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RESEARCH ARTICLE

Synthesis, Characterization, Antioxidant and Antibacterial Activities of Benzimidazole Compounds

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ABSTRACT

In this work, the synthesis of Azo compound [A1-A3] from different amines, was carried out and then converted to the 2-substituted benzimidazole compound [B1-B3], through the interaction of the resulting azo compounds with O-phenylenediamine. The structures of all new synthesized compounds were determined by FT-IR, 1H NMR and 13C NMR and their physicochemical properties, among them solubility, color, and melting point. The synthesized benzimidazole compounds were also evaluated for their antioxidant activity using the DPPH radical scavenging assay and for their antibacterial activity against selected Gram-positive and Gram-negative bacteria. The results demonstrated that the synthesized compounds exhibited antioxidant and antibacterial activities with different levels of activity among the tested compounds.

Keywords: Benzimidazole, Azo, antioxidant, antibacterial.

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1. Introduction

An organic reaction that typically occurs between a diazonium compound and another aromatic compound to form an azo compound (-N=N-) is known as an azo coupling. The manufacture of active antibiotics such as sulfonamides and numerous other powerful chemical compounds used in daily treatments depends on azo compounds. Derivatives of azo(1). The history of heterocyclic compounds commenced Alongside the advancement of organic chemistry in the 18th century. Heterocyclic chemistry constitutes the most extensive subfield of organic chemistry. The heterocyclic moieties exhibit significant structural variety and are found in the majority of physiologically active chemicals. According to Gupta et al., around 65% of the data in organic chemistry pertains to the chemistry of heterocyclic molecules(2). Many heterocyclic compounds have been reported in the literature such as histamine, proline, tryptophan and several vitamins and coenzymes such as riboflavin, vitamin B12, vitamin E and folic acid. Presently Pathan et al. reviewed the important role of heterocyclic compounds in medicinal research and study(3). Over 90% of Clinically accessible pharmaceuticals contain heterocyclic structures. This elucidates the crucial role of heterocyclic molecules in pharmaceutical and medical chemistry(4). The initial Benzimidazole was synthesized by Hobrecker in 1872(5). This distinctive structure is regarded as one of the significant compounds in organic and industrial chemistry, particularly in relation to corrosion resistance(6).

Benzimidazole serves as a multifaceted scaffold and a crucial intermediary in the synthesis of numerous bioactive chemicals(7). It constitutes the structural backbone of various therapeutic drugs utilised in anticancer applications(8,9) ,antiallergic(10),antiviral(11),antiAlzheimer's(12), and antidiabetic(13). It integrates into zeolitic frameworks to eliminate various colours from aqueous environments or connects to oxadiazole derivatives for application as antioxidants(14,15). The bioactivities of benzimidazole compounds may be enhanced via modifying the functional groups on the core structure. This is the predominant strategy for promoting novel cancer therapeutics, resulting in numerous commercially marketed anti-cancer agents derived from the benzimidazole framework, including osimertinib, navelbine, alectinib, nocodazole, abemaciclib, and vinblastine. Currently, benzimidazole research is a primary priority for numerous laboratories globally(16–18). Although a number of benzimidazole and azo derivatives have been described previously, the present work reports new 2-substituted benzimidazole derivatives containing azo linkages with substitution patterns that have not been reported earlier. In particular, the combination of functionalized aromatic rings (bromo and nitro substituents) with the azo-benzimidazole hybrid framework was designed for improved antioxidant and antibacterial properties. The structural changes intended to give better bioactivity profiles and higher stability than previously reported analogues

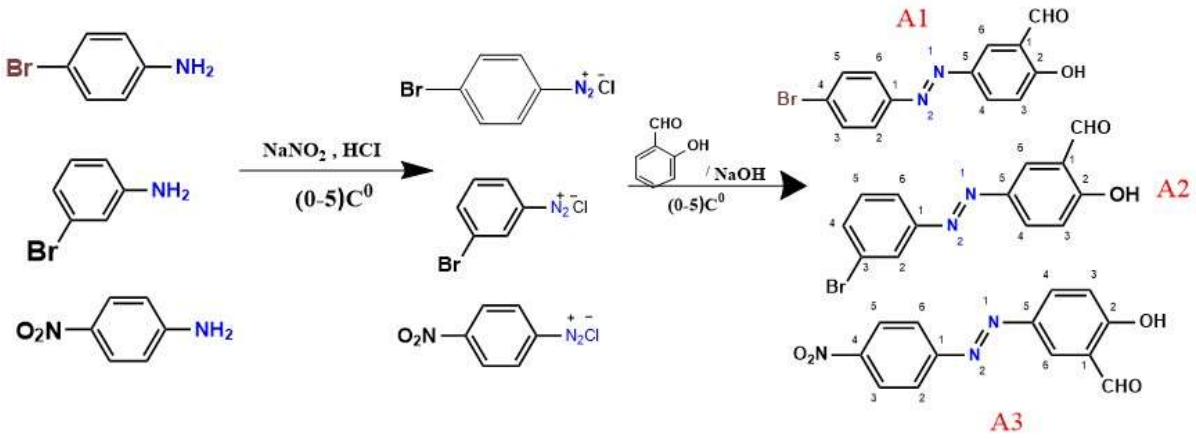
2. Experimental Section

2.1. Materials

All liquids and solid chemicals used in this study were obtained from Merek, Fluka and Aldrich with adequate purity. The FTIR spectra were recorded on Fourier Trans Infrared Spectrometer Promoter Shimadzu in the range 4000-400 cm^{-1}. ^{1}H NMR and ^{13}C NMR spectra were measured in DMSO-d^{6}.

2.2. Synthesis of Azo Compounds[A1-A3]

A1 was synthesized by diazotization of 4-bromoaniline (2.1 g, 0.01 mol) using HCl (2.8 mL) and $NaNO_2$ (1.0 g) at 0–5 °C. Urea (0.13 g) was added to remove excess nitrous acid, and the resulting mixture was stirred. The diazonium solution was then coupled with salicylaldehyde (1.3 mL, 0.01 mol) in the presence of NaOH (1.0 g) and allowed to stand for 45 min. The resulting precipitate was filtered, washed with distilled water, and dried.A2 and A3 were prepared by the same diazotization and azo-coupling procedure using 3-bromoaniline (1.34 mL, 0.01 mol) and 4-nitroaniline (1.69 g, 0.01 mol) (19), respectively, as illustrated in Scheme 1.



Scheme 1. Synthesis of compounds[A_1][A_2][A_3]

2.3. Synthesis of Benzimidazole Compounds[B1-B3]

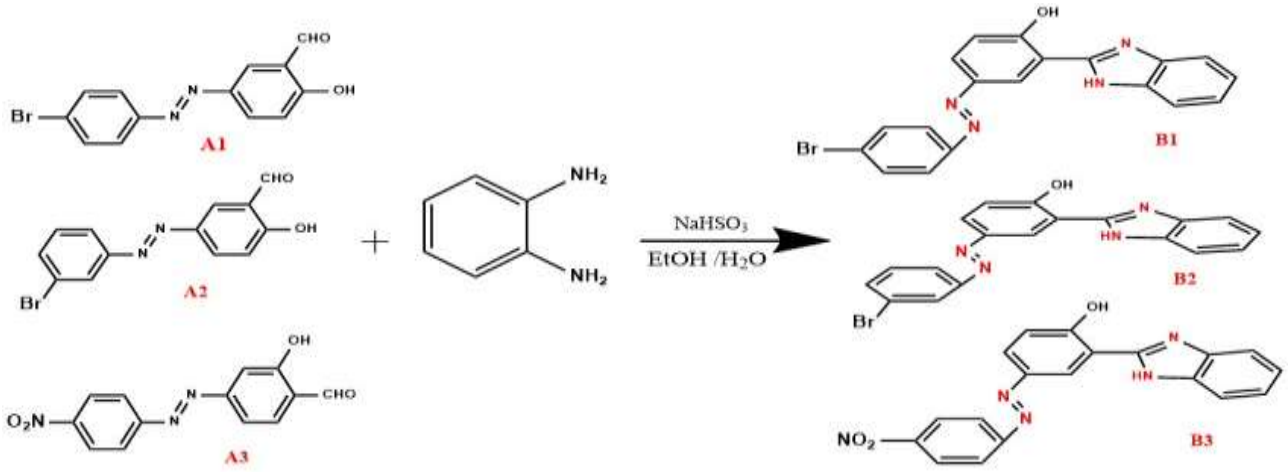
Synthesis of Compound B1:

A mixture of azo compound A1 (0.25 g, 0.0008 mol), o-phenylenediamine (0.08 g, 0.0008 mol), and $NaHSO_3$ (0.85 g, 0.0081 mol) in absolute ethanol (10 mL) and deionized water (10 mL) was refluxed for 10 h under continuous stirring. The reaction progress was monitored by TLC. The resulting precipitate was collected by

filtration and recrystallized from hot absolute ethanol to yield B1 (20).

Synthesis of Compounds B2 and B3:

Compounds B2 and B3 were synthesized similarly using the corresponding azo compounds A2 (0.25 g, 0.0008 mol) and A3 (0.25 g, 0.0009 mol), respectively, with the corresponding amounts of o-phenylenediamine (0.08 g, 0.0008 mol for B2 and 0.09 g, 0.0009 mol for B3) and $NaHSO_3$ (0.85 g, 0.0081 mol). The synthesis of compounds B1–B3 is illustrated in Scheme 2.



Scheme 2. Synthesis of compounds[B_1][B_2][B_3]

2.4. Antioxidant activity

Ascorbic acid was used as the positive standard in the DPPH method to evaluate the antioxidant activity of certain generated compounds. In compliance with the literature, the free radical scavenging activity was calculated(21). The compounds were tested at concentrations ranging from 50 to 250 ppm in 1% DMSO. Briefly, 0.4 mL of each sample was mixed with 2.6 mL of DPPH solution (40 ppm) and kept in the dark for 60 min at room temperature. Absorbance was recorded at 517 nm. Ascorbic acid (Vitamin C) was used as standard antioxidant. The scavenging activity was calculated according to the following equation:

Scavenging activity (%) = [(A − A_t) / A] × 100

where *A* is the control absorbance and A_t is the sample or standard absorbance.

2.5. Antibacterial activity

Biological activity of the synthesized compounds was evaluated by the agar disc-diffusion method against gram positive (Bacillus subtilis, and Staphylococcus aureus) and gram negative (Klebsiella pneumoniae, Pseudomonas aeruginosa, and E. coli) bacteria. The concentration of the investigated chemicals was 10^{-3} M, with dimethyl sulphoxide (DMSO) serving as the solvent control.

3. Results And Discussion

The amine (4-Bromo aniline) was react with salicylaldehyde in presence (HCI , $NaNO_2$, NaOH) at (0-5) ^{0}C to give azo dye [A_1] , Azo dye was react with o-phenylenediamine in presence $NaHSO_3$ to give 2-Sub benzimidazole [B1] , and amine (3-Bromoaniline) was react with salicylaldehyde in presence (HCI , $NaNO_2$, NaOH) at (0-5) ^{0}C to give azo dye [A_2] , Azo dye was react with o-phenylenediamine in presence $NaHSO_3$ to give 2-Sub benzimidazole [B_2], and amine (p-Nitro aniline) was react with salicylaldehyde in presence (HCI , $NaNO_2$, NaOH) at (0-5) ^{0}C to give azo dye [A_3] , Azo dye was react with o-phenylenediamine in presence $NaHSO_3$ to give 2-Sub benzimidazole [B_3]

3.1. Characterization of [A1-A3]

The molecular weight for azo compound A_1: (305.13g/mol),71 % yield of [A_1],m.p 202C . The FTIR spectrum exhibit anew absorption band at (1475) cm^{-1} corresponding to (-N=N-) and absorption band of (C=O) aldehyde at (1666) cm^{-1} absorption band at (3441) cm^{-1} for (O-H), and appearance absorption band of (C=C) aromatic at (1571) cm^{-1}, (2868) cm^{-1} for (C-H)aldehyde,^{1}HNMR(400MHz, DMSO), ppm: 10.25(s, 1H, CHO)aldehyde, 8.08-7.91(d, 2H, Ar-H), 7.71 -7.66 (m, 2H-Ar-H), 7.57-7.55 (d ,1H-Ar-H)7.29-7.08(m, 2H, Ar-H).

For the azo compound A_2 molecular formula ($C_{13}H_9BrN_2O_2$) and its molecular weight: (305.13g/mol),70 % yield of [A_2],m.p 144C . The FTIR spectrum exhibit anew absorption band at (1485) cm^{-1} corresponding to (-N=N-) and absorption band of (C=O) aldehyde at (1660) cm^{-1} absorption band at (3302) cm^{-1} for (O-H), and appearance absorption band of (C=C) aromatic at (1573) cm-1, (2852) cm-1 for (C-H) aldehyde1 HNMR(400MHz, DMSO), ppm:10.25(s,1H,CHO)aldehyde,8.07(d,1H,Ar-H),7.70-7.90(m,6H -Ar-H).

And molecular formula for A_3 $C_{13}H_9N_3O_4$ and molecular weight:271.23 g/mol, 43 % yield of [A_3],m.p 191C . The FTIR spectrum exhibit anew absorption band at (1477) cm^{-1} corresponding to (-N=N-) and absorption band of (C=O) aldehyde at (1658) cm^{-1} absorption band at (3442) cm^{-1} for (O-H), and appearance absorption band of (C=C) aromatic at (1577) cm^{-1}, (2852) cm^{-1} for (C-H) aldehyde. ^{1}H NMR (400 MHz, DMSO), ppm:10.25(s,1H, CHO) aldehyde, 8.0-8.3(d,4H,Ar-H), 7.0-8.0(d,3H-Ar-H) as shown in figure 1 to 3. The spectral data of the azo compounds [A_1–A_3] are consistent with their proposed structures. In the FTIR spectra, the formation of the azo group was supported by the presence of the characteristic (-N=N-) absorption band at 1475–1485 cm^{-1}, along with the aldehyde (C=O) band at 1658–1666 cm^{-1} and the broad phenolic (O-H) stretching band at 3302–3442 cm^{-1}. Furthermore, compound [A_3] exhibited characteristic absorption bands of the nitro group (-NO_2) at 1523 and 1340 cm^{-1}. In the ^{1}H-NMR spectra, the sharp singlet at δ 10.25 ppm was assigned to the aldehyde proton (-CHO), confirming the retention of the aldehyde group in all compounds. The aromatic protons appeared in the range of δ 7.00–8.30 ppm, with variations in their chemical shifts depending on the electronic effects of the substituents (Br and NO_2).

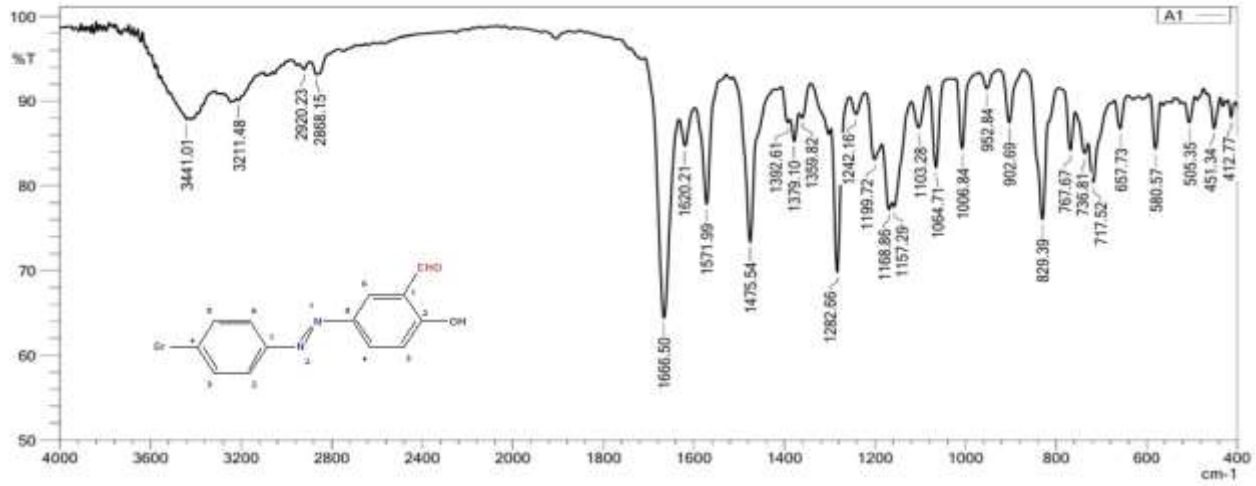


Figure 1. FTIR spectrum of compound[A_1]

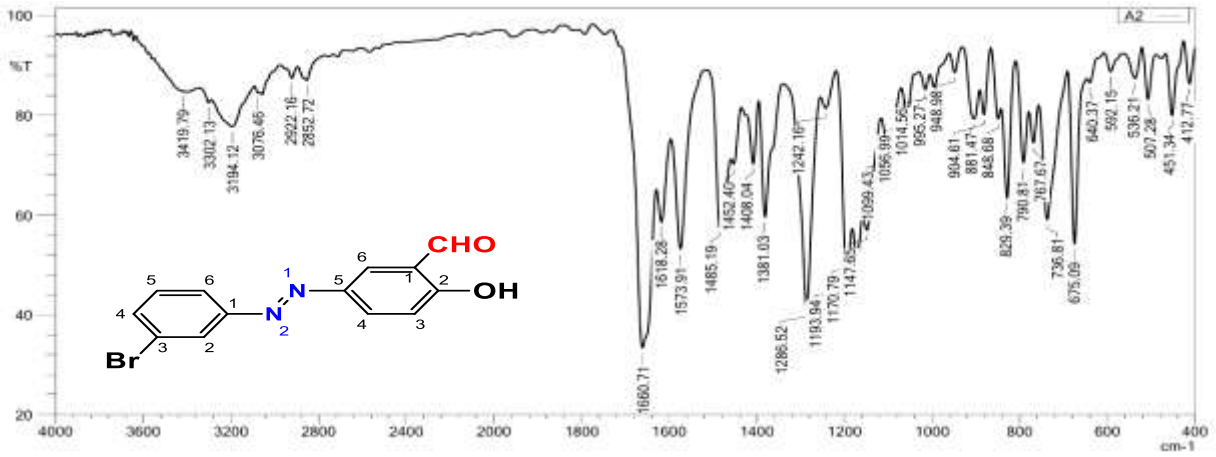


Figure 2. FTIR spectrum of compound[A_2]

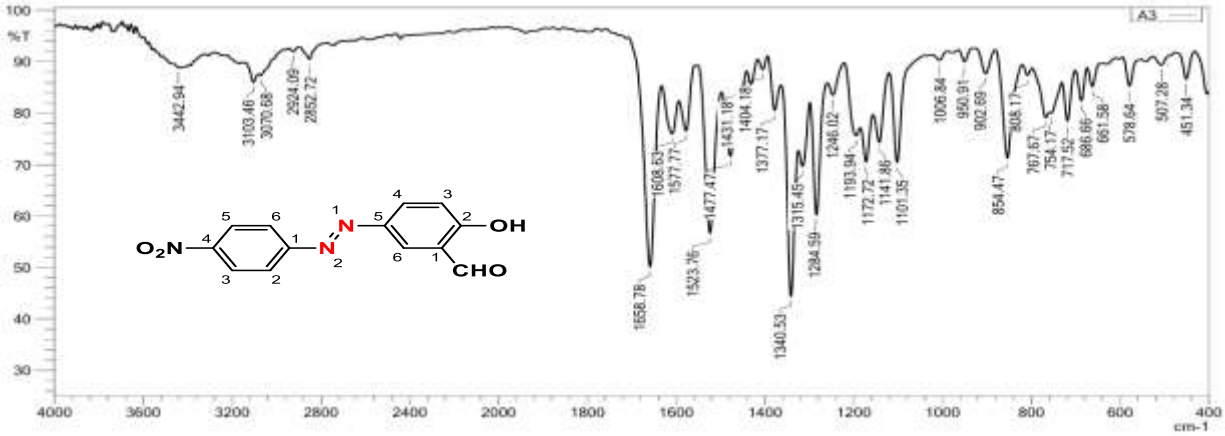


Figure 3. FTIR spectrum of compound[A_3]

3.2. Characterization of [B1-B3]

The molecular formula for B1 $C_{19}H_{13}BrN_4O$ with a molecular weight: (393.24 g/mol), 77% yield of [B1] , as Yellow-Orange powder, m.p 247C , FTIR νmax, cm^{-1}: (3352) cm^{-1} for (NH), N=N at (1477) cm^{-1}, (1631) cm^{-1} for (C=N), ; ^{1}H NMR (400 MHz, DMSO), ppm:10.28 (s ,1H,OH), 8..00-8.09(m,2H,Ar-H), 7.99-7.52 (m,8H,Ar-H),7.10(d,1H,Ar-H), The NH was not observed due to the exchange with the solvent, ^{13}CNMR (100 MHz, DMSO): δ190-195(C=N), δ 164 (C-OH), δ151(C-N=N), δ145(C-N=N), δ 136-129(Ar-C and C-Br), δ124-119(Ar-CH).

While,The molecular formula of B_2 $C_{19}H_{13}BrN_4O$ and a molecular weight of 393.24 g/mol, 65% yield of [B2] , as Yellowish-Brown powder, m.p 220C , FTIR νmax, cm^{-1}: (3352) cm^{-1} for (NH), N=N at (1496) cm^{-1}, (1602) cm^{-1} for (C=N),^{1}HNMR(400MHz,DMSO),ppm:14.02(s,1H,OH), 13.61 (s,1H,NH), 8.68(S,1H,ArH), 7.068.20(m,10H,Ar-H), ^{13}CNMR (100 MHz, DMSO):),^{13}CNMR (100 MHz, DMSO):δ162(C-OH),δ133(Ar-C),δ123(Ar-C),δ125 (Ar-CH), δ 118(Ar-CH), as shown in figure 4 to 6

The molecular formula for B_3 $C_{19}H_{13}N_5O_3$ molecular weight) (359.35 g/mol), 90% yield of [B3] , as Dark Red powder, m.p 284C , FTIR νmax, cm^{-1}: (3358) cm^{-1} for (NH), N=N at (1452)cm^{-1}, (1627) cm^{-1} for (C=N), ; ^{1}H NMR (400 MHz, DMSO), ppm:8.73(s ,1H,OH),8.36(d, 2H,Ar-H),8.08(m,2H,Ar-H),7.10-7.90(m,7 H ,Ar-H), The NH was not observed due to the exchange with the solvent),^{13}CNMR(100MHz,DMSO):δ162(C-OH),155(C-NO_2),151-145(A r-C), 126-113(Ar-CH).Overall, the good agreement among the FTIR, ^{1}H-NMR, and ^{13}C-NMR data, particularly the disappearance of the carbonyl (C=O) absorption of the starting azo compounds and the appearance of the characteristic imine (C=N) signals, together with the expected N–H, phenolic –OH, aromatic, and carbon resonances, provides strong spectroscopic evidence for the successful cyclization and formation of the proposed benzimidazole derivatives [B_1–B_3]. These findings are consistent

with the proposed molecular structures and confirm the successful transformation of the starting azo compounds into the corresponding benzimidazole derivatives.

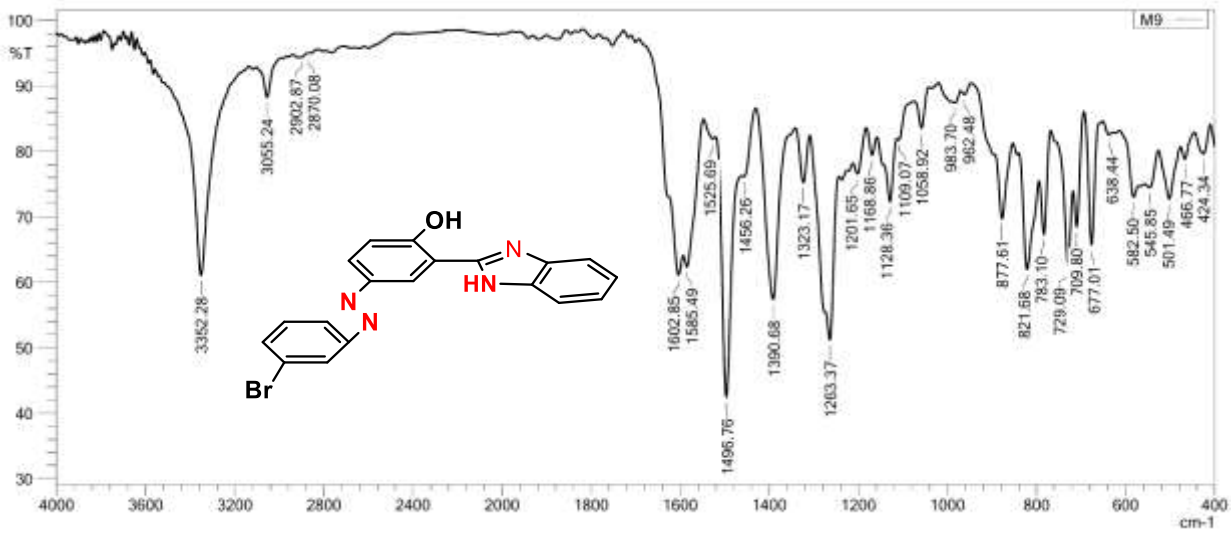


Figure 4. FTIR spectrum of compound[B_2]

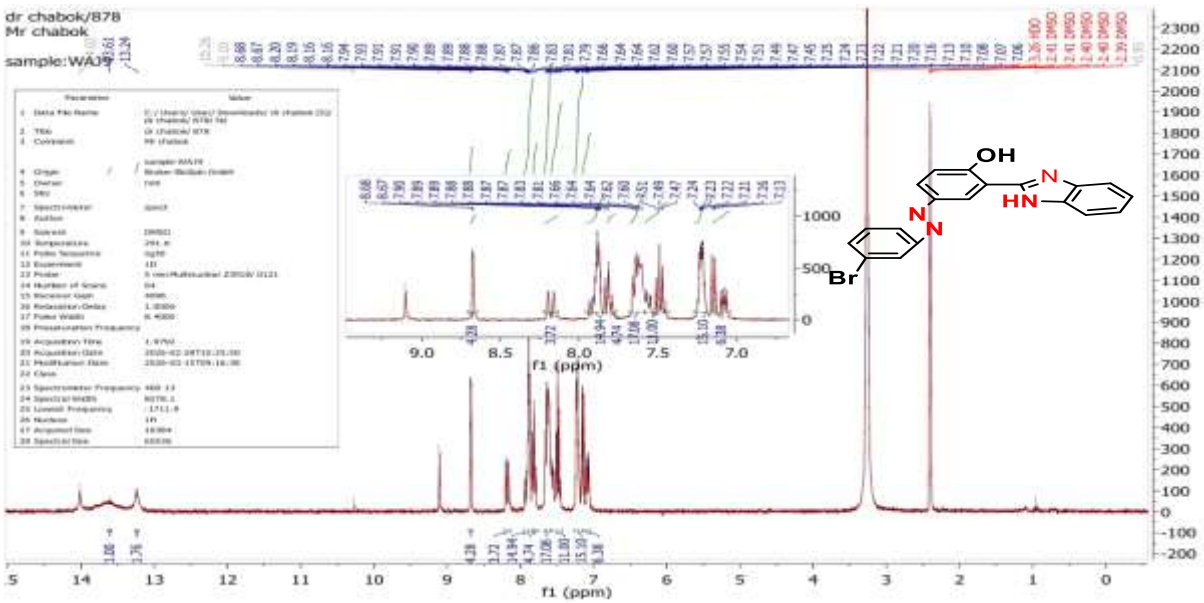


Figure 5. ^{1}H-MNR spectrum of compound[B_2]

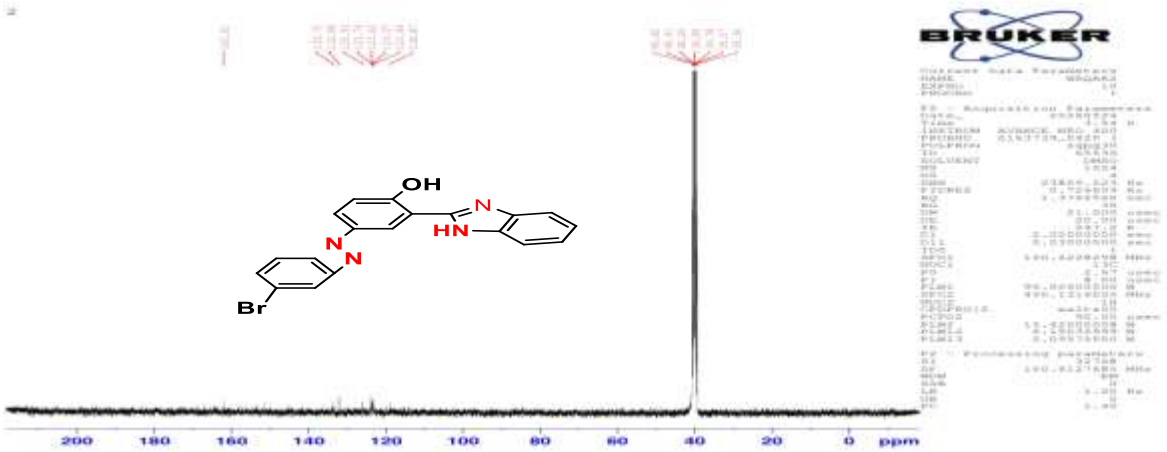


Figure 6. ^{13}C-MNR spectrum of compound[B_2]

3.3. Biological activity

3.3.1. Antioxidant Activity (DPPH Free Radical Scavenging Assay)

The antioxidant potential of the synthesized compounds [B1–B3], along with ascorbic acid as the standard reference, DPPH free radical scavenging activity was evaluated at different concentrations ranging from50 to 250 ppm. As shown in the table, all tested compounds exhibited a concentration-dependent increase in DPPH radical scavenging activity. Among the prepared derivatives, compound B2 showed the highest antioxidant activity in the investigated concentration range, reaching a maximum inhibition of 90.72% at 250 ppm. At the same concentration, B2 showed higher DPPH scavenging activity than the standard ascorbic acid, which exhibited 52.44% inhibition. In comparison, compounds B1 and B3 showed lower scavenging activities, with maximum inhibition values of 48.53% and 30.29%, respectively, at 250 ppm.

The relatively high antioxidant activity of B2 may be associated with the presence of phenolic hydroxyl (–OH) and N–H groups, which can contribute to free-radical stabilization through hydrogen atom or electron donation. In addition, the conjugated aromatic system and the electronic effects of the substituents may facilitate stabilization of the radical species formed during the scavenging process. The differences in antioxidant activity among B1–B3 may therefore be related to their structural and electronic characteristics, including the nature and position of the substituents, as demonstrated in Table 1 and Figure 7.

Table 1. Antioxidant activity for some of the synthesized compounds [B_1 -B_3]

	DPPH Scavenging (%)				
com	**50ppm**	**100ppm**	**150ppm**	**200ppm**	**250ppm**
B_1	29.8	36.81	38.11	42.51	48.53
B_2	56.84	68.08	82.41	87.3	90.72
B_3	13.19	17.26	20.36	28.18	30.29
Ascorbic acid	17.92	25.57	39.58	48.53	52.44

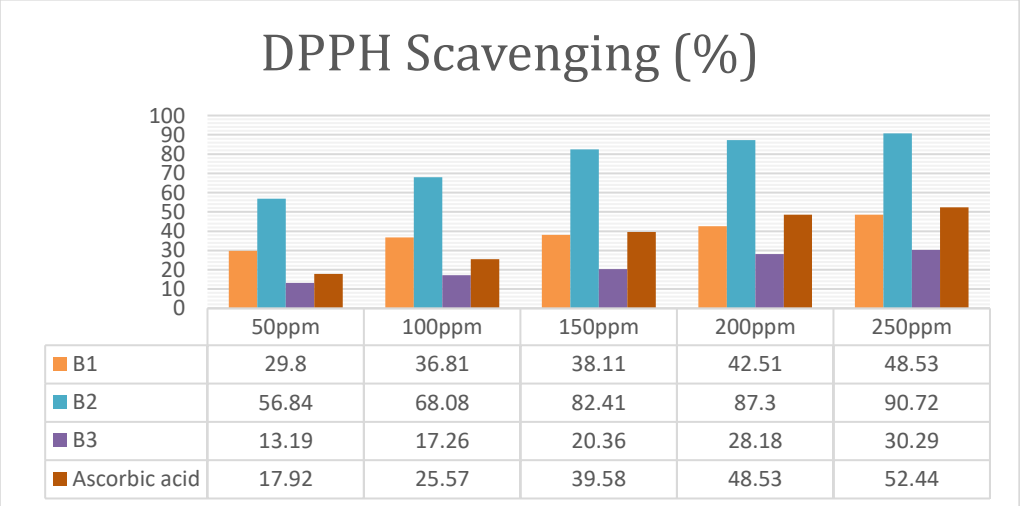


Figure 7. Antioxidant of compounds[B_1-B_3]

3.3.2. Antibacterial activity

The antibacterial activity of the synthesized compounds B1–B3 was evaluated against selected Gram-positive and Gram-negative bacteria using the inhibition zone method. As shown in Table 2 and Figures 8 and 9, the compounds exhibited different levels of antibacterial activity depending on the bacterial strain. B1 showed the highest inhibition zone against *Escherichia coli* (20 mm), while B2 showed the highest inhibition zone against *Staphylococcus aureus* (18 mm), followed by *Bacillus* (17 mm). B3 exhibited its highest inhibition zone against *S. aureus* (15 mm). Ciprofloxacin, used as the positive control, generally showed higher inhibition zones than the synthesized compounds, whereas DMSO showed no inhibition (0 mm) against all

tested bacteria. The results showed variable antibacterial activity of the synthesized compounds regarding the tested bacterial strains.

Table 2. Antibacterial activities of tested prepared compounds

	Antibacterial activity test(mm)				
Compound	**Bacillus**	**Pseudomonas**	**Staphylococcus aureus**	**E.coli**	**Klebsiella pneumoniae**
B_1	12	13	16	20	16
B_2	17	13	18	13	15
B_3	13	14	15	13	13
Ciprofloxacin	33	15	24	23	15
DMSO	0	0	0	0	0

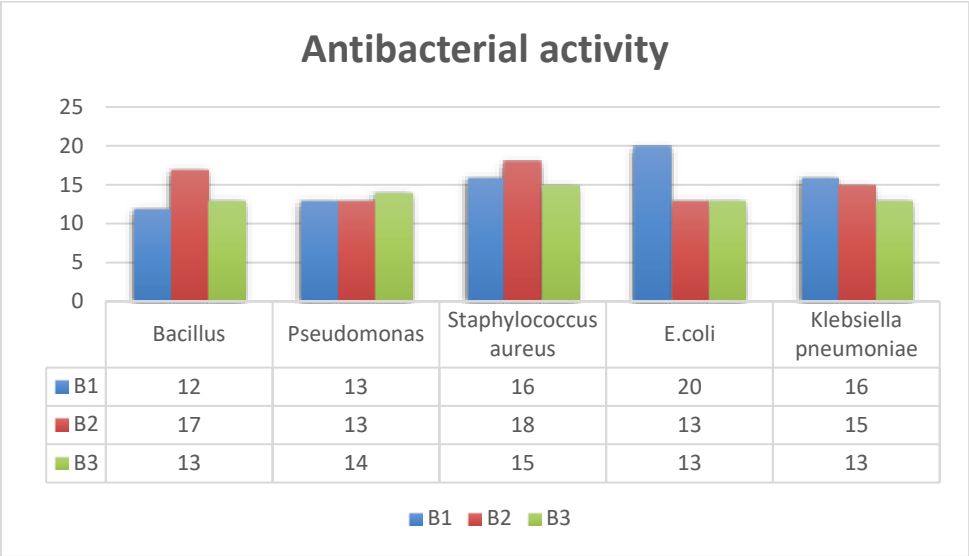


Figure 8. Antibacterial activity [B_1- B_3]

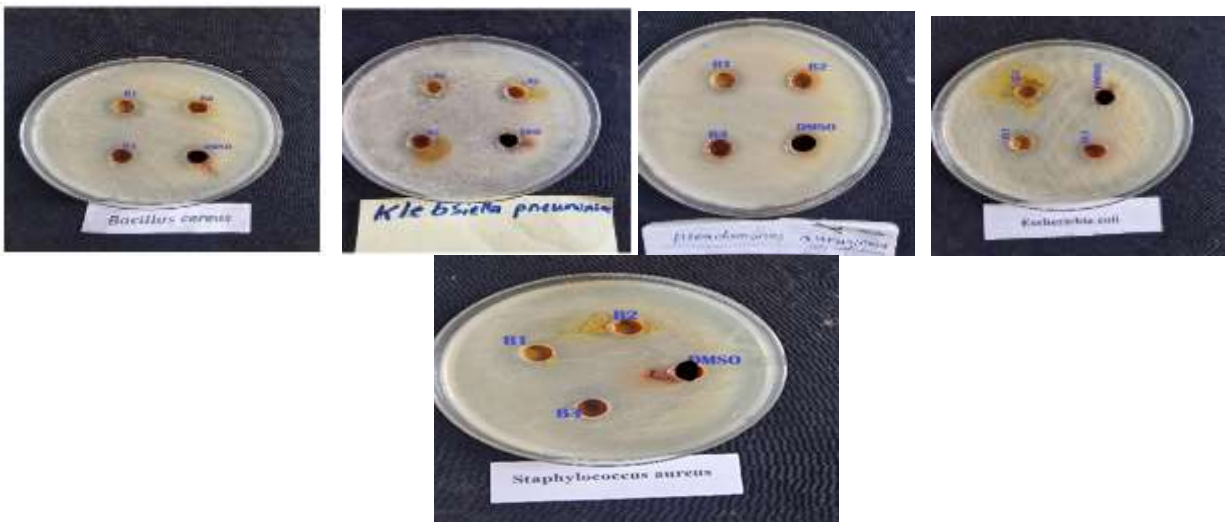


Figure 9. An antibacterial activity of compounds [B_1-B3]

4. Conclusion

In summary, a collection of novel benzimidazole derivatives [B1–B3] was successfully synthesized and characterized using FTIR, ^{1}H-NMR, and ^{13}C-NMR spectroscopic techniques. The biological evaluation revealed that the synthesized compounds showed antioxidant and antibacterial activities. Among the tested derivatives, B2 showed the highest DPPH radical scavenging activity, reaching 90.72% inhibition at 250 ppm, which was higher than that of the standard ascorbic acid (52.44%) at the same concentration. The antibacterial results also showed variable inhibitory activities against the tested bacterial strains, with B1 and B2 exhibiting notable activity against selected strains, while B3 showed pronounced inhibition against Staphylococcus aureus. Overall, these findings indicate that the novel benzimidazole derivatives possess promising biological activities and may serve as useful compounds for further biological and pharmacological investigations.

Conflict Of Interest

The authors assert that no conflicts of interest exist.Adherence to Ethical Standards: This article does not include any research involving human or animal subjects.

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