

## SYNTHESIS, QUANTUM MECHANICAL CALCULATION AND BIOLOGICAL EVALUATION OF 5-(4-SUBSTITUTED ARYL/ HETERO ARYL METHYLIDENE)-1, 3-THIAZOLIDINE-2, 4-DIONES

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### ABSTRACT

A series of 5-substituted aryl / heteroaryl methylidene-1,3-thiazolidine-2,4-diones (1a-1j) were synthesised by Knoevenagel condensation of 1,3-thiazolidine-2,4-dione with various aldehydes in toluene using piperidine as catalyst. Drug-likeness, protein binding energy calculation and docking simulations were carried out by *in silico* studies and the observed results suggest their possible hypoglycemic effect. Anti-oxidant potential of the series 1a-1j were examined by 2,2-diphenyl-1-picryl hydrazyl (DPPH) radical scavenging assay, superoxide anion radical scavenging assay and reducing power assay. Ascorbic acid and gallic acid are used as reference substances and their effects are compared with test compounds.

**Keywords:** Thiazolidine-2,4-diones, Drug-likeness, Binding energy studies, Antioxidant studies

### INTRODUCTION

Diabetes mellitus (DM) is characterized by deficiency in insulin secretion and action<sup>1</sup>. It is well associated with increase in the formation of free radicals and decrease in the bio-antioxidant potential<sup>2</sup>. Reactive oxygen species (ROS) play key role in nephropathy, neuropathy, retinopathy and atherosclerosis<sup>3</sup>. Antioxidants are also useful in the management of diabetes and anti-diabetic drugs glibenclamide, glipizide, metformin and repaglinide exhibit antioxidant properties<sup>4</sup>. So ameliorating oxidative stress along with hypoglycemic effect might be an effective therapeutic strategy for treating diabetes<sup>5</sup>.

Thiazolidinediones (TZDs) modulate glucose and fat metabolism by binding to the Peroxisome Proliferator Activated Receptor gamma (PPAR<sub>γ</sub>)<sup>6</sup>. Ligand binding domain (LBD) of a heterodimer molecule of PPAR<sub>γ</sub> is stimulated by thiazolidinedione class of drugs (eg: rosiglitazone) and regulates insulin action<sup>7,8</sup>. In light of above facts, ten 5-(4-substituted aryl/ heteroaryl methylidene)-1,3-thiazolidine-2,4-dione analogues<sup>9,10</sup> were synthesised and evaluated for their antioxidant potential. In addition drug-likeness, protein binding energy calculation and protein binding pattern of synthesised compounds to X-ray crystallized structure of PPAR<sub>γ</sub> (PDB 2PRG) were investigated using Molinspiration and ArgusLab 4.0.1.

### MATERIALS AND METHODS

All the chemicals were procured from Sd fine-chem Ltd and Himedia Pvt. Ltd. 2,2-diphenyl-1-picryl hydrazyl (DPPH) and nitro blue tetrazolium (NBT) were purchased from Sigma Aldrich (UK). All the solvents and starting materials were purified by standard methods. Melting points were determined in DBK melting point apparatus, expressed in °C and are uncorrected. Shimadzu digital balance, REMI magnetic stirrer for the synthesis, hot air oven of Biotech Company for drying and BIT incubator for incubation were used. Analytical thin layer chromatography (TLC) was performed on silica gel 60 plates (Merck) with visualization by UV Light and staining with iodine. The UV-visible spectrophotometric absorbance's are measured on Shimadzu UV-visible spectrophotometer and the IR spectrum was run on Shimadzu IR affinity 1 spectrophotometer. <sup>1</sup>H NMR (ppm δ, DMSO/CH<sub>3</sub>OH) was recorded on Advance 300 MHz spectrophotometer. Mass spectra were recorded on Shimadzu QP2010 PLUS GC-Mass spectrometer.

### Experimental

#### Synthesis of 1,3-thiazolidine-2,4-dione (TZD, 1)

Chloroacetic acid (14.4 g, 0.16 mole) in concentrated hydrochloric acid (32 mL, 36 %) was stirred for 15 min. Thiourea (12.16 g, 0.16 mole) was added to the stirred solution and the reaction mixture

was refluxed for 17 h. White needle like crystals of thiazolidinedione precipitated on cooling, filtered under vacuum, washed with water and recrystallized from ethanol.

White solid, Yield 70 %, m.p. 122-124 °C (124 °C), TLC (toluene:ethyl acetate: 4:1) R<sub>f</sub>: 2.2; IR (KBr): 3452 cm<sup>-1</sup>(NH), 1753 cm<sup>-1</sup> and 1658 cm<sup>-1</sup> (C=O), 1342 cm<sup>-1</sup> (C-N), 619 cm<sup>-1</sup> (C-S); <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>): δ 12.03 (s, 1H, NH), δ 4.14 (s, 2H, CH<sub>2</sub>); MS: m/z 118 (M<sup>+</sup>).

#### Synthesis of 5-(substituted aryl/ hetero aryl methylidene)-1, 3-thiazolidine-2,4-dione (1a -1j)

Substituted aryl / hetero aryl aldehyde (0.094 mole) and 1,3-thiazolidine-2,4-dione (0.094 mole, 10.9 g) suspended in dry toluene were taken in a flask equipped with a Dean-Stark apparatus fitted with calcium guard tube. Catalytic amount of piperidine (0.5 mL) was added and the mixture was refluxed with stirring for 6 h. On cooling the product was precipitated, filtered under vacuum, washed with cold dry toluene and dry ethanol.

#### 5-(Benzylidene)-1, 3-thiazolidine-2, 4-dione (1a)

Appearance: Half white solid; IR (KBr): 3442 cm<sup>-1</sup> (NH), 3034 cm<sup>-1</sup>(CH), 1698 and 1632 cm<sup>-1</sup> (C=O), 1431 cm<sup>-1</sup> (C-N), 638 cm<sup>-1</sup> (C-S); <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>): δ 12.62 (s, 1H, NH), δ 7.7 (s, 1H, =CH), δ 7.4-7.6 (m, 5H, Ar). MS: m/z 205 (M<sup>+</sup>).

#### 5-(4-Chlorobenzylidene)-1, 3-thiazolidine-2, 4-dione (1b)

Appearance: Pale yellow solid; IR (KBr): 3452 cm<sup>-1</sup> (NH), 3034 cm<sup>-1</sup>(CH), 1685 and 1632 cm<sup>-1</sup> (C=O), 1465 cm<sup>-1</sup> (C-N), 634 cm<sup>-1</sup> (C-S); <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>): δ 12.5 (s, 1H, NH), δ 7.7 (s, 1H, =CH), δ 7.0-7.6 (m, 4H, Ar).

#### 5-(4-Fluorobenzylidene)-1, 3-thiazolidine-2, 4-dione (1c)

Appearance: Yellowish orange solid; IR (KBr): 3445 cm<sup>-1</sup> (NH), 3043 cm<sup>-1</sup>(CH), 1753 and 1698 cm<sup>-1</sup> (C=O), 1445 cm<sup>-1</sup> (-C-N), 1146 cm<sup>-1</sup> (C-F), 639 cm<sup>-1</sup> (C-S); <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>): δ 12.7 (s, 1H, NH), δ 7.8 (s, 1H, =CH), δ 7.6 (d, 2H, Ar), δ 7.4 (s, 2 H, Ar).

#### 5-(2, 4-Dichlorobenzylidene)-1, 3-thiazolidine-2, 4-dione (1d)

Appearance: Pale yellow solid; IR (KBr): 3447 cm<sup>-1</sup> (NH), 3038 cm<sup>-1</sup>(CH), 1675 and 1616 cm<sup>-1</sup> (C=O), 1431 cm<sup>-1</sup> (C-N), 645 cm<sup>-1</sup> (C-S); <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>): δ 12.8 (s, 1H, NH), δ 7.8-7.85 (s, 2H, =CH, Ar), δ 7.5-7.65 (m, 2H, Ar).

#### 5-(4-Nitrobenzylidene)-1, 3-thiazolidine-2, 4-dione (1e)

Appearance: Brown solid; IR (KBr): 3445 cm<sup>-1</sup> (NH), 3034 cm<sup>-1</sup>(CH), 1678 and 1610 cm<sup>-1</sup> (C=O), 1411 cm<sup>-1</sup> (C-N), 631 cm<sup>-1</sup> (C-S); <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>): δ 12.4 (s, 1H, NH), δ 8.0 (s, 1H, =CH), δ 7.4 (s, 2H, Ar), δ 7.1 (s, 2 H, Ar).

**5-(4-Methoxybenzylidene)-1, 3-thiazolidine-2, 4-dione (1f)**

Appearance: Pale yellow solid; IR (KBr): 3423  $\text{cm}^{-1}$  (NH), 3072  $\text{cm}^{-1}$  (CH), 1728 and 1693  $\text{cm}^{-1}$  (C=O), 1504  $\text{cm}^{-1}$  (C=C), 1338  $\text{cm}^{-1}$  (C-O), 1317  $\text{cm}^{-1}$  (C-N), 628  $\text{cm}^{-1}$  (C-S);  $^1\text{H}$  NMR (DMSO- $d_6$ ):  $\delta$  11.77 (s, 1H, NH),  $\delta$  7.34 (s, 1H, =CH),  $\delta$  6.6-7.2 (m, 4H, Ar),  $\delta$  2.7 (s, 3H, CH<sub>3</sub>). MS: m/z 236 (M<sup>+</sup>1).

**5-[4-(Dimethyl amino) benzylidene]-1, 3-thiazolidine-2, 4-dione (1g)**

Appearance: Orange solid; IR (KBr): 3434  $\text{cm}^{-1}$  (NH), 3068  $\text{cm}^{-1}$  (CH), 1716 and 1617  $\text{cm}^{-1}$  (C=O), 1434  $\text{cm}^{-1}$  (C-N), 647  $\text{cm}^{-1}$  (C-S);  $^1\text{H}$  NMR (DMSO- $d_6$ ):  $\delta$  12.3 (s, 1H, NH),  $\delta$  7.6 (s, 1H, =CH),  $\delta$  7.4 (m, 2H, Ar), 6.8 (m, 2H, Ar), 2.9 (s, 6H, CH<sub>3</sub>). MS: m/z 248 (M<sup>+</sup>).

**5-[(Furan-2-yl) methylidene]-1, 3-thiazolidine-2, 4-dione (1h)**

Appearance: Yellow solid; IR (KBr): 3445  $\text{cm}^{-1}$  (NH), 3034  $\text{cm}^{-1}$  (CH), 1678 and 1610  $\text{cm}^{-1}$  (C=O), 1284  $\text{cm}^{-1}$  (C-O), 1411  $\text{cm}^{-1}$  (C-N), 636  $\text{cm}^{-1}$  (C-S);  $^1\text{H}$  NMR (DMSO- $d_6$ ):  $\delta$  12.4 (s, 1H, NH),  $\delta$  8.0 (s, 1H, =CH),  $\delta$  7.4 (s, 1H, Ar),  $\delta$  7.1 (s, 2 H, Ar). MS: m/z 195 (M<sup>+</sup>).

**5-[(5-Methyl Furan-2-yl) methylidene]-1, 3-thiazolidine-2, 4-dione (1i)**

Appearance: Yellow solid; IR (KBr): 3445  $\text{cm}^{-1}$  (NH), 3034  $\text{cm}^{-1}$  (CH), 1678 and 1610  $\text{cm}^{-1}$  (C=O), 1284  $\text{cm}^{-1}$  (C-O), 1411  $\text{cm}^{-1}$  (C-N), 686  $\text{cm}^{-1}$  (C-S);  $^1\text{H}$  NMR (DMSO- $d_6$ ):  $\delta$  12.4 (s, 1H, NH),  $\delta$  8.0 (s, 1H, =CH),  $\delta$  7.1 (s, 2 H, Ar)  $\delta$  2.7 (s, 3 H, CH<sub>3</sub>).

**5-[(Thiophen-2-yl) methylidene]-1, 3-thiazolidine-2, 4-dione (1j)**

Appearance: Brown solid; IR (KBr): 3445  $\text{cm}^{-1}$  (NH), 3034  $\text{cm}^{-1}$  (CH), 1678 and 1610  $\text{cm}^{-1}$  (C=O), 1411  $\text{cm}^{-1}$  (C-N), 686  $\text{cm}^{-1}$  (C-S);  $^1\text{H}$  NMR (DMSO- $d_6$ ):  $\delta$  12.5 (s, 1H, NH),  $\delta$  8.05 (s, 1H, =CH),  $\delta$  7.9 (s, 1H, Ar),  $\delta$  7.6-7.7 (s, 1H, Ar),  $\delta$  7.2-7.3 (s, 1H, Ar). MS: m/z 211 (M<sup>+</sup>).

**Drug-likeness**

Drug-likeness of a molecule is of prime importance in drug design and can be deduced by Lipinski rule of five (RO5)<sup>11</sup>. The RO5 predicts better oral absorption for the test molecule when the values of molecular weight (Mw) < 500, calculated Log P (cLog P) < 5, hydrogen bond donors (HBD) < 5 and hydrogen bond acceptors (HBA) < 10. Total polar surface area (TPSA)<sup>12</sup>, molecular volume (MV) and number of rotatable bonds (RB) of the molecules explain pharmacodynamic nature<sup>13</sup> of the test compound in biophase. The Lipinski parameters were calculated by using Molinspiration webME Editor<sup>14</sup>.

**Binding energy calculation**

The knowledge of the shape and electron density of a molecule helps to assess the nature of the binding of a drug to target site. The 2D structures of the compounds were geometry optimized using PM3 semi-empirical QM method. The Highest Occupied Molecular Orbital (HOMO), Lowest Unoccupied Molecular Orbital (LUMO) energy and GAP values<sup>15</sup> were estimated using Hamiltonian Zerner's Intermediate Neglect of Differential Overlap (ZINDO) and closed shell Restricted Hartree-Fock-Single Consistent Field (RHF-SCF) methods.

**Docking**

Human Peroxisome-Proliferator-Activated Receptor gamma (PPAR $\gamma$ ), 2PRG<sup>16</sup> was retrieved from protein data bank (PDB)<sup>17</sup>. The crystal structure of the human PPAR $\gamma$  is complexed with rosiglitazone (BRL) and is bound to the PPAR $\gamma$  active site through side-chain interactions. The binding site was defined from the coordinates of ligand in the original pdb2PRG. The residues, water and the hetero groups lie within the radius of 5 Å unit area of ligand was identified as binding site residue of 2PRG. Sixteen residues lie within 5 Å unit area of ligand that interacts with it through their side chain were considered as active site residues. 2D structures of the ligands are geometry optimised by PM3 semi empirical Quantum Mechanics (QM) method. The docking simulations between receptor and ligand were performed using the ArgusDock algorithm and BFGS geometry search. Both ligand and protein structures were flexibly treated and a spacing of 0.4 Å between the grid points was

used for docking simulations. At maximum 150 poses were allowed to be analysed and binding site box was set to 15 X 15 X 15 Å. Docking simulation with 2PRG exhibited ligand protein energies as kcal / mol. All the quantum mechanical calculations were carried out using Arguslab 4.0.1<sup>18</sup>. Molecular visualizations were applied using Argus Lab 4.0.1 and Molegro Viewer<sup>19</sup>. All the *in silico* studies were performed in Intel(R) Core (TM) i5 CPU @ 2.53 GHz with 4 GB RAM.

**DPPH free radical scavenging assay**

The DPPH radical scavenging activity was carried out by Lamaison method<sup>20</sup>. The antioxidant effect of compounds was assessed on the basis of radical scavenging effect on the DPPH stable free radical<sup>21</sup>. Test compounds with different concentrations (25  $\mu\text{g/mL}$ , 50  $\mu\text{g/mL}$ , 100  $\mu\text{g/mL}$ ) were prepared. The test compound solution (20  $\mu\text{L}$ ) was added to solution of DPPH in methanol (280  $\mu\text{L}$ , 0.1 mM) in a 96-well microtitre plate. The plate is incubated in dark room at 37 °C for 30 min and the absorbance of each solution was measured at 516 nm. The absorbance for blank also measured and used in the IC<sub>50</sub> calculation.

**Super oxide anion radical scavenging assay**

Super oxide was generated by riboflavin and NBT reduction by superoxide was measured<sup>22</sup> in the presence and absence of test compounds. The solution of test and standard compounds were added to the mixture containing EDTA (6 mM containing 3  $\mu\text{g}$  sodium cyanide), NBT (50  $\mu\text{M}$ ), riboflavin (2  $\mu\text{M}$ ) and phosphate buffer (58 mM, pH 7.8) to give the final volume of 300  $\mu\text{L}$ . The optical density of each solution was determined at 560 nm<sup>23</sup> after 15 min of uniform illumination. IC<sub>50</sub> values, the concentration that elicited the half maximal response were calculated.

**Reducing power assay**

The reducing power of the test compounds was determined by the method of Oyaizu<sup>24</sup>. Potassium ferricyanide (2 mL) was added to the test compounds (100  $\mu\text{g} / \text{mL}$ ) in dimethyl sulphoxide and was mixed with phosphate buffer (2 mL, pH 6.6). The mixture was incubated at 50 °C for 20 min, cooled and mixed with trichloroacetic acid (2 mL, 10%) and centrifuged at 3000 rpm for 10 min. Supernatant liquid (1 mL) was diluted with deionized water (1 mL) and then ferric chloride (1 mL, 0.1%) was added. The absorbance was read spectrophotometrically at 700 nm. Ascorbic acid was used as reference compound.

**RESULTS AND DISCUSSIONS**

Thiazolidine-2,4-dione was prepared by refluxing thiourea with chloroacetic acid in concentrated hydrochloric acid for 17 h. Condensation of various aryl and hetero aryl aldehydes in toluene using piperidine catalyst yielded ten different 5-(substituted aryl/hetero aryl methylidene)-1,3-thiazolidine-2, 4-diones (1a-1j). The TLC support for qualitative analysis and column chromatographic techniques for separation were utilized and the reaction was found to be completed after 6 h. The yields of the compounds were in the range of 39-81 % (Table 1). The compounds 1i, 1a and 1c were isolated with high yields (81 %, 79 % and 74 %). Disappearance of methylene peak at  $\delta$  4.14 and appearance of arylidene (=CH) peak in the range of  $\delta$  7.34-8.0 in  $^1\text{H}$ -NMR spectrum confirms the occurrence of Knoevenagel condensation between 1, 3-thiazolidine-2, 4-dione and aryl / heteroaryl aldehydes.

Drug-likeness study revealed that all the molecular descriptors are in compliance with the RO5. Binding energy calculations for 1a-1j were carried out after geometry optimization by PM3 QM calculations. The HOMO energies of the synthesized compounds are ranging between -9.45 eV to -8.03 eV, LUMO energies are in the range of -2.28 eV to -1.00 eV and GAP values are in the range of 6.88 eV to 8.30 eV (Fig.1, Table 2). These values suggest the chemical instability of molecules 1e and 1d among the series. Correlation is not found between these values and observed biological activity.

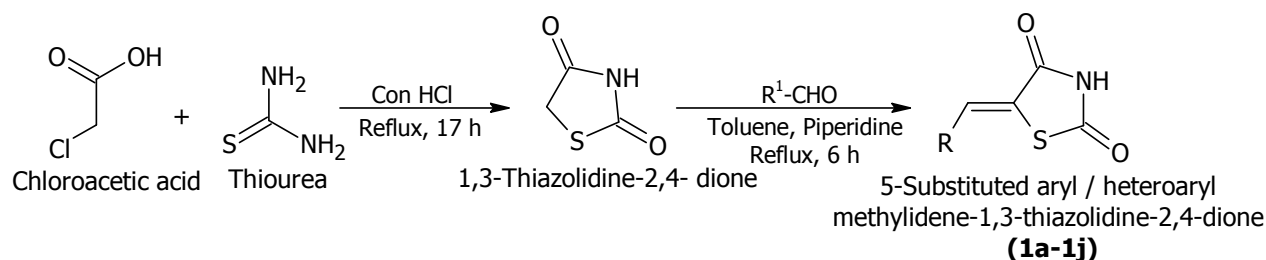
Docking of 2PRG with test molecules exhibited well established interactions with one or more amino acids in the receptor active pocket. *In silico* protein ligand studies of 2PRG with test molecules showed docking scores in the range of -10.49 kcal / mol to -8.34 kcal / mol while rosiglitazone gave docking score of -9.48 kcal / mol. Five

compounds (1a-1d and 1j) exhibited comparable binding energy to that of rosiglitazone (Table-2). Docking studies revealed the three important hydrogen bonding interactions between 2PRG and head group of TZDs. TZDs head group makes several specific interactions with amino acids of binding pocket. The carbonyl groups of TZD generate H-bond with His323 and His449 (Fig.2). The ring nitrogen of TZD forms H-bond with Tyr473 and Tyr327. The central benzene ring of the ligand occupies a very narrow pocket between Cys285 and Met364. Tyr473 also makes H-bond with carbonyl groups. Cys285 is bonded with nitrogen and sulphur of the ring extension groups in rosiglitazone and compound 1i.

The synthesized compounds (1a-1j) were tested for their *in vitro* antioxidant potential by DPPH free radical scavenging assay, super oxide anion scavenging assay and reducing power assay. In the DPPH assay, free radical scavenging effects of the synthesized

compounds (1a-1j) are determined by measuring the absorbance of DPPH radical at 517 nm. When they were tested at 25, 50 and 100 µg / mL concentration using ascorbic acid as reference, the radical scavenging potential of tested compounds was found to be very low. The super oxide anion scavenging effect of the test compounds (1a-1j) was also determined at 25, 50 and 100 µg / mL concentration and compared with gallic acid. Compounds 1b, 1d, 1e and 1h have shown moderate super oxide anion radical scavenging activity. Compound 1b with *p*- Chloro benzylidene moiety at 5-position of the TZD ring has shown maximum activity (IC<sub>50</sub>= 60.55 µg / mL) among the tested compounds (Table-3). Reducing capacity of the compounds was tested at 100 µg / mL concentration using ascorbic acid as the reference. A higher absorbance of the test compounds at 700 nm indicates their higher reducing power. The compounds 1a (0.229) 1b (0.244), 1f (0.234) and 1g (0.436) have shown significant reducing power (Table-4).

Scheme 1: The synthetic pathway for compounds 1a-1j



Compd Code	R	Compd Code	R
1a	C <sub>6</sub> H <sub>4</sub>	1f	4-OCH <sub>3</sub> -C <sub>6</sub> H <sub>4</sub>
1b	4-Cl- C <sub>6</sub> H <sub>4</sub>	1g	4-N(CH <sub>3</sub> ) <sub>2</sub> - C <sub>6</sub> H <sub>4</sub>
1c	4-F- C <sub>6</sub> H <sub>4</sub>	1h	2-Furyl
1d	2, 4-Cl- C <sub>6</sub> H <sub>3</sub>	1i	5-CH <sub>3</sub> -2-furyl
1e	4-NO <sub>2</sub> - C <sub>6</sub> H <sub>4</sub>	1j	Furfuryl Thiophen-2-carbyl

Table 1: Characterization and drug-likeness data of compounds 1a-1j

Compd	Mol formula	Yield %	m. p. °C	R <sub>f</sub>	Drug likeliness descriptors					
					M. Wt.	HBA	HBD	Log P	TPSA (Å <sup>2</sup> )	MV(Å <sup>3</sup> )
1a	C <sub>10</sub> H <sub>7</sub> O <sub>2</sub> NS	81	239-240	0.60 <sup>a</sup>	205	3	1	1.483	49.993	169.12
1b	C <sub>10</sub> H <sub>6</sub> ClNO <sub>2</sub> S	65	222-224	0.47 <sup>a</sup>	239	3	1	2.161	49.993	182.65
1c	C <sub>10</sub> H <sub>6</sub> FNO <sub>2</sub> S	74	216-217	0.64 <sup>a</sup>	223	3	1	1.646	49.993	174.05
1d	C <sub>10</sub> H <sub>5</sub> Cl <sub>2</sub> O <sub>2</sub> NS	58	219-220	0.53 <sup>a</sup>	274	3	1	2.757	49.993	196.19
1e	C <sub>10</sub> H <sub>6</sub> N <sub>2</sub> O <sub>4</sub> S	58	240-241	0.65 <sup>a</sup>	250	6	1	1.442	95.757	192.45
1f	C <sub>11</sub> H <sub>9</sub> NO <sub>3</sub> S	68	215-216	0.70 <sup>a</sup>	235	4	1	1.539	59.167	194.66
1g	C <sub>12</sub> H <sub>12</sub> O <sub>2</sub> N <sub>2</sub> S	72	274-276	0.66 <sup>a</sup>	248	4	1	1.585	53.171	215.02
1h	C <sub>8</sub> H <sub>5</sub> NO <sub>3</sub> S	73	234-236	0.44 <sup>a</sup>	195	4	1	0.74	63.073	150.68
1i	C <sub>9</sub> H <sub>7</sub> NO <sub>3</sub> S	81	228-230	0.70 <sup>a</sup>	209	4	1	0.962	63.073	167.25
1j	C <sub>8</sub> H <sub>5</sub> NO <sub>2</sub> S <sub>2</sub>	50	248-250	0.70 <sup>b</sup>	211	3	1	1.382	49.933	159.83

m.p: Melting point; R<sub>f</sub>: Retention factor; M. Wt.: Molecular weight; a = toluene: ethyl acetate (4: 1); b = toluene: ethyl acetate (7: 3); HBA: Hydrogen bond acceptor; HBD: Hydrogen bond donars; TPSA: Total polar surface area; MV: Molecular volume.

Table 2: Quantum mechanical calculation data of compounds 1a-1j

Ligand	E <sub>HOMO</sub> (eV)	E <sub>LUMO</sub> (eV)	GAP (eV)	Docking score (kcal / mol)	Interacting residues
Rosiglitazone	-8.33	-0.16	8.17	-9.48	His 323, His 449, Cys 285, Tyr 473
1a	-8.86	-1.07	7.79	-10.04	His 323, His 449, Ser 289, Tyr 327
1b	-8.89	-1.21	7.68	-9.65	His 323, Ser 289, Tyr 473
1c	-8.91	-1.33	7.58	-10.49	His 323, His 449, Ser 289, Tyr 473
1d	-9.26	-0.96	8.30	-9.83	His 323, Ser 289
1e	-9.45	-2.28	7.17	-9.02	His 449, Tyr 327
1f	-8.50	-1.12	7.38	-9.11	His 449, Tyr 327
1g	-8.24	-1.00	7.24	-8.86	His 323, His 449, Tyr 473, Gln 286
1h	-8.29	-1.24	7.05	-8.34	His 323, His 449, Ser 289
1i	-8.03	-1.15	6.88	-8.41	His 449, Cys 285
1j	-8.34	-1.34	7.00	-10.12	His 323, His 449, Ser 289, Tyr 473

Four out of ten synthesised compounds have H-bond formation with either His323 or His449 via their 2,4-dione groups while the other compounds forming H-bond with both His323 and His449.

Table 3: *In vitro* antioxidant activities of compounds 1a-1j

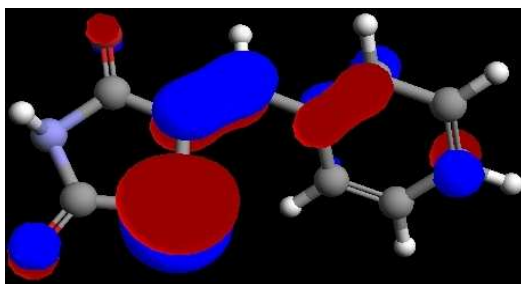
Compd	Dose ( $\mu\text{g/mL}$ )	DPPH free radical scavenging activity		Super oxide anion radical scavenging activity	
		Percent inhibition	IC <sub>50</sub> ( $\mu\text{g/mL}$ )	Percent inhibition	IC <sub>50</sub> ( $\mu\text{g/mL}$ )
1a	25	6.74	>100	9.80	>100
	50	7.62		17.74	
	100	9.61		44.42	
1b	25	14.08	>100	20.03	60.55
	50	12.51		32.76	
	100	10.53		90.72	
1c	25	13.56	>100	43.65	>100
	50	11.98		46.94	
	100	9.93		49.32	
1d	25	10.15	>100	15.54	95.19
	50	10.38		26.52	
	100	13.81		36.76	
1e	25	8.71	>100	12.04	68.90
	50	10.75		30.94	
	100	14.06		78.60	
1f	25	17.25	>100	30.40	>100
	50	15.50		31.53	
	100	14.53		37.31	
1g	25	12.51	>100	12.51	>100
	50	12.23		12.23	
	100	10.32		10.32	
1h	25	16.40	>100	53.60	99.17
	50	15.70		39.40	
	100	14.24		50.41	
1i	25	7.79	>100	35.16	>100
	50	8.29		37.63	
	100	13.35		49.61	
1j	25	9.97	>100	17.40	>100
	50	10.67		23.31	
	100	11.18		41.05	
Ascorbic acid	1	20.29			
	2.5	40.76	3.36	-	-
	5	69.59			
Gallic acid	1			34.81	
	2.5	-	-	47.42	0.54
	5			60.89	

Table 4: Reducing power of compounds 1a-1j

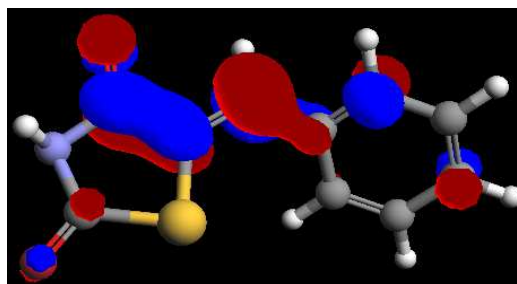
Compd Code	Absorbance (100 $\mu\text{g/mL}$ )
	O. D $\pm$ S. D
Control	0.068 $\pm$ 0.007
1a	0.229 $\pm$ 0.006
1b	0.066 $\pm$ 0.003
1c	0.206 $\pm$ 0.002
1d	0.101 $\pm$ 0.008
1e	0.244 $\pm$ 0.013
1f	0.234 $\pm$ 0.004
1g	0.436 $\pm$ 0.016
1h	0.071 $\pm$ 0.003
1i	0.079 $\pm$ 0.004
1j	0.112 $\pm$ 0.005
Ascorbic acid	0.635 $\pm$ 0.014

O. D: Optical density; S. D: Standard deviation;

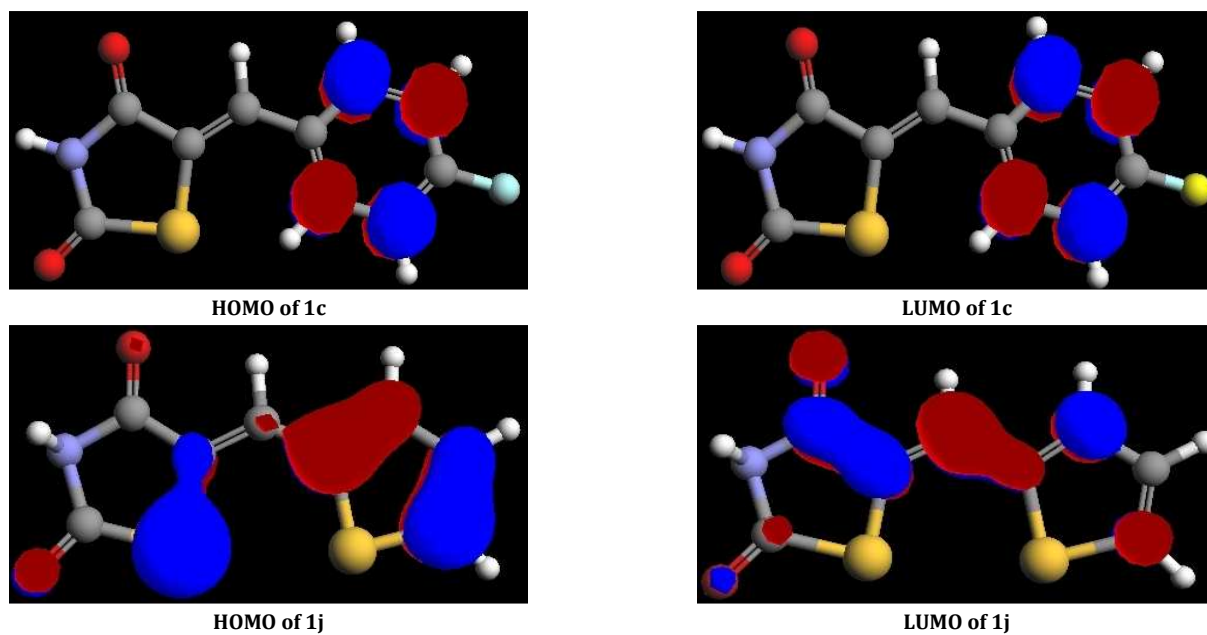
Base line correction with phosphate buffer pH 6.6



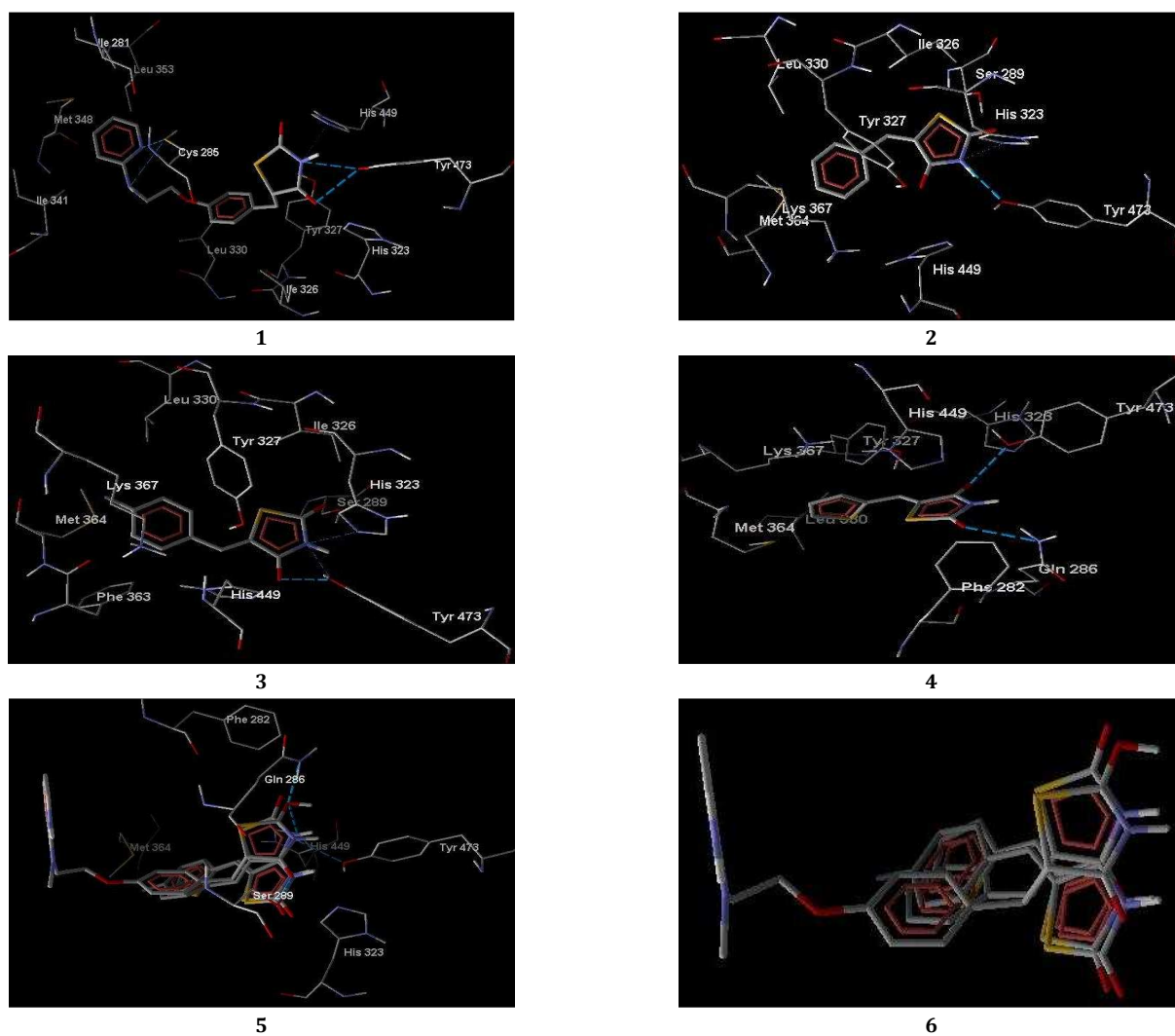
HOMO of 1a



LUMO of 1a



**Fig. 1: Image showing HOMO and LUMO energies of compounds 1a, 1c and 1j**



**Fig. 2: Docking poses of 2PRG with ligands in best of its conformation. 1- Rosiglitazone docked into 2PRG, 2- Compound 1a docked into 2PRG, 3- Compound 1b docked into 2PRG, 4 Shows compound 1a docked into 2PRG, 5 and 6- Overlay of compounds 1a,1c,and 1j in binding pocket of 2PRG along with rosiglitazone**

## CONCLUSION

In this study, we synthesized ten 5-substituted-1, 3-thiazolidine-2, 4-dione derivatives (1a-1j) by Knoevenagel condensation of substituted aryl / heteroaryl aldehydes with thiazolidine-2,4-dione. As expected these compounds exhibited mild to moderate antioxidant potential in DPPH free radical scavenging assay, superoxide anion radical scavenging assay and reducing power assay. Docking simulations of synthesized compounds with human 2PRG suggested that 2, 4-dione and NH are the key structural features of TZDs that interacting mostly with His323, His449, Ser289 and Tyr473. The series of 1a-1j found to have similar pattern of binding to the 2PRG as that of rosiglitazone. Further SAR study of TZDs along with various functional groups at the ring nitrogen and testing their *in vivo* efficacy is under investigation.

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