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Synthesis, Molecular Docking, and Biological Evaluation of the New Hybrids of 4-Thiazolidinone and 4(3*H*)-Quinazolinone Against Streptozotocin Induced Diabetic Rats

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Abstract—A series of new 3-(2-substituted)-4-(oxothiazolidin-3-yl)-2-methylquinazolin-4(3*H*)-ones **1a**–**1k** is synthesized by using the hybridization approach via one pot multicomponent reaction of 3-amino-2-methylquinazolin-4(3*H*)-one with substituted benzaldehyde, thioglycolic acid and *N,N*-dicyclohexylcarbodiimide in DMF media. Structures of the synthesized compounds are elucidated from the spectral data. Antidiabetic activity of the products is tested against streptozotocin induced diabetic rats at a dose of 200 mg/kg compared with standard Pioglitazone (15 mg/kg). Compounds **1b**, **1d**, **1f**, and **1i** demonstrate significant antidiabetic activity. Compounds **1b**, **1d**, **1f**, and **1i** are evaluated in vitro are tested for serum insulin, cholesterol, triglycerides, total protein, lipoprotein, and enzymes factors. Significant lowering of glycated hemoglobin level is induced by the compounds after 21 days of treatment. Mean±S.E.M. data accumulated are subjected to one-way analysis of variance (ANOVA) followed by Dunnett's *t*-test. $p < 0.001$ was considered statistically significant. Histopathological results accumulated for the rats treated by compounds **1b**, **1d** and **1f** confirm the significant recovery of pancreas destruction. Free energy of binding for all synthesized compounds is calculated using AutoDock 1.5.6 with peroxisome proliferator-activated receptor γ (PPAR γ; PDB ID: 4PRG). Among the synthesized compounds, **1d** demonstrates significant binding energy value of –11.46 kcal/mol. The current study is expected to provide useful insight into the design of potential agents that can act as a platform for the development of future antidiabetic drugs.

Keywords: diabetes mellitus, 4-thiazolidinone, 4(3*H*)-quinazolinone, synthesis, antidiabetic activity, molecular docking

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INTRODUCTION

Approximately 415 million people are affected by diabetes, and this number is predicted to increase to 642 million by 2040 [1]. Most of the currently used therapeutics for treatment of T2DM diabetes were discovered and developed in the absence of defined biological molecular targets [2] making development of new drugs for its treatment to be of considerable importance. One of the main molecular drug targets involved in the treatment of T2DM is (PPAR γ) peroxisome proliferator-activated receptor γ [3]. A number of thiazolidinediones (TZDs) is used in clinical treatment of T2DM. The TZD moiety containing sulfur atom in a five membered ring demonstrated antihyperglycemic activity and has been reported as insulin sensitizer, PPAR γ agonist. TZDs decrease the blood glucose concentration by ameliorating insulin resistance [4, 5]. TZDs such as rosiglitazone, pioglitazone, darglitazone, and troglitazone demonstrate high-affinities toward PPAR γ (Fig. 1) [6, 7]. 4-Thiazolidinone derivatives were found to be diverse anti-inflammatory [8, 9], antioxidant [10], antitubercular [11], anticancer [12, 13], and antidiabetic [14] agents. Quinazolinone is also the important heterocyclic unit possessing many pharmacological properties such as anticonvulsant [15–19], antibacterial [20, 21], antifungal [22], and antihyperglycemic [23].

As a part of our on-going research, here we report the molecular docking, synthesis and characterization of 3-(2-substituted)-4-(oxothiazolidin-3-yl)-2-methylquina-olin-4(3*H*)-ones **1a**–**1k** as new hybrids of 4-thiazolidinone and 4(3*H*)-quinazolinone. Their antidiabetic

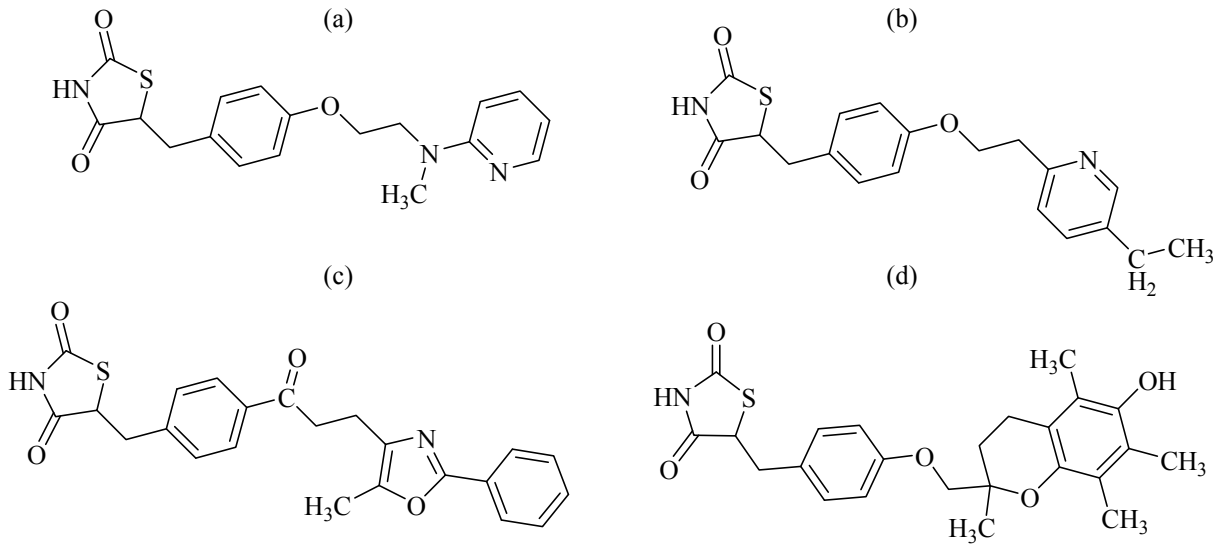


Fig. 1. Reported antidiabetic agents as a PPAR γ agonists containing thiazolidinedione scaffold: (a) rosiglitazone, (b) pioglitazone, (c) darglitazone, and (d) troglitazone.

activity as PPAR γ agonists was studied. In vitro analysis of such factors as serum insulin, cholesterol, triglycerides, total protein, lipoprotein, enzymes, and glycated hemoglobin was also carried out. Histopathological study was performed on pancreas.

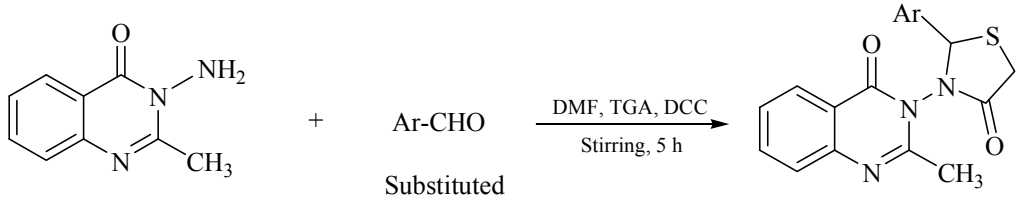
RESULTS AND DISCUSSION

3-(2-Substituted)-4-(oxothiazolidin-3-yl)-2-methyl-quinazolin-4(3*H*)-ones **1a**–**1k** were synthesized (Scheme 1) by a reaction of 3-amino-2-methyl-uinazolin-4(3*H*)-one with substituted aromatic aldehydes, thioglycolic acid and N, N-dicyclohexyl carbodiimide (DCC) in DMF media. Initially formation of imine and nucleophilic attack of thiol on the imino carbon atom was followed by intramolecular cyclization accompanied by loss of water molecule. DCC was used as a dehydrating agent which accelerated the intramolecular cyclization leading to faster reaction and higher yields [24, 25]. The synthesized compounds were characterized by physical and spectral data. IR (KBr) spectra displayed the C=O stretching vibrations bands of thiazolidinone at 1548.85–1828.58 cm^{-1}. In ^1H NMR spectra the singlet attributed to CH_2 in thiazolidinone was measured in the range of 2.10–3.25 ppm. The HRMS confirmed the structures of the synthesized compounds.

Drug likeness of the synthesized compounds **1a**–**1k** was analyzed using various parameters including molecular weight (MW), hydrogen bond donors (HBD) count, hydrogen bond acceptors (HBA) count and partition coefficient by manual application of Lipinski rule of Five [26]. According to the latter, an orally administered drug should have molecular weight MW ≤ 500, number of hydrogen bond acceptors HBA ≤ 10, number of hydrogen bond donors HBD ≤ 5. Its ClogP value should be ≤5. None of the synthesized compounds **1a**–**1k** violated the Lipinski rule of Five (Table 1).

All synthesized compounds demonstrated the potential of orally active agents.

Scheme 1. Synthesis of target compounds **1a**–**1k**.

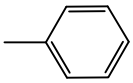
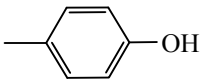
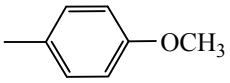
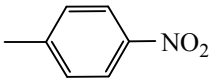
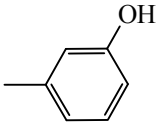
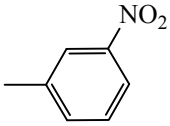
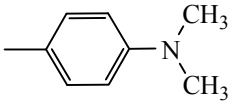
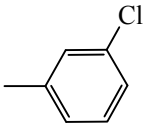
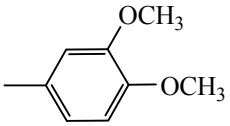
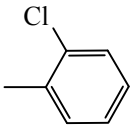
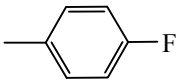


3-amino-2-methylquinazolin-4(3*H*)-one

Substituted aromatic aldehydes

3-(2-substituted)-4-(oxothiazolidin-3-yl)-2-methylquinazolin-4(3*H*)-one (**1a**–**1k**)

Table 1. Calculation of various descriptors for 3-(2-substituted)-4-(oxothiazolidin-3-yl)-2-methylluinazolin-4(3*H*)-one (**1a**–**1k**) according to Lipinski's Rule of Five

Comp. no.	Ar group	Number of HBD	Number of HBA	CLog*P*	M_W	Lipinski violation
1a		0	5	2.72	337.40	0
1b		1	6	2.05	353.39	0
1c		0	6	2.64	367.42	0
1d		0	8	2.46	382.39	0
1e		1	6	2.05	353.39	0
1f		0	8	2.46	382.39	0
1g		0	6	2.88	380.46	0
1h		0	5	3.43	371.84	0
1i		0	7	2.38	397.45	0
1j		0	5	3.43	371.84	0
1k		0	5	2.86	355.39	0

Biological profiling. Oral acute toxicity studies were performed on healthy rats treated with 2000 mg/kg body weight of the products [27, 28]. The animals were observed for behavioral changes for 48 h, and they did not show any sign of acute toxicity such as respiratory distress, convulsion, coma, or death. In oral glucose tolerance test [29–31], the target compounds exhibited significant increase in blood glucose level at 60 min intervals and noteworthy decrease at 60 min onwards. The decrease in blood glucose level induced by the synthesized compounds **1a**–**1k** treated with the dose of 200 mg/kg body weight administered orally was compared with the normal control with the dose of 10 mL/kg body weight and standard drug pioglitazone (15 mL/kg, i. p.). The synthesized compounds **1b**, **1d**, **1f**, **1i,** and **1k** revealed significant response to oral glucose tolerance test. The results were expressed as mean±S.E.M. data obtained were analyzed with one-way ANOVA followed by Dunnett's *t*-test (Table 2).

The non-insulin dependent diabetes mellitus (NIDDM) was experimentally induced in wistar albino

Table 2. Oral glucose tolerance test (OGTT) of 3-(2-substituted)-4-(oxothiazolidin-3yl)-2-methylluinazolin-4(3*H*)-one (**1a**–**1k**)[a]

Group	Treatment, mg/kg	Blood glucose concentration, mg/dL		
		0 min	60 min	120 min
Normal control (normal saline)	10	75.80±0.16	96.18±0.68	86.01±0.64
1a	200	69.59±0.44	89.73±0.63[b]	80.18±0.68
1b	200	67.38±0.33	88.67±0.49[c]	75.83±0.45[***]
1c	200	68.35±0.41	87.59±0.50	77.78±0.37
1d	200	70.55±0.36	84.1 6±0.74[d]	74.09±0.76[d]
1e	200	68.05±0.58	88.48±0.39	77.98±0.59
1f	200	67.94±0.35[b]	86.27±0.57[c]	76.33±0.76[d]
1g	200	69.19±0.58	88.96±0.64	79.99±0.61
1h	200	67.40±0.82	89.64±0.38	79.51±0.38
1i	200	69.75±0.37	87.09±0.68[b]	76.72±0.54[c]
1j	200	69.52±0.59	90.30±0.29	80.02±0.28
1k	200	65.82±0.58	87.28±0.49[c]	77.48±0.38[d]
Standard (Pioglitazone)	15	50.41±0.44	75.55±0.41[d]	70.66±0.46[d]

[a] The results are expressed as mean ± S.E.M; data obtained were analyzed with one-way ANOVA followed by Dunnett's *t*-test.
[b] $p < 0.05$.
[c] $p < 0.01$.
[d] $p < 0.001$.

rats by a single intraperitoneal injection of streptozotocin (50 mg/kg body weight). Elevated blood glucose level was determined and a blood glucose range of 200–300 mg/dL was considered as diabetic [32]. Antidiabetic activity of synthesized compounds **1a**–**1k** was studied by streptozotocin (STZ) induced diabetes test [27, 33]. The glucose production increased whereas its utilization decreased by the tissue which was fundamental basis for hyperglycemia in case of diabetes mellitus [34]. All the target compounds **1a**–**1k** were found to have remarkable antidiabetic activity (Table 3). Reduction in blood glucose level under the action of the synthesized compounds **1a**–**1k** (200 mg/kg) was compared with normal control (10 mg/kg normal saline), diabetic control (50 mg/kg STZ) and standard drug pioglitazone (15 mg/kg). The results were expressed as mean±S.E.M. The data obtained were analyzed with one-way ANOVA followed by Dunnett's *t*-test. Among all tested compounds **1b**, **1d**, **1f,** and **1i** were found to have most significant antidiabetic activity as compared to the standard. According to the obtained results the aryl moiety with methoxy and nitro substituents might enhance the acidic behavior of 4-thiazolidinone resulting in binding with active sites of PPAR γ with notable antihyperglycemic activity [1]. The structure activity relationship showed that presence of electron withdrawing groups such as $–NO_2$ at *p*- and *m*- positions increased the antidiabetic activity. The presence of the electrons rich groups such as –OH and $–OCH_3$ also supported significant antidiabetic activity of the products. The compounds **1c**, **1e**, **1g,** and **1j** with *p*-methoxy, *m*-hydroxy, *p*-dimethylamino, and *o*-chloro substituents exhibited moderate antidiabetic activity.

Biochemical analysis. The compounds **1b**, **1d**, **1f**, and **1i** with significant antidiabetic activity were tested for insulin and other biochemical parameters at the 21st day (Tables 4 and 5).

The alkaline phosphatase (ALP), alanine transaminase (ALT) and aspartate transaminase (AST) level of compound **1d** demonstrated statistically significant values [$p < 0.01$, $p < 0.001$] compared with diabetic control group. The liver enzymes (ALP, ALT and AST) levels were found to be elevated in diabetic rats

Table 3. Antidiabetic activity of 3-(2-substituted)-4-(oxothiazolidin-3yl)-2-methylluinazolin-4(3*H*)-one (**1a**–**1k**)[a]

Group	Treatment, mg/kg	Blood glucose concentration, mg/dL			
		0 day	7th day	14th day	21st day
Normal control (normal saline)	10	85.79±0.43	88.89±0.52	83.45±0.59	85.40±0.38
Diabetic control (STZ)	50	187.96±0.40[d]	219.80±0.36[b]	248.63±0.46	268.75±0.45
1a	200	182.79±0.22	147.49±0.19	133.75±0.44	128.90±0.27
1b	200	187.55±0.28	153.67±0.37[b]	136.78±0.49[d]	123.45±0.21[d]
1c	200	177.85±0.21	148.89±0.27	136.53±0.16[b]	125.67±0.08[d]
1d	200	180.87±0.49	152.70±0.36[c]	135.67±0.35[d]	121.49±0.47[d]
1e	200	178.68±0.37	150.66±0.31[b]	136.78±0.28[b]	127.78±0.22[b]
1f	200	186.65±0.16	152.75±0.24	135.61±0.21[d]	124.55±0.30[d]
1g	200	185.89±0.36	144.45±0.37	131.74±0.40	126.78±0.20[c]
1h	200	180.50±0.37	147.88±0.37	131.67±0.02	129.87±0.37[c]
1i	200	184.68±0.21	147.52±0.05	136.78±0.37[c]	124.55±0.37[d]
1j	200	186.54±0.45	145.23±0.65	135.63±0.66	127.80±0.21[b]
1k	200	182.79±0.22	148.78±0.70	140.43±0.42[b]	130.78±0.43
Standard (Pioglitazone)	15	177.56±0.34	135.77±0.36[c]	123.20±0.28[d]	117.63±0.29[d]

[a] The results are expressed as mean ± S.E.M; data obtained were analyzed with one-way ANOVA followed by Dunnett's *t*-test.
[b] $p < 0.05$.
[c] $p < 0.01$.
[d] $p < 0.001$.

Table 4. Effect of test compounds on biochemical parameters in STZ induced diabetic rats[a]

Group	Treatment, mg/kg	ALP, IU/L	ALT, IU/L	AST, IU/L	Insulin, μIU/mL	HbA1C, %
Normal control (normal saline)	10	92.00±0.34	62.40±0.49	126.70±0.43	7.45±0.29	2.72±0.22
Diabetic control (STZ)	50	156.90±0.37	89.00±0.42	161.80±0.44	14.28±0.53	7.93±0.23
1b	200	80.00±0.19[c]	60.40±0.29[b]	135.00±0.45[d]	9.16±0.21[b]	4.09±0.22[c]
1d	200	40.50±0.25[d]	53.90±041[c]	134.11±0.51[d]	8.63±0.36[c]	3.29±0.01[b]
1f	200	170.90±0.40	84.10±0.18[b]	145.14±0.34	10.41± 0.37[c]	4.44±0.30[b]
1i	200	198.20±0.36[c]	85.70±0.50[b]	166.00±0.58	13.09±0.06[b]	5.64±0.29[b]
Standard (Pioglitazone)	15	65.20±0.37[b]	65.40±0.36[d]	119.40±0.31[c]	8.22±0.28[c]	3.51±0.41[b]

[a] The results are expressed as mean ± S.E.M; data obtained were analyzed with one-way ANOVA followed by Dunnett's *t*-test.
[b] $p < 0.05$.
[c] $p < 0.01$.
[d] $p < 0.001$.

and significantly lowered by the tested compounds **1b**, **1d**, **1f,** and **1i**. The elevated level of these enzymes could be the indication of diabetic complications such as increased gluconeogenesis and ketogenesis [35]. HbA1C level (7.93±0.23%) was found to be increased in diabetic control group. Hyperglycemia and vascular complications of diabetes were associated with increased non-enzymatic and auto-oxidative

Table 5. Effect of test compounds on lipid profile in STZ induced diabetic rats[a]

Group	Treatment, mg/kg	Lipid parameters, mg/dL				
		HDL	LDL	cholesterol	triglycerides	total protein
Normal control (normal saline)	10	39.84±0.36	9.26±0.30	49.10±0.31	66.50±0.28	9.74±0.18
Diabetic Control (STZ)	50	54.23±0.30	18.47±0.33	62.70±0.17	90.60±0.32	9.28±0.18
1b	200	45.20±0.37[b]	11.10±0.37[c]	56.30±0.35[c]	63.30±0.21[b]	8.93±0.24[b]
1d	200	39.50±0.36[d]	10.33±0.36[b]	49.80±0.34[d]	62.90±0.26[d]	8.88±0.20[d]
1f	200	49.10±0.53[b]	11.20±0.16	60.30±0.16	64.50±0.23[b]	9.47±0.26
1i	200	55.88±0.32	15.72±0.25[b]	71.64±0.25[b]	69.60±0.37	9.61±0.23[b]
Standard (Pioglitazone)	15	44.10±0.37[c]	10.30±0.25[c]	54.40±0.22[d]	60.70±0.21[d]	8.51±0.41[b]

[a] The results are expressed as mean ± S.E.M; data obtained were analyzed with one-way ANOVA followed by Dunnett's *t*-test.
[b] $p < 0.05$.
[c] $p < 0.01$.
[d] $p < 0.001$.

glycosylation [36]. The compounds **1b**, **1d**, **1f,** and **1i** induced significant decrease in HbA1C level. In this study we also evaluated the lipid parameters such as HDL, LDL, cholesterol, triglycerides, and total proteins of compounds **1b**, **1d**, **1f,** and **1i** in STZ induced diabetic rats. Insulin activates lipoprotein lipase and hydrolyses triglycerides. Insulin deficiency results in inactivation of these enzymes and leads to hypertriglyceridemia [31]. Significant control in level of HDL, LDL, cholesterol, triglycerides, and total proteins of the tested compounds revealed their pronounced antidiabetic activity. HDL, LDL, cholesterol, triglycerides, and total proteins levels tested with **1d** were statistically significant [$p < 0.05$, $p < 0.01$, $p < 0.001$].

Histopathological study. Histopathological study of pancreas was performed for the compounds **1a, 1b, 1c,** and **1d,** and the results were compared with the standard normal control and disease control group of animals [37, 38]. Pancreas of normal control demonstrated normal histology with normal structure of islets of Langerhans (large arrow), acini (arrow head) and intercalated ducts (small arrow) (Fig. 2). The standard group also exhibited normal histology of pancreas with normal appearance of islets of Langerhans (large arrow), acini (arrow head) and intercalated ducts (small arrow) (Fig. 3). Cytoplasmic vacuolations at islets of Langerhans (large arrow) and lymphocytic infiltration (small arrow) were observed in diabetic control group (Fig. 4). The test compounds **1b**, **1d** and **1f** were found to have normal histology with normal structure of islets of Langerhans (large arrow), acini (arrow head) and intercalated ducts (small arrow) (Figs. 5, 6), respectively, whereas test compound **1i** was found to induce cytoplasmic vacuolations.

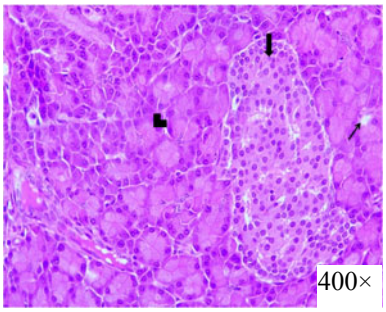


Fig. 2. Pancreas of normal control showing normal histology, islets of Langerhans (large arrow), acini (arrow head), intercalated duct (small arrow).

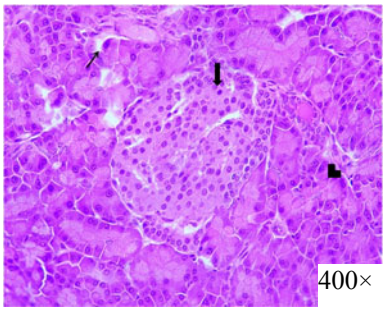


Fig. 3. Pancreas of standard showing normal histology, islets of Langerhans (large arrow), acini (arrow head), intercalated duct (small arrow).

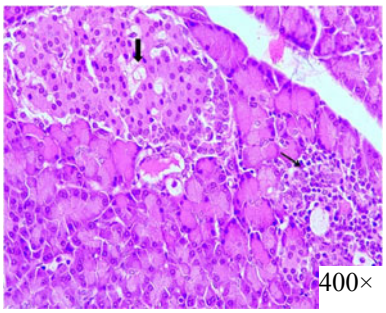


Fig. 4. Pancreas of diabetic control cytoplasmic vacuolations at islets of Langerhans (large arrow) and lymphocytic infiltration (small arrow).

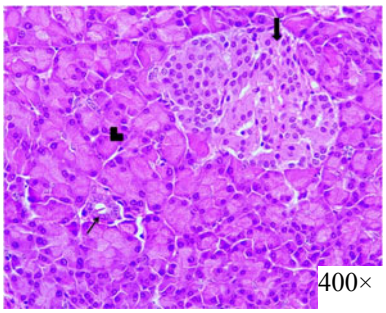


Fig. 5. Pancreas of compound **1b** showing normal histology, islets of Langerhans (large arrow), acini (arrow head), intercalated duct (small arrow).

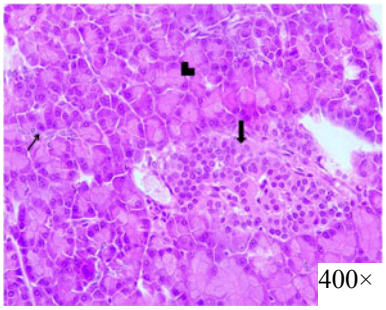


Fig. 6. Pancreas of compound **1d** showing normal histology, islets of Langerhans (large arrow), acini (arrow head), intercalated duct (small arrow).

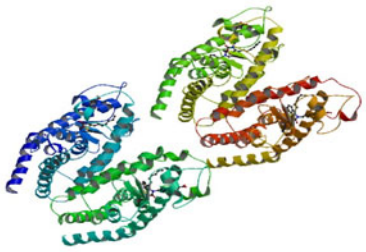


Fig. 7. 4PRG: Peroxisome proliferator-activated receptor γ.

Molecular docking studies. For the prediction of binding mode of target compounds, docking study of **1a**–**1k** was performed using AutoDock 1.5.6. Peroxisome proliferator-activated γ (PPAR γ) receptors demonstrated their role in regulation of glucose and lipid homeostasis, and were used as molecular targets for 2,4-thiazolidinone class of antidiabetic drugs. Agonist ligand activates PPAR γ by interacting with c-terminal region of ligand binding domain, which includes activation function 2 helix [39, 40]. The model of antidiabetic activity 3D crystal structure of PPAR γ with PDB ID: 4PRG is shown in Fig. 7. The binding energies and estimated inhibition constants of target compounds **1a**–**1k** were expressed in kcal/mol and *Ki*, μM respectively (Table 6).

According to docking analyses, *p*-nitro substituted derivative **1d** could demonstrate the highest affinity with binding energy –11.46 kcal/mol towards Peroxisome proliferator-activated γ (PPAR γ) receptors with interacting amino residues LYS263 and LYS265. Whereas, *p*-dimethyl amino substituted derivative **1g** would be of moderate binding affinity and unsubstituted derivative **1a** of the least binding affinity. The docking analysis results of the target compounds **1a**–**1k** were compared with the standard Pioglitazone having binding energy -9.09 kcal/mol and interacting amino acid residue LEU228. The binding energy of co-crystallized ligand (+/–)(2*S*,5*S*)-3-[4-(4-carboxy-phenyl)-butyl]-2-phenyl-4-oxo-5-thiazolidine was found to be –12.36 kcal/mol with interacting amino acid residues LYS265 and GLU343. The sample of predicted binding modes of the most active compound **1d** is presented in Fig. 8.

EXPERIMENTAL

All chemicals procured from Sigma-Aldrich, Sisco Research Laboratories (SRL) and SD fine chemicals (India) were of Laboratory Reagent (LR) and Analytical Reagent (AR) grade. Reaction progress was monitored by TLC on aluminium plates precoated with silica gel G (0.2 mm thickness, Merck, India) using chloroform : ethyl acetate (7 : 3) as an eluent and visualized under UV light. Melting points were determined in open capillary tubes and are uncorrected. FTIR spectra of KBr discs were recorded on a Shimadzu 8400-S spectrophotometer. ^{1}H NMR spectra were measured on a Bruker (India) 500 MHz spectrometer using DMSO-d_6 as a solvent and TMS as an internal standard. HRMS spectra were measured on a Bruker (India) spectrometer. Three dimensional

Table 6. Molecular docking analysis results for 3-(2-substituted)-4-(oxothiazolidin-3yl)-2-methylluinazolin-4(3*H*)-one (**1a**–**1k**)

Compound	Binding energy, kcal/mol	Inhibition constant K_i, μM	Number of hydrogen bonds involved	Interacting atoms of the protein
1a	–8.95	276.33	–	–
1b	–10.74	13.41	2	LYS263, LYS265
1c	–9.76	69.73	–	–
1d	–11.46	3.97	2	LYS263, LYS265
1e	–9.65	84.37	1	LYS265
1f	–10.65	15.6	1	LYS265
1g	–9.74	72.57	–	–
1h	–9.50	108.78	–	–
1i	–10.06	42.24	1	SER342
1j	–9.64	86.57	–	–
1k	–9.50	109.62	–	–
Co-crystallized	–12.36	875.56	2	LYS265, GLU343
Standard (Pioglitazone)	–9.09	216.62	1	LEU228

structure of PPAR γ (PDB ID: 4PRG) was obtained from RCSB protein data bank and used for molecular docking study. CS ChemDraw Ultra 8.0 was used for ligand preparation. AutoDock 1.5.6 was used for molecular docking studies. Discovery Studio 3.5 visualizer was employed for visualization and further analyses of results. Antidiabetic activity of synthesized compounds was screened by streptozotocin (STZ) induced diabetes model using male wistar albino rats. Blood glucose level was measured by Dr. Morepen BG 03 glucometer using Dr. Morepen glucose strips (India). Biochemical analysis and lipid profiles of cholesterol, triglycerides, total protein, low density lipoprotein (LDL), and high density lipoprotein (HDL) were performed by using automated biochemistry analyzer (Bioline technologies, India). Glycated haemoglobine (HbA1C) was analyzed by HPLC on a JASCO (India). Insulin levels were analyzed by chemiluminescent microparticle immunoassay (CMIA) technique [41, 42]. Triglyceride level was determined using the Hantzsch condensation method [43]. Cholesterol was determined according to the method of Liebermann Burchard reaction [44]. LDL and HDL levels were estimated according to Friedewald [45] and Assmann's [46] methods, respectively. Alanine transaminase (ALT), alkaline phosphatase (ALP), and aspartate transaminase (AST) levels were determined by auto analyzer (Erba Chem 5, India) using Erba diagnostic kits [47, 48].

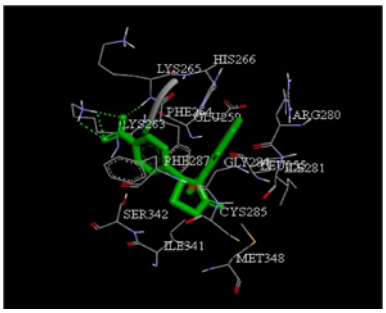


Fig. 8. Binding mode of the most active compound **1d** (binding energy –11.46 kcal/mol) with active site of PPARγ (PDB ID: 4PRG).

Synthesis of 3-(2-substituted)-4-(oxothiazolidin-3-yl)-2-methylquinazolin-4(3*H*)-ones (1a–1k) (*general pricedure*). A mixture of 0.01 mol of 3-amino-2-methyluinazolin-4(3H)-one with 0.012 mol of an aromatic aldehyde was stirred in 25 mL of DMF for 5 min at 0°C. This was followed by addition of thioglycolic acid (0.02 mol), and in 5 min DCC (0.012 mol) was added at 0°C. The resulting mixture was stirred for 5 h, dicyclohexylurea was filtered off. The final product was obtained upon addition of 25 mL of cold water to the filtrate. The corresponding crystalline products were recrystallized from acetone.

3-(4-Oxo-2-phenylthiazolidin-3-yl)-2-methylquinazolin-4(3*H*)-one (1a). Yield 80%, mp 214–216°C. IR spectrum, ν, cm^{-1}: 1652 (C=O), 1595 (C=O), 1444 (C–N),

1314 (C=N), 1283 (N–N), 752 (C–S–C). ^{1}H NMR spectrum, δ, ppm: 1.24 s (3H, CH_3, quinazolinone), 3.25 s (2H, CH_2, thiazolidinone), 5.95 s (1H, CH, thiazolidinone), 7.06–7.17 m (5H, C_6H_5, thiazolidinone), 7.55–7.59 m (4H, heterocyclic arom). MS: *m*/*z*: 337 [*M* + 1]. $C_{18}H_{15}N_3O_2S$.

3-[2-(4-Hydroxyphenyl)-4-oxothiazolidin-3-yl]-2-methylquinazolin-4(3*H*)-one (1b). Yield 75%, mp 194–196°C. IR spectrum, ν, cm^{-1}: 1616 (C=O), 1568 (C=O), 1438 (C–N), 1311 (C=N), 1244 (N–N), 1084 (O–H), 657 (C–S–C). ^{1}H NMR spectrum, δ, ppm: 1.52 s (3H, CH_3, quinazolinone), 2.10 s (2H, CH_2, thiazolidinone), 2.51 s (1H, CH, thiazolidinone), 5.59 s (1H, OH), 6.62–6.90 d.d (4H, C_6H_5, thiazolidinone), 7.09–7.10 m (4H, heterocyclic arom). MS: *m*/*z*: 355 [*M* + 2]. $C_{18}H_{15}N_3O_3S$.

3-[2-(4-Methoxyphenyl)-4-oxothiazolidin-3-yl]-2-methylquinazolin-4(3*H*)-one (1c). Yield 78%, mp 210–212°C. IR spectrum, ν, cm^{-1}: 1703 (C=O), 1620 (C=O), 1446 (C–N), 1317 (C=N), 1232 (C–O), 1082 (N–N), 732 (C–S–C). ^{1}H NMR spectrum, δ, ppm: 1.52 s (3H, CH_3, quinazolinone), 2.51 s (2H, CH_2, thiazolidinone), 3.36 s (1H, CH, thiazolidinone), 3.87 s (3H, OCH_3), 6.92–6.94 d.d (4H, C_6H_5, thiazolidinone), 7.13–7.32 m (4H, heterocyclic arom). MS: *m*/*z*: 368 [*M* + 1]. $C_{19}H_{17}N_3O_3S$.

3-[2-(4-Nitrophenyl)-4-oxothiazolidin-3-yl]-2-methylquinazolin-4(3*H*)-one (1d). Yield 80%, mp 200-202°C. IR spectrum, ν, cm^{-1}: 1662 (C=O), 1595 (C=O), 1521 (C–N), 1520 (Ar-NO_2), 1340 (C=N), 1230 (N–N), 756 (C–S–C). ^{1}H NMR spectrum, δ, ppm: 1.48 s (3H, CH_3, quinazolinone), 2.33 s (2H, CH_2, thiazolidinone), 3.23 s (1H, CH, thiazolidinone), 7.75–7.87 d.d (4H, C_6H_5, thiazolidinone), 7.89–7.95 m (4H, heterocyclic arom). MS: *m*/*z*: 384 [*M* + 2]. $C_{18}H_{14}N_4O_4S$.

3-[2-(3-Hydroxyphenyl)-4-oxothiazolidin-3-yl]-2-methylquinazolin-4(3*H*)-one (1e). Yield 75%, mp 226–228°C. IR spectrum, ν, cm^{-1}: 1691 (C=O), 1631 (C=O), 1427 (C–N), 1307 (C=N), 1247 (N–N), 1097 (O–H), 744 (C–S–C). ^{1}H NMR spectrum, δ, ppm: 1.51 s (3H, CH_3, quinazolinone), 2.51 s (2H, CH_2, thiazolidinone), 3.36 s (1H, CH, thiazolidinone), 6.71–6.84 m (4H, C_6H_5, thiazolidinone), 7.01–7.12 m (4H, heterocyclic arom), 9.92 s (1H, OH). MS: *m*/*z*: 355 [*M* + 2]. $C_{18}H_{15}N_3O_3S$.

3-[2-(3-Nitrophenyl)-4-oxothiazolidin-3-yl]-2-methylquinazolin-4(3*H*)-one (1f). Yield 75%, mp 228–230°C. IR spectrum, ν, cm^{-1}: 1828 (C=O), 1741 (C=O), 1575 (Ar-NO_2), 1435 (C–N), 1315 (C=N), 1236 (N–N), 754 (C–S–C). ^{1}H NMR spectrum, δ, ppm: 1.52 s (3H, CH_3, quinazolinone), 2.51 s (2H, CH_2, thiazolidinone), 3.37 s (1H, CH, thiazolidinone), 7.40–7.42 m (4H, C_6H_5, thiazolidinone), 7.96–8.06 m (4H, heterocyclic arom). MS: *m*/*z*: 383 [*M* + 1]. $C_{18}H_{14}N_4O_4S$.

3-[2-(4-(Dimethylaminophenyl)-4-oxothiazolidin-3-yl]-2-methylquinazolin-4(3*H*)-one (1g). Yield 79%, mp 206–208°C. IR spectrum, ν, cm^{-1}: 1619 (C=O), 1548 (C=O), 1455 (C–N), 1318 (C=N), 1240 (N–N), 825 (C–S–C). ^{1}H NMR spectrum, δ, ppm: 1.52 s (3H, CH_3, quinazolinone), 2.52 s (2H, CH_2, thiazolidinone), 3.35 s (1H, CH, thiazolidinone), 5.59 s (6H, 2CH_3), 7.56–7.86 m (4H, C_6H_5, thiazolidinone), 7.94–7.97 m (4H, heterocyclic arom). MS: *m*/*z*: 381 [*M* + 1]. $C_{20}H_{20}N_4O_2S$.

3-[2-(3-Chlorophenyl)-4-oxothiazolidin-3-yl]-2-methylquinazolin-4(3*H*)-one (1h). Yield 78%, mp 220–222°C. IR spectrum, ν, cm^{-1}: 1708 (C=O), 1616 (C=O), 1437 (C–N), 1315 (C=N), 1255 (N–N), 675 (C–S–C). ^{1}H NMR spectrum, δ, ppm: 1.52 s (3H, CH_3, quinazolinone), 2.52 s (2H, CH_2, thiazolidinone), 3.93 s (1H, CH, thiazolidinone), 7.42–7.43 m (4H, C_6H_5, thiazolidinone), 7.96–7.97 m (4H, heterocyclic arom). MS: *m*/*z*: 372 [*M* + 1]. $C_{18}H_{14}ClN_3O_2S$.

3-[2-(3,4-Dimethoxyphenyl)-4-oxothiazolidin-3-yl]-2-methylquinazolin-4(3*H*)-one (1i). Yield 79%, mp 270–272°C. IR spectrum, ν, cm^{-1}: 1761 (C=O), 1593 (C=O), 1433 (C–N), 1270 (C–O), 1265 (C=N), 1072 (N–N), 651 (C–S–C). ^{1}H NMR spectrum, δ, ppm: 1.52 s (3H, CH_3, quinazolinone), 2.51 s (2H, CH_2, thiazolidinone), 3.35 s (1H, CH, thiazolidinone), 5.57 s (6H, 2OCH_3), 6.91–7.04 m (3H, C_6H_5, thiazolidinone), 7.65–7.68 m (4H, heterocyclic arom). MS: *m*/*z*: 398 [*M* + 1]. $C_{20}H_{19}N_3O_4S$.

3-[2-(2-Chlorophenyl)-4-oxothiazolidin-3-yl])-2-methylquinazolin-4(3*H*)-one (1j). Yield 76%, mp 212–214°C. IR spectrum, ν, cm^{-1}: 1728 (C=O), 1618 (C=O), 1437 (C–N), 1315 (C=N), 1288 (N–N), 740 (C–S–C). ^{1}H NMR spectrum, δ, ppm: 1.51 s (3H, CH_3, quinazolinone), 2.51 s (2H, CH_2, thiazolidinone), 3.64 s (1H, CH, thiazolidinone), 7.30–7.45 m (4H, C_6H_5, thiazolidinone), 7.96–7.97 m (4H, heterocyclic arom). MS: *m*/*z*: 372 [*M* + 1]. $C_{18}H_{14}ClN_3O_2S$.

3-[2-(4-Fluorophenyl)-4-oxothiazolidin-3-yl]-2-methylquinazolin-4(3*H*)-one (1k). Yield 80%, mp 209–211°C. IR spectrum, ν, cm^{-1}: 1724 (C=O), 1645 (C=O), 1543 (C–N), 1323 (C=N), 1263 (N–N), 713 (C–S–C). ^{1}H NMR spectrum, δ, ppm: 1.52 s (3H, CH_3,

quinazolinone), 2.51 s (2H, CH_2, thiazolidinone), 3.36 s (1H, CH, thiazolidinone), 7.21–7.43 m (4H, C_6H_5, thiazolidinone), 7.44–7.96 m (4H, heterocyclic arom). MS: *m*/*z*: 357 [*M* + 2]. $C_{18}H_{14}FN_3O_2S$.

Biological profiling. Biological evaluation was performed by using healthy adult male wistar albino rats weighing 150-200 g. The animals were kept in polypropylene cages at the animal house. They were divided into different groups (control, test and standard) containing six animals in each. Experimental animals were kept in standard environmental condition with temperature 25±2°C and relative humidity 45–55%, maintained on a 12 h day/night cycle, were fed with standard food and water. The rats were acclimatized to standard laboratory conditions for one week prior to experiment. The experiment was approved by Institutional Animal Ethical Committee and the studies were conducted in accordance with Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines.

Oral acute toxicity. As per the guidelines set by Organisation for Economic Cooperation and Development (OECD), oral acute toxicity study was performed in healthy rats with six animals in each group. Rats fasted for 12 h and acute toxicity study was evaluated at the starting dose of compounds 2000 mg/kg body weight. The animals were continuously observed for behavioral changes and mortality over a period of 48 h. At this dose no mortality and signs of toxicity were observed.

Oral glucose tolerance test (OGTT). The animals were divided into thirteen groups containing six animals in each group. The animals were fasted overnight (18 h) for performing oral glucose tolerance test [49]. Group I served as normal control and was administered orally normal saline (10 mL/kg), groups II to XII were treated with test compounds **1a**–**1k** with 200 mg/kg. Group XIII was treated with standard Pioglitazone (15 mg/kg) intraperitoneally. Each group was fed with glucose (2g/kg) prior to administration of test compounds. Blood samples were collected from tail vein at the time interval of 0, 60, and 120 min to measure the blood glucose levels that were estimated with Dr. Morepen BG 03 glucometer using Dr. Morepen glucose strips.

Experimental induction of non-insulin dependent diabetes mellitus (NIDDM). Streptozotocin was prepared by dissolving in 0.1 N ice-cold sterile sodium citrate buffer (pH 4.5). The animals were fasted overnight and NIDDM was induced by a single intraperitoneal injection of streptozotocin 50 mg/kg body weight. The animals were allowed to drink 5% glucose solution overnight to overcome the drug induced hypoglycemia [50, 51]. Elevated blood glucose level was determined to confirm hyperglycemia after 72 h of streptozotocin injection. The blood glucose range of 200–300 mg/dL was considered as diabetic and used for experiments.

Experimental design for streptozotocin induced diabetes test. In the experiment wistar albino rats were divided into 14 groups containing six animals in each group. Group I served as normal control treated with normal saline 10 mL/kg orally. Group II served as diabetic control induced with STZ 50 mg/kg body weight. Groups III to XIII received test compounds **1a**–**1k** with the dose of 200 mg/kg. The suspensions of test compounds were prepared in 0.1% carboxymethyl cellulose (CMC) and administered intraperitoneally. Group XIV served as a standard treated with pioglitazone 15 mg/kg body weight intraperitoneally in normal saline solution. Diabetic rats were treated with standard and test compounds for 21 days continuously. Blood samples were withdrawn from tail vein and blood glucose was measured at 0, 7th, 14th, and 21st days interval. Blood samples were collected from tail vein by snip-cut at the tip of tail under mild anaesthesia and blood glucose level was determined by Dr. Morepen BG 03 glucometer using Dr. Morepen glucose strips. Blood samples were collected from total four test compounds **1b**, **1d**, **1f,** and **1i** with significant antidiabetic activity and serum was separated for estimation of insulin as well as other biochemical parameters at 21st day. The results obtained were compared with standard, disease control and normal control groups. Serum alanine transaminase (ALT), serum aspartate transaminase (AST) and alkaline phosphatase (ALP) levels were measured by auto analyzer using Erba diagnostic kits. Cholesterol level was estimated according to Liebermann Burchard reaction. Triglyceride and insulin levels were determined by using Hantzsch condensation method and chemiluminescent microparticle immunoassay (CMIA) technique, respectively. Glycated hemoglobine (HbA1C) was determined by HPLC. Total protein, low density lipoprotein (LDL) and high density lipoprotein (HDL) levels were determined by using automated biochemistry analyzer. All results were expressed as mean±S.E.M. Data obtained were analyzed with one-way ANOVA followed by Dunnett's *t*-test [$p < 0.05$, $p < 0.01$, $p < 0.001$].

Histopathological study. Histopathological study was performed for the compounds **1b**, **1d**, **1f,** and **1i** with significant antidiabetic activity and compared with standard, normal control and disease control groups of animals. The rats were sacrificed on the day 21 and whole pancreas from each animal was dissected out. The pancreas was washed with ice-cold saline immediately after removal. Pancreatic tissues were fixed in 10% formalin for histopathological studies. After fixation it was embedded in paraffin blocks. Thin slices of 5 μM were prepared by using microtome and placed on glass slides. It was stained by using (H&E) haematoxylin and eosin.

Molecular docking. The three dimensional crystal structure of PPAR γ with PDB ID: 4PRG [39] was downloaded from RCSB protein data bank (PDB; http://www.rcsb.org/pdb). Water molecules in 4PRG were removed followed by addition of Gasteiger charges and polar hydrogens using AutoDock 1.5.6 tools. The drugs like properties of ligands **1a**–**1k** were confirmed by manual application of Lipinski rule of Five and these were used for further studies. The structures of ligands **1a**–**1k** were drawn by using ChemDraw Ultra 8.0 (Cambridge Soft.com 100 Cambridge park drive, Cambridge, MA, 02140, USA).MarvinSketch 5.8.1 (Chemaxon Ltd; http:// www.chemaxon.com) programme was used to clean ligand structures in 2D and further in 3D with energy minimization. All structures were saved as pdb file format and then into pdbqt format for input into AutoDock 1.5.6 tools [52]. The grid box of 60×60×60 points with grid spacing of 0.375 A^0 were used for docking procedure. The maps were centered with centers x = 13.973, y = 67.424 and z = 14.538 on ligand binding sites. The Lamarckian Genetic Algorithm (LGA) was used to find the conformers with the lowest binding energies [53]. Final docking task was performed by using two commands: (1) autogrid4.exe –p 4PRG.gpf –l 4PRG.glg and (2) autodock4.exe –p ligand.dpf –l ligand.dLg. Discovery studio visualizer 3.5 was used for visualization and analysis of docking results.

CONCLUSIONS

A new series of 3-(2-substituted)-4-(oxothiazolidin-3-yl)-2-methylluinazolin-4(3*H*)-one derivatives is synthesized and characterized by IR, ^{1}H NMR, and mass spectra. All the compounds are analyzed for drug likeness by manual application of Lipinski rule of Five and found to be suitable for oral administration. The compounds **1b**, **1d**, **1f,** and **1i** demonstrate significant antidiabetic activity and lower activity than the standard. The compounds **1b**, **1d**, **1f,** and **1i** are tested for biochemical parameters like ALP, ALT, AST, Insulin, HbA1C and lipid profile such as HDL, LDL, cholesterol, triglycerides as well as total protein level that are found to be significant. According to molecular docking studies, the binding energies are determined to be in the range of –8.95 to –11.46 kcal/mol, with peroxisome proliferator-activated receptor γ (PPAR γ) receptors (PDB ID: 4PRG). The compound **1d** exhibits the highest binding affinity. The accumulated data can be helpful in designing and optimizing the studied hybrid molecules as new oral antidiabetic agents.

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CONFLICT OF INTEREST

No conflict of interest was declared by the authors.

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