Section A-Research paper

PASS ONLINE SCREENINING FOR ACTIVITY, INSILICO AND INVITRO ANTI-DIABETIC ACTIVITY OF SOME NOVEL THIAZOLIDINONE

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ABSTRACT

In sillico screening of the title compounds was done through PASS online web resource. It was found good probability for anti-diabetic activity. Novel Thiazolidinone derivatives were synthesized by simple

synthesis of benzohydrazide through nucleophilic acyl substitution reaction which involves reaction between benzoic acid and hydrazine hydrate. The compound formed is treated with substituted aromatic aldehyde in the presence of catalytic amount of concentrated hydrochloric acid with stirring for 10 min to give benzhydrazone, which is further treated with thioglycolic acid and N,N dimethylformamide in the presence of zinc chloride stirring the reaction at temperature 60^0C for 4hrs to give thiazolidinones. The obtained thiazolidinones undergone conventional synthesis in the presence of glacial acetic acid and sodium acetate anhydrous refluxed for 12hrs to give the title compounds. The newly synthesized derivatives were characterized by spectroscopical methods using IR, ^{1}H-NMR spectroscopy and Mass spectrometry. The selected derivatives of the title compounds Iva, IVb, IVc, IVe are screened for the antidiabetic activity by *in vitro* glucose uptake assay using Pioglitazone as standard. Among the selected derivatives IVe had shown potent antidiabetic activity.

The *in silico* antidiabetic screening of the title compounds showed good antidiabetic activity with reduced adverse effects compared to current marketed drugs.

KEYWORDS: Thiazolidinone derivatives, antidiabetic activity, *in silico* antidiabetic screening (PASS online).

INTRODUCTION

LEAD DISCOVERY:

Criteria for the selection of disease:

Diabetes is fast growing disease in India, with which more than 62 million diabetic individuals currently diagnosed with this disease. In 2000, India (31.7 million) topped the world with the highest number of people with diabetes mellitus that is followed by China (20.8 million) and United States with (17.7 million) in second and third place respectively. The prevalence of diabetes is predicted to double globally from 171 million in 2000 to 366 million in 2030 with a maximum increase in India. It is predicted that up to 79.4 million individuals in India may accomplishes by diabetes mellitus, while China (42.3 million) and the United States (30.3 million) will also see significant increases in those affected by the disease. The present investigational aetiology of diabetes in India is due to the multi

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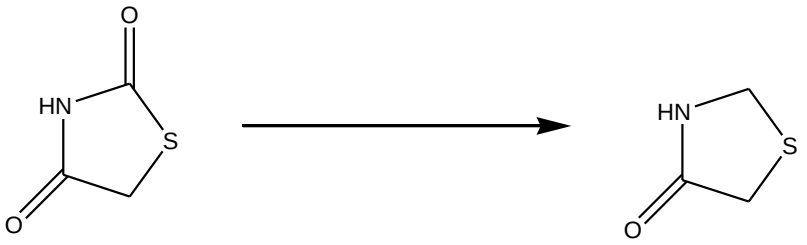
factorial and includes genetic factors coupled with environmental influences such as obesity associated with rising living standards, steady urban migration, and lifestyle changes.[1]

Criteria for the selection of Lead compound: For the present research work Thiazolidinone nucleus was selected as the lead compound due to its severe hypoglycemic effect. The current marketing antidiabetic drugs Rosiglitazone, pioglitazone and Citaglitazone are having the major adverse effect and less potency, which is due to the formation of sulphoxide ion with the ketonic oxygen in the skeleton of the thiazolidinediones which leads to the severe adverse effects like hepatotoxicity and anginopathy. The present selected nucleus thiazolidinone may over come the ADR of the current marketed drugs may be due to the presence of single ketonic group in its structure, and it shows more affinity towards the PPAR-γ activator receptors, which is a major cause for selecting thiazolidinones as antidiabetic targets[2].

LEAD MODIFICATION:

By the optimization of lead molecule from thiazolidinediones to thiazolidinones by the optimization of the one of the ketone group in the lead molecule may have the good antidiabetic activity with reduced adverse effects.



Thiazolidinedione Thiazolidinone

THIAZOLIDINONES-STRUCTURE

Thiazolidinones are derivatives of Thiazolidine with a carbonyl group at the 4-position.

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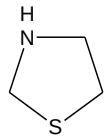
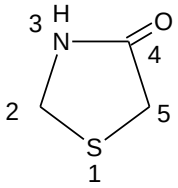


Thiazolidine

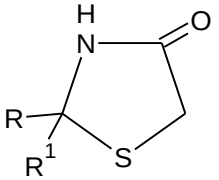


Thiazolidinone

1 **2**

Substituents in the 2-, 3-, and 5-positions may be varied, but the greatest difference in structure and properties is exerted by the group attached to the carbon atom in the 2-position (R and R' in 2 or X in3)[3].

Variations in the substituents attached to the nitrogen atom and the Methylene carbon atom are possible for the structures represented by 2 and 3.[4]



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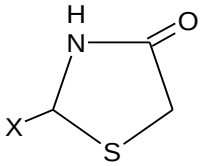
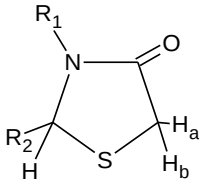
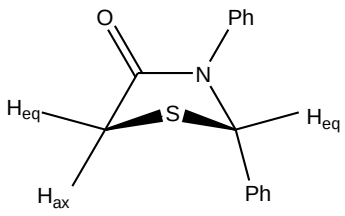


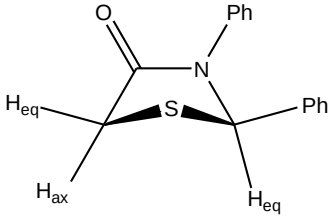
3

Stereochemistry of 4-thiazolidinones:

Theoretically, in the case of 2,3-disubstituted 4-thiazolidinones two diastereoisomers (I & II) are possible.







Stereo chemical orientation of 4-thiazolidinone.

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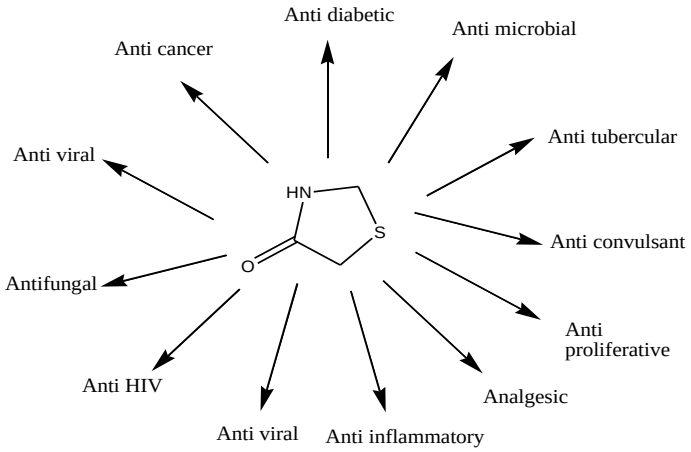
However, many researchers have done the conformational studies on various 2-aryl-3-(2-pyridyl)-4-thiazolidinones and have found that the preferred configuration (I) is that in which the C(2) proton and one of the Methylene protons are in cis1,3 di equatorial relationship. It is due to the fact that the phenyl group prefers the axial orientation to avoid the steric crowding with pyridyl group[5].

Heterocyclic compounds containing the thiazolidinone ring have reported to demonstrate a wide range of pharmacological activities which include antimicrobial, antifungal activity, anti-tubercular, antitumor, antidiabetic activity, anti-inflammatory, anticonvulsant etc.

Physical Properties: The 3-unsubstituted 4-thiazolidinones are usually solids, often melting with decomposition, but the attachment of an alkyl group at the nitrogen lowers the melting point. The 4-thiazolidinones that do not contain aryl or higher alkyl substituents are somewhat soluble in water.

Therapeutic Importance

The Thiazolidinone ring system represents a privileged structure in drug discovery. A large number of bioactive compounds containing this ring system are so vast that the complete range of their biological activities can be hardly classified. The nucleus contain the following therapeutic importance as shown in the figure.[6-8]



FigureNo.1.Therapeutic importance of Thiazolidinone

SYNTHETIC WORK

The title compounds were synthesized in four steps.

General planning

In the present work benzoic acid and hydrazine hydrate have been chosen as starting materials. The formation of the final products was monitored by TLC. The completed products show significant color under UV light. All the compounds prepared were purified by recrystallization with suitable solvents.

Scheme of work :

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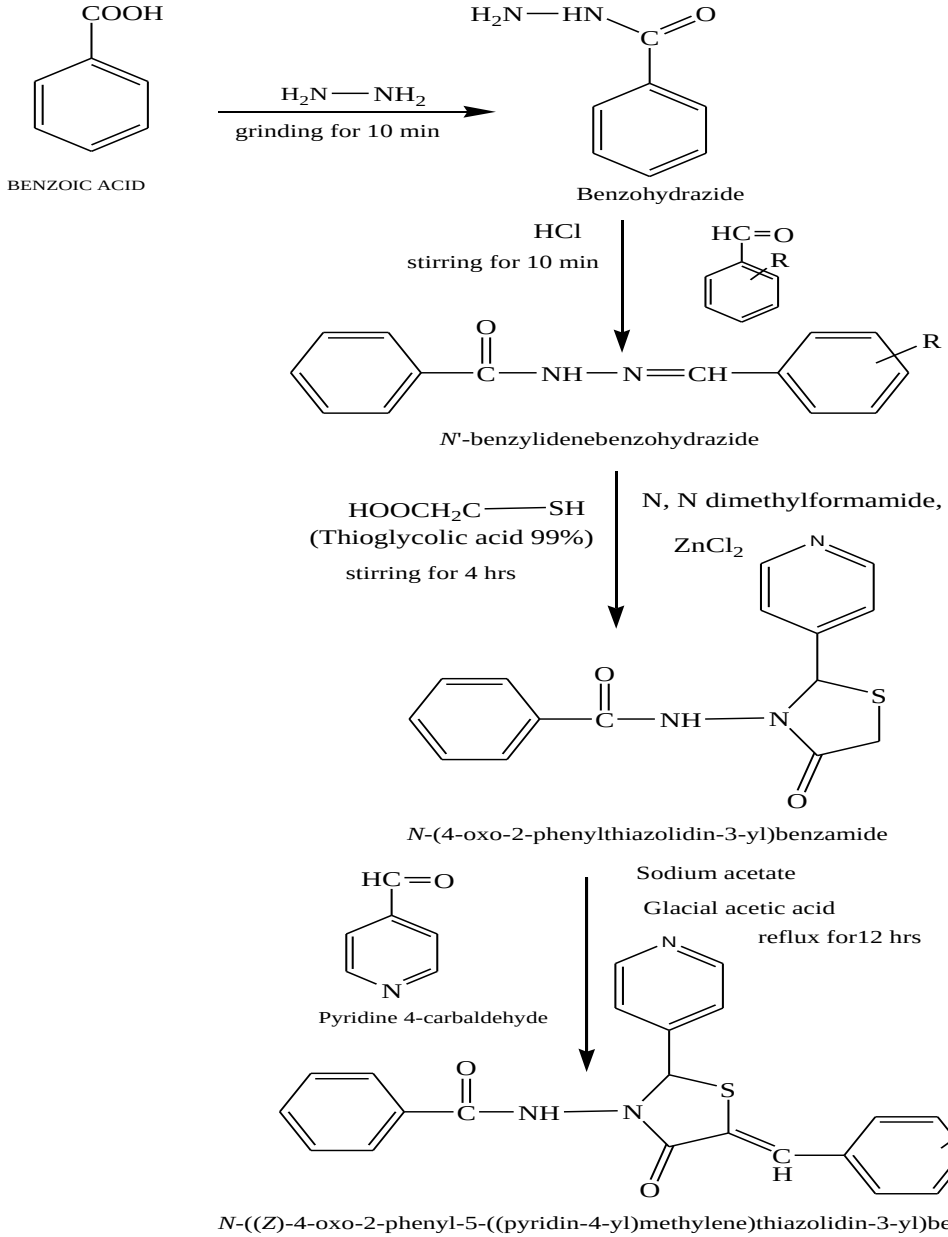

Compound code	Iva	IVb	IVc	IVd	IVe	IVf
R	H	Br	Cl	OCH_3	OH	OH-OCH_3

General method for the Synthesis of Title Compounds :

The procedure for the synthesis of compounds consists of four steps .

Step-1*: *Synthesis of Benzohydrazide:

The carboxylic acids(3.0mmol) was ground with hydrazine hydrate (80%3.75mmol) by mortar and pistel for 3-5 minutes and left for digestion for (10 minutes) when the reaction mixture set into a solid mass. The completion of the reaction was checked by a thin layer chromatography. The solid mass was crystallized from ethanol to give hydrazides.

Step-2: Synthesis of benzhydrazone: To the above mixture of benzohydrazide (0.01 mol)& aryl aldehyde (0.001) in 20 ml water was added three drops of concentrated Hydrochloric acid with continuous stirring for 10 min at room temperature and insoluble solid was generated & washed with water, after drying pure solid was obtained and recrystallized from ethanol.

Step-3: Synthesis of thiazolidinone:

0.01mol of substituted Schiff base and 0.01mol of Thioglycolic acid in 40 ml of N, N Dimethylformamide to the above mixture a pinch of $zncl_2$ was added and the reaction mixture was undergone for continuous stirring with the constant maintenance of temperature of 60^0 C for 4 hours, after the completion of reaction the reaction mixture was poured into crushed ice and the solid obtained was filtered, dried and recrystallised from ethanol.

Step 4: Synthesis of thiazolidinone derivatives :

A mixture of compound of above step 0.03mmol, pyridine-4carbaldehyde (0.01m mol.), anhydrous sodium acetate (0.5mg) in glacial acetic acid (50ml) was heated under reflux for 12 h. Concentrated, cooled and poured into the crushed ice. The solid thus separated was filtered, washed with water and recrystallized from ethanol.

ACTIVITIES SCREENING

***In vitro* Antidiabetic activity:**

Name of analysis method: glucose uptake assay

procedure:

3T3-L1 adipocytes, were seeded at a density of ~1500 cells per well in a 96-well plate, differentiated and maintained for another 10 days prior to use. To assay glucose uptake, adipocytes were starved in 100 µl serum free adipocyte medium overnight (to enhance glucose uptake) then washed with PBS, followed by a incubation (40 min) in an glucose free medium (100 µl Krebs-Ringer-Phosphate-HEPES (KRPH) buffer with 2 % BSA) then stimulated either with insulin (PGZ) (10 µM), compounds (10 µg/ml) or PBS. 10 µl of 10mM 2-Deoxy glucose (DG) was added and the cells incubated for 20 min. The amount of glucose uptake was determined as per manufactures protocol using the Glucose uptake kit from Biovision (glucose uptake colorimetric assay kit, the 2-DG6P is oxidized to generate NADPH, which can be determined by an enzymatic recycling amplification reaction, color generated can be quantified colorimetrically at 412 nm.). The calculation was carried out keeping 100% glucose uptake for Pioglitazones (PGZ) was used as a standard drug. [13]

2-DG uptake = Sa/Sv (pmol/µl or nmol/ml or µM)

Where: Sa is the amount of 2-DG6P (in pmol) in sample well calculated from Standard Curve.

Sv is sample volume (in 20 µl) added into the sample well.

***In silico* antidiabetic screening[9]**:

PASS Online:

This can be performed through an online web resource called PASS (Prediction of Activity Spectrum of Substances), which is a novel theoretical approach used to screen the novel pharmacological activities of the title compounds. PASS is an online programme, which compare the structure of the novel compound with the well known biologically active compounds and predicts the activity of the formulated compounds. By using this thousand's of compounds can be screened for their novel pharmacological activities. For the prediction of compounds for their novel pharmacological activities the chemical

formula was necessary and can be predicted by drawing in chemsketch and submitted into PASS online for the possible mechanism of actions.

Docking studies[10]:

AutoDock is an automated procedure for predicting the interaction of ligands with biomacromolecular targets. The motivation for this work arises from problems in the design of bioactive compounds, and in particular the field of computer-aided drug design.

Docking Protocol: AutoDock4.2 is parameterized to use a model of the protein and ligand that includes polar hydrogen atoms, but not hydrogen atoms bonded to carbon atoms. An extended PDB format, termed PDBQT, is used for coordinate files, which includes atomic partial charges and atom types. The current AutoDock force field uses several atom types for the most common atoms, including separate types for aliphatic and aromatic carbon atoms, and separate types for polar atoms that form hydrogen bonds and those that do not. PDBQT files also include information on the torsional degrees of freedom. In cases where specific sidechains in the protein are treated as flexible, a separate PDBQT file is also created for the sidechain coordinates. AutoDockTools, the Graphical User Interface for AutoDock, may be used for creating PDBQT files from traditional PDB files.

AutoDockTools includes a number of methods for analyzing the results of docking simulations, including tools for clustering results by conformational similarity, visualizing conformations, visualizing interactions between ligands and proteins, and visualizing the affinity potentials created by AutoGrid.

All the docking studies are done using AUTODOCK 4.2 version and the images are rendered using Accelry's Discovery studio visualizer v4.0 interface.

***In silico* antidiabetic screening:**

Results of reference standards and synthesized derivatives

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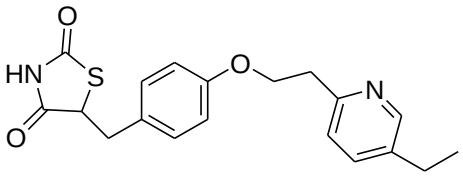
PIOGLITAZONE:

Estimation of propability of activity



Pa>Pi Pa>0,3 Pa>0,7

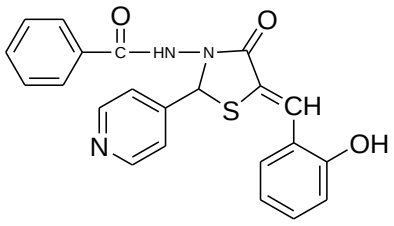
0,976	0,003	Antidiabetic
0,945	0,002	Peroxisome proliferator-activated receptor gamma agonist
0,923	0,007	CYP2C12 substrate
0,905	0,003	CYP2C8 substrate
0,886	0,002	Peroxisome proliferator-activated receptor agonist
0,883	0,001	Insulin sensitizer
0,872	0,002	CYP2C8 inhibitor
0,856	0,005	Hypolipemic
0,827	0,000	Thiazolidinedione
0,813	0,009	CYP2C substrate
0,746	0,004	Antidiabetic symptomatic
0,731	0,002	Glycogen synthase stimulant

0,730	0,005	Antidiabetic (type 2)
0,721	0,005	Reductant
0,712	0,038	Nootropic
0,666	0,005	Antipsoriatic
0,658	0,002	Antidiabetic (type 1)

Synthesized derivatives:

Compound code: IV e

Estimation of propability of activity



○ Pa>Pi ○ Pa>0,3 ○ Pa>0,7

0,569	0,006	PfA-M1 aminopeptidase inhibitor
0,511	0,029	5 Hydroxytryptamine uptake stimulant
0,489	0,024	Threonine aldolase inhibitor
0,464	0,050	Muramoyltetrapeptide carboxypeptidase inhibitor
0,475	0,094	Nicotinic alpha4beta4 receptor agonist

0,409	0,029	Antituberculosic
0,417	0,039	Antidiabetic
0,417	0,073	Antiarthritic
0,397	0,054	Dementia treatment
0,382	0,076	Kinase inhibitor
0,421	0,123	Taurine dehydrogenase inhibitor
0,306	0,018	Antineoplastic (liver cancer)
0,336	0,051	Diuretic inhibitor
0,294	0,010	Follicle-stimulating hormone agonist
0,385	0,111	Thioredoxin inhibitor
0,335	0,061	Antimycobacterial

Docking studies results:

QSAR, DRUGLIKENESS AND AUTODOCK 4.2 RESULTS

Molecular docking studies of PPAR-γ using Autodock (PDB ID: 1ZGY)

Standard drug- Pioglitazone:

S. No.	Compound Code	Binding Energy (Kcal/mol)
1	Pioglitazone	-8.7

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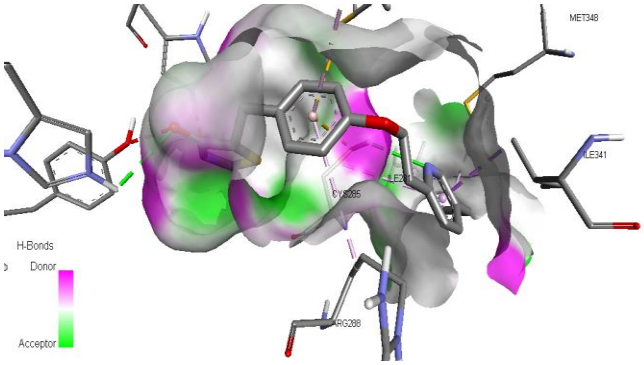
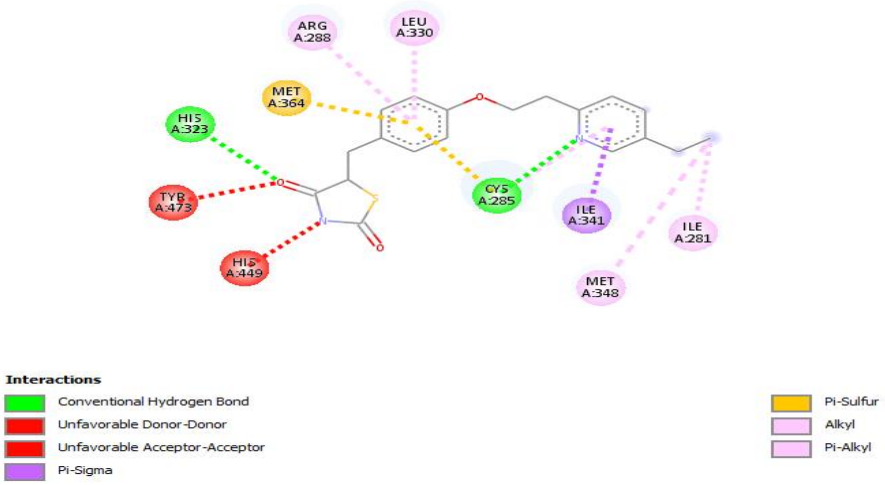
Docking Poses of the Synthetic Derivatives with 1ZGY



3D-Docked pose of Pioglitazone with 1ZGY



2D-Docked pose of Pioglitazone with 1ZGY

Synthesized derivatives:

IVa compound docking interactions with targeting PPAR (PDB ID: 2HWQ) for potential anti-diabetic activity:

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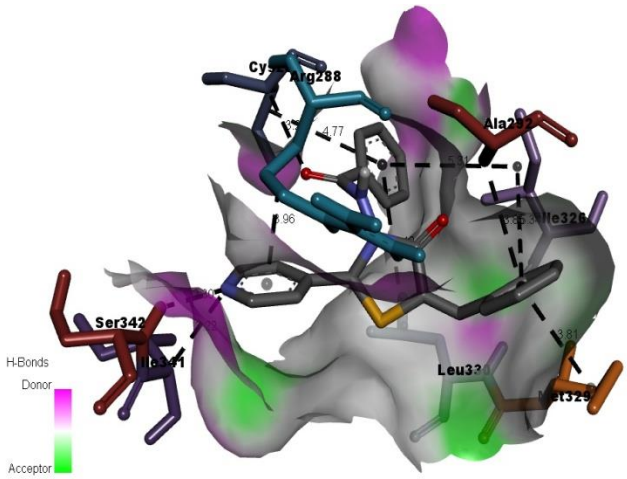
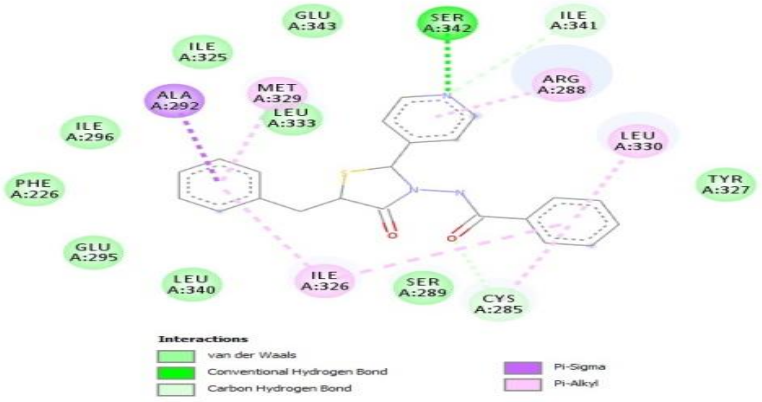
3D interaction formed by the compound IV a:



2D interactions of compound IV a:



IVb compound docking interactions with targeting PPAR (PDB ID: 2HWQ) for potential anti-diabetic activity):

3D interaction formed by the compound IV b:

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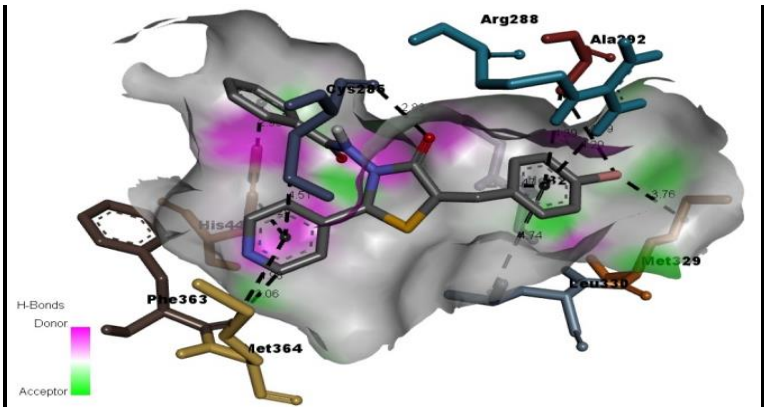
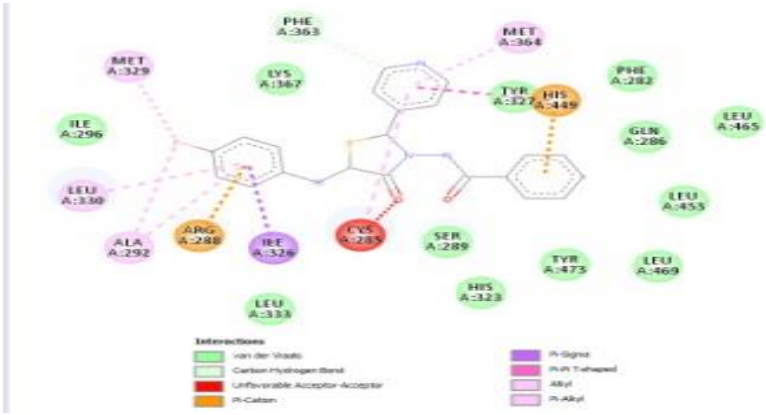


2D interactions of compound IV b :



IVc compound docking interactions with targeting PPAR (PDB ID: 2HWQ) for potential anti-diabetic activity):

3D interaction formed by the compound IV c:

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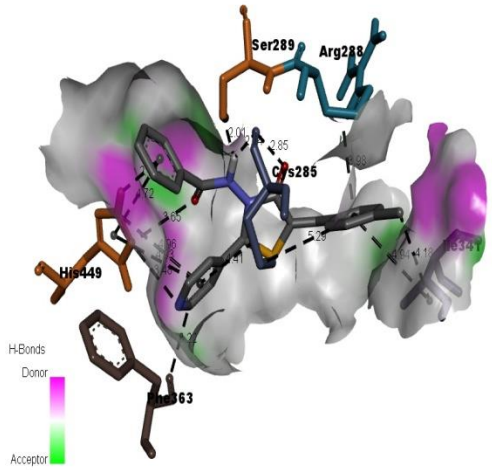
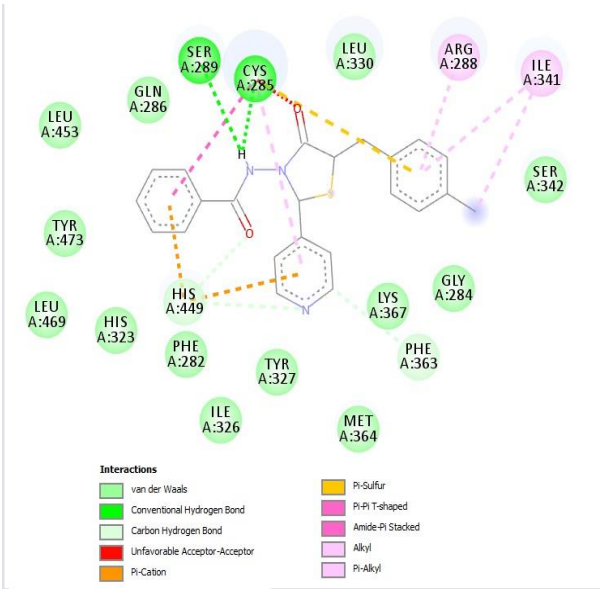
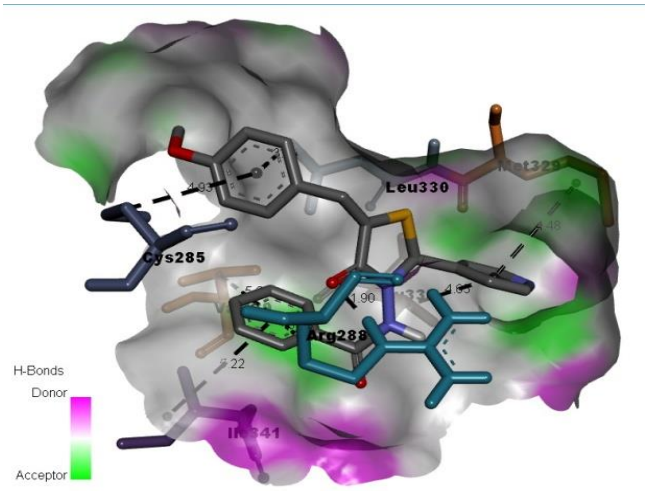


2D interactions of compound IV c:

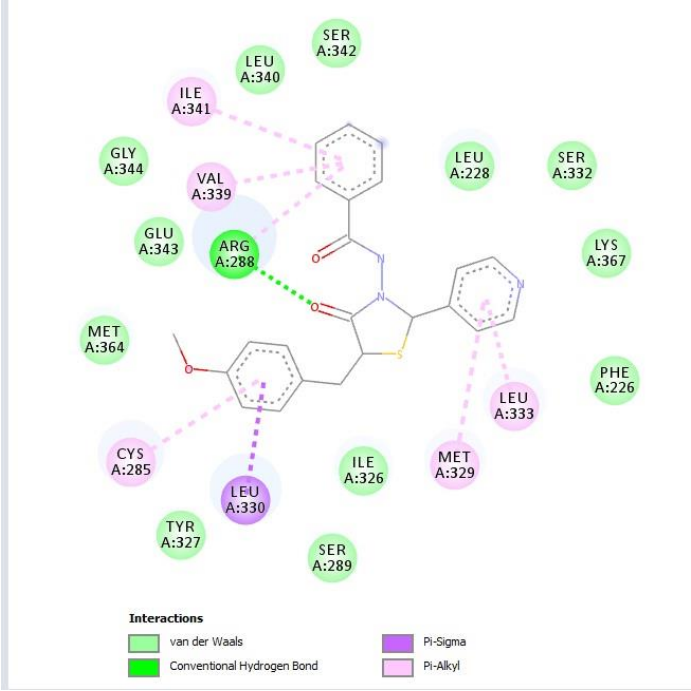


IVd compound docking interactions with targeting PPAR (PDB ID: 2HWQ) for potential anti-diabetic activity):

3D interaction formed by the compound IV d:



2D interactions of compound IV d:



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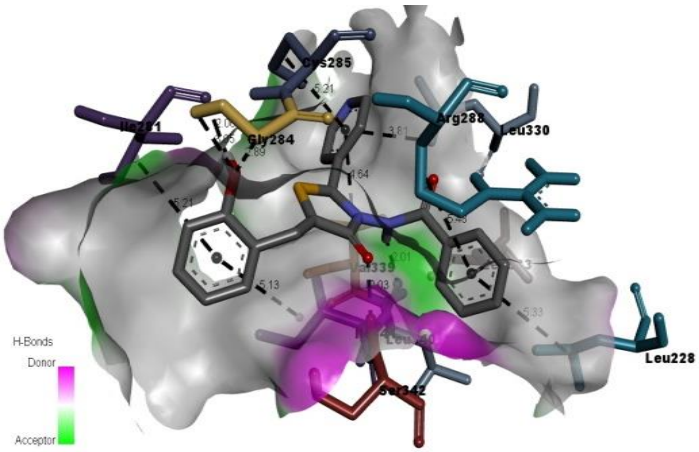
IVe compound docking interactions with targeting PPAR (PDB ID: 2HWQ) for potential anti-diabetic activity):

3D interaction formed by the compound IV e:



2D interactions of compound IV e:

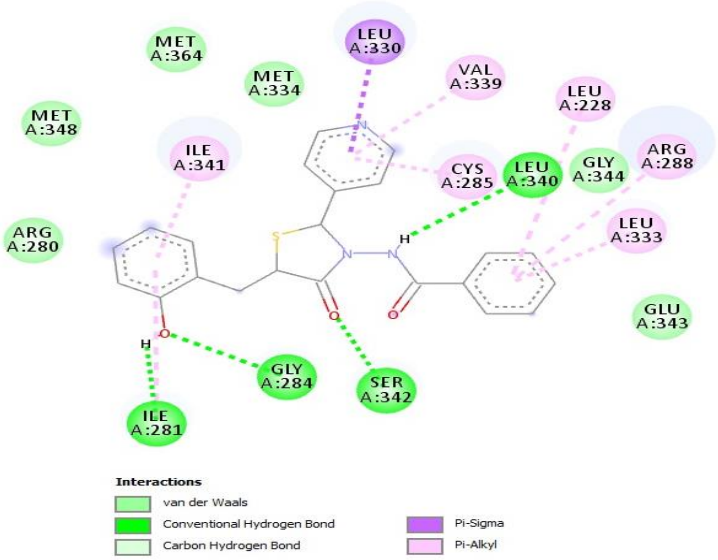


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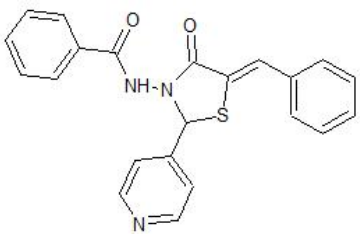
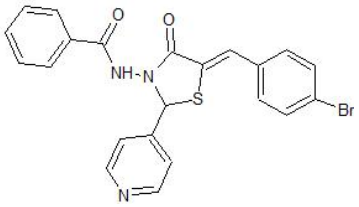
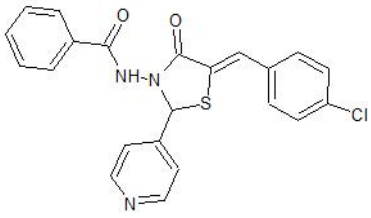
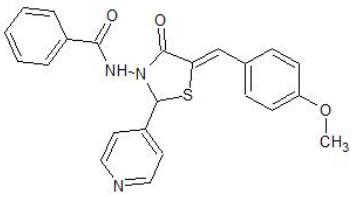
Table 1: Docking results of Compound-IVa to IVe targeting PPAR (PDB ID: 2HWQ) for potential anti-diabetic activity:

S.No	Drug Target	Compound name	Structure	Binding Energy in Kcal/mol	Predicted IC50 value(nano molar)
1	PPAR (PDB ID: 2HWQ)	IV a		-9.54	101.24 nM
2	PPAR (PDB ID: 2HWQ)	IV b		-10.13	37.34 nM
3	PPAR (PDB ID: 2HWQ)	IV c		-9.51	106.11 nM
4	PPAR (PDB ID: 2HWQ)	IV d		-8.68	434.71 nM

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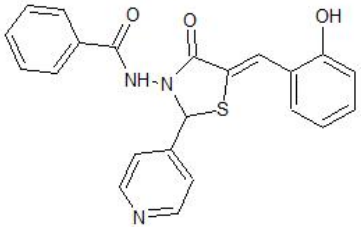
5	PPAR (PDB ID: 2HWQ)	IV e		-8.92	288.43 nM

In-vitro Antidiabetic Activity: antidiabetic activity of the synthesized derivatives was performed by the Glucose uptake assay and the results were tabulated below.

Table no:2 *in-vitro* antidiabetic results of 2-DG6P Standard:

S.No	2 DG6P (pmol)	OD (412 nm)
1	20	0.5
2	40	0.9
3	60	1.4
4	80	1.9
5	100	2.4
6	120	2.7
7	140	3.2
8	160	3.7

Table no:3 Effect of compounds on 2-DG uptake in 3T3-L1 presence and absence of insulin:

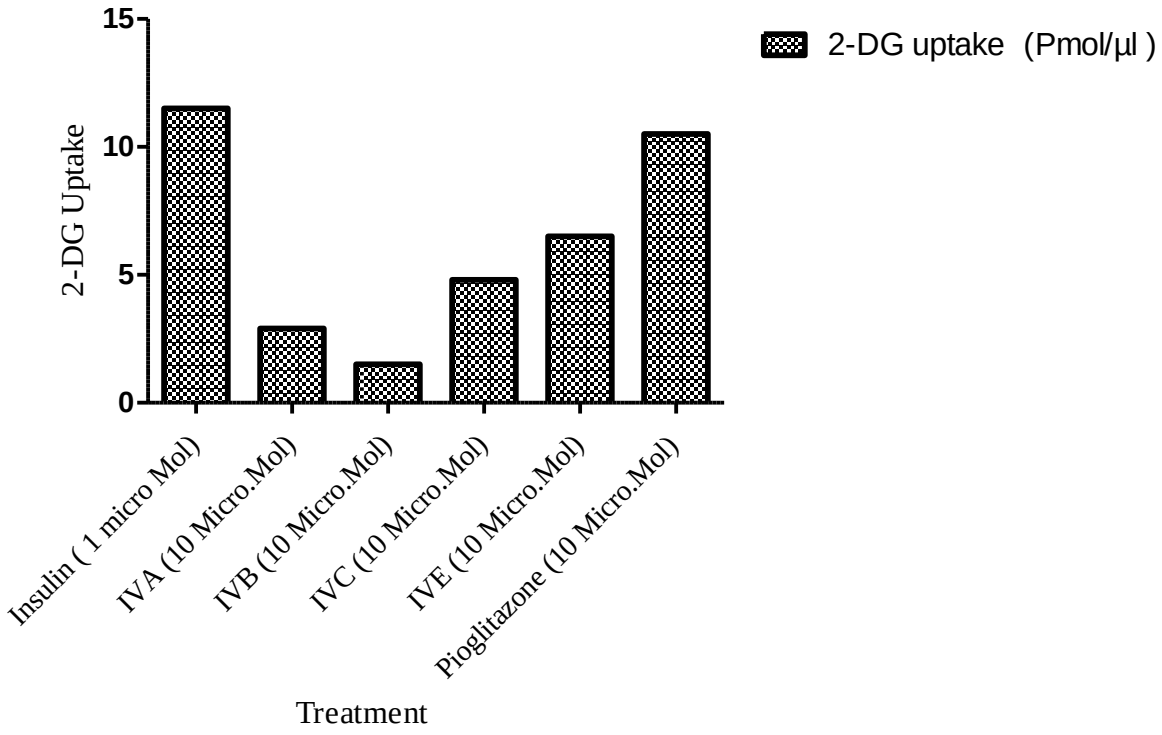
S.No	Compound	OD (412)	2 DG6P (pmol)	2-DG uptake (Pmol/µl)
1	Insulin (1 micro Mol)	3.04	174	11.5

2	IVA	1.4	58	2.9
3	IV A +Insulin	4.3	220	11
4	IVB	0.6	30	1.5
5	IV B+Insulin	2.9	135	6.75
6	IVC	2.1	95	4.8
7	IV C +Insulin	4.3	250	-
8	IVE	3.2	130	6.5
9	IV E +Insulin	7.5	350	17.5
10	Pioglitazone (10 Micro.Mol)+ Insulin (1 Micro.Mol)	10.4	450	22.5
11	Pioglitazone (10 Micro.Mol)	3.1	210	10.5

Table no:4 Effect of compounds on 2-DG uptake in 3T3-L1 absence of insulin:

S.No	Compound	OD (412)	2 DG6P (pmol)	2-DG uptake (Pmol/µl)
1	Insulin (1 micro Mol)	4.2	230	11.5
2	IVA	1.4	58	2.9
3	IVB	0.6	30	1.5
4	IVC	2.1	95	4.8
5	IVE	3.2	130	6.5
6	Pioglitazone (10 Micro.Mol)	3.8	210	10.5

Fig:12 Effect of compounds on 2-DG uptake in 3T3-L1 absence of insulin:



CONCLUSION

Novel thiazolidinone derivatives were synthesized, characterized and screened for *in-vitro* antidiabetic and *in-silico* antidiabetic activities using respective standards. *In-silico* antidiabetic screening was performed by PASS online web resource.

The results of *in-vitro* antidiabetic screening revealed that IV e compound shown good activity when compared to standard among all the derivatives. This is may be due to the fact that the target,PPAR gamma is hydrophilic in nature and highly polar, but the designed titled compounds are lipophilic in nature and are less polar this may be the one of the reason but not the only reason for rejecting null hypothesis.

The results of *in-silico* antidiabetic screening revealed that the synthesized derivatives have good antidiabetic activity when compared that of current marketed drugs.

Further research work need to be carried out to know the relationship between structure and biological activity. The further scope of present research work is to establish the antidiabetic activity of the synthesized derivatives on other targets, especially on *in-vivo* antidiabetic activity and QSAR studies.

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