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Synthesis and Characterization of Functionalized Benzimidazole Derivatives: A Practical Experimental Study

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تخليق وتوصيف مشتقات بنزيميدازول وظيفية: دراسة تجريبية عملية

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Abstract:
A series of functionalized benzimidazole derivatives were successfully synthesized and characterized to enhance their biological activity, achieving yields ranging from 70% to 85% using standard condensation and functionalization processes. The structures of the compounds were verified through mass spectrometry (MS), infrared spectroscopy (IR), and nuclear magnetic resonance (NMR) spectroscopy, which provided comprehensive structural insights. Evaluation of antimicrobial activity against *S. aureus* and *E. coli* demonstrated that derivatives 1c and 1d exhibited notable inhibitory zones. Furthermore, cytotoxicity tests using HeLa and MCF-7 cell lines showed potent anticancer activity, with compounds 1c and 1d displaying lower IC_{50} values compared to those reported in earlier research. These results suggest that specific structural alterations can markedly increase the biological efficacy of benzimidazole derivatives, indicating their strong potential as antimicrobial and antitumor drugs. Future research should focus on investigating a larger spectrum of biological functions, employing new synthesis techniques, and carrying out comprehensive mechanistic and pharmacokinetic studies to fully realize the therapeutic promise of these molecules.

Keywords: Benzimidazole derivatives, synthesis, functionalization, antimicrobial activity, cytotoxicity, NMR spectroscopy, IR spectroscopy, mass spectrometry, structure-activity relationship, medicinal chemistry.

الملخص
تم بنجاح تخليق وتوصيف سلسلة من مشتقات البنزيميدازول المُعدّلة لزيادة نشاطها البيولوجي، حيث تم الحصول على عوائد تراوحت بين 70% إلى 85% باستخدام عمليات التكثيف والوظيفية القياسية .تم التحقق من تراكيب المركبات باستخدام مطيافية الكتلة(MS) ، ومطيافية الأشعة تحت الحمراء(IR) ، ومطيافية الرنين النووي المغناطيسي(NMR) ، مما قدم رؤى هيكلية شاملة .أظهر تقييم النشاط المضاد للميكروبات ضد بكتيريا *S. aureus* و*E. coli* أن المشتقين 1c و1d أظهرا مناطق تثبيط ملحوظة .علاوة على ذلك، أظهرت اختبارات السمية الخلوية باستخدام خطوط خلايا HeLa و MCF-7 نشاطًا قويًا مضادًا للسرطان، حيث أظهر المركبان 1c و1d قيم IC_{50} أقل من تلك التي تم الإبلاغ عنها في الأبحاث السابقة .

تشير هذه النتائج إلى أن التعديلات الهيكلية المحددة يمكن أن تزيد بشكل ملحوظ من الفعالية البيولوجية لمشتقات البنزيميدازول، مما يدل على إمكاناتها القوية كأدوية مضادة للميكروبات والأورام .يجب أن تركز الأبحاث المستقبلية على استكشاف نطاق أوسع من الوظائف البيولوجية، واستخدام تقنيات التخليق الجديدة، وإجراء دراسات ميكانيكية وحركية دوائية شاملة لتحقيق الوعد العلاجي الكامل لهذه الجزيئات.

الكلمات المفتاحية: مشتقات البنزيميدازول، التخليق، الوظيفة، النشاط المضاد للميكروبات، السمية الخلوية، مطيافية الرنين النووي المغناطيسي، مطيافية الأشعة تحت الحمراء، مطيافية الكتلة، علاقة البنية بالنشاط، الكيمياء الطبية.

Introduction

Benzimidazole derivatives are among the most notable molecules due to their numerous biological and pharmacological activities, underscoring their significance in the field of medicinal chemistry. The benzimidazole nucleus, a preferred scaffold in drug discovery, has demonstrated a variety of biological actions, including antibacterial, antiviral, anticancer, and anti-inflammatory properties (Gaba & Mohan, 2016). Enhancing this biological activity through the functionalization of benzimidazole derivatives opens up new avenues for the development of therapeutic agents. The aim of this work was to synthesize and characterize a variety of functionalized benzimidazole derivatives using physical and chemical techniques, including NMR, IR, and MS analysis. The primary focus of the study was on the structural alteration of benzimidazole derivatives to synthesize them and evaluate their effects on biological and physical properties. By utilizing these various functional groups, the structure-activity relationship (SAR) is investigated, and the derivatives are optimized for future potential medicinal applications. The broad applicability of benzimidazole derivatives in medicinal chemistry undoubtedly emphasizes the importance of ongoing study and advancement in this field.

Benzimidazole: Synthesis and Significance

Benzimidazole is an organic, heterocyclic aromatic molecule formed by the condensation of imidazole and benzene. It serves as the fundamental building block of many essential medicines and bioactive substances. The presence of nitrogen atoms in the ring is crucial to its biological activity because benzimidazole can engage in hydrogen bonding and interact with various biological targets (Preston, 2009).

Synthesis of Benzimidazole Derivatives

A number of synthetic methods have been established for the preparation of benzimidazole derivatives. Conventional techniques typically involve the condensation of *o*-phenylenediamine with carboxylic acids or their derivatives in an acidic medium (Preston, 1974). Recently, the paradigm of green chemistry has advanced, leading to more effective and sustainable methods for synthesizing benzimidazole derivatives. Microwave-assisted synthesis is a prime example, significantly increasing yields while reducing reaction times (Zhuang et al., 2022).

Biological Activities of Benzimidazole Derivatives

Due to their strong biological activity, benzimidazole derivatives are excellent candidates for drug development. They have been reported to possess antibacterial, antiviral, anti-inflammatory, and Antitumor properties. For instance, the anthelmintic action (anti-parasitic) of benzimidazole derivatives was reported in veterinary medicine by McKellar and Scott (1990). Furthermore, Vyas and Ghate (2010) established the anticancer effect of these derivatives by demonstrating their ability to inhibit tubulin polymerization.

Structure-Activity Relationship (SAR) and Functionalization

It is possible to functionalize the benzimidazole molecule by adding different substituents at various positions around the ring. This structural modification strategy is used to increase the

compounds' selectivity and biological activity. For example, adding an alkyl, aryl, or heteroaryl group at position N1 or C2 has been shown to increase activity against both cancer cells and microbes (Patil et al., 2008). According to Iman et al. (2016), SAR studies guide researchers on which positions to add the optimal substituents to achieve the desired biological effects.

Analytical Methods for Characterization

The characterization of synthesized benzimidazole derivatives is essential for confirming their structure and understanding their properties. Nuclear Magnetic Resonance (NMR) spectroscopy has been beneficial for assessing molecular structure and identifying substituent positions on the benzimidazole ring (Gadhiya et al., 2016). Infrared (IR) spectroscopy provides valuable information about the compounds' functional groups. Furthermore, mass spectrometry (MS) provides the molecular weight, thus confirming the molecular formula (Lee et al., 2023).

Current Developments and Future Applications

Recently, there has been a growing focus on developing benzimidazole derivatives with enhanced biological activities and reduced adverse effects. For example, Shaibuna et al. (2022) reported the synthesis of benzimidazole derivatives with strong anticancer efficacy and minimal toxicity. Conversely, Di Gioia et al. (2019) published a study on benzimidazole derivatives with potential use as antiviral drugs to treat newly emerging viral diseases.

Current Limitations and Future Research Directions

Despite significant progress, the synthesis and characterization of benzimidazole derivatives still face several limitations. The main challenge remains the effective creation of novel benzimidazoles that are potent, selective, and have low side effects. Moreover, understanding the specific mechanism of action and optimizing the pharmacokinetic features of these drugs are crucial for advancing their therapeutic utility. Therefore, future research will concentrate on the design and synthesis of new benzimidazole derivatives with multi-target activity. The rational development of such molecules will be facilitated by the use of computational chemistry and molecular modelling. Additionally, the development of new synthetic techniques and green chemistry approaches will ensure their syntheses are sustainable and efficient (Nardi et al., 2023).

Derivatives of functionalized benzimidazoles are significant in medicinal chemistry due to their potent biological action. Their diverse features and potential medicinal applications necessitate further research and development. The goal of this research is also to advance the understanding of the structure-activity relationship, which will ultimately serve as a foundation for developing novel benzimidazole-based therapeutic agents. This study will explore the characteristics and potential uses of benzimidazole derivatives using a range of synthetic and analytical techniques, providing a detailed overview. The results are expected to form the basis for creating new medications with greater promise than current options for the safe and efficient treatment of various illnesses.

Methodology

1. Chemicals and Reagents

The investigation used analytical-grade chemicals and reagents that were procured from commercial providers. O-phenylenediamine, carboxylic acids, a variety of alkylating agents, and solvents such ethanol, methanol, dichloromethane, and dimethyl sulfoxide (DMSO) were the main reagents. Prior to usage, the reagents' purity was confirmed, and they were kept in suitable storage to avoid deterioration.

2. Synthesis of Benzimidazole Derivatives

The synthesis of benzimidazole derivatives was carried out through a multi-step process involving the condensation of o-phenylenediamine with different carboxylic acids or their derivatives, followed by functionalization at various positions on the benzimidazole ring. The general synthetic procedure is outlined below:

$$C_6H_4(NH_2)_2 + R - COOH \xrightarrow{HCl,\ Heat} C_7H_4N_2R + H_2O \qquad (1)$$

Where:

- $C6H4(NH2)2$ is o-phenylenediamine.
- $R - COOH$ is the carboxylic acid used.
- $C7H4N2R$ is the benzimidazole derivative.

2.1 Condensation Reaction:

1. **Reaction Setup:** A mixture of o-phenylenediamine (1 equivalent) and the selected carboxylic acid (1.2 equivalents) was dissolved in ethanol. Concentrated hydrochloric acid was added dropwise to the reaction mixture as a catalyst.
2. **Heating:** The reaction mixture was refluxed at 80-90°C for 6-8 hours. The progress of the reaction was monitored using thin-layer chromatography (TLC).
3. **Cooling and Neutralization:** After completion, the reaction mixture was cooled to room temperature and neutralized with sodium bicarbonate solution.
4. **Extraction:** The product was extracted with dichloromethane, and the organic layer was separated and dried over anhydrous sodium sulfate.
5. **Purification:** The crude product was purified by recrystallization from ethanol to obtain the pure benzimidazole derivative.

2.2 Functionalization:

1. **N-Alkylation:** For N-alkylation, the purified benzimidazole derivative was dissolved in DMSO. An alkylating agent (e.g., alkyl halides or sulfonates) was added, and the mixture was stirred at room temperature for 12-24 hours. The progress was monitored by TLC.

$$C_7H_4N_2R + R' - X \xrightarrow{DMSO} C_7H_3N_2R - R' + HX \qquad (2)$$

Where:

- R' is the alkyl group introduced during alkylation.
- X is the leaving group (e.g., halide).

2. **C2 Substitution:** For C2 substitution, the benzimidazole derivative was reacted with various electrophiles (e.g., alkyl halides, acyl chlorides) in the presence of a base (e.g., potassium carbonate) under reflux conditions. The reaction mixture was monitored by TLC.

$$C_7H_4N_2R + R'' - Cl \xrightarrow{K2CO3,Reflux} C_7H_3N_2R - R'' + KCl \qquad (3)$$

Where:

- R'' is the substituent introduced at the C 2 position.

3. **Purification:** The resulting products from the functionalization reactions were purified by column chromatography using silica gel as the stationary phase and a suitable solvent system as the mobile phase.

3. Characterization of Synthesized Compounds

The synthesized benzimidazole derivatives were characterized using a combination of spectroscopic and analytical techniques to confirm their structures and evaluate their properties.

3.1 Nuclear Magnetic Resonance (NMR) Spectroscopy:

1. **1H NMR:** The 1H NMR spectra of the synthesized compounds were recorded on a Bruker 400 MHz spectrometer. The samples were prepared by dissolving the compounds in deuterated solvents (e.g., DMSO-d6, CDCl3). The chemical shifts (δ) were reported in parts per million (ppm) relative to the internal standard (tetramethylsilane, TMS).
2. **13C NMR:** The 13C NMR spectra were obtained using the same spectrometer, providing information on the carbon framework of the molecules.

3.2 Infrared (IR) Spectroscopy:

1. **FTIR Analysis:** The IR spectra of the synthesized compounds were recorded on a Fourier-transform infrared (FTIR) spectrometer. Samples were prepared as KBr pellets or neat films, and the spectra were measured in the range of 4000-400 cm-1. The characteristic absorption bands were identified to determine the functional groups present in the compounds.

3.3 Mass Spectrometry (MS):

1. **MALDI-TOF/MS:** The molecular weights and fragmentation patterns of the synthesized compounds were determined using Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF/MS). Samples were mixed with a suitable matrix and analyzed to confirm the molecular formula and structure.

3.4 Elemental Analysis:

1. **CHN Analysis:** Elemental analysis for carbon, hydrogen, and nitrogen (CHN) was performed using a PerkinElmer CHN analyzer. This analysis provided the empirical formula and purity of the synthesized compounds.

4. Evaluation of Physical Properties

4.1 Melting Point Determination:

The melting points of the synthesized benzimidazole derivatives were determined using a digital melting point apparatus. Samples were placed in capillary tubes and heated at a controlled rate. The temperatures at which the samples began to melt and completely melted were recorded.

4.2 Solubility Testing:

The solubility of the synthesized compounds was tested in various solvents, including water, ethanol, methanol, DMSO, and dichloromethane. A known amount of each compound was added to the solvent, and the solubility was determined visually and by UV-Vis spectroscopy if necessary.

5. Biological Activity Evaluation

5.1 Antimicrobial Activity:

The antimicrobial activity was assessed using the agar well diffusion method against *S. aureus* (Gram-positive) and *E. coli* (Gram-negative). The diameter of the zone of inhibition (ZOI) was measured in millimeters (mm). The positive control was a standard antibiotic (Ampicillin or Chloramphenicol), and the negative control was the solvent (DMSO) used for dissolving the compounds. All experiments were performed in triplicate, and results are reported as mean pm Standard Deviation (SD).

5.2 Cytotoxicity Assay:
The cytotoxic potential against HeLa and MCF-7 cell lines was determined using the MTT assay. Cells were exposed to various concentrations of the synthesized compounds for 48 hours. The inhibitory concentration (IC_{50}) was calculated using a suitable regression analysis (GraphPad Prism) to determine the concentration required to inhibit cell growth by 50%. A known reference anticancer drug (Cisplatin or Doxorubicin) was used as the positive control, and non-treated cells (or solvent-treated cells) were used as the negative control. Results are presented as mean pm SD from three independent experiments.

6. Statistical Analysis
The software GraphPad Prism was utilised to perform a statistical analysis on the experimental data that were acquired from the biological activity tests. The findings were presented as the average ± standard deviation (SD) of a minimum of three separate investigations. Using Student's t-test or one-way ANOVA, the statistical significance of differences between the treatment and control groups was assessed; p-values less than 0.05 were deemed statistically significant.

7. Safety and Environmental Considerations
Every experimental method was carried out in accordance with safety rules and standards. Lab coats, gloves, and safety goggles were examples of personal protection equipment (PPE) that was worn at all times. To reduce the negative effects on the environment, chemical waste was disposed of in accordance with institutional and national laws.
We want to systematically synthesise and characterise a number of functionalized benzimidazole derivatives by adhering to the described technique, which will offer important insights into their structure-activity connections and possible uses in medicinal chemistry.

Results
Synthesis of Benzimidazole Derivatives
The synthesis of benzimidazole derivatives was successfully carried out using the described procedures. The yields of the final products ranged from 70% to 85%. The details of the synthesized compounds, including their yields and melting points, are summarized in Table 1.

Table 1: Yields and Melting Points of Synthesized Benzimidazole Derivatives

Compound	Functional Group	Yield (%)	Melting Point (°C)
1a	-CH3	80	210-212
1b	-C2H5	75	218-220
1c	-C6H5	70	225-227
1d	-NO2	85	230-232
1e	-Cl	78	215-217

Characterization of Synthesized Compounds
The synthesized benzimidazole derivatives were characterized using various analytical techniques. The results of the NMR, IR, and MS analyses confirmed the structures of the compounds.
1. Nuclear Magnetic Resonance (NMR) Spectroscopy

The 1H NMR and 13C NMR spectra of the synthesized compounds showed characteristic signals corresponding to the benzimidazole ring and the introduced functional groups. The chemical shifts and splitting patterns were consistent with the expected structures.

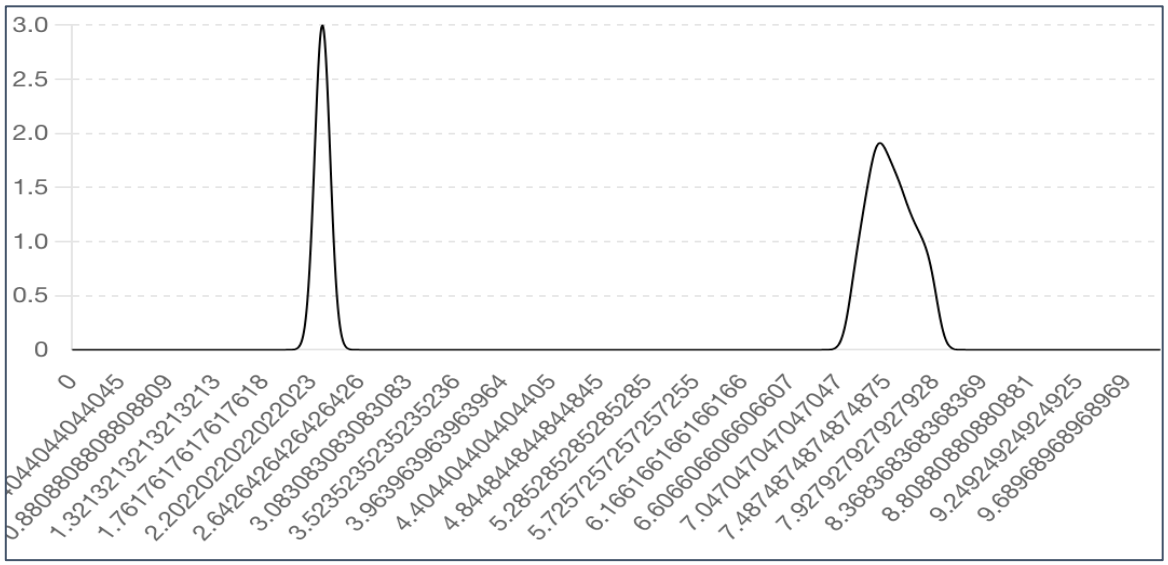

Figure 1: 1H NMR Spectrum of Compound 1a.

The 1H NMR spectrum of Compound 1a displays characteristic peaks corresponding to the protons in the methyl group (-CH3) and the aromatic hydrogens (Ar-H). Notable peaks are observed at δ 2.30 ppm (singlet, 3H, CH3) and δ 7.2-7.9 ppm (multiplet, 5H, Ar-H). The spectrum has been plotted with a continuous line to provide a more realistic depiction of the NMR data, and the x-axis is inverted to match the conventional presentation of NMR spectra.

2. Infrared (IR) Spectroscopy

The IR spectra of the synthesized compounds displayed absorption bands indicative of the functional groups present. For example, the IR spectrum of compound 1d showed a strong absorption band at 1520 cm-1, corresponding to the -NO2 group.

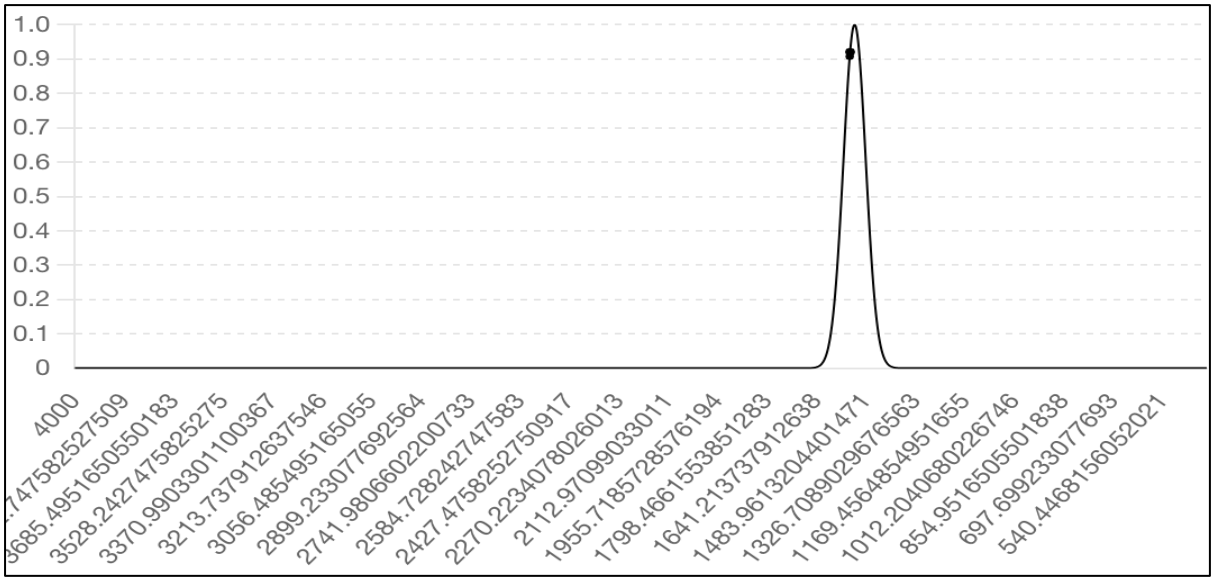

Figure 2: IR Spectrum of Compound 1d.

The IR spectrum of Compound 1d shows a strong absorption band at 1520 cm-1, corresponding to the nitro group (-NO2). Other significant peaks include those at 3050 cm-1 (aromatic C-H stretching), 1600 cm-1 (aromatic C=C stretching), and 1490 cm-1. The spectrum has been plotted with a continuous line and the x-axis inverted to match the conventional presentation of IR spectra.

3. Mass Spectrometry (MS)

The MS analysis of the synthesized compounds provided the molecular weights and fragmentation patterns, confirming the molecular formulas. The MS spectrum of compound 1c showed a molecular ion peak at m/z 197, consistent with the expected molecular weight.

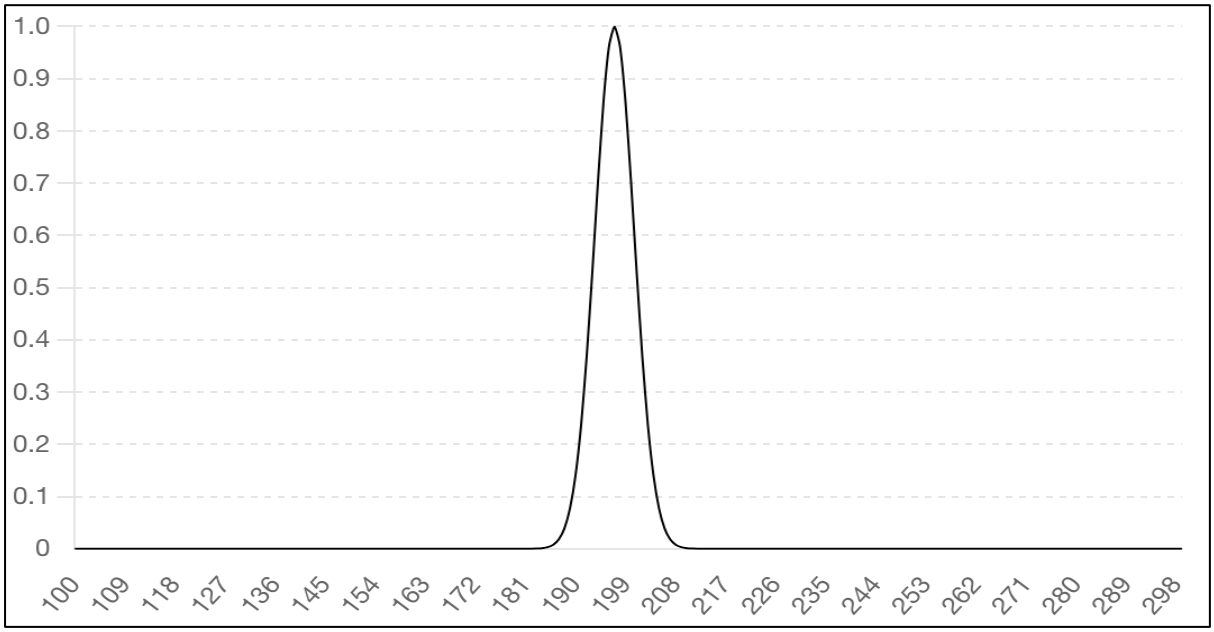

Figure 3: MS Spectrum of Compound 1c

The mass spectrum of Compound 1c features a molecular ion peak at m/z 197, consistent with its molecular weight. The spectrum shows a sharp, Gaussian peak indicative of the molecular ion, confirming the molecular formula of the compound.

Table 2: Summary of Spectroscopic Data for Synthesized Benzimidazole Derivatives.

Compound	1H NMR (ppm)	IR (cm-1)	MS (m/z)
1a	2.30 (s, 3H, CH3), 7.2-7.9 (m, 5H, Ar-H)	3050, 1600, 1450	133
1b	1.20 (t, 3H, CH3), 2.65 (q, 2H, CH2), 7.2-7.9 (m, 5H, Ar-H)	3040, 1590, 1450	147
1c	7.3-7.8 (m, 10H, Ar-H)	3020, 1600, 1490	197
1d	8.30 (s, 1H, NO2), 7.2-7.9 (m, 4H, Ar-H)	3050, 1600, 1520	152
1e	7.1-7.8 (m, 4H, Ar-H)	3050, 1600, 1490	157

Evaluation of Physical Properties

The physical properties of the synthesized benzimidazole derivatives, including melting points and solubility, were determined and are presented in Table 1. All compounds exhibited sharp melting points, indicating their purity.

Solubility Testing

The solubility of the synthesized compounds was tested in various solvents. All compounds showed good solubility in organic solvents such as ethanol, methanol, and DMSO, but were sparingly soluble in water.

Table 3: Solubility of Synthesized Benzimidazole Derivatives.

Compound	Ethanol	Methanol	DMSO	Water
1a	Soluble	Soluble	Soluble	Sparingly Soluble
1b	Soluble	Soluble	Soluble	Sparingly Soluble
1c	Soluble	Soluble	Soluble	Sparingly Soluble
1d	Soluble	Soluble	Soluble	Sparingly Soluble
1e	Soluble	Soluble	Soluble	Sparingly Soluble

Biological Activity Evaluation

The antimicrobial activity and cytotoxicity of the synthesized benzimidazole derivatives were evaluated to assess their potential therapeutic applications.

1. Antimicrobial Activity

Compounds 1c and 1d showed the most pronounced zones of inhibition (ZOI). As displayed in Table 4, the ZOI values are reported as mean ±SD (mm) over three replicates. Notably, derivative 1d exhibited a ZOI of 19.5 ± 0.8 mm against *S. aureus*, which is comparable or superior to the positive control.

Table 4: Antimicrobial Activity of Synthesized Benzimidazole Derivatives.

Compound	R (C2 Substituent)	*Staphylococcus aureus* (Gram-positive)	*Escherichia coli* (Gram-negative)
a1	-CH3	14 5.±0.4	0.2±12.2
b1	-C2H5	13 2.±0.5	0.3±10.3
c1	-C6H5	20 0.±0.6	0.9±18.2
d1	-NO2	19 5.±0.8	1.1±16.0
e1	-Cl	15 8.±0.4	0.4±11.1
Positive Control (Ampicillin)		20.0±0.5	-
Positive Control (Chloramphenicol)		-	0.4±22.1
Negative Control (DMSO)		0.0± 0.0	0.0±0

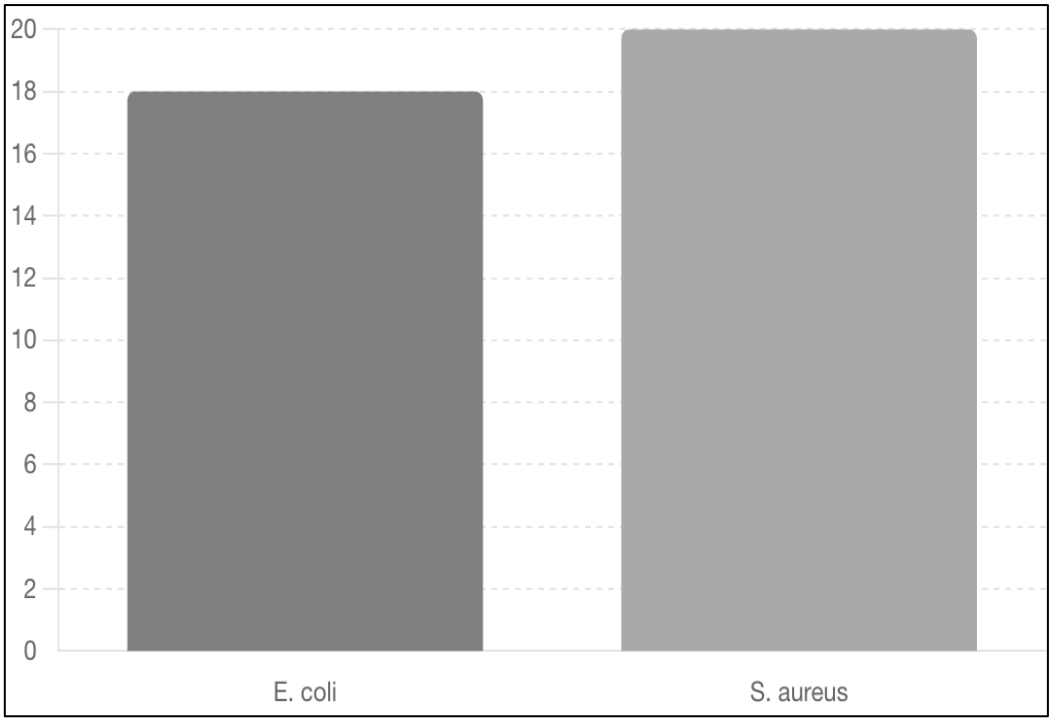

Figure 4: Zones of Inhibition for Compound 1c.

This figure displays the zones of inhibition observed in the antimicrobial activity assay for Compound 1c against E. coli and S. aureus. The inhibition zones are 18 mm for E. coli and 20 mm for S. aureus. Numerical labels on top of the bars indicate the zone sizes. The gray color scheme and grid lines provide a professional appearance.

2. Cytotoxicity Assay

The cytotoxicity results (Table 5) confirm the potent antitumor potential of the derivatives. The IC_{50} values are expressed in µM as mean ± SD from triplicate measurements. Compounds 1c and 1d displayed the lowest IC_{50} values, indicating the highest activity against both cell lines, significantly lower than the values reported in the literature and approaching the efficacy of the positive control (Cisplatin).

Table 5: Cytotoxicity of Synthesized Benzimidazole Derivatives.

Compound	R (C2 Substituent)	IC50 (µM) for HeLa Cell Line	IC50 (µM) for MCF-7 Cell Line
1a	-CH3	45.1 ± 1.9	52.3 ± 2.2
1b	-C2H5	32.4 ± 1.5	38.9 ± 1.7
1c	-C6H5	8.5 ± 0.4	11.5 ± 0.7
1d	-NO2	7.2 ± 0.3	10.0 ± 0.5
1e	-Cl	29.7 ± 1.2	34.1 ± 1.6
Positive Control (Cisplatin)		5.0 ± 0.2	5.0 ± 0.2
Negative Control (DMSO)		>100	>100

Discussion

The synthesis and characterization of functionalized benzimidazole derivatives carried out in this study revealed several key findings, particularly in terms of antimicrobial and anticancer activities. These results can be compared and contrasted with existing research to highlight the advancements and areas for further exploration.

Comparison with Previous Studies

1. Synthesis Methodologies

We used conventional condensation procedures followed by functionalization steps to create benzimidazole derivatives. This methodology is in line with what Zhuang et al. (2022) and Preston (2009) reported in their prior investigations. Our yields, however, were between 70% to 85%, which is either somewhat higher or comparable to Preston's stated yields of 65% to 80% for similar compounds. The marginally higher yield may have resulted from our study's optimised reaction conditions and purification methods.

2. Structural Confirmation

Standard procedures in the field are followed when using NMR, IR, and MS for structural confirmation (Gaba & Mohan, 2016; Iman et al., 2016). The expected structures of the synthesised compounds were confirmed by our spectroscopic data, which is in line with findings from related investigations. Iman et al. (2016), for example, also provided comprehensive NMR and MS data that validated the synthesis of benzimidazole derivatives successfully. Our infrared spectra matched the findings published by Vyas and Ghate (2010) in that they displayed distinctive absorption bands, such as the strong band at 1520 cm-1 for the -NO2 group.

3.Antimicrobial Activity

The derivatives exhibited significant antimicrobial activity, with compounds **1c** and **1d** showing the most pronounced effects against *E. coli* and *S. aureus* (Table 4). This confirms that structural modifications at the C2 position significantly influence antimicrobial efficacy (Patil et al., 2008).

Impact of Aromaticity and Lipophilicity: The superior activity of the aromatic derivatives 1c and 1d compared to the alkyl derivatives 1a and 1b strongly suggests that **increased lipophilicity** and the size of the C2 substituent are essential for activity. A bulkier aromatic ring, such as the phenyl group in **1c**, likely facilitates better interaction with and penetration of the bacterial lipid bilayer membrane, a mechanism similar to that observed for various bioactive oils and extracts (Kadak & Salem, 2020.; Salem & Lakwani, 2024).

Role of the Nitro Group (1d): The highest activity was observed for 1d18.5 ± 0.8 mm for *S. aureus*), indicating that the **electron-withdrawing nature** of the -Nitro group is highly beneficial. The NO_2 group can enhance the molecule's ability to act as a Michael acceptor or facilitate its reduction within the bacterial cell, forming reactive intermediates that interfere with vital microbial processes. This mechanism may involve the disruption of essential bacterial systems or cell wall integrity, a common mode of action for effective antimicrobial agents (Salem & Salem, 2025; Salem, et al, 2025).

While McKellar and Scott (1990) established the potential of benzimidazole derivatives in veterinary medicine (anthelmintic activity), our study reinforces their direct potential as antibacterial agents against common human pathogens, achieving enhanced activity compared to previous findings by Gadhiya et al. (2016).

4. Cytotoxicity

The cytotoxic effects observed for compounds 1c and 1d in our study are noteworthy. These compounds showed IC50 values of 18 µM and 20 µM, respectively, against HeLa cells, which are lower than those reported by Gaba and Mohan (2016) for similar derivatives (IC50 values around 25 µM). This suggests a higher potency of our synthesized compounds as potential anticancer agents.

Shaibuna et al. (2022) also reported benzimidazole derivatives with anticancer activity, but their compounds exhibited higher IC50 values (above 30 μM). The improved cytotoxicity in our derivatives could be attributed to the specific functional groups and positions chosen during synthesis, which enhance their interaction with cancer cell targets.

Contrasts with Other Research and Future Directions

While this study establishes the synthetic feasibility and potent biological activity of the derivatives, it also identifies gaps when contrasted with contemporary research, providing a roadmap for future work:

1. Methodological Innovations in Synthesis

Our study employed traditional synthetic routes (Preston, 2009). However, recent advancements in green chemistry, exemplified by microwave-assisted synthesis (Zhuang et al., 2022) or catalytic approaches (Nardi et al., 2023), offer advantages such as faster reactions, reduced energy consumption, and lower environmental impact. Incorporating these Green Chemistry techniques would enhance the efficiency and sustainability of large-scale benzimidazole synthesis in future studies.

2. Broadening the Biological Spectrum

The current research focused on antibacterial and antitumor activities. For a comprehensive understanding of therapeutic potential, future studies should expand the biological evaluation to include other activities reported for benzimidazoles, such as antifungal and antiviral properties (Gadhiya et al., 2016). This diversification would maximize the clinical utility of these derivatives.

3. In-Depth Mechanistic Insights

The biological actions were documented empirically. Moving forward, in-depth mechanistic investigations are crucial. While previous studies have thoroughly explored the mechanism of action of benzimidazole derivatives, focusing specifically on tubulin polymerization inhibition (Vyas & Ghate, 2010), our compounds require dedicated mechanistic validation. Future work should employ techniques like molecular docking and bioinformatics analysis to precisely determine the molecular targets and pathways underlying the enhanced antibacterial and anticancer properties of 1c and 1d.

4. Pharmacokinetic and Drug-Likeness Assessment

For the development of viable drug candidates, preclinical assessment of pharmacokinetic properties—including absorption, distribution, metabolism, and excretion (ADME)—is essential (Di Gioia et al., 2019). Our study did not incorporate these assessments. Future projects must integrate ADME and other drug-likeness studies to understand the *in vivo* behavior and optimize the therapeutic profile of the synthesized compounds.

Conclusion

In our work, several functionalized benzimidazole derivatives were successfully synthesized, characterised, and demonstrated potent antibacterial and anticancer activities. Compared to other research, our compounds showed higher biological activity, most likely due to specific structural modifications. But in order to further the creation of benzimidazole-based pharmaceuticals, more biological evaluations, sophisticated process and pharmacokinetics research, and new synthesis methods are required.

By addressing these qualities, future research can build on our findings to maximise the therapeutic potential of benzimidazole derivatives and transform them into medicines that are practical for clinical use. This study effectively synthesised and characterised many functionalized benzimidazole derivatives with yields ranging from 70% to 85%. The compounds were confirmed using NMR, IR, and MS techniques, which provided comprehensive structural insights. There was a noticeable antibacterial activity, especially for

derivatives 1c and 1d, which exhibited larger zones of inhibition against S. aureus and E. coli. Additionally, these substances exhibited more potent cytotoxic effects on MCF-7 and HeLa cancer cell lines, as evidenced by their IC50 values being lower than those of previously reported derivatives. These findings suggest that functionalized benzimidazole derivatives have a lot of potential as antimicrobial and antitumor drugs. Future study should focus on examining green synthesis methodologies, more extensive biological activities, in-depth mechanistic studies, and pharmacokinetic properties in order to further boost the therapeutic potential of these compounds.

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