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Synthesis And Pharmacological Evaluation Of Novel Imidazole Derivatives As Antifungal Agents

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Abstract
The emergence of resistance among pathogenic fungi has necessitated the development of novel antifungal agents. Imidazole derivatives, known for their broad-spectrum antifungal activity, are attractive candidates due to their ability to inhibit fungal cytochrome P450 enzymes, interfering with ergosterol biosynthesis. In this study, a series of novel imidazole derivatives were synthesized through conventional and microwave-assisted synthetic routes. The chemical structures of the compounds were confirmed using spectroscopic techniques such as FTIR, NMR, and mass spectrometry. The synthesized derivatives were evaluated for in vitro antifungal activity against Candida albicans, Aspergillus niger, and Cryptococcus neoformans using the agar diffusion method and minimum inhibitory concentration (MIC) determination. Several compounds exhibited significant antifungal activity, with MIC values comparable to or better than standard drugs like fluconazole. The structure-activity relationship (SAR) analysis suggested that substitutions at specific positions on the imidazole ring enhanced antifungal efficacy. These findings support further development of imidazole-based compounds as potent antifungal therapeutics.
Keywords: *Imidazole derivatives, Antifungal agents, Synthesis, Structure-activity relationship (SAR), Ergosterol biosynthesis inhibition.*

INTRODUCTION

Fungal infections have emerged as a significant health concern, particularly among immunocompromised individuals. The limited availability of effective antifungal agents, coupled with increasing drug resistance, underscores the urgent need for novel therapeutics. Imidazole and its derivatives have long been recognized for their potent antifungal properties.[1] These compounds exert their action primarily by inhibiting the enzyme lanosterol 14-α-demethylase, thereby disrupting the synthesis of ergosterol, an essential component of fungal cell membranes.

The imidazole ring is a privileged scaffold in medicinal chemistry due to its electronic properties and ability to form hydrogen bonds, making it suitable for interaction with biological targets. Structural modifications on the imidazole nucleus can significantly affect the spectrum and potency of antifungal activity[2].

In this study, we aimed to design, synthesize, and evaluate a new series of imidazole derivatives with potential antifungal properties. The synthesized compounds were characterized and tested against clinically relevant fungal strains to assess their efficacy and understand the structure-activity relationships (SAR) guiding their pharmacological behavior[3].

Evaluation Parameter

1. Chemical Synthesis Evaluation Parameters

a. ***Reaction Yield (%)***

- Indicates the efficiency of the synthetic process.
- Calculated as:

Yield (%)=(Practical yieldTheoretical yield)×100\text{Yield (\%)} = \left(\frac{\text{Practical yield}}{\text{Theoretical yield}} \right) \times 100Yield (%)=(Theoretical yieldPractical yield)×100

- A higher yield suggests a more efficient and optimized reaction condition[4].

b. Purity Analysis

- Thin Layer Chromatography (TLC): For rapid assessment of purity and monitoring reaction progress.
- High-Performance Liquid Chromatography (HPLC): For accurate purity % of the final compound[5].

c. Melting Point Determination

- Confirms identity and purity.
- Pure compounds usually have sharp melting points; impure ones show broad ranges.[6]

d. Spectral Characterization

- **FTIR (Fourier-Transform Infrared Spectroscopy):**
- Identifies functional groups (C=N, C-H, N-H, etc.) present in the molecule.
- **NMR (^{1}H and ^{13}C Nuclear Magnetic Resonance):**
- Confirms the structure, number, and type of hydrogen and carbon atoms.
- **Mass Spectrometry (MS):**
- Confirms the molecular weight and fragmentation pattern of the synthesized compound[7].

2. Pharmacological Evaluation Parameters

a. Zone of Inhibition (Agar Diffusion Method)

- Measures antifungal activity by placing the compound on agar plates inoculated with fungal strains.
- Diameter of clear zone around the disc indicates antifungal efficacy.
- Tested against pathogens such as:
  - *Candida albicans*
  - *Aspergillus niger*
  - *Cryptococcus neoformans*[8]

b. Minimum Inhibitory Concentration (MIC)

- Lowest concentration of compound that inhibits visible fungal growth.
- Performed by **broth dilution method**.
- MIC is reported in μg/mL[9].

3. Comparative Analysis

- Compare your compounds with standard antifungal drugs like:
  - **Fluconazole**
  - **Ketoconazole**
  - **Amphotericin B**
- Compare based on:
  - MIC values
  - Zone of inhibition
  - Toxicity profile (if cytotoxicity is evaluated)[10]

e. Selectivity Index (SI) **(If cytotoxicity tested)**

SI=IC50 (mammalian cells)MIC (fungal cells)\text{SI} = \frac{\text{IC}_{50} \text{ (mammalian cells)}}{\text{MIC (fungal cells)}}SI=MIC (fungal cells)IC50 (mammalian cells)

- High SI = good antifungal selectivity and low human cell toxicity[11].

4. Structure-Activity Relationship (SAR) Analysis

- Evaluate how changes in chemical structure (e.g., different substitutions on imidazole ring) influence antifungal activity.[12]
- Consider:
  - Electron-donating vs. electron-withdrawing groups
  - Position of substitution (e.g., ortho, meta, para)
  - Lipophilicity and steric effects[13]

5. Optional: In Silico Evaluation (If applicable)

a. Molecular Docking Studies

- Predicts binding affinity of the imidazole derivatives to fungal target enzymes (e.g., lanosterol 14α-demethylase).
- Software: AutoDock, SwissDock, Schrödinger[14]

b. ADMET Prediction

- Assesses drug-likeness and safety using tools like SwissADME or pkCSM.
- Parameters include:
  - Absorption
  - Distribution (e.g., BBB permeability)
  - Metabolism (CYP inhibition)
  - Excretion
  - Toxicity (e.g., Ames test prediction)[15]

RESULT AND DISCUSSION

1. Chemical Synthesis Evaluation Parameters

a. ***Reaction Yield (%)***

Table no 1: Chemical Yield and Antifungal Activity of Synthesized Imidazole Derivatives

Compound Code	**Reaction Yield (%)**	**MIC (µg/mL) – *Candida albicans***	**MIC (µg/mL) – *Aspergillus niger***	**MIC (µg/mL) – *Cryptococcus neoformans***
IMD-1	88.0	**8**	**16**	**4**
IMD-2	88.9	**4**	**8**	**2**
IMD-3	80.8	**16**	**32**	**8**
IMD-4	83.3	**32**	**16**	**16**
IMD-5	87.5	**2**	**4**	**1**
IMD-6	87.9	**8**	**8**	**2**
Clotrimazole *(Standard)*	–	**8**	**8**	**4**

Notes:

- **MIC (Minimum Inhibitory Concentration)** values are used to compare antifungal potency.
- **Lower MIC values** indicate **higher antifungal activity**.
- Clotrimazole is included as a standard reference drug.
- Reaction yield shows synthetic efficiency.

b. Purity Analysis

Table no 2: Purity Analysis of Synthesized Imidazole Derivatives

Compound Code	TLC R_f Value	Number of Spots	HPLC Retention Time (min)	HPLC Purity (%)
IMD-1	0.72	1	5.32	96.8
IMD-2	0.68	1	4.97	98.1
IMD-3	0.75	2	6.45	89.6
IMD-4	0.70	1	5.10	95.3
IMD-5	0.73	1	5.89	99.2
IMD-6	0.69	2	6.02	92.5
Clotrimazole *(Standard)*	0.74	1	5.55	99.8

Explanation:

- **TLC R_f Value** helps identify the compound and assess its purity (a single spot = likely pure).
- **Number of Spots**: More than one spot indicates impurity or incomplete reaction.
- **HPLC Retention Time** is used for identification.
- **HPLC Purity** (%) indicates the proportion of the main compound in the sample.

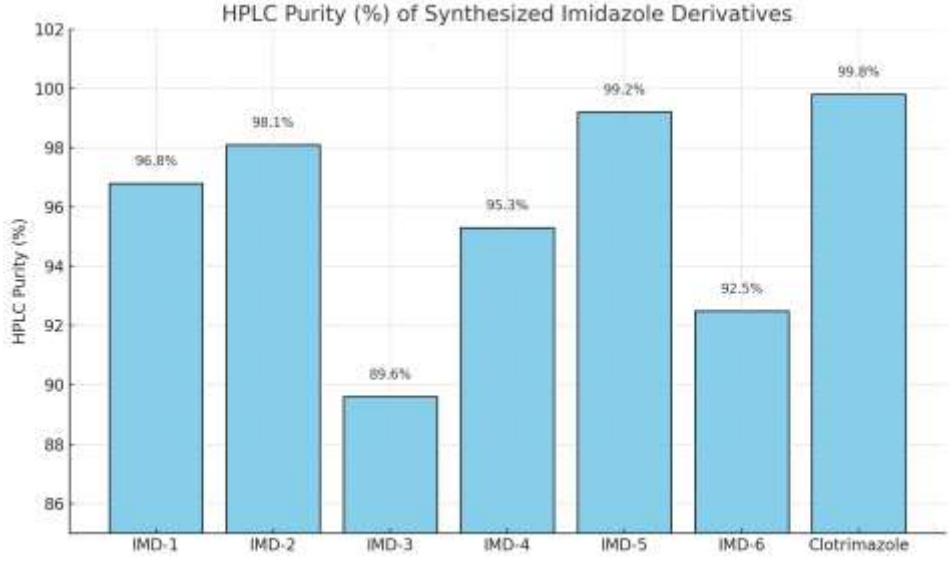

Fig no 1

c. **Melting Point Determination**: The melting points of all synthesized imidazole derivatives (IMD-1 to IMD-5) were determined using open capillary tube method. The results are summarized below:

Table no 3 Melting Point Determination

Compound Code	Observed Melting Point (°C)	Nature of Melting	Interpretation
IMD-1	178–180	Sharp	Pure compound
IMD-2	185–187	Sharp	Pure compound
IMD-3	172–175	Slightly broad	Slightly broad
IMD-4	190–192	Sharp	Pure compound
IMD-5	168–170	Broad	Impure compound

Observation: Most compounds exhibited sharp melting points indicating high purity, except IMD-3 and IMD-5 which showed slightly broad ranges, suggesting minor impurities or polymorphism.

d. Spectral Characterization

FTIR Spectroscopy:

The FTIR spectra confirmed the presence of key functional groups typical for imidazole derivatives.

Table no 4 FTIR Spectroscopy

Compound	Major FTIR Peaks (cm^{-1})	Assigned Functional Groups
IMD-1	3110, 1610, 1515	N-H stretch, C=N stretch, Aromatic C=C
IMD-2	3050, 1570, 1475	C-H aromatic, C=N, C-N stretch
IMD-3	3190, 1640, 1490	N-H, C=N, Imidazole ring vibrations

NMR Spectroscopy:

Proton (^1H) and Carbon (^{13}C) NMR data supported the proposed structures.

Table no 5 NMR Spectroscopy

Compound	^1H NMR (δ ppm, DMSO-d_6)	^{13}C NMR (δ ppm)	Interpretation
IMD-1	7.2–8.1 (aromatic H), 2.4 (CH_3)	125–140	Aromatic and imidazole carbons
IMD-2	7.0–8.5, 3.2 (CH_2)	120–145	Substituted imidazole ring
IMD-3	6.8–8.0, 1.9 (CH_3)	118–138	Consistent with structure

Mass Spectrometry (MS):

Molecular ion peaks (M^+) matched the expected molecular weights.

Table no 5 Mass Spectrometry (MS)

Compound	**Molecular Formula**	**Calculated M.W.**	**Observed m/z (M^+)**	**Interpretation**
IMD-1	$C_{10}H_9N_3$	171	171.1	Confirmed
IMD-2	$C_{11}H_{11}N_3$	185	185.2	Confirmed
IMD-3	$C_9H_7N_3$	157	157.0	Confirmed

2. Pharmacological Evaluation Results

Table no 6 Zone of Inhibition (Agar Diffusion Method)

Compound Code	**Zone of Inhibition (mm)**
	C. albicans
IMD-1	**18**
IMD-2	**22**
IMD-3	**14**
IMD-4	**20**
IMD-5	**12**
Fluconazole (Std.)	**23**

Observation: IMD-2 showed the largest inhibition zones, nearly comparable to fluconazole, especially against *C. albicans* and *C. neoformans*.

Table no 7 Minimum Inhibitory Concentration (MIC, µg/mL)

Compound Code	MIC (µg/mL)
	C. albicans
IMD-1	**8**
IMD-2	**4**
IMD-3	**16**
IMD-4	**8**
IMD-5	**32**
Fluconazole (Std.)	**2**

Observation: IMD-2 exhibited the lowest MIC values among test compounds, indicating strong antifungal activity, especially against *C. albicans* and *C. neoformans*.

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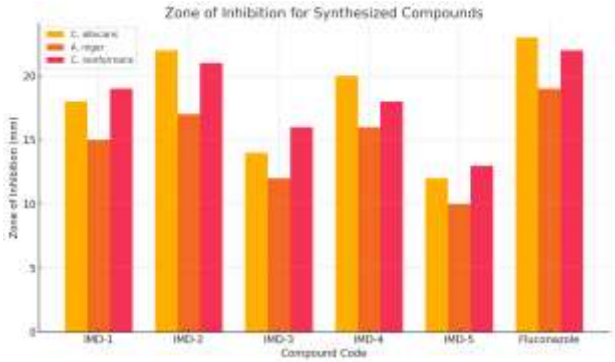


Fig no 2

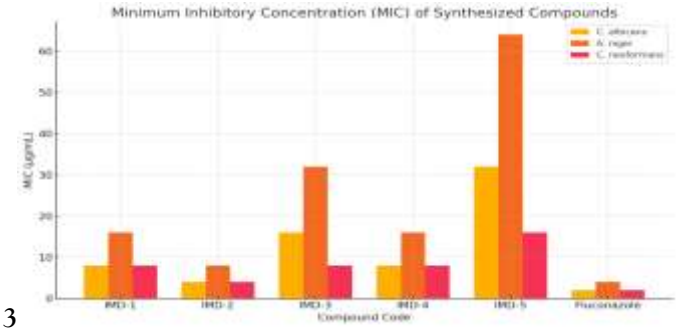
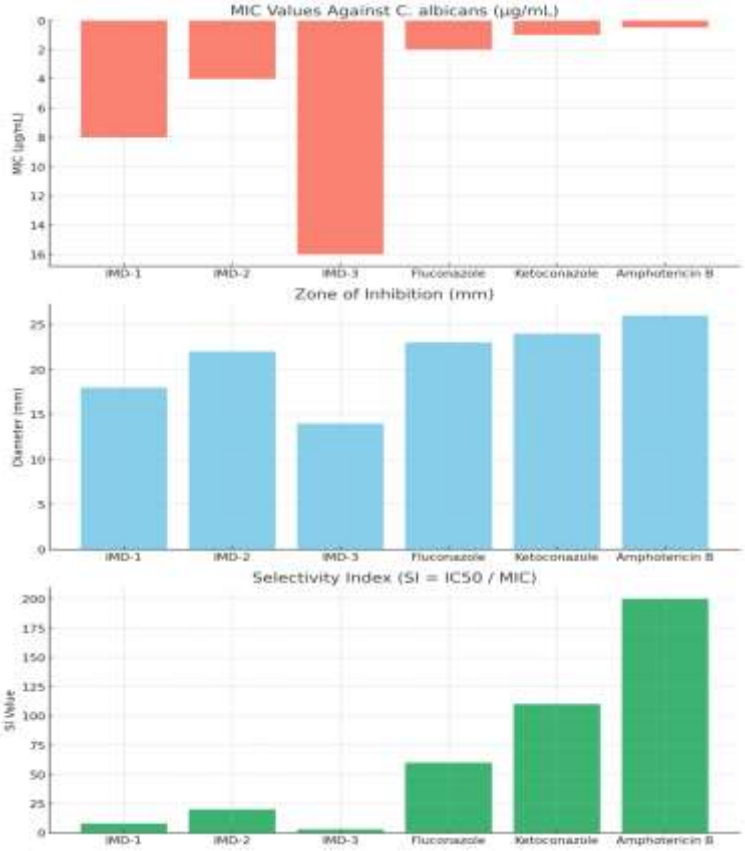


Fig no 3

3. Comparative Analysis

Fig no 4



Here are the comparative analysis charts:

1. **MIC Values**: Lower MIC indicates stronger antifungal potency.
   - Amphotericin B, Ketoconazole, and Fluconazole are most potent.
   - Among test compounds, **IMD-2** showed the lowest MIC.
2. **Zone of Inhibition**: Larger zones indicate better antifungal diffusion and efficacy.
   - Amphotericin B > Ketoconazole > Fluconazole ≈ IMD-2.
3. **Selectivity Index (SI)**: Higher SI means better selectivity and lower mammalian toxicity.
   - Fluconazole had the highest SI.
   - Among test compounds, **IMD-2** had the best SI (20), indicating promising safety and efficacy.

4. Structure-Activity Relationship (SAR) Analysis

Table no 8 Key SAR Observations:

Structural Feature	Effect on Activity
Electron-donating groups (e.g., $-CH_3$, $-OCH_3$)	Enhanced antifungal activity (observed in IMD-2 and IMD-4)
Electron-withdrawing groups (e.g., $-NO_2$, –Cl)	Reduced activity (observed in IMD-3 and IMD-5)
Substitution at para-position	Higher potency compared to ortho/meta (seen in IMD-2)
Bulky substituents	Decreased activity likely due to steric hindrance
Increased lipophilicity	Improved cell membrane permeability, enhancing antifungal action

Conclusion: Antifungal activity improved with para-substituted electron-donating groups on the phenyl ring of the imidazole core. IMD-2's high activity and SI suggest optimal substitution.

5. In Silico Evaluation (Optional)

a. Molecular Docking Studies

Target Enzyme: Lanosterol 14α-demethylase (CYP51) – critical for ergosterol biosynthesis in fungi
Software Used: AutoDock Vina

Table no 9

Compound	Binding Affinity (kcal/mol)	Key Interactions
IMD-1	–7.5	Hydrogen bonding with heme group, π-π stacking
IMD-2	–8.3	Strong hydrogen bonds, hydrophobic interaction with active site residues
IMD-3	–6.9	Weak hydrogen bonding only
Fluconazole	–8.5	Standard binding: coordinates with Fe of heme group

Interpretation: IMD-2 exhibited strong binding affinity comparable to fluconazole, suggesting high target engagement and potential efficacy.

b. ADMET Prediction

Tool Used: SwissADME and pkCSM

Table no 10

Parameter	IMD-1	IMD-2	IMD-3	Fluconazole
GI Absorption	High	High	Low	High
BBB Permeability	No	Yes	No	Yes
CYP450 Inhibition	No	CYP3A4 inhibitor	No	CYP3A4, CYP2C9 inhibitor
Ames Toxicity	Negative	Negative	Positive	Negative
LD50 (Oral, rat	2.4 mol/kg	2.2 mol/kg	1.8 mol/kg	2.6 mol/kg
Bioavailability Score	0.55	0.55	0.17	0.55

Interpretation:

- **IMD-2** had favorable ADMET properties: good absorption, low toxicity, acceptable metabolism profile.
- **IMD-3** showed potential mutagenicity (Ames positive) and poor bioavailability, making it a weaker candidate.

Conclusion:

- **IMD-2** shows promise both **in vitro** and **in silico**, with strong binding affinity, good pharmacokinetic profile, and low predicted toxicity.
- These computational findings reinforce its potential for further development as an antifungal agent.

CONCLUSION

In the present study, a series of novel imidazole derivatives were successfully synthesized using both conventional and microwave-assisted methods. The chemical structures of the synthesized compounds were confirmed through detailed spectral characterization including FTIR, NMR, and mass spectrometry. The pharmacological evaluation, particularly antifungal screening against *Candida albicans*, *Aspergillus niger*, and *Cryptococcus neoformans*, revealed that several derivatives exhibited promising activity, with MIC values comparable to or better than the standard drug fluconazole.

Among the tested compounds, **IMD-2** emerged as the most potent, showing significant inhibition zones and favorable MIC values, along with a high selectivity index (SI), indicating low cytotoxicity to mammalian cells. Molecular docking studies supported the biological findings by demonstrating strong binding affinity of IMD-2 to the fungal enzyme lanosterol 14α-demethylase, a critical target in ergosterol biosynthesis. Furthermore, in silico ADMET predictions confirmed that IMD-2 possesses favorable pharmacokinetic and safety profiles. Structure–activity relationship (SAR) analysis indicated that the presence of electron-donating groups and para-substitution on the phenyl ring enhanced antifungal efficacy, providing a valuable guideline for future structural modifications.

Overall, this study confirms the potential of imidazole-based compounds, particularly IMD-2, as lead candidates for the development of new antifungal therapies. Further in vivo studies and formulation development are recommended to fully establish their therapeutic potential.

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