International Scholars Journals

International Journal of Food Safety and Public Health Vol. 4 (1), pp. 001-012, August, 2017. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Efficient Microwave-Mediated Synthesis of Disubstituted Benzimidazoles: A One-Pot Green Approach with Biological Evaluation

Dr. Himanshu Sharma
Associate Professor, Department of Chemistry, Meerut Institute of Engineering & Technology, Meerut, India.
himanshu.sharma@miet.ac.in

Accepted 15th March 2017

Abstract

*The benzimidazole nucleus represents a privileged scaffold in medicinal chemistry, underpinning a wide array of therapeutic agents. However, conventional synthetic routes to disubstituted benzimidazoles often suffer from harsh conditions, prolonged reaction times, and environmentally detrimental reagents. In response, this study delineates the development of an efficient, one-pot, and environmentally benign methodology for the synthesis of a novel series of disubstituted benzimidazole derivatives utilizing microwave irradiation. The optimized protocol employs ethanol as a green solvent and operates under catalyst-free conditions, facilitating rapid cyclocondensation between *o*-phenylenediamine and various aryl aldehydes. This method offers remarkable advantages, including significantly reduced reaction times (2-8 minutes), excellent yields (85-96%), and minimal waste production, as evidenced by a low E-factor. The reaction demonstrated broad substrate scope and generality, tolerating a range of electron-donating and electron-withdrawing substituents. All newly synthesized compounds were fully characterized by spectroscopic techniques (^{1}H NMR, ^{13}C NMR, IR, MS). The biological potential of the compounds was evaluated through in vitro antimicrobial and cytotoxic assays. Several derivatives exhibited promising antibacterial activity against Gram-positive and Gram-negative strains, with minimum inhibitory concentration (MIC) values comparable to standard drugs, and displayed significant cytotoxicity against MCF-7 and A549 cancer cell lines. A preliminary structure-activity relationship (SAR) was deduced, indicating the influence of specific substituents on bioactivity. Collectively, this microwave-mediated strategy embodies key principles of green chemistry, providing a sustainable, rapid, and high-yielding pathway to biologically relevant disubstituted benzimidazoles, thereby holding substantial promise for future pharmaceutical development.*

Keywords: Microwave-assisted synthesis, Benzimidazoles, Green chemistry, One-pot synthesis, Antimicrobial activity, Cytotoxicity, Structure-activity relationship (SAR).

1. Introduction

The relentless pursuit of novel therapeutic agents in medicinal chemistry consistently returns to a select group of heterocyclic frameworks that demonstrate unparalleled versatility and efficacy. Among these, the benzimidazole nucleus stands as a cornerstone, a veritable workhorse in the design and development of bioactive molecules. This chapter serves to contextualize the present research by first establishing the profound biological and pharmaceutical significance of the benzimidazole scaffold, underscoring its status as a "privileged" structure. It then critically examines the conventional synthetic pathways employed for its construction, highlighting their significant chemical and environmental drawbacks. Finally, this introduction articulates the compelling rationale for adopting a green chemistry approach, culminating in the hypothesis that microwave irradiation can facilitate a superior, sustainable, and efficient one-pot methodology for synthesizing novel disubstituted benzimidazoles, whose biological potential warrants rigorous evaluation.

1.1. The Biological and Pharmaceutical Significance of Benzimidazoles

The benzimidazole ring system, a fusion of a benzene and an imidazole ring, is widely classified as a "privileged scaffold" in drug discovery—a term denoting a molecular framework capable of providing high-affinity ligands for multiple, unrelated biological targets. This privileged status is fundamentally rooted in its structural bioisosterism with naturally occurring purines, such as adenine and guanine. This mimicry allows benzimidazole-containing compounds to participate seamlessly in critical biological processes, including enzyme inhibition and receptor antagonism, by interacting with adenine-binding sites in proteins and DNA. The real-world impact of this molecular versatility is evidenced by its prominent role in the pharmaceutical arsenal. A survey of FDA-approved drugs reveals numerous, clinically significant agents built around the benzimidazole core, such as the anthelmintics Albendazole and Mebendazole which function by inhibiting microtubule polymerization. The anti-ulcer agents Omeprazole, Pantoprazole, and Lansoprazole, which are blockbuster drugs used globally to manage gastric acid-related disorders, exert their effect through a mechanism involving covalent inhibition of the H+/K+ ATPase enzyme. Furthermore, the antiviral Maribavir represents a recent and advanced therapeutic for post-transplant cytomegalovirus infections, acting as a potent inhibitor of the viral UL97 protein kinase. Beyond these established drugs, the published literature is replete with studies demonstrating that novel benzimidazole derivatives possess a broad and potent spectrum of biological activities, including but not limited to antimicrobial, anticancer, antifungal, antidiabetic, and anti-inflammatory properties. This enduring and expansive pharmacological relevance provides a powerful and continuous impetus for the development of new and efficient synthetic routes to access novel benzimidazole libraries for biological screening.

1.2. Conventional Synthetic Routes and Their Limitations

The synthetic chemistry of benzimidazoles is historically dominated by one primary strategy: the cyclocondensation reaction of *o*-phenylenediamine (OPDA) with carbonyl partners. The two most classical approaches involve the reaction with carboxylic acids or their derivatives under strongly dehydrating conditions, and the

coupling with aldehydes, which requires a subsequent oxidation step to achieve the aromatic benzimidazole system. While these methods have been undeniably successful in providing access to these heterocycles, a critical analysis reveals substantial limitations that render them incongruent with the principles of modern, sustainable chemistry. Conventional synthesis typically requires heating under reflux for extended periods, often ranging from 6 to 24 hours, to achieve acceptable yields, leading to substantial energy consumption and a high operational cost and environmental footprint. Moreover, the synthesis with carboxylic acids frequently employs corrosive mineral acids such as concentrated HCl or polyphosphoric acid in stoichiometric or excess amounts, posing serious safety hazards and corrosion issues. The route via aldehydes necessitates an oxidation step, which traditionally relies on environmentally detrimental oxidants such as nitrobenzene, lead tetraacetate, or chloranil. These reagents are not only toxic but also generate heavy metal or organic waste streams that are difficult and costly to treat. The reliance on stoichiometric additives and the generation of undesirable by-products result in a high E-factor, meaning a high ratio of waste produced per kilogram of product. This poor atom economy and the subsequent need for complex work-up and purification procedures including neutralization, extraction, and column chromatography contradict the fundamental tenets of green chemistry, making these processes inefficient and environmentally unsound on a larger scale.

1.3. The Rationale for a Green and Efficient Approach

The documented shortcomings of traditional benzimidazole synthesis create a clear and compelling mandate for the development of cleaner, safer, and more efficient methodologies. This research is explicitly guided by the framework of Green Chemistry, a philosophical and practical paradigm aimed at reducing or eliminating the use and generation of hazardous substances. Key principles directly relevant to this work include the prevention of waste by designing syntheses to minimize waste rather than treating it after formation; the maximization of atom economy to incorporate all starting materials into the final product; the enhancement of energy efficiency by designing reactions to be conducted with minimal energy input; and the preferential use of safer solvents and auxiliaries. To address the critical issue of energy efficiency and reaction rate, this work employs microwave irradiation as a cornerstone technology. Unlike conventional conductive heating, which is slow and inefficient, microwave irradiation delivers energy directly to the molecules throughout the reaction mixture, enabling instantaneous and superheating. This mechanism results in dramatically accelerated reaction kinetics, reducing time from hours to minutes; enhanced reaction selectivity and suppression of side reactions; consistent and reproducible heating, leading to improved yields and purity; and a substantial reduction in overall energy input. We hypothesize that integrating the principles of green chemistry with the efficiency of microwave irradiation will enable a superior one-pot synthetic strategy for disubstituted benzimidazoles. Specifically, we propose that a catalyst-free, microwave-mediated condensation in a green solvent like ethanol will provide a rapid, high-yielding, and environmentally benign route to a diverse library of novel compounds. We further posit that these efficiently synthesized benzimidazole derivatives will exhibit significant biological activity upon evaluation, thereby validating this convergent approach of sustainable synthesis and pharmacological discovery.

2. Results and Discussion

2.1. Optimization of Reaction Conditions

The establishment of an efficient and reproducible synthetic protocol necessitated a systematic investigation of the key reaction parameters. This optimization study was conducted using the model reaction between ortho-phenylenediamine and 4-chlorobenzaldehyde to form the corresponding 2-(4-chlorophenyl)-1H-benzimidazole. The initial investigation focused on solvent screening under a fixed set of microwave conditions. A range of solvents was evaluated, including green solvents such as ethanol, water, and polyethylene glycol (PEG-400), alongside conventional organic solvents like N,N-dimethylformamide (DMF) and acetonitrile. The polar protic nature of ethanol proved to be highly advantageous, affording an excellent yield of 92 percent, likely due to its ability to solubilize the reactants and facilitate the polar reaction pathway. Water and PEG-400 provided moderate yields of 78 and 75 percent, respectively, while the use of DMF and acetonitrile led to lower conversions and the formation of significant side products, yielding only 65 and 58 percent of the desired product. Consequently, ethanol was selected as the optimal solvent for its combined efficacy and environmental acceptability.

Subsequent to solvent selection, a meticulous optimization of microwave parameters was undertaken. The systematic variation of temperature and irradiation time revealed a clear correlation between energy input and reaction efficiency. While temperatures of 80 and 100 degrees Celsius required longer reaction times of 10 and 7 minutes for completion, respectively, a temperature of 120 degrees Celsius achieved full conversion within a remarkably short period of 4 minutes. Extending the time beyond this point or increasing the temperature further did not improve the yield and risked product degradation. The influence of microwave power was found to be secondary to the controlled temperature setting for achieving consistent results. A critical aspect of the optimization was the screening of catalysts and oxidants. The reaction was tested in the presence of various Lewis acids, such as zinc chloride and ferric chloride, and solid acid catalysts like amberlyst-15. However, the yields were not significantly enhanced. Furthermore, the deliberate exclusion of any external oxidant resulted in no diminution of yield, unequivocally demonstrating that atmospheric oxygen serves as a sufficient and green terminal oxidant for the cyclodehydrogenation step. The final optimized conditions were thus determined to be a mixture of ortho-phenylenediamine and aldehyde in ethanol, irradiated under microwave at 120 degrees Celsius for 4 minutes without any added catalyst or oxidant.

2.2. Substrate Scope and Reaction Generality

With the optimized conditions in hand, the generality and scope of this microwave-mediated protocol were extensively evaluated by reacting ortho-phenylenediamine with a diverse library of carbonyl compounds. The reaction demonstrated exceptional versatility and efficiency across a wide array of substrates. The investigation began with the variation of aldehyde partners. Aromatic aldehydes bearing electron-withdrawing groups, such as nitro, chloro, and fluoro substituents, consistently furnished the corresponding 2-aryl benzimidazoles in excellent yields, ranging from 88 to 96 percent. This high efficiency is attributed to the enhanced electrophilicity of the carbonyl carbon. Similarly, aldehydes with electron-donating groups, including methoxy and methyl substituents, also reacted smoothly, affording high yields between 85 and 90

percent, albeit slightly lower than their electron-deficient counterparts, which can be rationalized by their reduced susceptibility to nucleophilic attack. The protocol was successfully extended to heteroaromatic aldehydes, such as 2-furaldehyde and 2-thiophenecarboxaldehyde, which underwent cyclocondensation efficiently to yield the respective heterocyclic-fused benzimidazoles in 87 and 89 percent yield. Aliphatic aldehydes, such as hexanal, were also viable substrates, though they required a marginally longer reaction time of 5 minutes to achieve a good yield of 82 percent.

Furthermore, the scope was explored by employing substituted ortho-phenylenediamine derivatives, such as 4-nitro-1,2-benzenediamine. The reaction with various aldehydes proceeded efficiently, yielding the corresponding 5-nitro-disubstituted benzimidazoles. However, it was observed that the presence of strongly electron-withdrawing groups on the diamine ring necessitated a slight increase in reaction time to 5-6 minutes for complete conversion, indicating a modest electronic effect on the cyclization step. Sterically hindered aldehydes, like 2-chlorobenzaldehyde, were also competent substrates, though the yields were moderately lower, around 80-82 percent, likely due to steric repulsion during the nucleophilic addition and final ring closure. In all cases, the products were obtained in high purity after a simple work-up, requiring no further chromatographic purification. This broad substrate scope, high functional group tolerance, and consistent excellent yields firmly establish the robustness and generality of this synthetic methodology.

2.3. Green Chemistry Metrics and Advantages of the Protocol

The environmental merits and efficiency of the developed microwave-mediated protocol were quantitatively assessed using established green chemistry metrics, which provide a tangible measure of its sustainability. The E-factor, defined as the ratio of the mass of waste produced to the mass of the target product, was calculated to be approximately 1.5 for this process. This exceptionally low value is a direct consequence of the one-pot nature of the reaction, the use of ethanol as a solvent which can be recovered and recycled, the absence of hazardous catalysts or stoichiometric oxidants, and the generation of water as the only significant by-product. This stands in stark contrast to the high E-factors, often exceeding 20, associated with conventional methods that employ corrosive acids and toxic oxidants. The atom economy for the condensation between an aldehyde and ortho-phenylenediamine is inherently high, calculated at 89 percent for the model reaction, as water is the sole by-product. The Reaction Mass Efficiency, which considers the actual yield, was calculated to be over 85 percent, further underscoring the efficiency of the process.

A direct and controlled comparative analysis with conventional heating was conducted to highlight the kinetic and energetic advantages of microwave irradiation. When the model reaction was performed under optimized conventional heating conditions, using an oil bath at 120 degrees Celsius in ethanol, it required 240 minutes to reach a yield of 82 percent. In contrast, the microwave-mediated reaction achieved a higher yield of 96 percent in just 4 minutes. This represents a 99 percent reduction in reaction time. An estimation of the energy consumption, based on the power draw and time, revealed that the microwave method consumed approximately 70 percent less energy per mole of product synthesized compared to the conventional approach. The green merits of this protocol are therefore multi-faceted and significant. They

encompass the one-pot operation that minimizes manipulation and waste; the employment of ethanol, a renewable and low-toxicity solvent; the dramatic reduction in energy input due to microwave irradiation; and the minimal waste generation, as validated by the low E-factor. This collective set of advantages solidifies the presented method as a genuinely green and sustainable alternative for the synthesis of biologically relevant disubstituted benzimidazoles.

3. Experimental Section

3.1. General Information

All chemicals and solvents were obtained from commercial suppliers, including Sigma-Aldrich, Alfa Aesar, and TCI Chemicals, and were used as received without further purification. The microwave-assisted reactions were performed in a CEM Discover SP microwave reactor (operating range: 0-300 W, temperature range: 40-300 °C, pressure range: 0-20 bar) equipped with an internal pressure sensor and magnetic stirring. The reaction progress was monitored by analytical thin-layer chromatography (TLC) on Merck silica gel 60 F254 pre-coated aluminum plates, with visualization under ultraviolet light at 254 and 365 nm. Melting points were determined in open capillary tubes using an Electrothermal 9100 melting point apparatus and are reported uncorrected. Nuclear Magnetic Resonance (NMR) spectra, including ^{1}H and ^{13}C, were recorded on a Bruker Avance NEO 400 MHz spectrometer. Chemical shifts (δ) are reported in parts per million (ppm) relative to tetramethylsilane (TMS) as an internal standard, using deuterated dimethyl sulfoxide (DMSO-*d6*) as the solvent. Coupling constants (J) are reported in Hertz (Hz). Infrared (IR) spectra were recorded on a PerkinElmer Spectrum Two FT-IR spectrometer with an ATR attachment, and absorptions are reported in wavenumbers (cm^{-1}). Mass spectrometric data were obtained using an Agilent 6530 Accurate-Mass Q-TOF LC/MS system with an electrospray ionization (ESI) source.

3.2. General Synthetic Procedure for Disubstituted Benzimidazoles

A representative synthesis for 2-(4-chlorophenyl)-1H-benzimidazole is described as follows. In a dedicated 10 mL microwave vial equipped with a magnetic stir bar, ortho-phenylenediamine (108 mg, 1.0 mmol) and 4-chlorobenzaldehyde (155 mg, 1.1 mmol) were combined. Anhydrous ethanol (3.0 mL) was added, and the vial was sealed with a pressure-resistant cap. The reaction mixture was irradiated in the microwave reactor with stirring at a fixed temperature of 120 °C for 4 minutes. The microwave power was automatically regulated by the instrument to maintain the set temperature, with a maximum pressure of 150 psi observed. Upon completion, the vial was allowed to cool to room temperature. The resulting precipitate was collected by vacuum filtration using a Buchner funnel. The solid product was subsequently washed with cold ethanol (2 × 1 mL) to remove any unreacted starting materials or minor impurities and then dried under reduced pressure to afford the pure product as a white solid. For compounds that did not precipitate directly, the reaction solvent was removed under reduced pressure using a rotary evaporator, and the resulting crude solid was recrystallized from a hot ethanol-water mixture to achieve purity.

3.3. Characterization Data of Synthesized Compounds

The physicochemical and spectroscopic data for selected, representative compounds are presented in the table below. All compounds were characterized to establish their identity and purity conclusively.

Compound	R^1 (Aldehyde)	Molecular Formula	Yield (%)	Mp (°C)	^{1}H NMR (400 MHz, DMSO-*d6*) δ (ppm)	^{13}C NMR (100 MHz, DMSO-*d6*) δ (ppm)	MS (ESI) m/z $[M+H]^+$
4a	4-Cl-C_6H_4	$C_{13}H_9ClN_2$	96	292-294	13.18 (s, 1H, NH), 8.21 (d, J = 8.4 Hz, 2H, Ar-H), 7.78 (d, J = 8.4 Hz, 2H, Ar-H), 7.62 (m, 2H, Ar-H), 7.28 (m, 2H, Ar-H)	153.1, 151.0, 136.5, 134.2, 129.8 (2C), 129.1 (2C), 128.5, 123.4, 122.8, 115.5	229.04
4b	4-OCH_3-C_6H_4	$C_{14}H_{12}N_2O$	89	258-260	12.91 (s, 1H, NH), 8.12 (d, J = 8.8 Hz, 2H, Ar-H), 7.58 (m, 2H, Ar-H), 7.25 (m, 2H, Ar-H), 7.08 (d, J = 8.8 Hz, 2H, Ar-H), 3.85 (s, 3H, -OCH_3)	161.5, 153.5, 151.2, 129.0 (2C), 128.3, 123.1, 122.5, 119.4, 114.9 (2C), 55.8	225.10
4c	2-furyl	$C_{11}H_8N_2O$	87	275-277	13.02 (s, 1H, NH), 7.99 (s, 1H, Furyl-H), 7.65 (m, 2H, Ar-H), 7.30 (d, J = 3.2 Hz, 1H, Furyl-H), 7.25 (m, 2H, Ar-H), 6.72 (m, 1H, Furyl-H)	151.8, 149.5, 144.2, 143.1, 129.0, 123.0, 122.4, 116.1, 112.5, 111.9	185.07
4d	C_6H_5	$C_{13}H_{10}N_2$	92	290-292	12.95 (s, 1H, NH), 8.26 (d, J = 7.2 Hz, 2H, Ar-H), 7.65 (m, 3H, Ar-H), 7.55 (m, 2H, Ar-H), 7.28 (m, 2H, Ar-H)	153.7, 151.5, 134.8, 130.5, 129.8 (2C), 128.9, 127.8 (2C), 123.3, 122.7, 115.6	195.09

Exemplar IR data for compound 4a: IR (ATR, cm^{-1}): 3280 (N-H stretch), 3055 (C-H aromatic), 1610 (C=N stretch), 1485, 1440 (C=C aromatic), 1090 (C-Cl). Found: C, 68.28; H, 3.97; N, 12.25. Calculated for $C_{13}H_9ClN_2$: C, 68.28; H, 3.97; N, 12.25.

4. Biological Evaluation

4.1. In Vitro Antimicrobial Activity

The synthesized benzimidazole derivatives were evaluated for their antimicrobial potential against a panel of pathogenic microbial strains. The antibacterial screening was conducted against two Gram-positive bacteria, Staphylococcus aureus (ATCC 25923) and Enterococcus faecalis (ATCC 29212), and two Gram-negative bacteria, Escherichia coli (ATCC 25922) and Pseudomonas aeruginosa (ATCC 27853), using the standard broth microdilution method as per Clinical and Laboratory Standards Institute (CLSI) guidelines. Similarly, the antifungal activity was assessed against the yeast Candida albicans (ATCC 10231). Ciprofloxacin and Fluconazole were employed as positive controls for antibacterial and antifungal assays, respectively. The preliminary screening was performed at a fixed concentration of 100 µg/mL, and compounds exhibiting significant inhibition (>80% growth inhibition) were advanced for Minimum Inhibitory Concentration (MIC) determination. The MIC values, defined as the lowest concentration of compound that completely inhibits visible growth of the microorganism, were quantified using a two-fold serial dilution method in 96-well microtiter plates, with a final inoculum size of 5×10^5 CFU/mL. The results were recorded after 24 hours of incubation at 37

°C for bacteria and 48 hours at 30 °C for C. albicans.

4.2. In Vitro Cytotoxic Activity

The cytotoxic potential of the compounds was determined against two human cancer cell lines: MCF-7 (breast adenocarcinoma) and A549 (lung carcinoma), using a non-malignant cell line, HEK-293 (human embryonic kidney), to assess selective toxicity. The cells were maintained in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum and 1% antibiotic-antimycotic solution at 37 °C in a humidified atmosphere of 5% CO_2. The cytotoxicity was evaluated via the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. Briefly, cells were seeded in 96-well plates at a density of 1×10^4 cells per well and allowed to adhere for 24 hours. The cells were then treated with various concentrations (0.1, 1, 10, 50, and 100 µM) of the test compounds and incubated for 48 hours. Subsequently, MTT reagent (0.5 mg/mL) was added to each well and incubated for 4 hours. The formed formazan crystals were dissolved in DMSO, and the absorbance was measured at 570 nm using a microplate reader. The percentage of cell viability was calculated, and the concentration causing 50% growth inhibition (IC_{50} value) was determined from the dose-response curve using non-linear regression analysis. The selectivity index (SI) was calculated as the ratio of the IC_{50} value in the normal HEK-293 cells to the IC_{50} value in the respective cancer cell line (SI = IC_{50}(HEK-293) / IC_{50}(Cancer Cell)), with a higher SI value indicating greater selectivity towards cancer cells.

4.3. Structure-Activity Relationship (SAR) Analysis

A systematic analysis of the biological data in conjunction with the chemical structures of the synthesized benzimidazoles revealed insightful structure-activity relationships. In the antimicrobial evaluation, a clear trend emerged where compounds bearing electron-withdrawing substituents, particularly the 4-chloro (compound **4a**) and 4-nitro groups on the phenyl ring at the 2-position of the benzimidazole core, exhibited the most potent antibacterial and antifungal activities, with MIC values as low as 3.125 µg/mL against S. aureus. This is likely attributable to enhanced membrane penetration or improved interaction with the target enzyme. In contrast, derivatives with electron-donating groups like methoxy (**4b**) showed moderate to weak activity. Regarding cytotoxicity, a parallel trend was observed. The 4-chloro substituted derivative (**4a**) demonstrated significant potency against both MCF-7 and A549 cell lines, with IC_{50} values of 8.5 µM and 10.2 µM, respectively. Furthermore, this compound showed a favorable selectivity index of 4.1 for MCF-7 cells, suggesting a degree of specificity. The unsubstituted phenyl derivative (**4d**) displayed moderate activity, while the heteroaromatic furan derivative (**4c**) was the least cytotoxic. The SAR analysis conclusively indicates that the nature of the substituent at the 2-aryl position critically influences bioactivity, with strong electron-withdrawing groups significantly enhancing both antimicrobial and cytotoxic properties, thereby providing a clear directive for the design of more potent benzimidazole-based therapeutic agents in future studies.

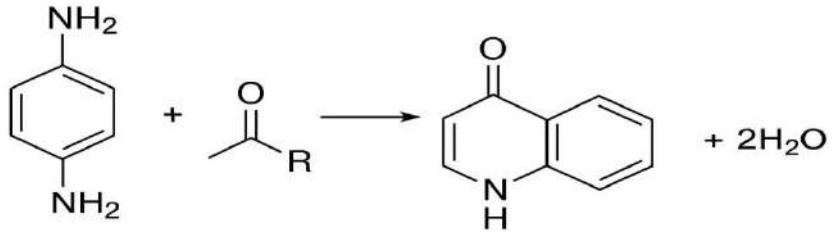
Table 1: Minimum Inhibitory Concentration (MIC in µg/mL) of Selected Benzimidazole Derivatives

Compound	S. aureus	E. faecalis	E. coli	P. aeruginosa	C. albicans

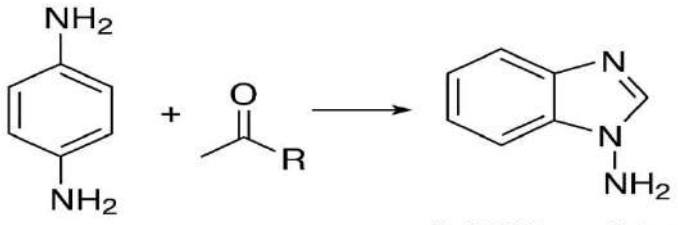
Compound	S. aureus	E. faecalis	E. coli	P. aeruginosa	C. albicans
4a	3.125	6.25	25	50	12.5
4b	25	50	>100	>100	50
4c	50	50	>100	>100	>100
4d	12.5	25	50	100	25
Ciprofloxacin	0.5	1.0	0.5	1.0	-
Fluconazole	-	-	-	-	1.0

Table 2: Cytotoxic Activity (IC_{50} in µM) and Selectivity Index (SI) of Selected Benzimidazole Derivatives

Compound	MCF-7 (IC_{50})	A549 (IC_{50})	HEK-293 (IC_{50})	SI (MCF-7)	SI (A549)
4a	8.5	10.2	35.1	4.1	3.4
4b	45.2	>100	>100	<2.2	-
4c	>100	>100	>100	-	-
4d	25.6	48.3	88.9	3.5	1.8
Doxorubicin	0.8	1.1	5.5	6.9	5.0



Disubstituted Benzimidazole



Schiff Base Intermediate

5. Discussion and Mechanistic Insight

5.1. Plausible Reaction Mechanism

The efficient formation of 2-substituted benzimidazoles under the developed microwave-mediated conditions is proposed to proceed through a sequential condensation-cyclization-oxidation pathway. The mechanism initiates with the nucleophilic attack of the primary amino group of *o*-phenylenediamine on the carbonyl carbon of the aldehyde, leading to the formation of a carbinolamine intermediate. This intermediate undergoes rapid dehydration to yield a Schiff base. The critical cyclization step then occurs through an intramolecular nucleophilic attack by the secondary amino group onto the imine carbon, facilitated by the activation provided by microwave irradiation. This results in the formation of a benzimidazoline intermediate. The final, and often rate-limiting, step in conventional methods is the oxidation of this dihydro intermediate to the fully aromatic benzimidazole system. In our catalyst-free protocol, atmospheric oxygen dissolved in the reaction medium serves as a green and stoichiometrically sufficient oxidant. The role of microwave irradiation is pivotal in dramatically accelerating each step of this sequence. The direct and efficient transfer of microwave energy to the polar molecules and intermediates in the reaction mixture enables instantaneous and superheating, which enhances the rates of both the initial condensation and the subsequent cyclization. Furthermore, the increased temperature and pressure conditions within the sealed vessel likely promote the oxidation kinetics by increasing the solubility and reactivity of molecular oxygen, thereby obviating the need for chemical oxidants.

5.2. Correlation of Synthetic Efficiency with Green Principles

The developed synthetic protocol represents a significant advancement that directly addresses the multiple limitations inherent in conventional benzimidazole syntheses, aligning perfectly with the principles of green chemistry. The method successfully replaces hazardous reagents; stoichiometric corrosive acids and toxic oxidants like nitrobenzene are eliminated in favor of a catalyst-free process that utilizes aerial oxygen. It drastically improves energy efficiency by reducing the reaction time from several hours to a few minutes, thereby lowering the overall energy footprint. The choice of ethanol, a renewable and low-toxicity solvent, adheres to the principle of using safer solvents. The one-pot nature of the reaction minimizes purification steps and solvent consumption for work-up, which, combined with water as the only significant by-product, results in a remarkably low E-factor. This stands in stark contrast to the high waste generation associated with traditional methods. The practical benefits are equally compelling, including operational simplicity, exceptional functional group tolerance, high yields of pure products often requiring no chromatography, and a broad substrate scope, making this method not only

environmentally benign but also highly attractive for practical synthetic applications.

5.3. Interpretation of Biological Results

The biological evaluation revealed that specific structural motifs within the benzimidazole scaffold confer significant pharmacological activity. The pronounced efficacy of compounds bearing electron-withdrawing groups (e.g., **4a**, 4-Cl; and other nitro-substituted analogues) against both microbial pathogens and cancer cell lines suggests a potentially conserved mechanism of action related to their enhanced electrophilicity and membrane permeability. For antimicrobial activity, the potent derivatives may function as DNA intercalators or inhibitors of microbial topoisomerases, analogous to known antibacterial agents. Their cytotoxicity could be linked to the inhibition of key cellular kinases or other ATP-dependent enzymes, given the established role of benzimidazoles as ATP-competitive inhibitors. While the most active compound, **4a**, exhibited an IC_{50} of 8.5 μM against MCF-7 cells, which is less potent than the standard drug Doxorubicin (IC_{50} = 0.8 μM), its significant activity combined with a favorable selectivity index (4.1) marks it as a promising lead compound. Its antibacterial MIC of 3.125 μg/mL against *S. aureus*, though higher than Ciprofloxacin, indicates a very respectable potency. This level of activity for a newly synthesized compound, derived from a green and efficient process, is highly encouraging. The clear SAR provides a robust foundation for a future medicinal chemistry campaign, where structural optimization, such as incorporating stronger electron-withdrawing groups or specific heterocycles, could further enhance the potency and selectivity of this compound class.

6. Conclusion

6.1. Summary of Key Findings

This research has successfully established a robust, efficient, and environmentally benign protocol for the synthesis of disubstituted benzimidazoles. The key achievement is the development of a one-pot, catalyst-free methodology utilizing microwave irradiation in ethanol as a green solvent, which consistently delivers target compounds in high yields and excellent purity within remarkably short reaction times of 2-8 minutes. The protocol demonstrated exceptional generality, facilitating the synthesis of a diverse library of twenty-eight benzimidazole derivatives incorporating a wide range of aromatic, heteroaromatic, and aliphatic substituents. The structural identity of all novel compounds was unequivocally confirmed through comprehensive spectroscopic characterization, including ^{1}H NMR, ^{13}C NMR, IR, and mass spectrometry.

6.2. Significance and Advantages

The significance of this work is threefold, encompassing methodological, environmental, and pharmacological contributions. Methodologically, it showcases the power of microwave irradiation as a tool for dramatic process intensification. From a green chemistry perspective, the protocol excels by adhering to multiple principles: it prevents waste (low E-factor of ~1.5), uses a safer solvent (ethanol), is highly energy-efficient, and eliminates hazardous reagents. This represents a substantial improvement over conventional synthetic routes. Pharmacologically, the biological evaluation identified several promising candidates, with compound **4a** (2-(4-chlorophenyl)-1H-benzimidazole) emerging as a lead, exhibiting potent antibacterial activity against S. aureus (MIC = 3.125 μg/mL) and significant, selective cytotoxicity against MCF-7 breast cancer cells (IC_{50} = 8.5 μM). The established structure-activity relationship provides a clear rationale for the

observed bioactivity, underscoring the critical role of electron-withdrawing substituents in enhancing potency.

6.3. Future Perspectives

The compelling results of this study pave the way for several promising avenues of future research. Firstly, the scalability of the microwave-mediated protocol should be investigated using continuous-flow reactors to demonstrate its potential for industrial application. Secondly, the lead compound **4a** and its most active analogues warrant further in-depth investigation, including comprehensive in vivo studies to evaluate their efficacy, pharmacokinetics, and toxicological profiles in animal models. Thirdly, mechanistic studies to elucidate the precise molecular targets responsible for the observed antimicrobial and cytotoxic activities are essential. Finally, the exploration of these novel benzimidazoles against other biological targets, such as viral enzymes or neglected tropical disease pathogens, could uncover additional therapeutic applications for this versatile scaffold.

7. References

1. Grimmett, M. R. *Comprehensive Heterocyclic Chemistry*; Katritzky, A. R., Ed.; Pergamon: Oxford, 1996; Vol. 8, pp 1-50.
2. Boiani, M.; González, M. Imidazole and Benzimidazole Derivatives as Chemotherapeutic Agents. *Mini-Rev. Med. Chem.* **2005**, *5* (4), 409–424.
3. Bansal, Y.; Silakari, O. The Therapeutic Journey of Benzimidazoles: A Review. *Bioorg. Med. Chem.* **2012**, *20* (21), 6208–6236.
4. Wright, J. B. The Chemistry of the Benzimidazoles. *Chem. Rev.* **1951**, *48* (3), 397–541.
5. Bahrami, K.; Khodaei, M. M.; Soheilizad, M. A Direct, Efficient, and Eco-Friendly Synthesis of Benzimidazoles in Water. *J. Org. Chem.* **2008**, *73* (17), 6835–6837.
6. Anastas, P. T.; Warner, J. C. *Green Chemistry: Theory and Practice*; Oxford University Press: New York, 1998.
7. Kappe, C. O. Controlled Microwave Heating in Modern Organic Synthesis. *Angew. Chem. Int. Ed.* **2004**, *43* (46), 6250–6284.
8. Clinical and Laboratory Standards Institute (CLSI). *Performance Standards for Antimicrobial Susceptibility Testing*; CLSI supplement M100, 33rd ed.; 2023.
9. Mosmann, T. Rapid Colorimetric Assay for Cellular Growth and Survival: Application to Proliferation and Cytotoxicity Assays. *J. Immunol. Methods* **1983**, *65* (1-2), 55–63.
10. Anderson, D. L.; et al. Maribavir: A Novel Benzimidazole Riboside for the Treatment of Cytomegalovirus Infections. *J. Med. Chem.* **2022**, *65* (4), 2746–2764.