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Molecular Docking Studies of p21-Activated Kinase-1 (PAK1) Inhibitors

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Abstract

The p21-activated kinase-1 (PAK1) has emerged as a potential target for anticancer therapy. It is overexpressed in ovarian, breast and bladder cancers. This suggests that PAK1 may contribute to tumorigenesis. 4-azaindole derivatives are reported as potent PAK1 inhibitors. The present work deals with the molecular docking studies of 4-azaindoles with PAK1. Probable binding mode of these inhibitors has been identified by molecular modeling. Docking results indicated that hydrogen bonding interactions with Glu345 and Leu347 are responsible for governing inhibitor potency of the compounds. Additionally, Val284, Val328, Met344 and Leu396 were found to be accountable for hydrophobic interactions inside the active site of PAK1

Keywords: PAK1, 4-azaindole, Cancer, Docking

1. Introduction

The p21-activated kinases (PAKs) are members of a family of serine/threonine protein kinases that function as downstream nodes for various oncogenic signaling pathways. They are stimulated by activated forms of the small GTPases, Cdc42 and Rac[1]. Paks are regulators of cytoskeletal remodeling and cell motility. They also promote cell proliferation, regulate apoptosis and accelerate mitotic abnormalities, which results in tumor formation[2]. PAK family comprised of six isoforms which are categorized into two subgroups based on architectural similarities. PAK1-3 belong to group I while PAK4-6 are the part of group II[3]. Paks are overexpressed and/or hyperactivated in numerous human tumors and their role in cell transformation makes them promising therapeutic targets[4]. PAK1 plays an important role in controlling cell motility by linking a variety of extracellular signals to changes in actin cytoskeleton organization, cell shape, and adhesion dynamics. It is involved in fundamental cellular processes beyond that of regulating the cytoskeleton, including regulation of apoptosis or programmed cell death. Overexpression of PAK1 in ovarian, breast and bladder cancers indicate that PAK1 may contribute to tumorigenesis. Consequently, PAK1 has emerged as an attractive target for anticancer therapy[5].

Determination of the binding mode and affinity between the ligand and receptor is crucial in understanding the interaction mechanisms and designing therapeutic molecules. 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. The main aim of the docking technique is to accurately predict the bioactive conformation of a ligand at the active site of the receptor and to correctly estimate the strength of binding. This technique explores several possible interactions in order to determine a set of ligand poses that represent local minimum-energy positions of the ligand. The calculated binding energy can be used to rank-order different ligands. This *in silico* approach provides useful information for proposing effective receptor inhibitors.

Our research group focuses on the application of computational techniques and reported several studies[6-10]. Recently, Lee *et al*. reported a series of 4-azaindole-containing PAK1 inhibitors[11]. The current study is to predict the binding mode of these inhibitors and to explore the binding interaction in the active site of PAK1. Binding mechanism was analyzed using molecular docking studies. Most potent compound **8** (K_i=4 nM)

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and least active compound **4** (K_i=360 nM) of this series were selected as representatives for the docking studies. Chemical structures of these selected compounds are shown in Table 1. These docking analyses could lead to the further development of novel and more potent PAK1 inhibitors for the treatment of cancer.

2. Methodology

2.1. Ligand Structure Preparation

The chemical structures of azaindole-containing PAK1 inhibitors were constructed using Sybyl software[12] and saved in Protein Data Bank (PDB) file format. These compounds were prepared for molecular docking by merging non-polar hydrogens, assigning Gasteiger charges, defining the rotatable bonds, assigning AutoDock type to each atom and finally saving them in PDBQT file format using AutoDock Tools (ADT)[13].

2.2. Protein Structure Preparation

Crystal structure of PAK1 (PDB code: 4O0R, 2.4 Å resolution) was obtained from the RCSB PDB (http://www.rcsb.org/pdb)[14]. Co-crystallized ligand, chain B, water molecules and ions were removed from the structure using PyMOL. Polar hydrogen atoms were added, Gasteiger charges were assigned and finally protein structure was saved in PDBQT file format using ADT. This structure was employed in docking studies.

2.3. Molecular Docking

Molecular docking studies were performed with the AutoDock[15]. All the rotatable bonds of ligands were considered as rotatable while protein was rigid during docking. AutoGrid program was used to define the search grid and generate grid maps. A grid box of 40 ×40×40 $Å^3$ dimension with 0.375 Å spacing was created and centered on co-crystallized ligand of the crystal structure. The docking parameter file and map files were created using ADT. Grid and docking parameter files were used later by AutoDock for running the docking simulations. Lamarckian Genetic Algorithm (LGA)[16] with default parameters was employed for docking. Hundred independent docking runs were carried out with population size of 150 individuals, 2.5 million energy evaluations, maximum of 27000 generations, number of top individuals to automatically survive to next generation of 1, mutation rate of 0.02 and crossover rate of 0.8. ADT provide various methods to analyze the results of docking simulations such as conformational similarity, visualizing the binding site and its energy, and other parameters like intermolecular energy and inhibition constant. The docked poses were analyzed on the basis of scoring functions and protein-ligand interactions. The selected pose was saved in PDBQT format and then converted to PDB file format using Python Molecular Viewer (PMV). The protein-ligand interactions were plotted using PyMOL. Molecular docking was performed on PC with Microsoft Windows 7 Home Premium version 2009, Intel(R) Core (TM)i5CPU (3.10 GHz) operating system.

3. Results and Discussion

Docking studies were carried out using AutoDock in order to gain insights into the most probable binding mode of the 4-azaindole-containing PAK1 inhibitors. Before running docking simulations, we evaluated our docking protocol for its reproducibility by re-docking the co-crystallized ligand of 4O0R. It can be seen in Fig. 1 that crystal and re-docked conformation of ligand occupied the same binding site. The docked pose of the co-crystallized ligand showed root mean square deviation (RMSD) value of 0.47 Å with co-crystal conformation. Moreover all the interactions made by co-crystal are also reproduced in the docked pose. This validated the docking protocol and indicated the reliability of the procedure in generating the accurate poses.

Same protocol was used to dock most potent compound **8** and least active compound **4** in the active site of PAK1. Docked poses of both the representative compounds were selected on the basis of the docking score and interactions with the active site residues. Both compounds were docked in a cavity lined by Ile276, Gly277, Gln278, Gly279, Ala280, Ser281, Gly282, Val284, Ala297, Val328, Met344, Glu345, Tyr346, Leu347, Ala348, Gly350, Leu396 and Thr406. Docking results showed similar binding mode for both the compounds. This observation was in accordance with the previous study[11]. As shown in Table 1, most potent compound **8** exhibited higher binding energy of -7.10 kcal/mol as compared to least potent compound **4** which demonstrated binding energy of -5.74 kcal/mol. Compound **8** displayed four hydrogen bonds in the binding

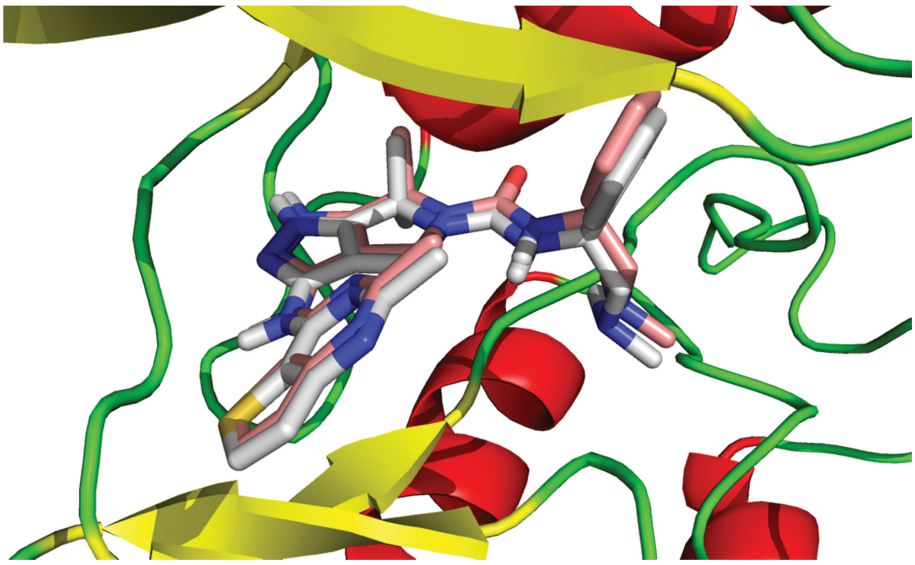
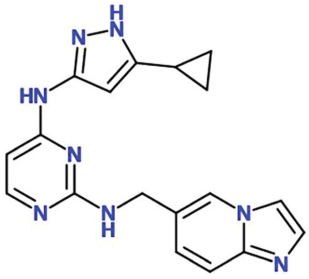
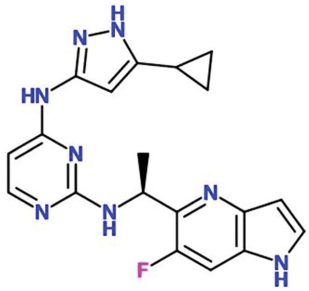


Fig. 1. Conformational comparison of co-crystallized ligand (gray) from X-ray crystal structure of PAK1 and from docking result (wheat). PLK2 is displayed as ribbon model and ligands are shown as stick models.

Table 1. Chemical structures and docking scores of PAK1 inhibitors

Compound name	Structure	K_i (nM)	AutoDock binding energy (kcal/mol)
Compound 4		360	-5.74
Compound 8		4	-7.10

site as shown in Fig. 2. Two hydrogen bond interactions were observed with Gly282 and Glu345 while two other bonds were formed with Leu347. Residues such as Val284, Val328, Met344 and Leu396 were involved in hydrophobic interactions with compound **8**. On the other hand, compound **4** exhibited only three hydrogen bonds as shown in Fig. 3. Two hydrogen bond interactions were observed with Leu347 whereas third bond

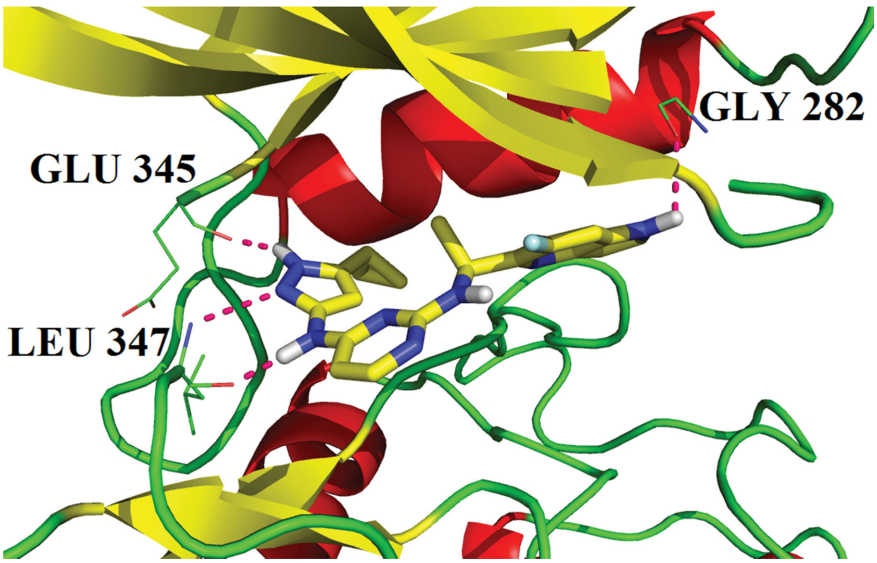


Fig. 2. Binding mode and interactions of compound **8** in the active site of PLK2. Main binding residues are shown as lines and ligand is displayed as stick model. Hydrogen bond interactions between ligand and protein are represented by red dashed lines. Non-polar hydrogens of the ligand are omitted for clarity.

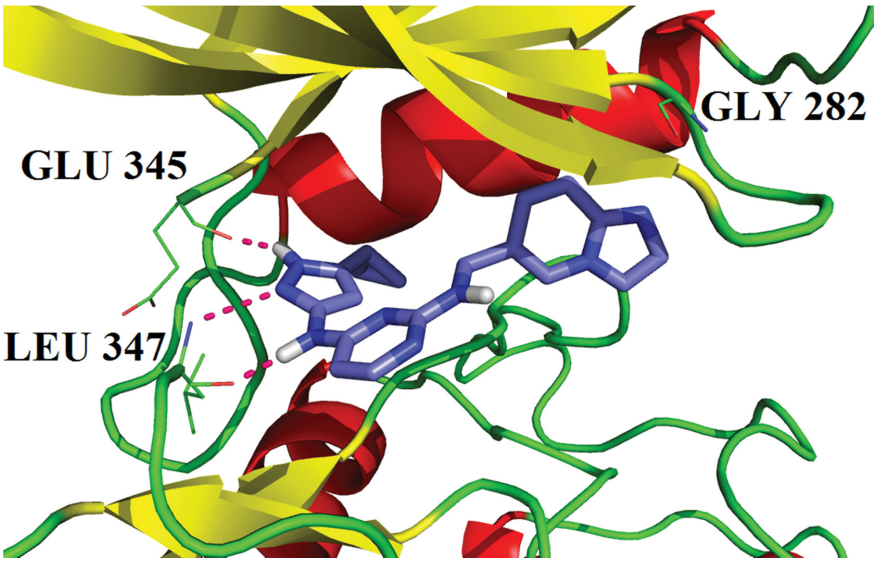


Fig. 3. Binding mode and interactions of compound **4** in the active site of PLK2. Main binding residues are shown as lines and ligand is displayed as stick model. Hydrogen bond interactions between ligand and protein are represented by red dashed lines. Non-polar hydrogens of the ligand are omitted for clarity.

was formed with Glu345. However, compound **4** demonstrated similar hydrophobic interactions with Val284, Val328, Met344 and Leu396. In case of highly potent compound **8**, indole NH group formed hydrogen bond with Gly282 while this interaction for compound **4** was missing due to the absence of indole NH group. This indicated that indole NH group is essential as a hydrogen bond moiety for PAK1 inhibitory activity.

4. Conclusion

Molecular docking studies of 4-azaindole derivatives with PAK1 demonstrated potential binding mode and binding interactions of these inhibitors. Results clearly indicated that hydrogen bond interactions with Glu345 and Leu347 are crucial for PAK1 binding. In addition, Val284, Val328, Met344 and Leu396 are important residues for hydrophobic interactions inside active site of PAK1. It was also found that compound **4** possess lesser inhibitory activity than compound **8** due to the absence of indole NH group. This group was involved in hydrogen bonding interaction with Gly282 in case of most potent compound **8**. Probably, indole NH group could be the determining factor for improving inhibitory activity.

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