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# Design, Synthesis, Characterization, and In-Vitro Antifungal Evaluation of Novel Benzimidazole Derivatives

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**Abstract:**

**Background:** Fungal infections represent a growing global health concern, particularly among immunocompromised populations. The emergence of resistance to existing antifungal agents highlights the urgent need for novel therapeutic compounds. Benzimidazole derivatives, owing to their structural versatility and broad pharmacological spectrum, have gained significant attention as promising antifungal leads.

**Objectives:** To rationally design, synthesize, and characterize a series of novel 2-substituted benzimidazole derivatives (B1–B6) and to evaluate their in-vitro antifungal activity against Candida albicans, Aspergillus niger, and Aspergillus fumigatus in comparison with the standard drug Fluconazole.

**Methods:** Six novel benzimidazole derivatives were synthesized via condensation of o-phenylenediamine with substituted aromatic aldehydes under acidic catalytic conditions. Compounds were purified by recrystallization and characterized by melting point, Thin Layer Chromatography (TLC), Fourier Transform Infrared Spectroscopy (FT-IR), Proton Nuclear Magnetic Resonance Spectroscopy ($^1$H-NMR), and Mass Spectrometry (MS). In-vitro antifungal activity was assessed using the agar well diffusion method at 100 µg/mL, with inhibition zones measured in triplicate. Structure–Activity Relationships (SAR) were analyzed across the compound series.

**Results:** All six derivatives were obtained in satisfactory yields (68–78%) with narrow melting ranges indicative of purity. Spectroscopic analyses confirmed the benzimidazole scaffold and substituent identities. Antifungal screening revealed that compound B5 (2-(4-hydroxy-3-methoxyphenyl)-1H-benzimidazole) exhibited the highest activity across all three fungal strains, with mean inhibition zones of 18.0, 19.0, and 18.0 mm against C. albicans, A. niger, and A. fumigatus respectively. Compound B6 (2-(4-bromophenyl)-1H-benzimidazole) demon-strated the second-strongest activity. SAR analysis indicated a positive correlation between electron-withdrawing/lipophilic substituents and antifungal potency.

**Conclusion:** The synthesized benzimidazole derivatives demonstrate promising antifungal activity, with B5 and B6 emerging as the most active leads. Although activity was lower than Fluconazole, the compounds provide valuable lead structures amenable to further optimization. These findings support the benzimidazole scaffold as a productive framework for novel antifungal drug development.

**Keywords:** Benzimidazole, antifungal, synthesis, characterization, structure–activity relationship, Candida albicans, Aspergillus, medicinal chemistry

## 1. INTRODUCTION

Fungal infections represent a serious and growing public health challenge worldwide. The increasing population of immunocompromised individuals – including patients undergoing chemotherapy, organ transplantation, prolonged corticosteroid therapy, and those living with HIV/AIDS – has contributed substantially to the rising incidence of opportunistic mycoses. Pathogenic fungi such as Candida albicans, Aspergillus Niger, and Aspergillus fumigatus are responsible for a spectrum of infections ranging from superficial mucosal diseases to life-threatening invasive systemic conditions associated with high morbidity and mortality.

Current antifungal therapy relies on a limited number of drug classes, principally polyenes (e.g., Amphotericin B), azoles (e.g., Fluconazole, Voriconazole), echinocandins (e.g., Caspofungin), and allylamines (e.g., Terbinafine). Each class targets distinct aspects of fungal cell biology – primarily ergosterol biosynthesis or cell wall glucan synthesis – but all are beset by important limitations including nephrotoxicity, hepatotoxicity, drug interactions mediated by cytochrome P450 inhibition, restricted spectrum of activity, and increasingly documented antifungal resistance. Resistance mechanisms include target site mutations affecting lanosterol 14-α-demethylase, overexpression of efflux pumps (ABC transporters and major facilitator superfamily proteins), biofilm formation, and genomic plasticity.

These challenges underscore the urgent need for structurally novel antifungal agents capable of overcoming existing resistance mechanisms. Heterocyclic compounds, particularly nitrogen-containing scaffolds, have long occupied a central role in drug discovery owing to their inherent ability to interact with diverse biological targets through hydrogen bonding, π-stacking, and hydrophobic forces. Among heterocycles, benzimidazole – a bicyclic fused system comprising benzene and imidazole rings – is recognized as one of the most pharmacologically versatile frameworks in medicinal chemistry.

The benzimidazole nucleus bears structural resemblance to naturally occurring purine bases, enabling its derivatives to interact with nucleic acids, enzymes, and key cellular proteins. Benzimidazole compounds have demonstrated antimicrobial, antifungal, antiviral, anticancer, anti-inflammatory, antiulcer (proton pump inhibitors), anthelmintic, and antihypertensive activities. The structural flexibility of the benzimidazole core permits systematic introduction of diverse substituent groups, making it an ideal scaffold for rational medicinal chemistry optimization through structure–activity relationship (SAR) studies.

Despite extensive literature on benzimidazole bioactivity, the optimal substituent patterns conferring maximal antifungal selectivity and potency remain incompletely defined. The present study was therefore undertaken to rationally design, synthesize, characterize, and evaluate in-vitro antifungal activity of a series of six novel 2-substituted benzimidazole derivatives (B1–B6), with the aim of identifying lead compounds and elucidating SAR trends to guide further antifungal drug development.

## 2. MATERIALS AND METHODS

### 2.1 Chemicals, Reagents, and Instruments

All chemicals including o-phenylenediamine, substituted aromatic aldehydes, hydrochloric acid, acetic acid, ethanol, methanol, acetone, dimethyl sulfoxide (DMSO), chloroform, and ethyl acetate were of analytical reagent grade. Microbiological media (Sabouraud Dextrose Agar, Sabouraud Dextrose Broth, and Potato Dextrose Agar) and Fluconazole (standard antifungal) were procured from standard commercial suppliers. Instruments employed included a digital analytical balance, magnetic stirrer with heating mantle, capillary melting point apparatus, TLC chamber with UV visualization, FT-IR spectrophotometer, $^{1}$H-NMR spectrometer (deuterated solvent), mass spectrometer, laminar airflow cabinet, autoclave, and incubator.

### 2.2 Molecular Design

Six 2-substituted benzimidazole derivatives were designed based on medicinal chemistry principles and published SAR data. Substituents were selected to investigate the influence of electron-donating groups (methoxy, methyl, hydroxyl), electron-withdrawing groups (chloro, bromo), and their combinations on antifungal activity. Structural modifications were expected to modulate lipophilicity, electronic distribution, hydrogen bonding capacity, and receptor binding affinity at fungal biological targets.

### 2.3 Synthesis

Benzimidazole derivatives were synthesized by condensation of o-phenylenediamine with appropriate substituted aromatic aldehydes in ethanol as solvent, using glacial acetic acid as catalyst. Stoichiometric quantities of reactants were refluxed under constant magnetic stirring at controlled temperature. Reaction completion was monitored by TLC (ethyl acetate: methanol: chloroform mobile phase system) until

disappearance of starting material spots. On cooling, precipitated products were filtered, washed with cold solvent to remove impurities, and recrystallized from appropriate solvents to yield purified compounds B1–B6.

### 2.4 Characterization

Purified compounds were characterized by: (i) physical observation of color, appearance, and crystal habit; (ii) melting point determination by capillary method; (iii) TLC for purity assessment and Rf determination; (iv) FT-IR spectroscopy for functional group identification; (v) $^1$H-NMR spectroscopy for proton environment confirmation; and (vi) Mass spectrometry for molecular weight verification and fragmentation pattern analysis.

### 2.5 In-Vitro Antifungal Screening

Antifungal activity was assessed using the agar well diffusion method. Fungal strains – Candida albicans, Aspergillus niger, and Aspergillus fumigatus – were inoculated uniformly on Sabouraud Dextrose Agar plates. Wells (6 mm diameter) were bored using a sterile cork borer, and 100 µg/mL solutions of each compound (dissolved in DMSO) were loaded into wells. Fluconazole (100 µg/mL) and DMSO (solvent control) were included on each plate. Plates were incubated at 28°C for 48–72 hours. Zones of inhibition (ZOI, mm) were measured in triplicate and expressed as mean ± SD.

### 2.6 Structure–Activity Relationship Analysis

SAR was analyzed by comparing antifungal activities across the six derivatives in relation to the nature, electronic character, and position of substituent groups on the 2-aryl ring of the benzimidazole nucleus.

## 3. RESULTS

### 3.1 Synthesized Compounds — Identity and Physical Properties

Six benzimidazole derivatives (B1–B6) were successfully synthesized. Table 1 provides compound identities, molecular formulae, molecular weights, key substituents, and physical appearance.

**Table 1:** Identity and physical characteristics of synthesized benzimidazole derivatives (B1–B6)

| Code | Compound Name | Mol. Formula | MW (g/mol) | Substituent | Appearance |
|---|---|---|---|---|---|
| B1 | 2-(4-Methoxyphenyl)-1H-benzimidazole | $C_{14}H_{12}N_2O$ | 224 | 4-Methoxy | Pale yellow crystals |
| B2 | 2-(4-Methylphenyl)-1H-benzimidazole | $C_{14}H_{12}N_2$ | 208 | 4-Methyl | Light brown needles |
| B3 | 2-(3,4-Dimethoxyphenyl)-1H-benzimidazole | $C_{15}H_{14}N_2O_2$ | 254 | 3,4-Dimethoxy | Off-white powder |
| B4 | 2-(4-Chlorophenyl)-1H-benzimidazole | $C_{13}H_9ClN_2$ | 228 | 4-Chloro | Light yellow granules |
| B5 | 2-(4-Hydroxy-3-methoxyphenyl)-1H-benzimidazole | $C_{14}H_{12}N_2O_2$ | 240 | 4-OH, 3-OMe | White crystalline |
| B6 | 2-(4-Bromophenyl)-1H-benzimidazole | $C_{13}H_9BrN_2$ | 273 | 4-Bromo | Cream powder crystals |

### 3.2 Yield and Melting Point

Percentage yields ranged from 68% to 78%, and all compounds showed narrow melting ranges (≤3°C interval), indicative of acceptable purity (Table 2).

**Table 2:** Percentage yield and melting point data

| Compound | Yield (%) | Melting Point (°C) |
|---|---|---|
| B1 | 72 | 182–184 |
| B2 | 68 | 188–190 |
| B3 | 75 | 175–177 |
| B4 | 70 | 191–193 |
| B5 | 78 | 180–182 |
| B6 | 73 | 186–188 |

### 3.3 Thin Layer Chromatography

All compounds exhibited single spots on TLC plates under the mobile phase system (ethyl acetate: methanol: chloroform), confirming satisfactory purity. Rf values ranged from 0.48 to 0.67 (Table 3), with variations attributable to differences in molecular polarity conferred by substituent groups.

**Table 3:** TLC analysis data of synthesized compounds

| Compound | Solvent System | No. of Spots | Rf Value |
|---|---|---|---|
| B1 | EA : MeOH : $CHCl_3$ | 1 | 0.54 |
| B2 | EA : MeOH : $CHCl_3$ | 1 | 0.61 |
| B3 | EA : MeOH : $CHCl_3$ | 1 | 0.48 |
| B4 | EA : MeOH : $CHCl_3$ | 1 | 0.67 |
| B5 | EA : MeOH : $CHCl_3$ | 1 | 0.57 |
| B6 | EA : MeOH : $CHCl_3$ | 1 | 0.63 |

### 3.4 FT-IR Spectral Data

FT-IR analysis of all compounds confirmed characteristic absorption bands corresponding to the benzimidazole nucleus and introduced substituents (Table 4). Key absorptions included N–H stretching (3388–3410 $cm^{-1}$), aromatic C–H stretching (~3052–3075 $cm^{-1}$), C=N stretching (1618–1630 $cm^{-1}$), aromatic C=C (1452–1461 $cm^{-1}$), and C–N stretching (1254–1275 $cm^{-1}$), consistent with expected structures.

**Table 4**: Summary of key FT-IR absorption bands ($cm^{-1}$)

| Compound | N–H Stretch | Ar C–H | C=N Stretch | C=C Aromatic | C–N Stretch |
|---|---|---|---|---|---|
| B1 | 3402 | 3068 | 1625 | 1456 | 1268 |
| B2 | 3388 | 3052 | 1618 | 1461 | 1254 |
| B3 | 3410 | 3075 | 1630 | 1452 | 1275 |
| B4 | 3395 | 3062 | 1622 | 1458 | 1260 |
| B5 | 3408 | 3071 | 1628 | 1459 | 1270 |
| B6 | 3398 | 3059 | 1624 | 1454 | 1263 |

### 3.5 $^1$H-NMR Spectral Data

$^1$H-NMR spectra confirmed the structural integrity of all derivatives (Table 5). Aromatic proton multiplets appeared at δ 7.05–7.96 ppm, consistent with the benzimidazole aromatic system and substituted phenyl ring. N–H singlets were observed at δ 8.88–9.10 ppm, characteristic of benzimidazole N–H environments. Substituent-specific proton signals (methoxy at δ ~3.76–3.95 ppm; methyl at δ ~2.42–2.56 ppm) confirmed successful incorporation of aromatic substituents.

**Table 5**: $^1$H-NMR chemical shift data (δ, ppm)

| Compound | Aromatic H (δ, ppm) | N–H (δ, ppm) | Substituent H (δ, ppm) | Assignment |
|---|---|---|---|---|
| B1 | 7.12–7.85 | 8.95 | 3.82 | $–OCH_3$ |
| B2 | 7.18–7.88 | 9.02 | 2.42 | $–CH_3$ |

| | | | | |
|---|---|---|---|---|
| B3 | 7.05–7.74 | 8.88 | 3.95 | –$OCH_3$ |
| B4 | 7.21–7.96 | 9.10 | – | –Cl (no H) |
| B5 | 7.08–7.82 | 8.91 | 3.76 | –$OCH_3$, –OH |
| B6 | 7.14–7.90 | 9.05 | – | –Br (no H) |

### 3.6 Mass Spectral Data

Mass spectral analysis confirmed molecular identities through observation of $[M+H]^+$ molecular ion peaks closely matching theoretical molecular weights (Table 6). Characteristic fragmentation patterns, including isotopic distributions for halogenated derivatives (B4: Cl pattern; B6: Br pattern), provided further structural evidence.

**Table 6:** Mass spectral data of synthesized compounds

| Compound | Molecular Formula | Expected MW (g/mol) | Observed $[M+H]^+$ (m/z) |
|---|---|---|---|
| B1 | $C_{14}H_{12}N_2O$ | 224 | 225 |
| B2 | $C_{14}H_{12}N_2$ | 208 | 209 |
| B3 | $C_{15}H_{14}N_2O_2$ | 254 | 255 |
| B4 | $C_{13}H_9ClN_2$ | 228 | 229 |
| B5 | $C_{14}H_{12}N_2O_2$ | 240 | 241 |
| B6 | $C_{13}H_9BrN_2$ | 273 | 274 |

### 3.7 In-Vitro Antifungal Activity

Table 7 presents the mean zones of inhibition (mm ± SD, n=3) of all synthesized compounds and Fluconazole against the three fungal strains at 100 µg/mL. DMSO (vehicle control) produced no inhibition (ZOI = 0 mm) for all strains.

**Table 7:** In-vitro antifungal activity — zones of inhibition (mean ± SD, mm) at 100 µg/mL

| Compound | C. albicans | A. niger | A. fumigatus |
|---|---|---|---|
| B1 | 12.0 ± 1.0 | 11.0 ± 1.0 | 10.0 ± 1.0 |
| B2 | 14.0 ± 1.0 | 13.0 ± 1.0 | 12.0 ± 1.0 |
| B3 | 15.0 ± 1.0 | 15.0 ± 1.0 | 14.0 ± 1.0 |
| B4 | 16.0 ± 1.0 | 16.0 ± 1.0 | 15.0 ± 1.0 |
| B5 | 18.0 ± 1.0 | 19.0 ± 1.0 | 18.0 ± 1.0 |
| B6 | 17.0 ± 1.0 | 18.0 ± 1.0 | 17.0 ± 1.0 |
| Fluconazole | 22.0 ± 1.0 | 23.0 ± 1.0 | 22.0 ± 1.0 |
| DMSO (control) | 0 | 0 | 0 |

All six synthesized derivatives demonstrated measurable antifungal activity against all three fungal strains. A clear progression of activity was observed from B1 (lowest) to B5 (highest) across all organisms. Compound B5 exhibited the greatest inhibition for all strains, while B1 showed the lowest. Activity levels were consistently lower than Fluconazole.

## 4. DISCUSSION

### 4.1 Synthetic Efficiency and Characterization

The condensation approach using o-phenylenediamine and substituted aromatic aldehydes proved efficient, yielding all target benzimidazole derivatives in 68–78% yields with satisfactory purity. The narrow melting point ranges (≤3°C interval) and single TLC spots confirmed adequate purity after recrystallization. The combination of FT-IR, $^1$H-NMR, and MS data provided unambiguous structural confirmation of all six compounds. Key infrared absorptions (N–H, C=N, aromatic C–H, C–N) were consistent with the benzimidazole framework, N–H chemical shifts in the δ 8.88–9.10 ppm range reflected the deshielding environment of imidazole N–H protons, and $[M+H]^+$ peaks confirmed molecular masses.

### 4.2 Antifungal Activity and SAR

The antifungal screening results reveal an informative SAR trend across the B1–B6 series. The parent unoptimized derivative with a methoxy substituent (B1) demonstrated the lowest activity (ZOI: 10–12 mm), while B5 bearing both hydroxyl and methoxy groups demonstrated the highest activity (ZOI: 18–19 mm), suggesting that the combination of hydrogen-bond donor (–OH) and electron-donating methoxy groups at the 4- and 3-positions respectively creates a favorable pharmacophoric arrangement for fungal target interaction.

Compound B6 (4-bromophenyl) exhibited the second-strongest activity (ZOI: 17–18 mm), consistent with the well-established principle that halogen substitution — particularly heavier halogens like bromine — increases lipophilicity, thereby enhancing passive diffusion across fungal membranes and improving intracellular target access. The bulkier bromine may also engage in halogen bonding interactions with enzyme active sites. B4 (4-chlorophenyl, ZOI: 15–16 mm) followed, with chlorine conferring similar but slightly less pronounced lipophilicity enhancement compared with bromine.

The dimethoxy derivative B3 (ZOI: 14–15 mm) showed moderate activity, while B2 bearing a 4-methyl group (ZOI: 12–14 mm) was marginally better than B1. The progressive activity ranking — B5 > B6 > B4 > B3 > B2 > B1 — suggests that lipophilicity and hydrogen-bonding capacity together are the most influential determinants of antifungal effectiveness within this series, in agreement with published SAR reports on 2-aryl benzimidazole antifungals.

While all synthesized derivatives demonstrated lower activity than Fluconazole, they serve as valuable lead structures. The gap between the most active compound (B5, ZOI ~18–19 mm) and Fluconazole (~22–23 mm) is not prohibitive and represents a reasonable starting point for further structural optimization through introduction of additional electron-withdrawing substituents, molecular hybridization with triazole or oxadiazole pharmacophores, or formulation improvements.

Consistent with previously published studies by Pathare et al., Pardeshi et al., Jasim et al., and Ibrahim et al., the present findings confirm that rational substituent modification within the 2-aryl benzimidazole series meaningfully modulates antifungal activity. The selectivity trend observed across C. albicans, A. niger, and A. fumigatus was broadly consistent, with A. niger showing slightly higher inhibition for B5 and B6, suggesting possible differences in membrane ergosterol composition or efflux pump expression between species.

## 5. CONCLUSION

Six novel 2-substituted benzimidazole derivatives (B1–B6) were rationally designed, successfully synthesized, and comprehensively characterized. All compounds demonstrated measurable in-vitro antifungal activity against Candida albicans, Aspergillus Niger, and Aspergillus fumigatus. Compound B5 (2-(4-hydroxy-3-methoxyphenyl)-1H-benzimidazole) emerged as the most potent derivative, followed by B6 (2-(4-bromophenyl)-1H-benzimidazole). SAR analysis established that substituents conferring favorable lipophilicity and hydrogen-bonding capability positively correlate with antifungal potency within this structural series.

Although the synthesized compounds exhibited activity below that of Fluconazole at the tested concentration, they represent promising lead molecules with substantial scope for structural optimization. Future investigations should focus on advanced molecular docking studies to delineate binding interactions with ergosterol biosynthesis enzymes, toxicity profiling, in-vivo efficacy studies, and rational second-generation designs incorporating molecular hybridization strategies. The benzimidazole scaffold remains a highly productive platform for discovery of novel antifungal therapeutic agents.

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## CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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