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Synthesis of some new substituted fluoro benzimidazoles and their antimicrobial screening

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ABSTRACT

A series of novel 5-fluoro-2-[(2'-(1-trityl tetrazol-5-yl) biphenyl-4-yl methyl) thio]-6-substituted-1H-benzimidazoles were prepared by alkylation of 6-substituted-2-mercapto-5-fluoro benzimidazoles with 5-[4'-(bromo methyl) biphenyl-2-yl]-1-trityl-1H-tetrazole. The synthesized compounds were screened against different strains of bacteria and fungi. The most active derivatives of the present series were the azole (imidazole and 1,2,4-triazole) substituted fluoro benzimidazoles and the para chloro phenyl ether analog indicating the importance of a halogenated diphenyl ether nucleus and the azole moiety at the 6th position. The results suggest that these three molecules are potential candidates for further development as antibacterial and antifungal agents.

Key Words: Fluoro benzimidazole, Antimicrobial activity, Arylether, Alkylation, Azoles.

INTRODUCTION

Despite advances in chemotherapy, severe life threatening systemic fungal infections like Candidiasis, Cryptococcosis and Aspergillosis have become a formidable opponent in the battle against infectious diseases causing morbidity and mortality in immunocompromised hosts, particularly in patients suffering from tuberculosis, Aids, cancer and in organ transplant cases [1-3]. Therapy is complicated due to the toxic side effects caused by antifungal agents and emergence of multi-drug resistant (MDR) bacteria and fungi [4]. A number of *Candida* species are encountered in Candidiasis such as *C. albicans*, *C. glabrata*, *C. tropicalis*, *C. krusei*, *C. dubliniensis* and *C. parapsilosis* [5]. These *Candida* species are opportunistic fungal pathogens that cause superficial and systemic Candidiasis in human and animal hosts [6].

The incidence of severe fungal infections caused by *Candida* species has increased in an alarming way and efforts are underway in the management of invasive fungal infections [7-9]. Considering that bacterial and fungal infections are also a health problem in india, a research project was undertaken in our laboratory with the purpose of developing new compounds with potential antibacterial and antifungal activity.

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 [10]. The azole class has become one of the most widely developed and investigated over the past 2 decades. Azole (benzimidazole) ring system, which is a core structure in various synthetic pharmaceuticals, displays a broad spectrum of biological activity, including antibacterial and antifungal properties [11,12]. Benzimidazole ring is one of the most common heteroaromatic scaffold present in bioactive molecules [13]. Both triclosan and chlorophenesin are halogenated aryl ether analogs with a broad-spectrum anti-bacterial and anti-fungal action. Incorporation of azole (imidazole/triazole/tetrazole) nucleus in compounds containing aryl phenyl ether group is an important synthetic strategy in the design of potent antibacterial and antifungal agents [14,15]. 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 [16]. 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 [17-22]. Based on the study of antifungal azoles bearing the imidazole, triazole and tetrazole moiety and the importance of aryl phenyl ether group as part of the structure for antibacterial and antifungal activity led to the design of biphenyl tetrazole based substituted fluoro benzimidazole derivatives. All the newly synthesized compounds were evaluated for their *in-vitro* antibacterial and antifungal activity against representative strains of bacteria and fungi.

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 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. Column chromatography was accomplished on silica gel (60-120 mesh) by gradient elution of the indicated solvent mixture.

The following compounds were prepared according to literature procedures: 5-(4'-(bromo methyl) biphenyl-2-yl)-1-trityl-1H-tetrazole **(4)** [23], **1** [24], **3b-j** [25].

*5-chloro-4-fluoro-2-nitro aniline **(1)***
Orange needle (85%); mp 143-145 °C; IR (KBr, cm^{-1}): 3493, 3319 (NH), 3050 (Ar CH), 1639, 1593, 1570 (C=C), 1502 (Ar NO_2), 1174 (Ar C-F), 1004 (Ar C-Cl). ^{1}H-NMR (DMSO): δ 6.00 (br.s, 2H, NH_2), 6.90-6.91 (d, 1H, H-3, *J* = 4.0 HZ), 7.91-7.94 (d, 1H, H-6, *J* = 12.0 HZ). ^{19}F-decoupled ^{1}H-NMR (DMSO): δ 4.75-6.20 (br.s, 2H, NH_2), 6.92 (s, 1H, H-3), 7.93 (s, 1H, H-6).

*General procedure for the synthesis of 4-fluoro-5-(substituted)-2-nitroaniline **(1b-j)***
The appropriate phenols or secondary amines (6 mmol) and anhydrous potassium carbonate (10 mmol) were added to a solution of 5-chloro-4-fluoro-2-nitro aniline (5 mmol) in dry DMF (14 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.

*General procedure for the synthesis of 4-chloro-5-fluoro-1,2-phenylenediamine **(2a)** and 4-(substituted)-5-fluoro-benzene-1,2-phenylenediamine **(2b-j)***

To a stirred solution of compound **1** and **(1b-j)** (5 mmol) in ethyl alcohol containing zinc dust (50 mmol) was slowly injected concentrated HCl (10 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. The crude solid was used for the next step without further purification.

*General procedure for the synthesis of 5-fluoro-6-(substituted)-1H-benzo[d]imidazole-2-thiol **(3b-j)***

To a stirred solution of 4-(substituted)-5-fluoro-benzene-1,2-phenylenediamine **(2b-j)** (5 mmol) in ethyl alcohol (25 ml) was added potassium hydroxide **(**15 mmol), water (5mL) and carbon disulphide (10 mmol). The resultant solution was heated with stirring under nitrogen atmosphere for 5-6 hours. After completion of the refluxing period the reaction mixture was cooled to room temperature and poured into water (100mL). The resultant solution was treated with 30% acetic acid solution to a pH 4-5. The product separated either as a solid or as oil. The solid material formed was filtered through Buchner funnel and washed with water. The crude product that separated as oil on treatment with dilute acetic acid solution was extracted with ethyl acetate. The combined organic layer was washed with water and dried over anhydrous sodium sulphate. The solvent was removed under reduced pressure to afford the crude product that was purified by recrystallization from aqueous ethanol to afford pure **3(b-j)**.

*6-chloro-5-fluoro-1H-benzo[d]imidazole-2-thiol **(3a)***

Cream colored solid (71%); mp 291-294 °C; IR (KBr, cm^{-1}): 3400 (NH), 3117, 3070 (Ar CH), 1625 (C=N), 1520, 1500, 1465 (C=C), 1155 (Ar C-F), 1050 (Ar C-Cl). ^{1}H-NMR (DMSO): δ 7.16-7.18 (d, 1H, *J* = 8.80 HZ, ArH), 7.24-7.26 (d, 1H, *J* = 6.8 HZ, ArH), 12.69 (s, 1H, NH or SH), 12.76 (s, 1H, NH or SH). MS (ESI) m/z: 202 (M+1), 203 (M+2). Anal. Calcd for $C_7H_4ClFN_2S$: C, 41.49; H, 1.98; N, 13.82. Found: C, 41.35; H, 1.90; N, 13.74.

*5-fluoro-6-phenoxy-1H-benzo[d]imidazole-2-thiol **(3b)***

Brown solid (70%); mp 232-234 °C; IR (KBr, cm^{-1}): 3446 (NH), 3132, 3070 (Ar CH), 1624 (C=N), 1593, 1510, 1475 (C=C), 1215, 1192 (C-O-C), 1157 (Ar C-F). ^{1}H-NMR (DMSO): δ 6.87-6.89 (d, 1H, *J* = 7.2 HZ, ArH), 6.93-6.95 (d, 2H, *J* = 8.0 HZ, ArH), 7.07-7.09, 7.09-7.10 (t, 1H, *J* = 7.2 HZ, *J* = 7.6 HZ, ArH), 7.14-7.17 (d, 1H, *J* = 10.0 HZ, ArH), 7.32-7.36 (m, 2H, ArH), 12.57 (br.s, 2H, SH and NH). MS (ESI) m/z: 260.1 (M+1). Anal. Calcd for $C_{13}H_9FN_2OS$: C, 59.98; H, 3.48; N, 10.76. Found: C, 59.80; H, 3.40; N, 10.78.

*6-(4-chloro phenoxy)-5-fluoro-1H-benzo[d]imidazole-2-thiol **(3c)***

Cream colored solid (77%); mp 237-239 °C; IR (KBr, cm^{-1}): 3490 (NH), 3130, 3082 (Ar CH), 1650 (C=N), 1593, 1471 (C=C), 1226, 1193 (C-O-C), 1170 (Ar C-F), 1101 (Ar C-Cl). ^{1}H-NMR (DMSO): δ 6.93-6.95 (d, 1H, *J* = 6.8 HZ, ArH), 6.97-6.99 (d, 2H, *J* = 8.8 HZ, ArH), 7.16-7.18 (d, 1H, *J* = 10.0 HZ, ArH), 7.37-7.40 (d, 2H, *J* = 9.2 HZ, ArH), 12.59 (s, 1H, NH or SH), 12.68 (s, 1H, NH or SH). MS (ESI) m/z: 294 (M+1), 295 (M+2). Anal. Calcd for $C_{13}H_8ClFN_2OS$: C, 52.97; H, 2.73; N, 9.50. Found: C, 52.81; H, 2.68; N, 9.53.

*5-fluoro-6-(p-tolyloxy)-1H-benzo[d]imidazole-2-thiol **(3d)***

Pale yellow solid (69%); mp 282-285 °C; IR (KBr, cm^{-1}): 3394 (NH), 3124, 3082 (Ar CH), 2989, 2955 (aliphatic CH), 1626 (C=N), 1606, 1508, 1476 (C=C), 1217, 1197 (C-O-C), 1159 (Ar C-F). ^{1}H-NMR (DMSO): δ 2.25 (s, 3H, CH_3), 6.80-6.81 (d, 1H, *J* = 7.2 HZ, ArH), 6.83-6.87 (d, 2H, *J* = 14.4 HZ, ArH), 7.12-7.14 (d, 1H, *J* = 7.2 HZ, ArH), 7.15-7.16 (d, 2H, *J* = 3.6 HZ, ArH), 12.50 (s, 1H, NH or SH), 12.65 (s, 1H, NH or SH). MS (ESI) m/z: 274.1 (M+1). Anal. Calcd for $C_{14}H_{11}FN_2OS$: C, 61.29; H, 4.04; N, 10.21. Found: C, 61.20; H, 3.95; N, 10.26.

*5-fluoro-6-(naphthalen-1-yl oxy)-1H-benzo[d]imidazole-2-thiol **(3e)***

Light brown solid (79%); mp 246-248 °C; IR (KBr, cm^{-1}): 3406 (NH), 3126, 3064 (Ar CH), 1626 (C=N), 1599, 1579, 1480 (C=C), 1259, 1232 (C-O-C), 1163 (Ar C-F). ^{1}H-NMR (DMSO): δ 6.78-6.80 (d, 1H, *J* = 7.6 HZ, ArH), 6.89-6.91 (d, 1H, *J* = 7.2 HZ, ArH), 7.20-7.22 (d, 1H, *J* = 10.0 HZ, ArH), 7.37-7.39, 7.39-7.41 (t, 1H, *J* = 8.0 HZ, *J* = 7.6 HZ, ArH), 7.56-7.62 (m, 2H, ArH), 7.66-7.69 (d, 2H, *J* = 8.4 HZ, ArH), 7.96-7.98 (m, 1H, ArH), 12.52 (s, 1H, NH or SH), 12.67 (s, 1H, NH or SH). MS (ESI) m/z: 310.1 (M+1). Anal. Calcd for $C_{17}H_{11}FN_2OS$: C, 65.79; H, 3.57; N, 9.02. Found: C, 65.71; H, 3.49; N, 8.98.

5-fluoro-6-(naphthalen-2-yl oxy)-1H-benzo[d]imidazole-2-thiol ***(3f)***
Light brown solid (75%); mp 253-255 °C; IR (KBr, cm^{-1}): 3410 (NH), 3123, 3069 (Ar CH), 1621 (C=N), 1599, 1579, 1480 (C=C), 1245, 1221 (C-O-C), 1155 (Ar C-F). ^{1}H-NMR (DMSO): δ 6.75 (d, 1H, *J* = 7.6 HZ, ArH), 6.88 (d, 1H, *J* = 7.6 HZ, ArH), 6.98 (s, 1H, ArH),7.23 (d, 1H, H-4, *J* = 10.2 HZ, ArH), 7.34 (t, 1H, *J* = 8.0 HZ, ArH), 7.54 (t, 1H, *J* = 8.0 HZ, ArH), 7.63 (d, 2H, *J* = 7.6 HZ, ArH), 8.21 (m, 1H, ArH), 12.50 (s, 1H, NH or SH), 12.62 (s, 1H, NH or SH). MS (ESI) m/z: 310 (M+1). Anal. Calcd for $C_{17}H_{11}FN_2OS$: C, 65.79; H, 3.57; N, 9.02. Found: C, 65.66; H, 3.52; N, 8.95.

5-fluoro-6-(1H-imidazol-1-yl)-1H-benzo[d]imidazole-2-thiol ***(3g)***
Cream colored solid (60%); mp 270-272 °C; IR (KBr, cm^{-1}): 3458 (NH), 3074 (Ar CH), 1627 (C=N), 1543, 1510, 1481 (C=C), 1347 (Ar C-N), 1155 (Ar C-F). ^{1}H-NMR (DMSO): δ 7.09 (d, 1H, , *J* = 1.6 HZ, imidazole-H), 7.21 (d, 1H, *J* = 10.4 HZ, ArH), 7.24-7.25 (d, 1H, *J* = 6.4 HZ, ArH), 7.49-7.50 (d, 1H, *J* = 1.6 HZ, imidazole-H), 7.95 (s, 1H, imidazole-H), 12.81 (s, 2H, SH and NH). MS (ESI) m/z: 234.1 (M-1). Anal. Calcd for $C_{10}H_7FN_4S$: C, 38.70; H, 2.27; N, 23.91. Found: C, 38.62; H, 2.21; N, 23.85.

5-fluoro-6-(1H-1,2,4-triazol-1-yl)-1H-benzo[d]imidazole-2-thiol ***(3h)***
Brick red colored solid (64%); mp 280-282 °C; IR (KBr, cm^{-1}): 3421 (NH), 3140, 3101 (Ar CH), 1629 (C=N), 1562, 1512, 1502, 1475 (C=C), 1381 (Ar C-N), 1149 (Ar C-F). ^{1}H-NMR (DMSO): δ 7.12 (s, 1H, NH or SH), 7.15 (s, 1H, NH or SH), 7.26 (s, 1H, H-7, ArH), 7.28 (s, 1H, H-4, ArH), 8.20 (s, 1H, triazole-H), 8.88 (s, 1H, triazole-H). MS (ESI) m/z: 235.1 (M+1). Anal. Calcd for $C_9H_6FN_5S$: C, 45.95; H, 2.57; N, 29.77. Found: C, 45.98; H, 2.51; N, 29.73.

5-fluoro-6-(piperidin-1-yl)-1H-benzo[d]imidazole-2-thiol ***(3i)***
Grey solid (61%); mp 168-170 °C; IR (KBr, cm^{-1}): 3421 (NH), 3124, 3088 (Ar CH), 2937 (aliphatic CH), 1620 (C=N), 1480, 1452 (C=C), 1234, 1180, 1124 (aliphatic C-N), 1139 (Ar C-F). ^{1}H-NMR (DMSO): δ 1.49-1.51 (m, 4H, piperidine CH_2), 2.85-2.92 (m, 6H, piperidine CH_2), 6.72-6.73 (d, 1H, *J* = 7.6 HZ, ArH), 6.90-6.93 (d, 1H, *J* = 11.6 HZ, ArH), 12.39 (s, 2H, NH and SH). MS (ESI) m/z: 251.1 (M+1). Anal. Calcd for $C_{12}H_{14}FN_3S$: C, 57.34; H, 5.61; N, 16.71. Found: C, 57.25; H, 5.53; N, 16.62.

5-fluoro-6-(pyrrolidin-1-yl)-1H-benzo[d]imidazole-2-thiol ***(3j)***
Off-white solid (59%); mp 152-155 °C; IR (KBr, cm^{-1}): 3450 (NH), 3000 (Ar CH), 2920 (aliphatic CH), 1632 (C=N), 1520, 1459, 1447 (C=C), 1215 (aliphatic C-N), 1141 (Ar C-F). ^{1}H-NMR (DMSO): δ 1.22 (m, 6H, pyrrolidine CH_2), 3.77-3.83 (m, 2H, pyrrolidine CH_2), 7.45-7.47 (d, 1H, *J* = 9.6 HZ, ArH), 7.61-7.63 (d, 1H, *J* = 9.6 HZ, ArH), 7.83 (s, 1H, SH), 12.94 (s, 1H, NH). MS (ESI) m/z: 237 (M+1). Anal. Calcd for $C_{11}H_{12}FN_3S$: C, 55.67; H, 5.09; N, 17.70. Found: C, 55.60; H, 5.14; N, 17.65.

5-(4'-(bromo methyl) biphenyl-2-yl)-1-trityl-1H-tetrazole ***(4)***
To a stirred solution of 5-(2-(4'-Methyl)-biphenyl)-1-trityl-1H-tetrazole (5 mmol) and N-bromosuccinimide (5 mmol) in dry carbon tetrachloride (25 ML), was added dibenzoyl peroxide (2 mmol). The resultant solution was refluxed for 10 hours. The mixture was cooled to room temperature and filtered. The filtrate was concentrated under pressure to afford **(4).** White solid (83%); mp 151-153 °C; IR (KBr, cm^{-1}): 3055 (Ar CH), 3028 (benzylic CH_2), 1606 (C=N), 1595 (N=N), 1529, 1510, 1491 (C=C), 1186 (CH_2Br), 1354, 1282 (Ar C-N), 609 (C-Br). ^{1}H-NMR (DMSO): δ 4.63 (s, 2H, benzylic CH_2), 6.81-6.87 (m, 6H, ArH), 7.04-7.06 (d, 2H, *J* = 8.4 HZ, ArH), 7.26 (d, 2H, *J* = 8.0 HZ, ArH), 7.29-7.39 (m, 15H, trityl), 7.45-7.48 (d, 1H, *J* = 12.0 HZ, ArH), 7.53 (t, 1H, *J* = 7.6 HZ, ArH),7.60 (t, 1H, *J* = 7.6 HZ, ArH), 7.79-7.82 (d, 1H, *J* = 12.0 HZ, ArH). MS (ESI) m/z: 557 (M+1).

General procedure for the synthesis of 5-fluoro-2-[(2'-(1-trityl tetrazol-5-yl) biphenyl-4-yl methyl) thio]-6-substituted-1H-benzimidazole ***(5a-j)***
To a stirred solution of **(3a-j)** (2 mmol) and anhydrous potassium carbonate (4 mmol) in 8ml of dry DMF at room temperature was added dropwise aryl alkyl halide (**4)** (2 mmol) dissolved in 8ml of dry DMF. After the addition was complete, the resulting solution was stirred at room temperature under nitrogen atmosphere over different periods till the completion of the reaction. When TLC revealed the absence of starting material, the reaction mixture was poured into water (100 mL), followed by extraction with ethyl acetate.The extract was washed with water, brine, dried over anhydrous sodium sulphate, filtered and concentrated under vacuum. The crude product was chromatographed on silica gel (60-120 mesh) with 2-20 % of ethyl acetate in petroleum ether / cyclohexane (gradient elution) as an eluent to afford **(5a-j)**.

*6-chloro-5-fluoro-2-[(2'-(1-trityl tetrazol-5-yl) biphenyl-4-yl methyl) thio]-1H-benzimidazole (**5a**)*
Yellow fluffy solid (72%); mp 101-103 °C; IR (KBr, cm^{-1}): 3460 (NH), 3061, 3032 (Ar CH), 2929 (benzylic CH_2), 1655 (C=N), 1626 (N=N), 1599, 1583, 1491, 1467 (C=C), 1340 (Ar C-N), 1149 (Ar C-F), 1030 (Ar C-Cl). ^{1}H-NMR (DMSO): δ 4.53 (s, 2H, benzylic CH_2), 6.83-6.87 (m, 6H, ArH), 7.00 (d, 2H, *J* = 8.0 HZ, ArH), 7.19-7.36 (m, 15H, trityl), 7.41-7.43 (d, 1H, *J* = 8.0 HZ, ArH), 7.52 (t, 1H, *J* = 7.6 HZ, ArH), 7.58 (t, 1H, *J* = 7.6 HZ, ArH), 12.83 (br.s, 1H, NH). MS (ESI) m/z: 678.2 (M+1). Anal. Calcd for $C_{40}H_{28}ClFN_6S$: C, 70.73; H, 4.15; N, 12.37. Found: C, 70.61; H, 4.19; N, 12.45.

*5-fluoro-2-[(2'-(1-trityl tetrazol-5-yl) biphenyl-4-yl methyl) thio]-6-(phenoxy)-1H-benzimidazole (**5b**)*
Light brown powder (74%); mp 86-88 °C; IR (KBr, cm^{-1}): 3427 (NH), 3075 (Ar CH), 2920 (benzylic CH_2), 1654 (C=N), 1600 (N=N), 1491, 1473 (C=C), 1365 (Ar C-N), 1220 (C-O-C), 1165 (Ar C-F). ^{1}H-NMR (DMSO): δ 4.54 (s, 2H, benzylic CH_2), 6.92 (d, 1H, *J* = 6.8 HZ, ArH), 6.98 (t, 1H, *J* = 13.2 HZ, ArH), 7.06 (d, 1H, *J* = 8.0 HZ, ArH), 7.14 (d, 1H, *J* = 10.2 HZ, ArH), 7.20-7.37 (m, 19H, ArH), 7.55 (t, 1H, *J* = 7.9 HZ, ArH), 7.60-7.65 (m, 6H, ArH), 12.72 (br.s, 1H, NH). MS (ESI) m/z: 736.3 (M+1). Anal. Calcd for $C_{46}H_{33}FN_6OS$: C, 74.98; H, 4.51; N, 11.40. Found: C, 74.89; H, 4.45; N, 11.34.

*5-fluoro-2-[(2'-(1-trityl tetrazol-5-yl) biphenyl-4-yl methyl) thio]-6-(4-chloro phenoxy)-1H-benzimidazole (**5c**)*
Cream colored solid (71%); mp 117-119 °C; IR (KBr, cm^{-1}): 3417 (NH), 3075 (Ar CH), 2930 (benzylic CH_2), 1660 (C=N), 1640 (N=N), 1485, 1473, 1431 (C=C), 1370 (Ar C-N), 1230 (C-O-C), 1160 (Ar C-F), 1100 (Ar C-Cl). ^{1}H-NMR (DMSO): δ 4.52 (s, 2H, benzylic CH_2), 6.83-6.85 (m, 6H, ArH), 6.92-6.94 (2H, d, *J* = 9.20 HZ, ArH), 7.00-7.02 (d, 2H, *J* = 8.0 HZ, ArH), 7.28-7.37 (m, 15H, trityl), 7.43-7.45 (d, 1H, *J* = 7.60 HZ, ArH), 7.51 (t, 1H, *J* = 13.80 HZ, ArH), 7.58-7.60, 7.60-7.62 (t, 1H, *J* = 7.60 HZ, *J* = 7.60 HZ, ArH), 7.77-7.79 (d, 1H, *J* = 6.80 HZ, ArH), 12.76 (br.s, 1H, NH). MS (ESI) m/z: 770.2 (M-1). Anal. Calcd for $C_{46}H_{32}ClFN_6OS$: C, 71.63; H, 4.18; N, 10.89. Found: C, 71.72; H, 4.25; N, 10.86.

*5-fluoro-2-[(2'-(1-trityl tetrazol-5-yl) biphenyl-4-yl methyl) thio]-6-(4-tolyloxy)-1H-benzimidazole (**5d**)*
Amber colored solid (68%); mp 107-109 °C; IR (KBr, cm^{-1}): 3408 (NH), 3124, 3064 (Ar CH), 2924 (benzylic CH_2), 2830 (aliphatic CH), 1627 (C=N), 1606 (N=N), 1506, 1450 (C=C), 1361 (Ar C-N), 1200 (C-O-C), 1157 (Ar C-F). ^{1}H-NMR (DMSO): δ 2.35 (s, 3H, CH_3), 4.54 (s, 2H, benzylic CH_2), 6.81-6.83 (m, 6H, ArH), 6.90 (d, 1H, *J* = 7.2 HZ, ArH), 7.02 (d, 1H, *J* = 8.0 HZ, ArH), 7.26-7.35 (m, 15H, trityl), 7.37 (d, 2H, *J* = 8.0 HZ, ArH),7.41 (d, 2H, *J* = 12.0 HZ, ArH), 7.52 (t, 1H, *J* = 13.40 HZ, ArH), 7.58 (t, 1H, *J* = 13.57 HZ, ArH), 12.70 (br.s, 1H, NH). MS (ESI) m/z: 750.4 (M+1). Anal. Calcd for $C_{47}H_{35}FN_6OS$: C, 75.17; H, 4.69; N, 11.19. Found: C, 75.05; H, 4.65; N, 11.14.

*5-fluoro-2-[(2'-(1-trityl tetrazol-5-yl) biphenyl-4-yl methyl) thio]-6-(naphthalene-1-oxy)-1H-benzimidazole (**5e**)*
Brown fluffy solid (72%); mp 97-99 °C; IR (KBr, cm^{-1}): 3416 (NH), 3075 (Ar CH), 2920 (benzylic CH_2), 1650 (C=N), 1597 (N=N), 1492, 1471, 1444 (C=C), 1354 (Ar C-N), 1259, 1228 (C-O-C), 1155 (Ar C-F). ^{1}H-NMR (DMSO): δ 4.53 (s, 2H, benzylic CH_2), 6.71 (d, 1H, *J* = 7.6 HZ, ArH), 6.82-6.86 (m, 6H, ArH), 6.99-7.03 (m, 2H, *J* = 8.0 HZ, ArH), 7.19 (d, 1H, *J* = 8.4 HZ, ArH), 7.24-7.40 (m, 15H, trityl), 7.40-7.47 (m, 1H, ArH), 7.53 (t, 2H, *J* = 7.2, 6.0 HZ, ArH), 7.58-7.72 (m, 2H, ArH), 7.77-7.79 (d, 1H, *J* = 8.0 HZ, ArH), 7.96 (d, 1H, *J* = 7.6 HZ, ArH), 12.68 (br.s, 1H, NH). MS (ESI) m/z: 786.2 (M+1). Anal. Calcd for $C_{50}H_{35}FN_6OS$: C, 76.31; H, 4.10; N, 10.67. Found: C, 76.18; H, 4.05; N, 10.71.

*5-fluoro-2-[(2'-(1-trityl tetrazol-5-yl) biphenyl-4-yl methyl) thio]-6-(naphthalene-2-oxy)-1H-benzimidazole (**5f**)*
Brown fluffy solid (76%); mp 90-92 °C; IR (KBr, cm^{-1}): 3412 (NH), 3072 (Ar CH), 2925 (benzylic CH_2), 1645 (C=N), 1592 (N=N), 1498, 1479 (C=C), 1350 (Ar C-N), 1253, 1221 (C-O-C), 1150 (Ar C-F). ^{1}H-NMR (DMSO): δ 4.53 (s, 2H, benzylic CH_2), 6.81-6.86 (m, 6H, ArH), 6.99-7.03 (m, 1H, ArH), 7.20-7.22 (d, 1H, *J* = 8.0 HZ, ArH), 7.27-7.37 (m, 15H, trityl), 7.51-7.56 (t, 2H, *J* = 9.2, 10.4 HZ, ArH), 7.58-7.72 (m, 6H, ArH), 7.77-7.80 (d, 1H, *J* = 12.0 HZ), 12.69 (br.s, 1H, NH). MS (ESI) m/z: 786 (M+1). Anal. Calcd for $C_{50}H_{35}FN_6OS$: C, 76.31; H, 4.10; N, 10.67. Found: C, 76.20; H, 4.03; N, 10.74.

*5-fluoro-2-[(2'-(1-trityl tetrazol-5-yl) biphenyl-4-yl methyl) thio]-6-(imidazole-1-yl)-1H-benzimidazole (**5g**)*
Off-white Solid (65%); mp 158-160 °C; IR (KBr, cm^{-1}): 3421 (NH), 3057, 3159 (Ar CH), 2924 (benzylic CH_2), 1636 (C=N), 1600 (N=N), 1599, 1583, 1492, 1473, 1440 (C=C), 1338 (Ar C-N), 1140 (Ar C-F). ^{1}H-NMR (DMSO): δ 4.51 (s, 2H, benzylic CH_2), 7.02 (d, 1H, *J* = 8.0 HZ, ArH), 7.15 (d, 1H, *J* = 12.0 HZ, ArH), 7.18-7.35 (m, 19H, ArH), 7.27 (d, 1H, *J* = 4.1 HZ, Imidazole-H), 7.40 (d, 2H, *J* = 4.8 HZ, ArH), 7.46 (d, 1H, *J* = 4.1 HZ, imidazole-H)

7.70 (d, 2H, *J* = 4.4 HZ, ArH), 8.03 (s, 1H, imidazole-H), 12.71 (br.s, 1H, NH). MS (ESI) m/z: 710.4 (M-1). Anal. Calcd for $C_{43}H_{31}FN_8S$: C, 72.65; H, 4.39; N, 15.76. Found: C, 72.50; H, 4.29; N, 15.79.

*5-fluoro-2-[(2'-(1-trityl tetrazol-5-yl) biphenyl-4-yl methyl) thio]-6-(1,2,4-triazole-1-yl)-1H-benzimidazole (**5h**)*
Merun colored Solid (63%); mp 145-146 °C; IR (KBr, cm^{-1}): 3431 (NH), 3080 (Ar CH), 2920 (benzylic CH_2), 1654 (C=N), 1610 (N=N), 1500, 1473, 1444 (C=C), 1360 (Ar C-N), 1150 (Ar C-F). ^{1}H-NMR (DMSO): δ 4.50 (s, 2H, benzylic CH_2),7.04 (d, 1H, *J* = 6.4 HZ, ArH),7.16 (d, 1H, *J* = 8.8 HZ, ArH), 7.20-7.36 (m, 19H, ArH), 7.38 (d, 2H, *J* = 8.0 HZ, ArH), 7.68 (d, 2H, *J* = 6.72 HZ, ArH), 8.41 (s, 1H, triazole-H), 8.60 (s, 1H, triazole-H), 12.70 (br.s, 1H, NH). MS (ESI) m/z: 711.3 (M+1). Anal. Calcd for $C_{42}H_{30}FN_9S$: C, 70.86; H, 4.24; N, 17.70. Found: C, 70.91; H, 4.35; N, 17.65.

*5-fluoro-2-[(2'-(1-trityl tetrazol-5-yl) biphenyl-4-yl methyl) thio]-6-(piperidine-1-yl)-1H-benzimidazole (**5i**)*
Grey colored solid (58%); mp 127-129 °C; IR (KBr, cm^{-1}): 3410 (NH), 3100 (Ar CH), 2929 (benzylic CH_2), 2880 (piperidine CH_2), 1650 (C=N), 1620 (N=N), 1480, 1460, 1440 (C=C), 1230 (aliphatic C-N), 1150 (Ar C-F). ^{1}H-NMR (DMSO): δ 1.50 (m, 6H, piperidine CH_2), 2.70 (m, 4H, piperidine CH_2), 4.55 (s, 2H, benzylic CH_2), 7.03 (d, 1H, *J* = 7.84 HZ, ArH), 7.19 (d, 1H, *J* = 12.88 HZ, ArH), 7.22-7.37 (m, 15H, trityl), 7.42-7.48 (m, 6H, ArH), 7.53 (t, 1H, *J* = 13.70 HZ, ArH), 7.55 (t, 1H, *J* = 13.32 HZ, ArH), 12.77 (br.s, 1H, NH). MS (ESI) m/z: 727.4 (M-1). Anal. Calcd for $C_{45}H_{38}FN_7S$: C, 74.25; H, 5.26; N, 13.46. Found: C, 74.18; H, 5.22; N, 13.49.

*5-fluoro-2-[(2'-(1-trityl tetrazol-5-yl) biphenyl-4-yl methyl) thio]-6-(pyrrolidine-1-yl)-1H-benzimidazole (**5j**)*
Light grey solid (55%); mp 133-135 °C; IR (KBr, cm^{-1}): 3453 (NH), 3090 (Ar CH), 2924 (benzylic CH_2), 2860 (pyrrolidine CH_2), 1650 (C=N), 1627 (N=N), 1500, 1450, 1430 (C=C), 1222 (aliphatic C-N), 1160 (Ar C-F). ^{1}H-NMR (DMSO): δ 1.60 (m, 4H, pyrrolidine-CH_2), 2.75 (m, 4H, pyrrolidine-CH_2), 4.55 (s, 2H, benzylic CH_2), 7.0 (d, 1H, *J* = 7.5 HZ, ArH), 7.19 (d, 1H, *J* = 9.8 HZ, ArH), 7.23-7.36 (m, 15H, trityl), 7.44-7.49 (m, 6H, ArH), 7.51 (t, 1H, *J* = 12.30 HZ, ArH), 7.57 (t, 1H, *J* = 12.79 HZ, ArH), 12.75 (br.s, 1H, NH). MS (ESI) m/z: 713.4 (M-1). Anal. Calcd for $C_{44}H_{36}FN_7S$: C, 74.03; H, 5.08; N, 13.73. Found: C, 73.91; H, 4.99; N, 13.65.

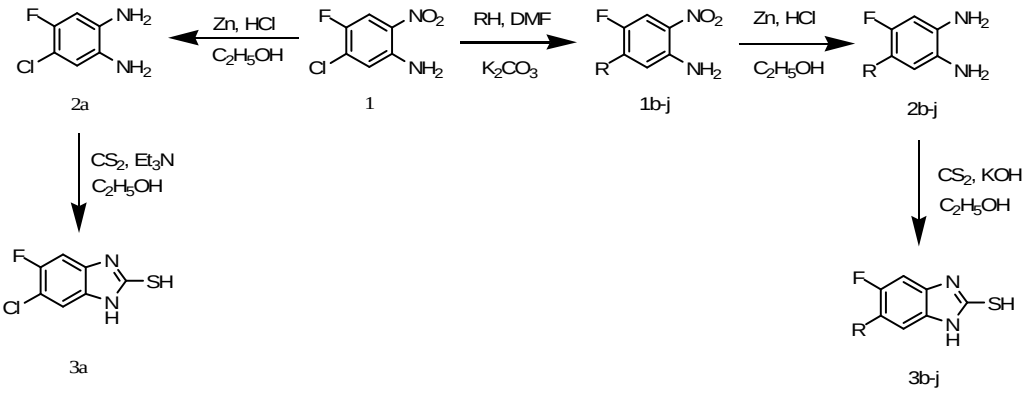
RESULTS AND DISCUSSION

Chemistry

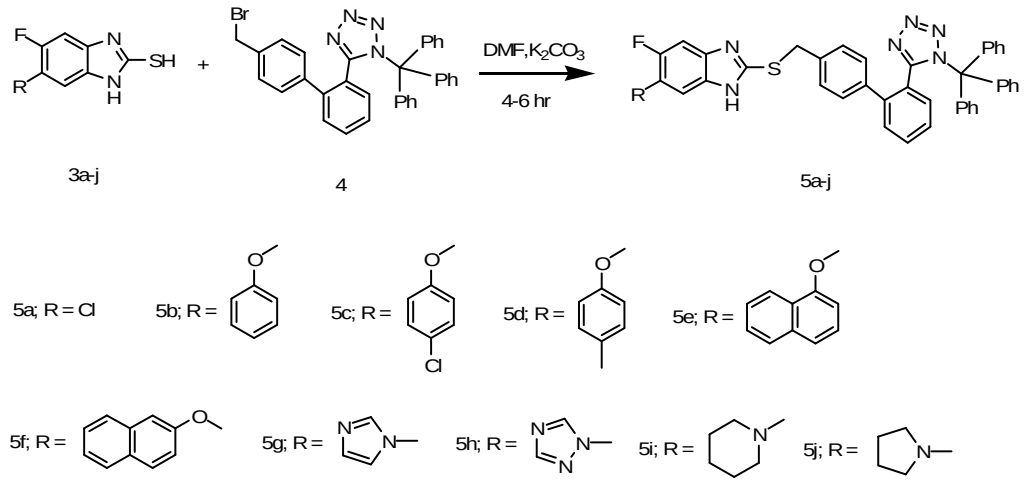
The synthetic pathways for the preparation of the title compounds **5a-j** are shown in the schemes 1 and 2. 5-chloro-4-fluoro-2-nitro aniline **1** was synthesized from the commercially available 3-chloro-4-fluoro aniline by nitration of the acetylated aniline followed by acid hydrolysis. The position of the NO_2 group in **1** was confirmed by comparative assessment of its ^{1}H-NMR and ^{19}F-decoupled ^{1}H-NMR. Synthesis of the appropriate substituted-2-mercapto benzimidazoles **3b-j** is depicted in scheme-1. Nucleophilic displacement of aryl chloride in 5-chloro-4-fluoro-2-nitro aniline **1** with appropriate phenols / secondary amines yielded nitro aniline **1b-j** which was reduced and immediately cyclocondensed with CS_2 in an EtOH-KOH solution to give **3b-j**. We found that treatment of **1** and **1b-j** 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 diamine **2a-j**. For the synthesis of unsubstituted-2-mercapto benzimidazole **3a** the corresponding diamine of **1** was immediately cyclocondensed with CS_2 in an EtOH-Et_3N solution to furnish 6-chloro-5-fluoro-1H-benzo[d]imidazole-2-thiol based on the reported method [26] as shown in scheme-1.

Compounds **3a-j** are the key intermediates for the synthesis of target compounds **5a-j**. A series of 5-fluoro-2-[(2'-(1-trityl tetrazol-5-yl) biphenyl-4-yl methyl) thio]-6-substituted-1H-benzimidazoles were prepared by alkylation of substituted mercaptobenzimidazoles with the aryl alkyl halide 5-(4'-(bromo methyl) biphenyl-2-yl)-1-trityl-1H-tetrazole in presence of potassium carbonate in dry DMF at room temperature (scheme-2). The crude product was chromatographed on silica gel (60-120 mesh) with 2-20% ethyl acetate in petroleum ether/cyclohexane to afford **5a-j**. Structures of synthesized compounds **3a-j** and **5a-j** were confirmed by IR, ^{1}H NMR, Mass spectra and their purity by elemental analysis. IR spectra of compounds **3a-j** showed a broad band at 3410-3420 cm^{-1} (NH stretching) while their ^{1}H NMR spectra showed singlets at δ 12.38-12.94 ppm corresponding to SH and NH which confirmed the cyclized structure. Higher nucleophilicity and polarizability of SH group as compared to the NH, favours the S-alkylation rather than the alkylation of NH in the benzimidazole ring [27]. The IR spectra of the products **5a-j** reveal the appearance of the benzylic S-CH_2 bands at 2920-2929 cm^{-1} and ^{1}H NMR spectra display the benzylic proton -CH_2- as a sharp singlet signal at δ 4.50-4.55 ppm and also disappearance of one of the two singlets for SH and NH seen in ^{1}H NMR spectra of 4(a-j) at δ 12.38-12.94 ppm is taken as an evidence for alkylation of the SH group. In the

[1]H NMR spectra of all the compounds of series **5a-j**, a NH proton appears at 12.65-12.85 ppm, accounting for the NH proton of the benzimidazole ring.



Scheme 1



Scheme 2

Antimicrobial activity

All the synthesized compounds were evaluated *in vitro* for their antibacterial and antifungal activity against representative strains of Gram-positive (*Bacillus subtilis*, *Staphylococcus aureus*) and Gram-negative (*Pseudomonas aeruginosa*, *Escherichia coli*) bacteria and four standard fungi (*Candida albicans*, *Candida krusei*, *Candida glabrata* and *Candida tropicalis*). The clinical samples of these strains were procured from National Centre for Industrial Microorganisms (NCIM) pune, India. Ampicillin and fluconazole were used as reference drugs. Table 1 summarizes the results of our antibacterial and antifungal studies of all the synthesized compounds.

Antifungal activity

In-vitro agar diffusion method and broth dilution assay were used to determine the effects of compounds **5a-j** on yeast growth. Yeasts were grown on sabouraud dextrose agar plate for 24 h at 35 °C. Initial screening of all the described benzimidazoles against fungal cultures showed that the compounds (**5e, 5f)** and (**5i, 5j)** were only poorly active against fungi with growth inhibition zone ≤6mm when tested at 1000 µg/mL by agar diffusion method [28]. However, compounds 6(a-d) and 6(g, h) showed good activity against *Candida* species with growth inhibition zone ≥9mm. The minimum inhibitory concentration (MIC) measurement was determined for the compounds 6(a-j) against *Candida* species using micro-broth dilution method in 96-well microtest plates as per NCCLS document M27-A with a slight modification [29].

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.

Antibacterial activity

All the synthesized compounds were evaluated in vitro against bacterial strains by paper disc diffusion and microdilution methods using liquefied Mueller Hinton agar and broth [30]. Preliminary antibacterial screening was performed by the agar diffusion method using paper discs impregnated with the test compounds. For the microdilution assay, the compounds to be tested were dissolved in dimethylsulphoxide (1.0 to 512µg/mL) serially diluted in Mueller Hinton broth, inoculated to the corresponding wells with the required amount of the inoculums to obtain a suspension of microorganism, which contains 10^6 CFU per mL. Wells containing only inoculated broth was kept as controls. After incubation for 24 h at 37 °C, the well with no growth of microorganism was recorded to represent MIC expressed in µg/mL.

Table 1 Antimicrobial activity of compounds 5(a-J) against different strains of bacteria and fungi

	GIZ[b] and MIC[c] of compounds and Std drugs against bacterial and fungal cultures															
	C.albicans		*C.glabrata*		*C.krusei*		*C.tropicalis*		*S.aureus*		*B.subtilis*		*E.coli*		*P.aeruginosa*	
Compd[a]	GIZ	MIC	GIZ	MIC	GIZ	MIC	GIZ	MIC	GIZ	MIC	GIZ	MIC	GIZ	MIC	GIZ	MIC
5a	14	16	17	16	16	16	17	16	4	>64	9	64	4	>64	-	-
5b	9	32	8	32	9	32	10	32	3	>64	9	64	4	>64	-	-
5c	**15**	**16**	**18**	**16**	**17**	**16**	**15**	**16**	**15**	**32**	**16**	**32**	**12**	**64**	**7**	**>64**
5d	11	32	10	32	12	32	10	32	9	64	8	64	5	>64	-	-
5e	6	64	5	>64	5	64	6	64	4	>64	9	64	5	>64	-	-
5f	6	64	6	64	5	64	5	>64	9	64	8	64	5	>64	-	-
5g	**17**	**16**	**19**	**8**	**17**	**8**	**16**	**16**	**18**	**16**	**19**	**16**	**15**	**32**	**6**	**>64**
5h	**18**	**8**	**20**	**4**	**17**	**8**	**17**	**8**	**19**	**16**	**18**	**16**	**15**	**32**	**7**	**>64**
5i	4	>64	3	>64	5	64	3	>64	-	-	5	>64	4	>64	-	-
5j	4	>64	4	>64	3	>64	3	>64	-	-	4	>64	5	>64	-	-
Flu[d]	22	2	23	1	20	4	19	4								
Amp[e]									25	2	26	2	23	4	17	64

[a] Detailed structures are shown in scheme 2; [b] Growth inhibition zone in mm; [c] Minimum inhibitory concentration in µg/ml
[d] Fluconazole.; [e] Ampicillin.

CONCLUSION

In this study we report the synthesis, characterization and screening of 10 new substituted fluoro benzimidazoles for antimicrobial activity against four strains of Gram-positive (*Bacillus subtilis*, *Staphylococcus aureus*) and Gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa*) bacteria and four standard fungi (*Candida albicans*, *Candida krusei*, *Candida glabrata* and *Candida tropicalis*). SAR study reveals that azole substituted compounds **5g** and **5h** with imidazole and 1,2,4-triazole at C-6 on the phenyl ring of fluoro benzimidazole were the most active of all the compounds and showed significant inhibitory activity against all the bacterial and fungal cultures. The presence of group like Cl in the phenyl ether analog **5c** and in the unsubstituted fluoro benzimidazole counterpart **5a** also play a significant role in imparting antimicrobial activity to the compound. Compounds **5a** and **5c** demonstrated moderate activity against bacterial and fungal cultures. In constrast, no significant activity was found for other aryl ether **(5b** and **5d-5f)** and amine substituted compounds **(5i, 5j)**. Therefore, one can propose these azole substituted novel

lipophilic compounds **5g** and **5h** as potential antibacterial and antifungal agents. Further structural optimization studies are in progress.

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The authors have declared no conflict of interest

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