

Synthesis and biological activities of piperazine derivatives as antimicrobial and antifungal agents

Hemant R. Suryavanshi¹ and Mithilesh M. Rathore^{*}

Department of Chemistry, Vidya Bharati Mahavidyalaya, C. K. Naidu Road, Camp,
Amravati. 444601 (MS) India

(Received May 31, 2017; Revised August 20, 2017; Accepted August 21, 2017)

Abstract: Apart from thiazole, benzimidazole, and tetrazole family, some of the piperazine analogs also show significant pharmacophoric activities. The synthesis of piperazine through intermediate **3** occurred via coupling of substituted benzenethiol with chloro-nitrobenzene. The nitro group of the isolated intermediate was reduced via an iron-acetic acid system. The aniline intermediate was cyclized with bis(2-chloroethyl)amine hydrochloride to obtain piperazine moiety. The synthesized substituted piperazine derivatives were screened for antibacterial and antifungal activities. The antibacterial activity was tested against *Staphylococcus aureus*, *Streptomyces epidermidis*, *Pseudomonas aeruginosa* and *Escherichia coli*, and antifungal activity was tested against *Candida albicans*, *Aspergillus niger*, *Aspergillus flavus* and *Aspergillus fumigatus*. As a result, many of the synthesized compounds showed significant antimicrobial and antifungal properties.

Keywords: Antibacterial activity; antifungal activity; piperazine derivatives. ©2017 ACG Publications. All rights reserved.

1. Introduction

Antibiotics are a class of drug that covers a wide range of pathogenic infections. Without causing any harm to the human organs or tissues, antibiotics interfere with the natural life cycle of bacteria, thus creating evolutionary pressure on them. Recently, many antibiotics-related researches have been conducted aiming at curing bacterial infections in humans. Antibiotics are now commonly used for medicinal purpose and during pre- and post-operation conditions. In critical operations involving use of oncology-based drugs, to prevent any infections, prompt antibiotic therapy is instituted.¹

Penicillin was the first antibiotic to be discovered in 1928, followed by clinically efficient sulfonamide. After regular use of penicillin, the microbes developed resistance towards the antibiotic. Breeding of a host of penicillin-fast organisms was reported, which were transferred to other individuals leading to pneumonic conditions, because of which penicillin became ineffective.² Periodic supervision of these drugs has been conducted to prevent transmission of infections; however, these parameters have often been considered as a reactionary tool rather than a piece of any strategic planning and vision.³ Hence, there is a need for a wide scope of research in finding new antibiotic molecules to overcome the bacterial infections. As the bacterium changes its nature regularly from one phase to another, studies with updated possible solutions are needed.

* Corresponding author: E-mail: mithsrathore@gmail.com

To address the absence of development in the field of new anti-toxins, initially the purposes behind the hindered improvement should be comprehended. Although antibacterial and antifungal illnesses exist for long time, their clinical analysis is still difficult. Since previous two decades, various classes of antibacterial⁴⁻¹⁰ and antifungal agents¹¹⁻¹⁷ have been found. Cotrimoxazole, Trimethoprim, sulfasalazine are few manufactured and semi-engineered antibacterial sulfa drugs. Penicillin drugs, such as Amoxicillin, Ampicillin, Penicillin G, and Cephalosporins have also been used for their bactericidal effects. Cephalosporins are known to eliminate microbes and work comparatively to penicillin. Antifungal drugs, such as Clotrimazole, Fluconazole and Amphotericin B, have been used to treat fungal infections. The most successive contagious pathogens are *Candida*, *Histoplasma*, *Stachybotrys*, *Aspergillus*, *Pneumocystis* and *Cryptococcus*, causing more than 1 million deaths globally every year. To restrict death rate, fast diagnosis and rapid execution of proper therapeutics need to be followed.¹⁸ Due to nosocomial bloodstream infections, many deaths are reported. Majority of cases (2/3) are due to gram positive organisms and remaining due to gram negative organisms and fungi.¹⁹

The most common microbial infections in the hematopoietic stem cell transplant (HSCT) recipients are is caused by *Aspergillus*.²⁰ Currently, almost half of the patients suffer from invasive *aspergillosis*die, and the mortality rate for *candidemia* also remains high at approximately 50%.²¹ Four classes of antifungal drugs, i.e. triazoles, polyenes, pyrimidine analogs and echinocandins, are available to treat fungal infections.²² Increasing incidence of vancomycin-resistant *Enterococcus faecalis* and methicillin-resistant *Staphylococcus aureus* have been marked as a serious menace. However, limitation to infusion-related reactions and nephrotoxicity has been exhibited by amphotericin B, an antifungal drug used to treat serious fungal illnesses.^{23,24}

Piperazines and substituted piperazines constitute an important class of pharmacophores. One of the well-known piperazine category is indinavir (trade name: Crixivan), which functions as an HIV protease inhibitor. Piperazinyl cross-linked ciprofloxacin dimers, when used against resistant bacteria strain, exhibited low antibacterial activity.²⁵ When piperazine derivatives were linked to benzimidazole and benzotriazole molecules, they showed antifungal activity. Similarly, piperazine derivatives, containing tetrazole nucleus, have been reported as antifungal agents.²⁶ The details of the scaffold synthesis included coupling reaction, which was followed by reduction of nitro group and then cyclization.²⁷⁻²⁸ In the present study, synthesis of a series of *N*-alkyl piperazine derivatives as a new class of synthetic antimicrobial and antifungal agents is disclosed.

2. Experimental

Analytical thin-layer chromatography (TLC) was performed on Merck 60 F254 silica gel plate (0.25 mm thickness) and visualized under UV light, and/or by spraying with 5% solution of phosphomolybdic acid (PMA) in ethanol, followed by charring with a heat gun. Following this, column chromatography was performed on Merck 60 silica gel (230-400 mesh). ¹H-NMR was recorded on Varian NMR 500 instrument at 500 MHz. Chemical shifts were reported in parts per million (ppm) relative to tetramethylsilane (TMS), which was used as internal and external standards for ¹H-NMR. Mass spectra were recorded on Shimadzu 2010s mass spectrometer.

2.1. Chemistry

2.1.1. Procedure for Synthesis of 1-3

(2,4-Dimethylphenyl)(2-nitrophenyl)sulfane (**1**) : To a solution of 1-chloro-2-nitrobenzene (**b**) (10.00 g, 63.47 mmol) in dry DMF (30 mL), K₂CO₃ (10.51 g, 76.16 mmol) and 2,4-dimethylbenzenethiol (9.03 mL, 66.64 mmol) were added, and the resulting reaction mixture was stirred at 25 °C for 18 h. Water (60 mL) was added and obtained mixture was stirred at 25 °C for 30 min. Yellow precipitate was then filtered off, washed with water (2 x 50 mL) and dried to afford the title compound **1** as yellow solid. Yield: 16.30 g, 99.08 %. ¹H NMR: (CDCl₃, 500 MHz) δ 8.25 (dd, *J* = 1.4, 8.2 Hz, 1 H), 7.46 (d, *J* = 7.8 Hz, 1 H), 7.32 (m, 1 H), 7.22-7.18 (m, 2H), 7.11 (m, 1H), 6.71 (dd, *J* = 1.2, 8.2 Hz, 1H), 2.40 (s, 3H), 2.30 (s, 3H).

2-((2,4-Dimethylphenyl)thio)aniline (2): To a mixture of (2,4-dimethylphenyl)(2-nitrophenyl)sulfane (**1**) (10.0 g, 38.6 mmol) and AcOH (100 mL), Fe (8.61 g, 154.4 mmol) was added, and the resulting reaction mixture was stirred at 30 °C for 16 h. The reaction mixture was then filtered through a bed of Celite, and the filtrate was concentrated. To the residue, 300 mL saturated NaHCO₃ and 100 mL EtOAc were added. Organic layer was separated, water layer was re-extracted with EtOAc (100 mL), the combined organic layers were dried over Na₂SO₄, and the solvent was evaporated to afford **2** as an orange oil. Yield: 8.85 g, 99%. ¹H NMR: (CDCl₃, 500 MHz) δ 7.38 (dd, *J* = 1.5, 7.6 Hz, 1 H), 7.23 (m, 1H), 7.02 (m, 1H), 6.87 (m, 1H), 6.75-6.81 (m, 2H), 6.71 (d, *J* = 8.0 Hz, 1H), 4.24 (br s, 2H), 2.40 (s, 3H), 2.28 (s, 3H).

1-(2-(2,4-Dimethylphenylthio)phenyl)piperazine (3): A mixture of 2-((2,4-dimethylphenyl)thio)aniline (**2**) (5.0 g, 21.8 mmol), bis(2-chloroethyl)amine hydrochloride (3.89 g, 21.8 mmol) and *N,N*-dimethylformamide (10 mL) was stirred at 110 °C for 48h. The reaction mixture was then cooled to 25 °C, the solvent was evaporated completely. Water (25 mL) and 25 mL ethyl acetate was added, and the mixture then further cooled to ~ 5°C while stirring. The formed white solid was filtered and washed with cold ethyl acetate (10 mL), and dried to afford **3** as a white powder as a HCl salt. ¹H NMR: (CDCl₃, 500 MHz) δ 7.39 (d, *J* = 7.8 Hz, 1H), 7.16 (m, 1H), 7.06-7.10 (m, 2H), 7.04 (m, 1H), 6.87 (m, 1H), 6.52 (m, 1H), 3.02-3.09 (m, 8H), 2.37 (s, 3H), 2.33 (s, 3H), 1.63 (br s, 1H, NH).

2.1.2. Procedure for Synthesis of **4a-4p**

2-(2-(4-(2-(2,4-Dimethylphenylthio)phenyl)piperazin-1-yl)ethoxy)ethanol (HS-4a): To a solution of 1-(2-(2,4-dimethylphenylthio)phenyl)piperazine (0.5 g, 1.67 mmol) in DMF (5 mL) was added di-isopropyl ethyl amine (0.6 mL, 3.35 mmol) and 2-(2-chloroethoxy)ethanol (0.31 g, 2.51 mmol), which was followed by addition of potassium iodide (0.069 g, 0.41 mmol). The contents were stirred at 100 °C for 16 h. It was cooled to room temperature, water was added and extracted with ethyl acetate. Brine washing was performed with the organic layer, which was dried over anhydrous sodium sulfate, filtered and the solvent was evaporated. The crude product was purified by column chromatography to afford **HS-4a**, 0.23 g, 35%. IR: 3537, 3240, 3192, 3180, 3080, 3051, 3007, 2978, 2941, 2914, 2891, 2875, 2864, 2833, 2816, 2785, 2750, 2706, 2684, 25511934, 1913, 1897, 1643, 1600, 1577, 1556, 1514, 1348, 1307, 1269, 1138, 1114, 1074, 1060, 1043, 954, 923, 885, 819, 779, 761, 752, 729 cm⁻¹. ¹H-NMR (CDCl₃, 500 MHz): δ 7.37 (d, *J* = 9.5 Hz, 1H), 7.22 (s, 1H), 7.11-7.07 (m, 3H), 6.90-6.86 (m, 1H), 6.37 (d, *J* = 10 Hz, 1H), 4.62 (m, 1H), 3.56-3.49 (m, 4H), 3.43-3.41 (m, 2H), 2.97 (br s, 4H), 2.59-2.56 (m, 4H), 2.51 (t, *J* = 10 Hz, 2H), 2.32 (s, 3H), 2.23 (s, 3H). MS [M+H]⁺ Calculated for C₂₂H₃₀N₂O₂S: *m/z* 387.21, found 387.20

1-(4-(2-(2,4-Dimethylphenylthio)phenyl)piperazin-1-yl)-2-methylpropan-2-ol (HS-4b): To a solution of 1-(2-(2,4-dimethylphenylthio)phenyl)piperazine (0.5 g, 1.67 mmol) in ethanol (10 mL) was added triethyl amine (0.7 mL, 5.01 mmol), which was followed by addition of 2,2-dimethyloxirane (0.31 g, 4.36 mmol) in a sealed tube. The contents were stirred at 90 °C for 6 h. The mixture was cooled to room temperature, the solvent was evaporated, water was added and extracted with ethyl acetate. Brine washing was performed with the organic layer, which was dried over anhydrous sodium sulfate, filtered and the solvent was evaporated. The crude product was purified by column chromatography to afford **HS-4b**, 0.4 g, 64%. IR: 3045, 2981, 2966, 1579, 1471, 1440, 1402, 1228, 1176, 1039, 960, 921, 804, 763, 754, 732 cm⁻¹. ¹H-NMR (CDCl₃, 500 MHz): δ 7.32 (d, *J* = 9.0 Hz, 1H), 7.15-7.02(m, 4H), 6.91 (t, *J* = 9.0 Hz, 1H), 6.49 (d, *J* = 9.5 Hz, 1H), 5.20-4.80 (br s, 1H, OH), 3.84-3.78 (m, 4H), 3.36-3.09 (m, 6H), 2.35 (s, 3H), 2.28 (s, 3H), 1.48 (s, 6H). MS [M+H]⁺ Calculated for C₂₂H₃₀N₂OS: *m/z* 371.22, found 371.15.

1-(Cyclopropylmethyl)-4-(2-(2,4-dimethylphenylthio)phenyl)piperazine (HS-4c): To a solution of 1-(2-(2,4-dimethylphenylthio)phenyl)piperazine (0.5 g, 1.67 mmol) in DMF (5 mL) was added di-isopropyl ethyl amine (0.6 mL, 3.35 mmol) and (bromomethyl) cyclopropane (0.31 g, 2.51 mmol), which was followed by addition of potassium iodide (0.069 g, 0.41 mmol). The contents were stirred at 100 °C for 12 h. It was cooled to room temperature, water was added and extracted with ethyl acetate. Brine washing was performed with the organic layer, which was dried over anhydrous sodium

sulfate, filtered and the solvent was evaporated. The crude product was purified by column chromatography to afford **HS-4c**, 0.15 g, 26%. IR: 2941, 2916, 2816, 1577, 1471, 1452, 1440, 1373, 1296, 1224, 1143, 1043, 1016, 813, 761, 750, 729 cm^{-1} . $^1\text{H-NMR}$ (CDCl_3 , 500 MHz): δ 7.38 (d, $J = 10.0$ Hz, 1H), 7.14 (s, 1H), 7.10-7.01 (m, 3H), 6.85 (t, $J = 8.5$ Hz, 1H), 6.49 (d, $J = 9.5$ Hz, 1H), 3.14 (br s, 4H), 2.75 (br s, 4H), 2.36-2.32 (m, 8H), 0.98-0.88 (m, 1H), 0.55-0.53 (m, 2H), 0.15-0.14 (m, 2H). MS $[\text{M}+\text{H}]^+$ Calculated for $\text{C}_{22}\text{H}_{28}\text{N}_2\text{S}$: m/z 353.21, found 353.15

1-(Cyclopentylmethyl)-4-(2-(2,4-dimethylphenylthio)phenyl)piperazine (HS-4d): To a solution of 1-(2-(2,4-dimethylphenylthio)phenyl)piperazine (0.5 g, 1.67 mmol) in DMF (5 mL) was added diisopropyl ethyl amine (0.6 mL, 3.35 mmol) and bromomethylcyclopentane (0.41 g, 2.51 mmol), which was followed by addition of potassium iodide (0.069 g, 0.41 mmol). The contents were stirred at 100 °C for 16 h. It was cooled to room temperature, water was added and extracted with ethyl acetate. Brine washing was performed with the organic layer, which was dried over anhydrous sodium sulfate, filtered and the solvent was evaporated. The crude product was purified by column chromatography to afford **HS-4d**, 0.12 g, 19%. IR: 3047, 2939, 2812, 1577, 1450, 1373, 1222, 1139, 1124, 1043, 1001, 931, 812, 765, 731 cm^{-1} . $^1\text{H-NMR}$ (CDCl_3 , 500 MHz): δ 7.38 (d, $J = 9.5$ Hz, 1H), 7.14 (s, 1H), 7.10-7.01 (m, 3H), 6.84 (t, $J = 8.0$ Hz, 1H), 6.49 (d, $J = 10$ Hz, 1H), 3.10 (br s, 4H), 2.65 (br s, 4H), 2.36-2.32 (m, 8H), 2.15-2.07 (m, 1H), 1.85-1.75 (m, 2H), 1.68-1.49 (m, 4H), 1.28-1.18 (m, 2H). MS $[\text{M}+\text{H}]^+$ Calculated for $\text{C}_{24}\text{H}_{32}\text{N}_2\text{S}$: m/z 381.24, found 381.30

2.1.3. General procedure for the synthesis of **4e, 4f, 4g, 4k, 4l, 4m, 4n, 4o, 4p**

To a solution of 1-(2-(2,4-Dimethylphenylthio)phenyl)piperazine (1 eq) in dry THF (10 mL) was added sodium hydride (2 eq) at 0 °C. The reaction mixture was stirred at the same temperature for 20 min, and then alkyl halide (1.5 eq) was added. The contents were stirred at room temperature for 4 h. The reaction was quenched using ice-water and extracted with ethyl acetate. Brine washing was performed with the organic layer, which was dried over anhydrous sodium sulfate, filtered and the solvent was evaporated. The crude product was purified by column chromatography.

1-(2-(2,4-Dimethylphenylthio)phenyl)-4-methylpiperazine (HS-4e): Yield: 0.03 g, 19%. IR: 2933, 2829, 2787, 1579, 1471, 1440, 1369, 1288, 1230, 1145, 1008, 923, 813, 763, 734 cm^{-1} . $^1\text{H-NMR}$ (CDCl_3 , 500 MHz): δ 7.40 (d, $J = 9.5$ Hz, 1H), 7.15 (s, 1H), 7.10-7.01 (m, 3H), 6.84 (m, 1H), 6.51 (d, $J = 10.0$ Hz, 1H), 3.12 (br s, 4H), 2.65 (br s, 4H), 2.39 (s, 3H), 2.36 (s, 3H), 2.32 (s, 3H). MS $[\text{M}+\text{H}]^+$ Calculated for $\text{C}_{19}\text{H}_{24}\text{N}_2\text{S}$: m/z 313.17, found 313.19

1-(2-(2,4-Dimethylphenylthio)phenyl)-4-ethylpiperazine (HS-4f): Yield: 0.2 g, 46%. IR: 2970, 2954, 2810, 2762, 1577, 1471, 1448, 1436, 1375, 1303, 1224, 1147, 1126, 1043, 1029, 952, 813, 752, 731 cm^{-1} . $^1\text{H-NMR}$ (CDCl_3 , 500 MHz): δ 7.39 (d, $J = 7.0$ Hz, 1H), 7.16 (s, 1H), 7.09-7.03 (m, 3H), 6.86-6.87 (m, 1H), 6.51 (d, $J = 7.5$ Hz, 1H), 3.14 (br s, 4H), 2.68 (br s, 4H), 2.52 (q, $J = 6.0$ Hz, 2H), 2.37 (s, 3H), 2.33 (s, 3H), 1.16 (t, $J = 6.0$ Hz, 3H). MS $[\text{M}+\text{H}]^+$ Calculated for $\text{C}_{20}\text{H}_{26}\text{N}_2\text{S}$: m/z 327.19, found 327.10

1-(2-(2,4-Dimethylphenylthio)phenyl)-4-isopropylpiperazine (HS-4g): Yield: 0.16 g, 35%. IR: 2960, 2918, 2816, 1577, 1448, 1438, 1377, 1328, 1224, 1145, 1043, 979, 765, 754, 731 cm^{-1} . $^1\text{H-NMR}$ (CDCl_3 , 500 MHz): δ 7.39 (d, $J = 10.0$ Hz, 1H), 7.16 (s, 1H), 7.11-7.03 (m, 3H), 6.88-6.85 (m, 1H), 6.51 (d, $J = 9.0$ Hz, 1H), 3.16 (br s, 4H), 2.82-2.73 (m, 5H), 2.37 (s, 3H), 2.33 (s, 3H), 1.13 (d, $J = 8.5$ Hz, 6H). MS $[\text{M}+\text{H}]^+$ Calculated for $\text{C}_{21}\text{H}_{28}\text{N}_2\text{S}$: m/z 341.21, found 341.20

1-(2-(2,4-Dimethylphenylthio)phenyl)-4-(2-(trifluoromethyl)benzyl)piperazine (HS-4k): Yield: 0.38 g, 62%. IR: 2951, 2808, 1577, 1469, 1456, 1373, 1355, 1311, 1220, 1170, 1111, 1058, 1010, 929, 817, 763, 748, 729 cm^{-1} . $^1\text{H-NMR}$ (CDCl_3 , 500 MHz): δ 7.83 (d, $J = 9.0$ Hz, 1H), 7.61 (d, $J = 9.5$ Hz, 1H), 7.52-7.49 (m, 1H), 7.36-7.29 (m, 2H), 7.12 (s, 1H), 7.05-6.99 (m, 3H), 6.82-6.81 (m, 1H), 6.48 (d, $J = 10.0$ Hz, 1H), 3.73 (s, 2H), 3.09 (br s, 4H), 2.67 (br s, 4H), 2.33 (s, 3H), 2.28 (s, 3H). MS $[\text{M}+\text{H}]^+$ Calculated for $\text{C}_{26}\text{H}_{27}\text{F}_3\text{N}_2\text{S}$: m/z 457.19, found 457.36

1-(2-(2,4-Dimethylphenylthio)phenyl)-4-(2-fluorobenzyl)piperazine (HS-4l): Yield: 0.31 g, 57%. IR: 2956, 2812, 1579, 1489, 1471, 1454, 1442, 1348, 1271, 1224, 1139, 1120, 1043, 1002, 941, 931, 815, 729. cm^{-1} . $^1\text{H-NMR}$ (CDCl_3 , 500 MHz): δ 7.44-7.36 (m, 2H), 7.25-7.24 (m, 1H), 7.14-7.01 (m, 6H), 6.87-6.83 (m, 1H), 6.50 (d, $J = 10.0$ Hz, 1H), 3.69 (s, 2H), 3.11 (br s, 4H), 2.71 (br s, 4H), 2.36 (s, 3H), 2.31 (s, 3H). MS $[\text{M}+\text{H}]^+$ Calculated for $\text{C}_{25}\text{H}_{27}\text{FN}_2\text{S}$: m/z 407.20, found 407.25

1-(2-(2,4-Dimethylphenylthio)phenyl)-4-(4-nitrobenzyl)piperazine (HS-4m): Yield: 0.21 g, 36%. IR: 2933, 2816, 1595, 1579, 1514, 1344, 1222, 1138, 1043, 1010, 943, 921, 850, 835, 763, 736 cm^{-1} . $^1\text{H-NMR}$ (CDCl_3 , 500 MHz): δ 8.19 (d, $J = 11.0$ Hz, 2H), 7.56 (d, $J = 10.5$ Hz, 2H), 7.37 (d, $J = 10.0$ Hz, 1H), 7.14 (s, 1H), 7.07-7.01 (m, 3H), 6.87-6.83 (m, 1H), 6.49 (d, $J = 9.5$ Hz, 1H), 3.68 (s, 2H), 3.11 (br s, 4H), 2.67 (br s, 4H), 2.35 (s, 3H), 2.30 (s, 3H). MS $[\text{M}+\text{H}]^+$ Calculated for $\text{C}_{25}\text{H}_{27}\text{N}_3\text{O}_2\text{S}$: m/z 434.19, found 434.26.

1-(2-(2,4-Dimethylphenylthio)phenyl)-4-(4-methylbenzyl)piperazine (HS-4n): Yield: 0.32 g, 59%. IR: 2953, 2814, 1579, 1469, 1450, 1438, 1224, 1139, 1124, 1043, 1043, 1008, 815, 750 cm^{-1} . $^1\text{H-NMR}$ (CDCl_3 , 500 MHz): δ 7.37 (d, $J = 9.0$ Hz, 1H), 7.25-7.23 (d, $J = 9.5$ Hz, 2H), 7.14-7.13 (m, 3H), 7.05-7.00 (m, 3H), 6.84-6.81 (m, 1H), 6.48 (d, $J = 9.5$ Hz, 1H), 3.55 (s, 2H), 3.09 (br s, 4H), 2.64 (br s, 4H), 2.35 (s, 3H), 2.34 (s, 3H), 2.30 (s, 3H). MS $[\text{M}+\text{H}]^+$ Calculated for $\text{C}_{26}\text{H}_{30}\text{N}_2\text{S}$: m/z 403.22, found 403.29.

1-(2-(2,4-Dimethylphenylthio)phenyl)-4-(3-methylbenzyl)piperazine (HS-4o): Yield: 0.38 g, 70%. IR: 2943, 2814, 1579, 1471, 1452, 1373, 1338, 1222, 1139, 1124, 1043, 1010, 929, 813, 761, 752, 729 cm^{-1} . $^1\text{H-NMR}$ (CDCl_3 , 500 MHz): δ 7.39 (d, $J = 9.5$ Hz, 1H), 7.25-7.15 (m, 4H), 7.10-7.02 (m, 4H), 6.87-6.83 (m, 1H), 6.50 (d, $J = 10.0$ Hz, 1H), 3.57 (s, 2H), 3.12 (br s, 4H), 2.68 (br s, 4H), 2.37 (s, 6H), 2.32 (s, 3H). MS $[\text{M}+\text{H}]^+$ Calculated for $\text{C}_{26}\text{H}_{30}\text{N}_2\text{S}$: m/z 403.22, found 403.35.

1-(4-Chlorobenzyl)-4-(2-(2,4-dimethylphenylthio)phenyl)piperazine (HS-4p): Yield: 0.32 g, 56%. IR: 3066, 2926, 2821, 1575, 1469, 1220, 1138, 1043, 1008, 817, 798, 763, 729, 688 cm^{-1} . $^1\text{H-NMR}$ (CDCl_3 , 500 MHz): δ 7.39 (d, $J = 10.0$ Hz, 1H), 7.33-7.31 (m, 4H), 7.16-7.14 (m, 1H), 7.07 (d, $J = 5.0$ Hz, 2H), 7.05-7.02 (m, 1H), 6.89-6.84 (m, 1H), 6.51 (d, $J = 9.5$ Hz, 1H), 3.57 (s, 2H), 3.11 (br s, 4H), 2.66 (br s, 4H), 2.37 (s, 3H), 2.32 (s, 3H). MS $[\text{M}+\text{H}]^+$ Calculated for $\text{C}_{25}\text{H}_{27}\text{ClN}_2\text{S}$: m/z 423.17, found 423.27.

1-(2-Chloroethyl)-4-(2-(2,4-dimethylphenylthio)phenyl)piperazine (HS-4h): To a solution of 1-(2-(2,4-dimethylphenylthio)phenyl)piperazine (0.4 g, 1.34 mmol) in dry DMF (10 mL) was added potassium carbonate (K_2CO_3) (0.54 g, 4.02 mmol) and 1-bromo-2-chloroethane (0.22 g, 1.60 mmol). The reaction mixture was stirred at the same temperature for 16 h and the reaction was quenched using water and extracted with ethyl acetate. Brine washing was performed with the organic layer, which was dried over anhydrous sodium sulfate, filtered and the solvent was evaporated. The crude product was purified by column chromatography. Yield: 0.048 g, 10%. IR: 2943, 2816, 1579, 1454, 1373, 1224, 1139, 1124, 1043, 1006, 929, 813, 758, 729 cm^{-1} . $^1\text{H-NMR}$ (CDCl_3 , 500 MHz): δ 7.39 (d, $J = 9.5$ Hz, 1H), 7.16 (s, 1H), 7.08-7.02 (m, 3H), 6.90-6.82 (m, 1H), 6.51 (d, $J = 7.5$ Hz, 1H), 3.66 (m, 2H), 3.13 (br s, 4H), 2.84 (m, 2H), 2.75 (br s, 4H), 2.37 (s, 3H), 2.33 (s, 3H). MS $[\text{M}+\text{H}]^+$ Calculated for $\text{C}_{20}\text{H}_{25}\text{ClN}_2\text{S}$: m/z 361.15, found 361.15.

2-(4-(2-(2,4-Dimethylphenylthio)phenyl)piperazin-1-yl)ethanol (HS-4i): To a solution of 1-(2-(2,4-dimethylphenylthio)phenyl)piperazine (0.35 g, 1.17 mmol) in dry acetone (10 mL) was added triethyl amine (0.23 mL, 1.17 mmol), which was followed by addition of 2-iodoethanol (0.09 mL, 1.17 mmol). The contents were stirred at room temperature for 6 h. The solvent was evaporated, water was added and extracted with ethyl acetate. Brine washing was performed with the organic layer, which was dried over anhydrous sodium sulfate, filtered and the solvent was evaporated. The crude product was purified by column chromatography to afford **HS-4I**. Yield: 0.05g, 12%. IR: 2914, 2818, 1579, 1469, 1454, 1438, 1311, 1224, 1126, 1043, 952, 813, 761, 750, 729 cm^{-1} . $^1\text{H-NMR}$ (CDCl_3 , 500 MHz): δ 7.37 (d, $J = 7.0$ Hz, 1H), 7.17 (s, 1H), 7.11-7.03 (m, 3H), 6.92-6.88 (m, 1H), 6.52 (d, $J = 9.5$

Hz, 1H), 3.88 (t, $J = 6.0$ Hz, 2H), 3.33-3.31 (m, 4H), 3.06 (m, 5H), 2.91 (t, $J = 6.5$ Hz, 2H), 2.37 (s, 3H), 2.32 (s, 3H). MS $[M+H]^+$ Calculated for $C_{20}H_{26}N_2OS$: m/z 343.18, found 343.20.

1-(2-(2,4-Dimethylphenylthio)phenyl)-4-propylpiperazine (HS-4j): To a solution of 1-(2-(2,4-dimethylphenylthio)phenyl)piperazine (0.4 g, 1.34 mmol) in dry acetone (10 mL) was added triethyl amine (0.19 mL, 1.34 mmol) followed by addition of 1-bromopropane (0.16g, 1.34 mmol). The contents were stirred at room temperature for 6 h. The solvent was evaporated, water was added and extracted with ethyl acetate. Brine washing was performed with the organic layer, which was dried over anhydrous sodium sulfate, filtered and the solvent was evaporated. The crude product was purified by column chromatography to afford **HS-4j**. Yield: 0.26 g, 65%. IR: 2956, 2812, 1579, 1471, 1373, 1224, 1147, 1122, 1043, 1008, 931, 813, 761, 750, 729 cm^{-1} . 1H -NMR ($CDCl_3$, 500 MHz): δ 7.38 (d, $J = 9.5$ Hz, 1H), 7.14 (s, 1H), 7.07-7.01 (m, 3H), 6.86-6.82 (m, 1H), 6.49 (d, $J = 10.0$ Hz, 1H), 3.11 (br s, 4H), 2.66 (br s, 4H), 2.41-2.35 (m, 5H), 2.31 (s, 3H), 1.61-1.52 (m, 2H), 0.93 (t, $J = 9.0$ Hz, 3H). MS $[M+H]^+$ Calculated for $C_{21}H_{28}N_2S$: m/z 341.21, found 341.25.

2.2. Biological Assay to Determine Antibacterial Activity

The final compounds (**4a-4p**) were tested for antibacterial and antifungal activities. The antibacterial activity was screened against Gram-positive bacteria, such as *Staphylococcus aureus* and *Streptomyces epidermidis*, and the Gram-negative bacteria, such as *Pseudomonas aeruginosa* and *Escherichiacoli*, in the agar disc diffusion method. The sample preparation was conducted by dissolving 1 mg of each sample in 1 ml of dimethyl sulphoxide (DMSO). For diffusion test against microbes, the agar plates were prepared by using agar solid medium. The media was poured into sterilized Petri dishes. The Petri dishes were then cooled for solidification. Then, a uniform mixture of inoculate was introduced to every Petri dish. The sterilized paper discs were loaded with samples of 1 mg/ml concentration and incubated at 37 °C. In successful reactions, clear zones of inhibition appeared around the discs. Ampicillin (19-21 mm, zone of inhibition) was used as a standard.

2.3. Biological Assay to Determine Antifungal Activity

Stock culture was prepared by transferring a loopful of fungal strain into the sterilized solid lipid micro-particles (SLM) with $25 \pm 1^\circ C$ incubation period for 48 h and 7 days for *Candida albicans* and *Aspergillus* species (*A. Niger*, *A. Fumigatus* and *A. flavus*), respectively. The stock was further sub-cultured by repeating the process mentioned above. *C. albicans* culture was harvested and diluted with the sterilized saline solution to obtain the spore count of about 1×10^7 CFU/mL. In the same manner, *Aspergillus* species cultures were harvested and the spore count was adjusted to about 1×10^7 CFU/mL with a sterilized saline solution. For inoculation of the culture, 0.1 mL of this saline solution consisting of fungal strain was used.²⁹⁻³¹

2.3.1. Determination of the MIC range

A set of seven sterilized culture tubes were taken, and 1.0 mL of sterilized double strength Sabouraud liquid medium (DSSLM) was transferred aseptically to Tube I and 1.0 mL of sterilized nutrient broth was transferred aseptically to the remaining six tubes. Different concentrations of all the compounds to be tested, including the standard drug, were prepared by serial dilution method to obtain the drug concentration of 5.0 $\mu g/mL$, 2.5 $\mu g/mL$, ... 0.078125 (0.08) $\mu g/mL$ in Tube I to Tube VII, respectively. A control tube was also prepared by aseptically transferring 0.5 mL of sterilized DSSLM and 0.5 mL of solvent (DMSO). The culture tubes were inoculated with 0.1 mL of fungal culture in a sterilized saline solution having microbial count of about 1×10^7 CFU/mL, so that the final microbial count in each culture tube would be about 1×10^6 CFU/mL. The tubes were incubated for 48 h at $25 \pm 1^\circ C$. To check the turbidity, tubes were monitored under the microscope. The lower and higher concentrations gave the MIC range for the compound.^{32,33}

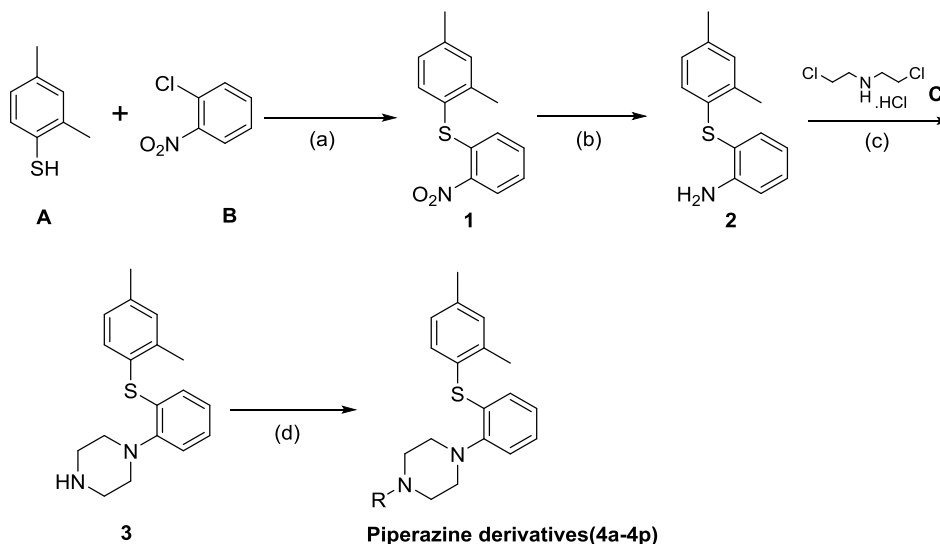
3. Results and Discussion

3.1. Chemistry

Earlier, the antimicrobial activities of piperazine dithiocarbonate derivatives were investigated.³³ The synthesized compounds had more potency to inhibit gram-negative bacteria than gram positive ones. Among the bacterial strains, *E. faecalis* (ATCC 51922) and *P. aeruginosa* (ATCC 27853) were found to be the most susceptible ones. Few final compounds (**6a-d**) including alkyl groups in their structure displayed the same potency like that of Chloramphenicol against *E. faecalis* (ATCC 51922).

1-Chloro-2-nitrobenzene and 2,4-dimethylbenzenethiol were used as starting materials for the synthesis of piperazine derivatives. Addition²³ of 1.1 eq. of 2,4-dimethylbenzenethiol (**A**) to 1-chloro-2-nitrobenzene (**B**) in the presence of potassium carbonate (K_2CO_3) in *N,N*-dimethylformamide for 18 h afforded (2,4-Dimethylphenyl)(2-nitrophenyl)sulfane (**1**) in good yield (99%). The nitro group of compound (**1**) was reduced in the presence of iron in acetic acid for 16 h at room temperature to give 2-((2,4-Dimethylphenyl)thio)aniline (**2**), which was cyclized using bis(2-chloroethyl)amine hydrochloride in *N,N*-dimethylformamide at 110 °C for 48 h. Structures of the compounds were explained on the basis of spectral data and comparison with their authentic samples (**3**).

The reaction of compound (**3**) with alcohol, oxirane, cyclic alkyl halide, alkyl halides and various substituted benzyl halides yielded piperazine analogs (**4a-4p**) (Scheme 1).



Scheme 1. Synthesis of compounds **4a-4p**. a) A, B, K_2CO_3 , DMF, 25°C, 18h b) Fe, AcOH, 30°C, 16h c) bis(2-chloroethyl)amine hydrochloride, DMF, 110°C, 48h d) Base, RX, solvent, 0-100°C, 4-16h.

Coupling reaction of (**3**) with different alkylating agents in the presence of base, such as sodium hydride, triethyl amine, di-isopropyl ethylamine, potassium carbonate, etc. also yielded the piperazine analogs. The products were separated on a silica gel column chromatography eluting with hexane/ethyl acetate. After the separation, structures of the compounds were determined by nuclear magnetic resonance (NMR), infrared (IR) and liquid chromatography/mass spectrometry (LCMS) studies. It was observed ~~seen~~ that the isolated products had high purity. Disappearance of a broad singlet at 1.63 ppm (piperazine NH) confirmed the coupling reaction yielding the desired products. The piperazine proton abstraction by base and nitrogen attack to the halide produced the final compounds. In piperazine, four CH_2 (8 protons) ranging from 3.02-3.09 confirmed the new product formation. Moreover, IR and LCMS data also confirmed the formation of the new compounds **4a-4p**.

3.2. Biological assay

3.2.1. In vitro Antibacterial Activity

All the isolated compounds were tested for their antibacterial activities against *Staphylococcus aureus*, *Streptomyces epidermidis*, *Pseudomonas aeruginosa* and *Escherichia coli* by agar disc diffusion method. For testing, the bacteria were obtained from agar slants. Loopful samples were grown in sterile nutrient broth medium, autoclaved at 121 °C at 15 atm for 15 min and left to grow for 48 h at 37 °C in an incubator. Ampicillin was used as reference drug for bacteria. The compound was tested at the concentration of 1 mg/mL, using DMSO as a solvent. From screening result, it was observed that most of the compounds were highly active against bacterial pathogens as well as fungus. The zone of inhibition (mm) of the final compounds against pathogenic bacterial strains is shown in Table 1.

Table 1. Antibacterial activity of newly synthesized piperazine derivatives

| Compounds | <i>S.aureus</i> | <i>S.epidermidis</i> | <i>P.aeruginosa</i> | <i>E. Coli</i> |
|--------------|-----------------|----------------------|---------------------|----------------|
| HS-4a | 20 | 19 | 15 | 16 |
| HS-4b | 17 | 17 | 20 | 18 |
| HS-4c | 21 | 18 | 14 | 20 |
| HS-4d | 15 | 20 | 18 | 19 |
| HS-4e | 20 | 17 | 18 | 20 |
| HS-4f | 20 | 19 | 15 | 16 |
| HS-4g | 20 | 17 | 07 | 18 |
| HS-4h | 21 | 18 | 14 | 20 |
| HS-4i | 15 | 20 | 18 | 19 |
| HS-4j | 20 | 17 | 18 | 20 |
| HS-4k | 20 | 19 | 15 | 16 |
| HS-4l | 17 | 17 | 20 | 18 |
| HS-4m | 04 | 18 | 14 | 18 |
| HS-4n | 15 | 20 | 13 | 19 |
| HS-4o | 20 | 17 | 18 | 20 |
| HS-4p | 15 | 17 | 18 | 20 |
| Ampicillin* | 21 | 20 | 19 | 20 |

*Positive control

In general, while most of the final compounds showed better antibacterial activities, the compounds **4g** and **4m** showed moderate antibacterial activities against *P. aeruginosa* and *S. aureus*, respectively. 1-(2-(2,4-dimethylphenylthio)phenyl)-4-methylpiperazine (**4e**), 1-(2-(2,4-dimethylphenylthio)phenyl)-4-propylpiperazine (**4j**), and 1-(2-(2,4-dimethylphenylthio)phenyl)-4-(3-methylbenzyl)piperazine (**4o**) had the best antibacterial activity against pathogenic bacterial strains with a zone of inhibition in the range of 17-20 mm. The other compounds also showed good activities, except **4g** and **4m**.

3.2.2. In vitro Antifungal Activity

Antifungal activities of the newly derived piperazine derivatives were screened against pathogenic fungal strains, such as *Candida albicans*, *Aspergillus niger*, *Aspergillus flavus* and *Aspergillus fumigates* where Clotrimazol was used as the standard drug. Antifungal activities of the final compounds were conducted by using two-fold serial dilution methods. Stock solutions of the final compounds and the standard drug having the concentration 10 µg/mL were prepared in dimethyl sulfoxide (DMSO). The required dilutions were made from these stock solutions. The MIC (µg/mL) of the screened compounds by pathogenic fungi is shown in Table 2.

Table 2. Antifungal activity of newly synthesized piperazine derivatives*

| Compounds | <i>C. albicans</i> | <i>A. niger</i> | <i>A. flavus</i> | <i>A. Fumigatus</i> |
|---------------------------|--------------------|-----------------|------------------|---------------------|
| HS-4a | 0.60 | 0.50 | 0.45 | 0.60 |
| HS-4b | 0.55 | 0.60 | 0.50 | 0.60 |
| HS-4c | 0.50 | 0.60 | 0.60 | 0.50 |
| HS-4d | 0.60 | 0.50 | 0.50 | 0.60 |
| HS-4e | 0.50 | 0.35 | 0.60 | 0.40 |
| HS-4f | 0.50 | 0.50 | 0.45 | 0.60 |
| HS-4g | 0.55 | 0.60 | 0.50 | 0.55 |
| HS-4h | - | 0.40 | 0.60 | 0.50 |
| HS-4i | 0.60 | 0.50 | 0.50 | 0.60 |
| HS-4j | 0.30 | 0.50 | 0.45 | 0.60 |
| HS-4k | 0.55 | 0.60 | - | - |
| HS-4l | - | 0.30 | 0.60 | 0.50 |
| HS-4m | 0.60 | 0.50 | 0.45 | 0.60 |
| HS-4n | 0.55 | 0.60 | 0.50 | 0.60 |
| HS-4o | 0.50 | 0.30 | 0.60 | 0.40 |
| HS-4p | 0.30 | - | 0.45 | 0.60 |
| Clotrimazole ^ϕ | 0.10 | 0.30 | 0.30 | 0.30 |

*MIC values are given as $\mu\text{g/mL}$; ^ϕPositive control.

While most of the synthesized compounds showed significant antifungal activities, the compounds **4h**, **4k**, **4l**, and **4p** showed moderate antifungal activities. The compounds **4a**, **4b**, **4c**, **4d**, **4e**, **4f**, **4g**, **4i**, **4j**, **4m**, and **4n** showed better activities against *C. albicans* and *Aspergillus*. All the compounds having better activities had MIC values ranging between 0.35-0.6 $\mu\text{g/mL}$. The activities of piperazine derivatives were observed due to the alkylation of piperazine derivatives. So, Considering overall activities of the compounds **4a**, **4b**, **4e**, **4j** and **4o**, they had good antibacterial and antifungal activities.

4. Conclusion

Novel 1-(2-(2,4-Dimethylphenylthio)phenyl)-4-piperazine derivatives (**4a-4p**) were obtained through the reaction of 1-(2-(2,4-dimethylphenylthio)phenyl)piperazine (**3**) with alcohols, alkyl halides and substituted aromatic benzyl halides in presence of suitable base. The structures of the obtained compounds were characterized using conventional spectroscopic methods (NMR, IR, LCMS). The compounds (**4a-4p**) were tested for their antibacterial and antifungal activities, which showed both antibacterial as well as antifungal activities. From the screening results, it was observed that the compounds **4a**, **4b**, **4c**, **4d**, **4f**, **4i**, **4j** and **4o** were found to have high activity against both Gram-positive and Gram-negative bacteria and fungi, while the other compounds possessed weak to moderate activities. Compounds **4a**, **4b**, **4e**, **4j** and **4o** showed maximum zone of inhibition. Thus, the compounds **4a**, **4b**, **4e**, **4j** and **4o** could be used for the antibacterial and antifungal applications.

Acknowledgements

Authors are thankful to the Head of Chemistry Department and Principal of Vidya Bharati Mahavidyalaya, Amravati for providing the necessary facilities.

ORCID

Hemant Suryavanshi: [0000-0001-5539-8472](https://orcid.org/0000-0001-5539-8472)

Mithilesh M. Rathore: [0000-0001-5711-658X](https://orcid.org/0000-0001-5711-658X)

References

- [1] Spellberg, B. B.; Blaser, M.; Robert J.G.; Boucher H.W.; Bradley, J.S; Eisenstein B.I; Gerding, D.; Lynfield, R.; Reller B.L.; Rex, J.; Schwartz D.; Septimus E; Tenover F.C. Combating antimicrobial resistance: policy recommendations to save lives. *Clin. Infect. Dis.* **2011**, *52*, (suppl 5), S397-S428.
- [2] Conly, J. M. Antimicrobial resistance -Judicious use is the key. *Can. J. Infect. Dis. Med. Microbiol.* **2004**, *15*, (5), 249-251.
- [3] Doron S.; Davidson L. E. Antimicrobial stewardship. *Mayo. Clin. Proc.* **2011**, *86*, 1113-23.
- [4] Appelbaum, P. C.; Hunter, P. A. The fluoroquinolone antibacterials: past, present and future perspectives. *Int. J. Antimicrob. Ag.* **2000**, *16*, 5-15.
- [5] Mizuki, Y.; Fujiwara, I.; Yamaguchi, T. Pharmacokinetic interactions related to the chemical structures of fluoroquinolones. *J. Antimicrob. Chemother.* **1996**, *37*, 41-55.
- [6] Ball, P. Quinolone generations: natural history or natural selection? *J. Antimicrob. Chemother.* **2000**, *46*, (Suppl), 17-24.
- [7] Sanz-Nebot, V.; Valls, I.; Barbero, D.; Barbosa, J. Acid-base behavior of quinolones in aqueous acetonitrile mixtures. *Acta Chem. Scand. (Copenhagen, Denmark: 1989)* **1997**, *51*, 896-903.
- [8] Kurath, P.; Jones, P. H.; Egan, R. S.; Perun, T. J. Acid degradation of erythromycin A and erythromycin B. *Cell. Mol. Life. Sci.* **1971**, *27*, 362-362.
- [9] Gregory, W. A.; Wang, C. L. J.; Hollis, S. K. Antibacterials. Synthesis and structure activity studies of 3-aryl-2-oxaooxazolidines.2. The "A" group. *J. Med. Chem.* **1990**, *33*, 2569-2578.
- [10] Brickner, S. J.; Hutchinson, D. K.; Barbachyn, M. R.; Manninem, P. R.; Ulanowicz, D. A.; Garmon, S. A.; Grega, K. C.; Hendges, S. K.; Topps, D. S.; Ford, C. W.; Zurenko, G. E. Two oxazolidinone antibacterial agents for the potential treatment of multidrug-resistant gram-positive bacterial infections. *J. Med. Chem.* **1996**, *39*, 673-679.
- [11] Andriole, V. T. B. Current and future therapy of invasive fungal infections. *Curr. Clin. Top. Infect. Dis.* **1998**, *18*, 19-36.
- [12] Groll, A. H.; Piscitelli, S. C.; Walsh, T. J. Clinical pharmacology of systemic antifungal agents: a comprehensive review of agents in clinical use, current investigational compounds, and putative targets for antifungal drug development. *Adv. Pharmacol.* **1998**, *44*, 343-500.
- [13] Georgopapadakou, N. H.; Walsh, T. J. Antifungal agents: chemotherapeutic targets and immunologic strategies. *Antimicrob. Agents. Chemother.* **1996**, *40*, 279-291.
- [14] Vazquez, J. A.; Sanchez, V.; Dmuchowski, C.; Dembry, L. M.; Sobel, J. D.; Zervos, M. J. Nosocomial acquisition of *Candida albicans*: an epidemiologic study. *J. Infect. Dis.* **1993**, *168*, 195-201.
- [15] Bodey, G. P. In *Candidosis: Pathogenesis, Diagnosis and Treatment*. Raven: New York **1993**, 371-406.
- [16] Andriole, V. T. B.; Gerald P. Pocket. *Guide to Systemic Antifungal Therapy*. Springfield, N.J. (505 Morris Ave., Springfield 07081): Sci. Ther. Information, © **1994**.
- [17] Dixon, G. K.; Copping, L. G.; Hollomon, D. W. In *Antifungal Agents: Discovery and Mode*. Oxford : Bios Scientific **1995**.
- [18] Brown, G. D.; Denning, D. W.; Gow, N. A. R.; Levitz, S. M.; Netea, M. G.; White, T. C. Hidden killers: Human fungal infections. *Sci. Transl. Med.* **2012**, *4*, (165), 165rv13-165rv13.
- [19] Wisplinghoff, H.; Bischoff, T.; Tallent, S. M.; Seifert, H.; Wenzel, R. P.; Edmond, M. B. Nosocomial bloodstream infections in US hospitals: analysis of 24,179 cases from a prospective nationwide surveillance study. *Clin. Infect. Dis.* **2004**, *39*, 309-317.
- [20] Kontoyiannis, D. P.; Marr, K. A.; Park, B. J.; Alexander, B. D.; Anaissie, E. J.; Walsh, T. J.; Ito, J.; Andes, D. R.; Baddley, J. W.; Brown, J. M. Prospective surveillance for invasive fungal infections in hematopoietic stem cell transplant recipients, 2001-2006: overview of the Transplant-Associated Infection Surveillance Network (TRANSNET) Database. *Clin. Infect. Dis.* **50**, 1091-1100.
- [21] Denning, D. W.; Bromley, M. J. How to bolster the antifungal pipeline. *Science* **2015**, *347*, (6229), 1414-1416.

- [22] Saglard, D. Emerging threats in antifungal-resistant fungal pathogens. *Front. Med.* **2016**, *3*, PMC4791369
- [23] Grasela, T. H.; Goodwin, D. S.; Walawander, M. K.; Cramer, R. L.; Fuhs, D. W.; Moriarty, V. P. Prospective surveillance of intravenous amphotericin B use patterns. *Pharmacotherapy: The J. Human Pharmacol. Drug Ther.* **1990**, *10*, 341-348.
- [24] Fanos, V.; Cataldi, L. Amphotericin B-induced nephrotoxicity: A review. *J. Chemother.* **2000**, *12*, 463-470.
- [25] Kerns, R. J.; Rybak, M. J.; Kaatz, G. W.; Vaka, F.; Cha, R.; Grucz, R. G.; Diwadkar, V. U. Structural features of piperazinyl-linked ciprofloxacin dimers required for activity against drug-resistant strains of *Staphylococcus aureus*. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 2109-2112.
- [26] Upadhayaya, R. S.; Sinha, N.; Jain, S.; Kishore, N.; Chandra, R.; Arora, S. K. Optically active antifungal azoles: synthesis and antifungal activity of (2R, 3S)-2-(2, 4-difluorophenyl)-3-(5-{2-[4-aryl-piperazin-1-yl]-ethyl}-tetrazol-2-yl/1-yl)-1-[1, 2, 4]-triazol-1-yl-butan-2-ol. *Bioorg. Med. Chem.* **2004**, *12*, 2225-2238.
- [27] Zupancic, B. New process for the synthesis of 1-(2-((2, 4-dimethylphenyl) thio) phenyl) piperazine. WO 2014191548 A1.
- [28] Zupancic, B. Synthesis of vortioxetine via (2-(piperazine-1-yl)phenyl)aniline intermediates. WO 2015107057 A1.
- [29] Öztürk, Ş.; Gürdere, B.; Gezen, H.; Ceylan, M.; Budak, Y. Potassium carbonate mediated one-pot synthesis and antimicrobial activities of 2-alkoxy-4-(aryl)-5H-indeno[1,2-b]pyridine-3-carbonitriles. *Org. Commun.*, **2016**, *9*, 125-132.
- [30] Narayana, K.V.; Rao, D.S.; Madhava, G., Venkateswarlu, N.; Vijaya, T.; Raju, C.N. Sulfonamide and carbamate derivatives of 6-chloropurine: synthesis, characterization and antimicrobial activity evaluation. *Org. Commun.*, **2016**, *9*, 42-53.
- [31] Mao, Y.; Jiang, L.; Chen, T.; He, H.; Liu, G.; Wang, H. A New and Practical Synthesis of Vortioxetine Hydrobromide. *Synthesis* **2015**, *47*, 1387-1389.
- [32] Tan, N.; Yazici-Tutunis, S. Yesil, Y.; Demirci, B.; Tan, E. Antibacterial activities and composition of the essential oils of *Salvia sericeo-tomentosa* varieties. *Rec.Nat. Prod.* **2017**, *11*, 456-461.
- [33] Mohsin, U. A. Synthesis and antimicrobial activity of some piperazine dithiocarbamate derivatives. *Turk. J. Pharm. Sci.* **2014**, *11*, 347-354.

ACG
publications

© 2017 ACG Publications