Characterization of single crystal by x-ray diffraction analysis and docking studies of 2-Mercapto-5-(3-Methoxyphenyl) 1, 3, 4 oxadiazole Thiones with focal adhesion kinase

K.S.Kiran[1, 2], M.K.Kokila[3], Guruprasad. R[4]

[1] Department of physics, Bangalore University, Bangalore, Karnataka, India
[2] Department of physics, School of Engineering and Technology, Jain University, Bangalore, India
[3] Department of physics, Bangalore University, Bangalore, Karnataka, India
[4] Scientific Director, Durga Femto Technologies & Research, Bangalore, Karnataka, India

Email:kiranxrd@gmail.com

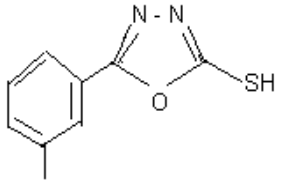
Abstract: **The main objective of the present work is to determine crystal structure of the ligand by x-ray methods and to perform molecular docking studies of the ligand 2-Mercapto-5-(3-methoxyphenyl) 1,3,4 oxadiazoles with protein focal kinase domain using software, autodock and pymol. A good correlation was observed in binding affinity of the ligand against the protein. The above titled receptor posses anticancer activity. Using different inhibitors for this enzyme can be used as an anticancer therapy target.**
Keywords-1,3,4 oxadiazole, docking, kinase, anticancer therapy target.

I. INTRODUCTION

Oxadiazole is a five membered heterocyclic aromatic compound.[1] Out of its four possible isomers, 1,3,4-oxadiazole is widely exploited for various applications[2]. These are azoles with oxygen and nitrogen containing derivatives play an important role in medicinal chemistry and pharmaceutical industries.1,3,4-oxadiazole is widely being exhibit biological activities like, anticancerous[3], antibacterial, anticonvulsant[4], fungicidal, anti-inflammatory[5], antimicrobial analgesic, antipyretic, anti-tubercular, and antisecretory properties[6,7].The lead compound has been synthesized by incorporating substituents at 2^{nd} and 5^{th}position of the 1,3,4-oxadiazole heterocyclic ring system. Since it is clear from various literatures that these derivatives possess remarkable inhibitor for cancer activity.[8,9]The molecular docking studies of 2-Mercapto-5-(3-Methoxyphenyl) with protein focal adhesion kinase (FAK) was performed by using Autodock. Docking studies have become nearly indispensable for study of macromolecular structures and interactions. Macromolecular modeling by docking studies provides most detailed possible view of drug receptor interaction and has created a new rational approach to drug design.

II. EXPERIMENTAL

In a clean dried 250ml round bottom flask Ethanol (80ml) and potassium hydroxide (1.12g, 0.02mol)(dissolved in 4ml of water) were taken. To this 3-Methoxy benzoic acid hyrazide (3.89g, 0.02mol) was added. Then to the above clear solution carbon disulfide (15.2g, 12ml and 0.4mol) was added and refluxed for 4 hours. A solid appears initially which dissolved on heating. Then 40ml of ethanol was distilled off and cooled to room temperature. The contents were poured onto water (approx20ml) and acidified with ice-cold concentration (Hcl, 2ml, 0.02mol). The white coloured compound is filtered and washed with ice cold water. The resulting compound is the 2-Mercapto-5-(3-methoxyphenyl)1,3,4-oxadiazole was recrystallized from aqueous ethanol.



2-Mercapto-5-(3methoxy phenyl) 1, 3, 4-oxadiazoles

III. MATERIALS AND METHODS

A. *Crystallization*

Crystallization is the creation of a dynamic equilibrium between the particles in fluid phase and solid phase from saturated solutions. To produce crystals of high quality for small molecules the procedure of Slow Evaporation is valuable and simplest method. In this method the compound is dissolved in a suitable solvent to get a saturated and clear solution. Slow warming is necessary if the compound is less soluble. If the single solvent solutions do not yield diffracting-quality crystals mixtures of solvents in particular ratio by volume may be useful. The solution is allowed to evaporate in a vibration free environment. The duration of crystallization takes either few days or few months to get crystals from the saturated solution depending upon the compound.

B. *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** and **Pymol**, File format conversion of the coordinates: **openbabel.**

C. *Preparation of ligand structure*

The crystals of the compound 2-Mercapto-5-(3-methoxyphenyl) 1, 3, 4-oxadiazole **[**10,11**]** were grown by slow evaporation technique using ethanol as solvent. The x-ray intensity data of the crystals were collected on a Bruker smart CCD diffractometer on graphite monochromatic Mok**α** radiation. The title molecule C9H8O2N2S crystallizes in orthorhombic space group Pn21a with unit cell dimensions, a=9.4447(18) Å b=6.7023(13) Å c=15.260 (3) Å and Z=4. The ortep diagram of the ligand showing 50% probability displacement ellipsoids is shown in figure 1. The atomic coordinates and equivalent displacement parameters are shown in table1.The hydrogen coordinates with isotropic displacement parameters shown in table 2.

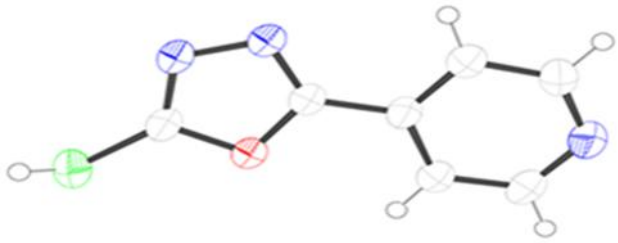


Figure1.Ortep diagram of 2-Mercapto-5-(3-Methoxyphenyl) 1,3,4 oxadiazole

TABLE1. Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters (A^2 x 10^3) of 2-Mercapto-5-(3-Methoxyphenyl) 1,3,4 oxadiazole. U (eq) is defined as one third of the trace of the orthogonal Zed Uij tensor.

Atoms	X	Y	Z	U(eq)
S(13)	3858 (1)	2422 (4)	6910 (1)	70 (1)
C(8)	3779(2)	2382(9)	9395(1)	42 (1)
O(9)	4509(2)	2333(7)	8619(1)	46 (1)
C(10)	3502(2)	2277(10)	7967(1)	45 (1)
O(1)	8144(2)	2383(9)	11141(1)	61 (1)
C(6)	6012(2)	2390(8)	10233(1)	40 (1)
N(11)	2273(2)	2413(9)	8384(1)	50 (1)
C(1)	4542(2)	2450(8)	10224(1)	42 (1)
C(5)	6713(2)	2361(10)	11035(1)	45 (1)
C(4)	5934(3)	2440(12)	11811(1)	58 (1)

N(12)	2428(2)	2392(7)	9282(1)	48 (1)
C(7)	9010(2)	2429(15)	10383(2)	65 (1)
C(3)	4479(3)	2557(12)	11789(2)	66 (1)
C(2)	3774(3)	2301(15)	11000(2)	58 (1)

TABLE 2.Hydrogen coordinates (x 10^4) and isotropic displacement parameters (A^2x 10^3) for 2-Mercapto-5-(3-Methoxyphenyl) 1,3,4 oxadiazole

H atoms	X	Y	Z	U (eq)
H(4)	6560(30)	2200(60)	9745(18)	50 (8)
H(1)	6400(30)	2680(70)	12386(17)	50 (9)
H(3)	2820(30)	2850(40)	10964(14)	30 (7)
H(2)	3900(20)	1950(50)	12354(19)	42 (9)
H(15)	4400(200)	1900(800)	7040(120)	750 (180)
H(11A)	9988	2414	10554	97
H(11B)	8816	3622	10055	97
H(11C)	8811	1284	10026	97

IV PREPARATION OF PROTEIN STRUCTURE

The 3D structure of FAK (PDB code: 2ETM) [12] was downloaded from protein data bank. The protein showed anticancer activity from the literature [13].By adding the polar hydrogen bond to the receptor FAK which is required to convert PDB to PDBQT format. The water molecules around ligand and cofactors were removed from the protein.

A .Protein ligand interaction using Auto dock version 1.5.6

Auto dock is a structural program which predicts the potential energies, molecular structures and geometry optimization of structure of atoms. FAK was docked against the 2-Mercapto-5-(3-methoxyphenyl) 1, 3, 4-oxadiazole using auto dock version 1.5.6. The interaction was carried out to find the favorable binding geometries of the ligand with the protein. Docking of the protein ligand complex was mainly targeted to the predicted active site. Protein- ligand interaction plays a significant role in structural based drug designing. The grid option with required area was selected to the receptor for docking programme to look for binding sites. Rotatable bond of the ligand were confirmed by using torsion tree and saved. The ligand was docked with the target protein, and the best docking energy were identified. The binding pose of ligand in binding site can be generated using pymol software.

V RESULTS AND DISCUSSION

The designed series of 1,3,4 oxadiazole were docked to the FAK protein with autodock software. Docking result shows the top 9 binding energy terms docked to the protein as shown in Table 3. Docked energy of -6.9 Kcal/mol with two hydrogen bond shown in figure2. There was a change in the ligand structure after binding to the receptor shown in figure 3. To understand pharmacological data on structural basis, we evaluate the designed compounds through docking technique. The molecular studies resulted in highlighting the ligands and their conformations which efficiently fit into the cavity of target protein. The higher the negative value of the energy of binding the better is affinity of the molecule to the receptor. The crystal structure have been refined to a final R=0.0544 for the 7720 observed reflections. The structure is stabilized by intermolecular C-H….S, C-H…N hydrogen bonds and intermolecular C-H…π interactions.

TABLE 3. Top 9 binding energy

mode	Affinity Kcal/mol	Distance from best mode Rmsd 1.b	Rmsdu.b
1	-6.9	0.000	0.000
2	-6.1	10.248	11.720
3	-5.9	41.250	42.451
4	-5.8	10.395	11.707
5	-5.6	42.690	44.150
6	-5.4	39.773	40.474
7	-5.3	41.298	42.345
8	-5.1	35.296	38.085
9	-5.0	12.605	13.837

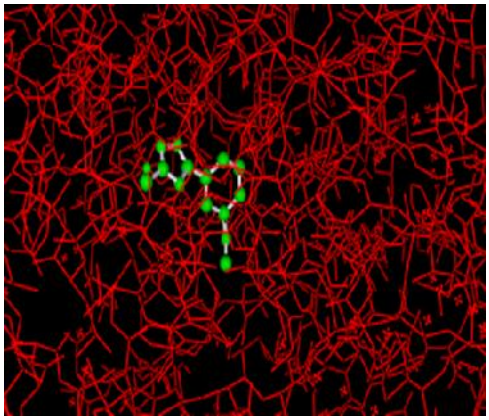


Figure 2. Binding pose of 1, 3,4oxadiazole in binding site

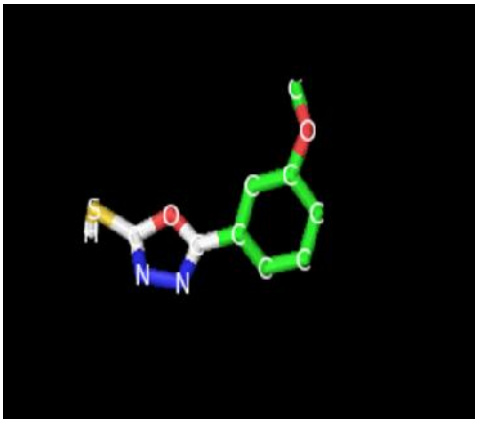


Figure 3. Ligand structure after binding to the receptor

VI CONCLUSION

The ligand synthesized for this study is considered as orally safe compound. The intermolecular interactions between the ligand and the protein were investigated. Synthesized chemical compound showed 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 selected compound which showed a maximum dock value were taken out for wet laboratory validations and made into an effective anticancer drug.

ACKNOWLEDGMENT

The authors are thankful to Department of Physics, Bangalore University and Durga Femto Technologies & Research, Bangalore. The authors would like to thank Department of Basic Sciences, SET, Director, Jain University for their valuable support and providing all necessary facilities to carry out this study.

REFERENCES

1. Elderfield RC, Hetrocyclic compounds, 5th edition Newyork;John wiley and sons, Inc, 1961, 525-39
2. C.Anisworth, Journal American chemical society, 87, 5800,1965
3. Singh Amarika, Mishra Upama, Proc. Indian Natl sci accad. Part A 573,1991
4. Hutt MP, Elslager EF, Werbel LM, Journal Hetrocyclic chem.. 7, 511, 1970
5. Ladva K, Patel P, Upadhyay P, Parekh H, Indian Journal Chem, 3513, 1062, 1996
6. RS.Bachav, V.S.Gulecha, &C.D.Upasani. Analgesic and anti-inflammatoryz activity of Argyreiaspeciosa root "Indian journal of pharmacology", 2009, 41,158-160.
7. Kucukguzel SG, Oruc EE, Rollas S, Sahin F, Ozbek A. synthesis characterization and biological acitivity of novel 1,3,4 oxadiazoles and some related compounds, European Journal of medicinal chemistry 2002.37, 197-206
8. SayanduttaGuptha, Harinarayanamoorthy, utpalsanyal.synthesis of cytotoxicevaluation, Insilico pharmacokinetic and QSAR study of benzotiazole derivatives.Journal of pharmacy and pharmaceutical sciences; 2010:3;57-62
9. Rand HP, Dale MM Ritter MM, Moore PK. Pharmocology 5th Edition. Newyork: 2003:51-63
10. Niti Bhardwaj, Saraf, Pankaj Sharma SK, Pradeepkumar, Synthesis, Evaluation and characterization of some 1,3,4 oxadiazoles as antimicrobial agents, European Journal of chemistry, 2009;6,4, 1133-38
11. Asif Husian, Mohammed Ajmal, synthesis of Novel 1,3,4 oxadiazole derivatives and their biological properties, Acta Pharma 2009;59;223-33
12. Andersen, C.B., Ng, K., Vu, C., Ficarro, S., Choi, H.-S., Gray, N., He, Y., Lee, C.C. Crystal Structure of Focal Adhesion Kinase Domain Complexed with ATP and novel 7H- Pyrrolo [2,3-d] pyrimidine scaffolds Journal to be Published.
13. Golubovskaya VM1, Kweh FA, Cance WG.focal adhesion kinase and cancer. Histol Histopathol. 2009 Apr;24(4):503-10.