World Journal of Pharmaceutical and Life Sciences
WJPLS
www.wjpls.org
SJIF Impact Factor: 4.223

INSILICO DESIGN AND MOLECULAR DOCKING STUDIES OF NOVEL 1, 2, 4- TRIAZOLE DERIVATIVES AS CYP-51 INHIBITORS

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Article Received on 06/12/2016 Article Revised on 26/12/2016 Article Accepted on 15/01/2017

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ABSTRACT

Molecular docking has become an increasingly important tool for drug discovery. It can be used to model the interaction between a small molecule and a protein at the atomic level, which allow us to characterize the behavior of small molecules in the binding site of target proteins as well as to elucidate fundamental biochemical processes. In the present investigation a new series of 1,2,4- triazole derivatives were designed as cytochrome P450 inhibitors based on docking studies and oral bioavailability scores based on Lipinski's rule evaluation. *Insilico* molecular docking was carried out using ArgusLab. To identify potential anti-fungal lead compounds among compounds 4a1-4a20, docking calculations were performed into the 3D structure of the catalytic site of CYP 51 enzyme (pdb code: 1EA1). Docking score of the novel compounds showed good fit against CYP 51 while compared with antifungal drug fluconazole.

KEYWORDS: 1,2,4-triazole, antifungal, Docking.

INTRODUCTION

Now days the growth of medicinal chemistry has reached a stage where the activity guided synthesis of compounds is possible rather than screening of synthesized compounds for different biological activity. The five membered triazole ring exists in two isomeric forms ie 1,2,3-triazole and 1,2,4-triazole. 1,2,4-triazole is one of a pair of isomeric chemical compounds with molecular formula $C_2H_3N_3$ called triazoles, which have a 5-membered ring

of two carbon atoms and three nitrogen atoms. 1,2,4-triazole is a basic aromatic heterocycle molecule. In the last few decades, several five membered heterocyclic compounds such as triazole derivatives have been studied extensively owing to their interesting biological activities such as antibacterial[1,2], antifungal[3], antiviral[4,5], anti convulsant[6], anti-oxidant[7], antitubercular[8], anticancer[9], anti-inflammatory and analgesic[10] activities. The development of antifungal agents has lagged behind that of antibacterial agents. Fungi, in contrast, are eukaryotes and consequently most agents toxic to fungi are also toxic to the host. This difficulty complicates experiments designed to evaluate the *in vitro* or *in vivo* properties of a potential antifungal agent. Invasive fungal infections are a major problem in immunocompromised patients.The recent expansion of antifungal drug research has occurred because there is a critical need for new anti fungal agents to treat these life threatening invasive infections. The overview of development of antifungal therapy which is provided here in reflects the increased interest in this very special area of infectious diseases. Azoles exert antifungal activity through inhibition of cytochrome P450 14α-demethylase (CYP51), which is crucial in the process of biosynthesis of ergosterol by a mechanism in which the heterocyclic nitrogen atom (N-4 of 1,2,4-triazole) binds to the heme iron atom. Selective inhibition of CYP51 would cause depletion of ergosterol and accumulation of lanosterol and other 14-methyl sterols resulting in the growth inhibition of fungal cells.[11]

Docking is a computational simulation of a candidate ligand binding to a receptor. In the field of molecular modeling, docking is a method which predicts the preferred orientation of one molecule to a second when bound to each other to form a stable complex. Knowledge of the preferred orientation in turn may be used to predict the strength of association or binding affinity between two molecules.[12] The present study aimed to develop molecules with improved antifungal activity. The possible effective molecules were designed by incorporating the triazole in to benzene derivatives. The objectives are to screen the 1,2,4-triazole derivatives by using Lipinski rule of five for oral bioavailability and carry out docking simulation by using ArgusLab and find out the derivative with higher docking scores. The present study aimed to develop molecules with improved antimicrobial activity.

MATERIALS AND METHODS

All the compounds were constructed using Chem Draw Ultra software, Cambridge Soft Corporation, USA. Version-12.0, 1997-2010. It is a Chem Tech tool used for the drawing of ligand molecules. The crystal structure of cyp 450 receptor used for docking was recovered

from the Brookhaven Protein Data Bank (http://www.rcsb.org/pdb/home) (entry code: 1EA1).

Docking study

Lead optimization

Lead optimization was done through insilico Lipinski filter. Molinspiration server was used for this purpose.[13] The structure drawn in the JME editor was subjected to calculate the druglikeness score through calculate the properties module. The datas are given in Table 2.

Input File Preparations for Energy Minimization of Protein

For each of the protein-ligand complexes chosen for the study, a "clean input file" was generated by removing water molecules, ions, ligands, and subunits not involved in ligand binding from the original structure file. Water molecules were removed because ArgusLab sometimes failed to dock the compounds having water molecules at their binding sites. All hydrogen atoms in the protein were allowed to optimize. The hydrogen locations are not specified by the X-ray structure but these are necessary to improve the hydrogen bond geometries, at the same time maintaining the protein conformation very close to that observed in the crystallographic model. The resulting receptor model was saved to a PDB file. Minimization was performed by geometry convergence function of ArgusLab software performed according to Hartree-Fock calculation method.

Ligand Input File Preparation and Optimization

Ligand input structure was drawn using Chem Draw software. The structure was cleaned in 3D format and energy was minimized. The resulting structure was then saved in "mdl mol" format for molecular docking studies.

Docking Methodology

After the preparation of the protein and ligand, molecular docking studies were performed by ArgusLab 4.0.1 to evaluate the interactions. The active site of protein was obtained from CASTp.[14]

ArgusLab 4.0.1

ArgusLab is implemented with shape based search algorithm. Docking has been done using "Argus Dock" exhaustive search docking function of ArgusLab with grid resolution of 0.40˚A. Docking precision was set to "Regular precision" and "Flexible" ligand docking

mode was employed for each docking run. The stability of each docked pose was evaluated using ArgusLab energy calculations and the number of hydrogen bonds formed.

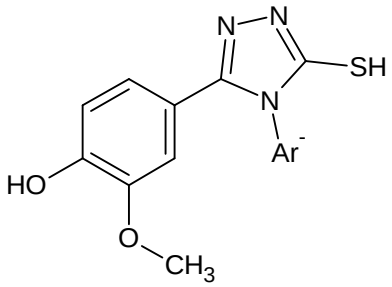
Molecular Docking Study

To perform docking one first needs to define atoms that make up the ligand and the binding sites of the protein where the ligand should bind. The prepared 3D structure of 1EA1 protein was downloaded into the ArgusLab program and binding sites were made by choosing "Make binding site for this protein" option. The ligand was then introduced and docking calculation was allowed to run using shape-based search algorithm and AScore scoring function. The scoring function is responsible for evaluating the energy between the ligand and the protein target. Flexible docking was allowed by constructing grids over the binding sites of the protein and energy-based rotation is set for that ligand's group of atoms that do not have rotatable bonds. For each rotation, torsions are created and poses (conformations) are generated during the docking process. For each complex 10 independent runs were conducted and one pose was returned for each run. The best docking model was selected according to the lowest AScore calculated by ArgusLab and the most suitable binding conformation was selected on the basis of hydrogen bond interactions between the ligand and protein near the substrate binding site. The lowest energy poses indicate the highest binding affinity as high energy produces the unstable conformations.[15]

RESULTS AND DISCUSSION

The least binding energy exhibits the highest activity which has been observed by the ranking of poses generated by AScore scoring function of ArgusLab and is given in Table 3.

Table 1: List of substituents used.



Sr. No	Compound code	Ar	Sr. No	Compound code	Ar
1	4a1	$C_7H_6N_2S$	11	4a11	$C_6H_6N_2O_2$
2	4a2	C_6H_7N	12	4a12	$C_6H_4F_2N_2O_2$
3	4a3	C_6H_6ClN	13	4a13	$C_6H_6N_2O_2$
4	4a4	$C_8H_{11}N$	14	4a14	C_7H_9N

5	4a5	$C_8H_{11}NO$	15	4a15	C_7H_9NO
6	4a6	$C_8H_{11}N$	16	4a16	$C_6H_5Cl_2N_2$
7	4a7	$C_{12}H_{11}N$	17	4a17	C_7H_8ClN
8	4a8	$C_9H_{13}N$	18	4a18	$C_6H_6N_2O$
9	4a9	C_6H_6ClN	19	4a19	C_6H_6ClN
10	4a10	C_6H_6BrN	20	4a20	C_6H_6BrN

Table 2: Lipinski Rule Analysis.

Sr.No	Compound code	Log p	H donor (nON)	H acceptor (nOHNH)	Mol. Wt	No of violation
1	4a1	3.88	6	1	356.43	0
2	4a2	2.90	5	1	299.36	0
3	4a3	3.58	5	1	333.80	0
4	4a4	4.13	5	1	327.41	0
5	4a5	3.76	6	1	343.41	0
6	4a6	3.94	5	1	327.41	0
7	4a7	4.70	5	1	375.45	0
8	4a8	4.53	5	1	341.44	0
9	4a9	3.74	5	1	333.80	0
10	4a10	3.71	5	1	378.25	0
11	4a11	3.02	8	1	344.35	0
12	4a12	3.47	8	1	380.33	0
13	4a13	2.86	8	1	344.35	0
14	4a14	3.35	5	1	313.38	0
15	4a15	2.96	6	1	329.38	0
16	4a16	4.37	5	1	368.25	0
17	4a17	4.38	5	1	347.83	0
18	4a18	1.13	8	2	343.37	0
19	4a19	3.77	5	1	333.80	0
20	4a20	3.90	5	1	378.25	0

Table 3: Binding energy of designed analogues.

Sr. No	Compound code	Binding energy (kcal/mol)	Sr. No	Compound code	Binding energy (kcal/mol)
1	4a1	-9.674	11	4a11	-8.769
2	4a2	-9.746	12	4a12	-8.677
3	4a3	-10.432	13	4a13	-8.732
4	4a4	-10.459	14	4a14	-10.404
5	4a5	-10.50	15	4a15	-8.860
6	4a6	-10.514	16	4a16	-10.628
7	4a7	-11.851	17	4a17	-10.590
8	4a8	-10.687	18	4a18	-8.463
9	4a9	-9.575	19	4a19	-10.799
10	4a10	-10.458	20	4a20	-9.845
Standard	fluconazole	-8.58			

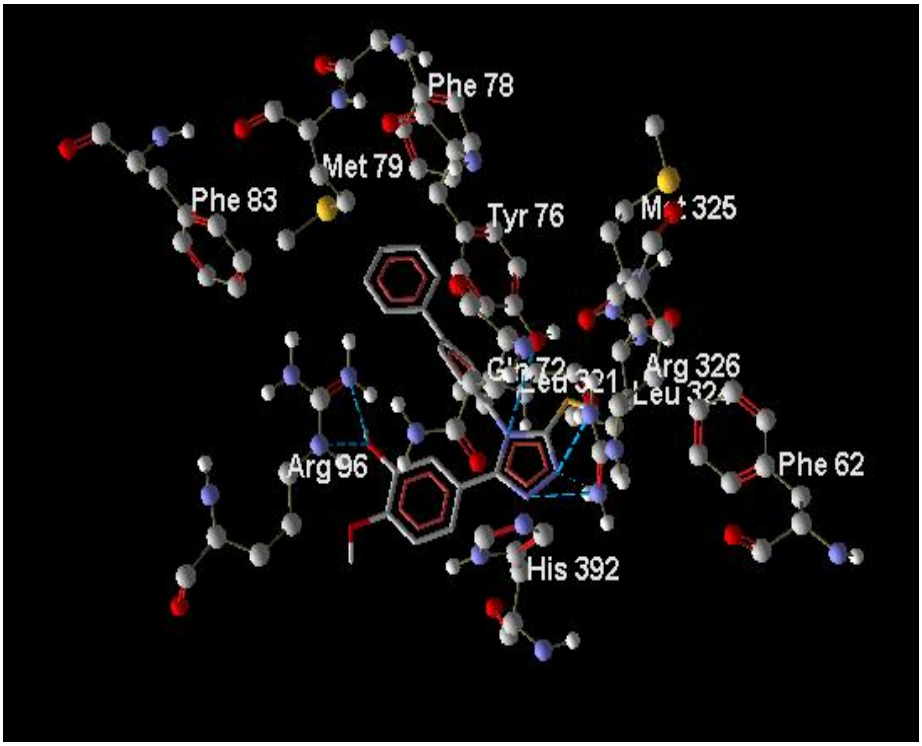


Figure 1: Hydrogen bond interaction of 4a7 with 1EA1.

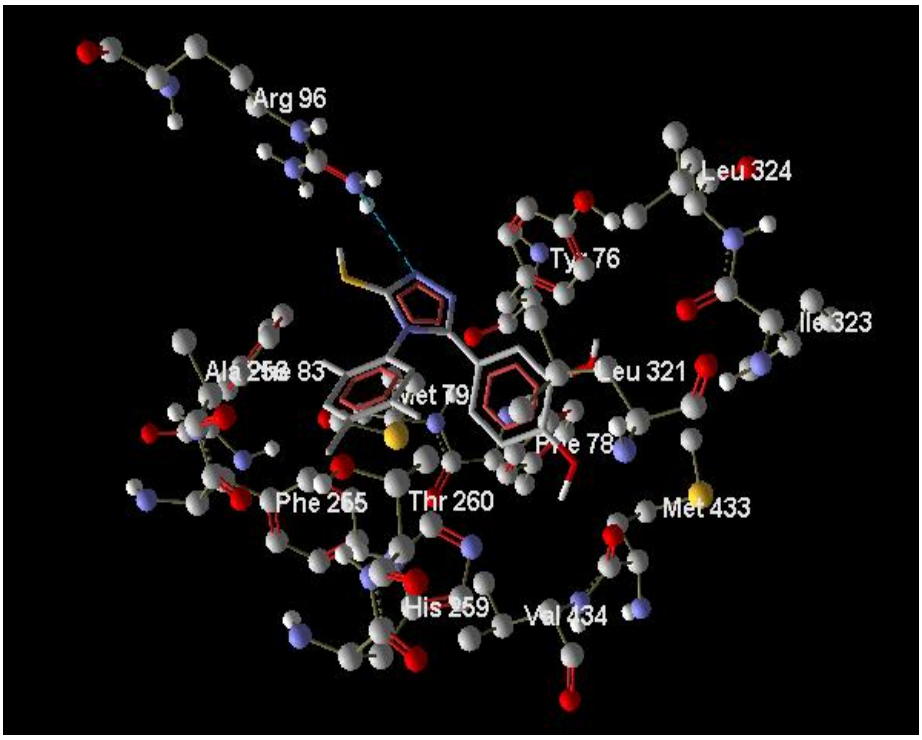


Figure 2: Hydrogen bond interaction of 4a8 with 1EA1

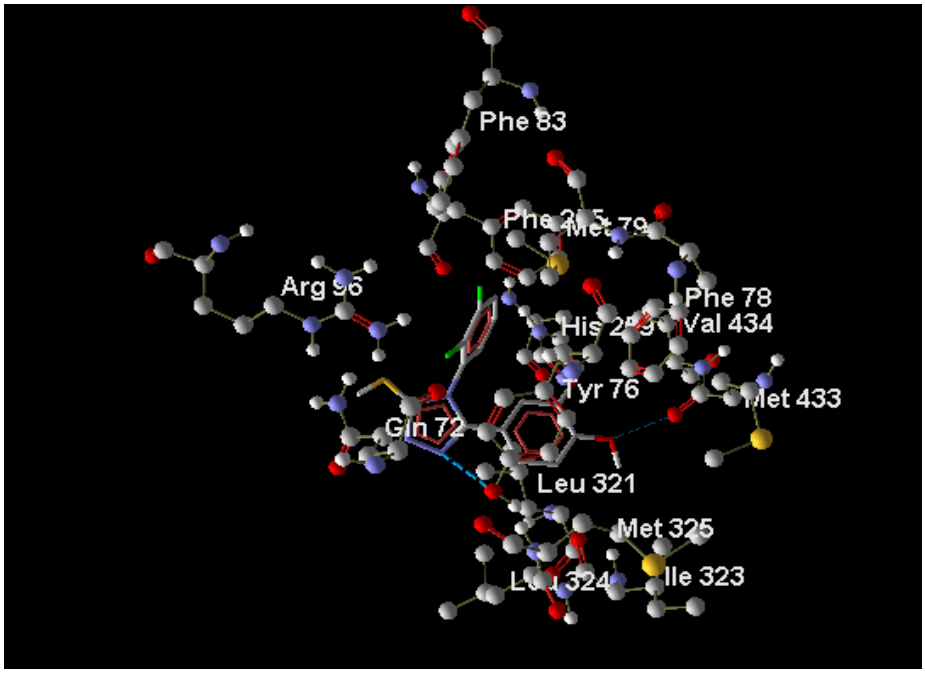


Figure 3: Hydrogen bond interaction of 4a16 with 1EA1.

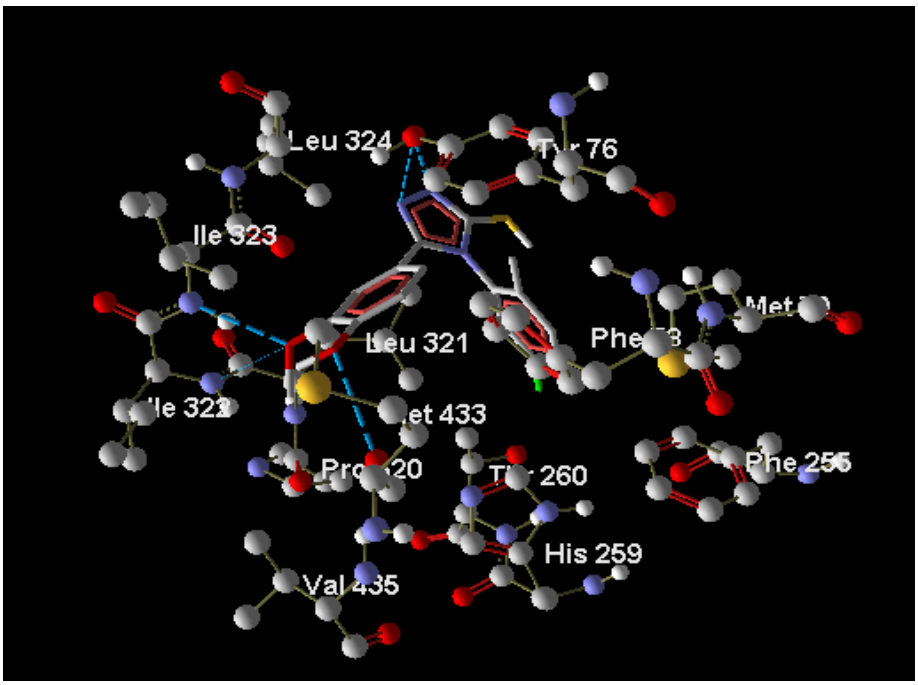


Figure 4: Hydrogen bond interaction of 4a17 with 1EA1.

CONCLUSION

Preliminary *in-silico* molecular modeling was carried out with the help of available softwares. All the proposed analogs obeyed Lipinski's Rule of Five. Docking studies were carried out on the proposed analogue to determine the affinity with the enzyme CYP 450 using Argus lab. The analogue 4a7 was found to have higher docking score and significant

binding interaction. Molecular docking studies shows that hydrogen bond interaction and hydrophobic interaction plays a crucial role in the biological activity of novel compounds. From the present study it can be concluded that the proposed triazole derivatives were found to possess good CYP 450 inhibition.

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