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RESEARCH ARTICLE

Molecular Docking Study of Heterocyclic Compounds for Antifungal Activity Against Granulomatous Amoebic Encephalitis

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ABSTRACT

Our study identified seven unique heterocyclic compounds: 2-(m Tolylthio) Chalcone, chitosan oligosaccharide, and 2-hydroxy chalcone. Six-chloropyridine, 4-naphthoquinone, Thiobenzimidazole, 2-thiobenzoxazole, 6-carboxylic acid ethyl ester, and Anthrimide are probable choices that may be found in a chemical database. Posaconazole and Isuvuconazole are reference compounds found in a literature study. The isuvuconazole-bound complex of *Acanthamoeba castellanii* CYP51 of PDBID 6UX0 is the focus of our investigation. Docking simulations were carried out to evaluate these drugs' binding affinity to the *Acanthamoeba castellanii* CYP51 complex, using Isuvuconazole as the reference compound. Vina Wizard and PyRX software were used to carry out the docking simulations. Anthrimide, the ligand, demonstrated a binding energy of -10.3 kcal/mol in our data, indicating great promise for treating antifungal diseases in the future. Auto dock further validated this value to be -10.2. Further *in vivo* testing will confirm the findings of this study.

Keywords: Docking; Amoebic; Encephalitis; Heterocyclic; Antifungal; PyRX

INTRODUCTION

We aim to determine if any particular heterocyclic compounds can treat granulomatous amebic encephalitis by acting as antifungal agents. Free-living amoebas, especially *Acanthamoeba* spp.[1] are the cause of the uncommon and frequently fatal brain illness known as granulomatous amebic encephalitis. Since GAE is so unusual, there is currently no recognized cure; however, a mix of drugs, such as Pentamidine, Flucytosine, azithromycin, Flucytosine, Miltefosine, and rifampin, has demonstrated efficacy in certain instances. Within the triazoles class of antifungal treatments, there is a fungal enzyme called lanosterol 14α-demethylase (LDM) that is targeted by the drugs Isuvuconazole, voriconazole, and potonazole[2] Chitosan, a copolymer made up of D-glucosamine and N-acetyl-d-glucosamine units, has antimicrobial properties that depend on the molecular weight[3] and the combination of these two units. However, the therapeutic potential of Chalcone derivatives has been explored. They demonstrated various medicinal properties, such as antibacterial, antimalarial, antiprotozoal, antitubercular, anticancer, and antifungal qualities[4]. Chalcone derivatives are an interesting topic for study in pharmacology because of their variety of functions. In the end, it has been discovered that conazoles, an antifungal medication that targets sterol 14-demethylase (CYP51), adversely affect the trophozoites and cysts of the organism castellani[5]. Many lipophilic compounds, including drugs, are metabolized by cytochromes, and antifungal medications such as econazole target CYP51 or sterol 14 α-demethylase[6] 2022A dimer is formed through the N-termini swapping of the enzyme target *Acanthamoeba castellanii* sterol 14 α-demethylase (AcCYP51), whereas AcCYP51 that is linked to a drug exists in a monomeric form. Within the AcCYP51-isavuconazole combination, the protein target cannot refold the 74 N-terminal[7] PyRX is free, open-source docking software that enables the docking of multiple ligands with a target protein. PyRX utilizes the Auto Dock Vina tool and Open Babel, and Biovia Discovery Studio serves as the visualizer.[8]

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MATERIALS AND METHODS

Protein Preparation

The protein structure with the identifier 6UX0 was obtained from the RCSB Protein Data Bank. After opening the PDB file, the protein's binding site was identified using relevant literature. HET atoms and ligand groups were removed, and the chains were eliminated to simplify the structure. The resulting file was saved in PDB format.

Preparation of Ligands

The ligands were downloaded from the PubChem database[9–12]. Moreover, it was redrawn using Chemsketch software**.** The ligand was converted to PDB format using PYMOL and saved accordingly. PyRX Software, developed in Python, was used for further processing. The hardware utilized for this process included an HP Intel Core i5 laptop with HD Graphics.

Input files

The PubChem database provided the ligands' 3D structure in Sdf format for download. The RCSB provided the 6UX0 Receptor protein for further investigation.

Docking executed with PyRX

The PyRX integration Auto Dock Vina was used to carry out the docking procedure. Following shipment, the ligand and protein's properties were determined. Docking requirements were specified using a grid box, and PDBQT files were established. Additionally, the amount of exhaustiveness of docking was established. Pose, a connection, and RMSD information were obtained after docking. PYMOL was used to evaluate the outcomes further. Redocking was also done using the same technique to determine the binding affinity.

RESULTS

Our study found that the ligand Anthrimide showed promising activity among seven specific heterocyclic compounds, with a binding energy of -10.3 kcal/mol. This is a higher binding energy than the Redocking score of Isuvaconazole, making it a potential lead compound for antifungal activity and drug development.

DISCUSSION

The heterocyclic compound anthrimide showed promising antifungal activity, which can be predicted from the compound's good docking score. Antifungal therapy is a strategy of granulomatous amebic encephalitis treatment. Anthrimide exhibited a docking score of -10.3 by the PyRX method and -10.2 by the auto dock 4.2.6 method, which is close together. The standard drugs used to compare docking scores were isavuconazole and posaconazole, with

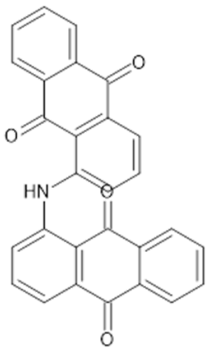

Fig. 1: Anthrimide (ligand)

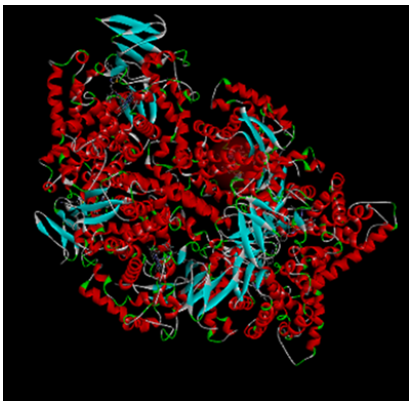

Fig. 2: Isuvuconazole-bound complex of *Acanthamoeba castellanii* (PDBID 6UX0)

Table 1: Pyrex Docking Results

Ligand	*Binding Affinity		RMSD / UB	RMSD / LB
	PyRX	Auto dock 4		
Chitosan oligosaccharide	-5.9		0	0
2 Hydroxy Chalcone	-6.3		0	0
2-(m Tolylthio)-6-Chloropyridine	-5.3		0	0
1-4- Naphoquinone, Thiobenzimidazole	-5.2		0	0
Thiobenzimidazole	-4.5		0	0
2- Thiobenzoxazole 6 Carboxylic acid ethyl ester	-5		0	0
Anthrimide	-10.3	-10.2	0	0
Posaconazole	-8.6	-8.5	0	0
Isavuconazole	-6.5		0	0

*N=9. Docking results showing binding energy, root mean square deviation, upper bound and lower bound (PyRX and Auto dock); less binding energy more activity predicted.

Table 2: Swiss ADME Prediction

Ligand	Lipophilicity	Water Solubility	Absorption	Drug Likeliness	Synthetic Accessibility
Anthracite	Log P 2.98	Log S -6.63	GI -High BBB-No	Lipinski- 0 Violation	3.29
Posaconazole	Log P 5.12	Log S -6.69	GI -High BBB-No	Lipinski- 2 Violation	6.02
Isuvuconazole	Log P3.26	Log S -4.91	GI -Low BBB No	Lipinski- 0 Violation	4.26

*N=9. Absorption distribution, metabolism distribution Prediction (Swiss ADME technique); log p indicates blood brain barrier penetration.

docking scores of-6.5 and -8.6, respectively. The absorption distribution metabolism excretion (ADME) parameters for the compound anthrimide were satisfactory. Synthetic accessibility was also found to be good. The log p value indicated the compound's lipophilicity, which predicted the blood-brain barrier penetration, an essential parameter for this disease. The mechanism underlying the antifungal activity is inhibiting the ergosterol production of fungal membranes. The enzyme 14α-de methylase is a critical factor in lanosterol synthesis, a fungal cell membrane component. As a proposed mechanism of action, the heterocyclic compound anthrimide may act by inhibiting this enzyme can be considered.

Further quantitative structure-activity relationship (QSAR) studies, followed by *in vitro* activity studies using cell lines and *in vivo* studies using animal models, may be conducted to establish the antifungal activity of this promising heterocyclic compound, anthrimide. Granulomatous amebic encephalitis is a rare but severe infection caused by amoebas living in freshwater lakes. This disease is spread by swimming and diving in lakes and ponds. Recently, the state of Kerala has witnessed several cases of granulomatous amebic encephalitis, and this is a matter of concern.

The compound of interest was chosen as anthrimide based on the docking score. The isavuconazole-bound complex of *Acanthamoeba castellanii* CYP51 of PDBID 6UX0, a prime target for antifungal drugs, was used as the receptor protein[12]. Biovia Discovery Studio was used as a visualizer for ligands and receptors. The standard medications for comparison of docking scores were Isuvuconazole and Posaconazole. The promising docking score obtained for the heterocyclic compound anthrimide predicts the possible mechanism of inhibition of ergosterol synthesis, which is a critical component of the fungal cell wall. These study findings provided valuable information on the pharmacological basis of heterocyclic compounds. The prediction of the absorption distribution metabolism excretion parameters was also found satisfactory, which further supports the feasibility of the antifungal compound.

CONCLUSION

Heterocyclic compounds possess diverse biological activities when tested in-vivo and in-vitro as per literature reported. In silico testing using computational tools provide a prediction of biological activity of compounds on a receptor based on their binding energies. Here we use two in-silico docking software's for prediction of antifungal activity of compounds selected from a chemical database based on structural similarity. The values of key compounds identified were compared using both software's and founds similar. Any differences in the quantum of values in both methods were due to the methods and different force fields employed. Antifungal activity is an inevitable criterion of drugs used for granulomatous amebic encephalitis treatment. The lead compound identified was anthrimide. Further structural modifications of the selected compound can be done by employing QSAR techniques to generate better drugs for antifungal therapy.

Any new drug developed for the cause of treating human ailments can be considered as a milestone of achievement.

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