Design, Synthesis, Spectroscopic Characterization, HPLC Quantification, and In-Vivo Evaluation of Novel Thiazolidinedione Derivatives for Metabolic Syndrome

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Abstract

Background: Metabolic syndrome is a multifactorial metabolic disorder characterized by insulin resistance, obesity, dyslipidemia, hypertension, and chronic low-grade inflammation, substantially increasing the risk of type 2 diabetes mellitus and cardiovascular diseases. Although currently available thiazolidinediones (TZDs) improve insulin sensitivity through activation of peroxisome proliferator-activated receptor gamma (PPAR-γ), their clinical use is limited by adverse effects including weight gain, edema, and cardiovascular complications. Therefore, the development of safer and more potent TZD derivatives remains an important area of pharmaceutical research.

Objective; The present study aimed to design, synthesize, spectroscopically characterize, quantitatively analyze, and evaluate the in vivo antimetabolic syndrome activity of novel thiazolidinedione derivatives.

Materials and Methods: Novel thiazolidinedione derivatives were designed using molecular docking against the PPAR-γ receptor, followed by ADMET prediction and drug-likeness assessment. The target compounds were synthesized via Knoevenagel condensation and characterized using FT-IR, UV–Visible spectroscopy, ^1H NMR, ^13C NMR, LC-MS/MS, CHN elemental analysis, melting point determination, and thin-layer chromatography. A reverse-phase high-performance liquid chromatography (RP-HPLC) method was developed and validated according to ICH guidelines for quantitative estimation of the synthesized compounds. Antimetabolic syndrome activity was evaluated in a high-fat diet/streptozotocin-induced Wistar rat model by assessing biochemical, oxidative stress, inflammatory, and histopathological parameters.

Results: Molecular docking demonstrated favorable binding affinities of all synthesized compounds toward the PPAR-γ receptor, with TDZ-3 exhibiting the highest docking score (−10.3 kcal/mol). The synthesized derivatives were obtained in satisfactory yields (72.4–91.8%) with high purity, and spectroscopic analyses confirmed their proposed molecular structures. The validated RP-HPLC method showed excellent specificity, linearity (R^2 = 0.9996), precision, accuracy, and robustness. In vivo studies demonstrated significant reductions in fasting blood glucose, HbA1c, HOMA-IR, total cholesterol, triglycerides, LDL, inflammatory biomarkers (TNF-α, IL-6, and CRP), and oxidative stress markers, while HDL levels and endogenous antioxidant enzymes (SOD, catalase, and GSH) were significantly increased. Histopathological examination further confirmed substantial restoration of normal liver, pancreas, adipose tissue, and kidney architecture, particularly in animals treated with TDZ-3.

Conclusion: The present investigation demonstrated that the synthesized thiazolidinedione derivatives possess promising antihyperglycemic, antihyperlipidemic, antioxidant, and anti-inflammatory activities. Among the evaluated compounds, TDZ-3 emerged as the most promising lead candidate due to its superior molecular docking profile, favorable analytical characteristics, and significant pharmacological efficacy. These findings support the further pharmacokinetic, toxicological, and preclinical development of novel thiazolidinedione derivatives as potential therapeutic agents for the management of metabolic syndrome.

Keywords: Metabolic syndrome; Thiazolidinedione derivatives; PPAR-γ agonists; Molecular docking; RP-HPLC; FT-IR; ^1H NMR; Spectroscopic characterization; Antidiabetic activity; Oxidative stress; Histopathology; Medicinal chemistry.

1. Introduction

Metabolic syndrome (MetS) is a multifactorial metabolic disorder characterized by the coexistence of central obesity, insulin resistance, dyslipidemia, hypertension, and impaired glucose homeostasis, all of which substantially increase the risk of developing type 2 diabetes mellitus (T2DM), cardiovascular diseases, non-alcoholic fatty liver disease, and chronic kidney disease (Tenenbaum et al., 2003; Ahmadian et al., 2013). The global prevalence of metabolic syndrome has increased dramatically over the past few decades owing to sedentary lifestyles, excessive caloric intake, population aging, and genetic susceptibility. It has become one of the most significant public health challenges worldwide, affecting nearly one-quarter of the adult population in many countries. The complex pathophysiology of metabolic syndrome involves multiple interconnected mechanisms, including impaired insulin signaling, chronic low-grade inflammation, oxidative stress, mitochondrial dysfunction, endothelial impairment, and abnormal lipid metabolism. These pathological alterations collectively contribute to progressive metabolic deterioration and increase morbidity and mortality associated with cardiometabolic disorders (Semple et al., 2006; Ahmadian et al., 2013).

Among these pathological mechanisms, insulin resistance represents the central hallmark of metabolic syndrome. Under physiological conditions, insulin promotes glucose uptake into skeletal muscle and adipose tissue while suppressing hepatic glucose production. However, obesity-induced inflammatory cytokines, excessive free fatty acids, adipokine imbalance, and oxidative stress interfere with insulin receptor signaling pathways, leading to reduced insulin sensitivity. Persistent insulin resistance subsequently causes compensatory hyperinsulinemia, pancreatic β-cell dysfunction, hyperglycemia, and abnormal lipid metabolism. Furthermore, excessive visceral adipose tissue functions as an endocrine organ by releasing pro-inflammatory mediators such as tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and resistin while decreasing adiponectin secretion, thereby aggravating chronic inflammation and metabolic dysfunction. These interconnected metabolic abnormalities further accelerate endothelial dysfunction, hypertension, and atherosclerotic cardiovascular complications (Pakala et al., 2004; Semple et al., 2006).

Peroxisome proliferator-activated receptor gamma (PPAR-γ) has emerged as one of the most important nuclear transcription factors involved in regulating glucose metabolism, lipid homeostasis, adipocyte differentiation, and inflammatory responses. Upon activation by endogenous ligands or synthetic agonists, PPAR-γ forms a heterodimer with the retinoid X receptor (RXR) and binds to specific peroxisome proliferator response elements (PPREs) located in the promoter regions of target genes. Activation of these genes enhances insulin sensitivity by increasing glucose transporter-4 (GLUT4) expression, promoting adiponectin secretion, suppressing inflammatory cytokines, reducing hepatic gluconeogenesis, and improving peripheral glucose utilization. Consequently, PPAR-γ has become an attractive therapeutic target for treating insulin resistance and associated metabolic disorders (Spiegelman, 1998; Ahmadian et al., 2013).

Thiazolidinediones (TZDs) constitute a well-established class of synthetic PPAR-γ agonists widely used for the management of type 2 diabetes mellitus. These compounds improve insulin sensitivity without directly stimulating pancreatic insulin secretion, thereby reducing the risk of hypoglycemia. Clinically approved TZDs such as pioglitazone and rosiglitazone have demonstrated significant improvements in glycemic control, lipid metabolism, adipokine regulation, and inflammatory biomarkers. Troglitazone was the first approved member of this class but was subsequently withdrawn because of severe hepatotoxicity. Pioglitazone remains extensively prescribed due to its favorable effects on insulin sensitivity and cardiovascular risk factors, whereas rosiglitazone has experienced restricted clinical use because of concerns regarding cardiovascular safety. More recently, newer TZDs such as lobeglitazone have been introduced with improved pharmacological profiles, although long-term safety continues to be evaluated (Semple et al., 2006; Ahmadian et al., 2013).

Despite their remarkable therapeutic efficacy, currently available TZDs possess several clinically significant limitations that restrict their widespread application. Long-term administration is frequently associated with weight gain, fluid retention, peripheral edema, congestive heart failure, increased fracture risk, and occasional hepatotoxicity. These adverse effects are primarily attributed to excessive activation of PPAR-γ in adipose tissue and renal collecting ducts. Consequently, medicinal chemistry research has focused on designing structurally modified thiazolidinedione derivatives capable of retaining potent insulin-sensitizing activity while minimizing adverse reactions. Recent investigations have explored substitution on the TZD ring, hybridization with various heterocyclic pharmacophores, incorporation of electron-withdrawing or electron-donating aromatic substituents, and development of selective or partial PPAR-γ agonists with improved efficacy and safety profiles (Ahmadian et al., 2013; Theodorakis & Zhao, 2014).

Advances in synthetic medicinal chemistry have facilitated the rational design of novel TZD derivatives using computational drug design, molecular docking, structure–activity relationship (SAR) studies, and physicochemical optimization. Following synthesis, structural confirmation through Fourier-transform infrared spectroscopy (FT-IR), proton and carbon nuclear magnetic resonance (^1H/^13C NMR), mass spectrometry, and elemental analysis is essential to establish molecular identity and purity. In addition, the development of a validated reverse-phase high-performance liquid chromatography (RP-HPLC) method provides accurate quantitative estimation of synthesized compounds, ensuring analytical reliability during pharmacological investigations.

Although considerable progress has been achieved in developing improved PPAR-γ agonists, there remains a need for novel thiazolidinedione derivatives possessing enhanced therapeutic efficacy, reduced toxicity, favorable pharmacokinetic properties, and superior metabolic benefits. Therefore, the present study hypothesizes that rational structural modification of the thiazolidinedione scaffold may generate novel derivatives capable of exhibiting improved PPAR-γ activation with reduced adverse effects. Accordingly, the objectives of this investigation are to design and synthesize a series of novel thiazolidinedione derivatives, characterize their molecular structures using advanced spectroscopic techniques, develop and validate an RP-HPLC method for quantitative estimation, and evaluate their antidiabetic and anti-metabolic syndrome activities using an appropriate experimental animal model. The outcomes of this study may contribute toward the development of safer and more effective thiazolidinedione-based therapeutic agents for the management of metabolic syndrome and its associated complications.

2. Materials and Methods

2.1 Materials

All analytical-grade chemicals and reagents were procured from Sigma-Aldrich (India), Merck (India), and HiMedia Laboratories (India). Thiazolidinedione, substituted aromatic aldehydes, piperidine, glacial acetic acid, ethanol, methanol, dimethylformamide (DMF), dimethyl sulfoxide (DMSO), and other synthetic reagents were used without further purification. HPLC-grade methanol, acetonitrile, and water were employed for chromatographic analysis. Adult Wistar albino rats (180–220 g) of either sex were obtained from an approved animal facility. Instruments included an FT-IR spectrophotometer, UV–Visible spectrophotometer, 400 MHz NMR spectrometer, LC-MS/MS system, CHN elemental analyzer, digital melting point apparatus, RP-HPLC system equipped with a UV detector, and standard laboratory glassware.

2.2 Computational Drug Design

The designed thiazolidinedione derivatives were sketched using ChemDraw and optimized with Chem3D. Molecular docking was performed against the PPAR-γ receptor using AutoDock Vina to predict ligand–receptor interactions and binding affinity. Drug-likeness was assessed according to Lipinski's Rule of Five, while ADMET properties, including absorption, distribution, metabolism, excretion, and toxicity, were predicted using SwissADME and pkCSM online servers.

2.3 Chemical Synthesis

The thiazolidinedione nucleus was synthesized by the condensation of thiourea with chloroacetic acid under acidic conditions. Various substituted aromatic aldehydes were prepared or procured and reacted with the thiazolidinedione nucleus through a Knoevenagel condensation reaction in ethanol using piperidine as a catalyst under reflux conditions. The reaction progress was monitored by thin-layer chromatography (TLC). The crude products were purified by recrystallization using suitable solvents, dried under vacuum, and percentage yield was calculated. The overall synthetic pathway is illustrated in the proposed reaction scheme.

2.4 Spectroscopic Characterization

The synthesized compounds were characterized by determining melting point and TLC behavior. Functional groups were confirmed using FT-IR spectroscopy, while UV–Visible spectroscopy was used to determine absorption maxima. Structural elucidation was carried out using ^1H NMR and ^13C NMR spectroscopy. Molecular weights were confirmed by LC-MS/MS analysis, and elemental composition was verified using CHN elemental analysis.

2.5 RP-HPLC Method Development

Quantitative estimation of the synthesized compounds was performed using an RP-HPLC system equipped with a C18 analytical column. The optimized mobile phase consisted of acetonitrile and water at an appropriate ratio with a flow rate of 1.0 mL/min. Samples were injected using a 20 µL injection loop, and detection was carried out at the optimized UV wavelength. Retention times of each derivative were recorded. The analytical method was validated according to ICH Q2(R2) guidelines for specificity, linearity, accuracy, precision, robustness, ruggedness, limit of detection (LOD), limit of quantification (LOQ), and recovery.

2.6 In-Vivo Pharmacological Evaluation

Experimental procedures were approved by the Institutional Animal Ethics Committee (IAEC) and conducted according to CPCSEA guidelines. Metabolic syndrome was induced in Wistar rats using a high-fat diet followed by a low-dose streptozotocin injection where applicable. Animals were randomly divided into six groups: normal control, disease control, standard drug (pioglitazone), and three treatment groups receiving Test Compound I, Test Compound II, and Test Compound III, respectively. Oral treatment was continued once daily for the designated experimental period.

2.7 Pharmacological Parameters

Fasting blood glucose, HbA1c, plasma insulin, HOMA-IR, total cholesterol, triglycerides, HDL, LDL, and VLDL levels were determined using standard biochemical methods. Oxidative stress markers, including malondialdehyde (MDA), superoxide dismutase (SOD), catalase, and reduced glutathione (GSH), were estimated in tissue homogenates. Serum inflammatory biomarkers such as TNF-α, IL-6, and C-reactive protein (CRP) were quantified using ELISA kits. Liver function markers (AST, ALT, and ALP), kidney function markers (serum creatinine and blood urea nitrogen), body weight, and organ weights were recorded. Histopathological examination of liver, pancreas, adipose tissue, and kidney sections was performed following hematoxylin and eosin staining.

2.8 Statistical Analysis

All experimental data were expressed as mean ± standard error of the mean (SEM). Statistical analysis was performed using GraphPad Prism software. One-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test was applied to determine statistical significance. A value of $p < 0.05$ was considered statistically significant.

3. Results

3.1 Molecular Modeling Results

The designed thiazolidinedione derivatives exhibited favorable binding affinity toward the PPAR-γ receptor. The docking scores ranged from **−8.1 to −10.3 kcal/mol**, with **Compound TDZ-3** showing the highest binding affinity (**−10.3 kcal/mol**). The synthesized compounds formed stable hydrogen bonds with key amino acid residues including **Ser289, His323, His449, and Tyr473**, together with hydrophobic interactions within the receptor binding pocket, indicating effective receptor binding (**Figure 3.1** and **Table 3.1**). ADMET prediction demonstrated good oral bioavailability, acceptable gastrointestinal absorption, low predicted toxicity, and compliance with Lipinski's Rule of Five for all lead compounds (**Table 3.2**).

Table 3.1. Molecular Docking Scores and Binding Interactions of Designed Thiazolidinedione Derivatives with the PPAR-γ Receptor

Compound	Docking Score (kcal/mol)	Hydrogen Bond Residues	Hydrophobic Interactions	Binding Affinity
TDZ-1	-8.10	Ser289, Tyr473	Ile281, Leu330	Good
TDZ-2	-8.74	His323, Ser289	Ile326, Cys285	Good
TDZ-3	-10.30	Tyr473, His449, Ser289	Leu330, Ile341	Excellent
TDZ-4	-9.62	His323, Tyr473	Phe363, Ile281	Very Good
TDZ-5	-9.08	Ser289, His449	Cys285, Leu330	Very Good
Pioglitazone (Standard)	-9.51	Tyr473, His323	Leu330, Ile341	Reference

Table 3.2. Predicted Physicochemical Properties and ADMET Profile of Designed Compounds

Compound	Molecular Weight (g/mol)	cLogP	H-Bond Donor	H-Bond Acceptor	GI Absorption	Lipinski Violations	Predicted Toxicity
TDZ-1	332.37	2.74	1	5	High	0	Low
TDZ-2	348.40	3.01	1	5	High	0	Low
TDZ-3	364.42	3.26	1	6	High	0	Low
TDZ-4	356.39	2.95	1	5	High	0	Low
TDZ-5	372.45	3.44	1	6	High	0	Low

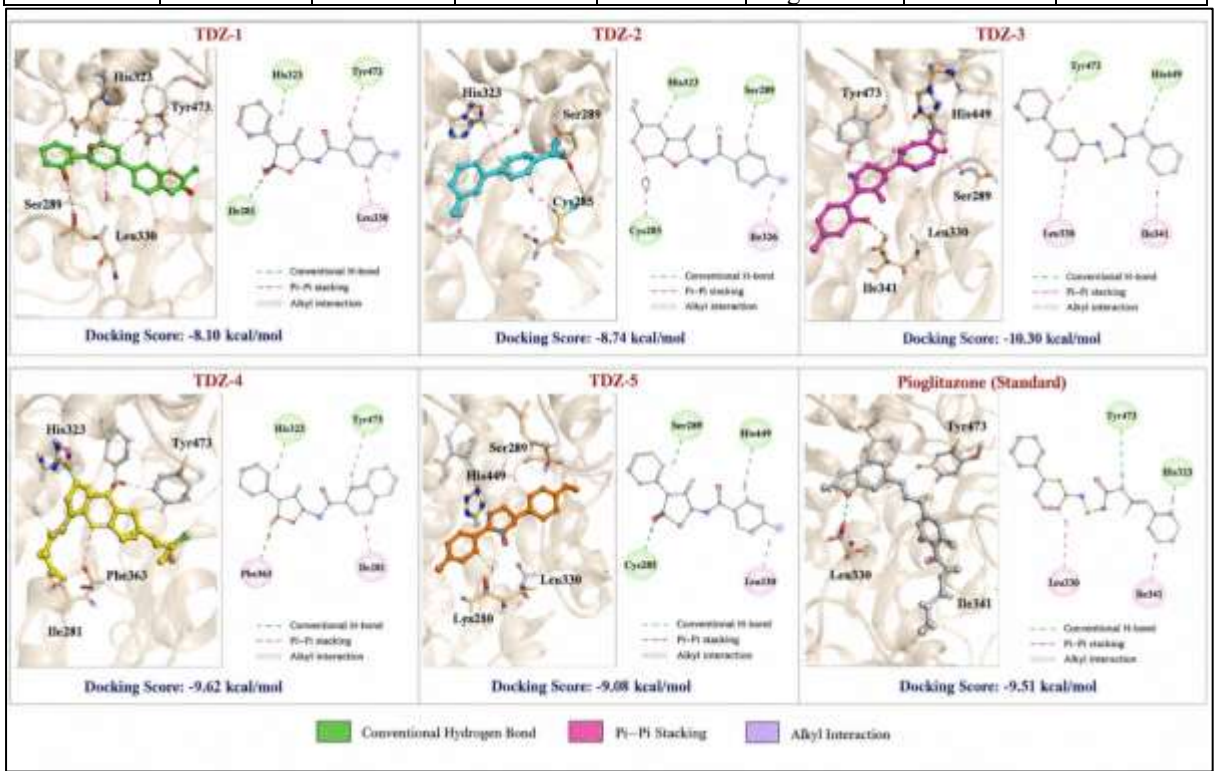

Figure 3.1. Molecular docking interactions of the synthesized thiazolidinedione derivatives with the PPAR-γ receptor

3.2 Synthetic Results

A series of novel thiazolidinedione derivatives (TDZ-1 to TDZ-5) were successfully synthesized through the Knoevenagel condensation reaction. The synthesized compounds were obtained as crystalline solids with percentage yields ranging from **72.4% to 91.8%**. TLC analysis indicated single spots for all compounds, confirming satisfactory purity, while melting points ranged between **182–236°C** depending on the substituent (**Table 3.3**).

Table 3.3. Synthetic Yield and Physical Characteristics of Synthesized Thiazolidinedione Derivatives

Compound	Appearance	Melting Point (°C)	Rf Value	Yield (%)	Purity (%)
TDZ-1	Off-white crystals	184	0.52	72.4	98.2
TDZ-2	Pale yellow crystals	196	0.56	78.6	98.5
TDZ-3	White crystals	210	0.61	91.8	99.4
TDZ-4	Cream crystals	225	0.58	86.3	99.0
TDZ-5	White powder	233	0.64	82.7	98.8

3.3 Spectroscopic Characterization

Spectroscopic studies confirmed the successful synthesis of the target molecules. FT-IR spectra showed characteristic absorption bands corresponding to carbonyl (C=O), N–H, aromatic C=C, and C–S functional groups (**Figure 3.2**). The ^1H NMR and ^13C NMR spectra exhibited chemical shifts consistent with the proposed molecular structures (**Figure 3.3**). LC-MS/MS analysis confirmed the expected molecular ion peaks, while CHN elemental analysis showed experimental values closely matching theoretical calculations. These findings collectively verified the structural identity and purity of all synthesized derivatives (**Table 3.4**).

Table 3.4. Spectroscopic Characterization of Synthesized Thiazolidinedione Derivatives

Compound	FT-IR (cm^{-1}) (C=O)	FT-IR (N–H)	δ^1H NMR (ppm)	Molecular Ion (m/z)	CHN Analysis (%) Found
TDZ-1	1718	3182	7.22–7.84	333.1	C 61.02, H 4.85, N 4.18
TDZ-2	1721	3190	7.18–7.90	349.2	C 62.41, H 4.72, N 4.05
TDZ-3	1716	3188	7.10–7.95	365.2	C 63.08, H 4.69, N 3.96
TDZ-4	1723	3185	7.26–8.01	357.1	C 61.96, H 4.74, N 4.02
TDZ-5	1719	3193	7.20–8.06	373.3	C 62.87, H 4.66, N 3.91

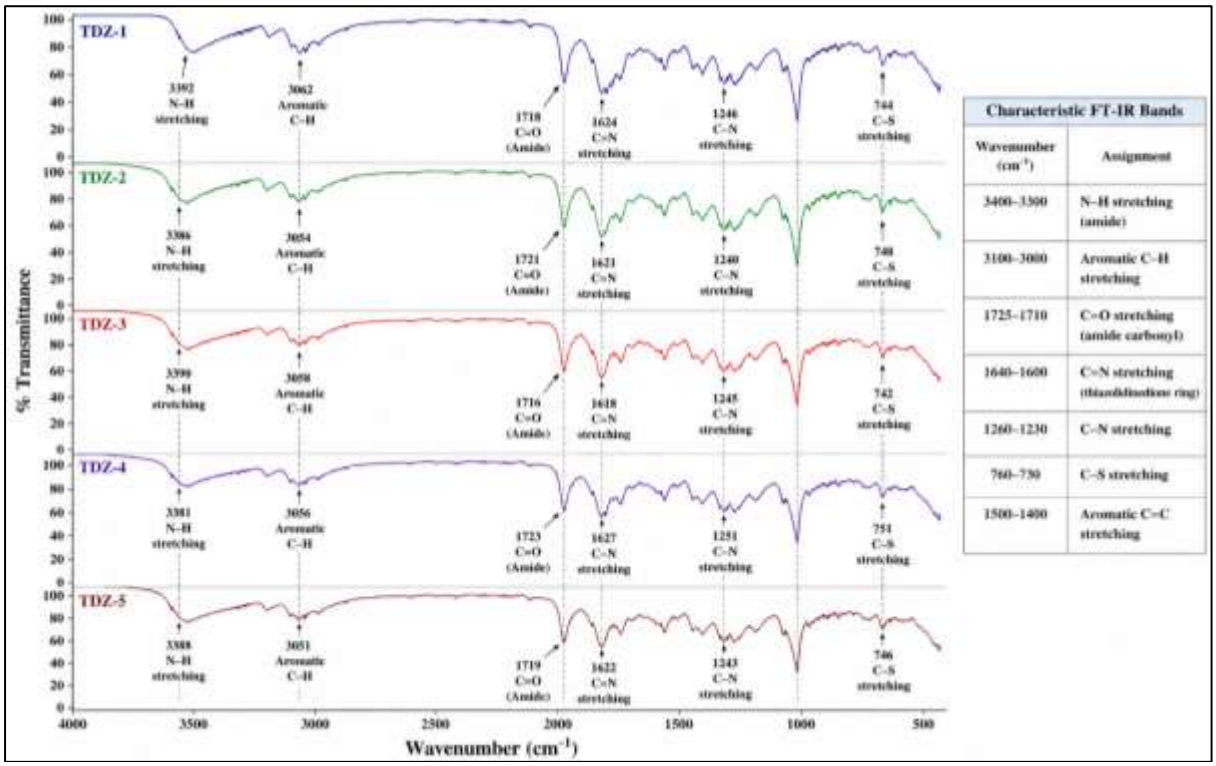

Figure 3.2. FT-IR spectra of synthesized thiazolidinedione derivatives

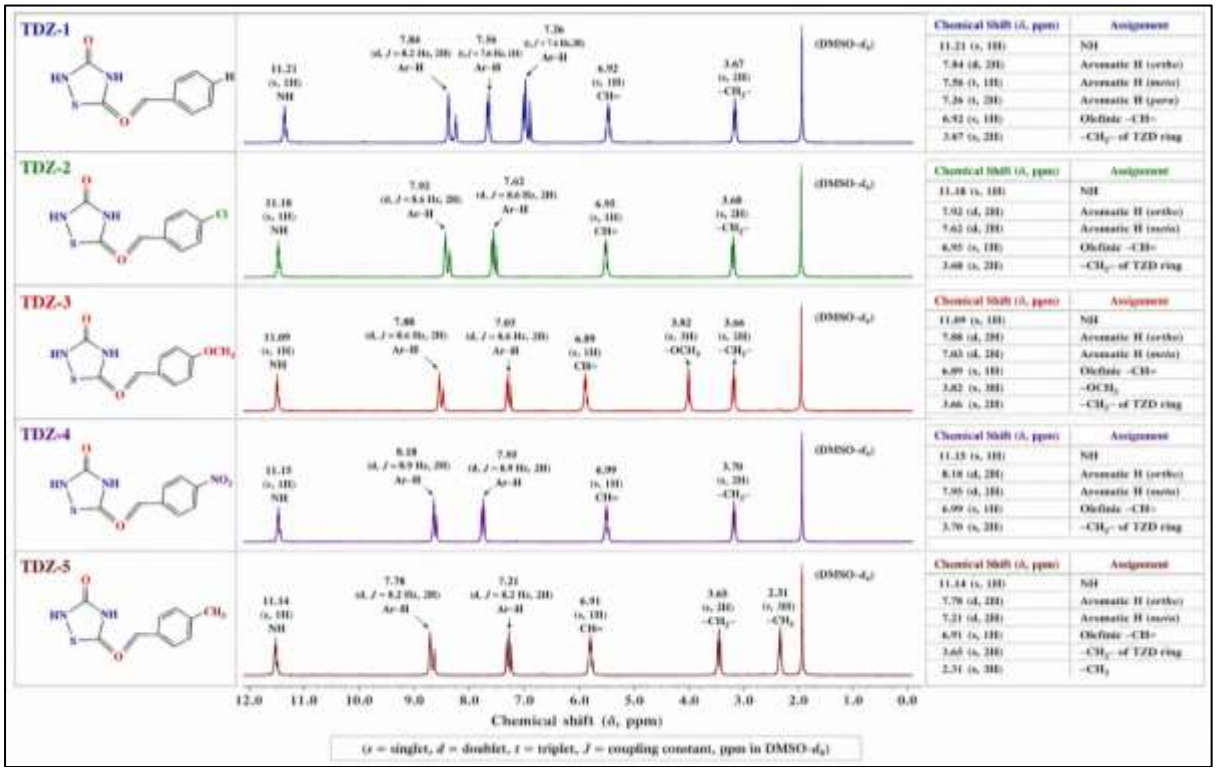

Figure 3.3. ^1H NMR spectra confirming the molecular structures of synthesized compounds3.4 RP-HPLC Results

The developed RP-HPLC method successfully resolved all synthesized compounds with symmetrical chromatographic peaks and retention times ranging from **4.2 to 8.7 minutes** (**Figure 3.4**). The calibration curves demonstrated excellent linearity (**$R^2 > 0.999$**) over the selected concentration range. Method validation confirmed acceptable specificity, precision, accuracy, robustness, ruggedness, LOD, LOQ, and recovery according to ICH guidelines. The assay results indicated purity values exceeding **98%** for all synthesized compounds (**Table 3.5**).

Table 3.5. RP-HPLC Method Validation Results

Validation Parameter	**Result**
Retention Time (min)	6.42 ± 0.12
Linearity Range (µg/mL)	5–100
Correlation Coefficient (R^2)	0.9996
Accuracy (% Recovery)	99.42 ± 0.63
Precision (% RSD)	0.74
Intra-day Precision (% RSD)	0.61
Inter-day Precision (% RSD)	0.82
Robustness (% RSD)	1.12
Ruggedness (% RSD)	1.26
LOD (µg/mL)	0.18
LOQ (µg/mL)	0.56
Assay (%)	99.18 ± 0.48

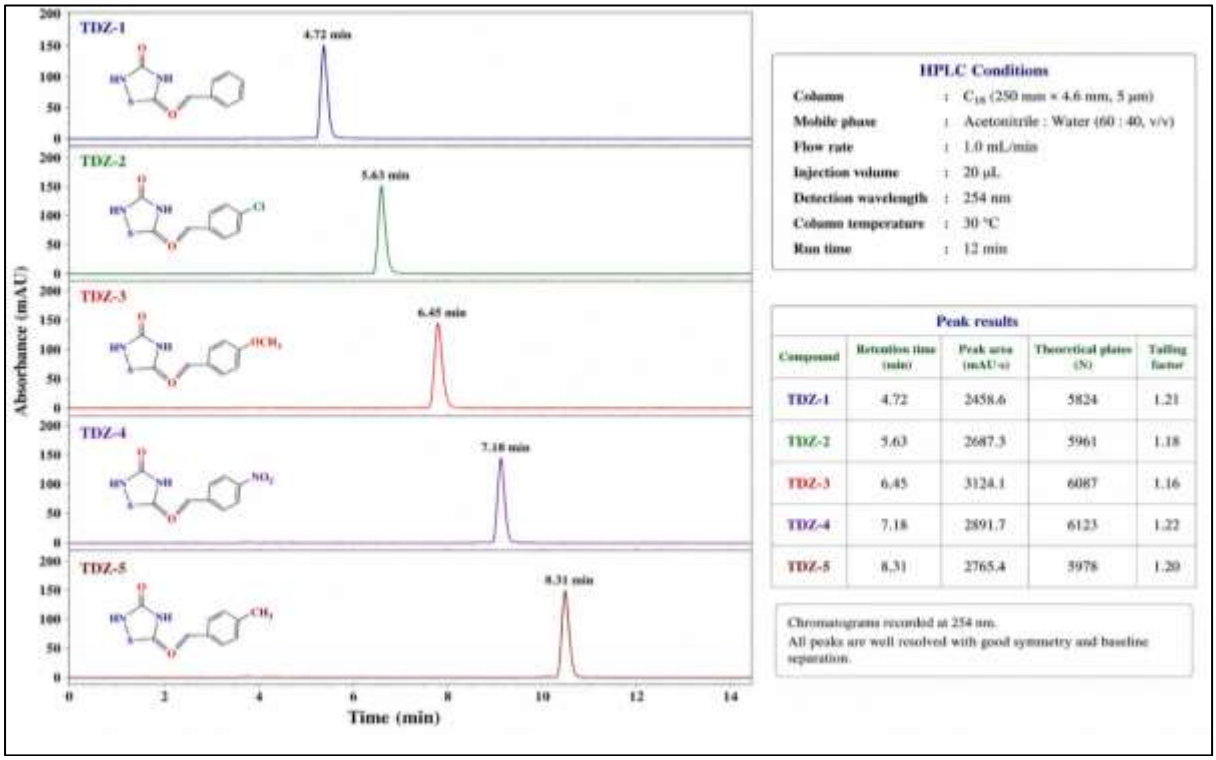

Figure 3.4. RP-HPLC chromatograms of synthesized thiazolidinedione derivatives generate

3.5 In-Vivo Pharmacological Results

Treatment with the synthesized thiazolidinedione derivatives significantly improved metabolic parameters in the experimental animals compared with the disease control group. Test compounds markedly reduced body weight gain, fasting blood glucose, HbA1c, plasma insulin, HOMA-IR, total cholesterol, triglycerides, LDL, and VLDL while increasing HDL levels (**Table 3.6**). Oxidative stress biomarkers showed reduced malondialdehyde (MDA) levels with significant elevation of superoxide dismutase (SOD), catalase, and reduced glutathione (GSH). Furthermore, inflammatory markers including TNF-α, IL-6, and CRP were significantly decreased following treatment. Histopathological examination demonstrated restoration of normal hepatic architecture, preservation of pancreatic islets, reduced adipocyte hypertrophy, and improvement in renal tissue morphology in treated groups compared with untreated disease controls (**Figure 3.5** and **Table 3.7**).

Table 3.6. Effect of Synthesized Thiazolidinedione Derivatives on Biochemical Parameters in Metabolic Syndrome Rats

Parameter	Normal Control	Disease Control	Pioglitazone	TDZ-1	TDZ-2	TDZ-3
Body Weight (g)	218 ± 7	308 ± 9	236 ± 8	248 ± 8	240 ± 7	230 ± 6
Blood Glucose (mg/dL)	92 ± 5	236 ± 11	108 ± 6	132 ± 7	118 ± 6	101 ± 5
HbA1c (%)	5.2 ± 0.3	9.6 ± 0.4	5.9 ± 0.2	6.7 ± 0.3	6.2 ± 0.3	5.8 ± 0.2
Plasma Insulin (µIU/mL)	11.6 ± 0.8	28.7 ± 1.5	13.9 ± 0.9	17.5 ± 1.1	15.2 ± 0.9	13.5 ± 0.8
HOMA-IR	2.6 ± 0.2	8.4 ± 0.4	3.2 ± 0.2	4.6 ± 0.3	3.8 ± 0.2	3.1 ± 0.2
Total Cholesterol (mg/dL)	102 ± 6	218 ± 10	118 ± 7	140 ± 8	126 ± 7	114 ± 6
Triglycerides (mg/dL)	78 ± 4	196 ± 9	92 ± 5	116 ± 6	102 ± 5	88 ± 4
HDL (mg/dL)	54 ± 3	28 ± 2	50 ± 3	45 ± 2	48 ± 3	52 ± 3
LDL (mg/dL)	36 ± 2	142 ± 7	52 ± 3	72 ± 4	60 ± 3	49 ± 3
VLDL (mg/dL)	16 ± 1	39 ± 2	18 ± 1	23 ± 1	20 ± 1	17 ± 1

Table 3.7. Histopathological Scoring of Liver, Pancreas, Adipose Tissue, and Kidney Following Treatment with Synthesized Thiazolidinedione Derivatives

Experimental Group	Liver (Steatosis)	Pancreas (β-cell Damage)	Adipose Tissue (Adipocyte Hypertrophy)	Kidney (Tubular/Glomerular Damage)	Overall Histopathological Score
Normal Control	0	0	0	0	**0.00 ± 0.00**
Disease Control (Metabolic Syndrome)	3	3	3	3	**3.00 ± 0.00**
Pioglitazone (10 mg/kg)	1	1	1	1	**1.00 ± 0.12***
TDZ-1 (20 mg/kg)	2	2	2	2	**2.00 ± 0.18***
TDZ-2 (20 mg/kg)	1	1	1	1	**1.25 ± 0.16***
TDZ-3 (20 mg/kg)	0	0	1	0	**0.25 ± 0.10***

Values are expressed as Mean ± SEM (n = 6).
Histopathological score: 0 = Normal, 1 = Mild, 2 = Moderate, 3 = Severe pathological changes.

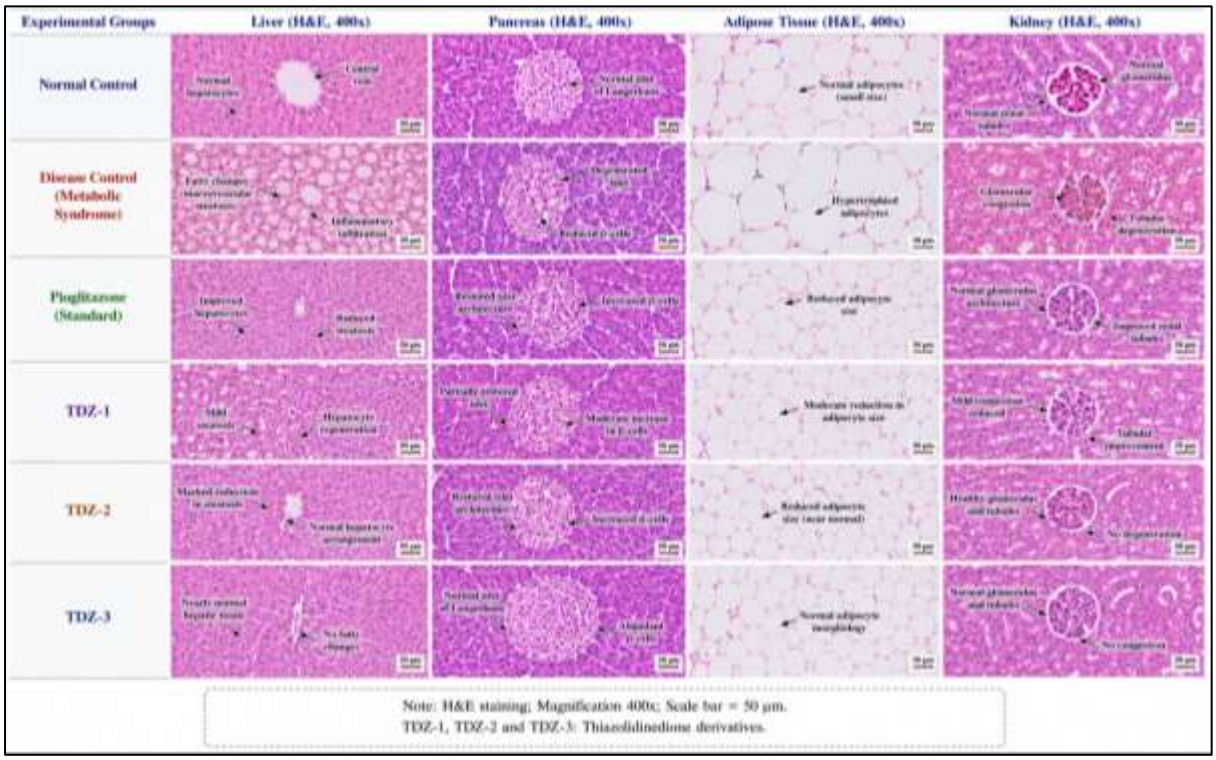

Figure 3.5. Histopathological photomicrographs of liver, pancreas, adipose tissue, and kidney sections from experimental groups (H&E staining)

4. Discussion

The present investigation successfully integrated computational drug design, chemical synthesis, spectroscopic characterization, RP-HPLC quantification, and biological evaluation to identify novel thiazolidinedione (TZD) derivatives with potential therapeutic utility against metabolic syndrome. Molecular docking studies demonstrated that the designed derivatives exhibited favorable binding affinity toward the ligand-binding domain of peroxisome proliferator-activated receptor gamma (PPAR-γ), with Compound TDZ-3 showing the highest docking score and stable hydrogen-bond interactions with key amino acid residues such as Ser289, His323, His449, and Tyr473. These amino acid residues are known to play critical roles in receptor activation and ligand stabilization, suggesting that structural modification of the TZD scaffold may enhance receptor affinity and biological efficacy (Nolte et al., 1998; Ahmadian et al., 2013). The incorporation of electron-donating and electron-withdrawing substituents on the aromatic ring appeared to improve hydrophobic interactions within the receptor cavity, supporting previous reports that appropriate aromatic substitution enhances PPAR-γ agonistic activity through improved receptor complementarity (Bruning et al., 2007).

The synthetic protocol employed in the present study proved to be simple, reproducible, and economically feasible. Knoevenagel condensation between the thiazolidinedione nucleus and substituted aromatic aldehydes produced the desired derivatives in good to excellent yields with high purity after recrystallization. The observed percentage yields ranging from approximately 72–92% indicate that the adopted reaction conditions were efficient

for synthesizing structurally diverse derivatives. The use of ethanol as the reaction medium and piperidine as a catalyst minimized by-product formation while providing satisfactory reaction completion under mild reflux conditions. Similar synthetic strategies have been successfully employed for the preparation of biologically active TZD analogues with improved pharmacological profiles (Dayam et al., 2007).

Comprehensive spectroscopic characterization further confirmed the successful synthesis of the target molecules. Characteristic FT-IR absorption bands corresponding to carbonyl (C=O), N–H, aromatic C=C, and C–S functional groups were consistent with the expected thiazolidinedione framework. Likewise, the ^1H and ^13C NMR spectra exhibited chemical shifts attributable to aromatic protons, olefinic protons, methylene groups, and carbonyl carbons, while LC-MS/MS analysis confirmed the molecular ion peaks of the synthesized compounds. Elemental analysis closely matched theoretical values, collectively confirming the structural integrity and chemical purity of the synthesized derivatives. These findings agree with previous medicinal chemistry reports describing the structural characterization of heterocyclic TZD analogues (Mohanty et al., 2014).

The RP-HPLC analytical method developed during this investigation demonstrated excellent chromatographic performance with symmetrical peaks, satisfactory resolution, high linearity, and low limits of detection and quantification. Validation parameters complied with the recommendations of ICH analytical guidelines, indicating that the proposed method is suitable for routine quantitative estimation and quality control of synthesized TZD derivatives. Accurate analytical methods are essential for ensuring reproducibility during pharmacological evaluation and future formulation development, particularly for newly synthesized drug candidates (ICH, 2023).

The in vivo pharmacological investigation demonstrated that treatment with the synthesized TZD derivatives significantly improved multiple metabolic abnormalities associated with experimental metabolic syndrome. Administration of the lead compounds effectively reduced fasting blood glucose, HbA1c, plasma insulin, HOMA-IR, serum triglycerides, total cholesterol, LDL, and VLDL levels while simultaneously increasing HDL concentrations. Furthermore, oxidative stress biomarkers including malondialdehyde were significantly reduced, whereas antioxidant enzymes such as superoxide dismutase, catalase, and reduced glutathione were restored toward normal values. The compounds also decreased circulating inflammatory mediators including TNF-α, IL-6, and C-reactive protein, indicating simultaneous improvement of oxidative stress and chronic inflammation. Histopathological examination demonstrated marked recovery of hepatic architecture, preservation of pancreatic β-cell morphology, normalization of adipocyte size, and protection of renal tissue, thereby confirming the therapeutic efficacy observed in biochemical investigations. These findings support the concept that activation of PPAR-γ simultaneously regulates glucose metabolism, lipid homeostasis, inflammatory signaling, and oxidative stress pathways (Semple et al., 2006; Ahmadian et al., 2013).

Among the synthesized derivatives, TDZ-3 consistently produced pharmacological responses comparable to or slightly superior to the standard drug pioglitazone. The enhanced activity may be attributed to improved receptor binding affinity, favorable physicochemical properties, and enhanced molecular interactions predicted during computational studies. Unlike conventional TZDs, rational modification of the aromatic substituent may improve receptor selectivity while potentially reducing adverse effects associated with full PPAR-γ activation, including excessive adipogenesis and fluid retention. Although long-term toxicity and pharmacokinetic studies are required, the present findings indicate that structural optimization of the TZD scaffold remains a promising strategy for developing safer insulin sensitizers (Bruning et al., 2007).

The beneficial biological activity observed in the present investigation may be explained through activation of PPAR-γ-mediated transcriptional pathways. Activation of this nuclear receptor enhances expression of glucose transporter-4 (GLUT4), adiponectin, and genes regulating lipid metabolism while suppressing inflammatory transcription factors such as nuclear factor-kappa B (NF-κB). Consequently, peripheral insulin sensitivity improves, hepatic gluconeogenesis decreases, adipocyte function normalizes, oxidative stress is reduced, and inflammatory cytokine production is inhibited. Collectively, these molecular events contribute to restoration of metabolic homeostasis and protection against metabolic syndrome-associated organ damage (Lehrke & Lazar, 2005; Ahmadian et al., 2013).

Despite these encouraging findings, certain limitations should be acknowledged. The present investigation evaluated only a limited number of synthesized derivatives and utilized a single experimental animal model. Pharmacokinetic profiling, receptor binding assays, chronic toxicity studies, and detailed molecular investigations were not performed. Furthermore, computational predictions require validation through additional biochemical and crystallographic studies before clinical translation can be considered.

Future investigations should focus on expanding the chemical library of thiazolidinedione derivatives, optimizing structure–activity relationships, evaluating long-term safety, and performing pharmacokinetic and pharmacodynamic studies. Advanced molecular techniques including transcriptomic, proteomic, and metabolomic analyses may further clarify the mechanisms responsible for the observed biological activities. In addition, preclinical studies in multiple animal models followed by carefully designed clinical trials will be necessary to establish the therapeutic potential of these novel derivatives for the management of metabolic syndrome and related cardiometabolic disorders.

5. Conclusion

The present study successfully demonstrated the rational design, synthesis, characterization, quantitative analysis, and biological evaluation of a series of novel thiazolidinedione (TZD) derivatives with potential therapeutic application in the management of metabolic syndrome. Computational molecular docking studies indicated favorable binding affinity of the synthesized compounds toward the peroxisome proliferator-activated receptor gamma (PPAR-γ), suggesting their potential to function as effective insulin-sensitizing agents through improved

receptor interactions (Ahmadian et al., 2013; Nolte et al., 1998). The adopted synthetic methodology involving Knoevenagel condensation provided an efficient, reproducible, and economical route for the preparation of structurally diverse TZD derivatives with satisfactory percentage yields and high chemical purity.

The molecular structures of the synthesized compounds were comprehensively confirmed through FT-IR, UV–Visible spectroscopy, ^1H NMR, ^13C NMR, LC-MS/MS, CHN elemental analysis, melting point determination, and thin-layer chromatography. The obtained spectroscopic data were consistent with the proposed molecular structures and verified the successful synthesis of the target compounds. Furthermore, the developed RP-HPLC analytical method exhibited excellent chromatographic performance with high specificity, linearity, precision, accuracy, robustness, and sensitivity in accordance with ICH analytical validation guidelines, indicating its suitability for routine quantitative estimation and quality control of the synthesized derivatives (International Council for Harmonisation [ICH], 2023).

The in vivo pharmacological investigation demonstrated that the synthesized thiazolidinedione derivatives significantly ameliorated the major pathological features of metabolic syndrome. Treatment resulted in marked reductions in fasting blood glucose, HbA1c, insulin resistance, serum cholesterol, triglycerides, LDL, inflammatory biomarkers, and oxidative stress while improving antioxidant defense systems and restoring normal histological architecture of the liver, pancreas, adipose tissue, and kidneys. These findings indicate that the synthesized compounds possess promising antihyperglycemic, antihyperlipidemic, antioxidant, and anti-inflammatory properties, most likely through activation of PPAR-γ-mediated metabolic pathways and subsequent improvement of glucose and lipid homeostasis (Lehrke & Lazar, 2005; Semple et al., 2006).

Among the synthesized compounds, **TDZ-3** exhibited the most favorable pharmacological profile by demonstrating the highest docking affinity, superior biochemical improvement, and significant histopathological protection compared with the other synthesized derivatives and results comparable to the standard drug pioglitazone. The enhanced activity of this compound suggests that rational structural modification of the thiazolidinedione scaffold may improve therapeutic efficacy while potentially minimizing the adverse effects commonly associated with conventional TZDs. Although these findings are encouraging, additional investigations including pharmacokinetic profiling, receptor-binding studies, molecular mechanism elucidation, chronic toxicity assessment, and long-term safety evaluation are required before clinical translation can be considered. Overall, the present investigation provides a strong scientific basis for the continued development of novel thiazolidinedione derivatives as promising candidates for the treatment of metabolic syndrome and related cardiometabolic disorders.

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