Anti-Cancer Potency of Cucurbitacin D through PI3K and AKT1: A Molecular Docking Studies

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Abstract. Cucurbitacin is a bitter-tasting compound found in the Cucurbitaceae family. Previously, cucurbitacin D was observed to have antitumor activity in several tumor cell lines *in vitro*. Several macromolecules have been shown affected by cucurbitacin treatment, including AKT1, JAK, and STAT3. Nevertheless, molecular docking study to predict the direct target of this phytochemical agent is still very limited. Our research aims to investigate the interaction of cucurbitacin D to AKT1 and PI3K, which are recognized as prominent targets in cancer research. The crystal structures of PI3K and AKT were retrieved from the PDB database and further validated using Autodock. The 3D structure of cucurbitacin D was prepared and optimized using Avogadro software. Molecular docking was performed using Autodock, and the interactions were visualized using Biovia Discovery. Cucurbitacin D exhibited low binding energy when binding to AKT1 and PI3K, with values of -10.46 and -2.57 kcal/mol, respectively. Biovia Discovery simulations indicated that cucurbitacin D interacts with PI3K through two conventional hydrogen bonds, one alkyl and 1 Pi-alkyl bonds. Cucurbitacin D binds Lys833 within ATP-binding pocket of PI3K, through conventional hydrogen bond. Cucurbitacin D interacts with AKT1 through five conventional hydrogen bonds and four alkyl and two Pi-alkyl bonds. The Pi-alkyl interaction also involves Trp80 of PH domain. These results suggest that cucurbitacin D possesses anti-cancer potential by targeting PI3K and AKT.

Keywords: cucurbitacin D, cancer, AKT1, PI3K, docking.

1 Introduction

1.1 A Subsection Sample

Cucurbitacin is a pharmacologically active compound found in Cucurbitaceae family, which consists mostly of vegetables and fruits, including pumpkin, cucumber, melon, and watermelon. Despite its bitter taste, Cucurbitaceae family has been traditionally used as herbal medicine in India and China as. In Indonesia, the plants are used as vegetables. The large empirical use of Cucurbitaceae plants have guided pharmacologists to study the pharmacodynamic of antiemetic, diuretic, antiepilepsy, antipyretic, antitussive, diuretic, antiobesity (fruit), bronchodilator and antibacterial cucurbitacin. The in vitro and in vivo research conducted has reveal several pharma-

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cological activities of cucurbitacin, including anti-inflammatory, antitumour, anti-atherosclerotic, and antidiabetic [1], [2].

Cucurbitacin D has gained attention for its antitumour activities reported on several cancer cell types, including breast, prostate, pancreas, gastric, endometrium, ovarium, and liver. Several signaling molecules are reported to be affected by cucurbitacin D treatment. It is reported to downregulate AKT1, STAT3 and JAK, that have been known to stimulate cell proliferation. Also, GLUT4 has been recently reported to be inhibited after cucurbitacin D treatment, causing glucose deprivation, that may mediate its cytotoxic activity to cancer cells [3]–[8].

Molecular docking studies of cucurbitacin with cancer-related macromolecules are still limited. Two molecular docking studies predicted cucurbitacin D direct targets, epidermal growth factor receptor (EGFR) [9] and GLUT1 [10]. Molecular docking study is important to predict the direct target of drug as well as its affinity to the target macromolecules [11].

AKT, also known as protein kinase B, is a kinase that is implicated in cell survival, proliferation, apoptosis, and angiogenesis. AKT is activated by growth factor and other stimuli. AKT blocks cell apoptosis by inactivating BAD, BIM, caspase 9, and FoxO1. During cell cycle progression, AKT phosphorylates and inhibits glycogen synthase kinase 3β (GSK3β) to prevent cyclin D1 degradation [12]. Mutation that causes its overactivation is involved in the pathogenesis of several cancer types, including breast, lung, ovarian, pancreatic, and gastric carcinomas [13]. Several anticancer drug candidates targeting AKT pathway are currently under clinical trials. These candidates are either ATP- competitive (which binds ATP-binding site) or allosteric inhibitors (which binds AKT phosphorylation and activation site). Some examples are ipatasertib and capivasertib [12].

There are four subfamilies of PI3K, classes I, II, III and IV. They are distinct in the catalytic and regulatory structures as well as substrate specificity. PI3K Class I are subdivided into PI3K1A and PI3K1B [14], [15]. PI3Kγ, the only member of PI3K1B, has been regarded as potential drug target in advance solid tumor, leukemia, and lymphoma [16]. At physiological condition, PI3K is important to stimulate cell growth and proliferation through activation of PDK1/AKT/mTOR, Ras/Raf/MEK/ERK/AKT/mTOR as well as JAK/STAT pathway. PTEN, on the other hand, inhibits PDK1/AKT/mTOR pathway by dephosphorylating PIP2 to PIP3 therefore prevents phosphorylation of PDK1. However, PI3K is reported over-activated in cancer. This deregulation may be caused by loss or inactivation of PTEN or mutation or amplification of PI3K. PI3K is activated by growth factor, cytokine, or hormones. Drug candidates targeting PI3K grouped into dual PI3K/mTOR inhibitors, pan-PI3K inhibitors and isoform-specific inhibitors. Dactolisib and buparlisib are two anticancer drugs currently under clinical trials, targeting dual PI3K/mTOR and PI3K class I [17].

One important constraint of cancer treatment is chemotherapy resistance. As pathogenesis of cancer involves various molecular signaling, cancer cells may overcome chemotherapy activity by over-activating other molecular signaling. Therefore, research to explore and develop new anticancer drugs are pivotal.

2 Materials and Method

2.1 Ligand and Target Protein Preparation

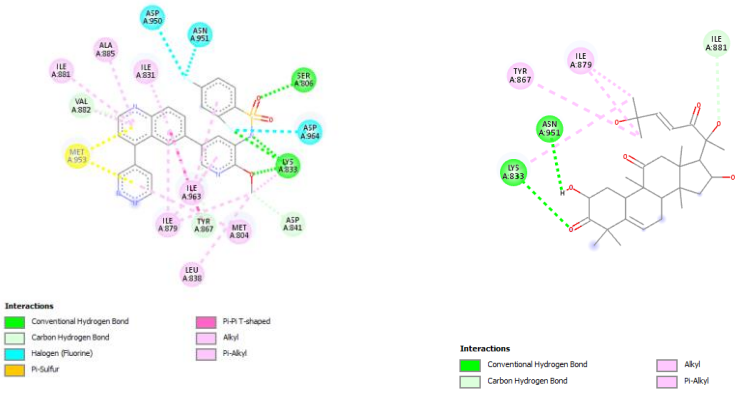
PDB database (PDB101.rcsb.org) [18] was used to obtain the crystal structures of the target protein, PI3K and AKT1. Several parameters were considered in the selection of the crystal structures. Firstly, it must be a bound structure of a target protein to a native ligand or small molecule inhibitor. Secondly, the binding site or catalytic site should not have mutation. Concurrently, the 3D configuration of cucurbitacin D were constructed with Avogadro software [19]. The structure was further optimized based on Density Function Theory using Gaussian 16, Revision D.01 [20].

2.2 Molecular Docking and Interaction Visualization

The method of docking was validated using Autodock 1.5.6 [21]. This validation was proceeded by redocking of the ligand or inhibitor into the binding site of the target protein. The validation process generated RMSD. RMSD value less than 2 Å indicates that the docking method is suitable for the docking process using the crystal structure. The sizes and positions of the grid box generated from the validation step was used for molecular docking with cucurbitacin D as the tested ligand [1]. The interaction between ligand and target proteins were simulated using the Biovia Discovery Studio software [22]. This visualized the interaction of each amino acid of the target macromolecule with chemical functional group of cucurbitacin D.

3 Results and Discussion

The crystal structures of PI3K and AKT1 used for docking were PDB ID 3L08 and PDB ID 3096, respectively. The two crystal structures are originated from *Homo sapiens.* Crystal structure PDB ID 3L08 is the structure of PI3K bound to omipalisib (GSK2126458). This drug candidates is a dual inhibitor of PI3K/mTOR that has completed phase I clinical trial dose-escalation study. Molecular docking showed that omipalisib binds PI3K with low binding energy, -11.02 kcal/mol.



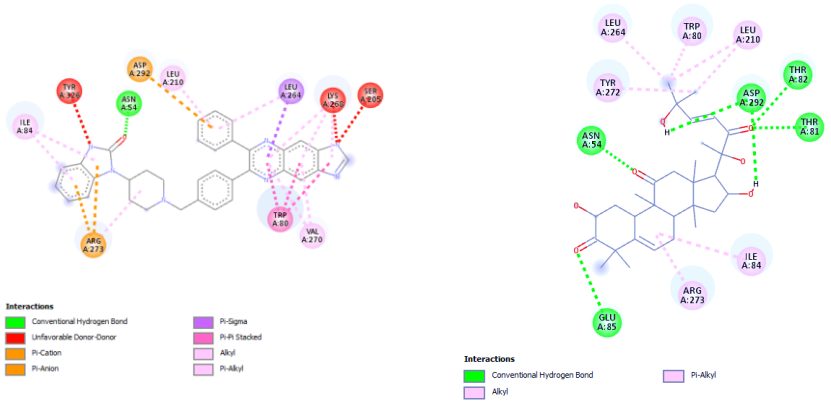
(a) **(b)**

Figure 1. Visualization of interaction between Omipalisib to PI3K (a) and Cucurbitacin D to PI3K (b)

Omipalisib is a competitive antagonist that interacts with PI3Kγ through its sulphonamide moiety at thiazolidindione ring (Lys833) within ATP-binding pocket of PI3Kγ. This interaction is shown in the interaction visualization generated by Biovia Discovery Studio (Figure 1a). It can be seen that omipalisib forms conventional hydrogen bond with LYS833 and SER806. It also interacts with Val882, Tyr867, and Asp841 through three carbon hydrogen bonds. It forms fluorine bond with Asp950, Asn951, Asp964, Pi-sulfur with Met953, alkyl bond with Ile879 and Leu838 and Pi-alkyl with Ile881, Ala885, Ile831, Ile963, Ile879, and Met804.

Cucurbitacin D provide low binding energy to PI3Kγ, -2.57 kcal/mol although still higher compared to the omipalisib. Like omipalisib, it binds the ATP-binding pocket at Lys833. While omipalisib binds Asn951 and Tyr867 with fluorine and carbon-hydrogen bond, respectively, cucurbitacin binds these amino acid residues with conventional hydrogen and Pi-alkyl bond, respectively. Like omipalisib, it interacts through Ile879 with alkyl bond. There is less interaction formed between Cucurbitacin D and PI3Kγ compared to omipalisib PI3Kγ, that may correlate with higher binding energy needed to form the interaction of cucurbitacin D and PI3Kγ, compared to omipalisib.

Crystal structure of PDB3096 consists of AKT1 structure bound with allosteric inhibitor IQO. While competitive inhibitor needs to bind AKT1 at kinase domain (catalytic site), previous studies have guided researchers to understand that allosteric inhibition to AKT1 needs interaction through PH domain, as well as catalytic kinase domain of this kinase protein. Molecular docking predicts IQO binds to AKT1 with binding energy -12.94. Cucurbitacin D also binds AKT1 with low binding energy, although still higher compared to IQO, -10.46 kcal/mol.



(a) **(b)**

Figure 2. Visualization of interaction between IQO to AKT1 (a) and Cucurbitacin D to AKT1 (b)

Biovia Discovery Studio predicts that IQO binds VL3 loop at Trp80 of AKT1 PH domain with Pi-Pi stacked bond, as can be seen in Figure 2a. This makes loop that is shifted to accommodate AKT1 binding to IQO or agonist IP4. The binding of IQO at PH domain prevents the phosphorylation of kinase domain by ATP thus maintaining this kinase protein in inactive form. IQO binds AKT1 with one conventional hydrogen bond (Asn54), one alkyl bond (Arg273), three Pi-alkyl bonds (Val270, Ile84, and Leu210), one Pi-Sigma (Leu264), and one Pi-Pi stacked (Trp80). More conventional hydrogen bond (Asn54, Glu85, Asp292, Thr82, Thr81) as well as more alkyl bond (Leu264, Leu210, Arg273, Ile84) is formed between cucurbitacin D and AKT1. Cucurbitacin D, but not IQO, binds to Glu85, Thr81, and Thr82. While interaction with Val270 is only found in IQO. Unfavorable interaction is shown in IQO, but not AKT1, through Tyr326, Lys268, and Ser205.

4 Conclusion

This study simulates cucurbitacin D interaction with PI3K and AKT1, signaling molecules important in cancer pathogenesis. The interaction is mediated through ATP-binding domain and PH domain of PI3K and AKT1, respectively. This shown that cucurbitacin D may be a potential anti-cancer candidate targeting PI3K/AKT1.

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