Design and synthesis of imidazolylmethyl substituted fluorobenzimidazoles for antitubercular and antifungal activity

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ABSTRACT

A series of novel 2-((1H-imidazol-1-yl)methyl)-5-fluoro-6-(substituted/unsubstituted)-1H-benzo[d]imidazoles were prepared by condensation of the dihydrochloride salts of the appropriate 1,2-phenylene diamines with 1-(cyano methyl) imidazole and screened for antitubercular activity against $H_{37}R_V$ strain and antifungal activity against Candida species. To examine the influence of imidazole and phenyl ether moiety on the activity chlorine substituted fluoro benzimidazole (8a) was synthesized. The newly synthesized compounds were characterized by I.R, ^{1}H-NMR, ^{13}C-NMR, Mass and elemental analysis. The most active derivatives of the present series were the imidazole substituted fluoro benzimidazole and the para chloro phenyl ether analog indicating the importance of a halogenated phenyl ether nucleus and the azole moiety at the 6th position. The results suggest that these two molecules are potential candidates for further development as antitubercular and antifungal agents.

Key words: Fluoro benzimidazole, Antitubercular activity, Antifungal activity, *Candida* species, H37Rv strain.

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INTRODUCTION

Despite advances in chemotherapy, the dramatically rising prevalence of multi-drug resistant life threatening mycobacterial and systemic fungal infection like Candidiasis poses a major health concern to world population [1-5]. Prolonged treatment regimen associated with significant toxicity and emergence of multi-drug resistant mycobacteria and fungi are some of the major limitations associated with the therapeutic agents currently employed for the treatment of tuberculosis and fungal infections. This has led to an ongoing need for new chemical moieties acting on novel targets with dual effects (benefits). As a consequence, there is a great need for novel compounds with both antitubercular and antifungal activities.

Benzimidazole ring system, which is a core structure in various synthetic pharmaceuticals, displays a broad spectrum of biological activity, including antimycobacterial and antifungal properties [6-14]. Clotrimazole, fluconazole and ketoconazole are some of the commercially available molecules containing aromatic scaffolds like imidazole and triazole with high antifungal potential. It is established that these compounds are potent inhibitors of ergosterol biosynthesis in *Candida albicans* by interacting with cytochrome p-450 dependent 14α-sterol demethylase, an important enzyme in ergosterol biosynthesis in fungi [15]. The enzyme p-450$_{DM}$ plays a vital role in the oxidative transformation of lanosterol to ergosterol and inhibition of this enzyme leads to accumulation of 14α-methylated sterols and thereby disrupting the membrane structure and function resulting in inhibition of growth [16].

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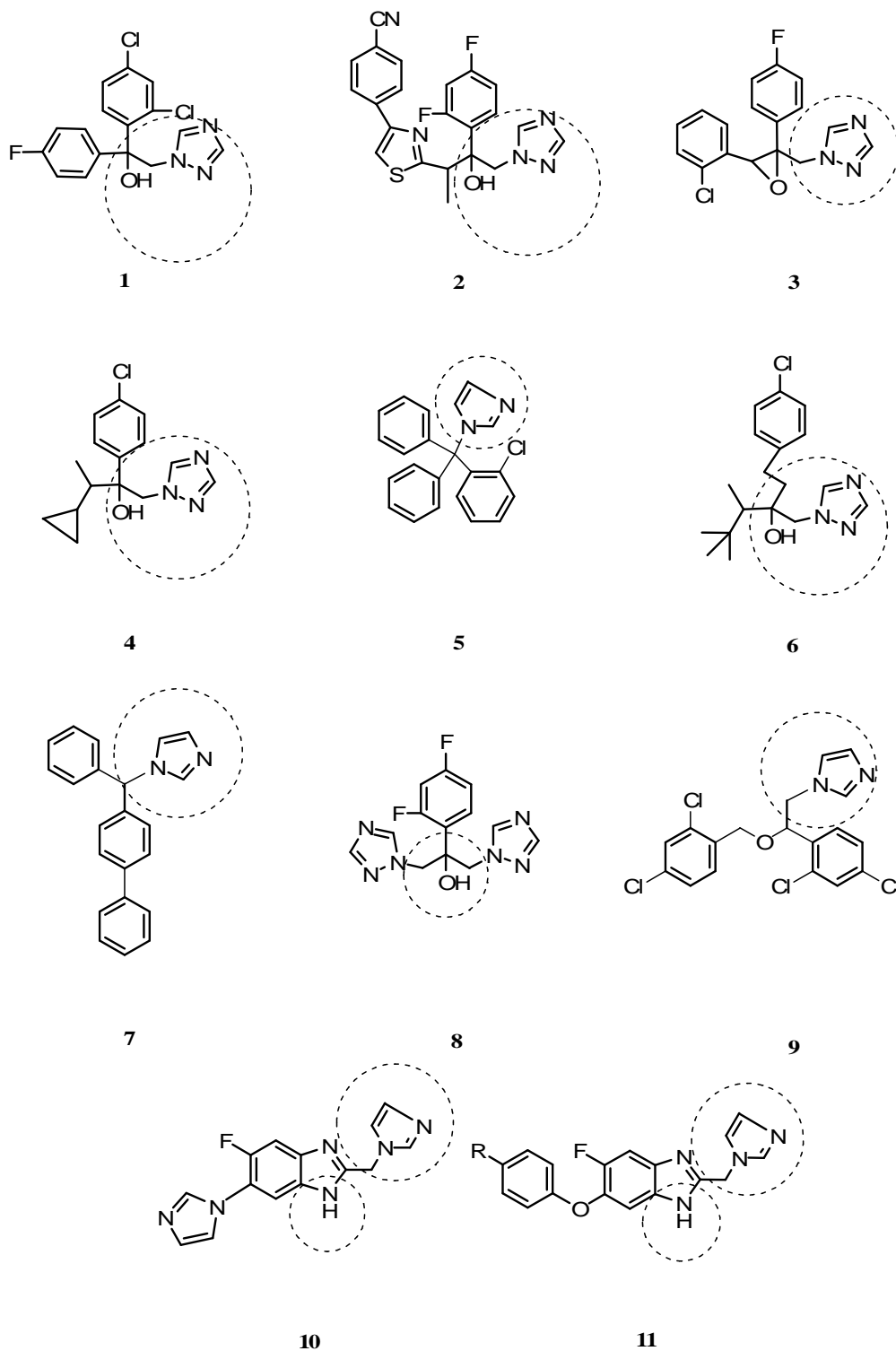
1 = ICI 153066; **2** = Ravuconazole; **3** = Epoxyconazole; **4** = Cyproconazole; **5** = Clotrimazole;
6 = Tebuconazole; **7** = Bifonazole; **8** = Fluconazole; **9** = Miconazole

Fig. 1: Representative antifungal agents **(1-9)** and target compounds **(10,11)**

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Furthermore, it was experimentally proved that several imidazole and triazole based antifungals were effective antitubercular agents and demonstrated the possibility that *Mycobacterium tuberculosis* (Mtb) cytochrome p-450 could be a novel Mtb drug target [17-19]. Numerous reports on compounds with imidazole, triazole and tetrazole moiety have appeared which are superior to or at least as active as the currently used class of antifungal or antibacterial agents [20-24]. The development of agents of this type was based on their mode of action that led to their desired bioactivity potential. As a part of our search for new antitubercular and antifungal agents, we have been exploring benzimidazole nucleus as potential agents for the treatment of mycobacterial and fungal infections. Recently we described the synthesis and evaluation of some new imidazole and triazole substituted fluoro benzimidazoles for antitubercular and antifungal activity against H37Rv strain and *candida* species, which afforded promising results [25, 26]. Our earlier work on the benzimidazole series identified azole and phenoxy substituent to be an important element of the pharmacophore. While retaining the desired substituents, we choose to examine substitution at the 2nd postion with imidazolylmethyl group, which is a common substructure seen in many potential azole antifungals. Based on the study of antifungal azoles bearing the imidazole and triazole moiety as part of the structure for antimycobacterial and antifungal activity has led to the design of imidazolylmethyl based substituted fluoro benzimidazole derivatives as depicted in figure 1. All the newly synthesized compounds were evaluated for their *in-vitro* antitubercular activity against the pathogenic *Mycobacterium tuberculosis* (Mtb) H37Rv strain and antifungal activity against *Candida albicans*, *Candida krusei*, *Candida glabrata* and *Candida tropicalis* as representatives of fungi.

EXPERIMENTAL SECTION

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$_4$Si (δ=0) in DMSO-d$_6$ and the J values in hertz (HZ). Signal multiplicities are represented by s (singlet), d (doublet), t (triplet), 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 (4a): 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 (4b-4e): The appropriate phenols or imidazole (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 h. When TLC revealed the absence of starting material, the reaction mixture was cooled to room temperature and poured into water (100 mL). The resulting 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.

5-(4-chlorophenoxy)-4-fluoro-nitrobenzenamine (4c): Yield (87%); mp 169-172 °C; IR (KBr) cm^{-1} : 3462, 3340 (Ar NH$_2$ str), 3072 (Ar CH str), 1600 (C=C ring str), 1518 (Ar NO$_2$ str), 1260 (Ar C-N str), 1230 (Ar C-O-C str), 1161 (C-F str), 1014 (C-Cl str). ^{1}H-NMR (400 MHZ, DMSO-d$_6$): δ 6.42-6.43 (d, 1H, J = 4.0 HZ, ArH), 7.27-7.29 (d, 2H, J = 8.0 HZ, ArH), 7.41 (br.s, 2H, NH$_2$), 7.54-7.57 (d, 2H, J = 12.0 HZ, ArH), 7.91-7.93 (d, 1H, J = 8.0 HZ, ArH). MS (ESI) m/z: 282.3 (M+1).

4-fluoro-5-(1H-imidazol-1-yl)-2-nitrobenzenamine (4e): Yield (89%); mp 188-189 °C; IR (KBr) cm^{-1} : 3473,3275 (Ar NH$_2$ str), 3146,3126 (Ar CH str), 1647 (C=N str), 1581 (Ar C=C ring str), 1527,1514,1500 (Asymmetric Ar NO$_2$ str),1383 (Ar 3$^°$ amine C-N str), 1301,1280,1257 (Ar NH$_2$ C-N str), 1211 (Ar C-F str),877 (Ar NO$_2$ C-N str). ^{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,

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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).

General procedure for the synthesis of 4-chloro-5-fluoro-1,2-phenylenediamine (5a) and 4-(substituted)-5-fluoro-benzene-1,2-phenylenediamine (5b-5e): To a stirred solution of compound 2(a-j) (10 mmol) in ethyl alcohol containing zinc dust (100 mmol) was slowly injected concentrated HCl (10 mL) via septum using glass syringe over a period of 2 h and continued stirring at room temperature under nitrogen atmosphere for another additional 2 h. 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$_2$SO$_4$ and evaporated. The crude solid was used for the preparation of the dihydrochloride salt without further purification.

General procedure for the synthesis of dihydrochloride salt of 4-chloro-5-fluoro-1,2-phenylenediamine (6a) and 4-(substituted)-5-fluoro-benzene-1,2-phenylenediamine (6b-6e): A solution of 4-chloro-5-fluoro-1,2-phenylenediamine (5a) (10 mmol) and 4-(substituted)-5-fluoro-benzene-1,2-phenylenediamine (5b-5e) (10 mmol) in absolute alcohol (25 ml) was treated with dry HCl in ether and stirred at 0-5 °C for 2 h. The solvent was evaporated under reduced pressure to afford the dihydrochloride salt of the corresponding 1,2-phenylenediamine. The dihydrochloride salt was stored at 0-5 °C in air tight container when not in use.

General procedure for the synthesis of imidazolylmethyl substituted fluoro benzimidazoles (8a-8e): An equimolar quantities of dihydrochloride salt of the corresponding 1,2-phenylenediamine (10 mmol) and cyanomethyl imidazole (10 mmol) was taken in a sealed tube and heated at 175-190 °C for 1h. The resultant mixture was refluxed with 2N HCl for 15 min and later neutralized with ammonia solution. After filtration, the precipitate obtained was purified by recrystallization from isopropyl alcohol. Reactions were monitored by TLC (10% Methanol: Dichloromethane) using precoated silica gel plates (Merck F $_{254}$) and the spots were visualized by exposing them to iodine vapour or Uv light was used for detection at λ 254.

2-((1H-imidazol-1-yl)methyl)-6-chloro-5-fluoro-1H-benzo[d]imidazole (8a): Yield (52%); mp 89-91 °C; IR (KBr) cm^{-1}: 3421 (Ar NH str), 3085 (Ar CH str), 2915 (CH$_2$ str), 1654 (C=N str), 1610, 1500, 1473,1444 (C=C ring str), 1360 (Ar C-N str), 1024 (C-Cl str), 1126 (C-F str). ^{1}H-NMR (400 MHZ, DMSO-d$_6$): δ 4.23 (s, 2H, CH$_2$), 7.17-7.19 (d, 1H, J = 8.0 HZ, ArH), 7.25-7.27 (d, 1H, J = 8.0 HZ, ArH), 8.16-8.18 (d, 2H, J = 8.0 HZ, imidazole), 8.56 (s, 1H, imidazole), 12.76 (s, 1H, NH). ^{13}C-NMR (100 MHZ, DMSO-d$_6$): δ 45.53 (CH$_2$), 115.59, 117.40, 123.40, 128.48, 130.58, 136.38, 141.28, 143.88, 153.44, 161.91 (Ar-C). MS (ESI) m/z: 250.1 (M+1). Anal. Calcd for C$_{11}$H$_8$ClFN$_4$: C, 52.70; H, 3.21; N, 22.35. Found: C, 52.49; H, 3.13; N, 22.29.

2-((1H-imidazol-1-yl)methyl)-5-fluoro-6-phenoxy-1H-benzo[d]imidazole (8b): Yield (49%); mp 110-112 °C; IR (KBr) cm^{-1}: 3460 (Ar NH str), 3095 (Ar CH str), 2905 (CH$_2$ str), 1600 (C=N str), 1491, 1467, 1446 (C=C ring str), 1263 (Ar C-O-C str), 1110 (C-F str). ^{1}H-NMR (400 MHZ, DMSO-d$_6$): δ 4.36 (s, 2H, CH$_2$), 7.22-7.25 (d, 1H, J = 12.0 HZ, ArH), 7.33-7.37 (d, 1H, ArH), 7.47-7.50 (d, 1H, ArH), 7.53-7.56 (d, 1H, J = 12.0 HZ, ArH), 7.60-7.63 (d, 1H, J = 12.0 HZ, ArH), 7.65-7.75 (m, 4H, ArH), 8.11 (s, 1H, imidazole), 13.11 (s, 1H, NH).. MS (ESI) m/z: 308.0 (M-1). Anal. Calcd for C$_{17}$H$_{13}$OFN$_4$: C, 66.22; H, 4.25; N, 18.17. Found: C, 66.01; H, 4.16; N, 18.11.

2-((1H-imidazol-1-yl)methyl)-6-(4-chlorophenoxy)-5-fluoro-1H-benzo[d]imidazole (8c): Yield (59%); mp 121-123 °C; IR (KBr) cm^{-1}: 3441 (Ar NH str), 3120 (Ar CH str), 2925 (CH$_2$ str), 1654 (C=N str), 1600, 1560 (C=C ring str), 1269 (Ar C-O-C str), 1070 (C-Cl), 1120 (C-F str). ^{1}H-NMR (400 MHZ, DMSO-d$_6$): δ 4.30 (s, 2H, CH$_2$), 7.20-7.23 (d, 2H, J = 12.0 HZ, ArH), 7.25-7.28 (d, 2H, J = 12.0 HZ, ArH), 7.42-7.45 (d, 2H, J = 12.0 HZ, ArH), 7.67-7.70 (d, 2H, J = 12.0 HZ, ArH), 8.00 (s, 1H, imidazole), 12.70 (s, 1H, NH). ^{13}C-NMR (100 MHZ, DMSO-d$_6$): δ 45.70 (CH$_2$), 104.01, 104.31, 107.91, 108.16, 116.49, 122.80, 128.50, 128.70, 136.92, 137.18, 150.50, 155.70, 157.87 (Ar-C). MS (ESI) m/z: 342.1 (M-1). Anal. Calcd for C$_{17}$H$_{12}$OClFN$_4$: C, 59.57; H, 3.52; N, 16.34. Found: C, 59.45; H, 3.41; N, 16.29.

2-((1H-imidazol-1-yl)methyl)-5-fluoro-6-(p-tolyloxy)-1H-benzo[d]imidazole (8d): Yield (55%); mp 97-99 °C; IR (KBr) cm^{-1}: 3430 (Ar NH str), 3010 (Ar CH str), 2922 (CH$_2$ str), 2890 (Aliphatic CH str), 1622 (C=N str), 1506,1476,1446 (C=C ring str), 1228,1203 (Ar C-O-C str), 1110 (C-F str). ^{1}H-NMR (400 MHZ, DMSO-d$_6$): δ 2.15 (s, 3H, CH$_3$), 4.50 (s, 2H, CH$_2$), 6.47-6.53 (m, 4H, ArH), 7.01-7.18 (m, 4H, ArH), 8.00 (s, 1H, imidazole), 12.53 (s, 1H, NH). ^{13}C-NMR (100 MHZ, DMSO-d$_6$): δ 20.93 (CH$_3$), 45.92 (CH$_2$), 98.22, 98.66, 109.20, 115.21, 117.88,

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122.65, 128.25, 129.55, 136.35, 138.00, 142.65, 150.55, 156.58, 161.50 (Ar-C). MS (ESI) m/z: 322.2 (M+1). Anal. Calcd for $C_{18}H_{15}OFN_4$: C, 67.07; H, 4.69; N, 17.38. Found: C, 66.87; H, 4.64; N, 17.30.

2-((1H-imidazol-1-yl)methyl)-5-fluoro-6-(1H-imidazol-1-yl)-1H-benzo[d]imidazole (8e): Yield (45%); mp 137-139 °C; IR (KBr) cm^{-1} : 3421 (Ar NH str), 3057,3159 (Ar CH str), 2924 (CH_2 str), 1636 (C=N str), 1600, 1492, 1473,1440 (C=C ring str), 1336 (Ar C-N str), 1149 (C-F str). ^{1}H-NMR (400 MHZ, DMSO-d$_6$): δ 4.21 (s, 2H, CH_2), 7.59-7.62 (d, 2H, *J* = 12.0 HZ, imidazole), 7.75-7.77 (d, 2H, *J* = 8.0 HZ, imidazole), 8.02 (s, 1H, imidazole), 8.16-8.18 (d, 1H, *J* = 8.0 HZ, ArH), 8.27-8.30 (d, 1H, *J* = 12.0 HZ, ArH), 8.46 (s, 1H, imidazole), 13.32 (br.s, 1H, NH). ^{13}C-NMR (100 MHZ, DMSO-d$_6$): δ 45.71 (CH_2), 107.91, 113.00, 115.49, 122.50, 122.80, 128.28, 136.33, 139.30, 141.91, 147.98, 155.28, 161.20 (Ar-C). MS (ESI) m/z: 282.3 (M+1). Anal. Calcd for $C_{14}H_{11}FN_6$: C, 59.56; H, 3.92; N, 29.77. Found: C, 59.37; H, 3.89; N, 29.67.

Bilological 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.

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.

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 [28]. 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 100 µL of *M. tuberculosis* inoculum 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 24 h post-reagent addition. Visual MICs were defined as the lowest concentration of test compound or drug that prevented a colour change.

Antifungal activity: The antifungal activities of compounds (8a-8e) 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 (8a) and (8b) were only poorly active against fungi with growth inhibition zone ≤14mm when tested at 1000 µg/mL by agar diffusion method [29]. However, compounds (8c-e) showed good activity against *Candida* species with growth inhibition zone ≥15mm. The minimum inhibitory concentrations (MICs) were determined for the compounds (8a-e) against *Candida* species according to standard micro-broth dilution method in 96 well microtest plates as per NCCLS protocol [30].

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

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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 512 µg/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 (10^6 CFU mL^{-1}) 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 h 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.

Table 1: Antitubercular activities of compounds (8a-e) against *M.tuberculosis* H37Rv

Compound[a]	MIC[b] (µg mL^{-1}) MABA[c]
8a	100
8b	50
8c	**25**
8d	50
8e	**12.5**
Isoniazid	0.78

[a] Detailed structures are shown in scheme 2
[b] Minimum inhibitory concentration in µg mL^{-1}
[c] Microplate Alamar Blue Assay (visual)

Table 2: Antifungal activity of compounds (8a-8e) against *Candida* Species

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

Compd[a]	*C.albicans* GIZ	MIC	MFC	*C.glabrata* GIZ	MIC	MFC	*C.krusei* GIZ	MIC	MFC	*C.tropicalis* GIZ	MIC	MFC
8a	11	50	50	12	50	50	11	50	100	10	100	100
8b	13	25	25	14	25	>25	14	25	25	13	25	>25
8c	**16**	**12.5**	**12.5**	**16**	**12.5**	**25**	**17**	**25**	**25**	**16**	**25**	**25**
8d	15	25	25	15	25	50	16	12.5	25	15	25	50
8e	**17**	**6.25**	**12.5**	**18**	**6.25**	**12.5**	**18**	**12.5**	**12.5**	**17**	**12.5**	**12.5**
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 2; (b) GIZ, growth inhibition zone (mm); (c) MIC, minimum inhibitory concentration (µg mL^{-1}); (d) MFC, minimum fungicidal concentration (µg mL^{-1}); (e) Clo, Clotrimazole; (f) Flu, Fluconazole.

RESULTS AND DISCUSSION

Chemistry: The synthetic pathways for the preparation of the title compounds (8a-8e) are shown in schemes 1 and 2. The starting material 5-chloro-4-fluoro-2-nitro aniline 4a was synthesized from the commercially available 3-chloro-4-fluoro aniline by nitration of the acetylated aniline (2) followed by acid hydrolysis [32]. Nucleophilic displacement of aryl chloride in 5-chloro-4-fluoro-2-nitro aniline (4a) with appropriate phenols and imidazole yielded nitro anilines (4b-4e)**.** We found that treatment of (4a) and (4b-4e) 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 (5a-5e). The hydrochloride salts of the corresponding diamines (6a-6e) were prepared by treating the diamines

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dissolved in absolute alcohol with dry HCl in ether at 0-5 °C (scheme-1). Compounds (6a-6e) are the key intermediates for the synthesis of target compounds (8a-8e). For the synthesis of target compounds (8a-8e) the dihydrochloride salts of the appropriate 1,2-phenylene diamines (6a-6e) was condensed with 1-(cyanomethyl) imidazole (7) at 175-190 °C in a sealed tube based on the reported method [33].

Structures of nitro anilines 4a, 4c, 4e and the synthesized compounds (8a-8e) 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-8e) showed a broad band at 3421-3460 cm^{-1} (NH stretching) while their ^{1}H NMR spectra showed singlets between δ 12.53-13.32 ppm corresponding to NH proton of the benzimidazole ring which confirmed the cyclized structure. The singlets between 4.21 and 4.52 ppm was typical in the ^{1}H NMR spectra of the compounds (8a-8e) accounting for the imidazolylmethyl CH_2 group and their IR spectras showed the absorption signals between 2905 and 2925 cm^{-1} (CH_2 stretching). The ^{13}C-NMR results showed that the compound (8d) with methyl group presented the expected signal at δ 20.93 ppm, while methylene signal for the compounds (8a-8e) appeared at δ 45.53-45.92 ppm. The carbons of imidazole rings were visible at δ 122.50-123.40 ppm and 136.33-136.92 ppm, while the aromatic carbons were observed at their usual chemical shifts.

Antitubercular and antifungal activity: The novel compounds were screened for antitubercular activity against $H_{37}R_V$ strain and antifungal activity against *Candida* species.

The compounds were synthesized and screened against *M. tuberculosis* H37Rv by MABA method. The chloro, imidazole and phenoxy 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. The unsubstituted compound at sixth position (8a) was poorly active and was synthesized to see its effect on activity profile. Among the aryl ether derivatives (8b-8d) the halogenated compound having 4-chloro substituent in aromatic ring was more active with MIC of 25 µg/mL. The imidazole substituted analog (8e) emerged as the most active compound with a MIC value of 12.5 µg/mL.

The *in vitro* antifungal activity data revealed that the substitution pattern at 6th position of the benzimidazole ring seemed to have different influence on the antifungal activity against various fungal strains. The fluoro chloro benzimidazole counterpart (8a) was found to be poorly active with negligible antifungal activity against all the *Candida* species. Compounds (8b-8e) having different substituents on the phenyl ring at 6th position demonstrated good to moderate antifungal activity against different *Candida* species with a MIC value of 6.25 to 100 µg/mL. Among the aryl ether derivatives, improvement in the antifungal activity was observed in compound (8c) having a chloro group at C-4 position against *Candida albicans* and *Candida glabrata* in comparison with compounds (8b) and (8d). The imidazole substituted analog (8e) exhibited better profile of antifungal activity against all tested *Candida* species, confirming the effectiveness of the imidazole substituent with the influence of imidazolylmethyl group at 2nd position of the benzimidazole 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.

Thus, at this stage all the modifications indicated that the imidazolylmethyl moiety at 2nd position and also an imidazole ring and halogenated phenoxy group at 6th position play a significant role in the antitubercular and antifungal activity with a dominating influence of the azole moiety.

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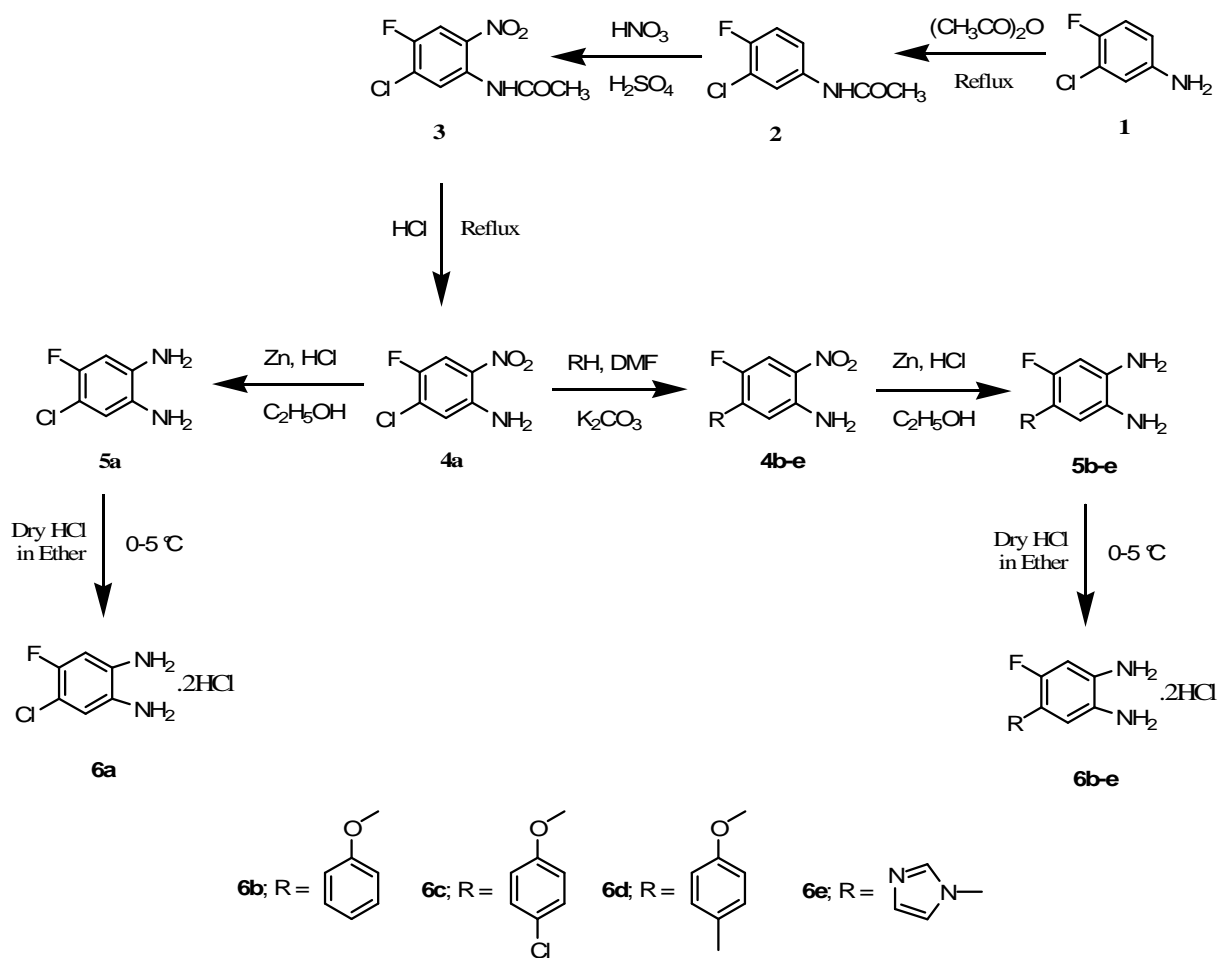
Scheme 1: Synthesis of dihydrochloride salts of appropriate 1,2-phenylenediames (6a-6e)
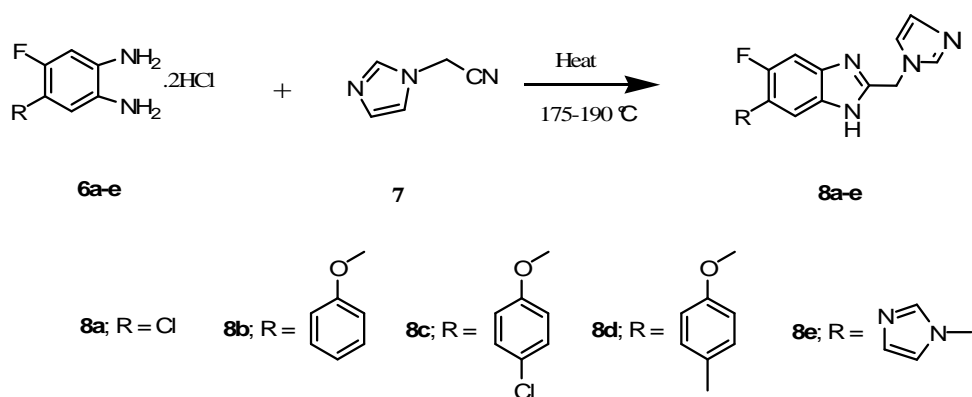
Scheme 2: Synthesis of imidazolylmethyl substituted fluoro benzimidazoles (8a-8e)

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CONCLUSION

In this study we report the design and synthesis of some new imidazolylmethyl substituted fluoro benzimidazoles for antitubercular activity against H37Rv strain and antifungal activity against *Candida* species. The invitro study reveals that imidazole substituted compound (8e) and para chloro phenyl ether analog (8c) were the most active of all the compounds and showed significant inhibitory activity against H37Rv strain and all the fungal cultures. The presence of group like Cl in the phenyl ether analog (8c) and in the unsubstituted fluoro benzimidazole counterpart (8a) also play a significant role in imparting antitubercular and antifungal activity to the compound. Compounds (8a) and (8b) demonstrated moderate activity against fungal cultures. In constrast, no significant antitubercular activity was found for the aryl ether (8b, 8d) and the unsubstituted analog (8a). Therefore, one can propose these imidazolylmethyl substituted novel compounds (8c) and (8e) as potential antitubercular and antifungal agents. Further structural optimization studies with modifications of the azole ring at 6th position of the benzimidazole ring are in progress.

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