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ARTICLE

Synthesis and Evaluation of Novel Fluorobenzimidazole Derivatives as Antibacterial and Antifungal Agents

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ABSTRACT

A new class of fluorobenzimidazole derivatives (**III**$_{a-j}$) was synthesized to investigate their antimicrobial potential. All the compounds were prepared by multiple step synthesis, initiating from the synthesis of 5-(difluoromethoxy)-1*H*-benzimidazole-2-thiol (**I**). The compound **I** was further reacted with different derivatives of 2-chloro-N-phenylacetamide (**II**$_{a-j}$) prepared by reacting differently substituted anilines with chloroacetylchloride and triethylamine in DMF (solvent); resulting in formation of fluorobenzimidazoles **III**$_{a-j}$. The compounds **III**$_{a-j}$ were characterized by spectral analysis *viz.* ^{1}H NMR, ^{13}C NMR, mass spectra, elemental analysis and IR. All these compounds were screened *in vitro* for their antimicrobial activity against Gram-positive (*S. aureus* and *E. faecalis*) and Gram-negative bacterial (*E. coli* and *P.aeruginosa*) strains as well as fungi (*A. niger* and *C. albicans*). Some of the compounds exhibited promising results (in MIC) against Gram-negative bacterial strains.

KEYWORDS

Benzimidazole, Fluorine, Antimicrobial activity.

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INTRODUCTION

In accordance with our earlier work of synthesizing bioactive molecules possessing benzimidazole nucleus [1] we have synthesized and present in this work a series of potent antimicrobial heterocyclic compounds bearing benzimidazole nucleus as the centre. The medicinal properties of benzimidazole as potent anticancer [2,3] and antimicrobial [4-6] have drawn our attention to concentrate research around it. Several other biological properties associated with different benzimidazole derivatives includes antiparasitic [7], analgesic [8], antibacterial [9] and molluscicidal [10] properties. It is also observed that benzimidazole derivatives are found to possess anti-HIV [11], anthelmintic [12], antimycobacterial [13], antidiabetic [14] and antioxidant properties [15]. Several biological properties exhibited by different benzimidazole scaffolds includes antiallergic [16], analgesic [17,18] and antihypertensive activities [19].

Drugs available in the market *viz.* albendazole, mebendazole and bendamustine are found to possess benzimidazole as its core. Literature survey also reveals that the benzimidazole ring containing molecules are potent growth inhibitors over a wide range of bacteria and fungi [20,21]. Synthesis and evaluation

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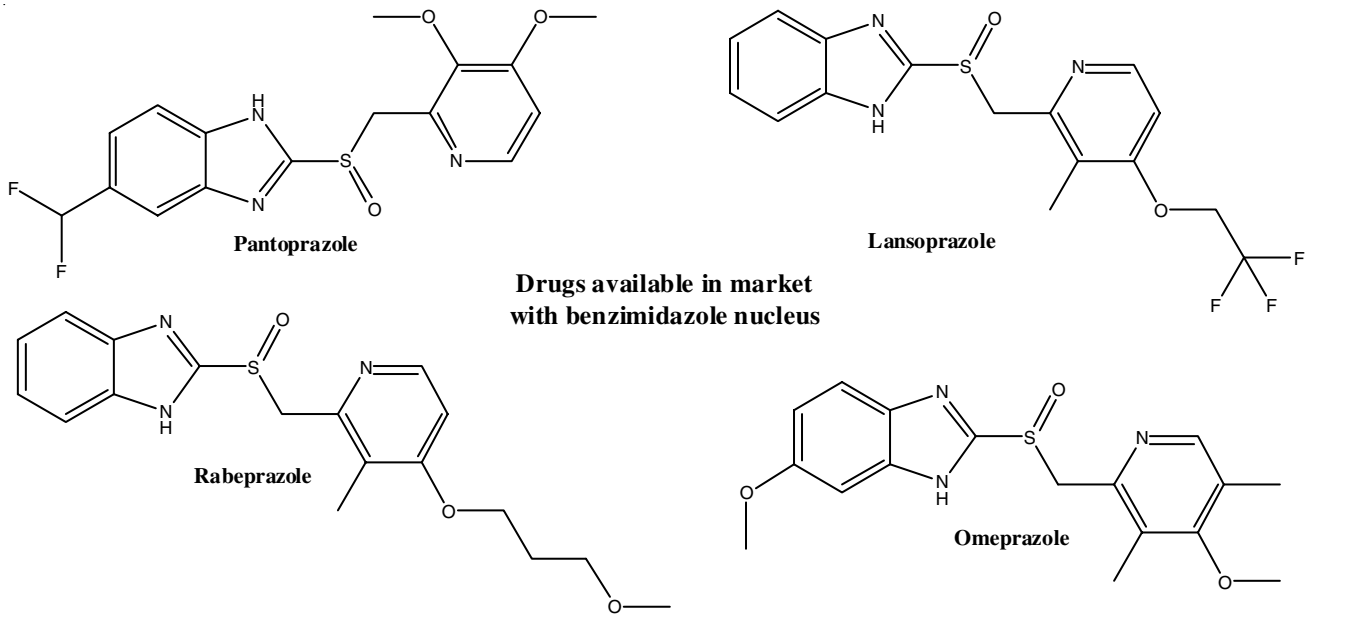


Fig. 1. Drugs with benzimidazole nucleus

of different substituted benzimidazole analogs resulted in the discovery of some important drugs *viz*. omeprazole, lansoprazole, rabeprazole and pantoprazole as shown in Fig. 1. Benzimidazole derivatives are used as CK2 inhibitors against the CK2 proteins responsible for neoplastic growth in animals [22]. Some fused heterocyclic benzimidazole derivatives are used as eukaryotic topisomerase II inhibitors [23]. Variedly substituted benzimidazoles exhibited excellent activity against a wide variety of virus including HIV and several leads possessing benzimidazole nucleus were developed and found capable of ceasing adenoviral replication [24]. The literature survey helped to understand the versatility of benzimidazole and its high potency as a therapeutic agent [25,26], which directed us to synthesize the fluorobenzimidazoles derivatives (**III**$_{a-j}$) as shown in Fig. 2.

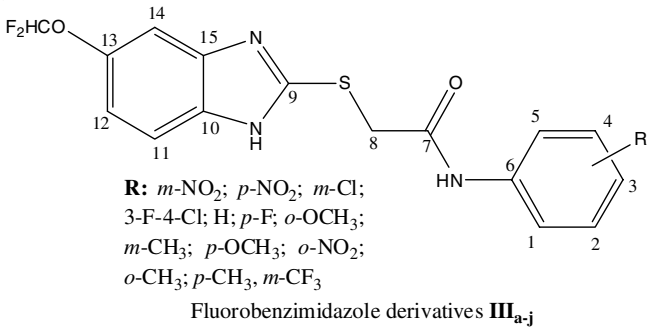


Fig. 2. General structure and carbon enumeration of titled compounds **III**$_{a-j}$

EXPERIMENTAL

All the chemicals and solvents required for the synthesis of fluorobenzimidazole derivatives and corresponding intermediates were purchased from Merck Ltd., SD Fine chemicals, LOBA Chemie and HIMEDIA. Melting points reported here were obtained using open end capillary method and are uncorrected. TLC plates (TLC Silica gel 60 F_{254}) used for monitoring the completion of reaction were purchased from Merck. The IR spectral data were measured by using Bruker FT-IR alpha-t (ATR). The ^{1}H NMR and ^{13}C NMR were obtained using Bruker Spectrophotometer-400 MHz and 100 MHz respectively, where DMSO-d_6 was used as solvent and TMS as reference. The mass spectral analysis was conducted on Shimadzu mass analyzer. The elemental analysis was carried out on Perkin-Elmer 2400 CHN Analyzer.

General procedure

Synthesis of 5-(difluoromethoxy)-1*H*-benzo[*d*]imidazole-2-thiol (I): This compound was obtained by literature procedure [27].

General procedure for the synthesis of 2-chloro-N-(aryl)acetamide derivatives (II$_{a-j}$): Various substituted amines (0.01 mol) were added to a solution of DMF (35 mL) containing triethylamine (3-4 drops). The mixture was stirred for 10 min at room temperature. Chloroacetylchloride (0.015 mol, 113 g/mol, 1.19 mL) was added to the above mixture, maintaining the temperature between 0 to 5 °C. The obtained solution was then stirred at room temperature for 4-6 h. The completion of reaction was monitored with TLC using toluene: acetone (8:2) as mobile phase. The solution was then added onto crushed ice and the separated precipitates were filtered and dried. The product was crystallized from methanol.

General method for the synthesis of final derivatives (III$_{a-j}$): 5-(Difluoromethoxy)-1*H*-benzo[*d*]imidazole-2-thiol (**I**) (0.01 mol, 180 g/mol, 1.8 g) was made soluble in acetone. To this well stirred solution different acetamide derivatives **II**$_{a-j}$ (0.01 mol) were added to the above solution. Potassium carbonate (0.02 mol, 138 g/mol, 2.76 g) was added to the solution containing mixture of 5-methoxy-1*H*-benzo[*d*]imidazole-2-thiol (**I**) and different acetamide derivatives **II**$_{a-j}$. The mixture was allowed to stir for 4 h at room temperature. The completion of reaction was monitored using TLC plate with mobile phase ethyl acetate: *n*-hexane (6:4). The final products thus obtained were poured into ice-cold water and stirred for 30 min. The

precipitates were filtered and washed occasionally. The final products **III$_{a-j}$** obtained were crystallized from alcohol.

Spectral data

5-(Difluoromethoxy)-1*H*-benzo[*d*]imidazole-2-thiol (I): Solid white; Yield: 56 %; m.p.: 241 °C; m.f.: $C_8H_6N_2OF_2S$; elemental analysis (%): Calculated: C, 44.44; H, 2.80; N, 12.96. Found: C, 44.47; H, 2.83; N, 12.99; FTIR (ATR, ν_{max}, cm^{-1}): 1176 (-C-F *str.* -$OCHF_2$), 1583 (-C=N *str.*, benzimidazole nucleus), 2996 (-CH *str.* -$OCHF_2$), 3400 (-NH *str.* sec. amine); ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 6.95 (1H, s, -$OCHF_2$), 6.742-7.91 (3H, m, Ar-H), 10.92 (1H, s, -NH); ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 102.8 (C_{14}), 111.9 (C_{12}), 116.9 (C_{11}), 139.4 (C_{15}), 147.7 (C_9); +ESI-MS (*m/z*): 217.

2-(5-(Difluoromethoxy)-1*H*-benzo[*d*]imidazol-2-ylthio)-N-(3-nitrophenyl)acetamide (III$_a$): Yield: 87 %; m.p.: 182 °C; m.f.: $C_{16}H_{12}N_4O_4SF_2$; elemental analysis (%): Calculated: C, 48.73; H, 3.07; N, 14.21; S, 8.13. Found: C, 48.75; H, 3.10; N, 14.25; S, 5.15; FTIR (ATR, ν_{max}, cm^{-1}): 1168 (-C-F *str.* -$OCHF_2$), 1586 (-C=N *str.* benzimidazole nucleus), 1681 (-C=O *str.*), 2842 (-CH_2 *str.* methylene), 3011 (-CH *str.* -$OCHF_2$), 3095 (-CH *str.* aromatic ring), 3408 (-NH *str.* sec. amine); ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 4.23 (2H, s, -CH_2), 6.95 (1H, s, -$OCHF_2$), 6.54-7.92 (7H, m, Ar-H), 10.96 (1H, s, -NH), 12.32 (1H, s, benzimidazole-NH); ^{19}F NMR (376 MHz, DMSO, ppm): -81.19 (2F, s, -$OCHF_2$); ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 39.1(C_8), 102.8 (C_{14}), 111.9 (C_{12}), 114.6 (C_1), 116.9 (C_{11}), 119.8 (C_3), 139.4 (C_{15}), 147.7 (C_9), 148.4 (C_2), 156.1 (C_{13}), 166.4 (C_7); ESI+MS (*m/z*): 395 (M^+).

2-(5-(Difluoromethoxy)-1*H*-benzo[*d*]imidazol-2-ylthio)-N-(4-nitrophenyl)acetamide (III$_b$): Yield: 66 %; m.p.: 194 °C; m.f.: $C_{16}H_{12}F_2N_4O_4S$; elemental analysis (%): Calculated: C, 48.73; H, 3.07; N, 14.21; S, 8.13. Found: C, 48.76; H, 3.11; N, 14.25; S, 5.16; FTIR (ATR, ν_{max}, cm^{-1}): 1171 (-C-F *str.* -$OCHF_2$), 1579 (-C=N *str.* benzimidazole nucleus), 1685 (-C=O *str.*), 2834 (-CH_2 *str.* methylene), 3001 (-CH *str.* -$OCHF_2$), 3087 (-CH *str.* aromatic ring), 3396 (-NH *str.* sec. amine); ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 4.19 (2H, s, -CH_2), 6.88 (1H, s, -$OCHF_2$), 6.62-8.10 (7H, m, Ar-H), 10.91 (1H, s, -NH), 12.36 (1H, s, benzimidazole-NH); ^{19}F NMR (376 MHz, DMSO, ppm): -81.23 (2F, s, -$OCHF_2$); ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 39.1(C_8), 102.8 (C_{14}), 111.9 (C_{12}), 116.9 (C_{11}), 119.9 (C_1), 124.3 (C_2), 124.3 (C_4), 139.4 (C_{15}), 143.8 (C_3), 147.7 (C_9), 156.1 (C_{13}), 166.4 (C_7); ESI + MS (*m/z*): 395 (M^+).

N-(3-Chlorophenyl)-2-(5-(difluoromethoxy)-1*H*-benzo-[*d*]imidazol-2-ylthio)acetamide (III$_c$): Yield: 89 %; m.p.:154 °C; m.f.: $C_{16}H_{12}N_3O_2SClF_2$; elemental analysis (%): Calculated: C, 50.07; H, 3.15; N, 10.95; S, 8.35. Found: C, 50.11; H, 3.18; N, 10.98; S, 8.38; FTIR (ATR, ν_{max}, cm^{-1}): 1167 (-C-F *str.* -$OCHF_2$), 1583 (-C=N *str.* benzimidazole nucleus), 1691 (-C=O *str.*), 2837 (-CH_2 *str.* methylene), 2998 (-CH *str.* -$OCHF_2$), 3091 (-CH *str.* aromatic ring), 3399 (-NH *str.* sec. amine); ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 4.22 (2H, s, -CH_2), 6.96 (1H, s, -$OCHF_2$), 6.51-8.02 (7H, m, Ar-H), 10.86 (1H, s, -NH), 12.41 (1H, s, benzimidazole-NH); ^{19}F NMR (376 MHz, DMSO, ppm): -81.24 (2F, s, -$OCHF_2$); ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 39.1(C_8), 102.8 (C_{14}), 111.9 (C_{12}), 116.9 (C_{11}), 122.2 (C_1), 128.1 (C_3), 134.7 (C_2), 139.4 (C_{15}), 147.7 (C_9), 156.1 (C_{13}), 166.4 (C_7); ESI+MS (*m/z*): 384 (M^+).

N-(4-Chloro-3-fluorophenyl)-2-(5-(difluoromethoxy)-1*H*-benzo[*d*]imidazol-2-ylthio)acetamide (III$_d$): Yield: 78 %; m.p.:166 °C; m.f.: $C_{16}H_{11}N_3O_2SClF_3$; elemental analysis (%): Calculated: C, 47.83; H, 2.76; N, 10.46; S, 7.98. Found: C, 47.86; H, 2.80; N, 10.49; S, 8.01; FTIR (ATR, ν_{max}, cm^{-1}): 1170 (-C-F *str.* -$OCHF_2$), 1587 (-C=N *str.* benzimidazole nucleus), 1695 (-C=O *str.*), 2829 (-CH_2 *str.* methylene), 3009 (-CH *str.* -$OCHF_2$), 3088 (-CH *str.* aromatic ring), 3410 (-NH *str.* sec. amine); ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 4.12 (2H, s, -CH_2), 6.89 (1H, s, -$OCHF_2$), 6.47-8.09 (6H, m, Ar-H), 10.91 (1H, s, -NH), 12.39 (1H, s, benzimidazole-NH); ^{19}F NMR (376 MHz, DMSO, ppm): -81.17 (2F, s, -$OCHF_2$); ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 39.1(C_8), 102.8 (C_{14}), 111.9 (C_{12}), 116.9 (C_{11}), 111.8 (C_1), 116.5 (C_3), 139.4 (C_{15}), 147.7 (C_9), 156.1 (C_{13}), 163.2 (C_2), 166.4 (C_7); ESI+MS (*m/z*): 402 (M^+).

2-(5-(Difluoromethoxy)-1*H*-benzo[*d*]imidazol-2-ylthio)-N-phenylacetamide (III$_e$): Yield: 56 %; m.p.:182 °C; m.f.: $C_{16}H_{13}N_3O_2SF_2$; elemental analysis (%): Calculated: C, 55.01; H, 3.75; N, 12.03; S, 9.18. Found: C, 55.05; H, 3.78; N, 12.05; S, 9.20; FTIR (ATR, ν_{max}, cm^{-1}): 1173 (-C-F *str.* -$OCHF_2$), 1592 (-C=N *str.* benzimidazole nucleus), 1696 (-C=O *str.*), 2833 (-CH_2 *str.* methylene), 3005 (-CH *str.* -$OCHF_2$), 3092 (-CH *str.* aromatic ring), 3398 (-NH *str.* sec. amine); ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 4.18 (2H, s, -CH_2), 6.91 (1H, s, -$OCHF_2$), 6.57-8.13 (8H, m, Ar-H), 10.93 (1H, s, -NH), 12.41 (1H, s, benzimidazole-NH); ^{19}F NMR (376 MHz, DMSO, ppm): -81.21 (2F, s, -$OCHF_2$); ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 39.1(C_8), 102.8 (C_{14}), 111.9 (C_{12}), 116.9 (C_{11}), 121.6 (C_1), 128.2 (C_3), 128.9 (C_2), 139.4 (C_{15}), 147.7 (C_9), 156.1 (C_{13}), 166.4 (C_7); ESI+MS (*m/z*): 350 (M^+).

2-(5-(Difluoromethoxy)-1*H*-benzo[*d*]imidazol-2-ylthio)-N-(4-fluorophenyl)acetamide (III$_f$): Yield: 85 %; m.p.:156 °C; m.f.: $C_{16}H_{12}N_3O_2SF_3$; elemental analysis (%): Calculated: C, 52.31; H, 3.29; N, 11.44; S, 8.73. Found: C, 52.34; H, 3.32; N, 11.47; S, 8.75; FTIR (ATR, ν_{max}, cm^{-1}): 1166 (-C-F *str.* -$OCHF_2$), 1586 (-C=N *str.* benzimidazole nucleus), 1672 (-C=O *str.*), 2847 (-CH_2 *str.* methylene), 3016 (-CH *str.* -$OCHF_2$), 3087 (-CH *str.* aromatic ring), 3398 (-NH *str.* sec. amine); ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 4.23 (2H, s, -CH_2), 6.93 (1H, s, -$OCHF_2$), 6.62-8.02 (7H, m, Ar-H), 10.96 (1H, s, -NH), 12.37 (1H, s, benzimidazole-NH); ^{19}F NMR (376 MHz, DMSO, ppm): -81.20 (2F, s, -$OCHF_2$); ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 39.1(C_8), 102.8 (C_{14}), 111.9 (C_{12}), 115.7 (C_2), 116.9 (C_{11}), 120.8 (C_1), 139.4 (C_{15}), 147.7 (C_9), 156.1 (C_{13}), 163.2 (C_3), 166.4 (C_7); ESI+MS (*m/z*): 368 (M^+).

2-(5-(Difluoromethoxy)-1*H*-benzo[*d*]imidazol-2-ylthio)-N-(2-methoxyphenyl)acetamide (III$_g$): Yield: 89 %; m.p.:135 °C; m.f.: $C_{17}H_{15}N_3O_3SF_2$; elemental analysis (%): Calculated: C, 53.82; H, 3.99; N, 11.08; S, 8.45. Found: C, 53.85; H, 4.03; N, 11.11; S, 8.48; FTIR (ATR, ν_{max}, cm^{-1}): 1176 (-C-F *str.* -$OCHF_2$), 1577 (-C=N *str.* benzimidazole nucleus), 1679 (-C=O *str.*), 2835 (-CH_2 *str.* methylene), 2997 (-CH *str.* -$OCHF_2$), 3094 (-CH *str.* aromatic ring), 3405 (-NH *str.* sec. amine); ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 4.16 (2H, s, -CH_2), 6.86 (1H, s, -$OCHF_2$), 6.54-8.06 (7H, m, Ar-H), 10.89 (1H, s, -NH), 12.39 (1H, s, benzimidazole-NH); ^{19}F NMR (376 MHz, DMSO, ppm): -81.22 (2F, s, -$OCHF_2$); ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 39.1(C_8), 102.8 (C_{14}), 111.9 (C_{12}), 113.2 (C_2), 116.9

(C_{11}), 128.2 (C_3), 139.4 (C_{15}), 147.7 (C_9), 149.8 (C_1), 156.1 (C_{13}), 166.4 (C_7); ESI+MS (*m/z*): 380 (M^+).

2-(5-(Difluoromethoxy)-1*H*-benzo[*d*]imidazol-2-ylthio)-N-*m*-tolylacetamide (III_h): Yield: 94 %; m.p.:194 °C; m.f.: $C_{17}H_{15}N_3O_2SF_2$; elemental analysis (%): Calculated: C, 56.19; H, 4.16; N, 11.56; S, 8.82. Found: C, 56.22; H, 4.20; N, 11.60; S, 8.85; FTIR (ATR, ν_{max}, cm^{-1}): 1169 (-C-F *str.* -$OCHF_2$), 1572 (-C=N *str.* benzimidazole nucleus), 1674 (-C=O *str.*), 2841 (-CH_2 *str.* methylene), 3001 (-CH *str.* -$OCHF_2$), 3091 (-CH *str.* aromatic ring), 3409 (-NH *str.* sec. amine); ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 4.23 (2H, s, -C<u>H</u>$_2$), 6.90 (1H, s, -$OCHF_2$), 6.47-7.99 (7H, m, Ar-<u>H</u>), 10.94 (1H, s, -N<u>H</u>), 12.31 (1H, s, benzimidazole-N<u>H</u>); ^{19}F NMR (376 MHz, DMSO, ppm): -81.17 (2F, s, -$OCHF_2$); ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 39.1(C_8), 102.8 (C_{14}), 111.9 (C_{12}), 138.8 (C_2), 116.9 (C_{11}), 124.8 (C_3), 139.4 (C_{15}), 147.7 (C_9), 120.4 (C_1), 156.1 (C_{13}), 166.4 (C_7); ESI+MS (*m/z*): 364 (M^+).

2-(5-(Difluoromethoxy)-1*H*-benzo[*d*]imidazol-2-ylthio)-N-(2-nitrophenyl)acetamide (III_i): Yield: 87 %; m.p.:195 °C; m.f.: $C_{16}H_{12}N_4O_4SF_2$; elemental analysis (%): Calculated: C, 48.73; H, 3.07; N, 14.21; S, 8.13. Found: C, 48.75; H, 3.10; N, 14.25; S, 8.15; FTIR (ATR, ν_{max}, cm^{-1}): 1176 (-C-F *str.* -$OCHF_2$), 1583 (-C=N *str.* benzimidazole nucleus), 1680 (-C=O *str.*), 2834 (-CH_2 *str.* methylene), 2995 (-CH *str.* -$OCHF_2$), 3096 (-CH *str.* aromatic ring), 3400 (-NH *str.* sec. amine); ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 4.20 (2H, s, -C<u>H</u>$_2$), 6.96 (1H, s, -$OCHF_2$), 6.51-8.06 (7H, m, Ar-<u>H</u>), 10.86 (1H, s, -N<u>H</u>), 12.36 (1H, s, benzimidazole-N<u>H</u>); ^{19}F NMR (376 MHz, DMSO, ppm): -81.24 (2F, s, -$OCHF_2$); ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 39.1(C_8), 102.8 (C_{14}), 111.9 (C_{12}), 125.5 (C_2), 116.9 (C_{11}), 125.1 (C_3), 139.4 (C_{15}), 147.7 (C_9), 142.7 (C_1), 156.1 (C_{13}), 166.4 (C_7); ESI+MS (*m/z*): 395 (M^+).

2-(5-(Difluoromethoxy)-1*H*-benzo[*d*]imidazol-2-ylthio)-N-(3-(trifluoromethyl)phenyl)acetamide (III_j): Yield: 84 %; m.p.:158 °C; m.f.: $C_{17}H_{12}N_3O_2SF_5$; elemental analysis (%): Calculated: C, 48.92; H, 2.90; N, 10.07; S, 7.68. Found: C, 48.95; H, 2.93; N, 10.11; S, 7.70; FTIR (ATR, ν_{max}, cm^{-1}): 1173 (-C-F *str.* -$OCHF_2$), 1581 (-C=N *str.* benzimidazole nucleus), 1674 (-C=O *str.*), 2840 (-CH_2 *str.* methylene), 3003 (-CH *str.* -$OCHF_2$), 3090 (-CH *str.* aromatic ring), 3403 (-NH *str.* sec. amine); ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 4.17 (2H, s, -C<u>H</u>$_2$), 6.93 (1H, s, -$OCHF_2$), 6.58-8.03 (7H, m, Ar-<u>H</u>), 10.92 (1H, s, -N<u>H</u>), 12.35 (1H, s, benzimidazole-N<u>H</u>); ^{19}F NMR (376 MHz, DMSO, ppm): -81.19 (2F, s, -$OCHF_2$), -61.89 (3F, s, -CF_3); ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 39.1 (C_8), 102.8 (C_{14}), 111.9 (C_{12}), 116.9 (C_{11}), 120.8 (C_3), 125.9 (C_1), 131.4 (C_2), 139.4 (C_{15}), 141.7 (C_6), 147.7 (C_9), 156.1 (C_{13}), 166.4 (C_7); ESI+MS (*m/z*): 418 (M^+).

RESULTS AND DISCUSSION

All the fluorobenzimidazole derivatives (III_{a-j}) were prepared by the procedure depicted in **Scheme-I**. Simultaneously two reactions were undertaken; 4-(difluoromethoxy)benzene-1,2-diamine was reacted with carbon disulphide and KOH in presence of ethanol, resulting in the formation of 5-(difluoromethoxy)-1*H*-benzo[*d*]imidazole-2-thiol (**I**) [27] and differently substituted anilines were reacted with chloroacetylchloride (CAC) and triethylamine (TEA) in presence of DMF, which resulted in the formation of 2-chloro-N-acetamide derivatives (II_{a-j}). The two intermediates thus obtained were condensed in presence of K_2CO_3 (scavenger) to yield titled molecules III_{a-j} in good quantity. The antimicrobial potency of the synthesized derivatives was reported in the form of minimum inhibitory concentration (MIC) values (Table-1).

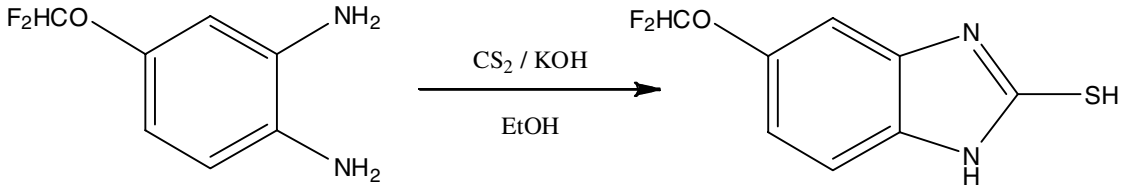
Characterization: The spectral data obtained for the final derivatives III_{a-j} helped to confirm their formation. Let us consider compound III_j and try to characterize its structure from the IR, ^{1}H NMR, ^{13}C NMR and mass analysis. IR spectral data for the compound 2-(5-(difluoromethoxy)-1*H*-benzo-[*d*]imidazol-2-ylthio)-N-(3-(trifluoromethyl)phenyl)acetamide (III_j) has shown a stretching vibration at 3403 cm^{-1} indicating clearly the presence of -NH (secondary amine) in the molecule. The presence of a band at 3003 cm^{-1} confirmed the presence of -C-H bond in the -$OCHF_2$ substitution. The -C-H bond in the aromatic ring can be depicted from a sharp band obtained at 3090 cm^{-1}. A weak absorption band observed at 2840 cm^{-1} helped to prove the formation of the -CH_2 linkage (methylene group) in the molecule. The presence of -C=O (carbonyl) group and -C=N was confirmed by a sharp intensified bands at 1674 and 1581 cm^{-1}, respectively. Also the presence of fluorine atom in form of -C-F band (in -$OCHF_2$) was proved by a sharp band at 1173 cm^{-1}.

The chemical shift observed in ^{1}H NMR spectral data added more confirmation to the formation of final derivatives. In the compounds III_j The absorption peak at δ = 12.35 helped to

TABLE-1
ANTIMICROBIAL DATA OF THE SYNTHESIZED DERIVATIVES (III_{a-j})

-R (Derivatives)	Minimum inhibitory concentration (µg/mL)					
	Gram-positive bacteria		Gram-negative bacteria		Fungi	
	S. aureus	*E. faecalis*	*E. coli*	*P. aeruginosa*	*C. albicans*	*A. niger*
-3-NO_2	250	250	62.5	125	250	250
-4-NO_2	250	500	250	125	500	500
-3-Cl	500	500	500	62.5	500	500
-3-F-4-Cl	500	500	500	62.5	500	500
-H	500	500	500	125	500	500
-4-F	125	250	62.5	125	125	125
-2-OCH_3	500	500	500	125	500	500
-3-CH_3	500	500	500	125	500	500
-2-NO_2	62.5	125	62.5	125	250	250
-3-CF_3	250	250	125	125	250	250
Fluconazole	–	–	–	–	125	62.5
Ciprofloxacin	62.5	125	125	125	–	–

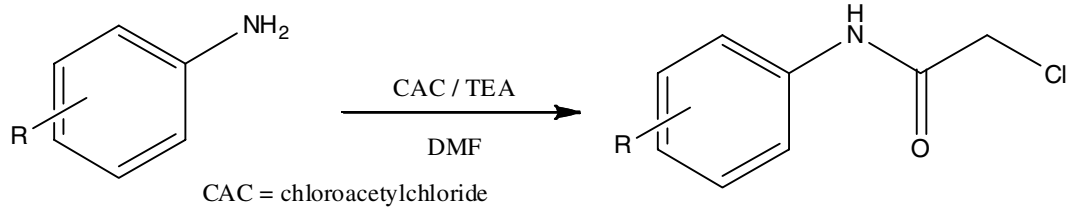
Synthesis of 5-(difluoromethoxy)-1H-benzimidazole-2-thiol I



4-(difluoromethoxy)benzene-1,2-diamine

5-(difluoromethoxy)-1*H*-benzo[*d*]imidazole-2-thiol

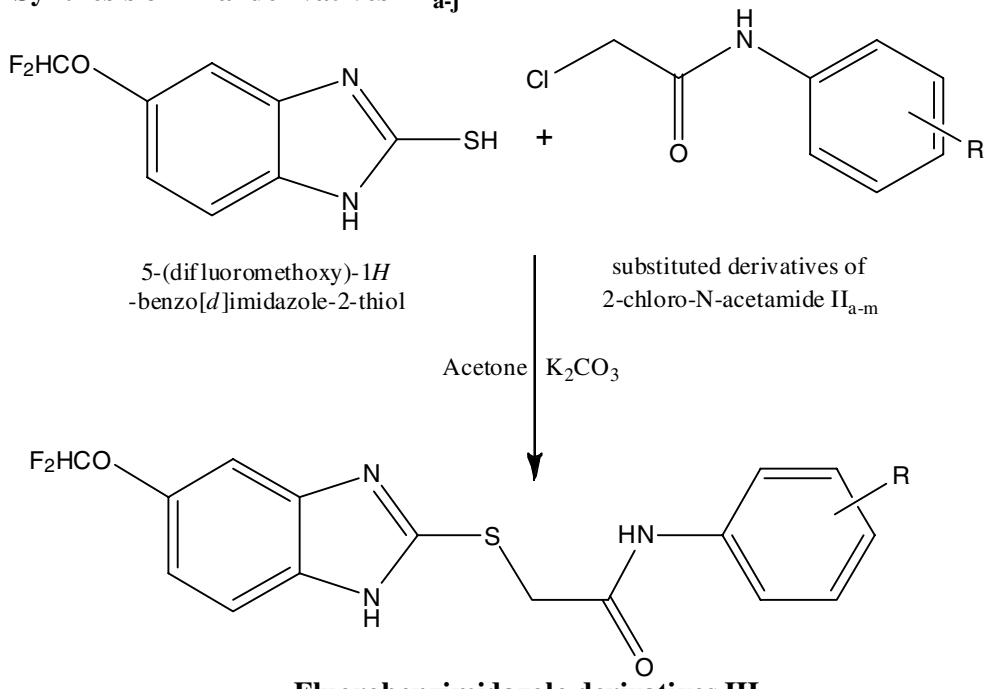
Synthesis of substituted 2-chloro-*N*-acetamide derivatives II_{a-j}



CAC = chloroacetylchloride
TEA = triethylamine

substituted derivatives of 2-chloro-N-acetamide II_{a-m}

Synthesis of final derivatives III_{a-j}



5-(difluoromethoxy)-1*H*-benzo[*d*]imidazole-2-thiol

substituted derivatives of 2-chloro-N-acetamide II_{a-m}

Fluorobenzimidazole derivatives III_{a-j}

Scheme-I: Synthetic route for titled derivatives $\mathbf{III_{a-j}}$

exhibit the presence of proton of secondary amino group (-NH) in the benzimidazole nucleus. The proton of -NH (secondary amine) group in between the methylene and the carbonyl group confirmed its presence with a absorption band at δ value 10.92 ppm. The aromatic protons were proved by a broad band between the δ values 6.58 to 8.03. A sharp absorption band appearing at δ = 4.17 confirmed the presence of $-CH_2$ linkage, as the δ value appeared corresponded to the two protons of the methylene group.

Different chemical shifts observed in the ^{13}C NMR helped to confirm the formation of the final molecules $\mathbf{III_{a-j}}$. The δ values in the spectra were seen to vary between 39.1 to 166.4 ppm values. The absorption peak at 39.1 ppm value proved the presence of $-CH_2$ group as the value correspond to the carbon of methylene group. The -C=O group (C-7) confirmed its presence in the molecules by a band observed at δ value 166.4. The carbon (C-9) present in the benzimidazole nucleus and in vicinity to the sulfur atom was confirmed by a absorption band at δ = 147.7 ppm. The carbon (C-6) of the phenyl ring attached to the -NH group was found at δ value 141.7 ppm. The carbon atom (C-13) attached to the $-OCHF_2$ substitution was seen at a downfield shift of δ = 156.1 ppm. It was observed that with the formation of different derivatives, the δ value of the carbon possessing the substituted functional group varied

accordingly. In compound **III$_a$** (*m*-NO_2) and **III$_b$** (*p*-NO_2), the shift for the carbon atom C-2 and C-3 was observed much downfield than that of a normal carbon bearing proton nearly at 148.4 and 143.8 ppm, respectively. The presence of -Cl in **III$_c$** shifted the absorption band for C-2 at δ = 134.7. Presence of fluorine atom at C-3 in structure **III$_d$** was confirmed by a absorption band at 163.2 ppm. Thus the ^{13}C NMR data helped to confirm the presence of various functional groups with their respective molecules. The carbon enumeration for the general structure of final derivatives is given in Fig. 1.

Antimicrobial activity: The synthesized compounds **III$_{a-j}$** were screened for their antibacterial and antifungal properties against a broad panel of Gram-positive bacteria, Gram-negative bacteria and fungi. The resulting MIC (µg/mL) values were reported in Table-1. It was observed that a few derivatives among the synthesized series were exhibiting excellent activity. Ciprofloxacin drug was used as a standard for antibacterial tests and fluconazole for antifungal activity. The MIC value for ciprofloxacin against Gram-positive bacteria *S. aureus* (ATCC no. 25923) was found to be 62.5 µg/mL. Against the other Gram-positive bacteria *E. faecalis* (ATCC no. 29212) and two Gram-negative bacteria *E. coli* (ATCC no. 25922) and *P. aeruginosa* (ATCC no. 27853); the MIC value for the standard drug ciprofloxacin was observed to be 125 µg/mL. Fluconazole when introduced as a standard drug for antifungal property against *C. albicans* (ATCC no. 10231) and *A. niger* (ATCC no. 1015), was found to show MIC value 125 µg/mL and 62.5 µg/mL respectively. The derivatives were screened for their biological activity by standard protocols like micro dilution/broth titer method. The tests were performed by diluting the samples and preparing the sets with different concentration initiating from 1000 µg/mL up to 7.8 µg/mL.

The newly synthesized compounds **III$_{a-j}$** were tested against the broad panel of Gram-positive and Gram-negative bacteria. A very few derivatives were found to be active against the Gram-positive bacterial strains *S. aureus* and *E. faecalis*. The compounds **III$_i$** (2-NO_2) was found to be exhibiting equivalent activity as that of the standard ciprofloxacin against *S. aureus* (62.5 µg/mL) as well as *E. faecalis* (125 µg/mL). Also compound **III$_f$** (4-F) was found to exhibit excellent activity (62.5 µg/mL) against *E. faecalis* as compared to the activity of standard drug ciprofloxacin against the same bacterial strain. The other derivatives were demonstrating poor activity against the Gram-positive bacterial strains. The same synthesized derivatives **III$_{a-j}$** when tested against Gram-negative bacteria *E. coli* and *P. aeruginosa* were found to exhibit much better activity than that shown against Gram-positive strains. The compounds **III$_a$** (3-NO_2) and **III$_f$** (4-F) showed excellent activity (62.5 µg/mL), even better than the standard (125 µg/mL) against *E. coli*. The compounds **III$_i$** (2-NO_2) and **III$_j$** (*m*-CF_3) also possessed activity (125 µg/mL) equivalent to that of standard drug ciprofloxacin when tested against *E. coli*. The complete series of synthesized motifs were found to exhibit best results against the Gram-negative strain *P. aeruginosa* as compared to the standard. Compounds **III$_c$** (3-Cl), **III$_d$** (3-F-4-Cl) and **III$_f$** (4-F) were found to show excellent activity (62.5 µg/mL) even better than the standard. The other compounds **III$_a$** (3-NO_2), **III$_b$** (4-NO_2), **III$_e$** (H), **III$_g$** (2-OCH_3), **III$_h$** (*m*-CH_3), **III$_i$** (2-NO_2) and **III$_j$** (3-CF_3) also exhibited activity equivalent (125 µg/mL) to that of the standard drug ciprofloxacin. The derivatives **III$_{a-j}$** when tested against the fungal strains *C. albicans* and *A. niger*; it was found that none of the compounds showed good activity even equivalent to that of the standard drug fluconazole. Overall it was found from the antimicrobial data that the derivatives synthesized were good antibacterial but none of them could be used as antifungal due to their poor MIC values as compared to the standard.

SAR study: The structure activity relationship study helped to analyze the impact of different functional group in the derivatives towards the resulting biological data against the broad panel of microorganisms. The variation in the structure of the final derivatives was made possible by the use of both electron-withdrawing and electron-donating substituents. The MIC values presented in Table-1 very well stated that the electron-withdrawing substituents like -NO_2, -F, -Cl and –CF_3 were responsible for the exhibition of excellent biological activity than the derivatives with electron-donating groups. Some of the derivatives bearing electron-withdrawing functional groups demonstrated even lower MIC (µg/mL) value than the standard proving them to be excellent antibacterial molecules.

Conclusion

The derivatives bearing fluorosubstituted benzimidazole nucleus presented in this research article up to a great extent have proved to be potent antibacterial agents. Also from the SAR study, it is much clear that the use of electron-withdrawing functional groups in the final derivatives as substituents has influenced the biological property of the synthesized motifs **III$_{a-j}$**. Also the concept of utilizing electron-withdrawing substituents will be kept in mind while undertaking other scientific work of the same kind. Further optimization will be undertaken in the structure to enhance the antimicrobial property of the derivatives reported.

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