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Research Article

DRUG REPURPOSING OF COMMERCIALLY AVAILABLE AZOLES AGAINST ASPERGILLOSIS PEROXIREDOXIN ASP F3 ALLERGEN DRUGS

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ABSTRACT

Objectives: *Aspergillus peroxiredoxin* is a significant contributor to a multitude of infections in both humans and animals infecting the respiratory system due to which lungs related illnesses and fatalities continue around the globe. This research examines various antifungal drugs which are effective against aspergillus.

Methods: The present study implements computational methods to assess the effectiveness of several phytochemicals toward the *A. peroxiredoxin* protein PyRx, the virtual screening tool was used to systematically perform molecular docking. It was done so to test the binding affinity with *A. peroxiredoxin protein* 5J9B, 10 phytocompounds were selected from Azole Antifungals which was based on previous knowledge. Using ADMET filters, the pharmacological evaluation of the ligands was performed.

Results: The ligands flutrimazole, clotrimazole, fluconazole, sertaconazole, and bifonazole demonstrated the best binding with the target protein peroxiredoxin Asp f3.

Conclusion: The broad-spectrum anti-fungal drug Flutrimazole demonstrated the least binding and best pharmacological parameters with the target proteins. This drug can be repurposed for other fungal infections.

Keywords: Drug repurposing, Azoles, Aspergillosis, Molecular docking, ADMET.

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INTRODUCTION

Aspergillus peroxiredoxin is a saprotrophic fungus that lives primarily in soil. The fungus has accomplished to adapt and proliferate in hostile environments in its ecological niche. This ability has enabled the fungus to resist and survive human host defenses, as well as to cause one of the most lethal lung infections in terms of morbidity and mortality [1]. The importance of this infection has grown dramatically as the number of patients with compromised immune systems associated with cancer, organ transplantation, and autoimmune conditions has increased; critically ill patients appear to be at a higher risk [2]. Invasive pulmonary aspergillosis (IPA) is a serious disease that can affect not only severely immunocompromised patients but also critically ill patients and those suffering from chronic obstructive pulmonary disease. Chronic necrotizing aspergillosis is a locally invasive infection that primarily affects patients with mild immunodeficiency or chronic lung disease. Non-invasive forms of Aspergillus lung disease include aspergilloma and allergic bronchopulmonary aspergillosis (ABPA). Aspergilloma is a fungus ball that develops in a pre-existing cavity within the lung parenchyma, whereas ABPA is a hypersensitivity manifestation in the lungs that almost always affects asthma or cystic fibrosis patients [3,4].

Asp f3 is one of the most abundant *Aspergillus fumigatus* proteins. This peroxiredoxin is a major fungal allergen as well as a virulence factor, vaccine candidate, and also reactive oxygen species (ROS) scavenger. Asp f3 protects *A. fumigatus* from immune cell killing, Asp f3 is a protein that is abundant among pathogenic *A. fumigatus*. It serves as a ROS scavenger and vaccine candidate. Among other components Asp f3 gene Afyap1 significantly reduces nuclear localization during ROS exposure, indicating that Asp f3 can act as an intracellular redox sensor for several target proteins [5]. This further corroborates that the Aspf3 mutant's avirulence in a murine infection model is linked to a low-iron growth defect of this mutant. According to our findings, Asp f3 is not required for iron homeostasis. Rather the key role of Asp f3 and its partially redundant homolog Af3l1 in overcoming the host's nutritional immunity [6].

A drug repurposing technique is used to locate eligible medications by virtual screening of drug libraries [7]. Several data-driven and experimental methodologies for identifying repurposable medication candidates have been proposed; nevertheless, there are significant technological and regulatory difficulties that must be solved [8,9]. The fundamental protocol in drug development is drug repurposing, which is delineated as exploring alternative uses for medications that have already received approval or previously investigated but unapproved medicines. Repurposing drugs is comparable to recycling as it includes using well-known medications for purposes other than their intended ones. Repurposing pharmaceuticals can be quicker, cheaper, less dangerous, and more productive than conventional drug development approaches. This is essential because investigators can circumvent the initial phases of research that determine drug safety as they are already accomplished [10]. In the present study, the commercially available azoles are repurposed against *aspergillosis peroxiredoxin,* and Asp f3 allergens to examine their antifungal potency.

METHODS

In pursuit of the most favorable therapeutic targets against the 5J9B protein, the primary causative factor for aspergillosis in man and animals, virtual screening, and molecular docking studies was done to find potential drugs that inhibit peroxiredoxin Asp f3.

Retrieval of ligands

The DRUGBANK online database (https://go.drugbank.com/categories/DBCAT003230) was used to select 26 promising antifungal azoles for the analysis [11]. The canonical SMILES and the PubChem

ID of the selected ligands were documented and the ligands were downloaded.SDF format (https://pubchem.ncbi.nlm.nih.gov/) [12].

Retrieval of protein

The protein peroxiredoxin Asp f3 with PDB ID 5J9B was downloaded from the RSCB PDB databank (https://www.rcsb.org/structure/5J9B) [13]. The protein is downloaded in PDB format and the missing residues were modeled with SWISS-MODEL [14]. The resolution of the downloaded protein is 2.10 Å and the method of retrieval of the protein is X-RAY DIFFRACTION (Fig. 1).

Protein purification

The protein 5J9B was purified by deleting the water molecules as the free energy of the water molecule does not match its crystallographic structure. Before docking, all water molecules were fully eliminated because they might affect docking results. All other additional chains were removed from the protein structure to simplify the structure and the A-chins were retained. The prebound heteroatoms and the complexed ligands were also deleted from the protein structure and the polar hydrogen atoms were added to upgrade the caliber of the purified protein (Fig. 1). The protein purification was achieved in the BIOVIA DS Discovery Studio [15]. The purified structures were subjected to secondary structure prediction and Ramachandran plot analysis in the PDBSumgenerate webserver (http://www.ebi.ac.uk/thornton-srv/databases/pdbsum/Generate.html) [16].

Pharmacological studies

SwissADME (http://www.swissadme.ch/) analysis is used to evaluate the pharmacological properties of the ligands based on their physicochemical parameters including lipophilicity, saturation, insolubility, flexibility, size, and polarity that are the physicochemical properties [17]. Then, the ligands were screened based on the LIPINSKI rule of 5. Utilizing ADMETlab 2.0 (https://admetmesh.scbdd.com/), the toxicity parameters of the ligands were evaluated [18].

Molecular docking

The PyRX virtual screening tools [19] were employed to evaluate the inhibitory properties of the antifungal drugs against the 5J9B protein. The PyRx software assumes the protein is a macromolecule by adding Kollman charges and assigning every atom as Autodock 4 type. Therefore, the purified 5J9B protein is converted to.pdbqt format before proceeding to docking. A total of 26 ligands were loaded in.sdf format. The ligands were subjected to energy minimization by the application of a universal force field and the torsions of the ligands were detected. The energy-minimized ligands were further converted into. pdbqt format in the OpenBabel feature of PyRx. The grid dimensions of X=41.9756Å, Y=37.6804Å, and Z=37.3425Å. were chosen for the docking. The ligands were docked independently against 5J9B. In the PyRx software, the ligands take up nine different conformations to attain the best binding conformation with the protein. The degree of binding is evaluated based on the binding affinity. The least binding affinity indicates best docking confirmations and therefore, the top 5 ligands with the least binding affinity scores at zero root mean square deviation values were visualized in the DS Biovia Discovery Studio. From the docking analysis, the most effective compounds were Flutrimazole, Bifonazole, Clotrimazole, Fluconazole, and Sertaconazole.

Visualization

The 2D and 3D models of the top five docked ligands with the best binding scores were generated and downloaded by utilizing Dassault Systems BIOVIA Discovery Studio Visualizer. The type of interactions, non-bond atoms, bond distances, and bond type were evaluated [15].

RESULTS

Protein structure analysis

Ramachandran plot

The Ramachandran plot may be used to see the energetically permissible areas where amino acid torsions are angled against one another in a protein structure. The Ramachandran plot for purified 5J9B, which is depicted in Fig. 2, was created using PROCHECK and the secondary structure was evaluated in the PROSITE (Fig. 3). The sterically permissible regions on the graph that allows for stable peptide conformation are shown by the red areas on the graph. For *in silico* investigations, amino acids must be in the sterically permissible zone to a greater extent than 88%. The approved section contains 88.6%, or 124 residues, of the amino acid residues, whereas the banned region has 0.7% or 1 residue. Sub-regions comprise the remaining 10.0%. Three end residues, 11 glycine residues, nine proline residues, and 140 non-proline and non-glycine residues make up the 163 total residues.

The protein peroxiredoxin Asp f3's predicted secondary structure includes two sheets, two beta hairpins, one beta bulge, seven strands, six helices, 13 beta turns, and one gamma turn, according to the PDBsum data.

SwissADME analysis

Drug likeliness and ADMET analysis

The drug similarity analysis uses the bioavailability score to determine the potency of the medication candidate for oral administration. The score criteria are built on the structural properties of small molecules.

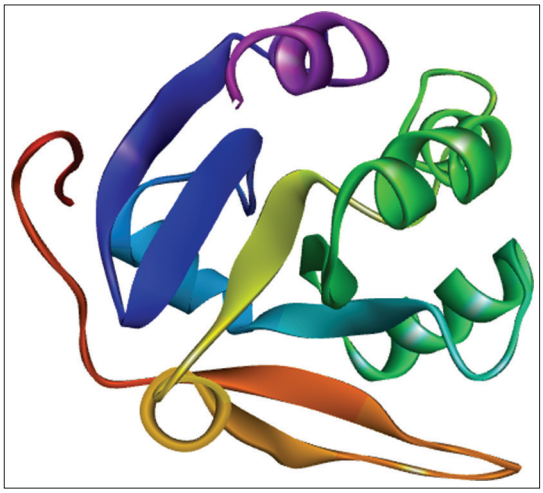

Fig. 1: Purified structure of Peroxiredoxin Asp f3 (PDB ID: 5J9B)

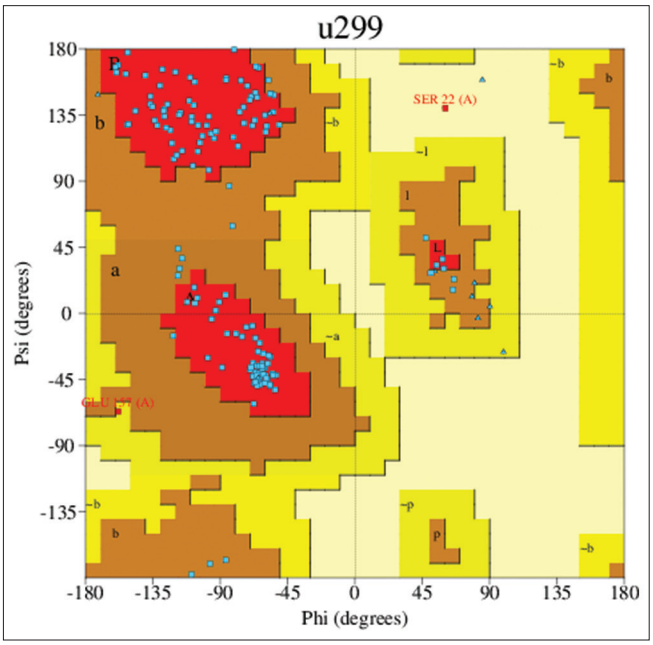

Fig. 2: Ramachandran plot of 5J9B protein using PDBsum

The Lipinski rule of five is used to screen out small compounds and evaluate how similar they are to medications. The PAINS score serves as a demonstration of the medicinal chemistry properties of therapeutic compounds by highlighting substructures in the studies that demonstrate strong responses independent of the protein target.

For the physicochemical evaluation, the ligands are screened based on the following parameters: Lipophilicity (xLogP: −0.7 to +5.0), Size (Molecular weight: 150–500 g/mol), Polarity (Topological Surface area: 20–130Å^2), Saturation (sp3 hybridization: not <0.25), Flexibility (Rotatable bonds: <9). The top five ligands fulfilled all five physicochemical screening parameters except Clotrimazole and Sertaconazole which exhibited slightly higher lipophilicity (Table 1).

The Lipinski Rule of 5 is stated as the golden rule for the pharmacological screening of candidate drug molecules. According to the Lipinski Rule, the drug should have a molecular weight of 150–500 Daltons, lipophilicity <4.15, the hydrogen bond donors should be <5, the hydrogen bond acceptors should be <10, and the molar refractivity should be between 40 and 130 Å^2. The top five ligands were subjected to Lipinski evaluation and the ligands fulfilled Lipinski parameters without any violations (Table 2).

ADME analysis

The degree of blood-brain permeation and human gastrointestinal absorption, glycoprotein permeability, and solubility of the drug is studied in the ADME analysis (Table 3). The blood brain barrier (BBB) determines the potential of the drug candidate to pass the blood-brain barrier. For the synthesis of medication, this knowledge is crucial. To increase the drug's effectiveness, gastrointestinal (GI) adsorption should be high. The oral drugs should indeed have high gastrointestinal absorption and solubility to exert optimal drug action.

Toxicity prediction

The essential elements of toxicity prediction include the following. Skin sensitivity, carcinogenicity, respiratory toxicity, AMES toxicity, rat oral acute toxicity, FDAMDD, hERG blockers, H-HT, and DILI are all factors that were considered (Table 4).

Molecular docking analysis

The ligands flutrimazole, clotrimazole, fluconazole, sertaconazole, and bifonazole demonstrated the best binding with the target protein peroxiredoxin Asp f3 (Table 5).

Visualization

The ligand Flutrimazole has the least binding energy with the target protein and therefore, this docked complex was visualized in Biovia. From the 3D and 2D interaction diagrams (Fig. 4a and b), it is evident that the ligand is bound to the amino groups including Asp 82, Val 83, Val 84, Asp 107, Asp 108, and Leu 110 in the A chain of the protein.

DISCUSSION

Micheli discovered a disease caused by saprophytic molds in the genus Aspergillus in 1729 this marked the first-time *Aspergillosis peroxiredoxin* was discovered as a fungal disease affecting humans Aspergillus causes a variety of health issues, including allergic responses, lung infections, and infections in other organs [1-3]. Depending on the immunological state or pulmonary anatomy of the host, patients with severe immunodeficiency are more likely to develop IPA. Individuals with an underlying condition, such as asthma, cystic fibrosis, or previous lung disease, or who have taken corticosteroid drugs for a long period, also in individuals with a weakened immune system, including people having low levels of neutrophils, a type of white blood cell that helps the body fight off infection and heal itself (neutropenia), or who are taking drugs that suppress the immune system, are much more likely to develop an infection. Aspergillosis often occurs when vulnerable individuals breathe in (inhale) Aspergillus spores [4,5]. Aspergillosis is not infectious and cannot be passed from person to person. The protein 5J9B (Fig. 1) used in this study provides insight into Oxidative Stress Resistance and Virulence of *A. fumigatus* [5].

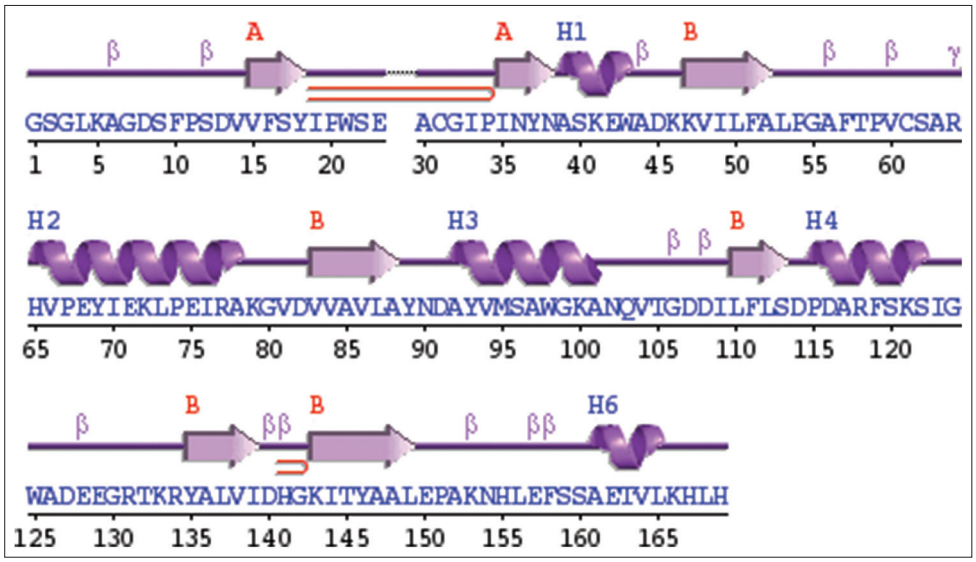

Fig. 3: Secondary structure of protein 5J9B

Table 1: Physicochemical properties of the ligand molecules

Ligands	Molecular weight	Fraction Csp3	Rotatable bonds	TPSA	Lipophilicity
Flutrimazole	346.37	0.05	4	17.82	4.99
Clotrimazole	344.84	0.05	4	17.82	5.41
Fluconazole	306.27	0.23	5	81.65	0.35
Sertaconazole	417.35	0.19	6	55.29	5.46
Bifonazole	310.39	0.05	4	17.82	4.77

TPSA: Topological polar surface area

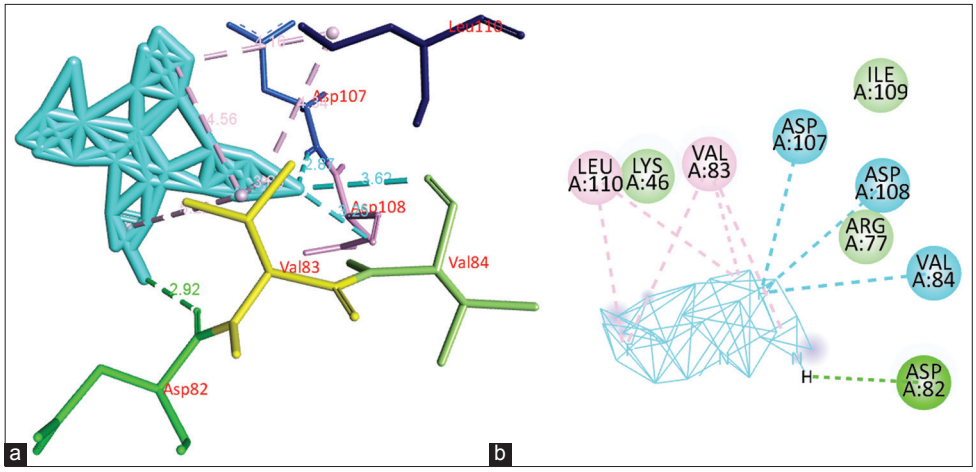

Fig. 4: Visualization of molecular interactions of 5J9b with Flutrimazole ligand. (a) 3D structure, (b) 2D structure

Table 2: Data for the properties of the Lipinski rule obtained using Swiss absorption, distribution, metabolism, excretion

Ligand	Molecular weight	MLogP	Hydrogen donors	Hydrogen acceptors	Molar refractivity
Flutrimazole	346.37	4.65	0	3	96.75
Clotrimazole	344.84	4.38	0	1	101.84
Fluconazole	306.27	1.47	1	7	70.71
Sertaconazole	417.35	4.11	0	2	113.53
Bifonazole	310.39	3.9	0	1	97.9

Table 3: Absorption distribution metabolism excretion data obtained using Swissabsorption distribution metabolism excretion

Ligands	Blood brain barrier penetration	GI absorption	PGP substrate	Solubility (LOGSw-SILICOS IT)
Flutrimazole	No	High	Yes	−8.53
Clotrimazole	Yes	High	Yes	−8.59
Fluconazole	No	High	Yes	−3.54
Sertaconazole	No	High	Yes	−8.51
Bifonazole	Yes	High	Yes	−7.99

GI: Gastrointestinal, PGP: P-glycoprotein

Table 4: Toxicity categorization

Ligands	hERG	H-HT	DILI	Ames	ROA	Carcinogenicity	Respiratory
Flutrimazole	0.67	0.193	0.979	0.5	0.028	0.491	0.055
Clotrimazole	0.103	0.054	0.981	0.729	0.109	0.424	0.041
Fluconazole	0.089	0.915	0.989	0.762	0.932	0.927	0.835
Sertaconazole	0.818	0.084	0.97	0.869	0.06	0.127	0.339
Bifonazole	0.854	0.099	0.982	0.452	0.051	0.079	0.014

hERG: Human ether-a-go-go related gene, DILI: Drug-induced liver injury, ROA: Rat oral acute, H-HT: Human hepatotoxicity

Table 5: Binding affinity of the ligands with protein peroxiredoxin *Aspergillus peroxiredoxin* f3

Ligand	Binding affinity
Flutrimazole	−8.5
Clotrimazole	−7.9
Fluconazole	−5.6
Sertaconazole	−5.4
Bifonazole	−5.1

The development of novel pharmaceuticals can be done more efficiently and easily. Drug repurposing is also known as medication repositioning or therapeutic shift. This approach is used to find new therapeutic agents from current FDA-approved clinically utilized medicinal compounds. It is regarded as an effective method for developing drug candidates with novel pharmacological activity or therapeutic characteristics [8]. Because drug discovery is an expensive, time-consuming, arduous, and high-risk procedure, the unique strategy of drug repositioning is used to boost the success rate of medication development. We are using azoles as the drug for repurposing. With the rising threat of invasive and life-threatening fungal infections, there is growing concern about the lower rate of development of antifungal medications compared to antimicrobial treatments [9]. Drugs commonly used in clinics are insufficient to address the rising number of fungal infections, particularly those resistant to fluconazole. Among the few antifungal medicines available in clinics, azoles have the most drug candidates in clinical trials. In this research, 10 phytocompounds from azole antifungals are selected and subjected to pharmacological analysis. The ligands with the best binding properties including, Flutrimazole, Clotrimazole, Fluconazole, Sertaconazole, and Bifonazole were subjected to pharmacological and molecular docking (Tables 1-4). analysis. Flutrimazole is a broad-spectrum antifungal medication. It is used topically to treat superficial mycoses of the skin. Flutrimazole is a kind of imidazole. *In vivo* and *in vitro* investigations have shown that it has antifungal activity equivalent to clotrimazole and greater than bifonazole [20,21].

These compounds serve as ligands and attach to the *Aspergillosis peroxiredoxin* which makes it harder for the protein to stick to the surface of the thus preventing fungal infection. The compound produced from azoles is an antifungal synthetic having broad-spectrum fungistatic action against yeasts and fungi, including candidal species. Azoles inhibit the formation of ergosterol, the primary sterol in fungal cell membranes, through inhibiting fungal cytochrome P450-dependent enzymes. where these enzymes play an important part in the pathogenesis of fungal infection. Hence, we can use these phytocompounds for drug repurposing against *Aspergillosis peroxiredoxin.*

CONCLUSION

A. peroxiredoxin, which is generated by a common mould, is a major contributor to a variety of illnesses in both people and animals (fungus). A condition that mostly affects the respiratory system, which contributes to the worldwide prevalence of lung-related diseases and mortality. In our study, we implemented computational techniques to evaluate the impact of several phytochemicals on the *A. peroxiredoxin* protein PyRx. A virtual screening tool was employed to carry out molecular docking in a systematic manner. The current research implemented a drug re-purposing method which is often referred to as drug repositioning, reprofiling, or retasking a technique for discovering new applications for authorized or experimental medications. This method is a new and emerging method where significant change will occur in the future with an increase in medical research. In this research, azole and its derivatives were repurposed to check their efficacy against the target protein. The broad-spectrum anti-fungal drug Flutrimazole demonstrated the least binding and best pharmacological parameters with the target proteins. This drug can be repurposed for other fungal infections.

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AUTHORS' CONTRIBUTION

All the authors have equally contributed to the research and manuscript.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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