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In Search of New Analogues as Anti-Fungal Agents

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ABSTRACT

Development of new Clotrimazole derivatives was studied here. Automated prediction of protein-small molecule interactions is one of the most challenging problems in structural biology as well as in medicinal chemistry. Many biological studies, both in academia and in industry, may benefit from credible high-accuracy interaction predictions. The molecular interactions with target protein have been discussed. A target based designing of new derivatives for future drug development presented, which can be further informative and marketed if their biological significance stand out better than the marketed one. The report concludes with a description of the initial efforts to prepare synthesized compounds to recognize as better antifungal agents than the regular prescribed drugs in market. The *in-silico* drug designing leading to initial successes are described along with future directions.

Keywords: Clotrimazole, Designed-derivatives, anti-fungal drugs, PATCHDOCK, Docking score

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INTRODUCTION

In the present scenario, fungal infections are associated with high case of mortality, and the availability of two important classes (**Figure 1**) of anti-fungal agents which are available for medication is found to be not enough to protect human life. In practice, existing antifungal agents mainly showed various side effects and toxicity after administration orally. The drug interactions along with the routes of administration majorly showed a downgrade tendency to make safer for mankind. An increasing prevalence of invasive fungal infections along with rising rates of resistance and the practical limitations of existing drugs developed a demand for the searching of new antifungal, particularly those with novel mechanisms of action.

Antifungal Drugs	
Imidazoles	**Triazoles**
Clotrimazole	**Fluconazole**
Oxiconazole	**Itraconazole**
Miconazole	**Terconazole**
Econazole	**Voriconazole**
Tioconazole	**Isavuconazole**
Ketoconazole	**Posaconazole**

Figure 1: Types of antifungal drugs available in market

Clotrimazole is one of such well-known anti-fungal agents (**Figure 2**). In the present study, we are trying to search for some new analogues of Clotrimazole by changing its side chain and functional groups to an entirely new derivative not reported earlier. Cytochrome P450 is a vulnerable target for this agent and the crystal structure of Clotrimazole complexes in CYT450 was reported. So, we have chosen Cytochrome P450 as our target protein.

Clotrimazole: In Antifungal medication, a common drug is known as Lotrimin among the other drugs, and which is nothing but Clotrimazole. The drug is normally used in oral thrush, diaper rash, and types of ringworm including athlete's foot and jock itch. It has also can use to treat vaginal infections and can be administrated by the mouth as tablet and as cream for vagina and other parts of human body like ankle, foot etc. Clotrimazole was first discovered in 1969 and it was included as a genericmedicine by the World Health Organization (WHO). It can be easily purchased on the market without any prescription as it is a very safe drug. The common side effects which were reported when it was taken by mouth are nausea and itchiness. When applied to the skin, common side effects include redness and burning. No such significant drug interactions are reported with Clotrimazole. Multiple drug interaction was found with CYP450 enzyme inhibitor. It is an imidazole derivative which basically inhibits the growth of fungal cell wall and binds to phospholipids in the cell membrane and can cause inhibition of the biosynthesis process of ergosterol which is responsible for cell membrane formation. It inhibits fungal growth and destroys the fungal cell finally.

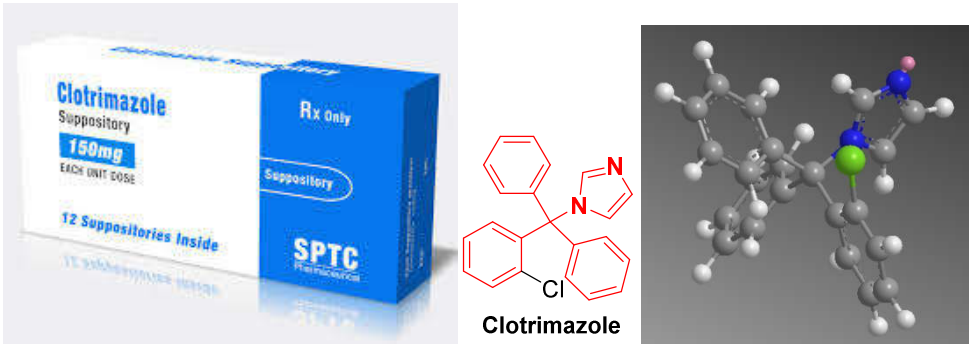


Figure 2: Clotrimazole

Present days, most of the pharmaceutical companies where medicinal drugs are developed, the concept of drug development has changed which are based on medicinal chemistry [1-5] and they are trying to find the inner interaction study of the marketed or designed drugs. Keeping in mind of this concept, our present projects is based on involvement of developing and find some better replacement of marketed drugs. Molecular docking is a bioinformatics tool where the interactions of one or sometime two or more small compounds form possible stable complexes by docking of these small moieties with macromolecules. The study of the designed molecules with their large, targeted molecules will be an interesting study and that can be obtained by analysis of that 3D-structure of the docked complex. Docking experiment generates not only single possibilities but a set of all the possible docked structures which depends on the docking software as well as algorithms followed in those. The whole docking study can generate a docking score based ranking list of the generated adducts which is basically obtained from summarizing of all possible interactions.

The 3D structures of target macromolecules can be analyzed with different software [6-10] available in the market. The molecular docking methodology is used here to identify the most

potent derivatives of Clotrimazole which is widely used by the medical practitioners. The molecular docking software can generate the best docked adduct among all the possible generated docked structures.

METHODS

A widely accepted molecular docking methodology for gathering best designed analogues of Clotrimazole had been used here. The initial designing of structurewere drawn by Chem Draw 15.0 initially.The energy minimized structures were obtained from Chem-3D Ultra software.For initial docking, all the 3D chemical structures of the novel analogues were saved in .PDB format file which was according to the basic requirement of docking.

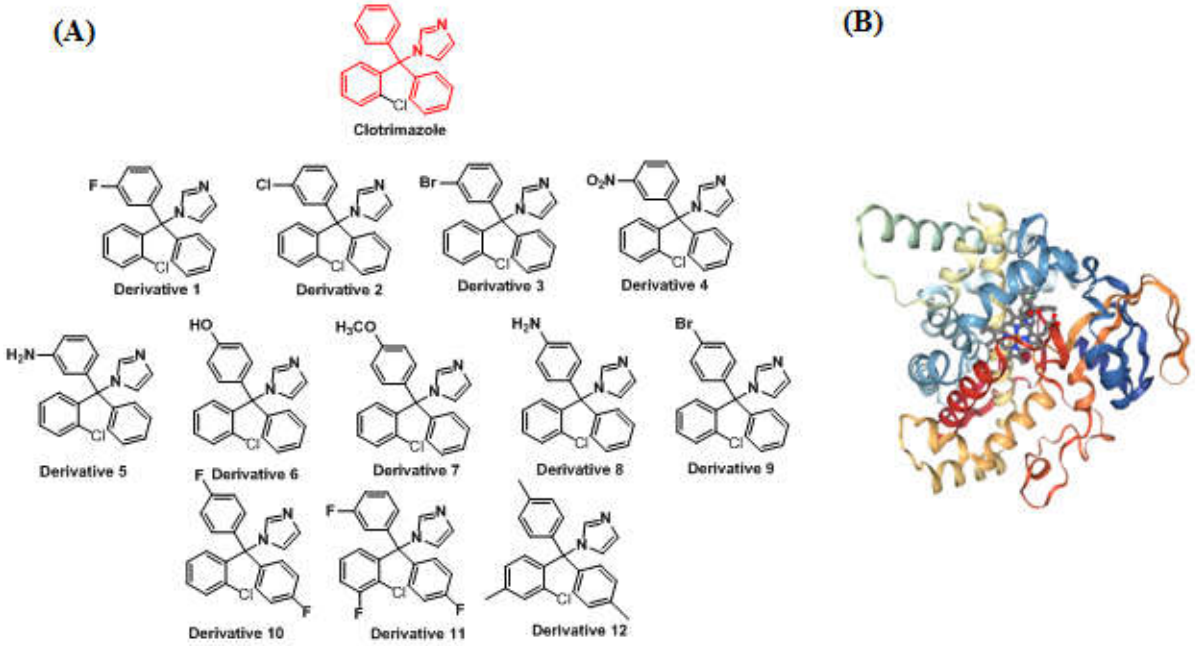


Figure 3: (A) Structures of designed analogues; (B) Three Dimensional target protein structure (PDB ID.3MDV)

Method followed during molecular docking: Protein data bank library (www.rcsb.in) was used to get crystal structure of target protein. At first all polar hydrogen atoms were added along with any missing atoms which were found during the initial process of docking. The crystal structure here 3MDV [11] was then ready for docking submission. All the water molecules and metal ions were deleted before submission for docking.

PATCHDOCK web server [8,12] was used for all the docking study. The PatchDock method performs structure prediction of protein–protein and protein–small molecule complexes. The SymmDock method predicts the structure of a homomultimer with cyclic symmetry given the structure of the monomeric unit. Submission of two files, one for ligand another for protein and those files should be in .PDB format had been done initially. After submission of each docking, an auto generated confirmation email containing the job status was obtained. The submitted job can be monitored from the link given after each job submission. The job completion information was obtained by email provided at the time of job submission along with the link for downloading the result and docked PDBs was obtained in the next step. In the initial page the best 20 docked complexes with their corresponding docking score (geometrically obtained) was tabulated. The three dimensional co-ordinates of each docked structures were also mentioned. UCSF Chimera was used to analyze the downloaded docked structures. The amino acids with close contact with the novel analogues were labelled and those are called as the interactive residues. All the possible

interactions which are present in each of the docked complex like H-bonding or other possible van der Waal interactions were checked and analyzed in different software like Biovia-Discovery studio, UCSF Chimera etc.

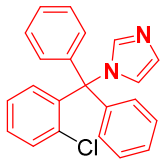
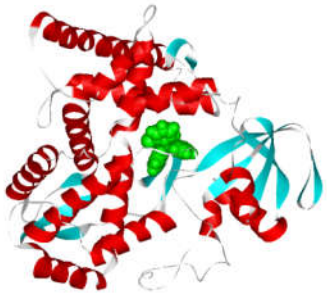
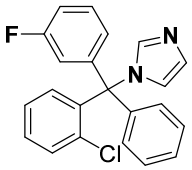
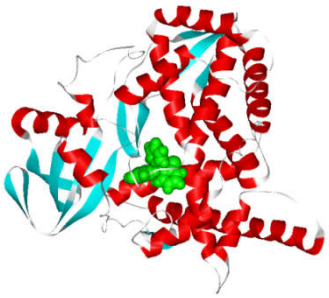
RESULT& DISCUSSIONS

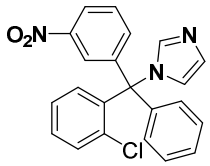
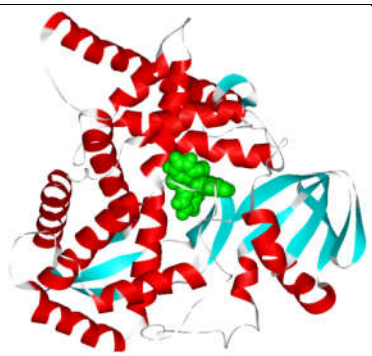
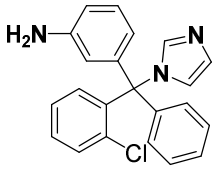
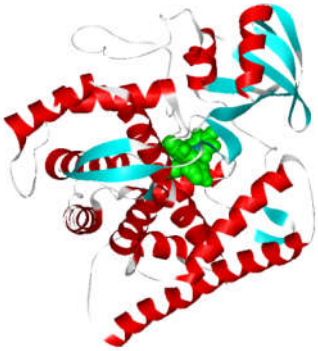
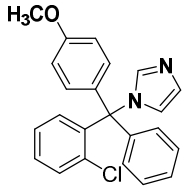
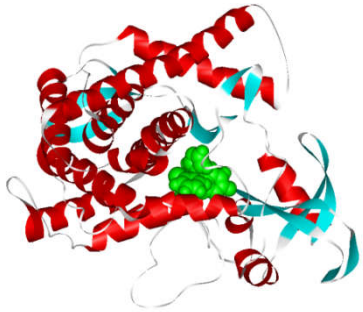
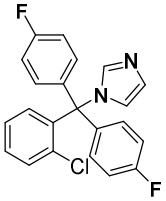
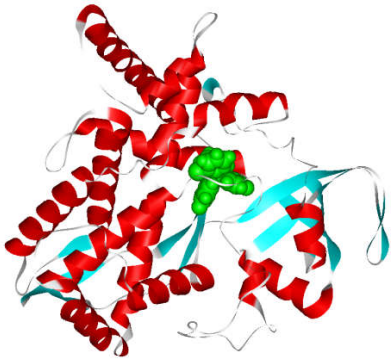
In the present work, twelve analogues of Clotrimazole were designed and submitted for initial screening via Molecular docking. The parent molecule (here Clotrimazole) shows a least docking score of 4752 with the targeted protein, Cytochrome P450 46A1. The best among the twelve derivatives was found to be Derivative 10 with the docking score of 5128 and the amino acids which are responsible for showing the good interaction are ARG139, HIS434, CYS437, HIS135, TYR131, CYS129, TYR102 and ASN103 (**Table 1**). Here we have chosen the best docked structure on the basis of docking score which can be calculated on the basis of binding free energy, van der Waal interactions, pi-pi interactions, enthalpy, entropy etc. The docking algorithm of the online server PATCHDOCK is widely acceptable.

The parent molecule shows a very few amino acids in the same binding pockets with CYS437, HIS135, TYR131, CYS129, TYR102 and ASN103 residues. The second-best derivative is no. 7 with a moderate docking score of 5110. After closely analyzing the data, we can conclude that all the designed and docked moieties have shown a better interaction in terms of docking score with the same targeted protein.

All twelve analogues are nitrogen-containing compounds and show better affinity for CYP46A1 yet differs in structure, the atom or the functional groups presented in the moieties. Structures of the co-complexes demonstrate that each derivative binds in a single orientation to the active site of the targeted protein.

Table 1: Docking Score along with interactive amino acids obtained after Docking of selected best compounds

2D Structure of compound	Docking Score	Amino acids present in close contact	Docked analogue* with targeted protein
 **Clotrimazole**	4752	CYS437, HIS135, TYR131, CYS129, TYR102, ASN103	
 **Derivative 1**	4946	HIS135, TYR131, CYS129, TYR102	

 Derivative 4	4980	HIS434, CYS437, HIS135, TYR131, CYS129	
 Derivative 5	5002	ARG139, HIS434, CYS437, HIS135, TYR131, CYS129, TYR102, ASN103	
 Derivative 7	5110	CYS437, HIS135, TYR131, CYS129, TYR102,TYR131	
 Derivative 10	5128	ARG139, HIS434, CYS437, HIS135, TYR131, CYS129, TYR102, ASN103	

* Docked analogues have shown in bright green coloured CPK models

In **Table 1**, five analogues among the twelve designed analogues along with Clotrimazole molecule were shown with their obtained docking score. The three-dimensional structures after docking was analysed and the interactive amino acid residues are identified. The docking score here proportionately increase with the increased number of neighbour amino acid residues in each case. The types of interactions like van der Waal and pi-pi interactions are also found in the docked structure of Derivative-10 which was reflected in the docking score as compared to the other derivatives designed.

CONCLUSION

In the present project we have designed twelve new derivatives of Clotrimazole, which is a marked antifungal drug. Five designed derivatives were found among the twelve with better potency in terms of molecular interaction between analogues and targeted protein. Derivative 10 was found to be the best among five designed new derivatives with a best docking score of 5128. Mostly all the new derivatives bind the same binding pocket of the targeted protein which validates the experimental procedure is an ideal one. After choosing a marketed antifungal agent for derivatization, we successfully designed few new agents which can be further synthesized and biologically evaluated for future scopes. A target based designing of new derivatives of a very well-known drug was studied here in the present project which can be further informative and marketed if its biological significance stand out better than the marketed one**.**

Abbreviations:
CYT450: Cytochrome P450; PDB: Protein Data Bank; CYS: Cysteine; HIS: Histidine; TYR: Tyrosine; ASN: Aspergine; ARG: Arginine

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Conflict of interest:
None

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