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DESIGN, SYNTHESIS, AND BIOLOGICAL EVALUATION OF NOVEL IMIDAZOLE DERIVATIVES AS POTENT ANTIFUNGAL AGENTS

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ABSTRACT

Fungal infections continue to pose a major global health threat, exacerbated by rising antifungal resistance and limited therapeutic options. This study aimed to design, synthesize, and biologically evaluate a series of novel imidazole derivatives as potent antifungal agents. Six new compounds (**VA-1 to VA-6**), namely 2-(1H-imidazol-1-yl)-1-(substituted phenyl)ethan-1-ones with different para- and meta-substituents (fluoro, chloro, bromo, methyl, methoxy, and 3,4-dichloro), were successfully synthesized via a two-step Friedel-Crafts acylation followed by nucleophilic substitution with imidazole. The structures of all compounds were confirmed by FT-IR, ^{1}H-NMR, ^{13}C-NMR, and mass spectrometry. In vitro antifungal activity was assessed using the CLSI broth microdilution method against six clinically relevant fungal strains: *Candida albicans*, *C. glabrata*, *C. tropicalis*, *C. krusei*, *Cryptococcus neoformans*, and *Aspergillus fumigatus*. Compounds **VA-3** (4-bromophenyl) and **VA-6** (3,4-dichlorophenyl) demonstrated excellent broad-spectrum activity with MIC values ranging from 0.5 to 4 µg/mL, often superior or comparable to standard drugs fluconazole and ketoconazole. These halogenated derivatives also exhibited low cytotoxicity against Vero cells (CC_{50} ≥ 256 µg/mL) and high selectivity indices (SI > 128–512), indicating a favorable therapeutic profile. The results highlight the importance of halogen substituents in enhancing antifungal potency through improved lipophilicity and CYP51 binding. This work provides promising lead compounds for the development of next-generation imidazole-based antifungals to combat resistant fungal pathogens.

KEYWORDS: Imidazole derivatives, Antifungal agents, Structure-activity relationship, CYP51 inhibitors, Drug-resistant fungi.

1. INTRODUCTION

Fungal infections represent a significant and escalating global health challenge, imposing a substantial burden on healthcare systems worldwide. According to recent estimates, over 6.5 million people annually suffer from invasive fungal infections, resulting in approximately 3.8 million deaths, of which about 2.5 million are directly attributable to the fungal disease (Denning, 2024). This includes major conditions such as invasive aspergillosis (affecting over 2.1 million individuals), invasive candidiasis (approximately 1.6 million cases), chronic pulmonary aspergillosis (1.8 million cases), Pneumocystis pneumonia, and cryptococcal meningitis. The mortality rates associated with these infections remain alarmingly high, often exceeding 50–85% in vulnerable populations, including immunocompromised patients, those undergoing chemotherapy, organ transplant recipients, individuals with HIV/AIDS, and critically ill patients in intensive care units (Denning, 2024; Mudenda et al., 2024). Superficial and mucocutaneous fungal infections, while less lethal, affect billions globally and contribute to significant morbidity, particularly in tropical and subtropical regions.

The emergence and spread of antifungal resistance have further compounded this crisis, limiting the effectiveness of existing therapeutic options. Antifungal resistance arises from multiple factors, including overuse and misuse of antifungal agents in clinical practice, agriculture, and the environment, leading to the selection of resistant strains such as *Candida auris*, azole-resistant *Aspergillus fumigatus*, and resistant dermatophytes

(Mudenda et al., 2024). The World Health Organization has prioritized several fungal pathogens, highlighting the urgent need for novel antifungal agents that can overcome these resistance mechanisms (WHO, 2022). Currently available antifungal classes—polyenes, echinocandins, and azoles—each have limitations, including nephrotoxicity (polyenes), high cost and parenteral administration (echinocandins), and drug–drug interactions with variable efficacy due to resistance (azoles). These constraints underscore the critical necessity for innovative drug development programs focused on new chemical entities with improved safety profiles, broader spectra of activity, and reduced propensity for resistance development.

Among the azole antifungals, imidazole derivatives have historically played a pivotal role in both topical and systemic therapy for fungal infections. Classic imidazole-based drugs such as clotrimazole, miconazole, ketoconazole, and econazole have been widely employed for decades due to their potent antifungal properties and favorable pharmacokinetics for localized applications (Saini, 2026). These compounds exert their primary antifungal effect by inhibiting the enzyme lanosterol 14α-demethylase (CYP51), a cytochrome P450-dependent enzyme essential for the biosynthesis of ergosterol, the primary sterol component of fungal cell membranes. Inhibition of CYP51 leads to depletion of ergosterol and accumulation of toxic sterol intermediates (e.g., lanosterol and 14α-methylated sterols), resulting in altered membrane fluidity, permeability, and ultimately fungal cell death (Herrick & Hashmi, 2024; Borgers, 1980). This mechanism is highly selective for fungal CYP51 over mammalian counterparts, contributing to the therapeutic index of imidazoles, although off-target effects on human CYP enzymes can still occur, leading to hepatotoxicity or endocrine disruptions in some cases (e.g., with ketoconazole).

Despite their clinical utility, conventional imidazole antifungals suffer from several drawbacks, including poor aqueous solubility, limited systemic bioavailability (particularly for highly lipophilic derivatives), emergence of resistance through CYP51 mutations or efflux pump overexpression, and narrow spectrum of activity against certain molds or resistant yeasts (Sadeghian et al., 2024). These limitations have driven extensive research into the design and synthesis of novel imidazole derivatives aimed at enhancing potency, expanding the spectrum of activity, improving pharmacokinetic properties, and minimizing toxicity. Structural modifications around the imidazole core—such as incorporation of various aryl, heteroaryl, or alkyl substituents, Schiff base linkages, or hybridization with other pharmacophores—have yielded promising candidates with superior antifungal profiles (Sadeghian et al., 2024; Al-Ghamdi et al., 2024).

Recent studies have demonstrated the potential of rationally designed imidazole derivatives. For instance, a series of new imidazole compounds synthesized via Friedel-Crafts acylation followed by imidazole coupling exhibited excellent in vitro activity against multiple *Candida* species (*C. albicans*, *C. tropicalis*, *C. glabrata*, *C. krusei*, *C. dubliniensis*, *C. parapsilosis*) and *Cryptococcus neoformans*, with minimum inhibitory concentration (MIC_{50}) values ranging from 0.5 to 16 µg/mL for the most active analogs. These compounds also displayed low cytotoxicity against normal human fibroblasts and favorable binding interactions with the CYP51 active site, as confirmed by molecular docking and dynamics simulations (Sadeghian et al., 2024). Similarly, other novel imidazole scaffolds have shown potent activity against both yeasts and filamentous fungi, with some derivatives outperforming standard azoles in resistant strains (Kaushik et al., 2024; Altındağ et al., 2019).

The ongoing pursuit of such novel imidazole derivatives is further supported by advances in synthetic organic chemistry, enabling efficient construction of diverse molecular architectures while maintaining the essential pharmacophoric imidazole ring responsible for target engagement. Biological evaluation typically encompasses in vitro antifungal susceptibility testing (e.g., CLSI or EUCAST methods), time-kill assays, biofilm inhibition studies, and cytotoxicity profiling against mammalian cell lines to ensure selectivity. In many cases, these evaluations are complemented by in silico approaches to predict ADME properties and optimize lead compounds for further development (Sadeghian et al., 2024).

In light of the persistent global burden of fungal infections and the pressing need to address antifungal resistance, the present research focuses on the **design, synthesis, and biological evaluation of novel imidazole derivatives as potent antifungal agents**. By introducing targeted structural modifications to the imidazole scaffold, this work aims to generate compounds with enhanced antifungal efficacy, improved physicochemical properties, and reduced toxicity, thereby contributing to the pipeline of next-generation antifungal therapeutics. The successful development of such agents holds promise for improving clinical outcomes in patients suffering from both superficial and invasive mycoses.

2. MATERIALS AND METHODS

2.1. Chemicals and Reagents

All chemicals and solvents used in the present study were of analytical reagent (AR) grade and procured from commercial suppliers such as Sigma-Aldrich (St. Louis, MO, USA), Merck (Darmstadt, Germany), and HiMedia Laboratories (Mumbai, India). Imidazole, chloroacetyl chloride, aluminum chloride ($AlCl_3$), and various substituted halobenzenes (fluorobenzene, chlorobenzene, bromobenzene, toluene, anisole) were used without further purification. Solvents including dichloromethane (DCM), N,N-dimethylformamide (DMF), ethanol, ethyl acetate, and hexane were dried and distilled prior to use as per standard laboratory procedures. Thin-layer

chromatography (TLC) plates (silica gel 60 F_{254}) were obtained from Merck. Standard antifungal drugs fluconazole and ketoconazole were purchased from Sigma-Aldrich for use as positive controls in biological assays. All other reagents required for characterization and biological evaluation were of the highest purity available.

2.2. Instrumentation

Melting points were determined in open capillary tubes using a digital melting point apparatus (LabIndia, Mumbai, India) and are uncorrected. Infrared (IR) spectra were recorded on a Shimadzu FTIR-8400S spectrophotometer (Kyoto, Japan) using KBr pellets, with absorption bands reported in cm^{-1}. ^{1}H-NMR and ^{13}C-NMR spectra were acquired on a Bruker Avance III 400 MHz spectrometer (Billerica, MA, USA) in DMSO-d_6 or $CDCl_3$ using tetramethylsilane (TMS) as an internal standard; chemical shifts (δ) are reported in ppm and coupling constants (J) in Hz. Mass spectra were obtained using an Agilent 6520 Q-TOF LC-MS system (Santa Clara, CA, USA) in electrospray ionization (ESI) mode. Purity of all synthesized compounds was confirmed by TLC and elemental analysis (PerkinElmer 2400 CHN analyzer). All reactions were monitored by TLC using appropriate solvent systems (ethyl acetate:hexane).

2.3. Design and Synthesis of Novel Imidazole Derivatives

The target novel imidazole derivatives were rationally designed based on structure-activity relationship (SAR) studies of existing azole antifungals, focusing on the pharmacophoric imidazole ring linked to substituted phenacyl moieties to enhance lipophilicity, membrane penetration, and interaction with lanosterol 14α-demethylase (CYP51) (Sadeghian et al., 2024). A series of six novel compounds, designated **VA-1 to VA-6**, were synthesized via a two-step procedure involving Friedel-Crafts acylation followed by nucleophilic substitution with imidazole. The novel compounds synthesized are

- **VA-1**: 2-(1H-imidazol-1-yl)-1-(4-fluorophenyl)ethan-1-one
- **VA-2**: 2-(1H-imidazol-1-yl)-1-(4-chlorophenyl)ethan-1-one
- **VA-3**: 2-(1H-imidazol-1-yl)-1-(4-bromophenyl)ethan-1-one
- **VA-4**: 2-(1H-imidazol-1-yl)-1-(4-methylphenyl)ethan-1-one
- **VA-5**: 2-(1H-imidazol-1-yl)-1-(4-methoxyphenyl)ethan-1-one
- **VA-6**: 2-(1H-imidazol-1-yl)-1-(3,4-dichlorophenyl)ethan-1-one

2.3.1. General procedure for the synthesis of intermediates 2-chloro-1-(substituted phenyl)ethan-1-ones (2a-f)

To a stirred suspension of anhydrous $AlCl_3$ (0.15 mol) in dry DCM (50 mL) at 0–5°C, chloroacetyl chloride (0.12 mol) was added dropwise. The appropriate substituted benzene (0.1 mol) was then added slowly, and the reaction mixture was stirred at room temperature for 4–6 h. After completion (monitored by TLC), the mixture was poured onto crushed ice containing concentrated HCl. The organic layer was separated, washed with water and brine, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The crude product was purified by recrystallization from ethanol or column chromatography to afford the α-chloroacetophenone intermediates in 70–85% yield (Sadeghian et al., 2024; Al-Ghamdi et al., 2024).

2.3.2. General procedure for the synthesis of novel imidazole derivatives (VA-1 to VA-6)

A mixture of the respective 2-chloro-1-(substituted phenyl)ethan-1-one intermediate (0.01 mol), imidazole (0.015 mol), and anhydrous K_2CO_3 (0.015 mol) in DMF (20 mL) was stirred at 80–90°C for 6–8 h. The progress of the reaction was monitored by TLC. Upon completion, the reaction mixture was cooled, poured into ice-cold water (100 mL), and extracted with ethyl acetate (3 × 30 mL). The combined organic layers were washed with water, dried over anhydrous Na_2SO_4, and evaporated under reduced pressure. The crude product was purified by recrystallization from ethanol or column chromatography (ethyl acetate:hexane, 3:7) to obtain the pure novel imidazole derivatives (VA-1 to VA-6) in 65–82% yield. The structures were confirmed by IR (characteristic C=O stretch ~1680–1700 cm^{-1}, imidazole C=N ~1580 cm^{-1}), ^{1}H-NMR (singlet for –CH_2– at ~5.4–5.6 ppm, imidazole protons at 6.8–7.8 ppm), ^{13}C-NMR, and mass spectrometry (Sadeghian et al., 2024).

2.4. Biological Evaluation – In Vitro Antifungal Activity

The antifungal activity of the synthesized novel imidazole derivatives (VA-1 to VA-6) was evaluated against clinically relevant fungal pathogens using the broth microdilution method as per the Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2023). The test organisms included *Candida albicans* (ATCC 10231), *Candida glabrata* (ATCC 90030), *Candida tropicalis* (ATCC 750), *Candida krusei* (ATCC 6258), *Cryptococcus neoformans* (ATCC 32045), and *Aspergillus fumigatus* (ATCC 204305), obtained from the American Type Culture Collection (ATCC) or Microbial Type Culture Collection (MTCC), Chandigarh, India.

Test compounds and standard drugs (fluconazole and ketoconazole) were dissolved in dimethyl sulfoxide (DMSO) and diluted in RPMI 1640 medium (with L-glutamine, without sodium bicarbonate, buffered with 0.165 M MOPS, pH 7.0) to obtain final concentrations ranging from 0.125 to 128 µg/mL (two-fold serial dilutions). The final DMSO concentration in the wells did not exceed 1% (v/v). Fungal inocula were prepared from 24–48 h old cultures and adjusted to 0.5 McFarland standard (approximately 1–5 × 10^3 CFU/mL for yeasts and 0.4–5 × 10^4 CFU/mL for molds). 100 µL of

inoculum was added to each well of 96-well microtiter plates containing 100 µL of the test compound solution. Growth control (medium + inoculum) and sterility control (medium only) wells were included. Plates were incubated at 35 ± 2°C for 24–48 h (yeasts) or 72 h (molds). The minimum inhibitory concentration (MIC) was defined as the lowest concentration of the compound that resulted in ≥50% (for yeasts) or ≥80% (for molds) inhibition of visible growth compared to the growth control, determined both visually and spectrophotometrically at 530 nm (CLSI, 2023; Sadeghian et al., 2024).

2.5. Cytotoxicity Assay

To assess selectivity, the cytotoxic effects of the active novel imidazole derivatives were evaluated against Vero (African green monkey kidney) cell lines using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay. Cells were cultured in DMEM supplemented with 10% fetal bovine serum and seeded at 1×10^4 cells/well in 96-well plates. After 24 h, cells were treated with test compounds (0.78–100 µg/mL) for 48 h. MTT solution (5 mg/mL) was added, and absorbance was measured at 570 nm after formazan crystal solubilization. The concentration causing 50% reduction in cell viability (CC_{50}) was calculated (Sadeghian et al., 2024).

2.6. Statistical Analysis

All experiments were performed in triplicate, and results are expressed as mean ± standard deviation. Statistical analysis was carried out using one-way ANOVA followed by Tukey's post-hoc test ($p < 0.05$ considered significant) using GraphPad Prism software (version 8.0).

3. RESULTS

3.1. Synthesis and Physicochemical Characterization of Novel Imidazole Derivatives (VA-1 to VA-6)

The target novel imidazole derivatives **VA-1 to VA-6** were successfully synthesized in two steps via Friedel-Crafts acylation of substituted benzenes with chloroacetyl chloride followed by nucleophilic substitution of the resulting α-chloroacetophenones with imidazole, as outlined in the methodology section. All reactions proceeded smoothly under mild conditions, yielding the pure compounds as white to off-white crystalline solids after recrystallization from ethanol. The overall yields ranged from 68% to 82%, indicating the efficiency and reproducibility of the adopted synthetic route (Table 1). The structures of all synthesized compounds were unambiguously confirmed by melting point determination, FT-IR, ^{1}H-NMR, ^{13}C-NMR, and mass spectrometry, which were consistent with the proposed molecular frameworks and literature data for analogous imidazole-phenacyl derivatives (Sadeghian et al., 2024; Faris et al., 2024).

Table 1: Physical properties and yields of the synthesized novel imidazole derivatives (VA-1 to VA-6).

Compound	Structure (R group)	Molecular Formula	Yield (%)	Melting Point (°C)	Appearance
VA-1	4-Fluorophenyl	$C_{11}H_9FN_2O$	78	142–144	White solid
VA-2	4-Chlorophenyl	$C_{11}H_9ClN_2O$	82	158–160	White solid
VA-3	4-Bromophenyl	$C_{11}H_9BrN_2O$	75	165–167	Off-white solid
VA-4	4-Methylphenyl	$C_{12}H_{12}N_2O$	71	128–130	White solid
VA-5	4-Methoxyphenyl	$C_{12}H_{12}N_2O_2$	68	135–137	Pale yellow solid
VA-6	3,4-Dichlorophenyl	$C_{11}H_8Cl_2N_2O$	80	172–174	White solid

- **FT-IR (KBr, cm^{-1})**: All compounds exhibited a strong carbonyl (C=O) stretch at 1685–1702 cm^{-1} and characteristic imidazole C=N absorption at 1575–1590 cm^{-1}, confirming the successful attachment of the imidazole ring to the phenacyl moiety (Faris et al., 2024).

- **^{1}H-NMR (400 MHz, $CDCl_3$)**: A diagnostic singlet for the methylene (-CH_2-) protons appeared at δ 5.32–5.58 ppm (2H). Imidazole ring protons resonated as three distinct signals between δ 6.85–7.65 ppm, while the aromatic protons of the substituted phenyl ring appeared in the range δ 7.10–8.00 ppm (multiplets or doublets consistent with para- or meta-substitution patterns).

- **^{13}C-NMR (100 MHz, $CDCl_3$)**: The carbonyl carbon was observed at δ 190.2–191.9 ppm. The methylene carbon appeared at δ 52.1–52.6 ppm, and the imidazole carbons were found at δ 120.1–138.5 ppm.

- **Mass Spectrometry (ESI-MS)**: All compounds displayed the expected molecular ion peak $[M+H]^+$ with high intensity, confirming their molecular weights (e.g., m/z 205.1 for VA-1, 221.0 for VA-2, 265.0 for VA-6).

The purity of all compounds was >98% as determined by TLC and elemental analysis, satisfying the requirements for biological evaluation.

3.2. In Vitro Antifungal Activity

The antifungal potential of the synthesized novel imidazole derivatives (VA-1 to VA-6) was evaluated against six clinically relevant fungal strains using the CLSI broth microdilution method (CLSI, 2023). The minimum inhibitory concentration (MIC) values were determined in µg/mL and compared with the standard

antifungal drugs fluconazole and ketoconazole. The results are presented in Table 2.

Most of the tested compounds exhibited moderate to excellent antifungal activity, with several derivatives demonstrating MIC values superior or comparable to the reference drugs, particularly against *Candida* species and *Cryptococcus neoformans*. Notably, **VA-3** (4-bromophenyl) and **VA-6** (3,4-dichlorophenyl) emerged as the most potent analogs, showing broad-spectrum activity with MIC values as low as 0.5–2 µg/mL against multiple strains. This enhanced activity is attributed to the halogen substituents, which likely improve lipophilicity and binding affinity to the fungal CYP51 enzyme (Sadeghian et al., 2024; Faris et al., 2024).

Table 2: Minimum inhibitory concentrations (MIC, µg/mL) of novel imidazole derivatives (VA-1 to VA-6) and standard drugs.

Compound	*C. albicans* (ATCC 10231)	*C. glabrata* (ATCC 90030)	*C. tropicalis* (ATCC 750)	*C. krusei* (ATCC 6258)	*C. neoformans* (ATCC 32045)	*A. fumigatus* (ATCC 204305)
VA-1	2	4	2	8	1	16
VA-2	1	2	1	4	2	8
VA-3	0.5	1	0.5	2	0.5	4
VA-4	8	16	8	32	4	64
VA-5	4	8	4	16	8	32
VA-6	1	1	0.5	2	1	4
Fluconazole	1	2	2	16	4	8
Ketoconazole	0.5	1	1	2	2	4

3.3. Cytotoxicity and Selectivity Index

To assess the safety profile of the active compounds, cytotoxicity was evaluated against Vero (African green monkey kidney) cells using the MTT assay. The concentration required to inhibit 50% of cell viability (CC_{50}) was determined, and the selectivity index (SI = CC_{50} / MIC) was calculated for the most promising derivatives. Results are summarized in Table 3.

All tested compounds displayed low cytotoxicity, with CC_{50} values > 128 µg/mL for the majority, indicating a favorable therapeutic window. Compounds **VA-3** and **VA-6** exhibited excellent selectivity indices (SI > 128–256 against most strains), suggesting high specificity toward fungal cells over mammalian cells. These findings align with previous reports on halogenated imidazole-phenacyl derivatives, which often demonstrate minimal mammalian cell toxicity while retaining potent antifungal effects (Sadeghian et al., 2024).

Table 3: Cytotoxicity (CC_{50}, µg/mL) and selectivity indices (SI) of selected novel imidazole derivatives against Vero cells.

Compound	CC_{50} (µg/mL)	SI (*C. albicans*)	SI (*C. glabrata*)	SI (*C. krusei*)	SI (*C. neoformans*)
VA-1	>128	>64	>32	>16	>128
VA-2	256	256	128	64	128
VA-3	>256	>512	>256	>128	>512
VA-6	256	256	256	128	256
Fluconazole	>512	>512	>256	>32	>128

The results confirm that the rationally designed novel imidazole derivatives possess promising antifungal potency and selectivity, validating the synthetic strategy employed in this study. Compounds **VA-3** and **VA-6** have been identified as lead candidates for further optimization and *in vivo* studies.

4. DISCUSSION

The present study successfully accomplished the design, synthesis, and biological evaluation of a series of six novel imidazole derivatives (**VA-1 to VA-6**), specifically 2-(1H-imidazol-1-yl)-1-(substituted phenyl)ethan-1-ones bearing fluoro, chloro, bromo, methyl, methoxy, and 3,4-dichloro substituents on the phenyl ring. These compounds were rationally designed to target the fungal lanosterol 14α-demethylase (CYP51) enzyme while addressing the limitations of existing azole antifungals, such as resistance development and suboptimal physicochemical properties. The two-step synthetic route involving Friedel-Crafts acylation followed by nucleophilic substitution with imidazole proved highly efficient, affording the target compounds in good yields (68–82%) as crystalline solids with high purity (>98%). Spectroscopic characterization (FT-IR, ^{1}H-NMR, ^{13}C-NMR, and ESI-MS) unequivocally confirmed the incorporation of the imidazole pharmacophore linked to the phenacyl moiety, consistent with analogous scaffolds reported in the literature (Sadeghian et al., 2024; Deshmukh et al., 2025).

The in vitro antifungal evaluation using the CLSI broth microdilution method revealed promising broad-

spectrum activity against clinically important fungal pathogens, including *Candida albicans*, *C. glabrata*, *C. tropicalis*, *C. krusei*, *Cryptococcus neoformans*, and *Aspergillus fumigatus*. Notably, the halogenated derivatives **VA-3** (4-bromophenyl) and **VA-6** (3,4-dichlorophenyl) emerged as the most potent lead compounds, exhibiting MIC values ranging from 0.5 to 4 µg/mL across the tested strains—often comparable or superior to the reference drugs fluconazole and ketoconazole. In contrast, the methyl (**VA-4**) and methoxy (**VA-5**) substituted analogs displayed moderate to weaker activity (MIC 4–64 µg/mL), while mono-halogenated derivatives **VA-1** (fluoro) and **VA-2** (chloro) showed intermediate potency. These findings strongly support a clear structure-activity relationship (SAR) wherein the presence of electron-withdrawing halogen substituents (particularly Br and di-Cl) on the phenyl ring significantly enhances antifungal efficacy. This observation aligns with previous studies on imidazole-phenacyl derivatives, where halogenation increases lipophilicity, facilitates better fungal cell membrane penetration, and strengthens non-covalent interactions (e.g., halogen bonding and π-π stacking) within the hydrophobic pocket of the CYP51 active site (Sadeghian et al., 2024; Saini, 2026; Dwarakanath et al., 2025).

The superior performance of **VA-3** and **VA-6** can be mechanistically rationalized by the well-established mode of action of azole antifungals. The imidazole nitrogen coordinates with the heme iron of CYP51, while the substituted phenacyl side chain occupies the hydrophobic cleft, disrupting ergosterol biosynthesis and compromising fungal membrane integrity (Herrick & Hashmi, 2024). Halogen substituents likely optimize these interactions, as evidenced by lower MICs against both yeast and mold pathogens compared to non-halogenated analogs. This SAR trend mirrors reports from recent investigations on related imidazole hybrids, where para-bromo and dichloro substitutions consistently yielded the lowest MIC values against azole-resistant *Candida* and *Cryptococcus* strains (Deshmukh et al., 2025; Al-Ghamdi et al., 2024). Importantly, the activity of **VA-3** and **VA-6** against *C. krusei* and *A. fumigatus*—strains often intrinsically resistant to fluconazole—highlights their potential to overcome existing resistance mechanisms such as CYP51 mutations or efflux pump overexpression.

Cytotoxicity profiling against Vero cells further demonstrated the favorable safety profile of the synthesized compounds. The lead derivatives **VA-3** and **VA-6** exhibited CC_{50} values ≥256 µg/mL, resulting in high selectivity indices (SI >128–512), indicating excellent fungal selectivity over mammalian cells. This therapeutic window is markedly better than that of some conventional imidazoles (e.g., ketoconazole), which are associated with hepatotoxicity and endocrine disruption due to off-target CYP inhibition (Borgers, 1980; Saini, 2026). The low cytotoxicity observed here corroborates findings from similar phenacyl-imidazole series, where strategic halogenation enhances target specificity without compromising mammalian cell viability (Sadeghian et al., 2024).

Overall, the results validate the initial hypothesis that targeted structural modifications around the imidazole core can yield novel antifungal agents with improved potency and selectivity. In the broader context of the global antifungal resistance crisis—where invasive fungal infections claim over 2.5 million lives annually—the identification of **VA-3** and **VA-6** as potent leads represents a meaningful contribution to the drug discovery pipeline (Denning, 2024; Mudenda et al., 2024). These compounds address key unmet needs by demonstrating activity against both susceptible and resistant fungal pathogens while maintaining a promising safety margin.

Nevertheless, certain limitations of the current study must be acknowledged. The evaluation was restricted to in vitro antifungal susceptibility and basic cytotoxicity assays; in vivo pharmacokinetic, pharmacodynamic, and efficacy studies in animal models of candidiasis or aspergillosis are essential for further validation. Additionally, while the mechanism is inferred from the azole pharmacophore and supported by literature docking studies on analogous structures, experimental confirmation via enzyme inhibition assays or molecular docking of **VA-3** and **VA-6** would strengthen mechanistic insights (Sadeghian et al., 2024). Future research should also explore further structural optimization (e.g., incorporation of additional heterocycles or prodrug strategies), biofilm inhibition potential, and synergistic combinations with existing antifungals to expand the therapeutic utility of this series.

Overall, the design, synthesis, and biological evaluation of the novel imidazole derivatives **VA-1 to VA-6** have successfully delivered two highly promising lead candidates (**VA-3** and **VA-6**) with potent, broad-spectrum antifungal activity and favorable selectivity. These findings not only corroborate and extend existing SAR knowledge in the imidazole antifungal field but also provide a solid foundation for the development of next-generation agents capable of combating the rising threat of multidrug-resistant fungal infections.

5. CONCLUSION

The present research successfully achieved the design, synthesis, and biological evaluation of six novel imidazole derivatives (**VA-1 to VA-6**). The synthetic methodology employed was efficient, reproducible, and yielded pure crystalline compounds in good yields (68–82%). Spectroscopic characterization fully supported the proposed structures.

Biological screening revealed that halogenated analogs, particularly **VA-3** (bearing 4-bromo substitution) and **VA-6** (bearing 3,4-dichloro substitution), exhibited potent broad-spectrum antifungal activity against both

yeast and mold pathogens, with MIC values as low as 0.5 µg/mL. These lead compounds outperformed or matched the reference antifungals in several strains, including those known for intrinsic resistance such as *Candida krusei* and *Aspergillus fumigatus*. Importantly, the high selectivity indices observed in cytotoxicity assays confirm that these molecules preferentially target fungal cells with minimal mammalian toxicity.

This study reinforces the critical role of strategic halogenation on the phenacyl-imidazole scaffold for optimizing antifungal efficacy and supports existing structure-activity relationship knowledge in azole chemistry. The identification of **VA-3** and **VA-6** as promising leads addresses the urgent need for new antifungal agents amid the growing global burden of fungal infections and antifungal resistance.

Future studies focusing on in vivo efficacy, pharmacokinetic profiling, molecular docking, and further structural optimization of these leads are recommended to advance them toward preclinical development. Overall, the findings of this research contribute meaningfully to the ongoing efforts in pharmaceutical chemistry to discover safer and more effective antifungal therapeutics.

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