RESEARCH PAPER

Synthesis, Characterization, and Antimicrobial Evaluation of Novel N-Substituted 2-(4′-Bromo-[1,1′-biphenyl]-4-yl)-1H-benzo[d]imidazole Derivative

Shashank V. Malge[1], Chandrakant U. Dhanwad[2], Rameshwar M. More[3], Gajanan G. Kadam[3], Gangadhar S. Bhopalkar[4]*

[1]Department of Chemistry, Yeshwant Mahavidyalaya, Nanded, India
[2]Department of Chemistry, M.S.P. and K.P.T. College, Manora, India
[3]Department of Chemistry, Shri Datta Arts, Commerce and Science College, Hadgaon, India
[4]Department of Chemistry, Indira Gandhi Sr. College, Cidco, India

Abstract
A series of ten novel N-alkylated benzimidazole derivatives (**JP1–JP10**) bearing a bromobiphenyl scaffold were synthesized through N-alkylation of 2-(4′-bromo-[1,1′-biphenyl]-4-yl)-1H-benzo[d]imidazole with various substituted alkyl halides. The synthetic protocol involved sodium hydride-mediated deprotonation in anhydrous DMF, followed by reaction with appropriate alkylating agents at 60°C for 6 hours, affording the target compounds in good-to-excellent yields ranging from 77.99% to 89.78%. The structures of all synthesized derivatives were unequivocally established through comprehensive spectroscopic analyses, including FT-IR, ^{1}H NMR, ^{13}C NMR, HRMS, and elemental analysis. The antimicrobial potential of the synthesized compounds was evaluated against a panel of pathogenic microorganisms comprising Gram-positive bacteria (Bacillus subtilis and Staphylococcus aureus), Gram-negative bacteria (Pseudomonas aeruginosa and Escherichia coli), and fungal strains (Candida albicans and Saccharomyces cerevisiae) using the agar well diffusion method. Among the tested derivatives, compound JP6 emerged as the most potent antimicrobial agent, exhibiting remarkable inhibition zones of 27–34 mm against bacterial strains and 30–34 mm against fungal strains, significantly surpassing the reference drugs streptomycin and fluconazole. Fluorinated derivatives **JP2** and **JP9** also demonstrated excellent broad-spectrum antimicrobial activity. The structure-activity relationship analysis revealed that the presence of electron-withdrawing fluorine substituents and appropriate alkyl groups significantly enhanced the biological efficacy, likely through improved membrane permeability and stronger target interactions. These findings position the synthesized benzimidazole derivatives, particularly JP6, as promising lead compounds for further development as antimicrobial agents.

Keywords: Benzimidazole derivatives; N-alkylation; Bromobiphenyl; Antimicrobial activity; Structure-activity relationship; Fluorinated compounds; Agar well diffusion method
How to cite this article: Malge SV, Dhanwad CU, More RM, Kadam GG, Bhopalkar GS. Synthesis, Characterization, and Antimicrobial Evaluation of Novel N-Substituted 2-(4'-Bromo-[1,1'-biphenyl]-4-yl)-1H-benzo[d]imidazole Derivative. Int J Drug Deliv Technol. 2026;16(65s):1076-1088. DOI: 10.25258/ijddt.16.65s.111

1. Introduction

Benzimidazole, a privileged heterocyclic scaffold, has garnered significant attention in medicinal chemistry due to its remarkable pharmacological versatility and structural relevance to biologically active natural products [1,2]. The benzimidazole nucleus, characterized by a fused benzene-imidazole ring system, serves as an essential pharmacophore in numerous therapeutic agents, exhibiting diverse biological activities including antimicrobial, anticancer, antiviral, anti-inflammatory, and antiparasitic properties [3,4]. The widespread occurrence of this heterocyclic framework in clinically approved drugs such as omeprazole, albendazole, and mebendazole underscores its therapeutic significance and drug-like characteristics [5,6]. The inherent ability of benzimidazole derivatives to interact with various biological targets through hydrogen bonding, π-π stacking interactions, and hydrophobic contacts makes them invaluable scaffolds for drug discovery and development programs [7,8].

*Author for Correspondence: Gangadhar S. Bhopalkar

The structural modification of benzimidazole derivatives through N-alkylation or N-arylation has emerged as a versatile strategy for optimizing their biological activities and physicochemical properties [9,10]. Introduction of diverse substituents at the N-1 position of the benzimidazole ring can significantly influence the electronic distribution, lipophilicity, and molecular conformation, thereby modulating their interaction with biological targets [11,12]. Among various substituents, fluorinated aryl groups have attracted considerable interest due to the unique properties imparted by fluorine atoms, including enhanced metabolic stability, improved membrane permeability, and increased binding affinity through halogen bonding and hydrophobic interactions [13,14]. The strategic incorporation of fluorine into drug molecules has become a well-established approach in medicinal chemistry, with approximately 20% of pharmaceutical drugs containing fluorine atoms [15].

The biphenyl moiety represents another privileged structural element frequently encountered in

pharmacologically active compounds [16]. The presence of a biphenyl system often contributes to increased lipophilicity, enhanced π-π stacking capabilities, and improved binding interactions with hydrophobic pockets of biological targets [17]. Compounds containing brominated biphenyl scaffolds have demonstrated significant biological activities, and the bromine atom serves as a valuable handle for further functionalization through cross-coupling reactions [18]. The combination of benzimidazole and biphenyl pharmacophores within a single molecular framework offers the potential for synergistic biological effects and improved drug-like properties [19].

In recent years, the emergence of antimicrobial resistance has become a critical global health concern, necessitating the development of novel antimicrobial agents with distinct mechanisms of action [20,21]. The alarming increase in multidrug-resistant bacterial and fungal pathogens has prompted intensive research efforts toward discovering new chemical entities capable of overcoming existing resistance mechanisms [22]. Benzimidazole derivatives have demonstrated promising antimicrobial activities against various clinically significant pathogens, including Gram-positive bacteria, Gram-negative bacteria, and fungal strains [23]. The diverse mechanisms of action exhibited by benzimidazole-based compounds, including inhibition of nucleic acid synthesis, disruption of membrane integrity, and interference with enzyme function, make them attractive candidates for combating drug-resistant microorganisms [24]. The present study reports the synthesis and characterization of a series of ten novel N-substituted benzimidazole derivatives bearing brominated biphenyl and various substituted phenyl groups (**JP1-JP10**). The synthetic strategy involved N-alkylation of 2-(4′-bromo-[1,1′-biphenyl]-4-yl)-1H-benzo[d]imidazole with appropriately substituted aryl halides under basic conditions. The synthesized compounds were comprehensively characterized using FT-IR, ^{1}H NMR, ^{13}C NMR, HRMS, and elemental analysis techniques. The antimicrobial activities of all derivatives were evaluated against a panel of clinically relevant bacterial and fungal strains using the agar well diffusion method. The findings of this study contribute to the expanding body of knowledge on benzimidazole-based antimicrobial agents and highlight the potential of N-substituted derivatives as promising candidates for further drug development.

2. Experimental

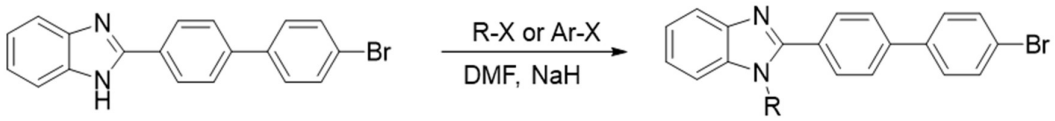
2.1. Materials and Methods

All chemicals and solvents employed in the present study were of analytical reagent grade and were used as received without further purification. The precursor, 2-(4′-bromo-[1,1′-biphenyl]-4-yl)-1H-benzo[d]imidazole, was synthesized according to a previously reported literature procedure [25]. Alkylating agents, including methyl iodide, ethyl bromide, and other substituted alkyl halides, were procured from Sigma-Aldrich and used without additional purification. Anhydrous dimethylformamide (DMF) was employed as the reaction solvent, while sodium hydride (60% dispersion in mineral oil) was used as the base for N-alkylation reactions. Thin-layer chromatography (TLC) was performed on silica gel 60 F254 plates to monitor reaction progress. Column chromatography was carried out using silica gel (60–120 mesh) as the stationary phase. Melting points were determined in open capillary tubes and are uncorrected. All moisture-sensitive reactions were conducted under a dry nitrogen atmosphere following standard inert reaction techniques [26].

2.2. General Procedure for the Synthesis of Alkylated Derivatives (NP1–NP10)

The synthesis of alkylated derivatives was accomplished through N-alkylation of 2-(4′-bromo-[1,1′-biphenyl]-4-yl)-1H-benzo[d]imidazole following a modified literature protocol [27]. In a typical procedure, 2-(4′-bromo-[1,1′-biphenyl]-4-yl)-1H-benzo[d]imidazole (1.0 mmol) was dissolved in anhydrous DMF (10 mL) under a nitrogen atmosphere and cooled to 0 °C. Sodium hydride (1.2 mmol) was added portionwise with continuous stirring. The reaction mixture was stirred for 30 min to generate the corresponding benzimidazole anion. Subsequently, the appropriate alkyl halide (1.5 mmol) was added dropwise, and the reaction mixture was heated at 60 °C for 6 h.

The progress of the reaction was monitored by TLC using hexane-acetate (7:3) as the mobile phase. After completion of the reaction, the mixture was poured into ice-cold water and extracted with ethyl acetate (3 × 20 mL). The combined organic layers were washed with water, dried over anhydrous sodium sulfate, and concentrated under reduced pressure. The resulting crude products were purified by silica gel column chromatography using suitable mixtures of hexane and ethyl acetate as eluents to afford the corresponding alkylated benzimidazole derivatives (**NP1–NP10**) in good yields ranging from 77.99–89.78 %. The synthetic route employed for the preparation of the target compounds is illustrated in **Scheme 1**.



Scheme 1: Synthesis of Alkylated Derivatives

Selected spectral data of the products:

*2-(4'-bromo-[1,1'-biphenyl]-4-yl)-1-(2-fluorophenyl)-1H-benzo[d]imidazole (**JP1**)*: Brown powder (83.67 %), M.P. = 199-201 °C. FT(IR) spectrum (KBr, □/cm^{-1}), 3197 (Ar C-H), 1603 (Ar C=N), 1576/1498 (Ar C=C), 1470 (Ar C-N), 1229 (Ar C-F), 626 (Ar C-Br). ^{1}H NMR (DMSO-d6, 400 MHz) δ ppm: 6.887 (*ddd*, 1H, Ar-H), 7.208 (*ddd*, 2H, Ar-H), 7.314 (*ddd*, 2H, Ar-H), 7.332 (*ddd*, 1H, Ar-H), 7.369 (*ddd*, 1H, Ar-H), 7.402 (*ddd*, 2H, Ar-H), 7.644 (*ddd*, 2H, Ar-H),7.697 (*ddd*, 1H, Ar-H). ^{13}C NMR (DMSO-d6, 400 MHz) δ ppm: 157.90 (C=N), 142.04 (Ph-Ph), 126.05 (C-Br). HRMS spectrum (m/z) 444.1016 $(M+2)^{+}$. (Anal. Calcd for $C_{25}H_{16}BrFN_2$: C, 67.73; H, 3.64; Br, 18.02; F, 4.29; N, 6.32 %. Found: C, 67.61; H, 3.62; Br, 17.97; F, 4.23; N, 6.28 %.

*2-(4'-bromo-[1,1'-biphenyl]-4-yl)-1-(3-fluorophenyl)-1H-benzo[d]imidazole (**JP2**)*: Brown powder (87.99 %), M.P. = 196-197 °C. FT(IR) spectrum (KBr, □/cm^{-1}), 3191 (Ar C-H), 1694 (Ar C=N), 1501 (Ar C=C), 1484 (Ar C-N), 1233 (Ar C-F), 690 (Ar C-Br). ^{1}H NMR (DMSO-d6, 400 MHz) δ ppm: 6.926 (ddd, 1H, Ar-H), 6.967 (ddd, 2H, Ar-H), 7.239 (ddd, 2H, Ar-H), 7.294 (ddd, 1H, Ar-H), 7.412 (ddd, 1H, Ar-H), 7.434 (ddd, 2H, Ar-H), 7.509 (ddd, 2H, Ar-H), 7.530 (ddd, 1H, Ar-H). ^{13}C NMR (DMSO-d6, 400 MHz) δ ppm: 158.08 (C=N), 146.26 (Ph-Ph), 126.22 (C-Br). HRMS spectrum (m/z) 444.1731 $(M+2)^{+}$. (Anal. Calcd for $C_{25}H_{16}BrFN_2$: C, 67.73; H, 3.64; Br, 18.02; F, 4.29; N, 6.32 %. Found: C, 67.69; H,3.60; Br, 17.95; F, 4.24; N, 6.29 %.

2-(4'-bromo-[1,1'-biphenyl]-4-yl)-1-(4-fluorophenyl)-1H-benzo[d]imidazole (***JP3***)*:* Brown powder (84.33 %), M.P. = 203-204 °C. FT(IR) spectrum (KBr, □/cm^{-1}), 3191 (Ar C-H), 1697 (Ar C=N), 1577 (Ar C=C), 1489 (Ar C-N), 1236 (Ar C-F), 718 (Ar C-Br). ^{1}H NMR (DMSO-d6, 400 MHz) 6.892 (ddd, 1H, Ar-H), 7.223 (ddd, 2H, Ar-H), 7.277 (ddd, 2H, Ar-H), 7.315 (ddd, 1H, Ar-H), 7.409 (ddd, 1H, Ar-H), 7.539 (ddd, 2H, Ar-H), 7.617 (ddd, 2H, Ar-H), 7.777 (ddd, 1H, Ar-H). ^{13}C NMR (DMSO-d6, 400 MHz) δ ppm: 158.33 (C=N), 143.09 (Ph-Ph), 126.39 (C-Br). ^{13}C NMR (DMSO-d6, 400 MHz) δ ppm: 167.57 (C=N), 148.93 (Ph-Ph), 127.21 (C-Br), 20.93 (-CH_3). HRMS spectrum (m/z) 444.1132 $(M+2)^{+}$. (Anal. Calcd for $C_{25}H_{16}BrFN_2$: C, 67.73; H, 3.64; Br, 18.02; F, 4.29; N, 6.32 %. Found: C, 67.66; H, 3.59; Br, 17.90; F, 4.25; N, 6.31 %.

*3-(2-(4'-bromo-[1,1'-biphenyl]-4-yl)-1H-benzo[d]imidazol-1-yl)phenol (**JP4**)*: Brown powder (83.58 %), M.P. = 206-207 °C. FT(IR) spectrum (KBr, □/cm^{-1}), 3192 (Ar C-H), 1670 (Ar C=N), 1574 (Ar C=C), 1440 (Ar C-N), 718 (Ar C-Br). ^{1}H NMR (DMSO-d6, 400 MHz) δ ppm: 6.916 (ddd, 1H, Ar-H), 6.830 (ddd, 2H, Ar-H), 6.890 (ddd, 2H, Ar-H), 7.256 (ddd, 1H, Ar-H), 7.540 (ddd, 1H, Ar-H), 7.781 (ddd, 2H, Ar-H), 9.952 (s, 1H, -OH). ^{13}C NMR (DMSO-d6, 400 MHz) δ ppm: 161.89 (C-OH), 159.96 (C=N), 143.07 (Ph-Ph), 126.39 (C-Br). HRMS spectrum (m/z) 442.1482 $(M+2)^{+}$.Anal. Calcd for $C_{25}H_{17}BrN_2O$: C, 68.04; H, 3.88; Br, 18.11; N, 6.35; O, 3.63 %. Found: C, 68.00; H, 3.83; Br, 18.07; N,6.31; O, 3.55 %.*4-(2-(4'-bromo-[1,1'-biphenyl]-4-yl)-1H-benzo[d]imidazol-1-yl)benzene-1,3-diol (**JN5**)*: Red-brown powder (80.36 %), M.P. = 202-205 °C. FT(IR) spectrum (KBr, □/cm^{-1}), 3245 (Ar C-H), 1616 (Ar C=N), 1583 (Ar C=C), 1485 (Ar C-N), 685 (Ar C-Br). ^{1}H NMR (DMSO-d6, 400 MHz) δ ppm: 6.965 (ddd, 1H, Ar-H), 7.014 (ddd, 2H, Ar-H), 7.152 (ddd, 2H,Ar-H), 7.289 (ddd, 1H, Ar-H), 7.329 (ddd, 1H, Ar-H), 7.396 (ddd, 2H, Ar-H), 8.074 (ddd, 2H, Ar-H), 8.211 (ddd, 1H, Ar-H), 11.506 (s, 1H, -OH), 11.714 (s, 1H, -OH). ^{13}C NMR (DMSO-d6, 400 MHz) δ ppm: 163.27/158.23 (C-OH), 156.69 (C=N), 147.27 (Ph-Ph), 127.33 (C-Br). HRMS spectrum (m/z) 458.1753 $(M+1)^{+}$. Anal. Calcd for $C_{25}H_{17}BrN_2O_2$: C, 65.66; H, 3.75; Br, 17.47; N, 6.13; O, 7.00%. Found: C, 65.52; H, 3.69; Br, 17.38; N, 6.11; O, 6.97 %.

*2-(4'-bromo-[1,1'-biphenyl]-4-yl)-1-(2,6-dimethylphenyl)-1H-benzo[d]imidazole (**JP6**)*: Brown powder (89.78 %), M.P. = 208-209 °C. FT(IR) spectrum (KBr, □/cm^{-1}), 3257 (Ar C-H), 1600 (Ar C=N), 1515 (Ar C=C), 1493 (Ar C-N), 739 (Ar C-Br). ^{1}H NMR (DMSO-d6, 400 MHz) δppm: 7.069 (ddd, 1H, Ar-H), 7.203 (ddd, 2H, Ar-H), 7.323 (ddd, 2H,Ar-H), 7.444 (ddd, 1H, Ar-H), 7.483 (ddd, 1H, Ar-H), 7.532 (ddd, 2H, Ar-H), 7.580 (ddd, 2H, Ar-H). ^{13}C NMR (DMSO-d6, 400MHz) δ ppm: 159.69 (C=N), 140.97 (Ph-Ph), 129.47 (C-Br), 13.20 (-CH_3). HRMS spectrum (m/z) 392.0761 $(M+1)^{+}$. Anal. Calcd for $C_{27}H_{21}BrN_2$: C, 71.53; H, 4.67; Br, 17.62; N, 6.18 %. Found: C, 71.44; H, 4.63; Br, 17.58; N, 6.15 %.

*2-(4'-bromo-[1,1'-biphenyl]-4-yl)-1-phenyl-1H-benzo[d]imidazole (**JP7**)*: Brown powder (77.99 %), M.P. = 196-197 °C. FT(IR) spectrum (KBr, □/cm^{-1}), 3207 (Ar C-H), 1620 (Ar C=N), 1514 (Ar C=C), 1468 (Ar C-N), 757 (Ar C-Br). ^{1}H NMR (DMSO-d6, 400 MHz) δ ppm: 6.791 (*ddd*, 2H, Ar-H), 7.113 (*ddd*, 2H, Ar-H), 7.135 (*ddd*, 3H, Ar-H), 7.285 (*ddd*, 3H, Ar-H), 7.448 (*ddd*, 3H, Ar-H), 7.529 (*ddd*, 2H, Ar-H), 7.733 (*ddd*, 2H, Ar-H). ^{13}C NMR (DMSO-d6, 400 MHz) δ ppm: 154.77 (C=N), 145.15 (Ph-Ph), 126.16 (C-Br). HRMS spectrum (m/z) 460.0711 $(M+1)^{+}$. Anal. Calcd for $C_{25}H_{17}BrN_2$: C, 70.60; H, 4.03; Br, 18.79;

N, 6.59 %. Found: C, 70.53; H, 4.00; Br, 18.71; N, 6.53 %.

*2-(4'-bromo-[1,1'-biphenyl]-4-yl)-1-(2,3-difluorophenyl)-1H-benzo[d]imidazole (**JP8**):* Brown powder (87.97 %), M.P. = 208-210 °C. FT(IR) spectrum (KBr, □/cm^{-1}), 3227 (Ar C-H), 1592 (Ar C=N), 1536 (Ar C=C), 1471 (Ar C-N), 1249 (Ar C-F), 691 (Ar C-Br). ^{1}H NMR (DMSO-d6, 400 MHz) δ ppm: 6.744 (*ddd*, 1H, Ar-H), 6.992 (*ddd*, 1H, Ar-H), 7.012 (*ddd*, 1H, Ar-H), 7.116 (*ddd*, 4H, Ar-H), 7.243 (*ddd*, 1H, Ar-H), 7.292 (*ddd*, 2H, Ar-H), 7.310 (*ddd*, 1H, Ar-H), 7.456 (*ddd*, 2H, Ar-H), 7.902 (*ddd*, 1H, Ar-H), 8.373 (*ddd*, 1H, Ar-H). ^{13}C NMR (DMSO-d6, 400 MHz) δ ppm: 156.65 (C=N), 146.31 (Ph-Ph), 129.90 (C-Br), 154.24 (Ar C-F). HRMS spectrum (m/z) 462.1594 (M+2)$^{+}$. Anal. Calcd for $C_{25}H_{15}BrF_2N_2$: C, 65.09; H, 3.28; Br, 17.32; F, 8.24; N, 6.07 %. Found: C, 64.94; H, 3.26; Br, 17.29; F, 8.21; N, 6.00 %.

*2-(4'-bromo-[1,1'-biphenyl]-4-yl)-1-(2,4-difluorophenyl)-1H-benzo[d]imidazole (**JP9**):* Yellow powder (85.14 %), M.P. = 200-202 °C. FT(IR) spectrum (KBr, □/cm^{-1}), 3172 (Ar C-H), 1645 (Ar C=N), 1598 (Ar C=C), 1456 (Ar C-N), 1206 (Ar C-F), 717 (Ar C-Br). ^{1}H NMR (DMSO-d6, 400 MHz) δ ppm: 6.739 (*ddd*, 3H, Ar-H), 7.007 (*m*, 8H, Ar-H), 7.135 (*ddd*, 3H, Ar-H), 7.775 (*ddd*, 1H, Ar-H). ^{13}C NMR (DMSO-d6, 400 MHz) δ ppm: 150.88 (C=N), 148.50 (Ph-Ph), 129.90 (C-Br), 158.14 (Ar C-F). HRMS spectrum (m/z) 462.0711 (M+1)$^{+}$. Anal. Calcd for $C_{25}H_{17}BrN_2O$: C, 68.04; H, 3.88; Br, 18.11; N, 6.35; O, 3.63 %. Found: C, 68.00; H, 3.83; Br, 18.09; N, 6.32; O, 3.61 %.

*2-(4'-bromo-[1,1'-biphenyl]-4-yl)-1-(2,6-difluorophenyl)-1H-benzo[d]imidazole (**JP10**):* Yellow powder (81.03 %), M.P. = 195-196 °C. FT(IR) spectrum (KBr, □/cm^{-1}), 3154 (Ar C-H), 1616 (Ar C=N), 1585 (Ar C=C), 1460 (Ar C-N), 1226 (Ar C-F), 707 (Ar C-Br). ^{1}H NMR (DMSO-d6, 400 MHz) δ ppm: 7.437 (*m*, 10H, Ar-H), 7.648 (*ddd*, 2H, Ar-H), 7.913 (*ddd*, 3H, Ar-H). ^{13}C NMR (DMSO-d6, 400 MHz) δ ppm: 150.90 (C=N), 148.52 (Ph-Ph), 129.70 (C-Br), 159.92 (Ar C-F). HRMS spectrum (m/z) 462.1440 (M+1)$^{+}$. Anal. Calcd for $C_{25}H_{15}BrF_2N_2$: C, 65.09; H, 3.28; Br, 17.32; F, 8.24; N, 6.07 %. Found: C, 65.01; H, 3.23; Br, 17.28; F, 8.20; N, 6.03 %.

2.3. Detection Method:

The synthesized compounds were characterized using a suite of spectroscopic techniques. Proton and carbon nuclear magnetic resonance (^{1}H NMR and ^{13}C NMR) spectra were recorded on a Bruker Avance 400 MHz spectrometer in $CDCl_3$ or DMSO-d6, with chemical shifts reported in ppm relative to tetramethylsilane (TMS)[24]. Infrared (IR) spectra were obtained using a Bruker Spectrum 100 FT-IR spectrometer in the range of 4000–400 cm^{-1}. High-resolution mass spectrometry (HRMS) was performed on a Waters Q-TOF Premier instrument using electrospray ionization (ESI) in positive ion mode. Melting points were determined using a Stuart SMP10 melting point apparatus and are uncorrected.

2.4. Antimicrobial Activity

The antimicrobial activity of compounds **NP1–NP10** was evaluated using the agar well diffusion (well cup) method, a widely accepted technique for preliminary screening of antimicrobial agents [28,29]. Test microorganisms included the Gram-positive bacteria *Bacillus subtilis* and *Staphylococcus aureus*, the Gram-negative bacteria *Pseudomonas aeruginosa* and *Escherichia coli*, and the fungal strains *Candida albicans* and *Saccharomyces cerevisiae*. Fresh microbial suspensions were prepared and standardized according to established microbiological protocols[30,31].

Sterile nutrient agar medium was employed for bacterial strains, while Sabouraud dextrose agar was used for fungal cultures. Wells of approximately 6 mm diameter were punched into the inoculated agar plates and filled with solutions of the synthesized compounds dissolved in DMSO. Streptomycin and fluconazole were used as reference antibacterial and antifungal drugs, respectively, whereas DMSO served as the negative control. Following incubation at appropriate temperatures, the diameters of the inhibition zones were measured in millimeters and recorded as indicators of antimicrobial efficacy [32].

3. Results and Discussion

The synthesized benzimidazole derivatives **JP1–JP10** were obtained as brown-to-yellow crystalline powders in good-to-excellent yields (77.99–89.78%) with sharp melting points ranging from 195–210 °C, indicating their high purity and successful formation [33,34]. The structures of all compounds were established through FT-IR, ^{1}H NMR, ^{13}C NMR, HRMS, and elemental analysis. The combined spectroscopic data were in excellent agreement with the proposed molecular structures and closely resembled previously reported N-substituted benzimidazole analogues [35,36].

The FT-IR spectra of all derivatives exhibited characteristic absorption bands corresponding to aromatic C–H stretching vibrations in the region 3154–3257 cm^{-1}, confirming the aromatic nature of the synthesized molecules [37]. A strong absorption band attributable to the benzimidazole azomethine (C=N) group appeared between 1592 and 1697 cm^{1}, providing clear evidence for the preservation of the benzimidazole framework after N-substitution [38].

Aromatic C=C stretching vibrations were observed within 1498–1585 cm^{1}, whereas the C–N stretching frequencies of the benzimidazole ring appeared in the range 1440–1493 cm^{-1} [39].

For the fluorinated derivatives **JP1**, **JP2**, **JP3**, **JP8**, **JP9**, and **JP10**, additional bands were detected at 1206–1249 cm^{-1}, corresponding to aromatic C–F stretching vibrations. The presence of these characteristic absorptions confirmed the successful incorporation of fluorinated phenyl substituents into the benzimidazole nucleus [40]. Likewise, all compounds exhibited diagnostic C–Br stretching vibrations between 626 and 757 cm^{1}, confirming retention of the bromobiphenyl moiety throughout the synthetic sequence [41].

The hydroxyl-containing compounds **JP4** and **JP5** displayed additional spectroscopic features. **JP4** exhibited a distinct singlet corresponding to the phenolic proton at δ 9.952 ppm, whereas **JP5** showed two highly deshielded hydroxyl resonances at δ 11.506 and 11.714 ppm. These downfield signals are characteristic of hydrogen-bonded phenolic hydroxyl groups and strongly support the proposed structures [42].

The ^{1}H NMR spectra of compounds **JP1–JP10** revealed complex multiplet patterns in the aromatic region, generally appearing between δ 6.74 and 8.37 ppm. The observed proton integrations were consistent with the number of aromatic hydrogens present in each molecule. The absence of an N–H resonance characteristic of the parent benzimidazole confirmed successful N-substitution in all derivatives [43]. Fluorinated derivatives displayed slight deshielding effects due to the strong electron-withdrawing nature of fluorine atoms, resulting in subtle variations in the aromatic proton chemical shifts [44].

Further structural confirmation was obtained from the ^{13}C NMR spectra. All compounds displayed a characteristic benzimidazole C=N carbon resonance between δ 150.88 and 159.96 ppm, which is consistent with literature values reported for substituted benzimidazole systems [45]. Signals corresponding to the biphenyl linkage carbons appeared in the range δ 140–149 ppm. The brominated aromatic carbon was consistently observed around δ 126–130 ppm, confirming the presence of the bromobiphenyl scaffold. In fluorinated compounds, additional downfield carbon resonances assigned to C–F bearing aromatic carbons were observed near δ 154–160 ppm, reflecting the strong inductive effect of fluorine substitution [40,45].

The methyl-substituted derivative **JP6** displayed a characteristic carbon resonance at δ 13.20 ppm corresponding to the two aromatic methyl groups attached to the 2,6-dimethylphenyl ring. Similarly, **JP5** exhibited two distinct phenolic carbon signals at δ 163.27 and 158.23 ppm, confirming the presence of two hydroxyl functionalities within the molecule [42,46].

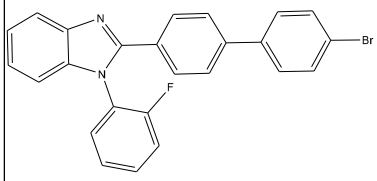
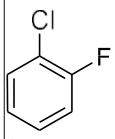
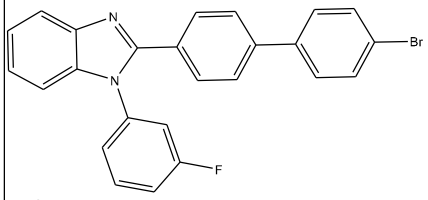
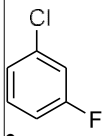
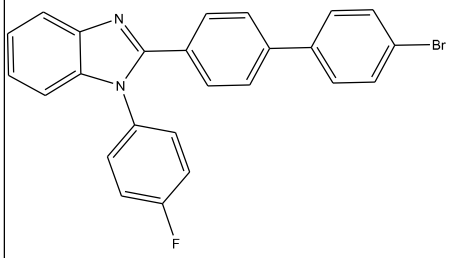
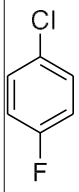
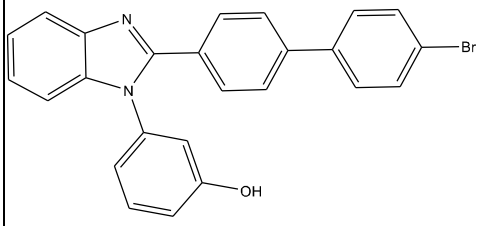
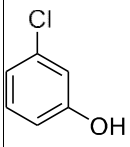
High-resolution mass spectrometric analysis provided definitive molecular confirmation for all synthesized compounds. The experimentally observed molecular ion peaks closely matched the calculated molecular masses. In addition, the characteristic isotopic pattern arising from the presence of bromine atoms was clearly visible in the spectra, particularly through the expected M and M+2 ion peaks with nearly equal intensity ratios, thereby confirming bromine incorporation within the molecular framework [47].

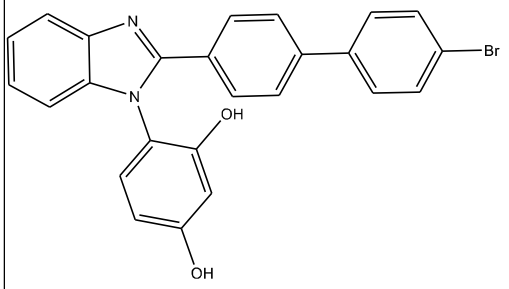
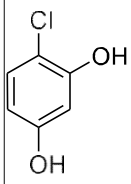
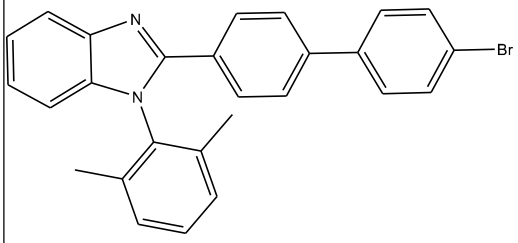
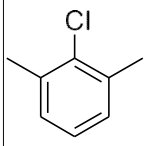
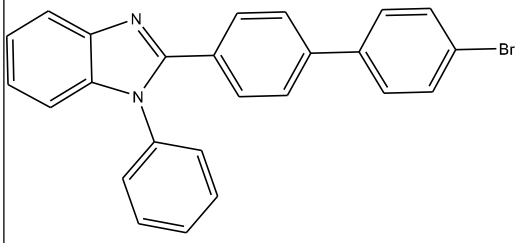
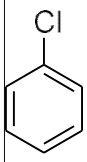
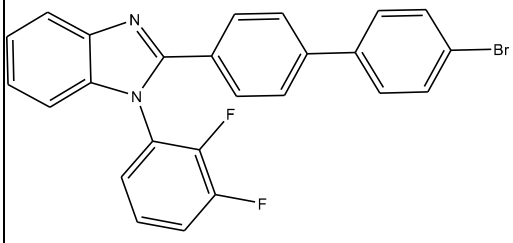
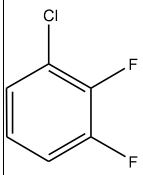
Elemental analysis data further supported the assigned structures. The experimentally determined percentages of carbon, hydrogen, nitrogen, bromine, fluorine, and oxygen were in close agreement with the theoretical values, generally within ±0.4% of the calculated compositions. Such close correspondence indicates high purity of the synthesized compounds and validates the proposed molecular formulas [48].

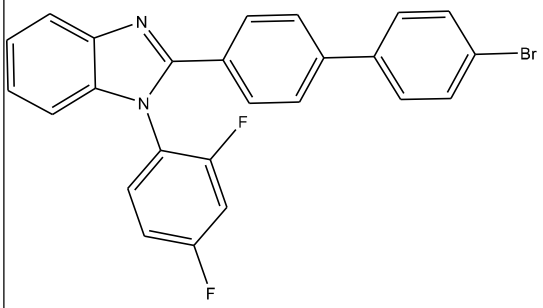
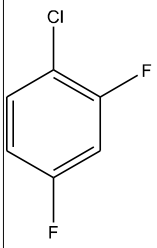
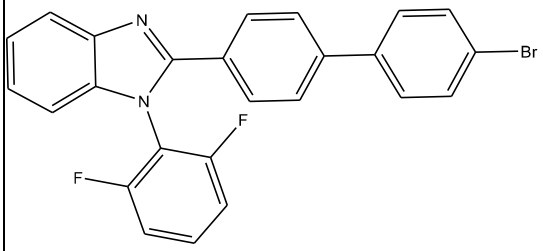
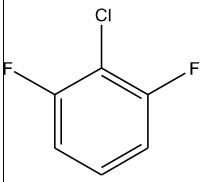
Among the synthesized derivatives, fluorinated compounds **JP1–JP3** and **JP8–JP10** displayed slightly higher melting points and enhanced crystallinity compared to the unsubstituted derivative **JP7**, likely due to stronger intermolecular interactions arising from fluorine substitution. Likewise, hydroxyl-substituted compounds **JP4** and **JP5** exhibited elevated melting points owing to intermolecular hydrogen-bonding interactions. The overall spectroscopic and analytical results conclusively demonstrate the successful synthesis of a structurally diverse series of N-substituted 2-(4′-bromo-[1,1′-biphenyl]-4-yl)-1H-benzo[d]imidazole derivatives, providing a solid foundation for subsequent biological evaluation and structure–activity relationship studies [33,35,48].

Table 1: Yield and physical constants of the product (**JP1-10**)

Products	**R =**	**Yield (%)**	**m.p. (□C)**

 **JP1**	 **1**	83.67	199-201
 **JP2**	 **2**	87.99	196-197
 **JP3**	 **3**	84.33	203-204
 **JP4**	 **4**	83.58	206-207

 **JP5**	 **5**	80.36	202-205
 **JP6**	 **6**	89.78	208-209
 **JP7**	 **7**	77.99	196-197
 **JP8**	 **8**	87.97	208-210

 **JP9**	 **9**	85.14	200-202
 **JP10**	 **10**	81.03	195-196

3.1. Antimicrobial Activity:

The antimicrobial screening results presented in **Table 2** demonstrated that several of the synthesized compounds possess significant antibacterial and antifungal properties. The observed biological activities may be attributed to the ability of heterocyclic frameworks to interact with microbial enzymes, nucleic acids, and cell membrane components, leading to inhibition of microbial growth [49,50].

Among all tested compounds, **NP6** exhibited the most potent and broad-spectrum antibacterial activity. Against *B. subtilis*, NP6 produced an inhibition zone of 27 mm, which was substantially greater than that of streptomycin (15 mm). Similarly, **NP2** (32 mm), **NP9** (29 mm), and **NP10** (28 mm) demonstrated excellent activity, indicating strong effectiveness against Gram-positive organisms. Enhanced activity against *B. subtilis* may result from improved penetration of the bacterial cell wall and stronger interactions with intracellular biological targets [50,51].

Remarkable activity was also observed against *P. aeruginosa*, one of the most challenging Gram-negative pathogens due to its intrinsic resistance mechanisms [51]. Compound **NP6** displayed the highest inhibition zone (34 mm), followed by **NP9** (30 mm) and **NP5** (28 mm), all exceeding the activity of streptomycin (20 mm). These results suggest that the synthesized compounds may possess structural features favorable for permeation through the outer membrane of Gram-negative bacteria and resistance to bacterial defense mechanisms [51-54].

In the case of *S. aureus*, **NP6** again showed the strongest antibacterial effect with an inhibition zone of 30 mm, followed by **NP9** (25 mm) and **NP10** (23 mm). The superior performance of these compounds compared to streptomycin indicates that they may interfere efficiently with essential cellular processes such as cell wall biosynthesis or protein synthesis [50,54]. Conversely, **NP2**, **NP7**, and **NP8** exhibited little or no activity against this organism, suggesting that subtle structural modifications significantly influence antimicrobial potency.

The activity against *E. coli* further confirmed the broad-spectrum nature of several derivatives. **NP6** exhibited the highest inhibition zone (30 mm), followed by **NP2** (28 mm), **NP5** (25 mm), and **NP3** (24 mm). These results compare favorably with streptomycin (18 mm) and suggest effective interaction with Gram-negative bacterial targets [51]. The inactivity of **NP7** and **NP8** may be associated with inadequate lipophilicity or unfavorable electronic properties that limit membrane permeability [49].

The antifungal evaluation revealed particularly encouraging results. Compounds **NP2** and **NP9** displayed inhibition zones of 32 mm and 30 mm, respectively, against *C. albicans*, significantly surpassing the activity of fluconazole (18 mm). **NP1** and **NP10** also exhibited excellent antifungal activity with inhibition zones of 30 mm and 29 mm,

respectively. The strong antifungal effects observed may arise from disruption of fungal membrane integrity or inhibition of ergosterol biosynthesis pathways, which are recognized targets for antifungal agents [52,53].
Against *S. cerevisiae*, compound **NP6** exhibited the highest activity (34 mm), followed by **NP4** and **NP9** (33 mm each), **NP3** (31 mm), and **NP2** (30 mm). These inhibition zones were substantially greater than that of fluconazole (17 mm), indicating excellent antifungal efficacy. Such enhanced activity may be related to favorable electronic and steric characteristics that facilitate stronger interactions with fungal cellular targets [52,53].

Compound	*B. subtilis*	*P. aeruginosa*	*S. aureus*	*E. coli*	*C. albicans*	*S. cere*
NP1	21	20	14	19	30	28
NP2	32	17	0	28	32	30
NP3	24	16	16	24	28	31
NP4	20	13	17	20	25	33
NP5	0	28	20	25	0	24
NP6	27	34	30	30	25	34
NP7	0	16	0	0	0	0
NP8	10	11	0	0	18	9
NP9	29	30	25	16	30	33
NP10	28	19	23	15	29	27
Streptomycin	15	20	20	18	-	-

The antimicrobial screening results indicate that **NP6** is the most promising derivative, displaying potent activity against all tested microorganisms. Compounds **NP2** and **NP9** also demonstrated excellent broad-spectrum antimicrobial profiles. The superior activities of these derivatives may be attributed to an optimal balance of electronic effects, molecular lipophilicity, and structural features that enhance microbial cell penetration and target binding [49-51]. The findings support further investigations involving minimum inhibitory concentration (MIC) determination, mechanistic studies, and structure–activity relationship analyses to establish these compounds as potential antimicrobial lead molecules [55]. **Table 2.** Antimicrobial Activity of Compounds **JP1–JP10** by the well cup method (Inhibition Zone Diameters in mm)

4. Conclusions

A structurally diverse library of ten novel N-substituted 2-(4′-bromo-[1,1′-biphenyl]-4-yl)-1H-benzo[d]imidazole derivatives (**JP1–JP10**) was successfully synthesized through an efficient N-alkylation protocol. The synthetic methodology proved highly effective, yielding the target compounds in good-to-excellent yields (77.99–89.78%) with high purity, as confirmed by sharp melting points and comprehensive spectroscopic characterization. FT-IR, NMR, HRMS, and elemental analysis data collectively validated the proposed molecular structures, with characteristic spectral features including the disappearance of N–H signals confirming successful N-substitution, and diagnostic C–F and C–Br stretching vibrations confirming the incorporation of key functional groups.

The antimicrobial evaluation revealed that several synthesized derivatives possess significant antibacterial and antifungal activities, with compound JP6 emerging as the most promising candidate. **JP6** exhibited remarkable broad-spectrum activity against all tested microorganisms, producing inhibition zones substantially greater than the reference drugs streptomycin and fluconazole. Notably, **JP6** demonstrated exceptional efficacy against the challenging Gram-negative pathogen P. aeruginosa (34 mm) and the fungal strain S. cerevisiae (34 mm), highlighting its potential as a broad-spectrum antimicrobial agent. Fluorinated derivatives **JP2** and **JP9** also displayed excellent antimicrobial profiles, suggesting that strategic incorporation of electron-withdrawing fluorine substituents enhances biological activity, likely through improved membrane permeability and stronger interactions with microbial targets.

The structure-activity relationship analysis indicated that antimicrobial potency is strongly influenced by the nature and position of substituents on the N-phenyl ring. Electron-withdrawing groups, particularly fluorine at specific positions, and appropriate alkyl substituents appeared to optimize the balance between lipophilicity, electronic effects, and steric factors, facilitating enhanced microbial cell penetration and target binding. The hydroxyl-containing derivatives **JP4** and **JP5** also demonstrated notable activity, possibly due to hydrogen-bonding interactions with biological targets.

These findings provide a solid foundation for future investigations, including the determination of minimum inhibitory concentrations (MICs), exploration of molecular mechanisms of action, evaluation of cytotoxicity profiles, and further structure-activity relationship optimization. The synthesized benzimidazole derivatives, particularly JP6, represent promising lead compounds for the development of novel antimicrobial therapeutics to address the growing challenge of antimicrobial resistance.

5. References

1. Yadav, G.; Ganguly, S. Structure activity relationship (SAR) study of benzimidazole scaffold for different biological activities: A mini-review. *Eur. J. Med. Chem.* **2015**, *97*, 419-443.
2. Akhtar, W.; Khan, M. F.; Verma, G.; Shaquiquzzaman, M.; Rizvi, M. A.; Mehdi, S. H.; Akhter, M.; Alam, M. M. Therapeutic evolution of benzimidazole derivatives in the last quinquennial period. *Eur. J. Med. Chem.* **2017**, *126*, 705-753.
3. Bansal, Y.; Silakari, O. The therapeutic journey of benzimidazoles: A review. *Bioorg. Med. Chem.* **2012**, 20, 6208-6236.
4. Gaba, M.; Singh, S.; Mohan, C. Benzimidazole: An emerging scaffold for analgesic and anti-inflammatory agents. *Eur. J. Med. Chem.* **2014**, 76, 494-505.
5. Boiani, M.; Gonzalez, M. Imidazole and benzimidazole derivatives as chemotherapeutic agents. *Mini-Rev. Med. Chem.* **2005**, *5*, 409-424.
6. Spasov, A. A.; Yozhitsa, I. N.; Bugaeva, L. I.; Anisimova, V. A. Benzimidazole derivatives: Spectrum of pharmacological activity and toxicological properties. *Pharm. Chem. J.* **1999**, *33*, 232-243.
7. Tahlan, S.; Kumar, S.; Kakkar, S.; Narasimhan, B. Benzimidazole scaffolds as promising antiproliferative agents: A review. *BMC Chem.* **2019**, *13*, 66.
8. Singla, R.; Gupta, K. B.; Upadhyay, S.; Dhiman, M.; Jaitak, V. Benzimidazole derivatives as anticancer agents: A review. *Mini-Rev. Med. Chem.* **2017**, *17*, 1695-1715.
9. Narasimhan, B.; Sharma, D.; Kumar, P. Biological importance of imidazole and benzimidazole derivatives: A review. *Med. Chem. Res.* **2011**, *20*, 1119-1140.
10. Ansari, K. F.; Lal, C. Synthesis and biological evaluation of some new benzimidazole derivatives. *Eur. J. Med.Chem.* **2009**, *44*, 2294-2299.
11. Gowda, N. R.; Kavitha, C. V.; Chiruvella, K. K.; Joy, O.; Rangappa, K. S.; Raghavan, S. C. Synthesis and biological evaluation of novel 1-(4-

substituted phenyl)-2-(4-aryl)benzimidazoles as anticancer agents. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 4594-4598.
12. El-Gohary, N. S.; Shaaban, M. I. Synthesis, antimicrobial, antiquorum-sensing and cytotoxic activities of new series of benzimidazole derivatives. *Eur. J. Med. Chem.* **2017**, *131*, 255-262.
13. Hagmann, W. K. The many roles for fluorine in medicinal chemistry. *J. Med. Chem.* **2008**, *51*, 4359-4369.
14. Shah, P.; Westwell, A. D. The role of fluorine in medicinal chemistry. *J. Enzyme Inhib. Med. Chem.* **2007**, *22*, 527-540.
15. Purser, S.; Moore, P. R.; Swallow, S.; Gouverneur, V. Fluorine in medicinal chemistry. *Chem. Soc. Rev.* **2008**, *37*, 320-330.
16. Proudfoot, J. R. Biphenyls as pharmacological agents: A review. *Bioorg. Med. Chem. Lett.* **2015**, *25*, 4766-4771.
17. Abdel-Halim, M.; El-Sayed, M. A.; El-Messery, S. M. Biphenyl derivatives as potential antimicrobial agents. *J. Pharm. Sci. Res.* **2019**, *11*, 234-241.
18. Dhiman, P.; Arora, N.; Thanikachalam, P. V.; Monga, V. Recent advances in the synthetic and medicinal perspective of benzimidazole derivatives. *Bioorg. Chem.* **2019**, *93*, 103321.
19. Kamal, A.; Dastagiri, D.; Ramaiah, M. J.; Reddy, J. S.; Bharathi, E. V.; Reddy, M. K.; Sagar, M. V.; Reddy, T. L.; Pushpavalli, S. N. C. V. L.; Pal-Bhadra, M. Synthesis and biological evaluation of new benzimidazole derivatives as anticancer agents. *Bioorg. Med. Chem.* **2010**, *18*, 6667-6673.
20. World Health Organization. Antimicrobial resistance: Global report on surveillance. **2014**, WHO Press, Geneva.
21. Ventola, C. L. The antibiotic resistance crisis: Part 1: Causes and threats. *P T* **2015**, *40*, 277-283.
22. O'Neill, J. Tackling drug-resistant infections globally: Final report and recommendations. *Review on Antimicrobial Resistance* **2016**.
23. Al-Trawneh, S. A.; El-Abadelah, M. M.; Zahra, J. A.; Al-Taweel, S. A.; Zani, F.; Incerti, M.; Cavazzoni, A.; Vicini, P. Synthesis and biological evaluation of some new benzimidazole derivatives. *Bioorg. Med. Chem.* **2010**, *18*, 5037-5045.
24. Husain, A.; Varshney, M.; Rashid, M.; Mishra, R.; Akhter, M. Benzimidazole: A versatile heterocyclic scaffold for drug discovery. *J. Pharm. Res.* **2011**, *4*, 4136-4140.
25. Siwach, A., & Verma, P. K. (2021). Synthesis and therapeutic potential of imidazole containing compounds. *BMC chemistry*, *15*(1), 12..
26. Armarego, W.L.F.; Chai, C.L.L. *Purification of Laboratory Chemicals*, 7th ed.; Butterworth-Heinemann: Oxford, UK, 2013.
27. Joule, J.A.; Mills, K. *Heterocyclic Chemistry*, 5th ed.; Wiley-Blackwell: Oxford, UK, 2010.
28. Bauer, A.W.; Kirby, W.M.M.; Sherris, J.C.; Turck, M. Antibiotic susceptibility testing by a standardized single disk method. *Am. J. Clin. Pathol.* 1966, 45, 493–496.
29. Perez, C.; Pauli, M.; Bazerque, P. An antibiotic assay by the agar-well diffusion method. *Acta Biol. Med. Exp.* 1990, 15, 113–115.
30. Balouiri, M.; Sadiki, M.; Ibnsouda, S.K. Methods for in vitro evaluating antimicrobial activity: A review. *J. Pharm.Anal.* 2016, 6, 71–79.
31. CLSI. Performance Standards for Antimicrobial Susceptibility Testing, 30th ed.; Clinical and Laboratory Standards Institute: Wayne, PA, USA, 2020.
32. Wiegand, I.; Hilpert, K.; Hancock, R.E.W. Agar and broth dilution methods to determine MIC of antimicrobial substances. *Nat. Protoc.* 2008, 3, 163–175.
33. Katritzky, A.R.; Rees, C.W.; Scriven, E.F.V. *Comprehensive Heterocyclic Chemistry II*; Elsevier, 1996.
34. Joule, J.A.; Mills, K. *Heterocyclic Chemistry*, 5th ed.; Wiley, 2010.
35. Bansal, Y.; Silakari, O. Benzimidazole derivatives: Biological activities and medicinal applications. *Bioorg. Med. Chem.* 2012, 20, 6208–6236.
36. Refaat, H.M. Synthesis and antimicrobial activity of benzimidazole derivatives. *Eur. J. Med. Chem.* 2010, 45, 2949–2956.
37. Silverstein, R.M.; Webster, F.X.; Kiemle, D. *Spectrometric Identification of Organic Compounds*; Wiley, 2005.
38. Socrates, G. *Infrared and Raman Characteristic Group Frequencies*, 3rd ed.; Wiley, 2004.
39. Pavia, D.L.; Lampman, G.M.; Kriz, G.S. *Introduction to Spectroscopy*; Cengage Learning, 2014.
40. Smart, B.E. Fluorine substituent effects in organic chemistry. *J. Fluorine Chem.* 2001, 109, 3–11.
41. Pretsch, E.; Bühlmann, P.; Badertscher, M. *Structure Determination of Organic Compounds*; Springer, 2009.
42. Lambert, J.B.; Gronert, S.; Shurvell, H.F.; Lightner, D.A. *Organic Structural Spectroscopy*; Pearson, 2010.
43. Claridge, T.D.W. *High-Resolution NMR Techniques in Organic Chemistry*; Elsevier, 2016.
44. O'Hagan, D. Understanding organofluorine chemistry. *Chem. Soc. Rev.* 2008, 37, 308–319.
45. Breitmaier, E. *Structure Elucidation by NMR in Organic Chemistry*; Wiley, 2002.
46. Field, L.D.; Sternhell, S.; Kalman, J.R. *Organic Structures from Spectra*; Wiley, 2013.
47. Gross, J.H. *Mass Spectrometry: A Textbook*; Springer, 2017.
48. Furniss, B.S.; Hannaford, A.J.; Smith, P.W.G.; Tatchell, A.R. *Vogel's Textbook of Practical Organic Chemistry*, 5th ed.; Pearson, 2008.
49. Cowan, M.M. Plant products as antimicrobial agents. *Clin. Microbiol. Rev.* 1999, 12, 564–582.
50. Silver, L.L. Challenges of antibacterial discovery.

Clin. Microbiol. Rev. 2011, 24, 71–109.
51. Ventola, C.L. The antibiotic resistance crisis. *Pharm. Ther.* 2015, 40, 277–283.
52. Pfaller, M.A.; Diekema, D.J. Epidemiology of invasive candidiasis. *Clin. Microbiol. Rev.* 2007, 20, 133–163.
53. Perfect, J.R. The antifungal pipeline: A reality check. *Nat. Rev. Drug Discov.* 2017, 16, 603–616.
54. Khameneh, B.; Diab, R.; Ghazvini, K.; Fazly Bazzaz, B.S. Breakthroughs in bacterial resistance mechanisms. *Microb. Pathog.* 2016, 95, 32–42.
55. Rai, M.; Kon, K. *Fighting Multidrug Resistance with Herbal Extracts, Essential Oils and Their Components*; Academic Press: London, UK, 2013.