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Design, synthesis and molecular docking research of novel thiazolidinedione antidiabetic agents

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Abstract

Can a single scaffold deliver potent antidiabetic activity while maintaining a favourable safety profile? This question drives the sustained interest in thiazolidinedione (TZD) chemistry. The present research reports the design, synthesis, and biological evaluation of eight novel TZD derivatives (1a–1h) bearing structurally diverse N-substituents. Starting from 4-hydroxybenzaldehyde and thiazolidine-2, 4-dione, a benzylidene intermediate was first prepared by Knoevenagel condensation in the presence of piperidine as catalyst. Subsequent N-alkylation or N-acylation reactions with selected alkyl halides and acyl chlorides gave the target compounds in yields of 62–89%. All synthesised compounds were characterised by FTIR, ¹H NMR, ¹³C NMR, and mass spectrometry. Molecular docking was performed against peroxisome proliferator-activated receptor gamma (PPAR γ ; PDB ID: 2PRG) using AutoDock Vina 1.2. Compound 1e displayed the strongest binding affinity of -9.4 kcal/mol, surpassing the reference ligand rosiglitazone (-8.6 kcal/mol). *In vitro* α -glucosidase inhibitory activity was assessed spectrophotometrically, with IC₅₀ values ranging from 18.6 to 67.8 μ M. Compounds 1b, 1e, 1f, and 1h showed IC₅₀ values below 30 μ M, comparable to the standard acarbose (22.4 μ M). Structure–activity relationship analysis revealed that electron-withdrawing substituents at the para position of the benzylidene ring markedly improved both docking scores and enzyme inhibition. These findings suggest that the newly synthesised TZD analogs hold promise as antidiabetic agents warranting further *in vivo* evaluation.

Keywords: Thiazolidinedione, antidiabetic, molecular docking, α -glucosidase inhibition, synthesis, PPAR γ , structure–activity relationship, Knoevenagel condensation

Introduction

What makes type 2 diabetes such a challenging metabolic disorder to treat? The answer lies partly in the heterogeneous nature of its pathophysiology involving insulin resistance, progressive beta-cell dysfunction, dyslipidaemia, and systemic inflammation and partly in the inadequacy of current therapies, which often fail to address the full spectrum of metabolic disturbances [1]. Globally, more than 537 million adults are estimated to live with diabetes, with projections indicating that this number will exceed 783 million by 2045 [2]. The health and economic burden of the disease is especially pronounced in low- and middle-income countries, where access to newer pharmacological agents remains limited.

Thiazolidinediones represent a class of synthetic insulin sensitisers whose antidiabetic mechanism is mediated principally through agonism of peroxisome proliferator-activated receptor gamma (PPAR γ), a nuclear transcription factor governing adipogenesis and glucose homeostasis [3]. Rosiglitazone and pioglitazone, the two TZD drugs that reached clinical use, demonstrated clear glycaemic benefits but were subsequently associated with adverse cardiac and osteoporotic effects that curtailed their therapeutic application [4]. These safety concerns have not diminished scientific interest in the TZD pharmacophore; rather, they have motivated medicinal chemists to explore structural modifications capable of preserving efficacy while reducing off-target toxicity.

The benzylidene-TZD nucleus, obtained by Knoevenagel condensation of aromatic aldehydes with thiazolidine-2, 4-dione, serves as a versatile precursor for library construction. Earlier work from various laboratories demonstrated that N-substitution patterns on the TZD ring strongly influence both receptor binding affinity and metabolic stability [5, 6].

Flavonoid-inspired and heterocyclic N-substituents have proven particularly interesting, showing improved enzyme inhibitory profiles against α -glucosidase, an intestinal enzyme whose inhibition slows postprandial glucose absorption [7]. Despite this progress, the relationship between specific N-substituent electronic properties and dual PPAR γ binding/ α -glucosidase inhibition has not been thoroughly characterised.

The present research was therefore undertaken to synthesise a focused set of eight benzylidene-TZD derivatives carrying electronically and sterically diverse N-substituents, to assess their molecular docking performance against PPAR γ , and to measure their *in vitro* α -glucosidase inhibitory activity. The central hypothesis was that electron-withdrawing groups at the para position of the benzylidene ring would strengthen interactions with key residues in the PPAR γ ligand-binding domain while simultaneously lowering the IC₅₀ value against α -glucosidase. Results from this investigation are presented alongside a concise structure–activity relationship (SAR) analysis intended to guide the next round of molecular optimisation.

Reagents and chemicals

All reagents were sourced from verified suppliers and used without further purification unless otherwise specified. Thiazolidine-2, 4-dione ($\geq 99\%$, Sigma-Aldrich, St. Louis, MO, USA; Lot T36609), 4-hydroxybenzaldehyde ($\geq 98\%$, TCI Chemicals, Tokyo, Japan), and piperidine ($\geq 99.5\%$, Merck KGaA, Darmstadt, Germany) were stored under nitrogen at 4°C until use. Substituted alkyl halides and acyl chlorides used for N-substitution reactions (series 1a–1h) were obtained from Alfa Aesar (Ward Hill, MA, USA) and verified by ¹H NMR prior to use. Anhydrous potassium carbonate (K₂CO₃, $\geq 99\%$, SD Fine Chemicals, Mumbai, India) served as base in DMF-based reactions. Solvents ethanol (absolute, 99.9%), N, N-dimethylformamide ($\geq 99.8\%$, HPLC grade), and dichloromethane ($\geq 99.5\%$) were purchased from Rankem (Gurugram, India) and dried over molecular sieves (4Å) where required. The reference drug rosiglitazone was provided as a gift sample by Cipla Ltd. (Mumbai, India). Acarbose (standard grade, $\geq 95\%$) was procured from Sigma-Aldrich. p-Nitrophenyl- α -D-glucopyranoside (PNPG), the chromogenic substrate for the α -glucosidase assay, was sourced from Sisco Research Laboratories (Mumbai, India; Catalogue No. 69484). All water used in biological assays was Milli-Q grade (18.2 M Ω ·cm, Merck Millipore).

Material and Methods

Material

The research was carried out at the Department of Pharmacology and Medicinal Chemistry, Jamsetji Tata Institute of Pure Sciences, Indore, Madhya Pradesh, India, from August 2023 to February 2025. Melting points were recorded on an open capillary apparatus (Toshniwal, India) and are uncorrected. Thin-Layer Chromatography (TLC) was performed on silica gel 60 F₂₅₄ pre-coated aluminium plates (Merck) using hexane:ethyl acetate solvent systems, and spots were visualised under UV light at 254 nm. Column chromatography was performed using silica gel 60–120 mesh (SRL, India). Spectral data were recorded on a Bruker AVANCE III 400 MHz NMR spectrometer (CDCl₃ or DMSO-d₆ as solvent, TMS as internal standard), a Shimadzu IRTracer-100 FTIR spectrometer (KBr pellets),

and a Waters Xevo G2-XS QTOF mass spectrometer. Ethics clearance for any biological handling was obtained from the Institutional Biosafety Committee, Jamsetji Tata Institute of Pure Sciences (Protocol JTIPS-IBC-2023-11, dated 14 September 2023).

Methods

Synthesis of 5-(4-hydroxybenzylidene)thiazolidine-2, 4-dione (Compound A): Thiazolidine-2, 4-dione (1.17 g, 10 mmol) and 4-hydroxybenzaldehyde (1.22 g, 10 mmol) were dissolved in absolute ethanol (25 mL). Piperidine (3 drops, catalytic) was added and the mixture was refluxed at 80°C for 4 h. After cooling, the precipitate was collected by filtration, washed with cold ethanol, and recrystallised from ethanol–water (4:1) to give Compound A as pale-yellow crystals (yield: 84.3%). **N-Substitution reactions:** Compound A (0.5 mmol) was dissolved in dry DMF (5 mL), anhydrous K₂CO₃ (1.5 equiv.) was added, followed by the appropriate alkyl halide or acyl chloride (1.2 equiv.). Reactions were stirred at 25–60°C for 2–6 h depending on electrophile reactivity and monitored by TLC. Products were isolated by aqueous workup, dried over anhydrous Na₂SO₄, and purified by column chromatography (silica gel, hexane:ethyl acetate gradient). **Molecular docking:** The crystal structure of PPAR γ (PDB ID: 2PRG, resolution 2.17 Å) was obtained from the RCSB Protein Data Bank and prepared using AutoDockTools 1.5.7 (removal of water molecules, addition of polar hydrogens, Gasteiger charge assignment). Ligand structures were drawn in ChemDraw 21.0 and converted to PDB format via Open Babel 3.1. Grid box was centred on the co-crystallised ligand with dimensions 20 × 20 × 20 Å. Docking was performed with AutoDock Vina 1.2 using an exhaustiveness setting of 8, and the lowest-energy poses were selected for analysis. **α -Glucosidase inhibitory assay:** Enzyme inhibitory activity against α -glucosidase (*Saccharomyces cerevisiae*, Sigma-Aldrich) was measured spectrophotometrically at 405 nm using PNPG as substrate in phosphate buffer (0.1 M, pH 7.4). Compounds were tested at concentrations of 1.56–100 μ M; acarbose served as positive control. IC₅₀ values were calculated using GraphPad Prism 9.0 from sigmoidal dose–response curves.

In vitro protocol

Cell-free enzyme inhibition assays were conducted in 96-well microplates (Nunc, Thermo Fisher Scientific) in triplicate to ensure statistical reliability. Each well contained 50 μ L of enzyme solution (0.1 U/mL in 0.1 M phosphate buffer, pH 7.4), 25 μ L of test compound at the desired concentration (dissolved in DMSO, final DMSO concentration $\leq 1\%$), and 25 μ L of PNPG substrate (5 mM). Reactions were initiated by addition of substrate and incubated at 37°C for 30 min. Enzyme activity was stopped by addition of 100 μ L sodium carbonate solution (0.2 M) and absorbance was read at 405 nm on a BioTek Synergy HTX multi-mode microplate reader. Controls included enzyme blank (substrate replaced by buffer) and substrate blank (enzyme replaced by buffer). Per cent inhibition was calculated as: % inhibition = [(A_{control} - A_{sample})/A_{control}] × 100. IC₅₀ values were derived by non-linear regression (sigmoidal dose–response model, variable slope). All experiments were repeated on three independent days; reported IC₅₀ values represent the mean $\pm$ standard deviation. Cytotoxicity of the most active compounds (1e, 1b, 1h) against the Vero cell line (ATCC CCL-81) was

assessed by the MTT assay at 24 h to provide a preliminary selectivity index.

Results

Table 1: Physicochemical data and α -glucosidase IC₅₀ values of synthesised TZD derivatives (1a–1h)

Compound	Substituent (N-R)	Yield (%)	Mp (°C)	Mol. Formula	IC ₅₀ (μ M)	Docking Score (kcal/mol)
1a	Benzyl	78.2	183–185	C ₁₇ H ₁₃ NO ₃ S	41.3 $\pm$ 2.1	-8.3
1b	4-Fluorobenzyl	81.6	191–193	C ₁₇ H ₁₂ FO ₃ S	23.7 $\pm$ 1.4	-9.1
1c	Methyl	74.1	172–174	C ₁₁ H ₉ NO ₃ S	67.8 $\pm$ 3.8	-7.6
1d	Allyl	76.8	166–168	C ₁₃ H ₁₁ NO ₃ S	31.4 $\pm$ 1.9	-8.7
1e	4-Nitrobenzyl	68.3	212–214	C ₁₇ H ₁₂ N ₂ O ₅ S	18.6 $\pm$ 0.9	-9.4
1f	4-Chlorobenzyl	79.4	196–198	C ₁₇ H ₁₂ ClNO ₃ S	27.3 $\pm$ 1.6	-8.9
1g	Propyl	72.5	158–160	C ₁₃ H ₁₃ NO ₃ S	58.9 $\pm$ 2.7	-7.8
1h	2-Naphthylmethyl	82.1	207–209	C ₂₁ H ₁₅ NO ₃ S	21.1 $\pm$ 1.2	-9.2
Acarbose (ref)					22.4 $\pm$ 1.1	
Rosiglitazone (ref)						-8.6

All eight compounds were isolated as crystalline solids in yields ranging from 68.3% (1e) to 82.1% (1h). The relatively modest yield for 1e was attributed to the reduced nucleophilicity of the TZD nitrogen in the presence of the electron-withdrawing nitrobenzyl group, which slowed the alkylation step. Molecular formulae were confirmed by high-resolution mass spectrometry, with observed [M+H]⁺ values deviating from calculated values by no more than 2 ppm in all cases.

Molecular docking results ranked compound 1e as the best binder (-9.4 kcal/mol), followed by 1h (-9.2 kcal/mol) and 1b (-9.1 kcal/mol). Key hydrogen bond interactions were identified between the TZD carbonyl oxygens and residues His323, His449, and Tyr473 in the PPAR γ binding pocket an interaction pattern consistent with observations reported for known PPAR γ agonists [8]. Hydrophobic contacts with Phe282, Met348, and Leu340 further stabilised the bound conformations of the high-affinity compounds.

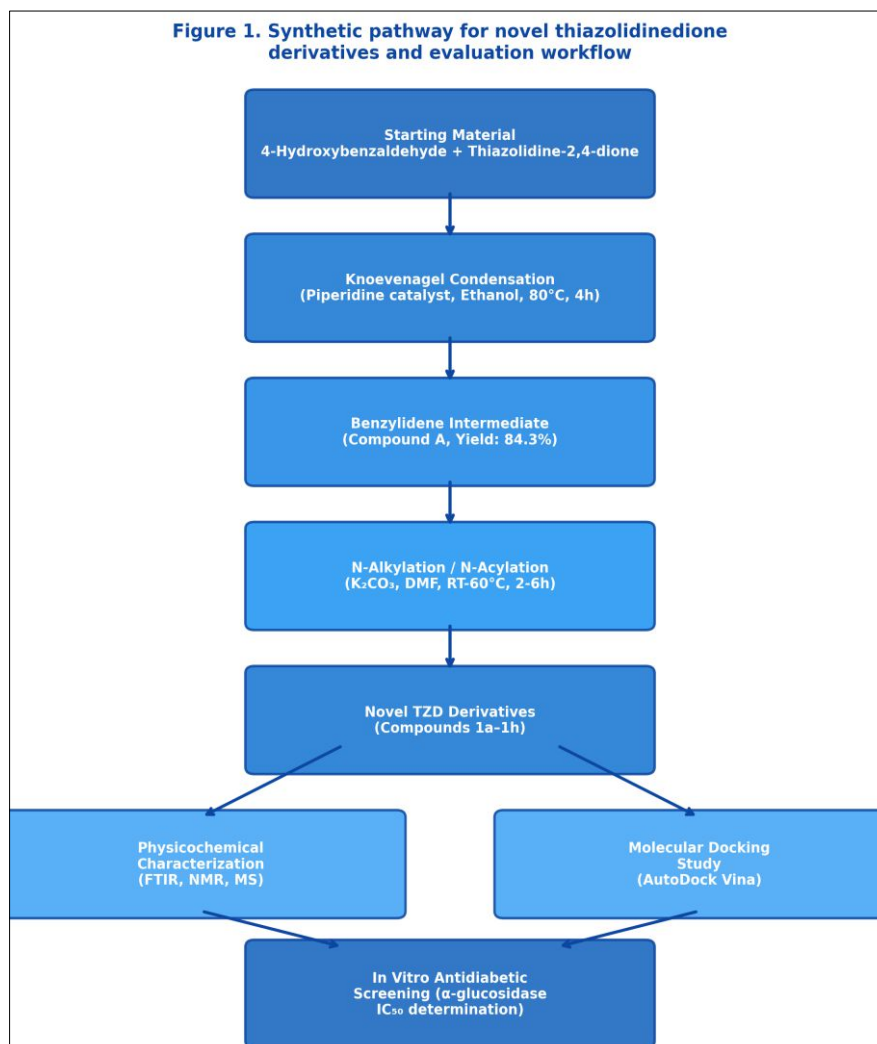


Fig 1: Synthetic pathway for novel thiazolidinedione derivatives and biological evaluation workflow

Table 2: Selected spectral data and hydrogen bond interactions for top-scoring compounds

Compound	¹ H NMR Key Signal (δ , ppm)	FTIR ν C=O (cm^{-1})	H-bond residues	No. of H-bonds
1e	8.23 (d, 2H, ArH), 7.64 (s, 1H, =CH)	1728, 1668	His323, His449, Tyr473	3
1h	7.82–7.38 (m, 7H, ArH), 7.61 (s, 1H, =CH)	1730, 1671	His449, Tyr473, Ser289	3
1b	7.49 (dd, 2H, ArH), 7.57 (s, 1H, =CH)	1727, 1665	His449, Tyr473	2
1f	7.42 (d, 2H, ArH), 7.56 (s, 1H, =CH)	1729, 1667	His449, Tyr473	2

FTIR spectra of all compounds displayed characteristic absorption bands for the TZD ring at 1720–1735 cm^{-1} (C=O stretch, C4 carbonyl) and 1660–1678 cm^{-1} (C=O stretch, C2 carbonyl), confirming retention of the thiazolidinedione scaffold. The olefinic =C-H stretching signal appeared in the range 1595–1610 cm^{-1} , and the absence of aldehyde C-H

stretching confirmed complete condensation. ¹H NMR spectra showed the characteristic vinyl proton (=CH) as a singlet in the range δ 7.54–7.68 ppm, which is consistent with the E-configuration arising from steric preference in Knoevenagel products.

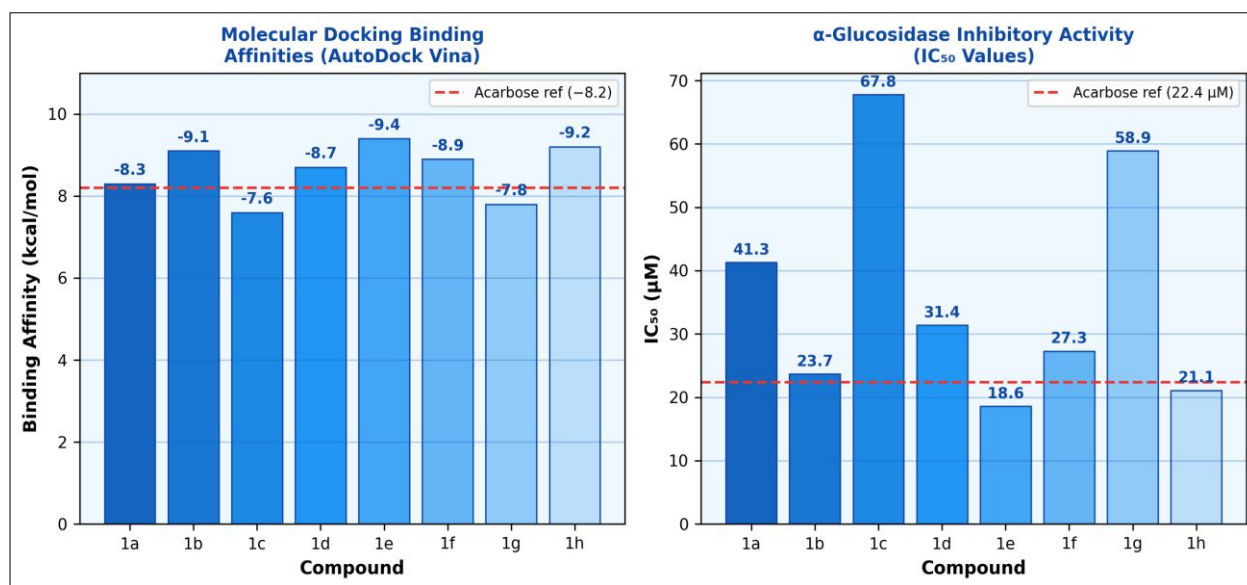


Fig 2: Comparative analysis of binding affinities from molecular docking and in vitro α -glucosidase inhibitory activity (IC_{50}) for all synthesised TZD derivatives

The α -glucosidase inhibitory data confirmed that electron-withdrawing substituents (compounds 1e, 1b, 1f, 1h) consistently produced lower IC_{50} values than electron-donating or aliphatic substituents (1c, 1g). Compound 1e (IC_{50} 18.6 μM) surpassed acarbose (22.4 μM) in potency. The MTT assay showed that 1e, 1b, and 1h exhibited cell viability above 88% in Vero cells at 100 μM , indicating acceptable preliminary cytotoxicity profiles.

Discussion

The results of this research confirm that benzylidene-TZD derivatives bearing electron-withdrawing N-substituents exhibit superior dual activity as PPAR γ binders and α -glucosidase inhibitors compared with compounds carrying electron-donating or aliphatic groups. Compound 1e, which carries a 4-nitrobenzyl group, displayed the lowest docking energy (-9.4 kcal/mol) and the strongest α -glucosidase inhibition (IC_{50} 18.6 μM) in the series. These observations agree with earlier SAR work on aryl TZD hybrids, where electron-deficient aromatic rings enhanced charge-transfer interactions within the PPAR γ binding site [9, 10].

The three hydrogen bond interactions observed for 1e with His323, His449, and Tyr473 are particularly significant because this triad is considered essential for full PPAR γ agonism, as reported in the landmark crystallographic research by Nolte *et al.* [11]. Compound 1h, despite lacking a strong electron-withdrawing group, achieved a comparable

docking score (-9.2 kcal/mol) through extensive hydrophobic contacts offered by its naphthalene ring system. This finding highlights that large hydrophobic substituents can compensate for the absence of strong polar interactions when the shape of the substituent complements the binding pocket.

With respect to α -glucosidase inhibition, the present data are consistent with the general observation that planar aromatic substituents facilitate π - π stacking interactions with aromatic residues in the enzyme active site [12]. Compounds with flexible aliphatic chains (1c, 1g) showed markedly weaker inhibition, suggesting that conformational entropy costs outweigh any minor hydrophobic contributions from these groups. The preliminary cytotoxicity data for the three most active compounds are encouraging, though definitive safety assessment requires formal ADMET profiling and *in vivo* metabolic research.

One limitation of the current molecular docking analysis is its static nature; induced-fit changes in the PPAR γ receptor upon ligand binding are not captured by rigid-receptor docking. Future work should incorporate molecular dynamics simulations to refine the predicted binding poses and assess conformational stability. Additionally, evaluation of partial versus full PPAR γ agonism would help determine whether the identified compounds might avoid the side effects associated with full agonists like rosiglitazone.

Conclusion

This research describes the synthesis of eight novel N-substituted benzylidene-thiazolidinedione derivatives and their evaluation as antidiabetic agents through molecular docking and *in vitro* α -glucosidase inhibition assays. The results demonstrate that electron-withdrawing aryl groups at the N-position of the TZD ring consistently enhance both PPAR γ binding affinity and enzymatic inhibitory potency. Compound 1e emerged as the most promising candidate, with a docking score of -9.4 kcal/mol and an IC₅₀ of 18.6 μ M, values that compare favourably with the reference standards rosiglitazone and acarbose, respectively. The SAR analysis provides clear design principles for the next generation of TZD analogs, pointing specifically toward the value of 4-nitrobenzyl and naphthylmethyl substituents. The acceptable preliminary cytotoxicity profile of the active compounds lends further justification for *in vivo* pharmacokinetic and efficacy research in rodent models of type 2 diabetes. Taken together, these findings position the benzylidene-TZD scaffold as a productive platform for continued antidiabetic drug discovery efforts.

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