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# Design, Synthesis and *In-vivo* Hypoglycemic Evaluation of Novel Non - TZD'S in a Type - 2 Diabetic Model

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**Abstract:** With a view to develop novel non-TZD anti-diabetic compounds, series of isoxazolidinediones were designed to target the PPAR-γ receptors. Docking studies were performed on co-crystallized protein structure of rosiglitazone with PPAR-γ receptor obtained from Protein Data Bank (2PRG). Interactions similar to that of rosiglitazone were observed for three molecules; 3a, 3b and 3c which were further synthesized and subjected to *in vivo* hypoglycemic, total cholesterol (CHL) and triglyceride (TG) evaluation. 14 days treatment revealed significant reduction in blood glucose levels but did not portray desirable results in terms of total CHL and TG lowering effect. The blood glucose reduction observed for 3a, 3b and 3c at 20 mg/kg/day was 53.96 %, 61.35%, 61.32% respectively as against 59.95% of the standard pioglitazone at 10mg/kg/day.

Keywords: 4-benzylidene-3,5-isoxazolidinedione, docking, HFD- low STZ model, hypoglycemic activity , Knoevenagel condensation.

# INTRODUCTION

Thiazolidinediones (TZDs) also known as 'Glitazones' have been extensively acknowledged for their anti-diabetic activity [1]. This category of molecules demonstrates prominent interactions with the Peroxisome Proliferator- Activity Receptors (PPARs), a subfamily of nuclear receptors (NRs). The NR family is one of the largest families of transcription factors. Three PPAR subtypes, i.e., PPARa, PPARy, and PPAR  $\delta$ , have been identified in humans, and their structures and functions are well known. PPARs play critical roles in the regulation of cellular differentiation and development and are, therefore, therapeutic targets in metabolic disorders such as obesity, type 2 diabetes, atherosclerosis, and cancer [2]. Glitazones are the representative family of ligands of PPARy. Ciglitazone and Troglitazone were the initial candidates of the TZD class that were marketed in the 1990's as anti-diabetic agents. Rosiglitazone and pioglitazone followed suit. The glitazones however portrayed discernible sideeffects like hepatotoxicity and cardiovascular toxicity. The basis for toxicity profile was unclear but previous references suggested that modifications other than the pharmacophore TZD were accountable for its toxicity. As also, there were evidences implying that acidic nature of the TZD is important for insulin-sensitizing activity and hypoglycemic activity [3, 4].

With the aforementioned inference, in our previous work [5], we synthesized two novel moieties wherein we retained the TZD ring; the pharmacophore and modified the non-pharmacophoric component by introducing amide linkage with a hope to alter the metabolism and thus the toxicity. Molecules screened for antidiabetic and hypolipidemic activ

ity in STZ-induced diabetic animal model showed comparable results with respect to the standard used viz. pioglitazone.

But, a recent literature survey provided evidence that the TZD ring of the troglitazone may be partially responsible for its hepatotoxicity due to formation of toxic reactive metabolites (RM). Five RM-GSH conjugates of troglitazone have already been identified in vitro using human liver microsomes and in vivo using Sprague-Dawley rats. One of the RMs is related to the quinone metabolite and four of the other metabolites arose from the TZD ring. The quinone metabolite however is not reported to be as cytotoxic as the parent drug in human and porcine hepatocytes. Hence, the authors tried an isosteric replacement of the TZD ring and evaluated troglitazone and the isosteric derivative on normal human hepatocytes cell line THLE-2. The study thus gave an insight into the drawback attributed to the TZD ring [6]. This gave us an impetus to modify the TZD ring based on the bioisosterism theory stating the reduction of adverse effects and optimization of pharmacokinetics in a view to build a new series of lead molecules containing isoxazolidinedione as the pharmacophore [7]. Isoxazolidinedione is a more acidic heterocycle (pka=1.86) as compared to TZD (pka= 6.82). Hence, we designed our molecules using the Molecular Operating Environment (MOE.2009.10) software. In this study, various structures of the desired compounds complexed with the PPARy Ligand Binding Domain 2PRG were modeled and their interactions were analyzed. Three molecules were synthesized and further screened for hypoglycemic and hypolipidemic activity.

# MATERIALS AND METHODS

# Materials

2-Amino-5-methyl thiazole was received as a gift sample from Ramdev Chemicals. 3-Aminobenzotrifluoride, 2aminobenzothiazole and chloroacetyl chloride were pur-

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Step 1: Synthesis of 1 (Knoevenagel condensation)



Step 2: Synthesis of 2a-2c (Chloroacetylation)





Step-3: Synthesis of 3a-3c (Condensation of 2a-2c and 1)



(i)Piperidinebenzoate, toluene, reflux, (ii)NH<sub>2</sub>OH;HCl,Na<sub>2</sub>CO<sub>3</sub> and THF:Methanol (1:1), (iii)K<sub>2</sub>CO<sub>3</sub> and Chloroform (iv)K<sub>2</sub>CO<sub>3</sub> and DMF



Fig. (1). Experimental scheme for synthetic work.

chased from Alfa Aesar. Piperidine was supplied by S.D Fine Chem. Ltd. Benzoic acid and anhydrous potassium carbonate were procured from Research lab. Fine Chem. Industries, while p-hydroxybenzaldehyde from CDH Laboratories. Streptozotocin (STZ) was purchased from Sigma-Aldrich, USA. Pioglitazone hydrochloride was obtained from Amoli Ltd., India as a gift sample. Sprague-Dawley female rats were gifted by Glenmark Pharmaceuticals Ltd. Solvents used were of LR grade. The animal feed ingredients were of veterinary grade. The Software MOE.2009.10 used for docking studies was procured from Chemical Computing Group, Canada.

### **METHODS**

#### **Docking Studies**

All computations for docking studies were carried out on a Pentium 2.67 GHz workstation, 256 MB memory with Windows operating system and Molecular Operating Environment (MOE 2009.10) as computational software. MOE identifies favorable poses of flexible ligands in rigid binding sites of macromolecules, typically proteins. MOE offers different routines for conformational sampling, placement and scoring.

2a-2c

The co-crystallized protein structure of rosiglitazone with PPAR-γ receptor was obtained from the Protein Data Bank (2PRG). The Rosiglitazone bound to the receptor was deleted. The crystal structure was then, protonated and the energy was minimized using AMBER99 forcefield. The active site was generated using atom selector and was labeled as the 'binding site'. All the designed molecules were built, energy minimized and used as ligands for the docking studies using the software. Docking was performed using Alpha PMI placement method with London dG scoring. The Alpha PMI method generates poses by aligning ligand conformations' principal moments of inertia to a randomly chosen subset of alpha sphere dummies in the receptor site. This method is preferred for tight pockets.

Different poses were scrutinized on the basis of suitable interactions and the best fitting moieties were considered on further studies.

# Chemistry

2-(4-Hydroxybenzylidene) malonic acid dimethyl ester was prepared by Knoevenagel condensation, wherein the aryl aldehydes were refluxed with dimethylmalonate in presence of a base; piperidinium benzoate. Then the condensed product was refluxed with hydroxylamine hydrochloride and sodium carbonate using methanol and tetrahydrofuran mixture to synthesize 4-(4-hydroxybenzylidene) isoxazolidine 3,5-dione [1]. The common intermediate 1 was further condensed at room temperature with various chloroacetylated amines to yield the desired molecules (**3a-3c**) (Fig. 1) [3,8].

# **BIOLOGICAL EVALUATION**

#### Animals

Female Sprague-Dawley rats with an average body weight of 180-200 g were selected for the study. Animals were housed in polypropylene cages at an ambient temperature of  $25 \pm 2^{\circ}$ C and 45-55% relative humidity with standard 12 hours light and dark cycle. They had free access to feed and water. Animals were examined properly for infection and metabolic disorders. CPCSEA guidelines for the use and care of laboratory animals were followed throughout the experiment and prior permission was sought from the institutional ethics committee for conducting the study.

#### Experimental Design

#### Table 1. Experimental Design for Animal Study

Groups	Administration of chemicals	No. of animals
Group1	Normal Control- received only vehicle	6
Group2	Normal +3a	6
Group3	Diabetic +3a	6
Group4	Normal +3b	6
Group5	Diabetic +3b	6
Group6	Normal +3c	6
Group7	Diabetic +3c	6
Group8	Diabetic +STD*	6
Group9 Diabetic Control –received only vehicle		6

\*STD -Standard drug Pioglitazone HCl

# Development of High Fat Diet (HFD) Fed and STZ Treated Type 2 Diabetic Rats

Except normal control animals rest all animals were given HFD for a period of two weeks. The composition of HFD is given below [9]:

Table 2. Composition and Calorie Value of Cafeteria Diet

Ingredients	Calorie Value (kcal/100g)
Condensed Milk	335
Bread	230
Chocolate	550
Biscuits	360
Cheese	320
Boiled potato	80
Dried coconut	660

# COLLECTION OF BLOOD AND ANALYTICAL METHODS

For blood glucose estimation, rats were given mild ether anesthesia followed by collection of blood from the retro orbital plexus. Blood was collected in sodium fluoride anticoagulant containing vials and glucose was analyzed by glucose oxidase method. For triglycerides (TG) and cholesterol (CHL) analysis, blood was collected in plain eppendorf's tubes to obtain serum and estimation was carried out using standard biochemical kits.

#### **Biological Activity**

Acute toxicity study for compounds **3a**, **3b** and **3c** was performed before testing the hypoglycemic activity of these compounds. Different doses upto 500mg/kg were tried. Hypoglycemic and hypolipidemic activities were carried out using a combination of high fat diet (HFD) and low dose of STZ-treated female Sprague - Dawley rats. The animals were fed HFD for a period of 14 days. After 14 days of dietary manipulation, rats were injected intraperitoneally (i.p.) with low dose of STZ (35mg/kg), while respective control rats were administered the vehicle; 1% Na-CMC (2ml/kg, p.o.). Blood glucose (BG) was estimated just before the study, 7 days after STZ injection and at the end of 14 days treatment. Rats with non-fasting plasma glucose level more than 250mg/dl were considered diabetic and selected for the study. For hypolipidemic activity, CHL and TG estimations were carried out prior to study, after 14 days of HFD and at the end of drug treatment.

Diabetic rats were randomly divided into five groups. The diabetic rats were either treated with test compound (20mg/kg/day for 14 days) or with pioglitazone hydrochloride (equivalent to 10 mg/kg/day for 14 days).

# **RESULT AND DISCUSSION**

### **Design of Novel Molecules**

The TZD ring of troglitazone has been reported to show liver toxicity in human via reactive metabolite formation (6). Acidity of the TZD moiety has been accounted for its insulin sensitizing effect [3, 4]. Hence, various research groups have attempted replacement of the TZD ring with different acidic

#### CENTRAL ARYL GROUP



Fig. (2). Generalized structure of novel benzylidene isoxazolidinediones.

groups. One of the attempts included, designing compounds containing malonate as a substitute for TZD which have been reported to possess antihyperglycemic activity similar to the thiazolidinediones [4, 10]. However, the free malonate group is susceptible to unspecific ester hydrolysis, converting it to malonic acid which is responsible for its toxicity [11]. To circumvent these negative aspects related to the molecule, cyclization of malonate moiety to isoxazolidinedione ring, a more acidic bioisostere of TZD was considered.

The non-pharmacophoric fraction of our previously mentioned TZD molecules were retained as they were expected to improve the lipophilicity of the molecules [5]. The novel lipophilic moiety was attached to isoxazolidinedione ring by using alkoxy linker and a benzylidine hydrophobic trunk. (Schematic representation in Fig. 2).

# **Docking Studies**

Structurally, PPAR $\gamma$  is composed of a DNA-binding domain (DBD), a hinge region, and a ligand-binding domain (LBD). Agonist binding to PPAR $\gamma$  regulates activity by causing conformational changes to the LBD, which is composed of approximately 250 amino acids near the C-terminal end of the protein. The LBD of PPAR $\gamma$  is a large, T-shaped cavity with a volume of approximately 1440 °A<sup>3</sup> which can easily accommodate many different ligands due to the dynamics of the ligand-binding pocket.

PPAR $\gamma$  agonists typically possess a small polar region and a hydrophobic region that form hydrogen bonds and hydrophobic interactions, respectively, within the LBD. Hydrogen bonding typically occurs between His323, Tyr473, Ser289 and His449 of the PPAR $\gamma$  LBD and carbonyl oxygen of the ligand. Hydrogen bonding of the ligand to Tyr473 is the key to the stabilization of the AF-2 region. The hydrophobic moiety interacts with other residues in the cavity, such as Leu465, Leu469, and Ile472, establishing hydrophobic interactions to stabilize the domain. Using the structural details of the receptor and the interactions we designed our new chemicals and docked them into the active pockets of the receptor.

Initiation of the study was done using rosiglitazone for which the binding mode has been determined experimentally. According to the crystal structure of rosiglitazone in the LBD of PPAR- $\gamma$  (PDB code-2PRG), the thiazolidinedione ring exhibits several specific interactions with neighboring amino acids of the LBD. These interactions include hydrogen bonding with amino acid residues His323, Tyr327 and His449 as reported in the literature [12, 13]. **MOE.2009.10** was successful in reproducing the binding position for rosiglitazone, showing a Root Mean Square deviation of 1.5 A° in comparison with the experimental geometry when this TZD is co-crystallized in the PPAR- $\gamma$ .

The binding profile of the 4-(4-hydroxybenzylidine) isoxazolidine-3.5-dione derivatives were compared with that of the rosiglitazone molecule. Interactions were distinct in the case of ligand N-(Benzo[d]thiazol-2-yl)-2-[4-[(3,5dioxoisoxazolidin-4-ylidene) methyl] phenoxy] acetamide (3a). The nitrogen atom of the isoxazolidinedione ring demonstrates hydrogen bonding with Tyr327 residue having 34% binding and bond length of 2.1 A° and the oxygen of carbonyl group exhibits hydrogen bonding with Ser289 having 25% binding and bond length 1.8 A°. As also, the amino acid residue Arg288 shows arene interactions with benzothiazole moiety and a hydrogen bond of percent binding 14%, bond length of 2 A° with oxygen of amide linker is also seen (Fig. 3A and 3B). The ligand 2-[4-[(3,5-dioxoisoxazolidin-4ylidene) methyl] phenoxy]-N-[(3-trifluoromethyl) phenyl] acetamide (3b) shows significant interactions with His449 and the Ser289 amino acid residues, forming hydrogen bonds with the two oxygen atoms of the carboxyl groups of the isoxazolidinedione ring. The percentage binding and the distance from the residues were found to be 12 %, 1.7 A° and 47 %, 2.7 A° respectively (Fig. 3C and 3D). Similarly, the ligand 2-[4-[(3,5-dioxoisoxazolidin-4-ylidene) methyl] phenoxy]-N-(5-methylthiazol-2-yl) acetamide (3c), interacts mainly with PPAR-y through the formation of hydrogen bonds between the two oxygen of the carbonyl groups of the isoxazolidinedione ring and the Tyr473 and the Ser289 residues, respectively (Fig. 3E and 3F). The percent binding with the amino acid residues Tyr473 and Ser289 and the distance through which binding occurs are 38%, 3.2 A° and 35%, 2.3 A° respectively.

### **BIOLOGICAL ACTIVITY**

#### **Statistics**

The results were calculated with the help of *GraphPad InStat* using one-way ANOVA and Dunetts' test and are expressed as mean  $\pm$  SEM. The percent reduction was calculated using the equation:  $[1 - (TT/OT)/(TC/OC)] \times 100$ , where TT is test day treated, OT is zero day treated, TC is test day control, and OC is zero day control.



**Fig. (3).** Ligand interactions of 3a, 3b and 3c with PPARγ (PDB 2PRG): Pink dashed lines represent bonding interactions between the hydrogens of the residue and the oxygen of ligand and green circles represent arene - arene interaction between aromatic rings of residue and ligand. [A] 3D interaction of 3a with 2PRG. [B] 2D interaction of 3a with 2PRG. [C] 3D interaction of 3b with 2PRG. [D] 2D interaction of 3c with 2PRG. [E] 3D interaction of 3c with 2PRG. [F] 2D interaction of 3c with 2PRG.

To study the insulin resistance in humans which is characterized by obesity, mild hyperglycemia and hypercholestrimia "HFD fed, low dose of STZ model" is used. Hypoglycemic activity in STZ-induced HFD fed rats was carried out in a 14 days study for compounds **3a**, **3b** and **3c** and they showed 53.96, 61.35 and 61.32% blood glucose reduction, respectively, when compared with disease control. Efficient control of Triglyceride (TG) and Cholesterol (CHL) level in type-2 diabetes mellitus (T2DM) is essential as diabetes is also associated with accelerated atherosclerotic macrovascular diseases such as myocardial infarction, stroke and limb amputation [10]. Thus, the drugs which effectively control TG and CHL levels would be more suitable candidates for further development for treatment of T2DM. In our study all three compounds were evaluated for its TG and CHL lowering activity. Slight alterations were observed in data during the study but the results are not significant as compared to disease control group, since observed TG and CHL lowering effect of pioglitazone hydrochloride are not reported in clinical trials [5].

Table 3. Body Weight in Grams Before and After HFD

Group	Normal rats	HFD rats
Group2	177.5±3.35	192.5±3.09
Group3	186.6±3.57	195.83±6.63
Group4	135.8±3.27	197.5±3.09
Group5	177.5±4.95	200±4.90
Group6	169.16±10.6	192.5±4.78
Group7	181.66±5.72	190±4.47
Group8	182.5±4.33	195.8±2.71
Group9	181.66±4.94	193.33±5.19

Table 4. Cholesterol in mg/dl Before and After HFD

Groups	Normal rats	HFD rats
Group2	74.33±4.94	81.66±6.17
Group3	80.16±3.42	89.33±6.88
Group4	81.83±4.57	86.33±4.60
Group5	73.33±5.81	82.33±9.17
Group6	73.165.02	83.33±4.8
Group7	72±5.79	81.5±6.14
Group8	75.66±5.73	87±6.48
Group9	80.83±4.97	91.8±6.46

Table 5. Triglyceride In mg/dl Before and After HFD

Groups	Normal rats	HFD rats
Group2	63.66±7.27	69.83±8.61
Group3	67.33±4.63	74.5±3.01
Group4	62.33±4.91	72.83±4.55
Group5	70.83±4.30	78.66±4.26
Group6	60.16±4.19	66.16±5.38
Group7	65.83±7.38	67.5±6.43
Group8	72.83±7.32	77.83±6.72
Group9	69.83±6.94	79±6.65

The order of drug molecules showing blood sugar lowering activity is  $3b \sim 3c > 3a$  with significant result. Thus, introducing the isoxazolidinedione ring may serve as a useful strategy in drug design in the area of antidiabetic non-TZDs.

# **EXPERIMENTAL SECTION**

<sup>1</sup>H NMR and <sup>13</sup>C NMR spectra were recorded in DMSOd6 on Bruker 400 MHz and Varian 300 MHz instrument

## Table 6. Statistical Analysis of Blood Glucose Mg/Dl After 2 Weeks of Drug Treatment

Groups	0 <sup>th</sup> day	14 <sup>th</sup> day	
Group 1	128.66±	123.5±	
Group2	130.67±7.07	111.5±3.97	
Group3	259.16±3.68	116.83±3.79	
Group4	119.17±1.99	104.5±1.41	
Group5	267.33±2.91	101.16±6.57	
Group6	137.15±7.96	108.83±8.93	
Group7	268±4.04	103.5±5.02	
Group8	265.6±3.73	100.5±2.90	
Group9	265.67±3.24	260.16±10.83	

**Table 7. Percent Reduction Values** 

Compound No.	Blood Glucose % Max Reduction	Triglyceride % max Reduction	Cholesterol % max Reduction
3a	53.96**	1 <sup>N.S</sup>	5.9 <sup>N.S</sup>
3b	61.35**	4 <sup>N.S</sup>	8.14 <sup>N.S</sup>
3c	61.32**	14.6 <sup>N.S</sup>	0.049 <sup>N.S</sup>
STD	59.95**	3.2 <sup>N.S</sup>	9.52 <sup>N.S</sup>

'% max reduction' is the maximum achieved reduction relative to diabetic control group. \*\* represents  $p\,{<}\,0.01$  (significant). N.S: Not significant

using TMS as internal standard. Chemical shift values are reported in  $\delta$ , ppm. All reactions as well as column chromatography were followed by TLC using Merck pre-coated silica gel 60 F254 plates and spots were visualized by observing in UV cabinet under short UV. IR spectra were recorded on FTIR-8400S instrument with KBr pellets and only the principal absorption levels (cm<sup>-1</sup>) have been listed. All reagents were used as received unless otherwise stated.

# Synthesis of the 4-(4-hydroxybenzylidine) Isoxazolidinedione-3,5-dione (1)

4-(4-Hydroxybenzylidine) isoxazolidine-3,5-dione was synthesized in two steps. First step was Knoevenagel condensation reaction wherein 4-hydoxybenzaldehyde (3.0 g, 5.0 mmol) and dimethyl malonate (2.82 ml, 5.0 mmol) in presence of 20 ml toluene and catalytic quantity of piperidinium benzoate were refluxed together to yield 2-(4hydroxybenzylidine) malonic acid dimethyl ester with continuous removal of water. Next step was cyclization of 2-(4hydroxybenzylidine) malonic acid dimethyl ester (4.3 g, 25.0 mmol) with hydroxylamine hydrochloride (1.8 g, 25.0 mmol) in presence of sodium carbonate (1.57 g, 5.0 mmol) using solvent THF: methanol (1:1) under reflux of 5-6 hrs. The cyclisation product of Knoevenagel condensation was unstable [4].

# Synthesis of N-(Benzo[d]thiazol-2-yl)-2-chloro Acetamide (2a)

2-Aminobenzothiazole (3 .0g, 1.0mol), anhydrous  $K_2CO_3$ (4.14g, 1.73mol) and 25 ml of chloroform were taken in RBF. Reaction mixture was cooled between 2-5°C and to this chloroacetyl chloride (4.12ml, 2.6mol) was added drop wise. The reaction mixture was allowed to stir overnight at room temperature. After completion of reaction, water was added to reaction mixture and organic layer was separated. Then, it was passed through anhydrous Na<sub>2</sub>SO<sub>4</sub>. Chloroform layer was distilled out and off white crystals were collected. Yield 92.10%, white crystals, mp 143-144°C IR [KBr v cm<sup>-1</sup>]: 3181 (N-H), 1660 (C=O). <sup>1</sup>H NMR [300 MHz,  $\delta$ , ppm, DMSO-d<sub>6</sub>]: 4.22 (2H, s), 7.35 (1H, t), 7.46 (1H, t), 7.77 (1H, d, J = 8.1Hz), 8.01 (1H, d, J = 6.0 Hz), 12.79 (1H, s).

# Synthesis of N-(Benzo[d]thiazol-2-yl)-2-[4-[(3,5-dioxoisoxazolidin-4-ylidene) Methyl] Phenoxy] Acetamide (3a)

Anhydrous K<sub>2</sub>CO<sub>3</sub> (0.42g, 1.5mol) was stirred in DMF for 10-15 min. To this 4-(4-hydroxybenzylidine) isoxazolidine-3,5-dione (0.43g, 1.0mol) and chloroacetylated 3aminobenzthiazole (0.47g, 1.0mol) were dissolved separately in DMF and added to the reaction mixture. The reaction was allowed to stir at room temperature till its completion. Reaction was monitored by TLC. Water was added to reaction mixture and further stirred for 30 minutes. Product was then extracted in chloroform and organic layer was passed through anhydrous Na<sub>2</sub>SO<sub>4</sub>. Chloroform layer was then distilled out to get crude product. Eluent used for column chromatography: Chloroform: Methanol (2:0.36). Yield 24.10%, off white powder, mp 234-239°C. IR [KBr v cm<sup>-1</sup>]: 3235 (N-H), 1705 (C=O), 1602 (C=O), 1182, 1132 (C-O). <sup>1</sup>H NMR (400 MHz, δ, ppm, DMSO-d<sub>6</sub>): 5.12 (2H, s, -CH<sub>2</sub>-), 7.31-7.35 (1H, m, benzothiazole proton), 7.48-7.44 (1H, m, benzothiazole proton), 7.59 (2H, t, phenyl proton), 7.71 (1H, t, benzylidene proton), 7.78 (1H, d, J= 6Hz, benzothiazole proton), 8.00 (1H, d, J= 6Hz, benzothiazole proton), 8.06 (2H, d, J= 6Hz, phenyl protons), 12.74 (1H, s, -NH-CO-).  $^{13}C$ NMR (100 MHz, DMSO-d<sub>6</sub>, δ): 167.26 (C=O), 165.89 (C=O), 134.28, 129.95, 129.35, 126.70, 124.22, 122.27, 121.16, 63.15.

# Synthesis of 2-Chloro-N-[3-(trifluoromethyl) Phenyl] Acetamide (2b)

3-Aminobenzotrifluoride (2.31ml, 1.0mol), anhydrous  $K_2CO_3$  (3.85g, 1.5mol) and 25 ml of chloroform were taken in RBF. Reaction mixture was cooled between 2-5°C and to this drop wise (2.20ml, 1.5mol) of chloroacetyl chloride was added. The reaction mixture was allowed to stir overnight at room temperature. After completion of reaction water was added to reaction mixture and organic layer was separated. Then, it was passed through anhydrous Na<sub>2</sub>SO<sub>4</sub>. Chloroform layer was distilled out and white crystals were collected. Yield 67.0%, white crystalline, mp 129-130°C. IR [KBr v cm<sup>-1</sup>]: 3305 (N-H), 1683 (C=O), 1070, 1124 (C-F). <sup>1</sup>H NMR [300 MHz,  $\delta$ , ppm, DMSO-d<sub>6</sub>]: 4.38(2H, s), 7.30-7.44 (2H, m), 7.95 (1H, d), 8.2 (1H, s), 10.05 (1H, s).

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# Synthesis of 2-[4-[(3,5-dioxoisoxazolidin-4-ylidene) methyl] Phenoxy]-N-[3-(trifluoromethyl) Phenyl] Acetamide (3b)

Anhydrous K<sub>2</sub>CO<sub>3</sub> (1.51g, 1.5mol) was stirred in DMF for 10-15 min. To this 4-(4-hydroxybenzylidine) isoxazolidine-3,5-dione (1.5g, 1.0mol) and chloroacetylated 3aminobenzotrifluoride (1.73g, 1.0mol) were dissolved separately in DMF and added to the reaction mixture. The reaction was continued to stir at room temperature till its completion. Reaction was monitored by TLC. Water was added to reaction mixture and further stirred for 30 minutes. Product was then extracted in chloroform and organic layer was passed through anhydrous Na<sub>2</sub>SO<sub>4</sub>. Chloroform layer was then distilled out to get crude product. Eluent for column chromatography: Benzene: Methanol (2:0.48). Yield 30%, color pale yellow colour powder, mp 96°C. IR [KBr v cm<sup>-1</sup>]: 3344 (N-H), 1724 (C=O), 1693 (C=O), 1158, 1128 (C-O). <sup>1</sup>H NMR (300 MHz, δ, ppm, DMSO-d<sub>6</sub>): 4.96 (2H, s, -CH<sub>2</sub>-), 7.43 (1H, d, aromatic), 7.55-7.60 (3H, m, aromatic and benzylidene proton), 7.69-7.78 (2H, m, aromatic protons), 8.03-8.0 (3H, m, aromatic protons), 10.58 (1H, s, -NH-CO). <sup>13</sup>C NMR (100 MHz, DMSO-d<sub>6</sub>, δ): 167.00 (C=O), 166.15 (C=O), 138.73, 133.22, 132.62, 131.27, 130.64, 130.32, 129.49, 129.27, 128.23, 128.05, 125.39, 122.98, 120.33, 119.98, 116.27, 62.83.

# Synthesis of 2- Chloro -N-(5-methlthiazol-2-yl) Acetamide (2c)

2-Amino-5-methylthiazole (3.0g, 1.0mol), anhydrous  $K_2CO_3$  (6.0g, 1.5mol) and 25ml of chloroform were taken in RBF. Reaction mixture was cooled between 2-5 °C and to this chloroacetyl chloride was added drop wise (5.0 ml, 1.5mol). The reaction mixture was allowed to stir overnight at room temperature. Water was added to reaction mixture and organic layer was separated. It was then passed through anhydrous Na<sub>2</sub>SO<sub>4</sub> layer. Chloroform layer was distilled out and off white colour powder was collected. Yield 82%, white needle shaped crystals, mp 191-192°C. IR [KBr v cm<sup>-1</sup>]: 3186 (N-H), 1704 (C=O), 1560 (C=N). <sup>1</sup>H NMR [300 MHz,  $\delta$ , ppm, DMSO-d<sub>6</sub>]: 2.43 (3H, s), 4.24 (2H, s), 7.5 (1H, s), 11.0 (1H, bs).

# Synthesis of 2-[4-[(3,5-dioxoisoxazolidin-4-ylidene) methyl] Phenoxy]-N-(5-methylthiazol-2-yl) Acetamide (3c).

Anhydrous  $K_2CO_3$  (1.01g, 1.5mol) was stirred in DMF for 5-10 min. To this 4-(4-hydroxybenzylidine) isoxazolidine-3,5-dione (1.0g, 1.0mol) and chloroacetylated 2amino-5-methylthiazole (0.92g, 1.0mol) were dissolved in DMF separately and added to the reaction mixture. The reaction was continued to stir at room temperature till its completion. Reaction was monitored by TLC. Water was added to reaction mixture and further stirred for 30 minutes. Product was then extracted in chloroform and organic layer was passed through anhydrous Na<sub>2</sub>SO<sub>4</sub>. Chloroform layer was then distilled out to get crude product. It was further purified by column chromatography. Yield 33.33%, buff colour powder, mp 130°C. IR [KBr v cm<sup>-1</sup>]: 2922 (N-H), 1726 (C=O), 1697 (C=O), 1595 (C=N), 1166, 1122 (C-O). <sup>1</sup>H NMR (400 MHz,  $\delta$ , ppm, DMSO-d<sub>6</sub>): 2.34 (3H, s, -CH<sub>3</sub>), 5.02 (2H, s, -CH<sub>2</sub>-), 7.15 (1H, s, thiazole proton), 7.55-7.62 (2H, m, phenyl protons), 7.71 (1H, t, benzylidene proton), 8.03 (2H, d, J=8Hz, phenyl protons), 12.27 (1H, bs, -NH-CO-). <sup>13</sup>C NMR (100 MHz, DMSO-d<sub>6</sub>,  $\delta$ ): 165.90 (C=O), 156.09 (C=O), 135.20, 134.22, 129.91, 129.42, 129.32, 127.07, 62.98, 11.56.

# CONCLUSION

In this project we have attempted a bioisosteric modification of the TZD ring to an isoxazolidinedione ring. Based on certain well established findings we derived a logical approach to design our molecules which were further docked using MOE.2009.10 software. Promising results for three molecules were obtained based on interactions similar to that of rosiglitazone on the PPAR- $\gamma$  receptor (PDB CODE: 2PRG). These three novel molecules were synthesized based on the scheme selected. All the final compounds and their intermediates have been characterized and subjected to biological evaluation. Molecules with benzotrifluoride and thiazole substituents were found to be equivalently active and they were superior than benzothiazole containing compound with regards to blood glucose lowering effect.

# **CONFLICT OF INTEREST**

The author(s) confirm that this article content has no conflicts of interest.

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