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DESIGN AND ANTIFUNGAL ACTIVITY OF NOVEL PYRIDYL TETRAZOLE DERIVATIVES AS CYP-51 INHIBITORS

Shiny George*[1] and P. Shanmugapandiyan[2]

[1]Department of Pharmaceutical Chemistry, Devaki Amma Memorial College of Pharmacy, Malappuram, Kerala- 673 634, India.

[2]Department of Pharmaceutical Chemistry, KVK College of Pharmacy, Surmaiguda, Hyderabad, India.

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***Correspondence for Author**
Shiny George
Department of Pharmaceutical Chemistry, Devaki Amma Memorial College of Pharmacy, Malappuram, Kerala, India.

ABSTRACT

Molecular docking is an important tool of molecular modelling system which provides the ligand-protein interaction through four force fields by orientation and translation. Docking is the identification of the low-energy binding modes of a small molecule or ligand, within the active site of a macromolecule or receptor whose structure is known. A new series of 2-(5-(substituted phenyl)-1H-tetrazol-1-yl) pyridine derivatives were designed as cytochrome P450 inhibitors based on docking studies and oral bioavailability scores based on Lipinski's rule evaluation. To identify potential anti-fungal lead compounds among compounds 5a1-5j2, docking calculations were performed using Autodock v3.0 into the 3D structure of the catalytic site of CYP 51 enzyme (pdb code: 1EA1). Autodock score of the novel compounds showed good fit against CYP 51. The compounds which showed good fit were synthesized and were screened for antifungal activity against *C. albicans* and *A. fumigatus*. Minimum Inhibitory Concentration was determined by broth dilution method and were in the range of 12.5-50 µg/ml.

Key Words: pyridine, tetrazole, antifungal, docking.

INTRODUCTION

The incidence of fungal infections has increased significantly in the past two decades [1]. The first generation of azoles antifungal inhibitors of CYP51, have revolutionized treatment of some serious fungal infections. Azole derivatives are currently the most widely studied class

of antifungal agents. Although we have newer less toxic antifungal agents that are available for clinical use, their clinical efficacy in some invasive fungal infections, is not optimal. Thus, intense efforts in antifungal drug discovery are still needed to develop more promising and effective antifungal agents for use in the clinical arena [2]. The cytocrome P450-dependent lanosterol 14α -demethylase (P450 14DM, CYP51) is the target of azoles. Azoles block ergosterol synthesis, and thereby fungal growth, by binding in the active site of the enzyme [3]. The azoles are potent inhibitors of 14α- demethylase. Tetrazole and its derivatives are reported show various biological activities like antibacterial[4,5], antifungal[6], anticonvulsant[7], analgesic[8], anti-inflammatory[9], antitubercular[10], anticancer[11], anti-hypertensive[12] and antidiabetic[13] activities. This study was performed through a preliminary computer modeling of drug/enzyme complexes in order to calculate relative free energies of association of these molecules to relate these values to experiment and to obtain mechanistic insight into binding modes and non covalent association. All these evidences can be of use in further experimental protein-ligand design.

This study was undertaken to design a new series of pyridyl tetrazole derivatives by docking in the active site of 14α-demethylase using Autodock program and evaluate the antifungal activity of the compounds on two species of fungi.

MATERIALS AND METHODS

The docking software used was autodock v3.0 and the lead optimization was done through mol inspiration server. The crystallographic structure of the enzyme CYP51 were obtained from the Brookhaven Protein Databank, accession number 1EA1. Missing atoms in the crystal structure were added and the structure was optimized.

Lead optimization

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

Molecular docking

The docking studies for all the derivatives were performed in the Autodock 3.0 version. The enzyme (PDB accession code: 1EA1) was refined by removing water molecules and polar hydrogens and kollmann charges were added. Energy minimized ligands in pdb were subjected to calculation of Gasteiger-Huckel charges. Grid box for docking simulations were

constructed with 60 points (with 0.375 A^{o} spacing) in x, y, and z direction to be centered in the active site using Autogrid utility of the Autodock programme. The enzyme ligand complex was subjected to 2.5 million evaluations. The binding energies are compared with the dock score of the standard (Table 3).

The proposed analogues were synthesized by cyclo addition method and reported earlier[14]. The synthesized pyridyl tetrazoles were subjected to antifungal activity.

***In vitro* antifungal susceptibility assay**

Minimum Inhibitory Concentration of compounds against *C. albicans* (NCIM 3471) and *A. fumigatus* (NCIM 902) was determined by broth microdilution testing[15]. Stock solutions were prepared in DMSO for fluconazole and the compounds. Serial twofold dilution of the compounds was made in RPMI1640 medium buffered to pH 7.0 with 0.165 M 4-morpholinepropanesulfonic acid (MOPS) buffer as outlined in NCCLS M27-A document [16]. Aliquots of 0.1 mL of each compound were dispensed into the wells of plastic microdilution microtiter plates so that the final concentration of solvent did not exceed 1% in any well. An inoculum of the organisms at 10^{6} CFU/mL (Colony Forming Unit/mL) concentration was prepared and 100 µL of the individual fungal inoculum was added to each well of the microtiter plate containing the reference drug or the compound. The plates were incubated at 25^{o}C for 72 hours. After the completion of incubation, the broth micro dilution wells were examined and the growth in each well was compared with that of the control. The MIC of each compound was defined as the lowest concentration that produced 80% inhibition in the growth of the organism compared with that of the control. All assays were performed in duplicate.

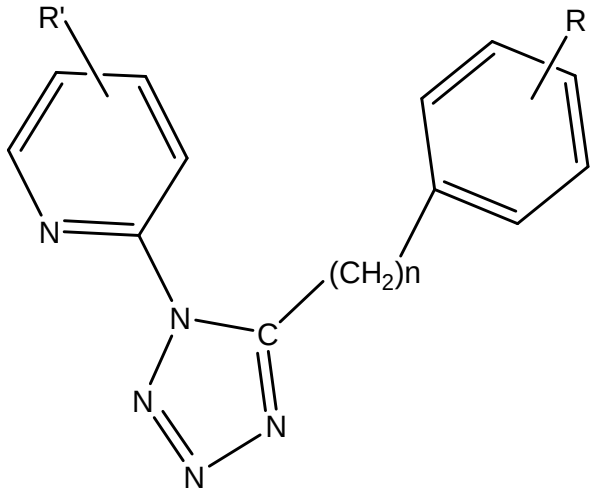

Table 1- Various substituents used for novel analogues

S. No	compound code	R'	R	n
1	5a1	H	H	0
2	5b1	H	4-NO_2	0
3	5c1	H	2-Cl	0
4	5d1	H	4-Cl	0
5	5e1	H	4-OCH_3	0
6	5f1	H	4-CH_3	0
7	5g1	H	3-Br	0
8	5h1	H	2,3-Cl	0
9	5i1	H	4-OH	0
10	5j1	H	H	1
11	5a2	5-Cl	H	0
12	5b2	5-Cl	4-NO_2	0
13	5c2	5-Cl	2-Cl	0
14	5d2	5-Cl	4-Cl	0
15	5e2	5-Cl	4-OCH_3	0
16	5f2	5-Cl	4-CH_3	0
17	5g2	5-Cl	3-Br	0
18	5h2	5-Cl	2,3-Cl	0
19	5i2	5-Cl	4-OH	0
20	5j2	5-Cl	H	1

Table 2 Molecular properties of novel derivatives

Compound	Log P	Mol. Wt	No of hydrogen Donors	No of hydrogen Acceptors	No of Violation
5a1	2.232	223.239	0	6	0
5b1	2.191	268.236	0	8	0
5c1	2.862	257.684	0	5	0
5d1	2.91	257.684	0	5	0
5e1	2.289	253.265	0	6	0
5f1	2.68	237.266	0	5	0
5g1	3.017	302.135	0	5	0
5h1	3.492	292.129	0	5	0
5i1	1.753	239.238	1	6	0
5j1	2.182	237.266	0	5	0
5a2	3.08	257.684	0	5	0
5b2	3.039	302.681	0	8	0
5c2	3.71	292.129	0	5	0
5d2	3.758	292.129	0	5	0
5e2	3.137	287.71	0	6	0
5f2	3.528	271.711	0	5	0

5g2	3.865	336.58	0	5	0
5h2	4.34	326.574	0	5	0
5i2	2.601	273.683	1	6	0
5j2	3.03	271.711	0	5	0

Table 3 Docking score of proposed analogues with 1EA1

Compound code	Binding Energy (Δ kcal/mol)	Inhibition constant
5a1	-8.68	532.05 nM
5b1	-9.53	104.38 nM
5c1	-9.13	112.05 nM
5d1	-5.28	133.39 µM
5e1	-5.58	81.92 µM
5f1	-5.01	214.25 µM
5g1	-5.81	54.93 µM
5h1	-8.76	379.46 nM
5i1	-7.25	4.87 µM
5j1	-7.40	3.75 µM
5a2	-8.37	732.99 nM
5b2	-6.52	16.60 µM
5c2	-11.46	3.99 nM
5d2	-8.54	548.05 nM
5e2	-7.30	4.47 µM
5f2	-9.94	51.37 nM
5g2	-11.06	7.77 nM
5h2	-8.95	275.72 nM
5i2	-9.65	84.47 nM
5j2	-11.07	7.65 nM
Fluconazole	-8.58	516.99 nM

Table 4 Antifungal activity of novel tetrazole derivatives

Cpd code	Minimum inhibitory concentration (in µg/ml)	
	A. fumigatus	*C. albicans*
5a1	50	25
5b1	25	12.5
5c1	50	50
5d1	25	50
5e1	25	25
5f1	50	12.5
5g1	25	12.5
5h1	50	25
5i1	50	50
5j1	50	12.5
5a2	50	25

5b2	25	25
5c2	12.5	25
5d2	12.5	25
5e2	50	50
5f2	50	50
5g2	50	25
5h2	25	12.5
5i2	12.5	12.5
5j2	50	25
Fluconazole	12.5	6.25

RESULTS AND DISCUSSION

The present work involves the preliminary *insilico* screening of various tetrazole analogues for quantifying their drug likeness using molinspiration software. Docking studies were carried out for a series of 20 tetrazole analogues for CYP 51 inhibition using Autodock v3.0. This study was very useful in deriving guidelines for the design of new inhibitors in this 1,5 disubstituted tetrazole series. The 100 independent docking runs which were carried out for each ligand generally converged to a small number of different positions ("cluster" of results differing by less than 2.0 ´ root mean square deviation (rmsd). The binding site for CYP51 inhibitors is located at residues PHE 63, GLN 72, ILE 323, TYR181, VAL 435 and the heme. As shown in Table 3, the calculated docking energy of compound 5c2 was -11.46 kcal/mol and the calculated Ki of compound 5c2 was 3.99×10^{-9}, which means that compound bound tightly to the CYP51 compared to Fluconazole (dock score= -8.58 kcal/mol, Ki = 516.99×10^{-9}). The methoxy group on the phenyl ring of compound 5e2 forms a hydrogen bond with the protonated nitrogen of VAL 435 with a bond length of 2.899Å (Figure 2).

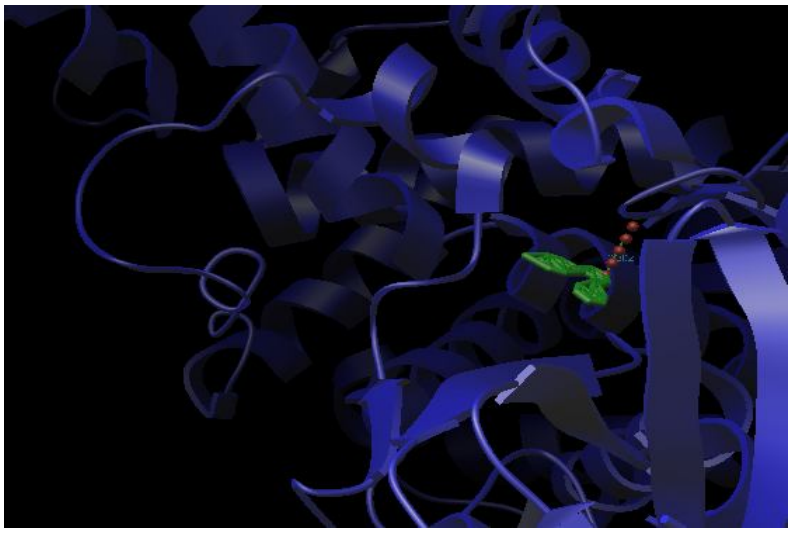

Figure 1 Hydrogen bonding interaction of 5i1 with VAL 435 of 1EA1

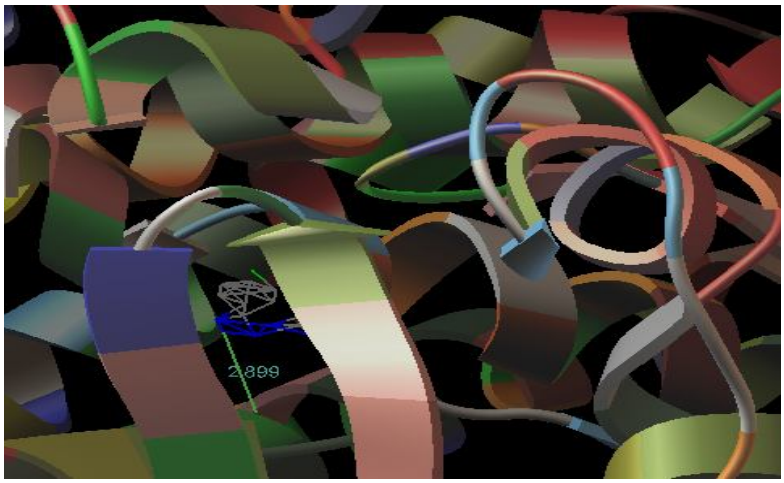

Figure 2 Hydrogen bonding interaction of 5e2 with VAL 435 of 1EA1

The antifungal activity of the compounds was tested against *C. albicans* and *Aspergillus fumigatus*. The minimum inhibitory concentration (MIC) was carried out using micro dilution susceptibility method**.** Fluconazole was used as a standard antifungal drug. The MIC values were determined as the lowest concentration that completely inhibited visible growth of the microorganisms. The investigation of antifungal screening (Table 4) revealed that some of the newly synthesized compounds showed moderate-to-good inhibition at 12.5–50 µg/mL in DMSO. Among the tested compounds 5b1, 5f1, 5g1,5j1, 5h2 and **5**i2 were found to be more active than other compounds against *C. albicans* (MIC: 12.5µg/mL). Compounds 5c2, 5d2 and 5i2 possess good activity against *A. fumigatus* (MIC: 12.5 µg/mL).

CONCLUSION

The present study has given an insight into the development of new CYP 51 inhibitors. Various *insilco* tools like Lipinski filter and molecular docking has been utilized to conclude the relevance in synthesizing the leads. Thus the *insilico* design has been helpful in synthesizing only promising molecules enabling the minimization of time spend for searching leads. We reported here a group of azoles acting as antifungal agents. The compounds exhibited higher and broader spectrum antifungal activities against tested fungi.

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