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Research Article

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ADME/T Prediction, Molecular Docking, and Biological Screening of 1,2,4-Triazoles as Potential Antifungal Agents

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Abstract

Clinical needs for new antifungal agents have gradually and steadily increased with the rise of AIDS-related mycoses, and the change in spectrum of fatal disseminated fungal infections. Triazoles are taken as potential antifungal molecules considering the existing portfolio of antifungal agents available in the market. This study was aimed to evaluate antifungal potential of 4-Amino 5-(2-Hydroxyphenyl)-1,2,4-Triazole-3-Thione and 4-(2-hydroxybenzalidine)amine-5-(2-hydroxy)phenyl-1,2,4-triazole-3-thiol using molecular docking and *in-vitro* antifungal screening approach. This study was further designed to evaluate potential drug likeness properties and ADME/T prediction of aforementioned experimental triazoles. 3D crystal model of cytochrome P450 lanosterol 14 α-demethylase enzyme was acquired from Protein Data Bank (PDB ID 5EQB). Docking studies revealed that both experimental compounds showed stable binding complex with lowest binding affinity values compared to reference standard Fluconazole (-6.8, -7.8 and -7.1Kcal/mol respectively for UI, UIA and Fluconazole) against cytochrome P450 lanosterol 14 α-demethylase, which is a key yeast target. *In-vitro* evaluation revealed that both experimental triazoles especially UIA showed promising MIC values, 24, 48 and 80 µg against *Candida albican, Candida tropicalis* and *Candida glabrata* respectively. Rat acute toxicity prediction using GUSAR model suggested that both experimental molecules were virtually nontoxic when computationally studied through IV, IP, SC and oral routes of administration. This experimental work concludes that both triazoles possess strong fungicidal potential and should be further explored. *In-silico* work in current study further proposes an exceptional strategy for drug discovery with minimum cost and time.

Keywords

1,2,4 Triazoles; Antifungal; Computer aided drug designing; *In-vitro;* Molecular docking

Introduction

Invasive, life-threatening fungal infections are an important cause of morbidity and mortality, particularly for patients with compromised immune function. The number of therapeutic options for the treatment of invasive fungal infections is quite limited when compared with those available to treat bacterial infections. Indeed, only three classes of molecules are currently used in clinical practice and only one new class of antifungal drugs has been developed in the last 30 years [1] As a matter of fact, arsenal of antifungal drugs is relatively unfilled, with fewer numbers of fungicidal molecules commercially available compared to antibacterial agents. At parallel emergence of resistant fungal strains is alarmingly increasing day by day. Frightening boost in fungal infections is mainly linked to distressing increase in reporting of AIDS, cancer and organ transplant patients. Since treatment of aforementioned ailments lead to compromised immune status, so the patients are always exposed to opportunistic fungal infections. Especially amplified incidence of AIDS is another gigantic challenge in which patient is at risk of catching up fungal infections because of the well-known fact that patients having AIDS are with weakened immune status [2]. These clinical challenges have amplified the consumption of antifungal agents which ultimately is resulting in emergence of drug resistance within leading pathogenic fungal strains. Top culprit responsible for systemic and local candidiasis includes, *Candida albican,* followed by *Candida glabrata* and *Candida tropicalis* isolated from patient samples in clinical settings. *Candida albican* is frequently responsible for oral candidiasis (oral thrush), vaginal candidiasis, and candidemia [3,4]. *Candida glabrata* has become clinically significant because of increasing prevalence globally, as it is second leading organism responsible for candidemia [5]. *Candida tropicalis* has been identified as most prevalent *Candida non-albican* group of yeasts. This pathogen can be proclaimed as emergent pathogenic fungal specie as it's reporting in causing Candidiasis has been dramatically increased [6]. Triazoles are biologically active moieties having various pharmacological properties including anti-inflammatory [7], analgesic [8,9] anti-cancer [10,11], anti-oxidant [12], anti-bacterial [13] and anti-fungal [14]. Many experimental 1,2,4 triazoles have shown promising antifungal potential in *in-vitro* and *in-silico* models [15-17]. Triazoles are backbone of currently available antifungal regimen. Most of commercially available antifungal molecules are triazoles in nature, including fluconazole, itraconazole and ketoconazole [18]. These triazoles target cytochrome P 450 dependent lanosterol 14 α-demethylase which is a key fungal target invovled in biosynthesis of sterols those are essential biochemical moeities responsible for carrying out various biological activitites within fungal cell. Its inhibition by different reported antifungal agents result in subsequent loss of normal sterols correlates with accumulation of 14 α-methyl sterols in fungi ultimately resulting in cell death [19].

In current study we evaluated two triazoles i.e. 4-Amino 5-(2-Hydroxyphenyl)-1,2,4-Triazole-3-Thione and 4-(2-hydroxybenzalidine)amine-5-(2-hydroxy)phenyl-1,2,4-triazole-3-thiol (Figure 1) for their antifungal potential. These experimental triazoles were already synthesized and characterized in Pharmaceutical Chemistry Lab of Riphah institute of pharmaceutical sciences [20], and were received as a gift for this experimental work. Evaluation of these triazoles was planned in three steps i.e. ADME/T prediction of experimental triazoles, docking studies against Cytochrome P 450 dependent lanosterol 14 α-demethylase enzyme and finally by *In-vitro* evaluation for antifungal activity.

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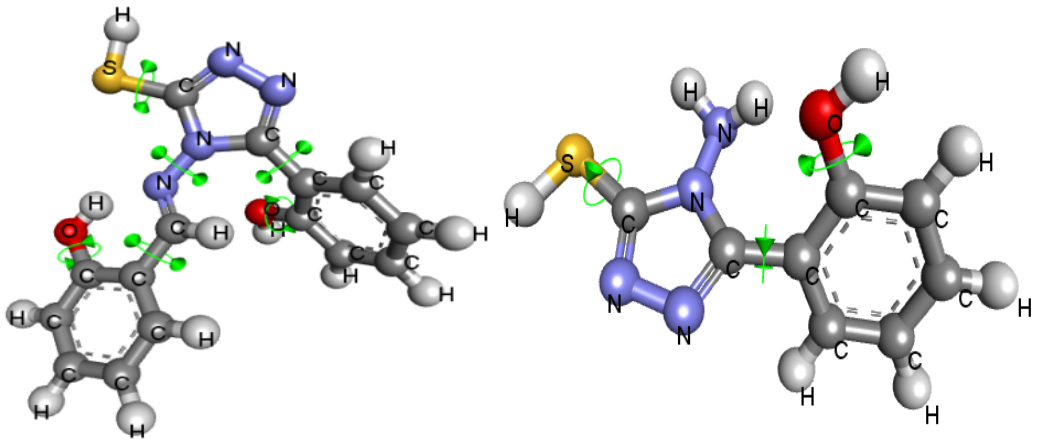

Figure 1: 3D structures of UI (A) and UIA.

Experimental Procedure

Prediction of physicochemical and ADMET properties

Phsyicochemical properties of both lead molceules were determined by using virtual predition tool FAF Drug 3 server [21]. UI and UIA compounds were screened through molinspiration online tool inorder to obtain their drug likeness characteristics. Rule of 5 also helps us to understand ADME/T properties of ligands. According to Rule of 5, ligand must carry following moecular characterisitcs inorder to be considered as a drug molecule: Hydrogen Bond Donor ≤5 (OH and NH Groups), Hydrogen Bond Acceptor ≤ 10 (N and O atoms), molecular weight ≤ 500 dalton and partition coefficient (Clogp) less than 5 [22,23] (Figure 2).

Toxicity prediction using GUSAR model: LD50 values for each lead molecules through different route of adminsteration including intravenous (IV), oral, subcutaneous (SC) and intraperitneal (IP) were predicted by using acute rat toxicity Generally Unrestricted Structure Activity Relationship (GUSAR) model [24].

Docking studies

Molecular modeling: Structures of both triazoles UI and UIA were drawn and cleaned into 3D models using Marvin Sketch Tool and were saved in PDB formats [25] (Figure 1). Mol formats of both ligand molecules were converted into PDB files using Discovery Studio and were finally prepared into PDBQT file using PyRx tool inorder to produce atomic coordinates. 3D crystal model of cytochrome P450 lanosterol 14 α-demethylase enzyme (CaCYP51) was acquired from Protein Data Bank (PDB ID 5EQB) (Figure 2). Using Drug Discvoery Studio, Ramachandran Plots for the downloaded protein model. Ramachandran Plot tells about specific low energy conformations for ϕ (phi) and (psi) or stable conformations of amino acid residues along with favorable and unfavorable regions for amino acid residues in plot. (Figure 3) Points on the plot indicate the ϕ (phi) and (psi), the torsion angles of amino acid residues in a 3D protein model [26]. Both target protein (CaCYP51) and ligand were uploaded in BioLip Virtual Tool in order to find out the coordinates of ligand in original target protein site [27]. BioLip is an online Ligand-Protein binding database used for prediction of ligand binding residues in a target protein.

Docking run: Docking is a computational tool to evaluate interactiuon of target with ligand. To closely monitor and evaluate possible residual interaction between ligand and target protein, both ligand and macromolecule (target protein CaCYP51) were uploaded in PyRx Virtual Screening tool which is an Autodock Vina option based on scoring functions. Residual interaction of triazoles with target enzyme was evaluated by viewing different poses of interaction using Discvoery Studio [28].

Antifungal susceptibility testing

In-vitro antifungal suceptibility testing was done using serial dilution method inorder to find out minimum inhibitory concentration values (MIC) against *C. albicans, C. glabratra* and *C. tropicalis* [29].

Results

Prediction of physicochemical and ADMET properties

Predicted water solubility values (in mg/L) showed that UI showed comparable water solubility i.e., 31681.67 mg/L Vs. 38565.40 mg/L. On VEBER and EGAN models, both UI and Fluconazole were proposed to have good oral bioavailability profile. With solubility 8704.71 mg/L UIA was predicted as having less oral bioavailability compared to both UIA and Fluconazole (Table 1). Both triaozles UI and UIA fulfilled criteria of Rule of Five without any violation. Molecular weight for UI was 208 and 312 Dalton for UIA. Similary all other pharmacokinetic parameters (ADME/T) were between normal values for all lead molecules with zero violations which revealed both triazles can be developed as drug molecules (Table 1).

Toxicity prediction using GUSAR model: Predicted LD_{50} values (in mg/kg) through IV, oral, SC and IP routes showed that both experimental triazoles are proposedly non toxic and thus can be further studied for their toxicity profile in animal models [30]. Toxicity prediction using GUSAR model further showed that LD_{50} values of UI and UIA were comparable with reference standard Fluconazole (Table 2)

Docking studies

Binding site with atomic coordinates of ligand was analyzed by using online server 3DLigandSite. HIS, LEU and ALA amino

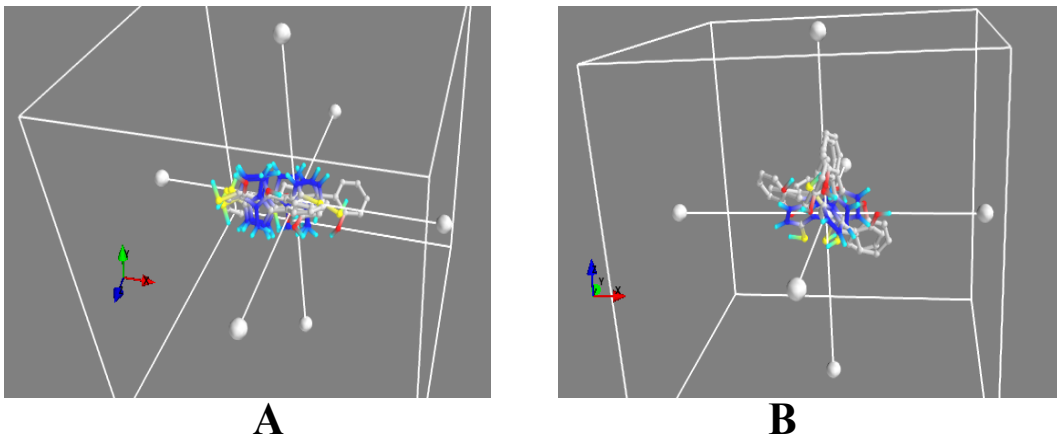

Figure 2: Different conformational positions of UI (A) and UIA (B) in the binding pocket of Cytochrome P 450 lanosterol 14 α demethylase enzyme, exhaustiveness was set at 8.

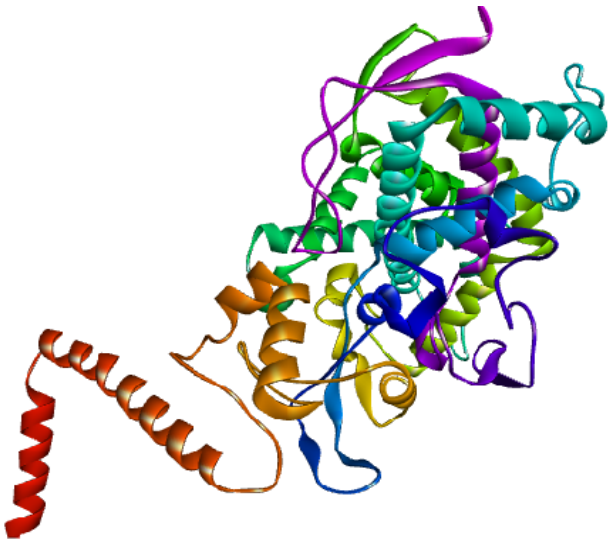

Figure 3: 3D model of Cytochrome P 450 lanosterol 14 α demethylase built acquired from PDB ((PDB ID 5EQB) and Viewed using Discovery Studio 4.1 in publication quality graphics.

acids were identified in the active binding pocket of target enzyme CaCYP51 (Figures 4 and 5). Docking interaction of both ligand against target protein (CaCYP51) revealed lowest binding affinity values indicating stable Ligand-Target complex which is a predictor of *insilico* inhibition of CaCYP51. Docking energies calculated using PyRx based on Autodock Vina were, -7.8 and -5.9 Kcal/mol with favorable RMSD values compared with -7.1 Kcal/mol with reference standard fluconazole (Table 3). Hydrogen interactions between ligand and target protein is an important predictor of strength of interaction between them which was also closely studied in our study and hydrogen bond donor and recepeint areas of both ligand and target was closely viewed as well as distance of interacting atoms were also monitored closesly (Figures 6 and 7).

Antifungal susceptibility testing

In-vitro antifungal susceptibility testing that all fungal strains including *C. albican, C. tropicalis* and *C. glabrata* were susceptible to both experimental triazoles and with serial dilution method UIA showed better MIC values than UI (Table 4). UIA showed least MIC values against *C. albicans* i.e., 24µg which unveils its promising potential as antifungal agent and requires further investigation in *in-vivo* models.

Conclusion

Both lead molecules (UI and UIA) complied with Lipinski Rule of Five with zero violation reflecting that both ligand molecules are excellent drug candidates. Docking studies suggested that both lead triazoles including UI and UIA showed excellent binding affinity values especially UIA showed most stable ligand-target complex hence we propose UIA as an excellent inhibitor of Cytochrome P 450 dependent lanosterol 14 α-demethylase with compareable docking energy values with reference standard fluconazole. Rat acute toxicity prediction using GUSAR model suggested that both experimental molecules were nontoxic when computationally studied through IV, IP, SC and oral routes of administration. Docking outcomes were validated by performing *in-vitro* antifungal susceptibility testing of both UI and UIA. Antifungal

Table 1: Physicochemical properties/ADME prediction.

Physicochemical Properties	UI	UIA	Fluconazole
Molecular Weight	210.25	312.34	306.27
Log_p	1.0	2.59	0.5
Log_D	0.92	3.35	0.56
log_{Sw}	1.89	3.58	-2.07
Topological Polar Surface Area	76.90	83.52	81.65
Rotatable Bonds	1	3	5
Rigid Bonds	11	18	16
Flexibility	0.083	0.14	0.24
Hydrogen Bond Donor	4	2	1
Hydrogen Bond Acceptor	5	6	7
Rings	2	3	3
Max size ring	6	6	6
Num Charges	0	1	0
Total Charge	0	-1	0
Heavy atoms	14	22	22
Carbons Atoms	8	15	13
Hetero Atoms	6	7	9
Ration H/C	0.75	0.46	0.69
Lipinski Violations	0	0	0
Solubility mg/L	31681.67	8704.71	38565.40
Solubility Forecast Index;	Good	Reduced Solubility	Good Solubility
Oral_Bioavailability_VEBER	Good	Acceptable	Good
Oral_Bioavailability_EGAN;	Good	Acceptable	Good

Table 2: Rat acute toxicity values of selected ligand molecules using GUSAR.

Route of Administration	LD_{50} mg/kg		
	UI	UIA	Fluconazole
Intra-peritoneal	280,900	481,900	708,400
Intravenous	64,320	193,600	200,400
Oral	764,600	1,548,000	584,000
Subcutaneous	521,990	1,212,000	511,300
Super toxic (<5 mg/kg), extremely toxic (5-50 mg/kg), very toxic (50-500 mg/kg), moderately toxic (500-5000 mg/kg), slightly toxic (5000-15,000 mg/kg), and practically non-toxic (>15000 mg/kg) [30].			

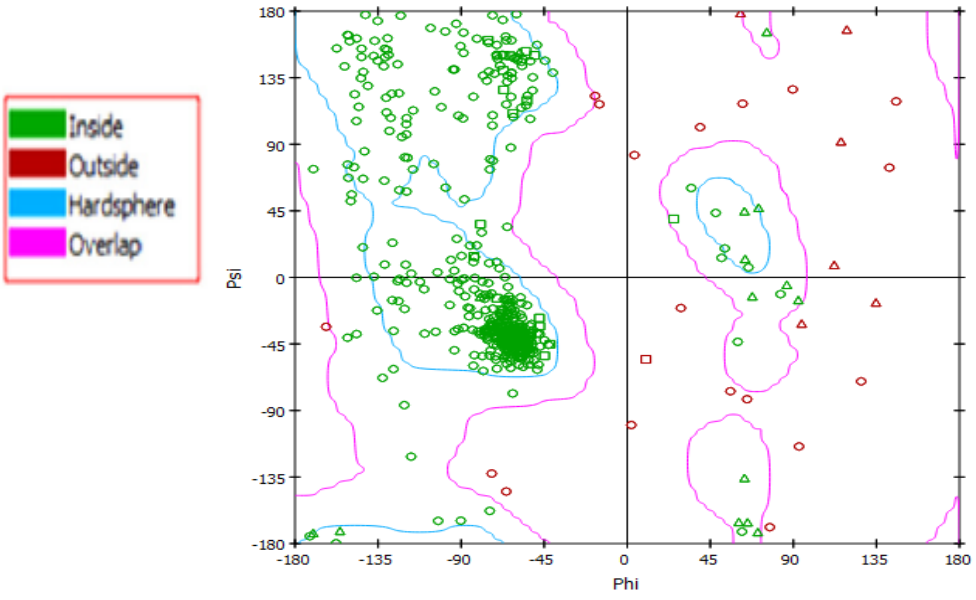

Figure 4: Ramachandran plot constructed by using Drug Discovery Studio 4.1 describing that whether amino acid residues reside in "accepted region" or "unaccepted" region. A better 3D protein modal should contain >90% amino acid residues in favored quadrant of Ramachandran Plot.

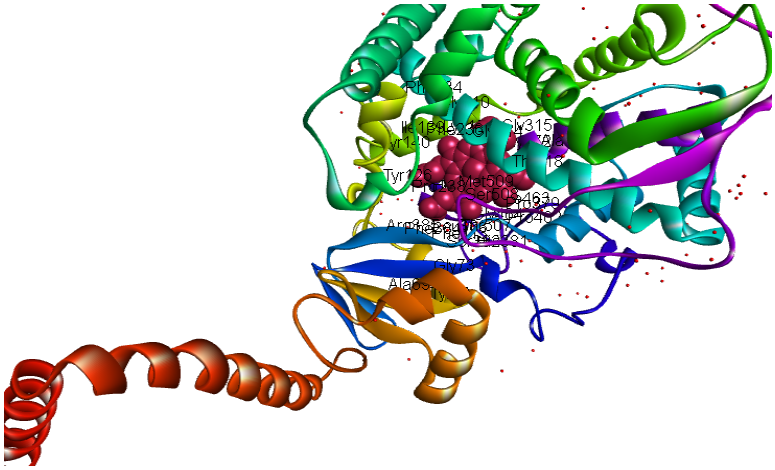

Figure 5: HIS, LEU, TYR amino acids highlighted in binding pocket of Cytochrome P 450 lanosterol 14 α-demethylase enzyme.

Table 3: Binding affinity energies and root Mean Square Deviation (RMSD) values obtained after docking run of ligands with target protein.

Ligand-Target Complex	Binding Affinity	RMSD/UB	RMSD/LB	Auto dock Elements
Q5AEK7_CANAL_U1	-6.8	0	0	A, NA, OA, N, SA, HD
Q5AEK7_CANAL_U1 Q5AEK7_CANAL_U1	-6.1 -6.1	1.399 2.308	1.338 1.818	
Q5AEK7_CANAL_U1	-5.6	2.026	1.371	
Q5AEK7_CANAL_U1A	-7.8	0	0 1.338	A, C, OA, N, SA, HD
Q5AEK7_CANAL_U1A Q5AEK7_CANAL_U1A	-7.0 -6.1	1.313 2.491	2.114 3.925	
Q5AEK7_CANAL_U1A	-6.1	3.383	0	
Q5AEK7_CANAL_Fluconazole	-7.1	0	2.722	A, C, F, OA, N, HD
Q5AEK7_CANAL_Fluconazole	-7.0	5.471	2.781	
Q5AEK7_CANAL_Fluconazole	-6.9	5.625	3.290	
Q5AEK7_CANAL_Fluconazole	-6.9	5.225		

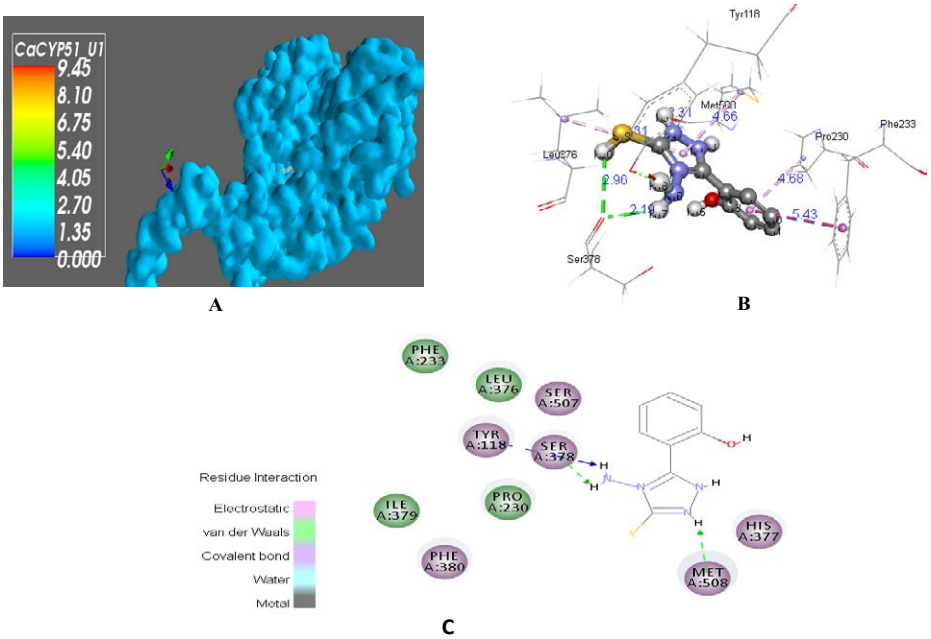

Figure 6: (A) UI entrapped in the binding pocket of yeast enzyme shown with three dimensional positions with heat map (B) Residual interaction of atoms of UI with serine and tyrosine amino acids of yeast enzyme in the binding pocket. OH group of ligand is interacting with amino acids at distance of 2.96 & 2.19 A°. (C) Two Dimensional interaction patterns of UI with target enzyme.

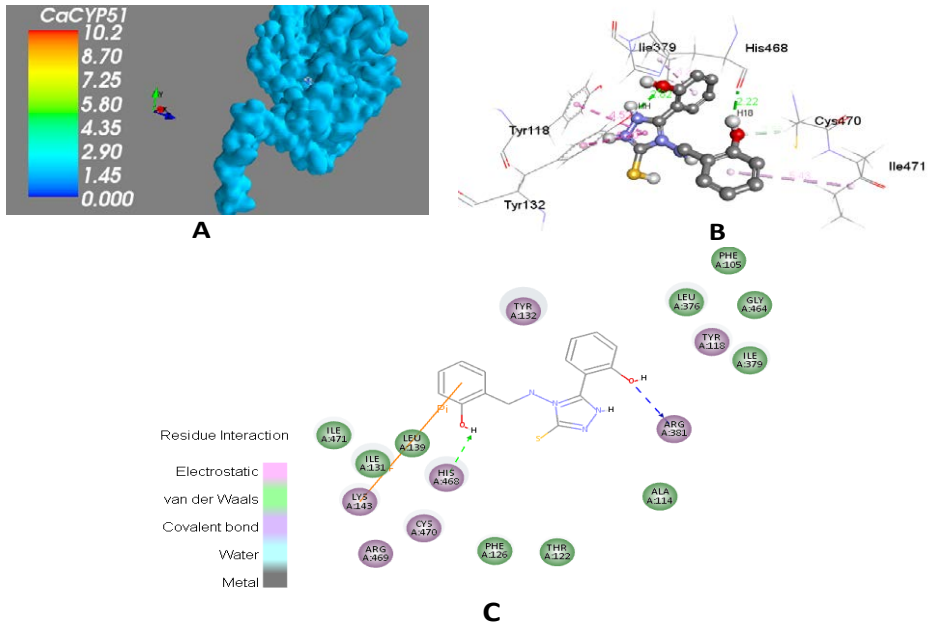

Figure 7: (A) UIA entrapped in the binding pocket of yeast enzyme shown with three dimensional positions with Heat Map (B) Residual interaction of atoms of UIA with serine and amino acid, Histine 468 of yeast enzyme in the binding pocket. OH group of ligand is interacting with amino acids at distance of 2.22 A°. (C) Two Dimensional interaction patterns of UIA with target enzyme.

Table 4: Minimum inhibitory concentration values.

Organisms	MIC Values (µg)		
	UI	UIA	Fluconazole
Candida albicans	42	24	5
Candida tropicalis	44	48	10
Candida glabrata	98	80	8

susceptibility testing revealed that all three pathogenic fungal strains were susceptible to both triazoles. UIA showed even promising MIC values compared with fluconazole against *Candida albican*. Furthermore, *Insilico* studies propose an outstanding framework to screen small molecules for their possible biological activities in dry lab in minimum cost and time.

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