Synthesis and evaluation of novel benzimidazole derivatives as potential antibacterial and anti fungal agents

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ABSTRACT

The novel analogs of the heterocyclic compound benzimidazole were synthesized by alkylation with different small alkylation groups. The antibacterial and antifungal activities of different synthesized benzimidazole compounds have been assessed with a zone of inhibition by well diffusion method, which has shown good activity. The synthesized molecules were subjected to molecular docking studies with a crystal structure of cytochrome P450 14 alpha-sterol demethylase (CYP51) coming through Mycobacterium tuberculosis in composite with fluconazole was collected from PDB ID: 1EA1 and molecular docking research work holding up the antifungal activity exhibiting inhibition and binding energy.

Introduction

Recently, the incidence of systemic fungal infection has become an important complication and a significant cause of disorder and fatality in immune-compromised individuals such as patients going through anticancer chemotherapy or organ transplants. In recent therapeutic chemistry and drug designing, benzimidazole is becoming the first choice for researchers and scientists because of its potential biological activity [3]. Therefore, it becomes an interesting impression for medicinal chemistry researchers. Most of the types of scaffolds are known for their multiple beneficial uses such as their anti-inflammatory [4–6] antibacterial [7–11] antifungal [12–15] antioxidant [16–21] antimalarial [22], anticancer [23,24], antiparasitic [25]. According to the mechanism and from the known six classes of antifungal agents: ergosterol (fungal) synthesis inhibitors (which are class of azoles: voriconazole, fluconazole, and ketoconazole) Figure 1, glucan mixture inhibitors (caspofungin and echinocandins), ergosterol interfering (polyenes antibiotics: amphotericin B), squalene epoxidase inhibitors (terbinafine and naftifine), chitin combination inhibitors (nikkomyacin), and nucleic acid synthesis inhibitors (5-fluorocytosine).

Among heterocyclic pharmacophore, the benzimidazole ring system is rather common. These substructures are often called ‘privileged’ due to their varied recurrence in bioactive compounds. In addition, there is great recognition for benzimidazole ligand and its structural chemistry compounds, the major focus is their biological activities. Benzimidazole-based [26,27] drugs display...
a broad range of different classes of biological activities as a result of substituting groups on central structure Figure 2. In the pharmaceutical, veterinary and agrochemical areas, many derivatives of benzimidazoles were brought to light including misonidazole, cimetidine, antihistamines, clotrimazole, azomycin, thiabendazole, anti-ulcerative (omeprazole), misonidazole and astemizole.

Experimental

Material and method

All the reagents and solvents used for the synthesis were obtained from Sigma Aldrich and Spectrochem and were used as such without further purification. The melting point of all compounds was determined on the Toshniwal apparatus and uncorrected. IR spectra were taken down on Shimadzu FTIR-8400S spectrophotometer KBr pellets. \(^{1}\)H and \(^{13}\)CNMR spectra were recorded in DMSO-\(d_6\) using the TMS as an internal standard on a Bruker spectrophotometer, respectively. Mass spectra of representative compounds were recorded on a JEOL SX-102 spectrometer at 70 eV. Elemental microanalyses were carried out on the model Carlo Erba 1108 CHN analyzer. Thin-layer chromatography was performed on pre-coated gel 60 F254 aluminum sheets (E. Merck, Germany) using various solvent systems and spots were identified by UV light, KMno4, Anisaldehyde and Iodine stains.

Synthesis of 2-(3-bromophenyl)-1 H-benzo[d] imidazole (2a)

A solution of 1,2-phenylenediamine (1) (1.60 g, 1.47 mmol) in acetic acid (10 mL) with 3-bromobenzaldehyde. The (3.28 g,1.77 mmol) was heated at 85°C overnight for 16 h. After completion of reaction was reduced under pressure to afford crude solid material, obtained which
Scheme 1. General synthetic approach to benzimidazole derivatives. Reagents and conditions: (a) (i) For 2a, R₁ = 3-Bromobenzaldehyde, AcOH, 85°C, overnight; For 2i, R₁ = CF₃, CF₂COOH, HCl, reflux; For 2o, R₁ = CH₃ Acetic acid; 4 N HCl reflux, 16 h. (b) R₂-X (1.1eq), Na₂CO₃ (1, 3eq), DMF, RT, 16 h.

was washed using dichloromethane (DCM) and dried to obtain 3.21 g of the title compound off white solid in pure state.

Synthesis of 2-(trifluoromethyl)-1 H-benzo[d]imidazole (2i)
The appropriate 1,2-phenylenediamine (1) (1.60 g, 1.48 mmol), 1.6 equivalents of CF₃COOH and one drop of concentrated HCl were heated under reflux in a N₂ atmosphere for 4 h. Reaction was monitored TLC. After completion was neutralized using sat NaHCO₃ solution, and crude was extracted using AcOEt. Organic layer was concentrated under pressure, and the resulting solid was recrystallized from ethanol.

Synthesis of 2-methyl-1 H-benzo[d]imidazole (2o)
A mixture of o-phenylenediamine (1) (3.24 g, 0.03 mol) in 4 N HCl (20 ml) and (0.09 mol) acetic acid, which was refluxed for 5 h. Reaction was monitored by TLC. After completion of the reaction, concentrated ammonia solution was gradually added and obtained precipitated was recrystallized using 10% aqueous ethanol.

General procedure for synthesis N-alkylation of benzimidazole (3a-3s):-
To a mixture of benzimidazole 2 (2a, 2i and 2o) (1mmol), sodium carbonate (Na₂CO₃) (1.3 mmol) under nitrogen atmosphere in dry DMF (5 ml) were taken in a 50 ml round bottom flask. To resulting mixture alkyl halide (1.1 mmole) was added drop-wise at rt. Reaction mixture was stirred at ambient temperature for 16 h. After completion of the reaction, it was diluted with water (10 ml), aqueous layer was extracted AcOEt (15 mlX2). Organic layer was separated and dried under sodium sulfate, concentrated under reduced pressure, which affords to give crude material purified by using column chromatography 100–200 silica gel. Pure compound was eluted in 10–30% EA: Hexane elute.

2-(3-bromophenyl)-1 H-benzo[d]imidazole (3a):
Off white solid. Yield: 79%, m.p. 254–256°C, FTIR (KBr) in cm⁻¹ 3368 (N-H), 1418 (C = N) ¹H NMR (DMSO-d₆, 500 MHz, δ ppm) 13.06 (s, 1 H, NH), 8.38 (s, 1 H), 8.20 (d, J = 7.8 Hz, 1 H, CH Ar), 7.74–7.63 (m, 2 H, CH Ar), 7.60–7.48 (m, 2 H, CH Ar), 7.24 (dt, J = 14.8, 6.9 Hz, 2 H, CH Ar); ¹³C NMR (DMSO-d₆, 500 MHz, δ ppm) δ 149.76 (s), 133.95 (s), 131.82 (s), 131.48 (d, J = 4.9 Hz), 126.14 (s), 121.41 (s) Calcd. for C₁₃H₉BrN₂ [M + H]⁺ 273.13 Found 273.08

2-(2-(3-bromophenyl)-1 H-benzo[d]imidazol-1-yl) acetonitrile (3b)
Brown Solid, Yield 88%, m.p. 325–327°C, FTIR (KBr) in cm⁻¹ 2260 (CN) 1231, 884,720 (Ar); ¹H NMR (DMSO-d₆, 500 MHz, δ ppm) 8.01 (t,
2-(3-bromophenyl)-1-(cyclopropylmethyl)-1 H-benzo[d]imidazole (3c)

Brown solid, Yield 89%; m.p.: 267–269°C, FTIR (KBr) in cm⁻¹ 1546, 1406, 1352, 1230, 1208, 1002, 889, 771, 731, 674 (Ar); ¹H NMR (DMSO-d₆, 500 MHz, δ ppm) 7.71 (d, J = 9.0 Hz, 2 H, CH Ar), 7.28 (tdd, J = 18.0, 12.8, 7.6 Hz, 2 H, CH Ar), 4.22 (t, J = 10.7 Hz, 2 H, CH2-N), 1.06–0.96 (m, 1 H, CH-CH₃), 0.42–0.33 (m, 2 H, CH₂-CH₂), 0.19–0.11 (m, 2 H, CH₂-CH₂); ¹³C NMR (DMSO-d₆, 500 MHz, δ ppm) 153.35 (s), 139.02 (s), 136.65 (s), 133.19 (d, J = 6.8 Hz), 130.96 (s), 130.30 (s), 125.27 (s), 124.26 (s), 123.05 (s), 121.20 (s), 120.31 (s), 112.52 (s), 46.55 (s), 11.15 (s), 9.01–8.61 (m), HRMS (ESI) Calcd. for C₁₃H₁₀BrN₃ [M + H]^+ 327.23 Found 327.11.

2-(3-bromophenyl)-1-propyl-1 H-benzo[d]imidazole (3d)

White solid, Yield 91%, m.p.: 278°C (dec), FTIR (KBr) in cm⁻¹ 1543, 1427, 1267, 1232, 1052, 877, 818, 780, (Ar); ¹H NMR (DMSO-d₆, 500 MHz, δ ppm) 7.97 (t, J = 1.7 Hz, 1 H, CH Ar), 7.81–7.75 (m, 2 H, CH Ar), 7.69 (dd, J = 11.8, 7.9 Hz, 2 H, CH Ar), 7.55 (t, J = 7.9 Hz, 1 H), 7.34–7.23 (m, 2 H, CH Ar), 4.39–4.18 (m, 2 H, N-CH₂), 1.70 (dd, J = 14.8, 7.4 Hz, 2 H, CH₂-CH₂), 0.74 (t, J = 7.4 Hz, 3H, CH₃-CH₂); ¹³C NMR (DMSO-d₆, 500 MHz, δ ppm) 153.02 (s), 138.86 (s), 136.58 (s), 133.19 (d, J = 6.8 Hz), 130.96 (s), 130.30 (s), 125.27 (s), 124.03 (s), 123.01 (s), 121.20 (s), 120.54 (s), 112.74 (s), 48.31 (s), 21.15 (s), 11.99 (s), HRMS (ESI) Calcd. for C₁₆H₁₅BrN₂ [M + H]^+ 315.21 Found 315.22.

2-(3-bromophenyl)-1-ethyl-1 H-benzo[d]imidazole (3e)

Brown solid, Yield 86%, ¹H m.p.: 310°C (dec), FTIR (KBr) in cm⁻¹ 1542, 1429, 1380, 1330, 1263, 1059, 786, 734, 675 (Ar); ¹H NMR (DMSO-d₆, 500 MHz, δ ppm) 7.97 (t, J = 1.7 Hz, 1 H, CH Ar), 7.82–7.76 (m, 2 H, CH Ar), 7.69 (dd, J = 17.7, 7.9 Hz, 3H, CH Ar), 7.56 (t, J = 7.9 Hz, 1 H, CH Ar), 7.35–7.21 (m, 2 H, CH Ar), 4.33 (q, J = 7.2 Hz, 2 H, N-CH₂), 1.33 (t, J = 7.2 Hz, 3H, CH₃-CH₂); ¹³C NMR (DMSO-d₆, 500 MHz, δ ppm) 152.70 (s), 138.71 (s), 136.50 (s), 133.19 (d, J = 6.8 Hz), 130.96 (s), 130.30 (s), 125.27 (s), 123.79 (s), 122.93 (s), 121.20 (s), 120.72 (s), 112.99 (s), 41.69 (s), 13.65 (s), HRMS (ESI) Calcd. for C₁₅H₁₃BrN₂ [M + H]^+ 301.19 Found 301.22.

2-(3-bromophenyl)-1-methyl-1 H-benzo[d]imidazole (3f)

White solid, Yield 93%, m.p.: 290°C (dec), FTIR (KBr) in cm⁻¹ 1497, 1440, 986, 820, 786, 730, 678 (Ar); ¹H NMR (DMSO-d₆, 500 MHz, δ ppm) 8.06 (dt, J = 3.4, 1.4 Hz, 1 H, CH Ar), 7.91–7.82 (m, 1 H, CH Ar), 7.81–7.74 (m, 1 H, CH Ar), 7.72 (dd, J = 14.0, 6.0 Hz, 1 H, CH Ar), 7.64 (d, J = 8.0 Hz, 1 H, CH Ar), 7.59–7.50 (m, 1 H, CH Ar), 7.37–7.17 (m, 3H, CH Ar), 3.90 (s, 3H, N-CH₃); ¹³C NMR (DMSO-d₆, 500 MHz, δ ppm) 153.34 (s), 136.66 (s), 136.46 (s), 133.39 (s), 133.07 (s), 131.02 (s), 130.34 (s), 125.34 (s), 124.16 (s), 123.41 (s), 121.29 (s), 120.45 (s), 109.91 (s), 29.62 (s), HRMS (ESI) Calcd. for C₁₅H₁₃BrN₂ [M + H]^+ 287.16 Found 287.2278.

1-allyl-2-(3-bromophenyl)-1 H-benzo[d]imidazole (3g)

Brown solid, Yield 87%, m.p.: 288°C (dec), FTIR (KBr) in cm⁻¹ 1497, 1440, 986, 820, 786, 730, 678 (Ar); ¹H NMR (DMSO-d₆, 500 MHz, δ ppm) 7.88 (s, 1 H, CH Ar), 7.63 (d, J = 29.2 Hz, 2 H, CH Ar), 7.53 (d, J = 5.9 Hz, 2 H, CH Ar), 7.34–7.23 (m, 3H, CH Ar), 5.81 (s, 1 H, CH = CH₂), 5.09 (d, J = 6.8 Hz, 2 H, CH₂ = CH), 4.65 (d, J = 6.7 Hz, 2 H, N-CH₂); ¹³C NMR (DMSO-d₆, 500 MHz, δ ppm) 153.09 (s),
2-(3-bromophenyl)-1-(oxetan-3-yl)-1 H-benzo[d]imidazole (3 h)

Yellowish solid, Yield: 84%; m.p.: 290–294°C, 1H (DMSO-d6, 500 MHz, δ ppm) 7.89 (s, 1 H, CH Ar), 7.65–7.46 (m, 3H, CH Ar), 7.33 (d, J = 5.0 Hz, 2 H, CH Ar), 7.24 (d, J = 12.8 Hz, 3H, CH Ar), 5.34–5.27 (m, 4H, O-CH2), 5.06–5.02 (m, 1 H), 4.92 (s, 1 H); 13C NMR (DMSO-d6, 500 MHz, δ ppm) 151.52 (s), 141.12 (s), 136.44 (s), 133.40 (s), 132.99 (s), 130.96 (s), 130.32 (s), 125.27 (s), 123.45 (s), 122.85 (s), 121.11 (s), 120.04 (s), 113.85 (s), 76.78–76.57 (m), 52.90 (s). HRMS (ESI) Calcd. for C16H13BrN2O [M + H]+ 329.20 Found 329.22

2-(trifluoromethyl)-1 H-benzo[d]imidazole (3i)

White solid, Yield: 80%; m.p.: 209–211°C; FTIR (KBr) in cm⁻¹ 3520 (N-H), 1620 (C = N), 1392, 1117, 745, 710,596,558 (Ar); 1H (DMSO-d6, 500 MHz, δ ppm) 14.15 (s, 1 H, NH), 7.81–7.47 (m, 2 H, CH Ar), 7.45–7.17 (m, 2 H, CH Ar); 13C NMR (DMSO-d6, 500 MHz, δ ppm) 139.52 (s), 137.69 (s), 137.50 (s), 123.62 (s), 123.33 (s), 118.49 (s), 116.43 (d, J = 327.1 Hz), 114.59 (s), HRMS (ESI) Calcd. for C6H13F3N2 [M + H]+ 186.14 Found 186.22

1-methyl-2-(trifluoromethyl)-1 H-benzo[d]imidazole (3 j)

Off white solid, Yield: 91%; m.p.: 95–97°C, FTIR (KBr) in cm⁻¹ 1650 (C = N), 1467, 1249, 1161,1105,825,792,714 (Ar); 1H NMR (DMSO-d6, 500 MHz, δ ppm) 7.80 (dd, J = 11.6, 8.2 Hz, 2 H, CH Ar), 7.51–7.45 (m, 1 H, CH Ar), 7.38 (ddd, J = 8.2, 7.2, 1.0 Hz, 1 H, CH Ar), 3.97 (d, J = 0.8 Hz, 3H, N-CH3); 13C NMR (DMSO-d6, 500 MHz, δ ppm) 140.58 (s), 136.66 (s), 136.46 (s), 124.50 (d, J = 34.3 Hz), 124.16 (s), 123.02 (t, J = 166.9 Hz), 120.45 (s), 109.91 (s), 31.68 (s). HRMS (ESI) Calcd. for C6H13F3N2 [M + H]+ 200.16 Found 200.44

1-(oxetan-3-yl)-2-(trifluoromethyl)-1 H-benzo[d]imidazole (3k)

Brown solid, yield: 94%, m.p.: 195–198°C, FTIR (KBr) in cm⁻¹ 1653 (C = N), 1102,826,792,712 (Ar); 1H NMR (DMSO-d6, 500 MHz, δ ppm) 7.61–7.45 (m, 2 H, CH Ar), 7.15–6.99 (m, 3H, CH Ar), 5.21 (dd, J = 10.7, 4.2 Hz, 2 H, O-CH2), 5.09 (dd, J = 13.6, 6.5 Hz, 2 H, O-CH2), 4.85 (t, J = 7.1 Hz, 1H, NH); 13C NMR (DMSO-d6, 500 MHz, δ ppm) 141.12 (s), 136.75 (d, J = 76.8 Hz), 123.45 (s), 122.85 (s), 120.68 (s), 120.04 (s), 113.85 (s), 76.78–76.57 (m), 55.59 (s), HRMS (ESI) Calcd. for C8H9F3N2O [M + H]+ 242.20 Found 242.22

1-propyl-2-(trifluoromethyl)-1H-benzo[d]imidazole (3 l)

Colorless liquid, yield: 90%, b.p.: 289–291°C, FTIR (KBr) in cm⁻¹ 1649 (C = N), 1413,1106,825,792,712 (Ar); 1H NMR (DMSO-d6, 500 MHz, δ ppm) 7.93–7.71 (m, 2H, CH Ar), 7.51–7.41 (m, 1H, CH Ar), 7.44–7.30 (m, 1H, CH Ar), 4.41–4.27 (m, 2H, N-CH2), 1.89–1.69 (m, 2H, N-CH2), 0.87 (dt, J = 35.4, 7.4 Hz, 3H, C-C2H3), 13C NMR (500 MHz, DMSO-d6) δ 140.46 (s), 138.86 (s), 136.58 (s), 124.03 (s), 123.01 (s), 122.81–112.66 (m), 112.74 (s), 112.74 (s), 51.49 (s), 21.15 (s), 11.99 (s), HRMS (ESI) Calcd. for C11H11F3N2 [M + H]+ 228.22 Found 282.20

1-allyl-2-(trifluoromethyl)-1H-benzo[d]imidazole (3 m)

Yellowish liquid, yield: 91%, b.p.: 263–265°C, 1H NMR (DMSO-d6, 500 MHz, δ ppm) 7.56 (d, J = 6.3 Hz, 2H, CH Ar), 7.22 (d, J = 14.3 Hz, 2H, CH Ar), 5.83 (m, 1H, CH = CH2), 4.98 (d, J = 6.8 Hz, 2H, CH2 = CH2), 4.48 (d, J = 30.8 Hz, 2H, CH2 = CH2), 13C (DMSO-d6, 500 MHz, δ ppm) 140.90 (s), 139.02 (s), 136.65 (s), 134.36 (s), 124.26 (s), 132.46–101.65 (m),...
117.27 (s), 114.89 (d, J = 593.9 Hz), 112.52 (s), 45.85 (s), HRMS (ESI) Calcd. for C_{11}H_{9}F_{3}N_{2} [M + H]^+ 226.20 Found 226.22

1-(cyclopropylmethyl)-2-(trifluoromethyl)-1H-benzo[d]imidazole (3n)
Colorless liquid, yield: 95%; b.p.: 310–313°C, 1H NMR (DMSO-d$_6$, 500 MHz, δ ppm) 7.57 (d, J = 13.1 Hz, 2 H, CH Ar), 7.25 (d, J = 18.5 Hz, 2 H, CH Ar), 4.27 (d, 1 H, N-CH$_2$), 3.65 (d, 1 H, N-CH$_2$), 1.11 (s, 1 H, CH-CH$_2$), 0.80–0.76 (m, 2 H, CH$_2$-CH$_2$), 0.47–0.43 (m, 2 H, CH$_2$-CH$_2$), 13C NMR (DMSO-d$_6$, 500 MHz, δ ppm) 140.51 (s), 139.02 (s), 136.65 (s), 124.26 (s), 123.05 (s), 121.10 (s), 120.31 (s), 112.52 (s), 50.12 (s), 11.15 (s), 9.01–8.61 (m), HRMS (ESI) Calcd. for C$_{12}$H$_{13}$F$_3$N$_2$ [M + H]^+ 240.23 Found 240.22

2-methyl-1H-benzo[d]imidazole (3q)
Colorless liquid, yield: 87%; b.p.: 314–316°C, FTIR (KBr) in cm$^{-1}$ 1678 (C = N), 1499, 1437, 1387,1343,1120,996,733 (Ar); 1H NMR (DMSO-d$_6$, 500 MHz, δ ppm) 7.50 (dd, J = 18.0, 7.4 Hz, 2H, CH Ar), 7.15 (dtdd, J = 18.7, 7.3, 1.2 Hz, 2H, CH Ar), 4.13 (t, J = 7.2 Hz, 2H, N-CH$_2$), 2.13 (s, 3H, CH$_3$-CH$_2$), 1.73 (dd, J = 14.6, 7.3 Hz, 2H, CH$_2$-CH$_3$), 0.87 (t, J = 7.4 Hz, 3H, CH$_3$-CH$_2$), 13C NMR (500 MHz, DMSO-d$_6$) δ 162.22 (s), 139.15 (s), 135.78 (s), 123.26 (s), 121.76 (s), 118.82 (s), 113.04 (s), 48.16 (s), 21.15 (s), 15.41 (s), 11.99 (s), HRMS (ESI) Calcd. for C$_{11}$H$_{14}$N$_2$ [M + H]^+ 174.20 Found 174.22

2-(2-methyl-1H-benzo[d]imidazol-1-yl) acetonitrile (3r)
Brown solid, yield: 89%; b.p.: 320(dec), FTIR (KBr) in cm$^{-1}$ 2269 (CN), 1646 (C = N), 1503, 1368, 1301,1267,998,732 (Ar); 1H NMR (DMSO-d$_6$, 500 MHz, δ ppm) 7.60–7.54 (m, 2H, CH Ar), 7.18 (dt, J = 7.6, 2.1 Hz, 2H, CH Ar), 5.31 (s, 1H, N-CH$_2$), 4.58 (d, J = 25.6 Hz, 1H, N-CH$_2$), 2.49 (s, 3H, CH$_3$-CH) 13C NMR (DMSO-d$_6$, 500 MHz, δ ppm) 159.12 (s), 139.20 (s), 136.04 (s), 123.50 (s), 121.14 (s), 118.23 (s), 116.86 (s), 112.43 (s), 37.13 (s), 15.41 (s), HRMS (ESI) Calcd. for C$_{10}$H$_9$N$_3$ [M + H]^+ 172.20 Found 172.22

1H-benzo[d]imidazole (3s)
White solid, yield: 96%; b.p.: 170–173°C, FTIR (KBr) in cm$^{-1}$ 1437 (C = N),1387,1346,1284, 1255,1227,1118,945,873,758,735,620,513 (Ar); 1H NMR (DMSO-d$_6$, 500 MHz, δ ppm) 7.99 (s, 1H, NH), 7.61–7.47 (m, 2H, CH Ar), 7.23 (d, J = 15.0 Hz, 2H, CH Ar), 6.83 (s, 1H, CH-CH) 13C NMR (500 MHz, DMSO-d$_6$) δ 144.34 (s), 139.25 (s), 137.02 (s), 122.45 (s), 121.71 (s), 117.85 (s), 114.01 (s), HRMS (ESI) Calcd. for C$_{7}$H$_{8}$N$_2$ [M + H]^+ 118.14 Found 3118.240

1, 2-dimethyl-1H-benzo[d]imidazole (3p)
Off white solid, yield: 94%; m.p.: 112–114°C, FTIR (KBr) in cm$^{-1}$ 1492,1429,1381,1311, 1270,1223, 990,721,560 (Ar); 1HNMR (DMSO-d$_6$, 500 MHz, δ ppm) 7.53 (t, J = 10.6 Hz, 1H, CH Ar), 7.43 (t, J = 10.0 Hz, 1H, CH Ar), 7.17 (dtdd, J = 16.2, 7.3, 1.2 Hz, 2H, CH Ar), 3.69 (s, 3H, N-CH$_3$); 13C NMR (DMSO-d$_6$, 500 MHz, δ ppm) 163.38 (s), 136.31 (s), 135.87 (s), 122.89 (s), 122.66 (s), 118.48 (s), 110.11 (s), 28.70 (s), 15.38 (s), HRMS (ESI) Calcd. for C$_{9}$H$_{10}$N$_2$ [M + H]^+ 146.19 Found 146.22
Biological activity

Antibacterial activity and antifungal activity

Antibacterial and antifungal activity [28] studies against Bacterial strains gram-positive Streptococcus pyogenes, Staphylococcus aureus gram-negative Pseudomonas aeruginosa, Escherichia coli and Fungal strains Candida albicans, Aspergillus clavatus were selected based on pharmacological and clinical significance. The bacterial microorganisms were cultured on nutrient agar/YPEP by making use of spread plate methodology and fungal stock cultures were incubated for 24 hours at 37°C on potato dextrose agar (PDA) medium (Microcare laboratory, Surat, India), following refrigeration storage at 4°C. Bacterial strains were developed in Mueller-Hinton agar (MHA) plates at temperature 37°C (bacteria developed in nutrient broth and retained as for nutrient agar slants at 4°C), while molds and yeasts were developed in PDA media and sabouraud dextrose agar respectively at 28°C. The typical stock cultures were kept at 4°C.

Method for determination of Zone of inhibition

Both antibacterial and antifungal activities in vitro were examined for sample by the Zone of inhibition method [29]. Activities of samples against two gram-positive, two-gram negative, two fungi, and pathogenic bacteria were investigated by the method of agar disk diffusion. Each compound was dissolved in DMSO (dimethyl sulfoxide), and after sterilization, it was filtered by making use of sintered glass filter, which was kept at 4°C. As for the calculation of zone of inhibition, Gram-negative, Gram-positive, and fungal strains were considered a standard antibiotic for collation of results. All the compounds were tested for their respective antibacterial and antifungal activities against gram-positive Streptococcus Pyogenes, Staphylococcus aureus, and gram-negative Pseudomonas aeruginosa, Escherichia coli, and fungal strains Candida albicans, Aspergillus clavatus. The compound dilutions (25 μg/ml) and respective standard drugs (5, 25, and 50 μg/ml) were provided in double-distilled water by utilizing nutrient agar tubes. The plates of Mueller-Hinton sterile agar were seeded by standard bacterial strains (108 cfu) and which were allowed to continue at 37°C for 3 hours with controlled experiments and under a similar environment using Cefixime and Griseofulvin as std. drugs. From the disks zone of developed inhibition were evaluate succeeding of 18 to 24 hours of incubation at temperature 37°C bacteria and 48 h to 96 h for fungi at 28°C. The sensitiveness of microorganism to plant extracts were calculated by using dimensions of inhibitory zones (counting diameter of a disk) of the agar surface region of the disk and value < 8 mm were measured as not active.

Disk diffusion test

It is also known as the Agar Diffusion test (Mueller-Hinton test) [30]. It is a test in which employs antibiotic-saturated wafers to test either certain bacteria are susceptible to distinct antibiotics. A known amount of bacteria are developed on agar plates in the existence of thin wafers carrying significant antibiotics. If the bacteria are sensitive to certain antibiotics, the region of clearing close around the wafer where bacteria are not effective of enlarging called zone of inhibition.

Biological assay

Antibacterial activity

For bacterial strain S. aureus, it can be seen that the compounds 3 f, 3p showed excellent inhibitory activity with MIC value 23 μg/mL each which is very close to Cefixime (MIC 29 μg/mL). For bacterial strain S. pyogenes, compounds 3b, 3 c, 3e, 3 j, and 3p exhibited very close two-fold antibacterial activity with MIC value 26 μg/mL and compounds 3 m,3 n, 3q and 3 r with MIC value 21 μg/
| Entry | Comp'd | R₁   | R₂   |
|-------|--------|------|------|
| 1     | 3a     | ![Image](3a) | H    |
| 2     | 3b     | ![Image](3b) | CN   |
| 3     | 3c     | ![Image](3c) | ![Image](3c) |
| 4     | 3d     | ![Image](3d) | ![Image](3d) |
| 5     | 3e     | ![Image](3e) | ![Image](3e) |
| 6     | 3f     | ![Image](3f) | ![Image](3f) |
| 7     | 3g     | ![Image](3g) | ![Image](3g) |
| 8     | 3h     | ![Image](3h) | ![Image](3h) |
| 9     | 3i     | ![Image](3i) | H    |
| 10    | 3j     | ![Image](3j) | ![Image](3j) |
| 11    | 3k     | ![Image](3k) | ![Image](3k) |
| 12    | 3l     | ![Image](3l) | ![Image](3l) |
| 13    | 3m     | ![Image](3m) | ![Image](3m) |
| 14    | 3n     | ![Image](3n) | ![Image](3n) |
| 15    | 3o     | ![Image](3o) | H    |
| 16    | 3p     | ![Image](3p) | ![Image](3p) |
| 17    | 3q     | ![Image](3q) | ![Image](3q) |
| 18    | 3r     | ![Image](3r) | ![Image](3r) |
| 19    | 3s     | H    | H    |
Griseoulvin. In addition, 3 h, 3 r showed 17 μg/mL, 3 f, 3 p showed 18 μg/mL, 3 g, 3 q 19 μg/mL. 3 e, 3 j and 3 o exhibited 20 μg/mL and finally 3 b, 3 l showed 22 μg/mL, which very close to onefold against fungicidal strains Aspergillus Clavatus (Table 3).

### Computational chemistry

#### Molecular modeling

Molecular modeling studies has constantly been proven to be a robust tool for justifying and ranking the conformation using a scoring function and also helps in finding the interactions for making this information available to virtual screening techniques. In addition, it also helps us to propose structural hypotheses of how the ligand inhibits the target. The enzymatic database that was developed from the total set of compounds was docked into the certain binding province. Molecular docking was achieved using the Surflex-Dock program that is associated with Sybyl-X 2.0 [31]. The crystal
Table 4. Surflex Docking score (kcal/mol) of the derivatives.

| Sr. No | Compounds | Total  | Crash | Polar | D Score<sup>a</sup> | PMF Score<sup>b</sup> | G Score<sup>c</sup> | Chem Score<sup>d</sup> |
|-------|-----------|--------|-------|-------|---------------------|----------------------|------------------|---------------------|
| 1     | Ligand    | 8.47   | −1.09 | 2.81  | −116.64             | −133.946             | −218.184         | −19.507             |
| 2     | 3b        | 4.34   | −1.07 | 0     | −79.64              | −85.631              | −202.769         | −30.803             |
| 3     | 3c        | 5.66   | −0.66 | 0     | −104.42             | −89.749              | −221.427         | −35.302             |
| 4     | 3d        | 5.48   | −1.09 | 0     | −89.533             | −86.446              | −224.838         | −33.728             |
| 5     | 3e        | 5      | −0.61 | 0.05  | −98.198             | −92.785              | −199.908         | −32.595             |
| 6     | 3f        | 4.58   | −0.72 | 0     | −73.397             | −72.915              | −172.924         | −32.079             |
| 7     | 3g        | 5.36   | −0.51 | 0     | −101.39             | −90.45               | −210.082         | −34.674             |
| 8     | 3h        | 5.03   | −0.73 | 0     | −105.51             | −97.253              | −201.074         | −32.868             |
| 9     | 3k        | 6.12   | −0.44 | 2.65  | −60.924             | −106.954             | −150             | −29.691             |
| 10    | 3l        | 6.39   | −0.95 | 0     | −62.375             | −99.239              | −200.954         | −26.048             |
| 11    | 3m        | 7.42   | −0.61 | 0     | −60.657             | −93.082              | −204.258         | −25.857             |
| 12    | 3n        | 7.48   | −0.77 | 0     | −65.599             | −92.922              | −214.385         | −27.588             |
| 13    | 3r        | 5.14   | −0.11 | 2.8   | −34.165             | −62.764              | −118.481         | −23.866             |

<sup>a</sup>Cscore (Consensus Score) integrates a number of popular scoring functions for ranking the affinity of ligands bound to active site of a receptor and reports the output of total score.

<sup>b</sup>Crash-score disclosing the unsuitable penetration into binding site. Crash scores close to 0 are favorable. Negative numbers specify penetration.

<sup>c</sup>Polar showing contribution of the polar interactions to total score. Polar score may be helpful for eliminating docking results that build no hydrogen bonds.

<sup>d</sup>D-score for charge and van der Waals reciprocal action between protein and ligand.

<sup>e</sup>PMF-score specifying Helmholtz free energies of synergies for protein-ligand atom pairs (Potential of Mean Force, PMF).

<sup>f</sup>G-score represents hydrogen bonding, complex (ligand-protein), and also inside (ligand-ligand) energies.

<sup>g</sup>Chem-score spikes for Hydrogen-bonding, lipophilic exposure, and rotational entropy, through an intercept term.

The structure of Cytochrome P450 14 alpha-sterol demethylase (CYP51) from Mycobacterium tuberculosis in composite with fluconazole was collected from PDB under code 1EA1 (X-Ray Diffraction; 2.21 Å) [32] and was extracted from the Brookhaven Protein Database (PDB: http://www.rcsb.org/pdb). The file was prepared for docking by adding a polar hydrogen atom with MMFF94 charges and water molecules were removed. The 3D structure of ligand, which was produced by the SKETCH module complies with the SYBYL application (Tripos Inc., St. Louis, USA) in addition its energy-reduced confirmation was acquired with the assistance of Tripos force field by using MMFF94 [1] charges and molecular docking was achieved with Surflex-Dock program that is interfaced with
Figure 4. Docked view of compound 3 \textit{n} at active site of enzyme PDB: 1EA1.

Figure 5. Docked view of compound 3 \textit{m} at active site of enzyme PDB: 1EA1.
**Figure 6.** Interaction of Flucanazole at the binding site of the enzyme (PDB ID: 1AE1).

**Figure 7.** A Hydrophobic amino acids enclosed to compounds 3 n (green color) and 3 m (cyan color). B Hydrophilic amino acids enclosed to compounds 3 n and 3 m.
Sybyl-X 2.0 and other miscellaneous parameters were allocated with default figures stated by the software.

**Results and discussion**

Surflex-docking was engaged to comprehend the interaction between the enzyme and different classes of synthesized benzimidazole derivatives and to elucidate the synergy in a mechanism.

As stated in antifungal activity section, compounds 3 m and 3 n were found to be the active derivatives against *S. aureus*, *S. pyrogenes*, *E. coli*, *C. albicans*, *A. Clavatus* with 16, 21, 18 and 16 μg/mL and 20, 25, 17 and 17 μg/mL MIC values, respectively. Thus, the main purpose of docking studies was to investigate the possible interaction of these compounds with enzyme. All inhibitors were docked [33] into the enzyme’s active site, and three-dimensional poses of the compounds are presented in **Figure 3(A-B)**. The forecasted binding energies of the synthesized benzimidazole compounds are recorded in **Table 4**. The docking poses in **Figure 4(A-C)** the compound 3 n makes two hydrogen-bonding interactions at the enzymes active site (PDB ID: 1EA1), interactions came from the fluorine atom of the trifluoromethyl group with hydrogen atoms of ARG96 and GLN72 (F – – H-ARG96, 2.66 Å; F – – H-GLN72, 2.67 Å). This evidence confirmed that suitable functional groups on imidazole ring second position were necessary for better antifungal activities in designing the molecule. Similarly, as shown in **Figure 5(A-C)**, compound 3 m, makes two hydrogen bonding interactions at the active site of the enzyme (PDB ID: 1EA1), interactions came from the two fluorine atoms of the trifluoro methyl group with hydrogen atoms of ARG96 and GLN72 (F – – H-ARG96, 2.41 Å; F – – H-GLN72, 2.54 Å). Thus, these results suggest that electron withdrawing groups play important roles in the antifungal activities of these tested compounds.

The binding interaction of standard Flucanazole (ligand) with Cytochrome P450 14 alpha-sterol demethylase (CYP51) active sites shows one bonding interaction, and the docked view of the same is shown in **Figure 6(A-C)**. The synthesized benzimidazole derivatives bind to the enzyme in a similar fashion as that of ligand. The compounds have the same H-bonding interaction with the same amino acid ARG96 as that of ligand. As shown in **Figure 7 (A-B)** both amino acids hydrophobic and hydrophilic adjacent to the studied compounds 3 m and 3 n.

**Conclusion**

In summary by the combination of benzimidazole with N-alkyl small pharmacophore. We obtained a novel class of benzimidazole derivatives endowed with significant antibacterial and antifungal activities as well as good potential molecular docking studies with a crystal structure. These results further encourage us in developing an enlarged synthetic study in order to highlight SAR in this interesting class of molecules.

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