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Catalytic *N*-formylation for synthesis of 6-substituted-2-benzothiazolylimino-5-piperazinyl-4-thiazolidinone antimicrobial agents

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Abstract A new class of piperazine-based 2-benzothiazolylimino-4-thiazolidinones has been efficiently prepared via highly accelerated *N*-formylation of *N*-isopropylpiperazine by the use of a mild heterogeneous catalyst, sulfated tungstate. Heterocyclization of *N*-(benzo[*d*]thiazol-2-yl)-2-chloroacetamides (**3a**–**j**) by use of NH_4SCN in ethanol under reflux efficiently furnished the intermediates 2-benzothiazolyliminothiazolidin-4-ones (**4a**–**j**). These were treated with 4-isopropylpiperazine-1-carbaldehyde (**2**) to prepare the final products **5a**–**j**. The structures of the new derivatives were confirmed by elemental analysis and use of spectroscopic data (FTIR and ^{1}H NMR). Their pharmacological potential as promising antimicrobial agents was determined in vitro against bacteria and a fungus; the lowest minimum inhibitory concentrations (MIC) observed were in the range 4–8 μg/mL.

Keywords *N*-Formylation · Sulfated tungstate · Piperazine · 2-Benzothiazolylimino-4-thiazolidinones · Antibacterial activity

Introduction

Rapid growth of bacteria and fungi in immune-compromised individuals quickly results in disease. The emergence of multidrug-resistant microbial strains as a result of comprehensive use of antibacterial and antifungal drugs is a serious global problem [1, 2]. Infections caused by resistant Gram-positive [3, 4] and Gram-negative [5, 6] bacterial strains often fail to respond to currently used drugs.

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Infection by these microorganisms is a serious problem for the medical community and emphasizes the immediate need for new, more effective, and, in particular, non-traditional antimicrobial agents. Discovery of new antimicrobial agents is an urgent priority to prevent worsening of the problem.

Numerous reports, including ours [7, 8], have emphasized the antimicrobial action of compounds containing a piperazine ring. We have also observed that combination of a piperazine ring with a thiazole ring significant inhibited growth of a wide range of bacteria and fungi [9]. In particular, the presence of a variety of 6-substituted benzothiazole nuclei has been established as integral to antimicrobial potency [10, 11]. Hence, during a search for new antimicrobial compounds, we have investigated a variety of derivatives with specific structures with known biological efficacy, including piperazines, 6-substituted benzothiazoles, and 4-thiazolidinones, the later ones of which have not yet been investigated. Much evidence of the importance of the 4-thiazolidinone ring in medicinal chemistry has emerged in recent decades [12, 13]. Among the important representatives of this class, 2-aryl/heteroarylimino-4-thiazolidinones have been observed to have high antimicrobial potency, and several 2-thiazolylimino-4-thiazolidinones were recently found to have antibacterial properties [14], with MIC ranging from 0.19 to >100 μg/mL. 2-Heteroarylimino-5-benzylidene-4-thiazolidinones have also been prepared and tested for in-vitro antimicrobial efficacy; their MIC ranged from 3 to >100 μg/mL [15]. A similar class of compounds had MIC in the range 0.3 to >100 μg/mL [16].

In an attempt to synthesize 6-substituted-2-benzothiazolylimino-5-piperazinyl-4-thiazolidinone derivatives for testing as antimicrobial agents, the first stage of introduction of piperazine groups into 4-thiazolidinones was *N*-formylation of piperazine derivatives. Previous research has revealed that sulfated tungstate is a highly efficient, recyclable, heterogeneous catalyst for facile *N*-formylation [17].

Materials and methods

Chemistry

Melting points were determined in open capillaries on a Veego VMP-D electronic apparatus and are uncorrected. IR spectra (4000–400 cm^{-1}) of synthesized compounds, as KBr pellets, were recorded on a Shimadzu 8400-S FTIR spectrophotometer. Thin-layer chromatography (TLC) was performed on glass slides (2 × 7.5 cm) coated with silica gel G and spots were visualized under UV irradiation. ^1H NMR spectra were recorded on a Varian 400 MHz spectrometer with dimethyl sulfoxide (DMSO) as solvent and TMS (Me_4Si) as internal standard. ^1H NMR chemical shifts are reported as parts per million (ppm) downfield from TMS. The splitting patterns are designated as follows; s, singlet; d, doublet; dd, doublet of doublets; q, quartet, m, multiplet.

Preparation of sulfated tungstate

A solution of chlorosulfonic acid (0.1 mol) in chloroform (75 mL) in a flask fitted with $CaCl_2$ drying tube was maintained at 0–5 °C in an ice bath, with continuous

stirring, while anhydrous sodium tungstate (0.05 mol) was added in portions. After completion of the reaction, the mixture was stirred for another 1.5 h. The yellowish-white solid obtained was filtered, and washed several times with Millipore water until the filtrate was neutral and free from chloride ions, as confirmed by testing with $AgNO_3$. The crude product was then dried in an oven at 100 °C for 2 h to furnish the final catalyst, sulfated tungstate. IR (KBr, cm^{-1}): 3451, 1616, 1222, 1089, 961, 654.

*Synthesis of 4-isopropylpiperazine-1-carbaldehyde (**2**)*

Sulfated tungstate (10 wt%) was added to a stirred solution of 1-isopropylpiperazine (**1**, 0.1 mol) and formic acid (0.12 mol) and the resulting mixture was heated in an oil bath at 70 °C for 4.5 h. The progress of the reaction was monitored by TLC with toluene–acetone 8:2 as mobile phase. Also, when conversion was complete the liquid reaction mixture automatically solidified, which is the main physical change confirming completion of the reaction. The mixture was left to cool then diluted with 20 mL ethyl acetate and the insoluble catalyst was recovered by filtration and washed. The combined filtrate and washings were washed with water (2 × 10 mL), dried over anhydrous sodium sulfate, and the pure product was obtained by evaporation of the solvent under reduced pressure. Yield 92 %, m.p. 102–103 °C, IR (KBr, cm^{-1}): 1731 (CHO). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.39 (s, ^{1}H, CHO), 7.35–7.28 (m, 4H, Ar–H), 4.34 (s, 3H, OCH_3), 3.87 (br s, 4H, piperazine ring), 3.39 (br s, 4H, piperazine ring).

*General procedure for synthesis of 2-amino-6-substituted benzothiazoles (**2a–j**)*

The appropriate *para*-substituted amine derivative (**1a–j**; 0.1 mol) and an equimolecular amount of potassium thiocyanate were added to 100 mL glacial acetic acid, with cooling of the reaction mixture in an ice bath. The mixture was left at this temperature for up to 20 min then bromine (0.1 mol) in glacial acetic acid was added very slowly, to maintain the temperature of the reaction mixture below 10 °C, then the mixture was stirred at room temperature for 2–4 h to furnish the hydrobromide (HBr) salt. The salt was then isolated by filtration, washed with acetic acid, dried in a vacuum oven, then dissolved in sufficient aqueous ammonia solution to ensure the pH was 11.0. The solid precipitate thus formed was filtered, washed with water, and dried in a vacuum oven to yield the intermediates **4a–j**. The progress of the reaction was monitored by TLC with toluene–acetone 8:2 as mobile phase.

*General procedure for synthesis of N-(benzo[d]thiazol-2-yl)-2-chloroacetamides (**3a–j**)*

Chloroacetyl chloride (0.06 mol) was added dropwise to a mixture of the appropriate 2-amino-6-Substituted benzothiazole, **2a–j** (0.05 mol) and K_2CO_3 (0.06 mol) in benzene (50 mL) at room temperature. The reaction mixture was heated under reflux for 6–12 h, then, after cooling to room temperature, it was

slowly poured into 100 mL ice water. The precipitate obtained was isolated by filtration, washed repeatedly with water, then dried under vacuum to furnish **3a–j**. The progress of the reaction was monitored by TLC with toluene–acetone 8:2 as mobile phase.

*General procedure for synthesis of 6-substituted-2-(benzo[d]thiazol-2-ylimino)thiazolidin-4-ones (**4a–j**)*

A solution of the appropriate *N*-(benzo[*d*]thiazol-2-yl)-2-chloroacetamide (**3a–j**; 5 mmol) and 10 mmol ammonium thiocyanate in ethanol was heated under reflux for 2–6 h. When completion of the reaction was confirmed by TLC, the mixture was left to stand overnight. The precipitate obtained was isolated by filtration, washed with water, then recrystallized, furnishing **4a–j**.

For example, **4a** [15]: Yield: Yield 59 %, m.p. 191–192 °C, IR (KBr, cm^{-1}): 3160 (N–H), 1728 (C=O), 1567 (N=C), 1659 (C=N, benzothiazole, str.), 647 (CS). ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.61 (s, ^{1}H, NH), 7.81 (d, ^{1}H, $J = 8.4$, H-7, benzothiazole), 7.71 (d, ^{1}H, $J = 8.1$, H-4, benzothiazole), 7.51 (t, ^{1}H, $J = 8.4$, H-5, benzothiazole), 7.35 (t, ^{1}H, $J = 8.2$, H-6, benzothiazole), 3.57 (s, 2H, CH_2, thiazolidin-4-one).

*General procedure for synthesis of 2-benzothiazolylimino-5-piperazinyl-4-thiazolidinones (**5a–j**)*

A well-stirred solution of 5 mmol 6-substituted-2-(benzo[*d*]thiazol-2-ylimino)thiazolidin-4-one (**4a–j**) in 40 mL acetic acid was buffered with 9 mmol sodium acetate. 4-Isopropylpiperazine-1-carbaldehyde (**2**; 6.5 mmol) was added and the mixture was heated under reflux for 6–9 h. When reaction was complete, as indicated by TLC, the mixture was poured into ice-cold water. The precipitate was isolated by filtration and washed with water, and the resulting crude product was purified by recrystallization from dioxane to furnish **5a–j** [15].

*2-(Benzo[d]thiazol-2-ylimino)-5-((4-isopropylpiperazin-1-yl)methylene)thiazolidin-4-one (**5a**)* Yield 70 %, m.p. 229–231 °C, IR (KBr, cm^{-1}): 3155 (N–H), 1726 (C=O), 1668 (C=N, benzothiazole, str.), 1571 (N=C), 647 (CS). ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.75 (s, ^{1}H, NH), 7.90 (d, ^{1}H, $J = 8.1$, H-7, benzothiazole), 7.72 (s, ^{1}H, CH), 7.68 (d, ^{1}H, $J = 7.6$, H-4, benzothiazole), 7.56–7.43 (m, 2H, H-5 and H-6, benzothiazole), 3.80 (br s, $4H_{pip}$), 3.44 (br s, $4H_{pip}$), 2.94–2.98 (m, ^{1}H, *N*–CH), 1.88 (d, $J = 6.6$ Hz, 6H, $-2CH_3$). Anal Calcd for $C_{18}H_{21}N_5OS_2$: C, 55.79; H, 5.46; N, 18.07; Found: C, 55.61; H, 5.64; N, 17.93.

*2-(6-Fluorobenzo[d]thiazol-2-ylimino)-5-((4-isopropylpiperazin-1-yl)methylene)thiazolidin-4-one (**5b**)* Yield 56 %, m.p. 259–261 °C, IR (KBr, cm^{-1}): 3170 (N–H), 1712 (C=O), 1661 (C=N, benzothiazole, str.), 1565 (N=C), 655 (CS). ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.81 (s, ^{1}H, NH), 7.78–7.42, 7.13–6.89 (m, 3H, benzothiazole), 7.73 (s, ^{1}H, CH), 3.76 (br s, $4H_{pip}$), 3.39 (br s, $4H_{pip}$), 2.94–2.98 (m,

^{1}H, *N*–CH), 1.91 (d, $J = 6.6$ Hz, 6H, –2CH_3). Anal Calcd for $C_{18}H_{20}FN_5OS_2$: C, 53.31; H, 4.97; N, 17.27; Found: C, 53.48; H, 5.11; N, 17.14.

*2-(6-Chlorobenzo[d]thiazol-2-ylimino)-5-((4-isopropylpiperazin-1-yl)methylene)thiazolidin-4-one (**5c**)* Yield 62 %, m.p. 248–250 °C, IR (KBr, cm^{-1}): 3160 (N–H), 1716 (C=O), 1653 (C=N, benzothiazole, str.), 1580 (N=C), 649 (CS). ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.69 (s, ^{1}H, NH), 7.69–7.39, 7.09–6.93 (m, 3H, benzothiazole), 7.78 (s, ^{1}H, CH), 3.85 (br s, 4H_{pip}), 3.48 (br s, 4H_{pip}), 2.88–2.92 (m, ^{1}H, *N*–CH), 1.82 (d, $J = 6.6$ Hz, 6H, –2CH_3). Calcd for $C_{18}H_{20}ClN_5OS_2$: C, 51.23; H, 4.78; N, 16.60; Found: C, 51.38; H, 4.91; N, 16.77.

*2-(6-Bromobenzo[d]thiazol-2-ylimino)-5-((4-isopropylpiperazin-1-yl)methylene)thiazolidin-4-one (**5d**)* Yield 79 %, m.p. 236–238 °C, IR (KBr, cm^{-1}): 3149 (N–H), 1723 (C=O), 1659 (C=N, benzothiazole, str.), 1570 (N=C), 642 (CS). ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.74 (s, ^{1}H, NH), 7.81–7.28 (m, 3H, benzothiazole), 7.69 (s, ^{1}H, CH), 3.79 (br s, 4H_{pip}), 3.41 (br s, 4H_{pip}), 2.90–2.95 (m, ^{1}H, *N*–CH), 1.78 (d, $J = 6.6$ Hz, 6H, –2CH_3). Anal Calcd for $C_{18}H_{20}BrN_5OS_2$: C, 46.35; H, 4.32; N, 15.02; Found: C, 46.48; H, 4.15; N, 15.19.

*5-(4-Isopropylpiperazin-1-yl)methylene)-2-(6-nitrobenzo[d]thiazol-2-ylimino)thiazolidin-4-one (**5e**)* Yield 49 %, m.p. 287–289 °C, IR (KBr, cm^{-1}): 3166 (N–H), 1709 (C=O), 1671 (C=N, benzothiazole, str.), 1575 (N=C), 639 (CS). ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.80 (s, ^{1}H, NH), 8.09–7.51 (m, 3H, benzothiazole), 7.74 (s, ^{1}H, CH), 3.87 (br s, 4H_{pip}), 3.42 (br s, 4H_{pip}), 2.87–2.92 (m, ^{1}H, *N*–CH), 1.93 (d, $J = 6.6$ Hz, 6H, –2CH_3). Anal Calcd for $C_{18}H_{20}N_6O_3S_2$: C, 49.98; H, 4.66; N, 19.43; Found: C, 50.14; H, 4.51; N, 19.28.

*5-(4-Isopropylpiperazin-1-yl)methylene)-4-oxothiazolidin-2-ylideneamino)benzo[d]thiazole-6-carbonitrile (**5f**)* Yield 44 %, m.p. 279–281 °C, IR (KBr, cm^{-1}): 3158 (N–H), 1699 (C=O), 1656 (C=N, benzothiazole, str.), 1560 (N=C), 650 (CS). ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.70 (s, ^{1}H, NH), 7.66–7.26 (m, 3H, benzothiazole), 7.68 (s, ^{1}H, CH), 3.80 (br s, 4H_{pip}), 3.45 (br s, 4H_{pip}), 2.94–2.99 (m, ^{1}H, *N*–CH), 1.86 (d, $J = 6.6$ Hz, 6H, –2CH_3). Anal Calcd for $C_{19}H_{20}N_6OS_2$: C, 55.32; H, 4.89; N, 20.37; Found: C, 55.42; H, 5.05; N, 20.51.

*5-(4-Isopropylpiperazin-1-yl)methylene)-2-(6-methylbenzo[d]thiazol-2-ylimino)thiazolidin-4-one (**5g**)* Yield 59 %, m.p. 271–273 °C, IR (KBr, cm^{-1}): 3171 (N–H), 1696 (C=O), 1649 (C=N, benzothiazole, str.), 1564 (N=C), 654 (CS). ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.78 (s, ^{1}H, NH), 7.65–7.09 (m, 3H, benzothiazole), 7.77 (s, ^{1}H, CH), 3.84 (br s, 4H_{pip}), 3.51 (br s, 4H_{pip}), 2.89–2.94 (m, ^{1}H, *N*–CH), 2.19 (s, 3H, CH_3), 1.88 (d, $J = 6.6$ Hz, 6H, –2CH_3). Anal Calcd for $C_{19}H_{23}N_5OS_2$: C, 56.83; H, 5.77; N, 17.44; Found: C, 56.71; H, 5.90; N, 17.29.

*5-(4-Isopropylpiperazin-1-yl)methylene)-2-(6-methoxybenzo[d]thiazol-2-ylimino)thiazolidin-4-one (**5h**)* Yield 66 %, m.p. 252–254 °C, IR (KBr, cm^{-1}): 3159 (N–H),

1712 (C=O), 1659 (C=N, benzothiazole, str.), 1572 (N=C), 646 (CS). ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.81 (s, ^{1}H, NH), 7.56–7.26, 6.85–6.96 (m, 3H, benzothiazole), 7.71 (s, ^{1}H, CH), 3.87 (br s, $4H_{pip}$), 3.76 (s, 3H, OCH_3), 3.46 (br s, $4H_{pip}$), 2.93–2.97 (m, ^{1}H, *N*–CH), 1.84 (d, $J = 6.6$ Hz, 6H, $-2CH_3$). Anal Calcd for $C_{19}H_{23}N_5O_2S_2$: C, 54.65; H, 5.55; N, 16.77; Found: C, 54.79; H, 5.38; N, 16.94.

*5-(4-Isopropylpiperazin-1-yl)methylene)-2-(6-ethoxybenzo[d]thiazol-2-ylimino)thiazolidin-4-one (**5i**)* Yield 71 %, m.p. 245–247 °C, IR (KBr, cm^{-1}): 3151 (N–H), 1721 (C=O), 1655 (C=N, benzothiazole, str.), 1579 (N=C), 647 (CS). ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.75 (s, ^{1}H, NH), 7.41–7.16, 6.81–6.90 (m, 3H, benzothiazole), 7.75 (s, ^{1}H, CH), 4.18 (q, $J = 6.6$ Hz, 2H, OCH_2CH_3), 3.77 (br s, $4H_{pip}$), 3.41 (br s, $4H_{pip}$), 2.91–2.95 (m, ^{1}H, *N*–CH), 2.21 (t, $J = 6.8$ Hz, 3H, CH_2CH_3), 1.78 (d, $J = 6.6$ Hz, 6H, $-2CH_3$). Anal Calcd for $C_{20}H_{25}N_5O_2S_2$: C, 55.66; H, 5.84; N, 16.23; Found: C, 55.52; H, 6.01; N, 16.11.

*N-5-(4-Isopropylpiperazin-1-yl)methylene)-4-oxothiazolidin-2-ylideneamino)benzo[d]thiazol-6-yl)acetamide (**5j**)* Yield 50 %, m.p. 239–241 °C, IR (KBr, cm^{-1}): 3168 (N–H), 1729 (C=O), 1669 (C=N, benzothiazole, str.), 1567 (N=C), 642 (CS). ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.79 (s, ^{1}H, NH), 9.42 (s, ^{1}H, –NH), 7.47–7.25 (m, 3H, benzothiazole), 7.68 (s, ^{1}H, CH), 3.83 (br s, $4H_{pip}$), 3.46 (br s, $4H_{pip}$), 2.93–2.98 (m, ^{1}H, *N*–CH), 2.19 (s, 3H, CH_3), 1.91 (d, $J = 6.6$ Hz, 6H, $-2CH_3$). Calcd for $C_{20}H_{24}N_6O_2S_2$: C, 54.03; H, 5.44; N, 18.90; Found: C, 53.92; H, 5.59; N, 19.07.

Microbiology

Derivatives **5a**–**j** were tested for antimicrobial activity against Gram-positive bacteria (*Staphylococcus aureus*, *Bacillus subtilis*), Gram-negative bacteria (*E. coli*, *P. aeruginosa*), and the fungus (*Candida albicans*). Ciprofloxacin and fluconazole were used as control drugs for antibacterial and antifungal activity, respectively. Details of the biological assays have been reported elsewhere [18]. Results were obtained as minimum inhibitory concentrations (MIC) by use of the agar streak dilution method [19]. A stock solution of the compound (100 μg/mL) was prepared in DMSO, and serial dilutions of the test compounds at μg/mL concentrations were incorporated in molten sterile agar (nutrient agar and Sabouraud dextrose agar for evaluation of antibacterial activity and antifungal activity, respectively). The medium containing the test compound was poured into a Petri dish to a depth of 4–5 mm and left to solidify under aseptic conditions. A suspension of the respective microorganism, approximately 10^5 CFU/mL, was prepared and applied to the plates, which were then incubated at 37 ± 1 °C for 24 h (bacteria) or 48 h (fungus). The lowest concentration of the substance that prevented development of visible growth was recorded as the MIC value. Zones of inhibition were determined by use of the paper disk diffusion technique [20].

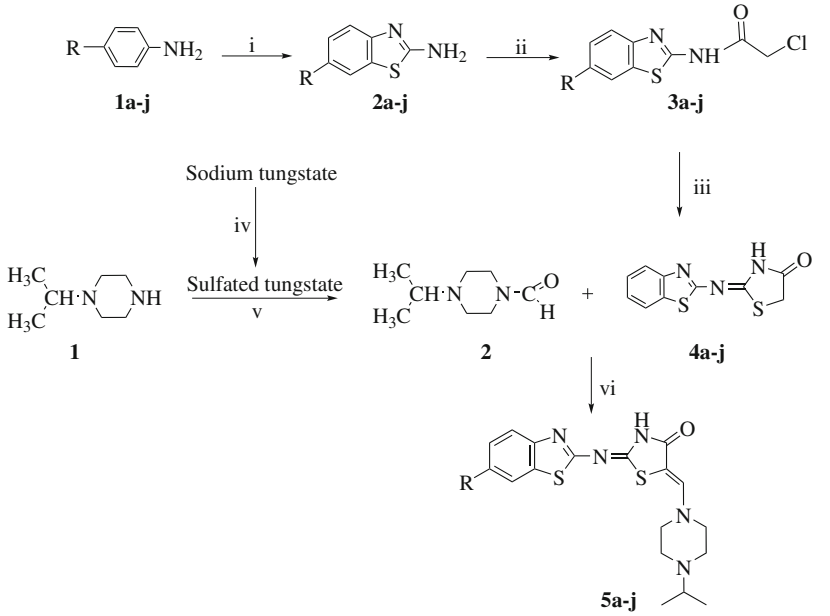
Results and discussion

Chemistry

The reactions outlined in Scheme 1 were used for synthesis of the intermediates and title compounds (**5a–j**). Sulfated tungstate as mild, heterogeneous, solid acid catalyst was obtained as a yellowish–white solid by reaction of anhydrous sodium tungstate with chlorosulfonic acid in chloroform. Its formation was confirmed by comparison of its FTIR spectrum with literature data. The catalyst (10 wt%) efficiently promoted rapid *N*-formylation of *N*-isopropylpiperazine with formic acid, yielding 4-isopropylpiperazine-1-carbaldehyde (**2**) [17]. Its FTIR and ^{1}H NMR spectra contained peaks at 1731 cm^{-1} and 8.39 ppm, respectively, indicative of CHO functionality. We have previously reported synthesis of 2-amino-6-substituted benzothiazoles **2a–j** from *para*-substituted amines in the presence of KSCN and bromine in glacial acetic acid, and their 2-chloroacetamide congeners **3a–j** obtained by use of chloroacetyl chloride [10]. The structures of the benzothiazole-based intermediates were confirmed by comparison of FTIR and ^{1}H NMR spectral data with literature results [21]. Heterocyclization of **3a–j** in the presence of NH_4SCN in ethanol under reflux furnished the 6-substituted-2-(benzo[*d*]thiazol-2-ylimino)thiazolidin-4-one intermediates **4a–j** in good yields and purity. The structures of **4a–j** were, again, confirmed by study of FTIR and ^{1}H NMR spectra. The spectra of derivative **4a** were in perfect agreement with the proposed structure. Peaks at 3160, 1728, and 1567 cm^{-1} in the FTIR spectrum confirmed cyclization to form an iminothiazolidinone. In addition, the ^{1}H NMR spectrum of **4a** contained an –NH proton signal at 12.61 ppm and a methylene proton signal of the cyclized ring at 3.57 ppm. Similar results confirmed successful synthesis of **4a–j**. The imino intermediates were then treated with *N*-(benzo[*d*]thiazol-2-yl)-2-chloroacetamides **3a–j** in the presence of anhydrous sodium acetate in glacial acetic acid to furnish 2-benzothiazolylimino-5-piperazinyl-4-thiazolidinones **5a–j**. Analytical data for **5a–j** confirmed the *Z* configuration—NH lactam proton peaks occurred at approximately 12.70 ppm rather than below 10 ppm (where signals from imine protons are observed). Moreover, because of the deshielding effect of the adjacent carbonyl functionality, the methine protons of exocyclic C=C bonds resonated at high chemical shifts, approximately 7.70 ppm, further confirming the *Z* isomerism [16, 22]. Furthermore, all other aspects of the ^{1}H NMR and FTIR spectra were indicative of the presence of benzothiazole and piperazine rings, in accordance with our previous research results [7, 23]. The purity of the synthesized compounds was monitored by TLC and by elemental analysis.

Pharmacology

Bioassay results from investigation of the activity of **5a–j** against two Gram-positive bacteria (*S. aureus* and *B. subtilis*), two Gram-negative bacteria (*E. coli* and *P. aeruginosa*), and the fungus *C. albicans* are summarized in Table 1. In general, the results for antibacterial action were encouraging with lowest MIC of 4–8 μg/mL for some of the derivatives. None of the derivatives had antifungal activity, however (MIC



Reagents & Conditions: i. KSCN, Br_2, AcOH, R.T.; ii. $ClCH_2COCl$, $CHCl_3$, K_2CO_3, reflux; iii. NH_4SCN, EtOH, reflux; iv. $ClSO_3H$, $CHCl_3$, 0-5°C; v. Sulfated tungstate (10 wt %), HCOOH, 70 °C; vi. anhydrous NaOAc in AcOH, 120 °C.

Scheme 1 Synthesis of piperazine-based benzothiazolylimino-4-thiazolidinones **5a–j**

>32 μg/mL). Gram-positive strains were more sensitive than Gram-negative strains (MIC 4 μg/mL and 4–8 μg/mL, respectively). Compounds with benzothiazole rings containing electron-withdrawing (EWD) fluorine (**b**) and nitro (**e**) and electron-releasing (ER) acetamide (**j**) were most potent. In general, however, EWD substituents resulted in better antibacterial activity than ER groups. The order of activity among EWD groups was $F > NO_2 > Br > Cl > CN$. Overall, SARs (Structure Activity Relationships) suggested that combined benzothiazole and thiazolidinone rings positively enhanced antibacterial action, and the presence of the piperazine ring was essential to reduce MIC compared with MIC reported in the literature.

The MIC of the 6-substituted-2-benzothiazolylimino-5-piperazinyl-4-thiazolidinones were determined by measurement of their growth inhibitory effects, as zone of inhibition obtained for a concentration of 32 μg/disk. Compound **5b** was the most potent of all the compounds (MIC 4 μg/mL against *S. aureus*, *B. subtilis*, and *P. aeruginosa* and 8 μg/mL against *E. coli*). These MIC suggest that the presence of the highly electronegative and EWD fluorine atom may be a crucial aspect of potency. In addition, **5e**, with an EWD nitro substituent was promisingly active (MIC 8 μg/mL against both Gram-positive bacteria and Gram-negative *E. coli*). Derivative **5j** with the ER acetamide group had an MIC of 4 μg/mL against both Gram-positive strains. Compound **5c** with the EWD chlorine atom and compound

Table 1 In-vitro antimicrobial activity of compounds **5a–j**

Compound (R) 32 μg/disk	MIC (zone of inhibition ± SD)[a]				
	S. aureus	*B. subtilis*	*E. coli*	*P. aeruginosa*	*C. albicans*
5a (H)	>32.0 (10 ± 0.5)	32.0 (12 ± 0.7)	32.0 (12 ± 1.0)	32.0 (10 ± 0.6)	>32.0 (10 ± 0.6)
5b (F)	*4.0* (*21* ± 0.4)	*4.0* (*17* ± 0.9)	*8.0* (*18* ± 0.6)	*4.0* (*19* ± 0.6)	>32.0 (10 ± 0.6)
5c (Cl)	>32.0 (10 ± 0.7)	32.0 (11 ± 0.4)	16.0 (14 ± 0.7)	8.0 (15 ± 0.8)	>32.0 (10 ± 0.7)
5d (Br)	16.0 (24 ± 0.6)	8.0 (19 ± 0.5)	16.0 (15 ± 1.1)	>32.0 (10 ± 0.9)	>32.0 (10 ± 0.5)
5e (NO_2)	8.0 (18 ± 0.5)	8.0 (19 ± 0.6)	*8.0* (*19* ± 0.7)	16.0 (14 ± 1.0)	>32.0 (10 ± 0.7)
5f (CN)	>32.0 (10 ± 0.6)	>32.0 (10 ± 0.6)	>32.0 (10 ± 0.8)	>32.0 (10 ± 0.8)	>32.0 (10 ± 0.9)
5 g (CH_3)	16.0 (16 ± 0.5)	16.0 (17 ± 0.6)	16.0 (16 ± 0.5)	8.0 (17 ± 0.6)	>32.0 (10 ± 0.6)
5 h (OCH_3)	>32.0 (10 ± 0.5)	>32.0 (10 ± 0.7)	>32.0 (10 ± 0.7)	>32.0 (10 ± 0.4)	>32.0 (10 ± 0.9)
5i (OC_2H_5)	32.0 (11 ± 0.3)	>32.0 (10 ± 0.5)	32.0 (12 ± 0.7)	>32.0 (10 ± 0.5)	>32.0 (10 ± 0.6)
5j ($NHCOCH_3$)	*4.0* (*22* ± 0.4)	*4.0* (*22* ± 0.4)	32.0 (12 ± 0.6)	32.0 (10 ± 0.8)	>32.0 (10 ± 0.7)
Ciprofloxacin (30 μg/disk)	3.12 (20 ± 0.6)	3.12 (24 ± 0.5)	6.25 (15 ± 0.5)	6.25 (16 ± 0.5)	
Fluconazole	–	–	–	–	6.25 (16 ± 0.7)

Values in italics represents MICs for the most potent analogues

[a] Each value is the mean from three independent experiments; SD, standard deviation

5g with the ER methoxy functional group on the benzothiazole ring had MIC of 8 μg/mL against *P. aeruginosa* and compound **5d** with the bromine substituent had a similar MIC against *B. subtilis*. Growth inhibitory effects of these compounds, as zone of inhibition, ranged from 17 to 22 mm compared with 15–24 mm for the control drug ciprofloxacin. Overall, the activity of the 6-substituted-2-benzothiazolylimino-5-piperazinyl-4-thiazolidinones was comparable with that of ciprofloxacin. Some were almost equipotent with ciprofloxacin whereas the others had MIC of 16–32 μg/mL against all the bacterial strains.

Conclusion

The purpose of this research study was rational development of a new class of compounds with promising antibacterial action. The novelty of the work includes attachment of piperazine at the C-1 position of the 4-thiazolidinone ring and use of more than one thiazole ring to enhance the overall potency of the compounds. As expected, the 6-substituted-2-benzothiazolylimino-5-piperazinyl-4-thiazolidinones were potent inhibitors of the growth of Gram-positive and Gram-negative bacteria with MIC of 4–8 μg/mL and inhibitory zones of 17–22 mm compared with 3.12–6.25 μg/mL and 15–24 mm, respectively, for the control drug ciprofloxacin. Growth of fungal species was not inhibited. These preliminary research results could aid discovery, by drug-discovery scientists, of other compounds with activity against several biological targets.

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Conflict of interest The authors report no conflicts of interest.

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