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A Molecular Docking Study of Aristolactam Analogues against Polo-Box Domain (PBD) of Human Polo Like kinase-1 in Cancer Progression

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Abstract

Polo like kinases (Plks) play a multiful role in the cell cycle progression and its mis-regulation in cancer believed as a promising anticancer-drug target. The N-terminal polo box domain(PBD) of plk1is another efficient target for potent plk1 inhibition. Natural Aristolactam analogues have shown anticancer activity,but their cellular target was unknown. In this work, molecular docking studies were performed in order to see the interaction of Aristolactam and its 11analogues with human plk1 in cancer. Results shown that among 11, three analogues exhibited good affinity to the PBD of plk1 with free energy of -8.10,-7.47 and -7.10 Kcal/mol and inhibition constant(Ki) of 1.16,3.33 and 6.61 µM. Active site residues of PBD interacting with Aristolactam analogues were Trp^{414},Lys^{540},Leu^{491},Asn^{533},Ser^{412},Arg^{516},Arg^{594},Asp^{371} andTyr^{510}.The inhibitor for the plk1,BI2523 was used as reference drug and it was shown poor docking energy compared to Aristolactam analogues. In binding mode, the length (Å) of H-bonds were varied from each analogue. These interactions indicated that stability of Aristolactams in PBD of plk1 and suggested as a potential drug candidates. Thus good affinity of Aristolactam analogues to plk1 may leads to synthesis of anticancer drugs.

Keywords: Cancer, Aristolactams, Polo like kinse-1, molecular docking and polo box domain.

Contents

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1. Introduction

Proper cell cycle progression is critical for maintaining genomic stability. Mitosis is particularly tightly regulated as deregulated mitosis would lead to improper segregation of chromosomes. Checkpoints including G2/M checkpoint, kinetochore and spindle checkpoint have therefore evolved to ensure proper onset of mitosis and correct

transmission of genetic material to daughter cells [1]. The mitotic progression is mainly promoted by cyclin-dependent kinases and further controlled by several critical mitotic kinases including Plk1 [2,3].Therefore, the expression level of these mitotic kinases must be tightly regulated. Over expression of polo like kinase causes many cancers and as promising target in cancer therapy. Polo like kinases are serine/threonine kinases .There is four different types of polo like kinases (plk1, plk2, plk3 and plk4), of which plk1 is the best studied. Plks possesses a conserved amino terminal catalytic kinase domain responsible for ATP-binding and enzyme activation. The catalytic kinase domain consists of a two lobes, each composed of a polypeptide chain.ATP molecules can bind at the gap between the two polypeptide chains [4].

In general all protein kinases reveal similar ATP binding pockets. This may pose problems in developing highly specific kinase inhibitors. The C-terminal region of all Plks have polo box domain(PBD) which consists of two polo boxes/motifs each consist of 80 residues [5,6] (Fig.1). Phospho peptide binds to the two motif-PBDs at the consus sequence S-Ps/Pt-p/x [7,8] (Fig.1)**.**The PBDs of the four Plks are similar, but not identical and have different binding affinities. It has been reported that plk1 interacts, through its PBD, with certain serine/threonine phosphorylated proteins localized at particular mitotic apparatuses, and binding of PBD to the primed phosphorylation sites not only serves for targeting the kinase domain to substrates but also simultaneously activates the kinase domain by relieving the inhibitory intermolecular interaction [9]. Therefore, in addition to blocking the ATP binding or the substrate binding site, targeting the PBD is also considered as another efficient target for the exploration of plk1 inhibitors.

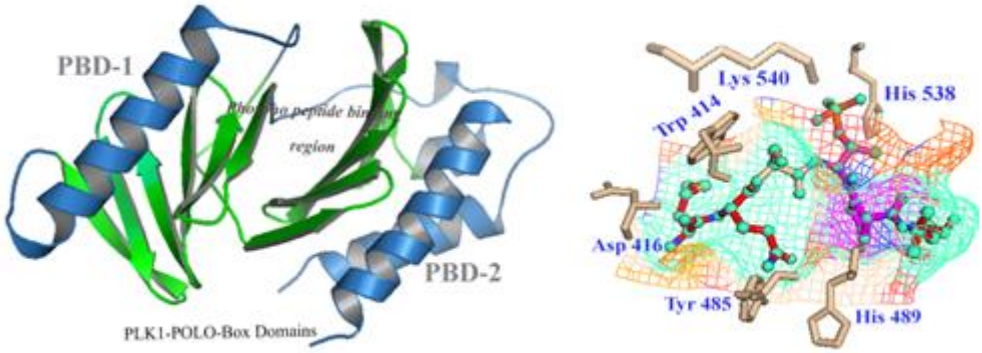

Figure 1: 3D structure of Polo like kinase1 represents the PBD domains (PBD1 & PBD2) and active site amino acid residues with its ligand, phospho-peptide.

Aristolactams (Fig.2), a Phenanthrine chromophores are a small group of compounds mainly found in the Aristolochiaceae together with the aristolochic acids and 4, 5-dioxoaporphines [10]. The natural product Aristolactam AIIIa, an Aristolactam derivative functions as a new type of ligand targeting the PBD of plk1. It could inhibit the proliferation of cancer cells and induce apoptosis and the mitotic arrest at G2/Mphase with spindle abnormalities [11]**.** We have been chosen the Phenanthrine ring containing Aristolactam derivatives for study of their anti cancer activity.

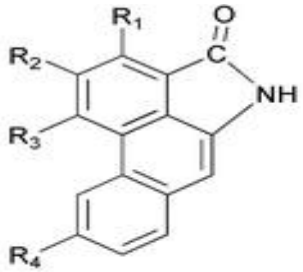

Figure 2 General structure of Aristolactam

In the present study, we have explored the binding affinities of Aristolactam derivatives against the human polo-like kinase, a crucial target in the cancer therapy. In this study we have been screened a potential Aristolactam derivative against plk1 in carcinogenesis, using molecular docking approach.

2. Materials and Methods

Preparation of protein structure

The crystal structure of the polo-box domain of human polo-like kinase-1(PDB ID: 1Q4K) in complex with phosphor-peptide solved by X-ray crystallography at 2.30Å was retrieved from the Protein Data Bank [12]. Before

initiating the docking simulations, all non-protein molecules were removed from 1Q4K; for any alternative atom locations only the first location (A) was retained. All the docking calculations were performed by using Autodock 4.0. 1Q4K was modified by adding polar hydrogens and then kept rigid in the docking process, whereas all the tensional bonds of ligands were set free by Ligand module in Autodock Tools-ADT.

Preparation of ligand structures

The sdf files of Aristolactam analogues were retrieved from PUBCHEM server [13]. The files were then converted into PDB format with help of an online molecular File Converter [14].

Binding site analysis

Plk1 active site prediction was performed using CASTp program for binding pocket analysis [15]. The largest surface area of cavity A was estimated as 441.7$Å^2$ with a volume of 665.2 $Å^3$. The remaining cavities B and C showed very less surface area and volume of 43.3, 34.9 $Å^2$ and 27.1, 23.9 $Å^3$ respectively (Fig.3).

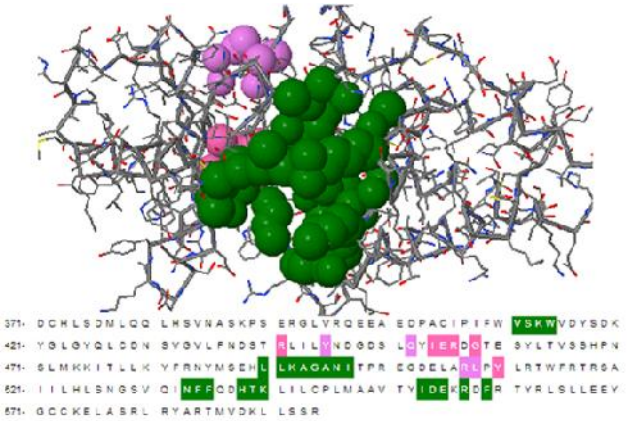

Figure 3. Plk-1 binding sites (green, light pink and red balls) and amino acid residues were predicted using CASTp server.

Molecular Docking:

All docking simulations were carried out in Auto dock (v4) using the Lamarckian genetic algorithm [16]. The targets, for each docking was kept rigid, therefore assuming there is no induced conformational change upon ligand binding. In contrast to the protein, tensional flexibility was permitted for the ligands via the side-groups and backbone of protein was kept rigid. Atomic affinity and electrostatic potentials were computed for a grid box positioned around the approximate centre of the binding site, with Dimensions 60.0x60.0x60.0A˚. The genetic algorithm (GA) population size was increased to 300 and the number of energy evaluations per trial was set to 5 x 10^6 .Genetic algorithms usually identify the notion of an individual and its genetic code with respect to the problem's solution space.

Autodock treats a particular orientation of the ligand inside the protein as an 'individual' in its GA. The increase here was due to the large number of flexible torsions that are explored during simulation, which increase the search space for the more flexible compounds. Each flexible docking simulation was repeated for 100 trials, yielding 100 docked conformations for ligand. Autodock 4.0 included the Lamarckian genetic algorithm [17], and experimental free energy function for estimating binding energy, inhibitory constant, docking energy, intermolecular energy, internal energy and tensional energy. The binding free energy was empirically calculated based on these energy terms and a set of co-efficient factors [17]. The value of binding energy was used to rank the docking positions of the molecules. The clusters with lowest binding energy were selected. After docking, the ligand-receptor complexes were analyzed by PyMOL program [18].

3. Results and Discussion

Protein kinases have amassed great interest as therapeutic targets because they are involved in numerous diseases including neurological, neoplastic and inflammatory diseases [19]. In malignant cells signal transduction is often deregulated due to mutated-activated protein kinases. Since polo like kinases are over expressed in many cancers, it represents an interesting target molecule for the development of specific inhibitors to selectively treat cancer while avoiding toxicity towards normal cell tissues. Plks are the only protein kinases that contain PBDs. To increase the specificity of plk1 inhibition, substances binding to the PBD and competing with natural plk1 substances have been developed.The compounds interact with the PBD of plk1 and compete with the Phospho-peptide for access to the kinase activity are the potent inhibitors of plk1 function in cancer. Aristolactams are the Phenanthrine ring containing alkaloids extracted from various plant sources having potential anticancer activity. The natural product

Aristolactam AIIIa functions as inhibitor of PBD of plk1. The target based PBD inhibition of Aristolactam AIIIa provides the basis for the screening of various analogues of Aristolactams against PBD of plk1 in cancer.

The docking studies of Aristolactam analogues were done by using Autodock (v4) tool and screening was performed by employing the scoring function. The Aristolactam analogues were found to bind with polo like kinase-1.The result validation was based on the score of estimated free binding energy (ΔG), estimated inhibition constant (Ki), vander wall energy, H-bond dissolve energy, electrostatic energy, total intermolecular energy and H-bond length between plk1 and ligands. Binding affinities between plk1 and Aristolactams analogues increased with decrease in free binding energy. Thus, a large value of Ki indicated a low affinity.

On comparing the free binding energy, best results was shown by 7-(Deoxyadenosin-N(6)-yl) Aristolactam I (CID 5486862), followed by CID 148745, CID 148657 and CID 6439485 having free binding energy of -8.10,-7.47,-7.10 and -6.87 Kcal/mol, respectively indicating high binding affinity towards plk1.The inhibition constant(Ki) of these compounds was 1.16,3.33,6.21 and 9.21µM respectively. In docking analysis, the total breakdown energy in terms of van der Walls and electrostatic energy (Table. 1) indicated the strong influence for the stable binding. The total intermolecular energy(The cumulative sum of van der Walla, H-bond, dissolve and electrostatic energy) of CID 5486862), followed by CID 148745, CID 148657 and CID 6439485 was found to be -7.87,-7.47,-7.32 and -7.86 Kcal/mol, respectively. The binding amino acid residues Trp^{414}, Lys^{540} and Tyr^{510} were common in analogues (Fig.4,A-C) and seemed to be important for binding of ligand.

Nucleoside analogues are the new potent chemotherapeutic agents against cancer especially in drug resistant tumors [20, 21]. The Aristolactam analogue CID 5486862showed high docking energy (-8.10 Kcal/mol) and low inhibition constant (1.16 µM), was the purine derivative, seemed to be best in the rest of analogues. The H-bond (strong dipole-dipole attraction) and its length determine the strength of attraction between molecules. Almost all Aristolactam analogues mentioned in the table were binds to the common amino acid residues of plk1 but, the H-bond length was varied (2.0 to 3.5 A˚) from each analogue. The purine analogue of Aristolactam possesses a strong interaction with plk1 with least H- bond length than remaining followed analogues. The standard drug for plk1, BI2536 shown strong interaction with least H- bond distance (Fig.4, D).

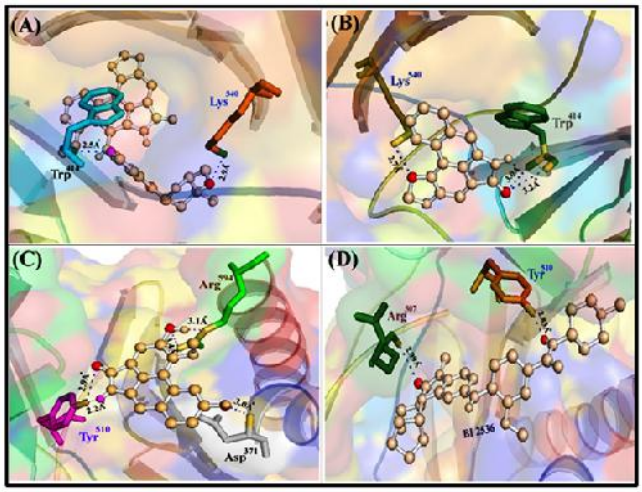

Figure 4. Docking conformation of plk1 PBD domain with Aristolactam analogues (A&B), Aristolactam-III (C) and BI2536 (D).Blue ball: Nitrogen, Red ball: Oxygen for ligands (ball and sticks) and yellow green for atoms in amino acid residues (sticks).

4. Conclusion

In conclusion, docking result shown that Aristolactam analogues viz, CID 5486862 and CID 148745 were seemed to be potential inhibitor of plk1 than BI 2536, a known plk1 inhibitor in cancer therapy. Thus natural Aristolactams could be a good alternative to control the expression levels of plk1 in cancer.

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