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Der Pharma Chemica, 2015, 7(9):268-273
(http://derpharmachemica.com/archive.html)

ISSN 0975-413X
CODEN (USA): PCHHAX

Crystal and molecular docking studies of 3-[Bis-(2-hydroxy-4,4-dimethyl-6-oxo-cyclohex-1-enyl)-methyl]benzonitrile with focal adhesion kinase inhibitors

K. S. Kiran[1,2*], M. K. Kokila[1], Guruprasad R.[3], Prashantha Karunakar[4], M. A. Pasha[5], Lokanath N.[6] and Naveen S.[7]

[1]*Department of Physics, Bangalore University, Bangalore, Karnataka, India*
[2]*Department of Physics, School of Engineering and Technology, Jain University, Bangalore, Karnataka, India*
[3]*Durga Femto Technologies & Research, Bangalore, Karnataka, India*
[4]*Department of Biotechnology, PES University, Bangalore, Karnataka, India*
[5]*Department of Chemistry, Central college campus, Bangalore University, Bangalore, Karnataka, India*
[6]*DOS in Physics, Manasagangothri, Mysore University, Mysore, Karnataka, India*
[7]*Centre for Excellence, Vigana Kendra, Manasagangotri, Mysore, Karnataka, India*

ABSTRACT

In the present study crystal structure of 3-[Bis-(2-hydroxy-4,4-dimethyl-6-oxo-cyclohex-1-enyl)-methyl]benzonitrile was determined using single crystal X-ray diffraction. Further the structural features was extrapolated to molecular docking studies with focal adhesion kinase (FAK) domain using Autodock to study its anticancerous property. The compound exhibited considerable bacterial inhibition of lower to moderate concentrations. We conclude that these derivatives can be used in medicine and have enormous potential as pharmaceutical agents due to their biological activities. The above titled receptor gain functional and structural insights into their mechanism of inhibition and explore its potential as an anticancer agent.

Keywords: Bis cyclohexyl diols, Docking, Focal adhesion kinase, anticancer therapy target.

INTRODUCTION

Cyclohexane is a non planar molecule and the shape of which is vaguely resembles a chair. The conformation of cyclohexane molecule is constantly changing with the atom on the left, which is currently pointing down, flipping up and the one on the right flipping down. During the process, another (slightly less stable) form of cyclohexane is formed known as the "boat" form [1,2]. In this arrangement, both of these atoms are either pointing up or down at the same time. It is a cyclic alkane that melts at 6°C and boils at 81°C. It is nearly insoluble in water. Cyclohexane is found naturally to some extent in petroleum, but is prepared commercially by catalytic hydrogenation of benzene. It is widely used as a solvent and in making certain compounds used in the preparation of nylon. The biological importance of cyclohexane has resulted in the synthesis of substituted derivatives, which show potent pharmaceutical activities like antimicrobial, antidiabetic, anticancer antipsychotic, anesthetic, expectorant activities [3-5]. Focal adhesion kinase is a cytoplasmic non-receptor protein tyrosine kinase, which was isolated for the first time by co-immunoprecipitation of tyrosine-phosphorylated proteins from cells transformed with Rous sarcoma virus v-Src. Non-receptor protein-tyrosine kinase (PTK2/FAK1) that plays an essential role in regulating cell migration, adhesion, spreading, reorganization of the actin cytoskeleton, formation and disassembly of focal adhesions and cell protrusions, cell cycle progression, cell proliferation and apoptosis. It is required for early embryonic and placenta development. It also required for embryonic angiogenesis, normal cardiomyocyte migration and proliferation, and normal heart development. It regulates axon growth and neuronal cell migration, axon branching and synapse formation; required for normal development of the nervous system. It Plays a role in osteogenesis and differentiation of osteoblasts. It helps in function of integrin signal transduction, but also in

signaling downstream of numerous growth factor receptors, G-protein coupled receptors (GPCR), EPHA2, netrin receptors and LDL receptors. The aberrant PTK2/FAK1 expression may play a role in cancer cell proliferation, migration and invasion, in tumor formation and metastasis. PTK2/FAK1 overexpression is seen in many types of cancer[6,7].

MATERIALS AND METHODS

For crystallographic analysis data collection and cell refinement was done using CAD-4, Data reduction using MOLEN, Structure solution and refinement using SHELX97[8], Molecular graphics using ORTEP [9] & PLATON[10], Material for publication using SHELX97 and for protein-ligand interaction using Autodock software. File format conversion of the coordinates using openbabel [11].

Crystallization of KCH1

The crystals of the compound 3-[Bis-(2-hydroxy-4,4-dimethyl-6-oxo-cyclohex-1-enyl)-methyl]benzonitrile (KCH1) was 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 Mokα radiation.

Protein-Ligand Interaction

The docking analysis of KCH1 with Crystal Structure of Focal Adhesion Kinase Domain Complexed with 7H-Pyrrolo [2,3-d] pyrimidine Derivative (PDB:2ETM) was carried using Autodock 4.2 [12]. The reference ligand 7PY (7-Pyridin-2-Yl-N-(3,4,5-Trimethoxyphenyl)-7h-Pyrrolo[2,3-D]pyrimidin-2-Amine) was selected for binding site analysis and respective amino acids are considered for gridbox construction. The docking site for KCH1 on 2ETM was defined at the position of the co-crystallized ligand 7PY by using PyRX 0.8 interface with grid box size of 56 x 55 x 56, spacing of 0.375, grid centre -1.220, 11.661 and 6.401 and assigning all possible “Degrees of Freedom” to all ligands. From the estimated free energy of ligand binding (ΔG), the inhibition constant (Ki) for each ligand was calculated Docking of the protein ligand complex was mainly targeted to 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 of the binding site. The molecules binding to a receptor, ideally must inhibit its function, and thus act as a drug. The collection of bis cyclohexyl diols derivatives and receptor complexes were identified via docking. The scoring functions with their parameters were read from the score obtained. The entire series of ligands in the data set was docked into the active site of FAK protein, using the same protocol. The docking poses were saved for the ligand and were ranked according to their score function. The pose possessing the highest dock score was selected for further analysis. PyMOL and Ligplot^{+} were used for docking conformation representation [13,14]. Only the best pose (the one with the lowest binding energy) was considered as a potential ligand.

RESULTS AND DISCUSSION

The molecule, $C_{24}H_{27}NO_4$ crystallizes in monoclinic space group $P2_1/c$ with unit cell dimensions, a=11.8048(13)Å, b=11.46769(13)Å, c=16.52539(19)Å and Z=4. The ORTEP diagram of the ligand showing 50% probability displacement ellipsoids is shown in figure 1. The crystal data of the compound are shown in table1.The bond lengths and bond angles are generally within the normal range. The cyclohexenone ring A (C6/C5/C4/C1/C2/C3) and the benzene ring B (C22-C27) are not planar. The cyclohexene ring constitutes a part of the tetra benzonitrile fused ring. The bond length O8-C13 is 1.26Å and C1-C2 is 1.38Å for the both cyclohexene ring. A part of the benzonitrile fused ring system has a flattened half-chair conformation that approximates an envelope conformation (in which the methylene C atom bearing the dimethyl substituent represents the flap) as five of the six atoms lie on a plane[15,16]. The mean plane of the cyclohexene ring with the hydroxyl substituent is approximately perpendicular to the mean plane of the tetra benzonitrile system. Adjacent molecules are linked by an O-H...O hydrogen bond to form a chain running along the b-axis of the monoclinic unit cell. The dihedral angle between the benzonitrile ring and cyclohexene ring is 70.8°. Relatively strong intramolecular O-H…O hydrogen bonds are observed. In the crystal, molecules are linked by C-H…O hydrogen bonds, forming a chain along the c-axis direction. There is an intramolecular hydroxy–ketone O-H…O interaction between the two substituted cyclohexane rings as well as a short intramolecular phenol–methoxy O-H…O interaction.The packing diagram C-H…O and O-H…O interactions is shown in Fig 2 [17].

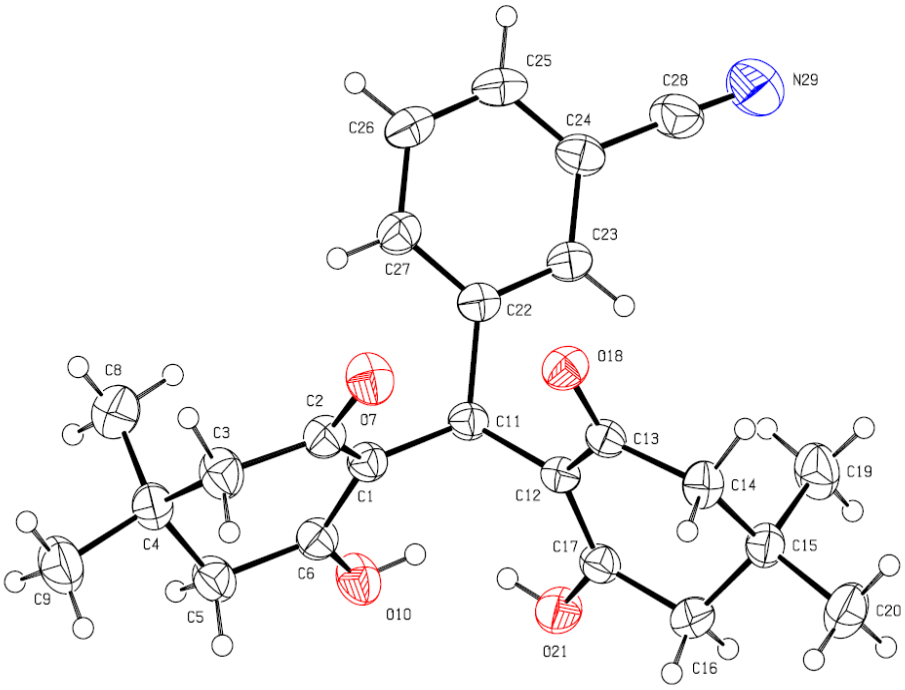

Fig1: Ortep diagram of 3-[Bis-(2-hydroxy-4,4-dimethyl-6-oxo-cyclohex-1-enyl)-methyl]benzonitrile

Table1: Crystal data

Identification and CCDC code	**KCH1 1016197**
Empirical formula	C_{24} H_{27} N O_4
Formula weight	393.46
Temperature	298(2) K
Wavelength	1.54 Å
Crystal system	monoclinic
Space group	$P2_1/c$
Unit cell dimensions	a=11.8048(13) Å α=90^0 b=11.46769(13) Å β=108.46^0 c=16.52539(19) Å γ=90^0
Volume	2121.9(4) A^3
Z	4
Calculated density	1.232Mg/m^3
Absorption coefficient	0.672mm^-1
F (000)	840
Crystal size	0.4 x0.35x0.2mm
Theta ranges for data collection	9.556 to 58.708 deg
Limiting indices	$-13 \le h \le 13$, $-5 \le k \le 13$, $-19 \le l \le 18$
Reflections collected / unique	13517/3489 [R(int)=0.0290]
Completeness to theta	98.2%
Absorption correction	None
Refinement method	Full matrix least squares on F^2
Data / restraints / parameters	3489/0/274
Goodness-of-fit on F^2	1.035
Final R indices [I>2sigma(I)]	R1=0.0379, wR2=0.0972
R indices (all data)	R1=0.0421 wR2=0.1005
Absolute structure parameter	0.8(2)
Extinction coefficient	0.0018 (16)
Largest diff. peak and hole	0.14 and -0.13 e A^-3

The *in silico* interaction of 7-pyridin-2-yl-n-(3,4,5-trimethoxyphenyl)-7h-pyrrolo[2,3-d]pyrimidin-2-amine (7PY) to 2ETM showed the least binding energy of -6.42kcal/mol. From the PDBsum, it is observed that 7PY forms two hydrogen bonds with CYS502 with bond length of 3.11Å and 2.91Å (Image not showed). The conformation with least binding energy and most stability based on cluster analysis was taken for docking analysis. In the Autodock predicted interaction, it shows the interaction as hydrophobic and due to this the inhibition constant value increased

from 0.212µM to 19.68 µM. Since Autodock calculates the inhibition constant from the binding energy and both 7PY and KCH1 has the same energy, we observe nearly the same IC50 values and may act by competitive inhibition. The interacting residues in both 7PY and KCH1 are almost conserved. KCH1 shows a hydrophobic interaction as showed in Figure 3 and Figure 4. The docking interaction details are represented in Table2. Since the synthesized chemical compound showed good fit with the protein, the bioactive compound may be used as a potent inhibitor to block the action of FAK protein.

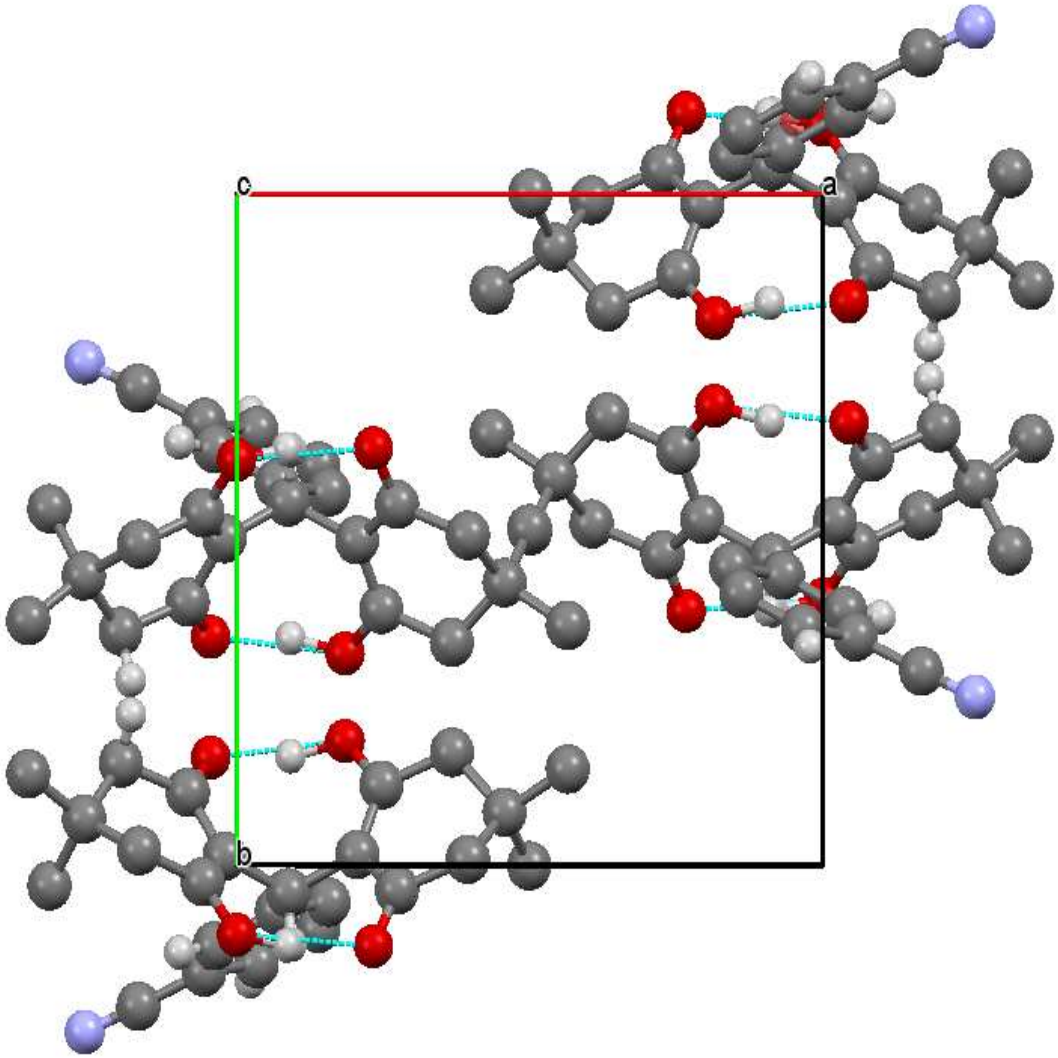

Fig.2 Packing diagram showing C – H … O and O --H…O interactions indicated as dashed lines. H atoms not involved in hydrogen bonding have been omitted

Table2: Docking interaction details of KCH1 and 7PY against FAK protein

Compound	Binding energy Kcal/mol	Intermol energy	Inhibition constant µM	Interacting residues of amino acids
7PY	-6.42	-7.91	19.68	ILE428, GLY429, VAL436, ALA452, GLU500, LEU501, CYS502, GLY505, LEU553
KCH1	-6.42	-7.91	19.76	ILE428, GLY429, VAL436, ALA452, GLU500, LEU501, CYS502, GLY505

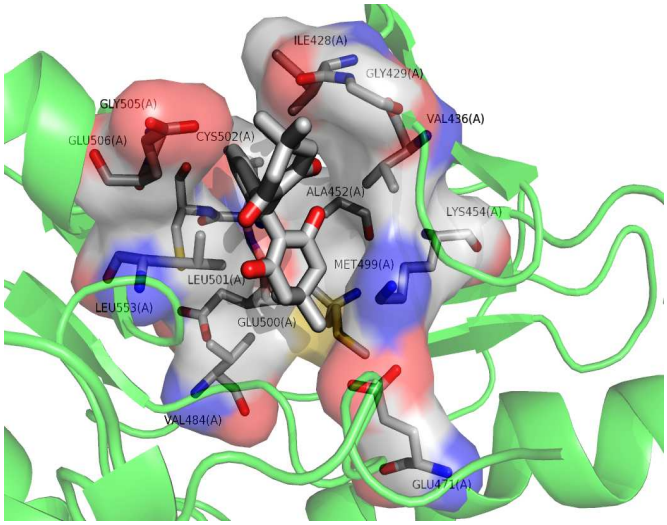

Fig3: Interaction of KCH1 with 2ETM

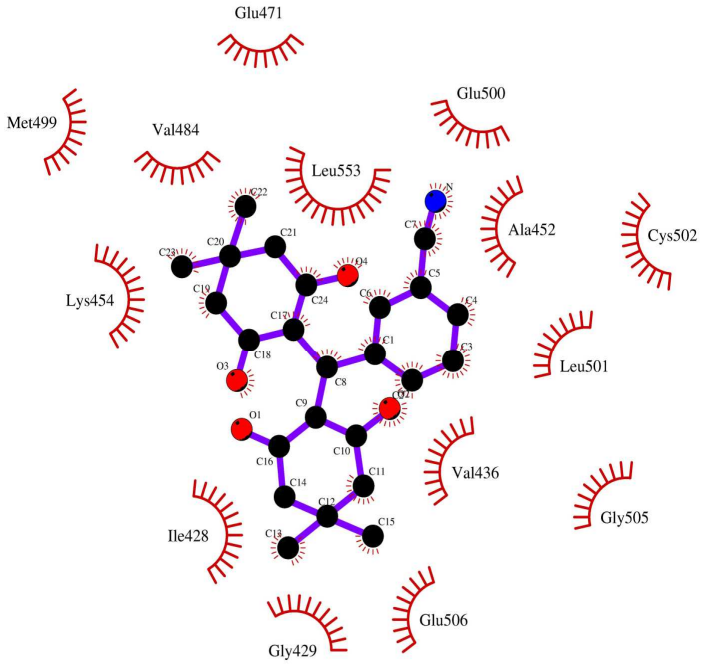

Fig4: Interaction of KCH1 in the active site of FAK protein

CONCLUSION

In the present study, topological analysis of 3-[Bis-(2-hydroxy-4,4-dimethyl-6-oxo-cyclohex-1-enyl)-methyl]benzonitrile is performed using crystallographic method. The results were extrapolated to molecular docking analysis to investigate the structure–activity relationship between the inhibitory features of KCH1 ligand. From the AutoDock result, it can be concluded that the bioactive compound KCH1 can be used as a potent inhibitor to block the action of FAK protein when it is over expressed. Further, the bioactive compound will be analyzed by molecular dynamics studies and then *in vivo* studies for detailed investigations. Further co-crystallization of protein-ligand complex and animal trials followed by biopharmaceutical scale up feasibilities could be encouraged.

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