WWW.IJRPR.COM

International Journal of Research Publication and Reviews

Journal homepage: www.ijrpr.com ISSN 2582-7421

In Silico Evaluation of Kaempferol-3-O-Rhamninoside as a Potential Inhibitor of *Trichophyton Rubrum* Sterol 14α-Demethylase: A Novel Topical Agent for Foot Health

Mehmet Veysi Cetiz [a]*

[a] *Department of Medical Biochemistry, Faculty of Medicine, Harran University, Sanliurfa, 63290, Turkey*

A B S T R A C T

Tinea pedis, caused primarily by Trichophyton rubrum, remains one of the most persistent superficial fungal infections globally. The enzyme sterol 14α-demethylase (CYP51), essential for ergosterol biosynthesis in fungal membranes, is a validated antifungal target. Given the increasing resistance to azole antifungals, exploration of natural compounds with novel binding modes is warranted. This study aimed to characterize, via molecular docking, the binding interaction between kaempferol-3-O-rhamninoside—a polyhydroxylated flavonol glycoside—and T. rubrum CYP51, in an effort to assess its potential as a lead candidate for antifungal development. The 3D structure of CYP51 was retrieved from AlphaFoldDB, and ligand preparation was conducted via PubChem and Avogadro. Docking was performed using AutoDock Vina, and protein–ligand interactions were visualized and analyzed with PyMOL and PLIP. The docking box was defined using POCASA-predicted pockets. Kaempferol-3-O-rhamninoside exhibited a strong binding energy and a well-converged pose. Key interactions included hydrogen bonds with LYS364, ASN426, ASN427, THR431, VAL433, GLY459, ARG464, and GLU468; a salt bridge with LYS364; π–π stacking with TYR455; and hydrophobic contacts with VAL430 and VAL432. Although lacking a heme-coordinating group, the ligand was predicted to sterically block substrate ingress at the channel entrance.

1. Introduction

Maintaining optimal foot health is of paramount importance for ensuring mobility and daily comfort. The foot, as a body part in direct contact with the external environment and responsible for bearing the entire body weight, is exposed to various factors such as mechanical stress, moisture, temperature fluctuations, inadequate hygiene, and circulatory problems. These factors increase an individual's susceptibility to a diverse range of infections, skin lesions, and chronic foot conditions. A wide spectrum of foot-related disorders—including athlete's foot (tinea pedis), nail fungus (onychomycosis), calluses, fissures, and diabetic foot ulcers—have been shown to not only adversely affect quality of life, but also to require considerable time and financial resources for effective management (Boulton et al., 2008; Navarro-Pérez et al., 2023; Salim, Mwita, & Gwer, 2018).

Among superficial mycoses, athlete's foot (tinea pedis) is one of the most prevalent infections worldwide, primarily caused by dermatophytes—particularly *Trichophyton rubrum*. These infections are characterized by a constellation of symptoms, including pruritus, burning, malodor, and alterations in skin color and texture. Notably, tinea pedis exhibits a high rate of recurrence and resistance to therapeutic interventions. In conventional treatment strategies, topical antifungal creams are generally utilized as the primary treatment modality. However, the limited efficacy of currently available topical agents, their potential for adverse effects with prolonged use, and the rapid development of antifungal resistance among pathogenic fungi underscore the urgent need for the discovery of novel and more effective compounds (Benedict, 2024; Leung, Barankin, Lam, Leong, & Hon, 2023; Poojary, 2017).

Ergosterol biosynthesis, the process that supplies the predominant structural sterol of the fungal plasma membrane, is a central pharmacotherapeutic target in the management of mycotic infections. Sterol 14α-demethylase (CYP51) performs the pivotal oxidative demethylation of lanosterol, an indispensable step for downstream ergosterol formation. The azole antifungals, including clotrimazole, ketoconazole, bifonazole, and econazole, are widely prescribed. These medications act through competitive inhibition of CYP51, leading to depletion or aberrant accumulation of sterol intermediates, loss of membrane integrity, and consequent fungal cell death. Nevertheless, prolonged and extensive clinical use of these drugs has hastened the development of antifungal resistance, thereby diminishing overall treatment efficacy. This underscores the necessity of identifying novel scaffolds, with particular emphasis on low-toxicity, naturally sourced compounds (Choi et al., 2019; Krishnan-Natesan, Chandrasekar, Alangaden, & Manavathu, 2008; Liu et al., 2012; Zhang et al., 2019).

Plant-origin phenolic compounds have gained prominence as multifunctional bioactive compounds. These compounds exhibit antimicrobial, antioxidant, and anti-inflammatory activities. This has led to an increase in the use of plant-origin phenolic compounds in ethnopharmacology and evidence-based therapeutics. Their generally low toxicity has facilitated their broad use in phytotherapeutic and cosmetic dermatology products, while a substantial body

of in vitro and in silico investigations documents their inhibitory action against a wide spectrum of microbial taxa. In particular, phenolics capable of perturbing fungal ergosterol (cell-membrane sterol) biosynthetic pathways are hypothesized to impose a selective pressure for resistance that is lower than that imposed by classical antifungal drugs (Daglia, 2012; Lepesheva & Waterman, 2007; Perfect, 2017).

Kaempferol-3-O-rhamninoside is a multi-glycosylated kaempferol-derived flavonol whose polyhydroxylated phenolic scaffold suggests a theoretical capacity for multi-target molecular interactions. To date, no experimental studies have conclusively demonstrated antimicrobial or anticancer activity for this specific derivative, nor its inhibitory effect on sterol 14α-demethylase (CYP51) in *Trichophyton rubrum (Duran et al., 2024)*. Accordingly, the present work provides an in silico characterization of its predicted binding mode and affinity toward fungal CYP51 as a hypothesis-generating step. These computational findings are intended to prioritize this molecule for subsequent biochemical validation and formulation studies rather than to claim established antifungal efficacy.

2. Materials and Methods

2.1 Molecular docking

Molecular docking analyses were performed to investigate the interactions between Kaempferol-3-O-rhamninoside and *Trichophyton rubrum of* CYP51 target, were retrieved from the Protein Data Bank. Ligand structures were obtained from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/). Three-dimensional structures of target CYP51 were retrieved from the AlphaFoldDB (A0A178F3C9), and ligand structure were obtained from PubChem. Protein preparation was carried out using BIOVIA Discovery Studio and AutoDockTools v4.2.6, while ligand optimization was performed with Avogadro v1.2.0 using Gasteiger charges. Docking simulations were run in AutoDock Vina v1.1.2 with an exhaustiveness of 32. Binding pockets were predicted via the POCASA v1.1 server. Protocol validation included re-docking of co-crystallized ligands and RMSD evaluation (Baloglu et al., 2025; Yildirim et al., 2025). Protein–ligand interactions, particularly hydrogen bonds, were analyzed using the Protein–Ligand Interaction Profiler (PLIP) (Angeles Flores et al., 2024; Cetiz et al., 2024). Visualization of docking poses and molecular interactions was performed using PyMOL v2.5.8.

3. Results and Discussion

3.1 Molecular docking result

In this in silico investigation, the binding interaction of kaempferol-3-O-rhamninoside—a highly glycosylated kaempferol derivative—with the sterol 14α-demethylase (CYP51) enzyme of *Trichophyton rubrum* was characterized using molecular docking. CYP51 is a well-validated antifungal drug target responsible for ergosterol biosynthesis, and inhibition of this enzyme disrupts fungal membrane integrity. Docking simulations were performed using AutoDock Vina, with the grid parameters set to CENTER_X = –6.236, CENTER_Y = –0.086, CENTER_Z = 0.109, and box dimensions of SIZE_X = 126, SIZE_Y = 126, and SIZE_Z = 126. The ligand exhibited a top-ranked binding energy of –10.0 kcal·mol⁻¹ and a root-mean-square deviation (RMSD) of 0.2 Å, indicating a well-converged and reproducible pose. Based on commonly accepted thresholds, binding energies ≤ –9.0 kcal·mol⁻¹ are generally considered indicative of strong affinity for drug-like compounds targeting cytochrome P450 enzymes.

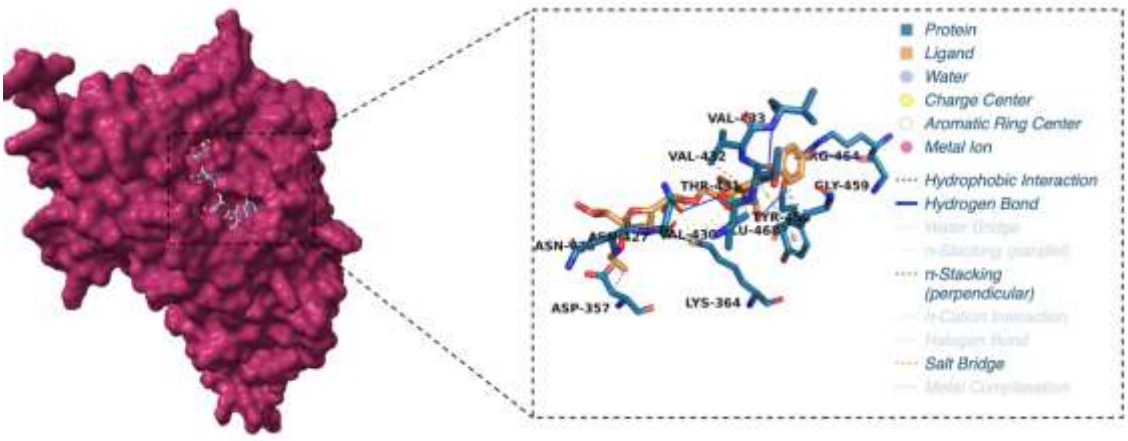

Fig. 1. Docking pose of Kaempferol-3-O-rhamninoside in *Trichophyton rubrum CYP51*.

The predicted binding conformation revealed an extensive hydrogen bonding network involving several catalytically and structurally relevant residues, including LYS364, ASN426, ASN427, THR431, VAL433, GLY459, ARG464, and GLU468 (Fig 1). In particular, a salt bridge interaction was observed between the ligand and LYS364, which may contribute to electrostatic stabilization of the complex. Additionally, π–π stacking interactions with TYR455 further reinforced the ligand's orientation, while hydrophobic contacts with ASP357, VAL430, VAL432, and the aromatic ring of TYR455 contributed secondary stabilization. Despite the absence of an iron-chelating heterocycle, which is typical for azole-based CYP51 inhibitors, the bulky polar head of kaempferol-3-O-rhamninoside appears to occupy the substrate channel entrance, potentially obstructing lanosterol access. This mode of interaction,

although distinct from classical heme-coordinating ligands, may represent a competitive or allosteric mechanism of inhibition. However, the high molecular weight, pronounced polarity, and elevated topological polar surface area of the ligand (likely >250 Å²) may limit its passive diffusion across the fungal membrane and its dermal permeability. These features also raise concerns regarding potential entropic penalties and overestimation of affinity due to scoring biases inherent to hydrogen bond-rich ligands in docking algorithms.

4. Conclusion

This computational study identifies kaempferol-3-O-rhamninoside as a stable and multi-anchored binder to Trichophyton rubrum CYP51, with a notable binding score of –10.0 kcal·mol⁻¹ and consistent RMSD convergence. The interaction fingerprint includes extensive hydrogen bonding, a stabilizing salt bridge, and π–π stacking, despite the absence of a canonical heme-coordinating moiety. The bulky glycosidic cap appears to obstruct the substrate access channel, suggesting a possible steric or allosteric mode of inhibition distinct from classical azole antifungals. However, the ligand's high molecular weight, extensive polarity, and predicted low membrane permeability raise important considerations regarding its pharmacokinetic feasibility. As such, these findings should be interpreted as hypothesis-generating rather than confirmatory. Future work should focus on experimental CYP51 inhibition assays, permeability testing, and the rational design of deglycosylated derivatives or formulation strategies to address delivery challenges. Collectively, this work establishes a structural rationale for prioritizing kaempferol-3-O-rhamninoside and its analogs as potential antifungal agents for further investigation.

Acknowledgements

Ethical approval: Not applicable

Conflict of interest: The authors declare no competing interests.

Author contributions: Conceptualization, M.V.C; **Supervision**, M.V.C; **Methodology**, M.V.C; **Writing- Original draft preparation**, M.V.C; **Writing - Review & Editing**, M.V.C.

Funding: The bioinformatics component of this study was supported by the Scientific and Technological Research Council of Türkiye (TUBITAK, Project No: 124C078).

Data Availability Data will be made available on request.

Code Availability: Not applicable

Consent to participate: Not applicable.

Consent for publication: Not applicable.

References

Angeles Flores, G., Cusumano, G., Zengin, G., Cetiz, M. V., Uba, A. I., Senkardes, I., . . . Emiliani, C. (2024). Using in vitro and in silico analysis to investigate the chemical profile and biological properties of polygonum istanbulicum extracts. Plants, 13(23), 3421.

Baloglu, M. C., Ozer, L. Y., Pirci, B., Altunoglu, Y. C., Soomro, S. I., Zengin, G., . . . Jekő, J. (2025). Decoding chemical profiles, biological functions, and medicinal properties of liquidambar orientalis extracts through molecular modeling and bioinformatic methods. Food Frontiers.

Benedict, K. (2024). Topical antifungal prescribing for medicare part d beneficiaries—united states, 2021. MMWR. Morbidity and Mortality Weekly Report, 73.

Boulton, A. J., Armstrong, D. G., Albert, S. F., Frykberg, R. G., Hellman, R., Kirkman, M. S., . . . Mueller, M. J. (2008). Comprehensive foot examination and risk assessment: A report of the task force of the foot care interest group of the american diabetes association, with endorsement by the american association of clinical endocrinologists. Diabetes care, 31(8), 1679-1685.

Cetiz, M. V., Isah, M., Ak, G., Bakar, K., Himidi, A. A., Mohamed, A., . . . Cespedes-Acuna, C. L. (2024). Exploring of chemical profile and biological activities of three ocimum species from comoros islands: A combination of in vitro and in silico insights. Cell Biochemistry and Function, 42(7), e70000.

Choi, M. J., Won, E. J., Joo, M. Y., Park, Y.-J., Kim, S. H., Shin, M. G., & Shin, J. H. (2019). Microsatellite typing and resistance mechanism analysis of voriconazole-resistant aspergillus flavus isolates in south korean hospitals. Antimicrobial agents and chemotherapy, 63(2), 10.1128/aac. 01610-01618.

Daglia, M. (2012). Polyphenols as antimicrobial agents. Current opinion in biotechnology, 23(2), 174-181.

Duran, T., Peron, G., Zancato, M., Zengin, G., Cetiz, M. V., Bouyahya, A., . . . Kljakić, A. C. (2024). Harnessing the chemical composition and anti-oxidant, anti-enzymatic, and anti-cancer activities of two corydalis species (c. Erdelii and c. Solida) by using in vitro and in silico analysis. Food Bioscience, 61, 104762.

Krishnan-Natesan, S., Chandrasekar, P. H., Alangaden, G. J., & Manavathu, E. K. (2008). Molecular characterisation of cyp51a and cyp51b genes coding for p450 14α-lanosterol demethylases a (cyp51ap) and b (cyp51bp) from voriconazole-resistant laboratory isolates of aspergillus flavus. International journal of antimicrobial agents, 32(6), 519-524.

Lepesheva, G. I., & Waterman, M. R. (2007). Sterol 14α-demethylase cytochrome p450 (cyp51), a p450 in all biological kingdoms. Biochimica et biophysica acta (BBA)-General subjects, 1770(3), 467-477.

Leung, A. K., Barankin, B., Lam, J. M., Leong, K. F., & Hon, K. L. (2023). Tinea pedis: An updated review. Drugs in context, 12.

Liu, W., Sun, Y., Chen, W., Liu, W., Wan, Z., Bu, D., & Li, R. (2012). The t788g mutation in the cyp51c gene confers voriconazole resistance in aspergillus flavus causing aspergillosis. Antimicrobial agents and chemotherapy, 56(5), 2598-2603.

Navarro-Pérez, D., Tardáguila-García, A., García-Oreja, S., López-Moral, M., García-Madrid, M., & Lázaro-Martínez, J. L. (2023). Onychomycosis associated with diabetic foot syndrome: A systematic review. Mycoses, 66(6), 459-466.

Perfect, J. R. (2017). The antifungal pipeline: A reality check. Nature reviews Drug discovery, 16(9), 603-616.

Poojary, S. A. (2017). Topical antifungals: A review and their role in current management of dermatophytoses. Clinical Dermatology Review, 1(Suppl 1), S24-S29.

Salim, A. O., Mwita, C. C., & Gwer, S. (2018). Treatment of madura foot: A systematic review. JBI Evidence Synthesis, 16(7), 1519-1536.

Yildirim, M. A., Sevinc, B., Paydas, S., Karaselek, M. A., Duran, T., Kuccukturk, S., . . . Acquaticci, L. (2025). Exploring the anticancer potential of dianthus orientalis in pancreatic cancer: A molecular and cellular study. Food Bioscience, 106183.

Zhang, J., Li, L., Lv, Q., Yan, L., Wang, Y., & Jiang, Y. (2019). The fungal cyp51s: Their functions, structures, related drug resistance, and inhibitors. Frontiers in microbiology, 10, 691.