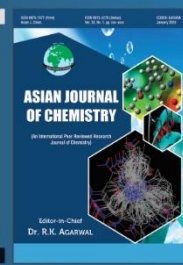




Asian Journal of Chemistry;

Vol. 38, No. 10 (2026), 2809-2827

ASIAN JOURNAL OF CHEMISTRY<https://doi.org/10.14233/ajchem.2026.36674>

Design, Computational Study, Synthesis, Characterization and Anti-diabetic Evaluation of Novel N-Substituted Thiazolidinedione Derivatives

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Received: 18 June 2026

Accepted: 5 October 2026

Published online: 9 October 2026

AJC-22513

In the present study, a series of novel N-substituted 2,4-thiazolidinedione derivatives (**PB-TZD1–PB-TZD14**) were rationally designed, synthesized and evaluated for their antidiabetic potential. The synthesized compounds were characterized by FT-IR, ¹H NMR, ¹³C NMR and mass spectrometry. Molecular docking revealed favourable interactions of the synthesized derivatives with key residues of the PPAR- γ receptor, particularly Cys285. *In vitro* glucose uptake studies revealed that **PB-TZD1** (36.00%), **PB-TZD3** (34.30%) and **PB-TZD6** (33.53%) exhibited significantly higher activity compared to the standard drug pioglitazone (24.77%). Among these, **PB-TZD3** exhibited the most pronounced glucose-lowering effect in *in vivo* antidiabetic studies using a high-fat diet STZ-induced rat model. Adipocyte differentiation studies showed that **PB-TZD3** significantly enhanced adipogenesis at higher concentrations, consistent with PPAR- γ activation. *In silico* ADME and toxicity predictions suggested favourable drug-like properties; however, these findings are preliminary and require further experimental validation. The structure–activity relationship analysis indicated that electronic, steric and hydrophobic factors play a crucial role in modulating biological activity. This study identifies **PB-TZD3** as a promising lead compound and highlights the significance of N-substitution in optimizing thiazolidinedione derivatives for improved antidiabetic activity.

Keywords: Thiazolidinediones, Glitazone derivatives, PPAR- γ , Antidiabetic activity.

INTRODUCTION

2,4-Thiazolidinedione (TZD) is an important heterocyclic scaffold in medicinal and synthetic chemistry due to its structural versatility and broad scope for chemical modification. The active methylene group at C-5 and the nitrogen atom at N-3 provide two key sites for functionalization, enabling the synthesis of diverse derivatives with distinct physico-chemical and biological properties [1,2]. Moreover, C-5 modification, particularly through Knoevenagel condensation, along with N-substitution, has been widely used to tailor the TZD framework and enhance its pharmacological properties [3,4]. The N-substituted derivatives have received particular attention since modification at the nitrogen atom can influence molecular stability, lipophilicity, receptor binding and biological activity [5,6].

Although benzylidene-TZD derivatives have been extensively investigated, comparatively fewer studies have systematically examined N-substitution as a means of modulating

receptor interactions and pharmacological responses. Introduction of substituted phenylacetamide moieties at the N-3 position provides an opportunity to incorporate additional hydrogen-bonding and hydrophobic interaction sites within the ligand structure. Such modifications may favour interactions with important residues in the PPAR- γ ligand-binding domain such as CYS285, TYR473 and HIS323. Molecular docking can provide structural insight into these ligand–receptor interactions, while *in silico* ADME analysis offers an early assessment of drug-likeness and pharmacokinetic characteristics, thereby supporting the selection of promising candidates before biological evaluation [7-9].

Based on this rationale, the present study explores dual modification of the TZD scaffold through benzylidene substitution at C-5 and tailored phenylacetamide substitution at N-3. This design was intended to examine whether simultaneous modification at these two positions could influence receptor binding, molecular properties and antidiabetic activity. The electron-donating and electron-withdrawing substituents

were introduced on the phenyl ring to assess the effect of electronic and structural variation on ligand–receptor interactions and biological response [10].

Although TZD derivatives and PPAR- γ agonists have been extensively studied, systematic exploration of N-substitution with tailored substitution patterns remains comparatively limited, as much of the previous work has focused on modifications of the aromatic ring and linker regions. The present work therefore focuses on structurally diverse N-substituted TZD derivatives and evaluates their potential as PPAR- γ directed antidiabetic agents. In the synthesized series, selected electron-withdrawing and hydrophobic substituents at the nitrogen-derived side chain were associated with improved glucose uptake activity relative to pioglitazone. These findings provide a basis for examining the contribution of N-substitution to receptor interactions and biological response and may help define structure–activity relationships within this class of TZD derivatives.

EXPERIMENTAL

All chemicals and solvents were obtained from the Loba Chemie and were purified and dried before use. The glassware was sourced from Agarwal Glassware Pvt. Ltd., Mathura, India. Aluminum-coated silica gel TLC from Merck was used. An oil bath (BTI-37) from BTI was used for heating. Infrared spectra were recorded on JASCO-ATR-FTIR. A Bruker Avance III HD 500 NMR spectrometer were acquired for NMR spectra and the Bruker Impact HD LC-MS/MS system was used to record mass spectra. Cell Line C2C12 and 3T3-L1 was procured from NCCS. Cell culture media was DMEM: Glucose from Gibco 11966025. Fetal bovin serum (FBS) Cell culture grade, Trypsin–EDTA solution (0.25% trypsin and 0.02% EDTA in PBS) from MP Biomedical System catalog no. 092910154. Insulin, dexamethasone and 3-isobutyl-1-methylxanthine (IBMX) purchase from Merck. All plastic wares are from Torsons Pvt Ltd. Biosafety cabinet Class-II from Alpha Linear, India. CO₂ Incubator from Thermo-Scientific. ELISA Plate reader from Byonoy GmbH (Absorbance 96), Streptozotocin were purchased from SRL and D-Glucose from Dabur. All other chemicals and solvent are analytical grades. Plasma glucose estimate using a glucometer (Accua-Check). High-fat diet (13% protein, 25% w/w fat and 55% w/w carbohydrate) made using mixing of butter having 55% saturated fat to normal laboratory animal diet. Cooling centrifuge from Borosil (100CLM C2400VT0) for separating plasma. Lipid profile estimate using a biochemistry analyzer from Fujifilm model NH500i. The animal cages and animal water feed bottle purchased from orchid scientific, Nashik. MS Excel and Graph Pad Software used for bio-statistics.

Design: A detailed literature and database review guided the design of 14 2,4-thiazolidinedione (TZD) derivatives with potential antidiabetic activity. The selected scaffolds were based on reported antidiabetic activity and their suitability for synthesis using simple, established methods. The designed compounds were evaluated *in silico* to assess their potential as antidiabetic candidates. Structural modifications focused mainly on key positions of the TZD moiety, particularly N-substi-

tution, to improve biological activity and pharmacokinetic properties [10].

Computational study: Ligands were prepared using the LigPrep module of Schrödinger's Maestro suite. The corresponding Mol files were imported, and the LigPrep settings were configured to generate relevant tautomers, ionization states within pH 2-7 and stereoisomers, followed by energy minimization using the OPLS4 force field. Up to 32 conformers were generated for each ligand. The prepared structures were then saved in the project table for subsequent molecular docking studies [11].

ADME analysis and drug likeness: ADME properties of the proposed 2,4-thiazolidinedione derivatives were predicted using the ligand-based ADME module in Maestro 24.3. All 14 ligands were prepared before analysis to ensure appropriate protonation states, formal charges and 3D geometries. The prepared ligand file was submitted to the ADME prediction module, which estimated key properties such as lipophilicity, solubility, permeability and metabolic stability. The hydrogen-bond properties were evaluated using the software's standard H-bond assignment procedure [12-14].

Protein preparation: Prior to the molecular docking investigation, protein preparation is an essential step. Protein preparation guarantees that proteins are optimized for usage in molecular research and prepared for computer analysis. The crystal structure of PPAR γ (PDB ID: 5Y2O) was retrieved from the Protein Data Bank (PDB; www.rcsb.org) and prepared using the Protein Preparation Wizard in Maestro 24.3.

Quality assessment of prepared protein: Prior to molecular docking experiments, the quality of the 3D protein created using protein preparation tools is evaluated utilising the Saves and ProSA online servers [15-17].

Molecular docking: Molecular docking was performed using the Glide module of the Schrödinger Suite in Maestro. A receptor grid was generated at the binding site defined by the co-crystallized ligand, and the grid parameters were saved for docking. The prepared ligand file containing 14 derivatives and the co-crystal ligand was then imported into the Ligand Docking panel. Docking was performed using the Extra Precision (XP) mode of Glide to evaluate ligand–receptor interactions. The resulting docking scores and poses were recorded from the Project Table [11].

Toxicity prediction: The Protox-III server was used to predict the toxicity of the synthesized molecules. The SMILES representation of each molecule was entered into the server, followed by selection of the appropriate toxicity endpoint [18].

Synthesis

Synthesis of novel N-substituted thiazolidinedione derivatives were achieved in multi-steps.

Synthesis of thiazolidinedione (1): Compound **1** was synthesized by dissolving 8.96 g of monochloroacetic acid in 12 mL of water in one beaker and 8.37 g of thiourea in 12 mL of water in another. The two solutions were combined in a 250 mL round-bottom flask and stirred for 15 min at room temperature. The reaction mixture was then cooled in an ice bath for 10-15 min, resulting in the formation of a white precipitate. Conc. HCl (12 mL) was added slowly to the cooled

mixture and the resulting solution was fitted with a reflux condenser and heated under reflux in an oil bath at 100-110 °C for 8-10 h. After completion of the reaction, as monitored by TLC using chloroform (8:2, v/v) as the mobile phase, the reaction mixture was allowed to cool, yielding white needle-shaped crystals. The solid was filtered, washed with cold water and recrystallized from hot water. Colour; white crystalline solid; Yield: 65%; m.p.: 124-127 °C (corrected); R_f : 0.60 [CHCl_3 : CH_3OH 8:2]; FTIR (ATR, ν_{max} , cm^{-1}): 3210 (N-H), 2950 (C-H), 1735 (C=O), 715 (C-S); ^1H NMR (500 MHz, $\text{DMSO}-d_6$, δ ppm): 4.16 (s, 2H, CH_2), 12.03 (s, 1H, NH); m.f.: $\text{C}_3\text{H}_3\text{NO}_2\text{S}$; calcd. mass: 117.92; MS (m/z): 119 [$\text{M}+\text{H}$] $^+$.

Synthesis of (5Z)-5-[(4-hydroxyphenyl)methylidene]-1,3-thiazolidine-2,4-dione (2): Then, compound **1** (11.71 g, 0.1 mol) and 4-hydroxybenzaldehyde (10.01 g, 0.082 mol) were added to a reaction flask and stirred for 15 min at room temperature. Toluene (60 mL), piperidine (2.56 mL, 2.21 g, 0.026 mol) and benzoic acid (2.44 g, 0.02 mol) were then added, and finally the reaction mixture was refluxed for 5 h. The reaction progress was monitored by TLC using CHCl_3 : CH_3OH (7.5:2.5) as the mobile phase. After completion, the resulting white crystalline product was collected by filtration and washed with methanol to obtain the crude solid [19,20]. The crude product was recrystallized from glacial acetic acid, and its melting point was determined. Off white solid, yield: 73%, m.p.: >300 °C (corrected); R_f : 0.52 [CHCl_3 : CH_3OH 8:2]; FTIR (ATR, ν_{max} , cm^{-1}): 3439 (O-H), 3180 (N-H), 3050 (C-H), 1768 (C=O), 1604 (C=C), ^1H NMR (500 MHz, $\text{DMSO}-d_6$, δ ppm): 6.92 (s, 2H, arom.), 7.46 (s, 2H, arom.), 7.7 (s, 1H, benzyldiene), 10.30 (s, 1H, OH), 12.50 (s, 1H, NH); m.f.: $\text{C}_{10}\text{H}_7\text{NO}_3\text{S}$; calcd. mass: 221.23; MS (m/z): 222.25 [$\text{M}+\text{H}$] $^+$.

Synthesis of N-phenylacetamide chloride (3): Glacial acetic acid (80 mL) was added to aniline (9.30 g, 0.1 mol) in a 250 mL flask. Anhydrous sodium acetate (8.2 g, 0.1 mol) was then added and the mixture was stirred for 30 min. Chloroacetyl chloride (8 mL, 0.1 mol) was added gradually under cooling, followed by continuous stirring at room temperature for 24 h. The reaction progress was monitored by TLC using chloroform as the mobile phase. After completion, the reaction mixture was cooled with water and the product was collected by vacuum filtration. The crude product was recrystallized from methanol, and its melting point was determined [21]. Off white, yield: 82.23%, m.p.: 119-120 °C (corrected); R_f : 0.42 [CHCl_3 : CH_3OH 8:1]; FTIR (ATR, ν_{max} , cm^{-1}): 3304 (N-H), 3271 (N-H), 3104 (C-H) 1693 (C=O), 761 (C-Cl); m.f.: $\text{C}_8\text{H}_8\text{ClNO}$; calcd. mass: 169.61; MS (m/z): 170.61 [$\text{M}+\text{H}$] $^+$.

2-Chloro-N-(2-chlorophenyl)acetamide: White crystalline, yield: 72.4%, m.p.: 67-70 °C (corrected); R_f : 0.50 [CHCl_3 : CH_3OH 8:1]; FTIR (ATR, ν_{max} , cm^{-1}): 3277 (N-H), 3043 (C-H), 2951 (C-C-H), 1690 (C=O), 670 (C-Cl); m.f.: $\text{C}_8\text{H}_8\text{Cl}_2\text{NO}$; calcd. mass: 204.05; MS (m/z): 204.59 [$\text{M}+\text{H}$] $^+$.

N-(4-Bromophenyl)-2-chloroacetamide: Yellow solid, yield: 90.12%, m.p.: 185-186 °C (corrected); R_f : 0.51 [CHCl_3 : CH_3OH 8:1]; FTIR (ATR, ν_{max} , cm^{-1}): 3271 (N-H), 2962 (C-H), 1762 (C=O), 1548 (C-H), 746 (C-Br); m.f.: $\text{C}_8\text{H}_7\text{BrClNO}$; calcd. mass: 248.51; MS (m/z): 249.52 [$\text{M}+\text{H}$] $^+$.

2-Chloro-N-(2-fluorophenyl)acetamide: White crystal, yield: 91.47%, m.p.: 90-91 °C (corrected); R_f : 0.61 [CHCl_3 : CH_3OH 8:1]; FTIR (ATR, ν_{max} , cm^{-1}): 3315 (N-H), 2953 (C-H), 1778 (C=O), 1182 (C-F), 700 (C-Cl); m.f.: $\text{C}_8\text{H}_7\text{NOClF}$; calcd. mass: 187.60; MS (m/z): 188.60 [$\text{M}+\text{H}$] $^+$.

2-Chloro-N-(2-nitrophenyl)acetamide: Orange, yield: 53.73%, m.p.: 79-80 °C (corrected); R_f : 0.65 [CHCl_3 : CH_3OH 8:1]; FTIR (ATR, cm^{-1}): 3309 (N-H), 3018 (C-H), 2964 (C-H), 1689 (C=O), 1504 (N=O), 1340 (N=O); m.f.: $\text{C}_8\text{H}_7\text{ClN}_2\text{O}_3$; calcd. mass: 214.60; MS (m/z): 215.60 [$\text{M}+\text{H}$] $^+$.

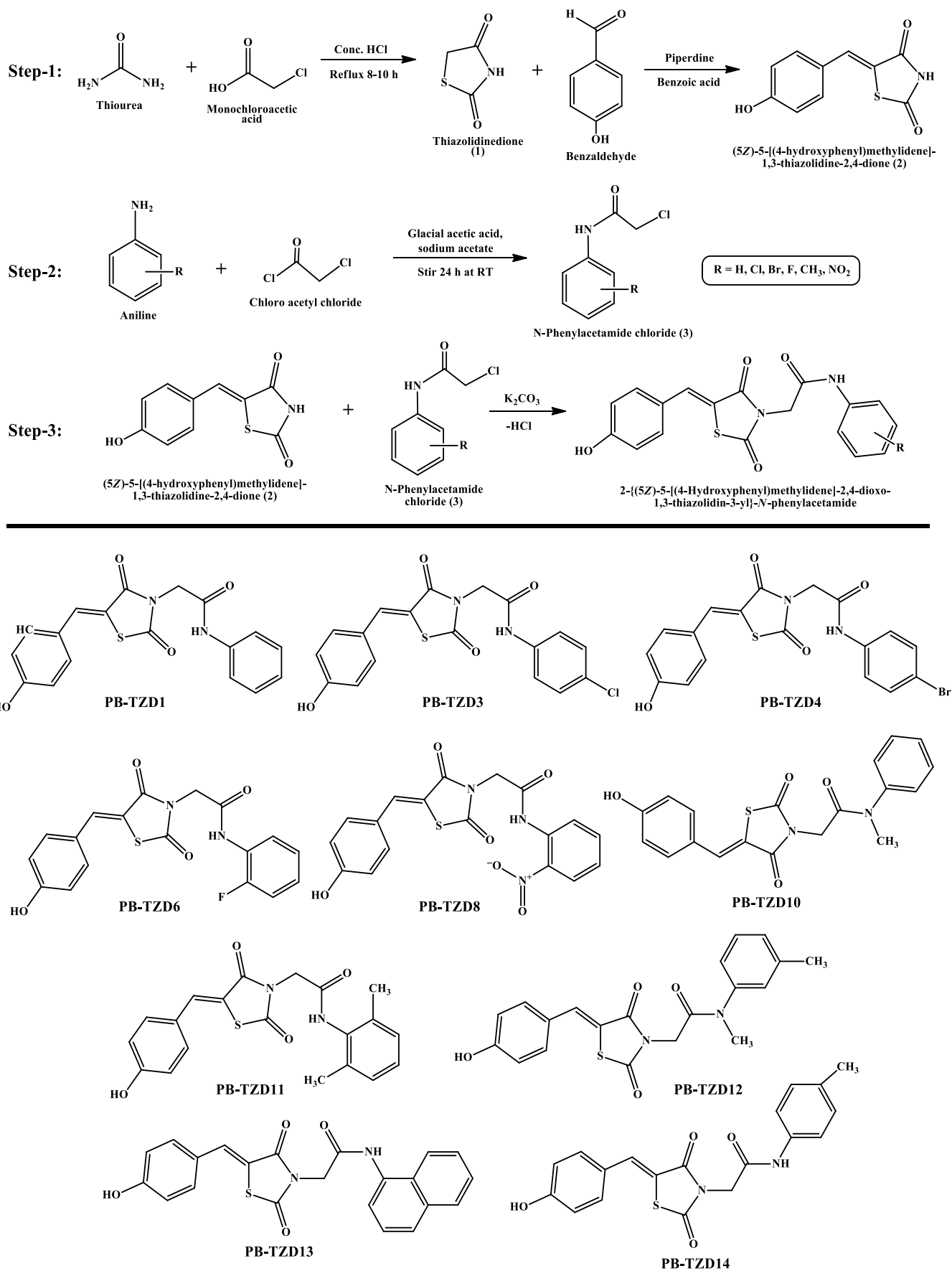
2-Chloro-N-methyl-N-phenylacetamide: Off white, yield: 86.38%, m.p.: 74-75 °C (corrected); R_f : 0.80 [CHCl_3 : CH_3OH 8:1]; FTIR (ATR, ν_{max} , cm^{-1}): 3319 (N-H), 3045 (C=C-H), 2954 (C-c-H), 1788 (C=O), 705 (C-Cl); m.f.: $\text{C}_9\text{H}_{10}\text{ClNO}$; calcd. mass: 184.63; MS (m/z): 185.64 [$\text{M}+\text{H}$] $^+$.

2-Chloro-N-(2,6-dimethylphenyl)acetamide: White, yield: 96.25%, m.p.: 150-151 °C (corrected); R_f : 0.59 [CHCl_3 : CH_3OH 8:1]; FTIR (ATR, ν_{max} , cm^{-1}): 3224 (N-H), 3054 (C-H), 2972 (C-H), 1735 (C=O), 1537 (C=C), 711 (C-Cl); m.f.: $\text{C}_{10}\text{H}_{12}\text{ClNO}$; calcd. mass: 197.66; MS (m/z): 198.6 [$\text{M}+\text{H}$] $^+$.

2-Chloro-N-(naphthalen-1-yl)acetamide: Off white, yield: 76.32%, m.p.: 157-158 °C (corrected); R_f : 0.74 [CHCl_3 : CH_3OH 8:1]; FTIR (ATR, ν_{max} , cm^{-1}): 3255 (N-H), 3055 (C-H), 2954 (C-H), 1657 (C=O), 1556 (C=C), 1504 (C=C), 769 (C-Cl); m.f.: $\text{C}_{12}\text{H}_{10}\text{ClNO}$; calcd. mass: 219.66; MS (m/z): 220.65 [$\text{M}+\text{H}$] $^+$.

Synthesis of 2-[(5Z)-5-[(4-hydroxyphenyl)methylidene]-2,4-dioxo-1,3-thiazolidin-3-yl]-N-phenylacetamide (PB-TZD1) (4): A clean and dry iodine flask was taken and 1.22 g (0.005 mol) of (5Z)-5-[(4-hydroxyphenyl)methylidene]-1,3-thiazolidine-2,4-dione (**2**) along with 0.84 g (0.005 mol) of N-phenylacetamide chloride was added. DMF (25 mL) was used as a solvent and 0.829 g (0.006 mol) of anhydrous K_2CO_3 was introduced into flask. The reaction allowed to react for 24 h under continuous stirring using a magnetic stirrer at room environment. The progress of the synthesis process was tracked using TLC with chloroform. After the reaction was completed, the reaction mixture was added to chilled water, leading to the rapid formation of a solid product. The precipitate was separated by vacuum filtration, dried to obtain the crude product and then further purified through recrystallization. Finally, the melting point of the purified product was determined [22] (Scheme-I). Yellow solid, yield: 67%, m.p.: 280-282 °C; R_f : 0.70; FTIR (ATR, ν_{max} , cm^{-1}): 3510 (O-H), 3140 (C-H), 2950 (C-H), 1730 (C=O), 1650 (C=O); ^1H NMR (500 MHz, $\text{DMSO}-d_6$, δ ppm): 4.47 (s, 2H, CH_2 of amide), 6.94 (d, 2H, arom.), 7.12 (d, 3H, arom.), 7.37 (d, 2H, arom.), 7.53 (d, 2H, arom.), 7.88 (s, 1H, CH benzyldiene), 10.28 (s, 1H, OH), 10.39 (s, 1H, NH); ^{13}C NMR (500 MHz, $\text{DMSO}-d_6$, δ ppm): 44.15, 115.83, 116.65, 122.22, 125.08, 127.07, 127.81, 128.06, 131.86, 132.45, 154.12, 159.05, 159.78, 167.16; m.f.: $\text{C}_{18}\text{H}_{14}\text{N}_2\text{O}_4\text{S}$; calcd. mass: 354.39; MS (m/z): 355.40 [$\text{M}+\text{H}$] $^+$.

2-[(5Z)-5-[(4-Hydroxyphenyl)methylidene]-2,4-dioxo-1,3-thiazolidin-3-yl]-N-(4-chloro phenyl)acetamide (PB-TZD3): Pale yellow solid, yield: 64%, m.p.: 254-258 °C; R_f : 0.52; FTIR (ATR, ν_{max} , cm^{-1}): 3600 (O-H), 2900 (C-H), 1737 (C=O), 1666 (C=O), 822 (C-Cl); ^1H NMR (500 MHz, $\text{DMSO}-d_6$, δ ppm): 4.50 (s, 2H, CH_2 of amide), 6.94 (d, 2H, arom.), 7.37 (d, 2H,



Scheme-I: Synthesis of novel design N-substituted thiazolidinedione derivatives

arom.), 7.53 (d, 2H, arom.), 7.56 (d, 2H, arom.), 7.89 (s, 1H, CH benzylidene), 10.399 (s, 1H, OH), 10.725 (s, 1H NH); ^{13}C NMR (500 MHz, DMSO- d_6 , δ ppm): 44.07, 101.64, 111.26, 115.84, 116.58, 125.37, 126.86, 128.36, 131.60, 132.25, 155.01, 158.57, 159.74, 167.63; m.f.: $\text{C}_{18}\text{H}_{13}\text{ClN}_2\text{O}_4\text{S}$; calcd. mass: 388.82; MS (m/z): 389.83 $[\text{M}+\text{H}]^+$.

2-{5-[(4-Hydroxyphenyl)methylidene]-2,4-dioxo-1,3-thiazolidin-3-yl}-N-(4-bromophenyl)acetamide (PB-TZD4): Yellow solid, yield: 87%, m.p.: 233-235 °C; R_f : 0.50; FTIR (ATR, ν_{max} , cm^{-1}): 3200 (O-H), 1674 (C=O), 1584 (C=C), 1246 (C-N), 1070 (C-O), 823 (C-Br); ^1H NMR (500 MHz, DMSO- d_6 , δ ppm): 4.5 (s, 2H, CH_2), 6.94 (d, 2H, arom.), 7.54 (m, 6H, arom.), 7.89 (s, 1H, CH benzylidene), 10.39 (s, 1H, OH phenolic), 10.52 (s, 1H, NH); ^{13}C NMR (500 MHz, DMSO- d_6 , δ ppm): 44.19, 115.79, 116.57, 124.28, 126.50, 129.37, 131.64, 132.25, 135.28, 138.78, 151.97, 158.77, 159.49, 167.09; m.f.: $\text{C}_{18}\text{H}_{13}\text{BrN}_2\text{O}_4\text{S}$; calcd. mass: 433.28; MS (m/z): 434.30 $[\text{M}+\text{H}]^+$.

2-{5-[(4-Hydroxyphenyl)methylidene]-2,4-dioxo-1,3-thiazolidin-3-yl}-N-(2-fluorophenyl)acetamide (PB-TZD6): Yellow solid, yield: 86%, m.p.: 218-220 °C; R_f : 0.75; FTIR (ATR, ν_{max} , cm^{-1}): 3416 (N-H), 3016 ((C=C-H), 1666 (C=O), 1575, 1509 (C=C), 751 (C-F); ^1H NMR (500 MHz, DMSO- d_6 , δ ppm): 4.58 (s, 2H, CH_2), 6.95 (d, 2H, arom.), 7.11 (m, 2H, aniline), 7.27 (m, 1H, aniline), 7.52 (d, 2H, arom.), 7.86 (d, 1H, arom.), 7.89 (s, 1H, CH benzylidene), 10.25 (s, 1H, OH), 10.40 (s, 1H, NH); ^{13}C NMR (500 MHz, DMSO- d_6 , δ ppm): 44.10, 114.37, 122.13, 123.71, 125.15, 126.60, 127.33, 129.14, 132.05, 135.25, 137.32, 149.39, 149.60, 166.77, 168.46, 169.95; m.f.: $\text{C}_{18}\text{H}_{13}\text{FN}_2\text{O}_4\text{S}$; calcd. mass: 472.37; MS (m/z): 473.33 $[\text{M}+\text{H}]^+$.

2-{5-[(4-Hydroxyphenyl)methylidene]-2,4-dioxo-1,3-thiazolidin-3-yl}-N-(2-nitrophenyl)acetamide (PB-TZD8): Yellow solid, yield: 56%, m.p.: 250-252 °C; R_f : 0.43; FTIR (ATR, ν_{max} , cm^{-1}): 3500 (O-H), 1740 (C=O), 1610 (C=C), 1578 (C=C); ^1H NMR (500 MHz, DMSO- d_6 , δ ppm): 4.54 (d, 2H, CH_2 of amide), 6.92 (d, 2H, arom.), 7.14 (t, 1H, arom.), 7.52 (d, 2H, arom.), 7.64 (d, 1H, arom.), 7.66 (m, 1H, arom.), 7.96 (s, 1H, CH benzylidene), 7.98 (d, 1H, arom.), 10.39 (s, 1H, OH), 10.69 (s, 1H, NH); ^{13}C NMR (500 MHz, DMSO- d_6 , δ ppm): 44.14, 114.11, 123.20, 123.62, 124.55, 125.03, 127.05, 127.57, 131.36, 134.80, 149.76, 156.34, 159.46, 166.48, 169.11, 168.95; m.f.: $\text{C}_{18}\text{H}_{13}\text{N}_3\text{O}_6\text{S}$; calcd. mass: 399.37; MS (m/z): 400.38 $[\text{M}+\text{H}]^+$.

2-{5-[(4-Hydroxyphenyl)methylidene]-2,4-dioxo-1,3-thiazolidin-3-yl}-N-methyl-N-phenylacetamide (PB-TZD10): Yellow solid, yield: 53%, m.p.: 230-233 °C; R_f : 0.64; FTIR (ATR, ν_{max} , cm^{-1}): 3090 (C-H), 2920 (C-H), 1740 (C=O), 1660 (C=C), 1552 (C=C); ^1H NMR (500 MHz, DMSO- d_6 , δ ppm): 3.19 (s, 3H, CH_3 (N-methyl), 4.11 (s, 2H, CH_2 amide), 6.93 (d, 2H, arom.), 7.51 (m, 7H, arom.), 7.83 (s, 1H, CH benzylidene), 10.38 (s, 1H, OH); ^{13}C NMR (500 MHz, DMSO- d_6 , δ ppm): 37.37, 44.21, 114.27, 119.45, 123.14, 123.52, 128.09, 130.04, 131.10, 139.98, 142.32, 149.56, 160.95, 166.56, 169.61; m.f.: $\text{C}_{19}\text{H}_{16}\text{N}_2\text{O}_4\text{S}$; calcd. mass: 368.40; MS (m/z): 369.40 $[\text{M}+\text{H}]^+$.

2-{5-[(4-Hydroxyphenyl)methylidene]-2,4-dioxo-1,3-thiazolidin-3-yl}-N-(2,6-dimethylphenyl)acetamide (PB-

TZD11): Yellow solid, yield: 79%, m.p.: 233-236 °C; R_f : 0.56; FTIR (ATR, ν_{max} , cm^{-1}): 3406 (O-H), 3354 (N-H), 3041 (C=C-H), 2953 (C-C-H), 1749 (C=O), 1541 (C=C); ^1H NMR (500 MHz, DMSO- d_6 , δ ppm): 2.14 (s, 6H, $2\times\text{CH}_3$), 4.56 (s, 2H, CH_2 amide), 6.92 (d, 2H, arom.), 7.07 (m, 2H, arom.), 7.529 (m, 1H, arom.) 7.52 (d, 1H, arom.), 7.7 (d, 1H, arom.), 7.89 (s, 1H, CH benzylidene), 7.96 (d, 1H, arom.), 9.7 (s, 1H, OH), 10.4 (s, 1H, 2 CH_3); ^{13}C NMR (500 MHz, DMSO- d_6 , δ ppm): 22.29, 44.21, 114.27, 123.52, 127.02, 128.09, 129.28, 130.04, 131.10, 139.98, 142.32, 149.37, 160.95, 166.56, 169.61; m.f.: $\text{C}_{20}\text{H}_{18}\text{N}_2\text{O}_4\text{S}$; calcd. mass: 382.43; MS (m/z): 383.45 $[\text{M}+\text{H}]^+$.

2-{5-[(4-Hydroxyphenyl)methylidene]-2,4-dioxo-1,3-thiazolidin-3-yl}-N-(3-methyl phenyl)acetamide (PB-TZD-12): Yellow solid, yield: 87%, m.p.: 251-255 °C; R_f : 0.50; FTIR (ATR, ν_{max} , cm^{-1}): 3560-3200 (OH), 3014 (C-H), 2910 (C-H), 1690 (C=O), 1658, 1581 (C=C); ^1H NMR (500 MHz, DMSO- d_6 , δ ppm): 2.73 (s, 3H, CH_3), 2.89 (s, 3H, N- CH_3), 4.58 (s, 2H, CH_2 of amide), 6.91 (d, 2H, arom.) 7.21 (t, 1H, arom.), 7.33 (t, 1H, arom.), 7.5 (d, 2H, arom.), 7.7 (t, 1H, arom.), 7.82 (s, 1H, arom.), 7.95 (s, 1H, CH benzylidene), 10.05 (s, 1H, OH); ^{13}C NMR (500 MHz, DMSO- d_6 , δ ppm): 19.52, 44.15, 115.83, 116.65, 122.22, 125.08, 127.07, 127.81, 129.06, 133.86, 144.45, 154.12, 159.05, 159.78, 167.16; m.f.: $\text{C}_{20}\text{H}_{19}\text{N}_2\text{O}_4$; calcd. mass: 383.43; MS (m/z): 384.40 $[\text{M}+\text{H}]^+$.

2-{5-[(4-Hydroxyphenyl)methylidene]-2,4-dioxo-1,3-thiazolidin-3-yl}-N-(naphthalen-1-yl)acetamide (PB-TZD13): Yellow solid, yield: 70%, m.p.: 320-322 °C; R_f : 0.45; FTIR (ATR, ν_{max} , cm^{-1}): 3600-3300 (OH), 3260 (N-H), 3014 (C-H), 1720 (C=O), 1658, 1581 (C=C); ^1H NMR (500 MHz, DMSO- d_6 , δ ppm): 4.69 (s, 2H, CH_2 amide), 6.94 (s, 2H, arom.), 7.52 (m, 5H, arom.), 7.6 (d, 1H, arom.), 7.65 (d, 1H, arom.), 7.91 (s, 1H, CH benzylidene) 7.95 (d, 1H, arom.), 8.11 (d, 1H, arom.), 10.38 (s, 1H, OH); ^{13}C NMR (500 MHz, DMSO- d_6 , δ ppm): 44.22, 96.13, 109.83, 116.10, 122.57, 123.56, 126.02, 126.59, 128.59, 129.37, 131.19, 132.36, 133.25, 134.17, 147.52, 151.38, 158.48, 159.84, 167.46; m.f.: $\text{C}_{23}\text{H}_{17}\text{NO}_4\text{S}$; calcd. mass: 404.45; MS (m/z): 405.45 $[\text{M}+\text{H}]^+$.

2-{5-[(4-Hydroxyphenyl)methylidene]-2,4-dioxo-1,3-thiazolidin-3-yl}-N-(4-methyl phenyl)acetamide (PB-TZD-14): Yellow solid, yield: 80%, m.p.: 300-303 °C; R_f : 0.57; FTIR (ATR, ν_{max} , cm^{-1}): 3400-3450 (OH), 3250 (N-H) 3050 (C-H), 1745 (C=O), 1580, 1510 (C=C); ^1H NMR (500 MHz, DMSO- d_6 , δ ppm): 2.25 (s, 3H, CH_3), 4.47 (s, 2H, CH_2 of amide), 6.94 (d, 2H, arom.), 7.12 (d, 2H, arom.), 7.35 (d, 2H, arom.), 7.88 (s, 1H, CH), 10.28 (s, 1H, OH), 10.39 (s, 1H, NH); ^{13}C NMR (500 MHz, DMSO- d_6 , δ ppm): 21.29, 44.21, 113.79, 123.52, 124.52, 127.02, 128.09, 129.28, 130.04, 131.10, 142.32, 149.37, 160.95, 166.56, 169.61; m.f.: $\text{C}_{19}\text{H}_{16}\text{N}_2\text{O}_4\text{S}$; calcd. mass: 368.41; MS (m/z): 369.42 $[\text{M}+\text{H}]^+$.

Biological activity

MTT assay: In this assay, the C2C12 cell line of interest was plated in a 96-well plate. The plate was cultured with a density of 5×10^4 cells per well. The 96-well plate was incubated at 37 °C in a 5% CO_2 environment for 24 h to facilitate cell adhesion. Following incubation, the culture medium was refreshed with DMEM included the test comp-

ounds at different concentrations (50, 100, 150, 250, 350, 450 and 550 μM) and the cells are incubated for further 24 h. After 24 h medium is carefully withdrawn from the well and 20 μL of 500 mg/100 mL.

The MTT solution prepared in phosphate-buffered saline (PBS) was added to each well. The 96-well plate was then incubated at 37 °C for 4 h to allow viable cells to convert MTT into insoluble formazan crystals. After incubation, the MTT solution was carefully removed and 100 μL of DMSO was added to each well. The plate was shaken for 10-15 min at room temperature to dissolve the formazan crystals completely. Absorbance was measured at 570 nm using an ELISA plate reader. The assay was performed in triplicate, with each concentration tested three times. Cell viability was calculated relative to the untreated control using the following formula [22,23].

$$\text{Cell viability (\%)} = \frac{\text{Control cells absorbance}}{\text{Treated cells absorbance}} \times 100$$

Glucose uptake assay: Glucose uptake was evaluated using C2C12 mouse myoblast cells cultured in DMEM supplemented with 10% FBS under 5% CO_2 . Cells were seeded in 96-well plates at a density of 1×10^5 cells/mL and incubated at 37 °C for 24 h in low-glucose DMEM. Test compounds (100 μM) and the reference compound (50 μM) were then added and incubated at 37 °C for 24-48 h. The culture medium was subsequently replaced with DMEM containing 0.1% BSA, 15 mM glucose and PBS, followed by incubation at 37 °C for 2 h. The incubation medium was transferred to a fresh 96-well plate and its glucose concentration was measured. The untreated cells served as negative control, while pioglitazone and insulin were used as positive controls. To calculate the enhancement in glucose uptake, the following equation was applied:

$$\text{Increase in glucose uptake (\%)} = \frac{\text{mg/dL Control} - \text{mg/dL Test}}{\text{mg/dL Control}} \times 100$$

where the mg/dL Test represents the glucose concentration in the test sample and mg/dL control sample represents glucose concentration in the control [24].

In vivo antidiabetic activity: A high-fat diet (HFD)- and streptozotocin (STZ)-induced Wistar albino rat model was used to evaluate the antihyperglycemic activity. The experiment was conducted according to the reported procedure [25].

Animals: Healthy Male albino Wistar rats, 5 to 6 weeks old and weight 150-180 g were purchased from LACSMI Bio Farms Pune, India. The animal growth under the controlled condition with 12 h dark and light cycle and at 23-25 °C in animal house facility of KBH Institute of Pharmacy Malegaon, India.

Animal ethical approval: The study received ethical clearance from the Institutional Animal Ethics Committee (IAEC) under approval no. KBH/IAEC/2024/12-09, dated December 10, 2024. The animal protocol was followed as per the guidelines of CPCSEA responsible for the research approval from small animal.

Induction of HFD-fed-STZ induces Type 2 diabetes in rats: Healthy Wistar rats were maintained on a high fat diet

(HFD) for 4 weeks. Following this period, the animals underwent a 6 hr fasting period prior to streptozotocin (STZ) administration. STZ was administered intraperitoneally at a dose of 50 mg/kg to the fasted rats. After 72 h of injection, blood was collected from the tail vein and fasting serum glucose (FSG) levels were measured using a glucometer. Rats with FSG ≥ 250 mg/dl were considered hyperglycemic and selected for further animal experiment. Rats continued with HFD for complete antidiabetic study period.

Anti-hyperglycemic study: The animals were divided into six groups, with six male Wistar rats in each group: normal control, diabetic control, positive control, **PB-TZD1**, **PB-TZD3** and **PB-TZD6**. The normal control group received a standard laboratory diet and water for 4 weeks without treatment. The remaining groups received a high-fat diet (HFD) for 4 weeks, followed by intraperitoneal administration of streptozotocin (STZ, 50 mg/kg) to induce diabetes. The positive control group was subsequently treated orally with pioglitazone (20 mg/kg/day) for 4 weeks. **PB-TZD1**, **PB-TZD3** and **PB-TZD6** compounds received the respective compounds orally at 20 mg/kg/day for 4 weeks. Body weight was recorded weekly, while food and water consumption were recorded daily and expressed as weekly averages. An oral glucose tolerance test (OGTT) was performed on day 28. Blood samples were collected on day 29 for biochemical analysis of total cholesterol, triglycerides, LDL and HDL levels.

Oral glucose tolerance test: The animal was deprived of food for 8 to 10 h prior to the initial blood collection from the tail tip at time zero. Following this, a glucose solution at a dose of 3 grams per kilogram of body weight was orally administered using a gavage. Blood samples were then taken from the tail at 30-min intervals specifically at 0, 30, 60, 90 and 120 min for the purpose of measuring serum glucose levels.

Biochemical estimation (endpoint): On day 29th, upon completing the treatment regimen, all rat cohorts were subjected to an 8-10 h fast. A total of 3.0 mL of blood was then drawn into a syringe and immediately distributed: 0.5 mL was placed into EDTA coated tubes and the remainder into serum-separator tubes for downstream biochemical assays. Insulin resistance was subsequently assessed by computing the Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) using the equations below:

$$\text{HOMA-IR} = \frac{\text{Fasting insulin } (\mu\text{U/mL}) \times \text{Fasting glucose (mg/dL)}}{405}$$

Adipocyte differentiation and PPAR- γ activity: The 3T3-L1 cells were cultured in DMEM supplemented with 10% (v/v) fetal bovine serum at 37 °C in a humidified 5% CO_2 atmosphere. The medium was replaced after every 48-72 h and cells were maintained below 80% confluency. For sub-culturing, cells were washed with PBS and detached using 0.25% trypsin-EDTA for 1-2 min at 37 °C. Complete medium was added to terminate trypsinization followed by centrifugation and cell counting. Cells were seeded in 12-well plates at 5×10^4 cells/well and cultured until confluent. Adipocyte differentiation was initiated on Day 4 using medium containing insulin (1 $\mu\text{g/mL}$), dexamethasone (0.25 μM), IBMX and test compounds. After 3 days, the medium was replaced

with differentiation medium containing insulin (1 µg/mL) alone and incubated for a further 3 days. Fresh culture medium was subsequently added on Days 10 and 12, with cells incubated for an additional 2 days after each replacement to obtain mature adipocytes.

For Oil Red O staining, differentiated cells were fixed with 10% formalin in PBS, washed with 60% isopropanol, and stained with Oil Red O working solution for 10 min at room temperature. Excess stain was removed with distilled water, and the cells were examined microscopically. Images were analyzed using ImageJ software. PPAR-γ activity was evaluated using the same differentiation protocol without fixation or staining. After differentiation, cells were harvested by trypsinization, washed with PBS and lysed using an appropriate lysis buffer. PPAR-γ activity was determined from the cell lysates using a commercial ELISA kit according to the manufacturer's instructions [26,27].

In vitro cytotoxicity study: The synthesized PB-TZD derivatives (**PB-TZD1–PB-TZD14**) were evaluated for preliminary biological properties using standard *in vitro* assays. Cytotoxicity was assessed by the MTT assay on C2C12 cell lines, based on the reduction of tetrazolium salt to formazan by metabolically active cells.

Statistical analysis: All *in vitro* experiments were performed in triplicate (n = 3) and results are expressed as mean ± standard deviation. Statistical analysis was carried out using one-way ANOVA followed by Dunnett's test. A *p*-value less than 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

All the designed PB-TZD derivatives (**PB-TZD1–PB-TZD14**) were developed through structural modification of the 2,4-thiazolidinedione core, with emphasis on *N*-substitution and C-5 functionalization. Substituted phenylacetamide groups were introduced at the *N*-position to increase the structural diversity and provide additional sites for molecular interactions. The synthetic accessibility scores ranged from 3 to 4 (Table-1) suggest that the designed compounds were amenable to synthesis using conventional laboratory procedures. The predicted physico-chemical properties, particularly lipophilicity and solubility, fell within the evaluated acceptable ranges, which support their suitability for further *in silico* and experimental assessment as potential antidiabetic candidates.

TABLE-1
PREDICTED SYNTHETIC ACCESSIBILITY OF
PB-TZD THIAZOLIDINEDIONE DERIVATIVES

Compound	Synthetic accessibility	Compound	Synthetic accessibility
PB-TZD1	3.32	PB-TZD8	3.53
PB-TZD2	3.33	PB-TZD9	3.68
PB-TZD3	3.29	PB-TZD10	3.43
PB-TZD4	3.35	PB-TZD11	3.53
PB-TZD5	3.38	PB-TZD12	3.53
PB-TZD6	3.32	PB-TZD13	3.54
PB-TZD7	3.29	PB-TZD14	3.42

The target compounds were designed through structural modification of the 2,4-thiazolidinedione core, with particular

attention to substitution at the N-3 and C-5 positions. Substituted phenylacetamide groups were introduced at the nitrogen atom to expand structural diversity and provide additional sites for molecular interactions. The synthetic route (**Scheme-1**) comprised three main steps: (i) synthesis of 2,4-thiazolidinedione; (ii) Knoevenagel condensation of 2,4-thiazolidinedione with 4-hydroxybenzaldehyde to obtain the corresponding benzylidene intermediates; and (iii) nucleophilic substitution with substituted chloroacetamides to furnish the final N-substituted derivatives. The reactions proceeded under the optimized conditions and afforded the desired compounds in moderate to good yields ranging from 53% to 87%.

The structures of the synthesized compounds were established by FT-IR, ¹H NMR, ¹³C NMR and mass spectrometric analyses. The FT-IR spectra displayed characteristic carbonyl stretching bands of the TZD ring within 1750-1700 cm⁻¹, along with aromatic C=C stretching and O–H vibrations consistent with the proposed structures. In the ¹H NMR spectra, the disappearance of the N–H proton signal of the parent TZD supported N-substitution. The methylene protons of the acetamide linkage appeared as a singlet at approximately δ 4.4-4.6 ppm, while the benzylidene proton resonated as a singlet near δ 7.8 ppm. The ¹³C NMR spectra contained the expected signals for the TZD carbonyl carbons and aromatic carbon atoms. Mass spectrometric analysis gave molecular ion peaks corresponding to [M+H]⁺, which in agreement with the calculated molecular masses and supporting the assigned molecular structures.

ADME analysis and drug-likeness: The ADME properties of the synthesized PB-TZD derivatives were predicted using Maestro 24.3 (Tables 2 and 3). All compounds exhibited acceptable physico-chemical parameters within recommended ranges. The calculated QPlogP values (-2.0 to 6.5) indicated suitable lipophilicity, essential for membrane permeability. The QPlogBB values (-3.0 to 1.2) also suggested limited blood–brain barrier penetration, which reflect the controlled distribution behaviour.

The predicted metabolic transformations ranged from 2 to 5, falling within the evaluated range for the compounds. QPlogS values of -4.2 to -6.6 reflected their predicted aqueous solubility. These findings place the derivatives within a reasonably balanced physico-chemical range and warrant further evaluation as potential drug-like candidates.

Protein preparation and validation: The protein structure was prepared by removing non-essential molecules, adding missing atoms, hydrogens and optimizing protonation states at physiological pH. Energy minimization was performed using the OPLS4 force field. The structural quality was assessed using the Ramachandran plot, ERRAT, Verify 3D, ProSA and residue-score analyses (Fig. 1). The Ramachandran plot placed 94.4% of residues in the favoured region, while the ERRAT score was 98.1%. Verify 3D showed 92% compatibility of residues with their three-dimensional environment, and the ProSA Z-score was -8.90. These results support the structural quality of the prepared protein for subsequent molecular docking studies.

Molecular docking and structure–activity relationship (SAR): Molecular docking analysis showed favourable binding of the synthesized derivatives within the PPAR-γ active

TABLE-2
In silico PREDICTED ADME ANALYSIS OF PB-TZD DERIVATIVES

Molecule	Absorption	Distribution		Metabolism	Excretion
	% Human oral absorption	QPlogP _{o/w}	QPlogBB	#metab	QPlogS
Pioglitazone	100	3.548	-0.87	5	-4.299
PB-TZD1	83.317	2.65	-1.591	3	-4.76
PB-TZD2	86.085	3.138	-1.46	3	-5.435
PB-TZD3	86.138	3.133	-1.454	2	-5.477
PB-TZD4	86.593	3.208	-1.45	2	-5.588
PB-TZD5	81.746	4.221	-0.997	2	-6.644
PB-TZD6	84.716	2.89	-1.496	3	-5.105
PB-TZD7	84.662	2.881	-1.491	2	-5.114
PB-TZD8	69.869	2.05	-2.46	4	-4.694
PB-TZD9	86.346	1.395	-3.464	4	-4.734
PB-TZD10	87.739	2.907	-1.437	3	-4.806
PB-TZD11	90.836	3.27	-1.304	5	-5.166
PB-TZD12	88.677	3.232	-1.419	4	-5.242
PB-TZD13	89.599	3.58	-1.596	2	-5.804
PB-TZD14	85.537	2.947	-1.642	3	-5.306

 TABLE-3
 DRUG LIKENESS PREDICTION BY LIPINSKI'S RULE OF FIVE OF PB-TZD DERIVATIVES

Molecule	Mol_MW	QPlogP _{o/w}	DonorHB	AccepHB	Violence of Lipinski's rule	Rule of three
Pioglitazone	357.426	3.548	1	5.25	0	0
PB-TZD1	354.380	2.650	2	6.25	0	0
PB-TZD2	388.825	3.138	2	6.25	0	0
PB-TZD3	388.825	3.133	2	6.25	0	0
PB-TZD4	433.276	3.208	2	6.25	0	0
PB-TZD5	591.068	4.221	2	6.25	1	1
PB-TZD6	372.370	2.890	2	6.25	0	0
PB-TZD7	372.370	2.881	2	6.25	0	0
PB-TZD8	399.377	2.050	2	7.25	0	0
PB-TZD9	444.375	1.395	2	8.25	1	1
PB-TZD10	368.406	2.907	1	6.75	0	0
PB-TZD11	382.433	3.270	2	6.25	0	0
PB-TZD12	382.433	3.232	1	6.75	0	0
PB-TZD13	404.439	3.580	2	6.25	0	1
PB-TZD14	368.406	2.947	2	6.25	0	0

site (PDB ID: 5Y2O). The docked compounds formed hydrogen bonding interactions with Cys285, along with hydrophobic contacts involving Leu, Ile and Phe residues and π - π interactions within aromatic regions. The binding mode of co-crystallized pioglitazone is shown in Fig. 2.

SAR analysis suggested that electronic, steric and hydrophobic features influenced the observed activity. Electron-withdrawing substituents may favour interactions within the PPAR- γ binding pocket, while aromatic groups can contribute hydrophobic contacts. Bulky substituents may alter ligand orientation and receptor interactions depending on their position. Some compounds showed differences between docking scores and experimental activity. Such variation may arise from factors not captured by docking, such as solubility, membrane permeability and intracellular availability. The biological activity was therefore interpreted alongside the docking results rather than from docking scores alone.

Correlation of structure with activity: The observed activity trends (Table-4) were associated with several structural features. *N*-substitution may improve ligand-receptor inter-

actions, while electron-withdrawing groups can influence binding within the PPAR- γ pocket. Aromatic substituents may contribute π - π interactions and the benzylidene linkage provides planarity and conjugation (Fig. 3). These features may contribute to the biological activity of the derivatives.

Toxicity prediction: The ADME and toxicity properties of the synthesized compounds were evaluated using *in silico* prediction tools. These results provide preliminary insights into pharmacokinetic behaviour and potential safety profiles, however, they should not be interpreted as definitive evidence of *in vivo* safety or efficacy. The predictions related to hepatotoxicity, nephrotoxicity, cardiotoxicity and drug-likeness are indicative in nature and require further experimental validation through *in vitro* and *in vivo* studies.

Predicted toxicity class and LD₅₀ values: Most compounds in the PB-TZD series had a predicted oral LD₅₀ of 1000 mg/kg, consistent with a relatively low level of acute toxicity. The predicted oral LD₅₀ was 500 mg/kg for **PB-TZD-4**, **PB-TZD-17**, **PB-TZD-18** and **PB-TZD-22**, whereas most compounds in the series had an LD₅₀ of 1000 mg/kg. Further toxi-

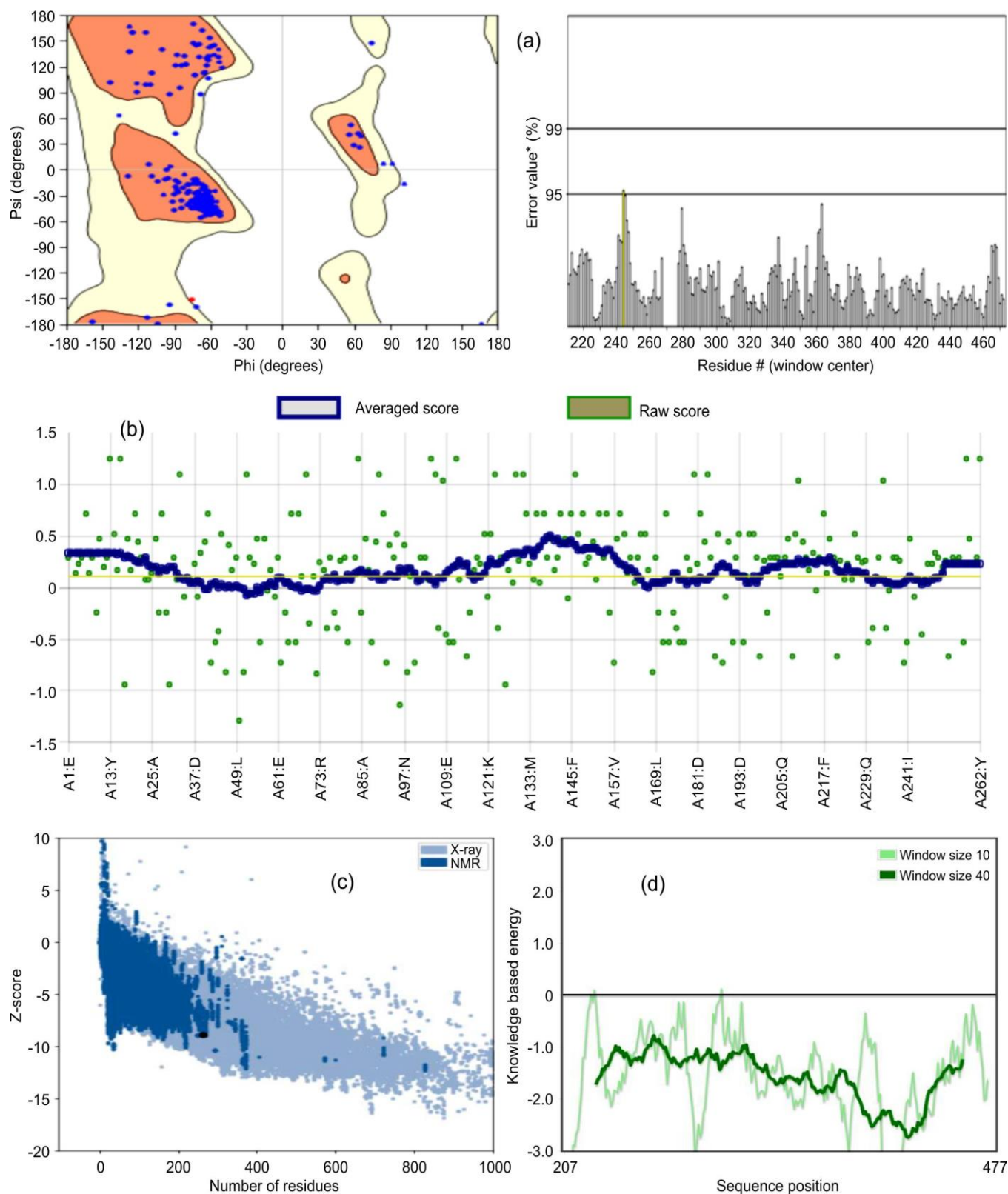


Fig. 1. Validation of the modelled protein structure: (a) Ramachandran plot, (b) Z-score plot, (c) verify3D profile and (d) ERRAT quality plot

colological studies are required to establish the safety profile of these 4 compounds. The relevant data are provided in Table-5.

Biological evaluation

***In vitro* cytotoxicity study:** All tested compounds exhibited low cytotoxicity, with IC_{50} values above 250 μ M. Cell

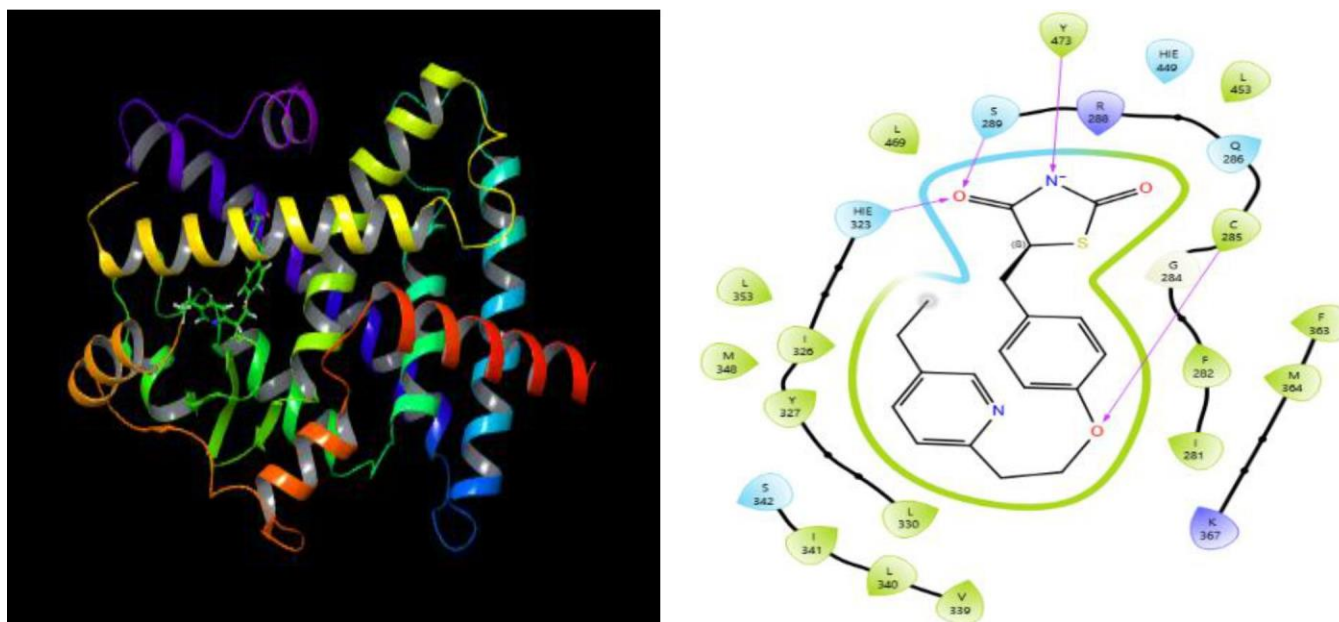


Fig. 2. PPAR- γ (PDB ID: 5Y2O) crystal structure complexed with the ligand pioglitazone, with polar interactions involving Ser289, His323, and Tyr473

TABLE-4
MOLECULAR DOCKING SCORE OF PB-TZD SERIES AND PARAMETER CONTRIBUTE FOR THE DOCKING SCORE OF COMPOUNDS USING PPAR- γ (PDB ID-5Y2O) DOWNLOADED FROM THE PROTEIN DATA BANK

Compound	Docking score	Electro	H-Bond	Lipophilic	Low M.W.
Pioglitazone	-10.282	-0.435	-1.799	-6.903	-0.312
PB-TZD5	-10.094	-0.327	-1.33	-7.318	-0.312
PB-TZD1	-8.727	-0.401	-1.232	-6.694	-0.400
PB-TZD6	-7.94	-0.541	-0.524	-5.739	-0.175
PB-TZD11	-7.714	-0.538	-1.059	-5.381	-0.175
PB-TZD3	-7.477	-0.44	-0.71	-5.498	-0.229
PB-TZD7	-6.92	-0.381	-1.49	-4.463	-0.265
PB-TZD4	-6.848	-0.501	-0.836	-4.174	-0.272
PB-TZD13	-6.641	-0.453	-0.814	-4.808	-0.265
PB-TZD12	-6.507	-0.049	-0.545	-5.331	-0.152
PB-TZD9	-6.434	-0.103	-0.552	-5.17	-0.225
PB-TZD10	-6.334	-0.139	-0.657	-5.165	-0.019
PB-TZD2	-6.292	-0.099	-0.552	-4.955	-0.272
PB-TZD14	-6.248	-0.069	-0.547	-4.827	-0.319
PB-TZD8	-6.102	-0.059	-0.55	-4.852	-0.265

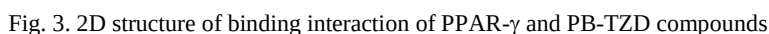
viability remained largely unaffected at concentrations up to 100 μ M, which support a favourable *in vitro* cytocompatibility profile. The IC₅₀ values are listed in Table-6, while the nonlinear logarithmic plots generated from the mean of three replicate measurements are shown in Fig. 4.

Glucose uptake: Glucose uptake activity was evaluated in C2C12 cells to assess the functional response of the synthesized derivatives (**PB-TZD-1–PB-TZD-14**). Several compounds demonstrated moderate to enhanced glucose uptake compared to control, which indicate their potential for further investigation. The activity was concentration-dependent and the results were expressed as percentage increase relative to untreated cells. The results of the glucose uptake assay are presented in Table-7.

The biological activity of the synthesized compounds was compared with the standard drug pioglitazone. Notably, **PB-TZD1** (36.00%), **PB-TZD3** (34.30%) and **PB-TZD6**

(33.53%) exhibited higher glucose uptake activity compared to pioglitazone (24.77%). Molecular docking analysis further supported these findings, with the synthesized compounds showing favourable binding interactions with key residues such as CYS285. These results suggest that the newly synthesized derivatives may offer improved biological performance compared to the standard drug. Enhancement of glucose absorption is seen by multinuclear formation (Fig. 5).

In vivo anti-hyperglycaemic activity: From the synthesized series, **PB-TZD1**, **PB-TZD3** and **PB-TZD6** were selected for detailed biological evaluation based on their higher *in vitro* glucose uptake activity, favourable docking scores and distinct structural features. These compounds represent the most promising candidates within the series and were therefore chosen for further validation to establish their potential as lead molecules. The antihyperglycaemic potential of the synthesized PB-TZD derivatives was evaluated using a high-fat diet



Compd.	Pred. toxicity class	Pred. LD ₅₀ (mg/Kg)	Organ toxicity			End point toxicity	
			Hepato-toxicity	Nephro-toxicity	Cardio-toxicity	Active for	Inactive for
PB-TZD1	4	1000	Inactive	Inactive	Inactive		Carcinogenicity, cytotoxicity and BBB-barrier
PB-TZD2	4	1000	Inactive	Active	Inactive		BBB-Barrier, cytotoxicity and carcinogenicity
PB-TZD3	4	1000	Active	Active	Inactive		Cytotoxicity, immunotoxicity, carcinogenicity, BBB-barrier, mutagenicity
PB-TZD4	4	1190	Active	Inactive	Inactive	Immuno-toxicity	Mutagenicity, cytotoxicity, cardiotoxicity and BBB-barrier
PB-TZD5	4	1000	Inactive	Active	Inactive	–	Carcinogenicity, immunotoxicity, BBB-barrier, mutagenicity
PB-TZD6	4	1000	Active	Active	Inactive	–	Immunotoxicity, carcinogenicity, mutagenicity
PB-TZD7	4	1000	Active	Active	Inactive	–	Immunotoxicity, mutagenicity cytotoxicity BBB-barrier
PB-TZD8	4	1000	Inactive	Inactive	Inactive	Mutagenicity	Immunotoxicity, BBB-barrier
PB-TZD9	4	1000	Inactive	Inactive	Inactive	Mutagenicity	BBB-barrier
PB-TZD10	4	1000	Inactive	Inactive	Inactive	–	Immunotoxicity, carcinogenicity BBB-barrier, mutagenicity
PB-TZD11	4	1000	Inactive	Inactive	Inactive	–	Immunotoxicity, cytotoxicity, BBB-barrier, mutagenicity
PB-TZD12	4	1000	Inactive	Inactive	Inactive	–	Immunotoxicity, cytotoxicity, carcinogenicity, BBB-barrier, mutagenicity
PB-TZD13	4	1000	Inactive	Inactive	Inactive	–	Immunotoxicity, cytotoxicity, carcinogenicity, BBB-barrier
PB-TZD14	4	1000	Inactive	Inactive	Inactive	–	Mutagenicity, immunotoxicity, carcinogenicity, cytotoxicity, BBB-barrier

TABLE-6
IC₅₀ VALUE OF PB-TZD COMPOUNDS CALCULATED FROM THE TRIPLICATE READING OF MTT ASSAY USING THE C2C12 CELL LINE ASSAY

Compound	IC ₅₀ (μM)	Compound	IC ₅₀ (μM)
PB-TZD1	329	PB-TZD11	370
PB-TZD3	398	PB-TZD12	339
PB-TZD4	306	PB-TZD13	339
PB-TZD6	284	PB-TZD14	289
PB-TZD8	336	Pioglitazone	450
PB-TZD10	350	—	—

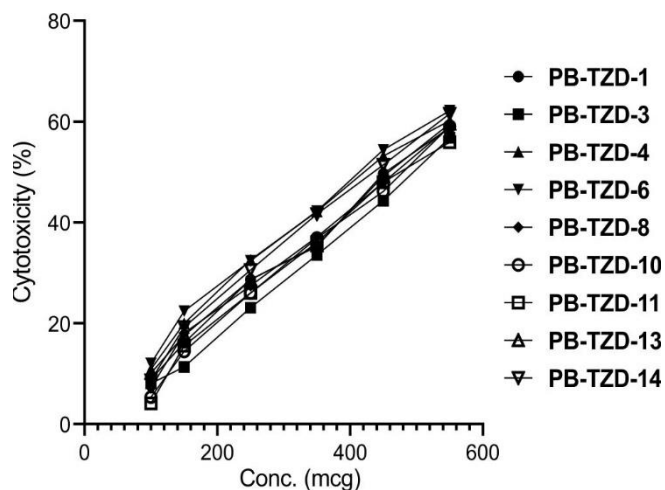


Fig. 4. Non-linear logarithm graph of average of triplicate reading of each conc. of derivatives in MTT assay on C2C12 cell line for the finding of IC₅₀ value

TABLE-7
GLUCOSE UPTAKE ACTIVITY OF PB-TZD COMPOUNDS IN C2C12 CELL LINE (mg/dL), EVALUATED IN TRIPLICATE WITH PERCENTAGE INCREASE RELATIVE TO BLANK MEDIA

Sample code	Average (mg/dl)	Standard deviation (SD)	Increase in glucose uptake (%)
Blank media	629.666	—	—
Negative control	606.333	2.0817	3.70
Pioglitazone	456.667	2.0817	24.77
Insulin	486.333	2.5167	19.79
PB-TZD-1	388.000	1.7300	36.00
PB-TZD-3	398.333	1.5200	34.30
PB-TZD-4	578.667	3.2100	4.56
PB-TZD-6	403.000	2.0000	33.53
PB-TZD-8	591.000	2.6400	2.52
PB-TZD-10	493.333	2.5100	18.63
PB-TZD-11	499.000	1.0000	17.70
PB-TZD-12	489.000	2.0000	18.75
PB-TZD-13	442.66	2.3100	26.99
PB-TZD-14	484.000	2.0000	20.17

(HFD) and streptozotocin (STZ)-induced diabetic rat model. The induction phase resulted in a significant increase in body weight (Table-8), confirming successful establishment of an insulin-resistant metabolic state. The experimental groups followed a similar pattern of body-weight change throughout the study, with no marked differences between groups.

During the treatment phase (Table-9), the diabetic control group experienced a marked decline in body weight, whereas the **PB-TZD1**, **PB-TZD3** and **PB-TZD6** groups showed pro-

TABLE-8
PERCENTAGE CHANGE IN BODY WEIGHT OF RATS DURING THE DIABETIC INDUCTION PHASE (WEEK 0 TO WEEK 4) UNDER HIGH-FAT DIET AND STZ PROTOCOL (MEAN ± SEM, n = 6)

Animal group	Body weight during rats on HFD (g); mean ± SEM					Increase in body weight (%)
	Day 0	Day 7	Day 14	Day 21	Day 28	
Normal control	161.00 ± 2.52	161.67 ± 2.56	163.83 ± 2.63	164.33 ± 2.56	165.00 ± 2.72	2.48
Diabetic control	167.00 ± 3.48*	176.67 ± 2.58*	185.50 ± 2.64*	194.50 ± 1.86*	202.67 ± 1.65*	21.39
Positive control	165.00 ± 2.54*	173.50 ± 2.31*	181.83 ± 2.33**	190.67 ± 2.19*	199.17 ± 2.06*	20.71
PB-TZD1	166.50 ± 1.41*	176.17 ± 1.62*	185.67 ± 1.43*	195.33 ± 1.17*	203.17 ± 0.95*	22.06
PB-TZD3	168.33 ± 0.92*	178.83 ± 1.01*	188.00 ± 1.15*	196.50 ± 1.02*	205.33 ± 0.67*	21.99
PB-TZD6	166.33 ± 1.03*	175.33 ± 1.05*	182.00 ± 1.05*	192.17 ± 1.06*	200.50 ± 0.90*	20.55

Data expressed in mean ± standard mean error of group of six animal. HFD = high feed diet, STZ = Streptozotocin. Statistical comparisons were made using one-way ANOVA analysis of variance. **p* < 0.05 vs. diabetic control, **p* < 0.05 vs. positive control, **p* < 0.05 vs. **PB-TZD1**, **p* < 0.05 vs. **PB-TZD3**, **p* < 0.05 vs. **PB-TZD6**. Data are statically significant.

TABLE-9
PERCENTAGE CHANGE IN THE RATS' BODY WEIGHT FROM WEEK 5 TO WEEK 8 AFTER THEY WERE GIVEN A HIGH-FAT DIET AND STZ INDUCTION (MEAN ± SEM, n = 6)

Animal group	Body weight during rats on HFD (g); mean ± SEM					Decrease or increase in body weight (%)
	On day 0	On day 7	On day 14	On day 21	On Day 28	
Normal control	165.00 ± 2.52	165.67 ± 2.56	168.17 ± 2.63	168.33 ± 2.56	167.00 ± 2.72	1.21
Diabetic control	202.67 ± 3.48*	187.67 ± 2.58*	178.33 ± 2.64*	172.50 ± 1.86*	168.17 ± 1.65*	-17.03
Positive control	199.17 ± 2.54*	181.5 ± 2.31**	173.17 ± 2.33*	163.50 ± 2.19*	173.17 ± 2.06*	-13.05
PB-TZD1	203.17 ± 0.95*	183.67 ± 1.26*	174.67 ± 0.67*	170.17 ± 0.60*	170.67 ± 0.95*	+16.00
PB-TZD3	205.33 ± 0.67*	189.33 ± 0.49*	180.67 ± 0.76*	173.83 ± 0.48*	175.17 ± 0.70*	+14.69
PB-TZD6	200.50 ± 0.92*	185.67 ± 1.36*	176.33 ± 0.99*	170.33 ± 0.92*	171.17 ± 0.95*	+14.63

HFD is for high-fat diet, while STZ stands for streptozotocin. Data expressed in mean ± standard mean error of group of six animal. One-way ANOVA analysis of variance was used for statistical comparisons. **p* < 0.05 vs. **PB-TZD1**, **p* < 0.05 vs. **PB-TZD3**, **p* < 0.05 vs. **PB-TZD6**, **p* < 0.05 vs. positive control, **p* < 0.05 vs. diabetic control and **p* < 0.05 vs. **PB-TZD6**. The information is statistically significant.

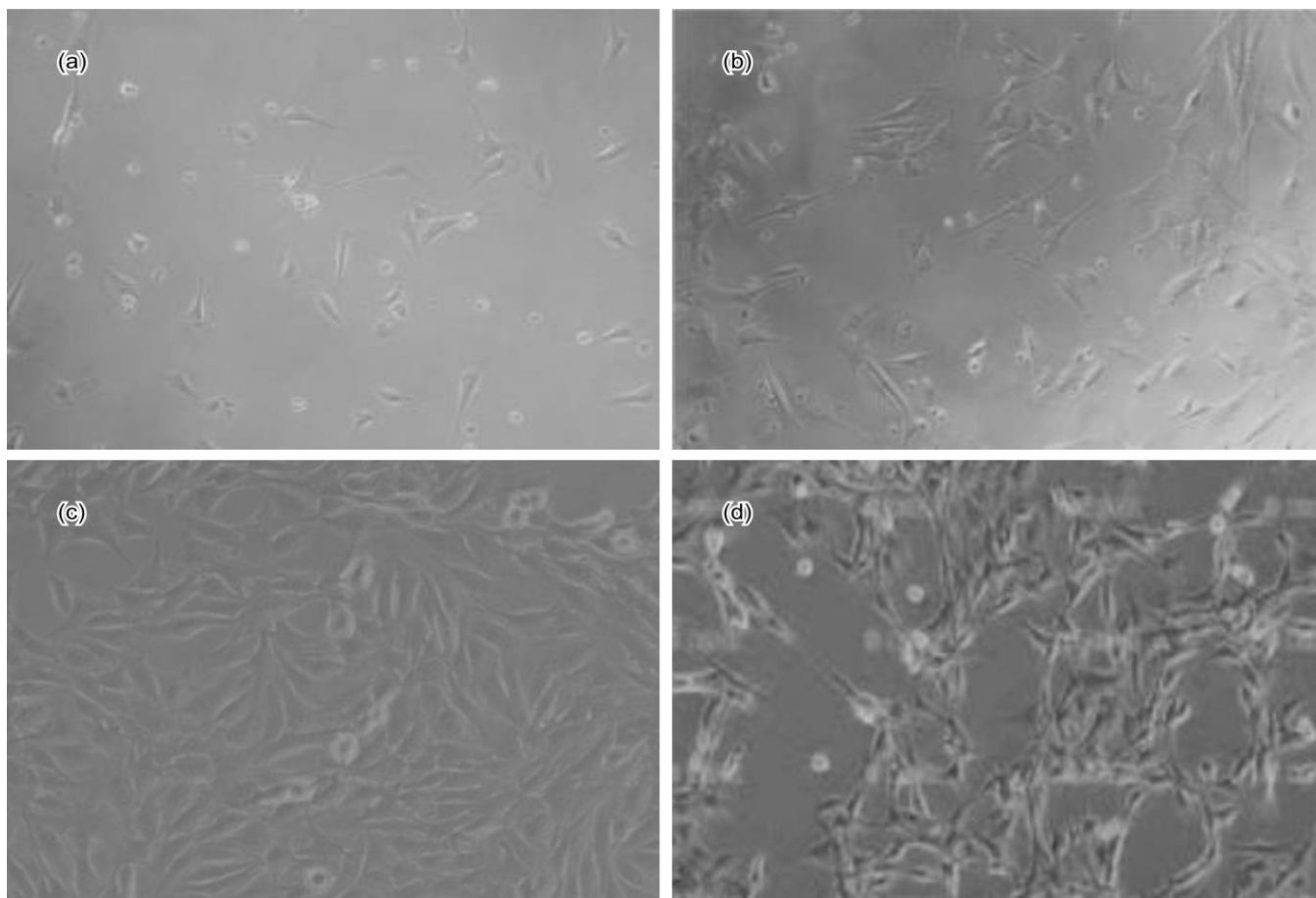


Fig. 5. (a) Negative control, cell is constricted due to serum withdrawal, (b) Cell with pioglitazone show multinuclear cell, (c) Cell with PB-TZD1 and (d) Cell with PB-TZD3 showing more multinuclear active cell

gressive recovery. This pattern was associated with improved maintenance of body weight following treatment with the synthesized compounds.

Successful diabetic induction was established by the marked elevation of fasting blood glucose (FBG) following STZ administration (Table-10). Comparable baseline hypergly-

TABLE-10
ASSESSMENT OF STZ-INDUCED DIABETES BASED ON FASTING BLOOD
GLUCOSE LEVELS BEFORE AND 72 H AFTER STZ ADMINISTRATION

Groups	Number of animals	Fasting blood glucose level (mg/dl)		Groups	Number of animals	Fasting blood glucose level (mg/dl)	
		Before STZ	After 72 h			Before STZ	After 72 h
Normal control	1	72	74	PB-TZD1	1	79	275
	2	80	78		2	78	281
	3	85	80		3	81	283
	4	79	83		4	75	274
	5	90	91		5	74	266
	6	88	87		6	78	288
Diabetic control	1	90	280	PB-TZD3	1	73	263
	2	100	293		2	84	273
	3	87	274		3	82	283
	4	84	260		4	81	272
	5	92	279		5	78	265
	6	93	287		6	76	258
Positive control	1	83	265	PB-TZD6	1	80	253
	2	91	282		2	78	268
	3	88	293		3	83	291
	4	90	279		4	84	284
	5	78	273		5	75	261
	6	76	285		6	76	290

caemia was recorded across the diabetic groups. During treatment, each tested derivative produced a significant reduction in FBG compared with the diabetic control group (Table-11). **PB-TZD3** exhibit the highest reduction in blood glucose among the tested compounds, with activity approaching that of the standard drug.

Biochemical analysis further supported the glucose lowering response as observed in the treated groups (Table-12). Treatment with the PB-TZD derivatives increased insulin levels and reduced HOMA-IR values, which is consistent with the improved insulin sensitivity. The structural features of the derivatives, particularly N-substitution and the nature of the aromatic substituents may influence their interaction with the biological target and contribute to the observed response.

Lipid profile analysis (Table-13) revealed an improvement in diabetes-associated dyslipidemia following treatment. PB-TZD3 produced a favourable lipid response, with lower triglyceride, total cholesterol and LDL levels together with an increase in HDL levels. The oral glucose tolerance test (OGTT) further supported improved glucose handling in the treated groups (Table-14). The strongest responses were

observed with **PB-TZD3** and **PB-TZD6**, which may promote peripheral glucose utilization and metabolic control. The *in vivo* antihyperglycaemic response agrees with the molecular docking results. **PB-TZD3** exhibited the strongest biological response and exhibited favourable binding within the PPAR- γ active site, with interactions involving key amino acid residues. Electron-withdrawing substituents and appropriate lipophilic character may have contributed to these interactions and the associated biological response. The agreement between the docking and experimental data provides a basis for related structural modifications of the PB-TZD derivatives to their PPAR- γ binding behaviour and antidiabetic activity.

All biological experiments were performed in sextuplicate ($n = 6$) and the results are expressed as mean $\pm$ standard deviation (SD). Statistical analysis was carried out using one-way analysis of variance (ANOVA) followed by a Dunnett's test to determine significance. A p -value of less than 0.05 was considered statistically significant.

Adipocyte differentiation: Further assessment of the antidiabetic potential of PB-TZD derivatives was performed by evaluating PPAR- γ activation through adipocyte differen-

TABLE-11
EFFECT OF TEST COMPOUNDS ON FASTING BLOOD GLUCOSE LEVELS DURING THE TREATMENT PHASE (WEEKS 5–8) IN STZ-INDUCED DIABETIC RATS

Group	Fasting blood glucose (mg/dl) during anti-diabetic study period (Means $\pm$ SEM)				
	Day 0	Day 7	Day 14	Day 21	Day 28
Normal control	82.17 $\pm$ 2.57	83.50 $\pm$ 2.41	84.00 $\pm$ 1.84	82.83 $\pm$ 2.29	81.50 $\pm$ 1.76
Diabetic control	278.83 $\pm$ 4.66	301.33 $\pm$ 4.13	317.17 $\pm$ 3.77	329.67 $\pm$ 3.55	340.17 $\pm$ 2.44
Positive control	279.50 $\pm$ 4.15****	237.83 $\pm$ 1.65****	204.67 $\pm$ 2.02****	163.50 $\pm$ 2.19****	122.33 $\pm$ 1.67****
PB-TZD1	277.83 $\pm$ 3.18****	242.33 $\pm$ 1.54****	204.5 $\pm$ 6.05****	171.0 $\pm$ 5.75****	138.17 $\pm$ 2.29***
PB-TZD3	269.0 $\pm$ 3.62****	230.33 $\pm$ 4.64****	194.83 $\pm$ 7.39****	155.67 $\pm$ 3.28****	124.5 $\pm$ 2.63****
PB-TZD6	274.5 $\pm$ 6.56****	229.83 $\pm$ 5.2****	185.17 $\pm$ 6.42****	155.83 $\pm$ 4.21****	131.5 $\pm$ 3.16****

Data expressed in mean $\pm$ standard mean error of group of six animal. Statistically comparisons were made using one-way ANOVA analysis of variance. ** $p < 0.0001$ vs. positive control, * $p < 0.0001$ vs. **PB-TZD1**, ** $p < 0.0001$ vs. **PB-TZD3** and ** $p < 0.0001$ vs. PB-TZD. All control and four test compounds groups have adjusted p -values < 0.0001 , so data are statistically highly significant.

TABLE-12
EFFECT OF TREATMENTS ON PLASMA INSULIN, GLUCOSE LEVELS AND HOMA-IR AT DAY 29 OF THE TREATMENT PHASE IN STZ-INDUCED DIABETIC RATS ($n = 6$ PER GROUP)

Group	Mean insulin (μ U/mL)	SEM insulin	Mean glucose (mg/dL)	SEM glucose	Mean HOMA-IR	SEM HOMA-IR
Normal control	15.00	0.26	79.50	1.34	2.95	0.08
Diabetic control	7.50	0.34	337.67	2.65	6.26	0.31
Positive control	12.33	0.33	121.33	0.92	3.70	0.12
PB-TZD1	9.3	0.43	138.5	1.99	3.19	0.14
PB-TZD3	11.0	0.37	126.7	1.91	3.44	0.13
PB-TZD6	10.0	0.51	135	4.66	3.33	0.22

TABLE-13
EFFECT OF TEST COMPOUNDS ON SERUM LIPID PROFILE IN EXPERIMENTAL RATS: COMPARISON OF TG, TC, LDL AND HDL LEVELS IN NORMAL, DIABETIC AND TREATMENT GROUPS

Group	TG (Mean $\pm$ SEM)	TC (Mean $\pm$ SEM)	LDL (Mean $\pm$ SEM)	HDL (Mean $\pm$ SEM)
Normal control	1.17 $\pm$ 0.05	2.51 $\pm$ 0.06	1.34 $\pm$ 0.08	1.43 $\pm$ 0.08
Diabetic control	1.87 $\pm$ 0.05	4.44 $\pm$ 0.18	2.99 $\pm$ 0.199 $\pm$ 0	0.73 $\pm$ 0.073 $\pm$ 0
Positive control	1.38 $\pm$ 38 $\pm$ 0	3.2121 $\pm$ 021 $\pm$ 0	1.72 $\pm$ 072 $\pm$ 0	1.28 $\pm$ 28 $\pm$ 0
PB-TZD1	1.42 $\pm$ 0.04	3.39 $\pm$ 0.07	1.72 $\pm$ 0.03	1.08 $\pm$ 0.03
PB-TZD3	1.28 $\pm$ 0.05	3.21 $\pm$ 0.05	2.08 $\pm$ 0.03	1.19 $\pm$ 0.04
PB-TZD6	1.36 $\pm$ 0.05	3.29 $\pm$ 0.06	1.94 $\pm$ 0.04	1.07 $\pm$ 0.02

TG: Triglycerides, TC: Total cholesterol, LDL: Low-density lipoprotein, HDL: High-density lipoprotein. Values are expressed as mean $\pm$ standard error of the mean (SEM), $n = 6$ animals per group.

TABLE-14
MEAN AND STANDARD ERROR OF MEAN OF RESULTS OF THE ORAL GLUCOSE
TOLERANCE TEST ON RATS CONDUCTED ON 28th DAYS

Group	0 min	30 min	60 min	90 min	120 min
Normal control	81.50 ± 1.77	88.50 ± 2.26	89.83 ± 1.22	82.67 ± 1.09	77.33 ± 1.23
Diabetic control	340.17 ± 2.55	355.67 ± 3.50	363.00 ± 3.58	353.50 ± 2.86	340.50 ± 2.03
Positive control	122.33 ± 1.94**	150.67 ± 3.14**	178.33 ± 1.41**	163.00 ± 1.59**	147.50 ± 1.59**
PB-TZD1	138.17 ± 2.29*	171.83 ± 1.92*	189.50 ± 1.45*	215.83 ± 2.02**	179.17 ± 2.50*
PB-TZD3	124.50 ± 2.63**	163.67 ± 2.36**	182.83 ± 1.58**	182.67 ± 1.09*	161.00 ± 2.00**
PB-TZD6	131.50 ± 3.16**	165.00 ± 2.50**	185.17 ± 3.39**	204.50 ± 2.26**	175.00 ± 1.98**

Data expressed in mean ± standard mean error of group of six animal. Statically comparisons were made using one-way ANOVA analysis of variance. ** $p < 0.01$ vs. positive control, * $p < 0.05$ vs. PB-TZD1, ** $p < 0.01$ vs. PB-TZD3 and ** $p < 0.01$ vs. PB-TZD. All control and three test compounds groups have adjusted p -values < 0.0001 , so data are statistically highly significant.

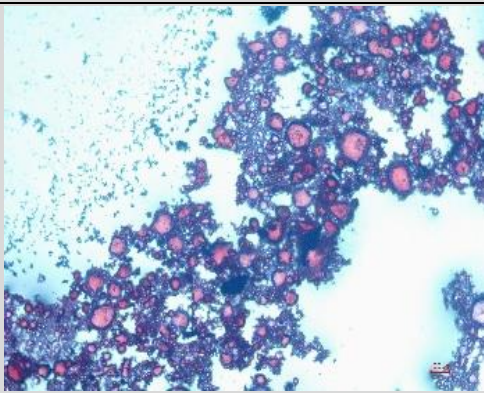
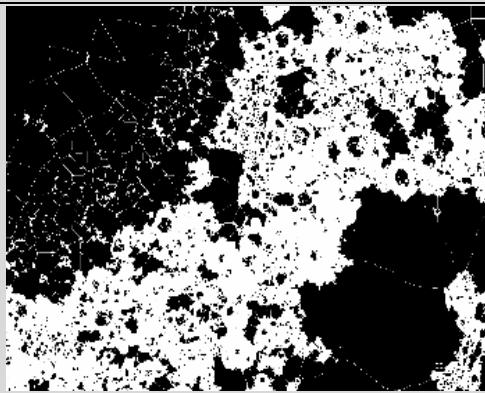
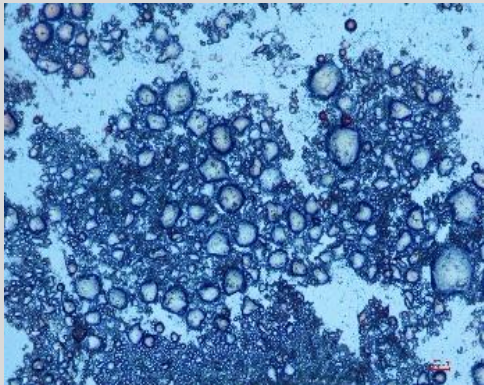
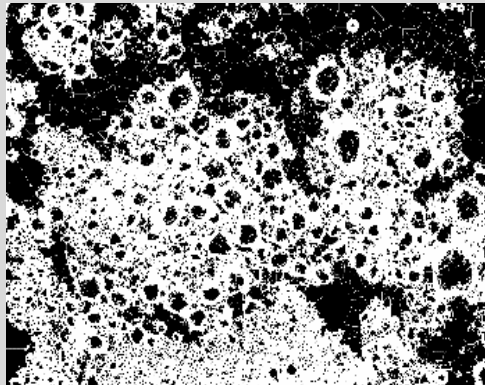
tiation in 3T3-L1 cells. Oil Red O staining was used to assess lipid accumulation, and the results are shown in Table-15. The control group exhibited moderate lipid accumulation with a balanced droplet distribution. Pioglitazone treatment increased the droplet count, with a higher proportion of small lipid droplets, indicating enhanced adipogenic activity and altered droplet morphology.

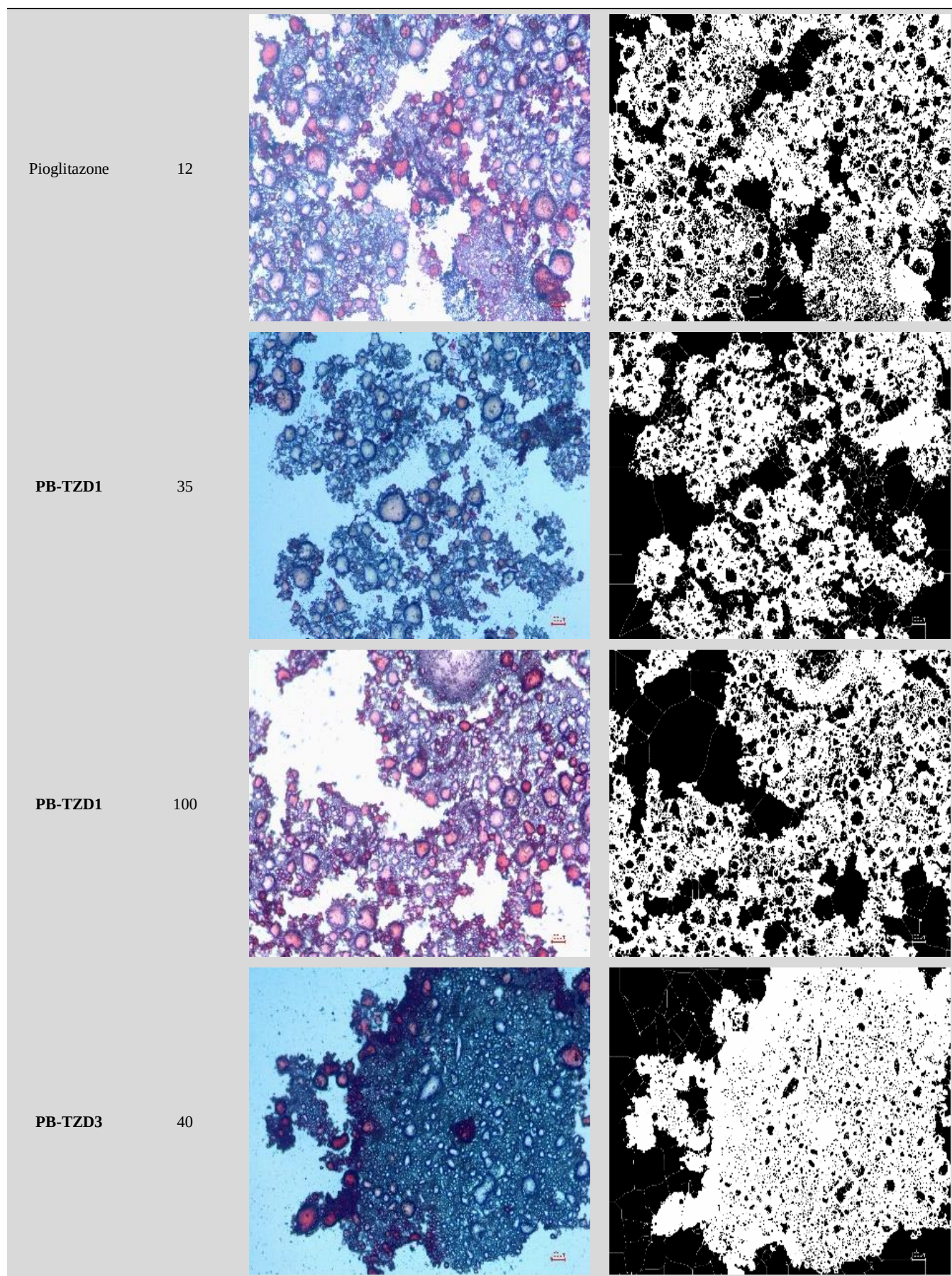
PB-TZD1 exhibit a concentration-dependent response. At the lower concentration, lipid accumulation was comparable to the control, whereas the higher concentration increased droplet count with a predominance of smaller droplets. **PB-TZD3** produced a biphasic response, with reduced lipid accumulation at the lower concentration and superior adipogenesis

at the higher concentration, accompanied by increased lipid coverage and droplet size. Table-16 presents the adipogenic activity of the PB-TZD derivatives based on droplet count, lipid coverage and size distribution. **PB-TZD6** showed a concentration-dependent adipogenic response. The lower concentration was associated with moderate droplet formation, lower lipid coverage, and a predominance of small droplets (80.30%). At the higher concentration, droplet count and lipid coverage increased, with large droplets reaching 26.46%, which may be associated with a more mature adipocyte.

PPAR- γ activity: PPAR- γ activity was significantly higher in all treated groups than in the control group. Pioglitazone exhibited a dose-dependent increase, which is consistent with

TABLE-15
MICROSCOPIC AND IMAGEJ-BASED ANALYSIS OF ADIPOGENESIS ACTIVITY OF
PB-TZD DERIVATIVES IN 3T3-L1 CELLS USING OIL RED O STAINING

Compound	Conc. ($\mu\text{g/mL}$)	Microscopic images	Images processed by ImageJ Software
Control	—		
Pioglitazone	4		



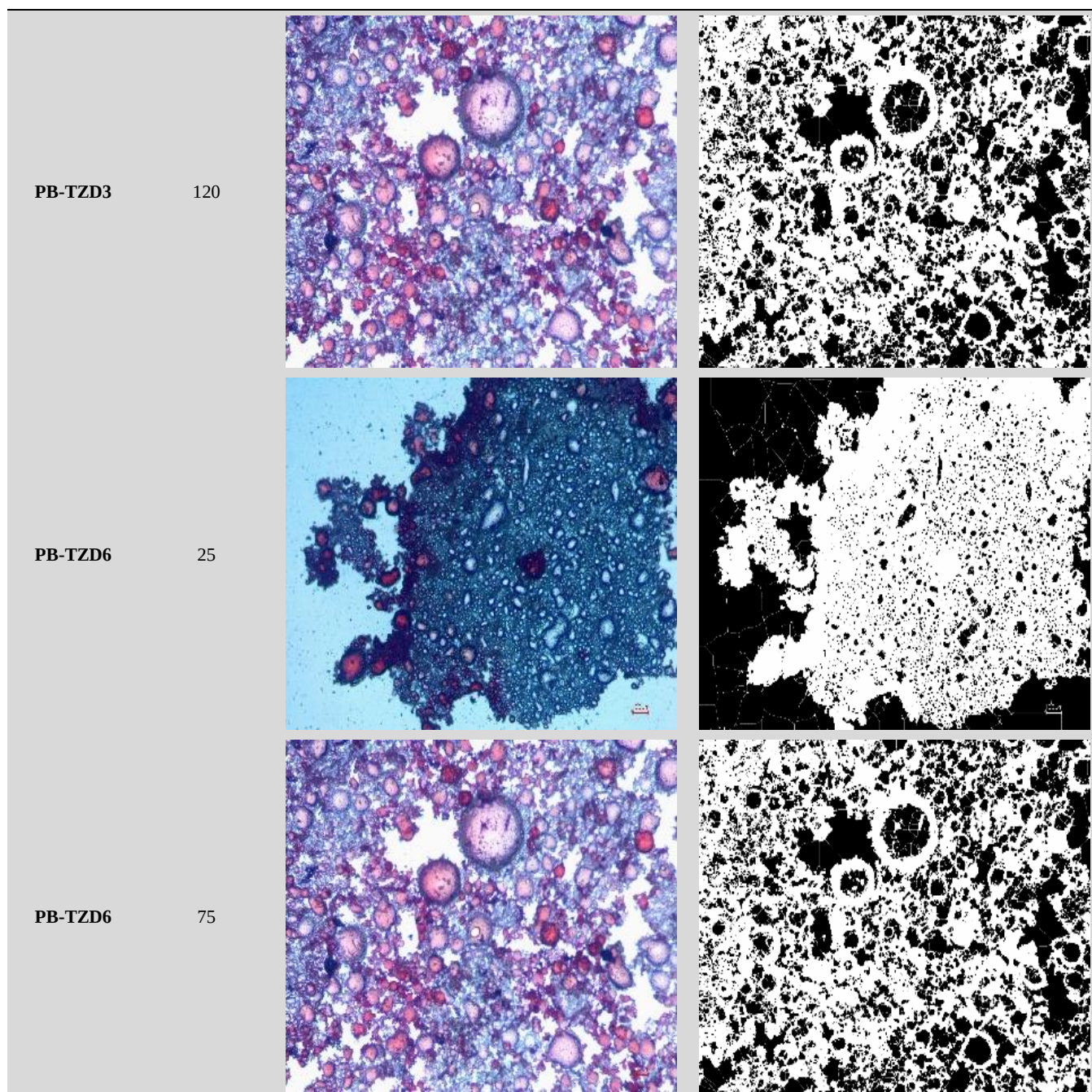


TABLE-16
ADIPOGENESIS ACTIVITY DATA OF PB-TZD DERIVATIVES IN 3T3-L1 CELLS: LIPID DROPLET
COUNT, COVERAGE AND SIZE DISTRIBUTION ANALYSIS USING OIL RED O STAINING

Compound	Conc. ($\mu\text{g/mL}$)	Droplet count	Coverage (%)	Droplet distribution (%)		
				Small (0-120 μm)	Medium (120-240 μm)	Large (>240 μm)
Control	—	2926	58.20	66.47	9.43	24.09
Pioglitazone	4	8874	56.03	75.88	8.54	15.57
Pioglitazone	12	10426	72.18	79.93	8.38	11.68
PB-TZD1	35	3555	56.41	71.42	8.30	20.28
PB-TZD1	100	4908	66.09	87.98	5.64	6.38
PB-TZD3	40	3725	36.55	78.39	8.19	13.42
PB-TZD3	120	5448	63.33	72.67	7.51	19.82
PB-TZD6	30	4441	37.41	80.30	8.60	11.10
PB-TZD6	90	5745	41.98	61.22	12.32	26.46

its established role as a PPAR- γ agonist. **PB-TZD1** and **PB-TZD3** exhibited increased PPAR- γ activity and enhanced adipogenic signalling. **PB-TZD3** at the higher concentration recorded the highest activity among the tested derivatives. The standard calibration curve for PPAR- γ activity was generated by plotting concentration against absorbance at 450 nm (Fig. 6) using the values listed in Table-17. The calibration equation was then used to calculate PPAR- γ activity for the test compounds, with the results presented in Table-18.

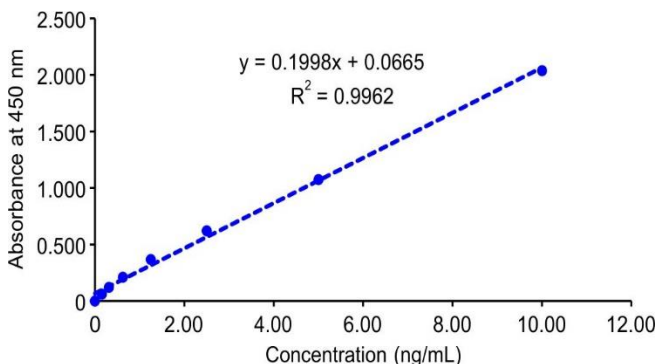


Fig. 6. Standard calibration curve for the prediction of PPAR- γ activity of samples

TABLE-17

ABSORBANCES OF STANDARD CONCENTRATION OF COMMERCIALY AVAILABLE ELISA KIT ACCORDING TO THE MANUFACTURER'S INSTRUCTIONS

Standard conc. (ng/mL)	Absorbance 1	Absorbance 2	Average	Corrected absorbance
10.00	2.154	2.116	2.135	2.039
5.00	1.121	1.221	1.171	1.075
2.50	0.711	0.726	0.719	0.623
1.25	0.465	0.461	0.463	0.367
0.63	0.301	0.313	0.307	0.211
0.31	0.213	0.221	0.217	0.121
0.16	0.15	0.165	0.158	0.062
0.00	0.096	0.096	0.096	0.000

TABLE-18

PPAR- γ ACTIVITY DATA OF STANDARD AND TEST COMPOUNDS USING 3T3-L1 CELL

Compound	Conc. (μ g/mL)	Absorbance	PPAR- γ activity (ng/mL)
Control	—	0.2865	1.10
Pioglitazone	4	0.4954	2.15
Pioglitazone	12	0.5701	2.52
PB-TZD1	35	0.3687	1.51
PB-TZD1	100	0.5509	2.42
PB-TZD3	40	0.3910	1.62
PB-TZD3	120	0.6220	2.78
PB-TZD6	30	0.2959	0.67
PB-TZD6	90	0.3670	1.02

Lipid accumulation and PPAR- γ activity varied across the tested compounds, with differences in adipogenic response and lipid droplet morphology. Pioglitazone and high-dose **PB-TZD3** exhibited pronounced adipogenic characteristics, whereas **PB-TZD1** altered lipid accumulation despite incre-

ased PPAR- γ activity. **PB-TZD6** had lower PPAR- γ activity than the other tested derivatives.

All experiments were performed in triplicate ($n = 3$) and data are expressed as mean $\pm$ standard deviation (SD). Statistical analysis was performed using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test in GraphPad Prism. Differences were considered statistically significant at $p < 0.05$.

Conclusion

A series of novel N-substituted thiazolidinedione derivatives (**PB-TZD1**–**PB-TZD14**) was designed, synthesized and evaluated for antidiabetic activity. N-substitution influenced the biological activity and receptor interactions of the derivatives. **PB-TZD1**, **PB-TZD3** and **PB-TZD6** exhibited higher *in vitro* glucose uptake activity than the reference drug pioglitazone. **PB-TZD3** exhibited the most pronounced glucose lowering activity in the high-fat diet/STZ-induced *in vivo* model. At higher concentrations, **PB-TZD3** enhanced adipocyte differentiation and increased PPAR- γ activity, which provide further evidence of its effect on the PPAR- γ pathway. The synthesized N-substituted TZD derivatives provide a basis for further structural optimization and evaluation of related analogues as potential antidiabetic agents.

ACKNOWLEDGEMENTS

The authors convey their gratitude to Vice-Chancellor of Dr. Babashaeb Ambedkar Technological University, Lonere, India for the financial support for the research work. The authors gratefully acknowledge Assoc. Prof. Santosh Chhajed, MET Institute of Pharmacy, Nashik, for his valuable support in conducting *in vitro* antidiabetic assay. Sincere thanks are also to Prof. Vinod Bairagi, KBHSS Institute of Pharmacy, Malegaon, for his assistance in animal studies.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

DECLARATION OF AI-ASSISTED TECHNOLOGIES

During the preparation of this manuscript, the authors used an AI-assisted tool(s) to improve the language and no data, results or scientific conclusions were generated by AI. All analyses, interpretations and conclusions are the authors' own. The authors reviewed and edited the content and take full responsibility for the published work.

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