Molecular Docking and Screening of Small Active Agents against β-1,3-glucan Biosynthatase as a Potential Target in the Management of Mucormycosis

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Abstract

A black fungus that was formerly known as zygomycosis, or mucormycosis, had ravaged several Indian states, including Maharashtra, and was considered a pandemic. The word "mucormycosis" refers to a group of infections of the Mucorales order of the Zygomycetes class that can be chronic, subacute, and often advance quickly. Based on statistical data, there is a 50% overall death rate for this rare fungal disease that affects 1 in 10,000 people. This opportunistic infection primarily affects people with cancer, uncontrolled diabetes, an immunocompromised viral illness (HIV), and more recently, COVID-19 patients. It may be brought on by the indiscriminate use of steroids in COVID-19 treatment. A variety of clinical presentations can occur with mucormycosis, such as disseminated, pulmonary, cutaneous, gastrointestinal, sinusitis (including pansinusitis, rhino-orbital, or rhino-cerebral), and other unusual manifestations. Management of mucormycosis infection remains difficult despite recent advancements in antifungal agents such as azole derivatives in addition to Amphotericin B. This is because of the cytotoxicity associated with the kidneys and liver, which includes the production of reactive oxygen species and increased fungal resistance. Given the current situation, the necessity for the development of novel antifungals and/or alternative therapy to effectively control mucormycosis is worrying. attempts to repurpose drugs as alternatives to combat this fungal infection.

In this study, HTVS was carried out by utilizing the Zinc13 Database (molecules) against β-1,3-glucan biosynthatase, an essential enzyme involved in the development of fungal cell walls. ZINC23504872 was identified as a potential candidate against Mucumycosis with binding score of -9.358 The in-silico ADME properties of identified ligand was also determined & molecule comply the Lippinski's Rule of five & does not violate any parameter of drug likeliness. Hence, ZINC23504872 may represent potential promising lead molecule as aβ-glucan synthase inhibitor for the treatment of Mucormycosis.

Key words: Mucormycosis, Covid -19, Amphotericin B, Posaconazole, drug-repurposing.

Introduction

A fatal pandemic was experienced across the world as a consequence of the Coronavirus Disease (Covid-19) outbreak, which was linked to numerous bacterial and fungal co-infections. There were increased incidences of fungal infection Known as "mucormycosis". It was also reported in patient who got infected with covid-19 [1-3]. Based on statistical data, there is a 50% overall death risk for this uncommon fungal illness that affects 1 in 10,000 people. In India, black fungal infection cases were observed in a few states, including Rajasthan, as well as Maharashtra and Gujarat, hence WHO had declared it as a pandemic. This opportunistic infection is most common in individuals with cancer, uncontrolled diabetes, immunocompromised viral disorder (HIV), and Covid-19 patients; it may be brought on by excessive consumption of steroids in the treatment of COVID-19.[4-6]. It was reported that the use of steroids in COVID-19 patients decreased lung inflammation when the body's immune system battled the virus, but excessive steroid use also decreased immunity and raised blood glucose levels in both diabetics and non-diabetics, increasing the possibility of causing a black fungus condition. Sporangiospores inhaled in the air is the main way that mucorales are spread.

Additional methods of transmission are spore consumption or fungal inoculation from wounds or trauma. Ventilation, medical equipment, and contaminated bandages are linked to nosocomial infections. The location and intensity of the infection determine the method by which the fungus spreads from one individual to another. Whereas cutaneous mucormycosis spreads through intimate personal contact, rhino cerebral mucormycosis primarily spreads through droplet inhalation.

In extremely immunocompromised individuals, black fungus infections can be fatal because they damage the brain, lungs, and sinuses. The symptoms that are most frequently noticed are fever, headache, stuffy nose, bleeding, and a blackish discoloration surrounding the nose. Swelling vicinity of eyes, eyelids drooping, teeth loosening, jaw involvement, black crusts in the nostrils, and worsening of respiratory symptoms changes in mental state, coughing up blood or dark fluids sometimes, shortness of breath, diffuse stomach discomfort, dark or bloody vomitus, flank pain, and an ulcer with a dark centre and well-defined edges may all occur[7]. Complex instances can lead to blindness, organ failure, infection-related tissue loss, debridement, and even death.

Typically, posaconazole, or isavuconazole, amphotericin B are the antifungal medications used in treatment. Surgery is necessary for mucormycosis in order to remove the affected tissue[8].

According to published research, employing inhibitor medications such as amphotericin/ echinocandins to block β-1,3-glucan production resulted in a reduction of fungal growth and replication[9]. The main drug compounds that function as β-1,3-glucan production inhibitors have been promoted as possible therapeutics for the treatment of fungal infections[10]. It is an enzyme called glucosyl-transferase that aids fungus to produce beta-glucan, an essential building block for their cell walls. medication "repurposing," sometimes referred to as "redirecting," "repositioning," or "reprofiling," has emerged as a tactic to expedite and lower the cost of medication research while simultaneously raising the success rates of therapy. In fact De novo medication repurposing speeds up the introduction of the medicine with the new indication into clinical practice, even though it takes 10–17 years and has a less than 10% success rate. Repurposing expands on earlier studies and the development of well-known drugs that have undergone toxicological and pharmacological testing on people. [11,12].
We present here our perspective on in-silico screening on the potential use of small molecules as a possible method of treating black fungus that targets the fungus 1,3-beta-glucan synthase using the software Wizard of Schrodinger Suite 9.2 (Schrodinger, Inc.).

Experimental:

Zinc Database 2013 virtual screening of 1,3-beta-glucan synthase (PDB ID: 4m80):

Protein Structure: 1,3-beta-glucan synthase's X-ray crystal structure (PDB ID: 4m80) was downloaded from www.rcsb.org.

Ligand database: Zinc Database 2013 Clean Drug-Like which contains **13,195,609** molecules.

Molecular docking:

Protein preparation: (4m80): Protein 4m80's X-ray crystal structure was obtained from www.rcsb.org. The protein was prepared using the Protein Preparation Wizard of Schrodinger Suite 9.2 (Schrodinger, Inc.). Schrodinger Suite 9.2's Protein Preparation Wizard (Schrodinger, Inc.) was used to prepare the protein. To create the hetero-state for the co-crystallized ligand, bond orders were given, hydrogens were

added, metals were treated, water molecules were eliminated, and Protassign was used to assign the protonation state and optimize the H bonding of the protein side chain. Impref minimization (energy minimization) was performed with the OPLS2001 force field.

Receptor Grid generation: Without restrictions and with default values of the scaling factor and partial charge cutoff, the receptor grid has been prepared. The chosen residues have a designated centroid at this site.

Ligand preparation: The Schrodinger Suite's Ligprep tool was used to prepare molecules from the Zincdata base using the default settings. The ligands underwent desalting, created states at pH 7 ± 2, and up to 32 tautomers and stereo isomers while maintaining designated chiralities. If any tautomers were generated, they were minimised using OPLS 2005 Forcefield.

Ligand-Docking: GLIDE (5.7) was used to dock the prepared ligands. Ligands with less than 200 atoms and less than 35 rotatable bonds were chosen. The potential charge cutoff of 0.25 and the Vdw scaling factor of 1.0 were applied. Here, constraints and core were not applied. Post dock minimization was performed after the ligand-docking. For residues that are within 12.0 Å of the grid centre, record the interaction score for each residue. There should be five poses for each ligand. Using Maestro's Pose Viewer tool, the output file is generated with the poses that score the highest first.

In-silico ADME calculation: Many promising compounds fail in the final stage of clinical trial (CT) because of poor pharmacokinetics, which makes pharmacokinetic property-based screening (ADME) an essential step in the drug discovery process. Schrödinger, LLC, New York, 2005's QikProp, version 3.0, was utilised for the virtual prediction of ADME properties. The Lipinski’s Rule of Five and the Jorgensen Rule of Three, which take into account a number of factors including molecular weight, LogP, hydrogen bond acceptor and donor were used to determine the drug likelihood of a ligand molecule. Numerous other factors were also taken into account, including Caco-2 permeability, polar surface area, anticipated water solubility, and human oral absorption.

Results and Discussion

Molecular docking

The docking programme was confirmed to be verified for continued usage with an RMSD (Root Mean Square Deviation) value of 0.062 Å. To determine the significant interactions that occur between the molecule and the amino acid residues at the β-glucan synthase active site, additional precision molecular docking studies were conducted. Since these interactions control the ligand-protein complex's stability, they suppress the catalytic activity of glucagon synthase. Through docking, a chemical moiety that best fits into the receptor pocket and is complementary to the catalytic site of the receptor can be identified. Out of all the top HITS compounds, compound ZINC23504872 was found to possess the highest docking score of -9.389. The fact that this score is negative indicates that the process of bond formation is exothermic, meaning that energy is produced when the ligand attempts to fit into the protein molecule's active pocket. **Figure 1** A and B shows the reference molecule's 3D and 2D interactive diagram, whereas Figures C and D show the top scoring hit that was found after the glucagon synthatase amino acid residues were complexed. The two polar H bonds with Tyr29, Glu27, and Arg312 were visible in the molecule. The hydrophobic interactions Tyr255, Phe229, Trp373, and Tyr29 stabilised these polar H bonds. Additionally, the molecule showed polar contact with residues of amino acids Arg312 and Arg309. Overall, it was discovered that all of these interactions were comparable to the reference molecules. The potential for these interactions to result in the inhibition of β-glucan synthase's catalytic activity.

In-silico ADME calculation

Since 60% of desirable candidates fail clinical trials due to poor ADME properties, predicted ADME properties are crucial to the drug design and development process. The detected HIT was taken into account for the in-silico ADME computation to verify the molecule's drug-likeness characteristics. The factors that defined the physicochemical feature of the molecule were taken into account. The analysis of Lipinski's Rules of Five (Ro5) was done for oral active molecules. It describes molecules with a molecular weight (MW) of less than 500D, hydrogen bond donor (HBD) groups of less than 5, and hydrogen bond acceptor (HBA) values that should not exceed 10. The molecule should have a logPo/w (oil/water partition coefficient) of less than 5. Table 1 summarises the outcomes . both molecules (the reference and the

top hit) comply with the Lipinski, Rule of Five. Blood-brain coefficient (plogBB) was used to predict CNS toxicity. The results show that the detected molecules have values within the range, indicating that ZINC23504872 cannot pass the blood-brain barrier to cause CNS toxicity. These findings clarified the ADME of HIT. A potent molecule must absorb, reach its target, and remain in its original form in order to have the desired effect.

Table 1 Docking analysis of Reference and Top hit molecule

ADME Properties	**Reference**	**ZINC23504872**
Molecular Weight	437.466	281.357
HB Donor	1	3
HB Acceptor	6.75	4.5
$LogP_{O/W}$	4.169	1.557
PCaco	457.592	160.237
logBB	-1.065	-0.311
#Metabolism	4	4
% Human oral Absorption	1	3
PSA	78.694	70.365
Ro5 Violation	0	0
Ro3 Violation	1	3

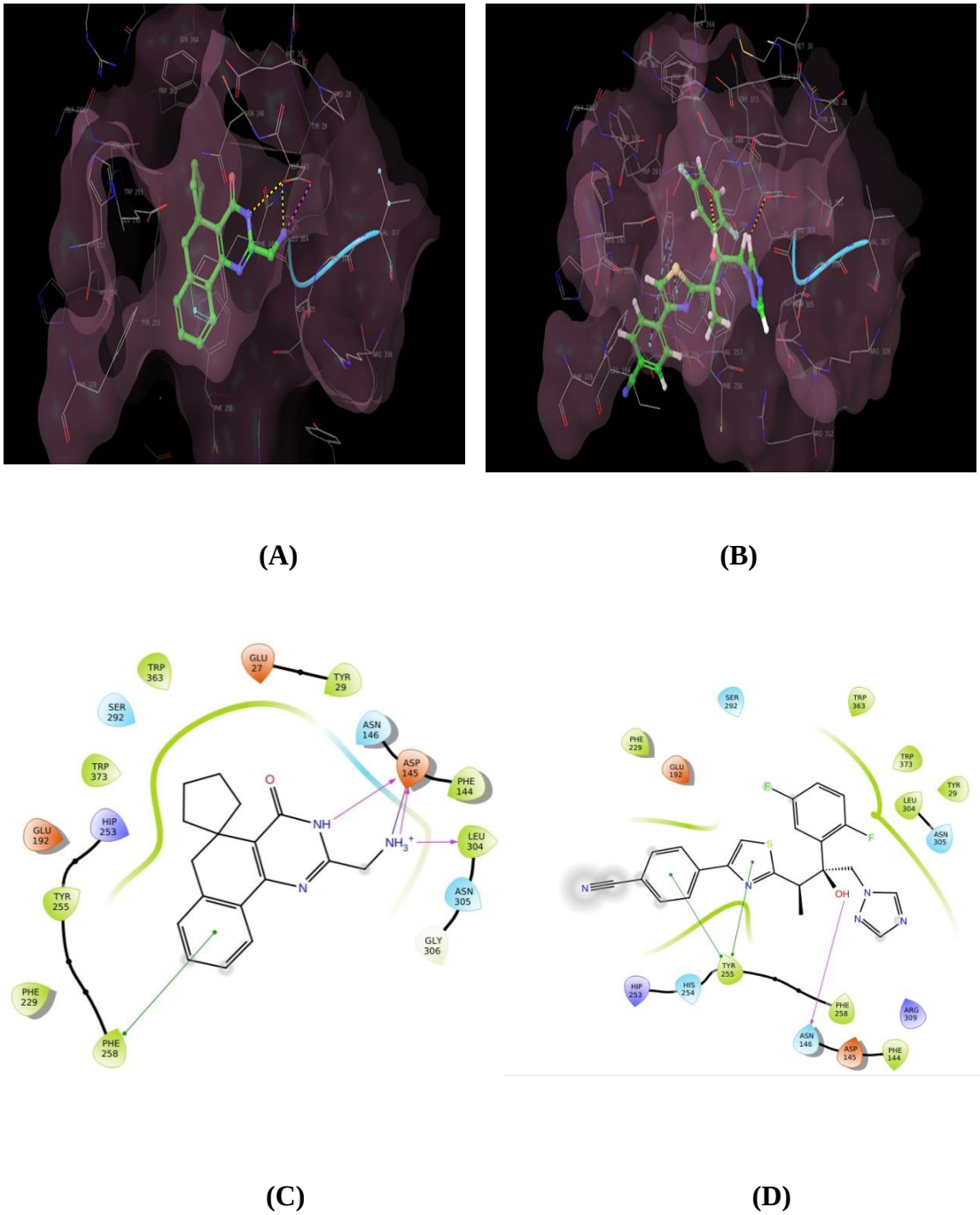

Figure 1. (A) An interactive 3D view of the compound ZINC23504872 with active site amino acid residue of 1,3-beta-glucan synthase (PDB ID: 4m80) (B) 3D overlay of reference molecule at the active pocket of 1,3-beta-glucan synthase (PDB ID: 4m80) (C) 2D interactive view of compound ZINC23504872 with key interactions (D) 2D diagram of reference molecule.

Conclusion:

In this instance, we employed computational technique to get more confirmed in silico results and explored a genetically ensured therapeutic target for the design of a 1,3-beta-glucan synthase inhibitor (PDB ID: 4m80). Therefore, a combination of computational methods, including site mapping, molecular modelling, shape, electrostatic comparison, and ZINC13 database-based in-silico ADME computations, which contains 13,195,609 molecules, could produce computational data that is more reliable. It is determined that ZINC23504872 is a potential lead molecule. A strong candidate to inhibit β-glucan synthase was suggested for this chemical based on its ADME characteristics and interaction profile. According to in silico ADMET calculations, every molecule met the Lipinski rule of five and had all the characteristics of a drug. The HIT that has been discovered exhibits great potential as a β-glucan synthase inhibitor for the management of proliferative disorders.

Future scope:

Further the protein-ligand complex will be subjected to molecular dyanamics studies to find out the stability of complex and key interaction. Lead optimization and the synthesis of molecule will be done after the generation of validated in-silico data.

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