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Molecular Docking Studies of Novel Aminopyrimidines as Potent Antifungal Agents

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ABSTRACT

Candida albicans is an opportunistic fungal pathogen that causes candidiasis in human hosts. Candidiasis includes a multitude of fungal infections, including invasive fungal infections, where most patients are immunocompromised; hence, the success of treatment is determined by the efficacy of the antifungal agent. However, with the increase in resistance to the existing drugs, the availability of effective antifungal agents is becoming scarce.

Many pyrimidine derivatives exhibit powerful antifungal activity. In this study, In silico antifungal activity was carried out on twenty novel aminopyrimidine derivatives to identify the specificity of the pyrimidine analogues for the antifungal targets using 'Glide'. Molecular docking studies were conducted on two antifungal targets; Dihydrofolate reductase of *C. albicans* (PDB ID: 4HOE); *N*-myristoyl transferase of *C. albicans* (PDB ID: 1IYK); energy minimization of title compounds was carried out using LigPrep, the protein targets were optimized and minimized, a 3-dimensional grid was generated at the active site, and molecular docking was carried out at both the standard precision (SP) and extra precision (XP) modes. The docking poses were ranked according to their docking scores (GScore) and their binding energy with the enzyme (Emodel). The obtained results for the docking of the title compounds with dihydrofolate reductase of *C. albicans* are quite promising. Molecular docking suggest that compounds 2N and 2A are potential inhibitors of dihyfrofolate reductase and are specific in binding at the active site of the enzyme. They form pi-pi stacking interactions with PHE 36 at the active site of the protein, similar to the standard drug. However the test compounds show lower docking scores against *N*-myristoyl transferase of *C. albicans* indicating that they may not be effective against the fungal protein.

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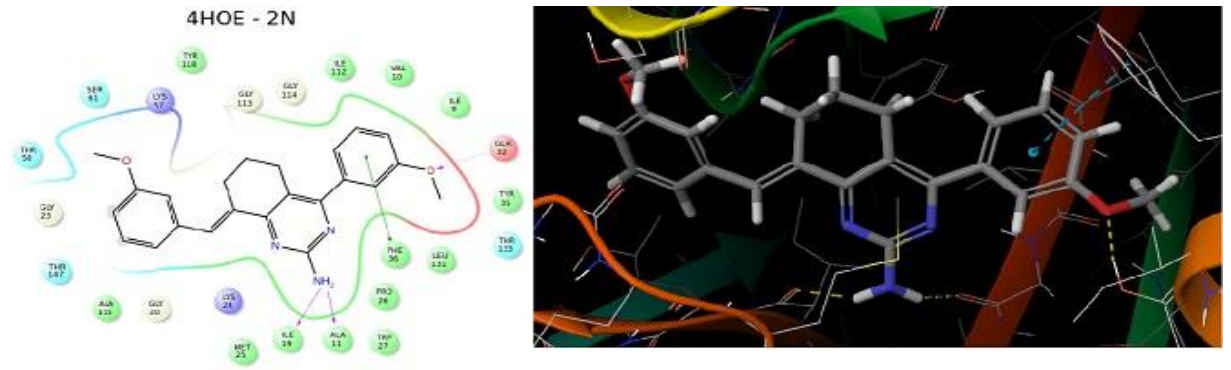
Graphical Abstract



Docked pose of compound 2N at the active site of Dihydrofolate reductase of *Candida Albicans*

Introduction

Candida albicans which is an adaptable fungal pathogen is the most common of the twenty types of *Candida* that are responsible for causing candidiasis in human hosts. Candidiasis includes a host of fungal infections, including invasive fungal infections (Candidemia), where most patients are immunocompromised. Candidemia is the fourth most common bloodstream infection among hospitalized patients in the United States. People at high risk for developing candidemia include intensive care unit (ICU) patients, surgical patients, patients with a central venous catheter, people whose immune systems are weakened (such as people with HIV/AIDS), and very low-birth-weight infants [1]. Hence, the success of the treatment depends more on the effectiveness of the antifungal agent. Many synthetic compounds containing the aminopyrimidine moiety exhibit a wide spectrum of pharmacological activities like, antibacterial, antifungal, antiparasitic, anti-tumor and urease-inhibitory activities [2-6]. In this perspective, novel aminopyrimidines were designed with the objective of obtaining more potent antifungals.

Molecular docking studies are used to explain the binding of the synthesized compounds with the target proteins. This helps to obtain knowledge for optimization of the structure. All the designed aminopyrimidines were docked onto the active site of the crystal structure of the antifungal enzymes. The mechanism of interaction of the novel aminopyrimidines were studied on two target proteins-*N*-myristoyl transferase of *C. albicans* (PDB ID: 1IYK) and dihydrofolate reductase of *C. albicans* (PDB ID: 4HOE).

N-myristoyl transferase (NMT) catalyzes the transfer of the rare fatty acid myristate from myristoyl-CoA to the *N*-terminal glycine of substrate proteins, and is found only in eukaryotic cells.

NMT activity is essential for vegetative growth. Recent *N*-myristoyltransferase inhibitors show in *vivo* antifungal activity and are promising selective fungal *N*-myristoyltransferase inhibitors [7-8]. Dihydrofolate reductase catalyzes the reduction of dihydrofolate into tetrahydrofolate, and is critical for the biosynthesis of purines, thymidylate and some amino acids. These are important for cell proliferation and cell growth. As the DHFR enzyme is found in both humans and microorganisms, the ideal antifungal agent should selectively inhibit the fungal enzyme [9]. Fungal DHFR is different from the human enzyme [10]. Hence, inhibitors of fungal DHFR may be useful as antifungal agents [11].

Experimental

Materials and methods

Molecular modeling and scoring

Molecular modeling was carried out using GLIDE 2.0 [12], running on Intel® Core TM i3-2130 CPU@ 3.40GHz processor using Linux professional workstation.

Preparation of ligands

The structures were drawn in 2D. The corresponding 3D structures were generated using the Chem draw 3D software. By using the LigPrep module [13], the geometry optimization of the drawn ligands was carried out using the standard bond lengths and bond angles, with the help of standard OPLS_2005 force field. All the conformations were optimized for minimum energy.

Preparation of protein

The crystal structures of dihydrofolate reductase of *C. albicans* complexed with NADPH and 5-[3-(2,5-dimethoxy-4-phenylphenyl)but-1-yn-1-yl]-6-methylpyrimidine-2,4-diamine (PDB code 4HOE) and, *N*-myristoyltransferase of *C. albicans*, complexed with myristoyl-CoA and peptidic inhibitor (PDB entry code 1IYK), was downloaded from the Protein Data Bank (PDB extracted from the Brookhaven Protein Database http://www.rcsb.org/pdb) and used for docking studies. Co-crystalized ligand and water molecules were removed from the structure, *H*-atoms were added, disulphide bonds were created and side chains were fixed during protein preparation. The structure was then subjected to an energy refinement and energy minimization procedure [14].

Receptor Grid generation

The optimized protein with co-crystallized ligand was taken to generate a 3D grid at the active site of the target protein as per the standard protocol of glide manual [12-14]. The co-crystallized ligand

molecule is removed and the prepared ligand is docked in its place. Receptor grid generation allows defining the position and size of the active site for ligand docking.

Docking protocol

The binding of the aminopyrimidine derivatives was estimated using a variety of scoring functions that have been compiled into the single score (GScore) [14-16]. The GScore integrates a number of popular scoring functions for ranking the affinity of ligand bound to the active site of a receptor.

GScore = 0.065*vdW+0.130*Coul +Lipo + Hbond + Metal + buryP + rotB +site.

VdW=Van der Waals energy, Coul= Coulonb energy; Lipo=lipophilic term; Hbond=hydrogen bonding term; metal=metal binding term; buryP=penalty for buried polar groups; rotB=penalty for freezing rotatable bonds; site=polar interactions in the active site.

Glide XP mode combines a powerful sampling protocol with a value of a custom scoring function designed to identify ligand poses. The chief purpose of XP method is to weed out false positives and provide a better correlation between good poses and good scores. The XP scoring function includes additional terms over SP scoring function.

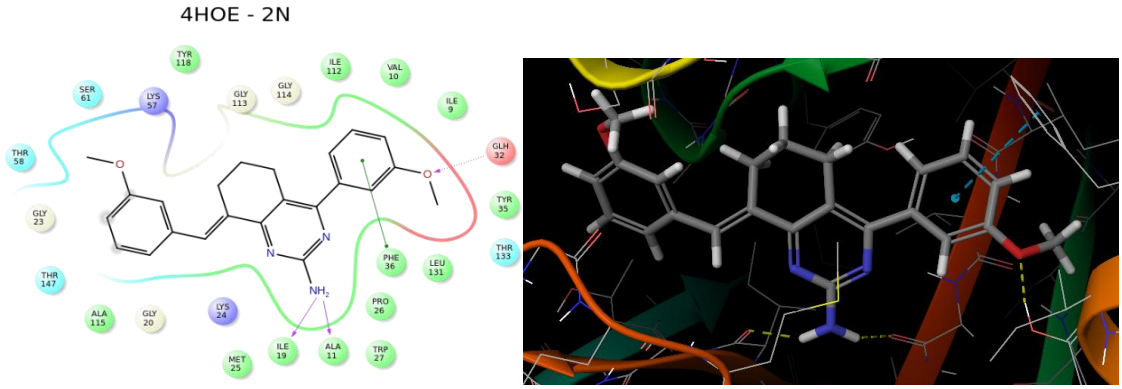


Figure1. Docked pose of compound 2N at active site of DHFR of *C. albicans* (PDB ID: 4HOE)

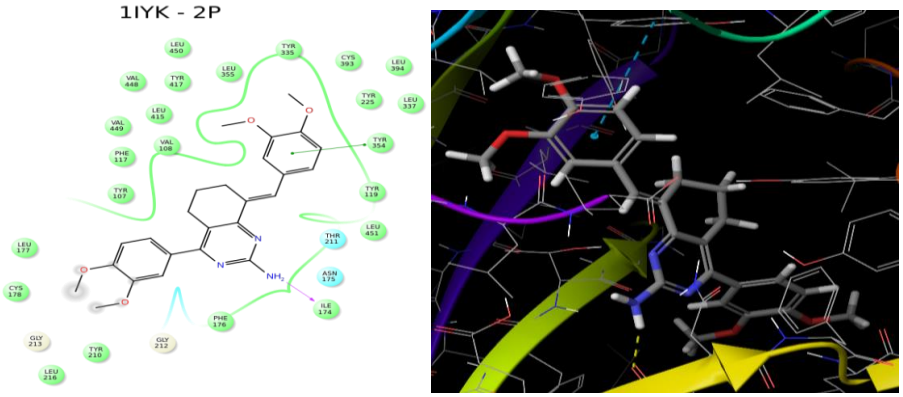


Figure 2. Docked pose of compound 2P at active site of NMT of *C. albicans* (PDB ID: IYK)

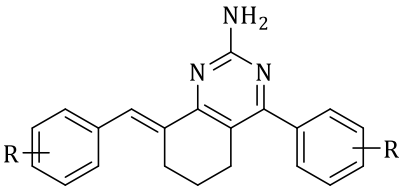


Table 1. Molecular docking results of pyrimidine derivatives with the target protein **4HOE**

Comp Code	R	SP model		XP model		
		GScore	Emodel	GScore	Emodel	Interactions at active site
2A	H	-8.334	-52.584	-9.205	-44.838	Phe 36, Ala 11, Ile 19
2B	furfuryl	-7.232	-51.941	-8.813	-40.48	Phe 36, Ala 11, Ile 19
2C	4-hydroxy	-6.378	-50.066	-5.737	-49.35	Phe 36
2D	2-hydroxy	-7.062	-59.728	-6.446	-53.013	Phe 36, Lys 24
2E	4-methyl	-6.447	-45.623	-5.701	-48.452	Ile 112
2F	4-ethyl	-6.539	-46.824	-6.342	-42.712	Phe 36, Lys 24
2G	4-nitro	-6.538	-60.513	-6.106	-61.083	Ile 112
2H	3-nitro	-6.797	-59.875	-5.629	-57.037	Phe 36, Phe 66, Ser 61
2I	4-dimethyl amino	-6.616	-54.655	-5.8	-50.284	Ile 112
2J	4-chloro	-6.307	-52.562	-6.616	-54.151	Ile 112
2K	3-chloro	-6.974	-55.809	-6.019	-52.41	Ile 112
2L	2,4-dichloro	-6.753	-56.897	-5.875	-56.776	Ile 112
2M	2,6-dichloro	-4.897	-45.531	-6.106	-38.541	Phe 36
2N	3-methoxy	-6.814	-51.898	-10.309	-49.832	Glh 32, Phe 36, Ala 11, Ile 19
2O	4-methoxy	-6.46	-52.077	-5.604	-47.472	Phe 36, Ser 61
2P	3,4-dimethoxy	-6.784	-60.278	-6.448	-52.792	Phe 36
2Q	3,4,5-trimethoxy	-6.687	-63.444	-6.613	-54.865	Ile 112
2R	2-nitro	-6.613	-56.631	-9.091	-52.692	Phe 36, Ala 11, Ile 19,
2S	3-methoxy-4-hydroxy	-7.403	-65.463	-8.017	-58.473	Phe 36, Glh 32
2T	3-ethoxy-4-hydroxy	-6.543	-57.068	-7.067	-60.208	Phe 36, Ser 61
Miconazole		-6.680	-57.104	-7.334	-63.555	Phe 36
Ketoconazole		-7.582	-67.082	-6.020	-64.858	Ala 115, Glh 32
Clotrimazole		-4.718	-28.043	-4.154	-34.119	Phe 36, Ile 112

Table 2. Molecular docking results of the pyrimidine derivatives with the target protein **1IYK**

Comp Code	R	SP model		XP model		
		GScore	Emodel	GScore	Emodel	Interactions at active site
2A	H	-5.54	-49.003	-5.031	-48.428	Phe 339, His 227
2B	furfuryl	-5.395	-44.974	-4.583	-45.867	Phe 117, Leu 451
2C	4-hydroxy	-6.737	-59.214	-6.293	-54.639	Phe 240, Tyr 354, Phe 339
2D	2-hydroxy	-6.146	-53.462	-6.378	-62.08	Ile 174, Thr 211
2E	4-methyl	-5.733	-50.134	-5.545	-48.628	-
2F	4-ethyl	-7.035	-53.153	-6.908	-50.328	Ile 174
2G	4-nitro	-5.531	-58.228	-6.42	-61.12	Ile 174
2H	3-nitro	-6.913	-66.568	-5.431	-68.124	Asp 412, Phe 240, His 227, Phe 339, Phe 117
2I	4-dimethyl amino	-6.016	-55.725	-5.933	-56.488	Phe 240, Phe 339
2J	4-chloro	-5.966	-53.579	-5.684	-51.996	Phe 240, Phe 339
2K	3-chloro	-6.524	-58.353	-5.415	-57.05	-
2L	2,4-dichloro	-5.687	-57.113	-5.545	-63.003	Ile 174
2M	2,6-dichloro	-4.657	-45.912	-6.181	-51.447	Phe 117, Tyr 119, Tyr 107
2N	3-methoxy	-5.664	-53.966	-5.275	-52.594	Phe 117, Tyr 107
2O	4-methoxy	-6.052	-54.478	-6.809	-51.867	Ile 174, Tyr 354
2P	3,4-dimethoxy	-5.565	-59.123	-7.477	-59.214	Ile 174, Tyr 354
2Q	3,4,5-trimethoxy	-7.075	-57.189	-7.003	-66.627	Leu 177
2R	2-nitro	-6.294	-54.903	-4.017	-56.254	Ile 174, Tyr 354, Leu 450, Leu 177
2S	3-methoxy-4-hydroxy	-5.797	-60.035	-7.098	-66.78	Phe 117, Leu 451, Ile 174
2T	3-ethoxy-4-hydroxy	-6.911	-62.146	-7.366	-74.583	Phe 117, Ile 174, Leu 451, Asp 412
Miconazole		-5.736	-68.807	-7.065	-66.578	Leu 451, Tyr 354
Ketoconazole		-7.503	-88.869	-6.357	-79.190	Phe 240,Asp 412
Clotrimazole		-5.372	-30.007	-3.889	-66.518	Tyr 225

Results and Discussion

To understand the molecular basis of interaction and affinity of binding of the aminopyrimidine analogues with the *N*-myristoyltransferase and dihydrofolate reductase proteins, the ligands were docked into the active site of the respective proteins. Flexible ligand docking was carried out in the

(SP) standard precision and XP (extra precision) modes. The XP results are discussed as the SP mode may give some false positives. The GLIDE score can be used as a semi-quantitative descriptor for the ability of ligands to bind to a specific conformation of the protein receptor. Usually good ligand affinity for the receptor may be expected for low GLIDE score.

Docking results of the ligands with DHFR are given in Table 1. The title compounds have presented very significant results for the XP mode of docking with DHFR at its' active site. The Gscore for the derivatives varied from -5.629 to -10.309 against the enzyme. While the GScore for docking of the standards Miconazole, Ketoconazole, and clotrimazole was -7.334, -6.020 & -4.154 respectively. Compound **2N** (3-methoxy derivative) with the XP Gscore -10.309 and Emodel -49.832 indicated the best inhibition for the DHFR enzyme among the derivatives. The amino group of the compound has formed *H*-bonds with Ile 19 and Ala 11, while the methoxy group formed *H*-bond with Glh 32. Furthermore, the phenyl ring has displayed pi-pi stacking interaction with Phe 36 at the active site of the protein. Compound 2A (unsubstituted derivative) with Gscore -9.205 has also exhibited significant results. Moreover, 2A exhibits similar binding interactions with the amino acids Phe 36, Ile 19 and Ala 11 at the active site. Interestingly the GLIDE score for many of the analogues were better than the GLIDE score of the standard drugs. There was good agreement between the localization of the inhibitor upon docking and from the crystal structure of the protein. Conformational analysis of different docked complexes also shows that binding with the residue Phe 36 play an important role in binding with the receptor. Docking studies executed by GLIDE has confirmed that the analogues fit well into the binding pocket of dihydrofolate reductase.

The docking results of the derivatives with *N*-myristoyltransferase is given in Table 2. The XP mode has given significant results with good Glide scores and Emodel. The docking score for the ligands varied from -4.017 to -7.477, whereas the GScore for docking of the standards miconazole, ketoconazole, and clotrimazole with *N*-myristoyltransferase was -7.065, -6.357 & -3.889 respectively. Compound **2P** with Glide Score -7.477, Emodel -59.214 was found with highest score. The ligand formed H-bonds with Ile 174 and pi-pi stacking interaction with Try 354 at the active site, which made the compound to fit well into the pocket. Moreover Emodel indicates sufficient stability of the ligand 2P. Surprisingly, the compounds having similar scaffold were exhibiting interactions with different amino acid residues at the active site, while the 3-chloro and 4-methyl derivatives have not formed any interactions.

Conclusions

Molecular docking studies were performed for twenty aminopyrimidine derivatives on two antifungal target proteins of *C. albicans* - DHFR and *N*-myristoyltransferase.

Energy minimization of the title compounds was carried out, the protein was optimized and minimized, a 3-dimensional grid was generated at the active site, and molecular docking was carried out using the SP and XP docking modes of Glide module.

The docking poses were ranked according to their docking scores (GScore) and their binding energy with the enzyme (Emodel). If the binding energy is less, compound is more active.

The results obtained from molecular docking of title compounds with DHFR of *C. albicans* are quite promising. Based on the docking studies, it can be concluded that compound 2N (3-methoxy derivative) and 2A (unsubstituted derivative) is predicted to have good antifungal activity. The study suggests that the compounds are specific in binding at the active site of the dihydrofolate reductase enzyme of *C. albicans*, and they could be potential anti-fungal drugs.

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