RESEARCH PAPER

Molecular Docking Analysis of Second-Generation Triazole Antifungal Agents Against CYP51 and Squalene Epoxidase Targets Associated with Dermatophytosis

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ABSTRACT
Fungal infections pose a significant threat around the globe because they mainly infect individuals with weak immune systems. This indicates that there is an urgency to develop new antifungals and evaluation of their efficacy in clinical trials. The study in this paper applied molecular docking analyses of the binding affinity of three triazole antifungal drugs (posaconazole, ravuconazole, voriconazole) at two major fungal enzyme targets involved in ergosterol biosynthesis (CYP51 PDB ID: 6C6P, squalene epoxidase PDB ID: 6E8Q), and determined how well each enzyme interacted with the ligand binding via evaluation of docking scores and the interaction profile; thus, measuring binding stability of each drug to the target enzymes. The compounds analyzed through docking studies, the highest binding affinity for CYP51 (−7.7 kcal/mol) was observed with posaconazole, whereas the highest binding affinity for squalene epoxidase (−8.5 kcal/mol) was observed with ravuconazole. The detailed analysis of the protein-ligand interaction demonstrated that all of the ligand-enzyme complexes formed between the triazole antifungal agents and either of the two targets were held together via the following interaction types: conventional hydrogen bonds; π-cation interactions; π-anion interactions; halogen bonds; π-π stacking interactions; hydrophobic interactions; and van der Waals forces. Voriconazole exhibited relatively weak binding affinities for both enzyme targets due to its reduced size and surface area available for interaction based on its molecular structure. The findings suggest that posaconazole and ravuconazole have superior binding properties, when compared to voriconazole, at their respective fungal target enzymes that participate in the ergosterol biosynthetic pathway and may represent potential therapeutic alternatives to voriconazole for the treatment of invasive fungal infections. The molecular basis of the maximum activity of these clinical and laboratory-based antifungal agents provides valuable information for future designs or refinement of drugs to inhibit the target enzymes involved in fungal CYP51 and squalene epoxidation.

Keywords: Molecular docking, Posaconazole, Ravuconazole, Voriconazole, CYP51, Squalene epoxidase, Antifungal agents, Ergosterol biosynthesis.
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Introduction

Dermatophytosis is a fungal infection affecting both humans and animals, caused by pathogenic keratinolytic fungi known as dermatophytes that invade keratinized tissues of the skin. [1,2]. Pathogenic dermatophytes were initially classified into the genera *Microsporum*, *Trichophyton*, and *Epidermophyton*, and are further grouped as anthropophilic, zoophilic, or geophilic according to their ecological niche and transmission pattern [3]. Anthropophilic species were identified as the predominant dermatophytes associated with human infections, accounting for 92.6% of cases. Dermatophytes comprise a group of more than 30 species of common and rare infectious filamentous fungus that can penetrate keratinized tissues and trigger superficial infections of the skin, nail and hair [4]. Dermatophyte-caused infections have increased significantly in recent years, and superficial mycoses are thought to affect around 20–25% of the global population [5]. In clinical isolates from central and northern Europe, Trichophyton rubrum is the most prevalent dermatophyte, while T. mentagrophytes is more frequently recorded from Asia. However, reports of zoophilic dermatophytes, such as Microsporum canis or Trichophyton verrucosum, from the Middle East and Southern Europe are common [6]. The prevalence of dermatophytosis in India has been reported to range from 36.6% to 78.4%, with Trichophyton species being the major causative agents. Among these, Trichophyton verrucosum has been identified as an important zoophilic dermatophyte, particularly in rural populations and individuals with close animal contact. Studies from India have reported variable isolation rates of *T. verrucosum*, including approximately 20.69% in West Bengal and up to 38% among dermatophyte isolates in Eastern Uttar Pradesh [7-9]. Three factors influence the distribution of dermatophytes isolated from skin diseases around the world, leading to the reintroduction of the infection [10]:

a) Poor hygienic conditions of living
b) densely populated urban regions

c) the rising movement of people from contaminated areas

The incidence of dermatophytosis is significantly higher in tropical and subtropical regions such as India, primarily due to elevated humidity and favorable environmental temperatures that support fungal growth. Furthermore, factors such as increasing urbanization, the adoption of occlusive footwear, and tight-fitting clothing all contribute to an increased infection rate [11].

An ideal antifungal therapy should have a high cure rate with little relapse, robust anti-inflammatory activity, a rapid commencement of action, and efficacy with minimal side effects and systemic absorption. Topical antifungal drugs are still the preferred therapeutic choice for localized and newly diagnosed dermatophytic infections, whereas systemic antifungal therapy is often suggested for extensive or severe infections [12].

Topical antifungal therapies are recommended as the first-line treatment for uncomplicated superficial dermatomycoses due to their high therapeutic efficacy and low risk of systemic side effects. Azoles, polyenes, and allylamine/benzylamine derivatives are the main types of topical antifungal drugs. In recent years, various novel antifungal drugs with increased efficacy and anti-inflammatory qualities have been introduced in India, enhancing the therapeutic choices available for the treatment of chronic dermatophytosis [13]. Triazole antifungal agents were introduced in the early 1990s. They greatly helped in developing antifungal drugs. Triazoles are synthetic compounds that contain one or more azole rings, each with three nitrogen atoms in a five-membered ring structure. There are two types of triazoles: first-generation agents such as itraconazole and fluconazole and second-generation agents such as voriconazole, posaconazole, and ravuconazole. All triazoles inhibit fungal growth by inhibiting cytochrome P450-dependent 14α-demethylase. This enzyme plays an important role in converting lanosterol to ergosterol, a vital step in the biosynthesis of the fungal cell membrane. [14].

In computer-aided drug design and molecular modeling, molecular docking is a well-known computational method. It involves predicting the preferred orientation and interaction of a small molecule ligand within the binding site of a target protein using scoring functions and conformational sampling techniques. This method helps identify the binding affinity and stability of ligand–protein complexes, thereby facilitating lead identification and optimization. In structural molecular biology, molecular docking plays a crucial role in predicting the most favorable ligand–protein binding modes. Furthermore, it serves as a cost-effective and time-efficient preliminary tool for evaluating the therapeutic potential of compounds prior to experimental studies and drug development processes [15,16].

In the present study, molecular docking analysis was performed to evaluate the binding affinity and interaction profile of second-generation triazole antifungal agents against important fungal target proteins involved in ergosterol biosynthesis. The selected targets included lanosterol 14α-demethylase (CYP51), represented by the PDB structure 6E8Q, which is the primary molecular target of azole antifungal drugs and plays a critical role in fungal cell membrane synthesis [17]. Additionally, docking studies were carried out using the squalene epoxidase structure 6C6P, an essential enzyme involved in sterol biosynthesis. Squalene epoxidase (SQLE), also known as squalene monooxygenase, is a key enzyme involved in ergosterol biosynthesis that catalyzes the conversion of squalene to 2,3-(S)-oxidosqualene. Due to its essential role in sterol synthesis, SQLE is considered an important therapeutic target for fungal infections and other diseases. Although experimentally resolved fungal squalene epoxidase structures are currently unavailable, previous studies have reported that the active-site residues and ligand-binding regions are highly conserved between fungal and human enzymes, making 6C6P a suitable model for qualitative antifungal docking analysis [18]. Therefore, the present study aims to investigate the molecular interactions and binding efficiencies of second-generation triazole compounds against these therapeutically important targets to explore their potential application in the management of dermatophytosis caused by *Trichophyton verrucosum.*

Materials and Methods

Ligand Preparation

The three-dimensional (3D) structures of the second-generation triazole antifungal compounds, namely voriconazole, posaconazole, and ravuconazole, were retrieved from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) in SDF format. The downloaded structures were subsequently converted into PDBQT format for molecular docking analysis [19].

Receptor preparation

Lanosterol 14α-demethylase (CYP51) (PDB ID: 6E8Q) and squalene epoxidase (PDB ID: 6C6P) 3D crystal structure were retrieved from the RCSB Protein Data Bank. BIOVIA Discovery Studio Visualizer (v17.2.0.16349) were used to assess the protein structures obtained from PDB. Preparation of proteins was carried out by removing the co-crystallized ligands, water molecules, and other heteroatoms present in the structures. PDB format was then used to store the cleaned receptor structures. AutoDock tools were used for further preparation of the protein, by adding polar H-atoms, assigning Kollman charges, and calculating partial charges on the protein. The final preparation of the protein files were saved as pdbqt files to be used in docking experiments. [20].

Molecular docking

Antifungal agents voriconazole, posaconazole and ravuconazole were docked into two critically important fungal proteins, lanosterol 14α-demethylase (PDB ID: 6E8Q) and squalene epoxidase (PDB ID: 6C6P), through conducting molecular docking studies. A primary focus of our studies was to determine how well these agents can bind to specific binding sites of lanosterol 14α-demethylase and squalene epoxidase (the regions of the proteins that are known to have ligands bound), and to analyze the interactions between the antifungal agents and the target proteins with the AutoDock molecular docking application software followed by visualizing and analyzing the docked compounds using the BIOVIA Discovery Studio Visualizer. [21].

Results

In this case, molecular docking study was employed to study the binding affinities and interaction patterns of voriconazole, posaconazole, and ravuconazole triazole antifungals against the fungal CYP51 target proteins (PDB IDs: 6C6P) and squalene epoxidase (PDB IDs: 6E8Q).Some docking scores and 2D interaction analysis display different ligand-binding behavior suggesting variations in the inhibitory potential. The binding energies discovered with the aid of molecular docking are illustrated in Table 1. The lower (more negative) binding energy values refer to stronger ligand-protein interactions and greater stability in binding.

Table.1: Docking scores for second-generation triazole antifungal drugs and their interactions with the enzyme.

Ligand	Protein (PDB ID)	Binding Energy (kcal/mol)	Major Binding Pocket Residues
Posaconazole	6C6P	-7.7	Tyr555, Glu475, Lys556, Phe392, Leu564, Pro563, Val560
	6E8Q	-7.9	Gln361, Tyr357, Ser437, His474, Tyr477, Leu158, Thr159, Ala436
Ravuconazole	6C6P	-7.1	Glu475, CPS602, Pro563, Ser567, Leu564
	6E8Q	-8.5	Ser230, Tyr227, Thr223, Ala226, Leu221, Ile205, Phe206, Asp233, Gln316
Voriconazole	6C6P	-6.1	Thr480, Arg349, Lys556, Leu484, Ala479, Glu475
	6E8Q	-7.1	Ser230, Ala226, Ile205, Leu221, Phe206, Glu202, Asp233, Gln316

The chemical with the strongest binding affinity was posaconazole (-7.7 kcal/mol) compared to voriconazole (-6.1 kcal/mol) and ravuconazole (-6.2 kcal/mol), whilst ravuconazole had a significantly lower binding affinity than posaconazole (both -7.9 kcal/mol), whereas 6E8Q had a significantly fewer binding value for both (6.2 and 7.3) than either, therefore it can be assumed that posaconazole would be a better inhibitor of 6C6P than ravuconazole, but it is certainly possible that ravuconazole may provide better binding properties against 6C8Q than posaconazole and voriconazole were able to provide at their respective binding affinities.

According to the docking analysis, the three triazole antifungal medications effectively used a combination of hydrogen bonding, hydrophobic contacts, halogen bonding, and aromatic interactions to occupy the active areas of CYP51 proteins. Nonetheless, notable variations in their binding strengths were noted. The interaction profiling revealed that all ligands occupied the active binding cavity of CYP51 proteins through multiple stabilizing contacts. In both proteins, posaconazole showed robust and stable binding due to its substantial hydrogen bonding and aromatic interactions. Ravuconazole showed the strongest affinity for 6E8Q based of the binding pocket's strong hydrophobic, π-sigma, and halogen interactions. Voriconazole less binding affinity may be attributed to the low number of stabilizing interactions it showed. Hydrophobic contacts, halogen interactions, and hydrogen bonding all interacted together in order to stabilize the ligand–protein complexes.

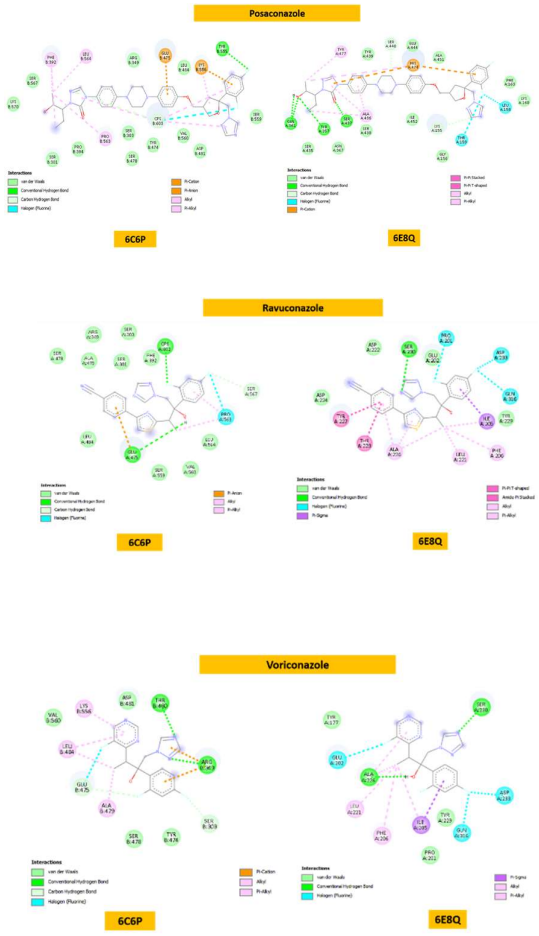

Figure.1: Comparative two-dimensional molecular interaction diagrams of Posaconazole, Ravuconazole, and Voriconazole docked against fungal CYP51 target protein (PDB ID: 6C6P) and squalene epoxidase (PDB ID: 6E8Q).

Discussion:

Dermatophytosis was considered one of the most frequently encountered superficial fungal infections globally, with an especially high burden in tropical and subtropical regions like India, where the climate supports persistent fungal survival and spread. The rising incidence of chronic and treatment-resistant dermatophytosis has brought growing attention to antifungal drug resistance and the declining effectiveness of standard therapeutic regimens Molecular docking is garnering attention as a viable computational approach for analyzing the way in which the antifungal compounds interact with critical fungal proteins that are involved in biosynthesis of ergosterol [22,23]. In the present study, molecular docking was employed for assessing the binding potential of three second-generation triazole antifungals namely voriconazole, posaconazole, and ravuconazole against lanosterol 14α-demethylase (CYP51; PDB ID: 6E8Q) and squalene epoxidase (PDB ID: 6C6P) which are two imperative fungal targets for sterol biosynthesis. It was observed that with all the three compounds demonstrated favourable docking interactions with both the enzymes; however their binding energies varied considerably.

With CYP51 (PDB ID: 6C6P); posaconazole demonstrated the strongest affinity at -7.7 kcal/mol while ravuconazole and voriconazole depicted −7.1 kcal/mol and −6.1 kcal/mol respectively. The enhanced docking performance of posaconazole can be attributed to its larger molecular structure which aided in establishing vast interactions with active-site residues namely Tyr555, Glu475, Lys556, Phe392, and Leu564. Furthermore, the ligand-protein complex would have been strengthened further by stabilising forces such as hydrogen bonds, π-cation contacts, and hydrophobic associations which ultimately resulted in complete engagement of the active site. On the other hand, weakest affinity for CYP51 was depicted by voriconazole which could possibly due to its smaller scaffold, which restricted the number of productive interactions regardless of hydrogen bonding with Thr480 and Arg349 and π-cation contact with Arg349. In case of squalene epoxidase (PDB ID: 6E8Q), ravuconazole showed the highest binding affinity of −8.5 kcal/mol. The superior binding was attributed to the strong hydrogen bond formed with Ser230, hydrophobic interactions with Ile205, Leu221, Phe206, and Ala226 along with halogen interactions with Asp233 and Gln316, and aromatic π-interactions collectively indicating higher structure compatibility within the active site. In addition, posaconazole's docking score of −7.9 kcal/mol demonstrated its ability to bind to this enzyme effectively through a combination of hydrogen bonds, π–π stacking, π–cation interactions and hydrophobic interactions, whereas ravuconazole's interactions were generally stronger. The differing interaction profiles; however, imply that posaconazole was likely to bind in a well-ordered manner by virtue of its more significant potential for inhibition. [24,25].

A primary step in the synthesis of ergosterol biosynthesis is the oxidative removal of the 14α-methyl group from lanosterol, which is mediated by lanosterol 14α-demethylase (CYP51) primary pharmacological target for azole-class antifungals. Any disruption in this process directly affects the fungal membrane integrity, subsequently inhibiting growth and proliferation. The superior docking affinity manifested by posaconazole against CYP51 in the current study thus indicates at a potentially enhanced capacity of impairing the synthesis of fungal sterol pathways [26,27]. Another vital target in antifungal drug design is the squalene epoxidase, which is responsible for converting squalene to 2,3-oxidosqualene. Though, the resolved fungal squalene epoxidase crystal structures are limited, the evidences suggest remarkable conservation of

active-site residues between fungal and human enzymes which further supports the use of structurally homologous models for assessments of qualitative docking [18,28]. Apart from this, the present study comprises of several limitations such as the no molecular dynamics simulations were considered and the analysis was restricted to computational docking, in vitro susceptibility testing, or in vivo experimental validations were not taken into account. The human-derived squalene epoxidase structure (6C6P) was utilized as a surrogate model due to the scarcity of resolved fungal enzyme structures. However, for confirming the biological relevance of the binding interactions reported in the study, experimental follow ups are required.

It was thus inferred that for CYP51 posaconazole showed the most favourable interaction profile, while ravuconazole demonstrated strong affinity for squalene epoxidase. However, owing to the smaller molecular structure and reduced active-site contact surface; voriconazole demonstrated lower docking scores. The results so obtained point at posaconazole and ravuconazole as promising candidates for development as antifungal agents targeting enzymes involved in ergosterol biosynthesis. The binding patterns identified in the study contribute to an enhanced understanding of ligand interaction at these fungal sites related to ergosterol biosynthesis.

Conclusion

The present study aided in demonstrating the effective binding of posaconazole, ravuconazole, and voriconazole to two significant fungal targets, squalene epoxidase (PDB ID: 6E8Q) and CYP51 (PDB ID: 6C6P), that are critical for the production of ergosterol. Based on the comparison of docking scores and interaction profiles; it was inferred that posaconazole exhibited highest binding affinity of −7.7 kcal/mol for CYP51, whereas ravuconazole demonstrated strongest affinity of −8.5 kcal/mol with squalene epoxidase. According to the research it was suggested that the presence of various stabilising interactions (hydrogen bonds, hydrophobic contacts, aromaticity, halogen bonding and electrostatic interactions) at the active site's cavities had the greatest influence on a compound's binding ability. In contrast, voriconazole had weaker binding capabilities because of its smaller molecular size and fewer interactions with the target. This study provides insight into how posaconazole and ravuconazole bind to antifungal targets as well as demonstrating their strong inhibitory ability to inhibit key fungal enzymes. These findings support the further exploration of these compounds in advanced computational, in vitro, and in vivo studies for the development of improved antifungal therapeutic strategies.

References:

1. Begum J, Mir NA, Linga Raju MC, Buyamayum B, Dev K. Recent advances in the diagnosis of dermatophytosis. J. Basic Microbiol 2020;60:293-303.
2. Rinaldi MG. Dermatophytosis: epidemiological and microbiological update. J. Am. Acad. Dermatol 2000;43:S120-4.
3. Martinez-Rossi NM, Peres NT, Rossi A. Pathogenesis of dermatophytosis: sensing the host tissue. Mycopathologia 2017;182:215-27.
4. Segal E, Elad D. Human and zoonotic dermatophytoses: epidemiological aspects. Frontiers in Microbiology. 2021 Aug 6;12:713532.
5. Nweze EI. Dermatophytoses in domesticated animals. Revista do Instituto de Medicina Tropical de São Paulo. 2011 Apr 25;53(2):94-9.
6. Kumar P, Ramachandran S, Das S, Bhattacharya SN, Taneja B. Insights into changing dermatophyte spectrum in India through analysis of cumulative 161,245 cases between 1939 and 2021. Mycopathologia. 2023 Jun;188(3):183-202.
7. Rajagopalan M, Inamadar A, Mittal A, Miskeen AK, Srinivas CR, Sardana K, Godse K, Patel K, Rengasamy M, Rudramurthy S, Dogra S. Expert consensus on the management of dermatophytosis in India (ECTODERM India). BMC dermatology. 2018 Jul 24;18(1):6.
8. Upadhyay V, Kumar A, Singh AK, Pandey J. Epidemiological characterization of dermatophytes at a tertiary care hospital in Eastern Uttar Pradesh, India. Current medical mycology. 2019 Mar;5(1):1.
9. Chatterjee A, Chattopadhyay D, Bhattacharya D, Dutta AK, Gupta DS. Some epidemiological aspects of zoophilic dermatophytosis.
10. Sholto A, Lee S, Hoffman BM, Barrett AG, Ehrenberg B. Spectroscopy, binding to liposomes and production of singlet oxygen by porphyrazines with modularly variable water solubility. Photochem Photobiol 2008;84:764-73.
11. Havlickova B, Czaika VA, Friedrich M. Epidemiological trends in skin mycoses worldwide. Mycoses. 2008 Sep;51:2-15.
12. Grillot R, Lebeau B. Systemic antifungal agents. Antimicrobial Agents: Antibacterials and Antifungals. 2005 Aug 31:1260-87.
13. Sahni K, Singh S, Dogra S. Newer topical treatments in skin and nail dermatophyte

infections. Indian dermatology online journal. 2018 May 1;9(3):149-58.
14. Shalini K, Kumar N, Drabu S, Sharma PK. Advances in synthetic approach to and antifungal activity of triazoles. Beilstein journal of organic chemistry. 2011 May 25;7(1):668-77.
15. Harman CA, Turman MV, Kozak KR, Marnett LJ, Smith WL, Garavito RM. Structural basis of enantioselective inhibition of cyclooxygenase-1 by S-α-substituted indomethacin ethanolamides. Journal of Biological Chemistry. 2007 Sep 21;282(38):28096-105.
16. Shiau AK, Barstad D, Loria PM, Cheng L, Kushner PJ, Agard DA, Greene GL. The structural basis of estrogen receptor/coactivator recognition and the antagonism of this interaction by tamoxifen. Cell. 1998 Dec 23;95(7):927-37.
17. Monk BC, Keniya MV, Sabherwal M, Wilson RK, Graham DO, Hassan HF, Chen D, Tyndall JD. Azole resistance reduces susceptibility to the tetrazole antifungal VT-1161. Antimicrobial Agents and Chemotherapy. 2019 Jan;63(1):10-128.
18. Padyana AK, Gross S, Jin L, Cianchetta G, Narayanaswamy R, Wang F, Wang R, Fang C, Lv X, Biller SA, Dang L. Structure and inhibition mechanism of the catalytic domain of human squalene epoxidase. Nature communications. 2019 Jan 9;10(1):97.
19. Hussen NH, Hasan AH, Jamalis J, Shakya S, Chander S, Kharkwal H, Murugesan S, Bastikar VA, Gupta PP. Potential inhibitory activity of phytoconstituents against black fungus: In silico ADMET, molecular docking and MD simulation studies. Computational toxicology. 2022 Nov 1;24:100247.
20. Sharma S, Sharma A, Gupta U. Molecular Docking studies on the Anti-fungal activity of. Allium sativum. 2021.
21. Trott O, Olson AJ. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. Journal of computational chemistry. 2010 Jan 30;31(2):455-61.
22. Sharma V, Kumawat TK, Sharma A, Seth R, Chandra S. Distribution and prevalence of dermatophytes in semi-arid region of India. Adv Microbiol. 2015 Feb 10;5(2):93.
23. Dogra S, Uprety S. The menace of chronic and recurrent dermatophytosis in India: Is the problem deeper than we perceive?. Indian dermatology online journal. 2016 Mar 1;7(2):73-6.
24. Parker JE, Warrilow AG, Price CL, Mullins JG, Kelly DE, Kelly SL. Resistance to antifungals that target CYP51. Journal of Chemical Biology. 2014 Oct;7(4):143-61.
25. Lepesheva GI, Waterman MR. Sterol 14α-demethylase cytochrome P450 (CYP51), a P450 in all biological kingdoms. Biochimica et biophysica acta (BBA)-General subjects. 2007 Mar 1;1770(3):467-77.
26. Monk BC, Tomasiak TM, Keniya MV, Huschmann FU, Tyndall JD, O'Connell III JD, Cannon RD, McDonald JG, Rodriguez A, Finer-Moore JS, Stroud RM. Architecture of a single membrane spanning cytochrome P450 suggests constraints that orient the catalytic domain relative to a bilayer. Proceedings of the National Academy of Sciences. 2014 Mar 11;111(10):3865-70.
27. Warrilow AG, Parker JE, Kelly DE, Kelly SL. Azole affinity of sterol 14α-demethylase (CYP51) enzymes from Candida albicans and Homo sapiens. Antimicrobial agents and chemotherapy. 2013 Mar;57(3):1352-60.
28. Ryder NS. Terbinafine: mode of action and properties of the squalene epoxidase inhibition. British Journal of Dermatology. 1992 Feb;126:2-7.