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Design and synthesis of some new imidazole and 1,2,4-triazole substituted fluorobenzimidazoles for antitubercular and antifungal activity

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ABSTRACT

*A series of thirteen imidazole and 1,2,4-triazole substituted fluoro benzimidazoles (**8a-i**), (**9a-b**) and (**11a-b**) with phenyl and benzyl group at 2[nd] position were synthesized and screened for antitubercular activity against $H_{37}R_V$ strain and antifungal activity against Candida species. Both imidazole and 1,2,4-triazole substituted fluoro benzimidazoles with 2-benzyl and 4-$N(CH_3)_2$ substituted 2-phenyl/2-phenyl-1-benzyl counterpart were found to be the most active of all the compounds. To examine the influence of azole moiety on the activity chlorine substituted fluoro benzimidazole (**5**) was synthesized. The newly synthesized compounds were characterized by I.R, ^{1}H-NMR, ^{13}C-NMR, Mass and elemental analysis. In vitro antitubercular and antifungal activity data revealed that the benzimidazole scaffold with imidazole or triazole moiety were important for antitubercular and antifungal activity.*

Keywords: Fluoro benzimidazole, Antitubercular activity, Antifungal activity, *Candida* species, *Mycobacterium tuberculosis*.

INTRODUCTION

The major drawbacks with the therapeutic agents for mycobacterial and fungal infections are prolonged treatment regimen with combination of drugs associated with significant toxicity and emergence of multi-drug resistant (MDR) bacteria and fungi causing morbidity and mortality in immunocompromised hosts. The necessity for effective therapy has stimulated research into the design and synthesis of novel compounds which can treat both mycobacterial and fungal infections.

Tuberculosis is a deadly infectious disease caused by the pathogenic *Mycobacterium tuberculosis* (Mtb) and poses a major health concern to world population [1]. Despite advances in chemotherapy, the frequency of severe mycobacterial infection present major challenges for the effective control of TB and it still remains a leading cause of death worldwide [2-4]. Over the past two decades significant developments in pharmaceutical research on various species of tuberculosis has been made with focus on synthetic products to find potential drug candidates against the resistant strains. In recent years, the increasing emergence of resistant *Mycobacterium tuberculosis* against the currently used first line anti-TB drugs such as isoniazid, pyrazinamide and rifampicin highlight the need to develop efficient drugs with high antitubercular potential particularly effective against the persistent bacilli. The azole class has become one of the most widely developed and investigated over the past two decades. Azoles are the most

common heteroaromatic scaffolds present in bioactive molecules [5]. Benzimidazole ring system which is a core structure in various synthetic pharmaceuticals, display a broad spectrum of biological activity, including antibacterial and antifungal properties [6-8]. Di and tri substituted benzimidazoles with imidazole, triazole and pyrollidine moiety as part of the structure were screened for antitubercular activity against *Mycobacterium tuberculosis* $H_{37}Rv$ strain and have shown better activity comparable to that of standard drugs isoniazid and rifampicin [9-11]. Numerous reports on compounds with imidazole and triazole moiety have appeared which are superior to or at least as active as the currently used class of antitubercular or antifungal agents [12-16]. Nitroimidazole pyran PA824 derived from 5-nitro imidazole has a good in vitro and in vivio activity against *Mycobacterium tuberculosis* and is a particularly promising candidate for TB treatment with MIC as low as 0.015-0.250 µg/mL [17]. In a more recent report imidazole substituted and azole-fused quinolines were found to exhibit potential anti-TB activity against $H_{37}Rv$ strain with MIC of 0.39 µg/mL (1 µM) and 0.78 µgmL (2 µM) respectively [18, 19]. QSAR analysis for heterocyclic antifungals were performed on 96 compounds to study the effects and antifungal potencies against the *Candida* strain and arrived at the general structure of some potent heterocyclic antifungals. As an outcome of this study, benzimidazoles were one of the heterocyclics that were investigated with the conclusion that a correlation existed between the general structure and the observed antifungal activity [20]. Fluorinated pharmaceuticals accounts for nearly 20% of the commercially available drug substances and influence of the fluorine on physico-chemical characteristics of the organic compounds makes it a previliged atom in the design and synthesis of biologically active moieties [21, 22].

The design and synthesis of imidazole and 1,2,4-triazole substituted fluoro benzimidazoles was based on the study of antitubercular/antifungal benzimidazoles, and the recently reported imidazole substituted/azole-fused quinolines for their antitubercular activity against the pathogenic $H_{37}Rv$ strain (figure 1). The synthesized azole substituted fluoro benzimidazoles (FBIMS) were screened for their antitubercular activity against *M. tuberculosis* $H_{37}R_V$ and antifungal activity against different strains of *Candida* species.

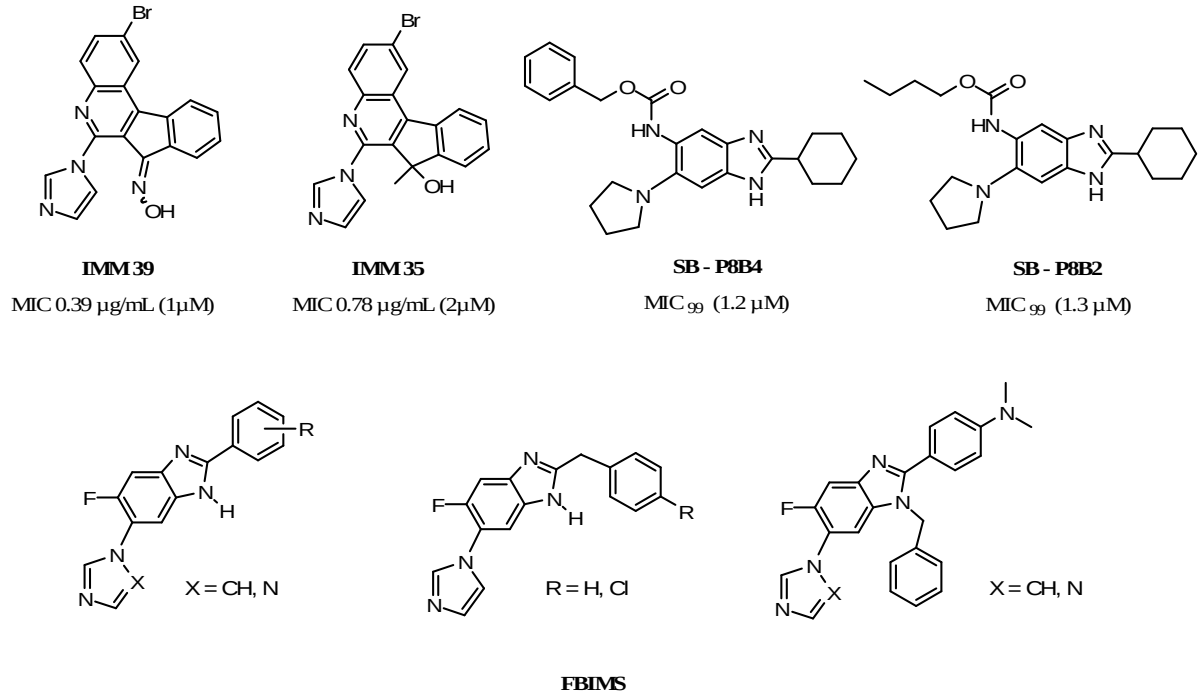


Figure 1. Structures of potent anti-tubercular compounds and azole substituted fluoro benzimidazoles (FBIMS)

MATERIALS AND METHODS

All chemicals and solvents used for this work were obtained commercially and used without further purification. Melting points of the synthesized compounds were determined in open capillaries and are uncorrected. All air-

sensitive reactions were carried out under nitrogen atmosphere. IR spectra were recorded on a shimadzu-5400 FT-IR spectrometer as KBr discs. ^{1}H-NMR, ^{13}C-NMR and ^{19}F-decoupled ^{1}H-NMR spectra were recorded on a Bruker Avance-400 MHZ spectrometer. The values of chemical shifts are expressed in ppm relative to Me_4Si (δ=0) in DMSO-d_6 and the *J* values in hertz (HZ). Signal multiplicities are represented by s [singlet], d [doublet], t [triplet], dd [double doublet], m [multiplet] and br.s [broad singlet]. Mass spectra were recorded on a LC/MS/MS 6410 triple quad mass spectrometer by electron spray ionization. Elemental analyses were performed on Perkin-Elmer 2400 CHN elemental analyzer and the found values were within ± 0.4% of the theoretical values. The progress of the reaction was monitored by thin layer chromatography with F_{254} silica-gel precoated sheets and the spots were visualized by exposing them to iodine vapour or Uv light was used for detection at λ 254.

5-chloro-4-fluoro-2-nitro aniline ***(3)***
Orange needle (85%); mp 143-145 °C; IR (KBr, cm^{-1}): 3493, 3319, 3050, 1639, 1593, 1570, 1502, 1479, 1465, 1445, 1334, 1242, 1074, 1004. ^{1}H-NMR (400 MHZ, DMSO-d_6): δ 6.00 (br.s, 2H, NH_2), 6.90-6.91 (d, 1H, H-3, *J* = 4.0 HZ, ArH), 7.91-7.94 (d, 1H, H-6, *J* = 12.0 HZ, ArH). ^{19}F-decoupled ^{1}H-NMR (DMSO): δ 4.75-6.20 (br.s, 2H, NH_2), 6.92 (s, 1H, H-3, ArH), 7.93 (s, 1H, H-6, ArH).

General procedure for the synthesis of 4-fluoro-5-(substituted)-2-nitroaniline ***(6a, 6b)***
Imidazole or 1,2,4-triazole (11 mmol) and anhydrous potassium carbonate (20 mmol) were added to a solution of 5-chloro-4-fluoro-2-nitro aniline (10 mmol) in dry DMF (20 mL). The reaction mixture was then stirred at 100 °C for 8 to 10 hours. When TLC revealed the absence of starting material, the reaction mixture was cooled to room temperature and poured into water (100 mL). The resultant solution was extracted with ethyl acetate. The extract was then washed with water and dried over anhydrous sodium sulphate. The solvent was removed under reduced pressure to afford 5-(substituted)-4-fluoro-2-nitro benzeneamine. The crude solid was used for the next step without further purification.

4-fluoro-5-(1H-imidazol-1-yl)-2-nitrobenzenamine ***(6a)***
Crystalline yellow solid (85%); mp 219-221 °C; IR (KBr, cm^{-1}): 3473, 3275, 3146, 3126, 1647, 1581, 1527, 1514, 1500, 1383, 1301, 1280, 1257, 1211, 877. ^{1}H-NMR (400 MHZ, DMSO-d_6): δ 7.15 (s, 1H, imidazole-H), 7.20-7.21 (d, 1H, H-6, *J* = 6.8 HZ, ArH), 7.48 (br.s, 2H, NH_2), 7.55 (s, 1H, H-3, ArH), 8.02 (d, 1H, J = 2.0 HZ, imidazole-H), 8.05-8.06 (d, 1H, *J* = 3.6 HZ, imidazole-H). MS (ESI) m/z: 222.1 (M-1).

4-fluoro-2-nitro-5-(1H-1,2.4-triazol-1-yl)benzenamine ***(6b)***
Yellow solid (85%); mp 260-262 °C; IR (KBr, cm^{-1}): 3493, 3282, 3155, 3136, 1647, 1583, 1525, 1502, 1394, 1260, 1295, 1141, 877. ^{1}H-NMR (400 MHZ, DMSO-d_6): δ 7.56 (s, 1H, triazole-H), 7.58 (br.s, 2H, NH_2), 8.07-8.10 (d, 1H, H-6, *J* = 12.0 HZ, ArH), 8.35 (s, 1H, triazole-H), 9.07-9.08 (d, 1H, H-3, *J* = 3.2 HZ, ArH). MS (ESI) m/z: 223.1 (M+1).

General procedure for the synthesis of 4-chloro-5-fluoro-1,2-phenylenediamine ***(4)*** *and 4-(substituted)-5-fluoro-benzene-1,2-phenylenediamine* ***(7a, 7b)***
To a stirred solution of compound (**3, 6a** and **6b**) (10 mmol) in ethyl alcohol containing zinc dust (100 mmol) was slowly injected concentrated HCl (20 mL) via septum using glass syringe over a period of 2 hours and continued stirring at room temperature under nitrogen atmosphere for another additional 2 hours. When TLC revealed the absence of starting material, the solution was filtered, made alkaline with 10% NaOH and then extracted with ethyl acetate. The extract was washed with water, dried over Na_2SO_4 and evaporated. Because of the instability of these diamines the reduced compounds were used for the next step without further purification.

General experimental procedure for the synthesis of ***(5)*** *and 2-(substituted phenyl)-5-fluoro-6-(1H-azole substituted)-1H-benzo(d)imidazole derivatives* ***(8a-i)***
To a stirred solution of 4-(substituted)-5-fluoro-benzene-1,2-phenylenediamine (**4, 7a** and **7b**) (10 mmol) in dry DMF was added the corresponding aldehyde (11 mmol) and sodium metabisulfite (11 mmol). The reaction mixture was heated at 125 °C with stirring under nitrogen atmosphere for 15 hours. TLC was used to monitor the reaction. After the completion of the reaction, the mixture was cooled to room temperature and poured into water (100 mL), followed by extraction with ethyl acetate. The organic layer was washed with saturated sodium bisulphite solution, brine, dried over anhydrous sodium sulphate, filtered and concentrated under vacuum to afford **5** and (**8a-i**). The obtained crude solid was triturated with diethyl ether and was pure enough for spectral analysis.

4-(6-chloro-5-fluoro-1H-benzo[d]imidazol-2-yl)-N,N-dimethylbenzenamine ***(5)***
Peach colored solid (85%); mp 252-254 °C; IR (KBr, cm^{-1}): 3441, 3107, 3093, 3070, 2920, 2899, 1620, 1506, 1452, 1435, 1394, 1373, 1361, 1209, 1155, 1030. ^{1}H-NMR (400 MHZ, DMSO-d_6): δ 2.98 (s, 6H, $N(CH_3)_2$), 6.80-6.82 (d, 2H, *J* = 8.8 HZ, ArH), 7.49 (br, s, 1H, ArH), 7.62-7.65 (d, 1H, *J* = 13.6 HZ, ArH), 7.94-7.97 (d, 2H, *J* = 9.20 HZ, ArH),12.77 (br.s, 1H, NH). MS (ESI) m/z: 289.1 (M-1). Anal. Calcd for $C_{15}H_{13}ClFN_3$: C, 62.28; H, 4.49; N, 14.53. Found: C, 62.07; H, 4.41; N, 14.48.

5-fluoro-2-phenyl-6-(1H-1,2,4-triazol-1-yl)-1H-benzo[d]imidazole ***(8a)***
Brown solid (85%); mp 150-152 °C; IR (KBr, cm^{-1}): 3440, 3090, 3050, 1650, 1510, 1470, 1450, 1145. ^{1}H-NMR (400 MHZ, DMSO-d_6): δ 7.51-7.59 (m, 3H, ArH), 7.65-7.67 (d, 1H, *J* = 16.0 HZ, ArH), 7.78-7.84 (m, 1H, ArH), 7.99 (br.s, 1H, triazole-H), 8.18-8.20 (d, 2H, *J* = 14.0 HZ, ArH), 8.28 (br.s, 1H, triazole-H), 8.96-8.99 (d, 1H, *J* = 18.40 HZ, ArH), 13.34 (s, 1H, NH). ^{13}C-NMR (100 MHZ, DMSO-d_6): δ 100.42, 100.95, 109.50, 117.12, 118.48, 122.64, 128.48, 135.86, 146.69, 149.66, 153.92, 157.86. MS (ESI) m/z: 279.1 (M+1). Anal. Calcd for $C_{15}H_{10}FN_5$: C, 64.51; H, 3.58; N, 25.08. Found: C, 64.36; H, 3.45; N, 25.01.

2-(4-chlorophenyl)-5-fluoro-6-(1H-1,2,4-triazol-1-yl)-1H-benzo[d]imidazole ***(8b)***
Brown solid (85%); mp 224-226 °C; IR (KBr, cm^{-1}): 3417, 3136, 3100, 1610, 1510, 1462, 1440, 1147, 1030. ^{1}H-NMR (400 MHZ, DMSO-d_6): δ 7.64-7.66 (d, 2H, *J* = 8.4 HZ, ArH), 7.78-7.83 (d, 1H, *J* = 18.42 HZ, ArH), 7.98 (br.s, 1H, ArH), 8.18-8.20 (d, 2H, *J* = 8.8 HZ, ArH), 8.27 (s, 1H, triazole-H), 8.97 (br.s, 1H, triazole-H), 13.42 (s, 1H, NH). ^{13}C-NMR (100 MHZ, DMSO-d_6) δ 97.96, 98.24, 110.03, 113.55, 122.20, 129.29, 131.55, 131.65, 137.60, 146.13, 152.27, 154.60, 157.77. MS (ESI) m/z: 313.1 (M+1). Anal. Calcd for $C_{15}H_9ClFN_5$: C, 57.50; H, 2.87; N, 22.36. Found: C, 57.56; H, 2.84; N, 22.30.

4-(5-fluoro-6-(1H-1,2,4-triazol-1-yl)-1H-benzo[d]imidazol-2-yl)-N,N-dimethylbenzenamine ***(8c)***
Pale yellow solid (85%); mp 200-202 °C; IR (KBr, cm^{-1}): 3441, 3130, 3076, 2916, 2901, 1610, 1510, 1471, 1363, 1359, 1309, 1200, 1149. ^{1}H-NMR (400 MHZ, DMSO-d_6): δ 2.98-3.02 (m, 6H, $N(CH_3)_2$), 6.76-6.80 (m, 1H, ArH), 6.82-6.84 (d, 2H, *J* = 8.0 HZ, ArH), 7.58-7.68 (m, 2H, ArH), 7.94-8.01 (m, 2H, ArH), 8.26 (s, 1H, triazole-H), 8.95 (s, 1H, triazole-H), 12.90 (br.s, 1H, NH). ^{13}C-NMR (100 MHZ, DMSO-d_6): δ 39.68, 110.99, 111.73, 116.34, 124.50, 127.72, 127.84, 131.47, 138.40, 145.20, 145.26, 151.57, 151.95, 155.73. MS (ESI) m/z: 322.2 (M+1). Anal. Calcd for $C_{17}H_{15}FN_6$: C, 63.35; H, 4.65; N, 26.08. Found: C, 63.25; H, 4.61; N, 26.03.

5-fluoro-6-(1H-1,2,4-triazol-1-yl)-2-(3,4,5,-trimethoxyphenyl)-1H-benzo[d]imidazole ***(8d)***
Brick red solid (85%); mp 136-138 °C; IR (KBr, cm^{-1}): 3406, 3383, 3207, 3176, 3113, 2968, 2931, 1593, 1504, 1470, 1433, 1278, 1240, 1134, 1026, 999. ^{1}H-NMR (400 MHZ, DMSO-d_6): δ 3.74 (s, 3H, OCH_3), 3.90 (s, 6H, $2OCH_3$), 7.53 (s, 2H, ArH), 7.69-7.76 (d, 1H, *J* = 16.20 HZ, ArH), 7.84-7.94 (d, 1H, *J* = 18.40 HZ, ArH), 8.27 (s, 1H, triazole-H), 8.97 (br.s, 1H, triazole-H), 13.25 (s, 1H, NH). ^{13}C-NMR (100 MHZ, DMSO-d_6) δ 55.68, 60.60, 100.43, 100.98, 108.13, 109.43, 115.98, 122.22, 128.13, 129.48, 136.43, 138.44, 145.68, 150.12, 155.66. MS (ESI) m/z: 369.2 (M+1). Anal. Calcd for $C_{18}H_{16}FN_5O_3$: C, 58.53; H, 4.33; N, 18.97. Found: C, 58.61; H, 4.25; N, 18.90.

5-fluoro-6-(1H-imidazol-1-yl)-2-phenyl-1H-benzo[d]imidazole ***(8e)***
Brown solid (85%); mp 171-173 °C; IR (KBr, cm^{-1}): 3415, 3178, 3117, 1650, 1510, 1470, 1450, 1380, 1120. ^{1}H-NMR (400 MHZ, DMSO-d_6): δ 6.86-7.16 (m, 4H, ArH), 7.45-7.47 (d, 1H, *J* = 8.0 HZ, ArH), 7.61-7.63 (d, 1H, *J* = 8.0 HZ, ArH), 7.83-7.87 (d, 2H, *J* = 16.0 HZ, imidazole-H), 8.29 (s, 1H, imidazole-H), 12.76 (s, 1H, NH). MS (ESI) m/z: 278.4 (M-1). Anal. Calcd for $C_{16}H_{11}FN_4$: C, 69.06; H, 3.95; N, 20.14 Found: C, 68.93; H, 3.86; N, 20.10.

2-(4-chlorophenyl)-5-fluoro-6-(1H-imidazol-1-yl)-1H-benzo[d]imidazole ***(8f)***
Brown solid (85%); mp 187-130 °C; IR (KBr, cm^{-1}): 3405, 3240, 3138, 3117, 1590, 1511, 1479, 1471, 1153, 1105. ^{1}H-NMR (400 MHZ, DMSO-d_6): δ 6.95 (s, 1H, ArH), 7.08 (s, 1H, ArH), 7.22 (s, 1H, ArH), 7.46-7.48 (d, 2H, *J* = 8.0 HZ, ArH), 7.61-7.63 (d, 2H, *J* = 8.0 HZ, ArH), 7.84-7.88 (d, 2H, *J* = 12.0 HZ, imidazole-H), 8.15 (s, 1H, imidazole-H), 12.95 (s, 1H, NH). ^{13}C-NMR (100 MHZ, DMSO-d_6): δ 103.02, 103.32, 106.92, 107.17, 115.59, 122.40, 128.29, 130.49, 139.25, 141.80, 153.50, 155.92, 159.59. MS (ESI) m/z: 312.1 (M+1). Anal. Calcd for $C_{16}H_{10}ClFN_4$: C, 61.53; H, 3.20; N, 17.94. Found: C, 61.45; H, 3.11; N, 17.88.

5-fluoro-6-(1H-imidazol-1-yl)-2-(4-methoxyphenyl)-1H-benzo[d]imidazole ***(8g)***
Buff colored solid (85%); mp 211-213 °C; IR (KBr, cm^{-1}): 3420, 3118, 3100, 2920, 1620, 1510, 1470, 1261, 1140, 1112. ^{1}H-NMR (400 MHZ, DMSO-d_6): δ 3.88 (s, 3H, OCH_3), 6.94-6.97 (d, 1H, *J* = 12.0 HZ, ArH), 6.98-6.99 (d,

2H, *J* = 4.0 HZ, ArH), 7.16-7.19 (d, 1H, *J* = 12.0 HZ, ArH), 7.37-7.39 (d, 2H, *J* = 8.0 HZ, ArH), 7.70 (s, 1H, ArH), 8.19-8.31 (m, 3H, ArH), 12.68 (s, 1H, NH). ^{13}C-NMR (100 MHZ, DMSO-d_6): δ 55.98, 100.40, 100.90, 109.55, 117.11, 122.92, 128.48, 132.66, 135.54, 138.08, 143.55, 153.90, 155.99. MS (ESI) m/z: 308.1 (M+1). Anal. Calcd for $C_{17}H_{13}FN_4O$: C, 66.23; H, 4.22; N, 18.18. Found: C, 66.16; H, 4.12; N, 18.12.

4-(5-fluoro-6-(1H-imidazol-1-yl)-1H-benzo[d]imidazol-2-yl)-N,N-dimethylbenzenamine ***(8h)***
Pale yellow solid (85%); mp 228-230 °C; IR (KBr, cm^{-1}): 3373, 3097, 2920, 2897, 2800, 1618, 1500, 1456, 1437, 1352, 1311, 1199, 1145. ^{1}H-NMR (400 MHZ, DMSO-d_6): δ 3.03 (s, 6H, N(CH_3)$_2$), 6.31-6.36 (d, 1H, ArH), 6.83-6.85 (d, 1H, *J* = 8.0 HZ, ArH), 6.97-7.00 (d, 1H, *J* = 12.0 HZ, ArH), 7.69-7.76 (m, 5H, ArH), 8.19 (s, 1H, imidazole-H), 13.32 (s, 1H, NH). MS (ESI) m/z: 321.2 (M-1). Anal. Calcd for $C_{18}H_{16}FN_5$: C, 67.28; H, 4.98; N, 21.80. Found: C, 67.20; H, 4.91; N, 21.75.

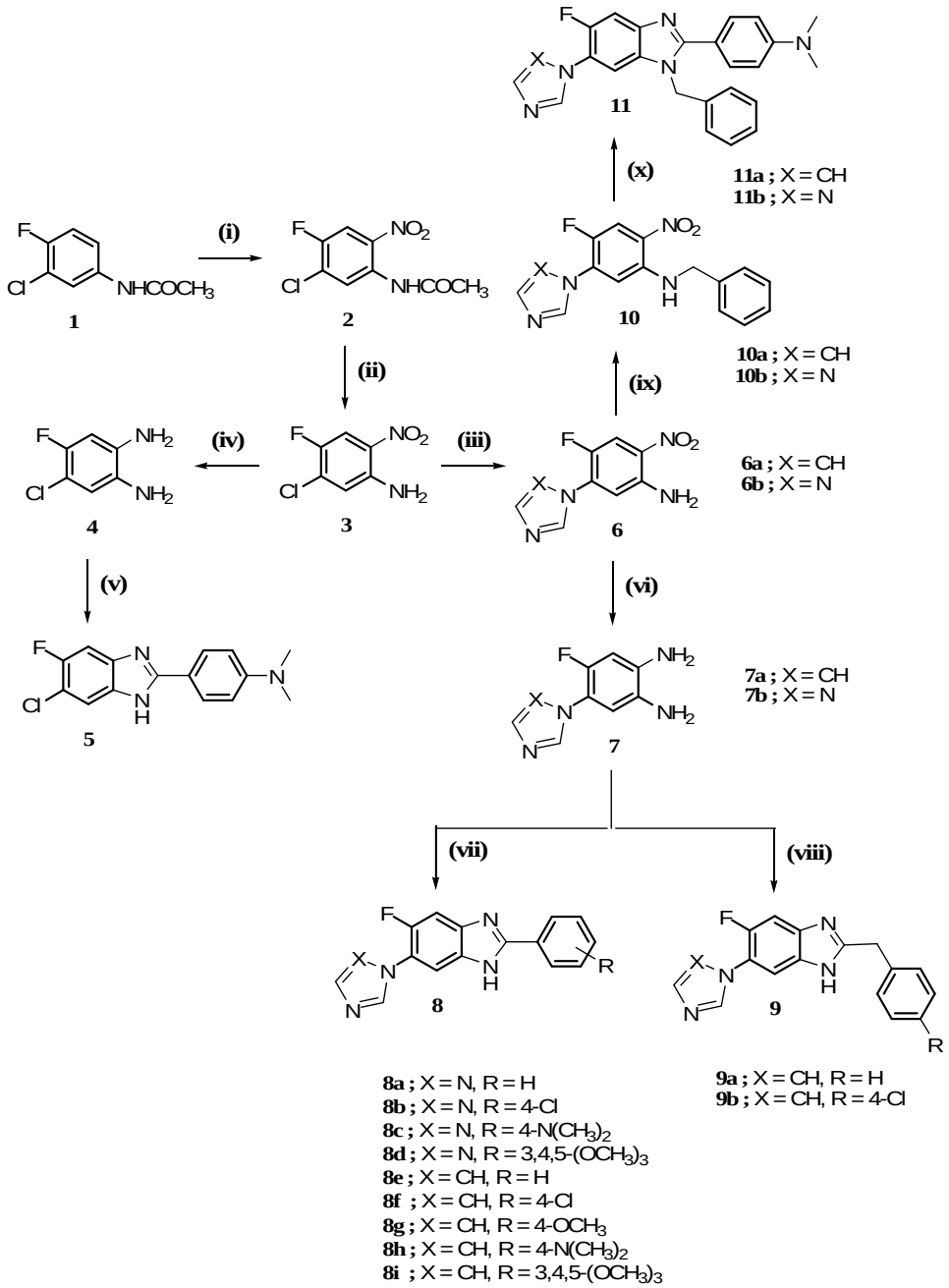
5-fluoro-6-(1H-imidazol-1-yl)-2-(3,4,5-trimethoxyphenyl)-1H-benzo[d]imidazole ***(8i)***
Brick red solid (85%); mp 256-258 °C; IR (KBr, cm^{-1}): 3440, 3120, 2925, 1591, 1494, 1467, 1275, 1238, 1125, 1055. ^{1}H-NMR (400 MHZ, DMSO-d_6): δ 3.73 (s, 3H, OCH_3), 3.89 (s, 6H, 2OCH_3), 7.12 (br.s, 1H, imidazole-H), 7.52 (s, 1H, ArH), 7.54 (s, 1H, ArH), 7.61-7.63 (d, 1H, *J* = 10.0 HZ, ArH), 7.72-7.75 (d, 1H, *J* = 11.2 HZ, ArH), 7.86-7.88 (d, 1H, *J* = 6.8 HZ, imidazole-H), 7.98-8.01 (d, 1H, *J* = 11.6 HZ, imidazole-H), 13.19 (s, 1H, NH). ^{13}C-NMR (100 MHZ, DMSO-d_6): δ 55.85, 60.08, 101.56, 101.79, 103.92, 122.01, 127.71, 128.81, 138.44, 145.19, 145.24, 149.32, 151.78, 152.98, 153.04. MS (ESI) m/z: 368.2 (M+1). Anal. Calcd for $C_{19}H_{17}FN_4O_3$: C, 61.95; H, 4.61; N, 15.21. Found: C, 61.72; H, 4.53; N, 15.15.

General experimental procedure for the synthesis of 2-benzyl-5-fluoro-6-(1H-imidazol-1-yl)-1H-benzo(d)imidazole ***(9a)***
To a stirred solution of 4-fluoro-5-(1H-imidazol-1-yl)benzene-1,2-diamine (**7a**) (10 mmol) in 35% HCl was added phenylacetic acid (11 mmol). The reaction mixture was refluxed with stirring under nitrogen atmosphere for 24 hours. Completion of the reaction was monitored by TLC. The contents were cooled to room temperature and neutralized with saturated solution of sodium bicarbonate. The solid separated was collected by filtration. The crude product was purified by recrystallization from ethanol.

White solid (76%); mp 197-199 °C; IR (KBr, cm^{-1}): 3425, 3118, 2921, 1645, 1550, 1478, 1152, 1045. ^{1}H-NMR (400 MHZ, DMSO-d_6): δ 4.52 (s, 2H, CH_2), 6.42-6.43 (d, 1H, *J* = 4.0 HZ, ArH), 7.27-7.29 (d, 2H, *J* = 8.0 HZ, ArH), 7.41(s, 2H, ArH), 7.54-7.57 (d, 2H, *J* = 12.0 HZ, ArH), 7.91-7.93 (d, 2H, *J* = 8.0 HZ, imidazole), 8.30 (s, 1H, imidazole), 13.30 (s, 1H, NH). ^{13}C-NMR (100 MHZ, DMSO-d_6): δ 45.50, 103.01, 103.31, 106.91, 107.16, 115.49, 122.50, 128.28, 130.48, 137.26, 139.81, 146.50, 153.40, 155.82. MS (ESI) m/z: 292.0 (M-1). Anal. Calcd for $C_{17}H_{13}FN_4$: C, 69.85; H, 4.48; N, 19.15. Found: C, 69.72; H, 4.44; N, 19.12.

2-(4-chlorobenzyl)-5-fluoro-6-(1H-imidazol-1-yl)-1H-benzo(d)imidazole ***(9b)***.
The compound **(9b)** was prepared as described for **(9a)**
Off-white solid (71%); mp 181-183 °C; IR (KBr, cm^{-1}): 3412, 3060, 3030, 2927, 1619, 1500, 1470, 1162, 1060. ^{1}H-NMR (400 MHZ, DMSO-d_6): δ 4.54 (s, 2H, CH_2), 7.26-7.37 (m, 3H, ArH), 7.50-7.51 (d, 2H, ArH), 7.93-7.94 (d, 1H, ArH), 7.99-8.01 (d, 1H, ArH), 8.14-8.18 (d, 1H, imidazole), 8.28 (s, 1H, imidazole), 13.51 (s, 1H, NH). ^{13}C-NMR (100 MHZ, DMSO-d_6): δ 45.73, 98.32, 98.56, 103.26, 110.12, 117.98, 121.22, 126.68, 128.68, 129.08, 129.70, 137.58, 139.08, 150.22, 156.12.MS (ESI) m/z: 326.1 (M+1). Anal. Calcd for $C_{17}H_{12}ClFN_4$: C, 62.49; H, 3.70; N, 17.13. Found: C, 62.40; H, 3.64; N, 17.05.

General experimental procedure for the synthesis of 4-(1-benzyl-5-fluoro-6-(1H-imidazol-1-yl)-1H-benzo(d)imidazol-2-yl)-N,N-dimethylbenzenamine ***(11a)***
To a cooled (0 °C) solution of **6a** (0.47 mmol) in DMF was added a suspension of sodium hydride (0.94 mmol) in dimethyl formamide and stirred for 30 min. To this reaction mixture benzyl bromide (0.48 mmol) was added and continued stirring to room temperature for 3-5 hours. After completion of the reaction as monitored by TLC, the reaction mixture was poured into ice cold water with stirring, followed by extraction with ethyl acetate. The organic layer was washed with brine and dried over anhydrous sodium sulphate, filtered and concentrated under vacuum to afford crude **10a** that was used for the next step without further purification. Compound **11a** was synthesized from the mono benzylated nitro compound **10a** by subjecting it to reduction and cyclocondensation with para dimethyl amino benzaldehyde as per the procedure mentioned for the synthesis of compound **5** from **3**.



Scheme 1. Reagents and conditions: (i) HNO_3/H_2SO_4, 0-5 °C, 7h; (ii) HCl/CH_3COOH, reflux, 5 h; (iii) Imidazole/1,2,4-triazole, DMF, K_2CO_3, stirring, 90 °C, 8h; (iv) Zn/HCl, C_2H_5OH, rt, 3h; (v) DMF, $Na_2S_2O_5$, para dimethyl amino benzaldehyde, stirring, 125 °C, 15h; (vi) Zn/HCl, C_2H_5OH, stirring, rt, 3h (vii) Substituded aldehydes, DMF, $Na_2S_2O_5$, stirring, 125 °C, 15h; (viii) Phenyl acetic acid/para chloro phenyl acetic acid, 35% HCl, stirring, reflux, 24h; (ix) Benzyl bromide, DMF, NaH, 0-5 °C to rt, stirring, 12h; (x) Zn/HCl, C_2H_5OH, rt, 3h and DMF, $Na_2S_2O_5$, para dimethyl amino benzaldehyde, stirring, 125 °C, 15h.

Grey solid (67%); mp 157-159 °C; IR (KBr, cm^{-1}): 3111, 3040, 2949, 1636, 1490, 1430, 1140, 1021. ^{1}H-NMR (400 MHZ, DMSO-d_6): δ 2.99 (s, 6H, $N(CH_3)_2$), 5.20 (s, 2H, CH_2), 6.25-6.27 (d, 2H, *J* = 8.0 HZ, ArH), 7.33 (br.s, 3H, ArH), 7.36-7.38 (d, 1H, *J* = 8.0 HZ, ArH), 7.56-7.63 (m, 3H, ArH), 7.87-7.89 (d, 1H, *J* = 8.0 HZ, ArH), 7.90-7.92 (d, 1H, *J* = 8.0 HZ, ArH), 7.96-7.99 (d, 1H, *J* = 12.0 HZ, ArH), 8.04-8.06 (d, 1H, *J* = 8.0 HZ, ArH), 8.20 (s, 1H,

imidazole). ^{13}C-NMR (100 MHZ, DMSO-d_6): δ 40.17, 47.75, 111.65, 115.42, 115.95, 116.02, 120.87, 120.93, 125.93, 126.00, 127.42, 127.50, 128.56, 128.61, 128.84, 129.80, 129.82, 131.54, 136.80, 151.16, 151.20. MS (ESI) m/z: 411.3 (M-1). Anal. Calcd for $C_{25}H_{22}FN_5$: C, 72.97; H, 5.38; N, 17.01. Found: C, 72.86; H, 5.32; N, 16.98.

4-(1-benzyl-5-fluoro-6-(1H-1,2,4-triazol-1-yl)-1H-benzo(d)imidazol-2-yl)-N,N-dimethylbenzenamine ***(11b).***
The compound **(11b)** was prepared from **6b** as described for **(11a)**
Grey solid (62%); mp 168-171 °C; IR (KBr, cm^{-1}): 3109, 3032, 2926, 1645, 1550, 1492, 1346, 1141, 1022. ^{1}H-NMR (DMSO-d_6): δ 3.10 (s, 6H, N(CH_3)$_2$), 5.30 (s, 2H, CH_2), 7.70-7.80 (d, 2H, ArH), 7.86 (s, 1H, ArH), 7.88-7.89 (d, 1H, *J* = 8.0 HZ, ArH), 7.99-8.10 (m, 3H, ArH), 8.11 (s, 1H, ArH), 8.16-8.21 (m, 3H, ArH), 8.26 (br.s, 1H, triazole), 8.93 (s, 1H, triazole). MS (ESI) m/z: 412.3 (M-1).Anal. Calcd for $C_{24}H_{21}FN_6$: C, 69.89; H, 5.13; N, 20.36. Found: C, 69.79; H, 5.02; N, 20.28.

RESULTS AND DISCUSSION

Chemistry

The synthetic pathway for the preparation of the title compounds **5,** (**8a-i),** (**9a-b**) and (**11a-b**) is shown in scheme 1. 5-chloro-4-fluoro-2-nitro aniline **3** was synthesized from the commercially available 3-chloro-4-fluoro aniline by nitration of the acetylated aniline **1** followed by acid hydrolysis [23]. Nucleophilic displacement of aryl chloride in 5-chloro-4-fluoro-2-nitro aniline **3** with imidazole and 1,2,4-triazole yielded nitro anilines **6a** and **6b.** We found that treatment of **3** and (**6a-b**) with zinc dust in the presence of HCl at r.t. effected a clean reduction of the nitro group to provide a good yield of the corresponding O-phenylene diamines **4** and (**7a-b**). Compounds **4** and (**7a-b**) are the key intermediates for the synthesis of target compounds **5,** (**8a-i),** (**9a-b**) and (**11a-b**). Among the available strategies for benzimidazole synthesis, condensations of ortho phenylene diamines with aldehydes under oxidative conditions or with carboxylic acids under acidic conditions are the generally adopted methods [24, 25]. For the synthesis of target compounds **5** and (**8a-i**) the corresponding diamines of **3** and (**6a-b**) was immediately cyclocondensed with aromatic aldehydes in DMF using $Na_2S_2O_5$ at 125 °C. The benzyl counterpart of azole substituted fluoro benzimidazoles (**9a-b**) were synthesized by refluxing the corresponding O-phenylene diamine **7a** with phenyl acetic acid / para chloro phenyl acetic acid in the presence of concentrated HCl according to the well known Phillips method [26]. The synthesis of Compounds (**11a-b**) were accomplished by benzylation of the imidazole substituted nitro anilines (**6a-b**) followed by reduction and cyclocondesation with para dimethyl amino benzaldehyde.

Structures of nitro anilines **3,** (**6a-b**) and the synthesized compounds (**8a-i),** (**9a-b**) and (**11a-b**) were confirmed by IR, ^{1}H NMR, ^{13}C NMR and Mass spectra. The purity of the title compounds was ascertained by elemental analysis. IR spectra of compounds (**8a-i**) and (**9a-b**) showed a broad band at 3441-3373 cm^{-1} (NH stretching) while their ^{1}H NMR spectra showed singlets between δ 12.68-13.51 ppm corresponding to NH proton of the benzimidazole ring which confirmed the cyclized structure and absence of same for the N-benzylated compounds (**11a-b**). The singlets at 4.52 and 4.54 ppm was typical in the ^{1}H NMR spectra of the compounds (**9a-b**) accounting for the benzylic CH_2 group and their IR spectras showed the absorption signals between 2920 and 2927 cm^{-1} (CH_2 stretching). The ^{13}C-NMR results showed that the compounds with methoxy and dimethyl amine groups presented the expected signals at δ 55.68-60.60 ppm and 39-41 ppm, while methylene signal for the compounds (**9a-b**) and (**11a-b**) appeared at δ 45.50-47.75 ppm. The carbons of imidazole and triazole rings were visible at δ 122.01-124.50 ppm and 135.86-138.44 ppm, while the aromatic carbons were observed at their usual chemical shifts.

Antimycobacterial activity

The advent of visual MABA method has facilitated the facile screening of compounds for antituberculosis activity making use of a thermally stable and nontoxic reagent. In comparison with the BACTEC and fluorometric MABA methods, visual MABA is an inexpensive alternative, providing nearly identical and rapid results without the use of specialized equipment. In addition to the aforementioned merits, visual Microplate Alamar Blue Assay (MABA) was adopted for the screening of test compounds against *M. tuberculosis* H37Rv in view of the high correlation between the MICs determined by BACTEC, fluorometric MABA and visual MABA methods [27]. The minimum inhibitory concentration (MIC, µg/mL) was defined as the lowest drug concentration that prevented a colour change from blue (no growth) to pink (growth). Isoniazid was used as positive control.

Three different series of compounds (**8a-i),** (**9a-b**) and (**11a-b**) were synthesized and screened against *M. tuberculosis* H37Rv by MABA method. The azole substituted fluorobenzimidazoles displayed antitubercular activity with MIC ranging from 12.5 to >100 µg/mL and the results of antitubercular activity are repoted in Table 1.

Table 1. Antitubercular activities of compounds 8(a-i), 9(a,b) and 11(a.b) against *M.tuberculosis* H37Rv

Compound[a]	MIC[b] (µg/ml) MABA[c]
8a	50
8b	50
8c	**25**
8d	>100
8e	50
8f	100
8g	>100
8h	**25**
8i	>100
9a	**25**
9b	**25**
11a	**12.5**
11b	**25**
Isoniazid	0.65

[a] *Detailed structures are shown in scheme 1*
[b] *Minimum inhibitory concentration in µg/ml*
[c] *Microplate Alamar Blue Assay (visual)*

Table 2. Antifungal activity of compounds 8(a-i), 9(a,b) and 11(a,b) against *Candida* Species

GIZ[b], MIC[c] and MFC[d] of compounds and Std drugs against fungal cultures

	C.albicans			*C.glabrata*			*C.krusei*			*C.tropicalis*		
Compd[a]	GIZ	MIC	MFC	GIZ	MIC	MFC	GIZ	MIC	MFC	GIZ	MIC	MFC
8a	**11**	**50**	**50**	**12**	**50**	**50**	**11**	**50**	**100**	**10**	**100**	**100**
8b	9	100	100	9	100	>100	8	100	>100	8	>100	>100
8c	**13**	**25**	**50**	**14**	**25**	**50**	**13**	**25**	**50**	**13**	**25**	**50**
8d	6	>100	>100	6	>100	>100	7	100	>100	6	>100	>100
8e	**11**	**50**	**50**	**11**	**50**	**50**	**12**	**25**	**50**	**12**	**25**	**50**
8f	10	50	100	10	50	100	9	100	100	10	100	100
8g	5	>100	>100	6	>100	>100	5	>100	>100	6	>100	>100
8h	**13**	**25**	**25**	**15**	**12.5**	**25**	**14**	**25**	**25**	**15**	**12.5**	**25**
8i	7	100	>100	8	100	100	10	50	100	8	100	100
9a	**15**	**25**	**25**	**15**	**25**	**50**	**16**	**12.5**	**25**	**14**	**25**	**50**
9b	**16**	**12.5**	**12.5**	**16**	**12.5**	**25**	**17**	**25**	**25**	**16**	**25**	**25**
11a	**17**	**6.25**	**12.5**	**18**	**6.25**	**12.5**	**18**	**12.5**	**12.5**	**17**	**12.5**	**12.5**
11b	**16**	**12.5**	**12.5**	**17**	**12.5**	**12.5**	**18**	**12.5**	**25**	**16**	**12.5**	**25**
DMSO	-	-	-	-	-	-	-	-	-	-	-	-
Clo[e]	23	3.12	6.25	23	6.25	6.25	22	3.12	6.25	21	3.12	12.5
Flu[f]	20	3.12	3.12	21	3.12	6.25	18	6.25	12.5	19	3.12	6.25

(a) Detailed structures are shown in scheme 1; (b) GIZ, growth inhibition zone (mm); (c) MIC, minimum inhibitory concentration (µg/ml); (d) MFC, minimum fungicidal concentration (µg/ml); (e) Clo, Clotrimazole; (f) Flu, Fluconazole.

The compound **5** without the azole moiety at 6th position was completely devoid of anti-tubercular activity and was synthesized to see its effect on activity profile. Among the compounds having 4-chloro, 4-dimethyl amino and methoxy substituent in aromatic ring, compounds with dimethyl amino subtituent **8c** and **8h** were more active with MIC of 25 µg/mL. Compounds with 4-chloro substituents (**8b, 8f**) were more active than compounds with methoxy substituent (**8d, 8g, 8i**), while the derivatives **8a** and **8e** without substituent in the aromatic ring showed moderate activity. Replacement of phenyl ring at 2nd position with benzyl group (**9a-b**) did offer a better result and comparable antituberculosis activity with compounds bearing a benzyl group at 1st position (**11a-b**). The imidazole substituted N-1 benzyl analog **11a** emerged as the most active compound with a MIC value of 12.5 µg/mL. Further studies with modifications of the azole ring at 6th position of the benzimidazole ring are in progress. Thus, at this stage all the modifications indicated that the 4-dimethyl amino group in the phenyl ring at 2nd position and also a benzyl group at 1st or 2nd position play a significant role in the antitubercular activity with a dominating influence of the azole moiety.

Antifungal activity

The antifungal activities of compounds (**8a-i**), (**9a-b**) and (**11a-b**) were evaluated by *in vitro* agar diffusion and broth dilution assay and the results of which are summarized in Table 2. The tested fungal strains of *Candida*

albicans, *Candida krusei*, *Candida glabrata* and *Candida tropicalis* were provided by National Centre for Industrial Microorganisms (NCIM) pune, India. Fluconazole and clotrimazole served as the standard drug controls. Initial screening of all the new azole substituted fluoro benzimidazoles against fungal cultures showed that the compounds (**8b, 8d**) and (**8g, 8i**) were only poorly active against fungi with growth inhibition zone ≤9mm when tested at 1000 µg/mL by agar diffusion method [28]. However, compounds (**9a-b**) and (**11a-b**) showed good activity against *Candida* species with growth inhibition zone ≥14mm. The minimum inhibitory concentrations (MICs) were determined for the compounds (**8a-i**), (**9a-b**) and (**11a-b**) against *Candida* species according to standard micro-broth dilution method in 96-well microtest plates as per NCCLS protocol [29].

In terms of structure-activity relationships (SARs), the substitution pattern of azole ring was explored using imidazole and 1,2,4-triazole in the benzimidazole skeleton. The type of azole ring linked to the 6th position of the benzimidazole ring seemed to have different influence on the antifungal activity against various fungi strains. The fluoro chloro benzimidazole counterpart **(5)** without the azole moiety at 6th position was found to be poorly active with negligible antifungal activity (not reported). Compounds (**8a-i**) having different substituents on the phenyl ring at 2nd position demonstrated good to moderate antifungal activity against different *Candida* species with a MIC value of 12.5 to 100 µg/mL. The *in vitro* antifungal activity data revealed that the presence of electron donating dimethyl amino group at 4th position of the phenyl ring enhances the activity against all strains. Among the benzyl derivatives, improvement in the antifungal activity was observed in compound **9b** having a chloro group at C-4 position against *Candida albicans* and *Candida glabrata* than the unsubstituted benzyl analog **9a**. The N-1 benzyl analogues (**11a-b**) were synthesized to expand our understanding of the structure-activity relationship among the benzimidazole derivatives. The imidazole substituted N-benzyl analog **11a** exhibited better profile of antifungal activity than the triazole analog **11b** against all tested *Candida* species, confirming the effectiveness of N-1 benzyl group and the imidazole substituent with the influence of dimethylamino group at para position of the phenyl ring. Using these as lead compounds, it is plausible that other modifications could uncover agents which are more active against these *Candida* species. Furthermore, minimum fungicidal concentrations (MFCs) of all the synthesized compounds were determined.

Biological evaluation

All the newly synthesized compounds were screened for their *in vitro* antitubercular activity against *M. tuberculosis* H37Rv strain and *in vitro* antifungal activity against *Candida albicans*, *Candida krusei*, *Candida glabrata* and *Candida tropicalis* as representatives of fungi.

***In vitro* evaluation of the antituberculosis activity**

The synthesized compounds were tested for their in vitro anti-mycobacterial activities against *M. tuberculosis* H37Rv using a broth microdilution assay, the Microplate Alamar Blue Assay (MABA) according to the reported method [30]. To prevent dehydration in experimental 96 well plates, the outer perimeter wells were filled with two hundred microliters of sterile deionised water. The test compounds dissolved in dimethyl sulfoxide (DMSO) were first diluted to the highest concentration (800 µg/mL) and these stock solutions were further diluted with appropriate volumes of Middlebrook 7H9 broth to yield final concentration of 1.562 to 800 µg/mL followed by addition of 100µL of *M. tuberculosis* inoculums to the plates (*M. tuberculosis* H37Rv in Middlebrook 7H9 supplemented with 0.05% Tween 80 OADC and the turbidity of the resultant suspension matched to a McFarland No.1). The wells in column 11 served as inoculums-only control and the solvent (DMSO) was included in every experiment as a negative control. The plates were sealed with parafilm and incubated at 37 °C for five days. After the incubation period, 25 µL of a freshly prepared 1:1 mixture of 10×Alamar Blue reagent and 10% Tween 80 was added to the plate and reincubated at 37 °C and results were recorded at 24h post-reagent addition. Visual MICs were defined as the lowest concentration of test compound or drug that prevented a colour change.

***In vitro* evaluation of the antifungal activity**

Determination of the minimum inhibitory concentration

Cultures on receipt were sub cultured in SDA plates and further stored in slants as stock cultures. For the experiments, stock culture was incubated at 35 °C for 24 h. The stock culture was adjusted to 0.5 McFarland standard turbidity and used for assay. Tests were performed in RPMI 1640 medium (Sigma-Aldrich) buffered to PH 7.0 with 0.165M 3-(N-morpholino)-propanesulphonic acid (MOPS, Sigma-Aldrich). The final concentrations of the test compounds ranged from 1.0 to 512µg/mL. In this assay, the minimum concentration of each test substance required to inhibit the growth of fungi was determined. For this assay, the compounds to be tested were dissolved in DMSO serially diluted in growth medium, inoculated with 100 µL of individual fungal inoculums (1x10^6 CFU per

mL) to each well of the micro titer plate and the sealed microplates were incubated at 35 °C for 48 h in a humid atmosphere. Solvent control (DMSO) and sterility controls were maintained throughout the experiment. The microdilution plates were inspected visually to determine the growth of the organism as indicated by turbidity (In fact, turbidity of the culture medium is indicative of the presence of a large number of cells). The wells in which the drug or test compound is present in concentration sufficient to inhibit fungal growth remain clear. In experimental terms the MIC is the concentration of the drug or test compound present in the well, i.e. in the well having the lowest concentration in which growth is not observed.

Determination of the minimum fungicidal concentration

The minimum fungicidal concentrations (MFCs) were determined according to a standard procedure as previously described [31]. Following an overnight incubation for the MIC determination, 100 µL was taken from each well showing no visible growth and further subcultured onto fresh sabouraud dextrose agar plates. The plates were incubated at 35 °C for 48 hours and then checked for viability. The concentration at which no growth or fewer than three colonies were obtained to give approximately 99 to 99.5% killing activity was considered to be the MFC.

CONCLUSION

In this study we report the design, synthesis, characterization and *in vitro* evaluation of azole substituted fluoro benzimidazoles for antitubercular and antifungal activities. The target compounds were obtained from chlorine, imidazole and 1,2,4-triazole bearing fluoro orthophenylene diamine by condensation with aromatic aldehydes and carboxylic acids. The synthesized compounds exhibited a high antimycobacterial activity associated with a remarkable antifungal activity. Both imidazole and 1,2,4-triazole substituted fluoro benzimidazoles with 2-benzyl and 4-N$(CH_3)_2$ substituted 2-phenyl/2-phenyl-1-benzyl counterpart were found to be the most active of all the compounds against H37Rv strain and *Candida* species. The N-benzylation was found to improve the antifungal potential against *Candida albicans* and *Candida glabrata*. However, in contrast compounds with benzyl group at 2nd position of the benzimidazole ring exhibited similar antitubercular activity. Among the N-benzylated analogs, imidazole substituted bezimidazole dervative **11a** emerged as the most active antitubercular compound and was endowed with selective antitubercular and antifungal potential. The SAR studies indicate that the azole (imidazole and 1,2,4-triazole) ring, the fluoro benzimidazole scaffold are important pharmacophores for antitubercular and antifungal activity. The present findings provide new opportunity for the development of novel antimicrobials to overcome the ever increasing problem of drug resistance and a prototype lead for further optimization and development.

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REFERENCES

[1] B. R. Bloom, C.J.L. Murray, *Science* **1992**, 257, 1055-1064.
[2] H. Tomioka, *Curr. Pharm. Des.* **2006**, 12, 4047-4070.
[3] V. Bhowruth, L. G. Dover, G. S. Besra, *Prog. Med. Chem.* **2007**, 45, 169-203.
[4] M. Laurenzi, A. Ginsberg, M. Spigelman, *Infect. Disord. Drug. Targets*. **2007**, 7, 105-119.
[5] P. Ertl, S. Jelfs, J. Muhlbacher, A. Schuffenhauer, P. Selzer, *J. Med. Chem*. **2006**, 49, 4568-4573.
[6] A. M. Soliman, S. K. Mohamed, M. A. A. EI-Remaily, H. Abdel-Ghany, *Eur. J. Med. Chem*. **2012**, 47, 138-142.
[7] H. Goker, C. Kus, D. W. Boykin, S. yildiz, N. Altanlar, *Bioorg. Med. Chem*. **2002**, 10, 2589-2596.
[8] T. H. Grossman, N. Mani, C. H. Gross, J. D. Parsons, B. Hanzelka, U. Muh, S. Mullin, Y. Liao, A. L. Grillot, D. Stamos, P. S. Charifson, *Antimicrob. Agents. Chemother*. **2006**, 50, 1228-1237.
[9] I. Ojima, K. Kumar, D. Awasthi, S. Lee, I. Zanardi, B. Ruzsicska, S. Knudson, P. J. Tonge, R. A. Slayden, *J. Med. Chem*. **2011**, 54, 374-381.
[10] C. H. Gill, G. R. Jadhav, M. U. Shaikh, R. P. Kale, M. R. Shiradkar, *Eur. J. Med. Chem*. **2009**, 44, 2930-2935.
[11] C. Gill, G. Jadhav, M. Shaikh, R. Kale, A. Ghawalkar, D. Nagargoje, M. Shiradkar, *Bio. Org. Med. Chem. Lett*. **2008**, 18, 6244-6247.
[12] M. Botta, D. Castagnolo, M. Radi, F. dessi, F. Manetti, M. Saddi, R. Meleddu, A. D. Logu, *Bioorg. Med. Chem. Lett*. **2009**, 19, 2203-2205.

[13] M. G. Mamolo, D. Zampieri, E. Laurini, G. Scialino, E. Banfi, L. Vio, *Bioorg. Med. Chem.* **2008**, 16, 4516-4522.
[14] G. Menozzi, L. Merello, P. Fossa, S. Schenone, A. Ranise, L. Mosti, F. Bondavalli, R. Loddo, C. Murgioni, V. Mascia, P. L. Colla, E. Tamburini, *Bioorg. Med. Chem.* **2004**, 12, 5465-5483.
[15] E. Banfi, G. Scialino, D. Zampieri, M. G. Mamolo, L.Vio, M. Ferrone, M. Fermeglia, M. S. Paneni, S. Pricl, *J. Antimicrob. Chemother*. **2006**, 58, 76-84.
[16] J. Chattopadhyaya, R. S. Upadhayaya, G. M. Kulkarni, N. R. Vasireddy, J. K. Vandavasi, S. S. Dixit, V. Sharma, *Bioorg. Med. Chem.* **2009**, 17, 4681-4692.
[17] A. J. Lenaerts, V. Gruppo, K. S. Marietta, C. M. Johnson, D. K. Driscoll, N. M. Tompkins, J. D. Rose, R. C. Reynolds, I. M. Orme, *Antimicrob. Agents. Chemother*. **2005**, 49, 2294-2301.
[18] J. Chattopadhyaya, R. S. Upadhayaya, S. V. Lahore, A. Y. Sayyed, S. S. Dixit, P. D. Shinde, *Org. Biomol. Chem*. **2010**, 8, 2180-2197.
[19] J. Chattopadhyaya, R. S. Upadhayaya, P. D. Shinde, A. Y. Sayyed, S. A. Kadam, A. N. Bawane, A. Poddar, O. Plashkevych, A. Foldesi, *Org. Biomol. Chem*. **2010**, 8, 5561-5673.
[20] P. R. Duchowicz, M. G. Vitale, E. A. Castro, M. Fernandez, J. Caballero, *Bioorg. Med. Chem.* **2007**, 15, 2680-2689.
[21] K. Muller, C. Fach, F. Diederich, *Science* **2007**, 317, 1881-1886.
[22] R. Filler, R. Saha, *Future. Med. Chem.* **2009**, 1, 777-791.
[23] M. El-Abadelah, M.Z. Nazer, N.S. El-Abadia, H. Meier, *Heterocycles* **1995**, 41, 2203-2219.
[24] Y. Jiang, Y. Chen, H. Liu, X. Chen, X. Luan, C. Zhang, C. Gao, C. Tan, Y. Li, *Bioorg. Med. Chem*. **2011**, 19, 4529-4535.
[25] O. Algul, A. Kaessler, Y. Apcin, A. Yilmaz, J. Jose, *Molecules* **2008**, 13, 736-748.
[26] M. A. Phillips, *J. Chem. Soc.* **1928**, 2393-2399.
[27] S. G. Franzblau, L. Collins, *Antimicrob. Agents.* Chemother, **1997**, 41, 1004-1009.
[28] S. K. Arora, R.S. Upadhayaya, S. Jain, N. Sinha, N. Kishore, R. Chandra, *Eur. J. Med. Chem*. **2004**, 39, 579-592.
[29] National Committee for Clinical Laboratory Standards (NCCLS), Reference method for broth dilution antifungal susceptibility testing of yeast, approved standard, M27-A, Wayne, PA **2002**.
[30] S. G. Franzblau, R. S. Witzig, J. C. Mclaughlin, P. Torres, G. Madico, A. Hernandez, M. T. Degnan, M. B. Cook, V. K. Quenzer, R. M. Ferguson, R. H. Gilman, *J. Clin. Microbiol*. **1998**, 36, 362-366.
[31] A. Espinel-Ingroff, A. Fothergill, J. Peter, M. G. Rinaldi, T. J. Walsh, *J. Clin. Microbiol*. **2002**, 40, 3204-3208.