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Original Article

Structure-based design of functionalized 2-substituted and 1,2-disubstituted benzimidazole derivatives and their *in vitro* antibacterial efficacy

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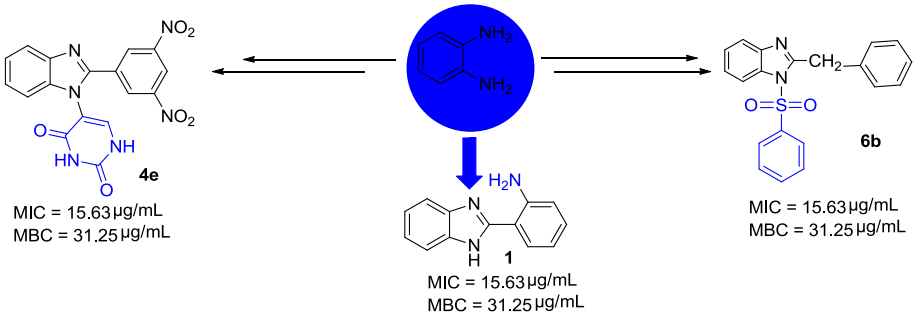
GRAPHICAL ABSTRACT



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ABSTRACT

The aim of this present study was to synthesize 2-substituted and 1,2-disubstituted benzimidazole derivatives to investigate their **antibacterial** diversity for possible future drug design. The structure-based design of precursors 2-(1H-benzimidazol-2-yl)aniline **1**, 2-(3,5-dinitro phenyl)-1H-benzimidazole **3** and 2-benzyl-1H-benzimidazole **5** were achieved by the condensation reaction of *o*-phenylenediamine with anthranilic acid, 3,5-dinitrophenylbenzoic acid, and phenylacetic acid, respectively. The precursors **1**, **3** and **5**, upon reaction with six different electrophile-releasing agents, furnished the corresponding 2-substituted benzimidazole, **2a-f** and 1,2-disubstituted benzimidazole derivatives **4a-f** and **6a-f**, respectively. The structural identity of the targeted compounds was authenticated by elemental analytical data and spectral information from FT-IR, UV, ^{1}H, and ^{13}C NMR. The outcome of the findings from the *in vitro* screening unveiled 2-benzyl-1-(phenylsulfonyl)-1H-benzimidazole **6b** as the most active derivative with lowest MIC value of 15.63 μg/mL.

Introduction

From time to time, heterocyclic templates have continued to gain respect and much interest among the medicinal chemists, because of their numerous therapeutic applications and effective reported druggability [1]. Benzimidazole is a heterocyclic aromatic

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organic compound that plays important functions in the development of theory in heterocyclic chemistry and organic synthesis [2]. Benzimidazole is a strongly acidic compound with a pKa of 12.75, while its conjugated acid has a pKa of 5.68, which is less basic than imidazole. Benzimidazole is readily prepared by [4+1]-cycloaddition of *o*-phenylenediamine with a one carbon donor source in the presence of various heterogeneous catalysts [3], such as H_2O_2/HCl [4], H_2O_2/CAN [5], H_2O/HCl [6], H_2O_2/$Fe(NO_3)_3$ [7], and H_2O_2/Bu_4NI [8] as efficient oxidative couples. In healthful analysis, the synthesis of novel benzimidazole derivatives remains a focus [9]. Diverse synthetic efforts for accessing benzimidazole derivatives have been documented, however, the commonest technique involves the reaction of *o*-phenylenediamine with alkanoic acids. From the evaluation of the works of various researchers, benzimidazole derivatives have been reported to possess antimalarial [10], anticancer [11], antimicrobial [12,13], antioxidant [14], and anticonvulsant [15] activities among others. Some derivatives of benzimidazole are well known in corrosion studies and their corrosion inhibition efficiencies are related to their adsorption properties [16].

The outbreak of new diseases and the increase in population of drug resistant strains of bacteria, such as methicillin-resistant *Staphylococcus aureus* [17], vancomycin-resistant *Enterococci* [18], ampicillin-resistant *Enterobacter aerogenes* [19], gentamicin-resistant *Escherichia coli* [20], and chloroquine-resistant *Plasmodium falciparum* [21], have posed great challenges to life and wellbeing of mankind. Based on the existence of antidrug multi-resistant bacteria strains [22], the occurrence of side effects to commercially available drugs [23], adverse drug reaction in elderly patient [24], the emergence of new diseases, and global health threat that have resulted in high mortality rate [25]; it has become highly imperative to consistently and continuously engage in the synthetic preparation of novel heterocyclic templates as highly dynamic biologically active substances for therapeutic uses. Therefore, it is beneficial to design some 2-substituted- and 1,2-disubstituted benzimidazole derivatives by ecofriendly method so as to examine their antimicrobial properties for possible future drug development.

Material and methods

Chemical compounds and reagents were purchased from Sigma-Aldrich Chemicals (St. Louis, Missouri, USA) apart from Tetrahydrofuran (THF), benzenesulfonyl chloride, and anthranilic acid which were supplied by the British Drug Houses (Poole, Dorset, England). All these compounds were then made available by Department of Chemistry, Covenant University for research use. All the chemicals are pure and they were used directly without further purification. The synthesized heterocyclic frameworks were evaluated for their melting point determination using Stuart equipment and the value obtained were recorded directly. Bruker fourier-transform (ft-ir) spectrophotometer was utilized to obtain infrared data. The UV spectra of the solution of the compounds in THF were run in UV Genesys 10 s. The ^1H and ^{13}C nuclear magnetic resonance of the heterocycles were NMR Bruker DPX 400 spectrometer at 400 M*Hz* and 100 M*Hz*, respectively in DMSO-d_6. The reference utilized was Tetramethylsilane (TMS). The reaction progress as well as the level of purity was routinely checked and monitored with Thin Layer Chromatography (TLC) using $CHCl_3$/CH_3OH (9:1, v/v) eluent. After reaction was completed, solvents were evaporated under reduced pressure using IKA® RV 10 Rotary evaporator. In a situation where more than one spots were observed, column chromatography was carried out to get a pure compound.

2-(1H-Benzimizadol-2-yl)aniline as precursor ***1***

o-Phenylene diamine (15.00 g, 140.00 mmol) was weighed and dissolved in 150 mL of ethanol in a round- bottomed flask. It was stirred for 5 min with the aid of magnetic stirrer after which anthranilic acid (19.20 g, 140.00 mmol) was gradually tipped into the solution followed by the addition of a catalytic amount of NH_4-Cl (0.75 g, 14.00 mmol). The resulting solution was then heated under reflux at 60–70 °C for 2 h. The TLC was utilized to ascertain the progress of reaction. Upon completion, the resulting solution was allowed to cool down. The flask content was evaporated to dryness and triturated with ice-cold water. The solid mass formed was separated by suction filtration to furnish 2-(1*H*-benzimizadol-2-yl)aniline **1** (72.68%), mp = 85–87 °C, colour = gray. ^1H NMR (400 M*Hz*, DMSO-d_6) δ_H: 4.50 (s-br, 2H, NH_2), 6.37–6.39 (dd, J_1 = 4.54 *Hz*, J_2 = 8.80 *Hz*, 1H, Ph-H), 6.49–6.50 (dd, J_1 = 4.21 *Hz*, J_2 = 8.87 *Hz*, 1H, Ph-H), 6.84–6.86 (d, *J* = 8.80 *Hz*, 1H, Ph-H), 7.12–7.14 (d, *J* = 8.00 *Hz*, 1H, Ph-H), 7.39–7.41 (m, 2H, Ph-H), 7.59–7.63 (d, *J* = 8.87 *Hz*, 1H, Ph-H). ^{13}C NMR (100 M*Hz*, DMSO-d_6) δ_C: 109.6, 115.2 (2 × CH), 116.9, 119.4, 123.1 (2 × CH), 125.5, 129.7, 141.9 (2 × C), 145.1, 155.0 ppm. λ_{max} in nm (log ε_{max}): 218 (4.2741), 253 (4.3096), 326 (3.6434). FT-IR ν in cm^{-1}: 3424 (N—H of NH_2), 3422 (N—H of NH_2), 3405 (N—H), 1620 (C=C aromatic). Anal. Calcd for $C_{13}H_{11}N_3$ (209.25): C, 74.62; H, 5.30; N, 20.08%. Found: C, 74.80; H, 5.47; N, 19.96%.

Overall protocol towards accessing 2-substituted benzimidazole ***2a-f***

Precursor **1** (4.00 g, 19.10 mmol) was dissolved in 20 mL of tetrahydrofuran (THF) in a round-bottomed flask at room temperature. The medium was basified by the addition of Na_2CO_3 (4.06 g, 38.30 mmol) and cooled to 0–5 °C in ice bath. The corresponding electrophile-releasing substrate **a-f** (19.10 mmol) was then added and the reacting mixture was maintained on ice bath for additional 15 min after which the medium was warmed up to room temperature and stirred there for 24 h. Monitoring of reaction progress was conducted using TLC and upon reaction completion, the solvent was evaporated at reduced pressure using rotary evaporator. Cold water was added to the resulting mass, filtered by suction, and air-dried to afford crude product which upon column purification afforded 2-substituted benzimidazole derivatives **2a-f.**

N-(2-(1H-Benzimidazole-2-yl)phenyl) acetamide ***2a***

When **a** = acetyl chloride, yield 57.70%, mp = 253–255 °C, colour = gray. ^1H NMR (400 M*Hz*, DMSO-d_6) δ_H: 2.67 (s, 3H, CH_3—CO), 6.36–6.39 (dd, J_1 = 4.58 *Hz*, J_2 = 8.82 *Hz*, 1H, Ph—H), 6.51–6.52 (dd, J_1 = 4.18 *Hz*, J_2 = 8.89 *Hz*, 1H, Ph—H), 6.86–6.88 (d, *J* = 8.82 *Hz*, 1H, Ph—H), 7.13–7.15 (d, *J* = 8.00 *Hz*, 1H, Ph—H), 7.39–7.43 (m, 2H, Ph—H), 7.62–7.65 (d, *J* = 8.89 *Hz*, 1H, Ph—H). ^{13}C NMR (100 M*Hz*, DMSO-d_6) δ_C: 26.4 (CH_3), 109.6, 115.2 (2 × CH), 116.8, 119.6, 123.6 (2 × CH), 125.6, 129.8, 141.7 (2 × C), 145.3, 156.1, 175.4 (C=O) ppm. λ_{max} in nm (log ε_{max}): 218 (4.2988), 248 (4.5563), 323 (3.8261), 464 (2.4771). FT-IR ν in cm^{-1}: 3405 (N—H), 1699 (C=O). Anal. Calcd for $C_{15}H_{13}N_3O$ (251.11): C, 71.70; H, 5.21; N, 16.72%. Found: C, 71.88; H, 5.09; N, 16.89%.

N-(2-(1H-Benzimidazole-2-yl)phenyl)benzenesulfonamide ***2b***

When **b** = benzenesulfonyl chloride, yield 97.21%, mp = N.D. (Oily), colour = black. ^1H NMR (400 M*Hz*, DMSO-d_6) δ_H: 6.35–6.38 (m, 3H, Ph—H), 6.46–6.49 (m, 2H, Ph—H), 6.84–6.86 (d, *J* = 7.16 *Hz*, 2H, Ph—H), 7.11–7.14 (d, *J* = 11.96 *Hz*, 2H, Ph—H), 7.40–7.42 (m, 2H, Ph—H), 7.62–7.65 (d, *J* = 11.88 *Hz*, 2H, Ph—H). ^{13}C NMR (100 M*Hz*, DMSO-d_6) δ_C: 109.8, 115.3 (2 × CH), 116.9, 119.5, 123.2 (2 × CH), 125.6, 127.5 (2 × CH), 128.9, 129.8

(2 × CH), 131.9, 154.8, 139.7, 141.6 (2 × C), 145.3 ppm. λ_{max} in nm (log ε_{max}): 221 (4.6609), 251 (4.7332), 317 (4.2878), 428 (3.9294). FT-IR ν in cm^{-1}: 3405 (N—H), 3266 (N—H), 1620 (C=C aromatic), 1575 (C=N imine), 1376 (SO_2), 1185 (SO_2). Anal. Calcd for $C_{19}H_{15}N_3O_2S$ (349.09): C, 65.31; H, 4.33; N, 12.03%. Found: C, 65.20; H, 4.15; N, 11.83%.

N-(2-(1H-Benzimidazole-2-yl)phenyl)-4-methylbenzenesulfonamide ***2c***

When **c** = *p*-toluenesulfonyl chloride, yield 80.03%, mp = N.D. (Oily), colour = brown. ^{1}H NMR (400 M*Hz*, DMSO-d_6) δ_H: 2.80 (s, 3H, CH_3-Ar), 6.36–6.39 (m, 3H, Ph—H), 6.49–6.51 (m, 2H, Ph—H), 6.84–6.86 (d, *J* = 7.04 *Hz*, 1H, Ph—H), 7.11–7.14 (d, *J* = 11.96 *Hz*, 2H, Ph—H), 7.40–7.42 (m, 2H, Ph—H), 7.62–7.65 (d, *J* = 11.88 *Hz*, 2H, Ph—H). ^{13}C NMR (100 M*Hz*, DMSO-d_6) δ_C: 21.3 (CH_3), 109.8, 115.3 (2 × CH), 116.9, 119.5, 123.2 (2 × CH), 125.6, 127.3 (2 × CH), 129.1, 129.8 (2 × CH), 131.9, 139.7, 141.8 (2 × C), 145.3, 155.0 ppm. λ_{max} in nm (log ε_{max}): 212 (4.6343), 233 (5.2124), 311 (4.6750). FT-IR ν in cm^{-1}: 3407 (N—H), 3263 (N—H), 1377 (SO_2), 1187 (SO_2). Anal. Calcd for $C_{20}H_{17}N_3O_2S$ (363.43): C, 66.10; H, 4.71; N, 11.56%. Found: C, 65.95; H, 4.63; N, 11.75%.

2-(1H-Benzimidazol-2-yl)-N-(3-chlorobenzyl)aniline ***2d***

When **d** = 3-chlorobenzyl chloride, yield 87.90%, mp = N.D. (Oily), colour = black. ^{1}H NMR (400 M*Hz*, DMSO-d_6) δ_H: 3.73 (s, 2H, CH_2), 6.31–6.35 (m, 3H, Ph—H), 6.48–6.50 (m, 2H, Ph—H), 6.84–6.87 (d, *J* = 8.00 *Hz*, 1H, Ph—H), 7.11–7.14 (d, *J* = 11.96 *Hz*, 2H, Ph—H), 7.61–7.63 (d, *J* = 7.96 *Hz*, 2H, Ph—H), 7.92–7.94 (d, *J* = 7.72 *Hz*, 1H, Ph—H), 8.21 (s, 1H, Ph—H). ^{13}C NMR (100 M*Hz*, DMSO-d_6) δ_C: 48.2 (CH_2), 112.3, 114.1, 115.1 (2 × CH), 117.4, 123.2 (2 × CH), 125.0, 125.4, 126.1, 126.8, 129.3, 129.9, 132.2, 134.3, 141.8 (2 × C), 145.4, 154.8 ppm. λ_{max} in nm (log ε_{max}): 224 (5.0026), 251 (4.9633), 302 (4.7279). FT-IR ν in cm^{-1}: 3460, 3354 (N—H), 3107 (C—H aromatic), 2924 (CH aliphatic), 2854 (CH aliphatic), 1624 (C=C Aromatic), 1581 (C=N). Anal. Calcd for $C_{20}H_{16}N_3Cl$ (333.81): C, 71.96; H, 4.84; N, 12.59%. Found: C, 72.14; H, 5.02; N, 12.38%.

5-((2-(1H-Benzimidazole-2-yl)phenyl)amino)pyrimidine-2,4(1H,3H)-dione ***2e***

When **e** = 5-bromouracil, yield 83.29%, mp > 300 °C, colour = -brown. ^{1}H NMR (400 M*Hz*, DMSO-d_6) δ_H: 6.36–6.39 (m, 1H. Ph—H), 6.50–6.52 (m, 1H. Ph—H), 6.83–6.86 (d, *J* = 11.88 *Hz*, 1H, Ph—H), 7.11–7.13 (d, *J* = 8.00 *Hz*, 2H, Ph—H), 7.39–7.43 (m, 2H, Ph—H), 7.62–7.65 (d, *J* = 11.88 *Hz*, 1H, Ph—H), 8.37–8.41 (d, *J* = 13.92 *Hz*, 1H, Ph—H), 11.02 (s, IH, NH), 11.56 (s, 1H, NH). ^{13}C NMR (100 M*Hz*, DMSO-d_6) δ_C: 109.2, 110.6, 115.6 (2 × CH), 119.3, 123.1 (2 × CH), 125.3, 125.7, 126.2, 129.4, 141.8 (2 × C), 145.0, 152.5, 169.0 (C=O), 169.8 (C=O) ppm. λ_{max} in nm (log ε_{max}): 202 (4.5646), 224 (4.9854), 254 (5.1392). FT-IR (ν_{max} in cm^{-1}): 3460, 3354 (N—H), 3107 (C—H aromatic), 2924 (CH aliphatic), 2854 (CH aliphatic), 1624 (C=C aromatic), 1581 (C=N). Anal. Calcd for $C_{17}H_{13}N_5O_2$ (319.32): C, 63.94; H, 4.10; N, 21.93%. Found: C, 63.90; H, 3.99; N, 22.01%.

N-(2-(1H-Benzimidazole-2-yl)phenyl)benzene-1,4-diamine ***2f***

When **f** = 4-chloroaniline, yield 80.74%, mp > 300 °C, colour = -gray. ^{1}H NMR (400 M*Hz*, DMSO-d_6) δ_H: 5.32 (s, 2H, NH_2), 6.33–6.38 (m, 2H, Ph—H), 6.49–6.53 (m, 2H, Ph—H), 6.83–6.86 (d, *J* = 9.92 *Hz*, 1H, Ph—H), 7.11–7.14 (d, *J* = 10.48 *Hz*, 2H, Ph—H), 7.39–7.41 (d, *J* = 8.50 *Hz*, 2H, Ph—H), 7.59–7.62 (d, *J* = 10.48 *Hz*, 2H, Ph—H). ^{13}C NMR (100 M*Hz*, DMSO-d_6) δ_C: 109.8, 115.3 (2 × C), 117.2 (2 × CH), 118.1, 119.5, 121.1 (2 × CH), 123.0 (2 × CH), 125.6, 129.8, 132.2, 137.9, 140.9, 141.8 (2 × C), 154.8 ppm. λ_{max} in nm (log ε_{max}): 224 (5.0228), 254 (1.9881), 308 (4.5453). FT-IR (ν_{max} in cm^{-1}): 3436 (N—H of 1° amine), 3406 (N—H of 1° amine), 3330 (N—H of 2° amine), 3060 (C—H aromatic), 1613 (C=C aromatic), 1577 (C=N). Anal. Calcd for $C_{19}H_{16}N_4$ (300.36): C, 75.98; H, 5.37; N, 18.65%. Found: C, 76.09; H, 5.40; N, 18.45%.

2-(3,5-Dinitrophenyl)-1H-benzimidazole as precursor ***3***

Procedure for the synthesis of precursor **1** was repeated for the reaction of *o*-phenylenediamine with 3,5-dinitrophenylbenzoic acid to afford precursor **3** (90.07%), mp = 177–179 °C, colour = yellow. ^{1}H NMR (400 M*Hz*, DMSO-d_6) δ_H: 7.40–7.42 (d, *J* = 8.00 *Hz*, 1H, Ph—H), 7.44–7.46 (d, *J* = 8.00 *Hz*, 1H, Ph—H), 7.72–7.74 (t, *J* = 7.58 *Hz*, 1H, Ph—H), 7.94–7.96 (m, 1H, Ph—H), 8.64 (s, 2H, Ph—H), 8.85 (s, 1H, Ar—H). ^{13}C NMR (100 M*Hz*, DMSO-d_6) δ_C: 115.2 (2 × CH), 125.8, 128.7 (2 × CH), 129.4 (2 × CH), 134.8, 144.2 (2 × C), 150.7 (2 × C), 156.1 ppm. λ_{max} in nm (log ε_{max}): 218 (4.6522), 248 (4.6365). FT-IR (ν_{max} in cm^{-1}): 3349 (N—H), 3172 (C—H aromatic), 3101 (C—H aromatic), 1606 (C=C), 1572 (C=N), 1543 (NO_2 asym.), 1344 (NO_2 sym.). Anal. Calcd for $C_{13}H_8N_4O_4$ (284.22): C, 55.13; H, 2.49; N, 19.78%. Found: C, 54.98; H, 2.54; N, 19.92%.

Overall protocol towards accessing 1,2-disubstituted-1H-benzimidazole ***4a-f***

Similar procedure for **2a-f** was repeated herein using 2-(3,5-dinitrophenyl)-1*H*-benzimidazole **3** as the precursor which reacted with substrates **a-f** to afford 1-substituted-2-(3,5-dinitrophenyl)-1*H*-benzimidazoles **4a-f**.

1-(2-(3,5-Dinitrophenyl)-1H-benzimidazole-1-yl)ethanone ***4a***

Yield 72.58%, mp = 250–252 °C, colour = brown. ^{1}H NMR (400 M*Hz*, DMSO-d_6) δ_H: 2.59 (s, 3H, CH_3—CO), 7.40–7.42 (d, *J* = 8.00 *Hz*, 1H, Ph—H), 7.44–7.46 (d, *J* = 8.00 *Hz*, 1H, Ph—H), 7.72–7.74 (t, *J* = 7.56 *Hz*, 1H, Ph—H), 7.94–7.96 (m, 1H, Ph—H), 8.64 (s, 2H, Ph—H), 8.85 (s, 1H, Ph—H). ^{13}C NMR (100 M*Hz*, DMSO-d_6) δ_C: 28.9 (CH_3), 116.6 (2 × CH), 126.1, 128.9 (2 × CH), 129.7 (2 × CH), 135.0, 144.3 (2 × C), 150.5 (2 × C), 156.3, 174.1 (C=O) ppm. λ_{max} in nm (log ε_{max}): 218 (4.3729), 248 (4.3050). FT-IR (ν_{max} in cm^{-1}): 3106 (C—H aromatic), 2854 (C—H aliphatic), 1699 (C=O amide), 1622 (C=C), 1580 (C=N), 1541 (NO_2asym.), 1346 (NO_2 sym.). Anal. Calcd for $C_{15}H_{10}N_4O_5$ (326.26): C, 55.22; H, 3.09; N, 17.17%. Found: C, 55.13; H, 2.89; N, 17.08%.

2-(3,5-Dinitrophenyl)-1-(phenylsulfonyl)-1H-benzimidazole ***4b***

Yield 62.77%, mp > 300 °C, colour = gray. ^{1}H NMR (400 M*Hz*, DMSO-d_6) δ_H: 7.15–7.19 (m, 5H, Ph—H), 7.40–7.46 (m, 2H, Ph—H), 7.72–7.74 (t, *J* = 7.60 *Hz*, 1H, Ph—H), 7.94–7.96 (m, 1H, Ph—H), 8.64 (s, 2H, Ph—H), 8.85 (s, 1H, Ph—H). ^{13}C NMR (100 M*Hz*, DMSO-d_6) δ_C: 116.6 (2 × CH), 119.6 (2 × CH), 126.1, 128.9 (2 × CH), 129.7 (2 × CH), 132.5, 135.0 (2 × C), 137.5 (2 × CH), 144.3 (2 × C), 150.5, 152.0, 156.3 ppm. λ_{max} in nm (log ε_{max}): 215 (3.9395), 251 (4.1492). FT-IR (ν_{max} in cm^{-1}): 3050 (C—H aromatic), 2855 (C—H aliphatic), 1620 (C=C), 1580 (C=N), 1541 (NO_2asym.), 1376 (SO_2), 1346 (NO_2 sym.), 1185 (SO_2 2nd band). Anal. Calcd for $C_{19}H_{12}N_4O_6S$ (434.39): C, 53.77; H, 2.85; N, 13.20%. Found: C, 53.95; H, 3.03; N, 13.31%.

2-(3,5-Dinitrophenyl)-1-tosyl-H-benzimidazole ***4c***

Yield 98.77%, mp = 112 °C, colour = brown. ^{1}H NMR (400 M*Hz*, DMSO-d_6) δ_H: 2.60 (s, 3H, CH_3—Ar), 6.90–6.92 (d, *J* = 8.00 *Hz*, 2H, Ph—H), 7.15–7.17 (d, *J* = 8.00 *Hz*, 2H, Ph—H), 7.40–7.44 (m, 2H, Ph—H), 7.73–7.75 (t, *J* = 7.54 *Hz*, 1H, Ph—H), 7.93–7.95 (m, 1H, Ph—H), 8.60 (s, 2H, Ph—H), 8.84 (s, 1H, Ph—H). ^{13}C NMR (100 M*Hz*, DMSO-d_6) δ_C: 21.7 (CH_3), 116.6 (2 × CH), 119.6 (2 × CH), 124.8, 156.3, 152.1, 150.5, 144.3 (2 × C), 137.5 (2 × CH), 135.0, 132.5, 129.5 (2 × CH), 126.0, 128.9 (2 × CH) ppm. λ_{max} in nm (log ε_{max}): 242 (4.6138). FT-IR (ν_{max} in cm^{-1}): 3105 (C—H aromatic), 2852 (C—H aliphatic), 1620 (C=C), 1575 (C=N), 1540 (NO_2 asym.), 1377 (SO_2), 1345 (NO_2 sym.). Anal. Calcd for $C_{20}H_{14}N_4O_6S$ (438.41): C, 54.79; H, 3.22; N, 12.78%. Found: C, 54.62; H, 3.16; N, 12.94%.

1-(3-Chlorobenzyl)-2-(3,5-dinitrophenyl-1H-benzimidazole ***4d***

Yield 71.16%, mp > 300 °C, colour = black. ^{1}H NMR (400 M*Hz*, DMSO-d_6) δ_H: 3.72 (s, 2H, CH_2—Ar), 7.11–7.14 (m, 1H, Ph—H), 7.25–7.27 (d, *J* = 7.56 *Hz*, 2H, Ph—H), 7.40–7.46 (m, 2H, Ph—H), 7.72–7.74 (t, *J* = 7.60 *Hz*, 1H, Ph—H), 7.94–7.96 (d, *J* = 7.22 *Hz*, 1H, Ph—H), 8.21 (s, 1H, Ph—H), 8.64 (s, 2H, Ph—H), 8.85 (s, 1H, Ph—H). ^{13}C NMR (100 M*Hz*, DMSO-d_6) δ_C: 48.0 (CH_2), 115.2, 116.2 (2 × CH), 119.2, 125.1, 126.1, 128.9 (2 × CH), 129.7 (2 × CH), 130.0, 135.0, 139.1, 144.0 (2 × C), 150.4 (2 × C), 156.1 ppm. λ_{max} in nm (log ε_{max}): 212 (3.7076), 248 (4.1986), 467 (2.6020), 470 (2.6020). FT-IR (ν_{max} in cm^{-1}): 2924, 2854 (CH aliphatic), 1612 (C=C Aromatic), 1584 (C=N imine), 1501 (NO_2 asym.). Anal. Calcd for $C_{20}H_{13}N_4O_4Cl$ (408.79): C, 58.76; H, 3.21; N, 13.71%. Found: C, 58.88; H, 3.32; N, 13.89%.

5-(2-(3,5-Dinitrophenyl)-1H-benzimidazol-1-yl)pyrimidine-2,4 (1H,3H)-dione ***4e***

Yield 61.04%, mp > 300 °C, colour = gray. ^{1}H NMR (400 M*Hz*, DMSO-d_6) δ_H: 7.40–7.42 (t, *J* = 7.24 *Hz*, 1H, Ph—H), 7.44–7.46 (m, 1H, Ph—H), 7.72–7.74 (d, *J* = 8.00 *Hz*, 2H, Ph—H), 7.94–7.96 (s, 1H, Ph—H), 8.64 (s, 2H, Ph—H), 8.85 (s, 1H, Ph—H), 11.05 (s, IH, NH), 11.55 (s, 1H, NH). ^{13}C NMR (100 M*Hz*, DMSO-d_6) δ_C: 116.6 (2 × CH), 126.1, 128.9 (2 × CH), 129.7 (2 × CH), 135.0, 138.7, 140.1, 144.3 (2 × C), 150.5 (2 × C), 156.3, 169.0 (C=O), 169.7 (C=O) ppm. λ_{max} in nm (log ε_{max}): 220 (4.0234), 242 (4.8730), 311 (4.0790). FT-IR (ν_{max} in cm^{-1}): 3362 (N—H), 3217 (N—H), 3050 (C—H aromatic), 1685 (C=O amide), 1612 (C=C aromatic), 1575 (C=N), 1536 (NO_2 asym), 1344 (NO_2 sym.). Anal. Calcd for $C_{17}H_{10}N_6O_6$ (394.30): C, 51.78; H, 2.56; N, 21.31%. Found: C, 51.97; H, 2.69; N, 21.51%.

4-(2-(3,5-Dinitrophenyl)-1H-benzimidazole-1-yl)aniline ***4f***

Yield 66.85%, mp > 300 °C, colour = gray. ^{1}H NMR (400 M*Hz*, DMSO-d_6) δ_H: 4.50 (s, 2H, NH_2), 6.90–6.92 (d, *J* = 8.00 *Hz*, 2H, Ph—H), 7.15–7.17 (d, *J* = 8.00 *Hz*, 2H, Ph—H), 7.40–7.44 (m, 2H, Ph—H), 7.73–7.76 (t, *J* = 7.84 *Hz*, 1H, Ph—H), 7.93–7.96 (m, 1H, Ph—H), 8.69 (s, 2H, Ph—H), 8.90 (s, 1H, Ph—H). ^{13}C NMR (100 M*Hz*, DMSO-d_6) δ_C: 115.2, 116.6 (2 × CH), 118.3 (2 × CH), 125.3, 126.1, 128.9 (2 × CH), 129.7 (2 × CH), 135.0, 144.1 (2 × C), 147.0, 147.8, 150.5 (2 × C), 155.4 ppm. λ_{max} in nm (log ε_{max}): 235 (5.4130), 302 (4.8040). FT-IR (ν_{max} in cm^{-1}): 3472 (N—H), 3363 (N—H), 3214, 1620 (C=C aromatic), 1536 (NO_2 asym), 1321, 1377 (NO_2sym). Anal. Calcd for $C_{19}H_{13}N_5O_4$ (375.34): C, 60.80; H, 3.49; N, 18.66%. Found: C, 61.00; H, 3.68; N, 18.84%.

2-Benzyl-1H-benzimidazole as precursor ***5***

Procedure for the synthesis of precursor **1** was repeated for the reaction of *o*-phenylenediamine with phenyl acetic acid to afford precursor **5** (79.12%), mp = 108–110 °C, colour = brown. ^{1}H NMR (400 M*Hz*, DMSO-d_6) δ_H: 3.56 (s, 2H, CH_2—Ar), 6.35–6.38 (dd, J_1 = 3.48 *Hz*, J_2 = 9.12 *Hz*, 1H, Ph—H), 6.48–6.50 (dd, J_1 = 3.48 *Hz*, J_2 = 8.00 *Hz*, 1H, Ph—H), 7.12 (s, 5H, Ph—H), 7.24–7.26 (d, *J* = 9.12 *Hz*, 1H, Ph—H), 7.29–7.31 (d, *J* = 8.00 *Hz*, 1H, Ph—H). ^{13}C NMR (100 M*Hz*, DMSO-d_6) δ_C: 34.9 (CH_2), 115.2 (2 × CH), 123.4 (2 × CH), 125.8, 128.7 (2 × CH), 129.4 (2 × CH), 136.9, 138.8 (2 × C), 142.7 ppm. λ_{max} in nm (log ε_{max}): 257 (5.4181), 275 (5.4133), 314 (4.4842). FT-IR (ν_{max} in cm^{-1}): 3415 (N—H), 2924 (C—H aliphatic), 2854 (C—H aliphatic), 1638 (C=C), 1587 (C=N imine). Anal. Calcd for $C_{14}H_{12}N_2$ (208.26): C, 80.74; H, 5.81; N, 13.45%. Found: C, 80.80; H, 6.01; N, 13.44%.

Overall protocol towards accessing 1,2-disubstituted-1H-benzimidazole ***6a-f***

Similar procedure for the synthesis of **2a-f** was repeated herein using 2-benzyl-1*H*-benzimidazole **5** as the precursor which reacted with substrates **a-f** to afford 1-substituted-2-benzyl-1*H*-benzimidazoles **6a-f**.

1-(2-Benzyl-1H-benzimidazole-1-yl)ethanone ***6a***

Yield 90.11%, mp = N.D. (Oily), colour = black. ^{1}H NMR (400 M*Hz*, DMSO-d_6) δ_H: 2.67 (s, 3H, CH_3CO), 3.56 (s, 2H, CH_2—Ar), 6.36–6.39 (dd, J_1 = 3.48 *Hz*, J_2 = 9.12*Hz*, 1H, Ph—H), 6.48–6.50 (dd, J_1 = 3.48 *Hz*, J_2 = 8.00*Hz*, 1H, Ph—H), 7.09 (s, 5H, Ph—H), 7.24–7.26 (d, *J* = 9.12 *Hz*, 1H, Ph—H), 7.29–7.31 (d, *J* = 8.00 *Hz*, 1H, Ph—H). ^{13}C NMR (100 M*Hz*, DMSO-d_6) δ_C: 28.9 (CH_3), 34.9 (CH_2), 115.2 (2 × CH), 123.5 (2 × CH), 125.9, 128.7 (2 × CH), 129.5 (2 × CH), 136.9, 138.9 (2 × C), 142.8 ppm. λ_{max} in nm (log ε_{max}): 227 (2.2300), 315 (2.5057). FT-IR (ν_{max} in cm^{-1}): 2922 (C—H aliphatic), 2855 (C—H aliphatic), 1685 (C=O), 1620 (C=C), 1575 (C=N imine). Anal. Calcd for $C_{16}H_{14}N_2O$ (250.30): C, 76.78; H, 5.64; N, 11.19%. Found: C, 76.94; H, 5.59; N, 11.00%.

2-Benzyl-1-(phenylsulfonyl)-1H-benzimidazole ***6b***

Yield 87.48%, mp = N.D. (Oily), colour = black. ^{1}H NMR (400 M*Hz*, DMSO-d_6) δ_H: 3.56 (s, 2H, CH_2—Ar), 6.35–6.38 (dd, J_1 = 3.60 *Hz*, J_2 = 9.26 *Hz*, 1H, Ph—H), 6.48–6.50 (dd, J_1 = 3.60 *Hz*, J_2 = 8.00 *Hz*, 1H, Ar—H), 6.91–6.95 (m, 3H, Ph—H), 7.12 (s, 5H, Ph—H), 7.13–7.17 (m, 3H, Ph—H), 7.22–7.24 (d, *J* = 9.26 *Hz*, 1H, Ph—H), 7.30–7.32 (d, *J* = 8.00 *Hz*, 1H, Ph—H), 7.44–7.46 (d, *J* = 8.66 *Hz*, 2H, Ph—H). ^{13}C NMR (100 M*Hz*, DMSO-d_6) δ_C: 34.7 (CH_2), 115.2 (2 × CH), 123.4 (2 × CH), 125.8, 128.1 (2 × CH), 128.7 (2 × CH), 129.0 (2 × CH), 129.9 (2 × CH), 133.9, 136.8, 137.9, 138.7 (2 × C), 142.9 ppm. λ_{max} in nm (log ε_{max}): 215 (4.4669), 254 (4.4540). FT-IR (ν_{max} in cm^{-1}): 2922, 2850 (CH aliphatic), 1620 (C=C aromatic), 1575 (C=N imine), 1375 (SO_2), 1185 (SO_2 2nd band). Anal. Calcd for $C_{20}H_{16}N_2O_2S$ (348.42): C, 68.94; H, 4.63; N, 8.04%. Found: C, 69.00; H, 4.82; N, 7.89%.

2-Benzyl-1-tosyl-1H-benzimidazole ***6c***

Yield 93.25%, mp = N.D. (Oily), colour = brown. ^{1}H NMR (400 M*Hz*, DMSO-d_6) δ_H: 2.76 (s, 3H, CH_3—Ar), 3.56 (s, 2H, CH_2—Ar), 6.35–6.38 (dd, J_1 = 3.60 *Hz*, J_2 = 9.26 *Hz*, 1H, Ph—H), 6.48–6.49 (dd, J_1 = 3.60 *Hz*, J_2 = 8.00 *Hz*, 1H, Ph—H), 6.92–6.94 (d, *J* = 8.76 *Hz*, 2H, Ph—H), 7.12 (s, 5H, Ph—H), 7.22–7.24 (d, *J* = 9.26 *Hz*, 1H, Ph—H), 7.30–7.32 (d, *J* = 8.00 *Hz*, 1H, Ph—H), 7.44–7.46 (d, *J* = 8.76 *Hz*, 2H, Ph—H). ^{13}C NMR (100 M*Hz*, DMSO-d_6) δ_C: 21.5(CH_3), 34.9 (CH_2),

115.2 (2 × CH), 123.4 (2 × CH), 125.8, 128.1, (2 × CH), 128.7 (2 × CH), 129.5 (2 × CH), 130.1 (2 × CH), 134.7, 136.7, 138.9 (2 × C), 139.6, 142.6 ppm. λ_{max} in nm (log ε_{max}): 233 (5.3679), 299 (4.7896). FT-IR (ν_{max} in cm^{-1}): 2924, 2854 (CH aliphatic), 1648 (C=C aromatic), 1562 (C=N imine), 1376 (SO_2), 1185 (SO_2 2nd band). Anal. Calcd for $C_{21}H_{18}N_2O_2S$ (362.44): C, 69.59; H, 5.01; N, 7.73%. Found: C, 69.51; H, 4.88; N, 7.82%.

2-Benzyl-1-(3-chlorobenzyl)-1H-benzimidazole ***6d***

Yield 91.83%, mp = N.D. (Oily), colour = brown. ^1H NMR (400 M*Hz*, DMSO-d_6) δ_H: 3.42 (s, 2H, CH_2—Ar), 3.58 (s, 2H, CH_2—Ar), 6.35–6.38 (dd, J_1 = 3.48 *Hz*, J_2 = 9.18 *Hz*, 1H, Ph—H), 6.48–6.50 (dd, Jm_1 = 3.48 *Hz*, J_2 = 8.00 *Hz*, 1H, Ph—H), 7.10 (s, 5H, Ph—H), 7.17–7.19 (dd, J_1 = 7.20 *Hz*, J_2 = 7.82 *Hz*, 1H, Ph—H), 7.23–7.25 (d, J = 9.18 *Hz*, 1H, Ph—H), 7.31–7.33 (d, J = 8.00 *Hz*, 1H, Ph—H), 7.37–7.38 (d, J = 7.82 *Hz*, 1H, Ph—H), 7.51–7.52 (d, J = 7.20 *Hz*, 1H, Ph—H), 8.21 (s, 1H, Ph—H). ^{13}C NMR (100 M*Hz*, DMSO-d_6) δ_C: 33.8 (CH_2), 48.0 (CH_2), 115.2 (2 × CH), 123.4 (2 × CH), 125.1 125.6, 125.8, 128.7 (2 × CH), 128.9, 129.5 (2 × CH), 130.2, 134.4, 136.7, 137.8, 138.9 (2 × C), 142.5 ppm. λ_{max} in nm (log ε_{max}): 233 (5.3877), 251 (5.0266). FT-IR (ν_{max} in cm^{-1}): 2984 (C—H aliphatic), 1646 (C=C). 1565 (C=N), 652 (C—Cl). Anal. Calcd for $C_{20}H_{17}ClN_2$ (332.83): C, 75.78; H, 5.15; N, 8.42%. Found: C, 75.70; H, 4.01; N, 8.25%.

5-(2-Benzyl-1H-benzimidazole-1-yl)pyrimidine-2,4(1H,3H)-dione ***6e***

Yield 80.58%, mp = N.D. (Oily), colour = black. ^1H NMR (400 M*Hz*, DMSO-d_6) δ_H: 3.56 (s, 2H, CH_2—Ar), 6.34–6.37 (dd, J_1 = 3.44 *Hz*, J_2 = 9.18 *Hz*, 1H, Ph—H), 6.48–6.50 (dd, J_1 = 3.44 *Hz*, J_2 = 8.00 *Hz*, 1H, Ph—H), 7.11 (s, 5H, Ph—H), 7.23–7.25 (d, J = 9.18 *Hz*, 1H, Ph—H), 7.30–7.32 (d, J = 8.00 *Hz*, 1H, Ph—H), 7.95–7.97 (s, 1H, Ph—H), 11.05 (s, IH, NH), 11.55 (s, 1H, NH). ^{13}C NMR (100 M*Hz*, DMSO-d_6) δ_C: 34.8 (CH_2), 115.2 (2 × CH), 119.9, 123.3 (2 × CH), 125.7, 128.7 (2 × CH), 129.4 (2 × CH), 136.7, 138.5 (2 × C), 142.9, 150.0, 169.2 (C=O), 169.7 (C=O) ppm. λ_{max} in nm (log ε_{max}): 218 (4.6532), 248 (4.5775), 293 (4.1399), 437 (3.4914). FT-IR (ν_{max} in cm^{-1}): 3360 (N—H), 3217 (N—H), 3050 (C—H aromatic), 1685 (C=O amide), 1615 (C=C aromatic), 1575 (C=N). Anal. Calcd for $C_{18}H_{14}N_4O_2$ (318.33): C, 67.91; H, 4.43; N, 17.60%. Found: C, 68.10; H, 4.57; N, 17.77%.

4-(2-Benzyl-1H-benzimidazole-1-yl) aniline ***6f***

Yield 86.24%, mp = N.D. (Oily), colour = brown. ^1H NMR (400 M*Hz*, DMSO-d_6) δ_H: 3.56 (s, 2H, CH_2—Ar), 4.50 (s-br, 2H, NH_2), 6.35–6.38 (dd, J_1 = 3.46 *Hz*, J_2 = 9.12 *Hz*, 1H, Ph—H), 6.48–6.50 (dd, J_1 = 3.46 *Hz*, J_2 = 8.00 *Hz*, 1H, Ph—H), 7.11 (s, 5H, Ph—H), 7.16–7.18 (d, J = 8.20 *Hz*, 2H, Ph—H), 7.24–7.26 (d, J = 9.12 *Hz*, 1H, Ph—H), 7.29–7.31 (d, J = 8.00 *Hz*, 1H, Ph—H), 7.44–7.46 (d, J = 8.20 *Hz*, 2H, Ph—H). ^{13}C NMR (100 M*Hz*, DMSO-d_6) δ_C: 34.9 (CH_2), 115.2 (2 × CH), 118.7 (2 × CH), 123.4 (2 × CH), 125.8, 126.2 (2 × CH), 128.7 (2 × CH), 129.4 (2 × CH), 136.9, 138.8 (2 × C), 142.7, 146.8, 148.7 ppm. λ_{max} in nm (log ε_{max}): 230 (5.2753), 251 (5.1322), 299 (4.8401). FT-IR (ν_{max} in cm^{-1}): 3404 (N—H interfered by OH), 2985 (C—H aliphatic), 1610 (C=C aromatic), 1572 (C=N). Anal. Calcd for $C_{20}H_{17}N_3$ (299.37): C, 80.24; H, 5.72; N, 14.04%. Found: C, 80.21; H, 5.90; N, 13.85%.

Antibacterial sensitivity testing of compounds ***1–6f***

All the synthesized compounds (**1–6f**) and gentamicin were screened for antibacterial activity on four bacterial strains using agar well diffusion method [26]. The detailed was as attached in Supplementary Materials.

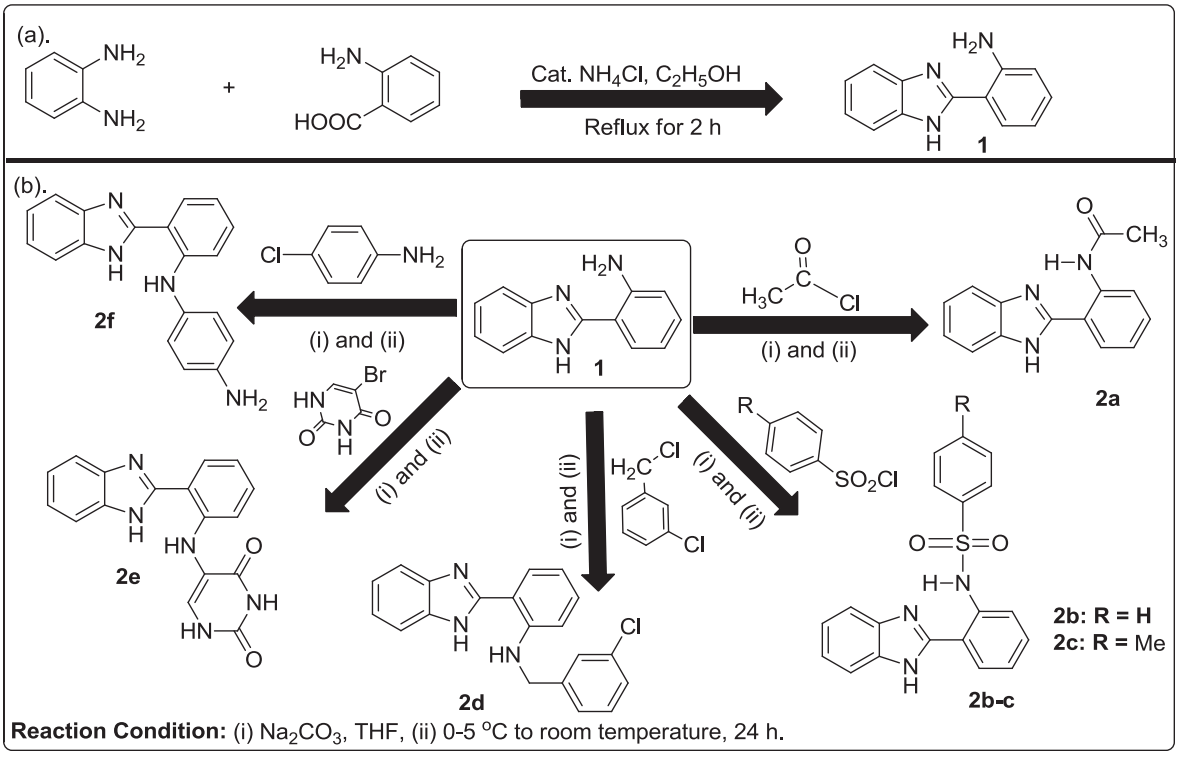
Minimum inhibitory/bactericidal concentration (MIC and MBC) testing

The minimum inhibitory concentration (MIC) test on the chosen organisms was carried out via serial dilution technique [27] and the concentration range was from 500.00 to 15.63 μg/mL, while the minimum bactericidal concentration (MBC) was determined by a standard method [26]. The detailed description of the procedures for the determination of MIC and MBC were as presented in the supplementary material.

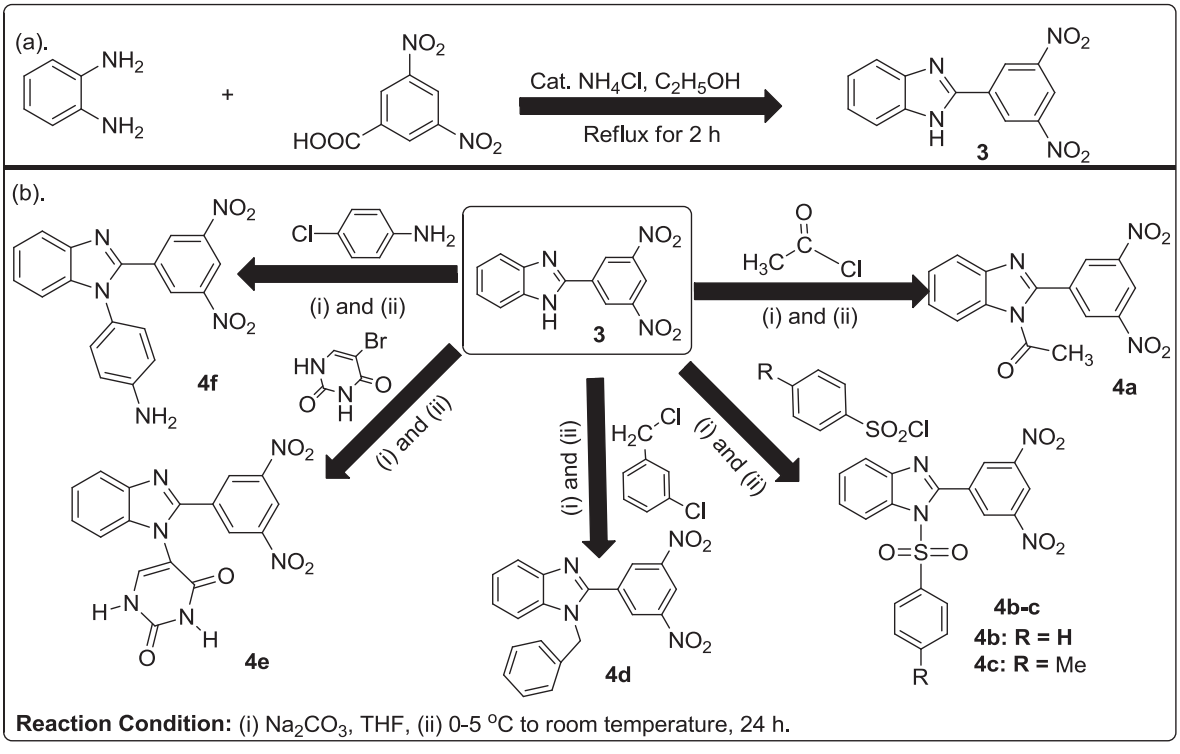
Results and discussion

Based on the enthusiastic outcome of an extensive review on functionalized benzimidazole [3] and in the continuation of the research effort in the area of benzo-fused imidazole moieties [6,28], the synthesis of functionalized 2-substituted and 1,2-disubstituted benzimidazole derivatives was herein reported to evaluate their antibacterial activities. Although, various derivatives of benzimidazole moieties have been synthesized and reported in literature; high temperature for the reflux process and the uses harsh reaction condition in the presence of strong and concentrated acids such as HCl [4,6] have been involved. On the contrary, the synthesis of 2-(1*H*-benzimizadol-2-yl)aniline precursor **1**, was achieved herein by the use of eco-friendly reaction of *o*-phenylenediamine with anthranilic acid using a catalytic amount of NH_4Cl in the presence of ethyl alcohol as a solvent at refluxing temperature of 60–70 °C (Scheme 1a). The synthetic modification of NH_2 functional side chain of the reactive intermediate **1** was conducted by reacting it with six different electrophile-releasing substrates to furnish **2a-f**. Prior to this, the reaction optimization study was carried out using two main parameters. First, solvent dependent condition was investigated using the synthesis of *N*-(2-(1*H*-benzimidazole-2-yl)phenyl)acetamide **2a** via the reaction of **1** with acetyl chloride in either tetrahydrofuran (THF), ethanol or acetonitrile at room temperature for 24 h; this gave yield of 87.7%, 30%, and 25% respectively. In addition, the thermodynamic dependent kinetic of the synthesis of **2a** was evaluated using the comparative study of the synthesis in THF at room temperature, 60 °C, and 120 °C. It was unveiled that room temperature gave the highest yield (87.71%) followed by 60 °C (38.24%), while there was no isolated product at 120 °C. Thus, the same reaction condition was adopted for reaction of **1** with the remaining five electrophile-releasing substrates **b-f** to produce diverse functionalized 2-substitutedbenzimidazole derivatives **2b-f** (Scheme 1b). According to another route [29], the *o*-phenylenediamine reacted with 3,5-dinitrobenzoic acid to achieve **3** Scheme 2a, which was subsequently treated with the earlier reported six electrophile-releasing substrates to furnish 1,2-disubstituted benzimidazole derivatives **4a-f** (Scheme 2b) in varying yields. Finally, the last precursor **5** in this present work was prepared by the condensation of *o*-phenylenediamine with phenyl acetic acid (Scheme 3a). Precursor **5** was eventually reacted with the six electrophile-releasing substrates to access the 1,2-disubstituted benzimidazole derivatives **6a-f** (Scheme 3b).

Physicochemical parameter data were presented in experimental section alongside the elemental analysis data. The result of elemental analysis for the % calculated agreed with % found for C, H N of all the synthesized compounds with high state of accuracy (the difference was not more that ±0.20 in all cases). Furthermore, the spectroscopic characterization of the targeted templates was carried out using IR and UV, ^1H, and ^{13}C NMR analysis. The ^1H NMR spectra of the compounds were run in DMSO-d_6 at 400 M*Hz* with chemical shift values recorded in ppm. The aryl-linked CH_3 of **2c**, **4c**, and **6c** resonated upfield at δ 2.60–2.80 ppm as 3H singlets and acetyl-linked CH_3 of **2a**, **4a**, and **6a** were seen upfield as 3H sin-

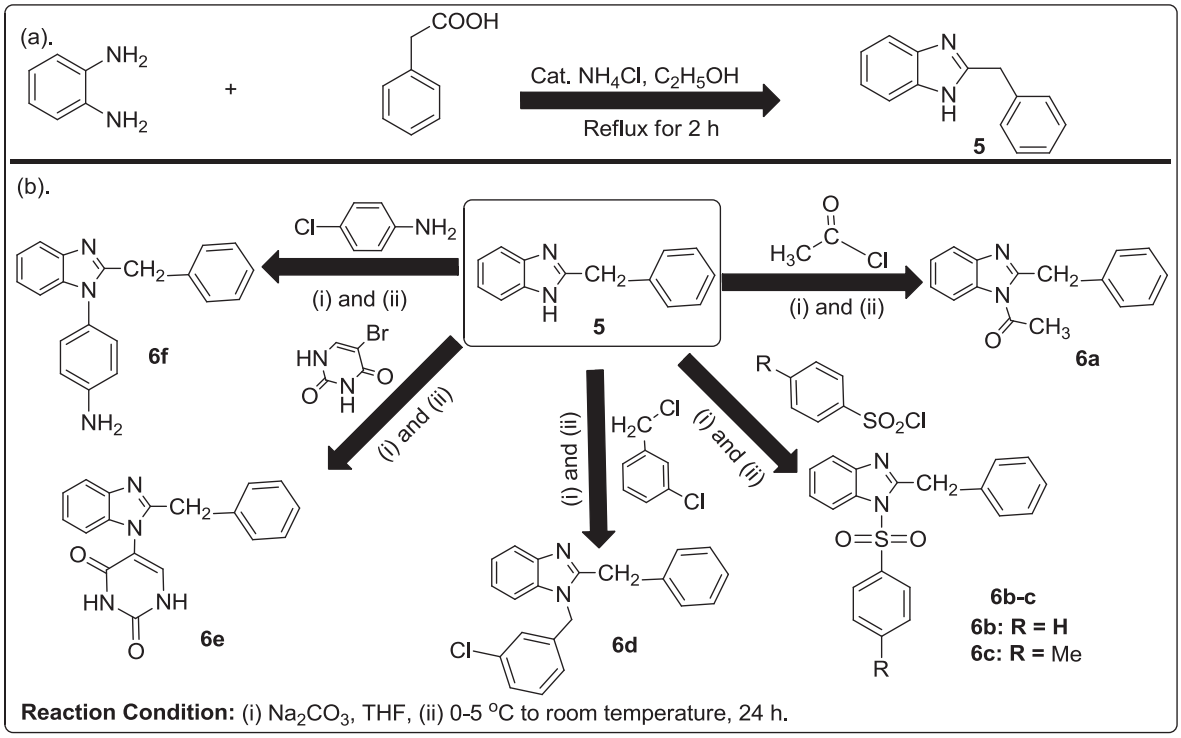


Scheme 1. (a) Synthesis of the precursor **1** (b) Synthesis of 2-substitutedbenzimidazoles **2a-f.**



Scheme 2. (a) Synthesis of the precursor **3** (b) Synthesis of 1,2-disubstitutedbenzimidazoles **4a-f.**

glets at δ 2.60–2.67 ppm. The aryl-linked CH_2 of **2d**, **4d**, and **5** as well as **6a-f** were noticed as singlets at δ 3.42–3.73 ppm. Signals of all aryl H appeared downfield of TMS around δ 6.31–8.90 ppm, NH_2 of amine in **1**, **2f**, **4f**, and **6f** were broad singlets at δ 4.50–5.32 ppm. The most downfield signals were that of N—H of amide found as singlets in compounds **2e**, **4e**, and **6e** at δ 11.02–11.56 ppm. The ^{13}C NMR spectra were run in DMSO-d_6 at 100 M*Hz* with chemical shift values recorded in ppm. On the overall, the ^{13}C NMR spectra of the structure-based benzimidazole derivatives varied from 21.3 ppm for CH_3 of compound **2c** to



Scheme 3. (a) Synthesis of the precursor **5** (b) Synthesis of 1,2-disubstitutedbenzimidazoles **6a-f.**

175.4 ppm for C═O of **2a**. Specifically, the aryl-linked CH_3 of **2c**, **4c**, and **6c** appeared at δ 21.7–21.3 ppm, whereas the CH_2 signals of **2d**, **4d**, and **6d** resonated at δ 48.0–48.2 ppm. The formation of acetamide in **2a**, **4a**, and **6a** was validated by presence of CH_3 intense singlet signal at δ 26.4–28.9 ppm, which was absent in the precursors **1**, **3** and **5**, respectively from which they were derived. The UV transition was run in tetrahydrofuran (THF) for the precursors **1**, **2**, and **3** and their final compounds **2a-f**, **4a-f**, and **6a-f**. The lowest wavelengths observed at 202–224 nm, were due to electronic excitation of $\pi \rightarrow \pi*$ peculiar to C═C, which depicted the presence of benzene ring in those structures. Bathochromic shifts observed herein led to the presence of other peaks at higher wavelengths (233 nm to 470 nm). Some of these shifts were because of $\pi \rightarrow n$ transition, which was attributable to the presence of auxochromic C═N group; which belong to K bands [28,30]. The FT-IR data of the benzimidazole derivatives **4a-f** revealed the stretching frequencies of C—H aromatic, C═C and C═N at 3106–3050, 1622–1600, and 1580–1575 cm^{-1}, respectively [28]. Additional bands were noticed in **2a**, **4a**, and **6a** at 1699–1685 cm^{-1}, which represents the C═O of amide. The two bands at 1377–1375 and 1187–1185 cm^{-1} which were domiciled in sulfonamide **2b-c**, **4b-c**, and **6b-c**, were peculiarly assigned to SO_2 functionality. The bands at 1543–1540 cm^{-1} depicted the presence of NO_2 (asym.) in compounds **3** and **4a-f**. Therefore, the extrapolated spectroscopic information of targeted benzimidazole motifs was in concordance with the proposed structures.

Antibacterial activity

The general sensitivity testing was evaluated using the *in vitro* screening of the synthesized compounds against four bacterial isolates (*Staphylococcus aureus*, *Bacillus licheniformis*, *Proteus vulgaris,* and *Pseudomonas aeruginosa*). Gentamicin was used as the positive control in this study. The justification of gentamicin as a clinical standard was due to the mode of action, which involved irreversible binding at ribosomal level, thereby signaling to obstruct and interrupt protein synthesis [31]. Agar diffusion method was used for the sensitivity testing and the diameters of zones of inhibition (Z. O. I) were documented in millimeter (Table 1). Although, large zones of inhibition were noticed for most of the targeted benzimidazole derivatives final products against the screened organisms, resistance was observed in few cases such as **6c** against *S. aureus*; **4d-f**, **6a** against *Bacillus licheniformis*; **2a**, **2c**, **4a**, **4d**, **and 6f** against *Proteus vulgaris*; **2e**, **4d-f**, **and 6a** against *Pseudomonas aeruginosa,* and **6f** developed resistant against the effect of gentamicin. Overall, the largest zone of inhibition (40.00 ± 0.10 mm) was recorded for **1** against *S. aureus,* while the lowest zone of inhibition (15.00 ± 0.08 mm) was recorded for **3** against *S. aureus*. In comparison with gentamicin, all synthesized compounds, except **3**, had better activity with higher zones of inhibition against growth potential of *S. aureus*. This means that the array of compounds synthesized herein might be a possible replacement for gentamicin on infectious disease caused by the *S. aureus* or enhance the potency of gentamicin where resistance issues occur. Compared to gentamicin, all compounds except **3** and **6c** showed larger zones of inhibition against growth of *S. aureus* (i.e. >16 mm); and except **4d-f** and **6c** against *B. licheniformis* (i.e. >16 mm), while **3** and **4c** exhibited approximately the same Z.O.I. as gentamicin against *S. aureus* (15 mm) and *B. licheniformis* (16 mm), respectively. Interestingly, more than 75% of the targeted benzimidazole derivatives were active on *P. vulgaris* with large zones of inhibition, whereas this organism was resistant to gentamicin (Table 1). Similarly, more than 75% of the targeted benzimidazole derivatives (Z.O. I = 25.00 ± 0.08 to 38.00 ± 0.12 mm) were more active than gentamicin (20.00 ± 0.08 mm) upon *P. aeruginosa*. In the present study, the choice of *S. aureus* and *E. coli* was due to the broad array of pathogenic infections and precarious health issue that are associated with these bacterial strains [32] and wide reported occurrence of resistant strain of *S. aureus* [1]. *S. aureus* has been reported to have strong relationship with death rate increase in human population, prolong admission of patients in hospitals, and the infections caused by this bacterial strain are expensive to treat [33].

Table 1
Antibacterial sensitivity testing with zones of inhibition in millimetre.

Compound No↓	*S. aureus*	*B. licheniformis*	*P. vulgaris*	*P. aeruginosa*
1	40.00 ± 0.10	26.00 ± 0.08	35.00 ± 0.09	38.00 ± 0.12
2a	27.00 ± 0.09	26.00 ± 0.08	R	28.00 ± 0.08
2b	30.00 ± 0.09	20.00 ± 0.08	25.00 ± 0.09	30.00 ± 0.08
2c	35.00 ± 0.09	25.00 ± 0.08	R	28.00 ± 0.08
2d	30.00 ± 0.10	28.00 ± 0.09	30.00 ± 0.09	30.00 ± 0.09
2e	32.00 ± 0.12	26.00 ± 0.08	35.00 ± 0.08	R
2f	30.00 ± 0.11	30.00 ± 0.12	28.00 ± 0.08	30.00 ± 0.08
3	15.00 ± 0.08	20.00 ± 0.08	30.00 ± 0.12	28.00 ± 0.09
4a	20.00 ± 0.10	24.00 ± 0.09	R	25.00 ± 0.08
4b	30.00 ± 0.10	18.00 ± 0.08	20.00 ± 0.08	25.00 ± 0.08
4c	25.00 ± 0.10	16.00 ± 0.08	20.00 ± 0.08	25.00 ± 0.08
4d	30.00 ± 0.09	R	R	R
4e	30.00 ± 0.10	R	20.00 ± 0.08	R
4f	30.00 ± 0.12	R	23.00 ± 0.08	R
5	30.00 ± 0.09	28.00 ± 0.09	32.00 ± 0.12	38.00 ± 0.08
6a	19.00 ± 0.08	R	18.00 ± 0.08	R
6b	35.00 ± 0.09	22.00 ± 0.08	30.00 ± 0.10	28.00 ± 0.08
6c	R	18.00 ± 0.08	32.00 ± 0.08	26.00 ± 0.08
6d	32.00 ± 0.10	26.00 ± 0.08	35.00 ± 0.12	30.00 ± 0.08
6e	25.00 ± 0.08	24.00 ± 0.08	25.00 ± 0.08	30.00 ± 0.09
6f	20.00 ± 0.08	26.00 ± 0.08	R	28.00 ± 0.08
Gentamicin	16.00 ± 0.09	16.00 ± 0.08	R	20.00 ± 0.08

R = Resistance. Mean ± SD of triplicate determination.

Due to heat stable toxin production, *S. aureus* is enlisted as one of the highly invasive organisms referred to as pyogenic cocci involved in numerous adverse infectious conditions in humans [28,32,34]. In view of the reported predicament aforementioned and large zones of inhibition experienced via action of the benzimidazole framework herein on *S. aureus*, motivation was enhanced to investigate the activity index (A.I.) of the synthesized compounds against this organism (Fig. 1). It is quite impressive to note that all the synthesized benzimidazole motifs herein, showed better activity indices than gentamicin against *S. aureus* except **3** and **6c**. The compound **3** competed favourably with gentamicin (A. I. ≈ 1.00) while **6c** developed resistance; hence could not have activity index.

Furthermore, based on high susceptibility of the organisms to the synthesized benzimidazole templates 1-6f and broad spectrum of activity observed herein, the MIC testing was carried out to determine the lowest concentration of the compound solution that conveniently inhibited the bacterial growth (Table 2). It was carried out using serial dilution method (500, 250, 125, 62.50, 31.25, and 15.63 μg/mL) via an earlier reported method [27]. The MIC of benzimidazole derivatives upon *S. aureus* varied from 15.63 ± 1.63 to 250 ± 2.66 μg/mL; against *B. licheniformis* varied from 62.50 ± 2.04 to 250 ± 2.65 μg/mL; against *P. vulgaris* varied from 31.25 ± 1.94 to 250 ± 2.65 μg/mL; and against *P. aeruginosa* ranged from 15.63 ± 1.63 to 125 ± 2.45 μg/mL. The best activity against *S. aureus* was observed in the precursor **1** among the series of the 2-substituted benzimidazoles **1-2f**. The presence of amino functionality in **1** and the ready availability of its lone pair of electron for coordination led to improved activity. The activity decreases after the NH_2 had underwent substitution as contained in **2a-f**, except for **2c** and **2e**, which competed favorably with **1**. This means that incorporation of *p*-toluenesulfonamido (in **2c**) and pyrimidinedione (in **2e**) played significant role as essential pharmacophores in increased activity observed in **2c** and **2e** against *S. aureus*. On the contrary, the series of 1,2-disubstituted benzimidazoles **4a-f** were more active than the precursor **3** from which they were derived. This showed that additional substitution on position 1 of compound **3** to afford 1,2-disubstitution in **4a-f**, was a worthwhile adventure in increasing the bioactivity of precursor **3**, since the series of 1,2-disubstituted benzimidazole products **4a-f** resulted in drastic improvement of growth inhibition in *S. aureus*. In addition to MIC testing, the minimum bactericidal concentration (MBC) testing was determined to authenticate the lowest concentration of the benzimidazole solution that causes death

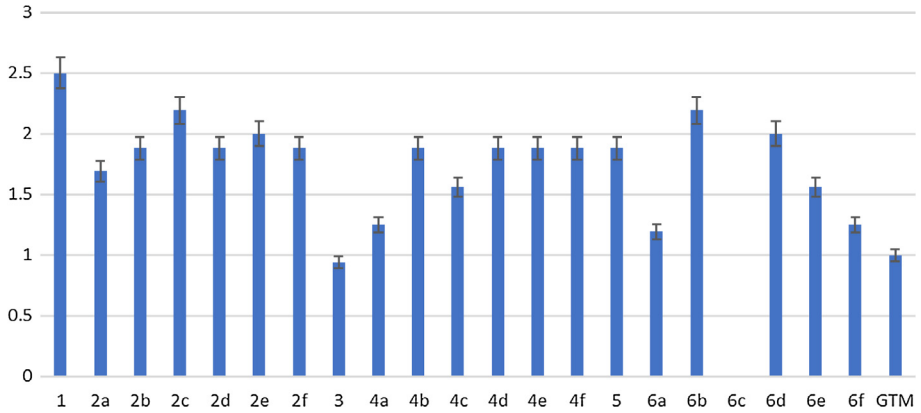


Fig. 1. Comparative study of the activity index of synthesized benzimidazoles and gentamicin.

Table 2
Minimum inhibitory concentration (MIC) in μg/mL of targeted benzimidazoles, **1-6f.**

Compound No↓	*S. aureus*	*B. licheniformis*	*P. vulgaris*	*P. aeruginosa*
1	15.63 ± 1.64	125.00 ± 2.44	62.50 ± 2.04	31.25 ± 1.98
2a	31.25 ± 1.98	125.00 ± 2.43	N.D.	62.50 ± 2.01
2b	31.25 ± 1.97	250.00 ± 2.67	125.00 ± 2.44	31.25 ± 1.94
2c	15.63 ± 1.67	125.00 ± 2.44	N.D.	62.50 ± 2.03
2d	31.25 ± 1.96	125.00 ± 2.44	62.50 ± 2.01	62.50 ± 2.03
2e	15.63 ± 1.63	250.00 ± 2.67	62.50 ± 2.01	N.D.
2f	31.25 ± 1.94	62.50 ± 2.04	125.00 ± 2.44	31.25 ± 1.94
3	250.00 ± 2.66	250.00 ± 2.67	125.00 ± 2.44	62.50 ± 2.01
4a	125.00 ± 2.45	125.00 ± 2.44	N.D.	62.50 ± 2.02
4b	31.25 ± 1.95	250.00 ± 2.66	250.00 ± 2.67	62.50 ± 2.01
4c	62.50 ± 2.01	250.00 ± 2.66	250.00 ± 2.66	31.25 ± 1.97
4d	31.25 ± 1.98	N.D.	N.D.	N.D.
4e	15.63 ± 1.65	N.D.	250.00 ± 2.65	N.D.
4f	31.25 ± 1.94	N.D.	250.00 ± 2.67	N.D.
5	15.63 ± 1.67	62.50 ± 2.04	62.50 ± 2.03	15.63 ± 1.63
6a	250.00 ± 2.66	N.D.	125.00 ± 2.44	N.D.
6b	15.63 ± 1.63	250.00 ± 2.65	62.50 ± 2.04	62.50 ± 2.02
6c	N.D.	250.00 ± 2.67	31.25 ± 1.94	125.00 ± 2.45
6d	31.25 ± 1.94	125.00 ± 2.44	31.25 ± 1.95	31.25 ± 1.96
6e	125.00 ± 2.45	125.00 ± 2.45	125.00 ± 2.44	62.50 ± 2.02
6f	125.00 ± 2.43	125.00 ± 2.45	N.D.	62.50 ± 2.01
Gentamicin	5.00 ± 1.23	2.5 ± 1.09	N.D.	7.5 ± 1.45

N.D. = Not Determined. Mean ± SD of triplicate determination.

of the bacterium targeted per time and the results are shown in Fig. 2. Apart from where the MBC was not determined (N.D.) due to occurrence of resistance, all other cases showed that the MBC values were double the MIC in each consideration, except in **6a** alone where MBC (500 μg/mL) was 4 times that of MIC (125 μg/mL) against *P. vulgaris*. From all indications, the lowest MBC trend was observed for the bio-assay screening of benzimidazole derivatives upon *S. aureus*, whereas the highest MBC was reported against *P. aeruginosa* as shown in Fig. 2.

Structure activity relationship (SAR) study

From the overview of the structure activity relationship (SAR) study, it was found that the nature of substituent on 1-position and 2-positions of the benzimidazole nucleus had significant effect on the antibacterial activity of the entire structures. The compounds series **2a-f** were structurally related in the core pharmacophoric 2-phenylbenzimidazole; the SAR study showed their activity against *S. aureus* to be in the order **2c** ≈ **2e** > **2a** ≈ **2b** ≈ **2d** ≈ **2f**. This means that the presence of electron donating CH_3 on *p*-toluenesulfonamide moieties in the 2-position of anilino side chain played a significant role in the improvement of activity, since its counterpart **2b** without CH_3 was far less active and stayed in the categories of **2a**, **2d**, and **2f** in its activity upon *S. aureus* growth inhibition. Considering **4a-f** on *S. aureus*, the activity varied in the order of: **4e** > **4b** ≈ **4d** ≈ **4f** > **4c** > **4a.** Thus, electron withdrawing ability and π-π stacking character in pyrimidine-dione at 1-position of 2-(3,5-dinitrophenyl)benzimidazole core worked synergistically with the electron withdrawing NO_2 on benzene at 2-position to increase the activity of **4e**, thereby causing it to exhibit outstanding activity against *S. aureus* among the **4a-f** series. Based on the *in vitro* screening of **6a-f** against *S. aureus*, the order of activity was **6b** > **6d** > **6e** ≈ **6f** > **6a** > **6c**. Hence, presence of benzenesulfonamido group on 1-position of 2-benzylbenzimidazole in series **6a-f** played a crucial role in activity boosting, making **6b** to be the most active among the group and more active as compared to its isomor-

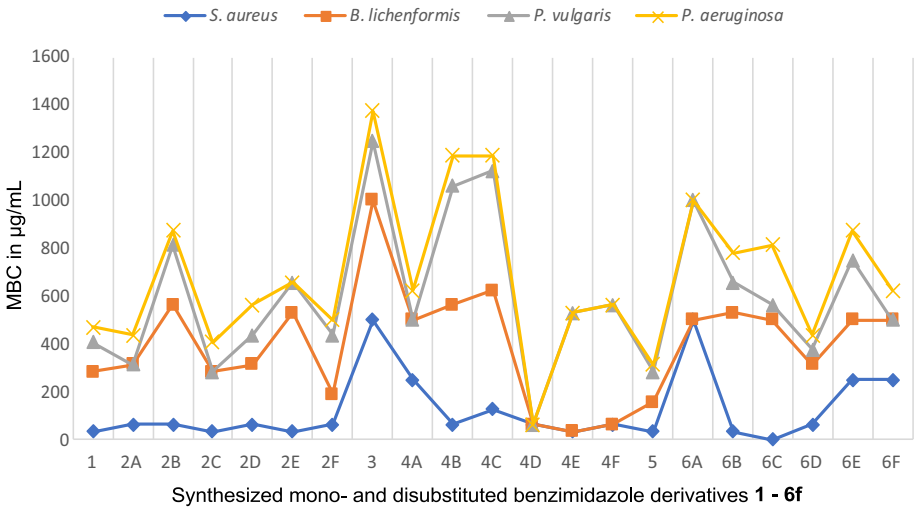


Fig. 2. Graphical representation of minimum bactericidal concentration (MBC).

phic template 6c where no activity was noticed. It was interesting to note that *p*-methyl group in **6c** which was the only group absent in **6b** provided the framework **6a-f** with antagonistic effect thereby causing total activity loss in **6c** as compared to **6b** against *S. aureus*.

Conclusions

Benzimidazole is an essential heterocyclic framework in agrochemicals, pharmaceuticals, and medicinal chemistry research. NH_4Cl catalyzed strategy was found to be efficient approach for accessing the reported benzimidazole precursor in good yield. Thus, mono- and disubstituted benzimidazole derivatives with improved medicinal potential were successfully synthesized via an elegant pathway. The findings of the *in vitro* screening unveiled the broad spectrum of activity of the synthesized benzimidazole templates in the present study. Among the series, the highest potency was exerted and experienced in 2-(1*H*-benzimizadol-2-yl)aniline, **1** and 2-benzyl-1-(phenylsulfonyl)-1*H*-benzimidazole, **6b**. It will be a worthwhile adventure to advance the work further for more guidance on the pharmacokinetic and pharmacodynamic study to ascertain probable candidature of the templates for future drug design.

Conflict of interest

The authors have declared no conflict of interest.

Compliance with Ethics Requirements

This article does not contain any studies with human or animal subjects.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.jare.2017.09.003.

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