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ORIGINAL RESEARCH

Computational Design of Targeted Inhibitors of Polo-Like Kinase 1 (Plk1)

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Abstract: Computational design of small molecule putative inhibitors of Polo-like kinase 1 (Plk1) is presented. Plk1, which regulates the cell cycle, is often over expressed in cancers. Down regulation of Plk1 has been shown to inhibit tumor progression. Most kinase inhibitors interact with the ATP binding site on Plk1, which is highly conserved. This makes the development of Plk1-specific inhibitors challenging, since different kinases have similar ATP sites. However, Plk1 also contains a unique region called the polo-box domain (PBD), which is absent from other kinases. In this study, the PBD site was used as a target for designed Plk1 putative inhibitors. Common structural features of several experimentally known Plk1 ligands were first identified. The findings were used to design small molecules that specifically bonded Plk1. Drug likeness and possible toxicities of the molecules were investigated. Molecules with no implied toxicities and optimal drug likeness values were used for docking studies. Several molecules were identified that made stable complexes only with Plk1 and LYN kinases, but not with other kinases. One molecule was found to bind exclusively the PBD site of Plk1. Possible utilization of the designed molecules in drugs against cancers with over expressed Plk1 is discussed.

Keywords: Plk1 kinase inhibitors, computational drug design

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Introduction

Protein kinases alter the activity of certain proteins by phosphorylation, a process in which a phosphate group is transferred from a donor molecule (for example, ATP[1]) to an amino acid with a free hydroxyl group. The study of kinases is important due to their involvement in many diseases, including cancer.[2] The cell cycle in cancer cells is often characterized by faulty signaling pathways.[3,4] Inhibiting cell cycle regulators offers promise for cancer treatment.[5]

Protein kinases control many complex cellular processes, such as signal transduction, and are thus highly regulated.[6] They can be switched on or off through interactions with other proteins or small molecules or by altering their positions within the cell in relation to their substrates.

Many protein kinases have a highly conserved catalytic domain.[7,8] The domain consists of two lobes made of a polypeptide chain. A segment known as the "hinge region" joins the two lobes and allows them to rotate.[8] The ATP molecule binds in a cavity created by the two lobes. The structure of this cavity is highly conserved among protein kinases.[6] Since different kinases have very similar ATP binding sites,[9,10] kinase inhibitors that target the site may not be sufficiently selective.[11,12]

The Polo-like kinase (Plk) family members are important cell cycle regulators.[3,4] The four known Polo-like kinases, Plk1, Plk2, Plk3, and Plk4, all have a polo-box domain (PBD) at their C-terminus.[13] This PBD consists of either one (Plk4) or two polo-box motifs, each containing about 80 residues.[14] Regulation of Plks during the cell cycle is accomplished through their expression levels,[15,16] positions within the cell influenced by PBD,[13,17] and phosphorylation in the catalytic domain.[18,19]

A previous study[20] showed that although the Polo box domains in each member of the Polo-like kinases family were similar, they were different and had specific binding affinities. According to this study, the PBD of Polo-like kinase 1 (Plk1), which consists of two polo boxes, is most similar to the one in Plk2, but not exactly alike. Plk3 is even less similar to Plk1. Plk4 has only one polo box and does not form a PBD binding pocket that is similar to the other Plk proteins. Therefore, potential Plk1 inhibitors could distinguish Plk1 from other Polo-like kinases.

Plk1, the best-characterized of the four Plk kinases, is a main regulator of mitosis.[5,21] The roles of the other three kinases are less defined.[5,21] Plk1 belongs to a group of highly conserved serine/threonine kinases. As a main mitotic regulator, Plk1 has an important role in cell proliferation.[5,21,22]

Plk1 has been implicated in certain human cancers.[21,23,24] It has been shown that Plk1 inhibition by RNA interference leads to mitotic arrest and apoptosis of cancer cells.[25,26] Plk1 is, therefore, an attractive target for cancer drug research. Plk1, expressed only in dividing cells, does not seem to play significant role in non-mitotic processes. Thus, a Plk1 inhibitor would act selectively on all rapidly dividing cells, while avoiding many side effects related to existing anti-mitotic drugs.[27,28]

Several Plk1 inhibitors have been identified so far.[21,29] These include scytonemin,[30] purvalanol A,[29] and wortmannin.[31] However, these have low selectivity and express similar potencies for binding with several other kinases aside from Plk1. Some Plk1 inhibitors, such as BI 2536,[32,33] 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 PBD mediates proper cellular positioning of Plk1,[13,17] which is necessary for mitosis.[40] The Plk1 PBD consists of two polo boxes, which are symmetrical halves connected by a somewhat flexible hinge structure.[41,38] Since PBD is unique to polo-like kinases, this domain may be a suitable target for development of selective Plk1 inhibitors.[21]

Poloxin, Thymoquinone, and Purpurogallin (PPG) were the only reported small molecules that potentially bind PBD of Plk1.[42] Their structures were published elsewhere[42] and shown here in Figure 1. Thymoquinone[43] was found to inhibit the Plk1 PBD in vitro, albeit less selectively that Poloxin. Although previous studies[44,45] found that Poloxin selectively bonded Plk1, Liao et al[14] experimentally showed that PPG, but not Poloxin, bonded to the PBD. Both Poloxin and Thymoquinone stopped mitosis[40] in cancer cells, apparently by impeding the correct positioning of Plk1.[45] However, Poloxin did not show Plk1 PBD

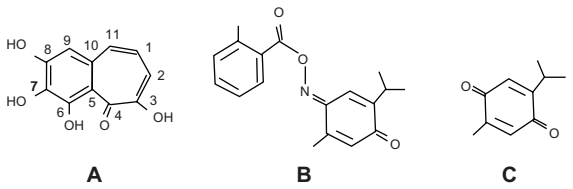

Figure 1. Structures of (**A**) PPG, (**B**) Poloxin and (**C**) Thymoquinone.

inhibition in another, ELISA-based, study.[42] PPG caused cellular delocalization of Plk1, which stopped mitosis in a way that suggested it might interrupt the Plk1 function in vivo.[46] Our goal was to computationally design and study novel druglike small molecules that would selectively bind to the PBD of Plk1, thus potentially promoting apoptosis in cancer cells.

A promising small molecule inhibitor should have toxicity, metabolic stability, and transport properties similar to existing drugs. These properties are influenced by the molecule's hydrophilicity, size, mass, flexibility, and electronic bond distributions.[47]

The molecule's hydrophilicity can be reliably evaluated[47] using its logP value (logarithm of the octanol-water partition coefficient). A low logP value indicates high hydrophilicity. It was shown previously[47] that a molecule with logP < 5 is generally well absorbed by an organism.

The absorption is also significantly determined by molecular solubility. High logS values (logarithm of the solubility measured in mol/liter, with units removed) generally indicate good absorption. More than 80% of known drugs have logS > -4 (this value corresponds to a solubility of 0.1 mmol/liter).[48] For most existing drugs $-5 <$ logS < 1.[47]

A PSA value, which is the total surface area of all polar atoms in a molecule, can predict the molecule's ability to permeate cell membranes, such as those in intestinal walls and the blood-brain barrier.[49] The PSA values of most commercial drugs are less than 120 Å^2.[49]

Molar masses of a vast majority of existing drugs are less than 500 g/moL.[48] Molecules with greater molar masses are harder to absorb. The number of rotatable bonds in a small molecule influences molecular flexibility, which in turn impacts the molecule's oral bioavailability.[50] Prior studies[50] showed that good oral bioavailability is indicated by 10 or fewer rotatable bonds. Similarly, a molecule with fewer than 6 hydrogen bond donors may be a viable drug candidate.[51]

Plk1, a main regulator of mitosis, is often over expressed in cancers.[21,52,53] In this work, we designed and studied drug like properties of putative new small molecules that would selectively bind to, and thus potentially inhibit, the polo-box domain of Plk1.

Methodology

Computational tools

The following software and servers were employed to design and evaluate molecules that could potentially inhibit Plk1 by interacting with its PBD: Protein Data Bank,[54] Deep View,[55] ArgusLab,[56] Molinspiration,[57] and Osiris Property Explorer.[48]

Information on experimental structures and functions of known molecules was obtained from Protein Data Bank (PDB). The Deep View program was employed to visualize and compare molecular structures. Molecular design and docking studies were done using the Argus Lab program.

The numbers of H-bond donors and rotatable bonds were obtained using the Molinspiration program. Osiris Property Explorer was employed to calculate drug like properties of designed molecules and to estimate their possible mutagenic, tumorigenic, or reproductive risks. When a designed molecule consists mostly of fragments frequently found in known drugs, the molecular Drug Likeness value calculated by the Osiris program is positive. The techniques and validation studies used to evaluate drug like properties by the Osiris and Molinspiration programs are given elsewhere.[57,58]

The Osiris program can alert if a molecule is likely to be toxic. Although the absence of an alert does not guarantee that a molecule is non-toxic, Osiris is a good prognostic tool. When tested on compounds known to cause mutations, Osiris properly indicated that 86% of them were toxic. Similarly, when tested on known drugs, the program predicted that only 12% of them were possibly toxic.[58]

No single drug like property guaranties that a molecule will be a viable drug candidate. Rather, a promising drug candidate should have optimized values of all drug like properties and no indicated toxicities. Osiris predicts overall molecular drug potential by using the values of logP, logS, molecular mass, Drug Likeness, and toxicity risks together to calculate the Drug Score of the molecule[58]

Dataset

Experimentally-known three-dimensional molecular structures of the protein kinases were obtained from Protein Data Bank (their PBD ID's are given in parentheses): Plk1 (1UMW); ABL (3NSV), AKT (3O96), CDK2 (3LFS), CHK1 (1IA8), CLK1 (1Z57), GSK3b (1H8F), LCK (3MPM), LYN (3A4O), NEK2 (2W5A), and PKA (3AGM). The structures of Purpurogallin (PPG), Poloxin, and Thymoquinone are shown in Figure 1.

Procedure employed

PPG, Poloxin, and Thymoquinone were docked in ArgusLab[56] to each of the 11 kinases. 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.

PPG was the most selective of the three molecules tested. It was found to bind only to Plk1 (at PBD) and LYN kinases. Thymoquinone and Poloxin formed more stable bonds with other kinases than with Plk1 and showed less selectivity than PPG. Different Plk1 binding environments are utilized for PPG, Poloxin and Thymoquinone interactions.[42] PPG binds to the PBD of Plk1, while Poloxin and Thymoquinone bind to the active, ATP-binding site of Plk-1. These are some of the reasons that PPG was chosen as a starting template to design new small molecules that would bind specifically to PBD of Plk1.

The binding environments of Plk1 PBD and LYN were carefully studied in the Deep View program[55] by examining properties of all amino acid residues within 10 Å of the PPG binding sites. The goal was to search for any chemical differences between the sites that could be exploited to design a molecule specific for Plk1 PBD that would not bind LYN or any other kinase.

The binding environments of LYN and the PBD of Plk1 were found to be remarkably similar to each other, introducing a challenge. Both binding sites contained 2 polar and neutral residues, 2 acidic residues, 1 or 2 basic residues, and 5 hydrophobic residues arranged similarly. The identities of these amino acids were also very similar. Both Plk1 and LYN contained one each of serine, glycine, lysine, leucine, phenylalanine, and aspartic acid residues. This made it difficult to target specific residues in the PBD for binding specificity.

However, there were spatial differences between the two sites which could be exploited (Fig. 2). Atomic distances between corresponding binding residues within each binding site were measured in Deep View. It was found that relevant atomic distances within the PBD Plk1 binding site were greater than those within LYN by approximately 44%–67% (see Fig. 2).

Therefore, a larger ligand could potentially fit into this space in Plk1 and would be excluded from LYN's smaller binding site. The flexible hinge structure of the PBD should also aid in allowing a larger ligand to fit into this space. LYN has no such flexible hinge structure and is therefore less likely to be able to change its conformation easily to accommodate a larger ligand.

Atoms with larger radii than those on the hydroxyl groups on PPG were added or substituted in the PPG molecule to create a larger molecule. Atoms were carefully chosen to achieve two goals: a sufficient increase in the size of the molecule without drastically decreasing its drug likeness by mass increase.

The Osiris and Molinspiration programs were employed to test drug like properties of the designed molecules. The programs assessed each molecule's mass, logP value, solubility, number of rotatable bonds, and hydrogen bond donors. Osiris also alerted for potential toxicities or mutagenicities and calculated a Drug Score for each molecule.

Three-dimensional structures of the designed molecules were optimized using the Molecule Builder and Semi empirical Geometry Optimization functions in the Argus Lab program. Docking of the molecules to the 11 kinases was done using the Argus Dock engine with flexible setting that permitted rotations of molecular bonds. Other docking parameters were: 0.4 Å grid resolution; AScore scoring function; and ascore.prm parameter set. ArgusLab calculated binding energies of stable configurations a ligand could assume when docked to a given kinase.

Results and Discussion

Atomic substitutions and additions in the side groups of PPG were made using the Osiris program to design molecules with improved drug like properties, better selectivity, and higher binding affinity to Plk1. Substitutions of the –OH group bonded to the carbon in position 1 (Fig. 1) yielded favorable increase of drug scores. In particular, Cl and I substitutions

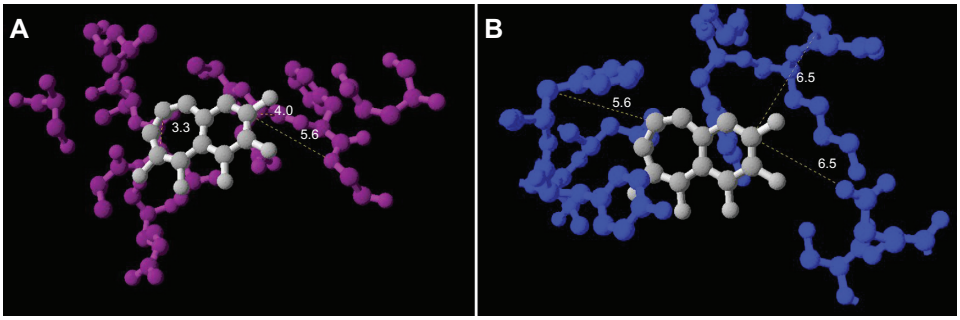

Figure 2. PPG (gray) binding to (**A**) Plk1 PBD (pink) and (**B**) LYN (blue) with relevant atomic distances shown.

at this position resulted in the increase of drug score by over 150%. On the other hand, substitutions with Br and F lowered drug likeness scores. Substitutions of the same ─OH group with Group 1A metals were also similarly favorable in terms of drug scores, but did not afford improved selectivity to Plk1.

Substitutions of the ─OH group by Si, P, and S all yielded lower drug likeness values and lower drug scores. Most drug likeness values were between −1 and −5. Drug scores were around 50%. Substitutions with C, H, and N gave drug scores of around 0.76 and drug likeness between 44%–64%.

Additions and substitutions of side-chains bonded to carbon atoms at positions 2–7 and 9–11 (Fig. 1) did not lead to improved drug like or selectivity properties of designed molecules. Addition of methyl and hydroxyl groups at position 8 yielded drug likeness scores of 40% and 17%, respectively. Additions of Si, S, and P at this position gave drug scores of less than 50% and drug likeness values between −1 and −6. The most favorable improvement of both drug like properties and Plk1 selectivity was achieved by simultaneous additions of side chains at position 8 and substitutions of the ─OH group at position 1, as shown in Figure 3.

Figure 3 shows the structures of ten designed small molecules, identified as having suitable drug like properties and binding only to PBD of Plk1, and possibly to LYN. Letters A–J designating each molecule in Figure 3 correspond to letters of designed molecules in Table 1. For comparison, the structure of PPG is shown in Figure 1.

The first five molecules in Figure 3 (A through E) are the most specific and drug like putative Plk1 inhibitors. Their chemical compositions are: $IC_{11}H_7O_4ONCl$ (molecule A); $IC_{11}H_6O_4OCl$ (molecule B); $ClOC_{11}H_6O_4I$ (molecule C); $NH_2OC_{11}H_6O_4I$ (molecule D); and $IOC_{11}H_6O_4OCl$ (molecule E). Molecules F through J are less specific to Plk1. Their chemical compositions are $N(CH_3)2C_{11}H_7O_4$ (molecule F); $NH_2C_{11}H_6O_4$ (molecule G); $CH_3C_{11}H_7O_4$ (molecule H); $ClC_{11}H_7O_4$ (molecule I); and $IC_{11}H_7O_4$ (molecule J). Only molecule A was predicted to bind exclusively to Plk1 and to no other tested kinase.

As can be seen in Figure 3, all ten designed molecules have the same basic double-ring structure as PPG, with different side-chain substitutions. Molecules A–E, which are more specific to the PBD of Plk1, have six side groups. Molecules F–J are less specific to the PBD of Plk1 and have five side groups. This is in agreement with the PBD Plk1 binding site being more spacious than the binding site of LYN.

The values of drug like properties of PPG and the ten designed molecules and their binding energies

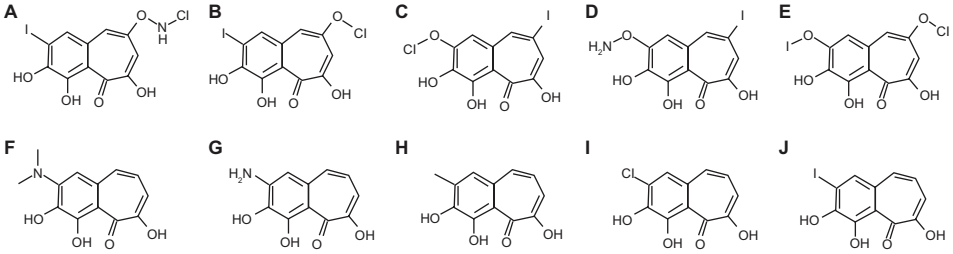

Figure 3. Structures of the ten designed molecules that bonded only to PBD of Plk1, and possibly to LYN kinase.

Table 1. Drug-related properties of the designed molecules.

Molecule	logP	logS	PSA (Å²)	MM (g/moL)	nrotb	nON	nOHNH	Drug likeness	Drug score	Energy[a] (kJ/moL)
A	3.26	–2.81	99.0	395	2	6	4	0.75	70%	–37.5
B	3.50	–3.48	87.0	380	1	5	3	0.75	68%	–26.5
C	3.50	–3.12	87.0	380	1	5	3	0.81	71%	–26.5
D	2.30	–3.85	113	360	1	6	5	0.97	69%	–27.0
E	3.46	–3.16	96.2	397	2	6	3	0.36	66%	–25.7
F	1.93	–2.44	81.0	247	1	5	3	1.9	87%	–37.2
G	1.31	–2.48	104	219	0	5	5	0.64	78%	–38.2
H	2.28	–2.75	77.8	218	0	4	3	0.44	74%	–43.8
I	2.50	–3.14	77.8	239	0	4	3	0.56	73%	–44.0
J	2.91	–3.42	77.8	330	0	4	3	1.1	72%	–44.7
PPG	1.31	–2.11	97.98	220	0	5	4	0.47	46%	–38.3

Note: [a]Binding energy of the most stable complex of Plk1 PBD with the designed molecules and PPG.

with PBD of Plk1 are given in Table 1. The Osiris Property Explorer and Molinspiration programs were used to calculate the values of logP, logS, Drug Likeness, Drug Score, and toxicity risks. Similar values for the molar masses (MM), logP, and PSA were predicted by both programs. The number of rotatable bonds (nrotb), H-bond acceptors (nON), and H-bond donors (nOHNH) were calculated with Molinspiration. Toxicity risks and the values of Drug Likeness and Drug Score were predicted by Osiris. The ten designed molecules had no indicated toxicity risks and optimal values for their drug-related properties.

For all designed molecules, logP $\leq$ 3.50 and $-3.85 \leq$ logS ≤ -2.44. As discussed in the Introduction section, these values are well within the established ranges for known drugs. The values for the polar surface area (PSA) of all ten molecules were between 77.8–113 Å². This is also within the "drug like" range (<120 Å²). All ten molecules had molar masses between 218–397 g/moL. They also had 2 or fewer rotatable bonds, 5 or fewer H-bond donors, and between 4–6 H-bond acceptors. All 10 molecules had positive Drug Likeness values, ranging from 0.44 to 1.9. The Drug Score values were between 66%–87%. As discussed earlier, these values should dependably predict[47,49,50] the overall drug potential of the designed molecules.

As described in the Methodology section, the Argus Lab program was then used to dock the designed molecules to the studied kinases. Binding energies of the complexes formed by the designed molecules and PBD of Plk1 are given in Table 1. More negative binding energies indicate more stable complexes. As can be seen from Table 1, the binding energies ranged between –44.7 and –25.7 kJ/moL. These values are comparable to the binding energies of –38.3 kJ/moL and –21.2 kJ/moL obtained for the PPG-Plk1 and PPG-LYN complexes, respectively.

The docking calculations showed that only molecule A (Fig. 3) was exclusively selective for PBD of Plk1. This molecule did not form stable configurations with any of the other 10 tested kinases, including LYN. Since the binding site of LYN is smaller than the binding site of Plk1, molecule A was designed to be too big to fit into the LYN binding site, but small enough to fit into the Plk1 binding site.

The binding energy of the most stable configuration of molecule A and PBD of Plk1 was –37.5 kJ/moL (Table 1). For molecule A, the logP value was 3.26, solubility –2.81, the PSA value 99.0 Å², molecular mass 395 g/moL, and its drug likeness 0.75. The molecule also had 2 rotatable bonds (nrotb), 6 H-bond acceptors (nON), and 4 H-bond donors (nOHNH). These factors combined to give a drug score of 70%. Figure 4, created in the Deep View program, shows molecule A bound to the PBD of Plk1.

Molecule B (Fig. 3) was similar in structure but slightly smaller in size than molecule A. However, it had only one stable pose when docked to LYN, compared to a dozen stable poses when docked to Plk1. Its binding energies with LYN and the most stable pose with Plk1 PBD were calculated to be –18.5 kJ/moL and –26.5 kJ/moL, respectively.

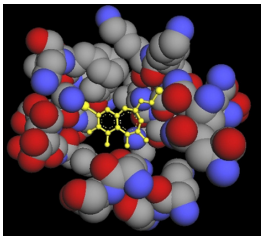

Figure 4. The designed molecule A (yellow ball-and-stick) bound to the PBD site of Plk1 (blue, red, and gray balls).

The other eight designed molecules, C–J in Figure 3, were less selective. However, they all bonded more favorably to Plk1 than to LYN. None of them bonded to any of the other nine studied kinases.

Molecules C, D, and E in Figure 3 bonded LYN with a relatively small number of stable configurations, making it less likely that these reactions would occur in an actual cellular environment. LYN made only 2 stable poses with each of the molecules C and D and 6 stable poses with molecule E.

Molecules F–J in Figure 3 formed numerous stable conformations with both Plk1 and LYN and were less specific than the first five molecules. However, each of these molecules had good drug scores and bonded more favorably to Plk1 than to LYN, but not to any of the other nine kinases.

All ten designed molecules bonded Plk1 at its PBD binding site, the same as PPG.[14] These findings suggest that the molecules might be able to interrupt the Plk1 cellular function in a similar manner as PPG.

PBD of Plk1 is as a phosphoresidue-binding domain. PBD binds to a phosphopeptide by employing hydrogen bonds.[59] The designed molecules presented in this paper are predicted to interact with the PBD not with hydrogen bonds, but via pi-pi stacking due to the aromatic ring structures found in each designed molecule. In these hydrophobic interactions, water is displaced from the binding pocket and is replaced by the ligand.[60] This is more energetically beneficial than the hydrogen bonding which would occur between the PBD and a phosphopeptide. As a result, the designed molecules are predicted to "oust" any phosphopeptide and thus interfere with the Plk1function. By preventing phosphopeptide binding of the PBD, centrosomal localization is prevented.[38]

The designed molecule A (Fig. 3) selectively binds the PBD of Plk1, has acceptable values of druglike properties, and no indicated toxicities. It is also structurally similar to PPG, which is known to bind PBD of Plk1. Therefore, molecule A may warrant further experimental studies to determine its potential usefulness as a drug.

Conclusions

In this research, several novel drug like small molecules were computationally designed. Studies indicate that the molecules could potentially make stable complexes with PBD of Plk1. One of the molecules, IC11H7O4ONCl, bonded selectively to Plk1 and to no other studied kinases.

All ten molecules were found to have drug like properties, such as $\log P \leq 5$, $-5 \leq \log S \leq 1$, PSA < 120 Å^2, molar masses below 500 g/moL, fewer than 10 rotatable bonds and hydrogen bond acceptors, and fewer than 6 hydrogen bond donors. All of the molecules had positive values of Drug Likeness and overall Drug Scores between 66%–87%. They all produced stable complexes with PBD of Plk1, and possibly with LYN. The binding energies of the complexes with Plk1 were between –44.7 and –25.7 kJ/moL. These results, coupled with prior experimental studies of PPG, strongly suggest that the molecules designed here might be good candidates for designing drug like Plk1 inhibitors.

One of the designed molecules ($IC_{11}H_7O_4ONCl$) bonded specifically to PBD of Plk1. Drugs based on this molecule could be useful against tumors with over expressed Plk1. Such drugs could selectively bind this kinase and thus lead to fewer side effects than a less selective drug.

Molecules designed here formed stable bonds with PBD of Plk1 in silico. While laboratory syntheses of the molecules have not been done, it should be noted that an experimentally known molecule, PPG, was used as a model to design all ten molecules. The merits of the designed molecules for anticancer applications eventually needs to be evaluated in vitro and, if warranted, in vivo.

Author Contributions

Conceived and designed the experiments: KSJ, DSD. Analyzed the data: KSJ. Wrote the first draft of the manuscript: DSD. Contributed to the writing of the manuscript: KSJ, DSD. Agree with manuscript results

and conclusions: KSJ, DSD. Jointly developed the structure and arguments for the paper: KSJ, DSD. Made critical revisions and approved final version: KSJ, DSD. All authors reviewed and approved of the final manuscript.

Disclosures and Ethics

As a requirement of publication author(s) have provided to the publisher signed confirmation of compliance with legal and ethical obligations including but not limited to the following: authorship and contributorship, conflicts of interest, privacy and confidentiality and (where applicable) protection of human and animal research subjects. The authors have read and confirmed their agreement with the ICMJE authorship and conflict of interest criteria. The authors have also confirmed that this article is unique and not under consideration or published in any other publication, and that they have permission from rights holders to reproduce any copyrighted material. Any disclosures are made in this section. The external blind peer reviewers report no conflicts of interest.

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