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Insilico Proportional Molecular Docking Study and Analysis of Insulinotropic Activity of TZD Derivatives by PPARγ Activation

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Abstract

Purpose: The thiazolidinediones (TZDs) have become one of the most commonly approved classes of medication for type 2 diabetes. In addition to glucose control, the TZDs have a number of pleiotropic effects risk factors for diabetes.

Method: In the present studies, we investigate and assess the insulinotropic prospective using binding energy and pharmacological interaction of TZD derivatives using insilico proportional molecular docking relation approach against roziglitazone and to investigate the mechanism of action of *TZD derivatives* as a hypoglycemic agent, both *in-vivo* and *in-vitro* experiments were conducted. Investigations were conducted on the intestinal level by delaying or inhibiting glucose absorption, the peripheral level on insulin-sensitive tissues by facilitating the entry of glucose into cells such as muscle, and the pancreatic level by stimulating insulin secretion.

Result: In this series, the most potent compounds were **6a and 6b** having methoxy group at C_5 position of TZD ring.

Conclusion: 5-(substituted benzylidene)-2-(4-chloro-2-fluoro-5-methoxybenzylidene) hydrazono) thiazolidin-4-one have shown better antidiabetic activity. However, clinical trials with standardized extracts and uniform protocols have been with experimental animals and validated TZD derivatives clinical applicability as an antidiabetic agent. The outcomes of such studies may be useful for the clinical applications in humans and may open up a new therapeutic avenue.

Keywords: Antidiabetic drugs, 2,4-thiazolidinediones, 4-chloro-2-fluoro-5-methoxybenzylidene) hydrazino, insulinotropic activity, in-silico molecular docking

INTRODUCTION

Thiazolidinediones (TZDs) are agonists for peroxisome proliferators-activated receptor (PPAR) γ, a member of the nuclear hormone receptor superfamily and are currently used therapeutically. Pioglitazone and Rosiglitazone are PPARγ agonists used for the management of hyperglycemia in Type 2 diabetes mellitus (T2DM). TZDs induced glycemic improvement is accompanied by a significant reduction in fasting insulin (1).

PPAR-gamma has also been recently identified as the major functional receptor for the thiazolidinedione class of insulin-sensitizing drugs. This appraisal examines the evidence that has concerned this transcription factor in the processes of systemic insulin action. Final discussion particularly involves the docking of cofactors in a ligand-dependent fashion, should lead to improved drugs that utilize the PPAR-gamma system for the treatment of insulin resistance (2). TZDs have provided an invaluable source of information pertaining to the physiological mechanisms of PPARγ. A better understanding of PPARγ ligand interactions is warranted considering the role of PPARγ in adipogenesis (3). Based on these issues the present study aimed to evaluate TZD derivatives as agonist of PPARγ by in silico proportional molecular docking studies and both *in-vivo* and *in-vitro* insulinotropic experiment.

DRUG DESIGN

During *In-silico* proportional molecular docking study and analysis of TZD derivatives the ligand and receptor are prepared as dicussed under materials and methods. We here in report the performance of 2D QSAR on the series of synthesized derivatives by using software V-Life MDS 3.5. It gives the output as an equation containing descriptors such as alignment independent parameters and as an indicative of physicochemical properties required to show biological activity i.e., anti-hyperglycemic activity. The correlation between independent variables (descriptors) and dependent variables (pharmacological activity) was established. The out-put is in the form of regression equation showing descriptors are in the form of positive and negative contributions by using the equation as an output from the QSAR study, we have designed the following derivatives. And further their synthesis has been done followed by anti-diabetic activity.

MATERIALS AND METHODS

The chemicals used in the present project work were purchased from Rankem, Merck and Spectrochem. The melting point of the synthesized compound was determined by open capillary with Thiel's melting point tube (capillary tube method). TLC plates were prepared by using Merck Silica Gel 60 GF 254. Visualization was done in UV light chamber at 254 nm, iodine chamber. The IR spectra of the synthesized compounds were recorded on a Fourier Transform Infra Red spectrometer (model Shimadzu 8400 S) in the range of 400-4000 cm^{-1} as KBr pellets. (^{1}H NMR) data of the compound was carried out in Bruker 200 spectrospin NMR at Astra Zeneca Pharma India Limited,

Bangalore and Bruker 400 spectrospin NMR at Indian Institute of Science, Bangalore. The solvent used for NMR was $CDCl_3$

Antidiabetic Drugs

Thiazolidinedione (TZDs) are a pharmacological insulin-sensitizing class of compounds those are high affinity ligands for PPAR-γ and widely used for treatment of type 2 diabetes (4).

Roziglitazone

Rosiglitazone is an antidiabetic drug in the thiazolidinedione class of drugs. It works as an insulin sensitizer, by binding to the PPAR gamma (5).

Preparations of Ligand and Receptor Protein

The selected ligands were sketched using Chemsketch and malformed into the 3D structure, intermolecular interactions of these ligands were optimized to attain a local minimum energy structure using Universal force fields (UFF) (6, 7). Considering the agonist for type II DM the indigenous docked complex of roziglitazone PPAR gamma Pdb-id – 1FM6 and the 3D-crystallographic structures was considered (8-10).

PPAR gamma receptor 3GBK is imported from protein data bank (RCSB PDB) with resolution $2.3A^0$, R- value free 0.98 and R-value work 0.230.

Active Site

The default active site were considered of docked complexes, Amino acid within 10 A by considering the ligand of interest in center.

Molecular Docking

Molecular interactions play an important role in all bio-chemical reactions. Drugs can either mimicking or extenuating the effect of natural ligands binding to the receptor by exerting the pharmacological reactions. Computational methods are used to identify and understand this approach of ligand to the receptor and their interaction and orientation. It is an attempt to find out the optimal binding between different a set of molecules: a receptor and a ligand. Genetic Algorithm (GA) based approach were used with the following parameters Population size = 200; Generations = 70 and number of solutions =3 in iGEM dock (11). Pharmacological points and interaction are the key features in drug binding ability and in formation of a stable complex. Hence, in the present study, TZD derivatives binding affinity was compared with the PPAR γ agonist roziglitazone and authenticate the pharmacological interactions between them.

***IN-VIVO and IN VITRO* SCREENING FOR ANTI-DIABETIC ACTIVITY (12, 13):**

Streptozocin induced insulin resistance in rats

Exogenous administration of streptozocin in rats causes hyperglycemia, hyperinsulinaemia, associated with insulin resistance. Institution of Animals Ethics Committee has approved the experimental protocol **(DSU/PhD/IAEC/09/2017-18)**

Animals

Male Sprague-Dawley (SD) rats (200-250 g) were obtained from the animal house of the School of Pharmaceutical Sciences, Dayananda sagar university, Bengaluru. They were housed in standard environmental conditions (24 ± 1 ºC) with 12 h light: 12 h dark cycles and fed a commercial diet and water *ad libitum.*

Streptozotocin-induced diabetic rats

Diabetes was induced by intraperitoneal injection of streptozotocin (Sigma aldrich) (65 mg/kg body weight in 0.9% NaCl, pH 4.5) to rats fasted for 16 h. Their diabetic conditions were confirmed by the symptoms of polydipsia, polyuria and a high fasting blood glucose concentration 72 h after injection of streptozotocin. Rats with a blood glucose level above15.0 mmol/L were considered to be diabetic and used in the experiment.14 day treatment with the 6a and 6b

Group I: Normal control - Received 0.25% CMC p.o and sterile water for injection i.m.

Group II: Streptozocin control - Received 0.25% CMC p.o and streptozocin 0.7 mg/Kg i.m.

Group III: Rosiglitazone treated - Received rosiglitazone 0.72 mg/Kg in 0.25% CMC p.o and streptozocin 0.7 mg/Kg i.m.

Group IV: IVa treated - Received **6a**, 0.72 mg/Kg in 0.25% CMC p.o and streptozocin 0.7 mg/Kg i.m.

Group V: IVb treated - Received **6b**, 0.72 mg/Kg in 0.25% CMC p.o and streptozocin 0.7 mg/Kg i.m.

Treatment was continued for 10 days. On day 10, after overnight fasting, blood samples were collected from all the animals by puncturing the retro orbital plexus under mild ketamine anaesthesia.

In vitro studies

RIN-5F cells were routinely cultured in RPMI 1640 supplemented with 2 mM L-glutamine, 10 mM HEPES, 1 mM sodium pyruvate, 4.5 g/L glucose, 1.5 g/L sodium bicarbonate and 10% fetal bovine serum. The cells were passaged 2-4 days before each experiment and plated in 24-well Nunclon multiwell plates (NUNC A/S, Denmark) at a density of 0.2 × 106 cells/well. Insulin secretion was measured as previously described by Gray and Flatt (1998, 1999). Multiwells were seeded with 0.2 × 106 cells and insulin release measured after 4-5 days as follows. Cells were washed three times with KRB (115 mM NaCl, 4.7 mM KCl, 1.28 mM CaCl2, 1.2 mM KH2PO4, 1.2 mM MgSO4, 24 mM NaHCO3, 10 mM HEPES-free acid, 1 g/L bovine serum albumin, 1.1 mM glucose; pH 7.4) and preincubated for 40 min at 37 ºC. Cells were then incubated for 20 min with 1 mL KRB and 1.1 mM glucose in the absence or presence of *G. procumbens* water extract (1, 5 or 10 mg/mL) and glibenclamide (0.2, 2 or 20 mM). Following incubation, aliquots were removed from each well and stored at -20 ºC for insulin assay. Insulin release was measured by rat an insulin ELISA kit (Crystal Chem, USA).

Cell viability

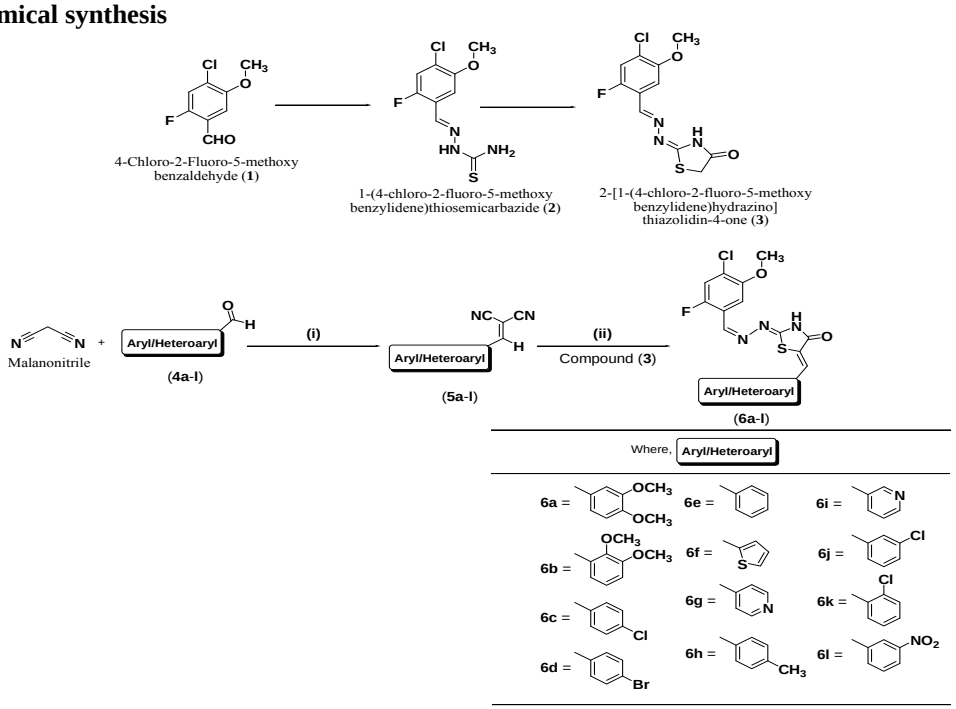
Cell viability was estimated by the MTT assay, which is based on the cleavage of a tetrazolium salt by mitochondrial dehydrogenases in viable cells (Hansen et al., 1989). V79-4 cells were seeded in a 96 well plate at a concentration of 1.2 ´ 105 cells/ml. Sixteen h after plating, cells were treated with concentrations of 100 mg/ml and 1 h later 1 mM H_2O_2 was added to the culture. Cells were incubated for an additional 24 h at 37^oC. During the last 4

h, cells were incubated with 20 ml of MTT stock solution (5 mg/ml) in 200 ml medium at 37^{o}C. Samples were then extracted with acidic isopropanol and the absorbance was measured with an ELISA reader (Bio-Rad, USA) at 570 nm. The relative cell viability was determined by the amount of MTT converted to the insoluble formazan salt.The results were used to construct a graph of percentage cell viability against concentration of fractions. The percentage of cell viability was calculated using formula as in Eq 1.

Percentage cell viability = 100 - {(At-Ab)/(Ac-Ab)} x 100

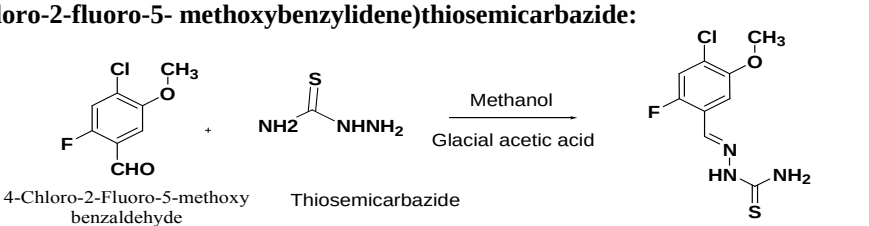
Where At is the absorbance of test sample or positive controls, Ab is the absorbance of blank and Ac is the absorbance of negative control.

Scheme of Chemical synthesis



EXPERIMENTAL PROCEDURE

Synthesis of 1-(4-chloro-2-fluoro-5- methoxybenzylidene)thiosemicarbazide:



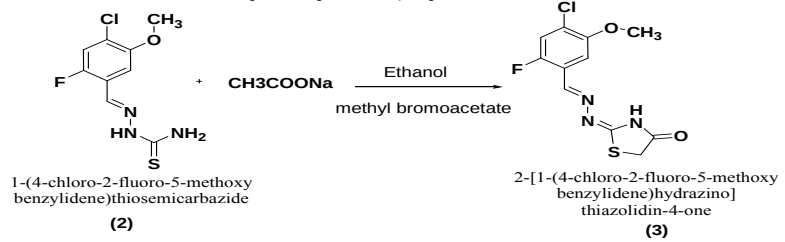
(**1**) (**2**)

Procedure:

Synthesis of 1-(4-chloro-2-fluoro-5-methoxybenzylidene)thiosemicarbazide

To a constantly stirred solution of compound **1** (4.0 g, 0.0191 mol, 1 equiv.) and thiosemicarbazide (1.91 g, 0.021 mol, 1.1 equiv.) in anhydrous methanol (40 mL), a catalytic amount of glacial acetic acid (0.15 equiv.) was added. The reaction mixture was refluxed for 4 h. After cooling to room temperature, the solid separated was filtered and washed with cold methanol to afford off white crystalline solid of compound (**2**)

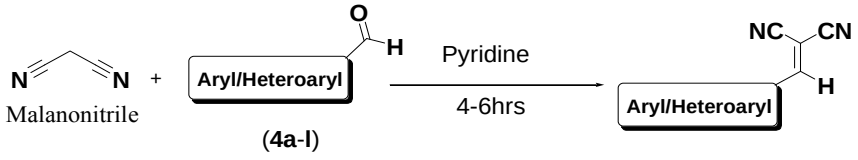
Synthesis of 2-[1-(4-chloro-2-fluoro-5-methoxybenzylidene) hydrazino]thiazolidin-4-one



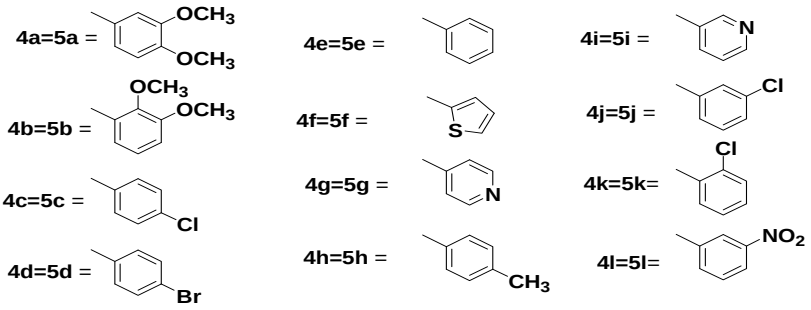
(**2**) (**3**)

General procedure for synthesis of substituted arylidinemalanonitriles (5a-l)

To a constantly stirred solution of malononitrile (0.5 g, 0.00757 mol.) in 10.0 mL of ethanol, an appropriately substituted aromatic/heteroaromatic aldehyde (**4a-l**; 0.00757 mol) and 2-4 drops of pyridine was slowly added. The reaction mixture was then either refluxed for 3-5 h (for substituted benzaldehydes) or was stirred at room temperature for 4-6 h (for substituted heteroaromatic aldehydes). The precipitate formed after cooling was filtered to get respective arylidinemalanonitriles (**5a-l**). The compounds so obtained were fairly pure to carry out the next step.

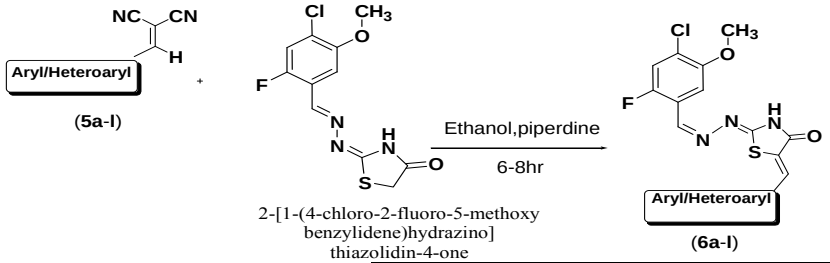


Where, Aryl/Heteroaryl

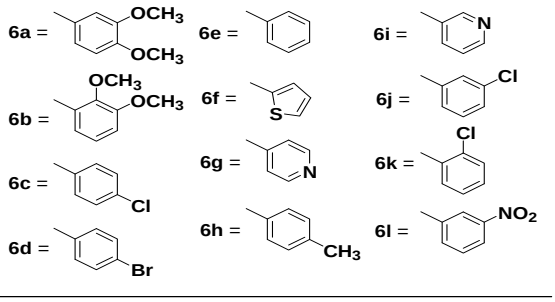


General procedure for synthesis of substituted 5-(substituted benzylidene)-2-(4-chloro-2-fluoro-5-methoxybenzylidene) hydrazono) thiazolidin-4-one (6a-l)

To a continuously stirred mixture of compound **2**(0.35 g, 0.00110 mol) and appropriate arylidinemalanonitriles (**5a-l**; 0.00110 mol) in ethanol (8 mL), few drops of piperidine were added. The reaction mass was refluxed for 6-8 h. The progress of the reaction was constantly monitored by TLC. After cooling, the separated solid or residue was filtered, washed with hot ethanol. All the compounds were further purified by recrystallized in ethanol in order to get the desired title compounds (**6a-l**).



Where, Aryl/Heteroaryl



RESULT

Table 1: Ligand optimized energy using Universal force field (UFF)

	Rosiglitazone		6a		6b	
	Initial energy	**Final energy**	**Initial energy**	**Final energy**	**Initial energy**	**Final energy**
MM bond	0.39	0.0045	1.05	0.025	0.98	0.0125
MM Angle	0.064	0.063	2.10	0.07	1.90	0.059
MM Dihedral	0.030	0.030	0.11	0.20	0.075	0.19
MM ImpTor	0.00	0.00	0.006	0.0075	0.005	0.0055
MM vdW	0.22	0.044	1331.79	0.10	1301.99	0.098
MM Coulomb	0.00	0.00	0.00	0.00	0.00	0.00
Total Energy	0.72 au	0.152a.u.	1333.93a.u.	0.28 a.u.	1322.94a.u.	0.18 a.u.
Total Energy in Kcal/mol	449.20 kcal/mol	90.10 kcal/mol	848090.10 kcal/mol	194.25 kcal/mol	838192.20 kcal/mol	204.35 kcal/mol

Table 2: Molecular interaction energy

Receptor (Pdb-id)	Ligand	Total Energy	VDW	H Bond	Elec
3GBK (PPAR gamma)	**Rosiglitazone**	103.791	89.7277	14.2633	0
	6a	102.841	93.6611	28.1449	-0.53564
	6b	105.847	79.1287	27.5678	-2.85088

Roziglitazone with indigenous receptor (Pdb-id: 3GBK):
Hydrogen bond interaction: HIS323, CYS285, BRL503, MET364, LEU330, GLY284, TYR327, HIS449, LEU453, SER289, TYR473, PHE282, LEU469, TYR327 and ILE 326.
Vanderwaal interactions: GLN-271; ILE-281; GLN-283; GLY-284; PHE- 287; ARG-288; LEU-340; ILE-341; GLU-343; and ROS-348.
6a with indigenous receptor PPAR gamma:
Electrostatic interaction: HIS-466
Hydrogen bond interactions: LEU-228; LEU-270; GLN-271; GLU-272; GLN- 283; GLU-343; SER-464 and HIS-466.
Vander-waal interactions: GLN-271; ILE-281; GLN-283; GLY-284; PHE- 287; ARG-288; LEU-340; ILE-341; GLU-343; ROS-348; SER-464; LEU-465 and HIS-466
6b with indigenous receptor PPAR gamma:
Hydrogen bond interaction: ARG-288; GLU-291; GLU-295; ILE-325; ILE-326;
SER-342 and GLU-343
Vander-waal interaction: ILE-281; GLY-284; CYS-285; PHE-287; ARG- 288; ARG-288; GLU-291; MET-329; LEU-330; ILE-341; SER-342 and GLU-343.

Table 3: Physical properties of synthesized compounds

Sl. no	C.C.*	State	% yield	MP
1.	**6a**	Yellow solid	45%	**232°C**
2	**6b**	Yellow solid	46%	**215 °C**
3	**6c**	Yellow solid	52%	**225-227°C**
4	**6d**	Yellow solid	42%	**245-247 °C**
5	**6e**	Yellow solid	61%	**202-204°C**
6	**6f**	Yellow orange solid	57%	**212-214°C**
7	**6g**	Yellow solid	49%	**238-240 °C**
8	**6h**	Yellow solid	50%	**213-215°C**
9	**6i**	Yellow solid	55%	**220-223 °C**
10	**6j**	Yellow solid	48%	**227-229 °C**
12	6l	Yellow solid	62%	**239-242°C**

Table 4: Elemental and spectral analysis data of synthesized compounds.

Comp. Code	Elemental Analysis (Calculated)	IR. values (cm^{-1})
6a	C= 30.76, H= 2.58, N= 11.96, O= 27.32, S= 27.38	3115.99 (N-H Str.), 3001.46 (Ar-H Str.), 2957.65 (CH Str. of CH_3), 1687.41 (C=O Str.), 1624.30 (C=C Str.), 1591.71 (C=N Str.)
6b	C= 53.24; H=3.78; N= 4.78; O=27.28; S=10.93	3109.88 (N-H Str.), 3040.73 (Ar-H Str.), 2948.43 (C-H Str. of CH_3), 1696.80 (C=O Str.), 1623.69 (C=C Str.), 1595.01 (C=N Str.)
6c	C= 47.31; H=3.25; N=15.05; O=22.92; S, 11.48	3077.23 (N-H Str.), 3052.86 (Ar-H Str.), 2932.41 (C-H Str. of CH_3), 1729.20 (C=O Str.), 1629.90 (C=C Str.), 1518.99 (C=N Str.)
6d	C= 64.14, H= 7.00, N= 7.48, O= 12.82, S= 8.56	3049.65 (N-H Str.), 3020.56 (Ar-H Str.), 2930.54 (C-H Str. of CH_3), 1728.47 (C=O Str.), 1628.72 (C=C Str.), 1518.65 (C=N Str.)
6e	C= 60.78, H= 6.71, N= 11.19, O= 12.78, S= 8.54	3112.43 (N-H Str.), 3013.70 (Ar-H Str.), 2959.85 (C-H Str. of CH_3), 1693.90 (C=O Str.), 1590.71 (C=C Str.), 1510.87 (C=N Str.)
6f	C= 63.31, H= 6.71, N= 7.77, O= 13.32, S= 8.90	3115.27 (N-H Str.), 3060.27 (Ar-H Str.), 2963.89 (C-H Str. of CH_3), 1694.20 (C=O Str.), 1590.52 (C=C Str.), 1514.47 (C=N Str.)
6g	C= 59.65, H= 6.12, N= 7.73, O= 17.66, S= 8.85	3011.90 (N-H Str.), 2935.45 (Ar-H Str.), 2837.61 (C-H Str. of CH_3), 1719.49 (C=O Str.), 1633.77 (C=C Str.), 1598.56 (C=N Str.)
6h	C= 61.31, H= 5.71, N= 6.77, O= 11.32, S= 7.90	3118.74 (N-H Str.), 3019.85 (Ar-H Str.), 2964.49 (C-H Str. of CH_3), 1697.55 (C=O Str.), 1592.59 (C=C Str.), 1514.23 (C=N Str.)
6i	C= 61.31, H= 6.31, N= 7.17, O= 13.12, S= 7.90	2999.21 (N-H Str.), 2929.09 (Ar-H Str.), 2833.81 (C-H Str. of CH_3), 1713.41 (C=O Str.), 1625.05 (C=C Str.), 1514.55 (C=N Str.)
6j	C= 59.31, H= 7.71, N= 6.77, O= 11.32, S= 7.90	3063.62 (N-H Str.), 2917.80 (Ar-H Str.), 2831.79 (C-H Str. of CH_3), 1720.58 (C=O Str.), 1628.09 (C=C Str.), 1599.95 (C=N Str.)
6l	C= 60.31, H= 6.01, N= 7.07, O= 13.12, S= 8.60	3106.33 (N-H Str.), 3039.40 (Ar-H Str.), 2957.11 (C-H Str. of CH_3), 1693.10 (C=O Str.), 1619.19 (C=C Str.), 1598.60 (C=N Str.)

Table 5: H^1NMR and C^{13}NMR data of synthesized compounds

Comp. Code	H^1NMR (400 MHz, DMSO-d_6, δ, ppm)	C^{13}NMR (100 MHz, DMSO, δ ppm)
6a	12.43 (s, 1H, NH), 7.55 (s, 1H, C=C-H), 7.44 (s, 1H, C=C-H),7.28 (s, 1H, Ar-H), 7.25 (s, 1H, Ar-H), 6.97-6.95 (d, 1H, *J* = 8.40 Hz),3.83 (s, 3H, -OCH_3), 3.83 (s, 3H, -OCH_3), 3.82 (s, 3H, -OCH_3)	167.25 (C=O, thiazolidin-4-one), 163.38 (C=N, 2-ylidene carbon), 156.96 (C=N of thiazolidone), 151.39, 149.69, 148.95, 136.31, 129.24, 128.88 (C-H,benzylidene carbon), 126.36, 122.55, 120.46, 114.14, 112.03, 55.63 (-OCH_3), 55.51 (-OCH_3), 55.47 (-OCH_3)
6b	12.55 (s, 1H, NH), 7.75 (s, 1H, C=C-H), 7.45 (s, 1H, C=C-H),7.34 (s, 1H, Ar-H),7.27 (s, 1H, Ar-H), 7.25-7.23 (d, 1H, *J* = 7.88 Hz), 7.20-7.15 (m, 4H), 3.84 (s, 3H, -OCH_3), 3.83 (s, 3H, -OCH_3), 3.78 (s, 3H, -OCH_3)	167.18 (C=O, thiazolidin-4-one), 163.68 (C=N, 2-ylidene carbon), 156.78 (C=N of thiazolidone), 152.72, 149.73, 147.86, 136.49, 128.87, 126.26, 124.41 (C-5 of thiazolidone), 123.08 (C-H, benzylidene carbon), 119.66, 114.69, 60.97, 55.84 (-OCH_3), 55.54 (-OCH_3), 55.48 (-OCH_3)
6c	12.59 (s, 1H, NH), 7.75 (s, 1H, C=C-H), 7.67-7.65 (d, 2H, *J* = 8.60 Hz), 7.45 (s, 1H, C=C-H),7.27 (s, 1H, Ar-H), 6.98-6.96 (d, 1H, *J* = 8.40 Hz), 3.83 (s, 3H, -OCH_3)	166.99 (C=O, thiazolidin-4-one), 163.84 (C=N, 2-ylidene carbon), 156.38 (C=N of thiazolidone), 149.76, 136.60, 134.17, 132.58, 129.24, 128.84, 127.41 (C-H, benzylidene carbon), 124.11 (C-5 of thiazolidone), 55.49 (-OCH_3)
6d	12.38 (s, 1H, NH), 7.70 (s, 1H, C=C-H), 7.58-7.56 (d, 2H, *J* = 8.34 Hz), 7.54 (s, 1H, C=C-H), 7.25 (s, 1H, Ar-H), 6.98-6.97 (d, 1H, *J* = 8.28 Hz), 3.80 (s, 3H, -OCH_3)	167.42 (C=O, thiazolidin-4-one), 164.13 (C=N, 2-ylidene carbon), 156.62 (C=N of thiazolidone), 150.57, 136.82, 133.56, 132.62, 129.65, 127.94 (C-H, benzylidene carbon), 126.97, 125.04 (C-5 of thiazolidone), 123.41, 79.64, 79.41, 79.19, 56.34 (-OCH_3)
6e	12.56 (s, 1H, NH), 7.66-7.64 (d, 2H, *J* = 7.56 Hz), 7.59 (s, 1H, C=C-H), 7.56-7.52 (t, 2H, *J* = 7.58 Hz), 7.47-7.43 (m, 1H), 7.29 (s, 1H, Ar-H), 6.98-6.96 (d, 1H, *J* = 6.88 Hz), 3.84 (s, 3H, -OCH_3)	167.13 (C=O, thiazolidin-4-one), 163.72 (C=N, 2-ylidene carbon), 156.70 (C=N of thiazolidone), 149.73, 148.96, 136.54, 133.65, 129.79, 129.71 (C-H, benzylidene carbon), 129.19, 128.58, 126.25, 123.29 (C-5 of thiazolidone), 55.54 (-OCH_3)
6f	12.51 (s, 1H, NH), 7.97-7.96 (d, 1H, *J* = 5.00 Hz), 7.86 (s, 1H, C=C-H), 7.62-7.61 (d, 1H, *J* = 3.44 Hz), 7.30 (s, 1H, Ar-H),	166.91 (C=O, thiazolidin-4-one), 163.70 (C=N, 2-ylidene carbon), 156.30 (C=N of thiazolidone), 149.75, 148.99, 137.82, 136.59, 133.23, 131.82, 128.87, 126.24, 122.18 (C-

Comp. Code	H^1NMR (400 MHz, DMSO-d_6, δ, ppm)	C^{13}NMR (100 MHz, DMSO, δ ppm)
	7.22-7.16 (m, 2H), 6.97-6.92 (m, 2H), 3.84 (s, 3H, -OCH_3), 3.78 (s, 3H, -OCH_3)	H, benzylidene carbon), 120.98 (C-5 of thiazolidone), 55.54 (-OCH_3)
6g	12.61 (s, 1H, NH), 8.72-8.71 (d, 2H, *J* = 5.04 Hz), 7.57-7.56 (d, 2H, *J* = 5.10 Hz), 7.53 (s, 1H, C=C-H), 7.27 (s, 1H, Ar-H), 7.18-7.17 (d, 1H, *J* = 8.28 Hz), 6.99-6.97 (d, 1H, *J* = 8.22 Hz), 3.84 (s, 3H, -OCH_3)	167.20 (C=O, thiazolidin-4-one), 164.59 (C=N, 2-ylidene carbon), 156.53 (C=N of thiazolidone), 150.97, 149.71, 141.31, 137.19, 129.51, 129.18 (C-5 of thiazolidone), 126.18 (C-H, benzylidene carbon), 123.70, 65.31, 56.18 (-OCH_3)
6h	12.50 (s, 1H, NH), 7.55 (s, 1H, Ar-H), 7.52 (s, 1H, C=C-H), 7.36-7.34 (d, 2H, *J* = 8.04 Hz), 7.29 (s, 1H, Ar-H), 7.21-7.15 (m, 2H), 6.98-6.92 (m, 2H), 3.84 (s, 3H, -OCH_3), 2.26 (s, 3H, -CH_3)	167.22 (C=O, thiazolidin-4-one), 163.59 (C=N, 2-ylidene carbon), 156.80 (C=N of thiazolidone), 149.71, 139.84, 136.46, 135.41, 130.88, 129.84 (C-H, benzylidene carbon), 129.80, 128.75, 126.29, 122.06 (C-5 of thiazolidone), 55.47 (-OCH_3), 21.04 (-CH_3)
6i	12.66 (s, 1H, NH), 8.85 (s, 1H, C=C-H), 8.60-8.59 (d, 1H, *J* = 4.76 Hz), 8.02-8.01 (d, 1H, *J* = 8.00 Hz), 7.62 (s, 1H, C=C-H), 7.58-7.55 (dd, 1H, *J* = 7.89, 4.24 Hz), 7.28 (s, 1H, Ar-H), 3.83 (s, 3H, -OCH_3)	166.85 (C=O, thiazolidin-4-one), 163.93 (C=N, 2-ylidene carbon), 156.39 (C=N of thiazolidone), 151.05, 149.76, 148.96, 136.69, 135.79, 129.81, 128.82, 126.16, 125.70 (C-5 of thiazolidone), 125.30 (C-H, benzylidene carbon), 124.09, 55.47 (-OCH_3)
6j	12.54 (s, 1H, NH), 7.68 (s, 1H, C=C-H), 7.56-7.48 (m, 5H, Ar-H), 7.14 (s, 1H, C=C-H), 7.02-7.00 (d, 1H, *J* = 8.76 Hz), 3.80 (s, 3H, -OCH_3)	167.06 (C=O, thiazolidin-4-one), 160.99 (C=N, 2-ylidene carbon), 155.76 (C=N of thiazolidone), 150.12, 148.95, 137.97, 135.87, 133.77, 131.01, 129.50, 129.23, 128.71, 127.01 (C-H, benzylidene carbon), 125.14 (C-5 of thiazolidone), 117.39, 55.43 (-OCH_3)
6l	12.70 (s, 1H, NH), 8.45 (s, 1H, Ar-H), 8.26-8.24 (d, 1H, *J* = 8.20 Hz), 8.06-8.04 (d, 1H, *J* = 7.92 Hz), 7.72 (s, 1H, C=C-H), 7.27 (s, 1H), 7.22-7.15 (m, 2H), 6.97-6.95 (d, 1H, *J* = 8.36 Hz), 3.83 (s, 3H, -OCH_3)	166.74 (C=O, thiazolidin-4-one), 164.18 (C=N, 2-ylidene carbon), 155.86 (C=N of thiazolidone), 149.78, 148.95, 136.83, 135.38, 135.25, 130.71, 128.79, 126.40 (C-5 of thiazolidone), 126.35 (C-H, benzylidene carbon), 126.05, 124.06, 123.76, 55.52 (-OCH_3)

Table 6: Effects of derivatives on body weight of streptozotocin-induced diabetic rats after 14-day treatment

Experimental group	Dose mg/kg	Body weight	
		Day 0	Day 14
Control		239.6 ± 15.5	200.6 ± 15.5*
Rosiglitazone	0.72	225.2 ± 10.1	190.0 ±4.4***
6a	0.72	202.4 ± 12.8	165.4 ± 13.5***
6b	0.72	204.0 ± 5.4	169.4 ± 2.2***

Table 1 shows the effects of *TZD derivatives* (0.72 mg/kg), rosiglitazone and (normal saline) control treatment on body weight changes in streptozotocin-induced diabetic rats. After 14-day treatment with the water extracts, rosiglitazone and normal saline, body weight was reduced significantly from day 0 (before treatment) to 14day (after treatment) ($P < 0.05$).

Each value represents the mean ± S.E.M (n = 5); * and *** indicate significant differences between day 0 and day 14 of same treatment group at $P < 0.05$ and $P < 0.001$, respectively.

Table 7: Effect of derivatives on blood glucose levels (mg/dl) in streptozocin induced insulin resistance model in rats.

GROUPS	GROUP 1 NORMAL CONTROL	GROUP 2 STREPTOCONTROL	GROUP 3 STREPTO+ Rosiglitazone	GROUP4 STREPTO + 6a	GROUP5 STREPTO + 6d
Serum glucose (mg/dl) MEAN ± SEM	86.08 ± 3.264	265.1 ±4.098 [a***]	112.5 ±2.023[b***]	102.47 ±1.145[b***]	108.9 ±1.230[b***]

The hypoglycemic effect of repeated oral administration of the **6a and 6b** in diabetic rats is shown in Figure 1. After two weeks, streptozotocin-induced diabetic rats that received *TZD derivatives* (0.72 mg/kg) and rosiglitazone had significantly decreased fasting blood glucose levels at $P < 0.001$.

Values are expressed as mean ± S.E.M., n = 6, DEXA = dexamethasone 0.7 mg/kg, i.m.

Rosiglitazone = Rosiglitazone 0.36 mg/kg, p.o., twice a day,

6a = **6a** 0.36 mg/kg, p.o., twice a day,

6b = **6b** 0.36 mg/kg, p.o., twice a day,

ANOVA and Tukey-kramer multiple comparisons carried out $^{***}p<0.001$ highly significant;

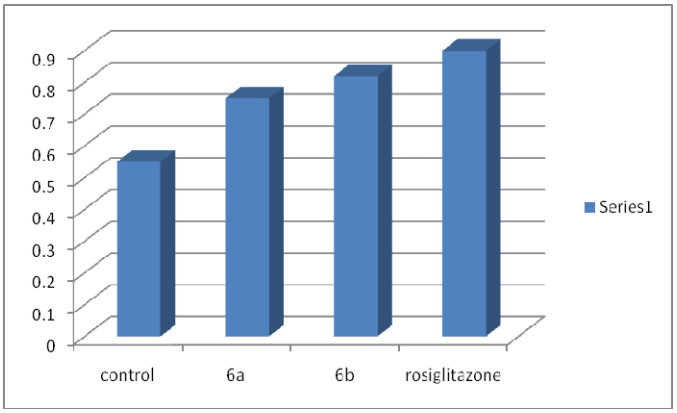


Fig 1: Effects of derivatives on insulin secretion by RIN-5F cells

Rosiglitazone (0.2-20 mM) produced a dose-dependent stimulatory effect on insulin secretion by RIN-5F cells incubated in 1.1 mM glucose. RIN-5F cells exposed to 20 mM of rosiglitazone for 20 min showed maximal levels of stimulation. However, concentrations of rosiglitazone less than 20 mM did not significantly enhance the insulin–releasing effect. As seen in treatment of RIN-5F cells with different concentrations of 6a and 6b (10 mg/mL) significantly increase the levels of insulin as compared with the control.Each value represents the mean ± S.E.M. (n = 6); * indicates significant difference between treated groups compared with control group without rosiglitazone at $P < 0.05$ 6a and 6b at concentrations of 10 mg/mL showed no cytotoxic effect in RIN-5F cells

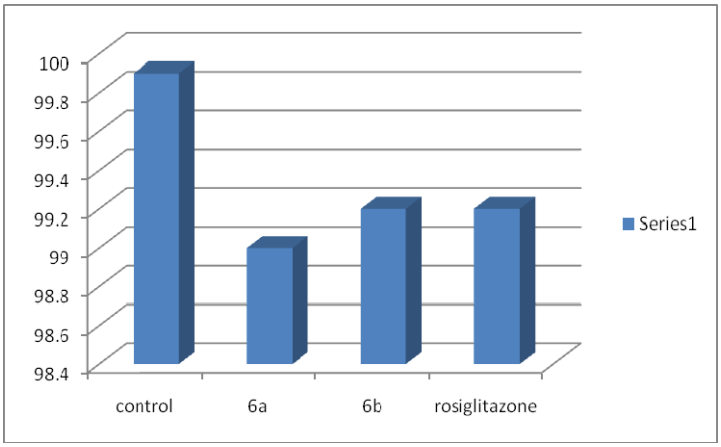


Figure-2:MTT assay/ Percentage cell viability of derivatives against rosiglitazone

DISCUSSION

The structure of new compounds prepared during present investigation has been authentically established by their UV, FTIR, H^1NMR, and C^{13} NMR. In following reaction the spectral studies of some selected compounds have been dealt.

Ligands were ingenuous from Chemsketch and twisted into the 3D structure, intermolecular interactions of these ligands were optimized to attain a local minimum energy structure using Universal force fields (UFF). The ligands were also subjected to toxicity estimation software (TEST) version 4.2.1.

The modelled PPAR-γ which was imported from protein data bank structure was validated using Procheck and from the Ramachandran plot it was inferred that the modelled protein contain 80.3% of amino acid residues in the most favoured region, 5.2% in additional allowed region, 1.8% in general allowed region and only 2.7% of amino acid residues in disallowed region. As the RMSD value is lower than 2.0 and more than 80% of the residues are in most favored region, the modeled structure can be considered to

be a good one. The active site residues of the modelled protein obtained using PBD Sum are HIS323, CYS285, BRL503, MET364, LEU330, GLY284, TYR327, HIS449, LEU453, SER289, TYR473, PHE282, LEU469, TYR327 and ILE 326. Molecular docking studies were performed for modeled PPAR-γ protein with the commercial available rosiglitazone. The results were analyzed based on the interaction of H-bonds, interacting residues and binding energy. The better interaction was selected by figuring out the minimum binding energy. The predictions results of all ligands were analyzed. The results indicated the two compounds **6a** and **6b** showed interaction with PPAR-γ protein than the standard rosiglitazone as shown in **(Table 1, 2).**

The synthesis of substituted 5-(substituted benzylidene)-2-(4-chloro-2-fluoro-5-methoxybenzylidene) hydrazono) thiazolidin-4-one was done by refluxing appropriate arylidinemalanonitriles in ethanol few drops of piperidine were added. The reaction mass was refluxed for 6-8 h. It was proved by the following peaks of IR 3115.99 (N-H Str.), 3001.46 (Ar-H Str.), 2957.65 (C-H Str. of CH_3), 1687.41 (C=O Str.), 1624.30 (C=C Str.), 1591.71 (C=N Str.) as soon in [Table 2]. Further proof was obtained from H^1NMR spectrum which clearly shows these prominent peaks at 12.43, 7.55, 7.28 and 3.83 Indicating the presence of –NH, C=CH, Ar-H and OCH_3. ^{13}C-NMR (100 MHz, DMSO, δ ppm): 167.25 (C=O, thiazolidin-4-one), 163.38 (C=N, 2-ylidene carbon), 156.96 (C=N of thiazolidone), 151.39, 149.69, 148.95, 136.31, 129.24, 128.88 (C-H,benzylidene carbon), Further substitution reaction with appropriate arylidinemalanonitriles leads to substituted 5-(substituted benzylidene)-2-(4-chloro-2-fluoro-5-methoxybenzylidene) hydrazono) thiazolidin-4-one [6a-l]. The formation of the product was determined from TLC by comparing R*f* values of starting materials and the product.

The other derivatives have been identified by similar manner. In chemexper data + sign indicate favourable drug and – sign indicate unfavorable drug and mole-inspiration shows vice-versa. Physical and spectroscopical data described in **(Table 3-5).**

The *in-vivo* and *in-vitro* anti-diabetic activity of 5-[4-(substituted) benzylidene] thiazolidinediones were evaluated. *In-vivo* studies were carried against streptozocin induced diabetes as shown in (**Table 7**) and simultaneously body weight was observed and recorded, most of the time diabetes induces obesity (**Table 6**)**.** The 5-[4-(substituted) benzylidene] derivatives were generally more active than the analogous thiazolidinediones. It has been seen that rosiglitazone showed a decrease in BGL in 30 min. while **6a** and **6b** shows a sudden decrease in BGL within a span of 30 min, and then a constant decrease appeared, While remaining showed a decrease in first 30 min, but after that they fail to show a decrease in the blood glucose level (BGL) and a further increase in BGL has been observed. After the above result the derivatives were also subjected to know the effect of insulin secretion by RIN-5F cells providing confirmation of insulinotropic effect and derivatives also showed less cytotoxicity. Based on all the above finding it is suggested that TZD derivatives is a promising source of antidiabetic that could have great importance as therapeutic agents in preventing or slowing the degenerative diseases such as cancer and various other human ailments. In view of the multiple

Bio-chemical activities of TZD derivatives, we believe that they deserve further investigations as potential multitarget-oriented remedies for diabetes mellitus and its pathological consequences.

CONCLUSION

In conclusion, the insulinotropic effect of substituted 5-(substituted benzylidene)-2-(4-chloro-2-fluoro-5-methoxybenzylidene) hydrazino) thiazolidin-4-one may be due to its ability to mimic or improve insulin action at the cellular level. In this series, the most potent compound is **6a** and **6b** having methoxy group of 2 substituted TZD ring in both test concentrations. Most studies with experimental animals and validates the ethnomedicinal use of TZD. However, clinical trials with standardized extracts and uniform protocols have been with experimental animals and validated TZD derivatives clinical applicability as an antidiabetic agent. The outcomes of such studies may be useful for the clinical applications in humans and may open up a new therapeutic avenue.

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DECLARATION OF INTEREST

The authors report no conflicts of interest.

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