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RESEARCH ARTICLE

Crystal Structure Determination and Molecular Docking Studies of 4-(5-Phenyl Pyrazin-2-Yl)-4h-1,2,4 Triazole-3-Thiol with Focal Adhesion Kinase Inhibitors

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Abstract: The main objective of the present work is to determine crystal structure of the ligand by x-ray methods and to perform molecular docking studies of the ligand 4- Phenyl -5-Pyrazinyl-3-mercapto 1,2,4 Triazole with protein focal adhesion kinase (FAK) domain using the software, Autodock and pymol. Macromolecular modeling by docking studies provides the most detailed view possible of drug receptor interaction. It has created a new rational approach to drug design, where the structure of drug is designed, based on its fit to three dimensional structures of receptor site, rather than basing it on analogies to other active structures. The above titled compound is binding with FAK protein. This may act as inhibitor to FAK and can be used for anticancer therapy target.

Keywords: Anticancer therapy target, Docking, Focal adhesion kinase, 1,2,4 Triazole.

INTRODUCTION

1,2,4 Triazole is one of the organic heterocyclic compounds containing a five membered di-unsaturated ring structure compound with three nitrogen atoms and two carbon atoms at nonadjacent positions. Although most triazoles are readily prepared and stored. 1,2,4-Triazole is a basic aromatic heterocycles and one of a pair of isomeric chemical compounds with molecular formula $C_2H_3N_3$. This compound is screened for its anti cancerous, antibacterial and anti-microbial activity [1 - 3]. The molecular docking studies of 4- Phenyl -5-Pyrazinyl-3-mercapto 1,2,4 triazole with protein FAK [4] were performed by using Autodock. Docking studies have become nearly indispensable for the study of macromolecular structures and interactions.

MATERIALS AND METHODS

Database and Software

Data collection and cell refinement: **CAD-4 software**; Data reduction: **MOLEN,** Structure solution and refinement: **SHELX97,** Molecular graphics: **ORTEP and PLATON** Material for publication: **SHELX97**, program used for docking: **Autodock** [5] and **Pymol**, File format conversion of the coordinates: **openbabel.**

PREPARATION OF LIGAND STRUCTURE

The crystals of the compound 4- Phenyl -5-Pyrazinyl-3-mercapto 1,2,4 triazole were grown by the slow evaporation

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technique using ethanol as solvent. The x-ray intensity data of the crystals was collected on a Bruker smart CCD diffractometer graphite monochromatic Mok**α** radiation. From the diffraction data,it was seen that the title molecule $C_{12}H_9N_5S$ crystallizes in monoclinic space group $P2_1/c$ with unit cell dimensions, a=9.630(4) Å b=15.023(6) Å c=9.022 (4) Å and Z=4. The ortep diagram [6, 7] of the ligand showing 50% probability displacement ellipsoids is shown in Fig. (**1**). The crystal data of the compound are shown in Table **1**.

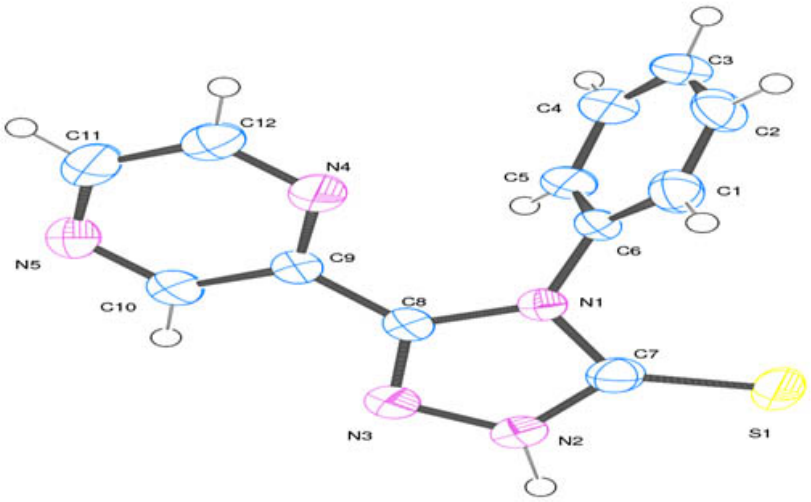

Fig. (1). Ortep diagram of 4- Phenyl -5-Pyrazinyl-3-mercapto 1,2,4 triazole.

Table 1. Crystal data.

Identification and CCDC code	**PYT3 1016195**
Empirical formula	C12 H9 N5 S
Formula weight	255.30
Temperature	295.15 K
Wavelength	0.71073 Å
Crystal system	Monoclinic
Space group	$P2_1/c$
Unit cell dimensions	a= 9.630(4)Å α=90 b=15.023(6) Å β=115.321 c=9.022(4)Å γ=90
Volume	1179.8(8)A^3
Z	4
Calculated density	1.437Mg/m^3
Absorption coefficient	0.262mm^{-1}
F (000)	528.0
Crystal size	0.25 × 0.15 × 0.12
Theta range for data collection	4.68 to 51deg
Limiting indices	-11 ≤ h ≤ 11, -18 ≤ k ≤ 18, -10 ≤ l ≤ 10
Reflections collected / unique	11408/2192 [R(int)=0.0157]
Completeness to theta	92.2%
Absorption correction	none
Refinement method	Full matrix least squares on F^2
Data / restraints / parameters	2192/0/164
Goodness-of-fit on F^2	1.070
Final R indices [I>2sigma(I)]	R_1 = 0.0612, wR_2 = 0.1105
R indices (all data)	R_1 = 0.1035, wR_2 = 0.1216
Absolute structure parameter	0.8(2)
Extinction coefficient	0.0414 (3)
Largest diff. peak and hole	0.19/-0.21 e $Å^{-3}$

PREPARATION OF PROTEIN STRUCTURE

The 3D structure of FAK (PDB code: 2ETM) [8] was downloaded from the protein data bank. By adding the polar hydrogen bond to the receptor FAK which is required to convert PDB to PDBQT format. The water molecules around the ligand and cofactors were removed from the protein.

PROTEIN LIGAND INTERACTION

Docking allows the user to virtually screen a database of compounds and predict the strongest binders based on various scoring functions. The docking analysis of the ligand 4-Phenyl-5-pyrazinyl-3-mercapto-1,2,4 triazole (PYT3) and their analogs with focal adhesion kinase receptor was carried by using Autodock 4.2 and FlexX docking software. The reference ligand 7PY (7-Pyridin-2-Yl-N-(3,4,5-Trimethoxyphenyl)-7h-Pyrrolo[2,3-D] pyrimidin-2-Amine) was selected for the binding site analysis [9, 10]. Docking of the protein ligand complex was mainly targeted at the predicted active site. The interaction was carried out to find the favorable binding geometries of the ligand with the protein. The selected residues of the receptor were defined to be part to the binding site. The molecules binding to a receptor, ideally must inhibit its function, and thus act as drug [11]. The collection of 1,2,4 triazole derivatives and receptor complexes was identified *via* docking. Molecular docking was carried out to find the favorable binding geometries of the ligand with the protein. 1,2,4 triazole derivatives were then docked at predicted active site of FAK, using Autodock4.2 (Lemarkian Genetic Algorithm) and Flexx (Incremental Docking Algorithm). The pose received with the highest dock score from Flexx as well as lowest binding free energy from Autodock4 was then selected for further analysis [12]. The best binding pose at the binding site is shown in Figs. (**2**-**4**). Table **2** shows different binding energies for different conformations of the ligand.

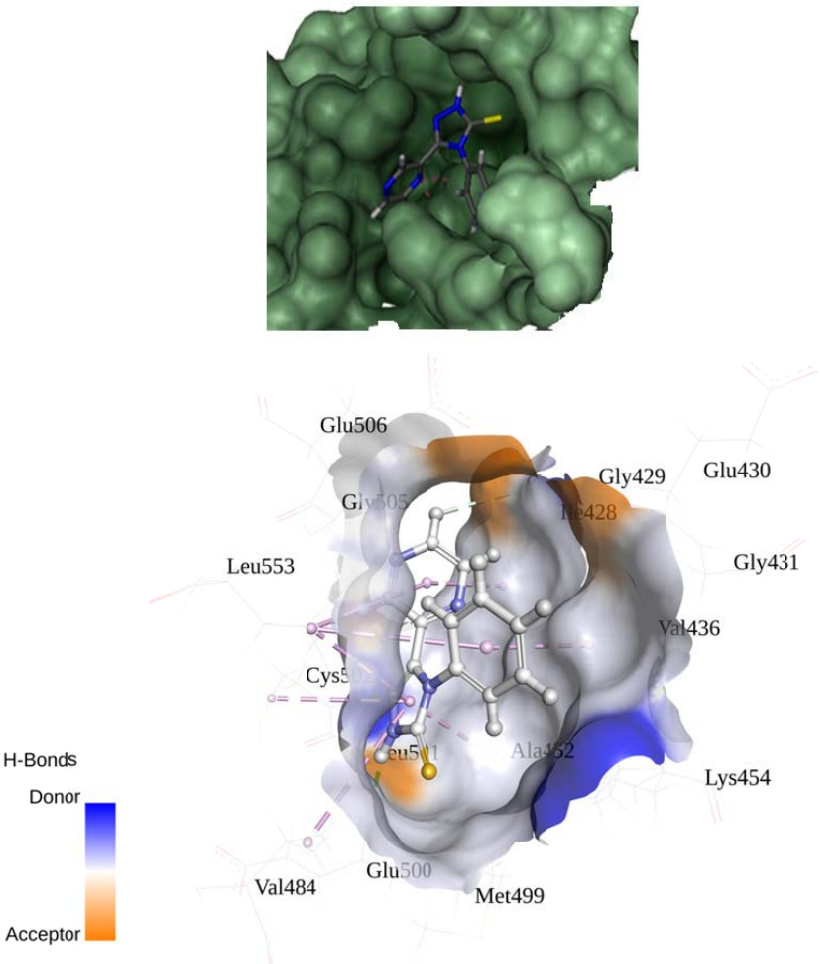

Fig. (2). Binding pose of ligand in binding site using flex.

Table 2. Best docking pose energy for ligand PyT3 with AutoDock.

Pose fitnees	Binding energy Kcal/mol	Intermol energy	Inhibition constant µM	Interacting residues of amino acids
1	-5.7	-6.29	66.68	LEU503,GLY505, CYS502, LEU501, GLU500, ALA452 VAL436
2	-5.69	-6.28	68.02	
3	-5.65	-6.24	72.45	
4	-5.62	-6.22	75.42	
5	-5.6	-6.2	78.35	
6	-5.15	-5.74	168.94	
7	-5.06	-5.66	193.97	
8	-5	-5.6	214.69	
9	-4.83	-5.43	287.10	
10	-4.32	-4.92	676.75	

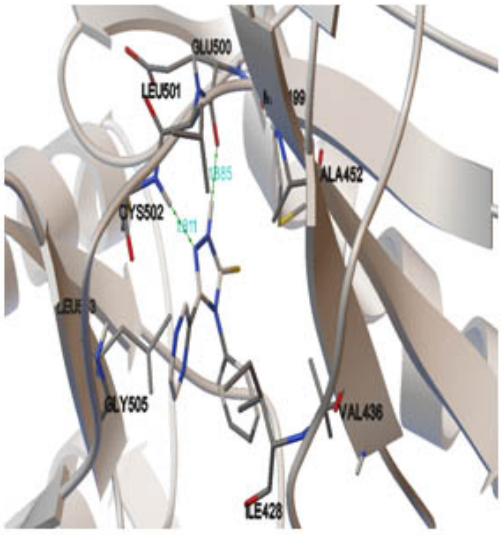

Fig. (3). Binding pose of ligand in binding site using Autodock.

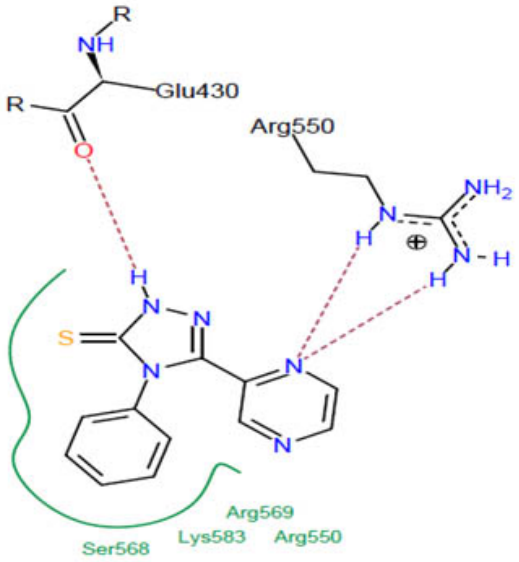

Fig. (4). The pharmacophore of the protein interacting with the ligand; residues forming hydrogen bonding.

RESULTS AND DISCUSSION

The docking result showed the best 10 binding sites. The best dock energy -5.7 kcal/mol was campared with the reference ligand (7PY) and the docked energy was -5.87 kcal/mol, which showed that the protein ligand interactions are good fit with the target protein. The molecular docking studies shows good affinity of the ligand towards the protein. The higher the negative value of the energy of binding, the better is the affinity of the molecule to the receptor. The intermolecular interactions between the ligand and the protein were investigated. 1,2,4 Triazole for the most part, subsists in the 1H-1,2,4 triazole tautomeric form and the 4H-1,2,4 triazole form does not exist nor in solid state neither in solution form. In general amino substituted 1,2,4 triazoles were electronically preferred in a tautomeric equilibrium dominantly in 1H form [13]. In the crystal lattice there were two intermolecular interactions, one potentially weak intramolecular and some C-π....π super molecular interactions [14]. In the solid state, supermolecular interactions stabilize the crystal structures.

CONCLUSION

The intermolecular interactions between the ligand and the protein were investigated. The synthesized chemical compound showed a good fit with the protein. Thus the bioactive compound interacting with the target can be used as a potent inhibitor to block the action of FAK protein. The docked value can be considered for further analysis.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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