the compounds possess good docking scores. Out of 12 compounds docked, 9 compounds were selected through Lipinski's rule of five for drug-likeness, which were acceptable for oral bioavailability and chosen for synthesis. The synthesized compounds were screened for anti-diabetic activity in vivo, which was carried out in alloxan induced diabetic mice models.

The results of active site prediction and cavity detection revealed that the structure of Ligand binding domain of PPAR-γ (PDB ID: 2PRG) is 316 residues long. The position & most prominent of the active site was found with volume of 185.856=Leu, Tyr, Cys (Figure 8). Active sites and cavities were detected for Chain A of 2PRG using Molegro Virtual Docker. Based on molecular docking carried out using Molegro virtual docker and Lipinski's rule of 5 for druglikeness, nine out of 12 ligands were selected for in vivo studies. The best analogue among the proposed 12 ligands was compound S7 with moldock score (-178.50) and the docking score (-131.92). The close contacts showed that

there is a high possibility of interaction of this analog with the amino acids of the active site of the protein. In addition, compound S7 showed good moldock and docking score than Rosiglitazone (-135.70 and -124.78 respectively)

Based on computer-aided drug design experiments, 9 analogs were synthesized as per the scheme and the procedure. These compounds were identified by their spectral data and were in accordance with the assumed structures.

The results of anti-diabetic activity were expressed as mean ± standard error of mean (SEM) for each group, $P<0.01$ was considered as statistically significant. Oral administration of compounds S1-3, S5-7 & S9-11 (30 mg/kg) reduced SG level in alloxan (70 mg/kg) induced diabetic mice significantly ($P<0.01$). Analogues S2, S5, S7 & S10 have shown good activity while others exhibited moderate hypoglycemic activity. In addition, out of nine synthesized compounds, S7, S2, S10 & S5 exhibited better anti-diabetic activity than Rosiglitazone in alloxan induced diabetic mice model, whereas compounds S1, S3, S6, S9 & S11 have shown less anti-diabetic activity than Rosiglitazone. Overall, compound S7 exhibited higher ability to reduce blood glucose in diabetic mice compared to all tested analogues and Rosiglitazone. The observed activity is well correlated with molecular docking studies of compound S7. In other words, there is a strong correlation of docking experiments with in vivo studies of Compound S7.

These analogues will provide a good platform for structure-based design of PPAR-γ agonists for the treatment of Diabetes Mellitus.

CONCLUSION

In view of all the mentioned observations and results, it is concluded that the synthesized compounds are of highest purity and standards. It is observed that there is a correlation between the molecular docking studies and the pharmacological activity of the title compounds. Many of the synthesized derivatives have shown comparable antidiabetic activity with the standard drug like Rosiglitazone and might be due to the presence of highly lipophillic benzisoxazole moiety.

ACKNOWLEDGEMENTS

Our sincere thanks to Dr. V. H. Kulkarni, Principal, S.E.T.'s College of Pharmacy-Dharwad, India and President, Soniya Education Trust, Dharwad, India for providing necessary support during the research work.

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