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RATIONAL DESIGN, SYNTHESIS AND PHARMACOLOGICAL EVALUATION OF NOVEL THIAZOLIDINONE ANALOGUES AS PPARγ AGONIST ANTI DIABETIC AGENTS

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ABSTRACT:

Diabetes is one of the most widespread and rising health issue worldwide, and researchers have been persevering to develop effective treatment strategies. In pursuit of potential and effective medications for diabetes millitus, we designed and screened some of the novel thiazolidinone moieties which were denoted by FCHSR 1-10 as peroxisome proliferator-acivated receptor-γ (PPAR-γ) modulators for anti-diabetic acitivity. Physicochemical properties of the designed derivatives were evaluated by *Insilico* investigations with the help of Swiss ADME, Molinspiration and Autodock. Swiss ADME predicted the Lipinski's rule of five parameters. Ligand interactions with its targeted receptor, its binding mode and binding affinity was predicted by Molecular Autodock 4.2 version. It was determined that all the derivatives demonstrated good drug likeness score than standard drug Pioglitazone. The selectively designed derivatives were synthesized through a concise four-step reaction process. The structural confirmation of the synthesized derivatives is evaluated by screening its physical parameters as well as characterized by IR, NMR spectroscopy, and mass spectrometry. The synthesized derivatives were evaluated for their *Invitro* acitivity against α-amylase enzyme involves in break down of carbohydrates. The *Invitro* study revealed that the derivative FCHSR-2 predicted to have the greatest effect against the α-amylase enzyme. The present study implies that the modified thiazolidinones may serve as potential lead for the advancement of novel antidiabetic agents.

Key words: Thiazolidinone, Pioglitazone derivatives, *In-silico* studies, Anti-diabetic activity.

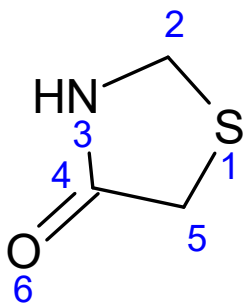
Introduction:

Diabetes mellitus is a metabolic disorder with numerous etiologies. The disease is marked by chronic hyperglycemia, which is accompanied with the disturbances of carbohydrate, protein and fat metabolism arising from deficiencies in insulin secretion, action, or from both [1]. Given the limitations associated with current therapeutic approaches for diabetes management, including adverse effects and suboptimal efficacy, there is a pressing demand to identify novel anti-diabetic agents capable of efficiently targeting by molecular pathways involved in glucose homeostasis [2]. According to the International Diabetes Federation (IDF) report, in 2024 it is estimated that 589 million adults aged 20-79 are currently grappling with diabetes and in 2024 it is witnessed that over 3.4 million people aged 20-79 died associated with the condition. It is anticipated that the total number of individuals living with diabetes would reach 853 million by 2050 [3]. In the arena of heterocyclic hybrids, 4-thiazolidinones stand out prominently due to their biological activities particularly within the category of glitazones.

Thiazolidinone or 4-Thiazolidinone:-

Thiazolidinone (TZ), a class of heterocyclic compounds characterized by a five-membered ring containing sulfur, nitrogen and a carbonyl group and serve as the structural framework for its 2nd and 5th substituted analogs (fig 1) eventually resulting in distinct compounds with unique pharmacological activities. Its molecular formula is C_3H_3NOS and molecular weight is 101.13 g/mol. It is an advantaged pharmacophore possessing multiple biological activities including anticancer, antibacterial, antifungal, antiviral, anti convulsant, sedative, antioxidant, anti hypertension and also antidiabetic [4,5]

Fig 1: chemical structure of 1,3-thiazolidin-4-one



Thiazolidinones [TZs] are widely recognized due their distinctive insulin sensitizing mechanisms, particularly as PPARγ agonists in the therapeutic management of type 2 diabetes [4]. TZs pertains to the glitazones class of antidiabetic agents and their administration can significantly modulate the transcription of various genes involved in lipid and glucose metabolism, as well as their regulation of energy balance [6].

Peroxisome Proliferator-Activated Receptor (PPAR) γ :-

The PPARs are classified as a members of the nuclear hormone receptor family and are also serves as ligand-activated transcription factors. They manipulate target genes by forming heterodimers with the retinoid X receptor (RXR) as well as interacting to specific PPAR response elements (PPREs) in the promoter domains of these genes. Three distinct variants of peroxisome proliferator-activated receptors (PPARs) have been discovered: PPAR-γ, PPAR-α, and PPAR-β/δ (referred to as PPAR-δ) [7].

Fig 2: PPARγ structure

PPARγ (Fig 2) is a promising target for discovering agonists for the management of metabolic disorders, especially type II diabetes, cancer, and atherosclerosis [8]. The activation of PPARγ by 4-Thiazolidinone enhances insulin sensitivity in both the liver and skeletal muscle, which results in the reduction of hepatic glucose production and increased absorption of glucose by skeletal muscle. This minimizes the amount of glucose in circulation, resulting in reduced blood glucose level in type II diabetes mellitus [7] (Fig 3).

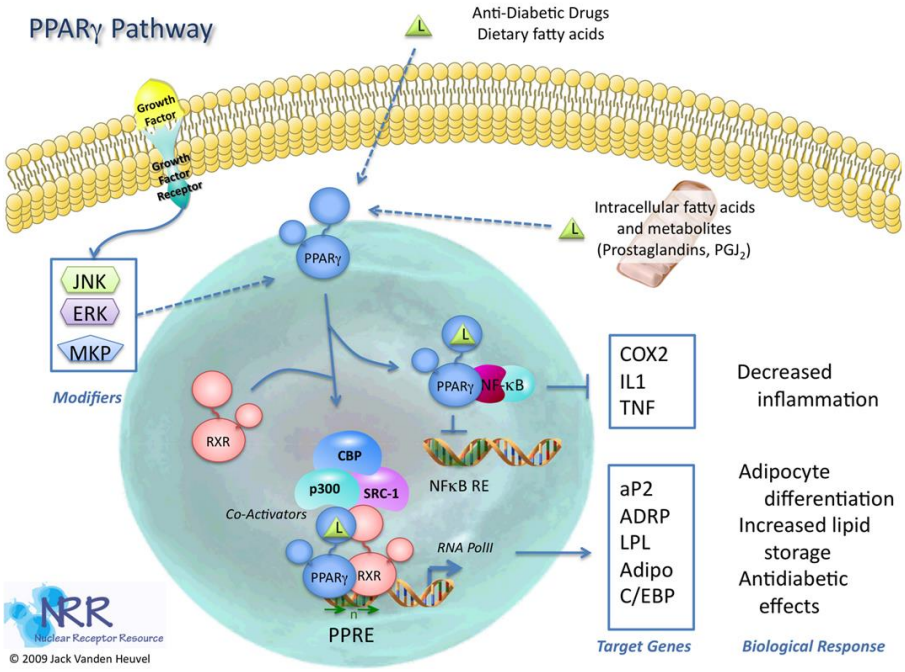


Fig 3: PPARγ pathway

***In-silico* studies:**

In-silico studies refer to the use of software techniques in the development of computational models or simulations which are used to predict, suggest hypotheses, and finally provide discoveries or advances in medicines and therapeutics. The word "*in-silico*" refers to computer-assisted experimentation, which is similar to biological terminology like *"in-vivo"* and *"in-vitro"*. *In-silico* methods, also known as computational methods, allow us to make predictions and digitally simulate any aspect of drug production and discovery, bringing the pharmaceutical industry closer to engineering-based disciplines. *In-silico* studies are one of the techniques used in computer-aided drug development [8].

Molecular docking Studies:

Molecular docking has emerged as a powerful tool for lead discovery and optimization. Over the past 3 decades numerous docking programs had been developed based on the various search algorithms and scoring functions. Molecular docking is a computational program, applied for the identification of possible interactions between the ligand and the target active site. The synthesized compounds were subjected to molecular docking investigation and their likely binding modes and the interactions with target proteins were anticipated. Among the Various Docking algorithms, Auto dock is the Golden version [8].

METHODOLOGY:

1. **General structure:-**

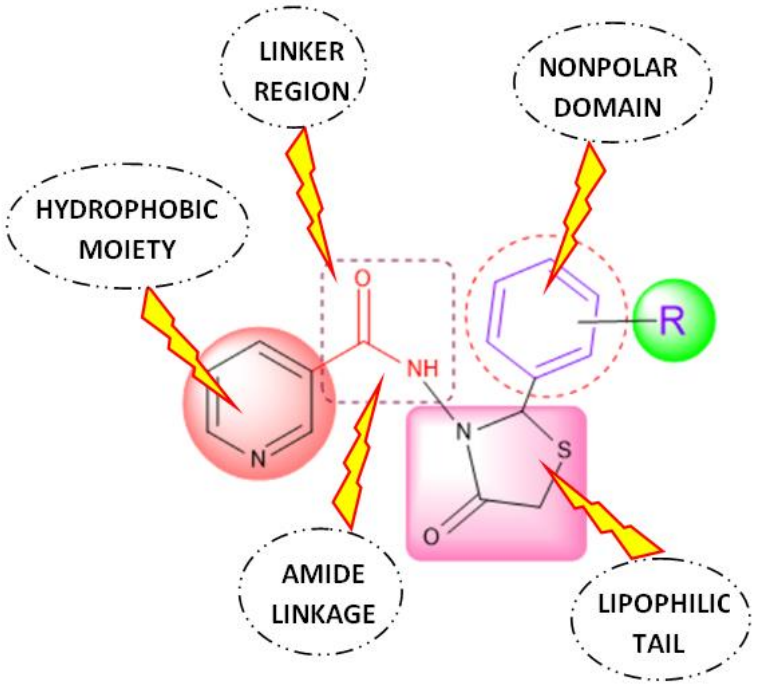


Fig 4: General Structure of Designed derivative

2. Designing strategy:-

Scheme:-

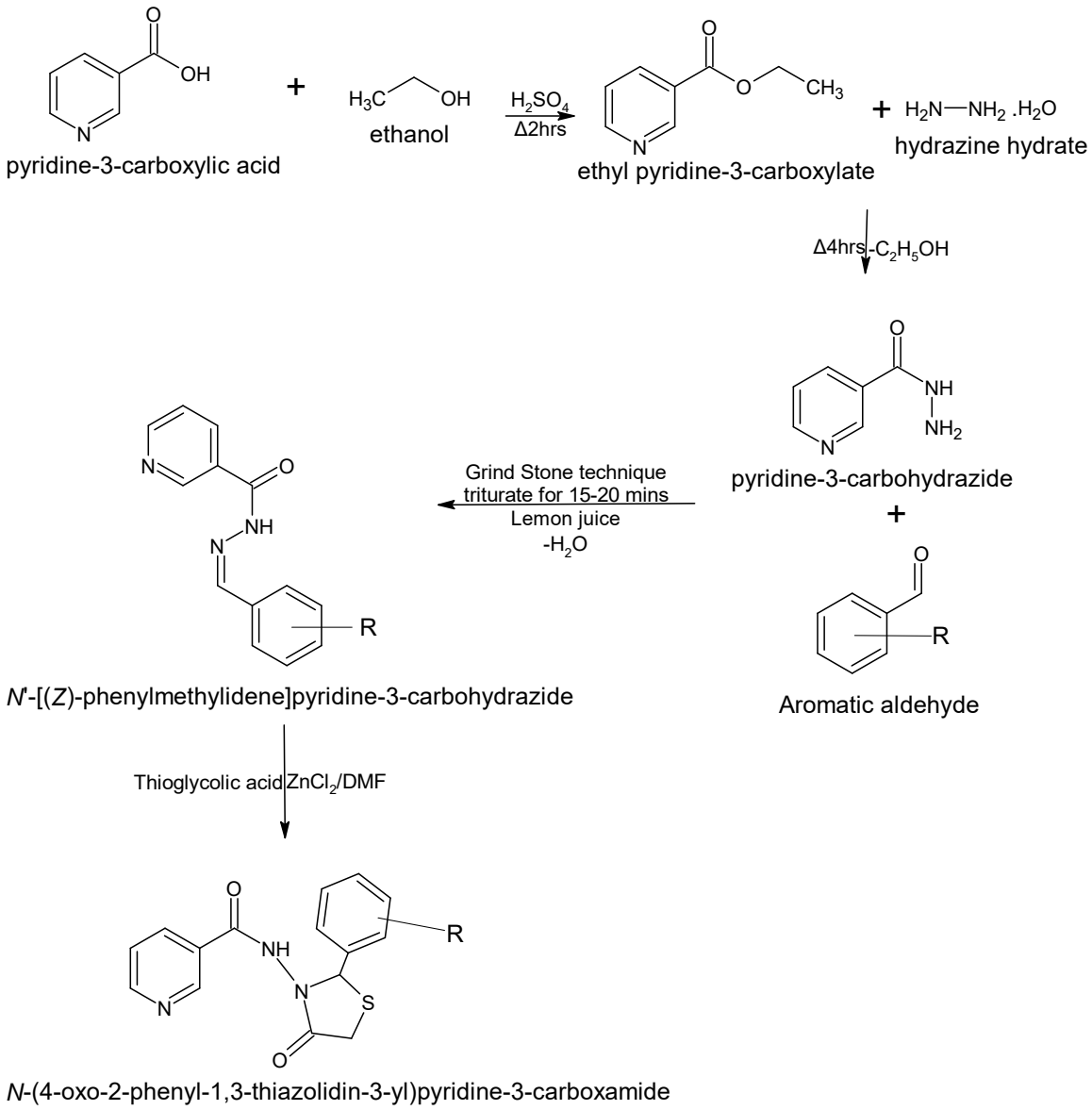


Fig 5: strategy for designing the thiazolidinone derivatives

3. Thiazolidinone Pharmacophore:-

A series of Novel Thiazolidinone Analogues were designed, featuring ten unique aromatic aldehydes in their structure. The FCHSR 1-10 series represents a sequence of designed thiazolidinone analogues.

Table 1: Thiazolidinone analogues

SLNO.	COMPOUND CODE	R
1	FCHSR 1	Benzaldehyde [-H]
2	FCHSR 2	Salicyladehyde [2-OH]
3	FCHSR 3	Anisaldehyde [4-OCH_3]
4	FCHSR 4	Vaniline [3-OCH_3 ,4- OH]
5	FCHSR 5	P-Nitro benzaldehyde [4-NO_2]
6	FCHSR 6	P-Chloro benzaldehyde [4- Cl]
7	FCHSR 7	P-Fluoro benzaldehyde [4- F]

8	FCHSR 8	P-Methyl benzaldehyde [4-CH_3]
9	FCHSR 9	O-Chloro benzaldehyde [2-Cl]
10	FCHSR 10	M-Chloro benzaldehyde [3-Cl]

Fig 5: General structure of designed derivative

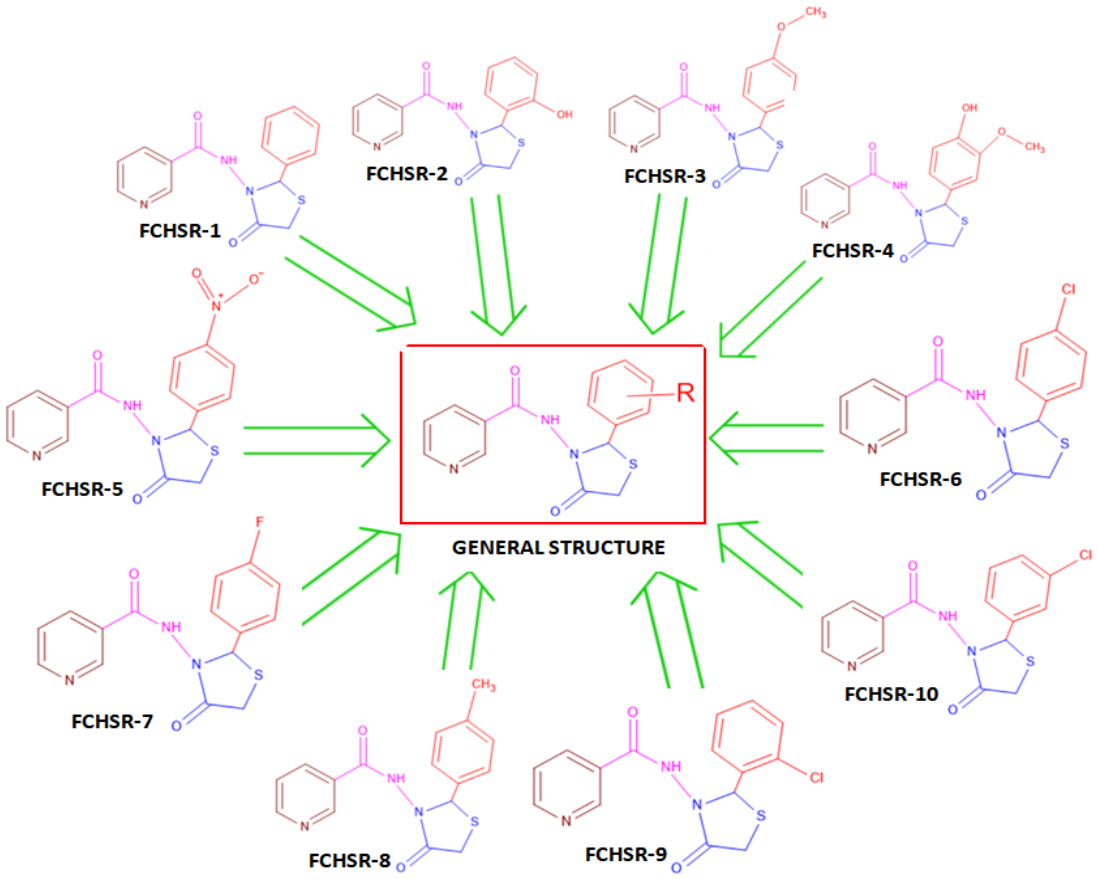


Fig 6: Design Of 1,3-thiazolidin-4-one Analogues.

4. Synthetic procedure:-

The synthesis of designed analogues was carried out according to the general procedure outlined in Fig.5.

A. STEP-1:- Synthesis of ethyl pyridine-3-carboxylate. (Fischer esterification reaction)

To the solution of Ethanol (0.74 M), Nicotinic acid (0.08 M) was added. A Substoichiometric amount of Hydrochloric Acid was introduced to the reaction mixture, which was then refluxed in a round bottom flask with reflux condenser for approximately 2-4 hours. The completion of reaction was evaluated by monitoring TLC. The reaction mixture was then cooled to ambient temperature. Excess amount of ice cold water was added which results in precipitation of the product, which was then filtered and the filtrate was dried. The resulting product was Ethyl Pyridine-3-carboxylate.

B. STEP-2:- Synthesis of Pyridine-3-carbohydrazide. (Electrophilic Substitution reaction)

A mixture of Ethyl Pyridine-3-carboxylate (0.01 M) and Hydrazine Hydrate (0.01 M) was added in a beaker containing ethanol. The reaction mixture was subjected for heating for up to 4-5 hours. Upon the completion of reaction, water was added. The formed precipitate is separated by filtration and the filtrate was dried. The product Pyridine -3-carbohydrazide was formed.

C. STEP-3:- Synthesis of *N*'-[(*Z*)-phenylmethylidene]pyridine-3-carbohydrazide. (Electrophilic addition reaction by grind stone technique)

The equimolar quantity of Nicotinic Hydrazide (0.01 M) and Respective aldehyde (0.01 M) was added in a motor. A catalytic amount of Lemon Juice was introduced in to the reaction mixture. Then it was triturated for about 15-20 mins, and upon the completion of reaction, water was added, the resulting product is separated and dried.

D. STEP-4:- Synthesis of *N*-(4-oxo-2-phenyl-1,3-thiazolidin-3-yl)pyridine-3-carboxamide. (Cyclisation reaction)

In a round bottom flask, 0.01 M of *N*'-[(*Z*)-phenylmethylidene]pyridine-3-carbohydrazide along with 0.01 M of Thioglycolic Acid was added in

DMF. The reaction mixture was subjected for reflux for about 5-7 hours, the reaction progress was monitored using TLC with a mobile phase of Chloroform and Ethanol (8:2 v/v). After the completion of the reaction, the contents were transferred into ice cold water, the resulting solid product was the filtered, the left solid product was dried.

RESULTS AND DISCUSSIONS:-

1. Chemistry:-

The designed Thiazolidinone analogues were synthesized by general procedure as described in Scheme. Briefly, the compounds FCHSR-1, FCHSR-2, FCHSR-4, FCHSR-7, FCHSR-9 were synthesized. The physical properties of the synthesized derivatives were recorded accordingly,

Table-2:- Physical properties of synthesized derivatives.

SL NO	COMPOUND CODE	PERCENTAGE YEILD	RF VALUE	SOLUBILITY	MELTING POINT
1	FCHSR-1	78.59%	0.48	water	143-145ºC
2	FCHSR-2	75.43%	0.51	water	146-148ºC
4	FCHSR-4	82.43%	0.45	water	144-146ºC
7	FCHSR-7	79.63%	0.41	water	157-159 ºC
9	FCHSR-9	72.18%	0.48	water	153-155 ºC

2. *In silico* studies:-

2.1. Molecular descriptors and drug likeness properties:-

Some physicochemical charecteristics of the synthesized compounds were anticipated and listed in Table 3. Molecular parameters which include partition coefficient, Molecular weight, H-bond acceptors and donors, Topological polar surface area and Rotatable bonds of a molecule were evaluated by Swiss ADME. From the results obtained, it was determined that majority of the compounds had complied with the Lipinski rule of five by possessing good oral bioavailability. Absorption and distribution parameters of newly designed analogues were listed in Table 4. Majority of the compounds proved good gastro intestinal absorption.

Table 3: Calculation of physicochemical properties of newly designed Thizolidinone analogues

Compound	Log P	TPSA	MW	HBA	HBD	N Viol	ROTB
FCHSR-1	1.77	87.60	299.35	3	1	0	4
FCHSR-2	1.37	107.83	315.35	4	2	0	4
FCHSR-4	2.02	117.06	345.37	5	2	**0**	5
FCHSR-7	1.88	87.60	317.34	4	1	0	4
FCHSR-9	1.83	87.60	333.79	3	1	**0**	4

*LOGP- Lipophilicity; TPSA- Topological Surface Area; MW - Molecular Weight; HBA – Hydrogen bond acceptors; HBD – Hydrogen bond donors; N Viol. - Number of Violations; ROTB- Number of Rotatable Bonds.

Table 4: Absorption and Distribution properties of Thizolidinone derivatives

Compound	G.I absn	BBB Permeation
FCHSR-1	High	No
FCHSR-2	High	No
FCHSR-4	High	No
FCHSR-7	High	No
FCHSR-9	High	No

*GI absn-Gastro intestinal absorption, BBB permeation-Blood Brain Barrier permeation.

2.2. Molecular docking studies:-

Peroxisome proliferator-activated receptor gamma (PPARγ) docking results:

Docking studies of the aforementioned compounds were computed by using AutoDock software. Docking scores displayed in the **Table 5** provides information regarding the affinities of the ligand with the receptor (target), as well as revealed the contribution and relevant significance of substituent towards target affinity.

By analyzing AutoDock results of Thiazolidinone series it represents that **FCHSR-2** (O-hydroxy) & **FCHSR-7** (p-fluoro) derivatives exhibited highest

affinity **-7.85K/Cal & -7.65K/Cal** respectively comparable to that of standard medication Pioglitazone. Figures 1–2 illustrate the interactions of FCHSR-2 at the active site of the PPARγ (pdb_00003qt0), while figures 3–4 depict the interactions of FCHSR-7. The various interactions of **FCHSR-2** includes two conventional Hydrogen bond with amino acid SER-A:342 and LEU-A:340, one Pi-sigma alkyl interactions with LEU-A:330, three pi-alkyl bonds with amino acids ARG-A:228, ILE-A:341, LYS-A:265 and one carbon hydrogen bond interaction with amino acid LYS-A:285. The interactions of **FCHSR-7** are one carbon hydrogen bond with amino acid GLN-B:98 and four Pi-alkyl interactions with amino acids VAL-B:99, LYS-B:103, LEU-B:10 and ILE-B:256. Other compounds were screened as having strong and significant anti-diabetic activity when compared with the standard drug Pioglitazone. Based on the Docking results it is indicated that the presence of hydroxyl group, methyl group and other electron-donating groups on the phenyl ring enhanced their biological activity.

Table 5: Docking results of title Thiazolidinone compounds for anti-diabetic activity

Sl.no	Docking score
FCHSR-1	-7.34
FCHSR-2	-7.85
FCHSR-4	-7.36
FCHSR-7	-7.65
FCHSR-9	-7.45
Pioglitazone	**-7.2**

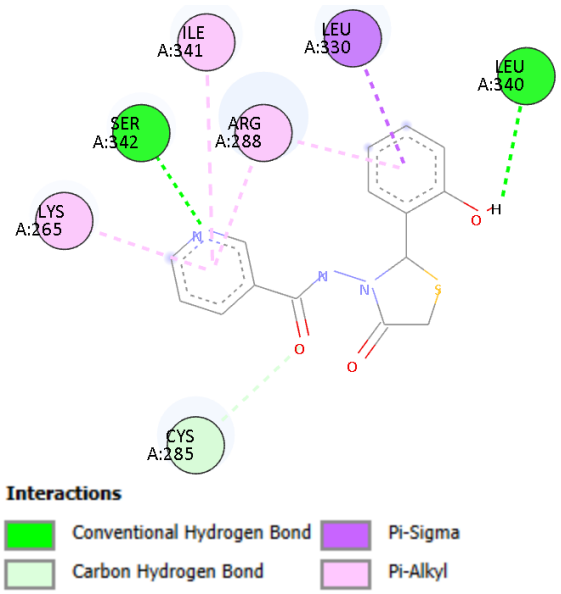


Fig 7: 2D docking interaction of FCHSR-2 with PPARγ

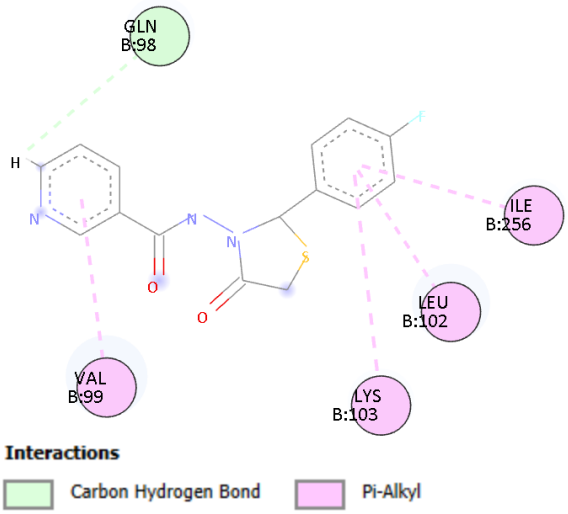


Fig 9: 2D docking interaction of FCHSR-7 with PPARγ

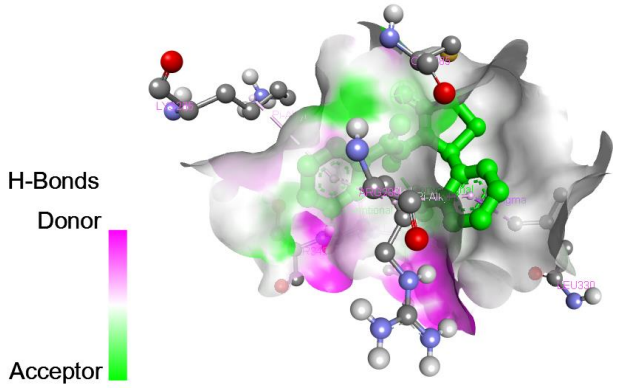


Fig 8: 3D docking interaction of FCHSR-2 with PPARγ

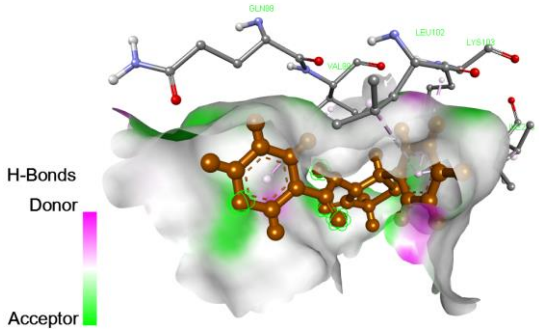


Fig 10: 3D docking interaction of FCHSR-7 with PPARγ

Interactions

Autodock 4.2 was the software used to perform docking investigations for FCHSR 1-10, PPARγ was a target protein that is utilized for screening anti-diabetic activity. On the basis of resulting docking scores, all of the Thiazolidinone derivatives binds with the PPARγ with less energy than standard. This indicates derivatives have potential anticancer activity than standard Pioglitazone. Among all the designed and screened Thiazolidinone derivatives **FCHSR-2** exhibited best interactions with the target PPARγ. The various interactions include conventional hydrogen bond, hydrophobic pi bond, carbon hydrogen bond interactions.

3. In vitro biological evaluation:-

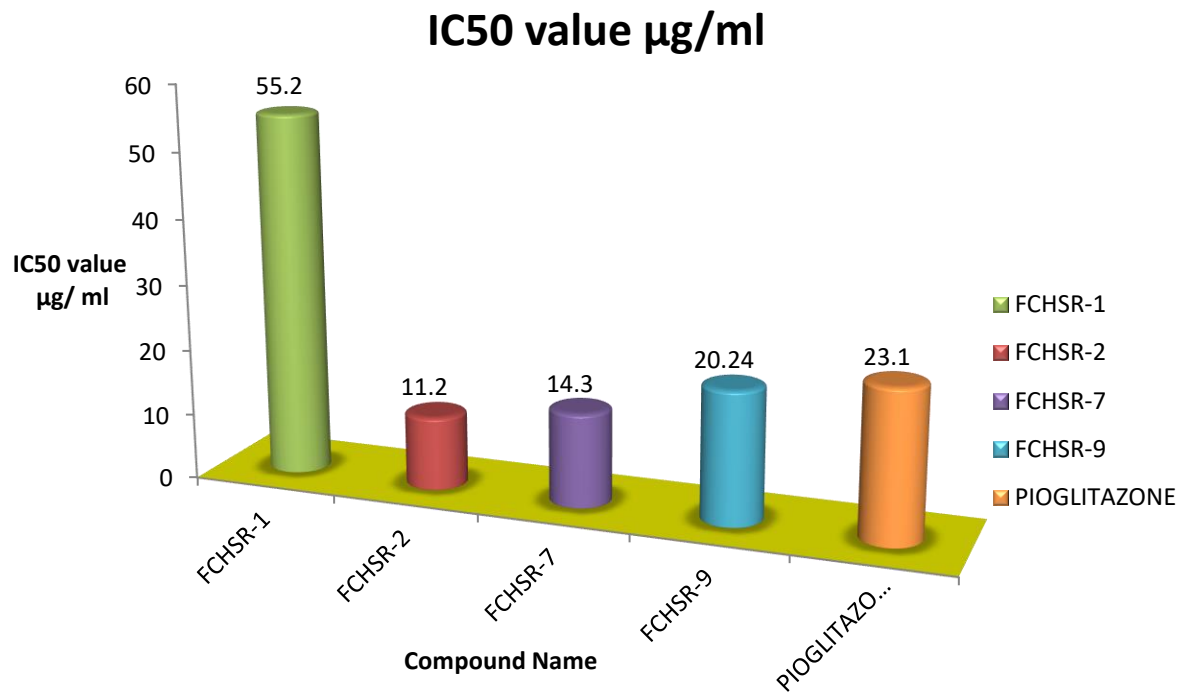
In vitro α-amylase inhibition assay:-

The aim of this study to evaluate the inhibitory potency of the newly designed and synthesized analogues **FCHSR-1, FCHSR-2, FCHSR-7** and **FCHSR-9** against the amylase enzyme, by using Pioglitazone as a standard drug. α-Amylase is a enzyme involves in carbohydrate digestion by breaking down starches into simpler sugars. The α-amylase inhibition properties of samples were performed as described in (Shankar Gharge, et al., 2024) [9].

It is estimated that the IC_{50} value of the compound **FCHSR-2** was found to be **11.2 µg/ml** which is comparatibly lesser than that of the standard drug **Pioglitazone (23.1 µg/ml).**

Table 6: The In Vitro α-Amylase Inhibitory Profile

Sl.no	Compound code	IC_{50} value
1	FCHSR-1	55.2 µg/ mL
2	FCHSR-2	11.2 µg/ mL
3	FCHSR-7	14.3 µg/ mL
4	FCHSR-9	20.24 µg/mL
5	PIOGLITAZONE (Standard Drug)	23.1 µg/mL



CONCLUSION:

In this present investigation, A series of novel thiazolidinone analogues were designed by using computational techniques. The designed analogues were docked with the target PPARγ (pdb_00003qt0) by using software Autodock 4.2 version for anti-diabetic activity by taking Pioglitazone as a

standard drug. From the docking results it was hypothesized that all the designed compounds had less binding energy and more binding affinity along with good interactions against PPARγ as compared to the standard drug Pioglitazone. All of the designed analogues followed Lipinski rule of five demonstrating intensifies oral bioavailability. Based on the results obtained from computational tools, the selectively five derivatives were synthesized and the structural confirmation is done by evaluating its physical parameters as well characterized by IR, NMR spectroscopy, and mass spectrometry. All the synthesized thiazolidinone derivatives was screened by *In Vitro* α-Amylase Inhibitory assay, the results of *Invitro* activity revealed that compound FCHSR-2 had excellent IC_{50} value there by it was concluded that it has potential anti-diabetic activity amongst all synthesized analogs and standard drug Pioglitazone. Present investigation indicated that all the synthesized compounds could be potential lead compounds in the discovery of potent and effective therapeutic agents for the treatment of diabetes mellitus.

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