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Synthesis, Molecular Docking Studies and *in vitro* and *in vivo* Hypolipidemic Activity of Thiazolidinedione Derivatives

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This work aimed to design, synthesize novel thiazolidinedione derivatives, which were tested for their antihyperlipidemic effects. Antihyperlipidemic activity was carried through *in silico* docking study for the 50 thiazolidinedione derivatives (SMI-IV-1 to 50 derivatives) in comparison with standard pioglitazone against the crystal structure of protein PPARgamma (PDB ID: 6QJ5) proteins. Based on the binding energy further, best three derivatives were selected and characterized with IR, NMR and MASS studies. Then, *in vitro* evaluation of each derivative's antihyperlipidemic activity was carried out by observing their effects on the reduction ability of mature 3T3-L1 adipocyte cells. These cells are susceptible to cytotoxicity because of their internal accumulation of lipids. The vitality of 3T3-L1 adipocytes was identified using the MTT assay. Finally, animal experiments were conducted in order to verify the antihyperlipidemic action of the substance in Wistar rats that had been genetically modified to develop hyperlipidemia. Initially, acute toxicity study was carried out and then based on further study was investigated. Simvastatin (4 mg/kg) was used as standard drug. All the selected three derivatives showed a reversal of the rise in blood triglycerides, cholesterol and LDL from 6 to 48 h and in VLDL from 24 h. All the derivatives showed satisfactory results but derivatives SMI-IV-23 followed by SMI-IV-4 and SMI-IV-31 were given very significant results in terms of binding energy and also *in vitro*, *in vivo* experiments. Overall, the results concluded that all the thiazolidinedione derivatives were potent against hyperlipidemic condition and among the all derivatives few derivatives were showed significant role as non-toxic and in mitigation of hyperlipidemia.

Keywords: Anti hyperlipidimic activity, Molecular docking study, MTT assay, Thiazolidinedione, Triton, 3T3-L1 adipocyte cell.

INTRODUCTION

Every year, heart disease-specifically coronary heart disease (CHD) ranks among the top killers on a global scale [1]. More than 30,000 more people died from coronary heart disease than projected between March 2020 and August 2022, equating to an average of 230 fatalities each week [2]. Being older (anything above 45 for men and 55 for women), smoking, having high blood pressure and low HDL cholesterol, being overweight (> 30% overweight), having diabetes, having high LDL cholesterol (> 160 mg/dl) and having a personal or family history of early coronary heart disease are all factors that raise the likelihood of coronary heart disease [3]. As an example of a risk factor, a high HDL level (> 60 mg/dl) presents itself [4]. As a result, the most effective treatment for coronary heart disease is anti-hyperlipidemic medicine.

Hypolipidemic drugs minimize blood levels of cholesterol and other lipids. These drugs are useful in treating hyperlipidemia or high blood lipid levels. Individuals who have hyperlipidemia are more likely to suffer from coronary artery disease (CAD), which is the leading cause of death among adults. Metabolic syndrome, which encompasses prothrombotic and inflammatory diseases, hypertension, insulin resistance and abdominal obesity, is associated with elevated levels of lipids and triglycerides [5]. The fatty streaks cause harm to the blood vessel lining by developing into plaques or atheromas. White blood cells and platelets are attracted to the area when the inflammatory reaction begins. These cells build up on the injured blood vessels, leading to the enlargement of atheroma, which further narrows the blood channel and reduces blood flow. Because of the damage, the vessels are less pliable, less extensible and less responsive to neurochemical stimuli. The oxygen

demand and blood flow these days are too much for coronary arteries to handle. This may induce arterial obstruction and disruption if it is not addressed by a medical professional.

Coronary artery disease, which is more common in people with hyperlipidemia, is the leading cause of mortality worldwide. Although the exact cause of coronary artery disease remains unknown, some risk factors have been identified. Factors such as menopause, age, gender, sedentary lifestyle, smoking, high-fat diet and diseases including diabetes, gout and hypertension are among these [6]. Well-known lipid-lowering medications called statins reduce LDLs and protect diabetics against cardiovascular events. Though promising results from animal studies support the use of statins to prevent neuropathy, there is little clinical data to support this approach. Thiazolidinediones or glitazones, are a well-known class of antidiabetic medications. Currently, fibrates (clofibrate, fenfibrate), statins (atorvastatin, simovastatin) and bile sequestrants (cholestipol, cholestiramine) are available as therapy options for hyperlipidemia [7,8]. Scientists are currently searching for alternative medications to treat hyperlipidemia and prevent cardio-cerebro vascular illnesses at a lower risk because the side effects and adverse consequences of these treatments may limit their long term use [9,10]. This study describes the synthesis and structural characterization of novel thiazolidinedione derivatives, continuing our effort to synthesize small molecules with biological activity, such as antihyperlipidemic activity.

EXPERIMENTAL

The purity of the starting materials used for the reactions was verified by TLC. All the chemicals procured from Sigma-Aldrich, Merck and CDH and were of laboratory grades.

Step-I: Synthesis of 1,3-thiazolidine-2,4-dione: A 250 mL three-necked flask was used to dissolve 56.4 g (0.6 M) of chloroacetic acid and 45.6 g (0.6 M) of thiourea in 60 mL water. White precipitates began to form after the mixture had been stirred for 15 min. A 60 mL of conc. HCl was added into the flask in small increments to dissolve the precipitates and then the mixture was refluxed at 100-110 ºC for 10-12 h. After cooling, the solidified contents appeared as white needle clusters. The product was filtered, washed thoroughly with distilled water and dired. The purity of the cmpound was confirmed by a single spot on a TLC plate after it was recrystallized from ethanol. The R_f value 0.9, boiling point 120-122 ºC and yield 8.32 g.

Step-II: Synthesis of (5*Z*)-5-[(pyridin-4-yl)methylidene]-1,3-thiazolidine-2,4-dione: After refluxing for 6 h in ethanol (15 mL) with five drops of glacial acetic acid, 1,3-thiazolidine-2,4-dione (**I**) (1.66 g, 0.005 mol) and pyridine-4-carbaldehyde (0.005 mol) were combined. The combination was then cooled. Filtered, dried and refined by recrystallization from methanol, the resulting solid was compound **II** (5*Z*)-5-[(pyridin-4-yl)methylidene]-1,3-thiazolidine-2,4-dionethe. A solitary spot on the TLC plate verified the product's purity. Production 8.14 g, m.p. 126-128 ºC and refractive index 0.84.

Step-III: Synthesis of (5*Z*)-3-acetyl-5-[(pyridin-4-yl)-methylidene]-1,3-thiazolidine-2,4-dione: To an agitated acid chloride solution (0.5 mmol, 1.0 equiv.) in (5*Z*)-5-[(methylidene)pyridin-4-yl]-1,3-thiazolidine-2,4-dione (**II**, 0.5 mL, 1 M) at 0 ºC For 1 h, the resulting mixture was left at room temperature. Following the addition of 5 mL of water, the mixture was vigorously stirred until the desired substance formed as a solid precipitate. The desired compound was obtained by filtering, washed the precipitate with distilled water and refined by recrystallization from methanol. One spot on the TLC plate verified the purity of product. Yield: 7.88 g, m.p.: 130-132 ºC, R_f value 0.96.

Step-IV: Synthesis of derivatives of (5*Z*)-3-[(2*E*)-3-phenyl-prop-2-enoyl]-5-[(pyridin-4-yl)methylidene]-1,3-thiazolidine-2,4-dione: In a round bottom flask, equimolar mixture of (5*Z*)-3-acetyl-5-[(pyridin-4-yl)methylidene]-1,3-thiazolidine-2,4-dione (**III**) and substituted aromatic aldehydes were dissolved in ethanol and then subjected for reflux on a water bath for 3.5 h in the presence of few drops of acetic acid. The resulting solid was filtered and dried after being put into ice-cold water from the reaction mixture. The derivatives of (5*Z*)-3-[(2*E*)-3-phenyl-prop-2-enoyl]-5-[(pyridin-4-yl)methylidene]-1,3-thiazolidine-2,4-dione (**SMI-IV**) was obtained (**Scheme-I**). After recrystallization from ethanol , a single spot on the TLC plate verified the purity of the product. The yield of compounds were in range of yield 65-90%.

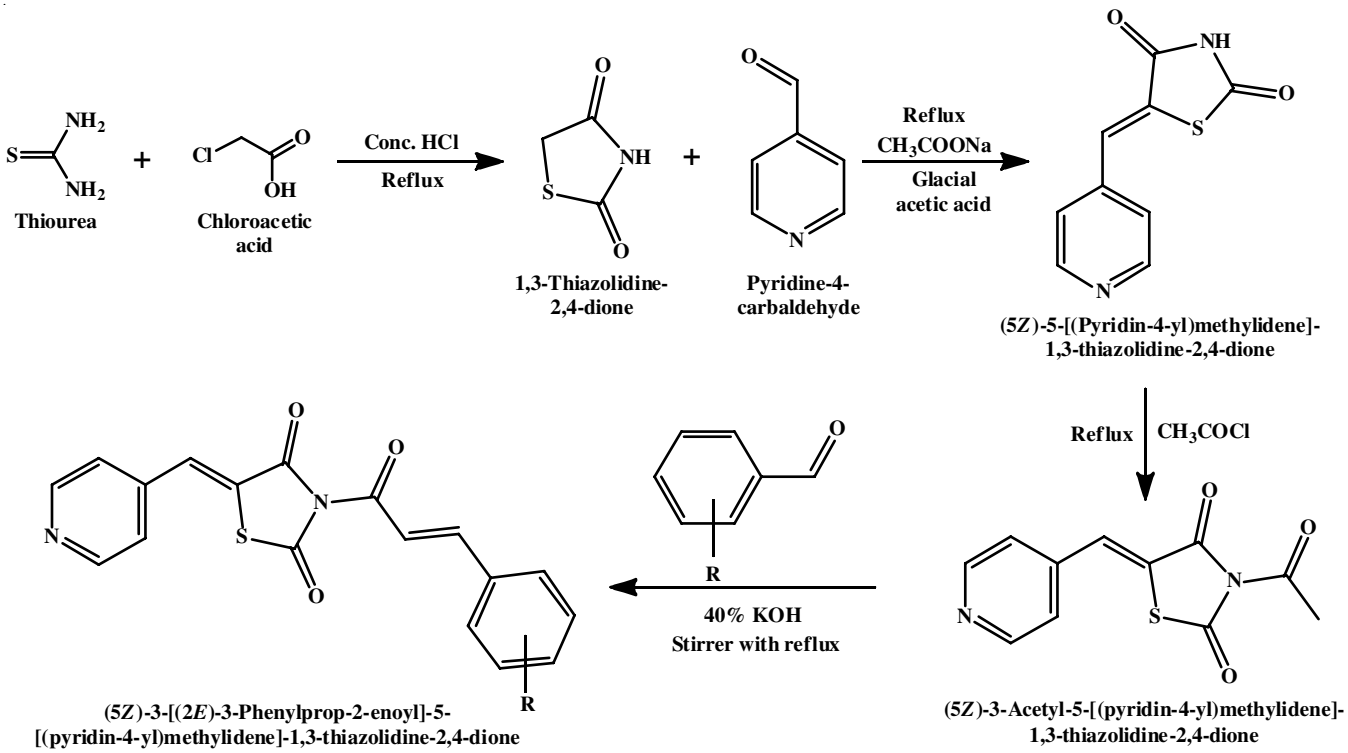
(5*Z*)-3-[(2*E*)-3-(3-Amino-4-hydroxyphenyl)prop-2-enoyl]-5-[(pyridin-4-yl)-methylidene]-1,3-thiazolidinedione-2,4-dione (SMI-IV-4): Yield: 79.09%, m.p.: 130-132 °C, m.f.: $C_{18}H_{13}N_3O_4S$ (m.w.: 367.39). IR (KBr, ν_{max}, cm^{-1}): 2380 (C=O). 800 (C-H), 1090 (C-S). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 4.315 (-NH_2 (2H)), 3.35 (-CH(3H)), 10.96 (-OH (1H)). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm: 32.35. 40.10. 74.25. 147.60. 154.10. 167.05. Mass *m/z*: 367.2 (M+1)*.

5*E*)-3-[(2*E*)-3-(4-Amino-3-hydroxyphenyl)prop-2-enoyl]-5-[(pyridin-4-yl)methylidene]-1,3-thiazolidine-2,4-dione (SMI-IV-23): Yield: 90.90%, m.p.: 130-132 °C, m.f.: $C_{18}H_{13}N_3O_4S$ (m.w.: 367.37). IR (KBr, ν_{max}, cm^{-1}): 2960 (O-H), 1400 (C=O), 1110 (C-S), 825 (C-H), 2800 (N-H). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 4.2 (-NH_2 (2H)), 3.1 (-CH(3H)), 6.6 (Ar-H). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm: 30.115. 170. Mass *m/z*: 367.1 (M+1)*.

(5*E*)-3-[(2*E*)-3-(4-Chlorophenyl)prop-2-enoyl]-5-[(pyridin-4-yl)methylidene]-1,3-thiazolidinedione-2,4-dione (SMI-IV-31): Yield: 92.72%, m.p.: 124-126 °C, m.f.: $C_{18}H_{11}ClN_2O_3S$ (m.w.: 370.80), IR (KBr, ν_{max}, cm^{-1}): 1435 (C=O), 690 (C-H), 1100 (C-S), 845 (C-Cl). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 2.3 (-CH(3H)), 6.6 (Ar-H). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm: 59.80, 66.78, 80.45, 166.96, 177.40, 179.45. Mass *m/z*: 369.2 (M+1)*.

Docking studies: To analyze the many kinds of biomolecular interactions and ligand receptor binding affinities, the docking experiments were conducted. The docking studies were carried out by means of Schrödinger. The docking study was performed on proteins namely crystal structures of PPAR-gamma (PDB ID: 6QJ5) protein.

Protein preparation: The RCSB protein data bank provided the PPARgamma protein structure (PDB ID: 6QJ5), which was generated by removing other ligands using the Swiss PDB viewer.



Scheme-I: Synthetic route of novel thiazolidinedione derivatives

Ligand preparation: The 3D structures of ligands were created with ChemSketch and imported into Schrödinger and BIOVIA Discovery Studio Visualizer-2020. The "SMALL MOLECULE" tool was used to minimize ligands and the cluster SDF file with the results was saved.

Afterwards, 50 different derivatives were prepared from (5*Z*)-3-[(2*E*)-3-phenylprop-2-enoyl]-5-[(pyridin-4-yl)methylidene]-1,3-thiazolidine-2,4-dione among them only 3 best derivatives were selected.

***In vitro* antihyperlipidemic activity:** 3T3-L1 preadipocytes were generated from mouse fibroblasts and subsequently cultivated in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS). A 10% (v/v) FBS, 1% (v/v) glutamine, a 25 mM solution of 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid buffer and 1% (v/v) antimycotic mixture were added to the medium. Subsequently, it was placed in an incubator set at 37 ºC with a 5% concentration of CO_2 in an atmosphere with controlled humidity. The media was removed after 3 days and the cells were kept in DMEM + 10% FBS medium and 10 µg/mL insulin until they were used, which typically happened within 1 to 3 days after the differentiation process was finished. The 3T3-L1 adipocytes were stained with oil-red-O for experimental purposes [11]. To assess lipid accumulation, the cells were stained with a working solution of oil-red-O at the concentration of 3 mg/mL in 60% (v/v) isopropyl alcohol. The staining process lasted for 1 h at 25 ºC and the cells were then examined under an optical microscope. After 1 h, lipids were removed from the cells using isopropyl alcohol in order to differentiate the control cells. A microplate reader tuned to 510 nm was then used to measure the lipids that had been collected.

Cell viability: The vitality of mature adipocytes was evaluated using the MTT assay to determine the impact of selected three thiazolidine derivatives. The experiments were conducted using 24 well plates. The mature adipocytes were subjected to 48 h incubation period in a solution containing 0.2% DMSO. Each well was treated with 50 µL of 5 mg/mL MTT solution and the plates were then incubated at 37 ºC for 3 h. After incubation, 200 µL of DMSO was added to each well to dissolve formazan dye and the wells were thereafter shaken gently. A microplate reader was employed to quantify the absorbance at a wavelength of 560 nm in order to determine the proportion of viable cells [12].

***In vivo* animal study for antihyperlipidemic activity:** Six albino male Wistar rats, ranging in weight from 150 to 200 g, were randomly assigned to separate groups (Ethical Ref. No: IAEC/Sangli/2023/74). The animals were given unlimited access to water after a 16 h fast before the experiment. Three thiazolidine derivatives (**SMI-IV**) were administered orally to Groups II-IV at dosages of 200 mg/kg body weight, 4 mg/kg of simvastatin and 20 mg/kg of fenofibrate, respectively. The first group served as a control. All animals in groups II–VI were given the drugs orally. Triton WR-1339 was administered *i.p.* (dose of 100 mg/kg b.w.) to all animals simultaneously. The animals used as controls only received 100 mg/kg of Triton WR-1339. At 6, 24 and 48 h, the AGAPPE diagnostic kits were used to assess HDL, triglycerides and blood cholesterol. Retro-orbital puncture was the technique utilized to collect blood samples. The CHOD-PAP method was used to determine cholesterol, the GPO-PAP method for triglycerides and the phosphotungstate magnesium acetate reagent method for HDL precipitation [13,14].

VLDL was calculated using the formula:

$$\text{VLDL} = \frac{\text{Triglycerides}}{5}$$

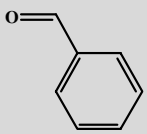
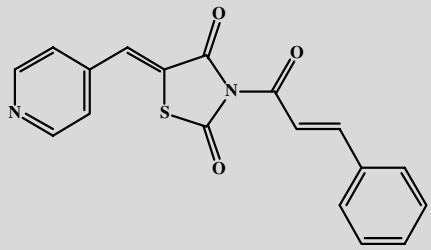
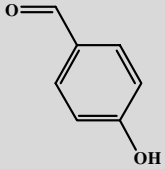
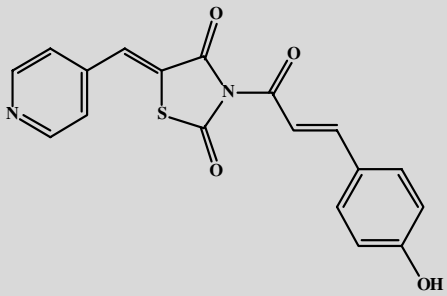
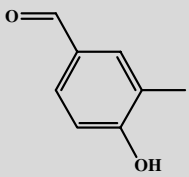
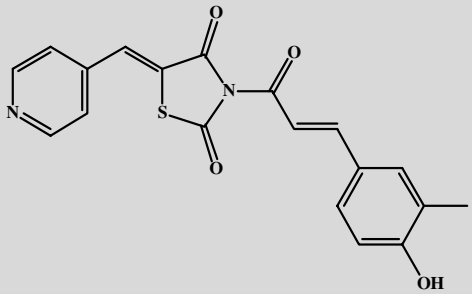
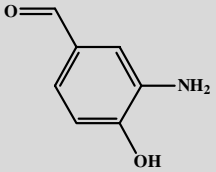
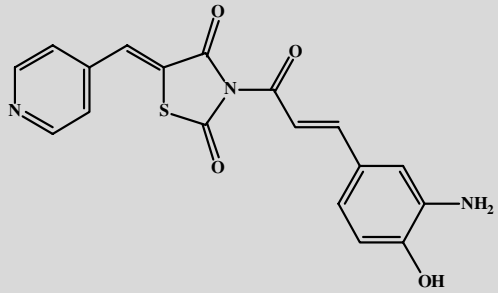
LDL cholesterol was calculated as:

$$\text{LDL} = \text{Total cholestrol} - \text{HDL} - \frac{\text{Triglycerides}}{5}$$

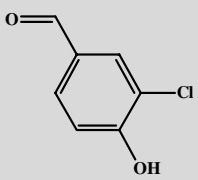
RESULTS AND DISCUSSION

The intermediates yielded good yields and high purity after converting into the respective derivatives. *In silico* docking study for all the 50 derivatives were performed with PPARgamma (PDB ID: 6QJ5) and binding energy for all the derivatives are tabulated in Table-1. It was observed that the docking scores were varied in the range of -2.8 to -5.9. Further, the 3D hydrogen

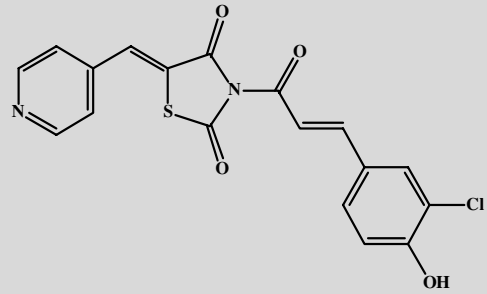
TABLE-1
DOCKING SCORE AND IUPAC NOMENCLATURE OF 50 DERIVATIVES OF THIAZOLIDINEDIONE

Compound	Substituted aromatic aldehydes	Thiazolidinedione derivatives	Docking score
SMI-IV-1	 Benzaldehyde	 (5*Z*)-3-[(2*E*)-3-Phenylprop-2-enoyl]-5-[(pyridin-4-yl)methylidene]-1,3-thiazolidine-2,4-dione	-3.8
SMI-IV-2	 4-Hydroxybenzaldehyde	 (5*Z*)-3-[(2*E*)-3-(4-hydroxyphenyl)prop-2-enoyl]-5-[(pyridin-4-yl)methylidene]-1,3-thiazolidine-2,4-dione	-4.4
SMI-IV-3	 4-Hydroxy-3-methylbenzaldehyde	 (5*Z*)-3-[(2*E*)-3-(4-Hydroxy-3-Methylphenyl)prop-2-enoyl]-5-[(pyridin-4-yl)methylidene]-1,3-thiazolidine-2,4-dione	-4.0
SMI-IV-4	 3-Amino-4-hydroxybenzaldehyde	 (5*Z*)-3-[(2*E*)-3-(3-Amino-4-hydroxyphenyl)prop-2-enoyl]-5-[(pyridin-4-yl)methylidene]-1,3-thiazolidine-2,4-dione	**-5.1**

SMI-IV-5



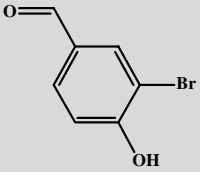
3-Chloro-4-hydroxybenzaldehyde



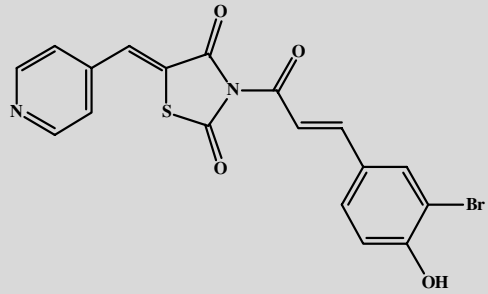
(5*Z*)-3-[(2*E*)-3-(3-Chloro-4-hydroxyphenyl)prop-2-enoyl]-5-[(pyridin-4-yl)methylidene]-1,3-thiazolidine-2,4-dione

-4.3

SMI-IV-6



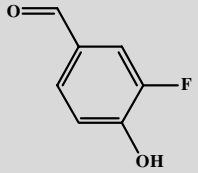
3-Bromo-4-hydroxybenzaldehyde



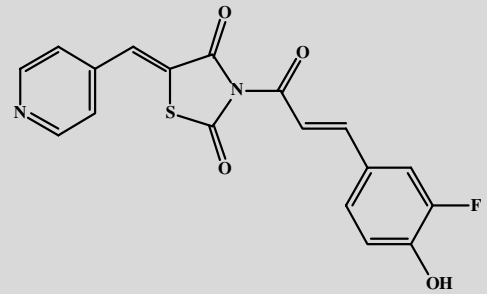
(5*Z*)-3-[(2*E*)-3-(3-Bromo-4-hydroxyphenyl)prop-2-enoyl]-5-[(pyridin-4-yl)methylidene]-1,3-thiazolidine-2,4-dione

-4.1

SMI-IV-7



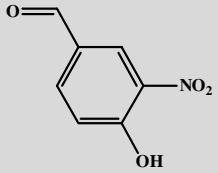
3-Fluoro-4-hydroxybenzaldehyde



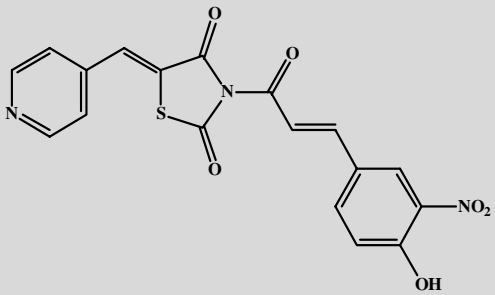
(5*Z*)-3-[(2*E*)-3-(3-Fluoro-4-hydroxyphenyl)prop-2-enoyl]-5-[(pyridin-4-yl)methylidene]-1,3-thiazolidine-2,4-dione

-4.4

SMI-IV-8



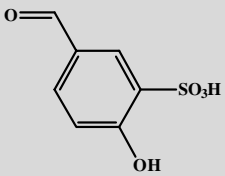
4-Hydroxy-3-nitrobenzaldehyde



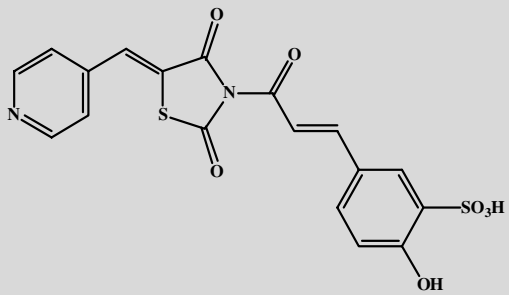
(5*Z*)-3-[(2*E*)-3-(4-Hydroxy-3-nitrophenyl)prop-2-enoyl]-5-[(pyridin-4-yl)methylidene]-1,3-thiazolidine-2,4-dione

-4.0

SMI-IV-9

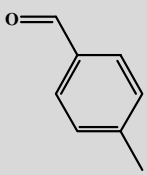
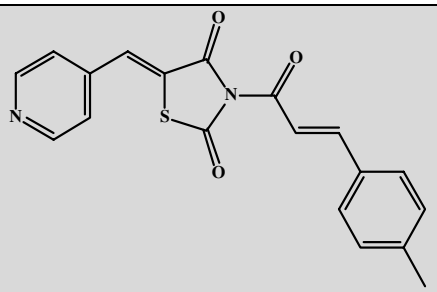
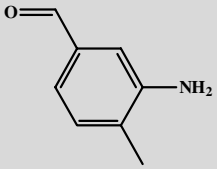
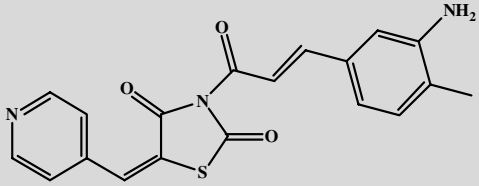
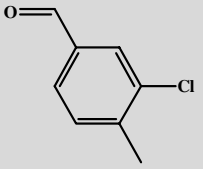
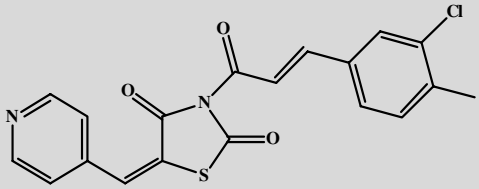
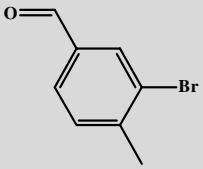
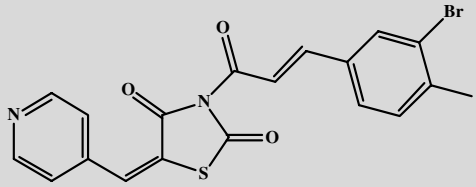
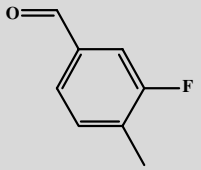
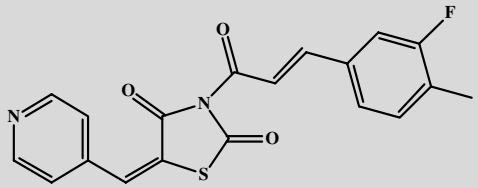
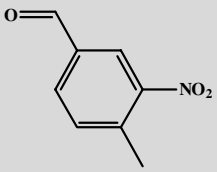
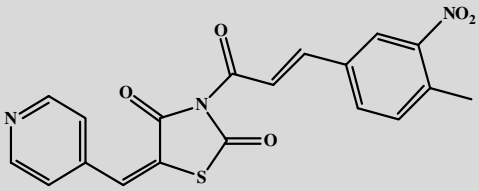
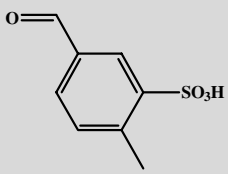
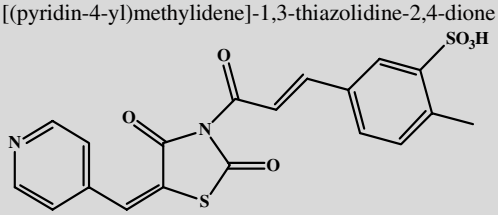


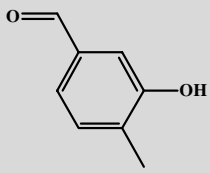
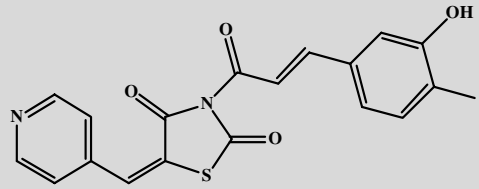
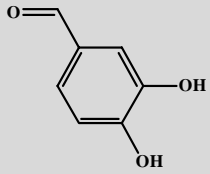
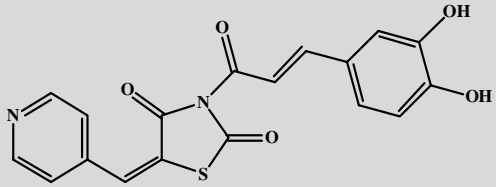
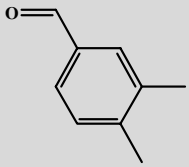
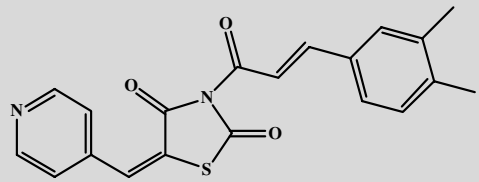
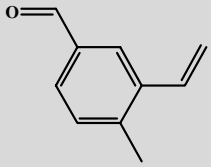
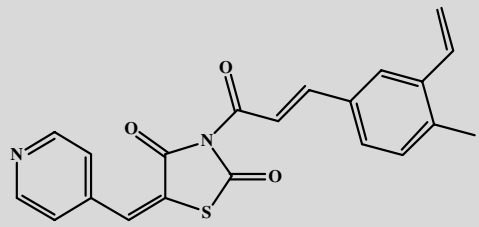
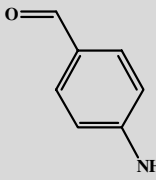
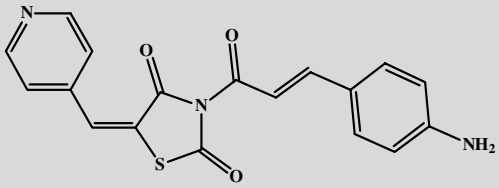
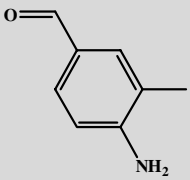
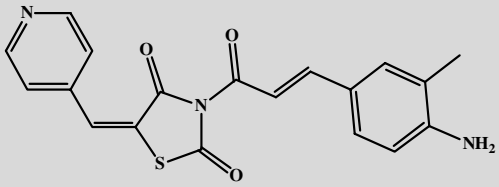
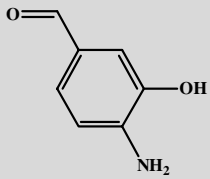
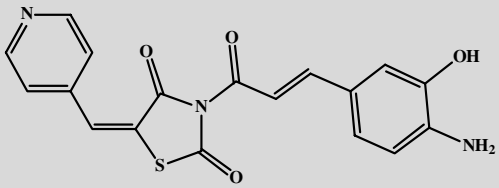
5-Formyl-2-hydroxybenzene-1-sulfonic acid

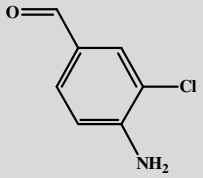
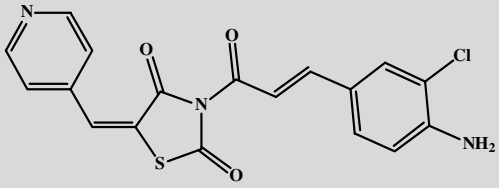
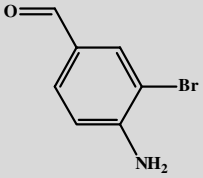
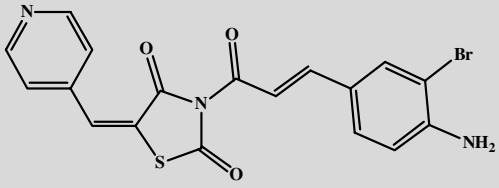
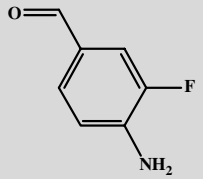
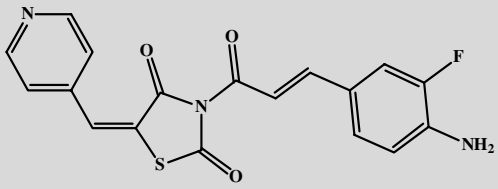
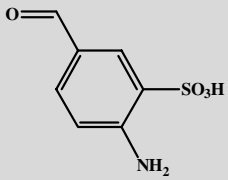
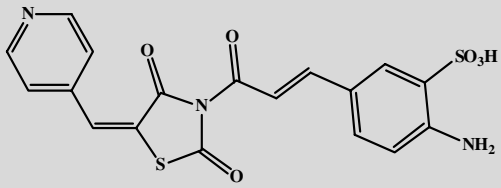
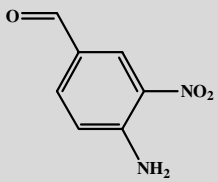
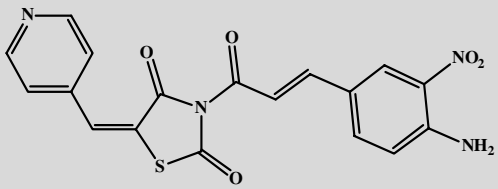
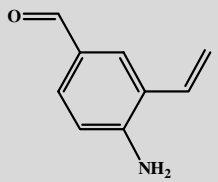
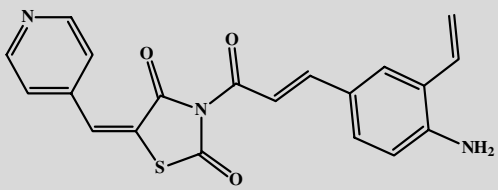
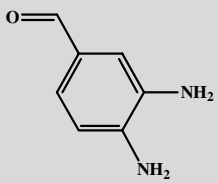
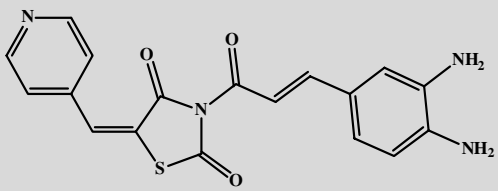


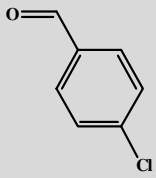
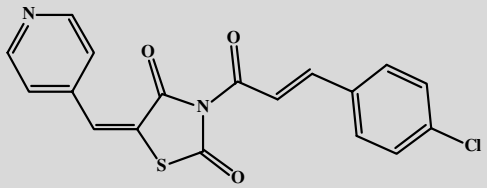
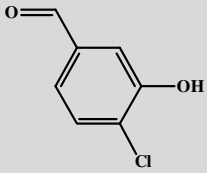
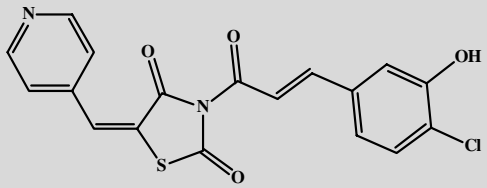
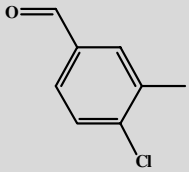
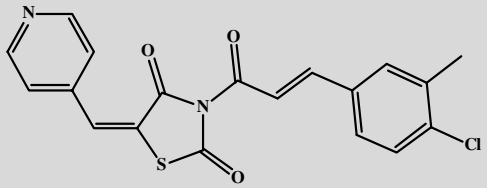
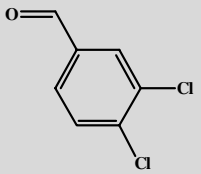
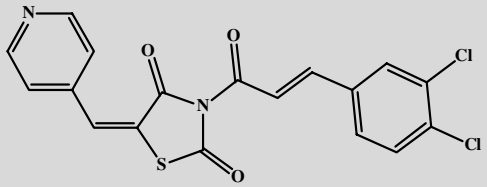
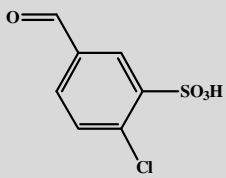
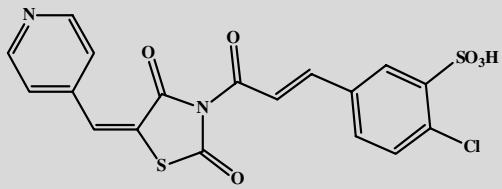
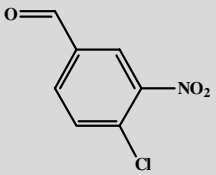
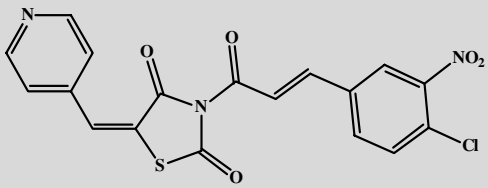
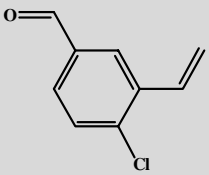
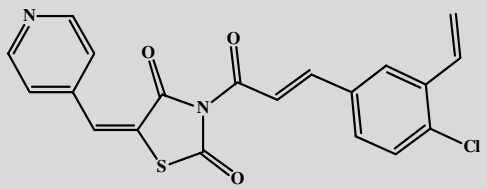
5-[(1*E*)-3-{(5*Z*)-2,4-Dioxo-5-[(pyridin-4-yl)methylidene]-1,3-thiazolidin-3-yl}-3-oxoprop-1-en-1-yl]-2-hydroxybenzene-1-sulfonic acid

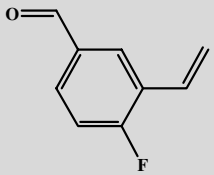
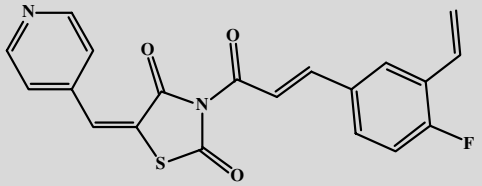
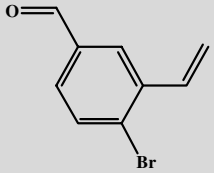
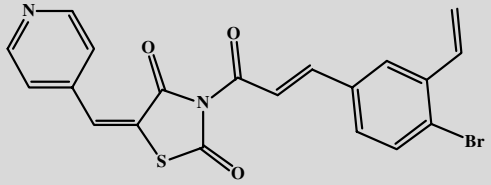
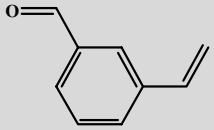
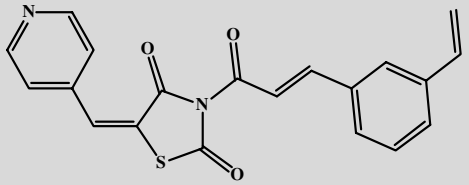
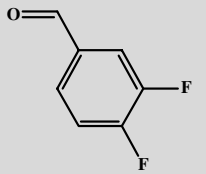
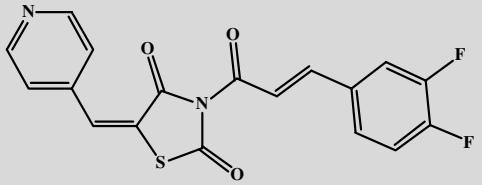
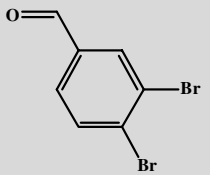
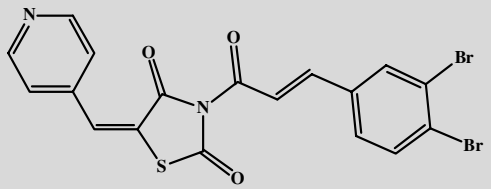
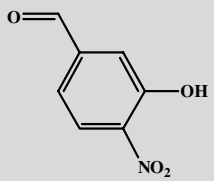
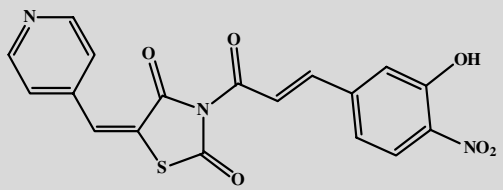
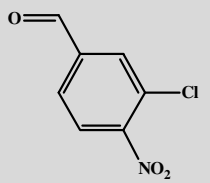
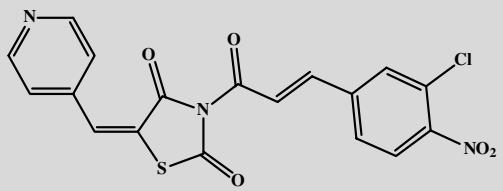
-4.3

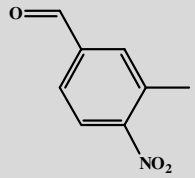
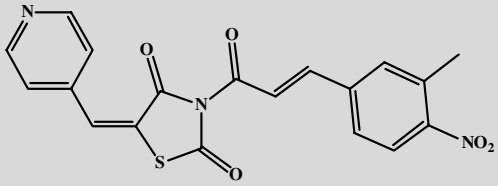
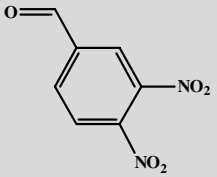
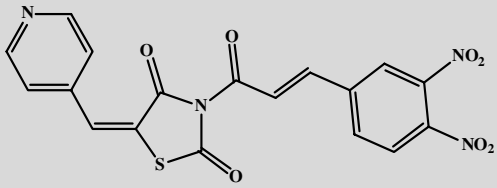
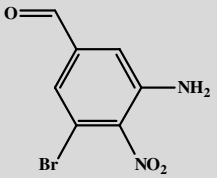
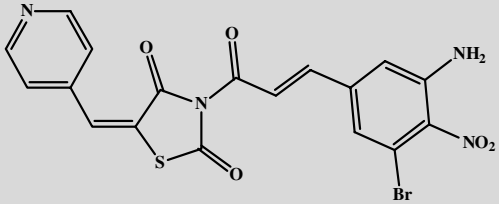
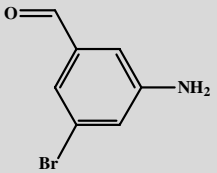
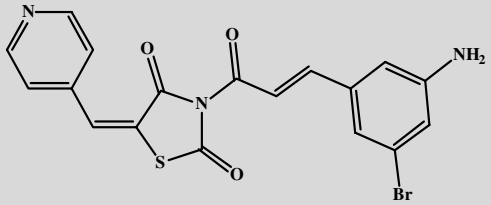
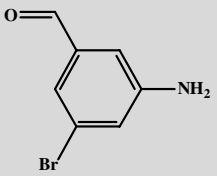
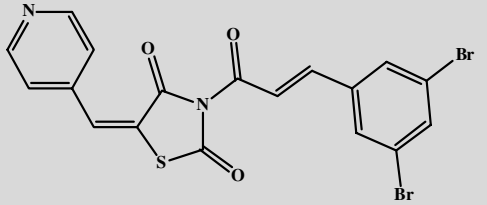
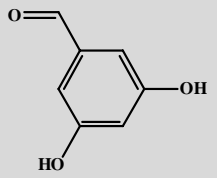
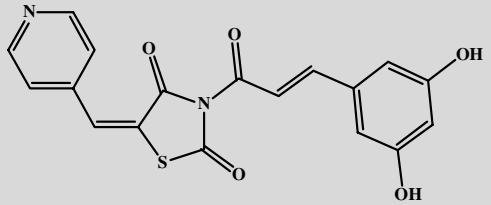
SMI-IV-10	 4-Methylbenzaldehyde	 (5*Z*)-3-[(2*E*)-3-(4-Methylphenyl)prop-2-enoyl]-5-[(pyridin-4-yl)methylidene]-1,3-thiazolidine-2,4-dione	-3.0
SMI-IV-11	 3-Amino-4-methylbenzaldehyde	 (5*Z*)-3-[(2*E*)-3-(3-Amino-4-methylphenyl)prop-2-enoyl]-5-[(pyridin-4-yl)methylidene]-1,3-thiazolidine-2,4-dione	-4.0
SMI-IV-12	 3-Chloro-4-methylbenzaldehyde	 (5*Z*)-3-[(2*E*)-3-(3-Chloro-4-methylphenyl)prop-2-enoyl]-5-[(pyridin-4-yl)methylidene]-1,3-thiazolidine-2,4-dione	-3.6
SMI-IV-13	 3-Bromo-4-methylbenzaldehyde	 (5*E*)-3-[(2*E*)-3-(3-Bromo-4-methylphenyl)prop-2-enoyl]-5-[(pyridin-4-yl)methylidene]-1,3-thiazolidine-2,4-dione	-3.0
SMI-IV-14	 3-Fluoro-4-methylbenzaldehyde	 (5*E*)-3-[(2*E*)-3-(3-Fluoro-4-methylphenyl)prop-2-enoyl]-5-[(pyridin-4-yl)methylidene]-1,3-thiazolidine-2,4-dione	-3.2
SMI-IV-15	 4-Methyl-3-nitrobenzaldehyde	 (5*E*)-3-[(2*E*)-3-(4-Methyl-3-nitrophenyl)prop-2-enoyl]-5-[(pyridin-4-yl)methylidene]-1,3-thiazolidine-2,4-dione	-3.8
SMI-IV-16	 5-Formyl-2-methylbenzene-1-sulfonic acid	 5-[(1*E*)-3-{(5*E*)-2,4-Dioxo-5-[(pyridin-4-yl)methylidene]-1,3-thiazolidin-3-yl}-3-oxoprop-1-en-1-yl]-2-methylbenzene-1-sulfonic acid	-3.5

SMI-IV-17	 3-Hydroxy-4-methylbenzaldehyde	 (5*E*)-3-[(2*E*)-3-(3-Hydroxy-4-methylphenyl)prop-2-enoyl]-5-[(pyridin-4-yl)methylidene]-1,3-thiazolidine-2,4-dione	-3.9
SMI-IV-18	 3,4-Dihydroxybenzaldehyde	 (5*E*)-3-[(2*E*)-3-(3,4-Dihydroxyphenyl)prop-2-enoyl]-5-[(pyridin-4-yl)methylidene]-1,3-thiazolidine-2,4-dione	-4.1
SMI-IV-19	 3,4-Dimethylbenzaldehyde	 (5*E*)-3-[(2*E*)-3-(3,4-Dimethylphenyl)prop-2-enoyl]-5-[(pyridin-4-yl)methylidene]-1,3-thiazolidine-2,4-dione	-4.6
SMI-IV-20	 3-Ethenyl-4-methylbenzaldehyde	 (5*E*)-3-[(2*E*)-3-(3-*E*thenyl-4-methylphenyl)prop-2-enoyl]-5-[(pyridin-4-yl)methylidene]-1,3-thiazolidine-2,4-dione	-3.0
SMI-IV-21	 4-Aminobenzaldehyde	 (5*E*)-3-[(2*E*)-3-(4-Aminophenyl)prop-2-enoyl]-5-[(pyridin-4-yl)methylidene]-1,3-thiazolidine-2,4-dione	-3.3
SMI-IV-22	 4-Amino-3-methylbenzaldehyde	 (5*E*)-3-[(2*E*)-3-(4-Amino-3-methylphenyl)prop-2-enoyl]-5-[(pyridin-4-yl)methylidene]-1,3-thiazolidine-2,4-dione	-3.4
SMI-IV-23	 4-Amino-3-hydroxybenzaldehyde	 (5*E*)-3-[(2*E*)-3-(4-Amino-3-hydroxyphenyl)prop-2-enoyl]-5-[(pyridin-4-yl)methylidene]-1,3-thiazolidine-2,4-dione	**-5.9**

SMI-IV-24	 4-Amino-3-chlorobenzaldehyde	 (5*E*)-3-[(2*E*)-3-(4-Amino-3-chlorophenyl)prop-2-enoyl]-5-[(pyridin-4-yl)methylidene]-1,3-thiazolidine-2,4-dione	-3.2
SMI-IV-25	 4-Amino-3-bromobenzaldehyde	 (5*E*)-3-[(2*E*)-3-(4-Amino-3-bromophenyl)prop-2-enoyl]-5-[(pyridin-4-yl)methylidene]-1,3-thiazolidine-2,4-dione	-2.8
SMI-IV-26	 4-Amino-3-fluorobenzaldehyde	 (5*E*)-3-[(2*E*)-3-(4-Amino-3-fluorophenyl)prop-2-enoyl]-5-[(pyridin-4-yl)methylidene]-1,3-thiazolidine-2,4-dione	-3.8
SMI-IV-27	 2-Amino-5-formylbenzene-1-sulfonic acid	 2-Amino-5-[(1*E*)-3-{(5*E*)-2,4-dioxo-5-[(pyridin-4-yl)methylidene]-1,3-thiazolidin-3-yl}-3-oxoprop-1-en-1-yl]benzene-1-sulfonic acid	-3.8
SMI-IV-28	 4-Amino-3-nitrobenzaldehyde	 (5*E*)-3-[(2*E*)-3-(4-Amino-3-nitrophenyl)prop-2-enoyl]-5-[(pyridin-4-yl)methylidene]-1,3-thiazolidine-2,4-dione	-4.5
SMI-IV-29	 4-Amino-3-ethenylbenzaldehyde	 (5*E*)-3-[(2*E*)-3-(4-Amino-3-ethenylphenyl)prop-2-enoyl]-5-[(pyridin-4-yl)methylidene]-1,3-thiazolidine-2,4-dione	-3.8
SMI-IV-30	 3,4-Diaminobenzaldehyde	 (5*E*)-3-[(2*E*)-3-(3,4-Diaminophenyl)prop-2-enoyl]-5-[(pyridin-4-yl)methylidene]-1,3-thiazolidine-2,4-dione	-3.7

SMI-IV-31	 4-Chlorobenzaldehyde	 (5*E*)-3-[(2*E*)-3-(4-Chlorophenyl)prop-2-enoyl]-5-[(pyridin-4-yl)methylidene]-1,3-thiazolidine-2,4-dione	**-4.9**
SMI-IV-32	 4-Chloro-3-hydroxybenzaldehyde	 (5*E*)-3-[(2*E*)-3-(4-Chloro-3-hydroxyphenyl)prop-2-enoyl]-5-[(pyridin-4-yl)methylidene]-1,3-thiazolidine-2,4-dione	-4.2
SMI-IV-33	 4-Chloro-3-methylbenzaldehyde	 (5*E*)-3-[(2*E*)-3-(4-Chloro-3-methylphenyl)prop-2-enoyl]-5-[(pyridin-4-yl)methylidene]-1,3-thiazolidine-2,4-dione	-3.3
SMI-IV-34	 3,4-Dichlorobenzaldehyde	 (5*E*)-3-[(2*E*)-3-(3,4-Dichlorophenyl)prop-2-enoyl]-5-[(pyridin-4-yl)methylidene]-1,3-thiazolidine-2,4-dione	-4.2
SMI-IV-35	 2-Chloro-5-formylbenzene-1-sulfonic acid	 2-Chloro-5-[(1*E*)-3-{(5*E*)-2,4-dioxo-5-[(pyridin-4-yl)methylidene]-1,3-thiazolidin-3-yl}-3-oxoprop-1-en-1-yl]benzene-1-sulfonic acid	-2.8
SMI-IV-36	 4-Chloro-3-nitrobenzaldehyde	 (5*E*)-3-[(2*E*)-3-(4-Chloro-3-nitrophenyl)prop-2-enoyl]-5-[(pyridin-4-yl)methylidene]-1,3-thiazolidine-2,4-dione	-3.7
SMI-IV-37	 4-Chloro-3-ethenylbenzaldehyde	 (5*E*)-3-[(2*E*)-3-(4-Chloro-3-ehenylphenyl)prop-2-enoyl]-5-[(pyridin-4-yl)methylidene]-1,3-thiazolidine-2,4-dione	-3.8

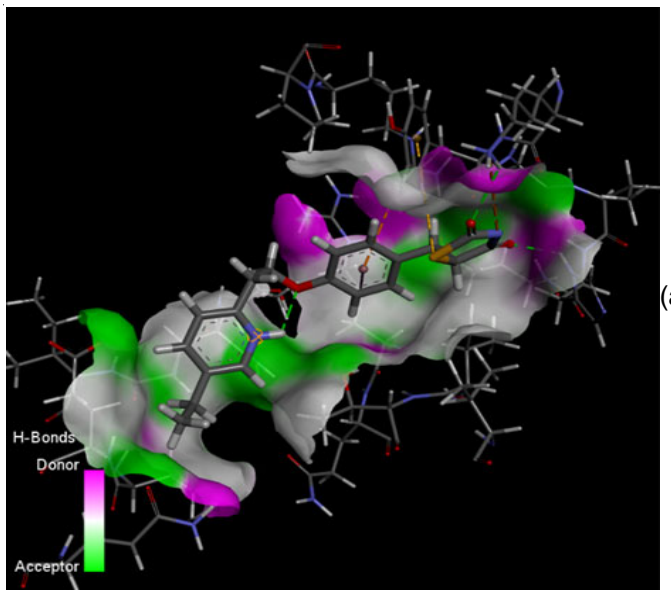
SMI-IV-38	 4-Ethenyl-4-fluorobenzaldehyde	 (5*E*)-3-[(2*E*)-3-(3-Ehenyl-4-fluorophenyl)prop-2-enoyl]-5-[(pyridin-4-yl)methylidene]-1,3-thiazolidine-2,4-dione	-3.2
SMI-IV-39	 4-Bromo-3-ethenylbenzaldehyde	 (5*E*)-3-[(2*E*)-3-(4-Bromo-3-ethenylphenyl)prop-2-enoyl]-5-[(pyridin-4-yl)methylidene]-1,3-thiazolidine-2,4-dione	-3.0
SMI-IV-40	 3-Ethenylbenzaldehyde	 (5*E*)-3-[(2*E*)-3-(3-Ethenylphenyl)prop-2-enoyl]-5-[(pyridin-4-yl)methylidene]-1,3-thiazolidine-2,4-dione	-3.3
SMI-IV-41	 3,4-Difluorobenzaldehyde	 (5*E*)-3-[(2*E*)-3-(3,4-Difluorophenyl)prop-2-enoyl]-5-[(pyridin-4-yl)methylidene]-1,3-thiazolidine-2,4-dione	-4.0
SMI-IV-42	 3,4-Dibromobenzaldehyde	 (5*E*)-3-[(2*E*)-3-(3,4-Dibromophenyl)prop-2-enoyl]-5-[(pyridin-4-yl)methylidene]-1,3-thiazolidine-2,4-dione	-3.7
SMI-IV-43	 3-Hydroxy-4-nitrobenzaldehyde	 (5*E*)-3-[(2*E*)-3-(3-Hydroxy-4-nitrophenyl)prop-2-enoyl]-5-[(pyridin-4-yl)methylidene]-1,3-thiazolidine-2,4-dione	-3.2
SMI-IV-44	 3-Chloro-4-nitrobenzaldehyde	 (5*E*)-3-[(2*E*)-3-(3-Chloro-4-nitrophenyl)prop-2-enoyl]-5-[(pyridin-4-yl)methylidene]-1,3-thiazolidine-2,4-dione	-3.5

SMI-IV-45	 3-Methyl-4-nitrobenzaldehyde	 (5*E*)-3-[(2*E*)-3-(3-Methyl-4-nitrophenyl)prop-2-enoyl]-5-[(pyridin-4-yl)methylidene]-1,3-thiazolidine-2,4-dione	-3.7
SMI-IV-46	 3,4-Dinitrobenzaldehyde	 (5*E*)-3-[(2*E*)-3-(3,4-Dinitrophenyl)prop-2-enoyl]-5-[(pyridin-4-yl)methylidene]-1,3-thiazolidine-2,4-dione	-3.6
SMI-IV-47	 3-Amino-5-bromo-4-nitrobenzaldehyde	 (5*E*)-3-[(2*E*)-3-(3-Amino-5-bromo-4-nitrophenyl)prop-2-enoyl]-5-[(pyridin-4-yl)methylidene]-1,3-thiazolidine-2,4-dione	-4.0
SMI-IV-48	 3-Amino-5-bromobenzaldehyde	 (5*E*)-3-[(2*E*)-3-(3-Amino-5-bromophenyl)prop-2-enoyl]-5-[(pyridin-4-yl)methylidene]-1,3-thiazolidine-2,4-dione	-3.2
SMI-IV-49	 3,5-Dibromobenzaldehyde	 (5*E*)-3-[(2*E*)-3-(3,5-Dibromophenyl)prop-2-enoyl]-5-[(pyridin-4-yl)methylidene]-1,3-thiazolidine-2,4-dione	-2.9
SMI-IV-50	 3,5-Dihydroxybenzaldehyde	 (5*E*)-3-[(2*E*)-3-(3,5-Dihydroxyphenyl)prop-2-enoyl]-5-[(pyridin-4-yl)methylidene]-1,3-thiazolidine-2,4-dione	-4.6

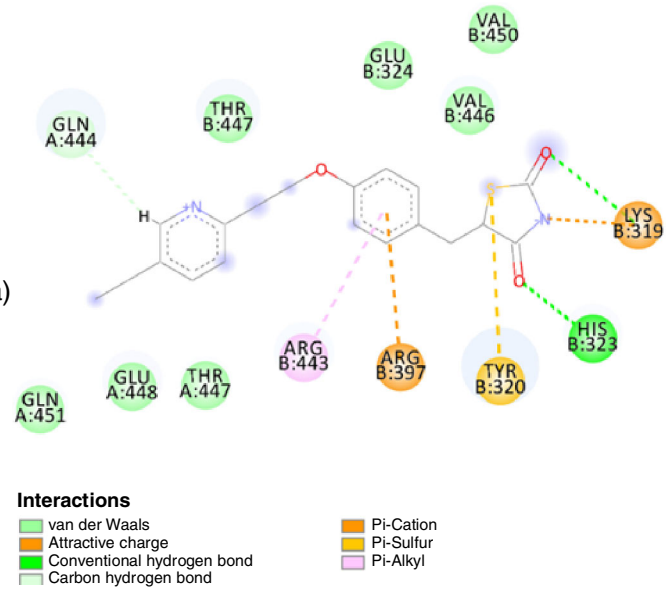
interactions and 2D interactions of the standard and the compounds with best three derivative's binding energies are shown in Fig. 1.

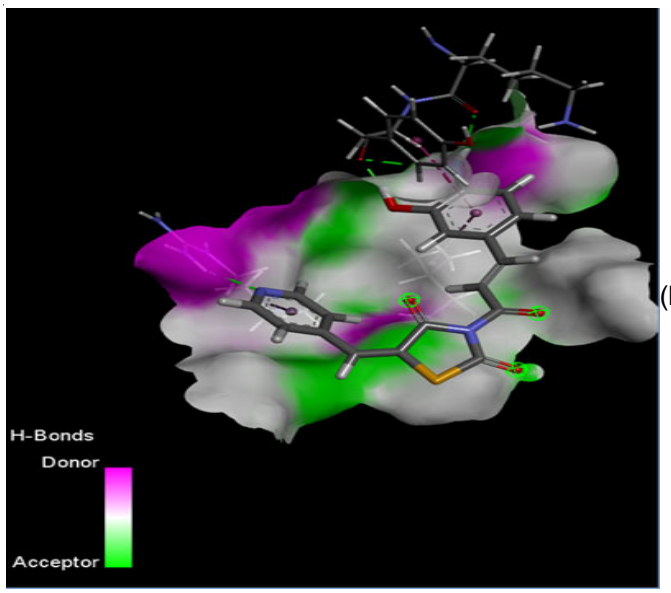
The FT-IR, NMR and mass techniques were characterized for best derived compound (SMI-IV-23) and the molecular wt. of the said compounds was 367.386 for SMI-IV-4, 366.398 for SMI-IV-31 and 367.386 for SMI-IV-23. Based on the characterization, various pharmacokinetics studies were performed and the results are tabulated in Table-2.

***In vitro* antihyperlipidemic activity:** In Table-3, the values are depicted for *in vitro* assay of antihyperlipidemic activity in in mature 3T3-L1 adipocytes. Compound SMI-IV-23 showed better result than other two compounds in terms of cell viability, triglycerides and cholesterol content.

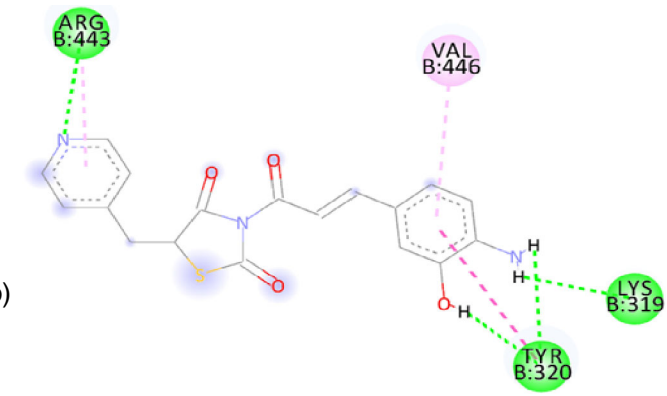

H-Bonds
Donor
Acceptor

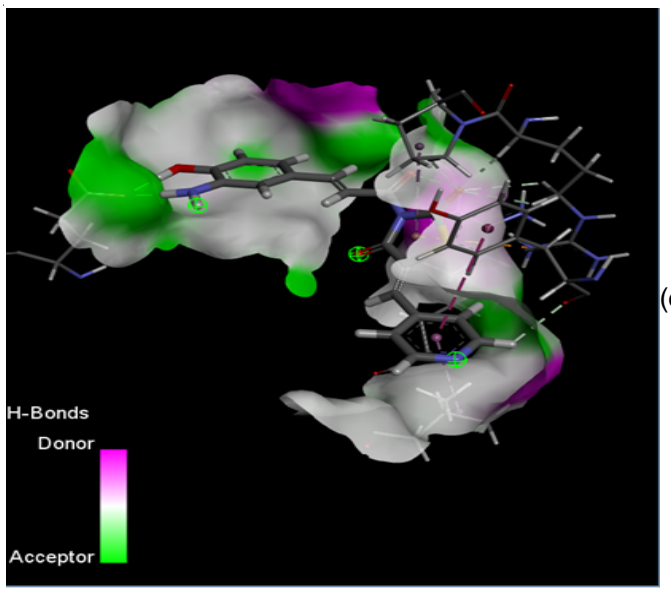
(a)


VAL B:450
GLU B:324
VAL B:446
THR B:447
GLN A:444
LYS B:319
HIS B:323
ARG B:443
ARG B:397
TYR B:320
GLU A:448
THR A:447
GLN A:451
Interactions
van der Waals
Attractive charge
Conventional hydrogen bond
Carbon hydrogen bond
Pi-Cation
Pi-Sulfur
Pi-Alkyl

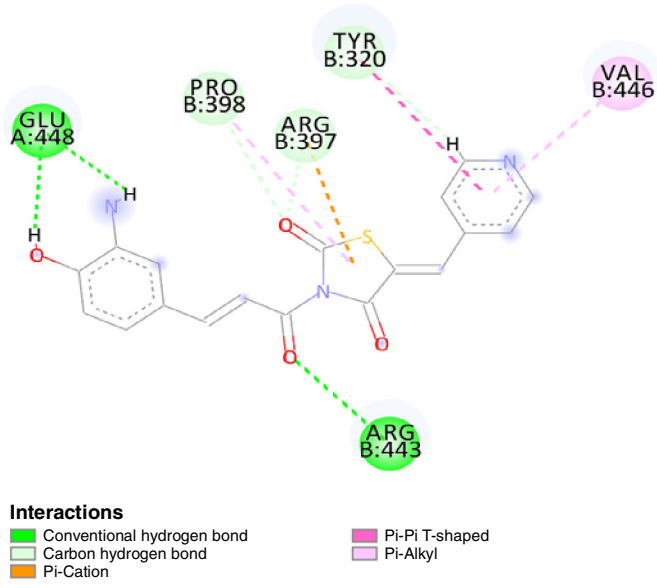

H-Bonds
Donor
Acceptor

(b)


ARG B:443
VAL B:446
LYS B:319
TYR B:320
Interactions
Conventional hydrogen bond
Pi-Pi T-shaped
Pi-Alkyl


H-Bonds
Donor
Acceptor

(c)


TYR B:320
VAL B:446
PRO B:398
GLU A:448
ARG B:397
ARG B:443
Interactions
Conventional hydrogen bond
Carbon hydrogen bond
Pi-Cation
Pi-Pi T-shaped
Pi-Alkyl

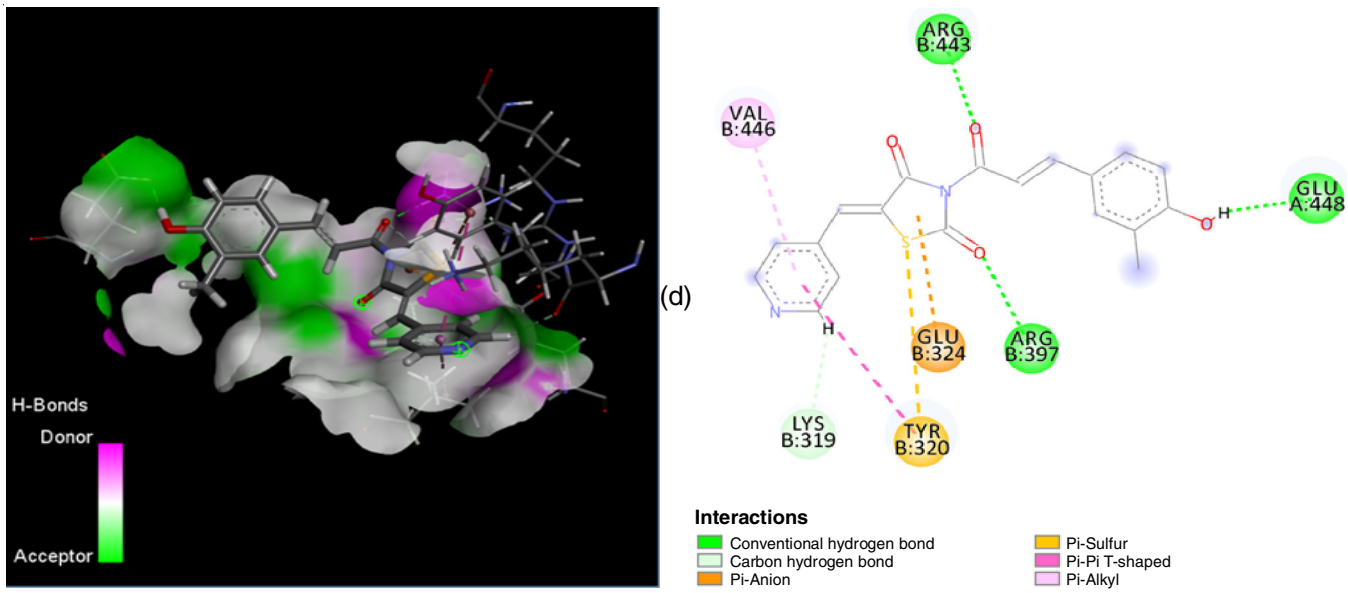


Fig. 1. 3D and 2D interaction of (a) pioglitazone, (b) SMI-IV-23, (c) SMI-IV-04 and (d) SMI-IV-03 with crystal structure of 6QJ5

TABLE-2
PHARMACOKINETICS PROPERTIES

Drug absorption properties				
Compound	Water solubility	$CaCO_2$ permeability	Intestinal absorption (human)	Skin permeability
SMI-IV-23	-3.916	0.891	85.697	-2.801
SMI-IV-4	-3.783	0.895	82.794	-2.808
SMI-IV-31	-4.516	1.193	93.524	-3.017
Drug distribution properties				
Compound	VDss (human)	Fraction unbound (human)	BBB permeability	CNS permeability
SMI-IV-23	-0.33	0.181	-1.295	-2.507
SMI-IV-4	-0.276	0.185	-1.236	-2.519
SMI-IV-31	-0.124	0.108	-0.442	-2.21
Drug metabolism properties				
Compound	CYP2D6 substrate	CYP3A4 substrate	CYP1A2 inhibitor	CYP2C19 inhibitor
SMI-IV-23	No	No	No	No
SMI-IV-4	No	No	No	No
SMI-IV-31	No	Yes	Yes	Yes
Drug excretion properties				
Compound		Total clearance		Renal OCT2 substrate
SMI-IV-23		-0.378		No
SMI-IV-4		-0.377		No
SMI-IV-31		-0.447		No

VDss = Volume of distribution; BBB = Blood brain barrier; OCT2 = Organic cation transporter 2

***In vivo* animal experimentation:** The administration of Triton led to elevated blood levels of cholesterol, triglycerides, VLDL and LDL. The animals treated with thiazolidine derivatives (SMI-IV-31, SMI-IV-4 and SMI-IV-23) showed a notable decrease in blood levels of cholesterol, triglycerides, VLDL and LDL compared to the control group. Simvastatin (standard) had the most pronounced impact in decreasing cholesterol and LDL levels after 6 h. Thiazolidine derivative (SMI-IV-23) exhibited a significant decrease in blood cholesterol and LDL levels. This reduction was shown to be much greater than that achieved by regular simvastatin at 6, 24 and 48 h as observed in Table-4.

TABLE-3
In vitro ASSAY OF ANTIHYPERLIPIDEMIC ACTIVITY IN MATURE 3T3-L1 ADIPOCYTES

Treatment adipocytes	Cell viability (% of the control)	Triglycerides (mg TGs/mg protein)	Cholesterol (% of the control)
Untreated adipocytes	99.8±0.21	0.317±0.023	100.0±0.30
SMI-IV-23	108.2±0.02	0.142±0.023*	54.2±0.52*
SMI-IV-4	103.9±0.41	0.170±0.11	62.8±1.43*
SMI-IV-31	98.6±0.26	0.202±0.40	70.4±0.42

The results are shown as the average ± standard error of the mean (SEM) from three separate studies. Disparities among groups in relation to untreated control cells. Significant at $*p < 0.05$.

Simvastatin achieved the greatest decrease in triglyceride and VLDL levels after 6 h. Thiazolidine derivative (SMI-IV-23) caused a notable reduction in triglyceride and VLDL levels at both the 24 h and 48 h time points as observed in Table-5. Simvastatin and three specific compounds demonstrated a statistically significant ($p < 0.01$) elevation in blood HDL levels at 6 and 48 h compared to the control group (Table-6).

Rats with hyperlipidemia caused by Triton WR-1339 and treated with thiazolidine derivatives (SMI-IV) saw a reversal of the elevated levels of blood cholesterol, triglycerides, LDL and VLDL. This reversal occurred from 6 h up to 48 h for cholesterol, triglycerides and LDL and from 24 h for VLDL. The result obtained in the present study was similar with the earlier report [15,16]. The control mice exhibited a significant increase in triglyceride levels as a result of the inhibition of lipoprotein lipase (LPL) by Triton. Administration of thiazolidine derivatives (**SMI-IV**) led to a decrease in triglyceride levels and activated LPL, resulting in a reduction of serum triglyceride levels. LPL is a key enzyme involved in the metabolism of triglycerides. VLDL levels were further lowered considerably after 48 h [17].

TABLE-4
IMPACT OF THIAZOLIDINEDIONE DERIVATIVES ON OVERALL CHOLESTEROL LEVELS AND LOW-DENSITY LIPIDS IN RATS WITH TRITON-INDUCED HYPERLIPIDEMIA

Compounds	6 h		48 h	
	Serum cholesterol (mg/dl)	Serum LDL (mg/dl)	Serum cholesterol (mg/dl)	Serum LDL (mg/dl)
Control	110.26 ± 0.25	101.45 ± 0.36	84.12 ± 0.10	65.25 ± 0.28
SMI-IV-23	91.20 ± 0.67	55.42 ± 0.46	60.38 ± 1.68	50.36 ± 0.98
SMI-IV-4	92.44 ± 0.25	77.23 ± 2.05	75.12 ± 0.27	56.35 ± 1.45
SMI-IV-31	95.76 ± 1.36	78.35 ± 1.12	76.30 ± 0.28	58.02 ± 0.40
Simvastatin	34.74 ± 0.60	38.40 ± 1.64	45.46 ± 0.70	34.52 ± 0.96

Mean ± S.D. (n = 6) is the way the values are presented. The concentrations of cholesterol and low-density lipoprotein (LDL) are calculated using the usual technique and are shown as mg/dl serum. Compared to the *Control group and #Simvastatin, there are significant differences ($p < 0.05$, $p < 0.01$, $p < 0.001$).

TABLE-5
EFFECTS OF THIAZOLIDINEDIONE DERIVATIVES ON TOTAL TRIGLYCERIDE AND VERY LOW DENSITY LIPIDS LEVELS IN TRITON-INDUCED HYPERLIPIDEMICRATS

Compounds	6 h		48 h	
	Serum cholesterol (mg/dl)	Serum LDL (mg/dl)	Serum cholesterol (mg/dl)	Serum LDL (mg/dl)
Control	72.15 ± 1.34	14.12 ± 0.42	64.72 ± 0.35	11.46 ± 0.32
SMI-IV-23	63.50 ± 0.67	15.08 ± 0.40	51.63 ± 0.40	12.55 ± 1.79
SMI-IV-4	69.28 ± 1.08	15.48 ± 0.65	59.22 ± 0.85	12.89 ± 1.22
SMI-IV-31	70.45 ± 0.84	17.85 ± 1.27	60.23 ± 0.22	14.62 ± 1.41
Simvastatin	40.75 ± 0.10	12.10 ± 0.24	22.45 ± 2.50	10.55 ± 0.45

Mean ± S.D. (n = 6) is the way the values are presented. The concentrations of cholesterol and low-density lipoprotein (LDL) are calculated using the usual technique and are shown as mg/dl serum. Compared to the *Control group and #Simvastatin, there are significant differences ($p < 0.05$, $p < 0.01$, $p < 0.001$).

TABLE-6
HYPOLIPIDEMIA PRODUCED BY TRITON AND ITS TREATMENT WITH THIAZOLIDINE DERIVATIVES (SMI-IV) AND ITS EFFECTS ON HDL LEVELS IN RATS

Compound	Serum HDL (mg/dl)	
	6 h	48 h
Control	42.58 ± 0.25	41.74 ± 0.60
SMI-IV-23	43.78 ± 0.46	28.52 ± 0.40*
SMI-IV-4	44.10 ± 0.55	31.46 ± 0.34
SMI-IV-31	44.75 ± 0.14	31.68 ± 0.25
Simvastatin	39.46 ± 0.20	20.35 ± 0.34*

Mean ± S.D. (n = 6) is used to express the values. The concentrations of cholesterol and low-density lipoprotein (LDL) are calculated using the usual technique and are shown as mg/dl serum. Compared to the control group and Simvastatin, there are significant differences (*# $p < 0.05$, $p < 0.01$, $p < 0.001$).

Conclusion

This study involved conducting *in silico* docking studies on newly synthesized thiazolidinedione derivatives to evaluate their potential as antihyperlipidemic medicines. The PPARgamma crystal structure (PDB ID: 6QJ5) was used as a reference for comparison. Significant docking scores indicated compounds SMI-IV-23, SMI-IV-4 and SMI-IV-31 showed significant scoring with H-bonding and found to be potent in mitigation of hyperlipidemic activity. Thereafter, pharmacokinetics study revealed the applied compounds are safe and may be used for further.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

REFERENCES

1. E. Jung, S.Y. Kong, Y.S. Ro, H.H. Ryu and S.D. Shin, *Int. J. Environ. Res. Public Health*, **19**, 8272 (2022); https://doi.org/10.3390/ijerph19148272
2. E. Mahase, *BMJ*, **379**, o2659 (2022); https://doi.org/10.1136/bmj.o2659
3. F.S. Ahmad, H. Ning, J.D. Rich, C.W. Yancy, D.M. Lloyd-Jones and J.T. Wilkins, *JACC Heart Fail.*, **4**, 911 (2016); https://doi.org/10.1016/j.jchf.2016.08.001
4. R. Hajar, *Heart Views*, **18**, 109 (2017); https://doi.org/10.4103/HEARTVIEWS.HEARTVIEWS_106_17
5. Y. Rochlani, N.V. Pothineni, S. Kovelamudi and J.L. Mehta, *Ther. Adv. Cardiovasc. Dis.*, **11**, 215 (2017); https://doi.org/10.1177/1753944717711379
6. J.L. Rodgers, J. Jones, S.I. Bolleddu, S. Vanthenapalli, L.E. Rodgers, K. Shah, K. Karia and S.K. Panguluri, *J. Cardiovasc. Dev. Dis.*, **6**, 19 (2019); https://doi.org/10.3390/jcdd6020019
7. C.E. Quinn, P.K. Hamilton, C.J. Lockhart and G.E. McVeigh, *Br. J. Pharmacol.*, **153**, 636 (2008); https://doi.org/10.1038/sj.bjp.0707452
8. B. Cariou, B. Charbonnel and B. Staels, *Trends Endocrinol. Metab.*, **23**, 205 (2012); https://doi.org/10.1016/j.tem.2012.03.001
9. Z.N. Zhu, Y.F. Jiang and T. Ding, *Bone*, **68**, 115 (2014); https://doi.org/10.1016/j.bone.2014.08.010
10. A.J. Scheen, *Drug Saf.*, **24**, 873 (2001); https://doi.org/10.2165/00002018-200124120-00002
11. B. García-Carrasco, R. Fernandez-Dacosta, A. Dávalos, J. Ordovás and A. Rodriguez-Casado, *Med. Sci.*, **3**, 38 (2015); https://doi.org/10.3390/medsci3020038

12. M. Ghasemi, T. Turnbull, S. Sebastian and I. Kempson, *Int. J. Mol. Sci.*, **22**, 12827 (2021); https://doi.org/10.3390/ijms222312827
13. I.M. Nagendranayak, R. Chinta, K.B. Nagaraju and R. Jetti, *Roman. J. Diabetes Nutr. Metab. Dis.*, **28**, 352 (2021); https://doi.org/10.46389/rjd-2021-1052
14. M.J. Zibaeenezhad, M. Shahamat, S.H. Mosavat, A. Attar and E. Bahramali, *Oncotarget*, **8**, 79636 (2017); https://doi.org/10.18632/oncotarget.18956
15. M.K. Wojczynski, S.P. Glasser, A. Oberman, E.K. Kabagambe, P.N. Hopkins, M.Y. Tsai, R.J. Straka, J.M. Ordovas and D.K. Arnett, *Lipids Health Dis.*, **10**, 181 (2011); https://doi.org/10.1186/1476-511X-10-181
16. S. Tiwari and S.A. Siddiqi, *Arterioscler. Thromb. Vasc. Biol.*, **32**, 1079 (2012); https://doi.org/10.1161/ATVBAHA.111.241471
17. L. Zhang, K. Reue, L.G. Fong, S.G. Young and P. Tontonoz, *Arterioscler. Thromb. Vasc. Biol.*, **32**, 2541 (2012); https://doi.org/10.1161/ATVBAHA.112.250571