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Design, synthesis, antitubercular and antimicrobial activities of novel thiazole substituted benzimidazole derivatives

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ABSTRACT

A series of novel N-(substitutedbenzylidene)-4-(1-((dimethylamino)methyl)-1H-benzimidazol-2-yl)thiazol-2-amine 5a-5o were designed and synthesized from o-phenylenediamine & lactic acid. All the synthesized compounds were characterized by FT-IR, 1H-NMR, Mass spectroscopy and bases of elemental analysis. In vitro M. tuberculosis method and agar streak dilution test were performed for screening antitubercular and antimicrobial activity, respectively. Results of biological studies revealed that all title compounds exhibited mild to good antitubercular and antibacterial activity. The relationship between the functional group variation and the biological activity of the screened compounds were discussed. The most active compound was found to be N-(4-Nitrobenzylidene)-4-(1-((dimethylamino)methyl)-1H-benzimidazol-2-yl)thiazol-2-amine 5b out of fifteen title compounds.

***Key Words:** Benzimidazole, Thiazole, Antitubercular, Antibacterial activity, Antifungal activity.*

INTRODUCTION

Tuberculosis (TB), a contagious infection caused by the airborne transmission of Mycobacterium tuberculosis, still remains the leading cause of the worldwide death among the infectious disease [1]. Being the airborne disease with no vaccine, it is the single largest disease encountered by both developing and developed countries. Two common problems are associated with

treatment, one is serious and life threatening adverse effect of existing anti tubercular drugs such as hepatotoxicity, neuritis, depression, asthma, anorexia etc, which many times forces to withdraw the treatment temporarily or change the treatment. Other one is development of resistance due to non completion of treatment regimen by patient and hence gene mutation by organisms made its management more difficult [2-3]. Therefore, the development of new drugs with activity against multi drug resistant (MDR) TB, extensively drug resistant (XDR) TB, and latent TB is a priority task [4]. The current TB treatment comprises of 3-4 drugs for a period of 6-9 months [5]. Hence, novel drugs are urgently required which can shorten this long treatment period and target MDR, XRD, and latent strains of TB. Infectious microbial disease remains a critical problem worldwide, because microbes have resisted prophylaxis or therapy longer than any other form of life. In recent decades, the prevalence of drug resistant microbe is growing at an alarming rate in both developing and developed countries [6]. The increase in microbial resistance has attracted considerable interest in the discovery and development of new classes of antimicrobial agents.

Heterocyclic species like benzimidazole derivatives have received much interest in the field of medicinal chemistry because of its synthetic utility and broad spectrum of biological activity. Among various benzimidazole derivatives, promising biological activities are exhibited by N-substituted benzimidazoles & disubstituted benzimidazoles. Literature survey indicates that the benzimidazole nucleus substituted at C-1 & C-2 position showed significant antitubercular [7-10] & antimicrobial [11-14] activities. In addition, various substituted thiazoles are reported to possess diverse biological activities particularly antitubercular [15-17] & antimicrobial [18-19] activities. Diverse examples of few antitubercular benzimidazoles (I – IV) & thiazoles (V – VII) are indicated in Figure 1 & diverse examples of few antimicrobial benzimidazoles (VIII – XI) & thiazoles (XII – XIII) are depicted in (Figure 2).

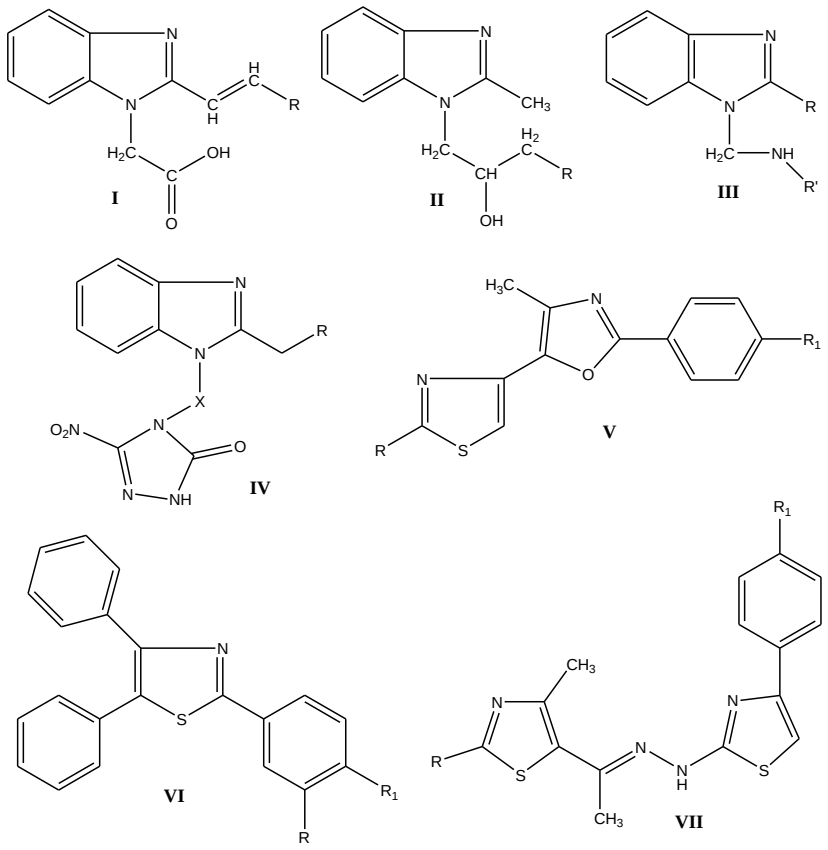


Fig. 1: Diverse examples of antitubercular benzimidazoles & thiazoles

The biological activities exhibited by compounds containing benzimidazole moiety has prompted chemists to synthesis more and more benzimidazole libraries and screen them for potential activities. Owing to the importance we planned to synthesize compounds with this functionality coupled with thiazole as possible antitubercular & antimicrobial agents which could furnish better therapeutic results. Based on these findings, we decided to synthesize some novel thiazole substituted benzimidazole derivatives and evaluated its antitubercular & antimicrobial activities with the hope to obtain more active and less toxic antimicrobial agents.

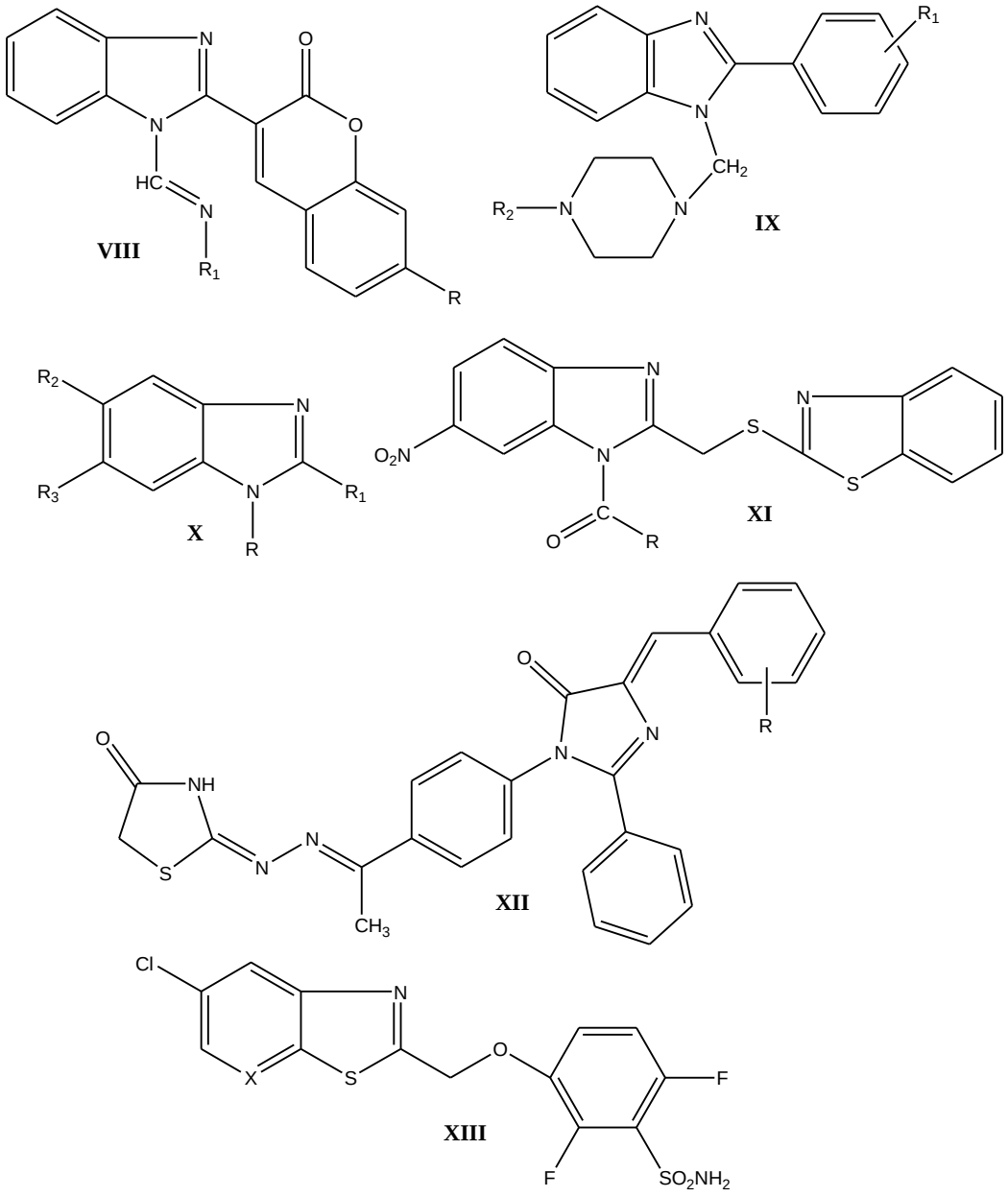


Fig. 2: Diverse examples of antimicrobial benzimidazoles & thiazoles

MATERIALS AND METHODS

Chemistry

The chemicals and reagents used were obtained from various chemical units Qualigens, E. Merck India Ltd., CDH, and SD Fine Chem. The solvents used were of LR grade and purified before their use. The silica gel G used for analytical chromatography (TLC) was obtained from E. Merck India Ltd. All the melting points were taken in open glass capillary and are uncorrected. All the IR spectra were recorded in KBr pellets on a Jasco FT-IR 410 spectrometer. 1H-NMR spectra were recorded at 500 MHz on Bruker Avance-500 NMR spectrometer in CDCl3 using tetramethylsilane (TMS) as an internal standard. The chemical shifts are reported in ppm scale. Mass spectra were obtained on a JEOL-SX-102 instrument using electron impact ionization. Elemental analyses were performed on a Perkin Elmer model 2400C analyzer and were within ± 0.4 % of the theoretical values.

Synthesis of 1-(1H-benzimidazol-2-yl)ethanol (1)

A mixture of o-phenylene diamine (10.8 g; 0.1 mol) and lactic acid (13.51 g; 0.15 mol) was taken in round bottomed flask. To this ethanol (20 ml) was added and refluxed for 5 h in a water bath. The resulting solution was cooled and 10 % sodium hydroxide was added slowly with stirring until it is alkaline to litmus. The product separated 1 was filtered, dried and recrystallised. Yield = 73 %, m.p. 181-183 °C. IR (KBr) cm-1: 3545 (OH), 3356 (NH), 3025 (Ar-CH), 2972 (CH3-CH), 1627 (C=N), 1587 (C=C). 1H-NMR (CDCl3, 500 MHz) δ ppm: 1.75 (d, 3H, CH3), 3.41 (s, 1H, OH), 4.24 (t, 1H, CH), 5.78 (s, 1H, NH), 7.12-7.90 (m, 4H, Ar-CH). EI-MS m/z: 162 (M+). Anal. Calcd for C9H10N2O: C, 66.65; H, 6.21; N, 17.27. Found: C, 66.81; H, 6.19; N, 17.25.

Synthesis of 1-(1H-benzimidazol-2-yl)ethanone (2)

To a solution of 1-(1H-benzimidazol-2-yl)ethanol 1 (8.1 g, 0.05 mol) in dilute sulphuric acid (40 ml), a solution of potassiumdichromate (9.8 g, 0.05 mol) in water (60 ml) was added at room temperature. To this mixture concentrated sulphuric acid (20 ml) was added in a drop wise fashion, over a period of 20 min. The reaction mixture was stirred vigorously during addition. The separated solid was filtered and wash with water (30 ml). The precipitate obtained was re-suspended in water (50 ml) and treated very carefully with aqueous ammonia to obtain a pH 6.0-6.5. The suspension was stirred for 0.5 h and filtered. The residue 2 was washed with water (3 X 10 ml) and dried. Yield = 70 %, m.p. 189-191 °C. IR (KBr) cm-1: 3358 (NH), 3024 (Ar-CH), 2971 (CH3-CH), 1740 (C=O), 1647 (C=N), 1601 (C=C). 1H-NMR (CDCl3, 500 MHz) δ ppm: 2.31 (s, 3H, CH3), 5.19 (s, 1H, NH), 7.342-7.86 (m, 4H, Ar-CH). EI-MS m/z: 160 (M+). Anal. Calcd for C9H8N2O: C, 67.49; H, 5.03; N, 17.49. Found: C, 67.68; H, 5.04; N, 17.44.

Synthesis of 1-(1-((dimethylamino)methyl)-1H-benzimidazol-2-yl)ethanone (3)

A mixture of 1-(1H-benzimidazol-2-yl)ethanone 2 (3.2 g; 0.02 mol), formaldehyde (0.9 g; 0.03 mol) and dimethylamine (1.35 g; 0.03 mol) in ethanol (30 ml) were stirred mechanically for the period of 1 h. The obtained mixture was then refluxed in a water bath for 4 h. The resulting reaction mixture was cooled and poured in ice cold water with vigorous stirring. The obtained product 3 was filtered and recrystallised using methanol. Yield = 76 %, m.p. 163-165 °C. IR (KBr) cm-1: 3014 (Ar-CH), 2950 (CH3-CH), 1734 (C=O), 1670 (C=N), 1614 (C=C). 1H-NMR (CDCl3, 500 MHz) δ ppm: 1.93 (s, 6H, N(CH3)2), 2.87 (s, 3H, CH3), 4.42 (s, 2H, CH2), 6.90-7.65 (m, 4H, Ar-CH). EI-MS m/z: 217 (M+). Anal. Calcd for C12H15N3O: C, 66.34; H, 6.96; N, 19.34. Found: C, 66.13; H, 6.98; N, 19.38.

Synthesis of 1-(1*H*-benzimidazol-2-yl)ethanol (1)

A mixture of *o*-phenylene diamine (10.8 g; 0.1 mol) and lactic acid (13.51 g; 0.15 mol) was taken in round bottomed flask. To this ethanol (20 ml) was added and refluxed for 5 h in a water bath. The resulting solution was cooled and 10 % sodium

hydroxide was added slowly with stirring until it is alkaline to litmus. The product separated **1** was filtered, dried and recrystallised. Yield = 73 %, m.p. 181-183 °C. IR (KBr) cm^{-1}: 3545 (OH), 3356 (NH), 3025 (Ar-CH), 2972 (CH_3-CH), 1627 (C=N), 1587 (C=C). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ ppm: 1.75 (d, 3H, CH_3), 3.41 (s, 1H, OH), 4.24 (t, 1H, CH), 5.78 (s, 1H, NH), 7.12-7.90 (m, 4H, Ar-CH). EI-MS *m/z*: 162 (M^+). *Anal*. Calcd for $C_9H_{10}N_2O$: C, 66.65; H, 6.21; N, 17.27. Found: C, 66.81; H, 6.19; N, 17.25.

Synthesis of 1-(1*H*-benzimidazol-2-yl)ethanone (2)

To a solution of 1-(1*H*-benzimidazol-2-yl)ethanol **1** (8.1 g, 0.05 mol) in dilute sulphuric acid (40 ml), a solution of potassiumdichromate (9.8 g, 0.05 mol) in water (60 ml) was added at room temperature. To this mixture concentrated sulphuric acid (20 ml) was added in a drop wise fashion, over a period of 20 min. The reaction mixture was stirred vigorously during addition. The separated solid was filtered and wash with water (30 ml). The precipitate obtained was re-suspended in water (50 ml) and treated very carefully with aqueous ammonia to obtain a pH 6.0-6.5. The suspension was stirred for 0.5 h and filtered. The residue **2** was washed with water (3 X 10 ml) and dried. Yield = 70 %, m.p. 189-191 °C. IR (KBr) cm^{-1}: 3358 (NH), 3024 (Ar-CH), 2971 (CH_3-CH), 1740 (C=O), 1647 (C=N), 1601 (C=C). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ ppm: 2.31 (s, 3H, CH_3), 5.19 (s, 1H, NH), 7.342-7.86 (m, 4H, Ar-CH). EI-MS *m/z*: 160 (M^+). *Anal*. Calcd for $C_9H_8N_2O$: C, 67.49; H, 5.03; N, 17.49. Found: C, 67.68; H, 5.04; N, 17.44.

Synthesis of 1-(1-((dimethylamino)methyl)-1*H*-benzimidazol-2-yl)ethanone (3)

A mixture of 1-(1*H*-benzimidazol-2-yl)ethanone **2** (3.2 g; 0.02 mol), formaldehyde (0.9 g; 0.03 mol) and dimethylamine (1.35 g; 0.03 mol) in ethanol (30 ml) were stirred mechanically for the period of 1 h. The obtained mixture was then refluxed in a water bath for 4 h. The resulting reaction mixture was cooled and poured in ice cold water with vigorous stirring. The obtained product **3** was filtered and recrystallised using methanol. Yield = 76 %, m.p. 163-165 °C. IR (KBr) cm^{-1}: 3014 (Ar-CH), 2950 (CH_3-CH), 1734 (C=O), 1670 (C=N), 1614 (C=C). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ ppm: 1.93 (s, 6H, $N(CH_3)_2$), 2.87 (s, 3H, CH_3), 4.42 (s, 2H, CH_2), 6.90-7.65 (m, 4H, Ar-CH). EI-MS *m/z*: 217 (M^+). *Anal*. Calcd for $C_{12}H_{15}N_3O$: C, 66.34; H, 6.96; N, 19.34. Found: C, 66.13; H, 6.98; N, 19.38.

Synthesis of 4-(1-((dimethylamino)methyl)-1*H*-benzimidazol-2-yl)thiazol-2-amine (4)

The entire reaction mixture of 1-(1-((dimethylamino)methyl)-1*H*-benzimidazol-2-yl) ethanone **3** (2.17 g; 0.01 mol), thiourea (1.52 g; 0.02 mol) and bromine (3.14 g; 0.02 mol) was boiled in a water bath for 12 h, and water was added to it and again heated until most of the solid has gone into solution. The solution was filtered when it was hot and the filtrate was cooled. Finally the filtrate was made alkaline with concentrated ammonium hydroxide to separate the product **4**. The formed product was

recrystallized with ethanol. Yield = 72 %, m.p. 207-209 °C. IR (KBr) cm^{-1}: 3357 & 3301 (NH_2), 3023 (Ar-CH), 2946 (CH_3-CH), 1660 (C=N), 1631 (C=C). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ ppm: 2.55 (s, 6H, $N(CH_3)_2$), 4.20 (s, 2H, NH_2), 4.73 (s, 2H, CH_2), 6.31 (s, 1H, C_5-H of thiazole), 6.72-7.38 (m, 4H, Ar-CH). EI-MS *m/z*: 273 (M^+). *Anal*. Calcd for $C_{13}H_{15}N_5S$: C, 57.12; H, 5.53; N, 25.62. Found: C, 56.95; H, 5.55; N, 25.69.

Synthesis of *N*-(substitutedbenzylidene)-4-(1-((dimethylamino)methyl)-1*H*-benzimidazol-2-yl)thiazol-2-amine (5a-5o)

To a well stirred mixture of different aromatic aldehydes (0.01 mol) in ethanol (30 ml) and glacial acetic acid (0.5 ml), 4-(1-((dimethylamino)methyl)-1*H*-benzimidazol-2-yl)thiazol-2-amine **4** (2.73 g; 0.01 mol) was in fraction with stirring. The resulting mixture was refluxed on water bath for 8 h and kept aside for some time. Then the mixture was poured in ice cold water with stirring and the product that separated out **5a-5o** was filtered, dried and recrystallized from ethanol. The method used for the preparation and isolation of the compounds gave materials of good purity, as evidenced by their spectral analyses.

***N*-Benzylidene-4-(1-((dimethylamino)methyl)-1*H*-benzimidazol-2-yl)thiazol-2-amine (5a)**

Yield = 75 %, m.p. 143-145 °C. IR (KBr) cm^{-1}: 3023 (Ar-CH), 2970 (CH_3-CH), 1640 (C=N), 1629 (C=C). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ ppm: 1.9 (s, 6H, $N(CH_3)_2$), 4.5 (s, 2H, CH_2), 6.6 (s, 1H, C_5-H of thiazole), 7.1-7.9 (m, 9H, Ar-CH), 8.8 (s, 1H, CH=N). EI-MS *m/z*: 361 (M^+). *Anal*. Calcd for $C_{20}H_{19}N_5S$: C, 66.46; H, 5.30; N, 19.37. Found: C, 66.66; H, 5.28; N, 19.31.

***N*-(4-Nitrobenzylidene)-4-(1-((dimethylamino)methyl)-1*H*-benzimidazol-2-yl)thiazol-2-amine (5b)**

Yield = 71 %, m.p. 117-119 °C. IR (KBr) cm^{-1}: 3021 (Ar-CH), 2970 (CH_3-CH), 1644 (C=N), 1593 (C=C), 1531 & 1329 (NO_2). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ ppm: 2.4 (s, 6H, $N(CH_3)_2$), 4.9 (s, 2H, CH_2), 6.6 (s, 1H, C_5-H of thiazole), 7.1-8.1 (m, 8H, Ar-CH), 8.6 (s, 1H, CH=N). EI-MS *m/z*: 406 (M^+). *Anal*. Calcd for $C_{20}H_{18}N_6O_2S$: C, 59.10; H, 4.46; N, 20.68. Found: C, 59.31; H, 4.44; N, 20.62.

***N*-(4-Chlorobenzylidene)-4-(1-((dimethylamino)methyl)-1*H*-benzimidazol-2-yl)thiazol-2-amine (5c)**

Yield = 78 %, m.p. 136-138 °C. IR (KBr) cm^{-1}: 3048 (Ar-CH), 2953 (CH_3-CH), 1655 (C=N), 1621 (C=C), 786 (C-Cl). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ ppm: 2.2 (s, 6H, $N(CH_3)_2$), 4.7 (s, 2H, CH_2), 6.8 (s, 1H, C_5-H of thiazole), 7.0-8.3 (m, 8H, Ar-CH), 8.9 (s, 1H, CH=N). EI-MS *m/z*: 397 (M^{+2}), 395 (M^+). *Anal*. Calcd for $C_{20}H_{18}ClN_5S$: C, 60.67; H, 4.58; N, 17.69. Found: C, 60.49; H, 4.60; N, 17.74.

***N*-(4-Bromobenzylidene)-4-(1-((dimethylamino)methyl)-1*H*-benzimidazol-2-yl)thiazol-2-amine (5d)**

Yield = 76 %, m.p. 112-113 °C. IR (KBr) cm^{-1}: 3065 (Ar-CH), 2930 (CH_3-CH), 1652 (C=N), 1599 (C=C), 685 (C-Br). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ ppm: 1.8 (s, 6H, $N(CH_3)_2$), 4.3 (s, 2H, CH_2), 6.4 (s, 1H, C_5-H of thiazole), 7.2-8.2 (m, 8H, Ar-CH), 9.2 (s, 1H, CH=N). EI-MS *m/z*: 441 (M^{+2}), 439 (M^+). *Anal*. Calcd for $C_{20}H_{18}BrN_5S$: C, 54.55; H, 4.12; N, 15.90. Found: C, 54.71; H, 4.13; N, 15.84.

_N_-(4-Methylbenzylidene)-4-(1-((dimethylamino)methyl)-1_H_-benzimidazol-2-yl)thiazol-2-amine (5e)

Yield = 79 %, m.p. 125-127 °C. IR (KBr) cm^{-1}: 3086 (Ar-CH), 2958 (CH_3-CH), 1683 (C=N), 1645 (C=C). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ ppm: 2.0 (s, 6H, $N(CH_3)_2$), 2.7 (s, 3H, CH_3), 5.1 (s, 2H, CH_2), 6.5 (s, 1H, C_5-H of thiazole), 7.2-7.9 (m, 8H, Ar-CH), 8.7 (s, 1H, CH=N). EI-MS *m/z*: 375 (M^+). *Anal*. Calcd for $C_{21}H_{21}N_5S$: C, 67.17; H, 5.64; N, 18.65. Found: C, 67.35; H, 5.62; N, 18.69.

_N_-(4-Methoxybenzylidene)-4-(1-((dimethylamino)methyl)-1_H_-benzimidazol-2-yl)thiazol-2-amine (5f)

Yield = 72 %, m.p. 105-106 °C. IR (KBr) cm^{-1}: 3094 (Ar-CH), 2947 (CH_3-CH), 1638 (C=N), 1616 (C=C), 1053 (C-O-C). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ ppm: 2.3 (s, 6H, $N(CH_3)_2$), 3.5 (s, 3H, OCH_3), 4.8 (s, 2H, CH_2), 6.2 (s, 1H, C_5-H of thiazole), 6.9-8.1 (m, 8H, Ar-CH), 8.3 (s, 1H, CH=N). EI-MS *m/z*: 391 (M^+). *Anal*. Calcd for $C_{21}H_{21}N_5OS$: C, 64.43; H, 5.41; N, 17.89. Found: C, 64.26; H, 5.43; N, 17.95.

_N_-(4-Hydroxybenzylidene)-4-(1-((dimethylamino)methyl)-1_H_-benzimidazol-2-yl)thiazol-2-amine (5g)

Yield = 77 %, m.p. 114-115 °C. IR (KBr) cm^{-1}: 3515 (OH), 3081 (Ar-CH), 2932 (CH_3-CH), 1656 (C=N), 1620 (C=C). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ ppm: 2.2 (s, 6H, $N(CH_3)_2$), 4.6 (s, 2H, CH_2), 5.3 (s, 1H, OH), 6.7 (s, 1H, C_5-H of thiazole), 6.8-7.9 (m, 8H, Ar-CH), 8.5 (s, 1H, CH=N). EI-MS *m/z*: 377 (M^+). *Anal*. Calcd for $C_{20}H_{19}N_5OS$: C, 63.64; H, 5.07; N, 18.55. Found: C, 63.88; H, 5.06; N, 18.50.

_N_-(4-(Dimethylamino)benzylidene)-4-(1-((dimethylamino)methyl)-1_H_-benzimidazol-2-yl) thiazol-2-amine (5h)

Yield = 75 %, m.p. 132-134 °C. IR (KBr) cm^{-1}: 3075 (Ar-CH), 2955 (CH_3-CH), 1644 (C=N), 1608 (C=C). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ ppm: 1.9 (s, 6H, $N(CH_3)_2$), 2.7 (s, 6H, $N(CH_3)_2$), 4.3 (s, 2H, CH_2), 6.8 (s, 1H, C_5-H of thiazole), 7.3-8.1 (m, 8H, Ar-CH), 8.6 (s, 1H, CH=N). EI-MS *m/z*: 404 (M^+). *Anal*. Calcd for $C_{22}H_{24}N_6S$: C, 65.32; H, 5.98; N, 20.77. Found: C, 65.11; H, 6.00; N, 20.83.

_N_-(3-Nitrobenzylidene)-4-(1-((dimethylamino)methyl)-1_H_-benzimidazol-2-yl)thiazol-2-amine (5i)

Yield = 70%, m.p. 108-110 °C. IR (KBr) cm^{-1}: 3067 (Ar-CH), 2969 (CH_3-CH), 1637 (C=N), 1612 (C=C), 1543 & 1318 (NO_2). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ ppm: 2.5 (s, 6H, $N(CH_3)_2$), 4.9 (s, 2H, CH_2), 6.9 (s, 1H, C_5-H of thiazole), 7.2-8.5 (m, 8H, Ar-CH), 9.0 (s, 1H, CH=N). EI-MS *m/z*: 406 (M^+). *Anal*. Calcd for $C_{20}H_{18}N_6O_2S$: C, 59.10; H, 4.46; N, 20.68. Found: C, 59.28; H, 4.48; N, 20.61.

_N_-(3-Chlorobenzylidene)-4-(1-((dimethylamino)methyl)-1_H_-benzimidazol-2-yl)thiazol-2-amine (5j)

Yield = 73 %, m.p. 121-122 °C. IR (KBr) cm^{-1}: 3055 (Ar-CH), 2901 (CH_3-CH), 1647 (C=N), 1609 (C=C), 766 (C-Cl). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ ppm: 2.4 (s, 6H, $N(CH_3)_2$), 4.2 (s, 2H, CH_2), 6.4 (s, 1H, C_5-H of thiazole), 6.7-7.5 (m, 8H, Ar-CH), 8.6 (s, 1H, CH=N). EI-MS *m/z*: 397 (M^{+2}), 395 (M^+). *Anal*. Calcd for $C_{20}H_{18}ClN_5S$: C, 60.67; H, 4.58; N, 17.69. Found: C, 60.89; H, 4.56; N, 17.64.

_N_-(3-Bromobenzylidene)-4-(1-((dimethylamino)methyl)-1_H_-benzimidazol-2-yl)thiazol-2-amine (5k)

Yield = 78 %, m.p. 148-150 °C. IR (KBr) cm^{-1}: 3072 (Ar-CH), 2967 (CH_3-CH), 1635 (C=N), 1614 (C=C), 680 (C-Br). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ ppm: 1.8 (s, 6H, $N(CH_3)_2$), 5.0 (s, 2H, CH_2), 6.9 (s, 1H, C_5-H of thiazole), 7.3-8.5 (m, 8H, Ar-CH), 8.7 (s, 1H, CH=N). EI-MS *m/z*: 441 (M^{+2}), 439 (M^+). *Anal*. Calcd for $C_{20}H_{18}BrN_5S$: C, 54.55; H, 4.12; N, 15.90. Found: C, 54.75; H, 4.10; N, 15.87.

_N_-(3-Methylbenzylidene)-4-(1-((dimethylamino)methyl)-1_H_-benzimidazol-2-yl)thiazol-2-amine (5l)

Yield = 74 %, m.p. 101-103 °C. IR (KBr) cm^{-1}: 3059 (Ar-CH), 2942 (CH_3-CH), 1658(C=N), 1620 (C=C). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ ppm: 2.2 (s, 6H, $N(CH_3)_2$), 2.9 (s, 3H, CH_3), 4.7 (s, 2H, CH_2), 6.8 (s, 1H, C_5-H of thiazole), 7.7-8.0 (m, 8H, Ar-CH), 8.3 (s, 1H, CH=N). EI-MS *m/z*: 375 (M^+). *Anal*. Calcd for $C_{21}H_{21}N_5S$: C, 67.17; H, 5.64; N, 18.65. Found: C, 66.95; H, 5.65; N, 18.71.

_N_-(3-Methoxybenzylidene)-4-(1-((dimethylamino)methyl)-1_H_-benzimidazol-2-yl)thiazol-2-amine (5m)

Yield = 79 %, m.p. 129-130 °C. IR (KBr) cm^{-1}: 3003 (Ar-CH), 2938 (CH_3-CH), 1641 (C=N), 1611 (C=C), 1057 (C-O-C). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ ppm: 2.6 (s, 6H, $N(CH_3)_2$), 3.4 (s, 3H, OCH_3), 5.1 (s, 2H, CH_2), 6.3 (s, 1H, C_5-H of thiazole), 6.6-7.9 (m, 8H, Ar-CH), 9.1 (s, 1H, CH=N). EI-MS *m/z*: 391 (M^+). *Anal*. Calcd for $C_{21}H_{21}N_5OS$: C, 64.43; H, 5.41; N, 17.89. Found: C, 64.20; H, 5.43; N, 17.93.

_N_-(3-Hydroxybenzylidene)-4-(1-((dimethylamino)methyl)-1_H_-benzimidazol-2-yl)thiazol-2-amine (5n)

Yield = 70 %, m.p. 159-161 °C. IR (KBr) cm^{-1}: 3482 (OH), 3070 (Ar-CH), 2955 (CH_3-CH), 1640 (C=N), 1606 (C=C). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ ppm: 2.4 (s, 6H, $N(CH_3)_2$), 4.5 (s, 2H, CH_2), 4.9 (s, 1H, OH), 6.7 (s, 1H, C_5-H of thiazole), 7.0-8.2 (m, 8H, Ar-CH), 8.7 (s, 1H, CH=N). EI-MS *m/z*: 377 (M^+). *Anal*. Calcd for $C_{20}H_{19}N_5OS$: C, 63.64; H, 5.07; N, 18.55. Found: C, 63.47; H, 5.09; N, 18.61.

N*-(3-(Dimethylamino)benzylidene)-4-(1-((dimethylamino)methyl)-1*H*-benzimidazol-2-yl)thiazol-2-amine (5o*)

Yield = 73 %, m.p. 140-142 °C. IR (KBr) cm^{-1}: 3067 (Ar-CH), 2940 (CH_3-CH), 1633 (C=N), 1629 (C=C). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ ppm: 2.1 (s, 6H, $N(CH_3)_2$), 3.2 (s, 6H, $N(CH_3)_2$), 4.8 (s, 2H, CH_2), 6.9 (s, 1H, C_5-H of thiazole), 7.2-8.1 (m, 8H, Ar-CH), 8.4 (s, 1H, CH=N). EI-MS *m/z*: 404 (M^+). *Anal*. Calcd for $C_{22}H_{24}N_6S$: C, 65.32; H, 5.98; N, 20.77. Found: C, 65.18; H, 5.99; N, 20.85.

Biological activity

Antitubercular activity

In vitro M. tuberculosis method (Agar dilution method) was performed to assess the antitubercular potency of test compounds [20-21]. 10 fold serial dilutions of each test compound/drug were incorporated into Middle brook 7H11 agar slants with OADC growth supplement. Inoculums of M. tuberculosis H37RV were prepared from fresh Middle brook 7H11 agar slants with OADC Growth Supplement adjusted to 1 mg/ml (wet weight) in tween 80 (0.05 %) saline diluted to 10-2 to give a concentration of approximately 107 cfu/ml. A 5 µl amount of bacterial suspension was spotted into 7H11 agar tubes containing 10 fold serial dilutions of drug per ml. The tubes were incubated at 37 °C, and final readings were recorded after 28 days. Tubes having the compounds were compared with control tubes where medium alone was incubated with H37RV. The concentration at which complete inhibition of colonies occurred was taken as active concentration of test compound. The minimum inhibitory concentration (MIC) is defined as the minimum concentration of compound required to give complete inhibition of bacterial growth. The MIC of the test compounds was compared with the standard Isoniazid (INH) and the results are presented in Table 1.

Antimicrobial activity

In this study, all the synthesized compounds were screened for antimicrobial activity by agar streak dilution method. The antibacterial activity of the compounds were evaluated against four Gram-positive bacteria Staphylococcus aureus ATCC 9144, Staphylococcus epidermidis ATCC 155, Micrococcus luteus ATCC 4698 and Bacillus cereus ATCC 11778 and three Gram-negative bacteria Escherichia coli ATCC 25922, Pseudomonas aeruginosa ATCC 2853 and Klebsiella pneumoniae ATCC 11298. The antifungal activities of the synthesized compounds were evaluated against two fungi Aspergillus niger ATCC 9029 and Aspergillus fumigatus ATCC 46645. Bacterial strains were cultured over night at 37 °C in Mueller-Hinton broth and the yeast was cultured overnight at 30 °C in YEPDE agar for antibacterial and antifungal activity tests. Test strains were suspended in nutrient agar to give a final density of 5 x 10-5 cfu/ml.

Minimum inhibitory concentration (MIC)

MIC of the compounds was determined by agar streak dilution method [22]. A stock solution of the synthesized compound in dimethyl formamide was prepared and graded quantities of the test compounds were incorporated in specified quantity of

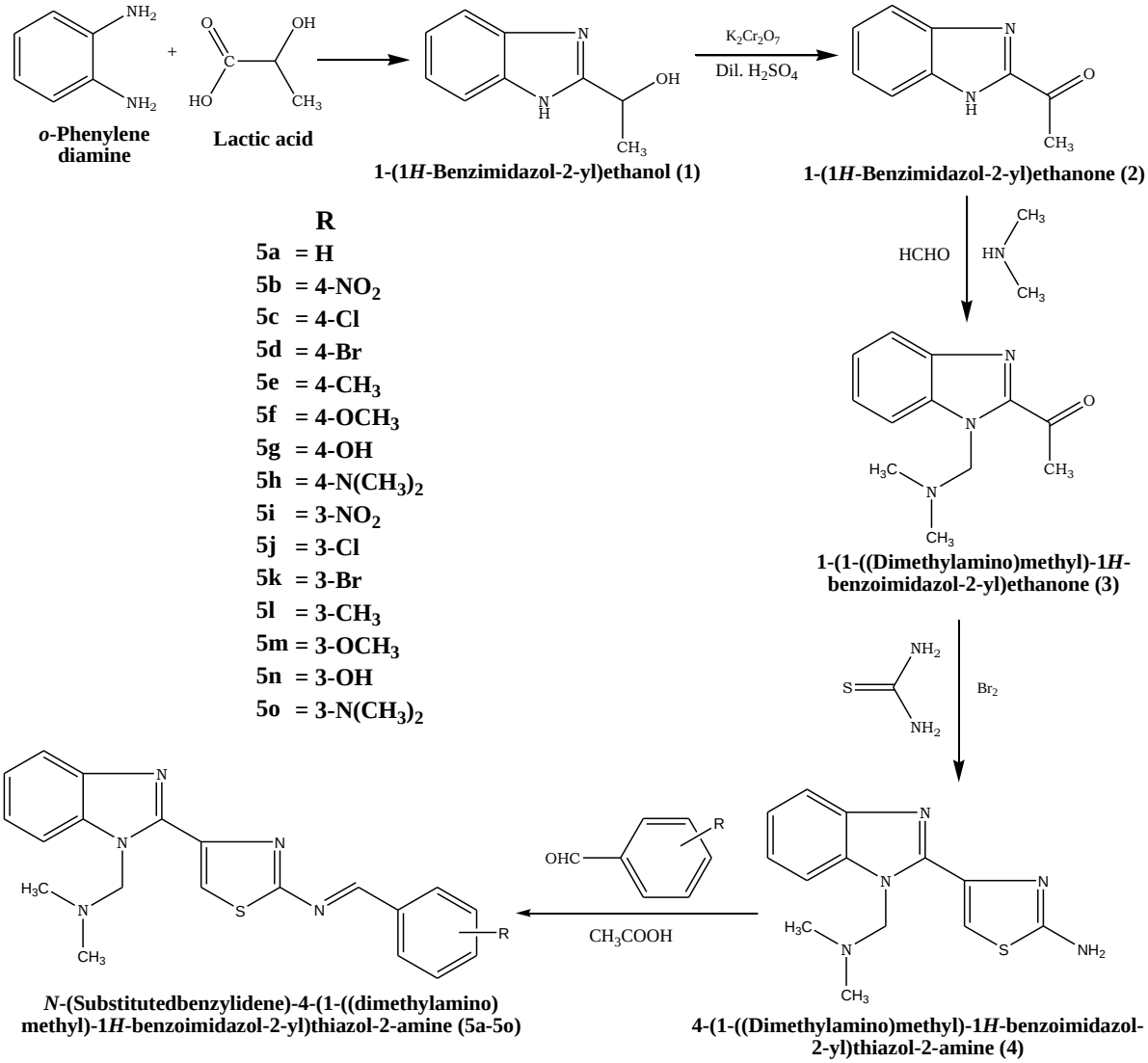
molten sterile agar (nutrient agar for antibacterial activity and Sabouraud's dextrose agar medium for antifungal activity). A specified quantity of the medium (40-50 ºC) containing the compound was poured into a petridish to give a depth of 3-4 mm and allowed to solidify. Suspension of the microorganism were prepared to contain approximately 5 x 10-5 cfu/ml and applied to plates with serially diluted compounds in dimethyl formamide to be tested and incubated at 37 °C for 24 h and 48 h for bacteria and fungi, respectively. The MIC was considered to be the lowest concentration of the test substance exhibiting no visible growth of bacteria or fungi on the plate. The observed MIC is presented in Table 1.

RESULTS AND DISCUSSION

Chemistry

The protocol for the synthesis of target compounds 5a-5o is shown in Scheme 1. In this study, a series of novel benzimidazole derivatives 5a-5o were synthesized by substituting different N-(substitutedbenzylidene)thiazol-2-amine at C-2 and dimethylaminomethyl groups at C-1 of benzimidazole. By a multistep synthesis, a sequence of new N-(substitutedbenzylidene)-4-(1-((dimethylamino)methyl)-1H-benzimidazol-2-yl)thiazol-2-amine 5a-5o were synthesized from o-phenylenediamine. Initially o-phenylenediamine was treated with lactic acid to obtain 1-(1H-benzimidazol-2-yl)ethanol 1 by a ring closure reaction with the elimination of two molecule of water. Latter, obtained 1-(1H-benzimidazol-2-yl)ethanol 1 undergone oxidation in presence of potassium dichromate and sulphuric acid and produced 1-(1H-benzimidazol-2-yl)ethanone 2. In the succeeding step, compound 2 was treated with dimethylamine and formaldehyde to produce 1-(1-((dimethylamino)methyl)-1H-benzimidazol-2-yl)ethanone 3 by Mannich base reaction. In the further step, reaction of -(1-((dimethylamino)methyl)-1H-benzimidazol-2-yl)ethanone 3 with thiourea and bromine yields a product called 4-(1-((dimethylamino)methyl)-1H-benzimidazol-2-yl)thiazol-2-amine 4 by a ring closure reaction. In the final step the title compounds 5a-5o were synthesized by reacting 4-(1-((dimethylamino)methyl)-1H-benzimidazol-2-yl)thiazol-2-amine 4 with various aromatic aldehydes through Schiff base reaction. The reaction preceded by dehydration through hemiaminal formation, a unique step in Schiff base reaction. The reaction optimization, completion and purity of the synthesized intermediates and

final compounds are confirmed by TLC.



R

5a = H
5b = 4-NO_2
5c = 4-Cl
5d = 4-Br
5e = 4-CH_3
5f = 4-OCH_3
5g = 4-OH
5h = 4-$N(CH_3)_2$
5i = 3-NO_2
5j = 3-Cl
5k = 3-Br
5l = 3-CH_3
5m = 3-OCH_3
5n = 3-OH
5o = 3-$N(CH_3)_2$

Scheme 1. Synthesis of *N*-(Substitutedbenzylidene)-4-(1-((dimethylamino) methyl)-1*H*-benzoimidazol-2-yl)thiazol-2-amine (5a-5o)

The structures of the novel synthesized compounds were confirmed by IR, 1H-NMR, mass spectra and elemental analyses data. Compounds spectral data are in accordance with the assigned structures. All the synthesized compounds showed some characteristic peaks in its IR spectra representing the presence of specific groups. Formation of the 1-(1H-benzimidazol-2-yl)ethanol 1 was confirmed by the presence of absorption peak at 3545 and 3356 cm-1 in IR due to presence of OH and NH stretching, respectively and appearance of singlet in its 1H-NMR spectra at δ 3.41 and 5.78 ppm for one proton which might be assigned to OH and NH proton, respectively. The formations of oxidized compound 2 was confirmed by the disappearance of absorption peak around 3500 cm-1 in IR due to absence of OH stretching and appearance of sharp peak at 1740 cm-1 in IR corresponds to C=O stretching. The formation of Mannich base 3 from compound 2 can be recognized by absence of strong absorption peak around 3350 cm-1 in IR due to absence of NH stretching and appearance of singlet in its 1H-NMR spectra at δ 4.42 ppm for two protons which might be assigned to CH2 linkage. In 1H-NMR spectra appearance of a singlet for two protons at δ 4.20 ppm due to NH2 group of compound 4. The IR spectrum of title compounds shows absorption bands at 1633-1683 cm-1, which can be assignable to C=N vibrations. Appearance of strong absorption band in IR spectrum of compound 5g & 5n

at 3515 & 3482 cm-1, respectively, which can be assigned to OH group. The entire title compounds 1H-NMR spectrum comparisons given the following conclusions. a) A singlet around δ 1.8-2.6 ppm for N(CH3)2 proton; b) A singlet in the range of δ 4.2-5.1 ppm which can be assignable to CH2 linkage protons; c) A singlet in the range of δ 6.2-6.9 ppm for C5-H of thiazole; d) A multiplet in the range of δ 6.6-8.5 ppm for aromatic proton; e) c) A singlet in the range of δ 8.3-9.2 ppm for CH=N proton; Further mass spectrum confirmed their purity and molecular weight.

Antitubercular activity

The entire series of compounds were screened for their in vitro antimycobacterial activity against H37Rv strain of M. tuberculosis and the results are expressed in terms of MIC. The MIC of INH was determined in parallel experiments in order to control the sensitivity of the test organisms. The results of antimycobacterial activity indicate that the test compounds inhibited the growth of mycobacterium in varying degree. Out of various tested compounds, compounds such as 5b and 5c inhibited the growth M. tuberculosis at 3.9 µg/ml concentrations. The potent activity of these compounds may be due to the presence of nitro/chlorine group at C-4 position of phenyl ring.

Compound	**Antitubercular activity**	**Antibacterial activity**							**Antifungal activity**	
	M. tuberculosis	***S. aureus***	***S. epidermidis***	***M. luteus***	***B. cereus***	***E. coli***	***P. aeruginosa***	***K. pneumoniae***	***A. niger***	***A. fumigatus***
5a	*31.25*	*31.25*	*31.25*	*62.5*	*31.25*	*62.5*	*31.25*	*31.25*	*125*	*62.5*
5b	*3.9*	*7.81*	*3.9*	*7.81*	*15.62*	*15.62*	*3.9*	*3.9*	*31.25*	*15.62*
5c	*3.9*	*7.81*	*7.81*	*15.62*	*7.81*	*15.62*	*7.81*	*3.9*	*31.25*	*31.25*
5d	*7.81*	*15.62*	*7.81*	*15.62*	*15.62*	*7.81*	*15.62*	*7.81*	*62.5*	*31.25*
5e	*62.5*	*31.25*	*62.5*	*62.5*	*31.25*	*62.5*	*62.5*	*31.25*	*125*	*62.5*
5f	*62.5*	*62.5*	*62.5*	*62.5*	*62.5*	*125*	*62.5*	*31.25*	*125*	*62.5*
5g	*62.5*	*62.5*	*125*	*62.5*	*62.5*	*125*	*62.5*	*62.5*	*>125*	*125*
5h	*125*	*125*	*125*	*62.5*	*62.5*	*125*	*125*	*62.5*	*>125*	*125*
5i	*15.62*	*15.62*	*7.81*	*15.62*	*31.25*	*15.62*	*15.62*	*15.62*	*62.5*	*31.25*
5j	15.62	*15.62*	*15.62*	*31.25*	*31.25*	*15.62*	*31.25*	*15.62*	*62.5*	*62.5*
5k	15.62	*31.25*	*15.62*	*31.25*	*31.25*	*31.25*	*31.25*	*15.62*	*125*	*62.5*
5l	*125*	*125*	*>125*	*62.5*	*125*	*125*	*125*	*62.5*	*>125*	*125*
5m	*125*	*125*	*>125*	*125*	*125*	*>125*	*125*	*125*	*125*	*>125*
5n	*>125*	*>125*	*>125*	*125*	*125*	*>125*	*125*	*125*	*>125*	*>125*
5o	*>125*	*>125*	*>125*	*>125*	*125*	*>125*	*>125*	*125*	*>125*	*125*
Standard*	0.97	*7.81*	*1.95*	*3.9*	*7.81*	*7.81*	*3.9*	*1.95*	*7.81*	*3.9*

Note: *Isoniazid used as a reference standard against *M. tuberculosis* whereas Ciprofloxacin used as a reference standard for other bacteria & Ketoconazole used as a reference standard for fungi.

Whereas compounds possessing bromine group at C-4 of phenyl ring 5d exhibited activity at the concentration of 7.81 µg/ml. Compounds 5i, 5j, and 5k possessing nito, chlorine, and bromo group, respectively at C-3 of phenyl ring, which inhibited the growth of M. tuberculosis at 15.62 µg/ml concentrations only. The MIC of unsubstituted derivative 5a was found to be 31.25

µg/ml. Rest of series (5e-5h, and 5l-5o) showed activity only at higher concentration (MIC: ≥62.5 µg/ml) might be due to the presence of methyl/methoxy/hydroxyl/dimethylamino group either at C-4 or C-3 of phenyl ring.

Antimicrobial activity

Agar streak dilution method was used to analyze the in vitro antimicrobial activity of title compounds 5a-5o. A comparison of antimicrobial activity of the synthesized compounds with that of standard drugs was effectively presented in Table 1. In order to control the sensitivity of the test organisms, MICs of standard drugs (Ciprofloxacin and Ketoconazole) were determined in parallel experiments. From the results it was observed that against S. aureus, compound 5b and 5c (MIC: 7.81 µg/ml) displayed similar activity like Ciprofloxacin; whereas rest of series exhibited lesser activity (MIC: ≥15.62 µg/ml). Compared to Ciprofloxacin, against B. cereus compounds 5c exhibited equal activity (MIC: 7.81 µg/ml) whereas rest of series exhibited lower activity (MIC: ≥15.62 µg/ml). While others demonstrated lesser activity than standard against E. coli, compounds 5d exhibited comparable activity (MIC: 7.81 µg/ml) as Ciprofloxacin. Compounds 5b showed the same activity (MIC: 3.9 µg/ml) as Ciprofloxacin, whereas rest of all compounds showed worse activity (MIC: ≥7.81 µg/ml) than standard against P. aeruginosa.

Among the various tested derivatives, the compound 5b displayed superior activity than rest of tested derivatives against all microorganisms except B. cereus, and E. coli. Among screened compounds against B. cereus, compound 5c exhibited highest activity; whereas against E. coli, compound 5d exhibited highest activity. The antifungal activities of the synthesized compounds were evaluated against A. niger and A. fumigatus. The title compounds exhibits varying degree of antifungal activity. None of the synthesized compounds displayed either equal/superior antifungal activity when compared to standard Ketoconazole against the tested fungi. Compounds 5b-5d displayed moderate antifungal activity; whereas rest of title compounds showed poor antifungal activity.

Structural activity relationship

In general, from the study it was found that compounds possessing electron withdrawing groups (5b-5d & 5i-5k) displayed better antimicrobial activity than compounds possessing electron donating groups (5e-5h & 5l-5o). The unsubstituted derivative 5a displayed intermediate activity. Within electron donating / withdrawing group compounds, the position of the group played important role while deciding the activity. Results of antitubercular and antimicrobial data revealed that in the phenyl ring presence of substituting group at C-4 position favors the antimicrobial activity than at C-3 position.

CONCLUSION

In conclusion, a variety of novel thiazole substituted benzimidazole derivatives were synthesized by a multi step synthesis. All synthesized compounds were characterized by FT-IR, 1H-NMR, Mass spectroscopy and elemental analysis. Entire title compounds were assessed for their in vitro antitubercular, antibacterial and antifungal activity. Compounds showed mild to good antibacterial activity and poor antifungal activity. From the SAR studies it was found that, nature of substituent's played major role in determining antimicrobial activity than position of the substituent. Electron withdrawing group substituted derivative exhibited better activity than electron donating group substituted analogs. Among several tested compounds, N-(4-nitrobenzylidene)-4-(1-((dimethylamino)methyl)-1H-benzimidazol-2-yl)thiazol-2-amine 5b showed better antibacterial activity which is almost equal to reference standard Ciprofloxacin. In addition, compound 5b and 5c also showed some excellent antibacterial activity against some pathogenic strains of microorganism. These compounds also displayed good antitubercular activity also. Hence, these analogs could be developed as a new class of antimicrobial agents. However, further structural modification is planned to enhance the antitubercular and antibacterial activity.

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