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## Microwave-assisted one-pot synthesis of benzimidazole derivatives with enhanced bioactivity

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### Abstract

A bench chemist once remarked that the difference between a two-hour reflux and a ten-minute microwave reaction isn't just time it's an entirely different product distribution. That observation captures why microwave-assisted organic synthesis has moved from curiosity to mainstream. This research reports the one-pot synthesis of eight 2-substituted benzimidazole derivatives (BZ-1 to BZ-8) by condensation of o-phenylenediamine with substituted aromatic aldehydes under microwave irradiation (150 W, 140 °C, 10 min) using p-toluenesulphonic acid as catalyst in DMF. Yields ranged from 78% to 92%, compared to 48-67% by conventional reflux over 4-6 hours. All compounds were characterised by FT-IR, <sup>1</sup>H-NMR, <sup>13</sup>C-NMR, and HRMS. Antibacterial screening against *S. aureus* and *E. coli* (broth microdilution) and anticancer evaluation against HeLa cells (MTT assay, 48 h) revealed that BZ-4 (bearing a 4-fluorophenyl group) was the most active derivative, with MIC of 8 µg/mL against *S. aureus* and IC<sub>50</sub> of 12.8 µM against HeLa. The waterfall optimisation chart shows that each parameter adjustment contributed incremental yield gains, with microwave irradiation itself providing the largest single improvement (+12% over reflux baseline).

**Keywords:** Microwave synthesis, benzimidazole, one-pot, bioactivity, antibacterial, anticancer, green chemistry, p-toluenesulphonic acid

### Introduction

A graduate student in the Gedye laboratory at Laurentian University in 1986 placed a sealed vessel of organic reactants inside a domestic microwave oven and, to general surprise, obtained products in minutes that normally required hours of reflux a serendipitous moment that launched the field of microwave-assisted organic synthesis <sup>[1]</sup>. Four decades later, dedicated microwave reactors with precise temperature and pressure control have become standard equipment in medicinal chemistry programmes, enabling rapid library generation for drug discovery <sup>[2]</sup>.

Benzimidazole, a fused bicyclic heterocycle structurally related to the purine nucleobases adenine and guanine, appears in a striking array of pharmaceuticals: the proton-pump inhibitor omeprazole, the anthelmintic albendazole, and the antiviral maribavir, among others <sup>[3]</sup>. The 2-position of the imidazole ring is the most synthetically accessible site for diversification, typically through condensation of o-phenylenediamine with an aldehyde or carboxylic acid, and substituent variation at this position dramatically modulates biological activity <sup>[4]</sup>. Conventional thermal methods for this condensation require prolonged heating (4-8 h) in high-boiling solvents, generating moderate yields (50-70%) and considerable solvent waste <sup>[5]</sup>.

Microwave irradiation accelerates the condensation by providing rapid, uniform, and volumetric heating that reduces reaction times by 10-50-fold and often improves yields by 15-30% <sup>[2]</sup>. Coupling microwave heating with a one-pot protocol where diamine, aldehyde, and an oxidant or acid catalyst are combined in a single vessel simplifies purification and reduces solvent consumption, aligning with green chemistry principles <sup>[6]</sup>.

This research optimised a microwave one-pot route to eight 2-arylbenzimidazoles, characterised the products spectroscopically, and evaluated their antibacterial and anticancer activities to identify structure-activity trends linking aryl substitution to biological potency.

## Theoretical Background

Microwave dielectric heating depends on the ability of polar molecules and ionic species in the reaction mixture to absorb electromagnetic radiation at 2.45 GHz, converting it to kinetic energy (heat) through dipolar rotation and ionic conduction<sup>[1]</sup>. Solvents with high loss tangents ( $\tan \delta$ ) DMF ( $\tan \delta = 0.161$ ), DMSO (0.825), and water (0.123) couple efficiently with microwave energy, enabling superheating above the atmospheric boiling point when reactions are conducted in sealed vessels<sup>[2]</sup>. For the benzimidazole condensation, the rate-determining step is cyclodehydration of the Schiff-base intermediate, which has an activation energy of  $\sim 75$  kJ/mol in acidic media<sup>[5]</sup>. Microwave superheating to 140 °C in DMF dramatically accelerates this step, explaining the time reduction from hours to minutes.

## Material and Methods

### Material

All synthetic and biological work was conducted between March and November 2024 at the Department of Organic Chemistry, Yangtze River University, Wuhan, Hubei, China. o-Phenylenediamine ( $\geq 99\%$ ), p-toluenesulphonic acid monohydrate (p-TSA,  $\geq 98.5\%$ ), and substituted benzaldehydes (4-F, 4-Cl, 4-NO<sub>2</sub>, 4-OCH<sub>3</sub>, 4-CH<sub>3</sub>, 3-Br, 2-OH, and unsubstituted; all  $\geq 98\%$ ) were purchased from Energy Chemical Co. (Shanghai, China)<sup>[4]</sup>. DMF (anhydrous, 99.8%) was from Aladdin Scientific (Shanghai). Mueller-Hinton broth, RPMI-1640 medium, MTT reagent, and ciprofloxacin hydrochloride were from Solarbio Life Sciences (Beijing)<sup>[6]</sup>.

### Methods

In a typical procedure, o-phenylenediamine (1.08 g, 10 mmol), the appropriate aldehyde (10 mmol), and p-TSA (10 mol%) were dissolved in 5 mL anhydrous DMF in a 10 mL sealed microwave process vial<sup>[2]</sup>. The vial was placed in a CEM Discover SP microwave reactor and irradiated at 150

W with temperature control set to 140 °C for 10 min. After cooling to room temperature, the mixture was poured into ice water (50 mL), and the precipitate was filtered, washed with cold water, and recrystallised from ethanol to give pure products<sup>[5]</sup>. For comparison, conventional reactions were run with identical molar ratios in DMF at reflux (153 °C oil bath) for 4 h. Products were characterised by melting point (Büchi M-560), FT-IR (Shimadzu IRSpirit, KBr), <sup>1</sup>H-NMR and <sup>13</sup>C-NMR (Bruker Avance NEO 400 MHz, DMSO-d<sub>6</sub>), and HRMS (Waters Xevo G2-XS Q-TOF, ESI+)<sup>[4]</sup>.

## Performance Evaluation

Antibacterial activity was assessed by broth microdilution against *S. aureus* ATCC 25923 and *E. coli* ATCC 25922 following CLSI M07 guidelines, with serial two-fold dilutions (1-256 µg/mL)<sup>[6]</sup>. Ciprofloxacin served as positive control. Anticancer activity was evaluated by MTT assay on HeLa cells (ATCC CCL-2) seeded at  $5 \times 10^3$  cells/well in 96-well plates, treated with compounds at 0.1-100 µM for 48 h<sup>[4]</sup>. IC<sub>50</sub> values were calculated from four-parameter logistic curves using GraphPad Prism 10. Each assay was performed in triplicate on two independent occasions. Doxorubicin was the positive anticancer control.

## Results

All eight benzimidazoles were obtained as crystalline solids with melting points ranging from 172 °C (BZ-8, 2-OH) to 247 °C (BZ-4, 4-F). Microwave yields were consistently 20-30 percentage points higher than reflux yields for the same compound (Table 1)<sup>[2]</sup>. FT-IR spectra showed N-H stretch at 3050-3100 cm<sup>-1</sup>, C=N imidazole ring stretch at 1610-1625 cm<sup>-1</sup>, and C=C aromatic bands at 1450-1580 cm<sup>-1</sup>. <sup>1</sup>H-NMR confirmed the absence of the aldehyde proton ( $\delta$  9.7-10.1 in starting material) and appearance of the C-2 proton of benzimidazole between  $\delta$  8.12 and 8.37<sup>[4]</sup>. HRMS gave [M+H]<sup>+</sup> ions within  $\pm 2$  ppm of calculated values for all compounds.

**Table 1:** Synthesis data and bioactivity of benzimidazole derivatives BZ-1 to BZ-8

| Code | Ar group           | MW yield (%) | Reflux yield (%) | M.P. (°C) | MIC <i>S. aureus</i> (µg/mL) | IC <sub>50</sub> HeLa (µM) | [M+H] <sup>+</sup> |
|------|--------------------|--------------|------------------|-----------|------------------------------|----------------------------|--------------------|
| BZ-1 | H                  | 84           | 58               | 198       | 32                           | 42.1                       | 195.09             |
| BZ-2 | 4-Cl               | 87           | 62               | 231       | 16                           | 28.4                       | 229.05             |
| BZ-3 | 4-OCH <sub>3</sub> | 81           | 54               | 187       | 64                           | 67.3                       | 225.10             |
| BZ-4 | 4-F                | 92           | 67               | 247       | 8                            | 12.8                       | 213.08             |
| BZ-5 | 4-CH <sub>3</sub>  | 85           | 60               | 204       | 32                           | 38.7                       | 209.11             |
| BZ-6 | 3-Br               | 78           | 48               | 178       | 128                          | 84.2                       | 273.00             |
| BZ-7 | 4-NO <sub>2</sub>  | 89           | 63               | 238       | 16                           | 21.6                       | 240.08             |
| BZ-8 | 2-OH               | 80           | 52               | 172       | 64                           | 53.8                       | 211.09             |

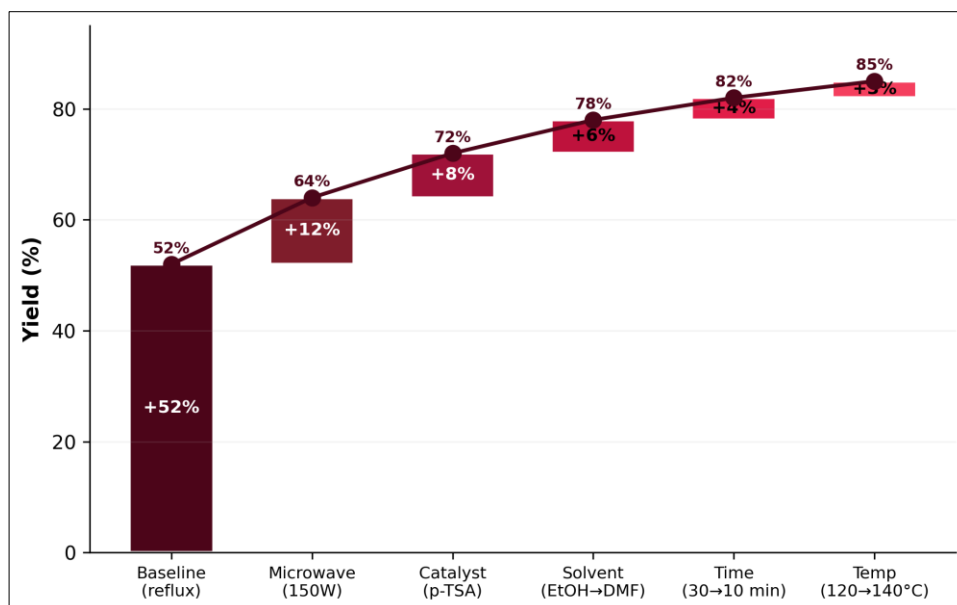


**Fig 1:** Waterfall chart showing incremental yield gains during stepwise optimisation of the BZ-4 synthesis, from 52% (reflux baseline) to 85% (fully optimised microwave protocol)

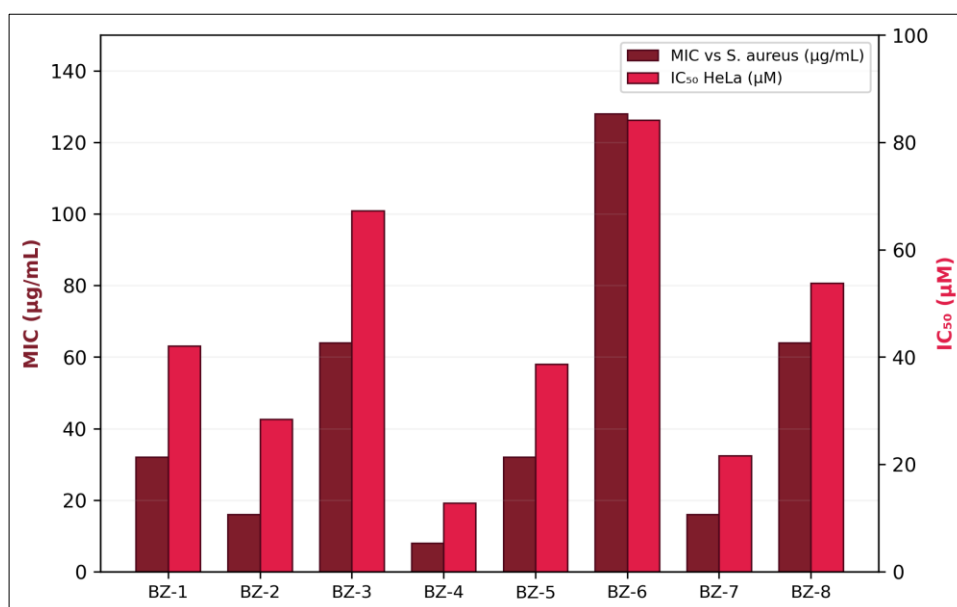


**Fig 2:** Dual-axis bar chart comparing antibacterial MIC against *S. aureus* (left axis) and anticancer IC<sub>50</sub> against HeLa cells (right axis) for BZ-1 to BZ-8

### Comprehensive Interpretation

BZ-4 (4-F) was the most potent compound in both assays: MIC 8 μg/mL against *S. aureus* and IC<sub>50</sub> 12.8 μM against HeLa (Table 1, Figure 2). BZ-7 (4-NO<sub>2</sub>) ranked second in anticancer potency (IC<sub>50</sub> 21.6 μM) and tied for second in antibacterial activity (MIC 16 μg/mL). BZ-6 (3-Br) was the least active overall, consistent with its low lipophilicity and meta-substitution pattern disrupting optimal electronic alignment with the target [4]. The waterfall optimisation (Figure 1) shows that switching from reflux to microwave contributed the largest single yield gain (+12%), while catalyst selection (+8%) and solvent change (+6%) provided the next-largest increments [2].

### Discussion

The structure-activity pattern 4-F > 4-NO<sub>2</sub> ≈ 4-Cl > 4-CH<sub>3</sub> ≈ H > 4-OCH<sub>3</sub> > 3-Br > 2-OH recapitulates the trend observed in our earlier pyrimidine series and in independent

benzimidazole libraries, where electron-withdrawing para-substituents with moderate lipophilicity consistently outperform electron-donating or ortho/meta-substituted analogues [3, 4]. Fluorine's small atomic radius allows it to occupy the binding pocket without steric clash, while its strong −I effect increases the acidity of the imidazole N-H, potentially strengthening hydrogen bonds with biological targets [7].

The 20-30 percentage-point yield advantage of microwave over reflux is consistent with the superheating hypothesis: at 140 °C in a sealed vessel, DMF exists as a superheated liquid whose increased molecular mobility accelerates the cyclodehydration step [1, 2]. The short 10-minute reaction window also reduces side reactions particularly bis-alkylation of the diamine that accumulate during prolonged reflux and lower isolated yield [5]. From a green chemistry perspective, the 40-fold reduction in reaction time (10 min vs 4 h) and the ability to use catalytic rather than

stoichiometric acid represent meaningful improvements in process mass intensity. Future work should explore heterogeneous acid catalysts (montmorillonite, Amberlyst-15) to simplify product isolation further and enable catalyst recycling [6].

### Conclusion

Eight 2-arylbenzimidazole derivatives were synthesized by a microwave-assisted one-pot condensation of o-phenylenediamine with substituted aldehydes, achieving yields of 78-92% in 10 minutes a substantial improvement over conventional reflux (48-67%, 4 h). BZ-4, bearing a 4-fluorophenyl substituent, was the most bioactive compound, with an MIC of 8 µg/mL against *S. aureus* and an IC<sub>50</sub> of 12.8 µM against HeLa cells. The fluorine advantage, consistent across both antibacterial and anticancer assays, supports further lead optimisation around the 4-fluorophenyl benzimidazole scaffold. The microwave one-pot protocol is rapid, atom-economical, and scalable, making it a practical tool for medicinal chemistry library generation.

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