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INVESTIGATION OF THIAZOLIDINEDIONE DERIVATIVES FOR ANTI-DIABETIC SCREENING: SYNTHESIS, IN SILICO ANALYSIS, AND IN VIVO EVALUATION

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ABSTRACT
Background: Diabetics possess inadequate amounts of insulin to control high glucose levels. Current WHO research estimates that 382 million people have diabetes mellitus, and by 2035, 592 million will. Thiazolidinedione, a physiologically active heterocyclic molecule, including thiazolidine-2,4-dione, is being studied for its anti-diabetic action. Thiazolidinediones, a class of hypoglycaemic drugs used to treat noninsulin-dependent diabetes, were first discovered as insulin-sensitive tissue stimulators. **Methodology:** In-silico applications like Lipinski's rule of five and Molinspiration evaluate physicochemical characteristics, and Molegro Virtual Docker docks molecules. Additionally, compounds are screened in vivo using alloxan as a diabetes inducer and pioglitazone as a comparator medication. **Result and Discussion:** Docking results determine the interactions of derivatives with 5U5L's active site (H2, H3, H5, H7, H9, and H14). Compound H3 interacts with Cys285, Tyr327, Ser289, His323, and Ala278, whereas compound H14 involves Ser289, Leu353, Phe360, Cys285, and Tyr473 with the PPAR-γ receptor, yielding docking scores of -128.341 and -129.766, respectively, and an RMSD value of 2.55 Å. Docking results showed anti-diabetic effects for H3 and H14. In animal screening, both compounds demonstrated efficacy against alloxan-induced diabetes models, supporting their computational findings. **Conclusion:** Pioglitazone interacts with hydrogen bonds involving Ser342, Tyr473, Ser289, Glu291, and Leu228 with a docking score of -118.485, while its co-crystal ligand interacts with Tyr327 and Tyr473 with -121.439. This work demonstrates that the thiazolidinedione pharmacophore is crucial for the discovery of anti-diabetic drugs. Both compounds showed significant anti-diabetic effectiveness in computational and in vivo screening. Thus, more research could prove the compound's anti-diabetic properties.

INTRODUCTION

"Diabetes" and "Mellitus" both have their roots in the Greek language. "Diabetes" signifies "a passer through; a syphon," whereas "Mellitus" denotes "sweet".The Greeks may have given it that name because flies and bees were drawn to the enormous volumes of urine generated by diabetics [1]. High glucose levels

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resulting from insulin deficiency are the hallmark of diabetes mellitus, a chronic endocrine disorder. Nerve damage and sensory problems are symptoms of diabetes. The beta cells of the islets of the pancreas secrete insulin, a peptide hormone regarded as the body's principal anabolic hormone [2-3]. According to a recent WHO research, 382 million individuals are thought to have diabetes mellitus, and by 2035, there will be 592 million diabetic patients worldwide. The prevalence of type 2 diabetes in adults in India is alarming, with around 77 million individuals currently affected and an additional 25 million at elevated risk of onset in the near future [4]. To facilitate the treatment of noninsulin-dependent diabetes mellitus, thiazolidinedione is a new class of hypoglycaemic medications. Initially, they were thought to be anti-diabetic medications that made tissues more sensitive to insulin. Hyperglycemia is a hallmark of diabetes mellitus, characterized by inadequate insulin release that results in impaired glucose utilization. Thiazolidinediones are utilized to manage type 2 diabetes due to their ability to normalize elevated blood glucose levels [5].

MATERIAL &METHODS

Materials

All synthetic-grade chemicals and reagents used in this study were acquired from Lobachemie Pvt. Ltd, Merck Pvt. Ltd, and Oxford Laboratory. The capillary open tube method has been used to determine melting points. Thin-layer chromatography (TLC) was employed to identify the reactions on Silica gel-G using an ethyl acetate: benzene (3:9) solvent mixture, and the resultant spots were analyzed using iodine vapors and ultraviolet light. The IR spectra, 1H NMR, 13C NMR, and mass spectra were conducted at IISER Bhopal.

Computational Study

A pharmacophore (Fig. no. 01) of thiazolidinedione was selected from previously published literature of Jiwane S. K. et al. [2009]. We used ChemDraw Ultra 8.0 to design a total of 65 derivatives (Table 1), utilizing various substitutions listed in Table 1, based on the documented structure-activity relationships published by Jiwane et al.

In silico computational studies were performed to evaluate the ADME (Absorption, Distribution, and Excretion) properties of chemical compounds. Utilizing computational tools like the Lipinski rule of five, Molinspiration, and PreADMET. Docking studies were also performed on all derivatives against the matching protein [PDB ID: 5U5L], downloaded from the Protein Data Bank, using the standard parameters in Molegro Virtual Docker version 6.0. [6-19].

Table 1: Designed Compounds

S. No.	Compounds	R_1	S. No.	Compounds	R_1	S. No.	Compounds	R_1
1	H1	4-Br	**23**	H23	4-CH_3SO_2	**45**	H45	4-CH_3NH
2	H2	2-Cl	**24**	H24	2-$C_6H_{12}O_3$	**46**	H46	2- $C_2H_4O_2$
3	H3	4-$(CH_3)2NH$	**25**	H25	4-$C_6H_{12}O$	**47**	H47	4-$C_2H_6O_2$
4	H4	2- $C_5H_4O_2$	**26**	H26	4-CH_3S	**48**	H48	3-CH_3Br
5	H5	4-CH_3O	**27**	H27	4-C_6H_5S	**49**	H49	2-$C_6H_{12}O_2$
6	H6	2-Cl,3-CH_3	**28**	H28	4-C_2H_5	**50**	H50	3-C_8H_5
7	H7	3-NO_2	**29**	H29	2-C_2H_5O	**51**	H51	4-$C_9H_{14}N_2O_3$
8	H8	4-$(CH_3)_2NH$	**30**	H30	2-OCH_3	**52**	H52	2-F_3C-S
9	H9	4-Cl	**31**	H31	2-$C_4H_{10}S$	**53**	H53	2-F_3C-O
10	H10	3-Cl	**32**	H32	4-$C_8H_{14}O$	**54**	H54	3-CF_2O
11	H11	3-OH	**33**	H33	3-CH_3Cl	**55**	H55	2-CF_2O
12	H12	4-OH	**34**	H34	4-CF_3	**56**	H56	2-$C_6H_5SO_2$
13	H13	2-NO_2	**35**	H35	3-C_7H_7O	**57**	H57	3- CF_3O
14	H14	4- NO_2	**36**	H36	2-$C_9H_{14}N_2O_3$	**58**	H58	4-$C_5H_{13}O_2$
15	H15	4-OH,3-OCH_3	**37**	H37	2-CH_3Cl	**59**	H59	4-C_4H_9
16	H16	3-C_3H_8-NH	**38**	H38	3-CH_3OH	**60**	H60	4-CH_3OH
17	H17	4-C_4H_8N	**39**	H39	4-C_2F_3O	**61**	H61	4-CH_3Cl
18	H18	4-C_7H_7O	**40**	H40	3-$C_2H_4O_2$	**62**	H62	4-$C_2H_4O_2$
19	H19	4-C6H5SO2	**41**	H41	4-C_7H_7O	**63**	H63	2-$C_5H_{11}O_2$
20	H20	4-CH_3OF_2	**42**	H42	2-$C_9H_{10}O_4S$	**64**	H64	4-CH_3OH
21	H21	4-C_8H_4O	**43**	H43	2-CH_3Br	**65**	H65	3-CH=CH_2
22	H22	2-$C_7H_8O_2$	**44**	H44	4-CH_3Br			

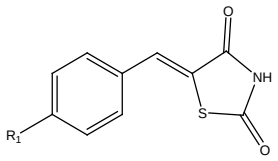


Figure 1: Pharmacophore of Thiazolidinedione

Synthesis

For synthesis, out of 65, we selected the four best compounds (Table 2) based on the results of the computational studies performed. A scheme (Fig. No. 02) was selected from the previously published literature by Jiwane S. K. et al. [2009].

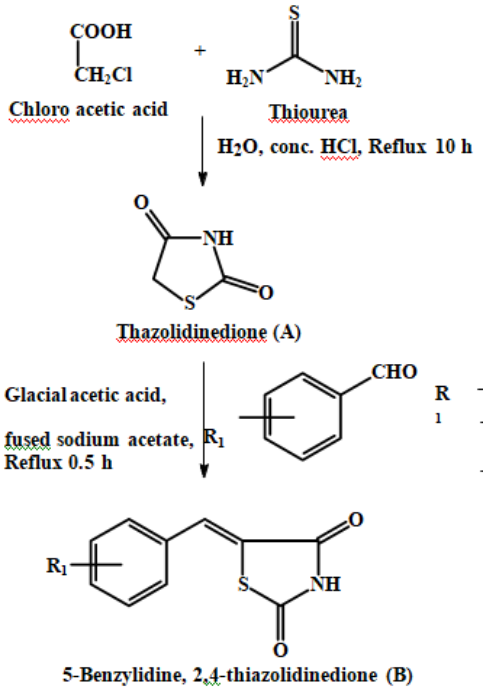


Figure 2: Routes for the synthesis of the substituted Thiazolidinedione compound.

Step I: General synthesis of intermediate (2, 4-Thiazolinedione)

A solution of chloroacetic acid was prepared by mixing 0.6 mol in 60 ml of water in a beaker. In another beaker, a solution of thiourea was prepared in the same manner. Both solutions were mixed and stirred for 15 minutes in an ice bath. Using a dropping funnel, 60 mL of hydrochloric acid was slowly added. After complete addition of acid, the mixture in the flask was refluxed for 8-10 hrs. at 100-110℃. TLC was used to evaluate the completeness of the reaction. The mixture was left to cool down at room temperature. The solid product was filtered off using a Buchner funnel and washed well with water. The pure crystals were recovered after recrystallization with ethanol.

Step-II: General synthesis of substituted compounds (5- (4-chlorobenzylidine)-2, 4- thiazolidinedione)

We added a mixture of fused sodium acetate (1.8 g), a solution of substituted benzaldehyde (0.25 mol), 50 ml of heated glacial acetic acid, and 2-thiazolidinedione (0.25 mol) into a beaker. The solution was stirred and refluxed at 100°C. The addition of 300 milliliters of water to the product resulted in the formation of precipitates upon cooling. The obtained product was filtered and subsequently rinsed with water for purification. Recrystallization was then performed using glacial acetic acid. [20]

***In vivo* Anti diabetic evaluation of the compounds against alloxan-induced diabetes in rats**

Experimental Animals

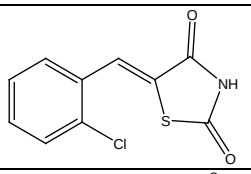
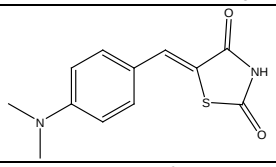
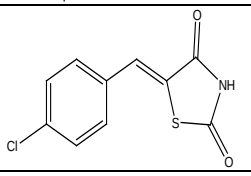
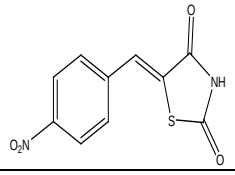
Healthy albino rats of either sex weighing about 150-200g were used during the study. The animals were acquired from the G.R.Y. Institute of Pharmacy, Borawan, dist. Khargone (Madhya Pradesh). Before the experiment began, the rats were acclimated for 5 days. Standard environmental conditions, such as temperature ranging from 18 to 32°C, relative humidity (70%), and 12 hours of dark/light cycles, were maintained during the quarantine. All the animals were fed with a rodent pellet diet and water under strict hygienic conditions. The experiment was conducted in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals, New Delhi, India, and the research protocol was approved by the Institute's Animal Ethics Committee (06/ac/01/CPCSEA).

Acute Oral Toxicity Study

In accordance with OECD 423 recommendations, an acute oral toxicity study was conducted to determine the minimal lethal dose of compounds H3 & H14. We utilized male & female Wistar albino rats ranging in weight from 150 to 200 grams. Before receiving oral administration of the extracts at doses up to 3000 mg/kg, the experimental animals were fasted overnight & given just water.

During the first 4 hrs. & at regular intervals throughout the first twenty-four hours, they were watched closely for any changes in behavior or toxic symptoms such as hyperactivity, abnormalities in the skin or fur, convulsions, vomiting, dilated pupils, drowsiness, hypothermia, or death. The animals were then observed nonstop for 7 days at predetermined times.

Table 2: Synthesized compounds

S. N.	Compound code	R_1	Structure	Compound Name	Melting Point(℃)	Rf Value	% Purity
1.	**H2**	2-Chloro Benzaldehyde		5-(2- chlorobenzalidene) thiazolidine-2, 4–dione	180-185	0.49	62±2%
2.	**H3**	4- Dimethyl amino Benzaldehyde		5-(4-Dimethyl aminobenzalidene) thiazolidine-2, 4–dione	100-110	0.67	64±2%
3.	**H9**	4-Chloro Benzaldehyde		5-(4-chlorobenzalidene) thiazolidine-2, 4–dione	182-184	0.57	59±4%
4.	**H14**	4- Nitro Benzaldehyde		5-(4-nitrobenzalidene) thiazolidine-2, 4–dione	115-120	0.51	64±2%

N= 3 runs

Induction of Diabetes

Male Wistar albino rats weighing 150-200 g that had fasted overnight were injected intraperitoneally with 120 mg/kg of alloxan monohydrate as a single dose to induce diabetes, except for the normal control. Animals were included in the study if their blood glucose levels were higher than 250 mg/dl after 72 hours, which is considered diabetic. Each of the five groups of animals consisted of six animals. The group I, designated as the normal control group of rats, was given 1ml of a gum acacia suspension (1% concentration). Group II is designated as the toxic control group treated with 1 ml of gum acacia suspension along with alloxan. The standard medicine, Pioglitazone (150 mg/kg), was administered to rats in Group III and designated as the standard group. In the fourth group, rats were administered 200 mg/kg of compound H3, and in Group V, rats received 200 mg/kg of chemical H14. For 21 days, the rats in this study received the same daily doses of the compounds. On days 0, 7, 14, and 21 after compound administration, glucometer readings were taken from the tail veins of non-fasted rats to determine blood glucose levels. On day 21, an auto-analyzer was used to measure the serum lipid profiles.

Blood glucose estimation

Animals were kept fasted overnight. Blood sampling was done by sterilizing the tail with 10% alcohol and then nibbling the tail at the start of the experiment. After the operation, the tips of the tails were sterilized by swabbing with 70% ethanol. Fasting blood glucose was estimated using commercially available glucose strips on a One Touch Glucometer and repeated on the 0th, 7th, 14th, and 21st days.

Histopathological studies

Pancreatic tissues from rats of all groups of Multi-dose (Sub-acute) treatments were subjected to histopathological studies. The whole pancreas from each animal was removed after sacrificing the animal under anesthesia and was collected in 10% formalin solution and immediately processed by the paraffin technique. Sections of 3-5 µm thickness were cut and stained by hematoxylin and eosin for histological examination.

Statistical analysis

Results of anti-diabetic studies were tabulated using mean values ± SEM. We found that the statistical significance of the data was analyzed by examining the variance and conducting group comparisons using the "Tukey-Kramer" multiple comparison test.

A significance level of $p<0.05$ was used. The toxic control group was evaluated in respect to the other treatment groups using in vivo techniques. In contrast, the normal control group was assessed in relation to the toxic control group.

RESULTS AND DISCUSSION

Spectrum studies, including FTIR, NMR, and mass spectrometry, were performed on synthesized compounds H3 and H14 at IISER, Bhopal. In FTIR (KBr) υ cm^{-1} interpretation for compound H3- a medium, sharp in the range of 3400 indicates the aliphatic primary amine group [N-H]. A medium band in the range of 2840–2980 shows an alkane group [CH_3]. The weak to medium, sharp, and stretching band in the range of 2222-2260 indicates the presence of the nitrile group [C-N]. An overtone combination band is observed in the range of 1650. The Stretched, strong, and sharp band in the range of 1250 states the aromatic ester [C=O].

Table 3: *In silico* studies of Synthesized compounds

Results of the Lipinski rule of five

Compound ID	Mass	Hydrogen bond donor	Hydrogen bond acceptor	Log P	Molar Refractivity
H2	239.500	1	3	1.8919	57.6276
H3	248.000	1	4	2.0765	69.8756
H9	239.500	1	3	1.8919	57.6276
H14	250.000	1	5	1.9187	62.2030

Results of Molinspiration properties

S.No.	Code	Mi log P	TPSA	No. of Atom	MW	nON	nOHNH	N violations	nrotb	Volume
1	**H2**	2.11	49.93	15	239.68	3	1	0	1	182.65
2	**H3**	1.58	53.17	17	248.31	4	1	0	2	215.02
3	**H9**	2.16	49.93	15	239.68	3	1	0	1	182.65
4	**H14**	0.61	49.93	15	204.23	3	1	0	1	178.81

Results of the predicted bioactive score of designed compounds.

S. No.	Code	GPCR ligand	Ion channel modulator	Kinase inhibitor	Nuclear receptor ligand	Protease inhibitor	Enzyme inhibitor
1	**H2**	-1.15	-1.17	-1.67	-1.90	-1.37	-1.52
2	**H3**	-0.86	-0.98	-0.38	-0.62	-0.97	-0.29
3	**H9**	-1.12	-1.12	-1.67	-1.90	-1.25	-1.44
4	**H14**	-1.12	-1.10	-1.63	-1.86	-1.17	-0.38

Results ofPreADME

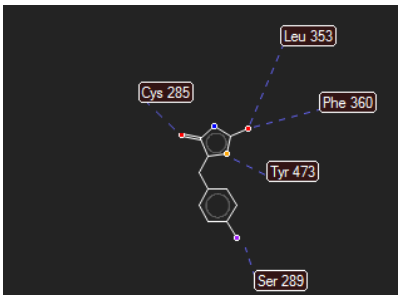
S. No.	Code	BBB	Caco2	HIA	Plasma Protein Binding	Skin Permeability	MDCK
1	**H2**	0.8654	15.8488	96.2925	93.4077	-2.9260	33.2318
2	**H3**	0.0183	18.4619	96.4222	98.2671	-3.3274	39.9290
3	**H9**	0.8654	20.7651	96.2925	96.9835	-2.9598	31.0947
4	**H14**	0.8630	27.1671	96.1880	97.0327	-2.7206	34.0827

Results of Toxicity

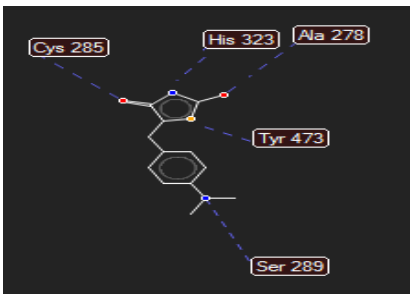
Toxicity		Compounds
Ames Test	**Mutagen**	H2, H3, H9, H14
	No Mutagen	-
Carcino Mouse	**Positive**	-
	Negative	H2, H3, H9, H14
Carcino Rat	**Negative**	H2, H3, H9, H14
	Positive	-
HERG Inhibition	**Medium risk**	H2, H3, H9, H14
	Low risk	-

Table 4: Results of docking study

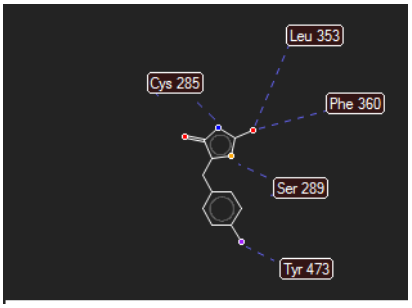
S. No.	Compounds	Mol Dock score	H-bond score	Interactions
1.	**H2**	-125.485	-6.83506	Cys285, Tyr327, Ser289, Phe360, Leu353
2.	**H3**	-128.341	-7.87713	Cys285, Tyr327, Ser289, His323, Ala278
3.	**H9**	-127.991	-6.81009	Cys285, Tyr327, Leu353, Phe360, Ser289
4.	**H14**	-129.766	-8.19274	Ser 289, Leu 353, Phe 360, Cys285, Tyr473
5.	**Standard (Pioglitazone)**	-118.485	-4.18665	Ser342, Tyr473, Ser289, Glu291, Leu228



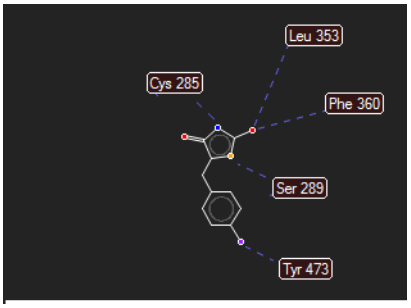
Compound H2



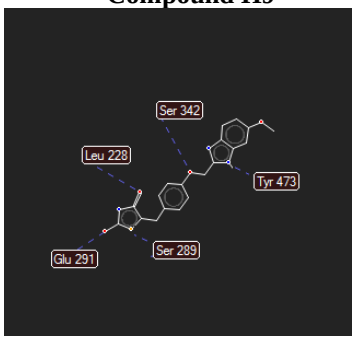
Compound H3



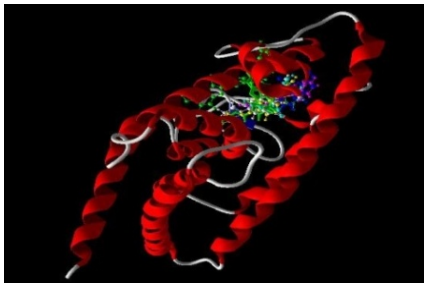
Compound H9



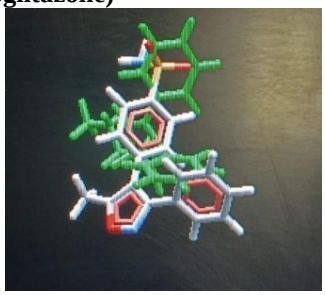
Compound H14



Standard (Pioglitazone)



Ribbon structure of Co-crystallized ligand with PDB



RMSD Pose (2.55 Å)

Figure 3: Hydrogen bond interaction of compounds with validation.

Structural analysis of synthesized compounds: FT-IR spectral data of compounds

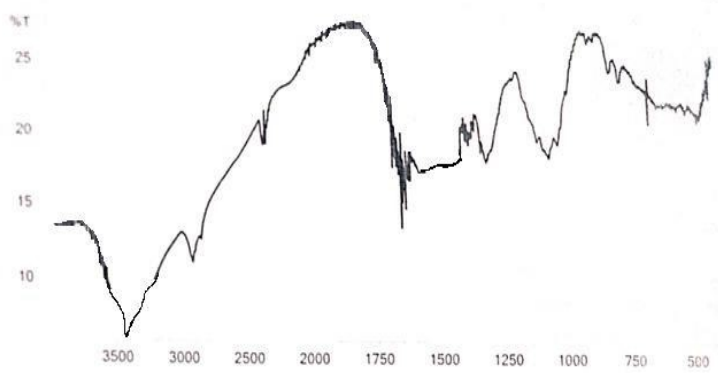


Figure 4: FT-IR Spectrum of 5-(4- Dimethylaminobenzalidene) thiazolidine- 2, 4- dione (H3)

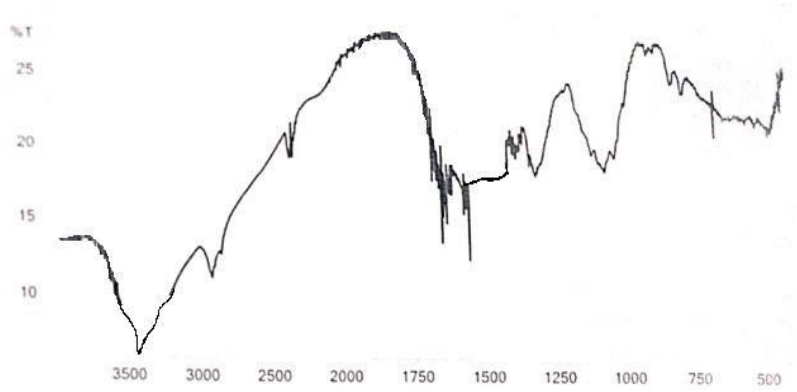


Figure 5: FT-IR Spectrum of 5-(4- Nitro benzalidene) thiazolidine - 2, 4- dione (H14)

MASS spectral data of compounds

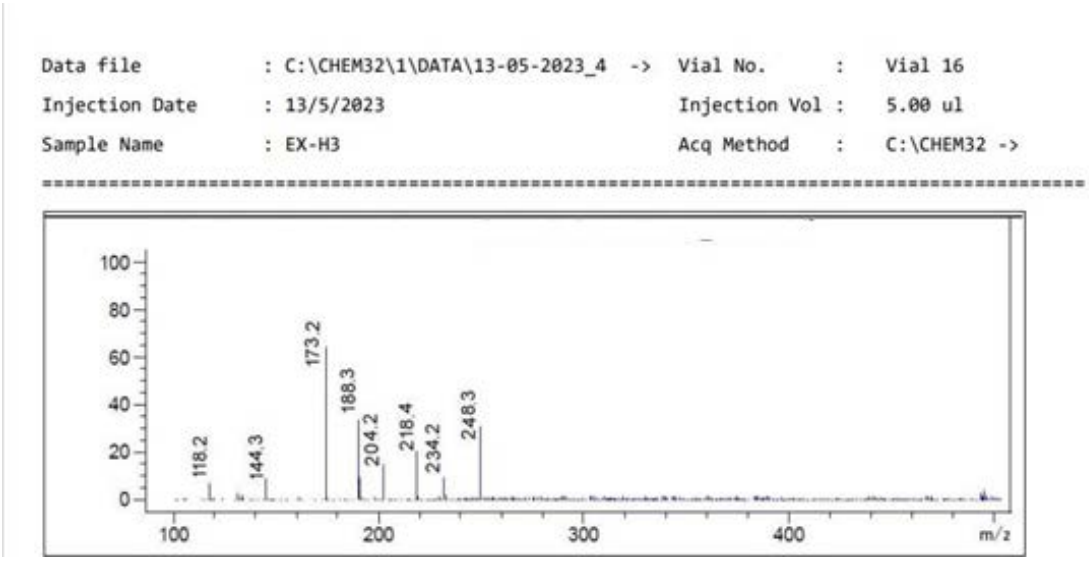


Figure 6: Mass Spectra of 5-(4- Dimethylaminobenzalidene) thiazolidine- 2, 4- dione (H3)

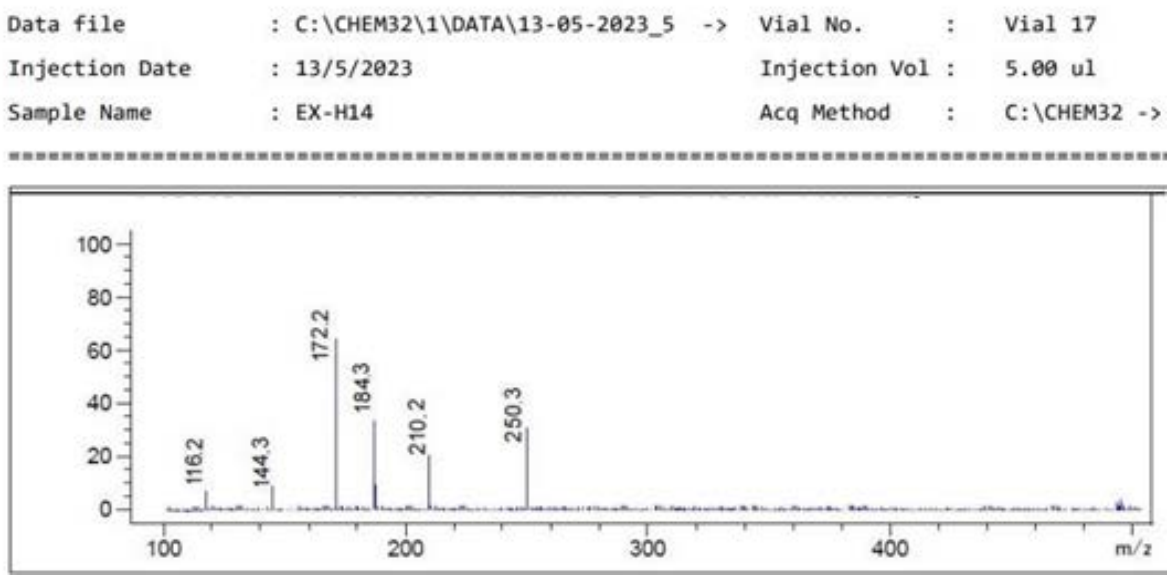


Figure 7: Mass Spectrum of 5-(4- Nitro benzalidene) thiazolidine - 2, 4- dione (H14)

1H NMR spectral data of compounds

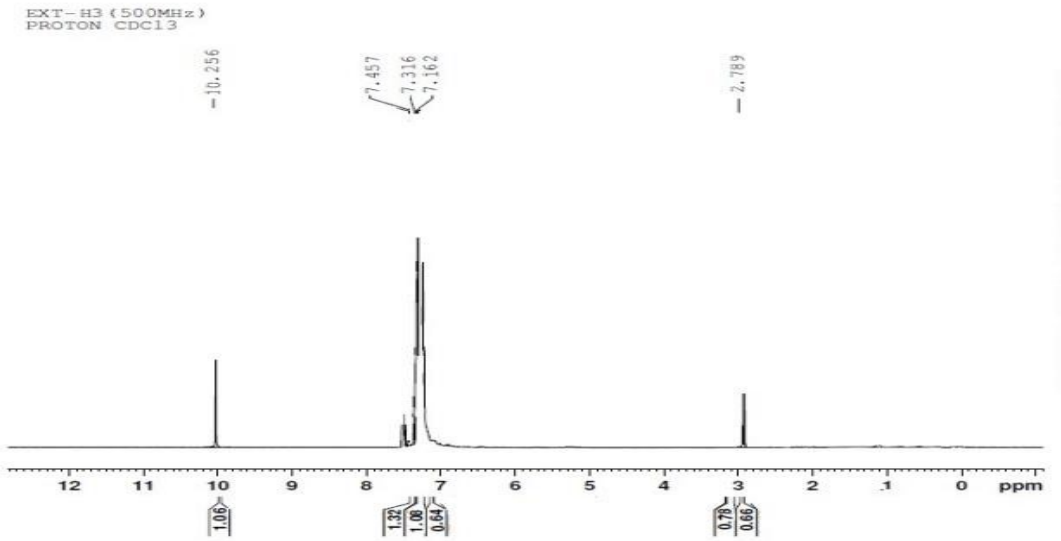


Figure 8: 1H NMR Spectrum of 5-(4- Dimethylaminobenzalidene) thiazolidine- 2, 4- dione (H3)

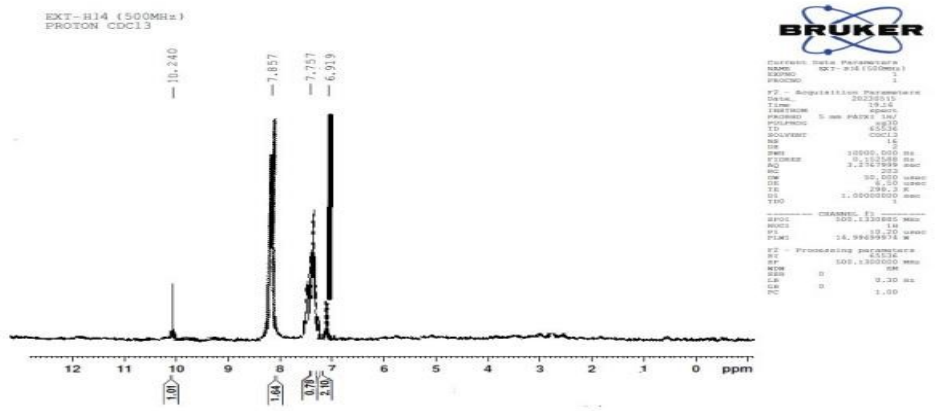


Figure 9: 1H NMR Spectrum of (5-(4- Nitro benzalidene) thiazolidine- 2, 4- dione (H14)

13C NMR spectral data of compounds

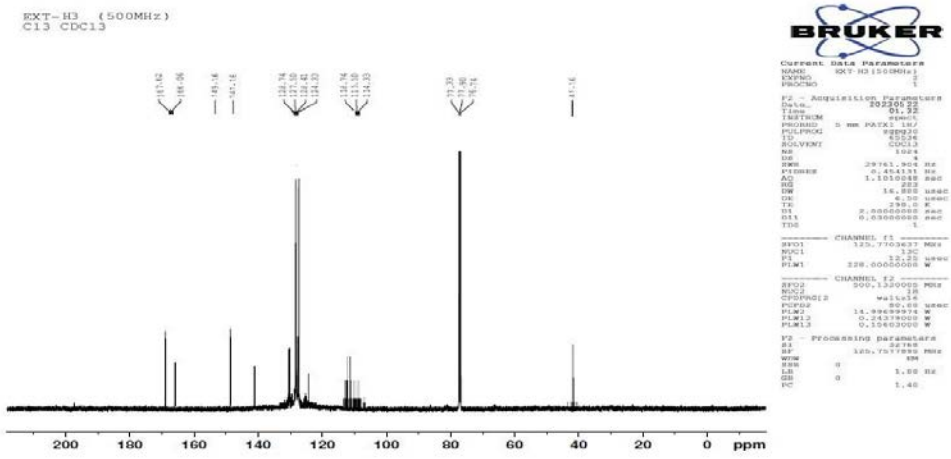


Figure 10: 13C NMR Spectrum of 5 -(4- Dimethylaminobenzalidene) thiazolidine- 2, 4- dione (H3)

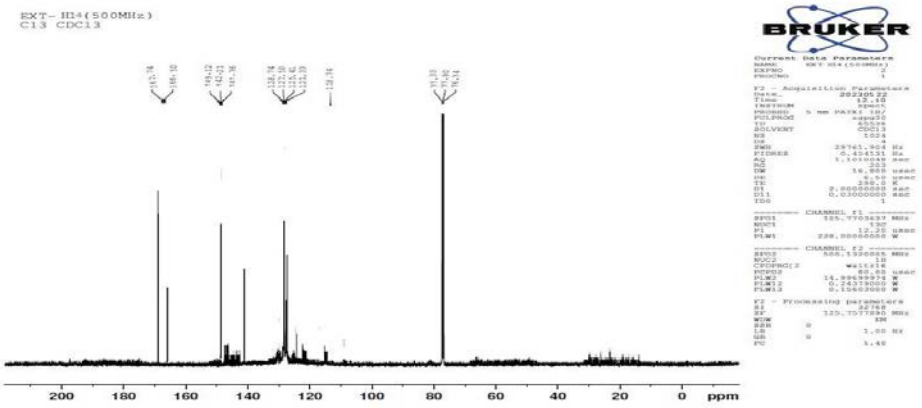


Figure 11: 13C NMR Spectrum of (5 -(4- Nitro benzalidene) thiazolidine- 2, 4- dione (H14)

In FTIR (KBr) υ cm^{-1} for H14 – a medium, sharp in the range of 3400 indicates the aliphatic primary amine group [N-H]. A medium band in the range of 2840–2980 shows an alkane group [CH_3]. The weak to medium, sharp, and stretching band in the range of 2222-2260 indicates the presence of the nitrile group [C-N]. An overtone combination band is observed in the range of 1650. A strong and stretched peak in the range of 1550 indicates nitro compounds [NO_2]. The Stretched, strong, and sharp band in the range of 1250 states the aromatic ester [C=O]. The strong band due to C=O in the region 1100 indicates aliphatic ether, and the small band in the region of 800 indicates sulfide group[C-S-C]. The graph was displayed in Figure 5.

The study of mass spectra (m/z) for compounds H3 and H14 revealed peaks at 248.3 (M+) and 250.3 (M+), respectively, confirming the designed structure. The graphs for mass studies of H3 and H14 were displayed in Figures 6 and 7, respectively. The interpretation of H^1NMR (500 MHZ) (ppm)for compound H3- The peak at 10.256 indicates the presence of the Thiazolidine N-H group. A peak range of 7.457-7.162 indicates that four hydrogens are present in the aromatic ring. A peak at 2.789 indicates an aromatic amine with two CH_3 groups. The graph is displayed in Figure 8. The interpretation of the C13 NMR (500 MHz) (ppm) for compound H3 is as follows: the peak range of 167.62-166.06 indicates the presence of a Thiazolidine ketone group. A peak range of 149.16-141.16 suggests the presence of a tertiary carbon group. A peak in the range of 126.74, 127.0, 126.41, and 124.33 indicates the presence of an aromatic carbon group. The peaks of 118.74, 115.50, and 11.33 indicate the thiazolidine carbons. The peaks at 77.33, 77.90, and 76.74 indicate the presence of chloroform. The peak range of 41.16 indicates the methylidyne [C-CH3]. The graph was shown in Figure 10.

The interpretation of H^1NMR (500 MHz) (ppm) for compound H14: The peak at 10.240 indicates the presence of a Thiazolidine N-H group. A peak range of 7.750-7.757 indicates that four hydrogens are present in the aromatic ring. And a peak at 6.919 indicates aromatic Hydrogen. The graph was displayed in Figure 9. The interpretation of the ^{13}C NMR (500 MHz) (ppm) for compound H14 suggests that the peak range of 167.62-166.06 corresponds to the Thiazolidine ketone group. A peak range of 149.16-141.16 indicates the presence of a tertiary carbon group. A peak in the range of 126.74, 127.0, 126.41, and 124.33 suggests the presence of an aromatic carbon group. The peaks of 118.74, 115.50, and 11.33 indicate the thiazolidine carbons. The peaks at 77.33, 77.90, and 76.74 indicate the presence of chloroform. The graph was shown in Figure 11.

The metrics for the Lipinski rule of 5 identify drug-like compounds from alternatives. Allow five hydrogen bond suppliers (nitrogen or oxygen atoms with hydrogen). Allow 10 hydrogen bond acceptors (nitrogen or oxygen) and a molecular weight under 500. No more than 5 octanol-water partition coefficient log P. Molar refractivity should be 40–130.

A web-based software called Molinspiration was used to measure parameters such as MiLogP, which is under the range of 5, TPSA is under the 140Å, MW is under the range of 500, nrotb, which is under 10, nON, which is under 10, nOHNH, which is under the range of 5, and Violations should be 0. Calculating activity scores for GPCR ligands, ion channel

modulators, nuclear receptor ligands, kinase inhibitors, protease inhibitors, and enzyme inhibitors can determine the bioactivity score of a drug. If the bioactivity score is larger than zero, the organic molecule is active; moderately active is between -5.0 and zero; and inert is below -5.0.

The *in silic*o prediction indicates that Compounds H2, H3, H9, and H14 comply with the rules of Lipinski, Molinspiration, PreADME, and Toxicity prediction, as shown in Table 2. Docking results (Table 3) demonstrate that compounds H3 and H14 exhibit significant affinity and hydrogen bonding interactions. Compound H3 interacts with Cys285, Tyr327, Ser289, His323, and Ala278, while compound H14 interacts with Ser289, Leu353, Phe360, Cys285, and Tyr473. The hydrogen bond scores for H3 and H14 are -7.87713 and -8.19274, respectively, with pdb 5U5L for the PPAR-γ receptor, resulting in docking scores of -128.341 and -129.766, respectively, and an RMSD value of 2.55 Å. [21-29].

Limitations of the docking study

MVD employs randomized search techniques, similar to those used in other molecular docking applications, which yield unpredictable outcomes. This implies that because investigating the huge conformational space is essentially random, subsequent runs with the same input may produce slightly different positions.

***In vivo* evaluation of anti-diabetic activity by Alloxan-induced diabetes in rats**

In this *in vivo* evaluation, alloxan is used to induce diabetes. An animal study found that the diabetogenic chemical alloxan caused insulin insufficiency and chronically high blood glucose levels by targeting and killing pancreatic beta cells. This model has been used extensively to investigate type 1 diabetes and evaluate new medications for the disease. This study highlights the potential of H3 and H14 as therapeutic agents by showing that after 21 days of treatment, there is a significant reduction of blood glucose level up to 38% and 45% respectively, with a significance value of $P<0.05$, when compared to the standard of 57% in rats induced with alloxan (Table 5). The acute toxicity analysis demonstrated that H3 and H14, taken orally at a dosage of 200 mg/kg, showed no signs of toxicity or death in the treated animals, thereby affirming their safety and suitability for further research. The anti-diabetic efficacy of the substances was assessed through the analysis of their biochemical and histopathological features. Our research suggests that H3 and H14 effectively modulate lipid metabolism by restricting the release of free fatty acids, resulting in decreased total cholesterol and triglyceride levels, alongside an elevation in HDL levels in diabetic rats. Importantly, during the 21st day of treatment, HDL levels rose. Additionally, compared to the reference, the produced compounds showed a considerable drop in VLDL and LDL levels. Alloxan destroys ß-cells in vitro by producing ROS, which in turn causes cell necrosis and a calcium surge in the cytoplasm. Tables 4 & 5 & Figure 12 reveal that ß-cell regeneration was enhanced by pioglitazone therapy, a finding also observed in H3 and H14, as indicated by the reduction in blood glucose levels and the plasma lipid profile.

The effectiveness of treatment against alloxan-induced diabetes in rats was demonstrated after 21 days, when all parameters exhibited a significant decrease, with an exceptional result of the compound H14 as compared to the compound H3 (table No. 06). When these animals' pancreas was examined Histopathological (Figure 13) it was shown that H3, H14 & Pioglitazone regenerated the Islets of Langerhans and ß cells, just as alloxan had previously caused necroses. On histopathology, the islets of Langerhans in the diabetic control group appeared abnormal, with significant damage evident. The cellular population was also low. The cellular population & size of islet cells were moderately expanded in diabetic rats treated with pioglitazone. Islet cell size and normal cellular population were partially restored in diabetic rats treated with H3 and H14 (200 mg/kg) [30-36].

Table 5: Pharmacological Effects on Glucose Levels in Alloxan-Induced Diabetes: A Multi-Dose Approach

Treatment Groups	Day 0	Day 7	Day 14	Day 21	Percentage reduction
Control	89.29±1.85	87.68±1.76	89.0±1.76	85.08±1.81	4.71%
Toxic Control	273.15±1.40	278.12±1.85	308.07±1.01	315±1.68	0%
Pioglitazone (150mg/kg)	265.58±1.76	175.35±1.33	140.32±1.75	111.61±2.48*	57.97%
H3	259.29±1.19	209.42±1.84	190.64± 2.02	160.56±2.06	38.07%
H14	261.79±1.99	198.13± 1.76	173.16± 2.53	143.22± 1.53*	45.29%

Data represented in the form of Mean ± SEM. Significantly different from control: * P<0.05

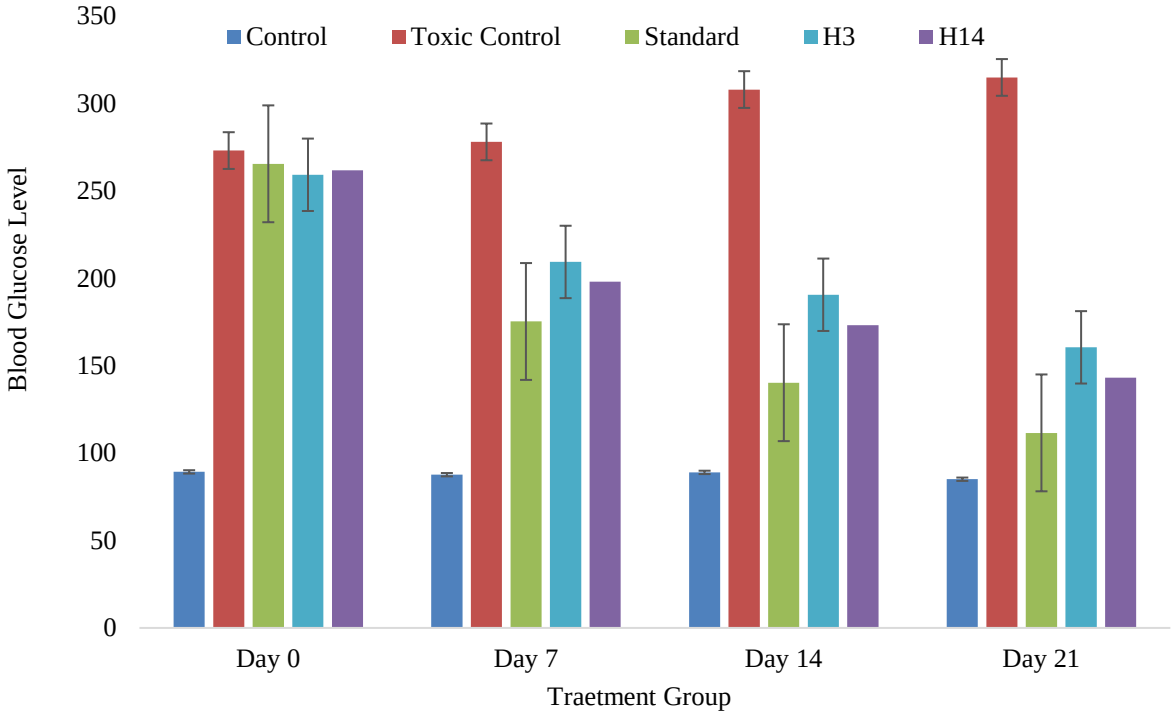
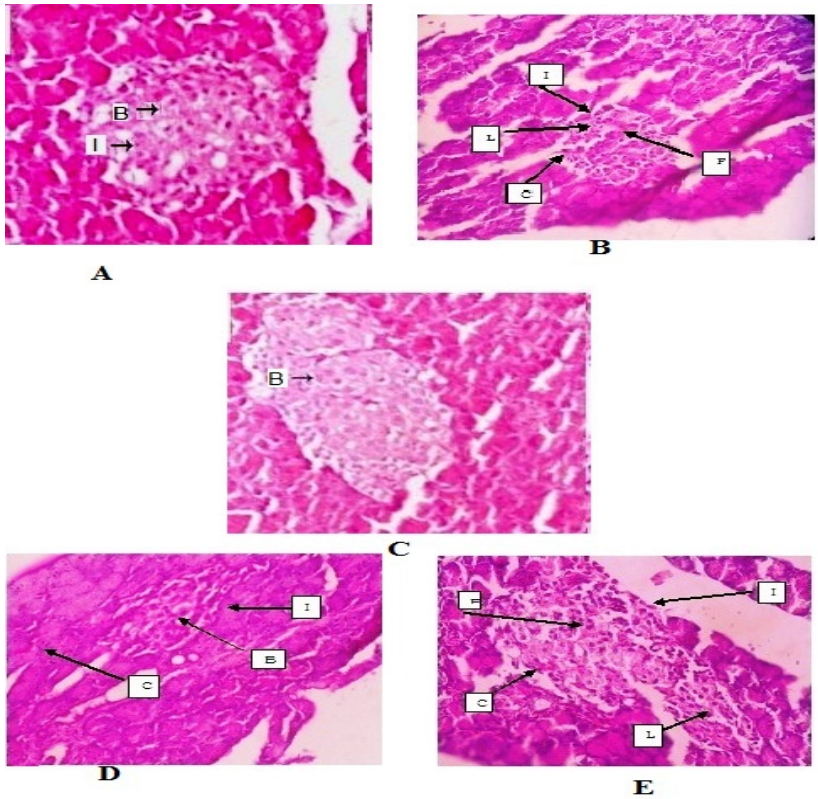


Figure 12: The impact of various substances and medications on the blood glucose levels of rats induced with diabetes using Alloxan



Results are expressed in Mean ± SEM. Significantly different from control: * P<0.05

Figure 13: Histopathology of pancreases after 21 days of treatment (A) Normal Control group, (B) Toxic Control group, (C) Standard group, (D) H3-treated group, (E) H14-treated group (B= Beta cells, I Islets of Langerhans, L lymphocytes, C Congestion, fibrosis

Table 6: Analysis of Alloxan-induced Diabetes in Rats: Medications' Impact on Plasma Lipid Profiles After the first 21 days of treatment

Treatment Groups	Plasma Lipid profile (mg/dl)				
	TG mg/dl	HDL mg/ dl	VLDL mg/dl	LDL mg/dl	Total Cholesterol mg/dl
Control	72.77±2.6	55.22±1. 5	26± 1.7	25.1±2.6	57.35 ±2.2
Diabetic Control	125±2.4	37.26±2. 9	37.28±2.3	35.38±3.2	90.3±4.2
Pioglitazone (150mg/kg)	87± 2.7	53±2.8	27.3±2.5	26.25± 2.1*	60.35 ±1.9
H3 (200mg/kg)	110±2.1	41.54±1. 4	37.6±3.1	36.16±3.6	73.30 ±2.3
H14 (200mg/kg)	95±2.8	50.32±0. 9	33.48±2.2	29.34±3.1*	68.58 ±3.1

CONCLUSION

This study underscores the potential anti-diabetic properties of thiazolidinedione derivatives, particularly molecules H3 and H14. Compound H3 and H14 exhibited good binding affinity with docking scores of -128.34 and -129.766, respectively, and exhibited significant interactions. Compound H3 showed interactions with Cys285, Tyr327, Ser289, His323, and Ala278, while H14 interacted with Ser289, Phe360, Cys285, and Tyr473. This is significant when compared with the standard drug Pioglitazone, which exhibited hydrogen interactions with Ser342, Tyr473, Ser289, Glu291, and Leu228 (docking score: -118.485). The synthesis of both derivatives was evaluated using IR spectroscopy, Nuclear Magnetic Resonance, and Mass spectrometry. The anti-diabetic efficacy of the compounds varied from moderate to substantial, and the addition of a nitro group enhanced the pharmacological activity. Because nitrogen is crucial for PPAR-γ's interaction with diabetes. Nitrogen atoms on TZD scaffolds generate hydrogen bonds in the PPAR-γ ligand-binding domain, impacting therapeutic efficacy and function upon design. The compound demonstrates significant ligand-protein interactions, advantageous physicochemical characteristics, and no indication of acute toxicity at a dosage of 200 mg/kg. In molecular docking and in vivo activity, H3 and H14 are more promising lead candidates than pioglitazone; however, toxicological and pharmacokinetic investigations are needed before clinical application. Many investigations have linked TZDs to multisystem organ failure, rhabdomyolysis, nephrotoxicity, and lethal hepatotoxicity. Without further study, in vivo rat evidence does not indicate human therapeutic acceptability, nor does acute oral safety guarantee chronic safety. To optimize the structural components associated with enhanced biological activity and forward these derivatives to clinical evaluation, subsequent research should concentrate on *in vivo* efficacy studies, as these results establish a robust foundation. Further research is needed to validate the compounds' effectiveness in a chronic anti-diabetic study that used streptozotocin as the diabetes-inducing agent. Future research on the toxicity of the chemicals under long-term use will also need to account for their synthetic character. Research has shown that thiazolidinedione derivatives hold promise as anti-diabetic medications, and our findings align with these findings.

FINANCIAL ASSISTANCE

NIL

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTION

The thiazolidinedione compounds were developed by Gourav Trivedi, who also performed the *in-silico* computational analysis. Bhoopendra Patidar and Aman Karma produced thiazolidinedione compounds using the computational data, while Prabhat Das oversaw the development of pharmacological tests for anti-diabetic activity. The findings were assessed and explained by Nilesh Mandloi. Gourav Trivedi and Prabhat Das drafted the final version of the manuscript. The final version of the manuscript was approved after all authors had reviewed it.

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