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Synthesis and antimicrobial activity of some new 1,2-*bis*-[1,3-thiazolidin-3-yl]ethane derivatives

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ABSTRACT

1,2-*Bis*-(2-(phenylimino)-4-oxo-1,3-thiazolidin-3-yl)ethane (**4**) was synthesized and its reaction with various aldehydes afforded the novel 5-arylidene derivatives **5a-e** and **7**. Reaction of compound **4** with phenyl isothiocyanate in the presence of potassium hydroxide, followed by addition of two equivalents of α-halo ketones furnished the corresponding 1,2-*bis*-[5-(thiazolidin-2-ylidene)thiazolidin-3-yl]ethane derivatives **10**, **14a-c**, and **17a**,**b**. The structures of the newly synthesized compounds were established by elemental and spectral analyses. Compounds **5a-e**, **7**, **10**, **14a-c** and **17a** were screened for their antimicrobial activity against eight microorganisms. All compounds showed high antibacterial and antifungal activities against all the test microorganisms except *E. coli* and *C. albicans*. The MIC of the active compounds was also evaluated, where; compounds **10** and **17a** were more potent active (minimum inhibitory concentration 0.49 and 0.98 µg/mL, respectively) against *S. racemosum* than Amphotericin B (MIC 1.95 µg/mL).

1. Introduction

Thiourea derivatives are valuable synthetic intermediates and are widely used in the synthesis of biologically active compounds [1]. Thioureas demonstrated a broad spectrum of biological activities such as anti-HIV [2], antiviral [3], vanilloid receptor antagonism [4], antibacterial [5], antifungal [6-8], anticancer [9], and anti-allergic [10] activities. Furthermore, bis-thiourea derivatives were extensively used as effective organocatalysts in asymmetric organic reactions [11-15]. In addition, thiazolidin-4-ones are interesting class of heterocyclic compounds that possess all types of biological activities [16]. Therefore, thiazolidin-4-ones are well known to have antimicrobial [17], antitubercular [18], anticancer [19], anti-inflammtory [20], anticonvulsant [21] and antiviral/anti-HIV [22,23] activities.

Bis-thiazolidin-4-one derivatives also showed interesting anti-inflammatory/analgesic activities together with better gastrointestinal safety profile than known NSAIDs [24]. As part of our research work towards synthesis of heterocycles with broad biologically activities [25-31], this work aims at synthesis of several new *bis*-thiazolidinone derivatives to evaluate their antibacterial and antifungal activities.

2. Experimental

2.1. Chemistry

2.1.1. Instrumentation

All melting points were measured on a Gallenkamp electrothermal melting point apparatus. The infrared (FT-IR) spectra were recorded for potassium bromide pellets on a Pye Unicam SP 3-300 and FT IR 8101 PC Shimadzu infrared spectrophotometers.

The ^{1}H NMR and ^{13}C NMR spectra were recorded in dimethylsulfoxide-d_6 (DMSO-d_6) as the solvent on Varian Mercury VXR-300 NMR spectrometer at 300 MHz (^{1}H NMR) and at 75 MHz (^{13}C NMR). Mass spectra were recorded at 70eV on a GCMS-QP 1000 EX Shimadzu mass spectrometer. Elemental analyses were carried out at the Microanalytical Center of Cairo University. The antimicrobial activity was performed at the Regional Center for Mycology and Biotechnology (Al-Azhar University, Cairo, Egypt). α-Bromo ketones **11a**,**b** [32], and **11c** [33] and pyrazole-4-carboxaldehyde **6** [34] were prepared according to procedures reported in the literature.

2.1.2. Synthesis of 1,2-bis-(2-(phenylimino)-4-oxo-1,3-thiazolidin-3-yl)ethane (4)

To a solution of the *bis*-thiourea derivative **1** (1 mmol, 0.33 g) in absolute ethanol (10 mL), ethyl chloroacetate (2.2 mmol, 0.24 mL) was added followed by triethylamine (0.2 mL). After complete addition, the reaction mixture was heated at reflux temperature for 6 hours then left to cool to room temperature. The solid formed that formed was filtered off, washed with ethanol, dried and finally recrystallized from DMF to afford the bis-thiazolidin-4-one derivative **4** (Scheme 1). Color: Yellow powder. Yield: 75% (0.31 g). M.p.: 100-101 °C. FT-IR (KBr, ν, cm^{-1}): 3063 (CH-arom), 2943, 2872 (CH-aliph), 1727 (C=O), 1637 (C=N), 1586 (C=C). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 3.93 (s, 4H, NCH_2), 4.11 (s, 4H, SCH_2), 6.91-6.94 (m, 4H, ArH), 7.11-7.14 (m, 2H, ArH), 7.31-7.37 (m, 4H, ArH). MS (EI, *m/z* (%)): 410 (M^+, 20.1), 218 (100), 205 (M^+/2, 5.3), 190 (18.9), 176 (68.6), 117 (31.4), 104 (58), 77 (95.3), 51 (29). Anal. calcd. for $C_{20}H_{18}N_4O_2S_2$: C, 58.52; H, 4.42; N, 13.65; S, 15.62. Found: C, 58.66; H, 4.48; N, 13.33; S, 15.57%.

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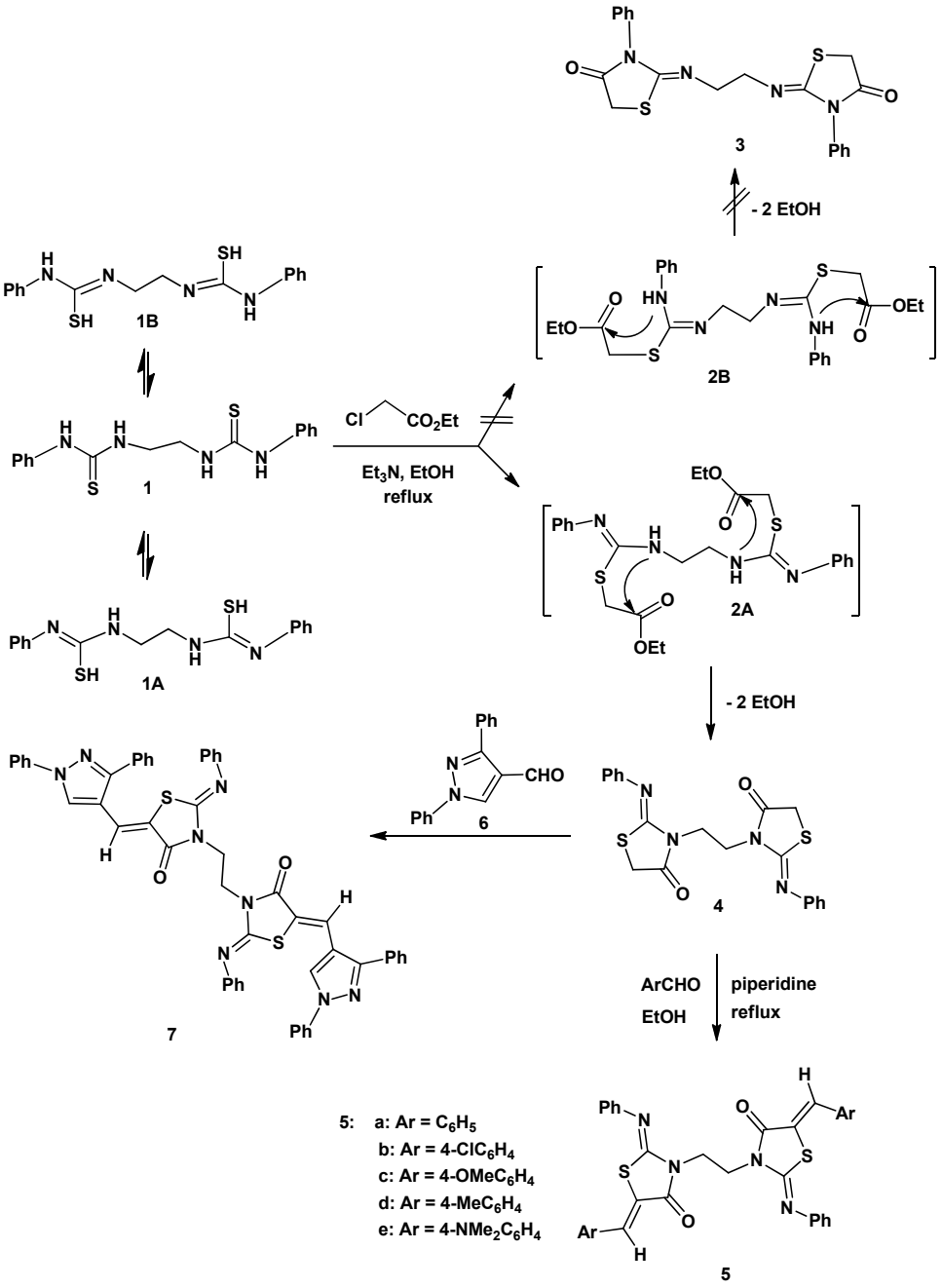


Scheme 1

2.1.3. Synthesis of 1,2-bis-[5-arylidene-4-oxo-2-phenylimino-1,3-thiazolidin-3-yl]ethane **5a-e** *and* **7**

General Procedure: To a mixture of the bis-thiazolidin-4-one derivative, **4**, (1 mmol, 0.41 g) and the appropriate aldehyde derivative (2 mmol) in absolute ethanol (10 mL), piperidine (0.2 mL) was added and the resulting mixture was refluxed for 6-8 hours, then left to cool to room temperature. The solid product so formed was collected by filtration, washed with ethanol, dried and finally recrystallized from DMF to afford the corresponding *bis*-(5-arylidene-thiazolidin-4-one) derivatives **5a-e** and **7**. The physical properties and spectral data of the obtained products are listed below (Scheme 1).

1,2-Bis-(5-benzylidene-4-oxo-2-(phenylimino)-1,3-thiazolidin-3-yl)ethane (**5a**): Color: Pale-yellow powder. Yield: 65% (0.38 g). M.p.: 196-198 °C. FT-IR (KBr, ν, cm^{-1}): 3055, 3012 (CH-arom), 2935, 2866 (CH-aliph), 1708 (C=O), 1646 (C=N). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 4.81 (s, 4H, NCH_2), 7.33-7.61 (m, 20H, ArH), 8.13 (s, 2H, C=CH-). ^{13}C NMR (75 MHz, DMSO-d_6, δ, ppm): 40.8, 113.3, 125.2, 129.7, 130.9, 131, 131.9, 132.1, 133.1, 134, 145, 166.2, 173.1. MS (EI, *m/z* (%)): 586 (M^+, 4.9), 306 (60.8), 293 (M^+/2, 5.4), 280 (4.1), 134 (100), 117 (28.1), 90 (7.4), 77 (25.4). Anal. calcd for $C_{34}H_{26}N_4O_2S_2$ (586.73): C, 69.60; H, 4.74; N, 9.55; S, 10.93. Found: C, 69.31; H, 4.68; N, 9.79; S, 10.78%.

1,2-Bis-[5-(4-chlorobenzylidene)-4-oxo-2-(phenylimino)-1,3-thiazolidin-3-yl]ethane (**5b**): Color: Pale-yellow crystals. Yield: 65% (0.43 g). M.p.: 182-184 °C. FT-IR (KBr, ν, cm^{-1}): 3065, 3023 (CH-arom), 2945 (CH-aliph), 1708 (C=O), 1637 (C=N), 1600 (C=C). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 4.80 (s, 4H, NCH_2), 7.29-7.36 (m, 8H, ArH), 7.43-7.62 (m, 10H, ArH), 8.09 (s, 2H, C=CH). MS (EI, *m/z* (%)): 657 (M^++2, 2.9), 656 (M^++1, 5), 655 (M^+, 3.3), 339 (74.5), 340 (85.6), 327 (3.3), 328 (M^+/2, 4.2), 273 (4.2), 212 (7.9), 168 (100), 144 (26.6), 131 (26.8), 117 (47.4), 89 (17.2), 77 (75.3). Anal. calcd for $C_{34}H_{24}Cl_2N_4O_2S_2$ (655.62): C, 62.29; H, 3.69; N, 8.55; S, 9.78. Found: C, 62.48; H, 3.64; N, 8.21; S, 9.66%.

1,2-Bis-[5-(4-methoxybenzylidene)-4-oxo-2-(phenylimino)-1,3-thiazolidin-3-yl]ethane (**5c**): Color: Pale-yellow crystals. Yield: 80% (0.52 g). M.p.: 206-208 °C. FT-IR (KBr, ν, cm^{-1}): 3025 (CH-arom), 2952 (CH-aliph), 1707 (C=O), 1636 (C=N). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 3.78 (s, 6H, OCH_3), 4.75 (s, 4H, NCH_2), 6.90 (d, 4H, *J* = 8.76 Hz), 7.32 (d, 4H, *J* = 8.76 Hz) 7.48-7.56 (m, 10H, ArH) 8.05 (s, 2H, C=CH-). ^{13}C NMR (75 MHz, DMSO-d_6, δ, ppm): 39.9, 54.2, 109.2, 114.7, 123.4, 124.4, 130.1, 131.1, 132.9, 133.2, 143.8, 163.5, 165.6, 171.8. MS (EI, *m/z* (%)): 646 (M^+, 7.4), 336 (38.3), 323 (M^+/2, 5.3), 310 (14.6), 164 (100), 149 (28.7), 121 (10.2), 77 (20.7). Anal. calcd for $C_{36}H_{30}N_4O_4S_2$ (646.78): C, 66.85; H, 4.68; N, 8.66; S, 9.92. Found: C, 66.55; H, 4.61; N, 8.89; S, 9.87%.

1,2-Bis-[5-(4-methylbenzylidene)-4-oxo-2-(phenylimino)-1,3-thiazolidin-3-yl]ethane (**5d**): Color: Yellow crystals. Yield: 75% (0.46 g). M.p.: 244-246 °C. FT-IR (KBr, ν, cm^{-1}): 3028 (CH-arom), 2940, 2858 (CH-aliph), 1708 (C=O), 1643 (C=N), 1602 (C=C). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 2.31 (s, 6H, CH_3), 4.34 (s, 4H, NCH_2), 6.87 (d, 4H, *J* = 7.2 Hz), 7.08-7.12 (m, 2H, ArH), 7.20-7.28 (m, 8H, ArH), 7.35-7.39 (m, 4H, ArH), 7.72 (s, 2H, C=CH-). MS (EI, *m/z* (%)): 614 (M^+, 8.4), 613 (7.7), 581 (2.1), 426 (3.8), 320 (70), 319 (54.7), 307 (M^+/2, 6.6), 277 (13.8), 148 (100), 117 (19.9), 105 (21.6), 91 (22.3), 77 (48.8). Anal. calcd for $C_{36}H_{30}N_4O_2S_2$ (614.78): C, 70.33; H, 4.92; N, 9.11; S, 10.43. Found: C, 70.10; H, 4.78; N, 8.89; S, 10.36%.

1,2-Bis-[5-(4-(dimethylamino)benzylidene)-4-oxo-2-(phenylimino)-1,3-thiazolidin-3-yl]ethane (**5e**): Color: Reddish-yellow crystals. Yield: 85% (0.57 g). M.p.: 298-300 °C. FT-IR (KBr, ν, cm^{-1}): 3060 (CH-arom), 2939, 2897 (CH-aliph), 1695 (C=O), 1636 (C=N), 1584 (C=C). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 2.95 (s, 12H, NMe_2), 4.30 (s, 4H, NCH_2), 6.74 (d, 4H, *J* = 9 Hz), 6.85 (d, 4H, *J* = 7.2 Hz), 7.04-7.09 (m, 2H, ArH), 7.18-7.23 (m, 4H, ArH), 7.30 (d, 4H, *J* = 9 Hz), 7.59 (s, 2H, C=CH-). MS (EI, *m/z* (%)): 673 (M^++1, 3.9), 672 (M^+, 4.7), 671 (M^+-1, 9.8), 349 (17.8), 336 (M^+/2, 5.2), 323 (15.2), 177 (100), 162 (7.5), 134 (7), 77 (7.1). Anal. calcd for $C_{38}H_{36}N_6O_2S_2$ (672.86): C, 67.83; H, 5.39; N, 12.49; S, 9.53. Found: C, 67.79; H, 5.30; N, 12.21; S, 9.44%.

1,2-Bis-[5-(1,3-diphenylpyrazol-4-yl)methylene-4-oxo-2-(phenylimino)-1,3-thiazolidin-3-yl]ethane (**7**): Color: Yellow crystals. Yield: 60% (0.52 g). M.p.: 240-242 °C. FT-IR (KBr, ν, cm^{-1}): 3054 (CH-arom), 2941 (CH-aliph), 1708 (C=O), 1637 (C=N), 1599 (C=C). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 4.31 (s, 4H, NCH_2), 6.91-6.94 (m, 4H, ArH), 7.06-7.09 (m, 2H, ArH), 7.21-7.26 (m, 4H, ArH), 7.34-7.53 (m, 18H, ArH), 7.88-7.91 (m, 4H, ArH), 8.55 (s, 2H, C=CH-). MS (EI, *m/z* (%)): 871 (M^+, 8.9), 590 (7.6), 494 (7.6), 447 (11.4), 355 (7.6), 327 (11.4), 275 (13.9), 146 (15.2), 121 (19), 105 (30.4), 91 (50.6), 77 (55.7), 64 (100). Anal. calcd for $C_{52}H_{38}N_8O_2S_2$ (871.04): C, 71.70; H, 4.40; N, 12.86; S, 7.36. Found: C, 71.99; H, 4.49; N, 13.03; S, 7.25%.

2.1.4. Synthesis of 1,2-bis-[5-(thiazolidin-2-ylidene) thiazolidin-3-yl]ethane derivatives 10, 14a-c and 17a,b

General procedure: A mixture of the *bis*-thiazolidin-4-one derivative, **4**, (1 mmol, 0.41 g) and KOH (2 mmol, 0.112 g) in DMF (10 mL) was stirred for 10 min. Then, phenyl isothiocyanate (2 mmol, 0.27 g) was added to the reaction mixture and stirring was continued for further 6 hour at room temperature. After that, the appropriate α-halo ketone derivative (2 mmol) was added and the stirring was continued overnight (10-14 hr). The solid formed was collected by filtration, washed with water then ethanol, dried and finally recrystallized from DMF to afford the corresponding 1,2-*bis*-[5-(thiazolidin-2-ylidene)thiazolidin-3-yl]ethane derivatives **10**, **14a-c** and **17a**,**b**. The physical properties and spectral data of the obtained products are listed below (Scheme 2 and 3).

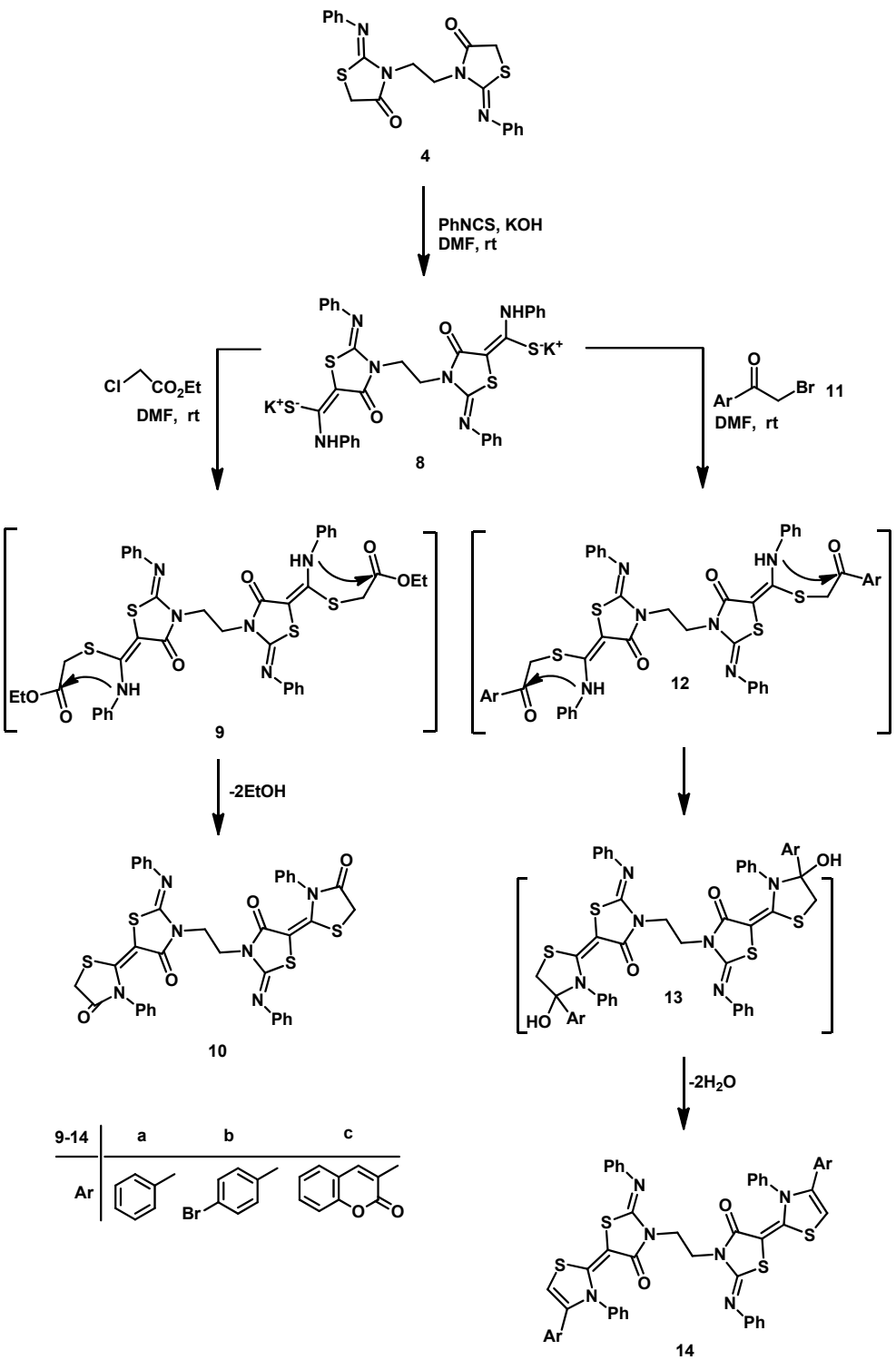
1,2-Bis-[4-oxo-5-(4-oxo-3-phenylthiazolidin-2-ylidene)-2-(phenylimino)thiazolidin-3-yl]ethane (**10**): Color: Purple powder. Yield: 70% (0.53 g). M.p.: >300 °C. FT-IR (KBr, ν, cm^{-1}): 3050 (CH-arom), 2959, 2925 (CH-aliph), 1730, 1644 (2 C=O), 1558 (C=N), 1546 (C=C). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 4.04 (s, 4H, -NCH_2), 4.07 (s, 4H, -SCH_2), 6.60 (m, 4H, ArH), 7.01-7.25 (m, 10H, ArH), 7.37-7.42 (m, 6H, ArH). MS (EI, *m/z* (%)): 761 (M^++1, 3.1), 760 (M^+, 6.1), 393 (49.3), 380 (M^+/2, 3.1), 366 (10.0), 249 (28.4), 221 (69.1), 192 (24.8), 178 (28.4), 148 (15.0), 135 (33.7), 103 (26.2), 93 (22.3), 77 (100). Anal. calcd for $C_{38}H_{28}N_6O_4S_4$ (760.93): C, 59.98; H, 3.71; N, 11.04; S, 16.86. Found: C, 59.67; H, 3.59; N, 11.30; S, 16.77%.

1,2-Bis-[5-(3,4-diphenylthiazol-2-ylidene)-4-oxo-2-(phenylimino)thiazolidin-3-yl]ethane (**14a**): Color: Yellow solid. Yield: 60% (0.52 g). M.p.: >300 °C. FT-IR (KBr, ν, cm^{-1}): 3104, 3049 (CH-arom), 2949 (CH-aliph), 1660 (C=O), 1615 (C=N), 1582 (C=C). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 4.10 (s, 4H, NCH_2), 6.64-6.68 (m, 4H, ArH), 6.90 (s, 2H, thiazole-5-CH), 6.95-7.00 (m, 2H, ArH), 7.13-7.28 (m, 24H, ArH). MS (EI, *m/z* (%)): 881 (M^+, 2.8), 880 (8.0), 644 (2.3), 453 (14.2), 440 (M^+/2, 4.6), 426 (12.5), 280 (36.8), 281 (100), 249 (21.7), 133 (10), 121 (22.8), 91 (12), 77 (36), 64 (21.1), 51 (24.8). Anal. calcd for $C_{50}H_{36}N_6O_2S_4$ (881.12): C, 68.16; H, 4.12; N, 9.54; S, 14.56. Found: C, 68.03; H, 4.07; N, 9.22; S, 14.34%.

1,2-Bis-[5-(4-(4-bromophenyl)-3-phenylthiazol-2-ylidene)-4-oxo-2-(phenylimino)-thiazolidin-3-yl]ethane (**14b**): Color: Yellow crystals. Yield: 55% (0.57 g). M.p.: >300 °C. FT-IR (KBr, ν, cm^{-1}): 3098, 3054 (CH-arom), 2938, 2853 (CH-aliph), 1655 (C=O), 1615 (C=N), 1581 (C=C). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 4.09 (s, 4H, NCH_2), 6.63-6.67 (m, 4H, ArH), 6.94 (s, 2H, thiazole-5-CH), 7.07 (d, 4H, ArH, *J* = 8.7 Hz), 7.13-7.30 (m, 16H, ArH), 7.41 (d, 4H, ArH, *J* = 8.7 Hz). MS (EI, *m/z* (%)): 964 (8), 963 (8), 533 (16), 530 (20), 507 (12), 361 (66), 359 (54), 329 (16), 327(22), 258 (12), 218 (16), 204 (14), 176 (38), 146 (36), 135 (38), 121 (50), 89 (38), 77 (100), 64 (84), 50 (64). Anal. calcd for $C_{50}H_{34}Br_2N_6O_2S_4$ (1038.91): C, 57.80; H, 3.30; N, 8.09; S, 12.35. Found: C, 57.42; H, 3.12; N, 8.32; S, 12.24%.

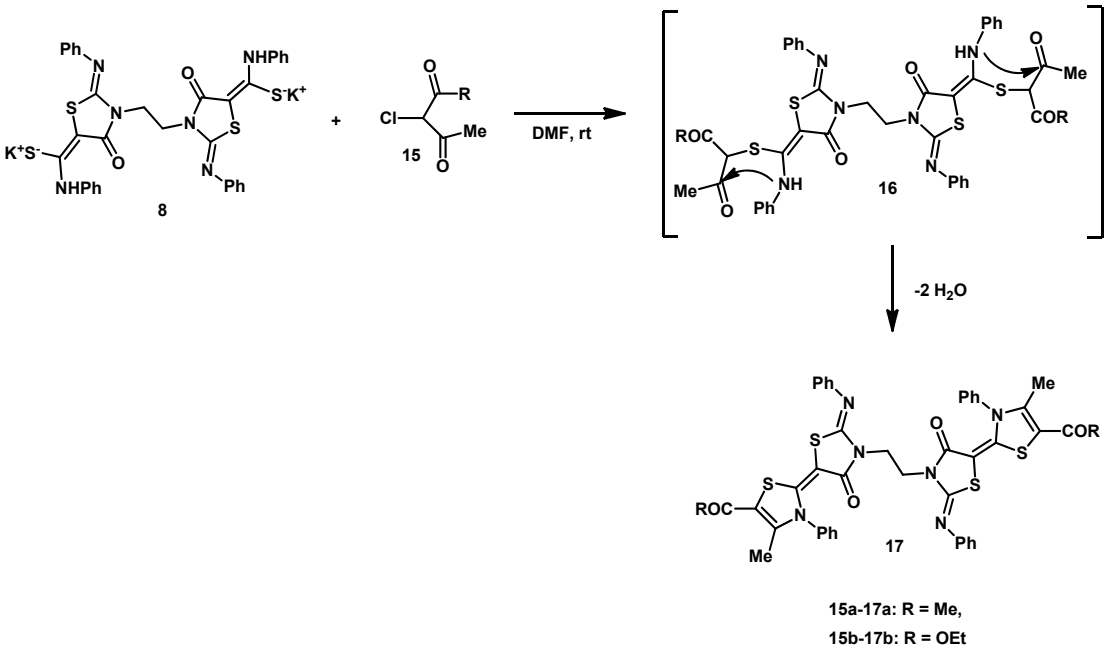
1,2-Bis-[5-(4-(3-coumarinyl)-3-phenylthiazol-2-ylidene)-4-oxo-2-(phenylimino)-thiazolidin-3-yl]ethane (**14c**): Color: Reddish-yellow crystals. Yield: 62% (0.63 g). M.p.: >300 °C. FT-IR (KBr, ν, cm^{-1}): 3033 (CH-arom), 2936 (CH-aliph), 1725, 1655 (2 C=O), 1609 (C=N). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 4.11 (s, 4H, NCH_2), 6.64-6.66 (m, 4H, ArH), 6.96-6.99 (m, 2H, ArH), 7.11 (s, 2H, thiazole-5-CH), 7.16-7.37 (m, 18H, ArH), 7.58-7.67 (m, 4H, ArH), 8.18 (s, 2H, coumarine-4-CH). MS (EI, *m/z* (%)): 1017 (M^+, 24), 996 (20), 976 (24), 923 (28), 824 (24), 770 (28), 589 (28), 508 (M^+/2, 20), 455 (52), 425 (36), 372 (48), 317 (60), 238 (40), 149 (40), 106 (40), 77 (100). Anal. calcd for $C_{56}H_{36}N_6O_6S_4$ (1017.18): C, 66.12; H, 3.57; N, 8.26; S, 12.61. Found: C, 65.93; H, 3.37; N, 8.41; S, 12.67%.

1,2-Bis-[5-(5-acetyl-4-methyl-3-phenylthiazol-2-ylidene)-4-oxo-2-(phenylimino)-thiazolidin-3-yl]ethane (**17a**): Color: Yellow crystals. Yield: 76% (0.64 g). M.p.: >300 °C. FT-IR (KBr, ν, cm^{-1}): 3064 (CH-arom), 2927 (CH-aliph), 1650 (broad, 2 C=O), 1620 (C=N), 1585 (C=C). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 2.06 (s, 6H, CH_3), 2.45 (s, 6H, $COCH_3$), 4.05 (s, 4H, NCH_2), 6.61-6.64 (m, 4H, ArH), 6.98-7.03 (m, 2H, ArH), 7.16-7.20 (m, 4H, ArH), 7.33-7.36 (m, 4H, ArH), 7.48-7.52 (m, 6H, ArH). MS (EI, *m/z* (%)): 841 (M^+, 9.9), 672 (18.5), 433 (13), 420 (M^+/2, 3.7), 349 (19.1), 261 (100), 218 (27.2), 185 (11.1), 177 (98.8), 147 (15.4), 121 (25.3), 91 (25.3), 84 (54.3), 77 (41.4), 54 (81.5). Anal. calcd for $C_{44}H_{36}N_6O_4S_4$ (841.05): C, 62.83; H, 4.31; N, 9.99; S, 15.25. Found: C, 62.66; H, 4.23; N, 9.72; S, 15.31%.



Scheme 2

1,2-Bis-[5-(5-ethoxycarbonyl-4-methyl-3-phenylthiazol-2-ylidene)-4-oxo-2-(phenylimino)thiazolidin-3-yl]ethane (**17b**): Color: Yellow solid. Yield: 72% (0.64 g). M.p.: >300 °C. FT-IR (KBr, ν, cm^{-1}): 3057 (CH-arom), 2976 (CH-aliph), 1699 (C=O), 1617 (C=N), 1584 (C=C). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 1.28 (t, 6H, $CO_2CH_2CH_3$, J = 6.9 Hz), 2.09 (s, 6H, CH_3), 4.05 (s, 4H, NCH_2), 4.26 (q, 4H, $CO_2CH_2CH_3$, J = 6.9 Hz), 6.63-6-68 (m, 4H, ArH), 6.97-7.03 (m, 2H, ArH), 7.18-7.23 (m, 6H, ArH), 7.37-7.39 (m, 2H, ArH), 7.46-7.52 (m, 6H, ArH). ^{13}C NMR (75 MHz, DMSO-d_6, δ, ppm): 13.5, 14.3, 61.1, 82.4, 105.7, 121.3, 123.6, 128.8, 129.7, 130.1, 131.1, 134.5, 146.7, 148.3, 148.8, 152.1, 161.4, 165.6.



Scheme 3

MS (EI, *m/z* (%)): 901 (M^+, 16.1), 899 (33.9), 754 (8.1), 450 ($M^+/2$, 9.7), 436 (24.2), 320 (16.1), 291 (100), 263 (75.8), 219 (53.2), 175 (22.6), 134 (29), 121 (43.5), 91 (29), 84 (40.3), 77 (61.3). Anal. calcd for $C_{46}H_{40}N_6O_6S_4$ (901.11): C, 61.31; H, 4.47; N, 9.33; S, 14.23. Found: C, 61.16; H, 4.41; N, 9.57; S, 14.31%.

2.2. Biological activity

2.2.1. Antimicrobial activity

Antimicrobial activity was determined using the agar well diffusion assay method as described by Holder and Boyce [35]. The tested organisms were sub-cultured on nutrient agar medium (Oxoid Laboratories, UK) for bacteria and Sabouraud dextrose agar (Oxoid Laboratories, UK) for fungi. Penicillin G and Streptomycin were used as a positive control for bacterial strains. Amphotericin B was used as a positive control for fungi. The plates were done in triplicate. Bacterial cultures were incubated at 37 °C for 24 h while the other fungal cultures were incubated at (25-30 °C) for 3-7 days. Antimicrobial activity was determined by measurement zone of inhibition [36].

2.2.2. Determination of minimum inhibitory concentration (MIC)

The minimum inhibitory concentration of the samples was estimated for each of the tested organisms in triplicates. Varying concentrations of the samples (1000-0.007 µg/mL), nutrient broth was added and then a loop full of the test organism previously diluted to 0.5 McFarland turbidity standard was introduced to the tubes. A tube containing broth media only was seeded with the test organisms to serve as control. Tubes containing tested organisms cultures were then incubated at 37 °C for 24 h while the other fungal cultures were incubated at (25-30 °C) for 3-7 days. The tubes were then examined for growth by observing for turbidity [37].

2.2.3. Media used

Sabouraud's glucose agar with antibiotic: The medium used for isolation of pathogenic yeasts has the following composition (g/L): Glucose, 20; peptone, 10; agar, 25 and distilled water, 1 L, pH was adjusted at 5.4. The medium was autoclaved at 115 °C for 15 min then 0.5 g/L. Chloramphenicol was added to avoid bacterial growth [38].

Nutrient agar (NA): The medium was used to cultivate tested bacteria. It contains (g/L) Beef extract, 3; Peptone, 5 and distilled water 1 L [39].

3. Results and discussion

3.1. Chemistry

N,N'-Diphenylethylene-*bis*-thiourea (**1**) was prepared as previously reported [40]. Then, reaction of the *bis*-thiourea derivative **1** with two equivalents of ethyl chloroacetate in refluxing ethanol in the presence of triethylamine furnished only one isolable product for which the *bis*-1,3-thiazolidin-4-one, **3** or **4**, can be assigned (Scheme 1). Formation of the bis-thiazolidin-4-ones, **3** and **4**, originated from the condensation of ethyl chloroacetate with the sulfur atom of two different intermediate thiols, **1A** and **1B**, generated from compound **1** by delocalization of the lone pairs of the two different nitrogen atoms on the adjacent thiocarbonyl group. However, the nature of solvent was reported to be determinant for the reaction course, where, in analogous example use of an alcohol at reflux directs the reaction towards formation of thiazolidin-4-one of type **4** from unsymmetrical thiourea [41]. Formation of 1,2-*bis*-(2-(phenylimino)-4-oxo-1,3-thiazolidin-3-yl)ethane (**4**) was evidenced by ^{1}H NMR where it revealed a singlet resonating at δ 3.93 for the highly deshielded -NCH_2 protons, if compared with -NCH_2 protons of compound **3**. This is in contrast to the reported ambiguous formation of compound **3** and its consequent reaction with aldehydes [42].

The 1,2-*bis*-(2-(phenylimino)-4-oxo-1,3-thiazolidin-3-yl) ethane (**4**) underwent a condensation reaction when treated with aromatic aldehydes in refluxing ethanol in the presence of catalytic amount of piperidine to furnish the corresponding 1,2-*bis*-(5-arylidene-2-(phenylimino)-4-oxo-1,3-thiazolidin-3-yl) ethane derivatives, **5a-e** (Scheme 1). The structures of the reaction products were assessed by elemental analysis and spectral data.

The ^{1}H NMR spectra of **5a-e** were free of 5-CH_2 protons of the starting substrate **4** at δ 4.1 ppm and revealed instead singlet signals around δ 8.0 ppm due to the methine *=CH* proton. The exocyclic C=CH bond in **5a-e** was assigned as *Z*-configuration based on the ^{1}H NMR spectroscopy of products **5a-e** where the methine proton, deshielded by the adjacent C=O, was observed around δ 8.2 ppm which is close to analogous (*Z*)-5-arylidene-thiazolidin-4-ones [43,44]. However, in the *E*-configuration such proton resonates at lower chemical shift values (δ < 7.5 ppm) [45].

Similar treatment of the *bis*-thiazolidin-4-one derivative **4** with pyrazole-4-barboxaldehyde **6** in refluxing ethanol in the presence of piperidine afforded 1,2-*bis*-[5-(1,3-diphenyl-pyrazol-4-yl)methylene-2-(phenylimino)-4-oxo-1,3-thiazolidin-3-yl]ethane (**7**) (Scheme 1). The structure of the reaction product **7** was ascertained from its elemental and spectral data.

Reaction of the *bis*-thiazolidin-4-one derivative **4** with phenyl isothiocyanate in dimethylformamide, in the presence of potassium hydroxide, at room temperature afforded the intermediate potassium salt **8** which reacted *in situ* with two equivalents of ethyl chloroacetate to give only one isolable product that was identified as 1,2-*bis*-[4-oxo-5-(4-oxo-3-phenylthiazolidin-2-ylidene)-2-(phenylimino)thiazolidin-3-yl]ethane (**10**) as depicted in Scheme 2. Elemental analysis and spectral data of the reaction product were in complete agreement with the assigned structure **10**. For example, the mass spectrum of compound **10** showed, beside further fragments, two characteristic fragments at 760 and 380 corresponding to its molecular ion (M^+) and half molecular ion ($M^+/2$), respectively.

In a similar manner, when the intermediate salt **8** was allowed to react *in situ* with the phenacyl bromide (**11a**) (in 1:2 molar ratio) at room temperature, it resulted in the formation of a single isolable product. According to Scheme 2, structure of the obtained product was established as 1,2-*bis*-[5-(3,4-diphenylthiazol-2-ylidene)-4-oxo-2-(phenylimino)thiazolidin-3-yl]ethane (**14a**) on the basis of its elemental and spectral analyses. The ^{1}H-NMR spectrum revealed two singlet signals resonating at δ 4.10 and 6.90 ppm corresponding to NCH_2 and thiazole-5-CH protons, respectively. Its mass spectrum showed ion peaks at *m/z* 881 and 440 assignable to (M^+) and ($M^+/2$), respectively. Similar reaction of the intermediate salt **8** with the α-bromoketones **11b**,**c** under the same experimental condition above furnished 1,2-*bis*-[4-oxo-2-(phenylimino)thiazolidin-3-yl]ethane derivatives **14b**,**c**, as shown in Scheme 2.

Next, treatment of the intermediate salt **8** with 3-chloropentane-2,4-dione (**15a**) in DMF at room temperature afforded only one isolable product which was analyzed correctly for $C_{44}H_{36}N_6O_4S_4$. The structure of the isolated compound was elucidated from spectral data as 1,2-*bis*-[5-(5-acetyl-4-methyl-3-phenylthiazol-2-ylidene)-4-oxo-2-(phenylimino)thiazolidin-3-yl]ethane (**17a**) (Scheme 3). The ^{1}H NMR spectrum of **17a** displayed three singlet signals resonating at δ 2.06, 2.45 and 4.05 ppm due to 4-Me, 5-COMe and NCH_2 protons, respectively, in addition to aromatic multiplets. Its mass spectrum exhibited two ion peaks at *m/z* 841 (M^+) and 420 ($M^+/2$). Formation of **17a** proceeded *via* intramolecular cyclization with loss of water from the intermediate **16a**. The intermediate salt **8** reacted similarly with ethyl 2-chloro-3-oxobutanoate (**15b**) to give the corresponding thiazole-ester derivative **17b** as outlined in Scheme 3. All spectral data (^{1}H NMR, ^{13}C NMR, IR and MS) are in complete accordance with the assigned structure **17b** as presented in experimental section.

3.2. Antimicrobial activity

All synthesized compounds were screened for their antifungal and antibacterial activities. Some of the synthesized compounds exhibited excellent antimicrobial activities with respect to the reference drugs. The results of the antifungal and antibacterial activities are outlined in Tables 1 and 2, respectively. The obtained results declared that most of the synthesized compounds showed variable degrees of inhibition against the tested microorganisms. Susceptibilities of the fungal and bacterial isolates to the synthesized thiazolidin-4-one derivatives were examined by measuring their inhibitory effect on the growth of microorganisms compared to the solvent used.

3.2.1. Antifungal activity

The results of antifungal screening of 1,2-*bis*-(4-oxo-2-(phenylimino)-1,3-thiazolidin-3-yl)ethane derivatives **5a-e**, **7**, **10**, **14a-c** and **17a** against the fungi (*Aspergillus fumigatus, Syncephalastrum racemosum, Geotricum candidum, Candida albicans*) are reported in Table 1, in comparison with those of the reference drug Amphotericin B. The inhibition zone of the compounds **5a-e**, **7**, **10**, **14a-c** and **17a** showed good to excellent activity ranged from 10.6~23.4 mm against *Aspergillus fumigatus, Syncephalastrum racemosum, Geotricum candidum,* if compared with the reference drug Amphotericin B. Definitely, compounds **5c**, **10** and **17a** showed the highest inhibition zones 20.4, 20.3 and 20.6 mm, respectively against *A. fumigatus* compared with 23.7 mm of the reference drug. Interestingly, compounds **10** and **17a** were more potent antifungal than the reference (Amphotericin B) showing higher inhibition zones 21.6 and 21.1 mm, respectively against *S. racemosum* than Amphotericin B (19.7 mm) (Table 1, column 2). Furthermore, compounds **5b**, **5c**, **10**, **14b** and **17a** were the most potent antifungal against *G candidum* with inhibition zones ranging from 21.4~23.4 mm comparing with 28.7 mm of the reference drug. However, all compounds **5a-e**, **7**, **10**, **14a-c** and **17a** were inactive against *Candida albicans*. It is interesting to point out that among the arylidene derivatives **5a-e**, the chloro- and methoxy-substituted compounds **5b** and **5c** are mostly endowed with higher activity with respect unsubstituted phenyl, 4-Me and 4-NMe_2 derivatives **5a**, **5d** and **5e**, respectively, against all tested microorganisms. In addition, the inhibitory activity of bromophenyl-thiazolidine derivative **14b** was also higher than the analogous derivatives **14a**,**c** against all tested microorganisms.

3.2.2. Antibacterial activity

The antibacterial activity of 1,2-*bis*-(4-oxo-2-(phenylimino)-1,3-thiazolidin-3-yl)ethane derivatives **5a-e**, **7**, **10**, **14a-c** and **17a** against Gram positive (*Streptococcus pneumonia, Bacillis subtilis*) and Gram negative (*Pseudomonas aeruginosa, Escherichia coli*) bacteria are outlined in Table 2. The results were compared with antibacterial activity of the reference drugs ampicillin, gentamicin. In comparison with those of ampicillin used as the standard, all the novel 1,2-*bis*-(4-oxo-2-(phenylimino)-1,3-thiazolidin-3-yl)ethane **5a-e**, **7**, **10**, **14a-c** and **17a** demonstrated a high inhibition of all the tested Gram positive microorganisms (*Streptococcus pneumonia* and *Bacillis subtilis*) (inhibition zones varied from 16~23.7 mm), as well as the Gram negative *Pseudomonas aeruginosa* bacteria (inhibition zones varied from 13~18.7 mm when compared with gentamicin). However, all the tested compounds were inactive against the Gram negative bacteria; *Escherichia coli* (Table 2, column 4). Compounds **5b**, **5c**, **10**, **14b** and **17a** showed the most potent effects against the Gram positive (*Streptococcus pneumonia* and *Bacillis subtilis*) bacteria when compared with ampicillin as reference drug (Table 2, columns 2 and 3). Notably, among the 5-arylidene derivatives **5a-e** the inhibitory effect appears to be dependent on the substitution at the benzene ring. The presence of chloro- and methoxy- groups in benzylidene thiazolidinones **5b**,**c** improved antibacterial activity in respect to compound **5a**.

Table 1. *In vitro* antifungal activity of the synthesized compounds *.

Compound	Inhibition zone [mm]			
	Aspergillus fumigatus	*Syncephalastrum racemosum*	*Geotricum candidum*	*Candida albicans*
5a	10.6±0.25	11.7±0.34	16.5±0.58	NA
5b	18.6±0.36	19.3±0.44	21.4±0.58	NA
5c	20.4±0.39	17.3±0.58	21.4±0.58	NA
5d	15.6±0.44	16.2±0.58	17.9±0.37	NA
5e	15.7±0.33	17.2±0.25	19.8±0.34	NA
7	13.6±0.25	16.8±0.34	16.5±0.58	NA
10	20.3±0.25	21.6±0.34	23.4±0.58	NA
14a	16.3±0.25	15.2±0.58	17.3±0.17	NA
14b	17.6±0.58	18.2±0.25	20.3±0.38	NA
14c	16.2±0.36	17.0±0.44	17.6±0.58	NA
17a	20.6±0.63	21.1±0.27	21.9±0.35	NA
Amphotericin B	23.7±0.1	19.7±0.2	28.7±0.2	25.4±0.1

* NA: No activity, data are expressed in the form of mean ± S.D.

Table 2. *In vitro* anti-bacterial activity of the synthesized compounds *.

Compound	Inhibition zone [mm]			
	Streptococcus pneumoniae	*Bacillis subtilis*	*Pseudomonas aeruginosa*	*Escherichia coli*
5a	16.0±0.44	18.3±0.67	NA	13.0±0.46
5b	19.6±0.63	20.0±0.32	NA	15.9±0.46
5c	19.6±0.44	23.7±0.63	NA	14.4±0.25
5d	16.9±0.44	19.3±0.25	NA	14.9±0.44
5e	16.2±0.15	19.8±0.42	NA	17.4±0.53
7	16.0±0.44	18.3±0.67	NA	13.0±0.46
10	20.6±0.44	23.4±0.67	NA	18.6±0.46
14a	15.2±0.44	17.4±0.25	NA	11.2±0.33
14b	20.3±0.43	21.4±0.53	NA	16.9±0.25
14c	18.1±0.63	20.0±0.32	NA	13.9±0.46
17a	20.6±0.34	23.7±0.25	NA	18.3±0.58
Ampicillin	23.8±0.2	32.4±0.3	-	-
Gentamicin	-	-	17.3±0.1	19.9±0.3

* NA: No activity, data are expressed in the form of mean ± S.D.

Table 3. Minimum inhibitory concentrations (MIC).

Microorganism	MIC [µg/mL]					
	5b	5c	10	14b	17a	Standard
Fungi						Amphotericin B
Aspergillus fumigatus	3.9	1.95	1.95	7.8	0.98	0.12
Syncephalastrum racemosum	3.9	15.63	0.49	7.8	0.98	1.95
Geotricum candidum	1.95	0.98	0.12	1.95	0.49	0.015
Gram positive bacteria						Ampicillin
Streptococcus pneumoniae	1.95	1.95	0.98	1.95	0.98	0.12
Bacillis subtilis	1.95	0.12	0.24	0.98	0.12	0.003
Gram negative bacteria						Gentamicin
Escherichia coli	31.25	125	3.9	15.63	7.8	1.95

3.2.3. Minimum inhibitory concentration

The minimum inhibitory concentration of the synthesized new compounds against highly inhibited organisms is reported in Table 3. Most of the tested compounds for MIC demonstrated an excellent antimicrobial activity against *A. fumigatus* (MICs 0.98-7.8 µg/mL), *S. racemosum* (MICs 0.49-15.63 µg/mL), *G. candidum* (MICs 0.12-1.95 µg/mL), *S. pneumoniae* (MICs 0.98-1.95 µg/mL), *B. subtilis* (MICs 0.12-1.95 µg/mL) and *E. coli* (MICs 3.90-31.25 µg/mL) as shown in *Table 3*. It is interesting to report here that the novel *bis*-(2-phenylylimino-thiazolidin-4-one)ethane derivatives **10** and **17a** exhibited higher activity (MIC 0.49 and 0.98 µg/mL, respectively) against *S. racemosum* than the reference antifungal drug Amphotericin B (MIC 1.95 µg/mL).

4. Conclusion

In conclusion, we have investigated a facile route to construction of novel bis-thiazolidine compounds from a very cheap starting material; ethylenediamine. The newly synthesized symmetrical *bis*-(4-oxo-2-phenylylimino-thiazolidin-3-yl)ethane derivatives were fully characterized by elemental and spectral analyses. Moreover, we studied the biological importance of the synthesized compounds by screening their antimicrobial activity against four fungi (*A. fumigatus, S. racemosum, G. candidum, C. albicans*) and two Gram +ve (*S. pneumonia, B. subtilis*) as well as two Gram -ve (*P. aeruginosa, E. coli*) bacteria. All the synthesized compounds **5a-e**, **7**, **10**, **14a-c** and **17a** showed high antibacterial and antifungal activities against all the test microorganisms except *E. coli* and *C. albicans*. Compounds **5c**, **10** and **17a** exhibited the highest antifungal and antibacterial activities against the test microorganisms. In addition, the MIC of the highly active compounds was also evaluated. It was found that compounds **10** and **17a** were more potent active (MIC 0.49 and 0.98 µg/mL, respectively) against *S. racemosum* than Amphotericin B (MIC 1.95 µg/mL).

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