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Identification of a high affinity selective inhibitor of Polo-like kinase 1 for cancer chemotherapy by computational approach

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ABSTRACT

Polo-like kinase (Plk)1 is a key regulator of the cell cycle during mitotic phase and is an attractive anti-mitotic drug target for cancer. Plk1 is a member of Ser/Thr kinase family which also includes Plk2-4 in human. Plk1 promotes the cell division whereas Plk2 and Plk3 are reported to act as tumour suppressors. The available inhibitors of Plk1 also suppress Plk2 and Plk3 activity significantly resulting in the cell death of normal cells in addition to the cancer cells. Hence, it is imperative to explore Plk1 specific inhibitors as anti-cancer drugs. In this work, a selective potential inhibitor of Plk1 has been identified by molecular docking based high throughput virtual screening. The identified compound exploits the subtle differences between the binding sites of Plk1 and other Ser/Thr kinases including Plk2-4. The predicted binding affinity of identified inhibitor is higher than available inhibitors with a 100-fold selectivity towards Plk1 over Plk2-4 and several cell cycle kinases. It also satisfies the Lipinski's criteria of drug-like molecules and passes the other ADMET filters. This triazole compound with aryl substituent belongs to a novel class of potential inhibitor for Plk1. The suggested potential lead molecule can thus be tested and developed further as a potent and selective anti-cancer drug.

1. Introduction

The regulatory proteins like cyclin-dependent kinases and Ser/Thr kinases in the cell cycle have recently been targeted for determining alternative suitable anti-cancer drug candidates with low levels of cytotoxicity. Amongst the Ser/Thr kinases, Polo-like kinase (Plk)1 has gained importance as an attractive anti-cancer drug target [1]. Plk1 is highly expressed in proliferating cells and has been seen to be overexpressed in 80% of human tumours and cancer cells of diverse origins including colorectal and lung carcinoma, glioma, melanoma, head and neck cancer, ovarian cancer and breast cancer [2,3]. Its overexpression has also been correlated with poor prognosis in a broad spectrum of malignancies [4,5]. The antisense oligonucleotides (siRNA) targeting the mRNA of Plk1 have been shown to reduce cell proliferation in several cancer cell lines and exhibit no visible effect on the viability of untransformed cells [6,7]. The inhibition of enzymatic activity of Plk1 is known to arrest the tumour cells in mitosis subsequently leading to apoptosis [8]. Hence, Plk1 serves as an important target for the development of anti-cancer drugs.

Till date, five members of human Plks have been reported: Plk1, Plk2/Snk, Plk3/Fnk/Prk, Plk4/Sak and Plk5. The Plk family is highly conserved from yeast to mammals and are structurally similar, yet, they exhibit significant differences in their cellular functions [9]. Out of these five Plks, the biological roles of Plk1, Plk2 and Plk3 have been extensively studied. Plk1 regulates the cell cycle during late G2 and M phases while Plk2 and Plk3 regulate the cell cycle during G1 and S phases. Plk1 is involved in activation of cyclin-dependent kinase, checkpoint kinase, mitotic entry, chromosome alignment, centrosome maturation, bipolar spindle formation, activation of anaphase promoting complex, chromatid separation, mitotic exit and initiation of cytokinesis [10–13]. Plk2 and Plk3 act as tumour suppressor. Plk2 is believed to be involved in DNA damage checkpoints [14] while Plk3 is required for the entry into S-phase [15,16]. Plk4 is less conserved compared to Plk1-3 and functionally it is involved in the duplication of centriole in the cell cycle [17]. Plk5 has recently been reported and unlike human Plk1-4, it does not play a role in the cell cycle [18]. Hence, a potent inhibitor of Plk1 that does not inhibit the enzymatic activity of other Plks or inhibits them with low potency as compared to Plk1 can act as an ideal anti-cancer drug candidate.

Structurally, the Plks are characterized by one or more polo box domain (PBD) at C-terminus and a catalytic domain of Ser/Thr kinase at N-terminus. In addition to the kinase domain, Plk1-3 each

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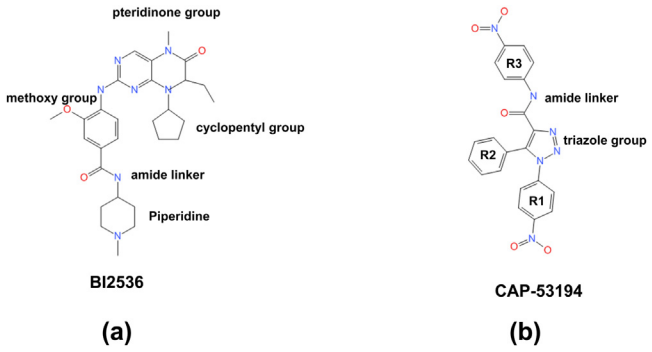

Fig. 1. Chemical structure of (a) known potent inhibitor BI2536 and (b) potential inhibitor CAP-53194 identified by virtual screening.

contain two PBDs whereas Plk4 possesses only one PBD. Plk5 is distinct as it contains one PBD and lacks the kinase domain. The PBD possesses regulatory activity and is responsible for the proper localization of Plks to their targets [19]. The catalytic domain of Ser/Thr kinase possesses an ATP binding pocket. Majority of the reported inhibitors till date developed against this catalytic domain are either ATP analogues or non-ATP small molecule inhibitors that target the ATP binding pocket [1]. The ATP binding site is highly conserved amongst all the Ser/Thr kinases including the four Plks. This makes the design and identification of Plk1 specific inhibitors a challenging task. Currently reported inhibitors of Plk1, to our knowledge, are not specific or sufficiently selective over other important cellular Ser/Thr kinases and produce side effects due to their cytotoxic effects on normal cells. Wortmannin, an ATP competitive inhibitor of Phosphatidylinositol 3-kinase (PI3K), inhibits Plk1 with an inhibition constant of 24 nM but is known to covalently modify the protein leading to toxicity [20]. Scytonemin, a natural product isolated from cyanobacteria, blocks Plk1 with a potency of 2 μM [21] but simultaneously suppresses cyclin dependent kinase, checkpoint kinase 1 and protein kinase C with nearly similar potency. Thiophene benzimidazole derivative is a novel class of Plk inhibitor which targets the ATP binding pocket of Plk1 but is also reported to inhibit Plk3 with similar potency [22]. ZK-Thiazolidinone, a non-competitive ATP inhibitor, possessing IC_{50} value of 19 nM for Plk1 has concurrently an IC_{50} value of approximately 100 nM for Plk2 and Plk3 [23]. High throughput screening followed by lead optimization has identified an ATP-competitive dihydropteridinone derivative based potent inhibitor of Plk1, BI2536 (Fig. 1a) [24]. This is reportedly one of the best known inhibitor of Plk1 in terms of its activity and selectivity (IC_{50} = 8 nM) with up to 50-fold selectivity over other Ser/Thr kinases. It, however, also prevents Plk2 and Plk3 activity to a similar extent [25]. Thus, the currently known inhibitors of Plk1 simultaneously inhibit Plk2 and Plk3 equally resulting in cytotoxicity. Hence, potent inhibitors of Plk1 selective over Plk2 and Plk3 and other cellular Ser/Thr kinases could provide a suitable lead molecule for the development of less toxic anti-cancer drugs.

This prompted us to explore the possibility of identifying a lead molecule based on sequence and structural information. The sequence analysis of the kinase domain of Plks revealed differences in the nature of residues between Ser/Thr kinase domain of Plk1 and other kinases including Plk2-4. The residues Leu132, Arg134 and Arg136 in the ATP binding pocket are relatively specific to Plk1. The corresponding residues in other kinases are generally Tyr/Phe, Ser/His and Gly, respectively. The selectivity determining methoxy group of ligand BI2536 occupies the small cavity provided by Leu132 in Plk1 [25]. This residue in Plk2 is replaced by the bulkier Tyr residue which reduces the binding space for BI2536 and results in steric hindrance to its methoxy group. Moreover, Plk1 has a large and positively charged residue Arg134 while the corresponding residue in Plk2 and Plk3 is relatively a smaller and neutral serine. In the present study, we have exploited these subtle differences in the key residues at the active site of Plks and other kinases to identify a potentially more selective inhibitor of Plk1 using the combined approach of molecular docking and virtual screening of chemical database.

2. Materials and methods

The X-ray crystal structures of several Plk1-ligand complexes are available. The structure with the highest resolution amongst the available crystal structure of human Plk1 complexed with inhibitors at 1.9 Å resolution with the highly potent inhibitor BI2536 (PDB: 2RKU) was chosen for this study [25,26]. This was used as receptor for identification of high affinity inhibitors of Plk1 during structure-based virtual screening of chemical library. Three-dimensional (3-D) structures of Plk2-4 were also crucial for the structure-based study as the potential inhibitors identified by virtual screening were screened for their selectivity against them. Since the structures of Plk2 and Plk3 are not available, these were homology modelled using the crystal structure of Plk1 as their Ser/Thr kinase domain shares more than 50% sequence homology with Plk1. The available crystal structure of Plk4–ATP analogue complex (PDB: 3COK) was used for selectivity study. Plk5 was excluded from this study as it lacks the kinase domain and is characterized by only the PBD domain. All the computational analyses has been carried out in the modelling environment of Discovery Studio (DS) 2.0 [27] using CHARMm 33.1 [28,29] force field and LigandFit [30] docking protocols.

2.1. Homology modelling of Plk2 and Plk3

The residues located at the ATP binding pocket in human Plk1 are more conserved in Plk2 and Plk3 than Plk4. The catalytic domains of Plk2 and Plk3 share a sequence identity of 52.6% (similarity 77.1%) and 53.0% (similarity 76.3%), respectively with Plk1. In the absence of crystal structures of Plk2 and Plk3 catalytic domains, their model structures were generated by homology modelling using Plk1 as template with the help of MODELLER 9v3 [31,32]. Ten homology models each for both Plk2 and Plk3 were generated and the best two models, one for each protein, were selected based on the Probability Density Function (PDF) energy [31] and Discrete Optimized Protein Energy (DOPE) score [33]. PDF energy is the sum of all the homology derived and stereochemical pseudo-energy terms while DOPE score is the atomic based statistical potential which is a measure of the stability of a conformation. As a lower value for both PDF Energy and DOPE score is indicative of a better model, the model with lowest PDF energy as well as DOPE score amongst the 10 generated models obtained for each protein (Plk2 and Plk3) was selected for further analysis. The stereochemical quality of the selected homology models was verified by PROCHECK [34]. The side chains of the selected models were refined using CHARMm force field 33.1 and conjugate gradient algorithm with convergence criteria of root mean square (r.m.s.) gradient less than 0.05 kcal mol^{-1} $Å^{-1}$. The two minimized models for Plk2 and Plk3 were further refined by short molecular dynamics (MD) simulations of 200 ps of equilibration followed by 200 ps of production run in the presence of water solvent model (TIP3P) to relax their model structures.

2.2. Receptor preparation

The binding site residues are nearly conserved amongst the four human Plks except in Plk4 where they are comparatively less conserved. Therefore, it was imperative to dock and cross