Research Article

Synthesis, Characterization and Discovery Novel Anti-diabetic and Anti-hyperlipidemic Thiazolidinedione Derivatives.

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ABSTRACT

Several therapeutic anti-diabetic drugs, were prepared using thiazolidinedione nucleus. Thus, some thiazolidinedione derivatives **2-5** were prepared. The structures of these compounds established on the basis of IR, ^{1}HNMR, ^{13}CNMR and MS data. Moreover, the optimization geometries for compounds **2-5** were discussed using DFT theory with B3LYP\6-311G base set. The molecular docking simulations into the active site of PPARγ were performed, and showed that, the compound (**5**) more suitable inhibitor against PPARγ, and can used as antidiabetic drug. The most suitable inhibitor (**5**) was evaluated as anti-diabetic and anti-hyperlipidemic activity (*in vivo*) using alloxan animal model, as well as antioxidant activity using DPPH radical scavenging activity method. The compound (**5**) with dose (50 mg/kg) showed, significant decrease in blood glucose, cholesterol, triglyceride and LDL levels, and increase in serum HDL level, as the time when compared with diabetic control group on day 2 and 4, after alloxan induced diabetes, moreover, compound (**5**) has antioxidant activity.

Keywords: antidiabetic, DFT, PPAR, Thiazolidinediones and rat.

INTRODUCTION

Diabetes mellitus type 2(DMII) is a common chronic, multi factorial and multi genetic disease, which occurs as combination metabolic disorder; insulin resistance and beta pancreas cell insufficiency[1].

The retinopathy, neuropathy, nephropathy and cataract are associated diabetic complications with DMII[2].

The stimulating insulin production from the pancreas or increasing the sensitivity of the body cells to insulin is the basic mechanism of the anti-diabetic drugs[3].

The peroxisome proliferator activated receptor-gamma (PPARγ), is a member of a super family of nuclear receptors[4-6], which play a pivotal role in regulating the expression of a large number of genes involved in lipid metabolism and energy balance[4-6].

The presence of (PPARγ) in the nucleus is able to promote the differentiation of lipocytes, and increasing the insulin sensitivity, and retard the occurrence of complications[7-9].

Thiazolidinediones (TZDs) are high-affinity selective agonists of (PPARγ), which normalizing glucose metabolism associated with insulin resistance without hypoglycemia[7-9].

Many thiazolidinediones derivatives (Figure 1), have been marketed for the treatment of DMII[10-14], these drugs are found to cause undesirable side effects including weight gain and edema[15,16].

The dual agonists of PPARα and PPARγ reported as useful treatment of hyperglycemia and hyperlipidemia[17-21], but, its agonists adverse effects like edema, carcinogenicity in rodent toxicity models, heart failure or cardiovascular deaths and elevated serum creatinine[22].

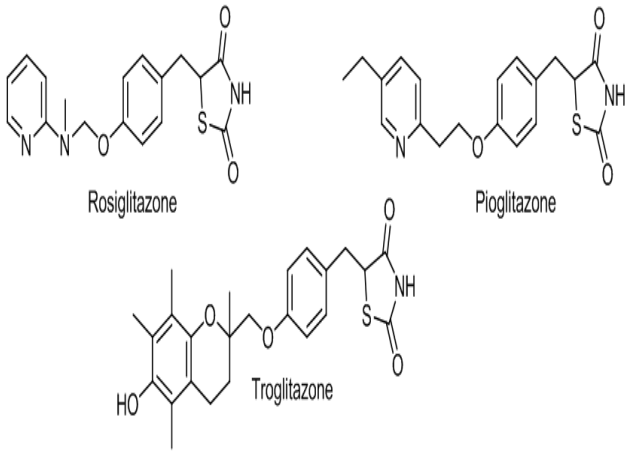


Figure 1

The unsuccessful efforts to develop dual agonist, so, the recent project aims to development synthetic activator of PPARα and PPARγ, and identify activities through, docking studies, and elevate lower triglycerides LDL, HDL and exert insulin-sensitizing effects remedy for disorders mediated by lipid and carbohydrate metabolism.

MATERIALS AND METHODS

Chemistry

Instrumentation and Materials

Melting points are uncorrected. IR spectra were recorded on a Shimadzu 440 infrared spectrophotometer (υ; cm^{-1}) using the KBr technique (Shimadzu, Japan). NMR spectra

were recorded on a Varian Gemini spectrometer (δ; ppm) 400 MHz using TMS as internal standard.

Mass spectra were recorded on a Jeol-JMS-600 mass spectrometer. Micro analytical data were obtained from the Micro analytical Research Centre, Faculty of Science, Cairo University.

Synthesis

5-(Benzo[d][1,3]dioxol-5-ylmethylene)thiazolidine-2,4-dione(2).

The titled compound were prepared as previous report[24].

Potassium-5-(benzo[d][1,3]dioxol-5-ylmethylene)-2,4-dioxothiazolidin-3-ide (3).

The KOH (2.49 gm., 0.01mol) in EtOH (20 mL) containing catalytic amount of piperidine (0.5 mL) was added to compound **(2;** 0.01mol). The reaction mixture was heated under reflux for 1h. The solid product formed was collected by filtration and recrystallized from ethanol to give **(3).** pale crystals, yield 90 %, m.p. >300 °C*. IR (KBr, cm^{-1}): 3096 (CH-aromatic), 1690 and 1646 (C=O thiazolidinone and amide). ^{1}H NMR (DMSO-d_6) δ 6.092 (s, 2H, CH_2-benzodioxol.), 7.01-7.17(*m*, 3H, CH-arm.), 7.51(s, 1H, CH-methine). ^{1}CNMR (DMSO-d_6) δ 101.7 (C-benzodioxol.), 108.8, 120.9, 125.1, 125.3, 128.1, 128.63, 145.29 (C-arm. + C-thiazld-2,4-dione), 148.4(s, C-methine), 170.3, 172.4(C-carbonyl).Anal. Calcd for $C_{11}H_6KNO_4S$ (286): C; 45.98, H; 2.09; N; 4.89. Found: C; 40.80, H; 2.10, N; 4.87.

5-(benzo[d][1,3]dioxol-5-ylmethylene)-3-(2-chloroacetyl) thiazolidine-2,4-dione (4)

To a solution of compound **(3;** 0.01mol) the chloroacetyl chloride (1.1 ml, 0.01mole) was added. The reaction mixture was stirred in DMF (50 ml) for 2h. The solid product formed was collected by filtration and recrystallized from acetic acid to give **(4).** yellow crystals, yield 65 %, m.p. 187-89 °C. IR (KBr, cm^{-1}): 2096(CH_2), 1706, and 1620 (C=O thiazolidinone and amide).^{1}H NMR (DMSO-d_6) δ 1.90 (s, 2H, CH_2-CH_2CO), 6.057 (s, 2H, CH_2-benzodioxol.), 7.01-7.10 (*m*, 3H, CH-arm.), 7.64 (s, 1H, CH-methine). ^{1}CNMR (DMSO-d_6) δ 40.28 (C-CH_2Cl), 101.9 (C-benzodioxol.), 101.9, 108.9, 109.0, 120.6, 120.9, 125.8, 127.0, 131.8 (C-arm. + C-thiazld-2,4-dione), 149.1 (s, C-methine), 167.2, 172.7, 172.9 (C-carbonyl). Anal. Calcd for $C_{13}H_8ClNO_4S$ (286): C; 45.98, H; 2.09; N; 4.89. Found: C; 40.80, H; 2.10, N; 4.87.

3,3'-(1-oxoethane-1,2-diyl)bis(5-(benzo[d][1,3]dioxol-5-ylmethylene) thiazolidine-2,4-dione)(5).

The compound **(4;** 0.01 mol) in DMF (20 mL) was reacted with (2; 0.01mol) in DMF (50ml.). The reaction mixture was heated for 3h. The solid product formed was collected by filtration and recrystallized from DMF to give **(5).** yellow crystals, yield 70 %, m.p. 290-292 °C. IR (KBr, cm^{-1}) : 1705, and 1650(C=O thiazolidinone and amide).7.10 (*m*, 3H, CH-arm.), 7.64 (s, 1H, CH-methine). ^{1}CNMR (DMSO-d_6) δ 40.28 (C-CH_2Cl), 101.9 (C-benzodioxol.), 101.9, 108.9, 109.0, 120.6, 120.9, 125.8, 127.0, 131.8 (C-arm. + C-thiazld-2,4-dione), 149.1(s, C-methine), 167.2, 172.7, 172.9 (C-carbonyl).640 (M^+, 0.98). Anal. Calcd for $C_{24}H_{14}N_2O_9S_2$ (538): C; 53.53, H2.60; N; 5.20. Found: C; 53.53, H; 2.62, N; 520.

Molecular Modeling Study

Generation of Ligand and Enzyme Structures

Docking study was carried out for the target compounds into (hPPAR-γ), the crystal structure of the (PDB: 2PRG) complexed with ***rosiglitazone*** was uploaded from the protein data bank PDB[33]**.**

Preparation of Small Molecule

All electronic structure calculations were performed using the MOE program. Geometry optimizations have been achieved using molecular orbital for density functional theory with aB3LYP\6-311G* basis set.

MOE Stepwise

The crystal structure of the (hPPAR-γ) with a Fid as inhibitor molecule, was used for the receptor molecule, Water and inhibitor molecules were removed, and hydrogen atoms were added. The parameters and charges assigned with MMFF94x force field. After alpha-site spheres were generated using the SITE FINDER module of MOE. The optimized 3D structures of molecules were subjected to generate different poses of ligands using triangular matcher placement method, which generating poses by aligning ligand triplets of atoms on triplets of alpha spheres representing in the receptor site points, a random triplet of alpha sphere centers is used to determine the pose during each iteration.

The pose generated rescored using London dG scoring function. The poses generated were refined with MMFF94x force field, also, the solvation effects were treated. The Born solvation model (GB/VI) used to calculate the final energy, and the finally assigned poses assigned a score based on the free energy in kcal/mol.

Pharmacological Study

Materials

Animals

Albino Wistar rats of either sex weighing 150-250 g purchased from the animal house colony of the National Research Center (Dokki, Giza, Egypt) and were kept in the animal house under conventional laboratory conditions.

Experiments were performed according to the National Regulations of Animal Welfare and Institutional Animal Ethical Committee (IAEC).

Drugs and Chemical Agents

Alloxan-monohydrate, Metformin and 2,2-Diphenyl-1-picrylhydrazyl radical (DPPH) were obtained from Sigma Aldrich Chemical Co. (USA). Reagent kits were obtained

from Biodiagnostic, Giza, Egypt. All other chemicals were of the highest available commercial grade.

Experimental Methods

Alloxan Induced Diabetes

Diabetes was induced by single subcutaneous injection of alloxan (100 mg / kg) [37]**.** The alloxan was freshly prepared by dissolving 100 mg in 1ml of normal saline solution. The animals were allowed to drink 5% glucose solution overnight to overcome the drug induced hypoglycemia. 48 hours after injection of alloxan, fasting plasma blood glucose was estimated. Animals with plasma glucose of >200 mg/dl were selected.

Rats were divided into 4 groups (n = 8), the animals were treated for 8 days as follows:

The Group I: Normal and serve as normal control.

The Group II: Alloxan (100mg/kg, s.c) and serve as disease control.

The Group III: Alloxan (100mg/kg, s.c) + Metformin (500 mg / kg) and serve as standard[38]**.**

The Group IV: Alloxan (100mg/kg, s.c) + compound5 (50 mg/kg, s.c).

Collection of Blood Samples

The blood samples were withdrawn on 0th, 2nd and 4th day from the retro orbital plexus of rats under anesthesia using a glass capillary tube after a fast of 6 hr and the blood was centrifuged (3000 rpm for 15 min) to get serum.

Biochemical Analysis

Determination of serum glucose level was evaluated according to the method of[39] and the level was expressed as mg/dl. Determination of lipid profile as serum cholesterol and triglycerides were done according to (**54,55)** as well as serum LDL and HDL according to **[56,57]** and the levels were expressed as mg/dl.

Statistical Analysis

Data are expressed as mean ± S.E. Data analysis was done using one way analysis of variance (ANOVA) followed by least significant difference (LSD) test for multiple comparisons. Difference was considered significant when *p* is less than 0.05. SPSS (version 11) program was used to carry out these statistical tests.

DPPH Radical Scavenging Activity

The assay of 2,2-Diphenyl-1-picrylhydrazyl radical (DPPH$^{\cdot}$) was performed according to the method adapted from[40,41]. DPPH (0.004% solution) in methanol was prepared and stored in dark until use. Preparation of the tested fractions at different concentrations was done in methanol. In a 96-well plate, addition of 20 µl of each concentration to 180 µl DPPH solution was carried out. Negative controls were done to correct for colored fractions. The resultant reaction mixtures were vortex-mixed and incubated at room temperature for 30 min. The absorbance of the reaction mixtures was measured at 520 nm. Methanol was used as blank and DPPH solution without addition of extract was used as control. Ascorbic acid was used as positive control. The scavenging activity was calculated by the following formula:

$$Scavenging\ Activity\ (\%) = \left(\frac{A_0 - A_1}{A_0}\right) \times 100$$

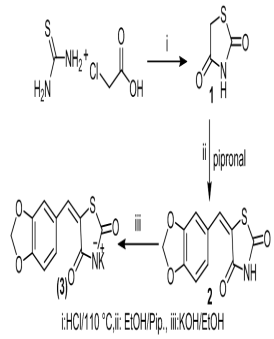
Where A_0 is the absorbance of the control and A_1 is the absorbance of the extract or reference standard. Concentrations yielding 50% inhibition (IC_{50}) values were determined from the graph of percentage of inhibition plotted against the concentration of extracts; using GraphPad Prism Software version 5.0.

RESULTS AND DISCUSSION

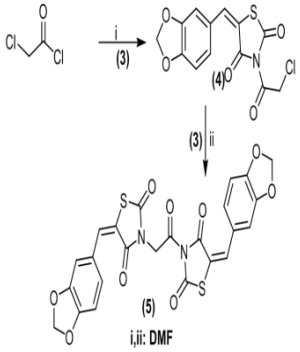
Chemistry

2,4-Thiazolidinedione **(1)** was used as our starting material[23], The compound **(2)** was prepared as reported later[24], (Scheme 1)**.**

The aryildine **(2)** was treated with alcoholic KOH to give corresponding potassium salt **(3),** Scheme 1.



Scheme 1



Scheme 2

The compound (**3**) reacted with chloroacetyl chloride, and affording 5-(benzo[d][1,3]dioxol-5-ylmethylene)-3-(2-chloroacetylthiazolidine-2,4- dione (**4**).

The bis-aryldine derivative (**5)** was obtained via the reaction of compound **(4)** with compound (**3**) in DMF, Scheme 2.

Molecular DOCKing Studies

Conformational Analysis

In trying to achieve better insight of the molecular structure, the optimization geometry were performed in vacuo using DFT withB3LYP\6-311G quantum mechanical level using MOE program for all synthesized compounds **3-5,** Figure 1.

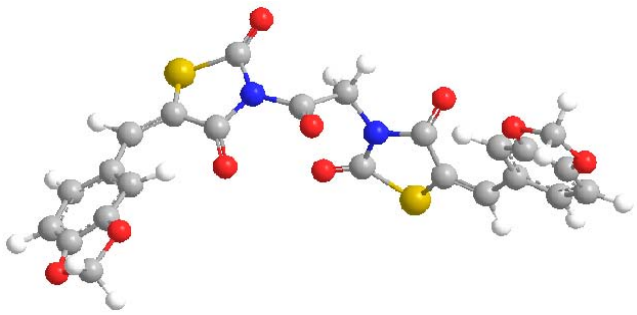


Figure 1: Optimization geometry of compound **5** using DFT with B3LYP\6-311G.

In most stable form of compound**5, F**igure 1, the two benzodioxolerings were arranged in plane withthiazolidine-2,4-dione rings, the two rings of thiazolidine-2,4-dione and benzodioxole, respectively, were stabilized with itself in parallel mode.

The deviation angle between two thiazolidine-2,4-dione and two benzodioxole rings are(~128° and 90°) respectively, the length of CC bond of $COCH_2$ linkage between two thiazolidine-2,4-dione moieties is(~1.54Å).

ADMET Factors Profiling

Oral bioavailability is playing an important role for the development of bioactive molecules as therapeutic agents. Many potential therapeutic agents fail to reach the clinic, because of their ADMET (absorption, distribution, metabolism, elimination and toxic) factors.

The computational study was performed, for prediction of ADMET properties of the molecules **3-5** including; determination of topological polar surface area (TPSA>140) have low oral bioavailability[25], a calculated percent absorption (% ABS) which was estimated by Zhao equation[26] and ''rule of five'' formulated by Lipinski[27].

Lipinski was established that, the chemical compound could be an orally active drug in humans, if no more than one violation of the following rule: (i) ClogP (partition coefficient between water and octanol) < 5. (ii) Number of hydrogen bond donors sites ≤ 5. (iii) Number of hydrogen bond acceptors sites ≤ 10. (iv) Molecular weight < 500 and molar refractivity should be between 40-130.

All calculated descriptors were performed using MOE Package[28], and their results were disclosed in (Table 1).

Our results revealed that, the CLogP (factor of the lipophilicity[29] less than 5.0, the molecular weight (MW < 500) except compound **5 (**MWt. = 538), hydrogen bond acceptors (5-10), hydrogen bond donors (0-5) and molar refractivity values ranged (~9-17), this data clear that, its compounds are fulfill Lipinski's rule.

The drug-likeliness calculated (Table 1), according to (ADME-T) algorithm[30,31], which examined the ability of compounds for transported across the intestinal epithelium, they probably have high affinity binding to the plasma proteins, and may be through the blood-brain barrier, and it necessary for ability drug transported throughout the body.

In general, No marked health effects in rodent toxicity profiles were observed, for its compounds **3-5** the compounds, and can be used as a good oral absorption.

Table 1: Pharmacokinetic parameters important for good oral bioavailability of compounds **3-5**

CPD	*TPSA*	*%ABS*	*Vol.*	*Logp*	*HBD*	*HBA*	*V*	*mr*	*LogS*	*D*
3	52.6	90.85	122	2.218	0	5	0	17.16	5	1
4	72.91	83.84	128	2.82	0	5	0	7.68	6	1
5	128.75	64.58	219	4.391	0	10	1	13.05	11	1
Ros	68.29	85.43	154	3.294	1	5	0	9.40	5	1

TPSA**: Polar surface area* A 2, ***%ABS: Absorption percentage, ***Vol:** Volume (A3), **M.wt.:** Molecular weight, **Log P**: Calculated lipophilicity., **Log S**: Solubility parameter, **HBA**: Number of hydrogen bond acceptor, **HBD**: Number of hydrogen bond donor, **V**: Number of violation from Lipinski's rule of five., mr: Molar Refractivity, D: drug likeness*

Table 2: Effect of compound 5 on serum cholesterol, triglyceride, low density lipoprotein (LDL) and high density lipoprotein (HDL) levels

Parameters	Normal control			Alloxan control (100mg/kg, s.c)			Cpd 5 (50 mg/kg, orally)		
	0th day	2nd day	4th day	0th day	2nd day	4th day	0th day	2nd day	4th day
Serum cholesterol	66.34 ± 0.91	67.47 ± 0.94	65.22 ± 0.48	106.87 ± 0.55[a]	107.69 ± 0.43[a]	107.47 ± 0.63[a]	106.34 ± 0.60[a]	84.80 ± 1.24[ab]	65.97 ± 1.05[b]
Serum triglyceride	83.95 ± 0.57	84.28 ± 0.70	83.31 ± 0.80	165.77 ± 1.59[a]	165.38 ± 1.54[a]	166.29 ± 1.63[a]	166.55 ± 1.69[a]	124.41 ± 0.66[ab]	117.99 ± 0.14[ab]
Serum LDL	153.58 ± 3.52	154.62 ± 4.39	150.99 ± 1.72	200.80 ± 1.76[a]	199.25 ± 0.78[a]	202.62 ± 2.9[a]	198.73 ± 0.64[a]	173.04 ± 7.04[ab]	153.58 ± 3.52[b]
Serum HDL	53.86 ± 2.81	56.77 ± 2.36	55.80 ± 2.55	27.17 ± 0.26[a]	27.42 ± 0.20[a]	28.87 ± 0.54[a]	27.42 ± 0.20[a]	42.98 ± 1.42[ab]	45.51 ± 2.28[ab]

Data represent the mean value ± S.E. of six rats per group, shown at 0th day (pre-drug values), 2nd and 4th day (Post - drug values) for each group and were analyzed by one-way ANOVA followed by LSD comparison test.

[a] Significantly different from normal control at P<0.05.; [b] Significantly different from diabetic control at P<0.05.

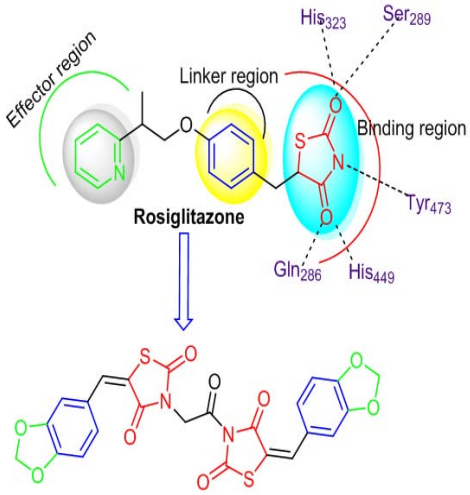


Figure 2: The compound **5** showing compliance of Rosiglitazone pharmacophore features which interact with PPARγ

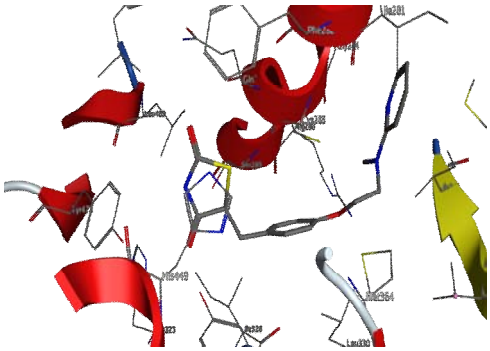


Figure 3: Interaction between ***rosiglitazone*** and binding site of hPPAR-γ (PDB: 2PRG), which red dot lines represented hydrogen bonding interaction of ligand with binding site. **Rosiglitazone** is represented in ball and stick mode, which carbon atoms are colored in dark grey, oxygen in red, nitrogen in blue and sulfur in yellow.

Docking Studies

The information analysis of 3D-QSAR studies, and the X-ray crystal structure of TZDs class of human PPARγ agonists revealed that, the TZD derivatives should have these structural elements to act as agonists for PPARγ: (i) acidic head fragment (TZD ring); to form H-bond interaction with amino acids protein residues ($His_{-449,}$ Tyr_{-473}, His_{-323}, Ser_{-289} and Gln_{-286}), Figure 2[32], (ii) a lipophilic side chain; which design to make antihyperglycemic agents are better, which modifies the pharmacokinetic and toxicity profiles of ligand[33], (iii) central aromatic linker region[13], Figure 2. So, the present project aimed to modification of effector site and linker region of pharmacophore, to development of better and safer antihyperglycemic drug than available ones (Figure 2).

The crystal structures of human PPAR-γ (hPPAR-γ) complexed with the agonist ***rosiglitazone*** (PDB: 2PRG)was employed[33]. In order to understand the binding mode for protein-ligand interactions, the docking study was carried out using MOE[38]. The tested compounds**2-5** docked into active site hPPAR-γ. The active site of the enzyme was defined to include residues within a 10.0 Å radius to any of the rosiglitazone atoms. The highest MOE scoring function of the most stable docking model for tested compounds applied to evaluate the binding affinities for the agonists **2-5**, which complexed with active site of hPPARγ (Table 3). The complexes were energy-minimized with molecular dynamic (MD) combined with molecular mechanics (MMFF94) force field[45], until the gradient convergence 0.05 kcal/mol reached. The ***rosiglitazone*** was re-docked into the active site of the enzyme, then replaced it with the tested compounds **2-5**, in order to compare the binding mode of ***rosiglitazone*** and the tested compounds.

The compounds docked successfully into the hPPAR-γ active site. The Dock scoring function was applied to evaluate the binding affinities between the (2PRG) and selected synthesized agonist **2-5**.

The original ligand reveals MOE score (-146.73 Kcal/mol) and forms **3** hydrogen bonds with: Ser-289, His-449 and Tyr-473, respectively, (Figure 3). The compound (**5**) gives highest binding affinity with MOE score (-155.08Kcal/mol) due to forming important interaction bonds with Ser-289, Ser-342 and His-449 (Figure 4), other members **2, 3** and **4** showed lower binding affinity values (~-134, -124.2 and -90.2), respectively, (Table 3).

Table 3: Docking energy scores (kcal/mol) derived from the MOE for new compounds **2-5**

Cpd.	dG	Int.	H.B.	*Eele*	*Evdw*	*E-sol*
Reg	-146.732	1.6	-146.73	-3.49	-146.73	200.71
2	-134.189	1	-134.18	-5.29	-134.18	207.83
3	-124.205	1.6	-124.20	-5.43	-124.20	199.18
4	-90.2141	1	-90.214	-3.33	-90.214	205.40
5	-155.085	-0.40	-155.08	-4.06	-155.08	241.62

d.G.: *free binding energy of the ligand from a given conformer*, **Int.**: affinity *binding energy of hydrogen bond interaction with receptor*, **H.B.:** *Hydrogen bonding energy between protein and ligand.* **Eele:** *the electrostatic interaction with the receptor*, **Evdw:** *van der Waals energies between the ligand and the receptor*, ***E-sol.****: energies between the ligand and the solvent for receptor.*

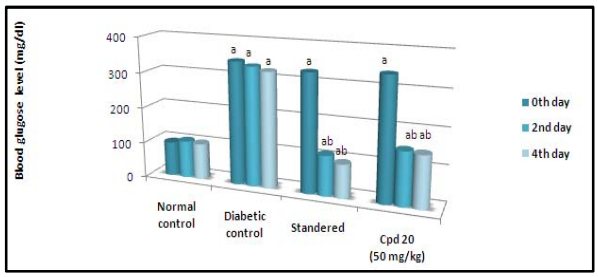
The results obtained clearly revealed that, the amino acid residues close to the reference molecules ***rosiglitazone*** mostly the same as those observed in the currently synthesized compounds **2-5,** which complexed with protein (Figure3 and 4). The higher binding energies and binding process interaction observed for compound **5** compared with the original inhibitor ***rosiglitazone*** indicate that, this compound **5** act as inhibitor against hPPAR-γ and considering more suitable inhibitor than ***rosiglitazone***.

Pharmacological Study

Anti-diabetic Activity

Blood Glucose Levels

The animals treated with alloxan (100 mg/kg, s.c) alone, showed a significant increase in blood glucose levels on 0th, 2nd and 4th day by 255.37, 218.55 and 217.91%, respectively (Figure 4), in comparing with normal animals,. The animal treated with compound **5** in (50 mg/kg, orally) caused a significant decrease in blood glucose levels on 2nd and 4th day (post-drug values) by 56.65 and 57.47%, respectively, while the reference drug metformine caused a significant decrease in blood glucose levels on 2nd and 4th day (post-drug values) by 66.08 and 71.47 %, respectively, when compared to 0th day (pre-drug values) Figure4.



[a] Significantly different from normal control at P<0.05.
[b] Significantly different from diabetic control at P<0.05.

Figure 4: The blood glucose levels of compound at 0th day (pre-drug values), 2nd and 4th day (Post-drug values) for each group of compound 5 and control, the mean value ± S.E. of six rats per group, the date analyzed by one-way ANOVA followed by LSD comparison test.

Level of serum cholesterol, triglyceride, low density lipoprotein (LDL) and high density lipoprotein (HDL).

It is well known that in, uncontrolled diabetes mellitus, there will be an increase in total cholesterol, triglycerides and LDL-C associated with decrease in HDL-C and contributes to coronary artery disease[34]. So, the animals treated with alloxan (100 mg/kg, s.c) alone showed, a significant increase in serum cholesterol level on 0th, 2nd and 4th day by 61.09, 59.62 & 64.79% and serum triglyceride level by 97.45, 96.23 & 99.61%, respectively, when compared to normal animals. While treatment with compound **5** with (50 mg/kg, orally), caused a significant decrease in serum cholesterol level on 2nd day and 4th day (post-drug values) by 20.25 and 37.97% and serum triglyceride level by 25.30 & 29.16%, respectively, when compared to 0th day (pre-drug values) Table 2.

The diabetic rats serum LDL level was significantly increased on 0th, 2nd and 4th day by 30.74, 28.86 & 34.19% while serum HDL level was significantly decreased by 49.55, 51.71 and 48.26%, respectively, when compared to normal animals. The diabetic rats treated with compound **5** in (50 mg/kg, orally) exhibited, a significant decrease in serum LDL level on 2nd day and 4th day (post-drug values) by 12.92 and 22.72 %, respectively, and increase in serum HDL level by 56.78 and 65.99%, respectively, when compared to 0th day (pre-drug values), Table 2.

The hypocholesterolemic effect may be explained, due to inhibition of fatty acid synthesis[35], which caused by increasing thiazolidinone group. In normal metabolism, insulin activates the enzyme lipoprotein lipase and hydrolyses triglycerides, the deficiency in resulting insulin, caused the inactivation of these enzymes thereby, which causing hypertriglyceridemia[36].

The significant reduction of serum lipid levels in diabetic rats after treated with compound **5,** may be directly attributed to improvements in insulin levels, or may inhibit the pathway of cholesterol synthesis, and this may be due to the activation of LDL receptors in hepatocyte, which is responsible for taken up LDL into the liver and reduce the serum LDL level.

Anti-oxidant Activity

The antioxidant activity of the compound **5** was measured in terms of their hydrogen donating or radical scavenging ability using the stable DPPH radical. The results were shown in Figure 6 (a) and (b). The IC_{50} for compound **5** and ascorbic acid measured at different concentrations of 0.16, 0.8, 4, 20, 40, 200 and 1000 µg/ml, showed marked antioxidant activity; the increasing scavenging activities of compound **5 (IC_{50} = 20 µg/ml.)** than ascorbic acid **(IC_{50} = 23.53 µg/ml.),** and generally increasing antioxidant activity of compound **5** (1.2, 2.5, 3.0, 15.4, 87.1, 95.8, 95.9%,) increased with increase of their concentration(0.16, 0.8, 4, 20, 40, 200 and 1000µg/ml). The increasing conjugated system in compound **5**, due to converted carbonyl groups in thiazolidine ring OH groups, led to increase the antioxidant activity, which explained by hydrogen atom transfer, and electron donating ability to DPPH.

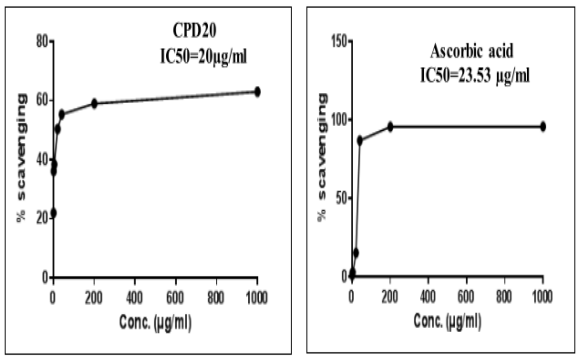


Figure 5: Antioxidant activity of (A) CPD 20, (B) Ascorbic acid, *in vitro*, using DPPH radical scavenging activity method. IC50 values were determined from the graph of percentage of inhibition plotted against the concentration; using Graph Pad Prism Software version 5.0. All statistical analyses used Microsoft Excel 2007 software.

CONCLUSION

The present work aimed to the development of novel anti-diabetes molecules containing thiazolidinedione pharmacophore.

The molecular docking study against PPARγ showed, the compound **5** have binding score more than reference drug.

The compound **5** was evaluated as anti-diabetic and anti-hyperlipidemic activity using alloxan animal model, as well as antioxidant activity using DPPH radical scavenging activity method.

The compound (**5**) with dose (50 mg/kg) showed, significant decrease in blood glucose, cholesterol, triglyceride and LDL levels, and increase in serum HDL level, in addition, compound **5** has antioxidant activity.

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