Molecular Docking Analysis of Plant-derived Small Molecules against Virulence Factors-Spore Coat Protein (CotH3) and High Affinity Iron Permease Protein (FTR1) of *Rhizopus Oryzae*

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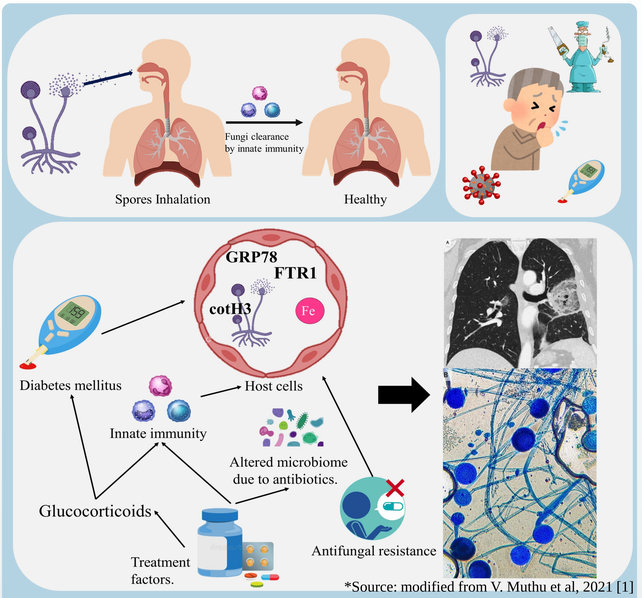
Introduction



*Source: modified from V. Muthu et al, 2021 [1]

Mucormycosis (Black Fungus) is a lethal fungal infection, caused by fungus belonging to the order Mucorales. *Rhizopus oryzae* is a main causative agent of mucormycosis with high mortality rate mainly in patients with neutropenia, hematopoietic and solid organ transplantation, iron overload and uncontrolled diabetes mellitus [2,3]. The treatment of mucormycosis is compromised by the limited spectrum of effective antifungal drugs. An alarming increase in resistant fungal strains and severe side effects of antifungals (hepatotoxicity, nephrotoxicity, and myelotoxicity) pose a severe challenge to the therapeutic strategies.

There are various virulence factors crucial in the infection process caused by *R. oryzae*. The spore coat protein homolog (CotH3) is a virulence factor i.e., detected universally on the spore surface of all Mucorales and plays a key role as invasins in the pathogenesis, disrupts and damages immune cells. The high-affinity iron permease (FTR1) has role in iron uptake and its transportation at the time of infection as acquisition of iron is essential and crucial pathogenic event during fungal infection. CotH3 and FTR1 proteins were selected for *in-silico* screening and molecular docking. The present study aims to screen and evaluate twelve natural bioactive molecules for their antifungal activity using *in-silico* approach.

Methodology

Retrieval of the target protein structures and their preparation.

- FASTA sequences of CotH3 and FTR1 protein of *R. oryzae* were retrieved from NCBI.
- Secondary structure of proteins was speculated using the Self-Optimized Prediction Method with Alignment (SOPMA).
- Homology modelling was conducted using Swiss Model via ExPasy and modelling structure was then evaluated using PROCHECK.

Preparation of ligands

- The 3D structures of 12 selected natural molecules were downloaded from the PubChem compound database.

Table 1: Plant-derived molecules selected for the study based on their antimicrobial properties

S.no.	Plant-derived molecules	Molecule code	S.no.	Plant-derived molecules	Molecule code
1.	Molecule-1	RO-01	7.	Molecule-09	RO-07
2.	Molecule-2	RO-02	8.	Molecule-08	RO-08
3.	Molecule-3	RO-03	9.	Molecule-09	RO-09
4.	Molecule-4	RO-04	10.	Molecule-10	RO-10
5.	Molecule-5	RO-05	11.	Molecule-11	RO-11
6.	Molecule-6	RO-06	12.	Molecule-12	RO-12

Molecular Docking

- The docking analysis was performed by program AutoDock4 Tool [4]. The Lamarckian genetic algorithm was utilized to perform the docking with total 50 poses.

ADME-Tox prediction

- The absorption, distribution, metabolism, excretion, and toxicity profile of selected molecules were predicted by SwissADME program [5].

Results

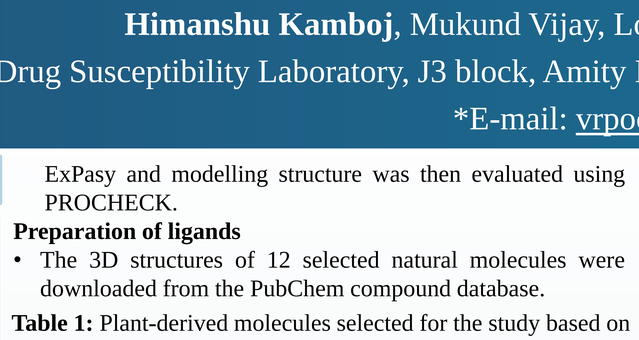


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Figure 1: The predicted secondary structure of the (a) CotH3 and (b) FTR1 protein of *R. oryzae* using SOPMA software.

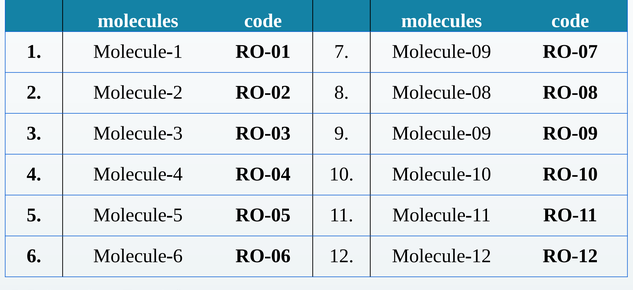

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Figure 2: Ribbon structure of (a) Spore coat protein CotH3 of *R. oryzae*. (b) Ramachandran plan analysis of modelled spore coat protein CotH3 generated by SwissMODEL.

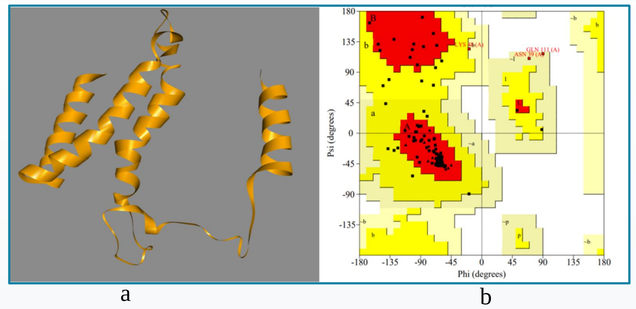

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Figure 3: Ribbon structure of (a) FTR1 protein of *R. oryzae*. (b) Ramachandran plot analysis of modelled protein FTR1 generated by SwissMODEL.

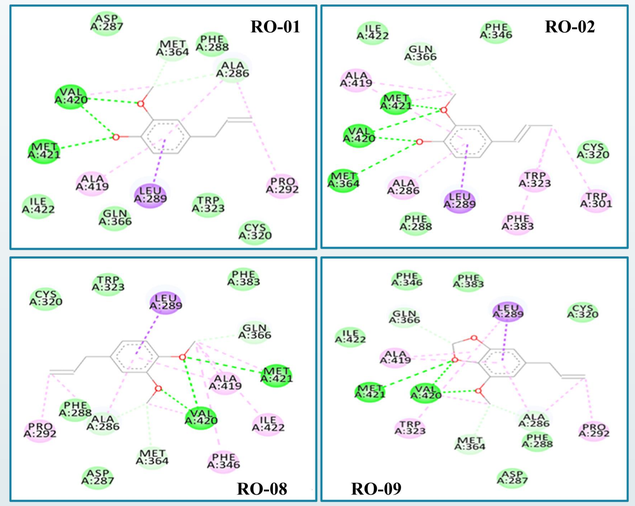


Figure 4: Binding interactions of RO-01, RO-02, RO-08 and RO-09 with active site of CotH3 protein of *R. oryzae*. Binding affinity for RO-01, RO-02, RO-08 and RO-09 was -6.75 Kcal/mol, -6.89 Kcal/mol, -6.60 Kcal/mol and -6.93 Kcal/mol respectively.

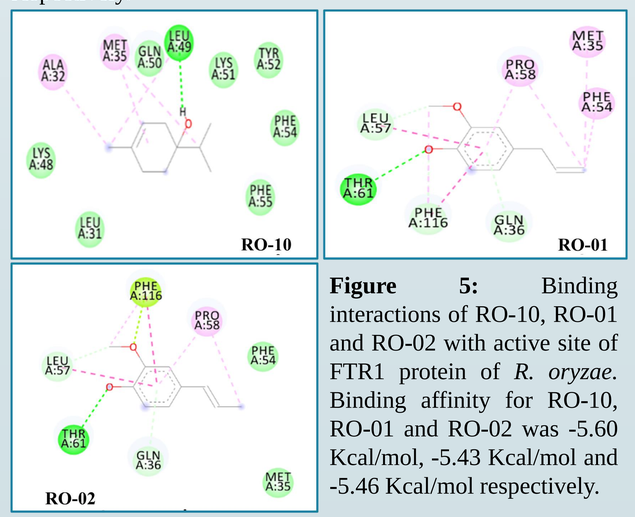
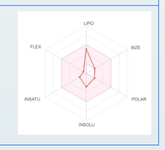


Figure 5: Binding interactions of RO-10, RO-01 and RO-02 with active site of FTR1 protein of *R. oryzae*. Binding affinity for RO-10, RO-01 and RO-02 was -5.60 Kcal/mol, -5.43 Kcal/mol and -5.46 Kcal/mol respectively.

Table 2. Physicochemical analysis of the potential inhibitor of CotH3 and FTR1 protein of *R. oryzae*

Compound	ADME properties (Lipinki's rule of five)	Molecular Weight	Radar diagram
RO-01	Molecular weight (<500g/mol)	164.20 g/mol	
	LogP (<5)	2.25	
	H-bond donor (<5)	1	
	H-bond acceptor (<10)	2	
	Violation	0	
RO-02	Molecular weight (<500g/mol)	164.20 g/mol	
	LogP (<5)	2.41	
	H-bond donor (<5)	1	
	H-bond acceptor (<10)	2	
	Violation	0	
RO-08	Molecular weight (<500g/mol)	178.23 g/mol	
	LogP (<5)	2.58	
	H-bond donor (<5)	0	
	H-bond acceptor (<10)	2	
	Violation	0	
RO-09	Molecular weight (<500g/mol)	192.21 g/mol	
	LogP (<5)	2.49	
	H-bond donor (<5)	0	
	H-bond acceptor (<10)	3	
	Violation	0	
RO-10	Molecular weight (<500g/mol)	154.25 g/mol	
	LogP (<5)	2.44	
	H-bond donor (<5)	1	
	H-bond acceptor (<10)	1	
	Violation	0	

Conclusion

CotH3 and FTR1 are potential drug targets that can be considered in antifungal drug designing to combat the mucormycosis infections. Out of the twelve molecules, RO-01 and RO-02 had interactions and binding energy at both the target proteins. These molecules could be further modified to enhance the efficacy against the target proteins. In addition, these molecules possess good *in silico* ADME properties that demonstrate their safety.

References

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*Contains Unpublished Data