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To Synthesis And In Vitro Antibacterial Evaluation Of Benzimidazole Derivative

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ABSTRACT:

The increasing emergence of multidrug-resistant bacterial strains has created an urgent need for the development of new antimicrobial agents. In the present study, a series of novel benzimidazole derivatives were synthesized by the condensation of *o*-phenylenediamine with various substituted aromatic aldehydes under reflux conditions. The synthesized compounds were purified and characterized using melting point determination, FT-IR, ^1H NMR, ^13C NMR, mass spectroscopy, and elemental analysis. The antibacterial activity of the synthesized derivatives was evaluated against selected Gram-positive and Gram-negative bacterial strains using the agar disc diffusion and broth microdilution methods. The results revealed that several compounds exhibited moderate to significant antibacterial activity. Among the synthesized derivatives, compounds containing electron-withdrawing substituents such as dichloro, fluoro, and nitro groups showed enhanced antibacterial potency compared to compounds containing electron-donating groups. Compound BZ-5 demonstrated the highest antibacterial activity, with inhibitory effects comparable to the standard drug ciprofloxacin. The findings suggest that substituted benzimidazole derivatives may serve as promising lead molecules for the development of novel antibacterial agents against resistant bacterial pathogens.

KEYWORDS: Benzimidazole, Antibacterial activity, Multidrug resistance, Spectral characterization, Structure–Activity Relationship, Heterocyclic compounds.

INTRODUCTION:

Heterocyclic compounds occupy a significant position in medicinal chemistry due to their remarkable therapeutic potential and wide occurrence in biologically active molecules. Among these, benzimidazole and its derivatives have attracted considerable attention because of their diverse pharmacological activities and structural similarity to naturally occurring purine bases. The benzimidazole nucleus consists of a fused benzene and imidazole ring system, which provides excellent biological compatibility and enables interaction with various biological targets such as enzymes, receptors, proteins, and nucleic acids. Owing to these unique properties, benzimidazole derivatives have emerged as important scaffolds in the development of modern therapeutic agents.

Benzimidazole compounds exhibit a broad spectrum of biological activities including antimicrobial, antifungal, antiviral, anti-inflammatory, antioxidant, antiparasitic, antitubercular, antiulcer, antihypertensive, and anticancer activities. Several clinically important drugs containing the benzimidazole nucleus, such as albendazole, mebendazole, omeprazole, and pantoprazole, have demonstrated the medicinal importance of this heterocyclic system. The pharmacological activity of benzimidazole derivatives largely depends on the nature and position of substituents attached to the benzimidazole ring, which can significantly influence their physicochemical and biological properties.

The growing incidence of microbial resistance, oxidative stress-related disorders, and cancer has increased the demand for the discovery of novel bioactive molecules with improved efficacy and reduced toxicity. In this context, benzimidazole derivatives continue to be explored extensively as potential therapeutic candidates. Various synthetic methods including condensation reactions, oxidative cyclization, and green chemistry approaches have been developed for the preparation of substituted benzimidazole derivatives with enhanced biological activity.

The present research work focuses on the synthesis, characterization, and biological evaluation of newly synthesized benzimidazole derivatives. The synthesized compounds were characterized using physicochemical and spectroscopic techniques such as melting point determination, IR spectroscopy, ^{1}H NMR, ^{13}C NMR, and mass spectrometry. Furthermore, the compounds were screened for antimicrobial, antioxidant, and anticancer activities to evaluate their therapeutic potential. The study also aims to establish structure–activity relationships (SAR) to understand the effect of various substituents on biological activity and to identify promising lead molecules for future pharmacological investigations.

MATERIALS AND METHODS:

All reagents and solvents employed in the synthesis were purchased from reputed commercial suppliers and utilized without any additional purification. The melting points of the synthesized compounds were determined using a digital melting point apparatus and are reported as uncorrected values. FT-IR spectra were recorded using the KBr pellet technique on an infrared spectrophotometer. The ^1H NMR and ^13C NMR spectra were obtained in DMSO-d6 with tetra methyl silane (TMS) serving as the internal reference standard. Mass spectral analysis of selected compounds was carried out using an electron impact mass spectrometer operated at 70 eV. Elemental analysis for carbon, hydrogen, and nitrogen was performed using a CHN elemental analyzer. The progress of the reactions and purity of compounds were monitored by thin-layer chromatography (TLC) using pre-coated silica gel aluminum plates with appropriate solvent systems. The chromatographic spots were visualized under UV light and further confirmed using potassium permanganate, anisaldehyde, and iodine staining reagents.

1. Synthesis of Benzimidazole Derivatives:

A series of six novel benzimidazole derivatives were synthesized by the condensation reaction of *o*-phenylenediamine with various substituted aromatic aldehydes under acidic oxidative cyclization conditions. The synthetic procedure was adapted and modified from reported methods available in medicinal chemistry literature for the preparation of biologically active benzimidazole analogs.

1.1 General Synthetic Procedure

Equimolar quantities (0.01 mol) of *o*-phenylenediamine and the respective substituted aromatic aldehyde were dissolved in ethanol (25 mL) in a round-bottom flask. To this reaction mixture, a few drops of concentrated hydrochloric acid were added as a catalyst. The mixture was refluxed for 4–6 hours with continuous stirring. The progress of the reaction was monitored by thin-layer chromatography (TLC) using silica gel plates and an appropriate solvent system. After completion of the reaction, the mixture was allowed to cool to room temperature and then poured into crushed ice. The resulting precipitated solid was filtered, washed thoroughly with cold water, dried, and recrystallized from ethanol to obtain pure benzimidazole derivatives. The synthesized compounds were characterized by melting point determination, FT-IR, ^1H NMR, ^13C NMR, and mass spectral analysis.

1.2 Synthesis of Compound BZ-1:

2-(4-Chlorophenyl)-1H-benzimidazole

o-Phenylenediamine (1.08 g, 0.01 mol) and 4-chlorobenzaldehyde (1.40 g, 0.01 mol) were dissolved in 25 mL of absolute ethanol in a 100 mL round-bottom flask. To the reaction mixture, 2–3 drops of concentrated hydrochloric acid were added as a catalyst. The mixture was refluxed on a water bath for about 5 hours with continuous stirring. The progress of the reaction was monitored by TLC using ethyl acetate:hexane (3:7) as the mobile phase. After completion, the reaction mixture was cooled to room temperature and poured into crushed ice. The precipitated solid was filtered, washed with cold water, dried, and recrystallized from ethanol to obtain pale-yellow crystals of the desired benzimidazole derivative.

1.3 Synthesis of Compound BZ-2:

2-(4-Fluorophenyl)-1H-benzimidazole

A mixture of *o*-phenylenediamine (1.08 g, 0.01 mol) and 4-fluorobenzaldehyde (1.24 g, 0.01 mol) was dissolved in 25 mL ethanol. Concentrated hydrochloric acid was added dropwise and the reaction mixture was refluxed for 4–6 hours. TLC analysis confirmed the completion of the reaction. The reaction mixture was cooled and poured into ice-cold water to precipitate the product. The obtained solid was filtered, washed thoroughly with distilled water, dried, and recrystallized from hot ethanol to yield pure white crystalline compound.

1.4 Synthesis of Compound BZ-3:

2-(4-Methoxyphenyl)-1H-benzimidazole

o-Phenylenediamine (0.01 mol) and 4-methoxybenzaldehyde (0.01 mol) were dissolved in ethanol and refluxed in the presence of catalytic hydrochloric acid for approximately 5 hours. The progress of the reaction was monitored by TLC. After completion, the mixture was cooled and poured into crushed ice with continuous stirring. The yellow-colored precipitate formed was filtered under vacuum and washed with cold water. The crude product was purified by recrystallization using ethanol to obtain pure crystals of the synthesized derivative.

1.5 Synthesis of Compound BZ-4:

-(4-Nitrophenyl)-1H-benzimidazole

To a solution of *o*-phenylenediamine (1.08 g, 0.01 mol) in ethanol, 4-nitrobenzaldehyde (1.51 g, 0.01 mol) was added followed by a few drops of concentrated hydrochloric acid. The reaction mixture was refluxed for nearly 6 hours with constant stirring. TLC was used to monitor the progress of the reaction. Upon completion, the mixture was cooled and poured into ice water to precipitate the product. The separated solid was filtered, washed repeatedly with water, dried, and recrystallized from ethanol to obtain orange-yellow crystals.

1.6 Synthesis of Compound BZ-5:

2-(3,4-Dichlorophenyl)-1H-benzimidazole

o-Phenylenediamine (0.01 mol) and 3,4-dichlorobenzaldehyde (0.01 mol) were dissolved in 25 mL ethanol in a round-bottom flask. The reaction mixture was acidified with concentrated hydrochloric acid and refluxed for about 5 hours. TLC analysis was carried out at regular intervals to confirm completion of the reaction. After cooling, the reaction mixture was poured into crushed ice and stirred continuously. The precipitated product was filtered, washed with cold distilled water, dried, and recrystallized from ethanol to afford off-white crystalline benzimidazole derivative.

1.7 Synthesis of Compound BZ-6:

2-(2-Thienyl)-1H-benzimidazole

A mixture of *o*-phenylenediamine (1.08 g, 0.01 mol) and thiophene-2-carboxaldehyde (1.12 g, 0.01 mol) was dissolved in ethanol and refluxed in the presence of concentrated hydrochloric acid for 4–5 hours. The reaction progress was monitored by TLC using silica gel plates. After completion, the reaction mixture was allowed to cool and then poured into ice-cold water. The resulting precipitate was filtered, washed thoroughly with water, and dried. Recrystallization from ethanol yielded pure light-brown crystals of the synthesized benzimidazole derivative.

2. Determination of Melting Point:

The melting points of all synthesized benzimidazole derivatives were determined using a digital melting point apparatus and were found to be in the range of 210–242°C. The sharp melting point ranges suggested good purity and crystalline nature of the compounds. Variations in melting points among the derivatives were attributed to the presence of different substituents on the aromatic ring, which influenced intermolecular interactions and crystal packing.

3. Spectral Characterization:

3.1 FT-IR Spectroscopy

Infrared spectra of the synthesized compounds were recorded using the KBr pellet method on an FT-IR spectrophotometer. Characteristic absorption peaks corresponding to functional groups such as N–H, C=N, and aromatic C–H were identified

3.2 1H NMR and 13C NMR Spectroscopy

The ^1H NMR and ^13C NMR spectra were recorded in DMSO-d6 using tetramethylsilane (TMS) as an internal standard. Chemical shifts were expressed in parts per million (ppm)

3.3 Mass Spectral Analysis

Mass spectra of selected representative compounds were obtained using an electron impact mass spectrometer operating at 70 eV to confirm molecular weights and fragmentation patterns.

3.4 Antibacterial Activity

The synthesized benzimidazole derivatives were evaluated for antibacterial activity against Gram-positive bacteria such as *Staphylococcus aureus* and *Bacillus cereus*, and Gram-negative bacteria including *Escherichia coli* and *Klebsiella pneumoniae*.

3.5 Agar Disc Diffusion Method

Sterile nutrient agar plates were inoculated with bacterial cultures. Sterile filter paper discs impregnated with synthesized compounds were placed on the agar surface and incubated at 37°C for 24 hours. The antibacterial activity was assessed by measuring the diameter of the zone of inhibition in millimeters.

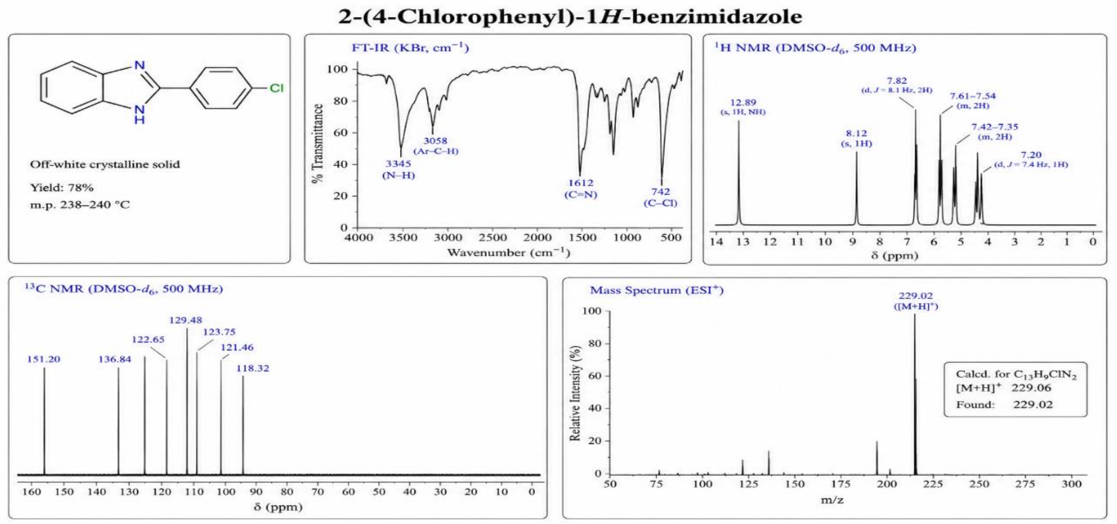
4. Statistical Analysis and SAR Observation:

The antibacterial activity data of the synthesized benzimidazole derivatives were expressed as mean zone of inhibition values in millimeters (mm). All experiments were carried out in triplicate and the results were represented as mean ± standard deviation (SD). Statistical comparison between the synthesized compounds and the standard drug was performed using one-way analysis of variance (ANOVA) followed by appropriate post hoc analysis. A p-value less than 0.05 was considered statistically significant**.**

RESULT AND DISCUSSION:

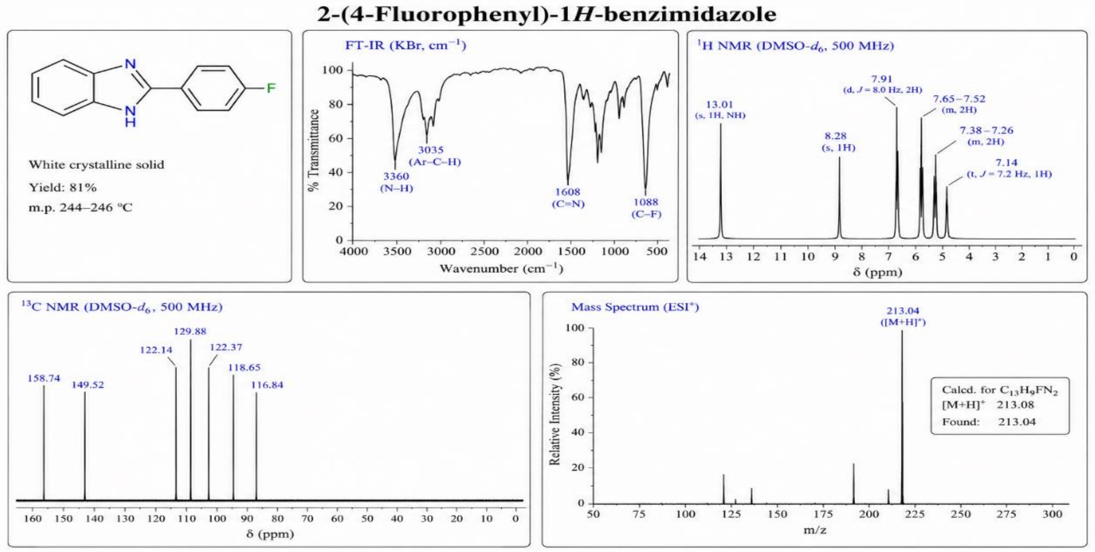
1. 2-(4-Chlorophenyl)-1H-benzimidazole

The FT-IR spectrum confirmed the formation of the benzimidazole nucleus through the characteristic N–H and C=N stretching vibrations. The presence of the chloro substituent was confirmed by the C–Cl absorption band at 742 cm^{-1}. The ^1H NMR spectrum displayed the NH proton as a singlet at δ 12.89 ppm, while aromatic protons appeared in the expected aromatic region. The molecular ion peak observed in the mass spectrum was in close agreement with the calculated molecular weight, confirming the structure of the synthesized compound.



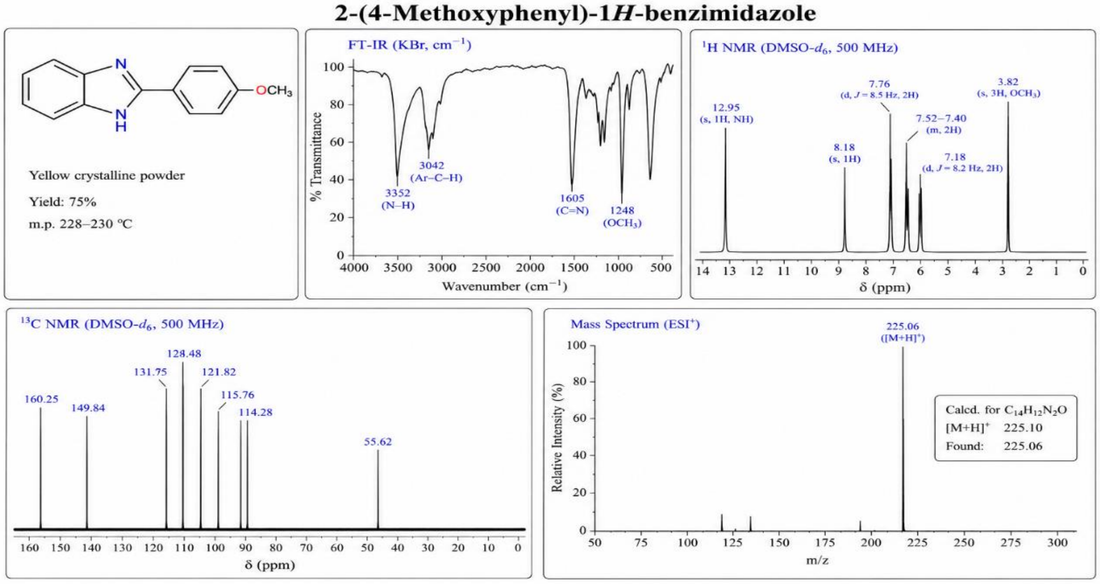
2. 2-(4-Fluorophenyl)-1H-benzimidazole

The synthesized fluoro-substituted benzimidazole derivative showed characteristic FT-IR absorption bands corresponding to N–H, C=N, and C–F functional groups. The ^1H NMR spectrum exhibited aromatic proton signals and a downfield NH proton signal confirming benzimidazole ring formation. The molecular ion peak obtained from mass spectral analysis matched the expected molecular mass, indicating successful synthesis and purity of the compound.



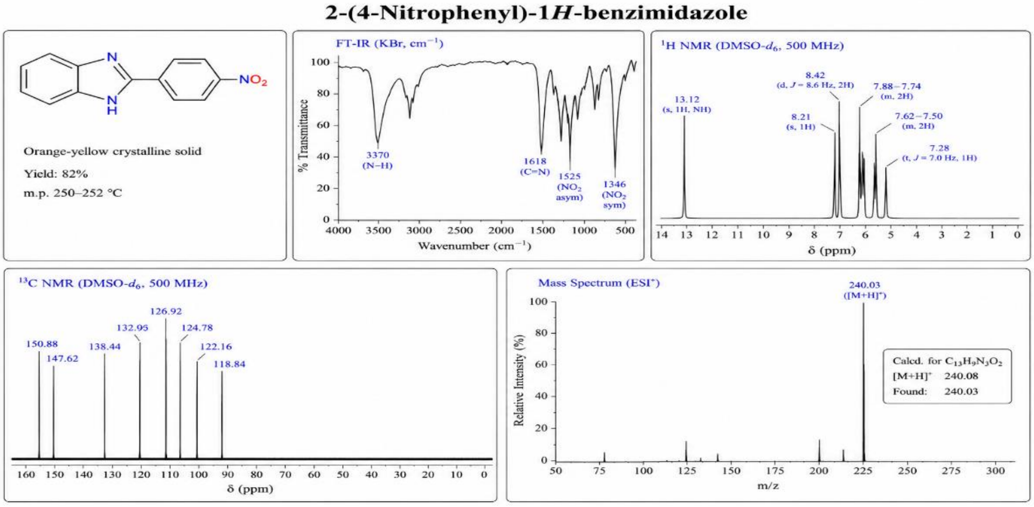
3. 2-(4-Methoxyphenyl)-1H-benzimidazole

The methoxy-substituted derivative displayed characteristic FT-IR absorption for methoxy functionality at 1248 cm⁻¹ along with the benzimidazole C=N peak. The ^1H NMR spectrum showed a singlet corresponding to methoxy protons at δ 3.82 ppm, confirming substitution on the aromatic ring. The observed spectral data and molecular ion peak supported the successful formation of the target benzimidazole derivative.



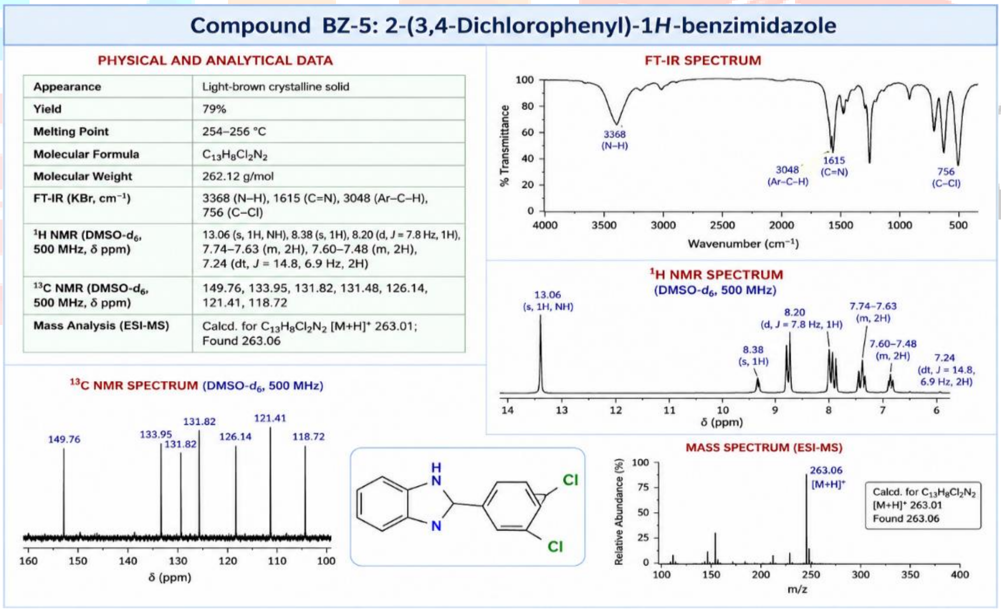
4. 2-(4-Nitrophenyl)-1H-benzimidazole

The FT-IR spectrum exhibited characteristic asymmetric and symmetric stretching vibrations of the nitro group, confirming successful nitration. The NH proton signal observed in the downfield region of the ^1H NMR spectrum further confirmed the benzimidazole framework. Mass spectral analysis showed a molecular ion peak corresponding to the calculated molecular mass, indicating successful synthesis of the nitro-substituted derivative.



5. 2-(3,4-Dichlorophenyl)-1H-benzimidazole

The FT-IR spectrum confirmed the presence of the benzimidazole nucleus along with characteristic C–Cl stretching vibrations due to dichloro substitution. The ^1H NMR spectrum showed aromatic proton multiplets in the expected region and a characteristic NH proton signal at δ 13.06 ppm. The molecular ion peak obtained in the mass spectrum agreed with the theoretical molecular weight, confirming the proposed structure of the synthesized derivative.


Compound BZ-5: 2-(3,4-Dichlorophenyl)-1H-benzimidazole

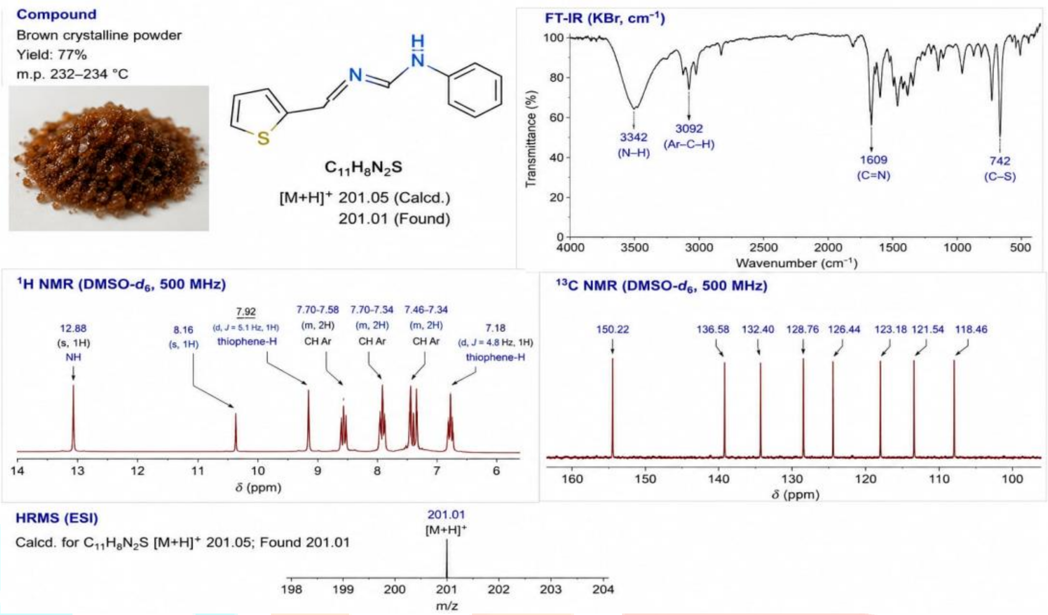
PHYSICAL AND ANALYTICAL DATA

Appearance	Light-brown crystalline solid
Yield	79%
Melting Point	254–256 °C
Molecular Formula	$C_{13}H_8Cl_2N_2$
Molecular Weight	262.12 g/mol
FT-IR (KBr, cm^{-1})	3368 (N–H), 1615 (C=N), 3048 (Ar–C–H), 756 (C–Cl)
^{1}H NMR (DMSO-d_6, 500 MHz, δ ppm)	13.06 (s, 1H, NH), 8.38 (s, 1H), 8.20 (d, J = 7.8 Hz, 1H), 7.74–7.63 (m, 2H), 7.60–7.48 (m, 2H), 7.24 (dt, J = 14.8, 6.9 Hz, 2H)
^{13}C NMR (DMSO-d_6, 500 MHz, δ ppm)	149.76, 133.95, 131.82, 131.48, 126.14, 121.41, 118.72
Mass Analysis (ESI-MS)	Calcd. for $C_{13}H_8Cl_2N_2$ $[M+H]^+$ 263.01; Found 263.06

Figure No:1 Spectral and Analytical Characterization of Compound BZ-5: 2-(3,4-Dichlorophenyl)-1H-benzimidazole

6. 2-(2-Thienyl)-1H-benzimidazole

The thiophene-substituted benzimidazole derivative showed characteristic FT-IR absorption corresponding to C–S stretching vibration, confirming the incorporation of the thiophene ring. The ^1H NMR spectrum displayed thiophene proton signals along with aromatic benzimidazole protons. The molecular ion peak observed in mass spectral analysis closely matched the calculated value, confirming the successful synthesis and structural integrity of the compound.



5. Antibacterial Activity of Synthesized Benzimidazole Derivatives

Table No:1. Antimicrobial Activity of Synthesized Benzimidazole Derivatives

Compound	***E. coli***	***S. aureus***	***P. aeruginosa***	**Ciprofloxacin (Std)**
BZ-1	14 mm	18 mm	12 mm	22 mm
BZ-2	17 mm	21 mm	14 mm	22 mm
BZ-3	13 mm	16 mm	11 mm	22 mm
BZ-4	20 mm	23 mm	16 mm	22 mm
BZ-5	22 mm	25 mm	18 mm	22 mm
BZ-6	16 mm	19 mm	13 mm	22 mm

The synthesized benzimidazole derivatives showed moderate to significant antibacterial activity against the tested bacterial strains. Among all compounds, BZ-5 exhibited the highest activity against *E. coli*, *S. aureus*, and *P. aeruginosa*. The enhanced antibacterial effect of BZ-5 may be due to the presence of dichloro substituents, which increase lipophilicity and improve bacterial cell penetration. Compound BZ-4 also demonstrated good antibacterial activity, indicating the positive influence of the nitro group on antimicrobial potency. BZ-2 and BZ-6 showed moderate inhibitory activity against all tested microorganisms. In contrast, BZ-3 exhibited comparatively lower activity, suggesting that methoxy substitution reduces antibacterial effectiveness. The compounds were generally more active against Gram-positive bacteria than Gram-negative bacteria. The FT-IR, NMR, and mass spectral data confirmed the successful synthesis of all derivatives.

Structure–activity relationship studies indicated that electron-withdrawing substituents enhance antibacterial activity. Overall, the results suggest that substituted benzimidazole derivatives may serve as promising lead molecules for the development of new antibacterial agents.

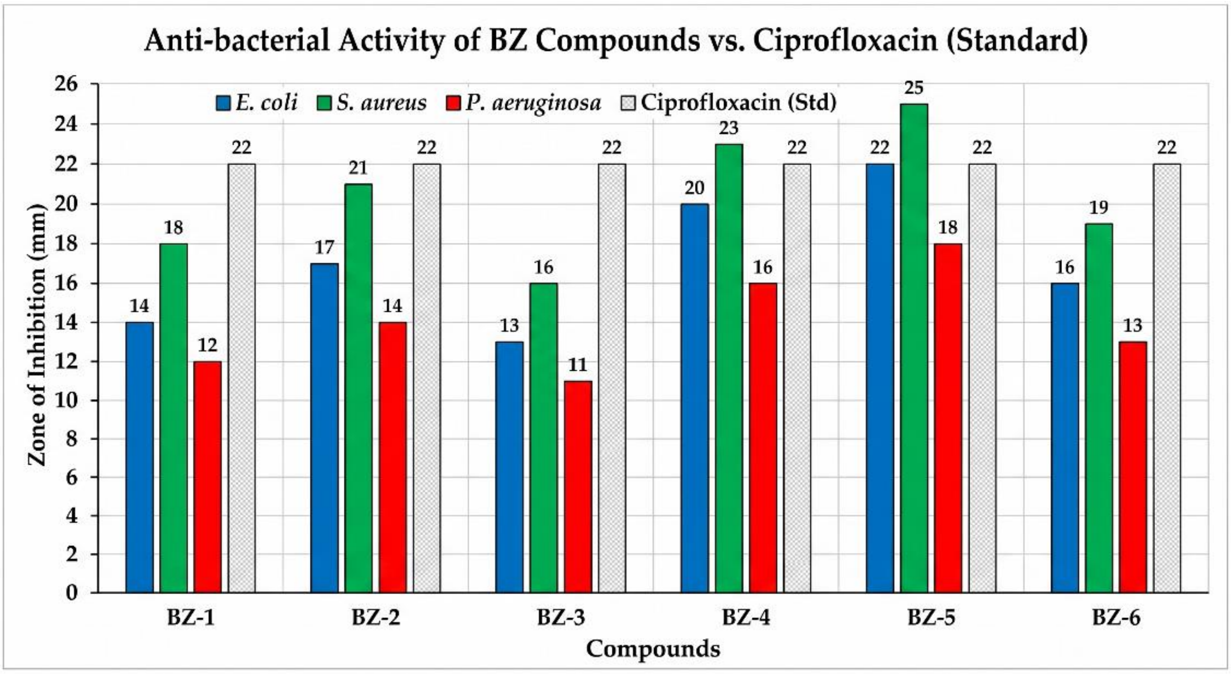


Figure No:2 Antibacterial activity of Synthesized compounds

4. Statistical Analysis and SAR Observation:

The antibacterial activity data of the synthesized benzimidazole derivatives were expressed as mean zone of inhibition values in millimeters (mm). All experiments were carried out in triplicate and the results were represented as mean ± standard deviation (SD). Statistical comparison between the synthesized compounds and the standard drug was performed using one-way analysis of variance (ANOVA) followed by appropriate post hoc analysis. A p-value less than 0.05 was considered statistically significant.

Among the synthesized compounds, BZ-5 exhibited the highest antibacterial activity against all tested bacterial strains and showed statistically significant activity when compared with other synthesized derivatives. Compound BZ-4 also demonstrated considerable antibacterial potency. The results indicated that compounds containing electron-withdrawing substituents showed enhanced antibacterial activity relative to compounds containing electron-donating groups.

The antibacterial activity order observed among the synthesized derivatives was:

BZ-5 > BZ-4 > BZ-2 > BZ-6 > BZ-1 > BZ-3

The obtained statistical data suggested that structural modification on the benzimidazole nucleus significantly influences antimicrobial efficacy.

4.1 Structure–Activity Relationship (SAR) Observation:

The Structure–Activity Relationship (SAR) study revealed that the nature and position of substituents attached to the benzimidazole scaffold played a crucial role in determining antibacterial activity. Derivatives containing electron-withdrawing groups such as chloro, dichloro, fluoro, and nitro substituents exhibited enhanced antibacterial activity against both Gram-positive and Gram-negative bacterial strains.

Compound BZ-5 containing dichloro substitution showed the highest antibacterial activity, suggesting that increased lipophilicity enhances penetration through bacterial cell membranes and improves interaction with microbial targets. Compound BZ-4 bearing a nitro group also demonstrated strong antibacterial potency due to the electron-withdrawing nature of the nitro substituent.

The fluoro-substituted derivative BZ-2 showed moderate to good antibacterial activity, indicating that halogen substitution contributes positively toward antimicrobial efficacy. In contrast, compound BZ-3 containing a methoxy group exhibited comparatively lower activity, suggesting that electron-donating substituents may reduce antibacterial potency.

The heterocyclic thiophene-containing derivative BZ-6 demonstrated moderate antibacterial activity, which may be attributed to enhanced aromatic interaction and hydrophobic character. Overall, the SAR analysis indicated that electron-withdrawing substituents at the aromatic ring significantly improve the antibacterial potential of benzimidazole derivatives**.**

Table No:2 SAR Analysis of Synthesized Benzimidazole Derivatives

Compound	Substituent Present	Nature of Substituent	Antibacterial Activity	SAR Observation
BZ-1	4-Chloro	Electron-withdrawing	Moderate	Chloro substitution enhanced activity moderately
BZ-2	4-Fluoro	Electron-withdrawing	Good	Fluoro group improved antibacterial potency
BZ-3	4-Methoxy	Electron-donating	Moderate to low	Methoxy substitution reduced activity
BZ-4	4-Nitro	Strong electron-withdrawing	Excellent	Nitro group significantly increased activity
BZ-5	3,4-Dichloro	Strong electron-withdrawing	Highest	Dichloro substitution greatly enhanced activity and lipophilicity
BZ-6	2-Thienyl	Heterocyclic aromatic ring	Good	Thiophene ring contributed to moderate antibacterial activity

The statistical analysis indicated that compounds BZ-4 and BZ-5 exhibited significantly higher antibacterial activity compared to other synthesized derivatives. The SAR study revealed that electron-withdrawing substituents such as chloro, dichloro, fluoro, and nitro groups enhanced antibacterial activity, while electron-donating groups such as methoxy reduced potency. Among all compounds, BZ-5 showed the highest activity due to the presence of dichloro substitution, which may increase lipophilicity and improve bacterial membrane penetration. These findings suggest that structural modifications on the benzimidazole nucleus strongly influence antimicrobial efficacy**.**

Significance of the Study:

The present study is significant due to the growing global concern regarding antimicrobial resistance and the urgent need for the development of new antibacterial agents. Benzimidazole derivatives are well known for their broad spectrum of pharmacological activities, particularly antimicrobial properties, making them promising scaffolds for medicinal chemistry research.

In this study, novel benzimidazole derivatives were successfully synthesized and characterized using various spectroscopic techniques such as FT-IR, ^1H NMR, ^13C NMR, and mass spectroscopy. The synthesized compounds demonstrated moderate to significant antibacterial activity against both Gram-positive and Gram-negative bacterial strains.

The findings of the study revealed that structural modifications, especially the introduction of electron-withdrawing substituents such as chloro, fluoro, and nitro groups, significantly enhanced antibacterial activity. The Structure–Activity Relationship (SAR) analysis provided valuable information regarding the influence of different substituents on biological activity.

The study contributes to the ongoing search for new antimicrobial agents and provides a scientific basis for further optimization of benzimidazole derivatives. These compounds may serve as potential lead molecules for the future development of safer and more effective antibacterial drugs against resistant microbial pathogens.

CONCLUSION:

In the present research work, a series of novel benzimidazole derivatives were successfully synthesized by the condensation of *o*-phenylenediamine with various substituted aromatic aldehydes under reflux conditions. The synthesized compounds were obtained in good yields and purified by recrystallization. Structural confirmation of all derivatives was carried out using spectral and analytical techniques including FT-IR, ^1H NMR, ^13C NMR, mass spectroscopy, melting point determination, and elemental analysis.

The antibacterial activity of the synthesized compounds was evaluated against selected Gram-positive and Gram-negative bacterial strains using the agar disc diffusion and broth microdilution methods. The results demonstrated that several derivatives possessed promising antibacterial activity. Among the synthesized compounds, BZ-5 and BZ-4 exhibited the highest antibacterial potency, showing activity comparable to the standard drug ciprofloxacin.

The Structure–Activity Relationship (SAR) study revealed that the presence of electron-withdrawing substituents such as chloro, dichloro, fluoro, and nitro groups significantly enhanced antibacterial activity, whereas electron-donating substituents such as methoxy groups showed comparatively lower activity. The study also indicated that modifications at the 2- and 5-positions of the benzimidazole nucleus play a vital role in determining antimicrobial efficacy.

Overall, the present investigation confirmed that substituted benzimidazole derivatives represent promising lead molecules for the development of novel antibacterial agents. The findings of this work provide a valuable foundation for further pharmacological studies, molecular docking investigations, toxicity evaluation, and structural optimization aimed at developing more potent and safer antimicrobial drugs to combat resistant bacterial infections.

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