Facile Synthesis, Characterization and *in vitro* Antibacterial Efficacy of Functionalized 2-Substituted Benzimidazole Motifs

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Abstract: *A series of functionalized 2-substituted benzimidazole motifs was designed and successfully synthesized via thermal cyclization of 1,2-diaminobenzene on COOH end of L- leucine to achieve benzimidazole derivatives* ***6*** *as the essential precursor. The coupling of the precursor* ***6*** *with benzaldehyde derivatives* ***a-h****, ketone series* ***i-k,*** *and aryl sulfonyl chlorides* ***l-n*** *led to the formation of the targeted 2-substituted benzimidazole motifs* ***7a-n*** *in improved yields. The targeted benzimidazole motifs were structurally authenticated through their spectral data and microanalytical parameters. The targeted final moieties were investigated for potential antimicrobial activity using the agar diffusion method with gentamicin as the clinical standard. All the compounds had a broad spectrum of activity with compound* ***7k*** *having the highest remarkable activity with MIC of 0.98 ± 0.02 µg/mL and MBC value of 3.91 ± 0.10 µg/mL. These findings suggest that compound* ***7k*** *containing camphor might be a good candidate for the design of new antimicrobial small-molecule drugs.*

Keywords: *benzimidazole; [4+1]-cycloaddition; serial dilution; SAR study; antibacterial*

■ INTRODUCTION

Over the years, heterocyclic compounds have been reported to be biomimetically-useful and pharmacophorically-sensitive frameworks with high essentiality in accessing biomolecular drugs and drug-like candidates in drug design [1]. One of the *N*-heterocyclic templates of high diversity in the pharmacological adventure are benzimidazole motifs, and they are known to be unavoidable rubrics in high through screening and identification of valuable lead targets in therapeutic research [2]. They possess observable biochemical interaction with many biomolecules in the body system because of their structural resemblance to some naturally existing nucleotides [3]. The natural occurrence of benzimidazole was reported in vitamin B_{12} to contain *N*-ribosyl-dimethyl benzimidazole, which functioned in the form of cobalt metal axial ligand [4]. The methods for the benzimidazoles design and preparation have gained topmost priority in the organic chemists' scale of preference due to the application of these scaffolds in many areas of human endeavors [5].

Although, numerous methods for harnessing benzimidazole derivatives are available in the current literature; nonetheless, the most common and easily adaptable method involves the [4+1]-cycloaddition of *o*-phenylene diamine to alkanoic acids [6], alkanal,

alkanols, and nitriles [7]. It is interesting to note that benzimidazole motifs have contributed immensely as core structures in many macrocyclic moieties with crucial medicinal applications in drug design [8-9]. They are widely available in commercially marketed drugs which include, but not limited to Omeprazole **1,** which is a proton pump inhibitor used as anti-ulcer [10]; Albendazole **2** as anthelmintic [11]; Bendamustine **3** as anticancer [12]; Telmisartan **4** as antihypertensive [13] and Pimozide **5** as antipsychotic agent [14] as shown in Fig. 1. A very recent study showed DNA binding and antiparasitic properties of benzimidazole bichalcophenes [15].

Many methods have been utilized in the preparation of benzimidazole and its functionalized derivatives some of which include reaction of ortho-phenylenediamine (o-PDA) with substituted carboxylic acid using concentrated HCl medium [16]; *p*-toluene sulphonic acid catalyst [17]; reaction of o-PDA with benzaldehyde derivative using $Na_2S_2O_5$ [18], which release SO_2 gas with pungent smell upon contact with water. Some of these methods involve the tedious work-up procedure and suffer some demerits such as the release of toxic chemicals, harsh reaction conditions, use of expensive reagents, prolonged reaction time, and corrosive nature.

Benzimidazole is a very important biologically active agent in therapeutic medicine because it possesses numerous biological and pharmacological activities. Due to confronting issues such as drug resistance and consistent increase in the outbreak of new diseases globally [19], there should be an uninterrupted quest for the design and preparation of novel heterocyclic moieties as efficacious antimicrobial drug candidates with the aid of synthetic technique with fast kinetic, eco-friendliness, and cost-effectiveness, which is part of focus of this present study. On this note, it is highly essential and strongly motivational to design and synthesize an array of 2-substituted benzimidazole derivatives with an eco-friendly approach for the possibility of preliminary new drug discovery.

■ EXPERIMENTAL SECTION

Materials

All reagents used were obtained from Sigma-Aldrich Chemicals (St. Louis, Missouri, USA) except ammonium chloride, L-leucine and dichloromethane which were purchased from BDH (Poole, Dorset, England), and concentrated hydrochloric acid and camphor which were obtained from Alfar Aesar (Chao Yang District, Beijing, China).

Instrumentation

Melting points determination was carried out with the Stuart melting point apparatus. Progress was monitored by thin layer chromatographic technique and visualization was done accordingly. Nuclear magnetic resonance (NMR) spectra for ^{1}H- and ^{13}C-NMR analysis

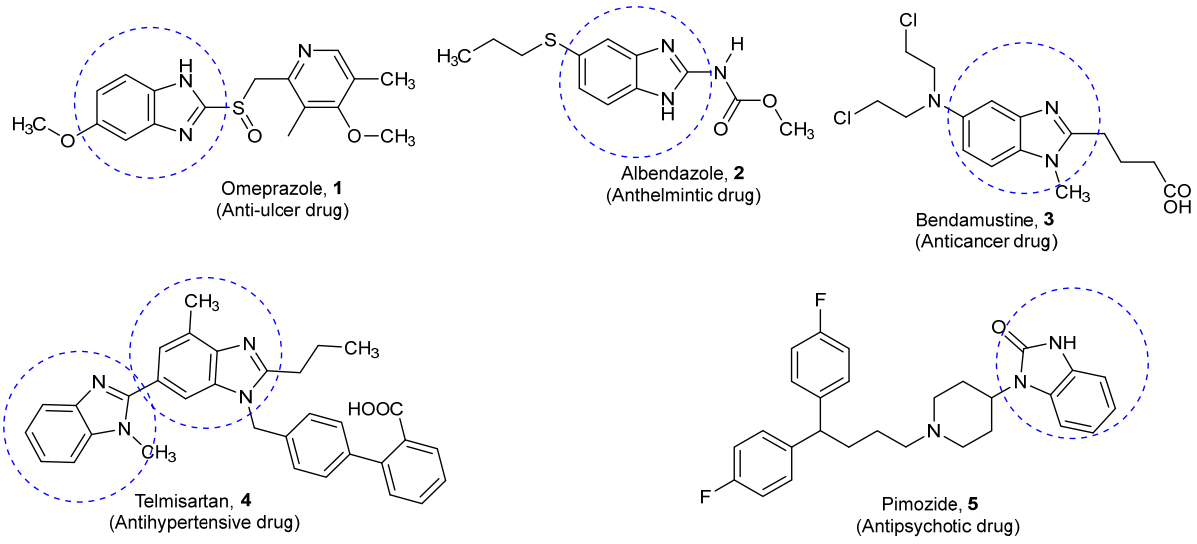


Fig 1. Selected commercially available benzimidazole based drugs

were recorded on Bruker DPX 400 NMR spectrometer at 400 MHz and 100 MHz, respectively, using DMSO-d_6 as the solvent and TMS as the internal standard. DEPT 135 NMR analysis was used for signal assignment to distinguish between methyl (CH_3), methylene (CH_2), and methine (CH) carbon atoms. The Infrared spectra for functional group identification were done with the Bruker FT-IR spectrophotometer; while ultraviolet-visible analysis on the synthesized compounds was carried out in a solution of ethanol (C_2H_5OH), using UV-Genesys spectrophotometer. Sample concentration via solvent removal was achieved with IKA® RV 10 Rotary evaporator and the vacuum-drying of the sample was done with DHG-9023A. Vacuum Oven. Flash EA 1112 Elemental Analyzer was used for carbon, hydrogen, and nitrogen elemental analyses.

Procedure

Synthesis procedure

Synthesis of 1-(1H-benzo[d]imidazol-2-yl)-3-methyl butan-1-amine (6). According to literature method [20], 15.00 g of o-phenylenediamine (138.00 mmol, 1.00 equiv.) in 80 mL of ethanol was added with ammonium chloride (0.74 g, 13.80 mmol, 10 mol%) as catalyst followed by gradual tipping of L-leucine (18.08 g, 138.00 mmol, 1.00 equiv.). The mixture was then stirred at ambient temperature for 5 min after which it was heated under reflux for 3 h (reaction was monitored with TLC). The resulting substrate was filtered while hot to remove the insoluble impurities. The filtrate obtained was evaporated to dryness at reduced pressure to get a crude compound which was recrystallized from isopropanol to afford **6** as brown solid. Yield: 72.57%. ^{1}H-NMR (400 MHz, DMSO-d_6) δ_H: 7.14-7.12 (d, J = 9.10 Hz, 2H, Ar-H), 6.51-6.49 (dd, J_1 = 3.52 Hz, J_2 = 9.10 Hz, 1H, Ph-H), 6.39-6.36 (dd, J_1 = 3.44 Hz, J_2 = 9.10 Hz, 1H, Ph-H), 4.50 (d, J = 4.24 Hz, 2H, NH_2-CH), 3.85-3.83 (m, 1H, C-H), 2.30-1.98 (m, 2H, C-H), 1.27-1.23 (m, 1H, C-H), 0.87-0.85 (d, J = 6.60 Hz, 6H, 2 × CH_3). ^{13}C-NMR (100 MHz, DMSO-d_6) δ_C: 158.4, 154.7, 147.2 (CH), 134.4 (CH), 130.7 (CH), 118.1, 116.0 (CH), 61.2 (CH), 47.3 (CH_2), 30.0 (CH), 15.6 (2 × CH_3). IR (cm^{-1}):3384 (N-H), 3363 (N-H), 3179, 3037 (C-H aromatic), 2957 (C-H aliphatic), 2865 (C-H aliphatic), 1627 (C=C), 1589 (C=N), 1457 (CH_2 deformation), 1407 (CH_3 deformation), 1294 (C=N bending), 1057 (C-N), 743 (Ar-H). UV-Vis.: λ_{max} (nm)/log ε_{max}: 210 (5.40), 236 (5.21), 290 (4.92), 401 (3.90).

(E)-1-(1H-benzo[d]imidazol-2-yl)-N-benzylidene-3-methylbutan-1-amine (7a). Compound **6** (1.00 g, 4.93 mmol, 1.00 equiv.) was dissolved in 10 mL of tetrahydrofuran at room temperature and allowed to stir for 5 min. Benzaldehyde (0.50 mL, 4.93 mmol, 1.00 equiv.) in 5 mL of tetrahydrofuran was added dropwise to the solution of **6** above and refluxed for 3 h. The resulting solution was concentrated under vacuum and the crude product obtained was recrystallized from ethanol to afford **7a** as brown solid. Yield: 61%. ^{1}H-NMR (400 MHz, DMSO-d_6) δ_H: 8.69 (s, 1H, N=C**H**), 7.90-7.88 (d, J = 9.10 Hz, 2H, Ar-H), 7.54-7.49 (m, 3H, Ar-H), 7.14-7.12 (d, J = 8.04 Hz, 2H, Ar-H), 6.51-6.49 (dd, J_1 = 3.52 Hz, J_2 = 9.10 Hz, 1H, Ar-H), 6.39-6.36 (dd, J_1 = 3.44 Hz, J_2 = 9.10 Hz, 1H, Ar-H), 5.87 (s, 1H, NH), 3.74-3.72 (t, J = 3.68 Hz, 1H, CH), 2.02-2.00 (dd, J_1 = 2.40 Hz, J_2 = 10.32 Hz, 1H, CH_a of CH_2), 1.96-1.94 (dd, J_1 = 2.74 Hz, J_2 = 10.32 Hz, 1H, CH_b of CH_2), 1.26-1.21 (m, 1H, CH), 0.87-0.85 (d, J = 6.60 Hz, 6H, 2 × CH_3). ^{13}C-NMR (100 MHz, DMSO-d_6) δ_C: 162.5 (CH), 158.3 (C), 154.7 (C), 147.2 (CH), 143.9 (CH), 140.0 (CH), 134.4 (CH), 130.7 (CH), 125.0 (CH), 124.1 (C), 118.1 (C), 116.0 (CH), 112.2 (CH), 108.1 (CH), 57.2 (CH), 46.8 (CH_2), 30.0 (CH), 15.3 (2 × CH_3). IR (cm^{-1}): 3056 (CH aromatic), 2956 (C-H aliphatic), 2868 (C-H aliphatic), 1610 (C=C), 1579 (C=N), 1444 (CH_2 deformation), 1405 (CH_3 deformation), 1294 (C=N bending), 1070 (C-N), 923 (=C-H bending), 742 (Ar-H). UV-Vis.: λ_{max} (nm)/log ε_{max}: 209 (5.25), 293 (4.82).

(E)-1-(1H-benzo[d]imidazol-2-yl)-3-methyl-N-(2-nitro benzylidene)butan-1-amine (7b). General procedure described for **7a** was used for reaction of **6** (1.00 g, 4.93 mmol) with 2-nitrobenzaldehyde (0.74 g, 4.93 mmol) to produce **7b** as red solid. Yield: 85%. ^{1}H-NMR (400 MHz, DMSO-d_6) δ_H: 8.71 (s, 1H, N=C**H**), 8.20-8.18 (d, J = 8.00 Hz, 1H, Ar-H), 7.69-7.67 (d, J = 8.02 Hz, 1H, Ar-H), 7.31-7.27 (m, 2H, Ar-H), 7.14-7.12 (d, J = 9.10 Hz, 2H, Ar-H), 6.51-6.49 (dd, J_1 = 3.52 Hz, J_2 = 9.10 Hz, 1H, Ar-H), 6.39-6.36 (dd, J_1 = 3.44 Hz, J_2 = 9.10 Hz, 1H, Ar-H),

5.88 (s, 1H, NH), 3.74-3.72 (t, J = 3.68 Hz, 1H, CH), 2.02-2.00 (dd, J_1 = 2.40 Hz, J_2 = 10.32 Hz, 1H, CH_a of CH_2), 1.96-1.94 (dd, J_1 = 2.74 Hz, J_2 = 10.32 Hz, 1H, CH_b of CH_2), 1.27-1.23 (m, 1H, CH), 0.87-0.85 (d, J = 6.60 Hz, 6H, 2 × CH_3). ^{13}C-NMR (100 MHz, DMSO-d_6) δ_C: 162.5 (CH), 158.3 (C), 154.7 (C), 150.2 (C), 147.2 (CH), 143.9 (CH), 140.0 (CH), 134.4 (CH), 130.7 (CH), 125.0 (CH), 124.1 (C), 118.1 (C), 116.0 (CH), 108.1 (CH), 57.2 (CH), 46.8 (CH_2), 30.0 (CH), 15.3 (2 × CH_3). IR (cm^{-1}): 3058 (C-H aromatic), 2957 (C-H aliphatic), 2869 (C-H aliphatic), 1607 (C=C), 1577 (C=N), 1514 (NO_2 asym.), 1440 (CH_2 deformation), 1406 (CH_3 deformation), 1295 (C=N bending), 1343 (NO_2 sym.), 1078 (C-N), 923 (=C-H bending), 746 (Ar-H). UV-Vis.: λ_{max} (nm)/log ε_{max}: 233 (4.78).

(E)-1-(1H-benzo[d]imidazol-2-yl)-N-(2-chlorobenzylidene)-3-methylbutan-1-amine (7c). General procedure described for **7a** was used for reaction of **6** (1.00 g, 4.93 mmol) with 2-chlorobenzaldehyde (0.55 mL, 4.93 mmol) to produce **7c** as brown solid. Yield: 81%. ^{1}H-NMR (400 MHz, DMSO-d_6) δ_H: 8.70 (s, 1H, N=C**H**), 8.28-8.25 (d, J = 8.06 Hz, 1H, Ar-H), 7.68-7.66 (d, J = 8.02 Hz, 1H, Ar-H), 7.27-7.25 (m, 2H, Ar-H), 7.14-7.12 (d, J = 9.10 Hz, 2H, Ar-H), 6.51-6.49 (dd, J_1 = 3.52 Hz, J_2 = 9.10 Hz, 1H, Ar-H), 6.39-6.36 (dd, J_1 = 3.44 Hz, J_2 = 9.10 Hz, 1H, Ar-H), 5.87 (s, 1H, NH), 3.74-3.72 (t, J = 3.68 Hz', 1H, CH), 2.02-2.00 (dd, J_1 = 2.42 Hz, J_2 = 10.32 Hz, 1H, CH_a of CH_2), 1.96-1.94 (dd, J_1 = 2.76 Hz, J_2 = 10.32 Hz, 1H, CH_b of CH_2), 1.27-1.23 (m, 1H, CH), 0.87-0.85 (d, J = 6.62 Hz, 6H, 2 × CH_3). ^{13}C-NMR (100 MHz, DMSO-d_6) δ_C: 162.5 (CH), 158.3 (C), 154.7 (C), 151.4 (C), 147.2 (CH), 143.9 (CH), 140.0 (CH), 134.4 (CH), 130.7 (CH), 125.0 (CH), 124.1, 118.1, 116.0 (CH), 108.1 (CH), 57.2 (CH), 46.8 (CH_2), 30.0 (CH), 15.3 (2 × CH_3). IR (cm^{-1}): 3058 (C-H aromatic), 2957 (C-H aliphatic), 2868 (C-H aliphatic), 1607 (C=C), 1573 (C=N), 1440 (CH_2 deformation), 1404 (CH_3 deformation), 1295 (C=N bending), 1051 (C-N), 922 (=C-H), 743 (Ar-H), 668 (C-Cl). UV-Vis.: λ_{max} (nm)/log ε_{max}: 206 (5.27), 281 (4.77).

(E)-1-(1H-benzo[d]imidazol-2-yl)-N-(3-methoxybenzylidene)-3-methylbutan-1-amine (7d). General procedure described for **7a** was used for reaction of **6** (1.00 g, 4.93 mmol) with 3-methoxybenzaldehyde (0.60 mL, 4.93 mmol) to produce **7d** as brown solid. Yield: 71%. ^{1}H-NMR (400 MHz, DMSO-d_6) δ_H: 8.69 (s, 1H, N=C**H**), 8.36 (s, 1H, Ar-H), 7.89-7.87 (d, J = 8.12 Hz, 1H, Ar-H), 7.71-7.69 (d, J = 8.02 Hz, 1H, Ar-H), 7.14-7.12 (d, J = 9.11 Hz, 2H, Ar-H), 6.86-6.84 (dd, J_1 = 8.02 Hz, J_2 = 8.12 Hz, 1H, Ar-H), 6.51-6.49 (dd, J_1 = 3.52 Hz, J_2 = 9.10 Hz, 1H, Ar-H), 6.39-6.36 (dd, J_1 = 3.44 Hz, J_2 = 9.12 Hz, 1H, Ar-H), 5.86 (s, 1H, NH), 3.74-3.72 (t, J = 3.68 Hz, 1H, CH), 3.13 (s, 3H, OCH_3), 2.03-2.00 (dd, J_1 = 2.42 Hz, J_2 = 10.32 Hz, 1H, CH_a of CH_2), 1.96-1.94 (dd, J_1 = 2.76 Hz, J_2 = 10.32 Hz, 1H, CH_b of CH_2), 1.27-1.23 (m, 1H, CH), 0.87-0.85 (d, J = 6.62 Hz, 6H, 2 × CH_3). ^{13}C-NMR (100 MHz, DMSO-d_6) δ_C: 163.4 (CH), 161.8 (C), 158.4 (C), 154.7 (C), 150.9 (C), 147.2 (CH), 142.9 (CH), 137.8 (CH), 134.4 (CH), 130.7 (CH), 118.1 (C), 116.0 (CH), 111.3 (CH), 108.3 (CH), 58.4 (CH), 53.5 (OCH_3), 46.0 (CH_2), 30.0 (CH), 15.6 (2 × CH_3). IR (cm^{-1}): 3057 (C-H aromatic), 2956 (C-H aliphatic), 2869 (C-H aliphatic), 1608 (C=C), 1579 (C=N), 1438 (CH_2 deformation), 1406 (CH_3 deformation), 1294 (C=N bending), 1160 (C-O), 1027 (C-N), 924 (=C-H), 743 (Ar-H). UV-Vis.: λ_{max} (nm)/log ε_{max}: 209 (5.25), 245 (4.99), 293 (5.06).

(E)-1-(1H-benzo[d]imidazol-2-yl)-N-(4-chlorobenzylidene)-3-methylbutan-1-amine (7e). General procedure described for **7a** was used for reaction of **6** (1.00 g, 4.93 mmol) with 4-chlorobenzaldehyde (0.69 g, 4.93 mmol) to produce Compound **7e** as black solid. Yield: 79%. ^{1}H-NMR (400 MHz, DMSO- d_6) δ_H: 8.71 (s, 1H, N=C**H**), 8.12-8.09 (d, J = 8.14 Hz, 2H, Ar-H), 7.55-7.53 (d, J = 8.02 Hz, 2H, Ar-H), 7.27-7.25 (m, 2H, Ar-H), 7.14-7.12 (d, J = 9.10 Hz, 2H, Ar-H), 6.51-6.49 (dd, J_1 = 3.52 Hz, J_2 = 9.10 Hz, 1H, Ar-H), 6.39-6.36 (dd, J_1 = 3.44 Hz, J_2 = 9.10 Hz, 1H, Ph-H), 5.89 (s, 1H, NH), 3.74-3.72 (t, J = 3.68 Hz, 1H, CH), 2.02-2.00 (dd, J_1 = 2.42 Hz, J_2 = 10.32 Hz, 1H, CH_a of CH_2), 1.96-1.94 (dd, J_1 = 2.76 Hz, J_2 = 10.32 Hz, 1H, CH_b of CH_2), 1.27-1.23 (m, 1H, CH), 0.87-0.85 (d, J = 6.60 Hz, 6H, 2 × CH_3). ^{13}C-NMR (100 MHz, DMSO-d_6) δ_C: 162.7 (CH), 158.2 (C), 154.1 (C), 150.2 (C), 146.8 (CH), 142.6 (C), 138.3 (2 × CH), 134.4 (CH), 130.7 (CH), 121.6 (2 × CH), 118.1 (C), 116.0 (CH), 58.6 (CH), 46.7 (CH_2), 29.7 (CH), 15.4 (2 × CH_3). IR (cm^{-1}): 1607 (C=C), 1579 (C=N), 1439 (CH_2 deformation), 1405 (CH_3 deformation), 1295 (C=N bending), 1084 (C-N),

921 (=C-H), 743 (Ar-H), 668 (C-Cl). UV-Vis.: λ_{max} (nm)/log ε_{max}: 209 (5.28), 296 (5.14).

(E)-4-((1-(1H-benzo[d]imidazol-2-yl)-3-methylbutylimino)methyl)phenol (7f). General procedure described for **7a** was used for reaction of **6** (1.00 g, 4.93 mmol) with 4-hydroxybenzaldehyde (0.60 g, 4.93 mmol) to produce **7f** as red solid. Yield: 99%; ^{1}H-NMR (400 MHz, DMSO-d_6) δ_H: 8.70 (s, 1H, N=C**H**), 7.95-7.93 (d, J = 8.00 Hz, 2H, Ar-H), 7.45-7.42 (d, J = 8.26 Hz, 2H, Ar-H), 7.14-7.12 (d, J = 9.10 Hz, 2H, Ar-H), 6.51-6.49 (dd, J_1 = 3.52 Hz, J_2 = 9.10 Hz, 1H, Ar-H), 6.39-6.36 (dd, J_1 = 3.44 Hz, J_2 = 9.10 Hz, 1H, Ar-H), 5.88 (s, 1H, NH), 3.74-3.72 (t, J = 3.68 Hz, 1H, CH), 2.02-2.00 (dd, J_1 = 2.42 Hz, J_2 = 10.32 Hz, 1H, CH_a of CH_2), 1.96-1.94 (dd, J_1 = 2.76 Hz, J_2 = 10.32 Hz, 1H, CH_b of CH_2), 1.27-1.23 (m, 1H, CH), 0.87-0.85 (d, J = 6.60 Hz, 6H, 2 × CH_3). ^{13}C-NMR (100 MHz, DMSO-d_6) δ_C: 163.4 (CH), 158.3 (C), 154.2 (C), 150.4 (C), 146.8 (CH), 144.4 (2 × CH), 141.5 (C), 134.5 (CH), 130.7 (CH), 122.3 (2 × CH), 118.0 (C), 116.0 (CH), 58.8 (CH), 46.7 (CH_2), 30.1 (CH), 15.6 (2 × CH_3). IR (cm^{-1}): 3380 (OH), 3050 (C-H aromatic), 2956 (C-H aliphatic), 2869 (C-H aliphatic), 1579 (C=N), 1439 (CH_2 deformation), 1405 (CH_3 deformation), 1295 (C=N bending), 1089 (C-N), 922 (=C-H), 743 (Ar-H). UV-Vis.: λ_{max} (nm)/log ε_{max}: 212 (5.31), 245 (5.21), 293 (5.29).

(E)-4-((1-(1H-benzo[d]imidazol-2-yl)-3-methylbutylimino)methyl)-N,N-dimethyl aniline (7g). General procedure described for **7a** was used for reaction of **6** (1.00 g, 4.93 mmol) with 4-(*N,N*-dimethylamino)benzaldehyde (0.74 g, 4.93 mmol) to produce **7g** as yellow solid. Yield: 80%. ^{1}H-NMR (400 MHz, DMSO-d_6) δ_H: 8.66 (s, 1H, N=C**H**), 7.77-7.75 (d, J = 8.08 Hz, 2H, Ar-H), 7.45-7.41 (d, J = 8.26 Hz, 2H, Ar-H), 7.14-7.12 (d, J = 9.10 Hz, 2H, Ar-H), 6.51-6.49 (dd, J_1 = 3.50 Hz, J_2 = 9.10 Hz, 1H, Ar-H), 6.39-6.36 (dd, J_1 = 3.42 Hz, J_2 = 9.10 Hz, 1H, Ar-H), 5.88 (s, 1H, NH), 3.74-3.72 (t, J = 3.68 Hz, 1H, CH), 3.32 (s, 6H, 2 × CH_3), 2.02-2.00 (dd, J_1 = 2.42 Hz, J_2 = 10.32 Hz, 1H, CH_a of CH_2), 1.96-1.94 (dd, J_1 = 2.76 Hz, J_2 = 10.32 Hz, 1H, CH_b of CH_2), 1.27-1.23 (m, 1H, CH), 0.87-0.85 (d, J = 6.60 Hz, 6H, 2 × CH_3). ^{13}C-NMR (100 MHz, DMSO-d_6) δ_C: 164.1 (CH), 158.4 (C), 156.2 (2 × CH), 154.5 (C), 150.4 (C), 147.2 (CH), 142.0 (C), 134.5 (CH), 130.7 (CH), 122.8 (2 × CH), 118.3 (C), 116.0 (CH), 59.8 (CH), 54.3 (2 × CH_3), 46.8 (CH_2), 30.0 (CH), 15.3 (2 × CH_3). IR (cm^{-1}): 3040 (C-H aromatic), 2956 (C-H aliphatic), 2868 (C-H aliphatic), 1607 (C=C), 1578 (C=N), 1439 (CH_2 deformation), 1405 (CH_3 deformation), 1295 (C=N bending), 1063 (C-N), 923 (=C-H), 746 (Ar-H). UV-Vis.: λ_{max} (nm)/log ε_{max}: 206 (5.10), 260 (4.75), 323 (4.94).

(E)-4-((1-(1H-benzo[d]imidazol-2-yl)-3-methylbutylimino)methyl)-N,N-diethylaniline (7h). General procedure described for **7a** was used for reaction of **6** (1.00 g, 4.93 mmol) with 4-(*N,N*-diethylamino)benzal dehyde (0.87 g, 4.93 mmol) to produce **7h** as orange solid. Yield: 91%. ^{1}H-NMR (400 MHz, DMSO-d_6) δ_H: 8.66 (s, 1H, N=C**H**), 7.77-7.75 (d, J = 8.06 Hz, 2H, Ar-H), 7.45-7.41 (d, J = 8.24 Hz, 2H, Ar-H), 7.14-7.12 (d, J = 9.10 Hz, 2H, Ar-H), 6.51-6.48 (dd, J_1 = 3.52 Hz, J_2 = 9.10 Hz, 1H, Ar-H), 6.38-6.36 (dd, J_1 = 3.44 Hz, J_2 = 9.10 Hz, 1H, Ar-H), 5.88 (s, 1H, NH), 4.53-4.50 (q, J = 6.70 Hz, 4H, 2 × CH_2), 3.74-3.72 (t, J = 3.68 Hz, 1H, CH), 2.02-2.00 (dd, J_1 = 2.42 Hz, J_2 = 10.32 Hz, 1H, CH_a of CH_2), 1.96-1.94 (dd, J_1 = 2.76 Hz, J_2 = 10.32 Hz, 1H, CH_b of CH_2), 1.66-1.60 (t, J = 6.70 Hz, 6H, 2 × CH_3), 1.27-1.23 (m, 1H, CH), 0.87-0.85 (d, J = 6.60 Hz, 6H, 2 × CH_3); ^{13}C-NMR (100 MHz, DMSO-d_6) δ_C: 164.1 (C), 158.3 (C), 156.2 (2 × CH), 154.5 (C), 150.4 (C), 147.2 (CH), 142.0 (C), 134.5 (CH), 130.7 (CH), 122.8 (2 × CH), 118.3 (C), 116.0 (CH), 62.4 (2 × CH_2), 59.2 (CH), 46.9 (CH_2), 30.0 (CH), 20.7 (2 × CH_3), 15.3 (2 × CH_3). IR (cm^{-1}): 3058 (C-H aromatic), 2962 (C-H aliphatic), 2929 (C-H aliphatic), 2870 (C-H aliphatic), 1609 (C=C), 1579 (C=N), 1451 (CH_2 deformation), 1405 (CH_3 deformation), 1276 (C=N bending), 1061 (C-N), 924 (=C-H), 744 (Ar-H). UV-Vis.: λ_{max} (nm)/log ε_{max}: 245 (5.38), 305 (5.38), 545 (-2.48), 578 (2.48).

(E)-1-(1H-benzo[d]imidazol-2-yl)-3-methyl-N-(propan-2-ylidene)butan-1-amine (7i). To a stirred solution of compound **6** (1.00 g, 4.93 mmol) in 10 mL of tetrahydrofuran, was added acetone (1.00 mL, 4.93 mmol) in 5 mL of tetrahydrofuran followed by the addition of two drops of concentrated HCl. The reacting mixture was then refluxed at 85 °C for 4 h as evident by reaction completion through complete consumption of starting material (monitored on TLC, CH_2Cl_2/CH_3OH → 9:1, v/v). The resulting solution was cooled and

concentrated to access crude product which was recrystallized from ethanol to afford **7i** as green solid. Yield: 57%. ^{1}H-NMR (400 MHz, DMSO-d_6) δ_H: 7.20-7.18 (d, J = 8.00 Hz, 2H, Ar-H), 6.51-6.48 (dd, J_1 = 3.52 Hz, J_2 = 8.00 Hz, 1H, Ar-H), 6.38-6.36 (dd, J_1 = 3.44 Hz, J_2 = 8.00 Hz, 1H, Ar-H), 5.88 (s, 1H, NH), 3.74-3.72 (t, J = 3.66 Hz, 1H, CH), 2.20 (s, 6H, 2 × CH_3), 2.03-2.00 (dd, J_1 = 2.42 Hz, J_2 = 10.32 Hz, 1H, CH_a of CH_2), 1.95-1.93 (dd, J_1 = 2.76 Hz, J_2 = 10.32 Hz, 1H, CH_b of CH_2), 1.28-1.23 (m, 1H, CH), 0.87-0.85 (d, J = 6.60 Hz, 6H, 2 × CH_3). ^{13}C-NMR (100 MHz, DMSO-d_6) δ_C: 162.0 (C), 158.4 (C), 154.5 (C), 147.2 (CH), 134.4 (CH), 130.7 (CH), 118.1 (C), 116.0 (CH), 60.5 (CH), 46.7 (CH_2), 43.4 (2 × CH_3), 30.0 (CH), 15.6 (2 × CH_3). IR (cm^{-1}): 3368 (N-H), 3061 (C-H aromatic), 2956 (C-H aliphatic), 2869 (C-H aliphatic), 1621 (C=C), 1554 (C=N), 1448 (CH_2 deformation), 1414 (CH_3 deformation), 1269 (C=N bending), 1020 (C-N), 926 (=C-H), 728 (Ar-H). UV-Vis.: λ_{max} (nm)/log ε_{max}: 239 (5.47), 269 (5.44), 431 (4.17).

(E)-3-((1-(1H-benzo[d]imidazol-2-yl)-3-methylbutylimino)indolin-2-one (7j). General procedure described for **7i** was used for reaction of **6** (1.00 g, 4.93 mmol) with isatin (0.73 g, 4.93 mmol) to produce **7j** as brown solid. Yield: 76%. ^{1}H-NMR (400 MHz, DMSO-d_6) δ_H: 11.56 (s, 1H, NH of amide), 7.92-7.89 (d, J = 8.96 Hz, 2H, Ar-H), 7.44-7.41 (m, 2H, Ar-H), 7.14-7.12 (d, J = 9.12 Hz, 2H, Ar-H), 6.51-6.48 (dd, J_1 = 3.52 Hz, J_2 = 9.12 Hz, 1H, Ar-H), 6.38-6.36 (dd, J_1 = 3.44 Hz, J_2 = 9.12 Hz, 1H, Ar-H), 5.88 (s, 1H, NH), 3.74-3.72 (t, J = 3.66 Hz, 1H, CH), 2.03-2.00 (dd, J_1 = 2.42 Hz, J_2 = 10.32 Hz, 1H, CH_a of CH_2), 1.95-1.93 (dd, J_1 = 2.76 Hz, J_2 = 10.32 Hz, 1H, CH_b of CH_2), 1.28-1.23 (m, 1H, CH), 0.87-0.85 (d, J = 6.60 Hz, 6H, 2 × CH_3). ^{13}C-NMR (100 MHz, DMSO-d_6) δ_C: 177.2 (C=O), 163.3 (C), 162.0 (C), 159.2 (C), 158.4 (C), 155.3 (C), 154.7 (C), 147.2 (CH), 145.6 (CH), 135.5 (CH), 134.4 (CH), 130.7 (CH), 116.0 (CH), 114.3 (CH), 111.5 (CH), 60.5 (CH), 46.7 (CH_2), 30.0 (CH), 15.6 (2 × CH_3). IR (cm^{-1}): 3396 (N-H), 3057 (C-H aromatic), 2926 (C-H aliphatic), 2869 (C-H aliphatic), 1612 (C=C), 1580 (C=N), 1454 (CH_2 deformation), 1404 (CH_3 deformation), 1274 (C=N bending), 1031 (C-N), 924 (=C-H), 745 (Ar-H). UV-Vis.: λ_{max} (nm)/log ε_{max}: 209 (5.36), 278 (4.92), 353 (4.49).

(E)-1-(1H-benzo[d]imidazol-2-yl)-3-methyl-N-(1,7,7-trimethylbicyclo[2.2.1]heptan-2-ylidene) butan-1-amine (7k). General procedure described for **7i** was used for reaction of **6** (1.00 g, 4.93 mmol) with camphor (0.75 g, 4.93 mmol) to produce **7k** as red solid. Yield: 80%. ^{1}H-NMR (400 MHz, DMSO-d_6) δ_H: 7.24-7.22 (d, J = 8.00 Hz, 2H, Ar-H), 6.51-6.49 (dd, J_1 = 3.50 Hz, J_2 = 8.00 Hz, 1H, Ar-H), 6.39-6.36 (dd, J_1 = 3.46 Hz, J_2 = 9.10 Hz, 1H, Ar-H), 5.86 (s, 1H, NH), 3.74-3.72 (t, J = 3.68 Hz, 1H, CH), 2.02-2.00 (dd, J_1 = 2.40 Hz, J_2 = 10.32 Hz, 1H, CH_a of CH_2), 1.96-1.94 (dd, J_1 = 2.74 Hz, J_2 = 10.32 Hz, 1H, CH_b of CH_2), 1.65 (s, 1H, CH), 1.30-1.27 (m, 1H, CH), 1.20 (s, 2H, CH_2), 1.18-1.15 (m, 4H), 1.10 (s, 3H, CH_3), 0.99 (s, 6H, 2 × CH_3), 0.87-0.85 (d, J = 6.60 Hz, 6H, 2 × CH_3). ^{13}C-NMR (100 MHz, DMSO-d_6) δ_C: 149.3 (C), 147.2 (CH), 134.4 (CH), 130.7 (CH), 128.1 (C), 124.5 (C), 118.5 (C), 116.2 (CH), 60.0 (CH), 46.4 (CH_2), 29.3 (CH), 27.8 (CH_2), 27.4 (CH_2), 26.9 (CH_2), 15.7 (2 × CH_3), 15.0 (2 × CH_3), 11.7 (CH_3). IR (cm^{-1}): 3058 (C-H aromatic), 2957 (C-H aliphatic), 2870 (C-H aliphatic), 1607 (C=C), 1578 (C=N), 1454 (CH_2 deformation), 1406 (CH_3 deformation), 1275 (C=N bending), 1046 (C-N), 923 (=C-H), 745 (Ar-H). UV-Vis.: λ_{max} (nm)/log ε_{max}: 209 (5.36), 254 (5.19), 437 (4.71).

(E)-N-(1-(1H-benzo[d]imidazol-2-yl)-3-methylbutyl) benzenesulfonamide (7l). Precursor **6** (5.08 g, 25.00 mmol) was transferred to Na_2CO_3 (5.57 g, 52.5 mmol) in H_2O (30.00 mL) with continuous stirring at 0 °C in ice-bath followed by addition of benzenesulfonyl chloride derivatives (3.83 g, 30.00 mmol) batch-wise for 1 h and the stirring continued at ambient condition for 5 h after which the reaction was terminated. The worked-up was done cautiously by adding 20% aq. HCl until complete neutralization was achieved. The collected crude product was purified by column chromatography on Merck silica gel F (Mesh 200-300) using $CHCl_3$/CH_3OH, (9:1, v/v) as eluting solvent to afford the benzimidazole-based sulfonamide, motifs **7l** as green solid. Yield: 84%. ^{1}H-NMR (400 MHz, DMSO-d_6) δ_H: 11.01 (s, 1H, N**H**), 7.94-7.92 (d, J = 8.08 Hz, 2H, Ar-H), 7.61-7.54 (m, 3H, Ar-H), 7.14-7.12 (d, J = 9.10 Hz, 2H, Ar-H), 6.51-6.49 (dd, J_1 = 3.52 Hz, J_2 = 9.10 Hz, 1H, Ar-H), 6.39-6.36 (dd, J_1 = 3.44 Hz, J_2 = 9.10 Hz, 1H, Ar-H), 5.87 (s, 1H, NH), 3.78-3.76 (t, J

= 3.70 Hz, 1H, CH), 2.02-2.00 (dd, J_1 = 3.70 Hz, J_2 = 10.32 Hz, 1H, CH_a of CH_2), 1.96-1.94 (dd, J_1 = 2.74 Hz, J_2 = 10.32 Hz, 1H, CH_b of CH_2), 1.26-1.21 (m, 1H, CH), 0.87-0.85 (d, J = 6.60 Hz, 6H, 2 × CH_3). ^{13}C-NMR (100 MHz, DMSO-d_6) δ_C: 159.2 (C), 158.3 (C), 155.6 (C), 147.8 (CH), 143.7 (CH), 140.2 (CH), 130.7 (CH), 125.7 (CH), 124.1 (C), 118.1 (C), 116.5 (CH), 113.9 (CH), 110.1 (CH), 59.4 (CH), 46.9 (CH_2), 30.0 (CH), 15.3 (2 × CH_3). IR (cm^{-1}): 3385 (N-H), 3208 (N-H), 1620 (C=C), 1595 (C=N), 1460 (CH_2 deformation), 1405 (CH_3 deformation), 1275 (C=N bending), 1213 (SO_2), 1147 (SO_2), 1032 (C-N), 916 (=C-H), 751 (Ar-H). UV-Vis.: λ_{max} (nm)/log ε_{max}: 206 (5.26), 227 (5.16), 293 (4.25), 452 (3.53).

(E)-N-(1-(1H-benzo[d]imidazol-2-yl)-3-methylbutyl)-2-methylbenzenesulfonamide (7m). General procedure described for **7l** was used for reaction of **6** (1.00 g, 4.93 mmol) with *o*-toluenesulfonyl chloride (0.71 mL, 4.93 mmol) to produce **7m** as red solid. Yield: 91%. ^{1}H-NMR (400 MHz, DMSO-d_6) δ_H: 11.00 (s, 1H, N**H**), 7.92-7.90 (d, J = 7.98 Hz, 1H, Ar-H), 7.44-7.42 (d, J = 7.84 Hz, 1H, Ar-H), 7.21-7.18 (m, 2H, Ar-H), 7.14-7.12 (d, J = 8.00 Hz, 2H, Ar-H), 6.51-6.49 (dd, J_1 = 3.52 Hz, J_2 = 8.00 Hz, 1H, Ar-H), 6.39-6.36 (dd, J_1 = 3.44 Hz, J_2 = 8.00 Hz, 1H, Ar-H), 5.87 (s, 1H, NH), 3.78-3.76 (t, J = 3.70 Hz, 1H, CH), 2.39 (s, 3H, CH_3), 2.02-2.00 (dd, J_1 = 3.70 Hz, J_2 = 10.32 Hz, 1H, CH_a of CH_2), 1.96-1.94 (dd, J_1 = 2.74 Hz, J_2 = 10.32 Hz, 1H, CH_b of CH_2), 1.26-1.21 (m, 1H, CH), 0.87-0.85 (d, J = 6.60 Hz, 6H, 2 × CH_3). ^{13}C-NMR (100 MHz, DMSO-d_6) δ_C: 151.2 (C), 147.8 (CH), 143.7 (CH), 140.2 (CH), 139.2 (C), 132.6 (CH), 130.7 (C), 125.9 (CH), 124.1 (CH), 118.1 (C), 116.3 (CH), 113.8 (CH), 110.9 (C), 59.4 (CH), 46.9 (CH_2), 30.0 (CH), 22.7 (CH_3), 15.3 (2 × CH_3). IR (cm^{-1}): 3384 (N-H), 3208 (N-H), 1621 (C=C), 1596 (C=N), 1461 (CH_2 deformation), 1406 (CH_3 deformation), 1275 (C=N bending), 1214 (SO_2), 1146 (SO_2), 1032 (C-N), 915 (=C-H), 750 (Ar-H). UV-Vis.: λ_{max} (nm)/log ε_{max}: 200 (5.21), 269 (5.05), 455 (4.83), 578 (3.43).

(E)-N-(1-(1H-benzo[d]imidazol-2-yl)-3-methylbutyl)-4-methylbenzenesulfonamide (7n). General procedure described for **7l** was used for reaction of **6** (1.00 g, 4.93 mmol) with *p*-toluenesulfonyl chloride (0.94 g, 4.93 mmol) to produce **7n** as yellow solid. Yield: 90%. ^{1}H-NMR (400 MHz, DMSO-d_6) δ_H: 11.03 (s, 1H, N**H**), 7.87-7.85 (d, J = 8.18 Hz, 1H, Ar-H), 7.40-7.38 (d, J = 8.22 Hz, 1H, Ar-H), 7.14-7.12 (d, J = 8.00 Hz, 2H, Ar-H), 6.51-6.49 (dd, J_1 = 3.52 Hz, J_2 = 8.00 Hz, 1H, Ar-H), 6.39-6.36 (dd, J_1 = 3.44 Hz, J_2 = 8.00 Hz, 1H, Ar-H), 5.87 (s, 1H, NH), 3.78-3.76 (t, J = 3.70 Hz, 1H, CH), 2.36 (s, 3H, CH_3), 2.02-2.00 (dd, J_1 = 3.70 Hz, J_2 = 10.32 Hz, 1H, CH_a of CH_2), 1.96-1.94 (dd, J_1 = 2.74 Hz, J_2 = 10.32 Hz, 1H, CH_b of CH_2), 1.26-1.21 (m, 1H, CH), 0.87-0.85 (d, J = 6.60 Hz, 6H, 2 × CH_3). ^{13}C-NMR (100 MHz, DMSO-d_6) δ_C: 151.4 (C), 147.2 (CH), 145.1 (2 × CH), 140.2 (C), 135.2 (CH), 130.7 (C), 125.9 (2 × CH), 124.1 (C), 118.1 (C), 116.3 (CH), 113.1 (CH), 59.4 (CH), 46.9 (CH_2), 30.0 (CH), 22.7 (CH_3), 15.3 (2 × CH_3). IR (cm^{-1}): 3384 (N-H), 3209 (N-H), 1621 (C=C), 1596 (C=N), 1462 (CH_2 deformation), 1407 (CH_3 deformation), 1275 (C=N bending), 1214 (SO_2), 1146 (SO_2), 1032 (C-N), 916 (=C-H), 751 (Ar-H). UV-Vis.: λ_{max} (nm)/log ε_{max}: 206 (5.23), 257 (5.06), 437 (4.66).

Antibacterial activity assay

The targeted functionalized benzimidazole motifs' antibacterial potential was evaluated using the agar diffusion method [20], while the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) testing were determined using serial dilution technique [21] with respect to four targeted organisms as shown in Supplementary materials.

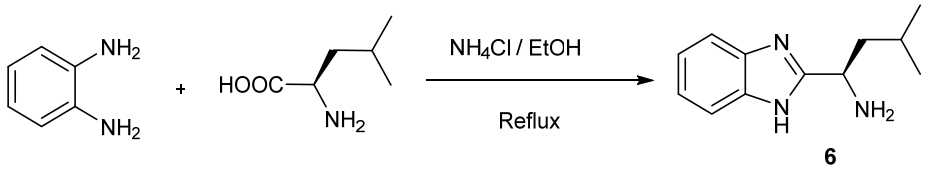
■ RESULTS AND DISCUSSION

Chemistry

Benzimidazole is a benzo-fused imidazole known to be highly versatile heterocyclic compounds in therapeutic medicine, agrochemicals, and catalysis study. In furtherance of our quest to design and evaluate the biological activity of benzimidazole motifs [2,20], we have herein synthesized novel functionalized benzimidazole scaffolds so as to unveil the antibacterial potential for probable future drug discovery. First, condensation reaction of *o*-phenylenediamine (o-PDA) on the COOH functionality of cheap and readily available amino acid, L-leucine was carried out in ethanol solvent using the catalytic amount of ammonium chloride (NH_4Cl) as an eco-friendly medium, as shown in Scheme 1, which was according to our recently reported procedure [20].

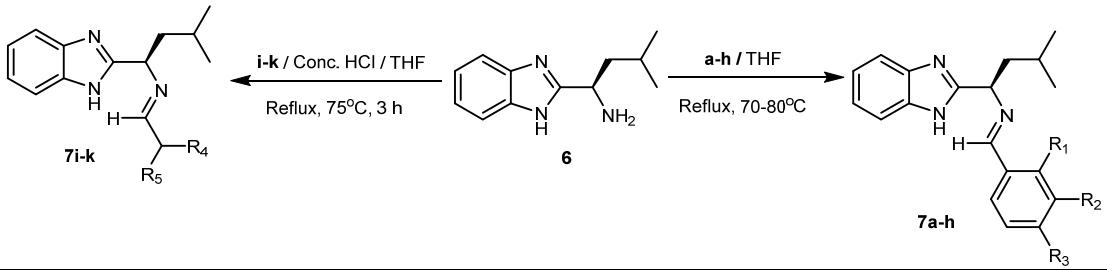
Some commonly used catalysts and the medium used for condensation of o-PDA in the synthesis benzimidazole led to the tedious work-up procedure and suffered some set back such as the release of toxic chemical, harsh reaction condition, use of expensive reagents, prolong reaction time and corrosive nature [16-18]. Thus, we have adopted the use of NH_4Cl as an environmentally friendly and cost-effective catalyst to achieve some functionalized benzimidazole with bioactive efficacy in this present study. It was worked-up as described in the experimental section to furnish **6** in a 73% yield.

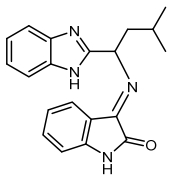
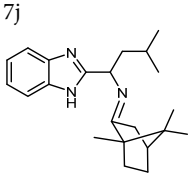
Compound **6** was then utilized as an essential building block by coupling it with eight benzaldehyde derivatives **a-h** in the presence of tetrahydrofuran (THF)



Scheme 1. Synthesis of 1-(1*H*-benzo[*d*]imidazol-2-yl)-3-methylbutan-1-amine, **6**

Table 1. Synthesis of 1-(1*H*-benzo[*d*]imidazol-2-yl)-*N*-(substituted-benzylidene/ketolidene)-3-methyl butan-1-amine, **7a-k**



Entry	R_1	R_2	R_3	R_4	R_5	Product	Yield [%]	Elemental Analysis % Calcd. (Found) C	H	N
1	H	H	H	-	-	7a	61	78.32(78.50)	7.26(7.46)	14.42(14.22)
2	NO_2	H	H	-	-	7b	85	67.84(67.04)	5.99(6.18)	16.66(16.80)
3	Cl	H	H	-	-	7c	81	70.04(69.88)	6.19(6.40)	12.90(12.77)
4	H	OCH_3	H	-	-	7d	71	74.74(74.82)	7.21(7.39)	13.07(12.96)
5	H	H	Cl	-	-	7e	79	70.04(70.22)	6.19(6.41)	12.90(12.73)
6	H	H	OH	-	-	7f	99	74.24(74.39)	6.89(7.08)	13.67(13.86)
7	H	H	NMe_2	-	-	7g	80	75.41(75.66)	7.84(7.67)	16.75(16.92)
8	H	H	NEt_2	-	-	7h	91	76.20(76.14)	8.34(8.14)	15.46(15.71)
9	-	-	-	CH_3	CH_3	7i	57	74.03(74.22)	8.70(8.53)	17.27(17.50)
10	-	-	-	-	-	 7j	76	72.27(72.45)	6.06(5.88)	16.86(16.89)
11	-	-	-	-	-	 7k	80	78.29(78.50)	9.26(9.37)	12.45(12.67)

Me = (CH_3); Et = (CH_2CH_3), Calcd. = Calculated

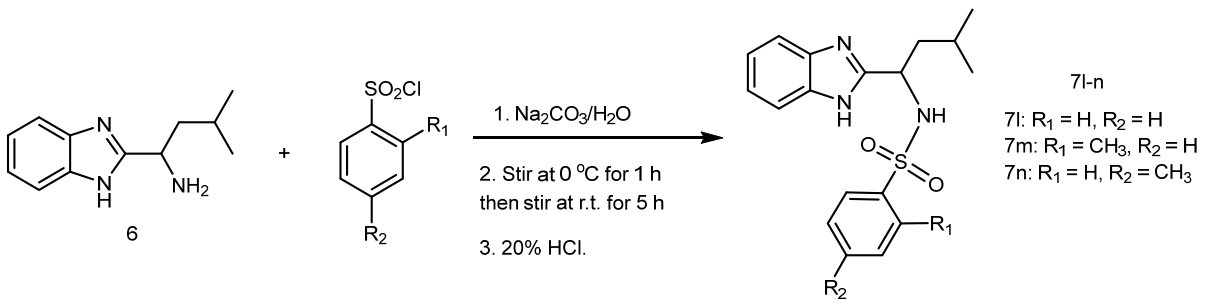
for the preparation of 1-(1*H*-benzo[*d*]imidazol-2-yl)-*N*-(s-benzylidene)-3methylbutan-1-amine, **7a-h**, as shown in Table 1. The reaction of precursor **6** with three distinct ketones **i-k** at a refluxing temperature of 75 °C in ethanol and concentrated HCl catalyst furnished **7i-k** as shown in Table 1. Finally, the coupling of **6** with three derivatives of aryl sulfonyl chlorides **l-n** in sodium carbonate basified medium at 0 °C to stirring at room temperature furnished **7l-n**, as shown in Scheme 2.

The spectroscopic characterization of the compounds was carried out for structural elucidation of the targeted compounds. The spectroscopic methods used involved ^{1}H-NMR, ^{13}C-NMR, FT-IR, and UV-Vis. spectrophotometric analyses. The series of products was represented by **7a** for a concise spectral discussion. The azomethine proton being the most downfield signal in **7a** resonated as a 1H singlet at 8.59 ppm. The most de-shielded aromatic proton was that of 2H doublet of the benzenoid nucleus portion of benzimidazole and they appeared at 7.90-7.88 ppm with a *J* value of 9.10 Hz, while their two neighboring protons on the same benzenoid nucleus resonated as 1H doublets of doublet at 6.51-6.49 ppm (J_1 = 3.52 Hz, J_2 = 9.10 Hz), and 1H doublets of doublet at 6.39-6.36 ppm (J_1 = 3.44 Hz, J_2 = 9.10 Hz). The five aromatic protons on the benzylidene portion resonated as a 3H multiplet at 7.54-7.49 ppm and a 2H doublet at 7.14-7.12 ppm with a *J* value of 8.04 Hz. The 1H singlet at 5.87 ppm depicted the presence of NH of benzimidazole.

Other signals in the ^{1}H-NMR spectrum of **7a** appeared more up-field because they were aliphatic protons which included 1H triplet at 3.74-3.72 ppm, followed by two germinal protons seen as 1H (CH_a of CH_2) doublets of a doublet at 2.02-2.00 ppm and 1H (CH_b of CH_2) which resonated as doublets of a doublet at 1.96-1.94 ppm. There was 1H multiplet of CH of aliphatic at 1.26-1.21 ppm. The most shielded signal was that of two chemically equivalent methyl groups, which resonated as a 6H doublet at 0.87-0.85 ppm with a *J* value of 6.60 Hz. The first evidence for completion of the reaction was the presence of NH_2 as a doublet at 4.50 ppm in the ^{1}H-NMR spectral data of precursor **6** which completely disappeared from this region in the spectrum of **7a** but reappeared at a more downfield region as 1H azomethine (C=N-H) singlet at 8.69 ppm in the spectrum of **7a**.

The ^{13}C-NMR spectrum of **7a** revealed it to contain nineteen carbon atoms with chemical shift values varied from 162.5 ppm to 15.3 ppm. This showed the presence of twelve CH carbons (162.5-30.0 ppm) and two chemically equivalent CH_3 carbon atoms (15.3 ppm) as positive signals, while the only negative signal at 46.8 ppm revealed that there was only one CH_2 carbon atom in the structure of **7a**. This showed that the remaining four carbon atoms (158.3, 154.7, 124.1, 118.0 ppm) were quaternary in nature.

The IR spectrum of **7a** showed the presence of CH aromatic, CH aliphatic, C=C, C=N as stretching vibrational bands which appeared at 3056, 2956, 1610, and 1579 cm^{-1}, respectively, which agreed with an earlier report with different scaffold but similar functionality [21]. The assignable bending vibrational bands and fingerprint region in agreement with the structure of **7a** were that 1294 cm^{-1} for C=N, 1070 cm^{-1} for C-N, 923 cm^{-1} for =C-H and 742 cm^{-1} for Ar-H. The UV–vis chart of **7a** unveiled its first peak at λ_{max} = 209 nm (log ε_{max} = 5.25)



Scheme 2. Synthesis of *N*-(1-(1*H*-benzo[*d*]imidazol-2-yl)-3-methylbutyl)-s-methylbenzene sulfonamide, **7l-n**

which depicted $\pi \rightarrow \pi^*$ transition traceable to C=C of the phenyl ring. The second signal found at λ_{max} = 293 nm (log ε_{max} = 4.82) was a bathochromic shift ascribable to the extensive conjugation and presence of C=N of imino functionality which originated from $n \rightarrow \pi^*$ transition exhibited by this auxochrome [22].

The physicochemical properties of the precursor **6** and the targeted products **7a-n** were reported to include molecular formula, molecular weight, melting point, % yields, and C, H, N analysis (Experimental). All the products had % yields ranging from 56.58% for **7i** to 99.00% for **7f**. The result of elemental analytical determination for C, H, N showed great correlation and concordance between percentage calculated and percentage observed with the difference of not more than ± 0.25 in all the synthesized benzimidazole motifs.

Biological Properties

The *in vitro* antibacterial properties of the designed motifs were carried out on four bacterial isolates comprising of *Staphylococcus aureus, Bacillus cereus*, *Escherichia coli,* and *Pseudomonas aeruginosa*. The screening was done alongside that of a typical clinical standard antibiotic (Gentamicin). The rationale beneath the choice of Gentamicin as a clinical reference drug was because of the mode of action, which entails the inhibition of protein synthesis [23].

The growth of *S. aureus* was inhibited by all the synthesized compounds with Z.O.I. ranging from 18.00 ± 0.06 mm for benzimidazole motif **7a** to 31.00 ± 0.09 mm for compound **7i**, whereas the Z.O.I. of Gentamicin against *S. aureus* was 20.00 ± 0.08 mm (Table 2). The other gram-positive organism used was *Bacillus cereus,* and it has been reported to be the causative agent for highly devastating non-gastrointestinal-tract infections. It has also been tagged with most of the food poisoning cases and secretion of tissue-destructive exoenzymes [24].

However, it is very interesting to note that, out of the four bacteria, *B. cereus* had the highest susceptibility to the efficiency of the synthesized targeted compounds with zones of inhibition ranging from 35.00 ± 0.09 mm for compound **7g** to 43.00 ± 0.12 mm for compound **7j**, and these Z.O.I. were twice as large as Z.O.I. from Gentamicin standard (20.00 ± 0.08 mm). It was discovered that *E. coli* was resistant to three compounds

Table 2. Result of general sensitivity testing on bacteria with zones of inhibition in (mm)

Comp. No	*S. aureus* (G^+)	*B. cereus* (G^+)	*E. coli* (G^-)	*P. aeruginosa* (G-)
6	25.00 ± 0.08	40.00 ± 0.12	22.00 ± 0.12	20.00 ± 0.09
7a	18.00 ± 0.06	38.00 ± 0.10	21.00 ± 0.09	20.00 ± 0.09
7b	20.00 ± 0.10	38.00 ± 0.09	20.00 ± 0.09	25.00 ± 0.08
7c	20.00 ± 0.07	37.00 ± 0.09	22.00 ± 0.09	25.00 ± 0.08
7d	30.00 ± 0.10	40.00 ± 0.10	R	20.00 ± 0.08
7e	25.00 ± 0.10	37.00 ± 0.09	31.00 ± 0.11	30.00 ± 0.10
7f	23.00 ± 0.10	40.00 ± 0.15	R	30.00 ± 0.08
7g	28.00 ± 0.09	35.00 ± 0.09	24.00 ± 0.09	26.00 ± 0.10
7h	29.00 ± 0.08	36.00 ± 0.09	22.00 ± 0.08	23.00 ± 0.08
7i	31.00 ± 0.09	37.00 ± 0.09	21.00 ± 0.09	22.00 ± 0.08
7j	30.00 ± 0.09	43.00 ± 0.12	30.00 ± 0.08	26.00 ± 0.08
7k	30.00 ± 0.10	42.00 ± 0.13	30.00 ± 0.10	26.00 ± 0.08
7l	25.00 ± 0.10	36.00 ± 0.10	R	30.00 ± 0.10
7m	30.00 ± 0.10	40.00 ± 0.13	29.00 ± 0.09	28.00 ± 0.08
7n	23.00 ± 0.09	40.00 ± 0.12	28.00 ± 0.08	30.00 ± 0.09
Gtm	25.00 ± 0.09	20.00 ± 0.08	25.00 ± 0.09	26.00 ± 0.08

S. aureus = *Staphylococcus aureus* (G^+), *B. cereus* = *Bacillus cereus* (G^+). *E. coli* = *Escherichia coli* (G^-). *P. aeruginosa* = *Pseudomonas aeruginosa* (G^-), Gtm. = Gentamicin. G^+ = Gram positive, G^- = Gram negative. Z.O.I. = Zone of Inhibition. R = Resistant.

7d, **7f** and **7l**, which is probably due to antagonistic effect of OCH_3 and OH as the EDG at meta and para positions respectively, but susceptible to the rest of the compounds including the precursor **6** with zones of inhibition varying from 20.00 ± 0.09 mm for compound **7b** to 31.00 ± 0.11 mm for compound **7e** (Table 2).

Lastly, *in vitro* screening against *P. aeruginosa*, revealed that all the compounds were active on this organism with the zones of inhibition ranging from 20.00 ± 0.09 mm for compounds **6**, **7a**, and **7d** to 30.00 ± 0.09 mm for compounds **7e**, **7f**, **7l**, and **7n**, whereas Z.O.I. of Gentamicin against this organism was 26.00 ± 0.09 mm. *Pseudomonas aeruginosa* is an opportunistic organism with low permeability and is responsible for infectious diseases in people experiencing altered immune systems, some of which are burned, HIV, nosocomial and neutropenic conditions [25]. Despite virulent possession in *P. aeruginosa* and its resistance to many antimicrobial drugs, it is quite interesting that this organism was susceptible to all the benzimidazole motifs designed herein and most of their Z.O.I. were higher than that of Gentamicin standard antibiotic used.

In addition, the activity index in this study was measured using the comparative study of inhibition zones of the synthesized compounds to that of Gentamicin upon all the organisms used (Fig. 2(a-d)). The activity index (A.I.) of the synthesized compounds against the growth of *S. aureus* was presented in Fig. 2(a). It was discovered therein that 47% of the benzimidazole products (**7d**, **7g-k**, **7m**) were more selective than Gentamicin against *S. aureus*; 20% of the benzimidazoles (**6**, **7e**, **7l**) had similar A.I. as Gentamicin,

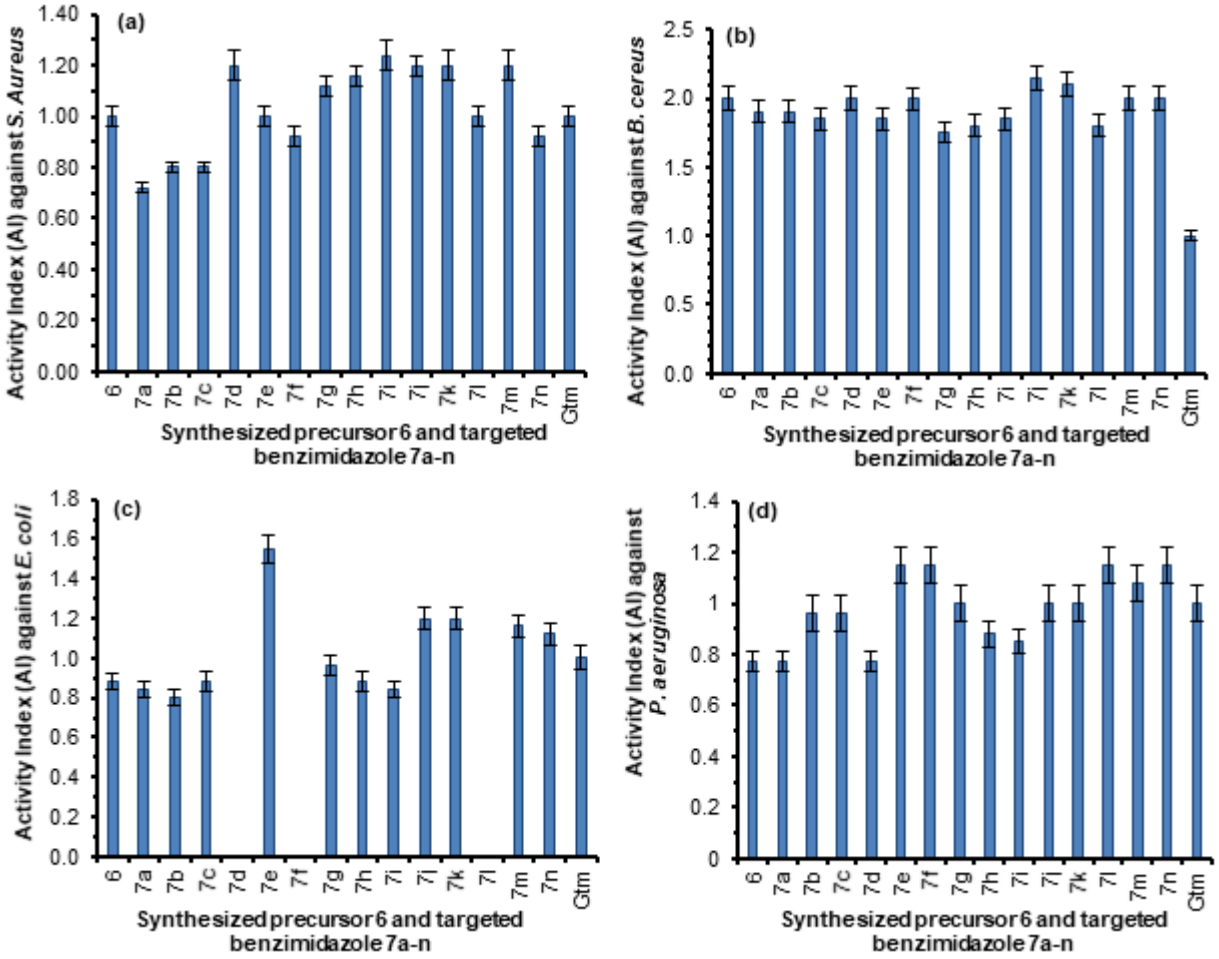


Fig 2. Activity index of the precursor 6 and targeted benzimidazole motifs **7a-n** as compared to Gentamicin clinical standard against (a) *Staphylococcus aureus*, (b) *Bacillus cereus*, (c) *Escherichia coli*, (d) *Pseudomonas aeruginosa*

while the remaining 33% (**7a**, **7b**, **7c**, **7f**, **7n**) were less active than Gentamicin against *S. aureus*. The activity index against the *B. cereus* showed that all the compounds (100%) were more selective than the Gentamicin (Fig. 2(b)), which buttressed the fact that synthesized compounds **7a-n** had impressive growth inhibitory activity against *B. cereus*. Activity index investigation against gram-negative *E. coli* unveiled that 33% of the compounds (**7e**, **7j**, **7k**, **7m**, **7n**) were more selective than Gentamicin, while the rest of the benzimidazoles were less active than Gentamicin apart from 20% of them (**7d**, **7f**, **7l**) where resistance was observed (Fig. 2(c)). Activity index against *P. aeruginosa* showed that 33% of the compounds (**7e**, **7f**, **7l-n**) were more active than Gentamicin (Fig. 2(d)); 20% of the benzimidazole motifs (**7g**, **7j**, **7k**) possessed the same activity as Gentamicin while the remaining 47% of the compounds (**6**, **7a-d**, **7h**, **7i**) were less active than gentamicin against *P. aeruginosa*.

Owing to the broad spectrum of activity, a further effort was made to determine the MIC values using serial dilution technique [21]. The result of the MIC of the precursor **6** and final benzimidazoles **7a-n** against the organisms varied from 0.98 ± 0.02 to 62.50 ± 0.59 μg/mL (Table 3). The MIC values of the screened motifs against *S. aureus* ranged from 0.98 ± 0.02 to 62.50 ± 0.57 μg/mL with compounds **7k** (MIC = 0.98 ± 0.02 μg/mL) being the most active. For activity on *B. cereus*, the MIC values varied from 1.95 ± 0.05 to 3.91 ± 0.08 μg/mL with all the screened compounds being strongly active at 1.95 ± 0.05 μg/mL except **7a**, **7d**, **7i** (MIC = 3.91 ± 0.08 μg/mL) and **7e** (15.63 ± 0.14 μg/mL). It was fascinating to note that among the four organisms, the best activity of the compounds was experienced on the *B. cereus*.

The screening against *E. coli* showed resistance to the compounds of **7d**, **7f**, and **7l**, while motifs **7e** and **7k** (MIC = 1.95 ± 0.05 μg/mL) possessed the highest activity against *E. coli*. The MIC values of the screened compounds against *P. aeruginosa* ranged from 3.91 ± 0.07 to 62.50 ± 0.59 μg/mL with compound **7k** and **7n** (MIC = 3.91 ± 0.07 μg/mL) being the most active on *P. aeruginosa*. The concise report from the MIC screened clearly identify benzimidazole motif **7k** to be the most outstanding molecular target among the series of compound **7a-n**; therefore, can be a hit for the new antimicrobial drug as compared to the positive control used herein. The efficiency of the precursor **6** and final products **7a-n** was

Table 3. Minimum inhibitory concentration (MIC) of **6** and final products **7a-n** (μg/mL)

Comp. No	*S. aureus*	*B. cereus*	*E. coli*	*P. aeruginosa*
6	1.95 ± 0.05	1.95 ± 0.04	15.63 ± 0.15	31.25 ± 0.29
7a	7.81 ± 0.09	3.91 ± 0.07	15.63 ± 0.14	15.63 ± 0.14
7b	31.25 ± 0.29	1.95 ± 0.05	31.25 ± 0.28	15.63 ± 0.15
7c	7.81 ± 0.09	1.95 ± 0.04	15.63 ± 0.14	15.63 ± 0.14
7d	62.50 ± 0.57	3.91 ± 0.08	N.D.	62.50 ± 0.57
7e	15.63 ± 0.14	15.63 ± 0.14	1.95 ± 0.05	31.25 ± 0.29
7f	1.95 ± 0.05	1.95 ± 0.05	N.D.	15.63 ± 0.14
7g	1.95 ± 0.05	1.95 ± 0.05	7.81 ± 0.09	62.50 ± 0.59
7h	1.95 ± 0.04	1.95 ± 0.05	7.81 ± 0.09	62.50 ± 0.59
7i	3.91 ± 0.07	3.91 ± 0.08	31.25 ± 0.29	31.25 ± 0.28
7j	1.95 ± 0.04	1.95 ± 0.04	7.81 ± 0.09	62.50 ± 0.59
7k	0.98 ± 0.02	1.95 ± 0.05	1.95 ± 0.05	3.91 ± 0.07
7l	7.81 ± 0.09	1.95 ± 0.04	N.D.	15.63 ± 0.15
7m	7.81 ± 0.09	1.95 ± 0.04	15.63 ± 0.15	15.63 ± 0.1
7n	7.81 ± 0.09	1.95 ± 0.05	31.25 ± 0.29	3.91 ± 0.07

S. aureus = *Staphylococcus aureus* (G^+), *B. lichenformis* = *Bacillus lichenformis* (G^+). *P. vulgaris* = *Proteus vulgaris* (G^-). *P. aeruginosa* = *Pseudomonas aeruginosa* (G^-), Gtm. = Gentamicin. G^+ = Gram positive, G^- = Gram negative, MIC = Minimum Inhibitory Concentration (μg/mL). N.D. = Not Determined

lowest against *P. aeruginosa* with very big MIC values of 15.63 to 62.50 μg/mL except for **7k** and **7n** (MIC = 3.91 μg/mL). This might be due to the presence of ABC transporters in the hardy cell wall of *P. aeruginosa*, which utilized the porins and efflux pumps to pump out some solution of synthesized compounds herein, immediately after the administration and before the effective distribution of the solution of these compounds **7a-n** takes place [23].

The minimum bactericidal concentration (MBC) test was carried out according to a known procedure [20], and the result was shown in Fig. 3. The MBC in all titled compounds **6**, **7a-n** were observed to be two-fold higher than the MIC against *S. aureus* and *B. cereus* and their MBC varied from 1.95 ± 0.04 to 125 ± 1.20 μg/mL, which was an indication that the final benzimidazole products had better potency as compared with the precursor **6**. Against gram-negative *E. coli*, the MBC of all the compounds were two-fold higher than MIC except for precursor **6** wherein MBC (62.50 ± 0.59 μg/mL) was four-fold higher than MIC (15.63 ± 0.15 μg/mL). The bioactivity of all the compounds was least against *P. aeruginosa* since MBC of three compounds **6**, **7i** (125 ± 1.20 μg/mL) and **7c** (62.5 ± 0.59 μg/mL) were four-fold higher than their MIC which was 31.25 ± 0.29 μg/mL and 15.63 ± 0.14 μg/mL respectively.

Structure Activity Relationship (SAR) Study

In order to optimize the inhibitory potential of molecules and to understand which residues and positions that are important for the activity [2,9,20], series of targeted 2-substituted benzimidazole motifs **7a-n** were herein evaluated for SAR study. Thus, SAR pattern identification is a crucial endeavor to harness a better comprehension of the trend of activities of biomolecules based on the pattern of substitution at the side chain to the structurally related pharmacophoric template portion [20]. The series of structurally related benzimidazole derivatives synthesized herein were evaluated for the SAR study to ascertain the effect of substitution pattern and nature of the substituent on the degree of bioactivity (Fig. 4). The choice of *S. aureus* as an organism for SAR discussion was due to its virulent and stubborn nature, difficulty to treat as well as the regular trend in the pattern of bioactivity observed as the moieties' positions are altered on the ring.

Considering the significance of substituents on the activity of the benzylidene side chain of benzimidazoles **7a-h** based on the MIC values, the order of activity against the growth of *S. aureus* was **7f** ≈ **7g** ≈ **7h** > **7a** ≈ **7c** > **7e** > **7b** > **7d**. The clear observation of this trend showed that the presence of electron donating group (EDG: -OH, -$N(CH_3)_2$ and -$N(CH_2CH_3)_2$) at the para-position of the benzylidene side chain of **7f**, **7g**, and **7h**, led to an increase in the antibacterial activity. This showed that the availability of EDGs on the para-position of benzylidene was an essential criterion for activity enhancement. On the contrary, there was no significant

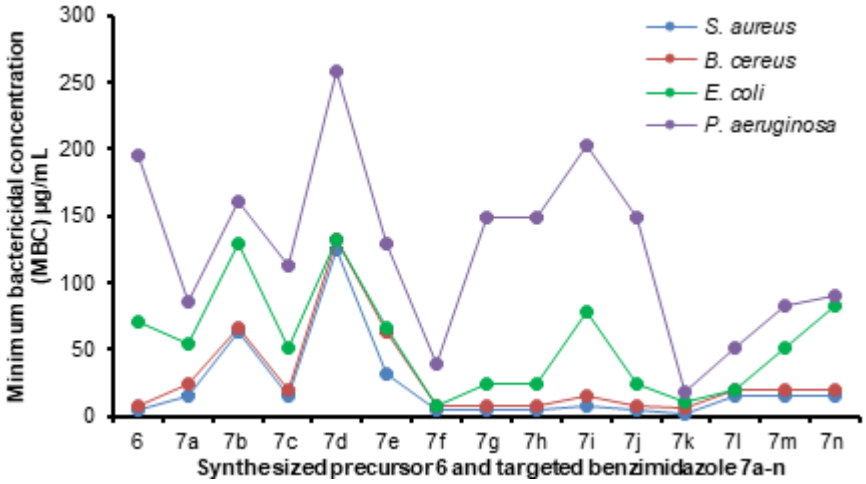


Fig 3. Minimum bactericidal concentration (MBC) result of synthesized benzimidazoles

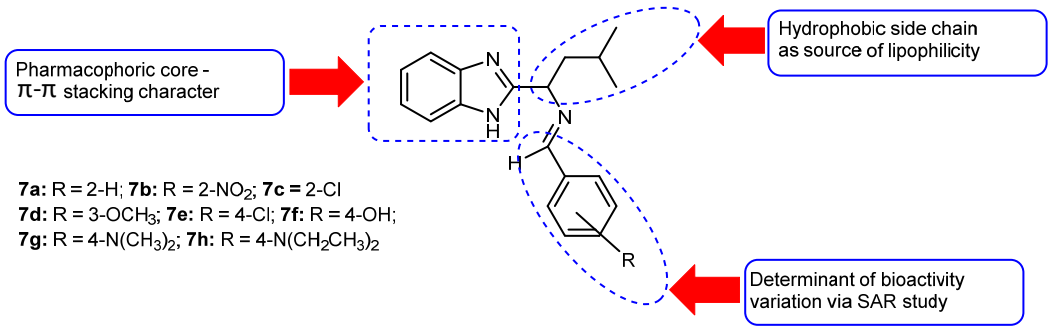


Fig 4. Structural moieties responsible for SAR study in the molecular template

bioactivity contribution to the availability of EWG (Cl, NO_2) at the ortho- or/and para-position led to the loss of antibacterial activity against *S. aureus*. This might be due to the fact that the depletion of electron density at the site of reaction led to the binding affinity reduction.

Considering the series of benzimidazole motifs **7i-k**, which had imine obtained from condensation from ketone, the order of antibacterial activity against *S. aureus* is **7k** > **7j** > **7i**. The highest activity was noticed in **7k** wherein the imine side chain was formed from a bicyclic ketone (camphor), which constituted the steric effect followed by **7j**, which contained bicyclic heterocyclic ketone (isatin). Meanwhile, the lowest activity among the three was from **7i** which was derived from straight chain, ketone acetone.

Thus, the bicyclic ring system of the imino arms contributed a significant function in the boosting of activity, which rendered **7k** to be the most active among **7i-k**. The π-π stacking character in the benzimidazole core also worked synergistically for the improvement of the antibacterial activity [20,26]. According to the activity behavioral pattern of the series of sulfonamido-based benzimidazole motifs **7l-n** against *S. aureus*, the order of activity includes **7l** ≈ **7m** ≈ **7n**. Since **7m** with ortho-substitution (2-CH_3) and **7n** with para-substitution (4-CH_3) had the same bioactivity with **7l** that had no substituent on its benzene nucleus, then the presence of methyl substituent on the benzene ring of sulfonamido portion played no significant role in activity change whether positive or negative against *S. aureus*. Benzylidene portion of benzimidazole series **7a-h** and bicyclic core orientation, as well as π-π stacking character in benzimidazole series **7i-k**, were accountable for diversity in bioactivity. Finally, no bioactive significant role could be ascribable to substitution on the series of benzimidazole with sulfonamide side group in **7l-n**.

■ CONCLUSION

In conclusion, NH_4Cl catalyzed approach was successfully utilized as an environmentally benign technique for the synthesis of novel benzimidazole precursor **6** in good yield. In this present study, the 2-functionalized derivatives of benzimidazole **7a-n** were successfully synthesized. Compound **7k** containing camphor (bulky group) was the most effective antibacterial agent. Thus, there is a need for further studies in order to appreciate the therapeutic efficacy displayed by this series of titled 2-substituted benzimidazole motifs.

■ ACKNOWLEDGMENTS

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■ REFERENCES

[1] Polshettiwar, V., and Varma, R. S., 2008, Greener and expeditious synthesis of bioactive heterocycles using microwave irradiation, *Pure Appl. Chem.*, 80 (4), 777–790.

[2] Ajani, O.O., Aderohunmu, D.V., Ikpo, C.O., Adedapo, A.E., and Olanrewaju, I.O., 2016, Functionalized benzimidazole scaffolds: Privileged

heterocycle for drug design in therapeutic medicine, *Arch. Pharm.*, 349 (7), 475–506.

[3] Srivastava, S., Pandeya, S.N., Yadav, M.K., and Singh, B.K., 2013, Synthesis and analgesic activity of novel derivatives of 1,2-substituted benzimidazoles, *J. Chem.*, 2013, 694295.

[4] Salahuddin, Salahuddin, Shaharyar, M., and Mazumder, A., 2017, Benzimidazoles: A biologically active compounds, *Arabian J. Chem.*, 10 (Suppl. 1), S157–S173.

[5] Alaqeel, S.I., 2017, Synthetic approaches to benzimidazoles from *o*-phenylenediamine: A literature review, *J. Saudi Chem. Soc.*, 21 (2), 229–237.

[6] Ajani, O.O., Ezeoke, E.K., Edobor-Osoh, A., and Ajani, A.O., 2013, Facile synthesis and characterization of new 2,3-disubstituted benzimidazole derivatives, *Int. Res. J. Pure Appl. Chem.*, 3 (1), 10–21.

[7] Rithe, S.R., Jagtap, R.S., and Ubarhande, S.S., 2015, One pot synthesis of substituted benzimidazole derivatives and their characterization, *Rasayan J. Chem.*, 8 (2), 213–217.

[8] Patil, S.A., Patil, S.A., and Patil, R., 2017, Medicinal applications of (benz)imidazole- and indole-based macrocycles, *Chem. Biol. Drug Des.*, 89 (4), 639–649.

[9] Maruthamuthu, Rajam, S., Stella, P.C.R., Dileepan, A.G.B., and Ranjith, R., 2016, The chemistry and biological significance of imidazole, benzimidazole, benzoxazole, tetrazole and quinazolinone nucleus, *J. Chem. Pharm. Res.*, 8 (5), 505–526.

[10] Chang, Y.S., 2012, Hypersensitivity reactions to proton pump inhibitors, *Curr. Opin. Allergy Clin. Immunol.*, 12 (4), 348–353.

[11] Ali, M.S., Saeed, K., Rashid, I., Ijaz, M., Akbar, H., Rashid, M., and Ashraf, K., 2018, Anthelmintic drugs: Their efficacy and cost-effectiveness in different parity cattle, *J. Parasitol.*, 104 (1), 79–85.

[12] Iwamoto, K., Uehara, Y., Inoue, Y., Taguchi, K., Muraoka, D., Ogo, N., Matsuno, K., and Asai, A., 2017, Inhibition of STAT3 by anticancer drug bendamustine, *PLoS One*, 12 (1), 1–18.

[13] Zhang, P., Wang, H., Sun, L., Zhang, J., Xi, Y., Wu, Y., Yan, L.L., Li, X., and Sun, N., 2017, Telmisartan and hydrochlorothiazide antihypertensive treatment in high sodium intake population: A randomized double-blind trial, *J. Hypertens.*, 35 (10), 2077–2085.

[14] Lieberman, L.A., and Higgins, D.E., 2009, A small-molecule screen identifies the antipsychotic drug pimozide as an inhibitor of *Listeria monocytogenes* infection, *Antimicrob. Agents Chemother.*, 53 (2), 756–764.

[15] Farahat, A.A., Ismail, M.A., Kumar, A., Wenzler, T., Brun, R., Paul, A., Wilson, W.D., and Boykin, D.W., 2018, Indole and benzimidazole bichalcophenes: Synthesis, DNA binding and antiparasitic activity, *Eur. J. Med. Chem.*, 143, 1590–1596.

[16] Anand, K., and Wakode, S., 2018, Synthesis, characterization and biological evaluation of benzimidazole derivatives, *Int. J. Pharm. Sci. Res.*, 9 (2), 617–624.

[17] Kadhim, A.J., and Kazim, A.C., 2018, Synthesis and characterization of benzimidazole by using O-phenylenediamine with different aldehydes and carboxylic acids in the presence of ρ-tsOh as a catalyst, *Orient. J. Chem.*, 34 (4), 2131–2136.

[18] Çevik, U.A., Nurpelin, S.B., Mina, A.C., Yusuf, Ö., and Özlem, A., 2018, Synthesis and evaluation of new benzimidazole derivatives with hydrazone moiety as anticancer agents, *Turk. J. Biochem.*, 43 (2), 151–158.

[19] Gupta, P.D., and Birdi, T.J., 2017, Development of botanicals to combat antibiotic resistance, *J. Ayurveda Integr. Med.*, 8 (4), 266–275.

[20] Ajani, O.O., Tolu-Bolaji, O.O., Olorunshola, S.J., Zhao, Y., and Aderohunmu, D.V., 2017, Structure-based design of functionalized 2-substituted and 1,2-disubstituted benzimidazole derivatives and their *in vitro* antibacterial efficacy, *J. Adv. Res.*, 8 (6), 703–712.

[21] Ajani, O.O., Obafemi, C.A., Nwinyi, O.C., and Akinpelu, D.A., 2010, Microwave assisted synthesis and antimicrobial activity of 2-quinoxalinone-3-hydrazone derivatives, *Bioorg. Med. Chem.*, 18 (1), 214–221.

[22] Komurcu, S.G., Rollas, S., Ulgen, M., Gorrod, J.W., and Cevikbas, A., 1995, Evaluation of some arylhydrazones of *p*-aminobenzoic acid hydrazide

as antimicrobial agents and their in vitro hepatic microsomal metabolism, *Boll. Chim. Farm.*, 135 (7), 375–379.

[23] Prescott, L.M., Harley, J.P., and Klein, D.A., 2005, *Microbiology*, 6th Ed., McGraw-Hill, Inc., New York.

[24] Bottone, E.J., 2010, *Bacillus cereus*, a volatile human pathogen, *Clin. Microbiol. Rev.*, 23 (2), 382–398.

[25] Arhin, A., and Boucher, C., 2010, The outer membrane protein OprQ and adherence of *Pseudomonas aeruginosa* to human fibronectin, *Microbiology*, 156 (5), 1415–1423.

[26] Ren, Y., Zhang, L., Zhou, C.H., and Geng, R.X., 2014, Recent development of benzotriazole-based medicinal drugs, *Med. Chem.*, 4 (9), 640–662.