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A structural insight into the inhibitory mechanism of an orally active PI3K/mTOR dual inhibitor, PKI-179 using computational approaches

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ABSTRACT

The PI3K/AKT/mTOR signaling pathway has been identified as an important target for cancer therapy. Attempts are increasingly made to design the inhibitors against the key proteins of this pathway for anti-cancer therapy. The PI3K/mTOR dual inhibitors have proved more effective than the inhibitors against only single protein targets. Recently discovered PKI-179, an orally effective compound, is one such dual inhibitor targeting both PI3K and mTOR. This anti-cancer compound is efficacious both *in vitro* and *in vivo*. However, the binding mechanisms and the molecular interactions of PKI-179 with PI3K and mTOR are not yet available. The current study investigated the exact binding mode and the molecular interactions of PKI-179 with PI3Kγ and mTOR using molecular docking and (un)binding simulation analyses. The study identified PKI-179 interacting residues of both the proteins and their importance in binding was ranked by the loss in accessible surface area, number of molecular interactions of the residue, and consistent appearance of the residue in (un)binding simulation analysis. The key residues involved in binding of PKI-179 were Ala-805 in PI3Kγ and Ile-2163 in mTOR as they have lost maximum accessible surface area due to binding. In addition, the residues which played a role in binding of the drug but were away from the catalytic site were also identified using (un)binding simulation analyses. Finally, comparison of the interacting residues in the respective catalytic sites was done for the difference in the binding of the drug to the two proteins. Thus, the pairs of the residues falling at the similar location with respect to the docked drug were identified. The striking similarity in the interacting residues of the catalytic site explains the concomitant inhibition of both proteins by a number of inhibitors. In conclusion, the docking and (un)binding simulation analyses of dual inhibitor PKI-179 with PI3K and mTOR will provide a suitable multi-target model for studying drug–protein interactions and thus help in designing the novel drugs with higher potency.

1. Introduction

Cancer is one of the most dreadful diseases in the world accounting for 8.2 million deaths in 2012 and the number is projected to rise to an estimated 13 million deaths within next two decades [1]. In view of the continuous hazardous impact on human health and economics, cancer poses a major and urgent challenge to the scientific community for discovery of novel anti-cancer chemical compounds. With the recent developments in understanding of the onset and progression of the cancerous conditions, the roles of various molecular pathways in this disease are becoming more explicit. The molecular pathway, PI3K/AKT/mTOR, is perhaps the most frequently dis-regulated pathway in human cancers [2–5]. Consequently, increasing attempts in recent years have been made to discover novel inhibitors of the key signaling molecules involved in the pathway including PI3K, AKT, and mTOR [6].

PI3Ks (Phosphatidylinositide 3-kinase, Phosphatidylinositol-4,5-bisphosphate 3-kinase) are intracellular lipid kinases involved in phosphorylation of PIP2 (Phosphatidylinositol-4,5-bisphosphate) to PIP3 (Phosphatidylinositol-3,4,5-trisphosphate) and regulate cellular functions through this second messenger PIP3 [7]. The wide array of cellular functions including proliferation, cell survival, vesicular trafficking, and cell migration are regulated by PI3Ks. These cellular functions are tightly regulated, however, any dis-regulation in these functions leads to cancerous state. Broadly PI3Ks are classified into 3 classes, *i.e.*, I, II, and III [2]. Out of the three classes, the most implicated in cancer is class-I PI3Ks [8].

Abbreviations: PI3K, phosphatidylinositide 3-kinase; PIP2, phosphatidylinositol-4,5-bisphosphate; PIP3, phosphatidylinositol-3,4,5-trisphosphate; mTOR, mammalian target of rapamycin; mTORC1, mTOR complex 1; mTORC2, mTOR complex 2; ASA, accessible surface area; ΔASA, loss in accessible surface area; PI3Kγ, class-IA PI3K p110γ.

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Class-I PI3K has further two groups: PI3K-IA and PI3K-IB. Class-IA PI3Ks are heterodimers and consist of two subunits namely p85 (85 kDa regulatory subunit) and p110 (110 kDa catalytic subunit). The class-IA p110 have four isoforms p110α, p110β, p110γ, and p110δ. All of these PI3K catalytic subunits have a PI3K core structure consisting of a C2 domain, a helical domain, and a catalytic domain [9]. The activating mutations of these class-1A PI3Ks are often observed in human cancer [3,10–14].

Another important signaling protein of PI3K/AKT/mTOR pathway is mTOR which is also considered in the current study. The mTOR (Mammalian target of rapamycin) protein is a serine-threonine kinase that belongs to the PI3K-related kinase family. The mTOR nucleates two multi-protein complexes, mTORC1 (mTOR complex 1) and mTORC2 (mTOR complex 2) with distinct input proteins and downstream effects [15,16]. The catalytic domain of mTOR called kinase domain (approx. 550 residues) has a typical bi-lobed fold which is characteristic of PI3Ks with a smaller N-terminal lobe (N lobe) and a larger C-terminal lobe (C lobe) [17]. There is a cleft between the two lobes of the protein and this cleft contains ATP binding site or the catalytic site of the protein. The mTOR regulates cellular metabolism, growth, and proliferation and, therefore, it is a target for the development of multitude of mTOR inhibitors for anti cancer therapy. The first discovered mTOR inhibitors were rapalogs (rapamycin and its analogs), however, their clinical results for anti-cancer therapy were not as promising as was expected initially [18]. The rapalogs bind to the FRB domain of mTOR to inhibit mTORC1. This inhibition of mTORC1 by rapalogs fails to repress a negative feedback loop which results in activation of PI3K/AKT/mTOR pathway [19,20]. These limitations led to the development of mTOR inhibitors which inhibit the catalytic function of mTOR [21]. So, the effective inhibitors were discovered which compete with ATP and inhibit kinase-dependent function of mTOR and, thus, act on both the mTOR complexes, mTORC1 and mTORC2 [22–24]. Various dual inhibitors have been discovered which inhibit PI3K p110 isoforms and mTOR and this provides therapeutic advantages [25]. The selective PI3K inhibition does not completely abolish mTOR function as the growth factors and nutrient signaling regulate mTOR. Whereas, the inhibition of mTOR alone leads to activation of PI3K signaling through S6K1-dependent negative feedback [26–28]. Thus, the concomitant inhibition of both PI3K and mTOR is more effective in blocking PI3K signaling. Various PI3K/mTOR dual inhibitors have entered clinical trials [29,30].

Recently, it was reported [31] that several bis-morpholino triazine based compounds acted as PI3K/mTOR dual inhibitors in which PKI-587 was a pan Class-I PI3K/mTOR inhibitor. When administered intravenously, the PKI-587 showed excellent anti-tumor activity *in vitro* and *in vivo* in xenograft tumor models [32]. However, the compound was not orally effective, *i.e.*, it was found to have poor plasma level when administered orally. Further, in an attempt to search for an orally effective compound, multiple compounds were generated after rationally modifying the PKI-587 [33]. Final analyses led to identification of a compound PKI-179 (Fig. 1) bearing a 4-pyridyl group, a pan Class-I PI3K/mTOR inhibitor, which was a highly potent and orally active compound. The efficacy of PKI-179 was checked *in vitro* by enzyme inhibition and cell proliferation inhibition. Also, when administered orally in nude mice with human tumor xenograft, the PKI-179 resulted in tumor growth arrest.

The present study is an attempt to understand the binding mechanism of a dual inhibitor PKI-179 with the two cancer signaling proteins PI3K and mTOR. Although, the inhibitory activity of PKI-179 with PI3K and mTOR is well established, yet the binding mechanism and the molecular interactions have not explored. The rationale of this study is that the better understanding of the binding mechanism and molecular interactions of the inhibitor with the binding site residues will help us in molecular modification of the existing inhibitors and to design novel ones targeting multiple proteins and providing higher efficacy against various cancers.

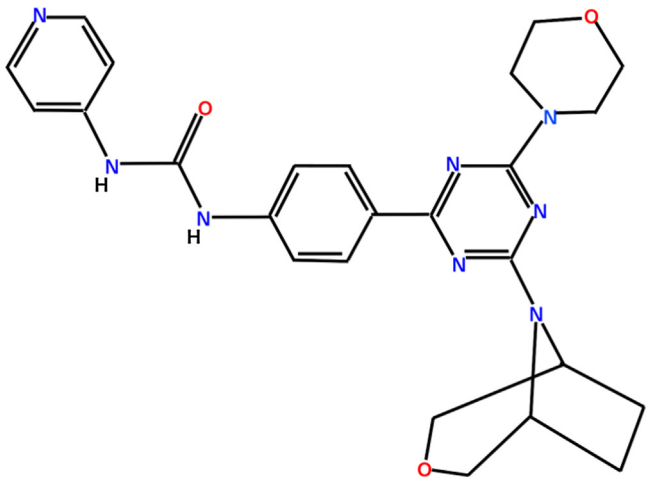

Fig. 1. Two dimensional structure of PKI-179. The IUPAC name of PKI-179 is "1-[4-[4-morpholin-4-yl-6-(3-oxa-8-azabicyclo[3.2.1]octan-8-yl)-1,3,5-triazin-2-yl]phenyl]-3-pyridin-4-ylurea". The nitrogen (N) and oxygen (O) atoms are shown in blue and red colors respectively.

2. Methods and computational details

2.1. Data retrieval

The molecular structure of PKI-179 was retrieved from PubChem compound database (CID, 46947264). The 3-D structure of human PI3Kγ p110 and mTOR were obtained from Protein Data Bank (PDB, http://www.rcsb.org/) with PDB IDs 3L54 and 4JT6, respectively. For mTOR, the kinase domain was considered and the stretch of the residues (1867–2436) was used for all analyses. Both of the PI3Kγ and mTOR PDB IDs were co-complex structures, *i.e.*, containing bound ligands LXX and PI-103 respectively. The bound ligands were used as clue for identifying catalytic sites which were used in molecular docking.

2.2. Molecular docking

The molecular docking of PKI-179 into the catalytic sites of PI3Kγ and mTOR were carried out by Dock v.6.5 (University of California, San Francisco, CA USA) [34]. The rigid body docking option of Dock was used which utilizes geometric matching algorithm to superimpose the ligand onto a negative image of the binding pocket. To obtain the lowest energy binding mode, the algorithm uses Random Conformation Search Strategy which involves the grid-based scoring functions of Coulombic and Lennard–Jones forces. The initial structure preparation of proteins and the drug required for docking and the visual analyses at different stages were performed by Chimera v.1.6.2 [35].

2.3. Analyses of docked protein-ligand complex

For visual analyses and illustrations of the protein-ligand complexes, PyMOL v.1.3 [36] was used. The polar and hydrophobic interactions between the proteins and the ligands were analyzed and the illustrations were generated by Ligplot+ v.1.4.3 program [37,38]. To score the extent of involvement of amino acid residues of the target in binding, ΔASA (loss in Accessible Surface Area) due to ligand binding was evaluated. For a residue to be involved in binding, it should lose more than 10 $Å^2$ ASA in the direction from