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MOLECULAR MODELING AND DRUG DISCOVERY OF POTENTIAL INHIBITORS FOR ANTI CANCER TARGET GENE PLK- *POLO LIKE KINASE1*

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ABSTRACT

The oncological target Polo like kinase 1 plays a key role in mitosis and cytokinesis .PLK 1 is associated with tumorigenesis and belongs to a family of disease relevant protein kinases that can be targeted by different drugs. It represents a promising approach for the development of novel anticancer therapies. Identification of novel inhibitors in polo like kinase 1 is important as it is closely correlated with malignant proliferative diseases such as lung cancer. PLK 1 is found to be over expressed in many lung cancer cell lines and human lung tumors. Thus, it is proposed that PLK1 might be a valuable diagnostic marker and a potential therapeutic target for lung cancer. However, only a few PLK1 inhibitors are in clinical development. Therefore, there is a high level of interest and an increasing effort to identify and develop novel PLK1 inhibitors. We have listed out 4 such potential inhibitors which could be promising inhibitors for Polo Like Kinase 1 namely Gemcitabine , paclitaxel, vorinostat and Etoposide. The interaction between the predicted structure of Polo like kinase 1 and its potential inhibitor is analysed *in silico* by Autodock. This study provides an insight into the structure of polo like kinase-1 in lung cancer and also gives an idea about potential sites responsible for inhibitory action that could further be substantiated by experimental investigations. This study facilitates initiation of the drug discovery process for polo like kinase 1 to present the scientific community with better inhibitors and/or drugs.

KEY WORDS: **Polo like Kinase 1, molecular modelling, Docking, Drug targets**

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1.0 INTRODUCTION

Polo-like kinases (PLKs) have gathered much attention as important elements that regulate cell cycle progression, particularly mitosis. There are four mammalian PLK family members identified thus far: PLK1, PLK2 (aka SNK), PLK3 (aka PRK, FNK and CNK) and PLK4 (aka SAK). More recently, PLK5 has been identified; however, it lacks a kinase domain and does not seem to function in cell cycle regulation [1]. The four mammalian PLK family members are structurally homologous, containing an N-terminal catalytic kinase domain and a C-terminal region composed of 'polo boxes' (only one in PLK4) from which the specificity for their targets is thought to be derived. PLK1 is the most investigated member of the family and has been widely pursued as an anti cancer target because it is over expressed in several human tumour types. Numerous studies have been published examining the potential of PLK1 as an anti tumour drug target, including work with antisense oligonucleotides, small interfering (si)RNA and small molecules [2–4].The best-characterized member of the human PLK family is PLK1. The activity and cellular concentrations of this kinase are crucial for the precise regulation of cell division [5] [6]. PLK1 is over expressed in a broad spectrum of cancer types, and its expression often correlates with poor patient prognosis [7]. So, drugs that target the kinase domain might not be specific to PLK1, whereas those that target the PBD are likely to be. Functional comparisons between drugs that target the two domains will help to address whether multi- or monokinase inhibitors are more beneficial for cancer patients with respect to an improved efficacy or side-effect relationship. Several reports have described the modulation of PLK1 activity by first-generation small-molecule inhibitors [8-14]. In this article, we discuss the use of PLK1 inhibitors as novel anticancer drugs. Numerous investigations have now established that PLK1 is a prime target for drug development in proliferative diseases such as cancer (Reference added) [15]. There are several reasons why PLK1 is a prime candidate. First, PLK1 is overexpressed in a broad range of human tumours. Second, the over expression of either wild-type PLK1 or kinase-inactive PLK1 (Lys82Met mutant) results in multinucleation [16].

1.1 Disease relevance of Polo like kinase-1

Polo-like kinase 1(PLK1) is a well characterized oncoprotein that is highly expressed in various cancer tissues. Polo like kinase 1 is the cancer relevant gene which attracts increasing attention in the field of cancer therapy. An increasing body of evidence suggests that the level of PLK 1 expression has prognostic value for predicting outcomes in patients with several cancers. Polo like kinases are a family of proteins highly conserved in terms of their structures and functions.PLK is involved in targeting cyclin B1, to the nucleus. The importance of PLK1 as a measure for the aggressiveness of a tumor seems to result from its important role for the mitotic checkpoints of cancer cells [17-20].No crystal structure is currently available for a PLK family kinase domain; however, given the high structural conservation of the protein kinase catalytic core in the active conformation, molecular models of kinase domains based on available crystal structures can be used to predict structural features of the active site that may be important for regulation and substrate selectivity.

2.0 MATERIALS AND METHODS

2.1 Sequence Retrieval from Swissprot

Amino acid sequences retrieved from swissprot/uniprot (www.uniprot.org) provides descriptions of a non redundant set of proteins including their function, domain structure, posttranslational modifications and variants [21] [22]. This database merges all proteins in single entry coded by one gene so as to minimize redundancy and improve reliability with fully featured information. Cross-references with others databases modemize swissprot entries to hold detailed expertise [23].The accession number of the retrieved sequence is Q58A51.

2.2 Template selection and Target Structure Modeling

Structural homologous entries were obtained for proteins through local alignment search using BlastP (Basic Local Alignment Search Tool)

[24], against Protein Data Bank (PDB) [25]. Comparison of homology models with known structure (Template) may also reveal similarities which allow biochemical and biological functions to be inferred. The alignment was used for comparative modeling to build 3D model by satisfaction of spatial restraints using Modeller9v7 [26] .The core modeling procedure begins with an alignment of the sequence to be modelled (Target) with related known 3D structures (templates). This alignment is usually input to the program. The output is a 3D model for the target sequence containing all main chain and side chain non hydrogen atoms. The model obtained after refining the protein structure was checked for its structural accuracy by the following program: Ramachandran Plot Analysis, using SAVES SERVER(Structural analysis and verification server). Ramachandran plot Analysis was performed to determine the stability of the modelled structure. Subsequently the model structure was validated using PROCHECK, which determine stereo chemical aspects along with main chain and side chain parameters with comprehensive analysis.

2.3 Target Structure prediction and Active site analysis

The 3-D crystal structure of the targeted lung cancer protein Polo Like Kinase 1, was modeled using suitable templates from the protein data bank (PDB) (www.rcsb.org/pdb). Structural and active site studies of the protein were done by using Q site finder and visualized using Rasmol software.

2.4 Preparation of ligand

Using Chemsketch Software the structures of the drugs and analogs were sketched and generated their MOL File followed by subsequent generation of their 3-D structures by using a molecule format converter tool. Fig.1. Inhibitors drawn in ACD/ChemSketch (Freeware).

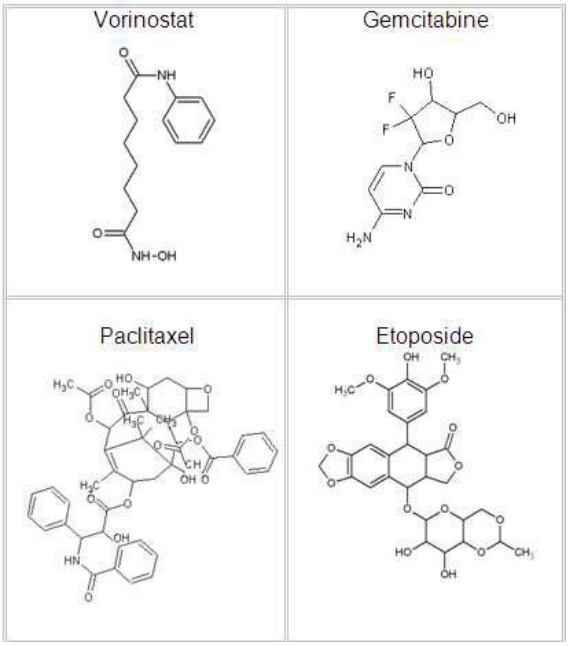

Figure1
Inhibitors drawn in ACD/ChemSketch (Freeware)

2.5 Receptor grid generation

Ligand docking jobs cannot be performed until the receptor grids have been generated. Receptor grid generation requires a prepared structure an all atom structure with appropriate bond orders and formal charges (Schrodinger, LLC).Receptor grids were calculated for polo like kinase 1 such that various ligand poses bind within the predicted active site during docking.

2.6 Active Site Prediction

After obtaining the final model, the possible binding sites of Polo like kinase 1 were searched using Q-SiteFinder (http://bmbpcu36.leeds.ac.uk/qsitefinder/).
Binding sites were obtained for Polo like kinase 1 from Q-SiteFinder. These binding sites were compared to the active site of the template to determine the residues forming the binding pocket as shown in the fig.5.

2.7. Docking using AUTODOCK

The molecular docking was performed using Auto Dock; a suite of automated docking tools. The software is used for modeling flexible small molecule such as drug molecule binding to receptor proteins of known three dimensional structures. It uses Genetic Algorithms for the conformational search and is a suitable method for the docking studies. The technique combines simulated annealing for conformation searching with a rapid grid based method of energy evaluation. Auto Dock tools are used to prepare, run and analyze the docking simulations, in addition to modeling studies. Auto Dock is the most cited docking software because it is very fast, it provides high quality predictions of ligand conformations and good correlations between inhibition constants and experimental ones. During the docking simulations, the inhibitors were regarded as flexible and subjected to an energy minimization. The ligand orientations were scored through the use of a force-field-based energy scoring function, and the top-scored binding structure was selected [27][28].

2.8. Drug Target and Inhibitor

We have listed out four potential ligands which could be promising inhibitors for polo like kinase 1namely Gemcitabine , paclitaxel, vorinostat and Etoposide. The interaction between the predicted structure of Polo like kinase 1 and its potential inhibitor is analysed *in silico* by Autodock. The vast literature review suggests specific drug targets for Polo like kinase 1 are Vorinostat, Gemcitabine, Paclitaxel and Etoposide.

3.0. RESULTS

Polo like kinase I (Q58A51) was subjected to homology search against PDB database using BlastP to identify significant structural homologs to be used as template for homology modelling. The results indicated the presence of Pkc like superfamily domain and the best homolog was 3KB7 with 99 % identity with the query protein and thus served as a template for modelling and the modelled protein obtained is shown in Fig2 and Validation was done using Ramachandran plot (Fig.3.) after loop refinement.

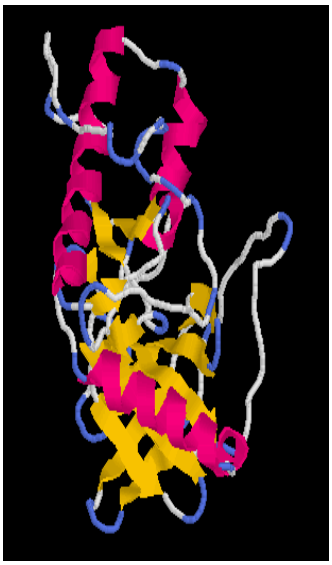

Figure 2
Structure of Polo like kinase I visualised using Rasmol

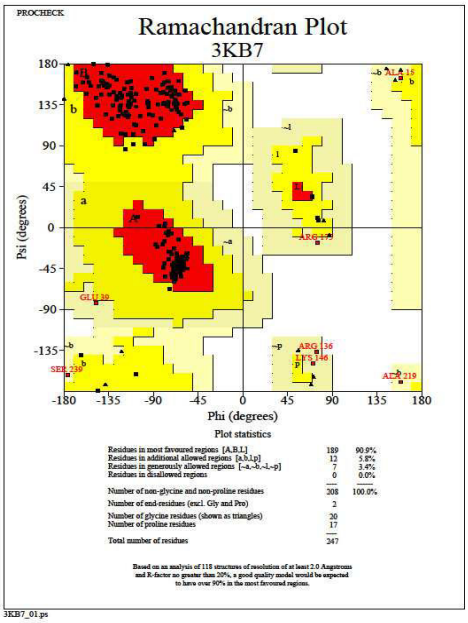

Figure 3
Ramachandran plot of Polo like kinase I

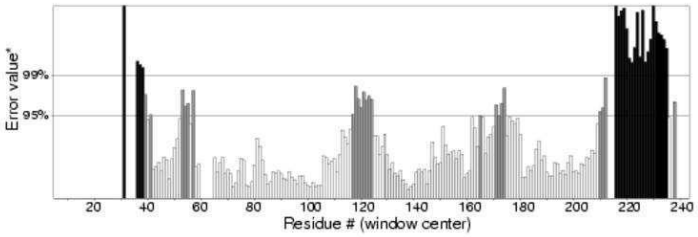

Figure 4
ERRAT result depicting overall quality factor of model protein

Geometric evaluations of the modelled 3D structure of PLK1 were performed using PROCHECK by calculating the Ramachandran plot (Fig. 2, 3 & 4). This plot represents the distribution of the phi and psi angles for the amino acid residues. The most favoured regions are coloured red; additional allowed, generously allowed and disallowed regions are shown as yellow, light yellow and white fields, respectively The plot value was found to be 90.9% in the most favoured region where as 5.8% of the amino acid residues are located in additional allowed region and 3.4% in the generously allowed region. No residues are located in the disallowed region. The statistics of non bonded interactions between different atom types were detected and value of the error function was analysed by ERRAT. Here the overall quality of the modelled structure was 75.897.

3.1 Active sites of polo like kinase 1:
***Q-SITE FINDER*:**

In order to find the inhibitors for polo like kinase 1, the active site where it binds to, on plk receptor must be known. For this purpose the structure obtained by SPDB viewer analysis was submitted to an online tool by Leeds University known as Q-Site Finder. It predicts the Active site of probes with the most favourable binding energy. Q-Site Finder allows you to upload a PDB file or select one from the Protein Database. The proteins are initially scanned for ligands Following are the active sites of Polo like kinase 1.

LEU59,GLY60,LYS61,CYS67,ALA80,LYS82,GLU101,HIS105,VAL114,LEU130,GLU131,LEU132,CYS133,ARG134,ARG135,ARG136,SER137,GLU140,GLY180,ASN181,PHE183,GLY193,ASP194,PHE195,VAL161,LEU162,CYS164,GLN1

65,LEU167,HIS168,VAL172,ILE173,HIS174,ARG175,ASP176,LEU177,PRO215,TYR217, ILE218, ALA219, PRO220, ALA221, PRO223, ARG232,LEU234

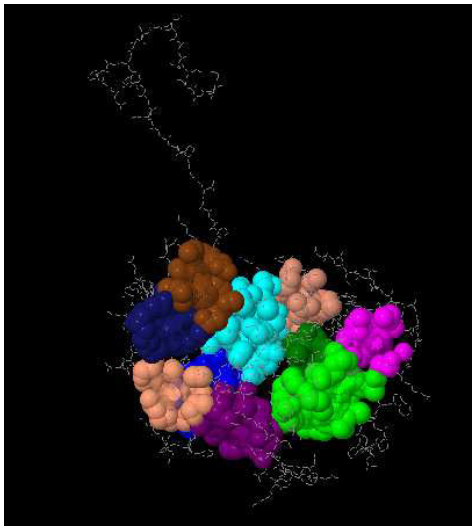

Figure 5
Ligand Binding Sites Prediction Using Q-Site Finder

Fig (5) shows the PLK1 protein and the Binding sites residue in different colors. The best binding site residues are highlighted in green and it was used for docking studies.

The vast literature review suggests specific drug targets for Polo like kinase 1 are Vorinostat, Gemcitabine, Paclitaxel and Etoposide. PUBCHEM reveals the biophysical properties of these compounds. Vorinostat has a Molecular Weight of 264.3202 [g/mol] with Molecular Formula $C_{14}H_{20}N_2O_3$,XLogP3: 1.9,H-BondDonor:3, H-BondAcceptor:3 and has SMILES notation as C1=CC=C(C=C1)NC(=O)CCCCCCC(=O)NO. Literature suggests the application of vorinostat in treatment of advanced non-small-cell lung cancer (NSCLC) that showed improved response rates and increased median progression free survival and overall survival. Gemcitabine has a Molecular Weight of 263.198146 [g/mol] with Molecular Formula $C_9H_{11}F_2N_3O_4$, XLogP3: -1.5,H-Bond Donor: 3,H-Bond Acceptor: 6 and has SMILES notation as 1=CN(C(=O)N=C1N)C2C(C(C(O2)CO)O)(F)F. Combination of gemcitabine and carboplatin has been found to be effective in treating several different types of cancer, but most commonly used to treat lung cancer. Paclitaxel has a Molecular Weight of 853.90614 [g/mol] with Molecular Formula: $C_{47}H_{51}NO_{14}$,XLogP3: 2.5,H-Bond Donor: 4,H-Bond Acceptor: 14 and SMILES notation as C1=C2C(C(=O)C3(C(CC4C(C3C(C(C2(C)C)(CC1OC(=O)C(C(C5=CC=CC=C5)NC(=O)C6=CC=CC=C6)O)O)OC(=O)C7=CC=CC=C7)(CO4)OC(=O)C)O)C)OC(=O)C. Paclitaxel is approved in the UK for ovarian, breast and lung cancers and Kaposi's sarcoma[29]. It is recommended in NICE guidance of June 2001 that it should be used for non small cell lung cancer in patients unsuitable for curative treatment, and in first-line and second-line treatment of ovarian cancer. Etoposide has Molecular Weight of 588.55658 [g/mol] with Molecular Formula $C_{29}H_{32}O_{13}$,XLogP3: 0.6,H-Bond Donor: 3,H-Bond Acceptor: 13 and has SMILES notation as CC1OCC2C(O1)C(C(C(O2)OC3C4COC(=O)C4C(C5=CC6=C(C=C35)OCO6)C7=CC(=C(C(=C7)OC)O)OC)O)O. Etoposide phosphate is an anticancer agent. It is known in the laboratory as a topoisomerase inhibitor. It is often given in combination with other drugs. SMILES notation was drawn using ACD Chemsketch and converted in to three dimensional PDB format using Molecular converter tool.The Key interacting sites of Polo like Kinase 1 are LYS61, HIS168, HIS174, ARG175, LEU177, LYS178, GLY180, ASN181, ASP194, TYR217,

and ALA219. The active site is docked with the four drug compounds. Polo Like Kinase 1 interacts with Gemcitabine forming 6 Hydrogen bonds and binds strongly with a docking score of –7.98 Kcal/mol, Paclitaxel formed 5 Hydrogen bonds with a docking score of –6.86 Kcal/mol, Vorinostat forming 1 Hydrogen bonds and docking score of –8.43 Kcal/mol, with Etoposide forming 4 Hydrogen bonds and docking score of –8.57 Kcal/mol. Therefore, it can be seen that Gemcitabine is the most effective inhibitor of Polo like kinase 1. (Fig.6.)

POLO LIKE KINASE 1				DOCKING SCORE (Kcal/mol)	H-BONDS	DOCKED STRUCTURE
LIGAND	RESIDUE	ATOM	ATOM			
GEMCITABINE	HIS174 LEU177 TYR217 ARG175 ARG175 ARG175	CG N N N	O O O O	-7.98	6	
PACLITAXEL	GLY160 ASN161 LYS178 LYS61 LYS61	O OD1 NZ O	O O O O	-6.86	5	
VORINOSTAT	ASP194	N	O	-8.43	1	
ETOPOSIDE	HIS158 ALA219 ARG175 ARG175	NE2 N O	O O H	-8.57	4	

Figure 6
Docking of Polo like Kinase 1 with potential inhibitors

These results suggest that all the four compounds are effective on their specific targets. The result of Lipinski's rule suggests that the drug targets are best therapeutic drugs. Docking study and *insilico* toxicity results proves the application of compounds as Potential and Natural Therapeutic agents to treat Lung Cancer.

4.0. DISCUSSION

Several PLK1 inhibitors have been identified so far [30-31].These include cytonemin [32], purvalanol A, and wortmannin [33].However, these have low selectivity and express similar potencies for binding with several other kinases aside from PLK1. Some PLK1 inhibitors, such as BI 536, inhibit PLK1 [34-35] by targeting its ATP binding site. The preserved structure of the site makes it difficult to selectively inhibit a specific kinase without the risk of nonspecific binding [36-37].Instead of relying on targeting the ATP binding site, this study focuses on designing a molecule which binds to the PBD of PLK1[38][39]in order to design a specific inhibitor. The goal of the docking studies was to determine the relative binding specificities and to identify any binding patterns that would help design selective PLK1 inhibitors. The PBD

mediates proper cellular positioning of PLK1 [40] which is necessary for mitosis. The PLK1 PBD consists of two polo boxes, which are symmetrical halves connected by a somewhat flexible hinge structure [41]. Since PBD is unique to polo-like kinases, this domain may be a suitable target for development of selective PLK1 inhibitors. The compounds taken are filtered according to anticancer drugs like properties (Molecular weight and Log P values) and selected those molecules which fulfilled the anticancer drug like properties. In order to find the basic range of anticancer drugs like properties, I evaluated their molecular weights and log P values. After finding the molecular weight and log P values, the compounds applied to the Lipinski rule of five for checking the drug likeliness property. The compound which satisfies the Lipinski's rule of five is taken as drug molecules and docking procedure is carried out.

The 3D structure of PLK 1 is generated by homology modeling and has selected this protein to design a specific and potent inhibitor by structure based drug designing. There are now a number of drugs whose development was heavily influenced by or based on structure-based design and screening strategies. So, Screening was carried out for finding novel ligands from Pubchem databases. The compounds docked with the protein which results in more negative energy score (kcal/mol) and more hydrogen bonds correspond to the more binding affinity. This data signifies that after further optimization process these probable leads can generate a potent inhibitor for the PLK1 protein. Nowadays, molecular docking approaches are routinely used in modern drug design to help understand drug–receptor interaction. Most biological processes are known to take place through protein-ligand interactions. The three-dimensional structure of a protein-ligand complex could serve as a significant source of understanding the way proteins interact with each other and perform biological functions. Therefore, knowing the detailed structure of protein-ligand complexes at the atomic level has been very important issues in biological sciences. However this detailed structure analysis is not an easy job. In the protein data bank [42] where experimentally determined 3D structures of proteins are stored, most of the protein structures are a single protein chain and only a small fraction (about 10 %) of the structures correspond to protein-ligand complexes. Two key elements are basically required for performing protein-ligand docking; an efficient conformational search algorithm and an accurate free energy function. The free energy function should be logically precise so that it can discriminate the native-like association of two constituent molecules from a variety of non-native associations. The search algorithm must be capable of exploring extensively the huge conformational space and can find conformations with free energy values near to the overall minimum [43]. There is always some imprecision in a free energy function. To overcome this discrepancy the search algorithm, instead of generating the conformation with lowest energy, it generates multiple low energy conformations. Based on the appropriate scoring function several structures are then selected, and proposed as candidates for the native-like structures for the complex. In our work we have done the comparative analysis of the interaction between the PLK1 protein and the ligands. Based on our results Polo Like Kinase 1 interacts with Gemcitabine forming 6 Hydrogen bonds and binds strongly with a docking score of –7.98 Kcal/mol. Therefore it can be seen that Gemcitabine is the most effective inhibitor of Polo like Kinase 1.

In most docking studies, conformational changes occur on ligand binding. This may only involve small side chain rotations to maximize interactions with the ligand [44], or the change may also be associated with small main chain movements. In extreme cases large loop movements or even domain shifts are induced on ligand binding. A more realistic goal would be a method robust enough to deal with relatively small changes in the active site when an analogous ligand binds. Hence knowledge of the protein–ligand interactions of the PLK1 protein with the specific inhibitors may give an important insight into the binding interactions and the drug relativeness. An extensive summary of currently available docking method has been presented. Comparisons suggest that the best algorithm for docking is probably a hybrid of various types of algorithm

encompassing novel search and scoring strategies. The most useful docking method will not only perform well, but will be easy to use and parametrise, and sufficiently adaptable such that different functionality may be selected, depending on the number of structures to be docked, the available computational resources, and the complexity of the problem. If the parameters cannot be generated quickly then although the algorithm may be computationally efficient, from a practical point of view it is limited. Conversely, a rapid scoring function may not necessarily be able to model some specific interactions. Algorithms that use the rigid receptor/flexible ligand approximation are well established and the most successful programs have achieved a success rate of between 70–80%. [45]

5.0. CONCLUSION

The Protein-Ligand interaction plays a significant role in structural based drug designing. Our approach in Molecular Docking analysis resulted in the identification of potential inhibitors. In the present work we have taken the Polo like kinase 1 as a key target that play a crucial role in lung cancer and identified the drugs that were used against Lung Cancer to study its efficacy. These drugs can be tested in wet lab and research can be further validated for clinical trials. This study provides an insight into the structure of polo like kinase-1 in lung cancer and also gives an idea about potential sites responsible for inhibitory action that could further be substantiated by experimental investigations. One of the molecule C1=CN(C(=O)N=C1N)C2C(C(C(O2)CO)O)(F)F. bonded specifically to PBD of PLK1. Molecules designed here formed stable bonds with PBD of PLK1. This study facilitates initiation of the drug discovery process for polo like kinase 1 to present the scientific community with better inhibitors and/or drugs thus reducing the time and cost in drug discovery process.

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