ABSTRACT

The globe has recently been fighting a battle with black fungus, also known as Mucormycosis, and with no immediate treatments available, the disease's devastation is spreading at an alarming rate. A large number of researchers are still looking for a promising new drug that could aid the medical care system in this fight. A docking-based screening employing quantum mechanical scoring of a library is shown, built from approved drugs and compounds that Ellagic acid, Hesperetin, Capsaicin, Concanavalin, Cinnamic acid, Quercetin, Citronellal, Limonene, Progoitrin, Sinigrin, Allicin, Curcumin, Indole, Resveratrol, Strigol, D-limonene, Benzoic acid, Panaxydol, Kaempferol and Berberine with Protein with PDB id 6VCT could display antifungal activity against Mucormycosis. Clearly, these compounds should be further evaluated in experimental assays and clinical trials to confirm their actual activity against the disease. We hope that these findings may contribute to the rational drug design against Mucormycosis.

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1. INTRODUCTION

Mucormycosis is a fungal infection that progresses gradually but has fatal consequences and has a high mortality rate (46-96%) [1]. It is caused by members of Mucorales and its symptoms include one-sided face swelling, black lesions and sinus congestion. The infection is caused by exposure to mucormycetes mold which can be contracted intradermally or via inhalation. The fungal spores are ubiquitous in nature, but in an individual with a compromised/weakened immune system or defects in phagocytic function, it may prove to be critical, and in some cases, a fatal infection [2].

This has challenged the Indian healthcare system amidst the 2nd wave of covid-19 pandemic [3]. India recorded 28,252 mucormycosis cases as of 8th June, 2021. According to the health experts, the overuse of medication is a major contributing factor for black fungus infection.

The European Conference on Infections in Leukemia (ECIL) published mucormycosis treatment guidelines in 2017 and the European Confederation of Medical Mycology (ECMM) provided an update in 2019 [4]. One issue to be addressed is the dose regimen of L-AmB. ECMM recommends 5-10 mg/kg, 10 mg/kg in the case of CNS involvement [5]. ECMM recommended dose should not be slowly increased over several days and a full dose must be given from the first day of daily treatment. SZ, a new azole, was approved in the United States and in Europe in 2015 for the treatment of mucormycosis [4,5].

Regarding central nervous system (CNS) infections, treatment is based on L-AmB due to clinical experience and in vitro data [6]. It has been demonstrated that ISZ penetrates the blood-brain barrier in animal models [7], while AmB displays limited penetration. However, concentration of ISZ in the necrotic center of brain abscess is very low despite high brain tissue penetration [8]. Some new antifungal drugs are under clinical evaluation include Rezafungin, SCY-078, orolofim, and encocleated amphotericin B [9]. Orolofim is a member of the orotomides, a new antifungal class inhibiting dihydroorotate dehydrogenase (DHODH), a key enzyme in pyrimidine biosynthesis. It is also poorly active against Mucorales [10]. Encocleated amphotericin B is a new oral formulation of amphotericin B [11].

The fungus secretes proteases which contribute to the invasion of host tissue and increased damage. Iron is an indispensable part for paving the way to infection of the host as it is fundamental for fungal cell growth and development. Maintaining low iron concentrations in host environment using iron binding proteins (transferrin, ferritin and lactoferrin) can be used as a defense mechanism. Phagocytes (both mononuclear and polymuclear) act as defense of host cells by generation of oxidative metabolites and cationic peptides [12].

2. PROCEDURE

2.1 Ligand Screening

For the initial Ligand screening purposes, a web-based tool named SwissADME (https://www.swiss adme.ch/) was used to eliminate a few compounds according to Lipinski’s rule of five parameters. For a compound to qualify as ligand it should Have < 500 Da molecular weight, a high lipophilicity i.e. value of Log P being less than 5, hydrogen bond acceptors being less than 10 and H-bond donors less than 5. Any compound with more than 2 violations was ruled out for further study (Lipinski2004).

2.2 Protein Preparation and Active site Determination

Required protein in pdb format was downloaded from the website rcsb.org, commonly known as the Protein Data Bank. 3D conformers of the ligand were downloaded from PubChem.

Using PyMOL (Version 2.4.1) software water molecules as well as native ligands from the protein were removed, defined as cleaning/purification of the protein for further application.

Using a web server called Deep Site Active Pockets of the proteins were calculated. The results calculated by the web server were in the form of different ids, centers and scores.

Scoring In deep site was using neural networking based on following instructions using DCNN architecture.

https://academic.oup.com/bioinformatics/article/33/19/3036/3859178 Center values for the grid were selected keeping score greater than 0.98.
UCSF Chimera (Version 1.14) was used to prepare the receptor using DockPrep function. Dock Prep prepared structures for Docking using these functions:

- deleting water molecules
- repairing truncated sidechains
- adding hydrogens
- assigning partial charges
- writing files in Mol2 format

2.3 In silico Docking Using Auto dock Vina

Auto dock Vina (Version 1.1.2) along with UCSF Chimera (Version 1.14) was used for molecular Docking Studies. Center values and size of the grid of different scores were used from DEEPSITE calculations done above. Following Parameters were set in auto dock vina.

2.4 Receptor Options

- **Add hydrogens in Chimera (true/false)** – whether to add hydrogens in Chimera before calling the script. The receptor prep script will check for hydrogens and add them if they are missing. AutoDock Vina needs the polar (potentially H-bonding) hydrogens to identify atom types for scoring purposes.
- **Merge charges and remove non-polar hydrogens (true/false)** – note AutoDock Vina does not use charges or nonpolar hydrogens, so this setting is not expected to affect results except for the presence or absence of nonpolar hydrogens in the processed receptor.
- **Merge charges and remove lone pairs (true/false)** – note AutoDock Vina does not use charges or lone pairs, so this setting is not expected to affect results except for the presence or absence of lone pairs in the ligand output files (and there may not have been any lone pairs to start with).
- **Ignore waters (true/false)**
- **Ignore chains of non-standard residues (true/false)** – ignore chains composed entirely of residues other than the 20 standard amino acids.
- **Ignore all non-standard residues (true/false)** – ignore all residues other than the 20 standard amino acids.

2.5 For Ligands

- **Merge charges and remove non-polar hydrogens (true/false)** – note AutoDock Vina does not use charges or nonpolar hydrogens, so this setting is not expected to affect results except for the presence or absence of nonpolar hydrogens in the ligand output files.
- **Merge charges and remove lone pairs (true/false)** – note AutoDock Vina does not use charges or lone pairs, so this setting is not expected to affect results except for the presence or absence of lone pairs in the ligand output files (and there may not have been any lone pairs to start with).

2.6 Docking Parameters

- **Number of binding modes (1-10, 10)** – maximum number of binding modes to generate.
- **Exhaustiveness of search (1-8, 8)** – thoroughness of search, roughly proportional to time.
- **Maximum energy difference (kcal/mol) (1-3.3)** – maximum score range, binding modes with scores not within this range of the best score will be discarded.

The docking results were calculated by Auto dock vina using its Scoring function and results were displayed in the form of Scores and RMSD values. Docking results with the highest value score accompanied by negative sign and least RMSD values were chosen for further studies.

2.7 Residue Analysis

PyMOL was used for visualization of interactions of the docked structure at the ligand sites. Discovery Studio 2020 was used to study the ligand interactions and total number of residues. It was also used to plot the 2D structure of the interactions and residues.

2.8 Statistical Analysis

Descriptive, estimation and Hypothesis testing with confidence interval of 95% was applied to data using formula 1 given below.

\[
CI = \bar{x} \pm z \frac{s}{\sqrt{n}}
\]

Where:
- \(CI\) = confidence interval
- \(\bar{x}\) = sample mean
- \(z\) = confidence level value
- \(s\) = sample standard deviation
- \(n\) = sample size
3. RESULTS AND DISCUSSION

3.1 ADMET Analysis

The ligand molecule qualified Lipinski’s rule of five. Furthermore, it also demonstrated high gastric retention properties. It exhibited poor skin permeability properties. Furthermore, it also demonstrated high plasma protein binding capacity with a score of 92.096762 (Table 2). The toxicity properties retrieved from the Pre-ADMET web server more or less demonstrated the least toxicity indications except for carcinogenicity in mice.

Table 1. Summarizes the results showing ligands with ADMET results

| Ligands            | Log P | Bioavailability score | Lipinski Violation |
|--------------------|-------|-----------------------|--------------------|
| CINNAMIC ACID      | 1.79  | 0.85                  | 0                  |
| CONCANAVALIN       | -0.06 | 0.56                  | 0                  |
| CAPSAICIN          | 3.43  | 0.55                  | 0                  |
| QUERCETIN          | 1.23  | 0.55                  | 0                  |
| CITRONELLA         | 2.93  | 0.55                  | 0                  |
| LIMONENE           | 3.35  | 0.55                  | 0                  |
| PROGOITRIN         | -1.71 | 0.11                  | 2                  |
| SINIGRIN           | -1.15 | 0.11                  | 0                  |
| KAEMFEROL          | 1.58  | 0.55                  | 0                  |
| HESPERITIN         | 1.91  | 0.55                  | 0                  |
| BERBERINE          | 1.91  | 0.55                  | 0                  |
| STRIGOL            | 2.12  | 0.56                  | 0                  |
| ELLAGIC ACID       | 1     | 0.55                  | 0                  |
| D-LIMONENE         | 3.37  | 0.55                  | 0                  |
| BENZOIC ACID       | 1.44  | 0.85                  | 0                  |
| PANAXYDOL ACID     | 4.61  | 0.55                  | 1                  |
| ALICIN             | 1.59  | 0.55                  | 0                  |
| CURCUMIN           | 3.03  | 0.55                  | 0                  |
| RESVERATOL         | 2.48  | 0.55                  | 0                  |
| INDOLE             | 1.98  | 0.55                  | 0                  |
| CINNAMIC ACID      | 1.79  | 0.85                  | 0                  |
| CONCANAVALIN       | -0.06 | 0.56                  | 0                  |
| CAPSAICIN          | 3.43  | 0.55                  | 0                  |
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| RESVERATOL         | 2.48  | 0.55                  | 0                  |
| INDOLE             | 1.98  | 0.55                  | 0                  |
3.2 Molecular Docking

The docking result was obtained from Auto dock vina in the form of Dock score for the protein docked with above mentioned ligands.

3.3 Mucormycosis Protein Docking Results

3.3.1 PDB-ID 6VCT

For 6VCT, one active site was selected with a deep site score of 0.991. The selection was made on the basis of the highest binding energy of the ligand-receptor. The docking results before statistics are shown in Table 1 and Table 2 shows the post statistical docking scores with Ligand Protein Interactions.

Table 2. shows the docking score evaluated from autodock vina

| Ligands      | Dock Score |
|--------------|------------|
| Ellagic acid | -5.9       |
| Hesperetin   | -5.4       |
| Capsaicin    | -6.3       |
| Concanavalin | -5.3       |
| Cinnamic acid| -6.2       |
| Quercetin    | -5.3       |
| Citronellal  | -5.6       |
| Limonene     | -5.5       |
| Progoitrin   | -5.5       |
| Sinigrin     | -6.3       |
| Allicin      | -4.6       |
| Curcumin     | -6.6       |
| Indole       | -6.0       |
| Reservatol   | -5.6       |
| Strigol      | -8.2       |
| D-limonene   | -5.4       |
| Benzoic acid | -5.9       |
| Panaxydol    | -5.9       |
| Kaempferol   | -5.3       |
| Berberine    | -4.9       |

Table 3. shows the ligand protein interactions by all the selected molecules in the library

| Ligand   | Dock Score | Interaction |
|----------|------------|-------------|
| Ellagic acid | -5.9      |             |
| Ligand   | Dock Score | Interaction |
|----------|------------|-------------|
| Hesperetin | -5.4       |             |
| Sinigrin  | -6.3       |             |
| Indole    | -6.0       |             |
| Citronellal | -5.6     |             |
| Curcumin  | -6.6       |             |
Table 4. summarizes the results showing ligands and their interacted proteins that were considered in the study for the targeted diseases

| Ligand       | Dock Score | Interaction |
|--------------|------------|-------------|
| Strigol      | -8.2       |             |
| Cinnamic acid| -6.2       |             |
| Capsaicin    | -6.3       |             |

| Ligand        | Proteins Interacted | Target Disease(s) |
|---------------|---------------------|-------------------|
| Curcumin      | 6VCT                | Mucormycosis      |
| Indole        | 6VCT                | Mucormycosis      |
| Reservatol    | 6VCT                | Mucormycosis      |
| Strigol       | 6VCT                | Mucormycosis      |
| D-limonene    | 6VCT                | Mucormycosis      |
| Benzoic acid  | 6VCT                | Mucormycosis      |
| Panaxydol     | 6VCT                | Mucormycosis      |
| Kaempferol    | 6VCT                | Mucormycosis      |
| Berberine     | 6VCT                | Mucormycosis      |
| Ellagic acid  | 6VCT                | Mucormycosis      |
| Hesperetin    | 6VCT                | Mucormycosis      |
| Capsaicin     | 6VCT                | Mucormycosis      |
| Concanavalin  | 6VCT                | Mucormycosis      |
| Cinnamic acid | 6VCT                | Mucormycosis      |
| Quercetin     | 6VCT                | Mucormycosis      |
| Citronellal   | 6VCT                | Mucormycosis      |
| Limonene      | 6VCT                | Mucormycosis      |
| Progoitrin    | 6VCT                | Mucormycosis      |
| Sinigrin      | 6VCT                | Mucormycosis      |
| Allicin       | 6VCT                | Mucormycosis      |
Table 3 shows the ligand protein interactions and residues that are formed using ligand library and receptor Mucor circinelloides FKBP12 protein bound with APX879 in C2221 space group. It was seen that the residue complex formed by strigol derivatives with the receptor mucor circinelloides FKBP12 protein bound with APX879 in C2221 space group consists of TYR-87, VAL-55, TRP-59, ILE-56, TYR-82 amino acids. Tyr-82 amino acid residue is suggested to be the native residue for protein structural integrity. It was seen that complete ligand directory used in the study was able to form TYR-82 residue suggesting that Ligand-Receptor formed in the study are similar amino acid interaction as that of the native ligand.

4. CONCLUSION

All twenty ligands were studied using bioavailability radar. Our results proposed Curcumin, Capsaicin, Cinnamic acid, Strigol, Ellagic acid, Hesperetin, Indole, Citronellal and Sinigrin showed the best docking result for Mucormycosis Proteins with PDB id 6VCT. To find the effectiveness and to propose the exact mechanism in-vitro studies can be encouraged on Curcumin, Capsaicin, Cinnamic acid, Strigol and Sinigrin targeting respective diseases that are discussed above to understand the mechanism and a potential cure for Mucormycosis.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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