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In silico Characterization, Identification, and Molecular-level analysis of Holotricin-3 (A Dynamic Study)

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ABSTRACT

Candida albicans, a prominent fungal infection, induces a wide range of illnesses, ranging from moderate mucosal irritation to fatal systemic problems. The yeast-to-hypha transformation is greatly studied in the *C. albicans* pathogenicity. It is, nevertheless, an unscrupulous disease that can induce lethal bloodstream infections. Fungi have major applications in industrial, medical and agricultural fields. They are utilized in the synthesis of peptides, micronutrients, metabolites, phenolics and other organic compounds. The current study has incorporated the implementation of bioinformatics techniques to analyse the molecular level binding of Holotricin-3 against the anti-fungal proteins. The findings of the study revealed that Holotricin-3 had highest binding with Bgl2p and the findings were further validated by molecular dynamic studies. These observations can be used to implement *In vitro* experiments.

Keywords: Molecular docking, *C. albicans*, Holotricin-3, Schrodinger, Pharmacoinformatics.

INTRODUCTION

Numerous studies have discovered that fungal pathogens are less prevalent than bacterial and viral infections. Fungi are remarkably, widely distributed microorganisms that shoulder essential roles in complex ecosystems. In addition to the synthesis of proteins, enzymes, antioxidants, acids, and medicines, fungi are used in a variety of industrial activities.[1,2] However, fungal infections, often known as mycoses, have emerged as a severe threat to health, causing a broad spectrum of ailments. It is estimated that 1.5 million people are killed and nearly a billion people are affected due to fungal infections[3]. These conditions vary from being a simple peripheral infection that impair living quality and damage the skin, keratinous tissues, and mucous membranes to severe systemic infections that damage the central nervous system, cardiovascular system, respiratory system, hepatic, renal and spleen tissues[4,5]. *Candida albicans, Cryptococcus neoformans*, and *Aspergillus fumigatus* are the most common human fungal pathogens, while non-albicans *Candida spp*. are a threat. Four primary types of antifungal drugs that dominate the market for were examined for a comprehensive overview of the most common

fungal diseases that impact individuals: azoles, which limit ergosterol production; polyenes, which physiochemically interact with fungal membrane sterols; echinocandins, which inhibit glucan synthesis; and fluorinated pyrimidines, which disrupt with pyrimidine metabolism, and suppresses DNA and RNA biosynthesis[6].

To prevent or cure fungal infections, new biologics such as monoclonal antibodies, cytokine immunotherapy, vaccines, and antimicrobial peptides have emerged[7]. Peptides are becoming increasingly important as promising new antibacterial medicines. Peptides can function as agonists or antagonists by acting as natural ligands. Peptides are extremely selective, efficacious, and well-tolerated in the context of therapeutic development[8]. The manner of action of antifungal peptides is classified. The first category uses lysis, which is accompanied by a number of processes[9]. The second peptide group prevents the formation of cell walls and critical cellular components including glucan and chitin[10]. The cell wall has been a continuous subject of investigation for *Candida albicans* throughout the last many decades. The study of *C. albicans* cell surface proteins is progressing in a variety of ways, utilizing various methods. In the cell wall, several proteins that are not linked to the polysaccharide matrix have enzymatic functions. Most enzymatically active proteins are thought to have an effect on cell wall construction. The following proteins are found in the cell wall:

- **Kre9p:** Kre9p belongs to the GH16 family, which also includes the covalently linked Crh11p, Crh12p, and Utr2p. Kre9p is involved in the production of 1,6-glucan. This polysaccharide's production necessitates combined functions of cytoplasmic and cell wall, and its biosynthesis is not as simple as that of the other two significant structural polysaccharides[11,12].
- **Bgl2p:** Mp65p and Scw4p are members of the same GH17 family as Bgl2p. Because glucan is a linear polymer, enzymes with the ability to produce branching from linear polymers are required for the branching of polysaccharides in the cell wall[13].
- **Xog1p:** The exoglucanase Xog1p is the only GH5 enzyme discovered in *Candida albicans*. In comparison to 1,6-glucoside couplings, Xog1p displays a strong preference for 1,3-glucoside linkages[14].
- **Eng1p:** The GH81 family includes the cell wall enzyme Eng1p as well as cytoplasmic Acf2p. Eng1p is a fungus-specific endo-1,3-glucanase that is used to separate cells. Endoglucanase activity was measured by the discharge of reducing sugars from the substrate which is exclusive for 1,3-glucan[15].
- **Chitinases:** In *C. albicans*, four chitinase genes have been identified. Three of them are connected to the cell wall and are all representatives of the GH18 family. The deletion has no effect on cytokinesis. Cht1p is a minor chitinase[16].

Methodology

Active binding Site

SWISS-MODEL was used to construct the three-dimensional structure for the selected proteins. The FT site server[17] was used to examine the identification of the protein binding site. The functional correlation between target proteins, drug design, and structure-based prediction are all part of the binding site analysis. In addition, the FT site server demonstrated a specific approach for Protein Binding Identification[17].

Structure validation and active site prediction

The active binding sites of the specified protein receptors were inferred or predicted using an FT site web server in this investigation. The SWISS-MODEL structure evaluation tool was used to evaluate the modeled structure. GROMOS96 was used to find the active site of the modeled structure using default parameters provided in Swiss PDB Viewer (version 4.0.4)[18], followed by a CASTp server[19] evaluation.

Identification of Ligands

Ligands are those molecules that attach to the protein's binding site. Holotricin-3 is a recombinant protein whose structure is obtained from Protein Data Bank (PDB)[20]. Both the ligands were used against selected protein receptors subjected to molecular docking simulation.

Retrieval of Target Receptors and Homology modeling

The 3D structures of target protein receptors (Kre9p, Bgl2p, Xog1p, Eng1p, and chitinase) were obtained from the NCBI database[20], while ligands were

obtained from the PubChem database[21] and the Protein Data Bank[20]. The structure's criteria were investigated using BLAST[22] and Protein Data Bank analysis. Utilizing the SWISS-MODEL website, the template for homology modeling was selected based on X-ray diffraction resolution and best sequence similarity.

Molecular Docking Analysis

The docking study was carried out using AutoDock Vina 4.0[23]. Based on the pre-calculation of grid maps utilizing various energies with a macromolecule, a grid box was created for each protein. The application was run using the default algorithm to find the best-docked configurations between the Holotricin-3 and the receptor. The prepared ligands were docked with the prepared protein receptors.

Molecular Dynamic Simulation

The compound with the highest docking score was subjected to molecular dynamic experiments to assess its stability towards the receptor of interest, proceed by evaluating the Root Mean Square Deviation (RMSD) and Root mean square fluctuation graphs. We have used Desmond Schrodinger v20.4 package for molecular dynamic simulation in Linux operating system with GUI. OPLS force field was used to prepare the protein topologies and simulation files using the standard parameters. The simulation run was for 100 nanoseconds to prepare the graph ratio.

RESULTS AND DISCUSSION

Active Binding Site

SWISS-MODEL was used to create the three-dimensional structure of selected proteins. The FT site server was used to scrutinize the identification of the protein binding site[17].

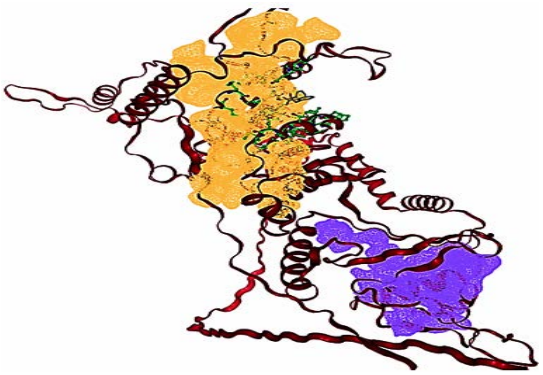

Fig. 1. Active Binding site Residues

Table 1: Physiological properties of Selected ligands

Ligand	Sequence position	Molecular weight (Da)	Sequence length (Nucleotide bases)
Holotricin-3	21-104; Full-Length protein	9,026 Da	566

Molecular Docking Analysis

The docking calculation was performed using Autodock vina 4.0. and the Holotricin-3 were docked in contrast to the receptors which are responsible for antifungal infections. In terms of comparative analysis, the synthesized natural compounds showed a better binding score compared to all co-crystals and drug molecules. The binding Affinities of both the compounds have shown in Table 2 below.

According to molecular docking analysis, the results exposed the good docking score against the selected protein receptors with ligands, respectively. Holotricin-3 showed a better docking score with the receptors.

Table 2: Molecular docking results using Patchdock

Receptors	Binding Affinity (kcal/mol) (Holotricin-3)
Kre9p	-8.1
Bgl2p	-8.9
Xog1p	-3.6
Eng1p	-5.2
Chitinase	-6.7

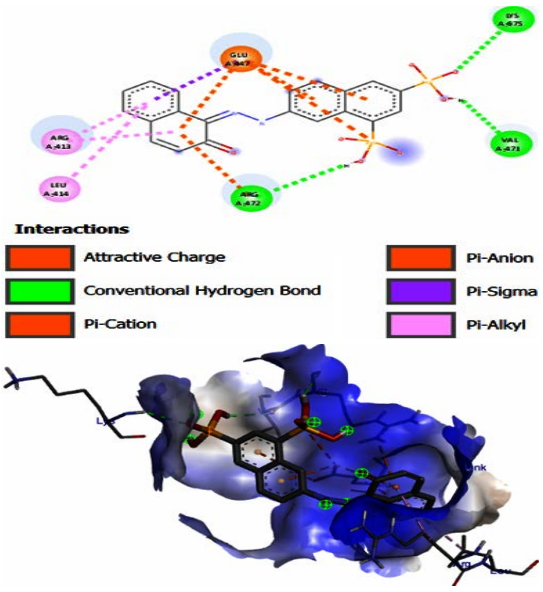

Fig. 2a. Discovery studio structural analysis of Bgl2p with Holotricin 3 receptor

Molecular Dynamic Simulation

In dynamic studies, Holotricin-2 was subjected to Desmond Schrodinger to analyse the free energy and Root Mean Square values through dynamic calculations. We have used the OPLS force field to analyze the protein followed by ligand properties. All the jobs were incorporated in the system builder of Schrodinger and directly subjected to molecular dynamics for 100ns to investigate the trajectories in every 20ns. Hence, RMSD and RMSF have exposed better fluctuation rates in trajectories as well as protein compatibility with the Holotricin-3 which can be shown in Figures 2, 3, and 4.

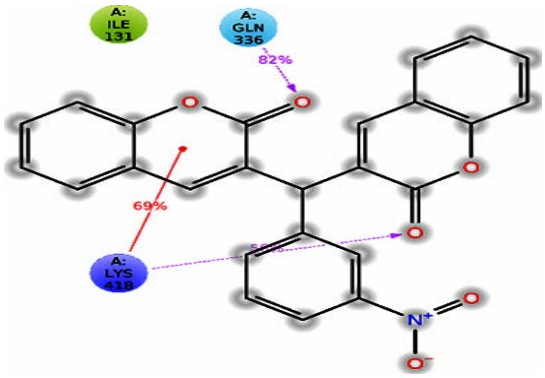

Fig. 2b. Amino acids contact with Holotricin-3

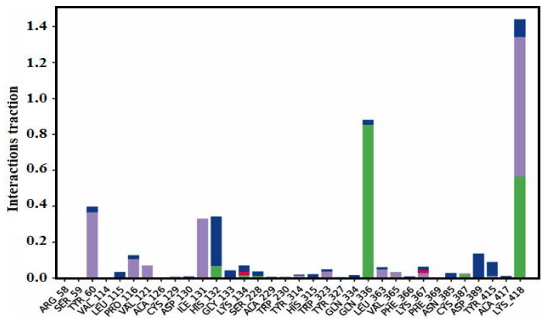

Fig. 3a. Protein-Ligand Contacts with interactive fraction on X-axis

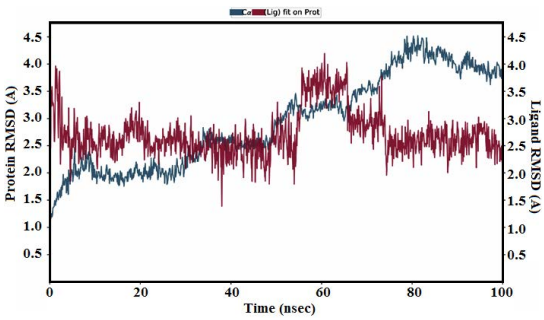

Fig. 3b. Root Mean Square Deviation graph of Bgl2p with Holotricin-3

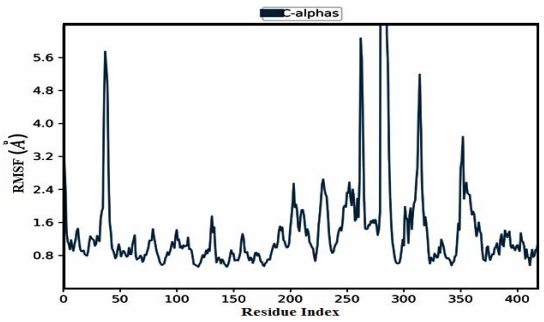

Fig. 4. Root Mean Square Fluctuation graph of Bgl2p

With respect to complicated chemicals, the force field was applied for 100 nanoseconds for a constant period. The stabilised trajectories were disclosed using the root mean square deviation (RMSD) and root mean square fluctuation (RMSF) before starting the simulation run at 100ns. The RMSD values for the conventional docked molecule were displayed on the Y-axis, while the ligand RMSD was displayed on the X-axis on the right. The fluctuation in ligand RMSF was detected over the first 30ns of the typical trajectory. The system was standardised, and no significant changes in density, volume, or kinetic energy were noted.

The Holotricin-3 wild-type strain has a multifunctional synthesis of viral ribonucleic acids that can often offset immunogenicity and immune response. Antiviral components in the RIG-I and FGI-103 IFN production were linked to holotricin-3. There are amino acids (LYS 37, ARG 34, VAL 85, HIS 47) that can manipulate the mutation rate in the Bglp2 receptor and inhibit the entire process during cyclization, resulting in changes in the Vander Waal force and binding energies, respectively, after high throughput screening with the selected phytoligands.

CONCLUSION

After analyzing the docking and trajectories analysis of Holotricin-3 with Bgl2p, we came to know that Holotricin-3 has exposed the inhibitory activity against antifungal protein and can be a lead molecule for *In vitro* studies. We have also shown the stability and binding energies of Holotricin-3 against antifungal receptors which showed tremendous outcomes towards the treatment of fungal activities and might be useful in future studies and investigation.

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Conflict of Interest

The authors declare no conflict of interest.

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