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Synthesis, *in vitro* and *in vivo* Antidiabetic Activity of N-Substituted Thiazolidinedione Derivatives

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A series of novel, N-substituted thiazolidinediones (TZD) were synthesized, characterized and evaluated for antidiabetic activity. Of the 22 designed molecules, 12 compounds were successfully synthesized and structurally validated by IR, ^{1}H NMR, ^{13}C NMR and mass spectra. The absence of the N-H peak in the NMR spectrum verified successful condensation at the nitrogen of the thiazolidinedione ring. *In vitro* toxicity was assessed *via* the MTT assay on C2C12 cell lines, with most of compounds exhibiting IC_{50} values above 250 µM/mL, indicating low cytotoxicity. Glucose uptake studies evaluated the efficacy of these derivatives, revealing that compounds **B-TZD-11** and **B-TZD-13** significantly enhanced glucose absorption, comparable to standard antidiabetic agents like pioglitazone. *In vivo* evaluated the anti-hyperglycemic activity of **B-TZD-11** and **B-TZD-13** in high fat diet-streptozotocin-induced diabetic model. Both compounds significantly reduced fasting blood glucose, improved insulin sensitivity (HOMA-IR) and favourably modulated lipid profiles. **B-TZD-13** showed superior efficacy, with the greatest glucose-lowering effect and improved glucose tolerance in oral glucose tolerance test (OGTT). Body weight analysis confirmed weight loss in the diabetic rats, mitigated by treatments. N-substituted thiazolidinedione derivatives bearing lipophilic moieties exhibited promising antidiabetic activity.

Keywords: Thiazolidinedione, Antidiabetic activity, Glucose uptake, Anti-hyperglycemic, Insulin sensitizer.

INTRODUCTION

In recent decades, the worldwide incidence of diabetes has escalated substantially. As of 2022, approximately 830 million people worldwide were affected by the diabetic mellitus, a sharp increase from 200 million in 1990. This rise is particularly evident in countries with limited financial resources, where factors such as urbanization, poor dietary habits and reduced physical activity contribute to the growing numbers. The WHO emphasizes the critical importance of implementing effective strategies for the prevention and control of this worldwide health crisis [1]. Diabetes in older adults is often complicated by a lack of exercise, reduced physical activity and obesity [2]. While the body produces sufficient insulin, it is not utilized effectively due to insulin resistance [3]. Thiazolidinedione derivatives, commonly referred to as glitazone, are a class of drugs designed to improve insulin sensitivity, making them effective in managing diabetes mellitus. These medications, referred to as insulin sensitizers, help the body respond better to insulin [4]. Glitazone is PPAR agonist compounds, two key drugs from this category, rosiglitazone and pioglitazone, were introduced in the U.S. market in 1999 for diabetes treatment. Nevertheless, their wide usage has been restricted because of safety concerns, including potential liver damage and a heightened risk of developing bladder cancer [5-8]. There is a growing global demand for a safer and more effective insulin sensitizer as an antidiabetic treatment. Based on the above rationale, we have designed novel N-substituted thiazolidinedione (TZD) derivatives in the search for new insulin sensitizers aimed at improving the quality of life of diabetic patients.

In our previous work [4], we designed 22 molecules and cond-ucted a comprehensive *in silico* evaluation, including molecular docking studies, ADME analysis, toxicity prediction and assessment of drug-likeness based on Lipinski's rule of 5. Several compounds demonstrated strong interactions with the target receptor and exhibited favourable drug-like properties. Most glitazone-class antidiabetic agents and their derivatives are structurally characterized by an acidic head group, a molecular

linker and an O-substituted lipophilic tail. In contrast, the present study focuses on the rational design and synthesis of N-substituted thiazolidinedione derivatives, wherein the lipophilic moiety is introduced *via* N-substitution instead of the conventional O-linked tail. The synthesized compounds were subsequently evaluated for their antidiabetic potential using both *in vitro* and *in vivo* biological assays.

EXPERIMENTAL

All chemicals were obtained from the Research Lab and Loba Chemie and were purified and dried before use. Aluminum coated silica gel TLC from Merck was used. Infrared spectra were recorded using an Jasco-ATR-FTIR spectrophotometer at the MET Institute of Pharmacy, Nashik, India. 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 at the Central Instrumental Facility, Department of Chemistry, Savitribai Phule Pune University, Pune, India. Cell line C2C12 were procured from NCCS, Pune, India. Cell culture media was DMEM: Glucose from Gibco 11966025. Fetal bovin serum (FBS)-cell culture grade from MP Biomedical System catalog no. 092910154. ELISA Plate reader from Byonoy GmbH (Absorbance 96), Streptozotocin were purchased from SRL and D-Glucose from Dabur. All other chemicals and solvents were of 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. Lipid profile were estimated using a biochemistry analyzer (make: Fujifilm model NH500i).

Thiazolidinedione (1): The synthesis of compound **1** was achieved by mixing monochloroacetic acid solution (8.96 g in 12 mL water) and thiourea solution (8.37 g in 12 mL water) in a 250 mL of beaker, stirred for 15 min at room temperature and then cool the mixture for 10-15 min using ice. A white precipitate was obtained then slowly added 12 mL conc. HCl and refluxed on oil bath for 8-10 h at 100-110 ºC. White needle crystals were obtained when the reaction mixture cool down. Reaction monitor with TLC using mobile phase chloroform: methanol (8:2). Upon completion, filtered the product, washed with cold water, recrystallized from hot water [9]. Yield: 65%; m.p.: 123-127 ºC; R_f: 0.61 [$CHCl_3$:CH_3OH 8:2)]; FTIR (ATR, cm^{-1}): 3380 (N-H), 2950 (alkane), 1750 (C=O), 715 (C-S); ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 4.16 (s, 2H, CH_2), 12.03 (s, 1H, NH); m.f.: $C_3H_3NO_2S$; calcd. mass: 117.92; MS (*m/z*): 119 $[M+H]^+$.

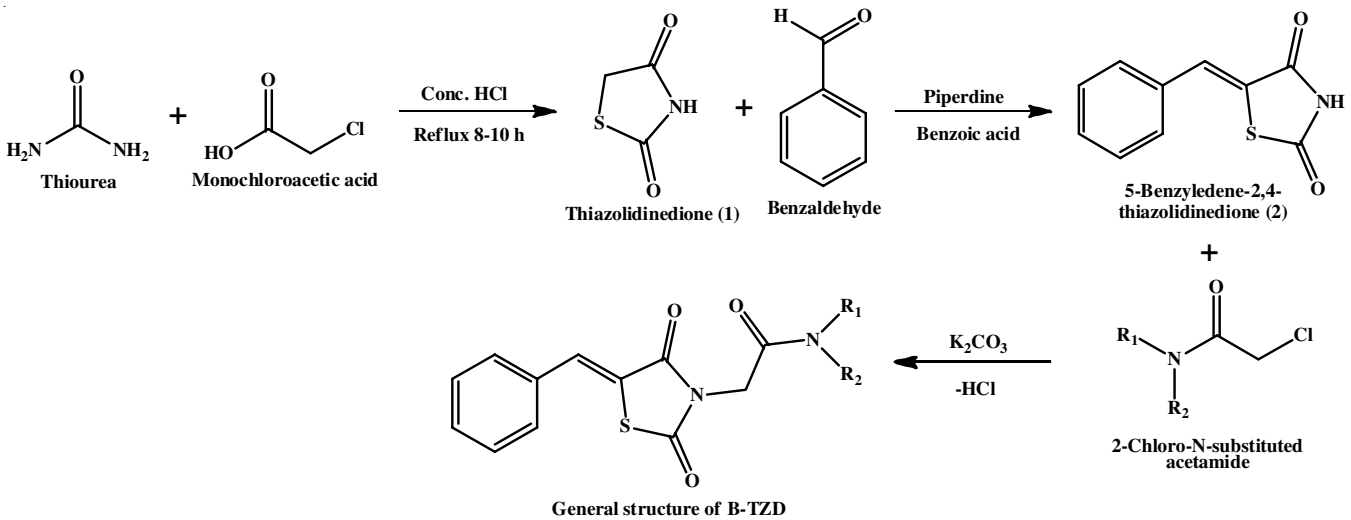
5-Benzylidene-1,3-thiazolidine-2,4-dione (2): Compound **2** was synthesized by adding 2,4-thiazolidinedione (11.71 g, 0.1 mol) and benzaldehyde (8.7 g or 8.3 mL, 0.082 mol) in the flask and shaked for 15 min at normal room temperature. Afterward, 60 mL of toluene, piperidine (2.56 mL or 2.21 g, 0.026 mol) and benzoic acid (2.44 g, 0.02 mol) were added to the reaction flask and subjected to reflux for 5 h. TLC was used to monitor the progress of reaction using chloroform:methanol (8:2) mobile phase. Once the reaction was completed, the white crystalline substance was isolated through filtration and rinsed with methanol and yielding a crude white solid [10,11]. Recrystallization of the crude product using glacial acetic acid and its melting point was determined. Colour: off white solid; yield: 70%; m.p.: 123-124 ºC; R_f: 0.54 [chloroform:methanol (8:2)]; FTIR (ATR, cm^{-1}): 1720 (C=O *str.*), 1592 (aromatic-H *str.*), 1600 (aromatic-H *str.*); ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 7.49 aromatic (t, 1H, CH), 7.53 aromatic (t, 2H, CH), 7.64 aromatic (d, 2H, CH), 7.80 (s, 1H, CH benzylidene), 12.64 (s, 1H, NH); m.f.: $C_{10}H_7NO_2S$; calcd. mass: 205.23; MS (*m/z*): 206 $[M+H]^+$.

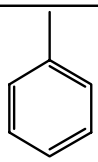
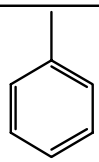
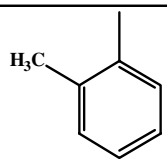
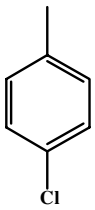
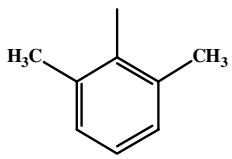
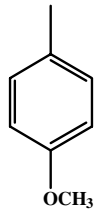
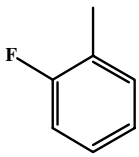
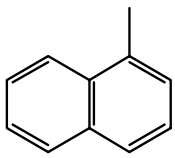
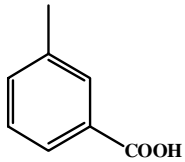
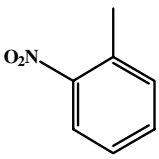
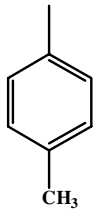
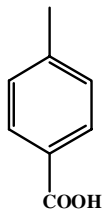
General procedure for 2-[5-benzylidene-2,4-dioxo-1,3-thiazolidin-3-yl]-*N*-phenylacetamide (B-TZD): Benzylidene-thiazolidinedione (**2**, 5 mmol) and 2-chloro-N-substituted-acetamide (5 mmol) was mixed in a iodine flask. Then, DMF (25 mL) as a solvent and 0.829 g (0.006 mol) of anhydrous K_2CO_3 was introduced into flask. The reaction allowed to react for 24 h under continuous stirring using a magnetic stirrer at room environment. The progress of 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 and then further purified through recrystallization [12] (**Scheme-I**).

2-[5-Benzylidene-2,4-dioxo-1,3-thiazolidin-3-yl]-N-(phenyl)acetamide (B-TZD-1): Benzylidene-thiazolidinedione (**2**, 1.02 g, 5.0 mmol) and 2-chloro-N-phenylacetamide chloride (0.676 g, 5.0 mmol) were subjected to the reaction under standard conditions. Colour: pale yellow solid; Yield: 80.51%; m.p.: 242-243 ºC; R_f: 0.45 (chloroform); FTIR (ATR, cm^{-1}): 3310 (N-H *str.*), 3021 (C-H *str.*), 1746 (C=O); ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 4.251 (s, 2H, CH_2 amide), 7.083 (t, 1H, Ar), 7.229 (d, 1H, Ar), 7.313 (t, 2H, Ar), 7.520 (m, 5H, Ar), 7.675 (d, 2H, Ar), 8.006 (s, 1H, CH benzylidene), 10.329 (s, 1H, NH); ^{13}C NMR (500 MHz, DMSO-d_6) δ ppm: 44.10, 114.22, 122.13, 123.71, 125.15, 126.6, 129.14, 132.05, 135.25, 137.32, 143.50, 149.60, 166.77, 168.72; m.f.: $C_{18}H_{14}N_2O_3S$; calcd. mass: 338.39; Mass (*m/z*): 339.06 $[M+H]^+$.

2-[5-Benzylidene-2,4-dioxo-1,3-thiazolidin-3-yl]-N-(4-chlorophenyl)acetamide (B-TZD-3): Benzylidene-thiazolidinedione (**2**, 1.02 g, 5.0 mmol) and 2-chloro-N-(4-chlorophenyl)acetamide (1.02 g, 5.0 mmol) were subjected to the reaction under standard conditions. Colour: pale yellow solid; yield: 54.67%; m.p.: 240-241 ºC; R_f: 0.54 (chloroform); FTIR (ATR, cm^{-1}): 3325.28-3408 (N-H *str.*), 3028-3059 (C-H, alkene), 2949 (C-H, alkane), 1743 (C=O carbonyl), 1543 (Ar ring), 727 (C-Cl); ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 4.529 (s, 2H, CH_2), 7.389 (d, 2H, aryl), 7.587 (m, 5H, Ar), 7.669 (d, 2H, Ar), 8.005 (s, 1H, CH benzylidene), 10.590 (s, 1H, NH); ^{13}C NMR (500 MHz, DMSO-d_6) δ ppm: 44.22, 116.01, 116.78, 119.44, 121.72, 125.16, 126.72, 130.70, 131.75, 132.43, 143.18, 165.74, 167.14, 168.91; m.f.: $C_{18}H_{13}ClN_2O_3S$; calcd. mass: 372.82; Mass (*m/z*): 373.0435 $[M+H]^+$.

2-[5-Benzylidene-2,4-dioxo-1,3-thiazolidin-3-yl]-N-(2-fluorophenyl)acetamide (B-TZD-6): Benzylidene-thiazolidinedione (**2**, 1.02 g, 5.0 mmol) and 2-chloro-N-(2-fluorophenyl)acetamide (0.937 g, 5.0 mmol) were subjected to the reaction under standard conditions. Colour: white solid; yield: 44.35%; m.p.: 293-295 ºC; R_f: 0.63 (chloroform); FTIR (ATR,



Compound	R^1	R^2	Compound	R^1	R^2	Compound	R^1	R^2
B-TZD-1	-H		**B-TZD-10**	$-CH_3$		**B-TZD-15**	-H	
B-TZD-3	-H		**B-TZD-11**	-H		**B-TZD-16**	-H	
B-TZD-6	-H		**B-TZD-13**	-H		**B-TZD-17**	-H	
B-TZD-8	-H		**B-TZD-14**	-H		**B-TZD-18**	-H	

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

cm^{-1}): 3406.29-3263.50 (N-H *str.*), 3061.03(C=C-H), 2924.09 (C-C-H), 1747.51 (NH-C=O), 1548.84 (aryl ring), 729.09 (C-F); ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 4.580 (s, 2H, CH_2-C=O), 6.935 (d, 2H, aryl), 7.159 (m, 3H, Ar), 7.184 (d, 1H, Ar), 7.269 (d, 2H, Ar), 7.892 (s, 1H, CH benzylidene, 10.257(s,1H, NH); ^{13}C NMR (500 MHz, DMSO-d_6) δ ppm: 44.19, 115.79, 116.57, 126.59, 128.22, 128.68, 129.04, 129.37, 131.64, 132.25, 135.28, 138.78, 151.97, 158.77, 159.49, 167.09; m.f.: $C_{18}H_{13}FN_2O_3S$; calcd. mass: 356.37; Mass (*m/z*): 357.07 $[M+H]^+$.

2-[5-Benzylidene-2,4-dioxo-1,3-thiazolidin-3-yl]-N-(2-nitrophenyl)acetamide (B-TZD-8): Benzylidene-thiazolidinedione (**2**, 1.02 g, 5.0 mmol) and 2-chloro-N-(2-nitrophenyl)-acetamide (1.07 g, 5.0 mmol) were subjected to the reaction under standard conditions. Colour: brownish yellow solid; yield: 13.03%; m.p.: 182 ºC; R_f: 0.55 (chloroform); FTIR (ATR, cm^{-1}): 1681 (C=O stretch), 1591 (C=C-H aryl), 1505 (C=C-H aryl); ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 4.565 (s, 2H, CH_2 amide), 7.306 (s, 1H, Ar), 7.537 (m, 1H, Ar), 7.434 (d, 1H, Ar), 7.537 (multiple, 3H, Ar), 7.813 (m, 5H, Ar), 7.982 (m, 1H, CH benzylidene), 10.715 (s, 1H, NH); ^{13}C NMR (500 MHz, DMSO-d_6) δ ppm: 44.15, 113.96, 114.48, 116.56, 123.26, 128.02, 128.27, 129.23, 134.78, 135.74, 135.87, 149.13, 149.70, 166.02, 166.64; m.f.: $C_{18}H_{14}N_2O_3S$; calcd. mass: 338.39; Mass (*m/z*): 384.06 $[M+H]^+$.

2-[5-Benzylidene-2,4-dioxo-1,3-thiazolidin-3-yl]-N-methyl-N-phenylacetamide (B-TZD-10): Benzylidene-thiazo-

lidinedione (**2**, 1.02 g, 5.0 mmol) and 2-chloro-N-methyl-*N*-phenylacetamide (1.07 g, 5.0 mmol) were subjected to the reaction under standard conditions. Colour: white solid; yield: 94.66%; m.p.: 241-242 ºC; R_f: 0.86 (chloroform:methanol 2:1); FTIR (ATR, cm^{-1}): 3057 (C-H *str.*), 1734 (C=O) 1574 and 1509 (aryl ring); ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 4.529 (s, 1H, CH_3 methyl), 7.389 (s, 2H, CH_2 amide), 7.587 (m, 10H, aryl), 8.005 (s, 1H, CH benzylidene); ^{13}C NMR (500 MHz, DMSO-d_6) δ ppm: 22.68, 44.20, 112.92, 115.90, 124.91, 125.25, 125.72, 126.47, 127.29, 132.13, 132.64, 143.38, 155.58, 159.44, 167.02; m.f.: $C_{19}H_{16}N_2O_3S$; calcd. mass: 352.41; Mass (*m/z*): 353.09 $[M+H]^+$.

2-[5-Benzylidene-2,4-dioxo-1,3-thiazolidin-3-yl]-N-2,6-dimethylphenyl)acetamide (B-TZD-11): Benzylidene-thiazolidinedione (**2**, 1.02 g, 5.0 mmol) and 2-chloro-N-(2,6-dimethylphenyl)acetamide (0.98 g, 5.0 mmol) were subjected to the reaction under standard conditions. Colour: white solid; yield: 39.41%; m.p.: 300-302 ºC; R_f: 0.70 (chloroform); FTIR (ATR, cm^{-1}): 3406-3354 (N-H *str.*), 3041 (C=C-H sp^2), 2953 (C-C-H sp^3), 1749.44 (C=O), 1541.12 (aryl ring); ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 2.126 (s, 6H, CH_3), 4.505 (s, 2H, N-CH_2-C=O), 6.980 (m, 2H, aryl), 7.028 (m, 2H, aryl), 7.070 (m, 2H, aryl), 7.142 (m, 2H, aryl), 7.980 (s, 1H, benzylidene), 10.790 (s, 1H, NH); ^{13}C NMR (500 MHz, DMSO-d_6) δ ppm: 18.54, 22.62, 67.37, 116.15, 116.80, 121.96, 126.79, 127.17, 128.16, 128.68, 131.64, 134.80, 135.84, 141.20, 159.82, 166.47, 168.69, 168.82, m.f.: $C_{20}H_{18}N_2O_3S$; calcd. mass: 366.43; Mass (*m/z*): 367.11 $[M+H]^+$.

2-[5-Benzylidene-2,4-dioxo-1,3-thiazolidin-3-yl]-N-(naphthalen-1-yl)acetamide (B-TZD-13): Benzylidene-thiazolidinedione (**2**, 1.02 g, 5.0 mmol) and 2-chloro-N-(naphthalen-1-yl)acetamide (1.098 g, 5.0 mmol) were subjected to the reaction under standard conditions. Colour: grey solid; yield: 86.42%; m.p.: 245-247 ºC; R_f: 0.72 (chloroform); FTIR (ATR, cm^{-1}): 3257-3402 (N-H *str.*), 3053.32 (C=C-H), 2978.09 (C-C-H), 1743.65 (C=O-NH), 1664-1691 (naphthylamine), 1500-1548 (aryl ring); ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 4.718 (s, 2H, CH_2 amide), 7.581 (m, 7H, naphthyline), 7.620 (t, 2H, aryl), 7.779 (t, 2H, aryl), 7.959 (d, 1H, aryl), 8.079 (t, 1H, CH benzylidene), 10.287 (s, 1H, NH), ^{13}C NMR (500 MHz, DMSO-d_6) δ ppm: 67.56, 116.10, 122.57, 122.89, 123.26, 126.02, 126.36, 126.42, 126.59, 126.91, 128.59, 129.37, 131.19, 132.36, 133.25, 134.17, 140.92, 159.84, 167.46, 169.24; m.f.: $C_{22}H_{16}N_2O_3S$; calcd. mass: 388.43; Mass (*m/z*): 389.05 $[M+H]^+$.

2-[5-Benzylidene-2,4-dioxo-1,3-thiazolidin-3-yl]-N-(4-methylphenyl)acetamide (B-TZD-14): Benzylidene-thiazolidinedione (**2**, 1.02 g, 5.0 mmol) and 2-chloro-N-(4-methylphenyl)acetamide (0.918 g, 5.0 mmol) were subjected to the reaction under standard conditions. Colour: off white solid; yield: 33.63%; m.p.: 251-252 ºC; R_f: 0.58 (chloroform); FTIR (ATR, cm^{-1}): 3300 (N-H *str.*), 1745 (C=O *str.*), 1693 (C=C-H aryl), 1638 (C=C-H Ar); ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 2.74 (s, 3H, CH_3), 4.593 (s, 2H, CH_2), 7.55 (d, 2H, Ar), 7.572 (d, 2H, Ar), 7.08(t, 1H, Ar), 7.137 (t, 2H, Ar), 7.26 (d, 1H, Ar), 8.00 (s, 1H, CH benzylidene), 10.307 (s, 1H, NH); ^{13}C NMR (500 MHz, DMSO-d_6) δ ppm: 16.63, 56.20, 114.30, 115.93, 116.08, 121.69, 123.79, 124.15, 125.00, 126.10, 127.25, 132.36, 149.63, 166.98, 167.96; m.f.: $C_{19}H_{16}N_2O_3S$; calcd. mass: 352.40; Mass (*m/z*): 353.09 $[M+H]^+$.

2-[5-Benzylidene-2,4-dioxo-1,3-thiazolidin-3-yl]-N-(2-methylphenyl)acetamide (B-TZD-15): Benzylidene-thiazolidinedione (**2**, 1.02 g, 5.0 mmol) and 2-chloro-N-(2-methylphenyl)acetamide (0.918 g, 5.0 mmol) were subjected to the reaction under standard conditions. Colour: white solid; yield: 32.65%; m.p.: 285 ºC; R_f: 0.44 (chloroform); FTIR (ATR, cm^{-1}): 3408-3267 (N-H *str.*), 3024(C=C-H), 2922 (C-C-H), 1587 (Ar). ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 2.198 (s, 3H, CH_3), 4.535 (s, 2H, CH_2-C=O), 6.742 (d, 2H, Ar), 7.103 (m, 3H, Ar), 7.218(d, 2H, aryl), 7.361 (d, 2H, Ar), 7.892 (d, 1H, Ar), 9.751 (s, 1H, CH benzylidene), 10.398 (s, 1H, NH); ^{13}C NMR (500 MHz, DMSO-d_6) δ ppm:14.67, 44.24, 94.36, 114.31, 119.25, 122.24, 123.72, 125.10, 127.29, 130.76, 131.91, 143.21, 149.66, 154.66, 165.74, 167.19; m.f.: $C_{19}H_{16}N_2O_3S$; calcd. mass: 352.40; Mass (*m/z*): 353.07 $[M+H]^+$.

2-[5-Benzylidene-2,4-dioxo-1,3-thiazolidin-3-yl]-N-(4-methoxyphenyl)acetamide (B-TZD-16): Benzylidene-thiazolidinedione (**2**, 1.02 g, 5.0 mmol) and 2-chloro-N-(4-methoxyphenyl)acetamide (0.998 g, 5.0 mmol) were subjected to the reaction under standard conditions. Colour: grey solid; yield: 41.23%; m.p.: 261-263 ºC; R_f: 0.61 (chloroform); FTIR (ATR, cm^{-1}): 3134-3282 (N-H *str.*), 3062 (C=C-H), 2933 (C-C-H), 2837.29 (C-H of CH_3), 1745 (C=O-NH), 1548 (aryl ring). ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 3.324 (s, 3H, O-CH_3), 4.489 (s, 2H, CH_2 amide), 6.995 (s, 1H, Ar), 7.117 (m, 1H, Ar), 7.356 (m, 1H, Ar), 7.505 (m, 4H, Ar), 7.680 (d, 1H, Ar), 7.734 (d, 1H, Ar), 8.024 (d, 1H, CH benzylidene), 10.250 (s, 1H, NH); ^{13}C NMR (500 MHz, DMSO-d_6) δ ppm: 44.14, 56.17, 114.11, 114.35, 123.62, 124.55, 127.05, 127.57, 131.36, 133.41, 134.80, 149.76, 156.34, 159.46, 166.48; m.f.: $C_{19}H_{16}N_2O_4S$; calcd. mass: 368.40; Mass (*m/z*): 369.09 $[M+H]^+$.

3-[2-[5-Benzylidene-2,4-dioxo-1,3-thiazolidin-3-yl]-acetamido]benzoic acid (B-TZD-17): Benzylidene-thiazolidinedione (**2**, 1.02 g, 5.0 mmol) and 3-(2-chloroacetamido)-benzoic acid (1.068 g, 5.0 mmol) were subjected to the reaction under standard conditions. Colour: white solid; yield: 89.89%; m.p.: 222-224 ºC; R_f: 0.41 (chloroform); FTIR (ATR, cm^{-1}): 3562 (OH carboxylic group), 3414-3462 (N-H *str.*), 3039 (C=C-H), 2954 (C-H, alkane), 1787 (C=O), 1556-1597 (aryl ring); ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 4.942 (d, 1H, Ar), 7.475 (d, 1H, Ar), 7.555 (m, 3H, Ar), 7.59 (t, 2H, Ar), 7.670 (t, 1H, Ar), 7.739 (t, 1H, Ar), 7.80 (s, 1H, CH benzylidene), 10.62 (s, 1H, NH), 12.65 [s, 1H, OH(COOH)]; ^{13}C NMR (500 MHz, DMSO-d_6) δ ppm: 44.13, 99.87, 114.23, 115.75, 115.93, 121.71, 122.57, 123.70, 127.37, 131.67, 135.28, 149.62, 149.65, 157.72, 159.63, 166.52, 169.05; m.f.: $C_{19}H_{14}N_2O_5S$: calcd. mass: 382.38; Mass (*m/z*): 383.07 $[M+H]^+$.

4-[2-[5-Benzylidene-2,4-dioxo-1,3-thiazolidin-3-yl] acetamido]benzoic acid (B-TZD-18): Benzylidene-thiazolidinedione (**2**, 1.02 g, 5.0 mmol) and 3-(2-chloroacetamido)-benzoic acid (1.068 g, 5.0 mmol) were subjected to the reaction under standard conditions. Colour: white solid; yield: 49.21%; m.p.: 252 ºC; R_f: 0.55 (chloroform); FTIR (ATR, cm^{-1}): 3292-3323 (N-H *str.*), 3035 (C=C-H), 1741 (C=O-NH), 3597 (OH-COOH), 1548 (aromatic ring); ^{1}H NMR (500 MHz, DMSO-

d_6) δ ppm: 4.959 (s, 2H, CH_2 amide), 7.499 (t, 2H, Aryl -phenyl), 7.577 (m, 4H, Aryl-benzoic acid), 7.07 (m, 1H, aryl), 7.801 (s, 2H, aryl), 7.914 (s, 1H, aryl), 8.024 (s, 1H, CH benzylidene), 10.664 (s, 1H, NH), 12.63(s,1H, COOH); ^{13}C NMR (500 MHz, DMSO-d_6) δ ppm: 44.10, 106.67, 114.17, 122.13, 123.71, 125.15, 126.13, 126.60, 127.33, 129.14, 132.05, 135.25, 137.32, 149.60, 166.77, 168.72. m.f.: $C_{19}H_{14}N_2O_5S$; calcd. mass: 382.38; Mass (*m/z*): 383.068 $[M+H]^+$.

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 ºC in a 5% CO_2 environment for 24 h to facilitate the cell adhesion. Following incubation, the culture medium was refreshed with DMEM included the test compounds at different concentrations (50, 100, 150, 250, 350, 450 and 550 µM) and the cells were incubated for further 24 h. After 24 h medium was carefully withdrawn from the well and 20 µL of 500 mg/100 mL.

The MTT solution, prepared in phosphate-buffered saline, was added to each well. Then, the 96-well plate was incubated at 37 ºC for 4 h, allowing living cells to transform MTT into insoluble formazan crystals. After incubation, the MTT reagent was carefully withdrawn and added 100 µL of DMSO to each well. The ELISA plate was thoroughly shaken for 10-15 min at room temperature to dissolve completely the formazan crystals. The absorbance of plate measure at 570 nm using ELISA plate reader. The assay was conducted in triplicate, with each concentration tested three times. Cell viability was determined relative to the untreated control by applying the following formula [13,14]:

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

Glucose uptake assay: The increase in glucose uptake was assessed using C2C12 cell lines (mouse myoblasts). These cells were grown in a 5% CO_2 incubator and maintained in DMEM supplemented with 10% FBS. For cell attachment, they were seeded into a 96-well plate at a density of 1×10^5 cells/mL and incubated at 37 ºC for 24 h in low-glucose DMEM.

After this initial incubation, test samples at a concentration of 100 µM, along with a reference compound at 50 µM, were introduced into the wells, followed by further incubation at 37 ºC for 24-48 h. Once the incubation period ended, the existing culture medium was removed and replaced with fresh incubation medium comprising DMEM with 0.1% BSA, 15 mM glucose and PBS. The cells were then incubated again at 37 ºC for an additional 2 h. Subsequently, the incubation medium was transferred to a new 96-well plate and the glucose concentration in the medium was measured. Cells not treated with any compounds served as a negative control, whereas pioglitazone and insulin were used as positive control. 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 [15].

Anti-hyperglycemic activity: High fat diet streptozotocin (STZ) induced Wistar albino rat model use for evaluating the anti-hyperglycemic activity. The experimental conducted as per the procedure described in literature [16].

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 ºC in animal house facility of KBH Institute of Pharmacy Malegaon, India.

Animal ethics: The study received ethical clearance from the Institutional Animal Ethics Committee (IAEC) under approval number 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 h fasting period prior to streptozotocin (STZ) administration. Streptozotocin 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 the further animal experiment. Rats continued with HFD for complete antidiabetic study period.

Anti-hyperglycemic study design: The animals were divided into five groups, normal control (negative control), diabetic control, standard control (positive control), derivative 1 (**B-TZD-11**) and derivatives 2 (**B-TZD-13**). Each group was allocated 6 male albino Wistar rats for the study. The normal control group received a standard laboratory diet and water for 4 weeks without any treatment. The diabetic control group was fed a high fat diet (HFD) for four weeks, followed by an intraperitoneal injection of STZ at a dose of 50 mg/kg to induce diabetes, with no further treatment. The positive control group underwent the same HFD and STZ protocol, followed by oral administration of pioglitazone at 20 mg/kg/day for 4 weeks as a reference drug. The **B-TZD-11** group received HFD for 4 weeks and STZ injection (50 mg/kg), then was treated orally with the test compound **B-TZD-11** at a dose of 20 mg/kg/day for another 4 weeks. In a similar manner, the **B-TZD-13** group underwent the same high-fat diet and STZ induction process, followed by oral administration of the **B-TZD-13** compound at a dosage of 20 mg/kg/day for 4 weeks to evaluate its effectiveness in combating diabetes.

The body weight of each animal was measured weekly. Further water and food consumption recorded daily for each group and averaged weekly. The oral glucose tolerance test (OGTT) was performed on day 28th of the study for all animals in each group. Blood of animal was withdrawn on the 29th day for the end point biochemical assay of total cholesterol, triglyceride, LDL and HDL.

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 g/kg 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 29, 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 following equation:

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

RESULTS AND DISCUSSION

The title compounds were synthesized according to the **Scheme-I**. The precursors, 2,4-thiazolidinedione (**1**) and benzylidene-2,4-thiazolidinedione (**2**) were synthesized by the reported procedure with the 65% and 75% yield, respectively. The synthesis of the final design targeted molecules by the condensation reaction of benzylidene-2,4-thiazolidinedione (**2**) and substituted 2-chloro-N-substituted acetamide chloride in DMF in presence of K_2CO_3 solution. The alkylation of thiazolidinedione at the nitrogen atom in the presence of a base has been previously reported [15]. However, when NaOH was used in place of K_2CO_3, the reaction did not proceed to completion and the desired product was not obtained. The crude product was purified by recrystallization in glacial acetic acid and form crystal of product. The absence of a singlet N-H peak at δ 12.62 ppm in the NMR spectrum confirms the condensation of 2-chloro-N-phenylacetamide chloride at the nitrogen atom of thiazolidinedione. The IR spectra show band between 3100 and 3000 cm^{-1} confirmation of presence of H-C=C at C-5. Also, we confirmed from the mass spectra that the molecular ion peak M^++H presence of targeted molecules. Out of the 22 target molecules, we obtained 12 molecules in purified form by recrystallization.

Toxicity studies: In order to validation of safety of synthesized N-substituted thiazolidinedione (TZD) as hypoglycemic agents, evaluated the preliminary *in vitro* toxicity by MTT assay. The IC_{50} value indicates the toxicity profile of synthesized compounds is the half maximum toxic concentration compounds was determined by MTT assay on C2C12 cell line. The cytotoxicity of the synthesized compounds was found to depend on the concentration because only metabolically active cells can reduce tetrazolium salts, while this process does not occur in non-living cells [14].

Most of the compounds did not show any inhabitation at 50 μM/mL and slight inhibition at 100 μM/mL. Most of the compounds have inhibitory concentration (IC_{50}) above 250 μM/mL in MTT assay. In Table-1, the IC_{50} of N-substituted thiazolidinediones was calculated from the triplicate readings of each derivative. According to results of toxicity study, we chosen 100 μM/mL conc. for further glucose uptake assay.

TABLE-1
IC_{50} VALUE CALCULATED FROM THE TRIPLICATE READING OF MTT ASSAY USING THE C2C12 CELL LINE

Compound	IC_{50} (μM)	Compound	IC_{50} (μM)
B-TZD-1	320	**B-TZD-14**	204
B-TZD-3	238	**B-TZD-15**	307
B-TZD-6	313	**B-TZD-16**	227
B-TZD-8	459	**B-TZD-17**	271
B-TZD-10	330	**B-TZD-18**	287
B-TZD-11	295	Pioglitazone	282
B-TZD-13	336	–	–

Glucose uptake assay in C2C12 cells: The glucose uptake test was performed on the C2C12 cell lines revealed that the PI3K-Akt signaling pathway is the main route for glucose absorption, while AMPK activation significantly contributes to the movement of GLUT4 to the cell membrane. The assay results revealed variability in glucose uptake across the **B-TZD** series. Compounds **B-TZD-1**, **B-TZD-3**, **B-TZD-6**, **B-TZD-8**, **B-TZD-10**, **B-TZD-16** and **B-TZD-17** exhibited the minimal glucose absorption, suggesting the limited efficacy in enhancing the glucose uptake. Conversely, **B-TZD-14**, **B-TZD-15** and **B-TZD-18** displayed moderate glucose absorption whereas **B-TZD-11** and **B-TZD-13** exhibited glucose uptake levels comparable to current antidiabetic drugs, suggesting their potential as efficacious glucose absorption enhancers.

Among the synthesized compounds, **B-TZD-11** and **B-TZD-13** exhibited the highest docking scores, indicate strong binding affinity. Structural analysis indicated that **B-TZD-11** possesses a 2,6-dimethyl phenyl substitution, whereas **B-TZD-13** features a naphthalene substitution at the nitrogen of the thiazolidinedione moiety. The presence of these hydrophobic groups may contribute to the enhanced receptor interaction and improved biological activity. Typically, adipose cells that do not efficiently absorb glucose exhibit 4-5 nuclei per cell. However, treatment with **B-TZD-11** and **B-TZD-13** resulted in the formation of 10-11 nuclei per cell, as observed in Fig. 1, further supporting their efficacy in glucose metabolism. The overall results of the glucose uptake assay present in Table-2.

***In vivo* anti-hyperglycemic activity:** The activity performs on Wistar male rats divided into five groups and each group have six animals for the study. In first four weeks, the animal were fed a high fat diet for to increase the weight and insulin resistance. Table-3 indicated that the high fat diet induced significant weight gain in all groups except the normal control.

The body weight of rats during the treatment phase varies in different groups. The results indicate that while all diabetic groups experienced weight loss due to HFD and STZ induced diabetes, treatment with pioglitazone and compounds **B-TZD-11** and **B-TZD-13** mitigated this effect to varying levels (Table-4). Notably, compounds **B-TZD-13** demonstrated the most significant protective effect, warranting further investigation into its therapeutic potential.

Biochemical parameters

Fasting blood glucose: Normal control group maintained the stable glucose levels, indicating no diabetic induction. The diabetic control group showed a progressive increase in glucose

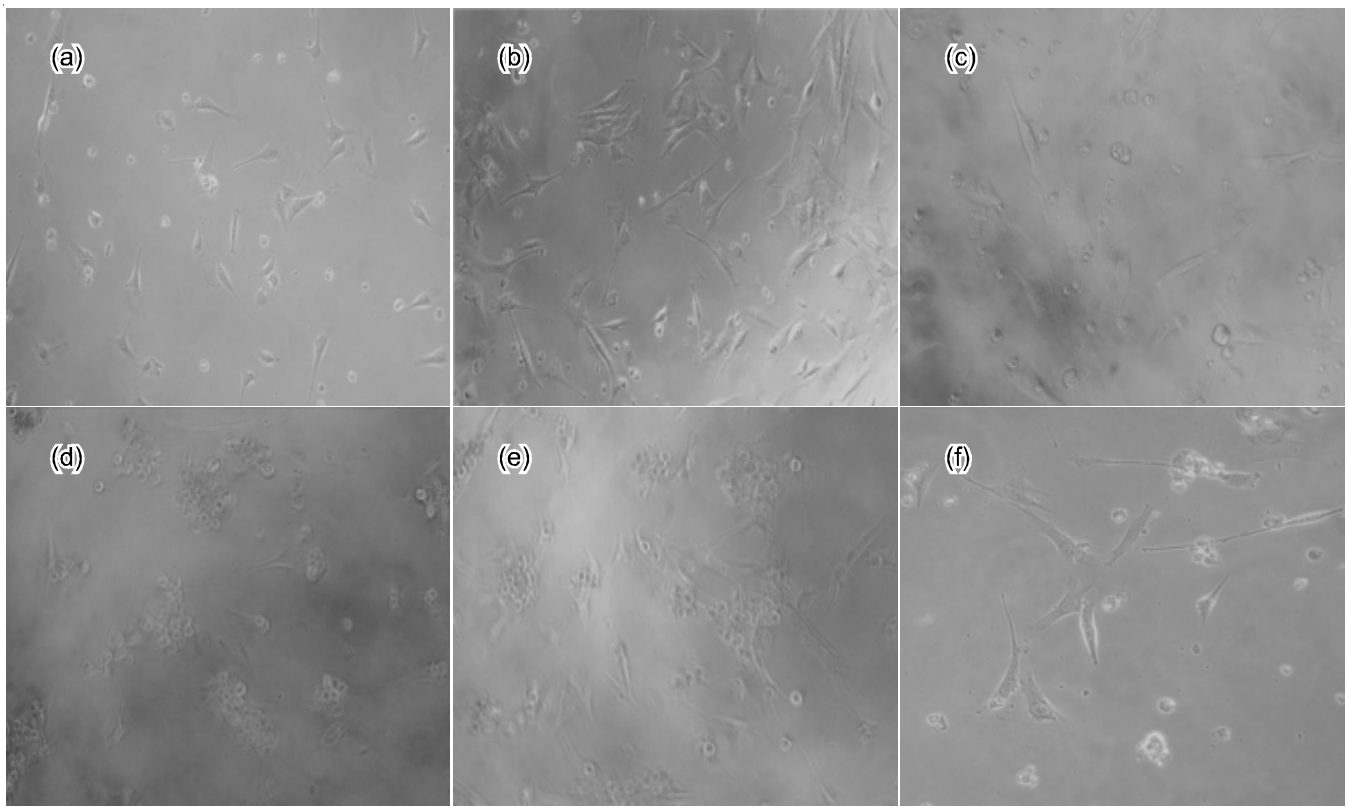


Fig. 1. (a) Negative control, cell is constricted due to serum withdrawal, (b) Cell with Pioglitazone show multinuclear cell, (c) Cell with B-TZD-16 show less cell active and (d) Cell with B-TZD-11 more multinuclear cell. (e) Cell with B-TZD-13 and (f) cell with insulin showing multinuclear active cells

TABLE-2
GLUCOSE UPTAKE ASSAY RESULTS FOR THE **B-TZD** SERIES OF COMPOUNDS WERE EVALUATED USING C2C12 CELL LINES (MOUSE MYOBLAST CELL LINE), WITH ALL ASSAYS CONDUCTED IN TRIPLICATE

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
B-TZD-1	577.333	3.0550	4.78
B-TZD-3	605.333	2.5167	0.16
B-TZD-6	557.666	1.5300	8.02
B-TZD-8	592.333	3.0550	2.30
B-TZD-10	589.000	2.0000	2.94
B-TZD-11	501.667	2.0867	17.26
B-TZD-13	485.000	2.0000	20.01
B-TZD-14	539.333	2.0800	14.34
B-TZD-15	545.333	1.5300	13.39
B-TZD-16	599.333	1.5275	1.15
B-TZD-17	596.667	1.5275	1.59
B-TZD-18	545.000	2.0000	13.44

levels, confirming sustained hyperglycemia. Positive control and test compound groups exhibited a significant reduction in glucose levels over time, suggesting the efficacy of the treatments in managing hyperglycemia. Compound **B-TZD-11** demonstrated a progressive and significant reduction in fasting blood glucose levels over the 28-day period. Starting from an initial level of approximately 279.33 mg/dL, the glucose levels decreased to 139.17 mg/dL by day 28. This indicates that compound **B-TZD-11** has a potent anti-hyperglycemic effect in the diabetic rat model, whereas compound **B-TZD-13** also exhibited a significant and consistent decrease in fasting blood glucose levels throughout the study. From an initial level of approximately 284.50 mg/dL, the levels reduced to 132.33 mg/dL by day 28. This indicate that compound **B-TZD-13** is effective in lowering the blood glucose levels in the diabetic rat model. The results of fasting blood glucose during the treatment phase are given in Table-5.

Insulin, glucose level and HOMA-IR: Biochemical analysis of blood at the end of study was carried out and it found that normal insulin level is 15 μU/mL, normal mean glucose level is 79.5 and HOMA-IR is 2.94. Compound **B-TZD-11** is the modest improvement in insulin level (9.3), while compound **B-TZD-13** shows a stronger effect in increasing insulin levels than compound **B-TZD-11**, implying better support of β-cell function or insulin release. Both the experimental compounds reduce blood glucose, but compound **B-TZD-13** is more effective and comparable to the standard treatment. Compounds **B-TZD-11** and **B-TZD-13** both improve insulin sensitivity, with compound **B-TZD-11** having a slightly better HOMA-IR, despite its lower effect on glucose and insulin. This could suggest a more targeted action on insulin signaling rather than insulin production. The mean values of insulin, glucose and

TABLE-3
PERCENTAGE OF BODY WEIGHT VARIANCE OF RATS DURING THE HFD INDUCTION PHASE (WEEK 0 TO WEEK 4)

Animal group	Body weight during rats on HFD (g) Mean ± SEM					% Increase in body weight
	On day 0	On day 7	On day 14	On day 21	On Day 28	
Normal control (standard diet, vehicle)	161.00±2.52	161.67±2.56	163.83±2.63	164.33±2.56	165.00±2.72	2.48
Diabetic control (HFD + STZ, vehicle)	167.00±3.48*	176.67±2.58*	185.50±2.64*	194.50±1.86*	202.67±1.65*	21.39
Positive control (HFD + STZ + pioglitazone 20 mg/kg)	165.00±2.54*	173.50±2.31*	181.83±2.33**	190.67±2.19*	199.17±2.06*	20.71
B-TZD-11 (HFD + STZ + **B-TZD-11** 20 mg/Kg)	171.50±2.28*	178.67±2.17*	187.17±2.01*	196.17±1.64*	204.67±1.61*	19.35
B-TZD-13 (HFD + STZ + **B-TZD-11** 20 mg/Kg)	174.33±1.12*	182.17±1.01*	191.17±0.98*	199.50±1.23*	207.67±1.12*	19.13

Data expressed in mean ± standard mean error, 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.* **B-TZD-11**, *$p < 0.05$ *vs.* **B-TZD-13**. Data are statically significant.

TABLE-4
PERCENTAGE OF BODY WEIGHT VARIANCE OF RATS DURING THE DIABETES INDUCTION AND TREATMENT PHASE (WEEK 5 TO WEEK 8)

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 (standard diet, vehicle)	165.00±2.52	165.67±2.56	168.17±2.63	168.33±2.56	167.00±2.72	1.21%
Diabetic control (HFD + STZ, vehicle)	202.67±3.48*	187.67±2.58*	178.33±2.64*	172.50±1.86*	168.17±1.65*	-17.03%
Positive control (HFD + STZ + pioglitazone 20 mg/kg)	199.17±2.54*	181.5±2.31**	173.17±2.33*	163.50±2.19*	173.17±2.06*	-13.05%
B-TZD-11 (HFD + STZ + **B-TZD-11** 20 mg/Kg)	204.67±2.28*	181.83±2.17*	174.50±2.01*	172.33±1.64*	175.67±1.61*	-14.18%
B-TZD-13 (HFD + STZ + **B-TZD-11** 20 mg/Kg)	207.67±1.12*	190.00±1.01*	181.50±0.98*	178.33±1.23*	182.33±1.12*	-12.21%

Data expressed in mean± standard mean error, HFD = high fat diet, STZ = streptozotocin. Statically 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.* **B-TZD-11**, *$p < 0.05$ *vs.* **B-TZD-13**. Data are statically significant.

TABLE-5
FASTING BLOOD GLUCOSE (FBG) DURING THE TREATMENT PHASE (5 TO 8 WEEK)

Group	Fasting blood glucose (mg/dL) during diabetic study period (Means ± SEM)				
	Day 0	Day 7	Day 14	Day 21	Day 28
Normal control	82.17 ± 2.57	83.50 ± 2.41	84.00 ± 1.84	82.83 ± 2.29	81.50 ± 1.76
Diabetic control	278.83 ± 4.66	301.33 ± 4.13	317.17 ± 3.77	329.67 ± 3.55	340.17 ± 2.44
Positive control	279.50 ± 4.15**	237.83 ± 1.65**	204.67 ± 2.02**	163.50 ± 2.19**	122.33 ± 1.67**
B-TZD-11	279.33 ± 4.52**	241.83 ± 3.94**	209.67 ± 3.38**	173.67 ± 3.99**	139.17 ± 3.38**
B-TZD-13	284.50 ± 2.54**	242.00 ± 2.31**	210.00 ± 2.33**	171.17 ± 2.19**	132.33 ± 2.06**

Data expressed in mean ± standard mean error. Statically comparisons were made using one-way ANOVA analysis of variance. ** $p < 0.01$ *vs.* positive control. **$p < 0.01$ *vs.* **B-TZD-11**. **$p < 0.01$ *vs.* **B-TZD-13**. All three, std and two test compounds have adjusted *p*-values < 0.01, so data are statistically highly significant.

calculated MOMA-IR at the 29th day of treatment phase are present in Table-6.

Lipid profile: Lipid profile was evaluated of all animals at the end of the treatment phase and the diabetic control group exhibited significantly elevated levels of triglycerides (TG), total cholesterol (TC) and low-density lipoprotein (LDL), along with reduced high-density lipoprotein (HDL) levels, indicating a dyslipidaemia profile. The results of lipid profile are present in Table-7. Both compounds **B-TZD-11** and **B-TZD-13** improved the lipid profiles of the subjects with the diabetic control

TABLE-6
MEAN VALUE OF INSULIN, GLUCOSE AND HOMA-IR AT THE 29th DAY OF THE TREATMENT PHASE IN RATS BLOOD PLASMA

Group	Mean insulin (μU/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
B-TZD-11	9.33	0.33	137.17	3.40	3.17	0.16
B-TZD-13	11.83	0.31	130.00	2.88	3.79	0.07

TABLE-7
EFFECT OF TREATMENTS ON SERUM LIPID PROFILE PARAMETERS IN THE EXPERIMENTAL GROUPS (MEAN ± SEM)

Group	TG (Mean ± SEM)	TC (Mean ± SEM)	LDL (Mean ± SEM)	HDL (Mean ± SEM)
Normal control	1.17 ± 0.05	2.51 ± 0.06	1.34 ± 0.08	1.43 ± 0.08
Diabetic control	1.87 ± 0.05	4.44 ± 0.18	2.99 ± 0.199 ± 0	0.73 ± 073 ± 0
Positive control	1.38 ± 38 ± 0	3.2121 ± 021 ± 0	1.72 ± 072 ± 0	1.28 ± 28 ± 0
B-TZD-11	1.5050 ± 050 ± 0	3.41 ± 041 ± 0	2.11 ± 11 ± 0	1.0808 ± 008 ± 0
B-TZD-13	1.33 ± 033 ± 0	3.20 ± 20 ± 0	1.7373 ± 05	1.29 ± 0.04

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

group. However, test drug 2 exhibited a more favourable effect, particularly in increasing HDL and reducing LDL levels are considered critical factors in cardiovascular risk management.

Oral glucose tolerance test (OGTT): The OGTT was conducted on day 28th of treatment to assay the long-term effect of test compounds on glucose tolerance. The normal control group demonstrated stable glucose levels with minimal fluctuations indicating the normal glucose metabolism. In contrast, the diabetic control group exhibited significantly elevated glucose levels at all time points, reflecting impaired glucose tolerance. After administering glucose, the glucose levels of positive control group increased somewhat indicated some improvement in glucose handling. Compound **B-TZD-11** group displayed a progressive increase in glucose levels, peaking at 90 min, which indicates delayed glucose clearance. Similarly, compound **B-TZD-13** group followed the same trend as compound **B-TZD-11** but with slightly lower glucose levels (Table-8). This suggests that compound **B-TZD-13** may be more effective in enhancing glucose metabolism.

Statistical analysis: Using One-Way ANOVA statistical analysis, a highly significant difference among the groups ($F(4,25) = 1287$, $p < 0.0001$) is revealed, which indicated that the treatment effects on blood glucose levels are statistically significant and not due to chance. This confirms the efficacy of the test compounds in managing hyperglycemia.

TABLE-8
RESULTS OF THE ORAL GLUCOSE TOLERANCE TEST ON RATS CONDUCTED ON 28th DAYS

Groups	No. of animals	Oral glucose tolerance test				
		0 min	30 min	60 min	90 min	120 min
Normal control	1	76	80	84	78	75
	2	80	87	90	82	73
	3	79	87	92	85	78
	4	81	88	91	84	80
	5	85	93	92	85	81
	6	88	96	90	82	77
Diabetic control	1	341	352	358	345	332
	2	344	355	360	362	344
	3	336	349	358	349	338
	4	331	347	355	348	340
	5	349	370	378	358	345
	6	340	361	369	359	344
Positive control	1	125	161	184	164	148
	2	121	156	175	162	144
	3	128	154	179	170	152
	4	118	147	180	163	148
	5	116	140	175	159	142
	6	126	146	177	160	151
B-TZD-11	1	148	176	192	218	188
	2	141	172	188	212	180
	3	138	176	182	208	192
	4	149	168	184	216	178
	5	130	164	178	221	184
	6	129	170	180	218	186
B-TZD-13	1	137	168	190	179	165
	2	133	164	188	175	162
	3	122	159	174	176	160
	4	145	162	173	178	164
	5	121	156	174	180	166
	6	136	158	176	176	168

Conclusion

In this study, few N-substituted thiazolidinedione (TZD) derivatives were designed, synthesized and assessed for their potential antidiabetic activity. N-substituted thiazolidinedione derivatives bearing lipophilic moieties exhibited the promising antidiabetic activity. *In vitro* toxicity studies using the MTT assay on C2C12 cell lines revealed that mostly all compounds exhibited low cytotoxicity, with IC_{50} values above 250 μM/mL, indicating a favourable safety profile. Glucose uptake assays demonstrated that compounds **B-TZD-11** and **B-TZD-13** significantly enhanced glucose absorption, showing activity comparable to that of standard drug, pioglitazone. Compound **B-TZD-13** demonstrated significant anti-hyperglycemic and lipid-lowering effects in diabetic rats, showing improved glucose control, insulin sensitivity and lipid profile compared to compound **B-TZD-11**. Its performance was also comparable to that of the standard drug pioglitazone, indicating strong potential as a therapeutic agent for type 2 diabetes. Overall, compounds **B-TZD-11** and **B-TZD-13** emerged as the most promising candidates with potent glucose uptake activity and low cytotoxicity, highlighting their potential as lead compounds for further development as insulin sensitizers for the management of diabetes mellitus.

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CONFLICT OF INTEREST

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

REFERENCES

1. B. Zhou, A.W. Rayner, E.W. Gregg, *et al.*, *Lancet*, **404**, 2077 (2024); https://doi.org/10.1016/S0140-6736(24)02317-1
2. D Kawade, N Jain, V Jadhav, *The Pharma Innov. J.*, **6**, 390 (2017).
3. M.U. Reddy and M.C.S. Reddy, *Asian J. Chem.*, **30**, 1231 (2018); https://doi.org/10.14233/ajchem.2018.21168
4. U. Jain, N.P. Jain and S. Amrutkar, *Int. J. Pharmaceut. Qual. Assur.*, **15**, 2472 (2024); https://doi.org/10.25258/ijpqa.15.4.46
5. U. Jain, N.P. Jain, S. Amrutkar and D. Kawade, *Int. J. Pharm. Sci. Drug Res.*, **17**, 203 (2025); https://doi.org/10.25004/IJPSDR.2025.170211
6. K. Mizushige, T. Tsuji and T. Noma, *Cardiovasc. Drug Rev.*, **20**, 329 (2002); https://doi.org/10.1111/j.1527-3466.2002.tb00100.x
7. M. Kobayashi and M.H.T. Iwata, *Cardiovasc. Drug Rev.*, **20**, 395 (2000).
8. J.D. Lewis, A. Ferrara, M. Hedderson, W.B. Bilker, C.P. Quesenberry Jr., T. Peng, D.J. Vaughn, L. Nessel, J. Selby and B.L. Strom, *Diabetes Care*, **34**, 916 (2011); https://doi.org/10.2337/dc10-1068
9. M. Shahnaz, P.K. Bhai and R. Bhai, *J. Drug Deliv. Ther.*, **2013**, 96 (2011).
10. N. Long, A. Le Gresley and S.P. Wren, *ChemMedChem*, **16**, 1716 (2021).
11. V. Patil, N. Upadhyay, K. Tilekar, H. Joshi and C.S. Ramaa, *Iran. J. Pharm. Res.*, **20**, 188 (2021); https://doi.org/10.22037/ijpr.2021.114969.15131
12. G. Marc, A. Stana, A. Pîrnău, L. Vlase, S. Oniga and O. Oniga, *J. Mol. Struct.*, **1241**, 130629 (2021); https://doi.org/10.1016/j.molstruc.2021.130629
13. S.S. Chhajed, P.E. Shinde, S.J. Kshirsagar, J.N. Sangshetti, P.K.P. Gupta, M.M. Parab and D. Dasgupta, *Struct. Chem.*, **31**, 1375 (2020); https://doi.org/10.1007/s11224-020-01500-4
14. T. Mosmann, *J. Immunol. Methods*, **65**, 55 (1983); https://doi.org/10.1016/0022-1759(83)90303-4
15. N.K. Verma, J. Singh and C.S. Dey, *Br. J. Pharmacol.*, **143**, 1006 (2004); https://doi.org/10.1038/sj.bjp.0706002
16. A.S.D. Wickramasinghe, A.P. Attanayake and P. Kalansuriya, *J. Pharmacol. Toxicol. Methods*, **113**, 107144 (2022); https://doi.org/10.1016/j.vascn.2021.107144