DOI 10.1007/s11094-024-03119-9
Pharmaceutical Chemistry Journal, Vol. 58, No. 1, April, 2024 (Russian Original Vol. 58, No. 1, January, 2024)

SYNTHESIS, *IN SILICO* ANALYSIS, ANTIBACTERIAL, RADICAL SCAVENGING AND ANTIDIABETIC ACTIVITIES OF CERTAIN BIS-AZETIDINONES AND BIS-THIAZOLIDINONES

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Original article submitted December 21, 2022.

A panel of 1,1-(phenylene)bis[3-chloro-4-(substituted phenyl)azetidin-2-ones] (**4a-m**) and 3,3′-(1,4-phenylene)bis[2-(substituted phenyl)thiazolidin-4-ones] (**5a-m**) were synthesized from Schiff base intermediates **3a-m**, that were in turn prepared from reaction between *p*-phenylenediamine and substituted benzaldehydes. The structures of title compounds and intermediates were confirmed by IR, ^{1}H NMR, ^{13}C NMR and mass spectral data. The compounds were screened for antibacterial, DPPH radical scavenging and antidiabetic activities. Compounds **4f** and **4a** exhibited good antibacterial activity against Gram-positive bacteria, but none showed appreciable activity against Gram-negative bacteria. In DPPH scavenging assay, compounds **5f**, **5e** and **5a** exhibited good activity. Compound **5a** displayed highly significant antidiabetic activity in fructose-induced diabetes in rats. The molecular docking studies of bis-thiazolidinones with PPAR-ã revealed the fit with high binding affinity and good interactions. Docking of compound **5a** was comparable to the standard drug pioglitazone. The *in silico* physicochemical, drug-likeness and ADME properties of title compounds were also performed and the majority of them displayed satisfactory results.

Key words: Schiff bases; MIC; DPPH; antioxidant; serum glucose; PPAR-γ; ADME; drug likeness.

The number of diabetics in the world in 2019 was 463 million and could rise to 629 million by 2045 [1]. The currently used antidiabetic agents suffer from moderate to severe adverse effects such as gastrointestinal discomfort (metformin and glucagon-like peptide-1 agonists), hypoglycemia and weight gain (sulfonylureas), an increased risk of bladder cancer, edema, heart failure, weight gain, and distal bone fractures in postmenopausal women (pioglitazone) that sometimes lead to treatment discontinuation [2]. Therefore, the development of effective and safer antidiabetic agents is the most sought-after endeavor of this time.

Thiazolidin-4-one is one of the privileged templates of biologically active compounds and closely resembles thiazolidindione in structure, which is a pharmacophore present in type 2 antidiabetic glitazone drugs. Hence, compounds with a thiazolidin-4-one scaffold were synthesized with the intention of showing good type 2 antidiabetic activity [3, 4]. In addition to antidiabetic activity, thiazolidin-4-one derivatives are also known for producing diverse pharmaceutical actions such as antioxidant [5], anti-inflammatory [6], anticancer [7], antitubercular [8], anticonvulsant [9], antimicrobial [10]. Likewise, azetidin-2-one commonly known as β-lactam, forms a key structural architecture in penicillins and cephalosporins [11], occupying a vital place among various pharmacophores due to their interesting medicinal properties such as antibacterial [12–15], antifungal [16], anticonvulsant [17], anti-inflammatory [18] and anticancer [19].

Bis-compounds play a significant role in medicinal chemistry as they offer two pharmacophore units to interact with biological targets and therefore, recently, there has been an increased interest in the synthesis of these compounds [20 – 23].

Encouraged by the aforementioned observations, it was sought to synthesize certain bis azetidin-2-ones and bis-thiazolidin-4-ones from *p*-phenylenediamine in anticipation of procuring good antibacterial and type 2 antidiabetic activities. A molecular docking study of bis-thiazolidin-4-ones with type 2 diabetes targeting peroxisome proliferative activated receptor-γ (PPAR-γ) was undertaken to understand the extent of binding. The *in silico* physicochemical, drug like-

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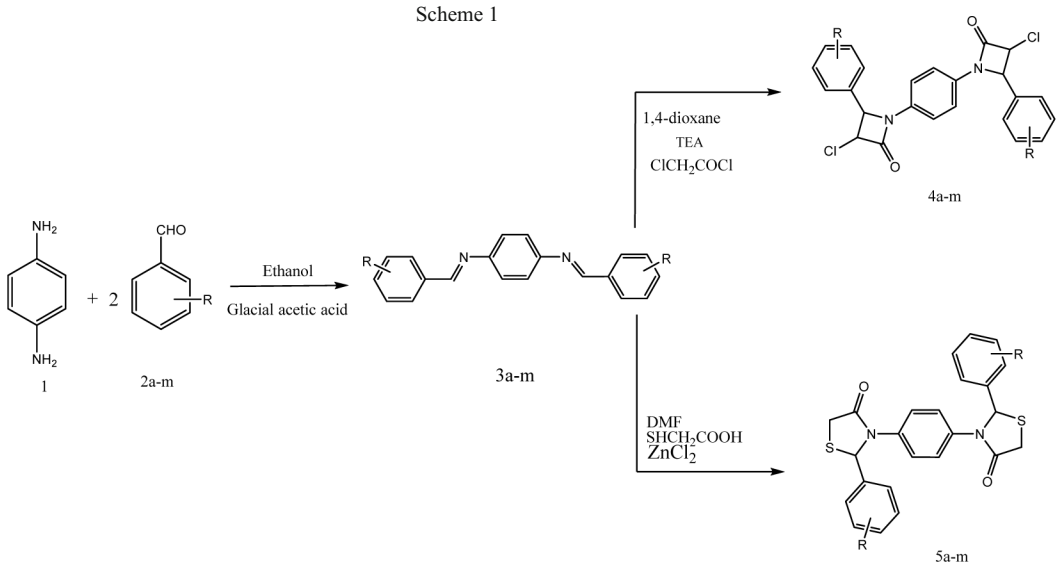
Scheme 1



R = a: H, b: 4-OH; c: 2-OH; d: 4-CH_3; e: 4-OCH_3; f: 4-$N(CH_3)_2$; g: 4-$N(C_2H_5)_2$; h: 3,4-$(OCH_3)_2$; i: 4-F; j: 4-NO_2; k: 4-Cl; l: 4-CF_3; m: 3,4-O-CH_2-O-

Synthetic route for bis-azetidinones **4a-m** and bis-thiazolidinones **5a-m**.

ness and ADME properties of title compounds were also studied. The findings of the study are embodied in this paper.

EXPERIMENTAL CHEMICAL PART

Chemicals

Chemicals used were of laboratory reagent grade without further purification. *p*-phenylenediamine, substituted benzaldehydes, 1,1-diphenyl-2-picryl-hydrazyl (DPPH), triethylamine, *N*,*N*-dimethylformamide (DMF), 1,4-dioxane, thioglycolic acid, zinc chloride, D-fructose, dimethyl sulfoxide and ascorbic acid were purchased from Loba Chemie Limited, Mumbai, SD Fine, Mumbai, Spectrochem, Mumbai, India and Sigma Aldrich, Steinheim, USA. The glucose kit was procured from Erba Mannheim, Sikkim, India. Standard antibacterial drugs ciprofloxacin and ampicillin were gifted from Wellona Pharma Private Limited, Surat, while standard antidiabetic drug pioglitazone hydrochloride came from USV Private Limited, Solan, India. Thin layer chromatography was carried out using aluminum sheets precoated with 0.2 mm thick silica gel 60 F_{254} (Merck, Darmstadt, Germany) and a combination of solvents in different ratios as the mobile phase. Spots were visualized by iodine vapors. The yield refers to crude product before recrystallization.

Instruments

Melting points were determined using open capillary apparatus (Veego VMP-DS, Mumbai, India) and are uncorrected. Ultraviolet (UV) spectra were taken on UV 1601 Shimazdu, Kyoto, Japan, infrared spectra (IR) on PerkinElmer-IF C94012 as KBr pellet. ^{1}H NMR (nuclear magnetic resonance) spectra were recorded in DMSO-d_6 on Agilent 400 MHz, California, USA while ^{13}C NMR at 50 MHz. Mass spectra were recorded by chemical ionization technique on Waters-Synapt G2, Massachusetts, USA. The following solvent systems were used to monitor reactions by TLC: (A) toluene : ethanol, 4.5:0.5 (v/v), (B) benzene : ethanol, 4:1 (v/v), (C) benzene : methanol, 4:1 (v/v).

Preparation of Schiff bases 3a-m

p-Phenylenediamine **1** (0.03 mol, 4.18 g) and substituted aldehydes **2a-m** (0.065 mol) were dissolved in 30 ml ethanol (95%), 5 ml of glacial acetic acid was added, and the mixture was refluxed for 4–5 h. After the completion of reaction as indicated by TLC (*n*-hexane:ethyl acetate 2:3), the excess ethanol was distilled off and allowed to cool. The reaction mixture was poured into a container with crushed ice and the solid obtained was filtered at the pump and washed with sufficient ice-cold water [24].

Synthesis of bis-azetidinones 4a-m

The mixture of Schiff bases **3a-m** (0.011 mol) in 10 ml 1,4-dioxane and triethylamine (0.025 mol) was stirred in an ice bath for 5-10 mins. To this solution, the mixture of chloroacetyl chloride (0.025 mol) in 1,4-dioxane (2–3 ml) was added dropwise at 0–5°C. The stirring was continued for 8 h after which the reaction mixture was allowed to stand in the dark at room temperature for 3 days. Then the mixture was poured into a container with crushed ice and the crude solid obtained was filtered and washed with ice-cold water and then recrystallized with alcohol [25].

Synthesis of bis-thiazolidinones 5a-m

To the mixture of Schiff bases **3a-m** (0.011 mol), thioglycolic acid (0.035 mol) and anhydrous zinc chloride (0.03 mol), 15 ml DMF was added, and the mixture was refluxed for 9–10 h. After the completion of the reaction, as monitored by TLC, the mixture was allowed to cool, and poured into a container with crushed ice. The solid obtained was filtered, washed with 10% $NaHCO_3$ solution until it was free from thioglycolic acid and recrystallized with alcohol [26].

1,1-(1,4-Phenylene)bis(3-chloro-4-phenylazetidin-2-one) (4a). Brown powder, yield 44.53%, R_f 0.53 (A); λ_{max} (DMSO) 294.5 nm; IR, cm^{-1}: 3098.45 (Ar-CH str), 2910.15 (Ali-CH str), 1691.24 (C=O str), 698.90 C-Cl str); ^1H NMR, δ, ppm: 7.79 – 7.77 (d, 4H, 2′& 6′ArH), 7.73 – 7.72 (d, 4H, 3′& 6′Ar-H), 7.31 (s, 4H, ArH), 7.14 – 7.01 (d, 2H, 3-CH Azt), 6.39 – 6.27 (d, 2H, 4-CH Azt); ^{13}C NMR, δ, ppm: 170.63 (2C, C=O Az), 150.63 (2C, 3′ArC), 149.43 (2C, 4′ArC), 148.46 (2C, 1′ArC), 134.84 (2C, substituted C of phenylene ring), 131.33 (4C, unsubstituted C of phenylene ring), 120.28 (2C, 2′ArC), 109.00 (2C, 3′ArC), 106.64 (2C, 6′ArC), 102.18 (2C, O-CH_2-O), 65.23 (2C, 4-C Az), 41.97 (2C, 3-C Azt); MS, *m/z*: 438.75 [M^{++}1].

1,1-(1,4-Phenylene)bis(3-chloro-4-(4-hydroxyphenyl)-azetidin-2-one) (4b). Brownish gray powder, yield 43.65%, R_f 0.30 (A); λ_{max} (DMSO) 294.50 nm; IR, cm^{-1}: 3562.54 (OH str), 3100.58 (Ar-CH str), 2905.32 (Ali-CH str), 1682.09 (C=O str), 735.76 (C-Cl); ^1H NMR, δ, ppm: 7.54 – 7.52 (d, 2H, 3-CH Azt), 6.64 – 6.54 (d, 2H, 4-CH Azt); ^{13}C NMR, δ, ppm: 170.75 (2C, C=O Az), 64.18 (2C, 4-C Az), 42.98 (2C, 3-C Az); MS, *m/z*: 469.89 [M^+].

1,1-(1,4-Phenylene)bis(3-chloro-4-(4-hydroxyphenyl)-azetidin-2-one) (4c). Brown crystals, yield 73.57%, R_f 0.70 (B); λ_{max} (DMSO) 292 nm; IR, cm^{-1}: 3576.25 (OH str), 3095.38 (Ar-CH str), 2915.89 (Ali-CH str), 1710.32 (C=O str), 833.52 (C-Cl); ^1H NMR, δ, ppm: 7.24 – 7.20 (d, 2H, 3-CH Azt), 6.34 – 6.12 (d, 2H, 4-CH Azt); ^{13}C NMR, δ, ppm: 168.75 (2C, C=O Az), 62.98 (2C, 4-C Az), 41.26 (2C, 3-C Az); MS, *m/z*: 470.19 [M^+ + 1], 471.04 [M^+ + 2].

1,1-(1,4-Phenylene)bis(3-chloro-4-tolyl)azetidin-2-one (4d). Brown crystals, yield 68.17%, R_f 0.88 (A); λ_{max} (DMSO) 294 nm; IR, cm^{-1}: 3047.38 (Ar-CH str), 2927.89 (Ali-CH str), 1691.32 (C=O str), 833.52 (C-Cl); ^1H NMR, δ, ppm: 7.79 – 7.77 (d, 4H, 2′& 6′ ArH), 7.73 – 7.72 (d, 4H, 3′& 6′Ar-H), 7.31 (S, 4H, H of phenylene ring), 7.24 – 7.09 (d, 2H, 3-CH Azt), 6.59 – 6.57 (d, 2H, 4-CH Azt), 2.36 (s, 6H, -CH_3); ^{13}C NMR, δ, ppm: 168.95 (2C, C=O Az), 66.78 (2C, 4-C Az), 42.71 (2C, 3-C Az); MS, *m/z*: 466.76 [M^+ + 1].

1,1-(1,4-Phenylene)bis(3-chloro-4-(4-methoxyphenyl)-azetidin-2-one) (4e). Black powder, yield 42.93%, R_f 0.35 (A); λ_{max} (DMSO) 294.9 nm; IR, cm^{-1}: 3089.68 (Ar-CH str), 2911.54 (Ali-CH str), 1720.25 (C=O), 1249.73 (Ar-C-O-C str), 834.09 (C-Cl str); ^1H NMR, δ, ppm: 7.48 – 7.44 (d, 2H, 3-CH Azt), 6.35 – 6.34 (d, 2H, 4-CH Azt), 3.92 (s, 6H, OCH_3); ^{13}C NMR, δ, ppm: 170.08 (2C, C=O Az), 65.48 (2C, 4-C Az), 55.4 (2C, OCH_3), 42.71 (2C, 3-C Az); MS, *m/z*: 497.98 [M^+].

1,1-(1,4-Phenylene)bis(3-chloro-4-(4-(dimethylamino)-phenyl)azetidin-2-one) (4f). Brown crystals, yield 73.57%, R_f 0.16 (B); λ_{max} (DMSO) 212.50 nm; IR, cm^{-1}: 3057.02 (Ar-CH str), 2908.45 (Ali-CH str), 1597.88 (C=O str), 719.28 (C-Cl str); ^1H NMR, δ, ppm: 7.53 – 7.49 (d, 2H, 3-CH Azt), 6.45 – 6.41 (d, 2H, 4-CH Azt), 3.89 (s, 6H, OCH_3); ^{13}C NMR, δ, ppm: 169.98 (2C, C=O Az), 62.48 (2C, 4-C Az), 42.71 (2C, 3-C Az); MS, *m/z*: 527.78 [M^+ + 1].

1,1-(1,4-Phenylene)bis(3-chloro-4-(4-(diethylamino)-phenyl)azetidin-2-one) (4g). Black powder, yield 38.16%, R_f 0.35 (A); λ_{max} (DMSO) 297 nm; IR, cm^{-1}: 597.88 (C=O str), 735.24 (C-Cl str); ^1H NMR, δ, ppm: 7.52 – 6.73 (m, 12H, Ar-H), 7.64 – 7.62 (s, 2H, 3-CH Azt), 6.75 – 6.73 (d, 2H, 4-CH Azt), 3.45 – 3.30 (q, 8H, CH_2), 1.12 – 1.05 (m, 12H, CH_3); ^{13}C NMR, δ, ppm: 168.78 (2C, C=O Az), 64.19 (2C, 4-C Az), 43.67 (2C, 3-C Az); MS, *m/z*: 580.12 [M^+ + 1].

1,1-(1,4-Phenylene)bis(3-chloro-4-(3,4-dimethoxyphenyl)azetidin-2-one) (4h). Brown powder, yield 61.07%, R_f 0.41 (A); λ_{max} (DMSO) 295.5 nm; IR, cm^{-1}: 3076.25 (Ar-CH str), 2940.25 (Ali- CH str), 1690.25 (C=O str), 1261.19 (Ar-C-O-C str), 813.25 (C-Cl str); ^1H NMR, δ, ppm: 7.58 (s, 2H, 3-CH Azt), 6.80 – 6.75 (d, 2H, 4-CH Azt); ^{13}C NMR, δ, ppm: 169.78 (2C, C=O Az), 66.19 (2C, 4-C Az), 45.67 (2C, 3-C Az); MS, *m/z*: 557.98 [M^+].

1,1-(1,4-Phenylene)bis(3-chloro-4-(4-fluorophenyl)-azetidin-2-one) (4i). Dark brown powder, yield 54.77, R_f 0.38(B); λ_{max} (DMSO) 295.5 nm; IR, cm^{-1}: 1664.25 (C=O str), 1245.19 (Ar-C-O-Cstr), 812.95 (C-Cl str); ^1H NMR, δ, ppm: 7.43 – 6.81 (m, 2H, 3-CH Azt & 12H, Ar-H), 4.20 (s, 2H, 4-CH Azt); ^{13}C NMR, δ, ppm: 170.78 (2C, C=O Az), 63.28 (2C, 4-C Az), 42.13 (2C, 3-C Az); MS, *m/z*: 470.76 [M^+], 471.41 [M^+ + 1].

1,1-(1,4-Phenylene)bis(3-chloro-4-(4-nitrophenyl)-azetidin-2-one) (4j). Reddish brown powder, yield 88.01%, R_f 0.28 (A); λ_{max} (DMSO) 297.00 nm; IR, cm^{-1}: 3056.59 (Ar-CH str), 2915.36 (Ali-CH str), 1665.25 (C=O), 1510.53 (NO asym str), 1342.04 (NO sym str), 691.07 (C-Cl str); ^1H NMR, δ, ppm: 7.35 (s, 2H, 3-CH Azt), 4.08 (s, 2H, 4-CH Azt); ^{13}C NMR, δ, ppm: 168.14 (2C, C=O Az), 61.92 (2C, 4-C Az), 43.83 (2C, 3-C Az); MS, *m/z*: 528.56 [M^+ + 1].

1,1-(1,4-Phenylene)bis(3-chloro-4-(4-chlorophenyl)-azetidin-2-one) (4k). Brownish gray powder, yield 66.66%, R_f 0.32 (C); λ_{max} (DMSO) 294.5 nm; IR, cm^{-1}: 3011.68 (Ar-CH str), 2971.31 (Ali-CH str), 1664.15 (C=O str), 830.12 (C-Cl); ^1H NMR, δ, ppm: 7.89 (s, 2H, 3-CH Azt), 4.20 (s, 2H, 4-CH Azt); ^{13}C NMR, δ, ppm: 170.78 (2C, C=O Az), 63.28 (2C, 4-C Az), 42.13 (2C, 3-C Az); MS, *m/z*: 470.76 [M^+], 471.41 [M^+ + 1].

1,1-(1,4-Phenylene)bis[3-chloro-4-(4-(trifluoromethyl)-phenyl)azetidin-2-one] (4l). Brownish gray powder, yield 71.42%, R_f 0.19 (C), λ_{max} (DMSO) 294 nm, IR, cm^{-1}: 3097.56 (Ar-CH str), 2918.25 (Ali-CH str), 1665.11 (C=O), 1124.23 (C-F str), 831.60 (C-Cl str); ^{1}H NMR, δ, ppm: 7.32 (s, 2H, 3-CH Azt), 3.98 (s, 2H, 4-CH Azt); ^{13}C NMR, δ, ppm: 168.18 (2C, C=O Az), 62.14 (2C, 4-C Az), 43.87 (2C, 3-C Az); MS, *m/z*: 572.56 [M^+], 573.89 [$M^+ + 1$], 574.95 [$M^+ + 2$].

1,1-(2,4-Phenylene)bis[4-(benzo[*d*][1, 3]dioxol-5-yl)3-chlroazetidin-2-one] (4m). Brown powder, yield 69.80%, R_f 0.46 (C); λ_{max} (DMSO) 296.5 nm; IR, cm^{-1}: 3056.59 (Ar-CH str), 2915.36 (Ali-CH str), 1665.25 (C=O), 1510.53 (NO asym str), 1342.04 (NO sym str), 691.07 (C-Cl str); ^{1}H NMR, δ, ppm: 7.53 – 7.02 (m, 4H, H of phenylene ring & 6H, 2′,5′& 6′ ArH), 6.14 (s, 4H, CH_2, O-CH_2-O), 6.10 – 6.09 (d, 2H, 3-CH Azt), 4.21(s, 2H, 4-CH Azt); ^{13}C NMR, δ, ppm: 164.63 (2C, C=O Az), 150.63 (2C, 3′ArC), 149.43 (2C, 4′ArC), 148.46 (2C, 1′ArC), 134.84 (2C, substituted C of phenylene ring), 131.33 (4C, unsubstituted C of phenylene ring), 120.28 (2C, 2′ArC), 109.00 (2C, 3′ArC), 106.64 (2C, 6′ArC), 102.18 (2C, O-CH_2-O), 43.97 (2C, 3-C Azt); MS, *m/z*: 524.81 [$M^+ + 1$].

3,3′-(1,4-Phenylene)bis(2-phenylthiazolidin-4-one) (5a). Grayish yellow powder, yield 89.69%, R_f 0.67 (A); λ_{max} (DMSO) 293.5 nm; IR, cm^{-1}: 3005.20 (Ar-CH str), 2920.20 (Ali-CH str), 1658.95 (C=O str), 615.19 (C-S str); ^{1}H NMR, δ, ppm: 6.43 (s, 2H, 3-CH Thiaz), 4.29 – 4.27 (d,4H, 5-CH_2 Thiaz); ^{13}C NMR, δ, ppm: 170.30 (2C, C=O Thiaz), 64.32 (2C, 2-C Thiaz), 33.05 (2C, 5-C Thiaz); MS, *m/z*: 434.56 [$M^+ + 2$].

3,3′-(1,4-Phenylene)bis(2-phenylthiazolidin-4-one) (5b). Brown crystals, yield 94.18%, R_f 0.46 (B); λ_{max} (DMSO) 293.10 nm; IR, cm^{-1}: 3075.36 (Ar-CH str), 2956.16 (Ali-CH str), 1613.07 (C=O str), 678.24 (C-S str); ^{1}H NMR, δ, ppm: 9.33 (s, 2H, OH), 6.48 (s, 2H, 2-C Thiaz), 3.16 – 3.10 (q, 4H, 5-CH_2Thiaz); ^{13}C NMR, δ, ppm: 168.42 (2C, C=O Thiaz), 64.32 (2C, 2-C Thiaz), 33.05 (2C, 5-C Thiaz); MS, *m/z*: 465.5 [$M^+ + 1$].

3,3′-(1,4-Phenylene)bis(2-(2-hydroxyphenyl)thiazolidin-4-one) (5c). Yellow crystals, yield 94.18%, R_f 0.60 (A); λ_{max} (DMSO) 293.5 nm; IR, cm^{-1}: 1658.95 (C=O str), 615.19 (C-S str); ^{1}H NMR, δ, ppm: 9.45 (s, 2H, OH), 6.39 (s, 2H, 2-C thiaz), 4.16 – 4.10 (q, 4H, 5-CH_2 Thiaz); ^{13}C NMR, δ, ppm: 170.86 (2C, C=O Thiaz), 63.27 (2C, 2-C Thiaz), 32.14 (2C, 5-C Thiaz); MS, *m/z*: 464.89 [M^+].

3,3′-(1,4-Phenylene)bis[2-(4-tolyl)thiazolidin-4-one] (5d). Brownish yellow powder, yield 91.61%, R_f 0.41 (A); λ_{max} (DMSO) 293.75 nm; IR, cm^{-1}: 1658.95 (C=O str), 615.19 (C-S str); ^{1}H NMR, δ, ppm: 6.39 (s, 2H, 2-CH Thiaz), 3.99 – 3.74 (q, 4H, 5-CH_2 Thiaz); ^{13}C NMR, δ, ppm: 170.30 (2C, C=O Thiaz), 64.32 (2C, 2-C Thiaz), 33.05 (2C, 5-C Thiaz); MS, *m/z*: 460.58 [M^+].

3,3′-(1,4-Phenylene)bis[2-(4-methoxyphenyl)thiazolidin-4-one] (5e). Brown crystals, yield 98.61%, R_f 0.26 (A); λ_{max} (DMSO) 293.53 nm; IR, cm^{-1}: 1695.26 (C=O), 1245.99 (Ar-C-O-C str), 610.84 (C-S str); ^{1}H NMR, δ, ppm: 6.27 (s, 2H, 2-CH Thiaz), 3.54 – 3.49 (q, 4H, 5-CH_2 Thiaz); ^{13}C NMR, δ, ppm: 167.56 (2C, C=O Thiaz), 66.12 (2C, 2-C Thiaz), 32.75 (2C, 5-C Thiaz); MS, *m/z*: 492.76 [M^+], 494.76 [$M^+ + 2$].

3,3′-(1,4-Phenylene)bis[2-(4-(dimethylamino)phenyl)-thiazolidin-4-one] (5f). Greenish gray powder, yield 98.05%, R_f 0.88 (B), λ_{max} (DMSO) 311.1 nm; IR, cm^{-1}: 1710.25 (C=O str), 1164.59 (C-N str), 610.15 (C-S str); ^{1}H NMR, δ, ppm: 6.43 (s, 2H, 2-CH Thiaz), 3.61 – 3.56 (q, 4H, 5-CH_2Thiaz); ^{13}C NMR, δ, ppm: 169.87 (2C, C=O Thiaz), 65.23 (2C, 2-C Thiaz), 31.25 (2C, 5-C Thiaz); MS, *m/z*: 519.3 [$M^+ + 1$].

3,3′-(1,4-Phenylene)bis[2-(4-(diethylamino)phenyl)-thiazolidin4-one] (5g). Brown powder, yield 95.45%, R_f 0.26 (A); λ_{max} (DMSO) 293.5 nm; IR, cm^{-1}: 1712.03 (C=O str), 1172.65 (C-N str), 635.32 (C-S str); ^{1}H NMR, δ, ppm: 6.35 (s, 2H, 2-CH Thiaz), 3.42 – 3.38 (q, 4H, 5-CH_2 Thiaz); ^{13}C NMR, δ, ppm: 171.07 (2C, C=O Thiaz), 63.94 (2C, 2-C Thiaz), 32.55 (2C, 5-C Thiaz); MS, *m/z*: 576.3 [$M^+ + 2$].

3,3′-(1,4-Phenylene)bis[2-(3, 4-dimethoxyphenyl)-thiazolidin-4-one] (5h). Yellow powder, yield 90.21%, R_f 0.67 (B); λ_{max} (DMSO) 293.54 nm; IR, cm^{-1}: 1658.95 (C=O str), 1261.19 (Ar-C-O-C str), 615.19 (C-S str); ^{1}H NMR, δ, ppm: 6.15 (s, 2H, 3-CH Thiaz), 4.12 – 4.06 (q, 4H, 5-CH_2Thiaz); ^{13}C NMR, δ, ppm: 171.06 (2C, C=O Thiaz), 74.52 (2C, 2-C Thiaz), 46.78 (2C, 5-C Thiaz); MS, *m/z*: 553.12 [$M^+ + 1$].

3,3′-(1,4-Phenylene)bis[2-(4-fluorophenyl)thiazolidin-4-one] (5i). Dark brown powder, yield 97.47%, R_f 0.19 (B); λ_{max} (DMSO) 295.57 nm; IR, cm^{-1}: 1686.56 (C=O str), 1222.70 (C-F str), 608.33 (C-S str); ^{1}H NMR, δ, ppm: 6.26 (s, 2H, 3-CH Thiaz), 4.21 – 4.17 (q, 4H, 5-CH_2 Thiaz); ^{13}C NMR, δ, ppm: 169.13 (2C, C=O Thiaz), 68.52 (2C, 2-C Thiaz), 45.78 (2C, 5-C Thiaz); MS, *m/z*: 470.12 [$M^+ + 2$].

3,3′-(1,4-Phenylene)bis[2-(4-nitrophenyl)thiazolidin-4-one] (5j). Brown powder, yield 99.04%, R_f 0.33 (A); λ_{max} (DMSO) 299.59 nm; IR, cm^{-1}: 1686.56 (C=O str), 1532.22 (NO asym str), 608.33 (C-S str); ^{1}H NMR, δ, ppm: 6.74 (s, 2H, 3-CH Thiaz), 3.90 – 3.80 (q, 4H, 5-CH_2 Thiaz); ^{13}C NMR, δ, ppm: 170.27 (2C, C=O Thiaz), 66.42 (2C, 2-C Thiaz), 42.96 (2C, 5-C Thiaz); MS, *m/z*: 471.14 [$M^+ + 1$].

3,3′-(1,4-Phenylene)bis[2-(4-chloropheyl)thiazolidin-4-one] (5k). Brownish yellow powder, yield 95.44%, R_f 0.31 (B); λ_{max} (DMSO) 294.34 nm; IR, cm^{-1}: 1653.47 (C=O str),1036.21 (Ar C-Cl str), 619.24 (C-S str); ^{1}H NMR, δ, ppm: 6.49 – 6.40 (m, 2H, 2-CH Thiaz), 3.87 – 3.76 (dd, 4H, 5-CH_2Thiaz); ^{13}C NMR, δ, ppm: 170.75 (2C, C=O Thiaz), 63.64 (2C, 2-C Thiaz), 33.09 (2C, 5-C Thiaz); MS, *m/z*: 502.35 [$M^+ + 1$], 503.78 [$M^+ + 3$].

3,3′-(1,4-Phenylene)bis[2-(4-(trifluoromethyl)phenyl)thiazolidin-4-one] (5l). Yellow powder, yield 89.26%, R_f 0.35 (B); λ_{max} (DMSO) 295.50 nm; IR, cm^{-1}: 1715.36 (C=O str), 1162.65 (C-F str), 1064.70 (C-N str), 611 (C-S str); ^{1}H NMR, δ, ppm: 6.58 (s, 2H, 2-CH Thiaz), 3.91 – 3.84 (dd, 4H 5-CH_2 Thiaz); ^{13}C NMR, δ, ppm: 170.83 (2C, C=O Thiaz), 65.05 (2C, 2-C Thiaz), 32.96 (2C, 5-C Thiaz); MS, *m/z*: 568.97 [M^+], 570.12 [$M^+ + 2$].

3,3′-(1,4-Phenylene)bis[2-(benzo[*d*][1, 3]dioxol-5-yl)-thiazolidin-4-one] (5m). Brown color powder, yield 94.25%, R_f 0.35 (B); λ_{max} (DMSO) 295.50 nm; IR, cm^{-1}: 1681.23 (C=O str) 1022.76 (C-N str), 634.86 (C-S str); ^{1}H NMR, δ, ppm: 6.45 (s, 2H, 2-C Thiaz), 5.94 (s, 4H, O-CH_2-O), 3.87 – 3.76 (dd, 4H, 5-CH_2 Thiaz);^{13}C NMR, δ, ppm: 168.94 (2C, C=O Thiaz), 64.97 (2C, 2-C Thiaz), 33.12 (2C, 5-C Thiaz); MS, *m/z*: 568.97 [M^+], 521.56 [$M^+ + 1$].

EXPERIMENTAL BIOLOGICAL PART

Bacterial strains

The clinical bacterial strains *Staphylococcus aureus* (ATCC 25923), *Enterococcus faecalis* (ATCC 29212) [Gram-positive], *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 25922) [Gram-negative] were used for antibacterial screening.

Animals

Male Sprague Dawley rats having body weight between 250 and 350g and Swiss albino mice of either sex having body weight 25 to 40 g were obtained from the central animal house of HSK College of Pharmacy, Bagalkote and were employed for antidiabetic and acute oral toxicity studies. The animals were housed under 25 ±1°C temperature, 50–55% relative humidity for 12 h dark and light cycle. Animals were acclimatized for 7 days before experimentation. They were given standard laboratory feed (Pranava Agro Industries Ltd., Sangli, India) and water *ad libitum.* The food was withdrawn 18 h before the experiment. The experiments were performed on randomly formed groups during the light phase and the animals were used for one experiment only. The test compounds and standard drugs were administered *p.o.* as aqueous suspension in 0.5% Tween 80. The animals in the vehicle control group received 0.5% aqueous Tween-80. The study was conducted according to the guidelines of the Institutional Animal Ethics Committee (IAEC) constituted as per committee for the purpose of control and supervision of experiments on animals, Ministry of Environment and Forestry, Government of India, after obtaining approval by IAEC (F. No. HSK College of Pharmacy Bagalkote/IAEC//HSKCOP/March 2020/PG10).

Antibacterial activity

Minimum inhibitory concentration (MIC) of the compounds was measured by the broth dilution method [27]. Compounds weighing 2 mg were dissolved in 1 ml DMSO and later the required dilutions were prepared in brain heart infusion (BHI) broth as 1/10 dilution i.e., 200 µg/ml. The 16–18 h old cultures were used, and 0.5 McFarland turbidity-adjusted inoculum was prepared. The 24-hour-old culture of the test bacterial strains was diluted 100 folds in BHI broth (100 µL bacterial cultures in 10 ml BHI broth). Increasing concentrations of the test compounds ranging from 12.5, 25, 50 and 100 µg were added to the test tubes containing the bacterial cultures. All the tubes were inoculated with prepared inoculum and incubated at 37°C for 24 h. The tubes were examined for visible turbidity against the BHI broth as a control. The MIC was recorded as the lowest concentration that inhibited visible growth of the tested organisms.

Free radical scavenging activity

A stock solution of DPPH (0.01 mM) in ethanol was prepared. The various concentrations such as 5, 10, 20, 40 and 80 µg/ml of bis-thiazolidinones **5a-m** were prepared. For all the sample solutions 2-ml stock solution of DPPH was added and the volume was made up to the 4 ml mark with ethanol. The reaction mixtures were allowed to stand for 30 min in dark and absorbance was recorded at 517 nm against reagent blank. Ascorbic acid was used as a reference compound. The experiment was carried out in triplicate [27].

The effective concentrations of the sample required to scavenge DPPH radical by 50% were obtained by linear regression analysis between concentrations. The free radical scavenging activity was calculated using the formula.

$$\% \text{ scavenging} = \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \times 100$$

Acute oral toxicity

Acute oral toxicity was performed according to the OECD guidelines 425 [28]. Mice of either sex were selected for this study and the animals were weighed and experimental compounds were administered initially at a dose of 2000 mg/kg b.w. in the form of aqueous suspension using 0.5% tween 80 as a suspending agent orally and observed for 4 h after administration and were observed for death for14 days.

Antidiabetic activity

The antidiabetic activity was carried out by fructose-induced diabetes model [29]. The adult health Sprague Dawley rats were fed 30% fructose in drinking water for 30 days and serum glucose level was estimated. The animals that exhibited hyperglycemia were selected for further treatment. The selected rats were allowed to receive fructose until the end of

the study. The animals were divided into 6 groups, each consisting of six rats. Group 1 (normal): received the water orally; Group 2 (control): received 30% fructose solution and 0.5 % aqueous tween 80 p.o; Group 3 (standard): received 30% fructose solution and pioglitazone (15 mg/kg b.w.) aqueous suspension with 0.5 % tween 80 p.o; Group 4 (Diabetic): received 30% fructose solution and compound 5a (15 mg/kg b.w.) in 0.5% tween 80 as aqueous suspension p.o; Group 5 (diabetic): received 30% fructose solution and compound 5f (200 mg/kg b.w.) in 0.5% tween 80 as aqueous suspension p.o; Group 6 (diabetic): received 30% fructose solution and compound 5g (200 mg/kg b.w.) in 0.5% tween 80 as aqueous suspension p.o. Serum glucose level was estimated by collecting approximately 1ml of blood from retro-orbital flexus of the animals under diethyl ether anesthesia and centrifuged at 3500 RPM for 15 min to obtain the serum [30]. Serum glucose was estimated as follows:

Pipette out into test tube and labeled as	Blank	Glucose Standard	Test
Sample serum	-	-	10 µl
Standard	-	10 µl	-
Glucose reagent	1 ml	1 ml	1 ml

Each addition was well mixed and incubated at 37°C for 10 min. The absorbances of the standard and the samples were read against the reagent blank at 505 nm.

TABLE 1. Antibacterial activity of bis-azetidinones **4a-m**

Compound	MIC, µg/ ml			
	Staphylococcus aureus	*Enterococcus faecalis*	*Escherichia coli*	*Pseudomonas aeruginosa*
4a	<12.5	50	100	-
4b	25	-	-	-
4c	-	-	-	-
4d	-	-	-	-
4e	100	50	-	-
4f	<12.5	25	25	100
4g	-	-	-	-
4h	-	-	-	-
4i	100	100	-	-
4j	-	-	-	-
4k	25	100	-	-
4l	-	-	-	-
4m	50	100	100	-
Ciprofloxacin	0.12 – 0.15	0.25 – 2	0.004 – 0.0016	0.12 – 1
Ampicillin	2.5 – 3.0	1.2 – 1.5	12.5 – 12.7	15.2 – 15.4

"-"indicates no activity or activity at >100 µg/ ml concentration.

$$\text{Concentration of glucose} = \frac{\text{Absorbances of sample}}{\text{Absorbances of standard}} \times \text{Concentration of standard (100 mg / dl)}$$

Statistics

The data of the animal experiments were expressed as mean± standard error of mean (SEM) and analyzed by Graphpad prism version 8.0.0 (Graphpad Software, San Diego, California USA). The statistical difference between the treatments and control were tested by analysis of variance, followed by Dunnett's multiples comparison test. Data with p-value < 0.01 was considered as significant.

Molecular Docking

The three-dimensional crystal structure of peroxisome proliferator activator receptor gamma (PPARγ) with PDB ID: 4PRG [31] was downloaded from RCSB protein data bank PDB. Water molecules in 4PRGwere removed followed by the addition of Kollaman's charges and polar hydrogens using AutoDock 1.5.6 tools [32]. The structures of the ligand were drawn by using ChemDraw 16.0 and copied as SMILES. The Avogadro tool [33] was used to generate the.pdb file format of the ligand with energy minimization. All structures were converted into.pdbqt format for input into AutoDock 1.5.6 tools. The grid file was generated and saved as.gpf file format. The Lamarckian Genetic Algorithm (LGA) was used to find the conformers with the lowest binding energies and the.dpf file was generated to dock. The final docking task was performed by using two commands: autogrid4.exe –p 4PRG.gpf –l 4PRG.glg and autodock4.exe –p ligand.dpf –l ligand.dlg. After completion of the docking

TABLE 2. DPPH scavenging activity of bis-thiazolidinones **5a-m**

Entry	IC_{50}, µg/ ml
5a	21.36
5b	48.94
5c	44.97
5d	47.76
5e	20.63
5f	20.19
5g	43.83
5h	34.75
5i	68.61
5j	118.4
5k	31.51
5l	51.99
5m	93.111
Ascorbic acid	12.38

TABLE 3. Antidiabetic activity of bis-thiazolidinones **5a, 5f** and **5g**

Groups	Serum glucose, mg/dl				
	0 day	3rd day	7th day	14th day	21stday
Normal (Normal saline 10 ml/kg b.w.)	85.74 ± 7.09	89.43 ± 5.40	85.28 ± 4.05	90.08 ± 3.605	86.46 ± 5.48
Control (30% w/v D-fructose in water *ad libitum*)	186.51 ± 19.37[a]	202.4 ± 11.13[a]	193.5 ± 8.39[a]	191.7 ± 7.664[a]	179.0 ± 3.14[a]
Compound **5a** (15 mg/kg b.w.p.o.)	147.8 ± 9.07	155.3 ± 3.11***	84.46 ± 1.78***	96.82 ± 0.77***	149.1 ± 4.211**
% reduction in serum glucose	20.75	23.27	56.35	49.49	20.05
Compound **5f** (100 mg/kg b.w.p.o.)	172.4 ± 14.14	168.9 ± 6.40***	117.3 ± 5.41***	172.7 ± 6.55*	156.0 ± 7.093*
% reduction in serum glucose	7.56	16.55	39.37	9.90	12.84
Compound **5g** (100 mg/kg b.w.p.o.)	181.7 ± 12.70	177.5 ± 5.03	162.8 ± 9.97**	171.9 ± 3.45*	156.2 ± 5.634*
% reduction in serum glucose	2.57	12.50	16.17	10.23	12.73
Standard Pioglitazone (15mg/kg b.w.p.o.)	152.7 ± 21.15	144.8 ± 9.23***	89.67 ± 3.96***	86.40 ± 4.18***	86.53 ± 2.737***
% reduction in serum glucose	18.12	28.45	53.35	54.92	51.65

All the values are expressed as mean ± SEM, $n = 6$, [a] $p < 0.05$ as compared to the normal group and *** $p < 0.0001$, ** $p < 0.001$ and * $p < 0.01$ as compared to the control group (One-way analysis of Variance (ANOVA) followed by Multiple Comparison Dunnett's test).

the file was saved in.dlg format and was analyzed for different binding conformations. The highest binding energy of conformation of the ligand was selected and visualized in Biovia Discovery Studio Client 2021 [34].

Physicochemical, Drug Likeness and ADME Properties

The ADME properties and drug likeness of title compounds were predicted by Swiss Prediction web tool [35]. The structures of synthesized compounds and standard drug were drawn in ChemDraw 16.0 and copied as SMILES and then submitted to Swiss prediction web tool to predict the physicochemical, ADME and drug likeness properties.

RESULTS AND DISCUSSION

A series of Schiff bases **3a-m** was prepared by condensing aromatic aldehydes with *p*-phenylenediamine in the presence of glacial acetic acid in good to excellent yields. The IR spectra of the compounds **3a-m** showed the characteristic imine C=N stretching frequency of Schiff bases between 1588–1622cm^{-1}, among others. The Schiff bases **3a-m** were allowed to react with chloroacetyl chloride in the presence of triethylamine in 1,4-dioxane by Staudinger′s reaction to yield 1,1′-(phenyene)bis[3-chloro-4-(R-substituted phenyl)azetidin-2-ones] **4a-m**. The compounds **3a-m** were also allowed to react with thioglycolic acid in the presence of zinc chloride in DMF to yield 3,3′-(1,4-phenylene)bis[2-(R-substituted phenyl)thiazolidin-4-ones] **5a-m**. The formation bis-azetidinones **4a-m** was confirmed by the presence of C=O stretching at 1665 – 1720 cm^{-1}, C-Cl stretching at 691 – 834 cm^{-1} in IR spectrum and the appearance of the protons of C-3 and C-4 of the azetidinone ring as a doublet in the range of δ 6.09 – 7.24 and 4.20 – 6.59, respectively, in ^{1}H NMR spectra. The structure of bis-thiazolidinones was confirmed by ^{1}H NMR, wherein the methylene protons of C-5 thiazolidinone ring resonated as either a doublet of a doublet or a quartet at δ 3.10 – 4.29. The proton of thiazolidinone C-2 resonated at δ 6.15 – 6.95. The presence of bands due to C-S stretching at 610.15 – 635.32 cm^{-1}, C=O stretching at 1658.95 – 1715.32 cm^{-1}, and C-N stretching at 1164.59 – 1172.65 cm^{-1} in IR spectrum, also suggested the formation of compounds **5a-m**. The synthesis of title compounds **4a-m** and **5a-m** is illustrated in Scheme 1.

TABLE 4. Interactions of **5a, 5f** and **5g** with PPAR-γ

Compound	Bindingenergy, Kcal/mol	Amino acid residues
5[a]	-7.68	LYS(C:265), GLU(C:343), ILE (C:281), ARG(C:288)
5f	-8.02	GLU(C:291), ILE (C:281), MET(C:348), ILE(C:341), PHE(C:287)
5g	-7.45	LEU(A:421), PHE (A:432) LYS(B:422), GLU(A:418), SER(B:428)
Pioglitazone	-7.12	GLU(B:343), LYS(C:263), LEU(C:255), MET(C:256), ILE(C:281), ARG(C:280), PHE(C:264)

Antibacterial activity

Since the compounds **4a-m** contain azetidinone moiety, a well-known pharmacophore present in β-lactam antibiotics, they were screened for antibacterial activity against *Staphylococcus aureus*, *Enterococcus faecalis*, *Pseudomonas aeru-*

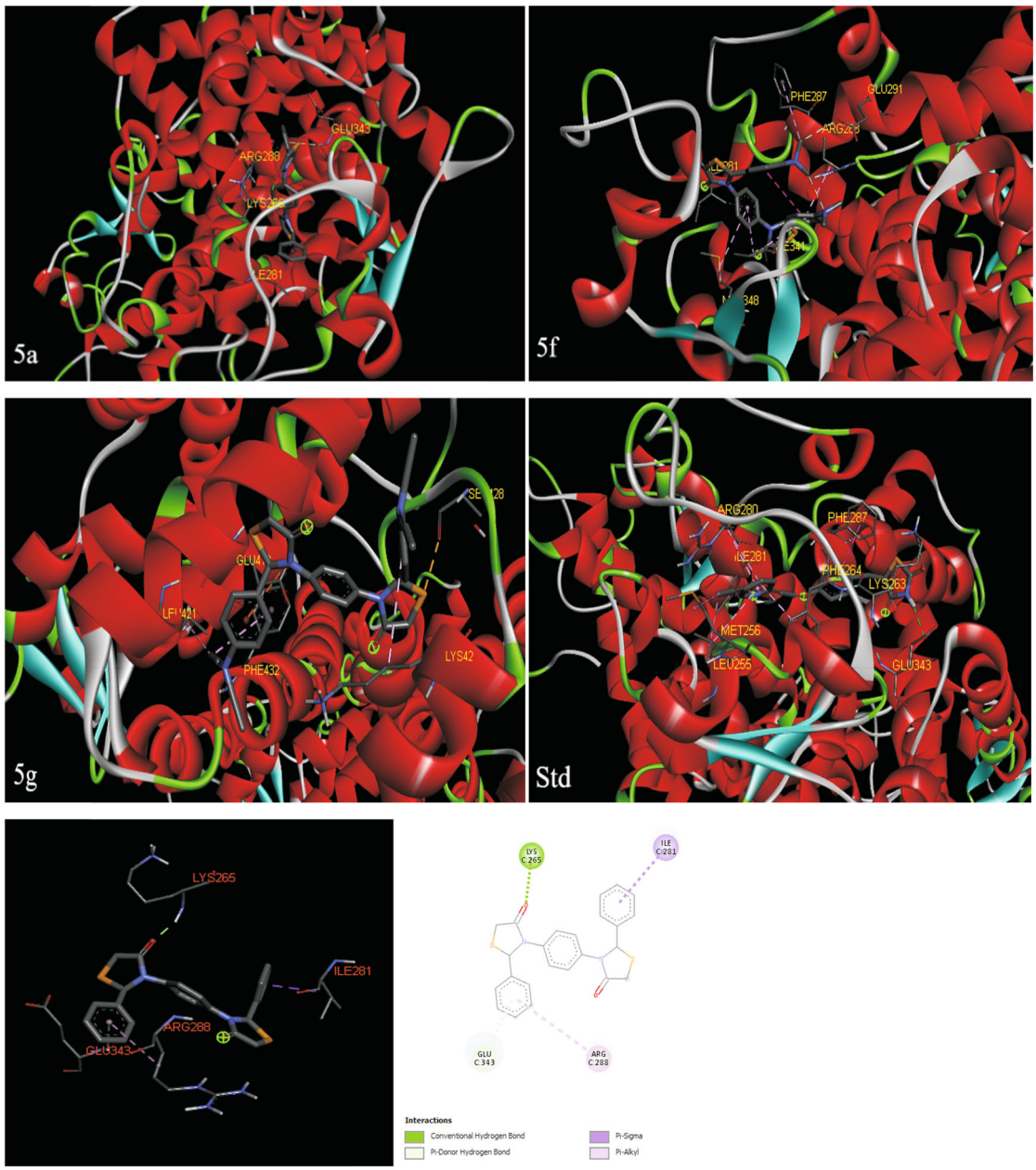
ginosa, *Escherichia coli* by the broth dilution method and the results are tabulated in Table 1. Compounds **4a** and **4f** have shown good activity against *Staphylococcus aureus* with MIC <12.5 µg/ ml followed by compounds **4b** and **4k** with 25 µg/ ml. Compounds **4f**, **4a** and **4e** exhibited moderate antibacterial activity against *Enterococcus faecalis* when compared with standard ciprofloxacin and ampicillin. The rest of the compounds have either shown weak or failed to show any appreciable antibacterial activity. Only compound **4f** displayed weak antibacterial activity against *E. coli*. None of the compounds exhibited any activity against *P. aeruginosa.* Overall, the bis-azetidinones **4a-m** did not exhibit antibacterial activity in the expected line.

DPPH radical scavenging assay

Increased oxidative stress is known to be associated with diabetes mellitus and plays an important role in neuropathy and microangiopathy [17], the long-term complications of diabetes. T2DM is associated with increased oxidative stress resulting from several abnormalities, including hyperglycemia, inflammation, and dyslipidemia. The compounds **5a-m** were tested for DPPH radical scavenging activity and the results are presented in Table 2. The dimethylamino derivative **5f** (IC_{50} = 20.19 µg/ ml), methoxy derivative **5e** (IC_{50} = 20.63 µg/ ml) and unsubstituted compound **5a** (IC_{50} = 21.36 µg/ ml) have shown good activity and chloro deriva-

TABLE 5. Predicted physicochemical, ADME and drug likeness properties of compounds **4a-m** and **5a-m**

Compounds	Physicochemical properties							Lipophilicity						Drug likeness					Pharmacokinetics	
	MW	Fsp3	RB	HBA	HBD	MR	TPSA	iLogP	XlogP3	WlogP	MlogP	Silicos-IT	CLogP	Lipinski	Ghose	Veber	Egan	Muegge	Log KP (cm/s)	F
4a	437.32	0.17	4	2	0	125.08	40.62	3.35	4.56	3.67	4.76	4.18	4.1	1	0	0	0	0	-5.73	0.55
4b	469.32	0.17	4	4	2	129.12	81.08	2.62	3.85	3.08	3.65	3.21	3.28	0	0	0	0	0	-6.43	0.55
4c	469.32	0.17	4	4	2	129.12	81.08	2.76	3.85	3.08	3.65	3.21	3.31	0	0	0	0	0	-6.43	0.55
4d	465.37	0.23	4	2	0	135.01	40.62	3.86	5.29	4.28	5.16	5.22	4.76	1	1	0	0	1	-5.38	0.55
4e	497.37	0.23	6	4	0	138.06	59.08	3.93	4.5	3.68	4.05	4.3	4.09	0	2	0	0	0	-6.14	0.55
4f	523.45	0.29	6	2	0	153.49	47.1	3.9	4.8	3.8	4.44	3.57	4.1	2	2	0	0	0	-6.09	0.17
4g	579.56	0.38	10	2	0	172.72	47.1	4.98	6.27	5.36	5.19	5.2	5.4	2	3	0	0	1	-5.38	0.17
4h	557.42	0.29	8	6	0	151.05	77.54	3.67	4.44	3.7	3.39	4.45	3.93	1	2	0	0	0	-6.55	0.55
4i	473.3	0.17	4	4	0	124.99	40.62	3.47	4.76	4.79	5.49	5.01	4.71	1	0	0	0	0	-5.81	0.55
4j	527.31	0.17	6	6	0	142.72	132.2	2.46	4.22	3.48	2.95	0.15	2.59	1	2	0	1	0	-6.52	0.55
4k	506.21	0.17	4	2	0	135.1	40.62	3.79	5.81	4.97	5.69	5.45	5.14	2	2	0	0	1	-5.26	0.17
4l	573.31	0.23	6	8	0	135.08	40.62	3.96	6.33	8.01	6.27	6.33	6.18	2	3	0	1	1	-5.3	0.17
4m	525.34	0.23	4	6	0	137.2	77.54	3.83	4.18	3.12	3.81	3.83	3.75	1	2	0	0	0	-6.54	0.55
5a	432.56	0.17	4	4	2	134.71	131.68	2.49	4.09	3.24	2.73	3.06	3.13	1	0	0	0	0	-5.73	0.55
5b	464.56	0.17	4	4	2	134.71	131.68	2.8	4.09	3.24	2.73	3.06	3.19	0	0	0	0	0	-6.43	0.55
5c	464.56	0.23	4	2	0	140.6	91.22	3.78	5.53	4.45	4.52	5.08	4.67	0	0	0	0	0	-6.43	0.55
5d	460.61	0.23	6	4	0	143.65	109.68	3.45	4.74	3.85	3.15	4.14	3.87	1	1	0	0	1	-5.38	0.55
5e	492.61	0.29	6	2	0	159.08	97.70	3.88	5.05	3.97	3.55	3.41	3.97	0	2	0	0	0	-6.14	0.55
5f	518.69	0.38	10	2	0	178.31	97.70	5.22	6.51	5.53	4.31	5.03	5.32	2	2	0	0	0	-6.09	0.17
5g	574.80	0.29	8	6	0	156.64	128.14	4.36	4.69	3.87	2.49	4.29	3.94	2	3	0	0	1	-5.38	0.17
5h	552.66	0.17	4	4	0	130.58	91.22	3.63	5	4.95	4.58	4.87	4.61	1	2	0	0	0	-6.55	0.55
5i	468.54	0.17	6	6	0	148.31	182.86	2.87	4.46	3.65	2.03	-0.31	2.54	1	0	0	0	0	-5.81	0.55
5j	522.55	0.17	4	2	0	140.69	91.22	3.98	6.06	5.14	4.79	5.3	5.05	1	2	0	1	0	-6.52	0.55
5k	501.45	0.23	6	8	0	141.06	91.22	3.31	6.74	8.81	5.52	6.17	6.11	2	2	0	0	1	-5.26	0.17
5l	568.55	0.23	4	6	0	142.79	128.14	3.68	4.43	3.29	2.91	3.67	3.6	2	3	0	1	1	-5.3	0.17
5m	520.58	0.17	4	4	2	134.71	131.68	2.49	4.09	3.24	2.73	3.06	3.13	1	2	0	0	0	-6.54	0.55
Ciprofloxacin	349.40	0.23	4	6	0	142.79	128.1	3.68	4.43	3.29	2.91	3.67	3.6	0	0	0	1	0	-9.23	0.55
Ampicillin	356.44	0.44	5	5	3	92.56	138.0	1.14	-1.13	-0.39	0.75	0.01	0.08	0	0	0	0	0	-5.81	0.55
Pioglitazone	356.44	0.32	7	4	1	102.17	93.59	2.61	3.75	2.78	2.01	4.28	3.09	0	0	0	0	0	-5.81	0.55



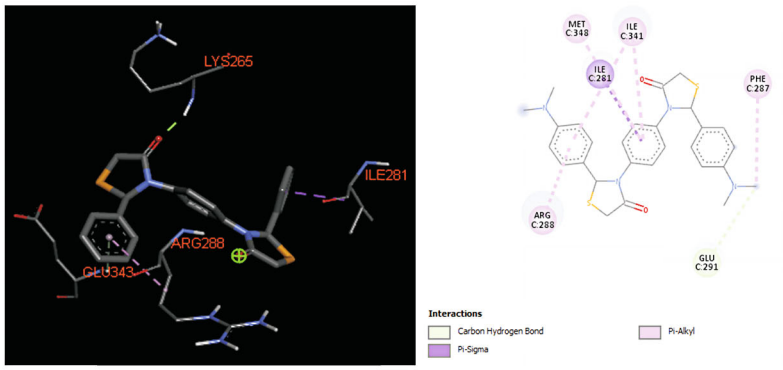
Compound **5a** (3D) 2D

Fig. 1. Docking of compounds and Standard drug with PPAR-γ.

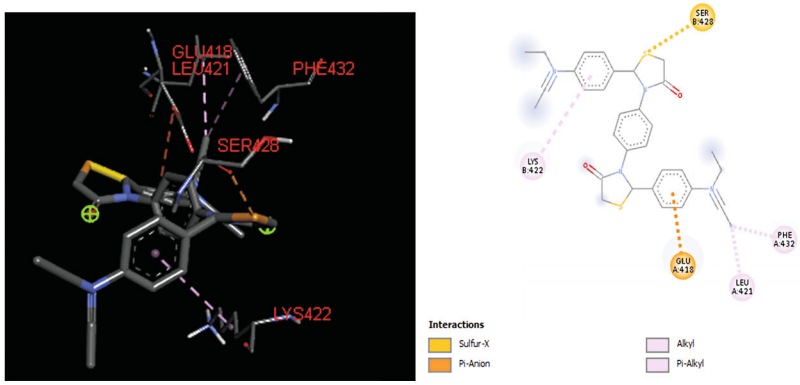
tive **5k** (IC_{50} = 31.51 µg/ ml), dimethoxy derivative **5h** (IC_{50} =34.75 µg/ ml), diethylamino derivative **5g** (IC_{50} = 43.83 µg/ ml), 2-hydroxy derivative **5c** (IC_{50} = 44.97 µg/ ml), methyl derivative **5d** (IC_{50} = 47.76 µg/ ml), 4-hydroxy derivative **5b** (IC_{50} = 48.97 µg/ ml), and trifluoromethyl derivative **5l** (IC_{50} = 51.99 µg/ ml) have displayed moderate DPPH radical scavenging activity while fluoro derivative **5i** (IC_{50} = 68.61 µg/ ml), methylenedioxy derivative **5m** (IC_{50} = 93.11 µg/ ml) and nitro derivative **5j** (IC_{50} = 118.4 µg/ ml) showed very weak activity when compared with the standard compound ascorbic acid (IC_{50} = 12.38 µg/ ml). It seems that the compounds containing electron-donating groups showed higher DPPH radical scavenging activity than those containing electron-withdrawing groups.

Antidiabetic activity

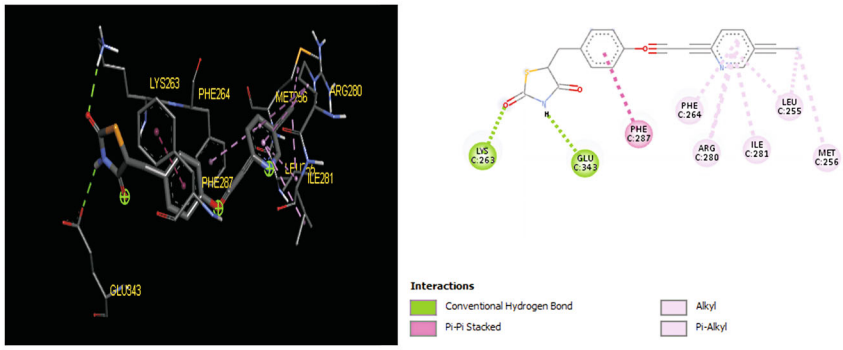
It was reported that, diabetes mellitus is associated with oxidative stress and compounds having the antioxidant prop-



Compound **5f** (3D)2D



Compound **5g** (3D)2D



Standard Pioglitazone (3D)2D

Fig. 1. (Continued)

erty will be of great value in the treatment of diabetes mellitus. On this basis, we have selected three bis-thiazolidinones **5a**, **5f** and **5g** that have exhibited the highest % inhibition of DPPH for antidiabetic activity screening. The antidiabetic activity was carried out by estimating the serum glucose level in fructose-induced diabetes rats. Since the synthesized bis-thiazolidinones possess thiazolidinone pharmacophore that closely resembles that of thiazolidinedione antidiabetic agents glitazones (example: pioglitazone, rosiglitazone), the antidiabetic activity of bis-thiazolidinones, therefore, was undertaken. The results are presented in Table 3.

Compound **5a** significantly reduced the serum glucose level on the 3rd, 7th, 14th and 21st days of its administration exhibiting incredibly significant antidiabetic activity when compared with standard pioglitazone. Compound **5f** also exhibited very significant antidiabetic activity on the 3rd and 7th days, while on 14th and 21stdays it exhibited weak antidiabetic activity. On the other hand, compound **5g** failed to show any activity on the 3rd day and displayed weak antidiabetic activity on the 7th, 14th and 21st days. It is interesting to

observe that the unsubstituted compound **5a** emerged as a good antidiabetic agent among the tested compounds. The substitutions either by dimethylamino or diethylamino at the 4th position of the aromatic ring resulted in lower antidiabetic activity.

Molecular Docking

The selected compounds that were screened for antidiabetic activity were subjected to molecular docking to explore their binding mode with PPAR-γ PDB ID: 4PRG using autodock tools 1.5.6. The PPAR-γ plays an important role in the regulation of glucose and homeostasis and was used as a molecular target for the glitazone class of type 2 antidiabetic agents. PPAR-γ agonists have been used in the treatment of hyperlipidaemia and hyperglycemia [36, 37]. Glitazones activate PPAR-γ as a means to lower serum glucose without increasing pancreatic insulin secretion [38]. The binding energies and amino acid residues with which ligands **5a**, **5f**, **5g** and standard drug pioglitazone interacted are presented in Table 4 and Figure 1. In docking studies, the compounds exhibited more binding affinity to PPAR-γ than the standard pioglitazone. The dimethylamino derivative **5f** demonstrated the highest binding affinity of -8.04 kcal/mol and formed one hydrogen bond with amino acid residue GLU (C:291) and formed other bonds by interacting with other amino acid residues ILE (C:281) and ARG (C:288). The unsubstituted derivative **5a** showed the good binding affinity of -7.68 kcal/mol and formed two hydrogen bonds with GLU (C:343) and LYS (C:265) and formed other bonds with amino acid residues ILE (C:281) and ARG (C:288). The compound **5g** with diethylamino substitution also displayed a good binding affinity of -7.45 kcal/mol and interacted with amino acid residues SER (B:428), GLU (A:418), PHE (A:432) and LYS (B:422). The standard drug pioglitazone exhibited a binding energy -7.12 kcal/mol and formed two hydrogen bonds with amino acid residues GLU (B:343) and LYS (C:263) and formed other bonds with amino acid residues PHE (C:287), LEU (C:255) MET (C:280), ILE (C:280), PHE (C:264) and ARG (C:280). In conclusion, compound **5f** exhibited good binding energy and interaction with PPAR-γ as that of standard pioglitazone.

Physicochemical, ADME and Drug Likeness Properties of Compounds 4a-m and 5a-m

To be effective as a potent drug, a molecule must reach its target in the body in sufficient concentration and stay there in bioactive form long enough for the expected biologic events to occur. Drug development involves assessments of pharmacokinetic properties such as absorption, distribution, metabolism, and excretion (ADME); thus, computer models constitute valid alternatives to experiments. The Swiss ADME web tool gives easy efficient inputs, free access to a pool of past yet robust predictive models for physicochemical properties, pharmacokinetics, and drug likeness of synthesized compounds. The ADME and drug likeness properties of the title compounds **4a-m** and **5a-m** were determined by using the Swiss ADME web tool and the results are tabulated in Table 5. The filters which are used to evaluate the drug likeness of title compounds are as follows: i) Lipinski filter [39]: MW ≤ 500; MLogP ≤ 4.15; HBA ≤ 10, HBD ≤ 5 ii) Ghose filter [40]:160 ≤ MW ≤ 480; -0.4 ≤ WlogP ≤ 5.6; 40 ≤ MR ≤ 130; 20 ≤ 70 iii) Veber (GSK) filter [41]: RB ≤ 10; TPSA ≤ 140, iv) Egan (Pharmacia) filters [42]: WLogP ≤ 5.88, TPSA ≤ 131.6 v) Muegge (Bayer) filter [43]: 200 ≤ MW ≤ 600, -2 ≤ XLogP ≤ 5, TPSA ≤ 157; HBD ≤ 5, RB ≤ 15, number of rings ≤7; number of carbons >4, number of heteroatoms ≤1. The results revealed that, all the synthesized compounds did not violate the Veber rul; however, some of the compounds violated Lipinski, Ghose, Egan and Muegge rules. In conclusion, the majority of the synthesized compounds met the criteria of drug likeness and exhibited satisfactory ADME parameters.

MW: Molecular Weight, FSP3: Fraction of sp^3carbon atoms, RB: Rotatable Bonds, HBA: Hydrogen Bond Accepter, HBD: Hydrogen Bond

Donor, MR: Molar Refractivity, TPSA: Topological Polar Surface Area LogP: Indicator of Lipophilicity, Log Kp: Skin peremiation, F: Bioavailability Score.

CONCLUSION

Understanding the biological importance and the present trend for bis compounds as molecules of therapeutic interest, two series of bis compounds namely 1,1′-(phenylene)bis[3-chloro-4-(substituted phenyl)azetidin-2-ones] **4a-m** and 3,3′-(1,4-phenylene)bis[2-(substituted phenyl)thiazolidin-4ones] **5a-m** were synthesized in this work, characterized by spectral data and evaluated for antibacterial and antidiabetic activities. The *in silico* analysis of these molecules was also carried out. The compounds exhibited good binding with the target PPAR-γ and fulfilled the criteria for drug likeness. They also exhibited moderate to good antibacterial and antidiabetic potential deserving further study.

Conflict of interest statement

The authors declare that there is no conflict of interest.

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