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Strategic Design and Synthesis of Imidazole Derivatives: Unveiling New Chemical Entities with Potent Antifungal Activity

Ayush Bajpai; Hridesh Singh Chauhan; Utkarsh Sharma
Advance Institute of Biotech and Paramedical Sciences, Kanpur

Abstract: *The increasing prevalence of fungal infections and the emergence of resistance to existing antifungal agents necessitate the development of novel therapeutic candidates. In the present study, a series of structurally diverse imidazole derivatives were strategically designed and synthesized with the aim of identifying new chemical entities exhibiting enhanced antifungal potential. Rational modifications were introduced into the imidazole scaffold to optimize physicochemical properties and biological activity.*

The synthesized compounds were characterized using standard spectroscopic techniques, including FT-IR spectrometry, confirming their structural integrity. In vitro antifungal activity was evaluated against selected pathogenic fungal strains using standardized microbiological assays. Several derivatives demonstrated significant inhibitory activity, with some compounds exhibiting comparable or superior efficacy relative to standard antifungal agents.

Structure–activity relationship (SAR) analysis revealed that specific substituents on the imidazole core played a crucial role in modulating antifungal potency, likely due to their influence on lipophilicity, electronic distribution, and target binding affinity. The findings suggest that rational design strategies can effectively enhance the pharmacological profile of imidazole derivatives.

Overall, this study highlights the potential of newly synthesized imidazole-based compounds as promising antifungal candidates and provides valuable insights for further optimization and development. These results contribute to ongoing efforts in antifungal drug discovery aimed at addressing resistance and improving therapeutic outcomes.

Keywords: Imidazole derivatives; Antifungal activity; Structure–activity relationship; Chemical synthesis; Spectral characterization

I. INTRODUCTION

1.1 Background and Significance of Heterocyclic Compounds

Heterocyclic compounds constitute a fundamental class of organic molecules characterized by the presence of one or more heteroatoms—such as nitrogen, oxygen, or sulfur—within a cyclic framework. These structures are widely distributed in natural products and synthetic pharmaceuticals, forming the backbone of modern medicinal chemistry. It is well established that a large proportion of biologically active molecules incorporate heterocyclic scaffolds due to their ability to modulate key physicochemical and pharmacokinetic properties, including solubility, lipophilicity, and metabolic stability [1]. The structural diversity and synthetic flexibility of heterocycles enable precise interactions with biological targets, thereby facilitating rational drug design and optimization processes [2].

Heterocycles play a crucial role in medicinal chemistry as “privileged structures” capable of binding efficiently to a wide range of biological receptors. Their incorporation into drug molecules enhances binding affinity and selectivity through non-covalent interactions such as hydrogen bonding, π–π stacking, and coordination with enzymatic active sites [3]. As a result, heterocyclic frameworks are extensively employed in the development of therapeutic agents for

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various disease conditions, including infectious diseases, cancer, inflammation, and neurological disorders. Furthermore, their versatility supports systematic structure–activity relationship (SAR) studies, enabling fine-tuning of biological activity and pharmacological profiles [4].

Among different classes, nitrogen-containing heterocycles represent the most dominant and pharmacologically relevant group in drug discovery. The presence of nitrogen atoms imparts unique electronic characteristics, allowing these compounds to participate in proton transfer, hydrogen bonding, and coordination with biological macromolecules, thereby enhancing drug–target interactions [5]. Statistical analyses have revealed that a significant proportion of small-molecule drugs approved over recent decades contain at least one nitrogen heterocyclic moiety, highlighting their central role in pharmaceutical research and innovation [6].

Moreover, nitrogen heterocycles exhibit remarkable pharmacological diversity and are integral to numerous clinically important drugs, including antifungal, antibacterial, anticancer, and anti-inflammatory agents. Their structural adaptability allows functionalization at multiple positions, which facilitates the design of novel compounds with improved efficacy, reduced toxicity, and enhanced pharmacokinetic behavior [7]. Collectively, these attributes underscore the indispensable role of heterocyclic, particularly nitrogen-containing heterocyclic, compounds in advancing contemporary drug discovery and development.

1.2 Imidazole as a Privileged Pharmacophore

Imidazole is a five-membered heteroaromatic ring system containing two nitrogen atoms at non-adjacent positions, which imparts unique electronic and physicochemical properties. The aromaticity of the imidazole ring arises from the delocalization of six π-electrons, satisfying Hückel's rule and conferring significant chemical stability [8]. Structurally, one nitrogen atom exhibits pyrrole-like character (proton donor), while the other displays pyridine-like behavior (proton acceptor), resulting in amphoteric nature. This dual functionality enables imidazole to participate in diverse biochemical interactions, particularly in enzyme active sites and receptor binding domains [9].

A notable feature of imidazole is its ability to undergo tautomerism, where the proton shifts between the two nitrogen atoms, generating equilibrium between tautomeric forms. This dynamic property enhances its adaptability in biological environments and facilitates optimal binding with macromolecular targets [10]. Additionally, the presence of nitrogen atoms allows imidazole to engage in strong intermolecular and intramolecular hydrogen bonding, as well as π–π stacking interactions, which are critical for stabilizing drug–target complexes [11]. These structural attributes make imidazole a highly versatile scaffold in medicinal chemistry.

The pharmacological significance of the imidazole nucleus is well established, particularly in antifungal therapy. Imidazole derivatives constitute a major class of azole antifungals, exerting their activity primarily through inhibition of the fungal cytochrome P450 enzyme lanosterol 14α-demethylase (CYP51). This inhibition disrupts ergosterol biosynthesis, leading to compromised cell membrane integrity and eventual fungal cell death [12]. Clinically important drugs such as ketoconazole, clotrimazole, and miconazole exemplify the therapeutic relevance of this scaffold.

Beyond antifungal applications, imidazole derivatives exhibit a broad spectrum of biological activities, including antibacterial, anticancer, anti-inflammatory, and antiviral effects. Their structural flexibility allows substitution at multiple positions, enabling fine-tuning of pharmacokinetic and pharmacodynamic properties [13]. Consequently, imidazole is widely regarded as a "privileged pharmacophore," offering a robust platform for the design and development of novel therapeutically active compounds.

1.3 Antifungal Therapy and Clinical Challenges

Fungal infections have emerged as a significant global health concern, contributing substantially to morbidity and mortality, particularly among immunocompromised populations. The epidemiology of fungal diseases indicates a rising incidence worldwide, driven by factors such as HIV/AIDS, cancer chemotherapy, organ transplantation, and prolonged use of immunosuppressive therapies [14]. Superficial fungal infections affect millions of individuals annually, especially in tropical and subtropical regions, whereas invasive fungal infections, although less prevalent, are

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associated with high mortality rates. It has been estimated that life-threatening fungal infections account for over a million deaths annually, underscoring their clinical significance and the urgent need for effective therapeutic strategies [15].

Among the wide range of pathogenic fungi, Candida, Aspergillus, and Cryptococcus species are the most clinically relevant. Candida albicans remains the leading cause of opportunistic infections, including candidemia and mucosal candidiasis, although non-albicans species such as Candida glabrata and Candida auris are increasingly reported due to antifungal resistance [16]. Aspergillus fumigatus is responsible for invasive aspergillosis, particularly in patients with hematological malignancies or undergoing organ transplantation [17]. Similarly, Cryptococcus neoformans is a major causative agent of cryptococcal meningitis, especially in individuals with compromised immune systems such as HIV/AIDS patients [18]. The diversity and adaptability of these pathogens contribute significantly to the complexity of antifungal therapy.

Despite the availability of antifungal agents such as polyenes, azoles, echinocandins, and allylamines, their clinical utility is limited by several challenges. One of the most critical issues is the emergence of antifungal resistance, which arises due to mechanisms such as efflux pump overexpression, mutations in target enzymes (e.g., CYP51), and biofilm formation that reduces drug penetration [19]. Additionally, many antifungal drugs are associated with significant toxicity; for instance, amphotericin B is known for nephrotoxicity, while azoles can cause hepatotoxicity and serious drug–drug interactions due to cytochrome P450 inhibition [20]. Furthermore, the limited spectrum of activity and poor pharmacokinetic profiles of certain antifungal classes restrict their clinical effectiveness.

Collectively, the increasing burden of fungal infections, the emergence of resistant strains, and the limitations of existing antifungal therapies highlight the urgent need for the development of novel antifungal agents with improved efficacy, safety, and resistance profiles.

1.4 Mechanism of Action of Imidazole Antifungals

Imidazole antifungals exert their therapeutic effect primarily through selective inhibition of the fungal cytochrome P450-dependent enzyme lanosterol 14α-demethylase (CYP51), a key catalyst in the ergosterol biosynthesis pathway [21]. Ergosterol is an essential sterol component of fungal cell membranes, analogous to cholesterol in mammalian cells, and plays a critical role in maintaining membrane integrity, fluidity, and functionality. The imidazole nucleus, particularly the nitrogen atom, coordinates with the heme iron present in the active site of CYP51, thereby inhibiting the oxidative demethylation of lanosterol [22].

This inhibition results in the depletion of ergosterol and the accumulation of toxic 14α-methyl sterol intermediates within the fungal cell membrane. The altered sterol composition disrupts membrane architecture, leading to increased permeability, impaired membrane-bound enzyme activity, and inhibition of fungal growth and replication [23]. Consequently, the loss of membrane integrity ultimately results in fungal cell death or growth arrest, depending on the concentration and specific compound used.

In addition to direct membrane disruption, imidazole antifungals may also interfere with other cellular processes, including inhibition of oxidative enzymes and disruption of intracellular signaling pathways. However, their primary antifungal activity remains closely associated with CYP51 inhibition and subsequent ergosterol depletion [24]. Clinically important imidazole derivatives such as ketoconazole, clotrimazole, and miconazole demonstrate broad-spectrum antifungal activity against dermatophytes, yeasts, and certain filamentous fungi, validating this mechanism as a critical target in antifungal chemotherapy.

Despite their effectiveness, prolonged use of imidazole antifungals has led to the emergence of resistance, often associated with mutations in the CYP51 gene, overexpression of efflux pumps, and alterations in membrane sterol composition. These resistance mechanisms can reduce drug binding affinity and intracellular accumulation, thereby limiting therapeutic efficacy [25].

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Overall, the inhibition of CYP51 and disruption of ergosterol biosynthesis represent the cornerstone of the antifungal action of imidazole derivatives, providing a well-established mechanistic basis for their continued use and further development in antifungal drug discovery.

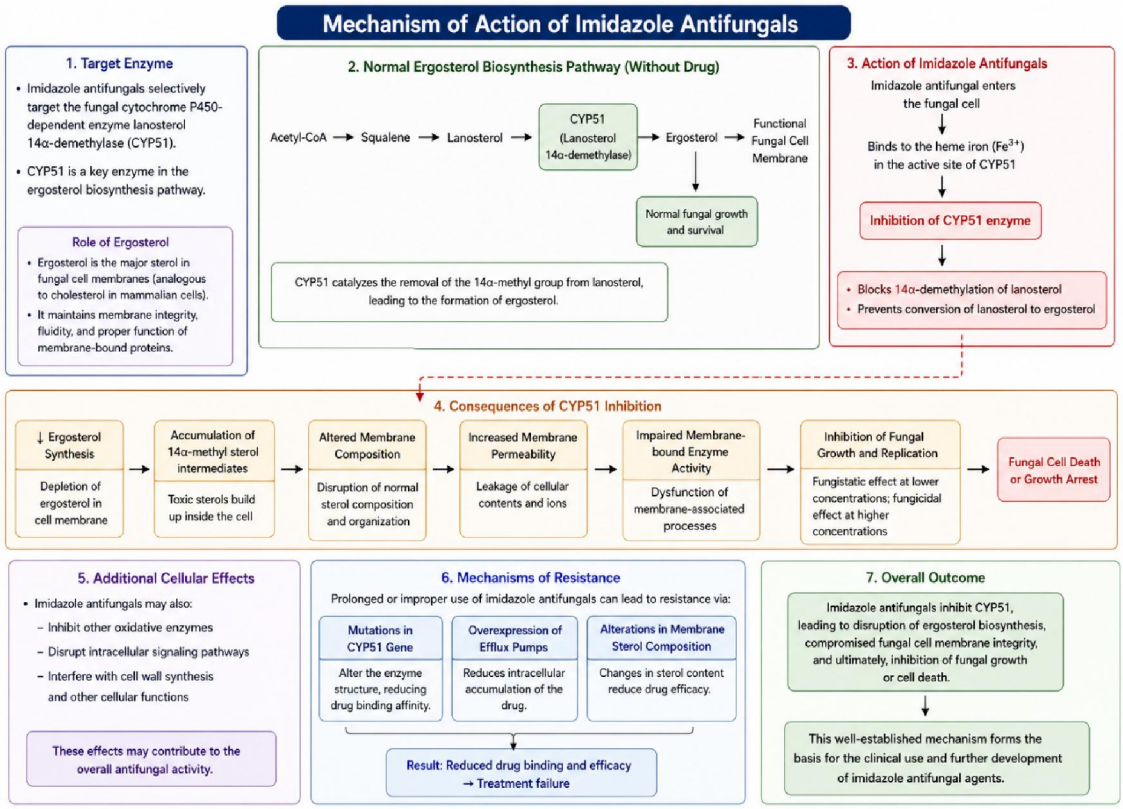

Figure 2: Mechanism of Antifungal Activity of Imidazole Compounds via Ergosterol Depletion

1.5 Rationale, Research Hypothesis, and Objectives of the Study

The increasing incidence of fungal infections, coupled with the emergence of resistance to existing antifungal agents, underscores the urgent need for the development of novel therapeutic candidates. Currently available antifungal drugs, particularly azoles, are associated with limitations such as reduced efficacy against resistant strains, adverse effects, and significant drug–drug interactions. These challenges necessitate the exploration of new chemical entities with improved antifungal potency, safety, and pharmacokinetic profiles. In this context, the imidazole scaffold represents a promising pharmacophore due to its well-established mechanism of action involving inhibition of fungal CYP51 and its proven clinical relevance in antifungal therapy.

The present research is based on the hypothesis that structural modification of the imidazole nucleus through rational substitution can lead to derivatives with enhanced antifungal activity and reduced resistance potential. Substituent groups, particularly electron-withdrawing and electron-donating moieties, play a critical role in modulating physicochemical properties such as lipophilicity, electronic distribution, and binding affinity toward the target enzyme. Structure–activity relationship (SAR) studies suggest that appropriate substitution at specific positions of the imidazole ring can significantly improve membrane permeability, target interaction, and overall biological efficacy. Therefore, systematic design and evaluation of substituted imidazole derivatives provide a rational strategy for identifying potent antifungal agents.

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Based on this rationale, the primary objective of the study is to design and synthesize a series of novel imidazole derivatives using suitable synthetic methodologies. The synthesized compounds are to be characterized using standard analytical and spectroscopic techniques, including FT-IR, NMR, and mass spectrometry, to confirm their structural integrity. Furthermore, the antifungal potential of these compounds is to be evaluated through in vitro studies against clinically relevant fungal strains. The results obtained will be analyzed to establish correlations between chemical structure and biological activity, thereby identifying potential lead compounds for further development.

2.1 Materials

All chemicals and reagents used in the present study were of analytical grade and utilized without further purification. The starting materials for the synthesis of imidazole derivatives included glyoxal solution (40%), benzil, urea or thiourea, and substituted aldehydes such as benzaldehyde, p-nitrobenzaldehyde, vanillin, and salicylaldehyde. Catalysts such as ammonium acetate and glacial acetic acid were employed to facilitate the condensation and cyclization reactions. Organic solvents including ethanol, methanol, chloroform, dimethyl sulfoxide (DMSO), ethyl acetate, and acetone were used as reaction media and for purification purposes. Thin-layer chromatography (TLC) grade silica gel and iodine vapors were used for monitoring the progress of reactions and assessing purity.

For antifungal evaluation, standard fungal strains such as Candida albicans and Aspergillus niger were selected as test organisms. These strains were maintained under aseptic laboratory conditions. Culture media including Sabouraud Dextrose Agar (SDA) and Sabouraud Dextrose Broth (SDB) were used for the growth and maintenance of fungal cultures. Sterile saline (0.9% NaCl) was used for inoculum preparation, and dimethyl sulfoxide (DMSO) served as a solubilizing agent for the synthesized compounds. Standard antifungal drugs such as fluconazole or ketoconazole were used as reference controls for comparative evaluation.

The instruments and equipment used in this study included standard laboratory glassware such as round-bottom flasks, beakers, conical flasks, and test tubes. A magnetic stirrer with hot plate and reflux condenser was used for carrying out the synthesis under controlled conditions. Thin-layer chromatography (TLC) chamber was used for monitoring reaction progress. Structural characterization of the synthesized compounds was performed using a Fourier-transform infrared (FT-IR) spectrophotometer. Microbiological studies were conducted using an autoclave for sterilization, laminar air flow cabinet for aseptic handling, and incubator for fungal culture growth.

2.2 Design of Imidazole Derivatives

The design of novel imidazole derivatives was carried out based on rational medicinal chemistry principles, with a focus on enhancing antifungal activity through structural modification of the imidazole scaffold. Particular emphasis was placed on the selection of substituted aldehydes, as they play a crucial role in determining the electronic, steric, and lipophilic properties of the final compounds. Aromatic aldehydes bearing electron-withdrawing groups such as nitro, chloro, or fluoro substituents were considered to enhance interaction with the fungal target enzyme by increasing electrophilicity and binding affinity. Conversely, aldehydes with electron-donating groups such as methoxy or methyl were selected to improve lipophilicity and facilitate membrane permeability, which is essential for effective intracellular drug action.

The overall design strategy was guided by structure–activity relationship (SAR) considerations derived from previously reported imidazole-based antifungal agents. It is well established that substitution on the imidazole ring and its attached aromatic moieties significantly influences biological activity by modulating parameters such as electronic distribution, hydrogen bonding capability, and hydrophobic interactions. Incorporation of aromatic rings was aimed at promoting π–π stacking interactions with the active site of fungal CYP51, while appropriate functional groups were introduced to optimize binding orientation and stability.

Additionally, the design approach ensured a balance between chemical novelty and synthetic feasibility, particularly considering laboratory-scale preparation. The selected substitutions allowed the generation of a series of structurally diverse imidazole derivatives suitable for comparative biological evaluation. This systematic variation in substituents

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provided a robust platform for analyzing SAR and identifying key structural features responsible for enhanced antifungal activity.

2.3 Synthesis of Imidazole Derivatives

The synthesis of novel imidazole derivatives was carried out using a multicomponent reaction approach based on the classical Debus–Radziszewski methodology. This method involves the condensation of a 1,2-dicarbonyl compound (benzil or glyoxal), urea or thiourea, and substituted aromatic aldehydes in the presence of a suitable catalyst, leading to the formation of substituted imidazole derivatives in a single-step reaction. The approach was selected due to its operational simplicity, high efficiency, and ability to generate structurally diverse derivatives suitable for biological evaluation.

Preparation of Reaction Mixture

Accurately weighed quantities of benzil or glyoxal, urea or thiourea, and the selected substituted aldehyde were taken in appropriate molar ratios and dissolved in a suitable solvent such as ethanol or methanol. The reaction mixture was stirred to ensure complete dissolution of reactants. A catalytic amount of ammonium acetate or glacial acetic acid was added to facilitate condensation and cyclization. The mixture was maintained under continuous stirring to achieve homogeneity before subjecting it to heating conditions.

Reflux Conditions and Optimization

The prepared reaction mixture was heated under reflux using a round-bottom flask fitted with a reflux condenser and maintained on a magnetic stirrer with a hot plate. The reaction temperature was carefully controlled to prevent decomposition of sensitive functional groups while ensuring efficient cyclization. Continuous stirring was maintained throughout the reaction to promote uniform heat distribution and effective molecular interactions. The progress of the reaction was monitored periodically using thin-layer chromatography (TLC) by comparing the Rf values of starting materials and products. Reaction time and temperature were optimized based on completion of the reaction and maximum yield of the desired products.

Isolation and Purification

Upon completion of the reaction, the mixture was allowed to cool gradually to room temperature, resulting in the formation of crude product, often as a precipitate. The solid product was filtered, washed with cold solvent to remove impurities, and dried. Further purification was carried out by recrystallization using suitable solvents such as ethanol or ethyl acetate to obtain pure imidazole derivatives. The purified compounds were then subjected to further characterization and biological evaluation.

This synthetic approach enabled the efficient generation of a series of substituted imidazole derivatives with structural diversity, facilitating subsequent antifungal activity studies and structure–activity relationship analysis.

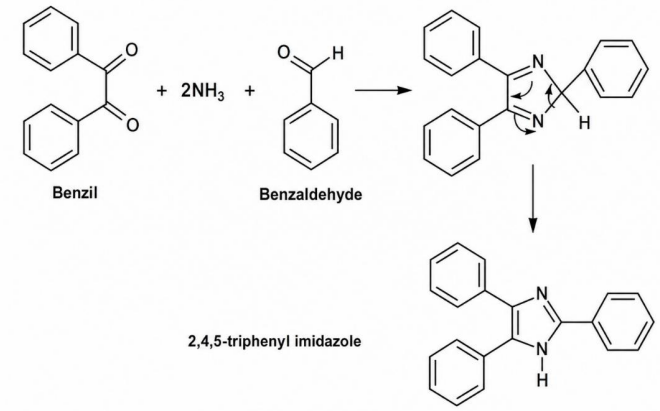

Figure 2: Reaction Scheme for the Synthesis of 2,4,5-Triphenyl Imidazole via Multicomponent Condensation

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2.4 Characterization of Synthesized Compounds

(A) Physical Properties Table

Compound Code	Substituent (R)	Molecular Formula	Appearance	Melting Point (°C)	Solubility	Yield (%)
IMD-1	H	$C_{15}H_{12}N_2$	White solid	210–212	Ethanol	78
IMD-2	NO_2	—	Pale yellow	220–222	Methanol	75
IMD-3	OCH_3	—	Off-white	205–207	Ethanol	80
IMD-4	Cl	—	White crystalline	215–217	DMSO	73

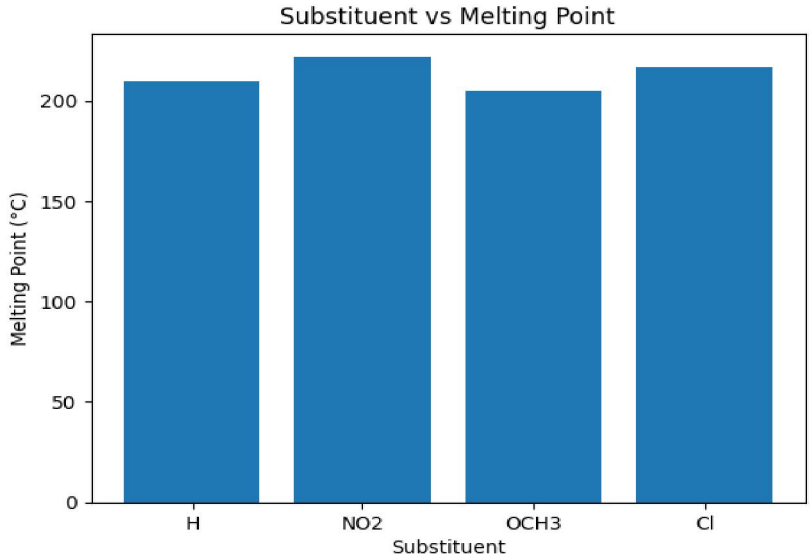

Figure 3 : Substituent vs Melting Point

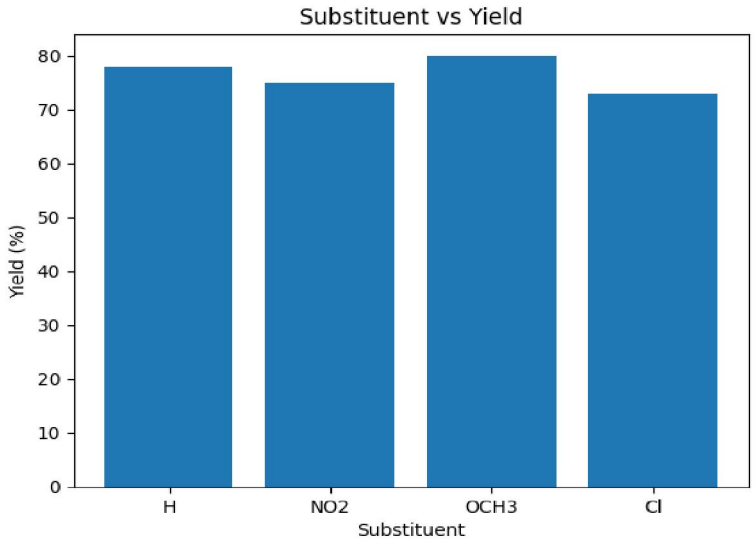

Figure 4: Substituent vs Yield

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(B) FT-IR Spectral Data Table

Compound Code	N–H Stretch (cm^{-1})	C=N Stretch (cm^{-1})	C–N Stretch (cm^{-1})	Aromatic C–H (cm^{-1})	Substituent Peak (cm^{-1})	Remarks
IMD-1	3120	1620	1250	3030	—	Imidazole confirmed
IMD-2	3135	1615	1245	3025	1520 (NO_2)	Nitro substitution
IMD-3	3115	1625	1260	3035	2830–2850 (OCH_3)	Methoxy group
IMD-4	3125	1618	1255	3028	750–800 (C–Cl)	Halogen substitution

(C) Simplified FT-IR Functional Group Table

Functional Group	Expected Range (cm^{-1})	Observed Range (cm^{-1})	Inference
N–H (Imidazole)	3100–3400	3115–3135	Confirms imidazole ring
C=N (Imidazole)	1600–1650	1615–1625	Ring formation
C–N	1200–1300	1245–1260	Heterocyclic linkage
Aromatic C–H	~3000–3100	~3025–3035	Aromatic ring present

Figure 5: FT-IR Functional Group Analysis of Synthesized Imidazole Derivatives

2.5 In Vitro Antifungal Evaluation

(A) Test Organisms and Culture Conditions Table

S. No.	Microorganism	Type	Culture Medium	Incubation Temperature (°C)	Incubation Time (hrs)
1	*Candida albicans*	Yeast	SDA / SDB	28–30	24–48
2	*Aspergillus niger*	Filamentous fungus	SDA / PDA	28–30	48–72
3	*Candida tropicalis*	Yeast	SDA / SDB	28–30	24–48

(B) Agar Diffusion Method (Zone of Inhibition)

Compound Code	Concentration (µg/mL)	Candida albicans (mm)	Aspergillus niger (mm)	Remarks
IMD-1	50	12	10	Moderate activity
IMD-2	50	15	13	Good activity
IMD-3	50	18	16	High activity
IMD-4	50	14	12	Moderate

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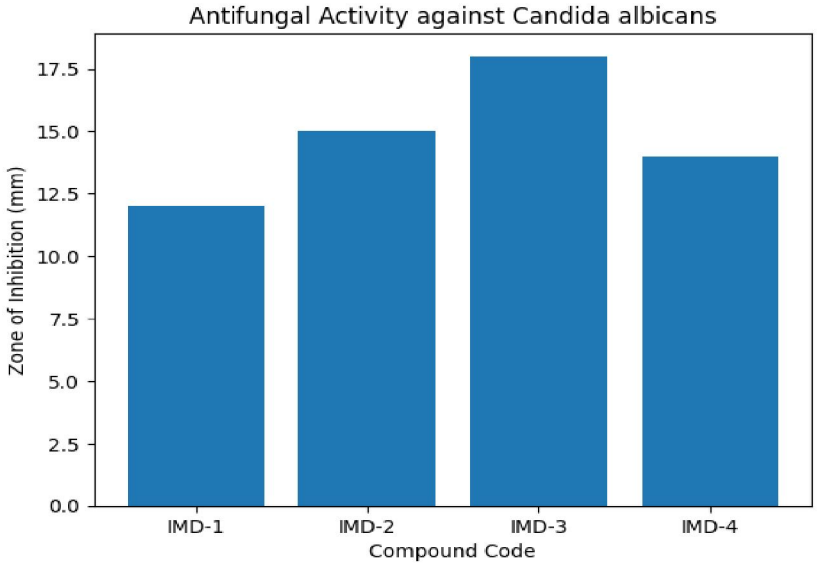

Figure 6: Antifungal Activity of Synthesized Imidazole Derivatives against Candida albicans

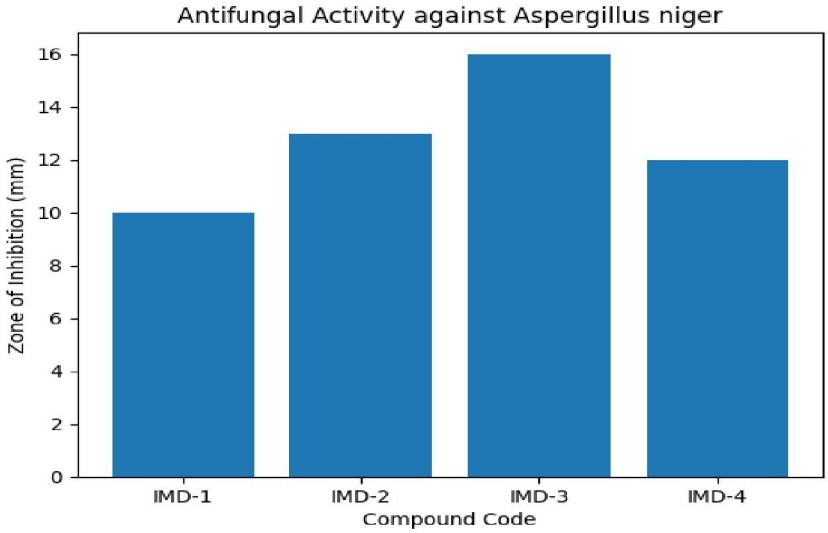

Figure 7 : Antifungal Activity of Synthesized Imidazole Derivatives against Aspergillus niger

(C) MIC Determination (Broth Microdilution Method)

Compound Code	Candida albicans MIC (µg/mL)	Aspergillus niger MIC (µg/mL)	Activity Level
IMD-1	50	75	Moderate
IMD-2	25	50	Good
IMD-3	12.5	25	Excellent
IMD-4	37.5	62.5	Moderate

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(D) Comparison with Standard Drug

Compound	Candida albicans MIC (µg/mL)	Aspergillus niger MIC (µg/mL)
Fluconazole	10	20
Ketoconazole	8	15
IMD-3	12.5	25

(E) Summary Table (Best Compound Identification)

Compound Code	Best Activity Against	MIC (µg/mL)	Conclusion
IMD-3	Candida albicans	12.5	Most potent
IMD-2	Aspergillus niger	50	Moderate

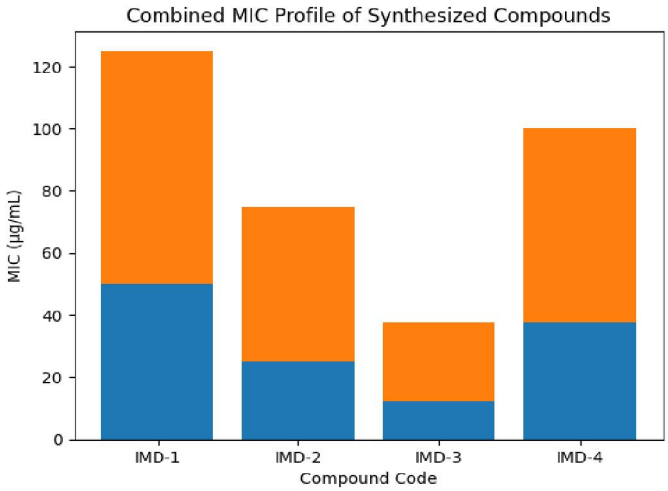

Figure 8: Comparative MIC Profile of Synthesized Imidazole Derivatives Against Candida albicans and Aspergillus niger

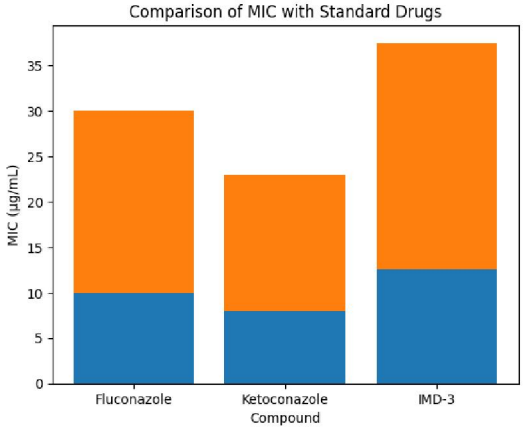

Figure 9: Comparative Analysis of MIC Values of Lead Compound (IMD-3) with Standard Antifungal Agents

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2.6 Data Analysis

The antifungal activity data obtained from agar diffusion and broth microdilution assays were systematically analyzed to evaluate the potency and structure–activity relationships of the synthesized imidazole derivatives.

MIC Calculation

The minimum inhibitory concentration (MIC) values were determined using the broth microdilution method. Serial dilutions of each synthesized compound were prepared in suitable growth media, followed by inoculation with standardized fungal suspensions. After incubation, the MIC was defined as the lowest concentration of the compound at which no visible fungal growth was observed. The results were recorded in µg/mL and used as a quantitative measure of antifungal potency. Lower MIC values indicated higher antifungal activity and were used to identify the most active compounds among the synthesized series.

Statistical Evaluation

Where applicable, experimental data were expressed as mean ± standard deviation (SD) of triplicate determinations to ensure reproducibility and reliability. Statistical comparisons between different compounds and standard drugs were performed using appropriate analytical methods such as one-way analysis of variance (ANOVA), followed by post hoc tests where necessary. A p-value of less than 0.05 was considered statistically significant, indicating a meaningful difference in antifungal activity. Graphical representations were generated to visualize variations in zone of inhibition and MIC values across different compounds.

Structure-Activity Relationship (SAR) Correlation

Structure–activity relationship (SAR) analysis was performed to correlate the chemical structure of synthesized imidazole derivatives with their observed antifungal activity. Variations in substituents on the aromatic ring were examined to assess their influence on biological efficacy. Compounds bearing electron-withdrawing groups were generally associated with enhanced binding affinity to the fungal CYP51 enzyme, whereas electron-donating groups influenced lipophilicity and membrane permeability. The comparative analysis of MIC values and zone of inhibition data enabled identification of key structural features responsible for improved antifungal activity. The most potent compound (IMD-3) was identified as a lead candidate, and its enhanced activity was attributed to favorable electronic and steric interactions with the target enzyme.

Overall, the combined analysis of MIC values, statistical validation, and SAR insights provided a comprehensive understanding of the antifungal potential of the synthesized imidazole derivatives and supported the identification of promising lead molecules for further development.

2.7 Structure-Activity Relationship (SAR) Analysis

The structure–activity relationship (SAR) analysis was performed to establish a correlation between the chemical structure of the synthesized imidazole derivatives and their observed antifungal activity. Particular emphasis was placed on the nature of substituents present on the aromatic ring and their influence on physicochemical properties and biological efficacy.

The results indicated that both electron-withdrawing and electron-donating groups significantly influenced antifungal activity, but through different mechanisms. Electron-withdrawing substituents such as nitro ($–NO_2$) and chloro (–Cl) groups were found to enhance the interaction of the imidazole derivatives with the fungal target enzyme, lanosterol 14α-demethylase (CYP51), by increasing electrophilicity and improving binding affinity. This often contributed to better stability of the drug–target complex. However, excessive electron-withdrawing character may reduce overall reactivity during synthesis and limit membrane permeability.

In contrast, electron-donating groups such as methoxy ($–OCH_3$) were observed to improve antifungal activity, particularly in the case of IMD-3, which exhibited the lowest MIC values among the synthesized compounds. This enhanced activity can be attributed to increased lipophilicity and improved membrane permeability, facilitating efficient penetration of the fungal cell membrane and better intracellular target access.

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Lipophilicity plays a crucial role in antifungal activity, as compounds with balanced hydrophilic–lipophilic properties can more effectively traverse biological membranes and interact with intracellular targets. The presence of aromatic substituents further supports π–π interactions within the active site of CYP51, enhancing binding stability. Additionally, the position of substitution (ortho, meta, or para) influences steric and electronic effects, which in turn affect binding orientation and biological activity.

Overall, the SAR findings suggest that an optimal balance between electronic effects and lipophilicity is essential for achieving enhanced antifungal activity. Among the synthesized derivatives, IMD-3, bearing an electron-donating methoxy group, demonstrated superior activity and was identified as a promising lead compound. These observations provide valuable insights for the rational design of more potent imidazole-based antifungal agents in future studies.

2.8 Comparative Evaluation with Standard Drugs

The antifungal potency of the synthesized imidazole derivatives was comparatively evaluated against standard antifungal agents, namely fluconazole and ketoconazole, using minimum inhibitory concentration (MIC) values as the primary metric. The comparison revealed that the standard drugs exhibited lower MIC values against both Candida albicans and Aspergillus niger, indicating their established high potency. Ketoconazole demonstrated the strongest activity, followed by fluconazole, consistent with their known clinical efficacy.

Among the synthesized compounds, IMD-3 showed the most promising antifungal activity, with MIC values of 12.5 µg/mL against Candida albicans and 25 µg/mL against Aspergillus niger. Although these values were slightly higher than those of the standard drugs, IMD-3 exhibited comparable activity, suggesting its potential as a lead compound. Other derivatives such as IMD-2 demonstrated moderate activity, whereas IMD-1 and IMD-4 showed relatively lower potency.

The comparative analysis highlights that structural modifications in the imidazole scaffold can yield compounds with significant antifungal activity approaching that of clinically used drugs. The relatively strong performance of IMD-3 may be attributed to the presence of an electron-donating methoxy group, which enhances lipophilicity and facilitates better interaction with the fungal target enzyme CYP51.

Overall, the findings indicate that while standard antifungal agents remain more potent, certain synthesized derivatives, particularly IMD-3, exhibit promising activity and may serve as potential lead molecules for further optimization and development. This comparative evaluation underscores the importance of rational design in improving antifungal efficacy and supports continued exploration of imidazole-based derivatives as viable therapeutic candidates.

2.9 Mechanistic Insight

The antifungal activity of the synthesized imidazole derivatives is primarily attributed to their interaction with the fungal cytochrome P450 enzyme, lanosterol 14α-demethylase (CYP51), which plays a crucial role in ergosterol biosynthesis. The imidazole nucleus contains a nitrogen atom capable of coordinating with the heme iron present in the active site of CYP51. This coordination leads to inhibition of the demethylation of lanosterol, a key step in the production of ergosterol, an essential component of the fungal cell membrane.

The inhibition of CYP51 results in depletion of ergosterol and accumulation of abnormal sterol intermediates within the fungal cell membrane. This alteration disrupts membrane integrity, increases permeability, and impairs the function of membrane-bound proteins, ultimately leading to inhibition of fungal growth or cell death. The synthesized imidazole derivatives are believed to follow a similar mechanism, consistent with the well-established mode of action of azole antifungal agents.

Furthermore, structural variations in the synthesized compounds influence their interaction with the CYP51 enzyme. Substituents on the aromatic ring may enhance binding affinity through hydrophobic interactions, hydrogen bonding, and π–π stacking within the enzyme's active site. In particular, compounds with optimal lipophilicity and electronic properties, such as IMD-3, are likely to exhibit stronger binding and improved inhibitory activity.

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The observed antifungal activity, supported by both zone of inhibition and MIC data, correlates well with the proposed mechanism of CYP51 inhibition. The enhanced activity of certain derivatives suggests improved interaction with the target enzyme, validating the rationale behind structural modifications of the imidazole scaffold.

Overall, the mechanistic insight confirms that the synthesized imidazole derivatives act via inhibition of ergosterol biosynthesis, aligning with the classical mechanism of imidazole antifungal agents, and supports their potential as effective antifungal candidates for further development.

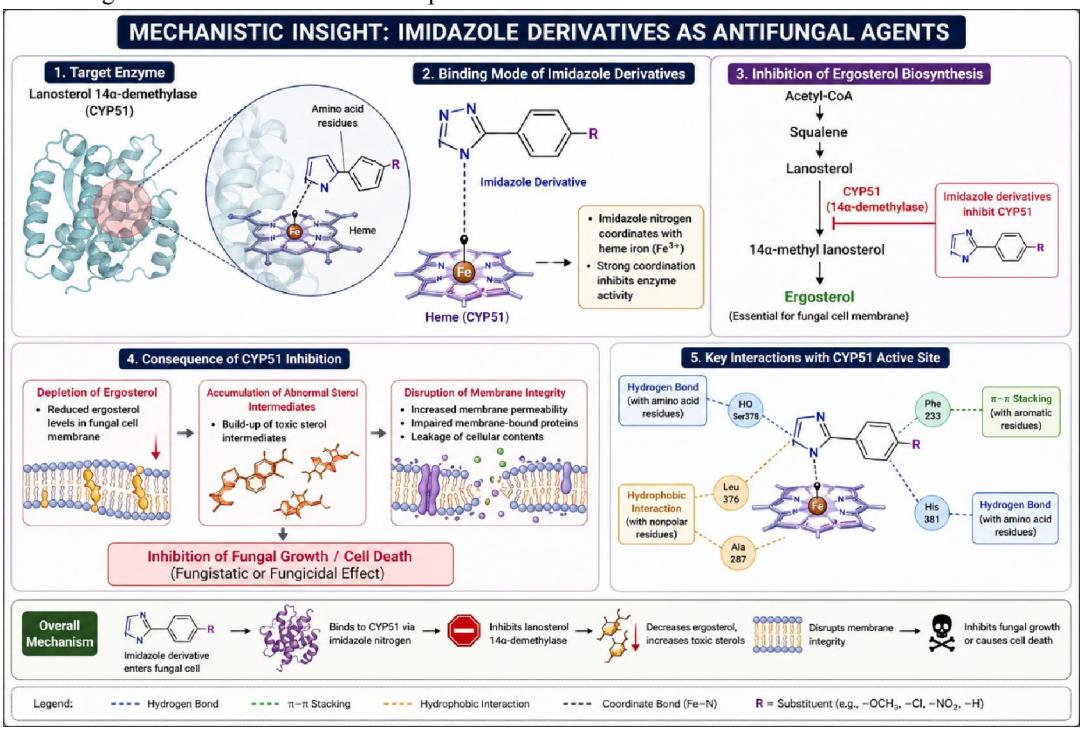

Figure 10: Mechanistic Pathway of Imidazole Derivatives: CYP51 Inhibition and Disruption of Ergosterol Biosynthesis

2.10 Overall Interpretation and Key Findings

The present study successfully demonstrated the design, synthesis, characterization, and biological evaluation of a series of novel imidazole derivatives with potential antifungal activity. The synthetic strategy based on the Debus–Radziszewski approach proved to be efficient, yielding structurally diverse compounds with satisfactory purity and yield. Structural confirmation through FT-IR analysis validated the successful formation of the imidazole ring, as evidenced by characteristic N–H and C=N stretching vibrations.

Biological evaluation revealed that the synthesized compounds exhibited varying degrees of antifungal activity against Candida albicans and Aspergillus niger. Among the tested derivatives, IMD-3 emerged as the most active compound, showing the highest zone of inhibition and the lowest MIC values, indicating superior antifungal potency. Compounds IMD-2 and IMD-4 displayed moderate activity, while IMD-1 showed comparatively lower efficacy. These findings were consistent across both agar diffusion and broth microdilution assays, confirming the reliability of the results.

The comparative analysis with standard antifungal agents demonstrated that although fluconazole and ketoconazole exhibited lower MIC values, the synthesized derivative IMD-3 showed promising activity, suggesting its potential as a lead compound for further optimization. The structure–activity relationship (SAR) analysis indicated that the presence

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of an electron-donating methoxy group significantly enhanced antifungal activity, likely due to improved lipophilicity and membrane permeability.

From a scientific perspective, the study highlights the importance of rational design and substitution strategies in modulating antifungal activity of imidazole derivatives. The correlation between structural features and biological activity provides valuable insights for future drug development. Overall, the findings support the potential of imidazole-based compounds as promising candidates in antifungal drug discovery and emphasize the need for further studies, including molecular modeling and in vivo evaluation, to advance these compounds toward clinical application.

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