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COMPUTATIONAL REPURPOSED LIGANDS: POTENTIAL CURE FOR BLACK FUNGUS

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Abstract

A rare but serious fungal infection, known as mucormycosis and colloquially as " Black fungus", is being detected relatively frequently among Covid 19 patients in some states of India. The disease often manifests in the skin and also affects the lungs and the brain. With no prompt medicines accessible the scourge brought about by the disease is expanding step by step. A ton of researchers are continuing for the potential medication up-and-comer that could help the medical care framework in this battle. We present a docking-based screening using a quantum mechanical scoring of a library built from approved drugs and compounds that Cefuroxime, Amoxicillin, Peramivir, Fluconazole, Itraconazol, Micafungin,Quercetin, Carvacrol, Xanthones,Curcumin,Thymol and Resveratrol with Proteins with PDB id 6VCT could display antibacterial activity against Black fungus. 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 Black fungus.

Introduction

Mucormycoses is a difficult-to-manage infection owing to limited diagnostic tools and therapeutic options. It is caused by fungi belonging to the order Mucorales. The major risk factors for mucormycosis include uncontrolled diabetes mellitus in ketoacidosis, other forms of metabolic acidosis, treatment with corticosteroids, organ or bone marrow transplantation, neutropenia, trauma and burns, malignant hematologic disorders, and deferoxamine therapy in patients receiving hemodialysis. Because of the increasing prevalence of diabetes mellitus, cancer, and organ transplantation in the aging Indian population, the number of patients at risk for this deadly infection is dramatically increasing. Clearly new strategies to prevent and treat mucormycosis are urgently needed. Repurposing of realized little particles is by all accounts an exceptionally productive path so as to create strong medications to battle diseases in this brief timeframe. As of late, various endeavors have been made to plan novel inhibitors or utilize drug repurposing ways to deal with recognition hostile to medications.

Procedure:

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. 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 was 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 the 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

1. 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.

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 processed receptor (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.
- **For Ligands**
- **Merge charges and remove non-polar hydrogens** (**true**/false) – note Auto Dock 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)
- **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 it's 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.

4. 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.

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

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

CI = confidence interval

$\bar{x}$ = sample mean

z = confidence level value

s = sample standard deviation

n = sample size

Formula 1: used for calculation of confidence interval

Results and Discussion:

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.

Black Fungus Protein Docking Results:

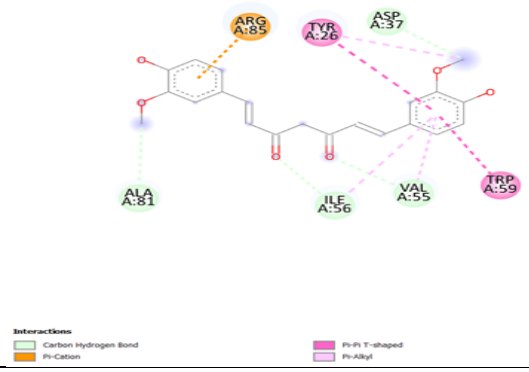
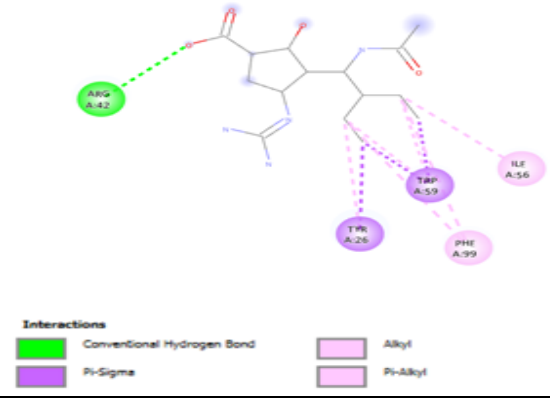
PDB-ID 6VCT

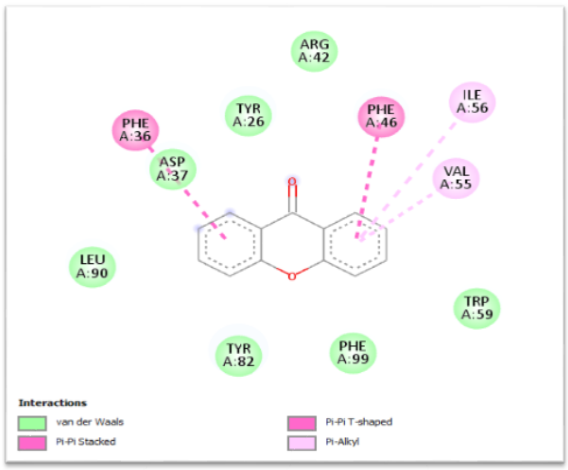
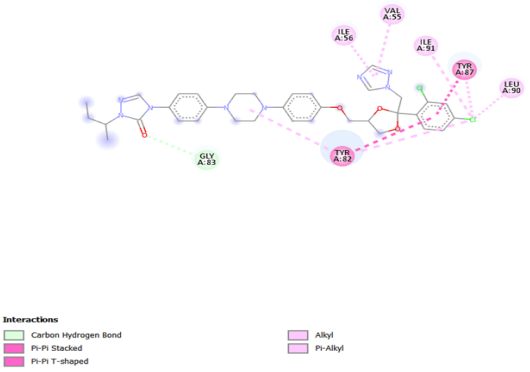
For 6VCT, two active sites were selected out of which the 2thactive site was selected with a Deep site score of 0.991. The selection was made on the basis of the highest binding energy of the ligand-receptor. The docking results before statistics are shown in Table 1 and Table 2 shows the post statistical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

Table 1:

Ligands	Dock score
Cefuroxime	-6.2
Amoxicillin	-6
Peramivir	-6.7
Fluconazole	-6.4
Itraconazol	-7.9
Micafungin	-6.3
Quercetin	-5.8
Carvacrol	-5.7
Xanthones	-7.9
Curcumin	-6.8
Thymol	-5.7
Resveratrol	-5.6

Table 2:

Ligands	Dock score	Interactions
Curcumin	**-6.9**	
Peramivir	**-6.7**	

Xanthone	**-7.9**		
Itraconazole	**-7.9**		

Conclusion:

All four ligands were studied using bioavailability radar. Our results proposed that Peramivir, Itraconazol, Xanthones and Curcumin showed best docking results with 6VCT. Besides this Itraconazol and Xanthones showed its best docking results with the protein 6VCT. To find the effectiveness and to propose the exact mechanism in-vitro studies can be encouraged on these ligands by targeting proteins of organisms responsible for mucormycosis that are discussed above to understand the mechanism and a potential cure for mucormycosis.

Ethics Approval and Consent To Participate.

Not applicable.

Human And Animal Rights

No Animals/Humans were used for studies that are the basis of this research.

Consent For Publication

Not applicable.

Availability of Data and Materials

The author confirms that the data supporting the findings of this research are available within the article.

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The authors whose names are listed immediately above certify that they have NO affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

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