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Molecular Docking Study of Imidazole as a Potential Anti-Fungal Agent Targeting CYP51 & SAP2 Enzymes

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Abstract: ***Imidazole derivatives, long valued for their antifungal efficacy, inhibit two critical fungal enzymes-lanosterol 14α-demethylase (CYP51), vital for ergosterol biosynthesis, and secreted aspartyl proteinase 2 (SAP2), a driver of fungal virulence and tissue invasion. In this in silico study, docking simulations were conducted using validated protein structures and docking platforms CB-Dock (for cavity-guided blind docking) and MzDOCK (a flexible GUI-based workflow). Results showed that imidazole binds strongly within the active sites of both CYP51 and SAP2, with favorable docking scores, hydrogen-bonding, and hydrophobic interactions. These findings highlight imidazole's promise as a dual-target antifungal scaffold and provide a rationale for advancing to in vitro and in vivo studies.***

Keywords: Antifungal agent, Imidazole, Lanosterol 14α-demethylase, Secreted aspartyl proteinase 2, Docking

1. Introduction

Fungal infections, or mycosis, are diseases caused by a fungus (yeast or mold). Fungal infections are most common on your skin or nails, but fungi (plural of fungus) can also cause infections in your mouth, throat, lungs, urinary tract and many other parts of your body.[1] Ergosterol is an important sterol component of cell and mitochondrial membranes in fungi. It helps in maintaining cell membrane structure, function, fluidity, and permeability. Proper cellular ergosterol levels are important in maintaining normal cellular functions that include environmental stress response, cellular detoxification, nutrient transport, and host-pathogen interactions. Hence, ergosterol biosynthesis is crucial for fungal cell growth and proliferation.[2] Like cholesterol, ergosterol is biosynthetically derived from acetyl-coenzyme A. The nine biosynthetic steps leading to the first sterol intermediate, lanosterol, are identical in both pathways; related enzymes in fungi and mammalian cells catalyze all but one of the remaining steps in either the cholesterol or ergosterol pathways. While there have been reports of compounds inhibiting the fungal-specific step of introduction of the 24-methyl group through sterol Δ24-methyltransferase, to date no drugs have arisen from this approach, hence in all cases selectivity for the fungal target is a key target to avoid toxicological consequences. The most successful targets have been squalene epoxidase (SE), lanosterol-14α-demethylase and Δ7-8 isomerase.[3] Until the 1970s, fungal infections were considered largely treatable and the demand for new medicines was very small. Today the situation for fungal pathogens is very different, and the need for potent new antifungal agents has probably never been greater. This need has arisen largely through the increase in the numbers of profoundly and chronically immunosuppressed patients. Serious invasive fungal infections caused by yeasts, such as Candida spp., and moulds, such as Aspergillus spp., represent an increasing threat to human health. The greatest predictor for fungal infection is the depth and duration of neutropenia, while other risk factors include the type and status of the primary illness and the use of broad-spectrum antibiotics. Environmental quality can also affect the likelihood of infection, as defective air handling systems and building work in facilities housing neutropenic patients have been implicated in the development of fungal infection. [4] The advent of HIV has also created a pool of patients who are susceptible to both serious invasive and superficial fungal infections. The relatively sudden increase in the numbers of patients with invasive fungal infection means that the supply of novel, potent antifungal agents has failed to keep e with the therapeutic need. This is illustrated by the fact that the antifungal that is arguably the standard at present-amphotericin B-was developed in the 1950s. To compound the poor choice of antifungals, toxicity, limited spectrum of activity, and the propensity to induce or select for resistant strains, create a need for novel effective agents to treat deep fungal infection.[5]

1.1 Imidazole and its antifungal relevance

Imidazole is a five membered heterocyclic compound having two nitrogen atoms at position 1 and 3.[6] Imidazole antifungals belong to the azole family, which also includes triazole and thiazole. An imidazole functional group, which is a five-membered ring containing two nitrogens, differentiates these drugs from other members of the azole family. Thiazole groups are similar rings with one nitrogen and one sulfur, while triazoles contain three nitrogens. Imidazole is used primarily to treat two types of yeast infections: oral thrush and vaginal candidiasis. They are as effective as polyene antifungals such as nystatin for oral thrush and somewhat more effective for vaginal candidiasis.[7]

1.2 Mechanism of action

Imidazole antifungals act similarly to triazole and thiazole antifungals. They work by inhibiting cytochrome P450 demethylase, an enzyme responsible for converting lanosterol to ergosterol. Because ergosterol is a major component of fungal cell membranes, blocking this conversion leads to the buildup of lanosterol. Lanosterol contains a 14 alpha-methyl group not present in ergosterol. Because of the different shape and physical properties of this sterol, the fungal cell

membrane exhibits permeability changes and becomes leaky. Key cellular components can leak, and cell death results.[8]

1.3 Common fungal targets

a) CYP51 [Lanosterol 14α-demethylase] The CYP51-involved catalytic reaction consists of three steps, each of which requires one molecule of oxygen and two molecules of NADPH-sourced reduction equivalent. The first two steps are typical cytochrome P450 monooxygenation processes, during which the 14α methyl is converted to methyl alcohol and further converted to methyl aldehyde. And in the last step, the aldehyde group is transformed into formic acid and detached, accompanied with the synthesis of the Δ-14, 15 double bonds. [9] CYP51 genes are commonly contained in the Ascomycotta Pezizomycotina genomes, including CYP51A and CYP51B. CYP51C is exclusive in Fussarium spp. Some Aspergillus spp such as A. fumigatus carries only one CYP51A and one CYP51B protein, while other Aspergillus species such as A. flavus and A. terreus carry a third paralogous gene, which is a copy of CYP51A or CYP51B.[10]

b) SAP2 [Secreted Aspartyl Proteinase 2]
It is a key virulence factor produced by Candida Albicans, a common fungal pathogen responsible for opportunistic infections in humans. As a member of the SAP (Secreted Aspartyl Proteinase) family, SAP2 plays a crucial role in fungal pathogenicity by facilitating tissue invasion, immune evasion, and nutrient acquisition. It achieves this by degrading host proteins such as keratin, collagen, immunoglobulins, and components of the immune system, thereby enabling the fungus to invade host tissues and evade immune responses. Additionally, SAP2 is highly expressed during biofilm formation, a state that enhances fungal resistance to antifungal therapies. Because of its central role in infection and virulence, SAP2 has emerged as a promising target for antifungal drug development. Inhibiting SAP2 could reduce fungal invasion and survival while minimizing tissue damage, making it an attractive candidate for therapeutic intervention.[11] Targeting SAP2 for antifungal action involves inhibiting its proteolytic activity, thereby disrupting critical processes essential for fungal survival and virulence. SAP2 is a secreted aspartyl protease that Candida albicans uses to degrade host proteins, enabling tissue invasion, immune evasion, and nutrient acquisition. By designing inhibitors that specifically bind to the active site of SAP2—particularly the catalytic aspartic acid residues—researchers can block its enzymatic function. This inhibition prevents the fungus from penetrating host tissues, weakens its ability to escape immune attacks, and deprives it of necessary nutrients, ultimately leading to reduced fungal growth and virulence. Additionally, blocking SAP2 can impair biofilm formation, making fungal cells more susceptible to host defenses and conventional antifungal therapies. Some classes of drugs, such as HIV protease inhibitors and synthetic peptidomimetics, have shown promise in inhibiting SAP2 activity. Thus, by neutralizing SAP2's action, antifungal strategies can effectively limit infection spread, enhance immune clearance, and improve clinical outcomes.[12]

c) TRR [Thioredoxin reductase]
It is an essential enzyme in the thioredoxin system of fungi, playing a critical role in maintaining cellular redox balance by reducing oxidized thioredoxin using NADPH as an electron donor. This reduction is vital for protecting fungal cells against oxidative stress, repairing damaged proteins, and supporting DNA synthesis and other metabolic processes. Targeting fungal TRR disrupts the redox homeostasis, leading to the accumulation of reactive oxygen species (ROS) and oxidative damage within the fungal cell, ultimately resulting in cell death. Importantly, fungal TRR differs structurally and functionally from its human counterpart, allowing for selective inhibition and minimizing toxicity to human cells. Small molecule inhibitors designed against fungal TRR can sensitize the pathogen to oxidative stress and enhance the effectiveness of host immune responses and other antifungal agents. Thus, TRR represents a promising target for the development of novel antifungal therapies, especially against drug-resistant fungal pathogens.[13]

d) β-1,3-GLUCAN SYNTHASE
It is a vital enzyme responsible for the synthesis of β-1,3-glucan, a major structural polysaccharide in the fungal cell wall that provides strength, rigidity, and protection against environmental stresses. Since β-1,3-glucan is essential for maintaining fungal cell wall integrity and is absent in human cells, β-1,3-glucan synthase represents an ideal and highly selective antifungal target. Inhibition of this enzyme disrupts the production of β-1,3-glucan, leading to weakened cell walls, osmotic instability, and ultimately fungal cell lysis and death. Echinocandins, a class of antifungal drugs such as caspofungin, micafungin, and anidulafungin, specifically inhibit β-1,3-glucan synthase, making them effective against a variety of pathogenic fungi, particularly Candida and Aspergillus species. By targeting a critical component of fungal cell wall synthesis without affecting mammalian cells, β-1,3-glucan synthase inhibitors offer a powerful and relatively safe therapeutic strategy for treating serious fungal infections.[14]

2. Molecular docking

Molecular docking is a method to identify the architecture of compounds generated by two or more distinct molecules computationally. The objective of docking studies is to anticipate the desired three-dimensional structures. Docking, in and of itself, generates only suitable incentive structures. Docking plays a critical role in rational drug design. Docking is a mathematical technique that anticipates the preferable orientation of one molecule relative to another when they are linked together to create a stable complex. Using scoring functions, it is possible to estimate the strength of the connection or binding affinity across two compounds based on their preferential orientation. Signal transduction is dependent on the interactions of physiologically significant substances such as proteins, nucleic acids, carbohydrates, and lipids. As a result, docking may be used to forecast both the intensity and type of signals generated.[15]

2.1 Types of docking

There are two distinct forms of docking. Rigid docking and Flexible docking. Rigid docking Assuming the compounds are inflexible, we are seeking a rearrangement of one of the compounds in three-dimensional space that results in the best

match to the other compounds in parameters of a scoring system. The ligand's conformation can be formed with or without receptor binding activity. Flexible docking In conjunction with transformation, we evaluate molecular flexibility to identify confirmations for the receptor and ligand molecules as they exist in the complex [16]

2.2 Molecular docking approaches

a) **Monte carlo approach** It creates a randomized conformation, translations, and rotation of a ligand in an active site. It assigns an initial configuration value. Then it develops and scores a new configuration. It determines if the new configuration is kept using the Metropolis criterion. (Metropolis criterion-If a new approach outperforms the prior one, it is approved instantly. If the arrangement is not novel, a likelihood study aimed at Boltzmann's law is employed. The resolution is acceptable if it satisfies the probability function test; otherwise, the arrangement is rejected).
b) **Matching approach** This strategy emphasizes redundancy, the optimal location of the ligand atom in the site is determined, resulting in a ligand-receptor arrangement that might also need improvement.
c) **Ligand fit approach** Ligand fit is a word that refers to a quick and precise methodology for docking small molecules ligands into protein active sites while taking shape complementarity into account.[17]

2.3 Applications of docking

Applications of molecular docking in drug development. Docking is most often employed for drug discovery, as the majority of medications are composed of tiny organic compounds. Docking may be used to:

- Hit identification Docking in conjunction with a score function enables rapid screening of vast databases of possible medications in silico to find compounds that are capable of binding to a particular target of interest.
- Lead optimization Docking can be used to anticipate the location and relative position of a ligand's interaction to a protein (also referred to as the binding mode or pose). This data can be utilized to develop more powerful and selective analogues.[18]
- ADMET Prediction Docking can predict the Absorption, Distribution, Metabolism, Excretion, and Toxicity (ADMET) properties of small molecules. This prediction helps in screening out compounds with unfavorable properties early in the drug discovery process.
- Structure Elucidation Molecular docking aids in elucidating the structure of proteins with unknown structures by predicting the binding modes of small molecules. This information can help in creating homology models of proteins based on the predicted binding mode and experimental data refinement.
- Drug Repurposing Docking techniques are employed to explore existing drugs for new therapeutic targets, accelerating treatment discovery. This approach was notably utilized during the COVID-19 pandemic to identify potential inhibitors for SARS-CoV-2 proteins.[19]

3. Molecular Docking Process

3.1 Selection of fungal proteins

According to several review of literatures, we have selected two fungal protein targets, namely:

CYP51-Lanosterol 14α-demethylase is a cytochrome P450 enzyme that plays a critical role in the biosynthesis of ergosterol, a key component of the fungal cell membrane. Fungi rely on ergosterol, but humans use cholesterol. This difference makes enzymes like CYP51 ideal targets. Imidazole Antifungals bind to the heme iron in the active site of CYP51.This binding prevents the enzyme from demethylating lanosterol, halting ergosterol synthesis. As a result, toxic sterol intermediates accumulate and cell membrane integrity is lost, leading to fungal death or growth inhibition.[20]

SAP2-Secreted Aspartyl Protease 2 (SAP2) is a key virulence protein playing a crucial role in fungal infection and pathogenicity. SAP2 is especially active in acidic, nutrient-poor environments such as the vaginal or oral mucosa. This enzyme is secreted during infection and facilitates tissue invasion by degrading host proteins like collagen, albumin, mucins, and immunoglobulins. It also helps the fungus evade immune responses and acquire nutrients, making it essential for colonization and persistence. Although SAP2 is not vital for fungal survival, its pivotal role in virulence makes it a promising antifungal target. Its extracellular nature and infection specific expression further enhance its value as a drug target in antifungal therapy.[21]

3.2 Target selection and prepration

We have selected two proteins namely CYP51 and SAP2. To execute the docking study, it is essential to have the receptor structures of these compounds. The structure of receptors should be downloaded in the PDB format from RCS PDB. The quality of receptor structure is important. Higher the resolution better the docking result.

Preparation of protein
Hydrogen atoms are added to the protein structure followed by the removal of water molecules except in the active site. After the addition of kollmann charges the protein is ready for docking, and it is saved in PDBQT format. The two selected proteins (CYP51, SAP2) are thus prepared.

3.3 Ligand selection and prepration

Select the ligand of interest The structures of our selected ligand should be downloaded as SDF format from PUBCHEM. The ligands we have selected for docking studies are Eberconazole, Flutrimazole, Lombazole, Parconazole Preparation of ligand The preparation of ligand is done by using Pymol software. As we are downloaded the SDF format of the ligands, it is converted to PDB format in the Pymol.

Figure (a): Eberconazole

Figure (b): Flutrimazo

Figure (c): Parconazole

Figure (d): Lombazole

3.4 Methodology

Ligand-protein docking ***Using MzDOCK***

Step 1: Prepare PDB Files
Clean the receptor and ligand structures (remove water, ligands).

Step 2: Convert to ZDOCK Format
Use the tool `pdb2zdock` to convert PDB files to ZDOCK compatible format.

Step 3: Create Symmetry File
Make a text file with the symmetry type (e.g., `C3` for cyclic 3-fold symmetry).

Step 4: Run MZDOCK
Execute docking using the MZDOCK Software.

Step 5: Get Docking Results
The Output file contains the top docking poses and scores.
Ligand-protein docking **using CB-DOCK**

Step 1: Prepare Input Files
Ensure protein and ligand files are ready.
Acceptable format: PDB for proteins and mol2/SDF for ligands.

Step 2: Open CB-Dock
Go to http://clab.labshare.cn/cb-dock/

Step 3: Upload Files
Upload the protein and ligand files.

Step 4: Auto Cavity Detection
CB-Dock automatically detects potential binding sites (cavities) on the protein.

Step 5: Run Docking
Click "Start Docking" – CB-Dock uses AutoDock Vina for docking.

Step 6: View and Download Results
View docking poses, binding affinities, and download results (PDB files +docking scores).

Using Swiss Dock

Step 1: Prepare Input Files
- Protein: PDB format (remove water molecules and heteroatoms).
- Ligand: mol2 format (can be converted using tools like Open Babel).

Step 2: Go to SwissDock Web Server
- Visit: http://www.swissdock.ch/

Step 3: Upload Files
- Upload the target protein and ligand files.

Step 4: Set Docking Options
- Choose docking region: blind docking or focused docking (specify binding site).
- You can use default setting or customize as needed.

Step 5: Submit Job
- Click-Start Docking‖ to begin the process.
- The server runs docking using the EADock DSS engine.
- You will receive an email or link once the docking is complete.

Step 6: Check Results
- View predicted binding modes, scores, and download PDB files.

Step 7: Visualize Results
- Use molecular visualization tools like SwissDock Viewer, UCSF Chimera, or PyMOL.
- The tools helps to analyze docking process and visualize interactions. We have done the docking by interacting CYP51 with Eberconazole, CYP51 with Lombazole, CYP51 with Flutrimazole, CYP51 with Parconazole, SAP2 with Eberconazole, SAP2 with Lombazole, SAP2 with Flutrimazole and Parconazole. The interactions can be visualized with **Pymol.**

Report

4.1 Results and Discussion

The quality of docking studies is measured by the binding affinity, which indicates the strength of interaction between the ligand and the target protein. In this in study molecular Docking was performed using MzDOCK and CBDOCK to evaluate the antifungal potential of selected imidazole derivatives against two fungal proteins: CYP51 and SAP2.

Binding affinity analysis
The binding affinity values (kcal/mol) of the ligands with protein are summarised in table 1 below:

Table 1: Binding affinity (kcal/mol) of ligands with protein

PROTIEN-LIGAND	AFFINITY (kcal/mol)	
	By MzDOCK	By CBDOCK
CYP51- Eberconazole	-7.8	-7.8
CYP51- Lombazole	-9.2	-8.6
CYP51- Flutrimazole	-7.8	-8.0
CYP51- Parconazole	-7.2	-7.0
SAP2- Eberconazole	-8.1	-8.5
SAP2- Lombazole	-7.8	-7.6
SAP2- Flutrimazole	-7.3	-7.0
SAP2- Parconazole	-6.6	-6.9

Observation:
CYP51 showed a stronger binding affinity with the ligands than SAP2. Lombazole demonstrated highest binding affinity towards CYP51 with values of-9.2 kcal/mol (MzDOCK) and-8.6kcal/mol (CBDOCK).

Protein-ligand interactions:
The following figures illustrate key hydrogen bonding and hydrophobic interactions between the imidazole derivatives and the CYP51 and SAP-2 active site residues. Notably, Lombazole forms multiple stabilizing interactions compared to other ligands. SAP2 interactions are comparatively weaker as reflected by the binding scores. Lombazole shows better interactions but not as significant with CYP51.

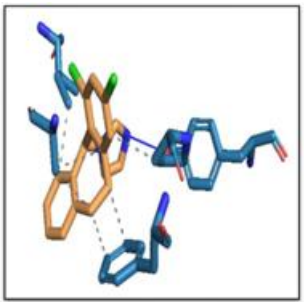
Fig (a): CYP51-Eberconazole

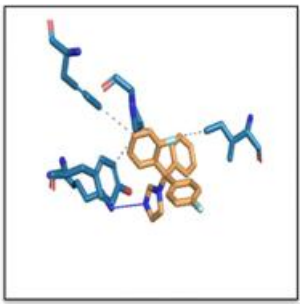
Fig (b): CYP51-Flutrimazole

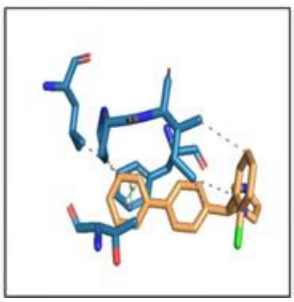
Fig (c): CYP51-Lombazole

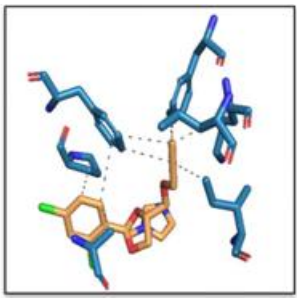
Fig (d): CYP51-Parconazole

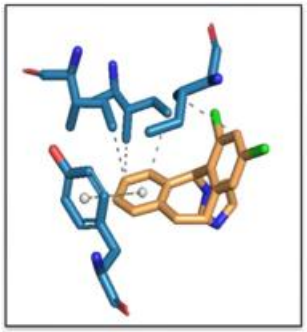
Fig (a): SAP2-Eberconazole

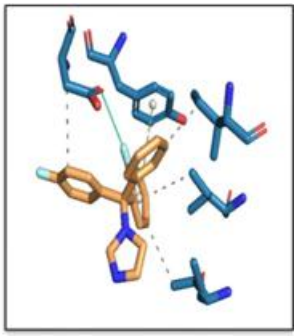
Fig (b): SAP2-Flutrimazole

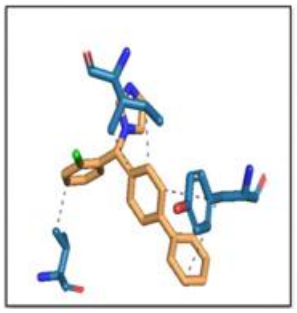
Fig (c): SAP2-Lombazole

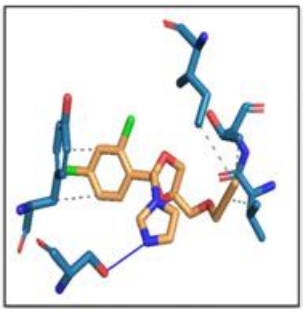
Fig (d): SAP2-Parconazole

Conclusion

This molecular Docking study demonstrates that imidazole derivatives, particularly Lombazole, exhibit strong potential as antifungal agents. The use of two docking platforms MzDOCK and CBDOCK allowed for cross validation of the binding site results.

Lombazole showed the strongest affinity towards CYP51 followed by Parconazole, Flutrimazole, Eberconazole.

Docking with SAP2 showed relatively weaker interactions, indicating CYP51 as more promising targets.

Future Aspects

1) In Vitro Validation
   The promising compounds, particularly Lombazole, should be subjected to in vitro antifungal assays against common fungal pathogens like Candida albicans, Aspergillus fumigatus, etc., to validate the docking results.
2) In Vivo Studies
   Animal model studies can help evaluate the pharmacokinetics, toxicity, and therapeutic potential of the compounds, particularly focusing on their ability to inhibit fungal growth in systemic infections.
3) Structure-Activity Relationship (SAR) Studies
   Detailed SAR studies can be conducted to optimize the chemical structure of the lead compounds, aiming to enhance their binding affinity, selectivity, and drug-likeness.
4) ADMET Profiling
   Further ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) analysis using computational and experimental models will help assess the safety profile and develop clinically viable drugs.
5) Target Expansion
   While CYP51 and SAP2 were used in this study, other fungal targets such as β-glucan synthase, chitin synthase, and protein kinases may be explored for broader spectrum activity.
6) Formulation Development
   Once active compounds are validated, research can progress toward developing suitable formulations such as topical gels, oral tablets, or nanoparticles for effective drug delivery.

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