Asian Journal of Chemistry; Vol. 38, No. 9 (2026), 2413-2418
ASIAN JOURNAL OF CHEMISTRY
https://doi.org/10.14233/ajchem.2026.36397

Synthesis and *in vitro* Biological Evaluation of 5-Benzylidene Derivatives of 3-(4-Fluorophenyl)-2,4-thiazolidinedione as Potential α-Glucosidase Inhibitors

LAY HSIEN TAN, LAI CHUN WONG and VASUDEVA RAO AVUPATI*

Department of Pharmaceutical Chemistry, School of Pharmacy, IMU University (Formerly known as International Medical University), 126, Jalan Jalil Perkasa 19, Bukit Jalil, 57000 Kuala Lumpur, Malaysia

*Corresponding author: E-mail: vasudevaraoavupati@imu.edu.my

Received: 8 May 2026 *Accepted*: 10 August 2026 *Published online*: 5 September 2026 AJC-22469

α-Glucosidase is an intestinal membrane-bound enzyme that catalyzes the final step in the digestion of carbohydrates. It cleaves the glycosidic bonds of oligosaccharides to release glucose and its inhibition results in delayed glucose absorption, thereby inhibiting post-prandial hyperglycemia and hyperinsulinemia in patients with type 2 diabetes. Therefore, in view of the biological role of α-glucosidase as an antidiabetic drug target, a series of 5-(substituted)-1,3-thiazolidine-2,4-diones (**C1-C6**) were designed, synthesized and characterized by FT-IR, ^{1}H NMR, ^{13}C NMR and mass spectral analyses. All the compounds were subjected to *in vitro* α-glucosidase inhibitory evaluation. Among the compounds tested for α-glucosidase inhibitory activity, compounds **C1** and **C3** exhibited significant inhibition, with IC_{50} values of 0.1363 ± 0.00085 µM and 3.63780 ± 0.00005 µM, respectively. Structure-activity relationship (SAR) analysis of the test compounds revealed the positive contribution of the 1,3-thiazolidine-2,4-dione moiety and the phenyl ring substituents at the 5-position of the 1,3-thiazolidine-2,4-dione scaffold towards the observed activity.

Keywords: Drug discovery, 5-Bezylidene-1,3-thiazolidine-2,4-dione, α-Glucosidase inhibitor, Type 2 diabetes.

INTRODUCTION

Diabetes mellitus (DM) is a group of metabolic disorders characterized by persistent hyperglycaemia resulting from the impaired insulin secretion, insulin action or both [1]. Prolonged hyperglycaemia damages multiple organ systems, particularly the cardiovascular system, kidneys, retina and nervous system, increasing the risk of complications such as kidney failure, stroke, myocardial infarction, blindness and lower-limb amputation [2]. According to the IDF Diabetes Atlas, diabetes affects approximately 536.6 million people (10.5% of the global population) and this number is projected to reach 783.2 million (12.2%) by 2045 [3]. DM is classified into three major forms *viz*. type 1 diabetes mellitus (T1DM), type 2 diabetes mellitus (T2DM) and gestational diabetes. T1DM results from autoimmune destruction of pancreatic β-cells, leading to absolute insulin deficiency and lifelong insulin therapy. T2DM is characterized by insulin resistance accompanied by impaired insulin secretion [4]. Gestational diabetes develops during pregnancy as a result of glucose intolerance and is associated with adverse maternal and fetal outcomes if left untreated.

Thiazolidinediones (TZDs), due to their ability to decrease insulin resistance by increasing insulin sensitivity in the liver, muscle and adipose tissue, have high potential for structural modification to improve their efficacy. According to Lebovitz *et al.* [5], rosiglitazone and pioglitazone both showed similar effects in reducing blood glucose levels by lowering HbA1c levels by approximately 0.5% and preserving β-cell function better than sulfonylureas. According to the DREAM (diabetes reduction assessment with ramipril and rosiglitazone medication ongoing follow-up) study [6], rosiglitazone was found to reduce the progression of β-cell dysfunction and decrease the long-term risk of diabetes development. However, existing thiazolidinediones could not successfully pass all clinical evaluations due to several adverse findings, including heart failure caused by fluid retention, potential fatal idiosyncratic hepatotoxicity, increased risk of acute ischemic events, risk of bladder cancer and other serious side effects. Besides contributing to glycaemic control, TZDs also provide

additional benefits. Studies have shown that rosiglitazone reduces fasting insulin levels, increases HDL levels and significantly decreases high-sensitivity C-reactive protein (hs-CRP) levels.

α-Glucosidases are vital enzymes that hydrolyse O- and S-glycosyl residues and are involved in the biosynthesis and processing of oligosaccharide chains of N-linked glycoproteins. During the hydrolysis of glycosidic bonds at the terminal glucose cleavage site, α- and β-glucosidases catalyse the reaction through the action of two carboxylic acid side chains [7]. The catalytic mechanisms of α- and β-glucosidases differ, where one residue attacks the anomeric centre as a catalytic nucleophile while the other weakens the C–O bond as an acid catalyst. Acarbose, voglibose and miglitol are among the clinically approved α-glucosidase inhibitors used for the management of diabetes. Therefore, the design and development of glucosidase inhibitors with improved selectivity and efficacy are still needed. α-Glucosidase inhibitors are also recognized for their anti-HIV and antiviral activities, as they can alter the glycosylation of envelope glycoproteins by interfering with the biosynthesis of N-linked oligosaccharides [8]. Recently, several synthetic ligands have been reported to exhibit inhibitory activity against α-glucosidase.

This study does not introduce a new thiazolidinedione scaffold. Its novelty lies in the systematic evaluation of the structure–activity relationship (SAR) and *in vitro* α-glucosidase inhibitory activity of a series of 5-(substituted)-1,3-thiazolidine-2,4-dione derivatives to identify promising hit molecules [9]. This work extends our previous efforts toward the development of novel α-glucosidase inhibitors [10-12] and provides valuable insights for the lead optimization of the thiazolidinedione scaffold rather than the discovery of a new chemotype.

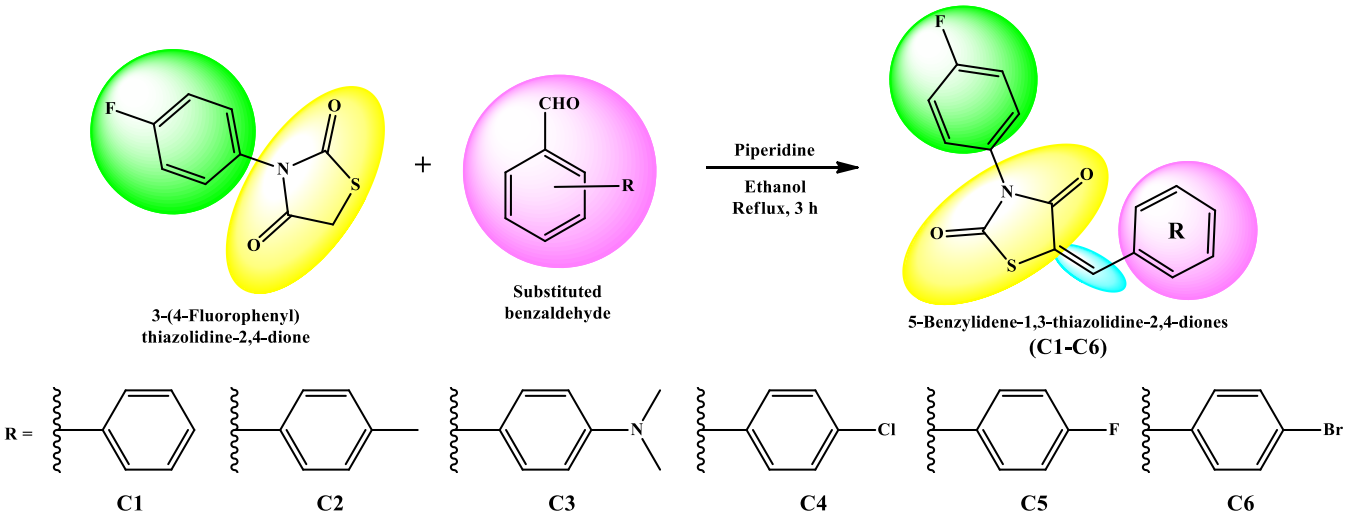
EXPERIMENTAL

All reagents and chemicals were of analytical grade and purchased from Sigma-Aldrich (St. Louis, MO, USA). These included 3-(4-fluorophenyl)-1,3-thiazolidine-2,4-dione, benzaldehyde, 4-dimethylaminobenzaldehyde, 4-methyl-/4-chloro/4-fluoro/4-bromobenzaldehyde, piperidine, acetone, methylene chloride, LC-grade methanol, hexane, ethyl acetate, absolute ethanol, dimethyl sulfoxide (DMSO) and 4-nitrophenyl-α-D-glucopyranoside.

The purity of the synthesized compounds was monitored by thin-layer chromatography (TLC) on pre-coated silica gel 60 F_{254} plates (0.25 mm; Merck, USA) using *n*-hexane/ethyl acetate as the developing solvent system. FT-IR spectra were recorded on a Shimadzu MIRAffinity-1S spectrometer (Japan). ^{1}H NMR spectra were acquired on a 500 MHz Varian NMR spectrometer (USA) using TMS as the internal standard. The compounds were weighed using a Mettler Toledo ML204 analytical balance (USA). High-resolution electrospray ionization mass spectra (HR-ESI-MS) were recorded on a Thermo Scientific Q Exactive Focus Orbitrap LC-MS/MS system (USA). Melting points were determined in open capillary tubes using a Stuart Scientific SMP1 melting point apparatus (United Kingdom) and are uncorrected.

General procedure for the synthesis of 5-benzylidene derivatives of 3-(4-fluorophenyl)-2,4-thiazolidinedione (C1-C6): 3-(4-Fluorophenyl)-2,4-thiazolidinedione (50 mg) was dissolved in 5 mL of absolute ethanol and 24 µL of piperidine was added using a pipette into the solution with continuous gentle stir. An equimolar amount of the appropriate substituted benzaldehyde was weighed (or pipetted for liquid derivatives) and added to the reaction mixture. The mixture was stirred at 75 ºC for 1 h, then allowed to cool. The resulting solid was collected by filtration and dried in an oven at 40 ºC for 20 min (**Scheme-I**). The purity of the product was examined by TLC using a solvent system of 30% ethyl acetate and 70% *n*-hexane.

5-Benzylidene-3-(4-fluorophenyl)thiazolidine-2,4-dione (C1): Cream coloured amorphous powder; yield: 65%; m.p.: 210 ºC; m.f.: $C_{16}H_{10}FNO_2S$; relative molecular mass: 299; FT-IR (ATR, ν_{max}, cm^{-1}): 3057.17 (=C-H *str.*), 1759.08 (C=O *str.*), 1678.07 (conjugated C=O *str.*); ^{1}H NMR (500 MHz, DMSO-d_6, δ ppm): 7.35-7.39 (t, 2H, J_1 = 10 MHz, J_2 = 10 MHz, Ar-H), 7.50-7.57 (m, 5H, Ar-H), 7.65 (d, 2H, J = 10 MHz, Ar-H), 7.98 (s, 1H, benzylidene -C=C-**H**); ^{13}C NMR (500 MHz, DMSO-d_6, δ ppm): 167.37, 165.60, 163.44, 161.48,



Scheme-I: Synthesis of 5-benzylidene derivatives of 3-(4-fluorophenyl)-2,4-thiazolidinedione (**C1-C6**)

157.38, 141.87, 133.46, 131.10, 130.83, 130.76, 130.56, 129.87, 129.67, 129.64, 121.94, 116.65, 116.46; ESI-HRMS (*m/z*): 300.0485 $[M+H]^+$ (positive-ion mode).

3-(4-Fluorophenyl)-5-(4-methylbenzylidene)thiazolidine-2,4-dione (C2): Pale yellow coloured amorphous powder; yield: 51%; m.p.: 226 ºC; m.f.: $C_{17}H_{12}FNO_2S$; relative molecular mass: 313; FT-IR (ATR, ν_{max}, cm^{-1}): 3078.39 (=C-H *str*.), 1755.22 (C=O *str*.), 1680.00 (conjugated C=O *str*.); ^{1}H NMR (500 MHz, DMSO-d_6, δ ppm): 2.37 (s, 3H, Ar-CH_3), 7.35-7.38 (m, 4H, Ar-H), 7.48-7.52 (m, 2H, Ar-H), 7.55 (d, 2H, *J* = 5 MHz, Ar-H), 7.94 (s, 1H, benzylidene -C=C-**H**); ^{13}C NMR (500 MHz, DMSO-d_6, δ ppm): 167.40, 165.67, 163.42, 161.46, 141.47, 133.55, 130.85, 130.77, 130.72, 130.65, 130.51, 129.71, 120.67, 116.63, 116.45; ESI-HRMS (*m/z*): 314.0652 $[M+H]^+$ (positive-ion mode).

5-(4-(Dimethylamino)benzylidene)-3-(4-fluorophenyl)-thiazolidine-2,4-dione (C3): Dark yellow coloured amorphous powder; yield: 27%; m.p.: 268 ºC; m.f.: $C_{18}H_{15}FN_2O_2S$; relative molecular mass: 342; FT-IR (ATR, ν_{max}, cm^{-1}): 3080.32 (=C-H *str*.), 1735.93 (C=O *str*.), 1676.14 (conjugated C=O *str*.); ^{1}H NMR (500 MHz, DMSO-d_6, δ ppm): 3.02 (s, 6H, N-(CH_3)$_2$), 6.83 (d, 2H, *J* = 5 MHz, Ar-H), 7.34-7.38 (t, 2H, J_1 = 10 MHz, J_2 = 10 MHz, Ar-H), 7.47-7.50 (m, 4H, Ar-H), 7.84 (s, 1H, Benzylidene -C=C-**H**); ^{13}C NMR (500 MHz, DMSO-d_6, δ ppm): 168.28, 165.53, 163.08, 161.21, 152.09, 134.70, 132.85, 130.88, 130.81, 130.18, 120.24, 116.56, 116.38, 113.62, 112.58; ESI-HRMS (*m/z*): 343.2963 $[M+H]^+$ (positive-ion mode).

5-(4-Chlorobenzylidene)-3-(4-fluorophenyl)thiazolidine-2,4-dione (C4): Light yellow coloured amorphous powder; yield: 46%; m.p.: 235 ºC; m.f.: $C_{16}H_9ClFNO_2S$; relative molecular mass: 333; FT-IR (ATR, ν_{max}, cm^{-1}): 3016.67 (=C-H *str*.), 1753.93 (C=O *str*.), 1681.93 (conjugated C=O *str*.); ^{1}H NMR (500 MHz, DMSO-d_6, δ ppm): 7.36-7.40 (t, 2H, J_1 = 10 MHz, J_2 = 10 MHz, Ar-H), 7.50-7.52 (m, 2H, Ar-H), 7.62 (d, 2H, *J* = 10 MHz, Ar-H), 7.69 (d, 2H, *J* = 10 MHz, Ar-H), 7.99 (s, 1H, Benzylidene -C=C-**H**); ^{13}C NMR (500 MHz, DMSO-d_6, δ ppm): 167.02, 165.52, 161.51, 161.22, 142.76, 135.71, 132.20, 132.07, 130.86, 130.79, 129.98, 123.12, 122.53, 116.68, 116.50; ESI-HRMS (*m/z*): 334.2255 $[M+H]^+$ (positive-ion mode).

5-(4-Fluorobenzylidene)-3-(4-fluorophenyl)thiazolidine-2,4-dione (C5): Greenish yellow coloured amorphous powder; yield: 22%; m.p.: 215 ºC; m.f.: $C_{16}H_9F_2NO_2S$; relative molecular mass: 317; FT-IR (ATR, ν_{max}, cm^{-1}): 2918.30 (=C-H *str*.), 1761.01 (C=O *str*.), 1685.79 (conjugated C=O *str*.); ^{1}H NMR (500 MHz, DMSO-d_6, δ ppm): 7.36-7.43 (m, 4H, Ar-H), 7.50-7.53 (m, 2H, Ar-H), 7.74-7.76 (m, 2H, Ar-H), 8.00 (s, 1H, Benzylidene -C=C-**H**); ^{13}C NMR (500 MHz, DMSO-d_6, δ ppm): 165.23, 164.26, 160.92, 155.71, 144.92, 137.25, 133.11, 130.87, 130.80, 129.30, 121.92, 121.26, 117.18, 117.01, 116.49; ESI-HRMS (*m/z*): 318.0400 $[M+H]^+$ (positive-ion mode).

5-(4-Bromobenzylidene)-3-(4-fluorophenyl)thiazolidine-2,4-dione (C6): Light yellow coloured amorphous powder; yield: 29%; m.p.: 246 ºC; m.f.: $C_{16}H_9BrFNO_2S$; relative molecular mass: 378; FT-IR (ATR, ν_{max}, cm^{-1}): 3082.25 (=C-H *str*.), 1735.93 (C=O *str*.), 1668.86 (conjugated C=O *str*.); ^{1}H NMR (500 MHz, DMSO-d_6, δ ppm): 7.36-7.40 (t, 2H, J_1 = 10 MHz, J_2 = 10 MHz, Ar-H), 7.50-7.53 (m, 2H, Ar-H), 7.61 (d, 2H, *J* = 10 MHz, Ar-H), 7.76 (d, 2H, *J* = 10 MHz, Ar-H), 7.97 (s, 1H, benzylidene -C=C-**H**); ^{13}C NMR (500 MHz, DMSO-d_6, δ ppm): δ 165.53, 161.50, 154.14, 151.69, 150.80, 140.31, 139.41, 135.10, 132.91, 132.34, 132.16, 130.86, 124.65, 122.86, 116.68, 116.50; ESI-HRMS (*m/z*): 379.9623 $[M+H]^+$ (positive-ion mode).

***In vitro* α-Glucosidase inhibitor screening:** The α-glucosidase inhibitory activity of compounds **C1-C6** were evaluated using *in vitro* α-glucosidase enzymatic kinetics. The α-glucosidase inhibitory activity was evaluated using *Saccharomyces cerevisiae* α-glucosidase (Type I), 4-nitrophenyl-α-D-glucopyranoside (pNPG) as the substrate, voglibose as the reference inhibitor and compounds **C1-C6** as the test samples (100-1 μM). Phosphate buffer (pH 7.3) was used as the reaction medium and molecular biology-grade dimethyl sulfoxide (DMSO) served as the solvent.

Phosphate buffer solution (PBS, pH 7.3) was prepared by dissolving a pre-adjusted buffer tablet in distilled water. The enzyme solution (0.0125-0.8 U/mL) and pNPG substrate solution (0.0125-0.8 mM) were prepared in PBS, while the test compounds and voglibose were dissolved in DMSO to obtain stock solutions (100 μM). A calibration curve ($r \geq 0.999$) was constructed using 0.1 U/mL enzyme and pNPG concentrations ranging from 0.0125 to 0.8 mM at 405 nm.

The assay was carried out in 130 μL reaction mixtures containing enzyme, substrate, buffer and either the test compound, voglibose or DMSO as appropriate. Control, reaction blank, solvent blank, test and standard wells were prepared using the corresponding reagent combinations. The release of *p*-nitrophenol was monitored by measuring the absorbance at 405 nm after 20 min of incubation. The percentage (%) enzyme inhibition calculated using the following formula:

$$\text{Enzyme inhibition (\%)} = \left(1 - \frac{\text{Abs}_{\text{test compound}} - \text{Abs}_{\text{solvent blank}}}{\text{Abs}_{\text{control}} - \text{Abs}_{\text{control blank}}}\right)$$

Statistical analysis was carried out using Microsoft Excel.

RESULTS AND DISCUSSION

The structures of synthesized compounds **C1-C6** were established based on the Knoevenagel condensation mechanism, in which substituted benzaldehydes react with the activated methylene group of 3-(4-halophenyl)thiazolidine-2,4-dione to form the corresponding 5-substituted thiazolidine-2,4-dione derivatives. The proposed structures were assigned from the expected reaction pathway and characterized through combined interpretation of HRMS, FT-IR, ^{1}H NMR, and ^{13}C NMR spectral data.

The electrospray ionization mass spectrum (ESI-MS) of compound **C1**, recorded in the positive-ion mode (*m/z* 100-1000) using methanol as the solvent, exhibited a pseudo-molecular ion peak at *m/z* 300.0485 corresponding to the $[M+H]^+$ ion. This value agreed with the calculated molecular formula $C_{16}H_{10}FNO_2S$ (*m.w.* 299). The FT-IR spectrum displayed characteristic absorption bands at 1759.08 and 1678.07 cm^{-1}, assigned to the two carbonyl (C=O) stretching vibrations of the thiazolidine-2,4-dione ring, while the band at 3057.17 cm^{-1} corresponded to the olefinic (=C–H) stretch-

ing vibration. The ^{1}H NMR spectrum matched the expected proton count for the proposed structure. A singlet at δ 7.98 ppm was assigned to the benzylidene proton, consistent with the formation of the Knoevenagel condensation product. The remaining nine aromatic protons appeared between δ 7.35 and 7.65 ppm. In the ^{13}C NMR spectrum, the benzylidene carbons resonated at δ 141.87 and 116.65 ppm, while the carbonyl carbons of the thiazolidine-2,4-dione ring appeared at δ 167.37 and 165.60 ppm. The remaining aromatic carbons resonated between δ 121.94 and 163.44 ppm. The HRMS, FT-IR, 1H NMR, and 13C NMR data were consistent with the proposed structure of **C1**, identified as 5-benzylidene-3-(4-fluorophenyl)thiazolidine-2,4-dione.

The ESI-MS spectrum of compound **C2**, recorded under identical conditions, exhibited a pseudo-molecular ion peak at *m/z* 314.0652 corresponding to the $[M+H]^+$ ion, in agreement with the calculated molecular formula $C_{17}H_{12}FNO_2S$ (*m.w.* 313). The FT-IR spectrum showed characteristic carbonyl stretching bands at 1755.22 and 1680.00 cm^{-1}, together with an olefinic (=C–H) stretching band at 3078.39 cm^{-1}. The ^{1}H NMR spectrum was consistent with the expected proton count. The benzylidene proton appeared as a singlet at δ 7.94 ppm, while the remaining eight aromatic protons resonated between δ 7.35 and 7.55 ppm. A singlet at δ 2.37 ppm integrating for three protons confirmed the presence of the aromatic methyl substituent. The ^{13}C NMR spectrum exhibited benzylidene carbon resonances at δ 141.47 and 120.67 ppm, whereas the carbonyl carbons appeared at δ 167.40 and 165.67 ppm. The remaining aromatic carbons resonated between δ 116.45 and 163.42 ppm. The combined spectroscopic data established the structure of **C2** as 3-(4-fluorophenyl)-5-(4-methylbenzylidene)thiazolidine-2,4-dione.

Compound **C3** gave a pseudo-molecular ion peak at *m/z* 343.2963 ($[M+H]^+$) in the positive-ion ESI-MS spectrum, consistent with the proposed molecular formula $C_{18}H_{15}FN_2O_2S$ (*m.w.* 342). The FT-IR spectrum displayed carbonyl stretching vibrations at 1735.93 and 1676.14 cm^{-1} along with an olefinic (=C–H) stretching band at 3080.32 cm^{-1}. The ^{1}H NMR spectrum agreed with the theoretical proton count. A singlet at δ 7.84 ppm corresponded to the benzylidene proton, while the remaining eight aromatic protons resonated between δ 6.83 and 7.50 ppm. A singlet at δ 3.02 ppm integrating for six protons confirmed the presence of the N,N-dimethylamino substituent. In the ^{13}C NMR spectrum, the benzylidene carbons resonated at δ 152.09 and 113.62 ppm, the carbonyl carbons appeared at δ 168.28 and 165.53 ppm and the remaining aromatic carbons resonated between δ 112.58 and 163.08 ppm.

The electrospray ionization mass spectrum (ESI-MS) of compound **C4**, recorded in the positive-ion mode (*m/z* 100-1000) using methanol as the solvent, exhibited a pseudo-molecular ion peak at *m/z* 334.2255 corresponding to the $[M+H]^+$ ion. The observed molecular ion agreed with the proposed molecular formula $C_{16}H_9ClFNO_2S$ (*m.w.* 333). The FT-IR spectrum displayed characteristic absorption bands at 1753.93 and 1681.93 cm^{-1}, assigned to the two carbonyl (C=O) stretching vibrations of the thiazolidine-2,4-dione ring, together with an olefinic (=C–H) stretching band at 3016.67 cm^{-1}. The ^{1}H NMR spectrum matched the expected proton count for the proposed structure. A singlet at δ 7.99 ppm was assigned to the benzylidene proton, consistent with the formation of the Knoevenagel condensation product, while the remaining eight aromatic protons resonated between δ 7.36 and 7.69 ppm. In the ^{13}C NMR spectrum, the benzylidene carbons appeared at δ 142.76 and 122.53 ppm, whereas the carbonyl carbons of the thiazolidine-2,4-dione ring resonated at δ 167.02 and 165.52 ppm. The remaining aromatic carbons were observed between δ 116.68 and 161.51 ppm. The combined HRMS, FT-IR, ^{1}H NMR and ^{13}C NMR data supported the proposed structure of **C4**, identified as 5-(4-chlorobenzylidene)-3-(4-fluorophenyl)thiazolidine-2,4-dione.

The ESI-MS spectrum of compound **C5**, recorded under identical conditions, exhibited a pseudo-molecular ion peak at *m/z* 318.0400 corresponding to the $[M+H]^+$ ion. This value agreed with the calculated molecular formula $C_{16}H_9F_2NO_2S$ (*m.w.* 317). The FT-IR spectrum showed characteristic carbonyl stretching bands at 1761.01 and 1685.79 cm^{-1}. The absorption band reported at 2918.30 cm^{-1}, assigned to the olefinic (=C–H) stretching vibration, should be verified using the original FT-IR spectrum, as olefinic C–H stretching vibrations are generally observed in the 3100-3000 cm^{-1} region. The ^{1}H NMR spectrum agreed with the expected proton count. The benzylidene proton appeared as a singlet at δ 8.00 ppm, while the remaining eight aromatic protons resonated between δ 7.36 and 7.76 ppm. The ^{13}C NMR spectrum exhibited benzylidene carbon resonances at δ 144.92 and 117.18 ppm. The carbonyl carbons of the thiazolidine-2,4-dione ring appeared at δ 165.23, 164.26 ppm, and the remaining aromatic carbons resonated between δ 116.49 and 160.92 ppm. Thus, based on these data the proposed structure of **C5** is assigned as 5-(4-fluorobenzylidene)-3-(4-fluorophenyl)thiazolidine-2,4-dione.

Compound **C6** gave a pseudo-molecular ion peak at *m/z* 379.9623 ($[M+H]^+$) in the positive-ion ESI-MS spectrum, consistent with the proposed molecular formula $C_{16}H_9BrFNO_2S$ (*m.w.* 378). The FT-IR spectrum displayed characteristic absorption bands at 1735.93 and 1668.86 cm^{-1}, assigned to the carbonyl (C=O) stretching vibrations of the thiazolidine-2,4-dione ring, together with an olefinic (=C–H) stretching band at 3082.25 cm^{-1}. The ^{1}H NMR spectrum matched the expected proton count. A singlet at δ 7.97 ppm corresponded to the benzylidene proton, while the remaining eight aromatic protons resonated between δ 7.36 and 7.76 ppm. In the ^{13}C NMR spectrum, the benzylidene carbons resonated at δ 140.31 and 122.86 ppm. The carbonyl carbons of the thiazolidine-2,4-dione ring appeared at δ 165.53 and 161.50 ppm, whereas the remaining aromatic carbons resonated between δ 116.50 and 154.14 ppm. Thus, based on these data the proposed structure of **C6** is confirmed as 5-(4-bromobenzylidene)-3-(4-fluorophenyl)thiazolidine-2,4-dione.

***In vitro* α-glucosidase inhibitory activity:** The α-glucosidase inhibitory activity results of the synthesized compounds **C1-C6** are shown in Table-1. The IC_{50} values indicate the concentration required for 50% inhibition of the α-glucosidase enzyme, where lower IC_{50} values correspond to stronger inhibitory potency. Among the compounds evaluated, compound **1** (0.1363 μM) demonstrated the most potent inhibitory

TABLE-1
In vitro α-GLUCOSIDASE INHIBITORY ACTIVITY (IC_{50}) OF 5-BENZYLIDENE DERIVATIVES OF 3-(4-FLUOROPHENYL)-2,4-THIAZOLIDINEDIONE (**C1**-**C6**)

Compound	IC_{50} (μM)	Molecule structural features			
		Core scaffold	Substituent on the 5-benzylidene-1,3-thiazolidine-2,4-dione	Electronic nature	N3-substituent at the position 3 of 1,3-thiazolidine-2,4-dione
C1	0.1363 ± 0.00085	1,3-Thiazolidine-2,4-dione	–	–	4-Fluorophenyl
C2	88.15760 ± 0.63095	1,3-Thiazolidine-2,4-dione	4-Methyl	EDG	4-Fluorophenyl
C3	3.63780 ± 0.00005	1,3-Thiazolidine-2,4-dione	4-Di-methyl-amino	EDG	4-Fluorophenyl
C4	4.58130 ± 0.00001	1,3-Thiazolidine-2,4-dione	4-Chloro	EWG	4-Fluorophenyl
C5	4.28490 ± 0.00001	1,3-Thiazolidine-2,4-dione	4-Fluoro	EWG	4-Fluorophenyl
C6	5.36620 ± 0.00001	1,3-Thiazolidine-2,4-dione	4-Bromo	EWG	4-Fluorophenyl
Voglibose	0.0434 ± 0.002				

EWG: Electron withdrawing group, EDG: Electron donating group

activity among the synthesized derivatives and showed activity comparatively close to the standard drug voglibose (0.0434 μM). In contrast, compounds **3** (3.6378 μM), **5** (4.2849 μM) and **4** (4.5813 μM) exhibited moderate inhibitory activity, while compound **6** (5.3662 μM) displayed comparatively lower potency. Compound **2** (88.1576 μM) showed the weakest inhibitory activity among all the tested compounds.

The structure activity relationship (SAR) analysis of compounds **C1**-**C6** showed that substitutions on the 5-benzylidene moiety of the 1,3-thiazolidine-2,4-dione scaffold had a marked influence on α-glucosidase inhibitory activity. All synthesized compounds contained the 1,3-thiazolidine-2,4-dione ring and a 4-fluorophenyl substituent at the N-3 position. These structural features are common to all derivatives and are likely to contribute to enzyme inhibition. Variations in inhibitory potency were mainly related to the substituents on the phenyl ring of the 5-benzylidene moiety, particularly their electron-donating (EDG) or electron-withdrawing (EWG) properties.

Among the synthesized compounds, **C1**, bearing an unsubstituted benzylidene phenyl ring, exhibited the highest inhibitory activity with an IC_{50} value of 0.1363 μM, approaching that of the reference inhibitor voglibose (IC_{50} = 0.0434 μM). The absence of a substituent on the benzylidene ring may provide a steric and electronic environment favorable for interaction with the α-glucosidase active site. Substitution of the phenyl ring generally reduced the inhibitory activity. Among the compounds containing EDGs, C3 bearing a 4-dimethylamino group exhibited moderate activity (IC_{50} = 3.6378 μM), whereas **C2**, containing a 4-methyl substituent, showed much weaker inhibition (IC_{50} = 88.1576 μM). The stronger electron-donating effect of the dimethylamino group may promote more favourable interactions with the enzyme active site, whereas the weaker electron-donating ability of the methyl group appears insufficient to maintain high inhibitory potency.

Compounds containing EWGs, namely **C4** (4-chloro), **C5** (4-fluoro) and **C6** (4-bromo), exhibited moderate α-glucosidase inhibitory activity with IC_{50} values of 4.5813, 4.2849 and 5.3662 μM, respectively. Among the halogen-substituted derivatives, **C5** displayed the highest activity, followed by **C4** and **C6**. The differences in inhibitory activity may be related to variations in electronegativity, atomic size, lipophilicity and steric effects. The lower activity of the bromo derivative may arise from the larger atomic radius of bromine, which can increase steric hindrance around the binding region.

The SAR analysis also identifies the nature of the substituent on the 5-benzylidene phenyl ring as a key factor governing α-glucosidase inhibitory activity. The 1,3-thiazolidine-2,4-dione scaffold and the 4-fluorophenyl substituent at the N-3 position were present in all active compounds and appear to provide the structural framework required for enzyme inhibition. Modifications at the 5-benzylidene phenyl ring influence inhibitory potency through electronic and steric effects. The unsubstituted benzylidene derivative (**C1**) exhibited the highest activity among the synthesized compounds, whereas weak electron-donating groups and bulky halogen substituents reduced the inhibitory potency. These results provide useful information for the future design and optimization of 1,3-thiazolidine-2,4-dione derivatives as α-glucosidase inhibitors.

Conclusion

In conclusion, a series of six 1,3-thiazolidine-2,4-dione derivatives bearing different substituents at the 5-benzylidene position were successfully synthesized while retaining a common 1,3-thiazolidine-2,4-dione core scaffold with a 4-fluorophenyl substituent at the N3 position. All synthesized compounds were evaluated for their α-glucosidase inhibitory activity. Among the synthesized derivatives, compound **C1** exhibited the most potent inhibitory activity, followed by compound **C3**, demonstrating promising potential as α-glucosidase inhibitors. SAR analysis revealed that the nature of the substituent on the 5-benzylidene phenyl ring markedly influenced the inhibitory activity. The unsubstituted benzylidene derivative (**C1**) displayed the highest potency, whereas substitution generally reduced inhibitory activity to varying extents depending on the electronic and steric properties of the substituent. These findings support the design and optimization of 1,3-thiazolidine-2,4-dione derivatives as α-glucosidase inhibitors.

ACKNOWLEDGEMENTS

This research was funded by the IMU University Joint-Committee on Research & Ethics, Project ID. MSc in Analytical and Pharmaceutical Chemistry: MAPC I-2019(01). All

authors are thankful to the IMU University Vice Chancellor, Director and Deputy Directors, Institute for Research, Development & Innovation (IRDI) and Dean and Associate Deans, School of Pharmacy, for providing the necessary research facilities to complete this research project.

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.

REFERENCES

1. M. Roden, K.F. Petersen and G.I. Shulman, in eds.: R.I.G. Holt, Insulin Resistance in Type 2 Diabetes, In: Textbook of Diabetes, John Wiley & Sons Ltd., edn 6, Chap. 17, p. 238 (2024); https://doi.org/10.1002/9781119697473.ch17
2. D. Mohajan and H.K. Mohajan, *Innov. Sci. Technol*., **3**, 23 (2024); https://doi.org/10.56397/IST.2024.01.04
3. P. Patel, D. Shah, T. Bambharoliya, V. Patel, M. Patel, D. Patel, V. Bhavsar, S. Padhiyar, B. Patel, A. Mahavar, R. Patel and A. Patel, *Med. Chem*., **20**, 503 (2024); https://doi.org/10.2174/0115734064264591231031065639
4. M. Sadeghi, M. Miroliaei and M. Ghanadian, *Int. J. Biol. Macromol*., **270**, 132164 (2024); https://doi.org/10.1016/j.ijbiomac.2024.132164
5. H.E. Lebovitz, M. Kreider and M.I. Freed, *Diabetes Care*, **25**, 815 (2002); https://doi.org/10.2337/diacare.25.5.815
6. Y. Fujita, D. Tamada, J. Kozawa, Y. Kobayashi, S. Sasaki, T. Kitamura, T. Yasuda, N. Maeda, M. Otsuki, K. Okita, H. Iwahashi, H. Kaneto, T. Funahashi, A. Imagawa and I. Shimomura, *Intern. Med*., **51**, 2581 (2012); https://doi.org/10.2169/internalmedicine.51.8171
7. R.R. Holman, J. Steemson and R.C. Turner, *Diabetes Res*., **18**, 149 (1991).
8. W.Y. Hua, N.J. Jing, W. Wan, C.X. Xuan and A.V. Rao, *Res. J. Biotechnol*., **20**, 213 (2025); https://doi.org/10.25303/203rjbt2130221
9. S.J. Seelan, D. Tharmalingam, V. Suresh, V.N.W. Yi, Y.R. Xin and V.R. Avupati, *Asian J. Chem*., **37**, 112 (2025); https://doi.org/10.14233/ajchem.2025.32826
10. A.N. Suresh, A.S.A. Luang, M.C.Y. Ling, T.N. Selvam and V.R. Avupati, *Asian J. Chem*., **36**, 2467 (2024); https://doi.org/10.14233/ajchem.2024.32383
11. A. Harunani, B.C.S. Chua, J.S. Cheong, J.Y. Chok, N.A.N. Azni, S. Santhiran, W. Shajahan, X.Y. Lai and V.R. Avupati, *Asian J. Chem*., **36**, 1429 (2024); https://doi.org/10.14233/ajchem.2024.31558
12. M.X.R. Mong, V.R. Avupati, H. Hussain and A.Q. Khalid, *Res. J. Chem. Environ*., **25**, 1 (2021); https://doi.org/10.25303/2510rjce001006