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Synthesis of New 5-Substituted –Aminomethylene-Thiazolidine-2,4-dione Derivatives As Potential Antibacterial Agents

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ABSTRACT

*2, 4-Thiazolidinedione moiety is the generic feature of the glitazone, antidiabetic agents and are a class of molecules that normalize elevated blood glucose level. 5-Ethoxymethylene-thiazolidine-2,4-dione (**1**) was condensed with various secondary amines (**2a–2m**) in acetonitrile to yield the corresponding thiazolidine-2,4-dione derivatives (**3a-3m**). The newly synthesized **TZD** analogues **3a – 3m** were characterized by ^{1}H NMR, ^{13}C NMR, Mass and HRMS spectral data and evaluated for their in vitro antibacterial activity against Escherichia coli and Pseudomonas aeruginosa representing Gram-negative bacteria and Staphylococcus aureus and Bacillus subtilis representing Gram-positive bacteria by agar well diffusion method. The antibacterial results revealed that, in general, compounds containing pyridine and piperazine substituent showed excellent to good antibacterial activity.*

Keywords: Antibacterial activity, 5-Ethoxymethylene-thiazolidine-2,4-dione, Synthesis, Thiazolidine-2,4-dione (TZD), Secondary amines.

INTRODUCTION

Thiazolidine-2,4-dione **(TZD)** pharmacore has been the subject of immense research because of its deep involvement of its regulation in various physiological processes [1].Thiazolidine-2,4-dione moiety is the generic feature of the glitazones [2]. TZDs are a class of molecules that normalize elevated blood glucose level. TZDs (e.g., troglitazone, rosiglitazone, pioglitazone) improve insulin sensitivity in liver, muscle and fat tissues and thus counter acts insulin resistance, TZDs are effective in reducing glycosylated haemoglobin (HbA1c). Thiazolidine-2,4-dione derivatives have a diverse array of pharmacological responses such as antidiabetic [3],antioxidant, analgesic, antiarthritic [4], anti-inflammatory [5],anticancer [6], antituberculosis [7], anti-HIV [8], antibacterial and antifungal [9]. It was reported that 5-arylidene-2,4-thiazolidinediones can act as potentially promising 15-hydroxyprostaglandin dehydrogenase inhibitors [10], inhibitors of MurD ligase [11] and antimicrobial agents [12-14].

Due to rapid development in drug resistance, tolerance, and side effects, there is a critical need for the development of a new generation of antimicrobial agents that exhibit improved pharmacological properties and drug-resistance profiles. Therefore the search of new and effective antimicrobial drugs is a very

important subject because of the manifestation of a large group of antibiotic resistant strains [15].Our hypothesis is to incorporate the certain pharmacophoric features and scaffolds on the templates of 2, 4-Thiazolidinedione molecule in order to design as novel antimicrobial agents. In sight of the particulars mentioned above and as part of our preliminary efforts to discover potentially active new medicinal therapeutic agents. We report herein the synthesis, characterization and evaluation of antimicrobial activity of thiazolidine- 2,4-dione embedded with various aliphatic cyclic amines such as pyrrolidine, morpholine, substituted piperidine, piperazine and N-methylated pyridines (derived from chloride intermediates of Omeprazole, Pantaprazole and Rabeprazole).

MATERIALS AND METHODS

Chemical and solvents used were purchased either from Fluka or Merck. All the reagents were of analytical grade. Thin-layer chromatography (TLC) was performed on E.Merck AL silica gel 60 F254 plates and visualized under UV light. IR spectra were recorded as KBr pellet with a PerkinElmer spectrum gx FTIR instrument and only diagnostic and/or intense peaks are reported. ^{1}H NMR spectra were recorded in $CDCl_3$ with a Varian Mercury plus 400 MHz instrument. ^{13}C NMR spectra were recorded in $CDCl_3$ with a Varian Gemini 100 MHz instrument. Signals due to the solvent (^{13}C NMR) or residual protonated solvent (^{1}H NMR) served as the internal standard. All the chemical shifts were reported in δ (ppm) using TMS as an internal standard. . The ^{1}H NMR chemical shifts and coupling constants were determined assuming first-order behaviour. Multiplicity is indicated by one or more of the following: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), br (broad); the list of coupling constants (*J*) corresponds to the order of multiplicity assignment. Mass spectra were recorded with a PE Sciex model API 3000 instrument. All the reactions were carried out under nitrogen atmosphere.

Synthesis of 5-(ethoxymethylene)thiazolidine-2,4-dione 1 [16]: Pale yellow solid**:** Yield: 98%; M.p: 91-83^{o}C; IR (KBr): υ_{max} 3383, 3137, 3043, 2989, 2432, 1721, 1677, 1473, 1392, 1229, 1105, 1010, 867, 740, 691 cm^{-1};^{1}H NMR (400 MHz, DMSO-d_6): δ 8.38 (br.s, 1 H), 7.65 (s, 1H), 4.20 (q, *J* = 5.4 Hz, 2H), 1.40 (t, *J* = 5.4 Hz, 3H); ESI-MS: m/z, 173.0 (M+1); ESI-HRMS *m/z*: calcd for $C_6H_7NO_3S$ ([M+H]$^{+}$): 174.0212; found: 174.0219.

General Experimental Procedure for the Synthesis of compound 2k, 2l and **2m:** To a solution of compounds **4-6** in water was added N-methyl amine (15 mmol) and stirred at room temperature for 24 h. After completion of the reaction, the reaction contents was extracted with dichloromethane and washed with water followed by brine solution. The organic layer was dried over anhydrous Na_2SO_4, filtered and evaporated to obtain compounds **2k-2m**. The crude compounds **2k-2m** were utilized in the subsequent step without any further purification.

General Experimental Procedure for the Synthesis of TZD derivatives (3a-3m): To a suspension of 5-(ethoxymethylene)thiazolidine-2,4-dione (100 mg, 0.58 mmol) in acetonitrile (5 vol) was added 2^{o} amines **2a – 2m** (0.5 mmol) in one lot. The reaction mixture was stirred at room temperature for 15- 20 min, the completion of the reaction was monitored by T.L.C. The precipitated solids were filtered and dried to obtain the pure compounds in quantitative yields.

(Z)-5-((pyrrolidin-1-yl)methylene)thiazolidine-2,4-dione (3a): White solid ; Yield: 98%; M.p: 81-82 ^{o}C; IR (KBr): υ_{max} 3129, 3014, 2970, 2874, 2750, 1702, 1664, 1589, 1477, 1453, 1379, 1362, 1321, 1284, 1244, 1176, 1133, 1047, 968, 857, 818, 741, 688, 669 cm^{-1};^{1}H NMR (400 MHz, DMSO-d_6): δ 11.33 (br.s, 1H), 7.74 (s, 1H), 3.56 – 3.50 (m, 4 H), 1.88-1.86 (m, 4 H);^{13}C NMR (400 MHz, DMSO-d_6): δ 168.9, 167.1, 140.2, 87.8, 40.1 (2C), 24.7 (2C); ESI-MS: m/z, 197.1 (M-1); ESI-HRMS *m/z*: calcd for $C_8H_{10}N_2O_2S$ ([M+H]$^{+}$): 199.0541; found: 199.0536.

(Z)-5-((piperidin-1-yl)methylene)thiazolidine-2,4-dione (3b): White solid ; Yield: 98%; M.p: 96-98 ^{o}C; IR (KBr): υ_{max} 3438, 3083, 2938, 2768, 1707, 1663, 1573, 1465, 1442, 1355, 1340, 1243, 1161, 147,

1100, 1021, 999, 957, 931, 849, 800, 739 cm^{-1};^{1}H NMR (400 MHz, DMSO-d_6): δ 11.47 (br.s, 1H), 7.55 (s, 1H), 3.46 – 3.38 (m, 4 H), 1.58-1.52 (m, 6 H);^{13} C NMR (400 MHz, DMSO-d_6): δ 168.0, 167.5, 142.9, 85.1, 85.1, 51.3 (2C), 25.8 (2C), 23.2; ESI-MS: m/z, 211.1 (M-1); ESI-HRMS *m/z*: calcd for $C_9H_{12}N_2O_2S$ ($[M+H]^+$): 213.0696; found: 199.0692.

(Z)-5-(morpholinomethylene)thiazolidine-2,4-dione (3c): White solid ; Yield: 98%; M.p: 111-112 ^oC; IR (KBr): υ $_{max}$ 3432, 3085, 3023, 2934, 2748, 1718, 1683, 1580, 1429, 1393, 1268, 1231, 191, 1113, 1020, 1069, 949, 926, 862, 738 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.56 (br.s, 1H), 7.58 (s, 1H), 3.68 – 3.62 (m, 4 H), 3.48-3.42 (m, 4 H); ^{13} C NMR (400 MHz, DMSO-d_6): δ 167.9, 167.6, 142.1, 86.4, 65.8 (2C), 49.7 (2C); ESI-MS: m/z, 213.1 (M-1); ESI-HRMS *m/z*: calcd for $C_8H_{10}N_2O_3S$ ($[M+H]^+$): 215.0490; found: 215.0485.

(Z)-5-((azepan-1-yl)methylene)thiazolidine-2,4-dione (3d): White solid ; Yield: 98%; M.p: 105-106^oC; IR (KBr): υ $_{max}$ 3400, 3085, 3015, 2935, 2857, 2764, 1704, 1663, 1595, 1465, 1448, 1406, 1386, 1321, 1291, 1244, 1198, 1135, 1010, 978, 967, 932, 906, 821, 740, 701 cm^{-1};^{1}H NMR (400 MHz, DMSO-d_6): δ 11.48 (br.s, 1H), 7.60 (s, 1H), 3.50 – 3.42 (m, 4 H), 1.70-1.62 (m, 4 H), 1.54-1.48 (m, 4 H); ESI-MS: m/z, 225.1 (M-1); ESI-HRMS *m/z*: calcd for $C_{10}H_{14}N_2O_2S$ ($[M+H]^+$): 227.0855; found: 215.0849.

1-((Z)-(2,4-dioxothiazolidin-5-ylidene)methyl)piperidine-4-carbonitrile (3e): White solid ; Yield: 98%; M.p: 125-126 ^oC; IR (KBr): υ $_{max}$ 3408, 3087, 2942, 2866, 2768, 2236, 1708, 1660, 1694, 1579, 1458, 1443, 1390, 1357, 1344, 1294, 1258, 1221, 1202, 1162, 1148, 1125, 1101, 1049, 1022, 997, 859, 809, 796, 739, 699 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.60 (br.s, 1H), 7.60 (s, 1H), 3.60-3.58 (m, 1 H), 3.55 – 3.50 (m, 4 H), 2.0-1.88 (m, 4 H);^{13} C NMR (400 MHz, DMSO-d_6): δ 167.9, 167.6, 142.9, 121.5, 85.4, 40.3, 28.0 (2C), 24.7 (2C); ESI-MS: m/z, 238.2 (M-1); ESI-HRMS *m/z*: calcd for $C_{10}H_{11}N_3O_2S$ ($[M+H]^+$): 238.0648; found: 238.0645.

methyl 1-((Z)-(2,4-dioxothiazolidin-5-ylidene)methyl)piperidine-4-carboxylate (3f): White solid ; Yield: 98%; M.p: 115-116 ^oC; IR (KBr): υ $_{max}$ 3436, 3113, 2947, 2771, 1726, 1700, 1655, 1438, 1362, 1344, 1322, 1290, 1206, 1181, 1165, 1139, 1030, 973, 940, 908, 858, 799, 741, 700 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.58 (br.s, 1H), 7.60 (s, 1H), 3.78-3.60 (m, 3 H), 3.38 – 3.20 (m, 4 H), 2.65 (m, 1 H), 1.60-1.55 (m, 2 H);^{13} C NMR (400 MHz, DMSO-d_6): δ 173.8, 167.9, 167.6, 142.9, 85.9, 51.6 (2C), 49.14, 27.9 (3C); ESI-MS: m/z, 271.2 (M-1); ESI-HRMS *m/z*: calcd for $C_{11}H_{14}N_2O_4S$ ($[M+H]^+$): 271.0742; found: 238.0747.

(Z)-5-((4-ethylpiperazin-1-yl)methylene)thiazolidine-2,4-dione (3g): White solid ; Yield: 98%; M.p: 88-89^oC; IR (KBr): υ $_{max}$ 63371, 3013, 2073, 2932, 2914, 2847, 2675, 2462, 1707, 1671, 1597, 1618, 1447, 1432, 1370, 1350, 1333, 1237, 1152, 1124, 1010, 1039, 949, 936, 922, 843, 825, 807, 770, 740, 696, 653 cm^{-1};^{1}H NMR (400 MHz, DMSO-d_6): δ 11.50 (br.s, 1H), 7.56 (s, 1H), 3.45 (t, *J* = 5.6 Hz, 4 H), 2.40 (t, *J* = 5.4 Hz, 4 H), 2.32 (q, *J* = 4.8 Hz, 2 H), 1.00 (t, *J* = 4.8 Hz, 3 H); ^{13} C NMR (400 MHz, DMSO-d_6): δ 167.9, 167.6, 142.7, 86.0, 52.0 (2C), 51.2 (2C), 49.7, 11.74;ESI-MS: m/z, 242.1 (M+1); ESI-HRMS *m/z*: calcd for $C_{10}H_{15}N_3O_2S$ ($[M+H]^+$): 242.0962; found: 242.0958.

(Z)-5-((4-phenylpiperazin-1-yl)methylene)thiazolidine-2,4-dione (3h): White solid ; Yield: 98%; M.p: 99-100^oC; IR (KBr): υ $_{max}$ 3428, 3086, 2968, 2770, 1714, 1669, 1586, 1476, 1426, 1364, 1285, 1262, 1249, 1176, 1010, 929, 866, 768, 739, 701 cm^{-1};^{1}H NMR (400 MHz, DMSO-d_6): δ 11.52 (br.s, 1H), 7.66 (s, 1H), 7.24 (t, *J* = 6.8 Hz, 2 H), 6.95 (d, *J* = 7.2 Hz, 1 H), 6.82 (t, *J* = 6.8 Hz, 1H), 3.60 (t, *J* = 5.2 Hz, 4 H), 3.22 (t, *J* = 5.2 Hz, 4 H);^{13} C NMR (400 MHz, DMSO-d_6): δ 167.9, 167.8, 150.3, 142.7, 129 (2C), 119.5 (2C), 115.9, 86.4, 49.4 (2C), 49.3 (2C);ESI-MS: m/z, 288.1 (M-1); ESI-HRMS *m/z*: calcd for $C_{14}H_{15}N_3O_2S$ ($[M+H]^+$): 290.0956; found: 290.0958.

tert-butyl 4-((Z)-(2,4-dioxothiazolidin-5-ylidene)methyl)piperazine-1-carboxylate (3i): White solid ; Yield: 98%; M.p: 72-78^oC; IR (KBr): υ $_{max}$ 3091, 2963, 2846, 2763, 1702, 1661, 1575, 1589, 1497, 1142,

1386, 1365, 1324, 1286, 1205, 1144, 1093, 1018, 930, 918, 842, 737, 761, 700, 689 cm^{-1} ; ^1H NMR (400 MHz, DMSO-d_6): δ 11.56 (br.s, 1H), 7.60 (s, 1H), 3.46-338 (m, 8 H), 1.40 (s, 9 H);^{13} C NMR (400 MHz, DMSO-d_6): δ 167.9, 167.2, 153.5, 142.8, 86.7, 79.3 (2C), 49.3 (2C), 27.11 (4C); ESI-MS: m/z, 312.1 $(M-1)^-$; ESI-HRMS *m/z*: calcd for $C_{13}H_{19}N_3O_4S$ $([M+H]^+)$: 314.1170; found: 314.1169.

(Z)-5-(((4aS,7aS)-octahydropyrrolo[3,4-b]pyridin-6-yl)methylene)thiazolidine-2,4-dione (3j): White solid ; Yield: 98%; M.p: 118-119^{o}C; IR (KBr): υ $_{max}$ 3435, 3330, 3399, 3098, 2874, 2830, 2939, 2855, 2291, 1705, 1671, 1467, 1453, 1427, 1356, 1341, 1309, 1252, 1241, 1142, 1168, 1118, 1026, 948, 926, 904, 850, 826, 801, 761, 738, 693, 656 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): 3.65-3.58 (m, 3 H), 3.40-3.35 (d, *J* = 8.2 Hz, 1H), 3.18 (br.s, 1 H), 2.80 (d, *J* = 8.2 Hz, 1 H), 2.48-2.40 (m, 1H), 2.20 (br.s, 1H), 1.70 – 1.30 (m, 4 H); ^{13} C NMR (100 MHz, DMSO-d_6): 20.8, 22.5, 44.3, 86.5, 141.9, 166.9, 169.0; ESI MS: m/z: 254.1 $[M+1]^+$.

(Z)-5-((((3,4-dimethoxypyridin-2-yl)methyl)(methyl)amino)methylene)thiazolidine-2,4-dione (3k): White solid ; Yield: 98%; M.p: 61-62 ^{o}C; IR (KBr): υ $_{max}$ 3430, 3096, 3006, 2950, 2845, 2737, 1707, 1624, 1599, 1489, 1421, 1350, 1300, 1275, 1233, 1121, 1064, 1043, 997, 916, 862, 843, 740,721 cm^{-1};^1H NMR (400 MHz, DMSO-d_6): δ 11.40 (br.s, 1H), 8.20 (s, 1H), 7.80 (s, 1H), 4.60 (s, 2 H), 3.98 (s, 3 H), 3.80 (s, 3 H), 3.05 (s, 3 H); ^{13} C NMR (100 MHz, DMSO-d_6): δ 56.0, 60.4, 86.9, 108.4, 128.7, 142.8, 145.5, 145.7 (2C),148.65, 158.14, 167.54, 168.71;ESI-MS: m/z, 310.1 $(M+1)^+$; ESI-HRMS *m/z*: calcd for $C_{13}H_{15}N_3O_4S$ $([M+H]^+)$: 310.0852; found: 310.0856.

(Z)-5-((((4-methoxy-3,5-dimethylpyridin-2-yl)methyl)(methyl)amino)methylene)thiazolidine-2,4-di one (3l): White solid ; Yield: 98%; M.p: 122-124^{o}C; IR (KBr): υ $_{max}$ 3438, 3214, 3000, 2939, 2752, 1717, 1664, 1590, 1474, 1450, 1421, 1311, 1283, 1267, 1095, 999, 855, 741,717 cm^{-1};^1H NMR (400 MHz, DMSO-d_6): δ 11.58 (br.s, 1H), 8.22 (s, 1H), 7.78 (s, 1H), 4.62 (s, 2 H), 3.78 (s, 3 H), 3.10 (s, 3 H), 2.20 (s, 6 H); ESI-MS: m/z, 308.1 $(M+1)^+$; ESI-HRMS *m/z*: calcd for $C_{14}H_{17}N_3O_3S$ $([M+H]^+)$: 308.1065; found: 310.1063.

(Z)-5-((((4-(3-methoxypropoxy)-3-methylpyridin-2-yl)methyl) (methyl)amino) methylene) thiazol idine-2,4-dione (3m): White solid ; Yield: 98%; M.p: 131-132^{o}C; IR (KBr): υ $_{max}$ 3367, 3089, 2976, 2825, 2878, 2742, 1694, 1668, 1600, 1461, 1453, 1424, 1318, 1291, 1237, 1266, 1189, 1051, 1136, 1124, 1088, 996, 951, 906, 867, 850, 742, 708, 654 cm^{-1};^1H NMR (400 MHz, $CDCl_3$): δ 8.25 (d, *J* = 7.2 Hz, 1 H), 7.82 (s, 1 H), 6.80 (d, *J* = 7.2 Hz, 1 H), 4.62 (s, 2 H), 4.22 (t, *J* = 5.8 Hz, 2 H), 3.62 (t, *J* = 5.8 Hz, 2 H), 3.40 (s, 3 H), 3.18 (s, 3 H), 2.22 (s, 3 H), 2.10 (t, *J* = 5.8 Hz, 2 H) ; ^{13} C NMR (100 MHz, $CDCl_3$): δ 10.3, 29.4, 29.9, 58.9, 65.6, 69.2, 88.4, 106.8, 121.4, 146.1, 148.3, 153.0, 164.1, 169.0, 170.4;ESI-MS: m/z, 352.2 $(M+1)^+$; ESI-HRMS *m/z*: calcd for $C_{16}H_{21}N_3O_4S$ $([M+H]^+)$: 352.1315; found: 352.1326.

BIOLOGICAL ASSAYS

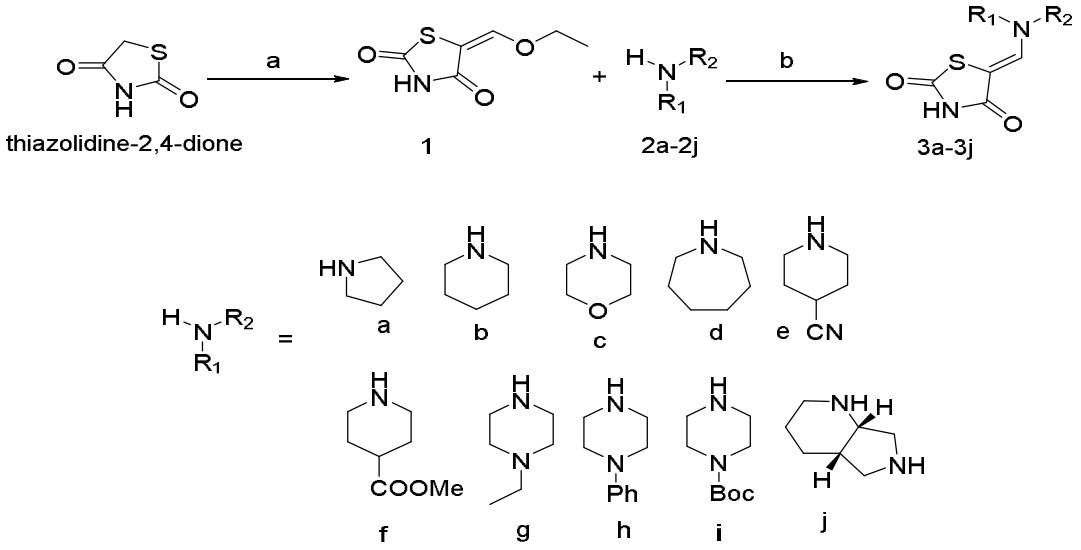
Antimicrobial test: MIC is the lowest concentration of an antimicrobial compound that will inhibit the visible growth of a microorganism after overnight incubation. Minimum Inhibitory Concentration (MIC) of newly synthesized compounds against tested bacterial strains was determined using macro dilution tube method [17,18]. In this method, various test concentrations of newly synthesized compounds were prepared from 128 to 0.25 µg mL^{-1} in sterile tubes No. 1 to 10. 100 µL sterile Mueller Hinton Broth (MHB) was poured in each sterile tube followed by addition of 200µL test compound in tube 1. Two fold serial dilutions were carried out from tube 1 to tube 10 and excess broth (100 µL) was discarded from the last tube No. 10. To each tube, 100 µL of standard inoculums (1.5 x 08 cfu mL^{-1}) was added [20]. Ciprofloxacin was used as control. Turbidity was observed after incubating the inoculated tubes at 37 ^{o}C for 24 h.

The antibacterial activity of newly synthesized compounds was evaluated by agar well diffusion method [17, 18, and 19]. Using sterile cork borer of 8 mm diameter, wells were bored into seeded agar plates and these were loaded with a 100 µL volume with concentration of 4.0 mg mL^{-1} of each compound

reconstituted in dimethylsulphoxide (DMSO). Antibacterial activity of thirteen newly synthesized compounds was evaluated by measuring the zone of growth inhibition against the test bacteria. DMSO was used as a negative control whereas ciprofloxacin was used as a positive control. All the plates were incubated at 37 ^{o}C for 24 h. The experiments were performed in triplicates. The antibacterial activity of the compounds was compared with ciprofloxacin as standard.

RESULTS AND DISCUSSION

Chemistry: The synthetic strategy for the target compounds, (Z)-5-(substituted aminomethylene)thiazolidine-2,4-dione derivatives (**3a-3m**) is illustrated in Scheme **1** and Scheme **2**. In the present work, 5-Ethoxymethylene-thiazolidine-2, 4-dione (**1**) was condensed with various aliphatic cyclic amines (**2a – 2j**) such as pyrrolidine, morpholine, substituted piperidine, piperazine and N-methylated pyridines, **2k – 2m** (derived from chloride intermediates of Omeprazole, Pantaprazole and Rabeprazole) in acetonitrile to yield the corresponding thiazolidine-2,4-dione derivatives (**3a-3m**). The reaction proceeds through addition of 5-Ethoxymethylene-thiazolidine-2,4-dione(1) with 2^{o} amines **2 (a-m)** to afford **3 (a-m).** We prepared a library of compounds **3(a-m)** in quantitative yields and the structures of the products were determined by ^{1}HNMR, ^{13}C NMR, Mass, IR and High resolution mass spectral (HRMS) analyses. The details of the synthetic procedures, the physical data, the yields and the spectral characterizations of the synthesized compounds are presented in experimental section. As a representative example, the ^{1}H NMR spectra of compound **3c**, showed one signal for methylidene proton as a singlet at 7.58 ppm and a broad singlet signal at 11.56 ppm corresponding to -NH proton while the morpholine ring proton are found to be in the expected region (multiplet signals at 3.68 – 3.62 ppm and 3.48-3.42 ppm). The ^{1} HNMR, ^{13} C NMR, IR, mass and HRMS spectral data of the remaining compounds in the series are in agreement with the desired structures. The 5-Ethoxymethylene-thiazolidine-2,4-dione was prepared by the reaction of thiazolidinedione with Triethyl orthoformate in acetic anhydride according to the procedure reported in the literature [16].

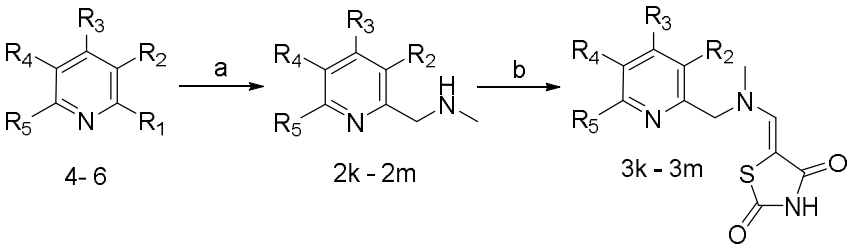


Scheme 1. Synthesis of TZD derivatives (**3a – 3j**) embedded with cyclic aliphatic 2^{o} amines

Experimental conditions: a): triethyl orthoformate, acetic anhydride; b): 2^{o} amines **2a – 2j**, acetonitrile, r.t., 15 - 20 min.

Biological evaluation : All the newly synthesized 5-substituted –aminomethylene-Thiazolidine-2,4-dione derivatives (**3a-3m**) were evaluated for their *in vitro* antibacterial activity against *Escherichia coli* and

Pseudomonas aeruginosa representing Gram negative bacteria and *Staphylococcus aureus* and *Bacillus subtilis* representing Gram positive bacteria (**Table 1 and Table 2**) by agar well diffusion method [17] using ciprofloxacin as the standard drug. The results were recorded for each tested compound and the average diameter of inhibition zones of bacterial growth surrounding the well in millimetres. The Minimum Inhibitory Concentration (MIC) measurements were performed using a macrodilution tube method [17, 18] (Table 2).



4. R_1 = -CH_2-Cl, R_2 = -OCH_3, R_3 = -OCH_3, R_4 = R_5 = H
5. R_1 = -CH_2-Cl, R2 = -CH_3, R_3 = -OCH_3, R_4 = -CH_3, R_5 = H
6. R_1 = -CH_2-Cl, R_2 = -CH_3, R_3 = -$O(CH_2)_3$-OCH_3, R_4 = R_5 = H

Scheme 2. Synthesis of TZD derivatives (**3k – 3m**) bearing pyridine moiety
Experimental conditions: a) methyl amine, water, r.t., 24 h; b) amines **2k – 2m**, 5-(ethoxymethylene)thiazolidine-2,4-dione, acetonitrile, r.t., 15 – 20 min

Table-1 Results of *In vitro* antibacterial activity of compounds **3a – 3m**

Compound[a]	**2° amines (NHR_1R_2)**	*Gram negative*		*Gram positive*	
		E.coli ***MTCC 443***	*P.aeruginosa* ***MTCC 424***	*S.aureus* ***MTCC 96***	*B.subtilis* ***MTCC 121***
		Diameter of growth of inhibition in ***zone (mm)*** [b]			
3a	a	--	--	15.0	17.0
3b	b	--	--	15.5	17.0
3c	c	15.6	15.8	16.8	17.4
3d	d	--	--	17.6	18.3
3e	e	17.3	16.6	18.5	19.6
3f	f	17.0	17.2	19.0	19.6
3g	g	18.6	18.0	19.6	20.8
3h	h	19.3	18.6	20.3	20.5
3i	i	20.6	20.2	21.6	20.8
3j	j	22.3	23.3	25.3	25.6
3k	k	24.6	25.3	27.6	26.3
3l	l	25.3	24.6	27.3	25.6
3m	m	25.3	25.0	26.6	26.3
Standard Drug	Ciprofloxacin	25.3	25.0	27.6	26.3

[a] Concentration 4.0 mg mL^{-1}. [b] mean ± SD of three independent experiments

Table-2 Results of Miminimum inhibitory concentration (µg/mL) of compounds **3a – 3m**

Compound[a]	**2° amines**	*Gram negative*		*Gram positive*	
		E.Coli ***MTCC 443***	*P.aeruginosa* ***MTCC 424***	*S.Aureus* ***MTCC 96***	*B.Subtilis* ***MTCC 121***
		Minimum inhibitory concentration (MIC) (µg/mL)			
3a	a	128	64	128	128
3b	b	128	128	64	128
3c	c	64	128	128	64
3d	d	64	128	128	128
3e	e	128	64	128	64
3f	f	128	128	128	128
3g	g	128	32	64	64
3h	h	64	64	128	128
3i	i	32	32	32	32
3j	j	32	32	32	32
3k	k	16	16	16	16
3l	l	16	16	16	16
3m	m	16	16	16	16
Standard Drug	Ciprofloxacin	5	5	5	5

[a] Concentration 4.0 mg mL^{-1}

The results of the antibacterial activity data revealed that in general, all the tested compounds displayed moderate to good antibacterial activity against Gram negative (*E. coli, P. aeruginosa*) and Gram positive bacteria (*S. aureus, B. subtilis*). On the basis of zone of inhibition against the tested bacterial strains, it is observed that in case of *E.coli*: compounds **3j**, **3k**, **3l** and **3m** was found to be significantly most active (zone of inhibition: 22.3 – 25.3) as compared with the reference drug Ciprofloxacin (zone of inhibition: 25.3), while compounds **3g**, **3h** and **3i** showed good activity (zone of inhibition: 18.6 – 20.6). Compounds **3c**, **3e** and **3f** showed moderate activity and the compounds **3a**, **3b** and **3d** were inactive against. Against *P.aeruginosa*, compounds **3a** to **3m** exhibited similar trend of antibacterial activity as in the case of E.coli bacterium.

In case of *S.aureus*: compounds **3k, 3l** and **3m** nearly equipotent activity showing the zone of inhibition 26.6 – 27.6) when compared with the Ciprofloxacin (zone of inhibition: 27.6) and compounds **3e, 3f, 3g, 3h, 3i** and **3j** showed good activity (zone of inhibition: 18.5 – 21.6) while the remaining compounds **3a - 3d** were found to be moderately active. In case of *B.subtilis*, compounds **3a – 3d** were found to be moderately active and compounds **3e – 3i** (zone of inhibition: 19.6 – 20.8) showed good activity and compounds **3j – 3m** showed nearly equipotent activity when compared with the ciprofloxacin as the reference drug.

In terms of MIC, amongst all the compounds, the MIC ranged between 16 (**3k, 3l, 3m**) and 128 µg/mL against Gram negative bacteria and Gram positive bacteria (**Table 2**). It is observed that compounds **3k, 3l** and **3m** exhibited excellent activity against all the tested bacterial strains, while the compounds **3i** and **3j** showed good activity. Within the individual series, no correlation between the antibacterial activities with

respect to the substituent on the **TZD** is observed. However, in general, it is observed that **TZD** core embedded with a pyridine and piperazine moiety showed excellent to good antibacterial activity than the remaining compounds in the series. The preliminary in vitro antimicrobial screening of the **TZD** derivatives **3a-3m** revealed that most of the compounds in the series showed potent activity. Therefore, the present study is valuable for finding the new drugs against bacterial diseases.

APPLICATIONS

In the present study the derivatives which we have synthesized were screened for their antibacterial activity, which are promising as active pharmacophore. Further studies are undergoing to explore the scope of the various biological activities.

CONCLUSIONS

The present study describes the synthesis of thiazolidine-2,4-dione derivatives (**3a-3m**) prepared by condensing 5-Ethoxymethylene-thiazolidine-2, 4-dione (**1**) with various secondary amines (**2a – 2m**) in acetonitrile. The synthesized **TZD** analogues **3a – 3m** were characterized by ^{1}H NMR, ^{13} C NMR and Mass spectral data and evaluated for their *in vitro* antibacterial activity against Escherichia. coli and *Pseudomonas aeruginosa* representing Gram-negative bacteria and *Staphylococcus aureus* and *Bacillus subtilis* representing Gram-positive bacteria by agar well diffusion method. The antibacterial study revealed that, in general, it is observed that **TZD** core embedded with a pyridine and piperazine moiety showed excellent to good antibacterial activity than the remaining compounds in the series. The preliminary in vitro antimicrobial screening of the **TZD** derivatives **3a-3m** revealed that most of the compounds in the series showed potent activity. Therefore, the present study is valuable for finding the new drugs against bacterial diseases.

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