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Pharmacophore Modeling, Synthesis And Analytical Characterization Of Novel Ligands For Diabetes Mellitus

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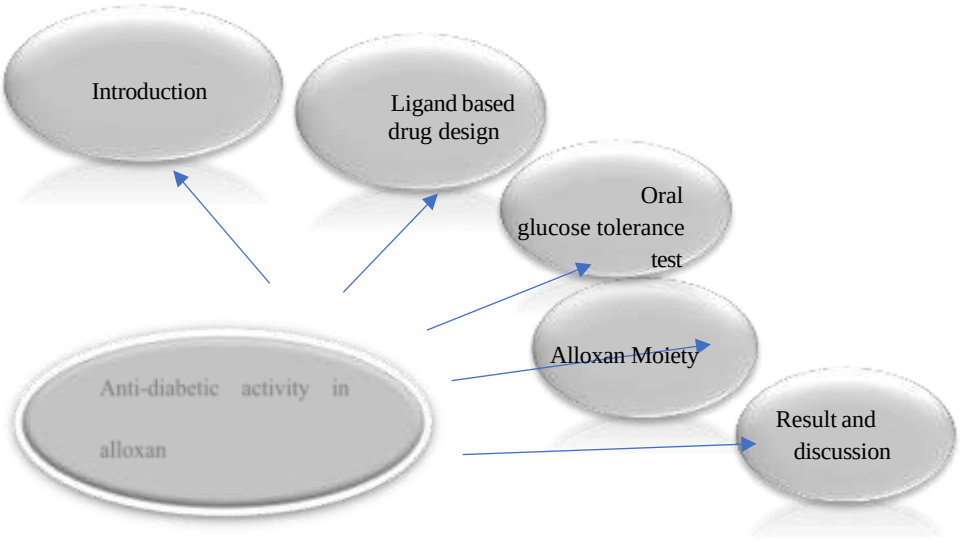
The study highlights the potential of thiazolidinedione (TZD) derivatives of 7-flavonols in treating diabetes mellitus through computational and experimental approaches. TZD-linked flavonols were evaluated for their hypoglycaemic and glucose tolerance effects in normoglycemic and alloxan-induced diabetic rats. The results demonstrated significant blood glucose reduction, particularly in hyperglycaemic conditions, indicating their ability to maintain glucose homeostasis and reduce the risk of severe hypoglycaemia. The alloxan-induced diabetic model suggests these compounds enhance insulin secretion, likely by stimulating surviving β-cells. The antioxidant properties of flavanols, coupled with the well-established diabetic pharmacophore TZD, proved effective in lowering blood glucose levels, supported by both computational analysis and wet lab experiments. The study also involved molecular modelling and docking studies targeting diabetic proteins, which further validated the therapeutic potential of these compounds. ADMET analysis confirmed their minimal toxicity, making them strong candidates for anti-diabetic drug development. The structural diversity of TZD derivatives, combined with their favorable therapeutic properties, emphasizes their promise as potent agents for diabetes treatment. The study concludes that these compounds warrant further investigation due to their broad spectrum of biological activities, minimal toxicity, and potential to serve as effective, low-risk treatments for diabetes mellitu

GRAPHICAL ABSTRACT



KEYWORDS: Anit-diabetic, Ligand based design, Oral Glucose Tolerance, TZD

1. INTRODUCTION

Drug discovery is mostly portrayed as a linear, consecutive process that starts with target and lead discovery, followed by lead optimization and pre-clinical in vitro and in vivo studies to determine if such compounds satisfy a number of pre-set criteria for initiating clinical development. For the pharmaceutical industry, the number of years to bring a drug from discovery to markets is approximately 20 years, costing up to US$ 1200 million per individual drug[1].

A traditional drug development process has resulted in high attrition rates with failures attributed to poor pharmacokinetics (39%), lack of efficacy (30%), animal toxicity (11%), adverse effects in humans (10%) and various commercial and miscellaneous factors. Today, the process of drug discovery has been revolutionized with the advent of genomics, proteomics, bioinformatics and efficient technologies like, combinatorial chemistry, high throughput screening (HTS), virtual screening, de novo design, in vitro, in silico ADMET screening and structure- based drug design. Computational tools offer the advantage of delivering new drug candidates more quickly and at a lower cost. In silico drug design utilizes computational methods to aid drug discovery by identifying targets, screening molecules, and predicting drug behavior. Key techniques include virtual screening and de novo design for generating drug-like molecules, in silico ADME/T prediction to evaluate pharmacokinetics and

toxicity, and protein-ligand binding analysis to predict molecular interactions. Accelrys Discovery Studio automates lead design by optimizing molecular geometry, docking candidates, and performing molecular dynamics simulations. [2] Ligand-based drug design relies on known molecules binding to biological targets to guide new drug candidates,[3] while fragment-based drug design uses small molecular fragments optimized into selective candidates via linking, fusion, or growth.[4] Molecular descriptors and drug- like filters like molecular weight, hydrogen bonds, log P, and rotatable bonds help screen viable drug candidates.[5] Computational simulations, such as Monte Carlo and Molecular Dynamics (MD), model molecular interactions under various conditions, with MD providing insights into protein-ligand interactions in physiological conditions. Molecular docking predicts ligand binding modes and estimates binding affinity, with advanced methods like CDOCKER aiding virtual screening. Energy minimization stabilizes molecular structures by reducing strain, and Lipinski's Rule of Five outlines key molecular properties for drug-like compounds. Ligand modeling using Accelrys Discovery Studio supports lead identification and optimization. Protein purification and minimization ensure stability for accurate drug modeling, while ligand screening, including primary screening based on interaction energy, hydrogen bond interaction screening, and ADMETOX screening, evaluates key pharmacokinetic parameters, reducing clinical trial failure rates.

ADMET Descriptors	**Values**				
	0	1	2	3	4
Aqueous solubility	Extremely low	Very low,	Low	Good	Optimal
BBB penetration	Very high	High	Medium	Low	Undefined
Hepatotoxicity	Non-toxic	Toxic	---	---	---
Absorption level	Good	Moderate	Poor	Very poor	---
logP	Less than or equal to 3				

Table-1. ADMET parameters and values

Diabetes mellitus is a metabolic disorder that affects carbohydrate metabolism, with current antidiabetic drugs like sulfonylureas and biguanides unable to fully restore insulin sensitivity or β-cell function, emphasizing the need for new PPARγ ligands. Molecular modeling has advanced the design of PPARγ agonists, particularly thiazolidinediones (TZDs) and their analogs, by improving understanding of ligand binding. TZDs, key in improving glucose and lipid metabolism, also bind to multiple targets, making them versatile but promiscuous ligands. Diabetes, affecting 285 million people and projected to rise to 435 million by 2030, is primarily caused by insulin resistance and secretion defects, worsened by factors like obesity and poor diet. Complications include neuropathy, cardiovascular diseases, and kidney damage. Research

models like the alloxan-induced diabetes model help in studying therapies, while natural compounds such as flavonoids and sterols show potential in reducing blood glucose and regenerating β-cells.

2. MATERIALS AND METHODS

2.1 Protain Data Bank – PDB

The Research Collaboratory for Structural Bioinformatics (RCSB) became responsible for the management of the PDB and the pattern of structures is updated weekly by Tuesday. The PDB is a principle repository of experimentally-determined structures of proteins, nucleic acids, and complex assemblies. Perceptive of a molecule helps to understand the role played in a disease condition and also in drug development[8].

The three-dimensional structures of large biological macromolecular molecules are provided with information on electron microscopy, X-ray crystallography, NMR data related, deposition of validation server and related tools. In this fragment-based drug design work we used eleven diabetic binding proteins for characterization from PDB.

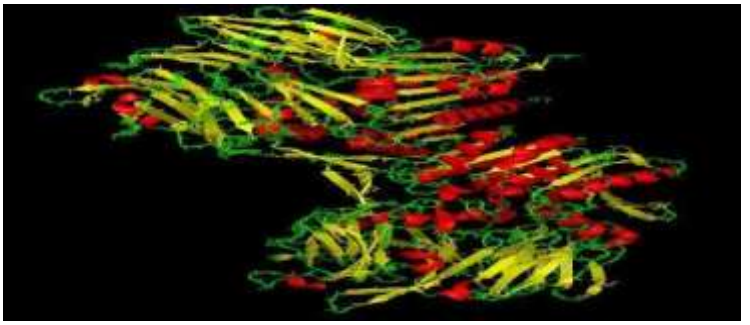


Figure-1. Selected protein targets and their PDB ID (1TOW, 1X70, 1KZW, 1UVQ, 2I78, 2WBB, 2YOQ, 2ZNO, 4A5S, 3RSW, 1JK8)

2.2 Target Characterization Tools

Effective drug design requires systematic target analysis. Characterization of selected diabetic protein targets was performed using PDB data.

2.2.1 Primary Structure Analysis – ProtParam

ProtParam, an ExPASy tool, was used to analyze 11 diabetic protein domains, computing key physico-chemical parameters:

2.2.1.1 Aliphatic Index: Measures protein thermostability based on aliphatic side chains.

2.2.1.2 Instability Index: Predicts protein stability; values <40 indicate stability.

2.2.1.3 GRAVY: Assesses hydropathy by averaging amino acid hydropathy values.

2.2.1.4 Half-life: Estimates protein stability in vivo based on the N-terminal residue.

2.2.1.5 Isoelectric Point (pI): Determines the pH at which the protein is electrically neutral.[9]

2.2.2 Secondary Structure Analysis – SOPMA

SOPMA predicted secondary structures (α-helix, β-sheet, and coil) from the FASTA sequences[10].

2.2.3 Protein Surface Analysis – CASTp

CASTp identified and measured surface pockets and interior cavities, providing insights into protein binding and active sites[10].

2.2.4 Motif Identification – PROSITE

PROSITE detected protein motifs, domains, and functional sites, aiding in functional annotation[11].

2.2.5 Subcellular Localization – UNIPROT

UNIPROT predicted the subcellular location of target proteins using FASTA sequences[12].

2.3 Interaction Study with Natural Ligand

Interaction study explicates the complete information about the target's affinity and binding behavior towards the ligands[13].

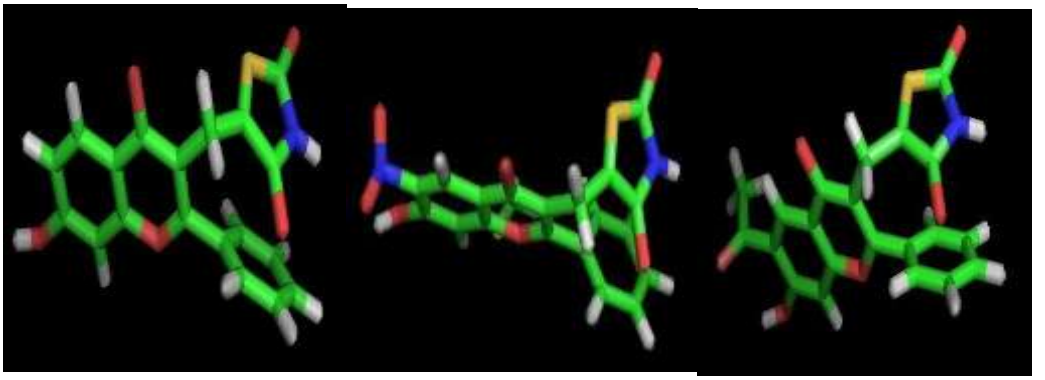
2.3.1 Hotspot identification-MOE

Hot spots are interacting amino acid residues accounting to the majority of the binding energy / interaction energy. These residues are critical in understanding the principles of protein interactions. The protein – natural ligand interactions and the hot spots were analyzed during the interaction study[13].

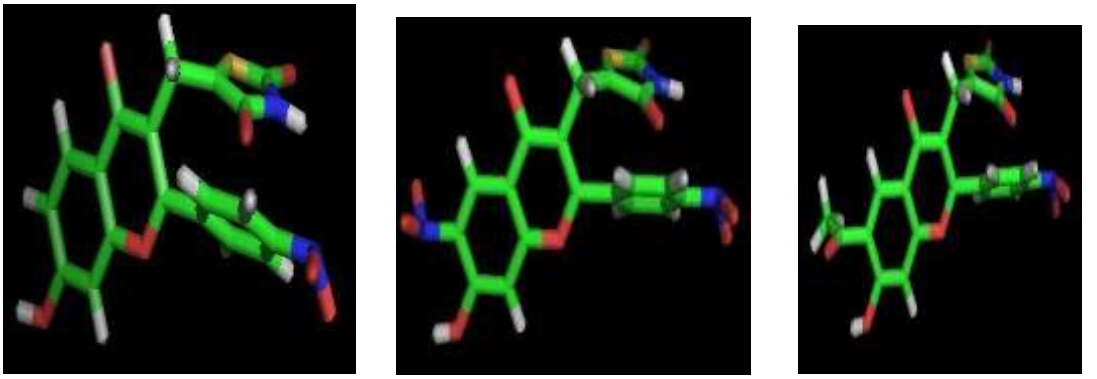
2.4 Molecular Modeling-PYMOLE

The docking studies were carried out to make sure that the amino acid hotspots identified previously were correct. For the docking studies, all the 9 diabetic protein targets were docked using Accelry's discovery studio

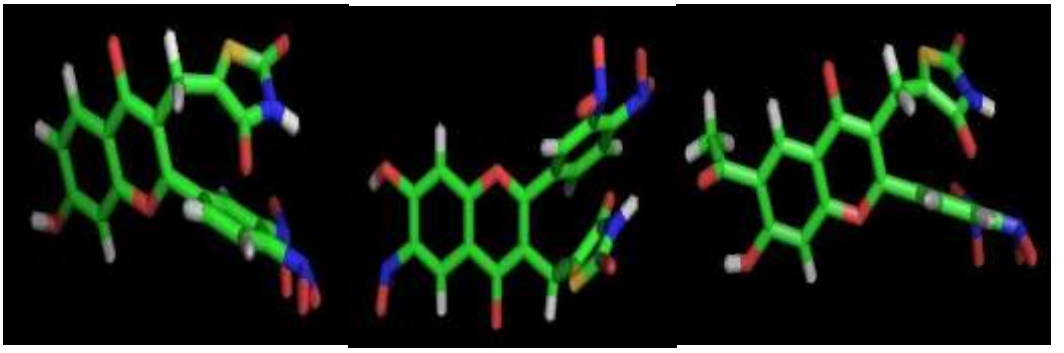
2.1. CDOCKER is a grid-based molecular docking method that employs CHARMm force field. It provides a refinement docking studies using a set of ligands against a single target. From this interaction study, we can also conclude whether the target protein molecules were 'conformationally biased' or not[14].



TZD of 7-flavonol **(IIIa)** TZD of 6-nitro,7-flavonol **(IIIb)** TZD of 6-acetyl, 7-flavonol **(IIIc)**



TZD of 4'nitro, 7-flavonol **(IIId)** TZD of 6, 4'-dinitro, 7-flavonol **(IIIe)** TZD of 6-acetyl, 4'nitro, 7-flavonol **(IIIf)**



TZD of 3'4'-dinitro, 7-flavonol **(IIIg)** TZD of 6,3'4'trinitro, 7-flavonol **(IIIh)** TZD of 6-acetyl, 3'4'-dinitro, 7flavonol **(IIIi)**

Figures-2(a-i). PYMOL structures of FBDD led templates

2.5 Ligand Modeling Using ACCELRYS DISCOVERY STUDIO 2.1

Discovery Studio provides the most advanced modeling and simulation software solutions to visualize, analyze, modify and simulate protein structures[15].

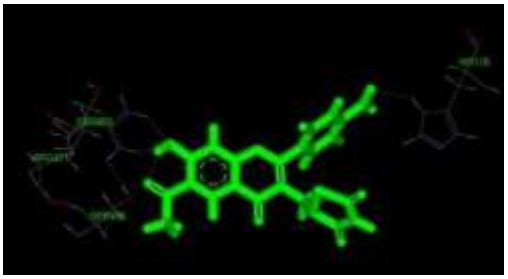


Figure-3. Ligand Modeling Using ACCELRYS DISCOVERY STUDIO 2.1

2.6 Protein Purification & Minimization

Diabetic proteins were purified using Discovery Studio 2.1, with removal of alternate conformers, ligands, heteroatoms, and residue corrections. CHARMm force field was used for energy minimization[16].

2.7 Ligand Screening

- Primary screening: Based on interaction energy.
- Hydrogen bond interaction: Identifies key molecular interactions.
- ADMETox screening: Shortlists ligands based on toxicity and pharmacokinetics.
- Docking: Final ligand-target protein docking to evaluate interaction energy[3].

2.8 Diabetes Animal Models

- Normoglycemic model: Evaluates oral hypoglycemics[17].
- Glucose loading model: Uses glucose tolerance testing[18].
- Chemical induction: Alloxan & STZ induce diabetes in rodents[7].

2.9 Animal Grouping & Drug Administration

Rats were divided into 8 groups, receiving either control, standard drug (Ciglitazone), or thiazolidinedione derivatives of 7-flavonol (30 mg/kg b.w.).

2.10 Hypoglycemic & Anti-Diabetic Studies

- Blood glucose monitoring: Collected at 0.5, 1, 2, and 3 hours post-drug administration[19].

- Oral glucose tolerance test (OGTT): Conducted at multiple time points after glucose load.
- Alloxan-induced diabetic study: Uses Sprague Dawley rats, maintaining ethical protocols.
- Synthetic test compound administration: Tested in single-dose & multiple-dose studies over 21 days[20].

2.11 Statistical Analysis

Data analyzed using one-way ANOVA and Dunnett's multiple comparisons, with significance at $P \leq 0.05$. Software: GraphPad Prism 5.01[22].

3 RESULTS AND DISCUSSION

Protein Characterization:

Most target proteins, except for a few (e.g., 1X70, 1UVQ, 2I78), exhibited high structural stability (instability index <40). Aliphatic index (70–100) indicated high thermodynamic stability. A negative GRAVY value confirmed the proteins' hydrophilic nature. Isoelectric point (pI) values (4.8–6.9) suggest their presence in the Zwitter ionic form, which may be beneficial in slightly acidic cancer tissues. Subcellular localization varied across the apical cell membrane, nucleus, cytoplasm, and extracellular space. Most proteins (except a few) had a high half-life, indicating kinetic and mutational stability.

Secondary Structure and Binding Analysis

Proteins showed structural stability with efficient folding, supported by a low β-turn and high α-helix percentage. CASTp9 analysis revealed active site pockets (volume: 300–6931 $Å^3$). Strong hydrogen bonding interactions were observed between ARG 125, ARG 126, ARG 185, and anionic groups of thiazolidinedione derivatives of 7- flavonols.Hydrophobic interactions with TYR 662, TYR 631, TYR 473, GLU 205, GLU 206, SER 209, SER 289 facilitated ligand binding.

Ligand Screening and ADMET Analysis

Out of 81 templates, 25 highly interacting ligands were identified using CDOCKER scoring. Further pharmacokinetic screening narrowed them down to 9 optimal ligands. ADMET filtering (toxicity, solubility, absorption) identified 4 lead compounds as non-mutagenic, non-toxic, and non-carcinogenic, with high target interaction potential.

Hypoglycemic and Anti-Diabetic Studies

Thiazolidinedione derivatives of 7-flavonols (IIIa-i) were tested in normal and alloxan-induced diabetic SD Wistar rats. Compared to Ciglitazone (standard drug), the synthesized compounds showed dose-dependent hypoglycemic and anti-diabetic effects.

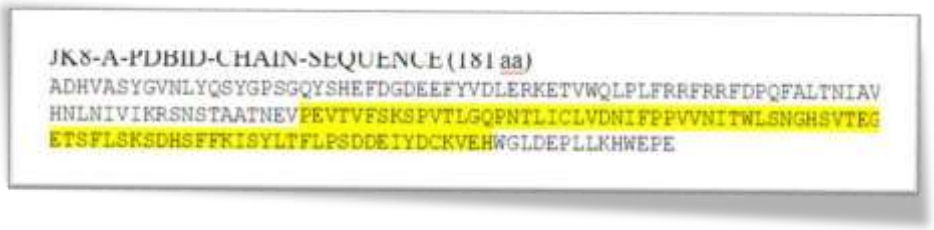
Sulfonylureas and Biguanides:

Sulfonylureas increase β-cell glucose sensitivity and insulin release through multiple pathways (cAMP stimulation, Ca^{2+} influx, catecholamine inhibition). Biguanides stimulate peripheral glucose utilization by inhibiting hepatic gluconeogenesis and increasing anaerobic glycolysis.

Thiazolidinedione Mechanism (TZDs)

TZDs increase insulin sensitivity via PPARγ receptor activation (primarily in adipose tissue).This leads to enhanced fatty acid uptake and storage, reducing circulating free fatty acids, which protects β-cells, liver, and skeletal muscles.This mechanism is known as the "fatty acid steal hypothesis."

Statistical Analysis and Findings Blood glucose levels were measured at various time points after oral administration using a glucometer. ANOVA with Dunnett's multiple comparison test confirmed statistically significant differences in hypoglycemic and anti-diabetic activity between synthesized compounds and the control group.Results were detailed in Tables (5-7) and Figures (11-13), demonstrating dose-dependent hypoglycemia and anti-diabetic effects of



TZD 7-flavonols.

Figure-4. Motif Identification of the targeted diabetic protein domains using PR

Sl. No..	PDB I.D.	Theoretical pI	Half-life	Instability Index	Aliphatic Index	Gravy	Subcellular Location
1	1TOW	6.8	1.2> 20> 10 hrs	22.32 (S)	80.92	-0.27	Cytoplasm, Nucleus
2	1X70	5.56	7.2> 20> 10 hrs	44.69 (US)	76.53	-0.41	Apical cell membrane; Single-pass type II membrane protein.
3	1KZW	6.87	4.4> 20> 10 hrs	32.01 (S)	79.54	-0.59	Cytoplasm
4	1UVQ	4.79	1 hr >30 min> 10 hrs	50.58 (US)	70.34	-0.48	Secreted

5	2I78	5.49	1.9> 20> 10 hrs	45.18 (US)	76.39	-0.42	Apical cell membrane; Single-pass type II membrane protein.
6	2WBB	5.69	30> 20> 10 hrs	26.44 (S)	87.03	-0.17	Cytoplasm
7	2YOQ	6.03	1 hr >30 min> 10 hrs	50.87 (US)	71.71	-0.63	Secreted
8	2ZNO	5.25	30> 20> 10 hrs	45.12 (US)	102.29	-0.17	Cytoplasm, Nucleus
9	4A5S	5.6	1.9> 20> 10 hrs	44.89 (US)	76.24	-0.41	Apical cell membrane; Single-pass type II membrane protein.
10	3RSW	6.39	30> 20> 10 hrs	23.40 (S)	80.41	-0.43	Cytoplasm
11	1JK8	6.29	1 hr >30 min> 10 hrs	24.33 (S)	68.17	-0.46	Secreted, Extracellular space

Table-2. Primary Structure Analysis of the targeted diabetic protein domains using PROTPARAM

S.No.	PDB ID	ALPHA HELIX (%)	EXTENDED STRAND (%)	BETA TURN (%)	RANDOM COIL (%)
1	**1TOW**	20.61	37.4	11.45	30.53
2	**1X70**	21.63	32.21	32.21	36.2
3	**1KZW**	19.08	38.17	12.21	30.53
4	**1UVQ**	23.13	30.37	11.68	34.81
5	**2I78**	22.07	33.37	11.74	32.82

6	**2WBB**	35.02	22.49	11.91	30.58
7	**2YOQ**	16.67	27.41	12.15	43.77
8	**2ZNO**	52.62	13.99	8.04	25.35
9	**4A5S**	21.28	31.15	10.2	37.36
10	**3RSW**	25.63	28.8	10.76	34.81
11	**1JK8**	18.7	32.21	12.21	36.88

Table-3. Secondary Structure Analysis of the targeted diabetic protein domains using SOPMA

Sl.No.	PDB	Max.ID	Max.area	Max. volume	Min.ID	Min.area	Min. volume
1	**1KZW**	22	300.7	237.8	1	8.7	4
2	**1TOW**	16	637.2	925.9	1	16.5	8.7
3	**1UVQ**	61	458.2	893.2	1	16.9	7.6
4	**1X70**	182	6931.6	19025	1	27.3	13.1
5	**2I78**	132	6547.1	18043	1	25.4	12.6
6	**2WBB**	344	8657	21798	1	26.1	12.4
7	**2YOQ**	73	1298.6	3348.1	1	15	11
8	**2ZNO**	68	3010.3	5551.8	1	27	13
9	**4A5S**	184	6863.7	19238	1	35.1	18.6
10	**3RSW**	37	833	1310.4	1	27.3	12.6

Table-4. Protein Surface Scanning of the targeted diabetic protein domains using CASTP

Sl.No.	PDB ID	INTERACTING AMINOACIDS
1	1TOW	ARG 126, TYR 128
2	1X70	ARG 125, SER 209, ARG 358, TYR 662, ASN 710, GLU 205, GLU 206
3	2I78	ARG 125, TYR 662, GLU 205, GLU 206
4	2WBB	GLY 28, LEU 30, THR 31, GLY 26

5	2YOQ	ARG 185, ASP 157, SER 144
6	2ZNO	SER 289, TYR 473, TYR 327
7	4A5S	TYR 631, TYR 662, GLU 205
8	1JK8	ASN 78

Table-5. Interaction study with Natural ligands using ACCELRYS DISCOVERY STUDIO 2.1

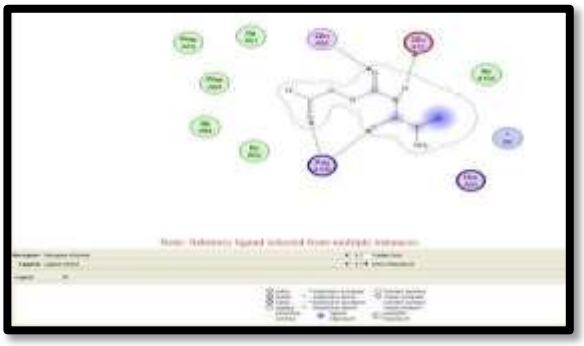


Figure-5. Hotspot Identification for the natural ligands using MOE

Sl.No.	PDB ID	CDOCKER Score								
		3 -	3 -	3 -	3 -	3 -	3 -	3 -	3 -	3 -
1	1TOW	0.971	21.248	29.681	15.138	21.765	11.765	18.329	23.924	25.143
2	1X70	1.987	10.65	18.329	18.975	26.987	3.765	17.269	31.23	21.527
3	1KZW	3.654	4.87	12.324	7.867	13.87	12.111	8.186	15.876	13.625
4	1UVQ	2.453	9.78	10.765	10.361	11.98	9.876	6.784	13.765	11.684
5	2I78	0.765	negative	negative	18.975	15.345	negative	18.723	21.142	19.637

6	2WBB	1.067	13.76	13.43	16.303	24.321	5.771	21.487	25.45	19.129
7	2YOQ	3.786	18.4	8	12.8	13.342	negative	11.347	19.786	12.681
8	2ZNO	2.223	24.381	23.65	21.788	26.32	23.694	27.453	32.67	27.483
9	4A5S	4.231	negative	negative	20.236	21.32	negative	24.12	15.87	26.006
10	3RSW	0.345	7.923	4.765	13.577	9.345	4.675	11.12	21.134	14.307
11	1JK8	2.342	negative	negative	11.765	7.435	7.986	12.342	14.331	12.487

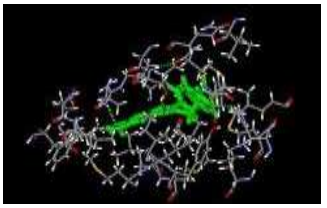
Table-6. CDOCKER Score of the Generated Lead Molecule with target proteins

Sl. No.	Templates	BBB	Absorption	Solubility	Hepato-toxicity	Molecular weight	Log P
1	zolidine-2''4''-dionyl)methyl)7-flavanol **(IIIa)**	2	1	2	0	411	0.474
2	iazolidine-2''4''-dionyl)methyl)6-nitro,7-flavanol **(IIIb)**	1	0	3	0	456	1.629
3	'-(thiazolidine-2'',4''-dionyl) methyl) 6- acetyl, 7-flavanol **(IIIc)**	2	1	2	0	501	0.473
4	iazolidine-2'',4''-dionyl) methyl) 4'- nitro, 7-flavanol **(IIId)**	1	0	3	0	366	3.199
5	iazolidine-2'',4''-dionyl)methyl) 6,4'- dinitro, 7-flavanol **(IIIe)**	0	0	3	0	411	2.782
6	3-(5''-(thiazolidine-2'',4''-dionyl)methyl) 6- acetyl, 4'-nitro, 7-flavanol **(IIIf)**	1	1	3	0	456	3.044
7	iazolidine-2'',4''-dionyl)methyl) 3',4'- dinitro, 7-flavanol **(IIIg)**	0	0	3	0	408	3.199

8	iazolidine-2’’,4’’-dionyl)methyl) 6,3’,4’- trinitro, 7-flavanol **(IIIh)**	0	0	3	0	421	2.987
9	3-(5’’-(thiazolidine-2’’,4’’-dionyl)methyl) 6 – acetyl, 3’,4’-dinitro, 7-flavanol **(IIIi)**	1	1	2	0	498	2

Table-7. ADMET values of the Generated Lead Molecules with target proteins

TZD of 4’-nitro,7-flavonol (**IIId**) TZD of 6, 4’-dinitro,7-flavonol (**IIIe**)



TZD of 6-acetyl, 4’-nitro,7-flavonol (**IIIf**) TZD of 3’4’-dinitro,7-flavonol (**IIIg**) TZD of 6, 3’4’-trinitro,7-flavonol (**IIIg**)

Figure-6. Docking analysis of generated templates with target proteins using ACCELRYS DISCOVERY STUDIO 2.

3.1 Hypoglycemic effect in normal rats

Synthesized thiazolidinedione derivatives were evaluated for their hypoglycemic and anti-diabetic effects in normal and alloxan-induced diabetic SD Wistar rats.

- Hypoglycemic Effect in Normal Rats: The compounds (30 mg/kg) caused only slight reductions in blood glucose levels (5.17%–27.88%), with di- and tri-nitro derivatives showing higher reductions than acetyl or un-substituted derivatives. This suggests they maintain glucose homeostasis without excessive lowering.

- **Oral Glucose Tolerance Test (OGTT):** Following glucose administration, the di- and tri-nitro derivatives showed significant glucose reductions (58.04%–60.84%), comparable to Ciglitazone (63.18%), while other derivatives had moderate effects. Nitro derivatives were the most effective, likely due to their antioxidant properties.

- **Anti-Diabetic Study in Alloxan-Induced Diabetic Rats**:

Single Dose Study: Blood glucose peaked at 1 hour and gradually declined. The most effective compounds reduced glucose levels by 40.10%–58.67%, closely matching Ciglitazone (63.01%).
Multiple Dose Study: Anti-diabetic effects became prominent from Day 7, stabilizing over 15 days, and showed maximum reductions by Day 24 (64.44%–69.46%), surpassing Ciglitazone (62.86%)

Sl. No.	**Treatment**	**Blood glucose level (mg / dl) (Hours)**				
		0	**0.5**	**1**	**2**	**3**
01.	Control (0.3 ml / 100 g)	68.17 ± 1.11	66.83 ± 1.87	63.67 ± 1.84	58.50 ± 1.93	58.00 ± 1.96
02.	Standard drug, Ciglitazone (20 mg / kg)	58.50± 1.78	56.50±1.78 *	47.00±1.53** (26.18 %)	44.33±1.38***	47.33±2.28***(18.39 %)
03.	Compund **IIId**	60.17±2.67	57.67±2.52 *	47.67±1.52** (16 %)	44.83±1.65***	41.83± 2.42*** (27.88)
04.	Compund **IIIe**	58.83± 5.74	52.83±2.41 *	45.17±1.82**(29.01 %)	43.50±1.66***	42.50± 2.81***(26.72 %)
05.	Compund **IIIf**	66.67±4.43	64.50±4.48	59.83±3.51* (6.0 %)	58.33±3.27**	53.67± 2.06**(7.47 %)
06.	Compund **IIIg**	67.00±2.95	62.00±1.95	53.67± 3.47*(15.77 %)	52.67±1.60**	52.17± 2.60** (10.05 %)
07.	Compund **IIIh**	63.17±3.68	61.00±2.66 *	51.00±1.66**(10.48 %)	49.00±1.68***	47.67±1.83***(17.81 %)
08.	Compund **IIIi**	63.33±2.41	61.50±1.42	56.50±1.42*(11.26 %)	55.67±1.75**	55.00±1.77**(5.17 %)

Table-8. Hypoglycemic activity of 2", 4"-thiazolidinedione derivatives of 7-flavonols in normal SD Wister rats

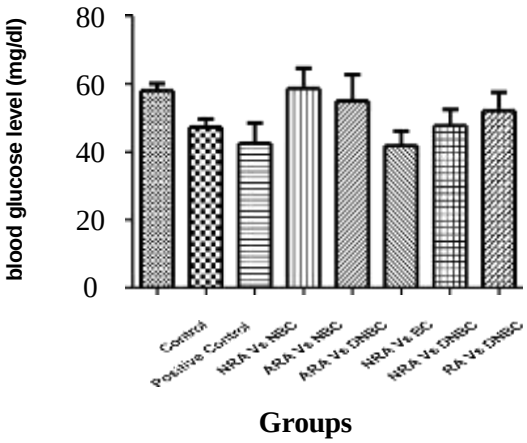


Figure-7. Hypoglycemic activity of 2", 4"-thiazolidinedione derivatives of 7-flavonols in normal SD Wister rats

Sl. No	Treatment	Blood glucose level (mg / dl) (hours)						
		0	**0.5**	**1**	**1.5**	**2**	**2.5**	**3**
1.	Control	74.0±4.0	150±1.50	245.5±13.5	208.0±12.0	203.0±3.0	201.0±3.0	201.0± 2.0
2.	Standard drug, Ciglitazone (20 mg/kg)	84.0±1.0	88.0±2.0	102.0±2.0	83.5±2.5**(59.85%)	81.0±1.0**	80.0±0.7***	74.0±0.4***(63.18 %)
3.	Compound **IIId**	74.5±3.5	89.0±1.0	136.0±10.0	97.0±5.2**(53.37 %)	95.0±5.0**	94.0±3.0***	93.0±1.0***(53.23 %)
4.	Compound **IIIe**	59.5±6.5	85.0±5.0	116±4.0	100.5±0.50*(51.68 %)	97.5±0.50**	88.5±0.40**	80.4±0.40***(60.04 %)
5.	Compound **IIIf**	91.0±6.0	97.5±7.5	134.5±11.5	99.5±1.5**(52.16 %)	95.0±5.0**	94.0±0.60**	94.0±0.6**(53.23 %)
6.	Compound **IIIg**	75.0±9.5	92.5±7.5	156±4.0	102.5±2.5**(50.72 %)	105±5.0**	101.5±4.0**	99.0±4.0***(50.75 %)
7.	Compound **IIIh**	77.0±1.0	85.0±3.0	115.0±5.0	103.0±2.0*(50.48	101.5±1.5*	87.2±1.0**	78.7±2.5**(60.84 %)

					%)	*		
8.	Compound **IIIi**	86.0±7.0	104 ±1.0	129.0±3.0	128.5±9.50*(56.32 %)	102.0±2.0*	94±2.0*	85.5±1.8**(58.0 %)

Table-9. Effect of 2", 4"- thiazolidinedione derivatives of 7-flavonols in Oral Glucose Tolerance Test in normal SD wister rats

OGTT in normal rats

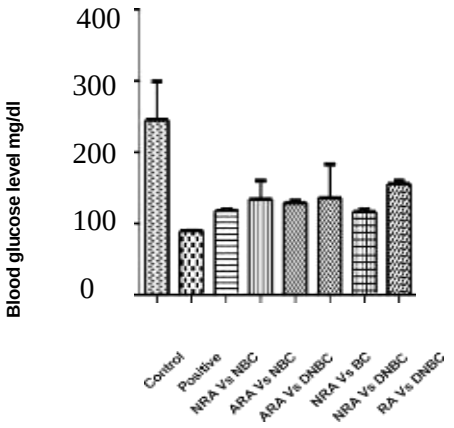


Figure-8. Effect of 2", 4"- thiazolidinedione derivatives of 7-flavonols in Oral Glucose Tolerance Test in normal SD wister rats

Sl. No.	**Treatment**	**Blood glucose level (mg / dl) (Hours)**			
		0	**1**	**2**	**3**
01.	Control (0.3 ml / 100 g)	301.3±0.92	305.2±1.45	308.2±1.62	309.2±2.10
02.	Standard drug, Ciglitazone (20 mg/kg)	239.5±11.90	277.2±6.53*	205.3±4.81** (33.39 %)	121.2±1.96*** (61.10 %)
03.	Compound **IIId**	229.2±6.11	237.2±4.92	199.7±2.90* (35.20 %)	179.3±2.42** (42.01 %)
04.	Compound **IIIe**	247.3±11.42	257.5±10.39*	201.7±10.70** (34.56 %)	148.3±4.60*** (52.04 %)
05.	Compound **IIIf**	251.8±10.4	252.0±12.86	182.8±9.99** (40.69 %)	150.8±9.00*** (51.23 %)

		8			
06.	Compound **IIIg**	240.0±10.10	249.2±9.89	205.2±3.61* (33.42 %)	185.2±3.00** (40.10 %)
07.	Compound **IIIh**	234.2±2.30	242.5±2.67*	166.2±3.54** (46.07 %)	127.8±4.62*** (58.67 %)
08.	Compound **IIIi**	269.3±8.48	277.7±8.34*	207.0±7.48** (32.83 %)	167.3±6.70*** (45.89 %)

Table-10. Effect of 2", 4"-thiazolidinedione derivatives of 7-flavonols in Single dose study on Alloxan induced diabetic animals

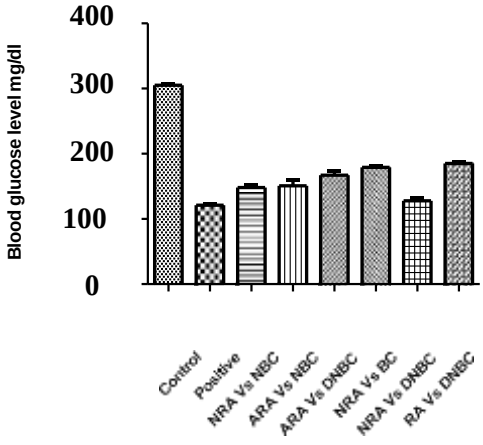


Figure-9. Effect of 2", 4"-thiazolidinedione derivatives of 7-flavonols in Single dose study on Alloxan induced diabetic animals

Sl. No.	**Treatment**	**Blood glucose level (mg dl) (days)**				
		0	**1**	**7**	**14**	**21**
01.	Control (0.3 ml / 100 g)	79.83±2.83	296.7±5.13	285.7±4.01	296.7±4.94	296.7±4.00

02.	Standard drug, Ciglitazone (20 mg / kg)	63.67± 2.99	240.8±7.54**	127.3±9.02** (55.44 %)	122.2±7.94** *	110.2±6.94*** (62.86 %)
03.	Compund **IIId**	96.17± 2.12	228.8±7.99**	151.5±11.22** (46.97 %)	121.5±7.12** *	100.4±6.12*** (66.16 %)
04.	Compund **IIIe**	81.83± 2.39	246.0±8.49*	134.5±12.16** (52.92 %)	111.2±5.44** *	90.6±4.4*** (69.46 %)
05.	Compund **IIIf**	94.50± 3.10	245.2±11.98*	209.5±5.127* (26.67 %)	267.0±6.23**	160.0±5.23** (45.87 %)
06.	Compund **IIIg**	85.17± 3.74	262.3±11.24	206.3±13.45* (79.4 %)	267.7±2.51**	90.7±2.61** (69.43 %)
07.	Compund **IIIh**	80.50± 2.93	238.3±3.33**	168.2±14.70** (41.1 %)	131.0±6.24** *	105.5±5.34*** (64.44 %)
08.	Compund **IIIi**	85.50± 9.93	250.3±9.94*	233.2±3.30* (18.38 %)	226.3±5.45**	160.2±2.45*** (46.01 %)

Table-11. Effect of 2", 4"- thiazolidinedione derivatives of 7-flavonols in multiple dose study on Alloxan induced diabetic animals

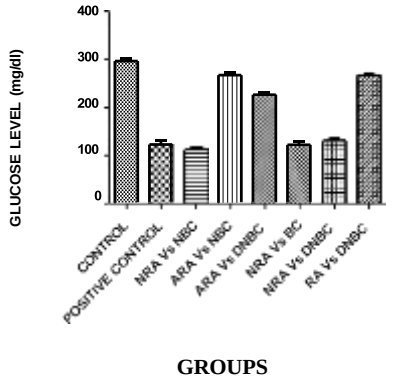


Figure-10. Effect of 2", 4"- thiazolidinedione derivatives of 7-flavonols in multiple dose study on Alloxan induced diabetic animals

3.4 Oral Glucose Tolerance Test in alloxan induced diabetic rats

The results obtained in these studies were tabulated in Tables-12 and were expressed in Figure-

17.The blood glucose levels of the alloxan induced diabetic rats reached a peak at 1.5 hrs after the oral administration of glucose and gradually decreased to pre-glucose load level. Of the six different compounds, the compounds IIId, IIIf and IIIg executed a significant attenuation in the blood glucose at 3rd hr and showed a significant reduction ($P < 0.001$) in blood glucose of 56.39 %; 60.66 % and 57.81 % respectively at 3rd hour when compared to the alloxan-treated diabetic control group ($P<0.05$). The percentage reduction of blood glucose levels produced by other compounds in diabetic SD rats were given as 47.32 %; 52.74 % and 50.45 % for compounds IIId, IIIh and IIIi respectively. Nevertheless, the un-substituted compounds also exhibited hypoglycemic activity when compared to the control. Compounds IIId, IIIf and IIIg gave almost equal results to that of the standard, Ciglitazone (30 mg/kg, 63.1 %).

Compounds with 6-nitro, 4'-nitro, 6,4'-dinitro, 3'4'-dinitro and 6,3',4'-trinitro derivatives were found to be showing more or equipotent hypoglycemic activity to the standard than the acetyl substituted or un-substituted forms of 3-(5''-(thiazolidine-2'', 4''-dionyl) methyl) derivatives of 7-flavonol. These compounds significantly decreased **($p<0.01$) blood glucose levels in diabetic rats and the results supported to be efficient. Like α- glucosidase type of anti-diabetic drugs, the presently dealt TZDs of 7-flavonols too have got a negligible role in hypoglycemia in normal individuals and mimics α-glucosidase type drugs in this aspect.

Sl. No	**Treatmen t**	**Blood glucose level (mg / dl) (hours)**						
		0	**0.5**	**1**	**1.5**	**2**	**2.5**	**3**
1.	Control	300.0 ± 7.30	325.0 ± 4.28	355.0 ± 6.95	362.5 ± 3.82	250.0 ± 0.92	333.3 ± 6.67	323.3 ± 4.41
2.	Standard drug, iglitazone (20 mg / kg)	153.3 ± 6.67	182.7 ± 3.07	217.0* ± 4.01	237.0** ± 4.69	205.6** ± 11.9 (41.26 %)	184.7*** ± 5.12	121.4*** ± 2.96 (63.1 %)
3.	Compund **IIId**	133.4 ± 4.35	141.2 ± 2.99	149.0* ± 3.92	158.2* ± 4.69	200.0** ± 5.2 (42.86 %)	171.0*** ± 9.59	141.0*** ± 6.47 (56.39 %)
4.	Compund **IIIe**	158.3 ± 5.40	174.0 ± 4.58	188.0 ± 3.72	202.5* ± 7.35	247.3*± 11.2 (29.34 %)	182.5** ± 6.29	170.3**± 4.52 (47.32 %)
5.	Compund **IIIf**	129.2 ± 3.75	142.3 ± 4.10	164.2* ± 4.10	157.0* ± 8.36	200.0**± 3.42 (42.86 %)	145.0*** ± 6.83	127.2***± 6.00 (60.66 %)
6.	Compund **IIIg**	160.0 ± 5.77	167.2 ± 5.63	180.2 ± 6.06	187.2 ± 4.48	240* ± 5.00 (31.43 %)	175.0** ± 4.83	155.8** ± 4.17 (57.81 %)

7.	Compund **IIIh**	151.5 ± 0.75	162.5 ± 6.30	177.3 ± 4.67	187.5 ± 10.53	199.0 *± 5.1 (43.13 %)	177.5** ± 10.84	152.8*** ± 8.66 (52.74 %)
8.	Compund **IIIi**	141.5 ± 4.94	155.5 ± 4.21	179.8 ± 4.22	189.5 ± 4.65	229.2*± 6.2 (34.51 %)	174.5** ± 3.36	160.2** ± 3.66 (50.45 %)

Table-12. Effect of 2", 4"- thiazolidinedione derivatives of 7-flavonols in oral glucose tolerance test on Alloxan induced diabetic animals

OGTT in diabetic rats

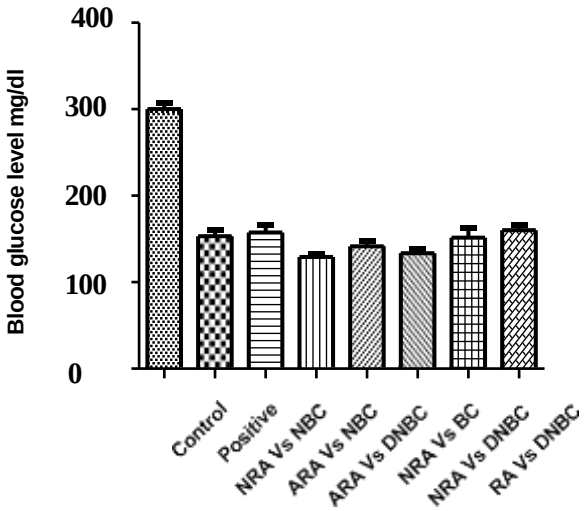


Figure-11. Effect of 2", 4"- thiazolidinedione derivatives of 7-flavonols in oral glucose tolerance test on Alloxan induced diabetic animals

As the knowledge on the heterogeneity of the diabetes mellitus disorder is advanced, the need for more appropriate therapy increases (Baily and Flatt, 1986). Lesser hypoglycemic effect, of the test samples in normoglycemic rats compared to hyperglycemic ones, is a beneficial feature, this because of the emphasize laid on glucose homeostasis as a severe hypoglycemia can result in life threatening situation. Alloxan a beta-cytotoxin induces a chemical diabetes in a wide variety of animal species through damage of insulin secreting cell (Rerup, 1970). Alloxan-treated animals receiving the test samples of thiazolidinediones of 7-flavonols showed rapid normalization of blood glucose levels in comparison to the control and this could be due to the possibility that some β-cells are still surviving to exert their insulin releasing effect by test samples. This suggests that the mode of action of the samples is probably mediated by an enhanced secretion of insulin. These TZD derivatives of 7- flavonols seem to have a promising value for the development of potent drug for diabetes.

A flavanol, being a very good and prevalently obtainable antioxidant, when coupled with a marketedly proven diabetic pharmacophore, thiazolidinedione had thus found to be effective for a significant blood glucose reduction both by computational works and by wet lab.

Henceforth, thiazolidine-2,4-dione derivatives have been studied extensively and found to have diverse chemical reactivities and broad spectrum of biological activities. We, in our laboratory, have been investing our efforts in developing novel TZD molecules and have explored and achieved success in establishing their anti- hyperglycemic potential. Even though these molecules have been established for a long time now, their structural and therapeutic diversity makes them interesting enough to be explored in depth. The attracting significance of thiazolidine diones can be better explored in future as a potent candidate for diabetes mellitus. It is also noteworthy that the toxicity studies have been carried out for these compounds and least toxicity is being found in all these compounds.

1. CONCLUSION

In conclusion, the exploration of thiazolidinedione (TZD) derivatives, specifically TZD-linked 7-flavonols, shows promising potential in the treatment of diabetes mellitus. The beneficial hypoglycemic effects observed, particularly in hyperglycemic models, highlight their role in maintaining glucose homeostasis and reducing the risk of life-threatening hypoglycemia. Alloxan-induced diabetic models suggest that these compounds enhance insulin secretion, possibly by stimulating surviving β-cells. The antioxidant properties of flavonols, when combined with the proven diabetic efficacy of thiazolidinediones, present a promising avenue for developing potent anti-diabetic drugs. Extensive research has shown that these novel TZD molecules possess diverse chemical and therapeutic properties with minimal toxicity, warranting further investigation for future diabetes therapies.

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