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Review

Molecular and enzoinformatics perspectives of targeting Polo-like kinase 1 in cancer therapy

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ABSTRACT

Cancer is a disease that has been the focus of scientific research and discovery and continues to remain so. Polo-like kinases (PLKs) are basically serine/threonine kinase enzymes that control cell cycle from yeast to humans. PLK-1 stands for 'Polo-like kinase-1'. It is the most investigated protein among PLKs. It is crucial for intracellular processes, hence a '*hot*' anticancer drug-target. Accelerating innovations in Enzoinformatics and associated molecular visualization tools have made it possible to literally perform a '*molecular level walk*' traversing through and observing the minutest contours of the active site of relevant enzymes. PLK-1 as a protein consists of a kinase domain at the protein N-terminal and a Polo Box Domain (PBD) at the C-terminal connected by a short inter-domain linking region. PBD has two Polo-Boxes. PBD of PLK-1 gives the impression of "a small clamp sandwiched between two clips", where the two Polo Boxes are the 'clips' and the 'phosphopeptide' is the small 'clamp'. Broadly, two major sites of PLK-1 can be potential targets: one is the adenosine-5′-triphosphate (ATP)–binding site in the kinase domain and the other is PBD (more preferred due to specificity). Targeting PLK-1 RNA and the interaction of PLK-1 with a key binding partner can also be approached. However, the list of potent small molecule inhibitors targeting the PBD site of PLK-1 is still not long enough and needs due input from the scientific community. Recently, eminent scientists have proposed targeting the 'Y'-shaped pocket of PLK-1-PBD and encouraged design of ligands that should be able to concurrently bind to two or more modules of the 'Y' pocket. Hence, it is suggested that during molecular interaction analyses, particular focus should be kept on the moiety in each ligand/drug candidate which directly interacts with the amino acid residue(s) that belong(s) to one of the three binding modules which together create this Y-shaped cavity. This obviously includes (but it is not limited to) the 'shallow cleft'-forming residues i.e. Trp414, H538 and K540, as significance of these binding residues has been consistently highlighted by many studies. The present article attempts to give a concise yet critically updated overview of targeting PLK-1 for cancer therapy.

1. Introduction

Cancer as a disease continues to remain the focus of scientific research and discovery [1–3]. PLK-1 stands for 'Polo-like kinase-1'. It is a crucial protein for intracellular processes, hence an interesting anticancer drug-target. Polo- like kinases (PLKs) are basically serine/threonine kinase enzymes that control cell cycle from yeast to humans [4,5]. In mammals, four different PLK family members have been identified: PLK-1, PLK-2, PLK-3 and PLK-4, which are structurally homologous. PLKs have a catalytic kinase domain at N-terminal and

Abbreviations: PLKs, Polo like kinases; PBD, Polo-Box-Domain; PLK-1, Polo-like kinases-1; PBD, Protein Data Bank

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polo-box domains (PBDs) at C-terminal and a short linking region connecting them [6]. PBDs regulate the phosphorylation, substrate binding, and localization of PLK; and therefore are favorite domain to experiment with when one wants to inhibit PLK [7]. Although PLK5 has also been identified, but as it lacks a kinase domain, it does not seem to function in cell cycle regulation [8]. The present article attempts to give a concise yet critically updated overview of targeting PLK-1 for cancer therapy with a focus on the PBD site.

2. Polo-like kinase 1 (PLK-1) and its association with cancer

As stated, PLK-1 belongs to the serine-threonine kinase family of proteins that play a key role in the regulation of crucial intracellular processes. This list includes DNA replication, mitotic cell division and also response to stress [9]. Among the PLK family members, it is PLK-1 that is the most exhaustively studied and is perceived as a '*hot*' oncology target since its overexpression is seen in many kinds of tumors [10,11]. PLK-1 plays an important role in the progress of the cell cycle in the cells that are dividing normally. At the same time, PLK-1 is over-expressed in many cancerous cells, be it breast, colorectal, prostate, pancreatic, ovarian, or of other types [12,13]. PLK-1 expression is low during interphase in normal cells, while PLK-1 gets localized to the nucleus in cancer cells [13], although remaining low in surrounding normal, non-dividing tissue. However, no chromosomal translocations or mutations have been found in the PLK-1 gene [13] and therefore the tumorigenic potential of PLK-1 cannot be attributed to them. One way that PLK-1 may be employing to direct cells on a cancerous path might be by regulating p53 protein. This has been suggested to occur via two additional targets of PLK-1, namely 'G2 and S phase expressed protein 1' or 'GTSE1' and 'DNA topoisomerase 1 binding protein' or 'Topors' [14,15]. GTSE1 acts as a negative regulator of p53. It directly attaches and shuttles p53 out of the nucleus which leads to its degradation thereby permitting for G2 checkpoint recovery [15]. It has been shown that PLK-1 phosphorylates GTSE1 and promotes its nuclear localization. This in turn facilitates the 'GTSE1-p53-interaction' thereby resulting in checkpoint recovery. Topors, basically possesses a dual function for p53. First, it acts as an 'E3 ubiquitin-protein ligase'. Secondly it is also an 'E3 SUMO1-protein ligase' for p53. Here, p53-ubiquitination leads to its degradation whereas sumoylation of the same leads to an increase in its stability [14]. Activity of Topors gets modulated due to its phosphorylation by PLK-1. This modulated state of Topors ultimately leads to a decrease in sumoylation of p53 and an increase in ubiquitination of the same. The essence of the above facts is that PLK-1 is a significant negative regulator of both p53 protein stability and nuclear localization [14,15]. The p53 protein is a tumor suppressor. It is a critical regulator of DNA damage checkpoints and cell cycle control. Hence, negative regulation of p53 by PLK-1 may very well be a potential mechanism for the tumorigenic potential of PLK-1. In this context, depletion of PLK-1 using small interfering RNA in human cancer cell lines was characterized by activation of the p53 pathway and resulted in apoptosis [16–18]. Importantly, normal cells were shown to be largely unaffected by PLK-1 depletion in these studies, supporting the feasibility of PLK-1 as a potential target for cancer therapy [16–18]. Preclinical data already demonstrates that PLK-1 is a valid target for cancer therapy. While normal human cells are largely unaffected by loss of PLK-1, apoptosis is seen in cancer cells [18].

3. Enzoinformatics insight into the structure of PLK-1, a '*molecular level walk*'

With the advancement of Bioinformatics and accelerating innovations in molecular visualization tools it has become possible to literally perform a '*molecular level walk*' traversing through and observing the minutest contours of the active site of relevant enzymes. Here, it is pertinent to explain the scientific term, ENZOINFORMATICS, since the term is a relatively new one. The term was coined in the year 2012 by Shakil and Kamal [19]. As for now, the term has been widely accepted by the global scientific community with an array of scientific papers citing this term [2,20–24]. Precisely, it is defined as "*A sub-discipline of Bioinformatics which specifically deals with computational enzyme-ligand binding studies*" [19,20]. This in turn involves (but is not limited to) much discussion of structural features crucial to successful molecular interaction between the two. In this context, it is important to have information about the sequence and functionally important regions of PLK-1. The human PLK-1 gene encodes a polypeptide having 603 amino acid residues (Fig. 1). As stated, the PLK-1 protein consists of a kinase domain at the protein N-terminal and a PBD located at the C-terminal connected by a short inter-domain linking region. The kinase domain is obviously a serine-threonine kinase domain in accordance with the protein family. This domain is a highly conserved one. It is composed of 252 amino acid-residues and extends up to 303 amino acid position [10,25,26]. Three amino acid residues of the kinase domain, namely Lys82, Glu131, and Asp194 are regarded as crucial to ATP-binding. Another noteworthy feature of the kinase domain is the presence of T-loop involving threonine at 210th position. As expected, T-loop-phosphorylation is unswervingly linked to the kinase activity of the protein [27]. The 'activation loop' and the 'D-box' (responsible for targeting the protein for proteasomal-degradation in anaphase) have been shown to cover amino acid residue positions 194–221 and 337–340, respectively as per the Uniprot entry P53350 for human PLK-1 (http://www.uniprot.org/uniprot/P53350#family_and_domains; accessed on 16-09-2017). In a 2007 study, Kothe et al. described the X-ray co-crystal structure of the catalytic domain of human PLK-1 (T210V; 2.1 Å) bound to the pyrrolo-pyrazole inhibitor PHA-680626 [28]. PLK-1 was observed to display the typical fold resembling the kinase domain folds of other kinases. The authors also compared the kinetic parameters for the wild-type PLK-1, and also the mutants (T210V and T210D). It was found that the mutations primarily affect the kcat value and there was a small alteration in the apparent Km [28]. Further to this, the authors also highlighted distinct active site hotspots which could be targeted to design PLK-1 inhibitors possessing due 'specificity' and 'selectivity' over other kinases as well as the PLK variants. Importantly, these hotspots included phenylalanine residue (located at the bottom of the ATP pocket), a cysteine residue (located in the pocket rooftop), a subsite produced due to L132 (the hinge region-residue), and a bunch of amino acid residues bearing positive charges located in the protein surface which was exposed to the solvent near the hinge area [28]. We downloaded the concerned pdb file [PDB ID: 2OWB] and explored these hotspots using 'show ligand binding' command of 'ligand interactions' under 'scripts' menu of Discovery Studio Visualizer 4.1 (Biovia-Accelerys). Fig. 2 shows the ligand (PHA-680626) within the kinase domain of human PLK-1 (Fig. 2). The 'roof' of the ATP-binding pocket is formed by C67, while the residue F183 contributes to its 'bottom' or 'floor'. K82 and E131 which represent two of the three crucial ATP-binding residues of the kinase domain are clearly marked while the third residue (D194) is not visible. The 'hinge-region' is formed by L132. Two residues namely F58 and R134 have been displayed in a rectangular box (Fig. 2). This has been done to highlight their importance as among all the amino acid residues that line the ATP pocket, these residues are unique to PLK-1.

The PBD which is located at the protein C-terminal is a salient feature of the PLKs. The PBD of human PLK-1consists of around 60–70 amino acid residues (number of amino acids may vary to some extent). Hence this domain is less conserved. There are two Polo-Boxes in the PBD with a flexible 'connecting-region' in between. Polo box 1 extends from 411th to 489th amino acid position while Polo Box 2 location extends from amino acid positions 511–592. The phosphopeptide binding is aided by one residue (Trp414) located in Polo Box 1 and two residues (His538, Lys540) located in Polo Box 2; within the PBD of PLK-1. In fact, the protein (PLK-1) could make binding interactions with phosphopeptide of other proteins via the PBD. In the scenario, whilst PLK-1 comes at a specified cell position, the kinase domain is released

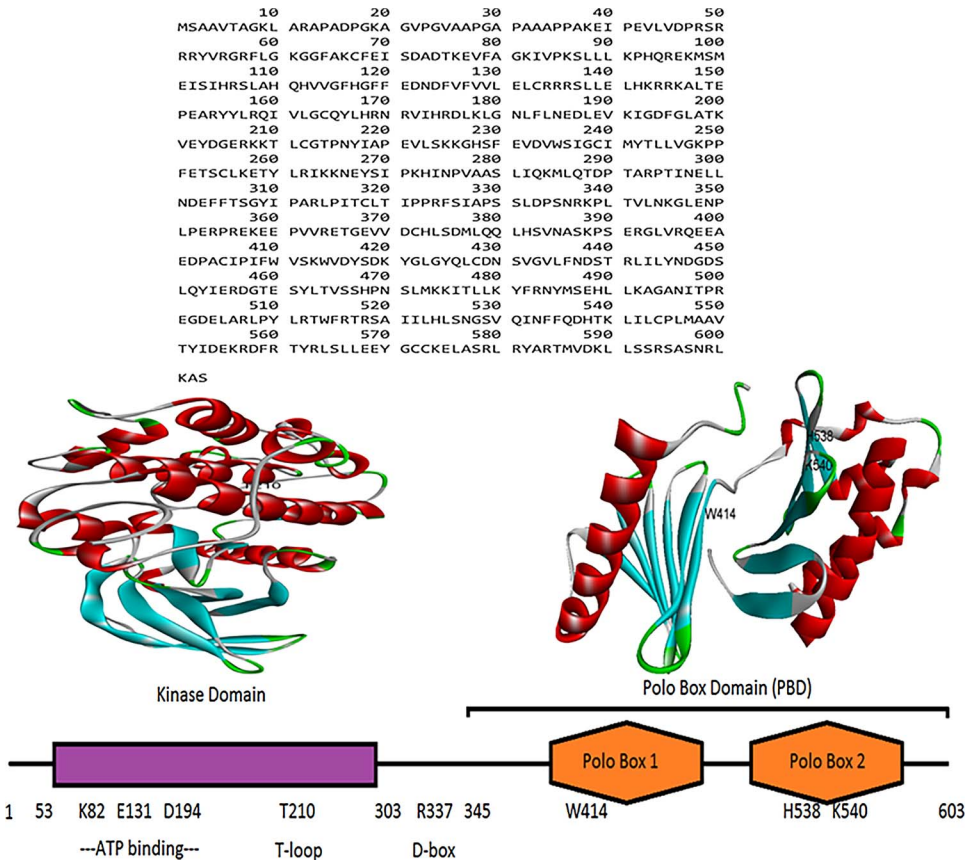

Fig. 1. A representative illustration including linear amino acid sequence of human PLK-1, schematic diagram and 3-D structure of Kinase Domain and Polo-Box-Domain.

as it interacts with different phosphopeptide. Accordingly, varied protein molecules and also varied moieties within a single protein may get phosphorylated. Normally, the PBD present at the C-terminal is kept in an interacting state to the kinase domain present at the N-terminal to obstruct the T210-phosphorylation. In this manner, the kinase activity remains inhibited. However, PLK-1 gets activated by the ligand binding of the PBD [29]. In a noteworthy 2003 study, Cheng et al. had described structure of PBD of PLK-1 by X-ray crystallography covering a residue range of 367–603 amino acids at a resolution of 2.2 Å and that of a phosphopeptide-PLK-1-PBD complex (residues 345–603; 2.3 Å resolution) [30]. The authors reported that the structure of the two polo boxes displayed identical folds based on a 6-stranded β-sheet and an α-helix, although the sequence identity was mere 12%. The phosphopeptide was observed stuck between the two polo boxes as it made a short anti-parallel β-sheet connection as well as key interactions to four amino acid residues, namely Trp414, Leu490, His538 and Lys540 [30]. We analyzed the published crystal structure of Polo-box Domain of Polo-like kinase 1 reported in 2015 by Ahn et al. [31] by various functions of Discovery Studio Visualizer 4.1 (Biovia-Accelerys). The corresponding Protein Data Bank (PDB) reference is 4WHH. We specifically generated a '2-D-binding diagram' for the PDB crystal (4WHH) using the 'ligand interactions' function of the said software (Fig. 3). A layman look at the three dimensional molecular structure (kindly refer to the crystal 4WHH available in Protein Data Bank) of PBD of PLK-1 gives the impression of "a small clamp sandwiched between two clips", where the two Polo Boxes are the 'clips' and the 'phosphopeptide' is the small 'clamp'. Trp414, His538 and Lys540 which are significantly crucial amino acid residues for ligand interaction with the shallow cleft of the PBD of PLK-1 are clearly labelled in the figure (Fig. 3). It is worth mentioning that there are almost 69 structures of PLK-1 available on PDB of different organisms in complex with different ligands. Hence, a comparative analysis is warranted to enhance the importance of specified regions and residues. Accordingly, the trend of interactions for binding of different ligands with the kinase domain and the PBD of PLK-1 accompanied by relevant illustrations has been described in the following sections of the article. This has been done as per the expert suggestion of a learned referee during the revision process of this manuscript.

4. Strategies for inhibition of PLK-1

For treating cancer, proliferation of cells has to be stopped and cell death needs to be inducted. Although antimitotic therapy is standard for many cancer types, but there is no selectivity involved, which results in adverse effects, like neurotoxicity and myelosuppression [12]. Broadly, two major sites of PLK-1 can be potential targets: one is the adenosine-5′-triphosphate (ATP)–binding site in the kinase domain and the other is PBD [32]. Targeting PLK-1 RNA and the interaction of PLK-1 with a key binding partner can also be approached. Furthermore, there is compelling evidence regarding the huge significance of PBD-phosphopeptide-interaction as a PLK-1 targeting-strategy [33]. This very interaction is held critical both for substrate-recognition as well as PLK-1-regulation [33]. Several scientists are working across the globe to discover potent PLK-1-inhibitors against kinase domain [10,34,35] and PBD binding site as well [36]. However, the list of potent small molecule inhibitors targeting the PBD site of PLK-1 is still not long enough and needs due input from the scientific community [10]. Here, we would discuss each strategy in separate subheadings for easy comprehension.

4.1. The RNAi approach

As of now, PLK-1 has established itself as a promising therapeutic target against cancer. Knocking it out by various strategies could lessen survival of the cancerous cells, cause apoptotic induction as well as enhance sensitivity towards chemotherapeutic agents, albeit, at the cost

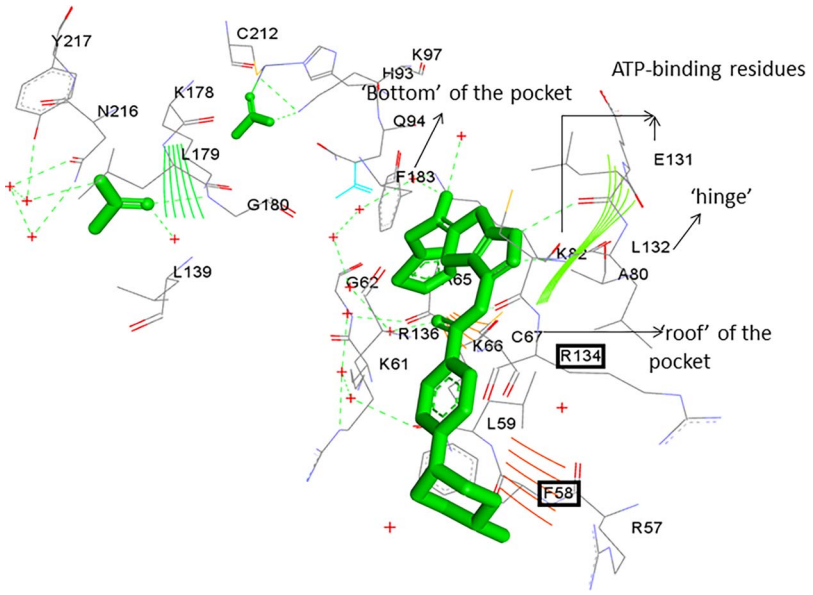

Fig. 2. Molecular interaction of PHA-680626 with the kinase domain of human PLK-1. The residues forming the 'roof', 'floor' and 'hinge region' of the ATP-binding pocket are clearly marked. Residues unique to PLK-1 ATP groove are displayed in a rectangular box.

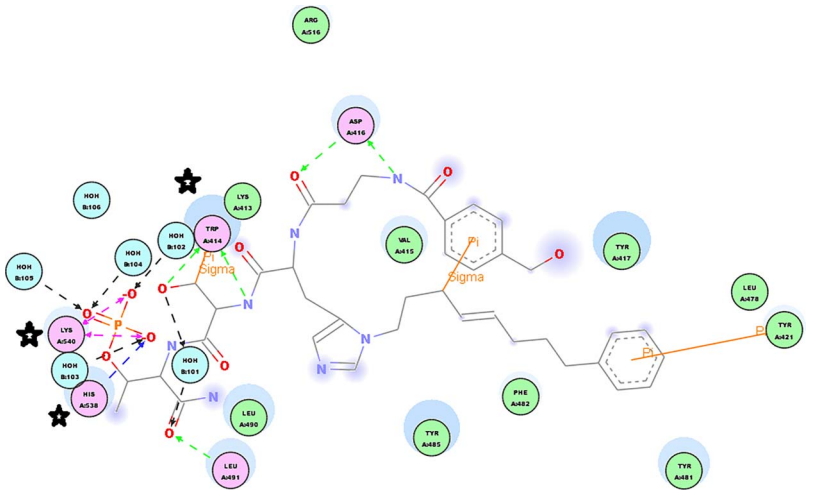

Fig. 3. '2-D-binding diagram' corresponding to the PDB crystal (4WHH) generated by Discovery Studio Visualizer 4.1 (Biovia-Accelerys) using the 'ligand interactions' function. The three important binding residues belonging to Polo Box Domain of PLK-1 (Trp414, located in Polo Box 1; His538 and Lys540, located in Polo Box 2) are marked by 'star' shapes in the figure.

of small effect on normal cells [37–40]. In a 2017 study by Wright et al. [41] the authors concluded that a combination of RNAi with AZD1775 treatment suggested PLK-1 as one of the most pertinent proteins for facilitating anticancer functions of the aforementioned inhibitor [41]. Previously also, many authors have highlighted that RNAi mediated inhibition of PLK-1 expression/function as an effective approach to contain proliferation of cancerous cells [42,43]. Spänkuch-Schmitt et al. reported reduction in proliferation of cancerous cells via siRNAs (small interfering RNAs) based targeting of PLK-1 while no significant effect was observed for mammary epithelial cells [44]. McCarroll et al. is reported to have targeted PLK-1 using RNA-interfering nanoparticle-7. A reduction in non–small cell lung cancer cell proliferation was observed in this case while using a mouse model [45]. There are numerous reports in which scientists have argued 'siRNA mediated PLK-1-targeting' as a feasible strategy for cancer treatment [45–47]. But, since there are genuine stability related issues associated with this technique (e.g. Ribozyme acting on oligonucleotides), many scientists believe that small molecule inhibitors could be a superior alternate to RNAi for PLK-1 targeting based cancer therapy.

4.2. Targeting the kinase domain of PLK-1

N-terminal kinase domain of PLK-1 regulates cell signaling via phosphorylation of serine or threonine residues present on the target protein. Most of the inhibitors of PLK-1 are potential anticancer drugs and blockers of its kinase domain are mostly ATP-competitive compounds having low selectivity. We had previously studied effect of plant extract components in inhibition of PLK-1 kinase domain [2]. Yun et al. reported novel non-ATP-competitive compounds employing molecular-docking-based virtual screening method along with in vitro enzyme assay with full-length PLK-1 and direct binding assay with PLK-1 kinase domain. Compound 4, which was chemically 3-((2-oxo-2-(thiophen-2-yl)ethyl)thio)-6-(pyridin-3-ylmethyl)-1,2,4-triazin-5(4H)-one was found to be the most potent compound among different test candidates displaying significant inhibitory activity [48]. ATP-competitive inhibitors interact with the deep groove in the kinase domain which binds ATP [49]. BI2536 may be regarded as a representative of ATP-competitive type inhibitors as it was suggested to perform a strong and also selective PLK-1- inhibition [50]. BI2536 was shown to inhibit cancer cell proliferation by checking it in mitotic metaphase thereby causing apoptosis [51]. Among the most noteworthy small molecule inhibitors of PLK-1, there is the FDA "Breakthrough Therapy'-declared 'Volasertib'. It is also referred as BI6727. Numerous experiments have confirmed it be a highly potent molecule for induction of tumor regression [52,53]. It has been declared as a 'Breakthrough' by Food and Drug Administration, USA due its promising benefits with reference to treatment of patients suffering from acute-myeloid-

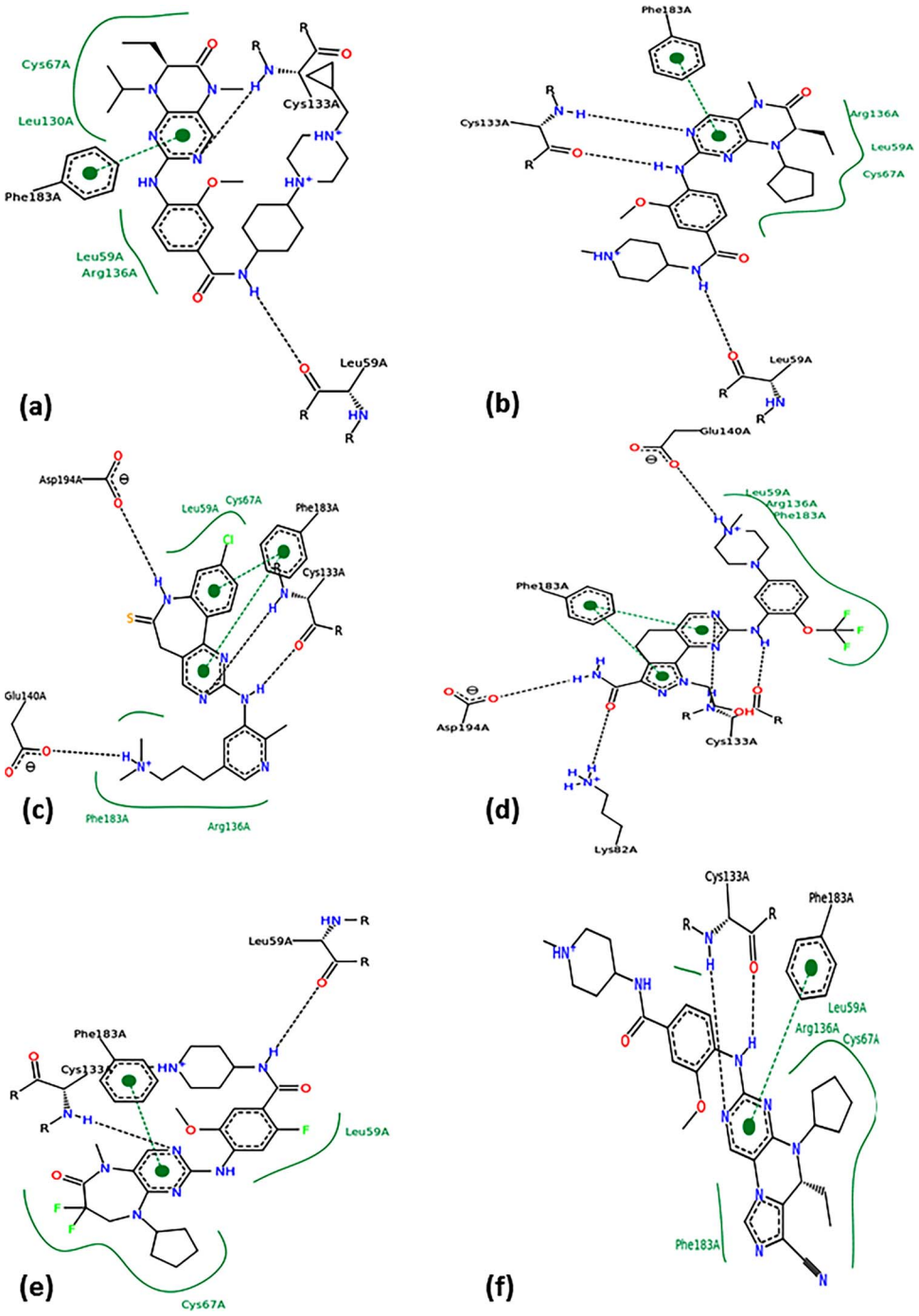

Fig. 4. Ligand interactions of Kinase Domain of human PLK-1 with extensively studied inhibitors: (a) Volasertib (b) BI-2536 (c) MLN0905 (d) NMS-P937 (e) TAK-960 (f) 79D. Black broken lines represent H-bonds. Hydrophobic interactions are displayed as intact green lines while pi-pi and cation-pi interactions appear as green broken lines.

leukemia [54]. Fig. 4 represents how different inhibitors (volasertib or BI-6727, BI-2536, MLN0905, NMS-P937, TAK-960, 79D) bind to the kinase domain, and residues crucial to the interactions are labelled (Fig. 4). Notably, Volasertib, the 'Breakthrough Therapy', interacts with the kinase domain of PLK-1 through six important residues namely, Leu 59, Cys 67, Leu 130, Cys 133, Arg 136 and Phe 183. It is worth mentioning that in the figure, three residues, Leu 59, Cys 133 and Phe 183 can be seen as common to the displayed interactions of these extensively studied inhibitors (Fig. 4). Still, there is serious specificity issue associated with most of the ATP-competitive type inhibitors as there is always a possibility to display off-target effects by binding to unintended similar grooves present in other kinases. Hence specificity for PLK-1 remains questioned. This has further significance to the close isoforms, PLK-2 and PLK-3 which may possess tumor-suppressor function [55]. We generated a superimposition view of ligand bound crystals of PLK1, PLK2 and PLK3 to highlight similarity of the binding groove. The PDB IDs were 3FC2, 4I6H and 4B6L, respectively. The Backbone RMSD of PLK2 and PLK3 with PLK1 was found to be 0.799 and 0.866 A respectively (Fig. 5).

4.3. Targeting the signal localization domain of PLK-1

A bipartite nuclear localization signal, also referred as NLS is needed for nuclear accumulation during S and G2 phases. In a 2017 study by Chen et al. [56], authors reported a compound designated as 'compound D110′ which was claimed to possess appreciable potential for inhibition of PLK-1 kinase function. Moreover 'compound D110′ also played crucial role in blocking the nuclear localization during concerned phases (i.e. S and G2). Further to this, the authors argued that

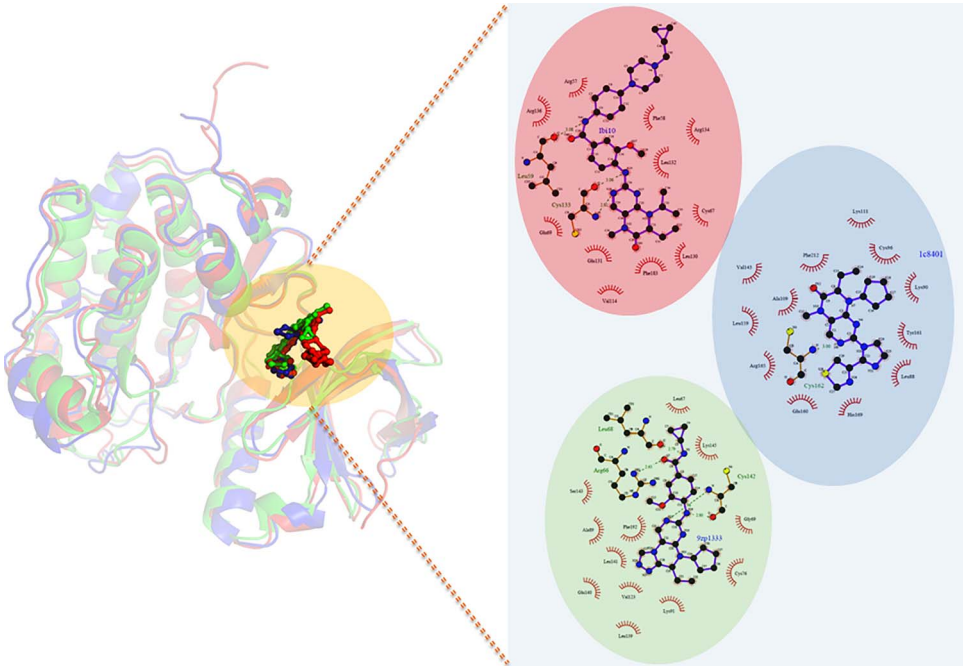

Fig. 5. Superimposition of ligand bound crystals of PLK1, PLK2 and PLK3 to highlight similarity of the binding groove. The ligand bound to PLK1 is shown in red color while blue and green colors represent the ligands bound to PLK2 and PLK3, respectively.

D110 was significantly selective for PLK-1 as opposed to PLK2 or PLK3, the reason being its dual binding potency to ATP site as well as the nuclear localization signal present in PLK-1 [56].

4.4. Targeting the Polo-Box-Domain of PLK-1

Eventually, it has become evident to the scientific community that targeting the "Polo Box Domain" (PBD) is a more fruitful approach since the specificity of binding remains questioned when the kinase domain is targeted by putative drugs/ligands. 'Peptidomimetics' category ligands have expressed commendable in vitro specificity and affinity. However issues related to their poor membrane permeability and restricted bioavailability lead to a parallel search for small-molecule inhibitors. It is important to mention that screening and/or designing small-molecule inhibitors against the PLK-1-PBD binding site is a challenging one since it is a small rather shallow cleft interactions with which have to be relied upon for successful binding. Trp414, H538 and K540 are among the most important amino acid residues belonging to this binding site which are the focus of targeted binding by putative drug molecules. The polo-box domain, being specific to PLKs is an obvious target for drug screening or PLK-1-inhibitor design Poloxin, thymoquinone, and purpurogallin are known to be significantly selective inhibitors directed against the PBD of PLK-1 [57]. Poloxin as well as thymoquinone possess the ability to alter the proper orientation of PLK-1. In this manner, they contain cancer cell division [58]. Poloxin-2 is a poloxin analogue. In a recent work using cultured cancer cells (human), the authors suggested that it possessed expressively enhanced efficacy along with commendable selectivity over its parent molecule poloxin in terms of induction of mitotic arrest as well as causing apoptosis [59]. Still, so far, the small molecule PLK-1-inhibitors are yet to achieve display of practically satisfactory therapeutic results in clinical trials, a prominent cause being the dose-limiting toxicities associated with PLK-1-inhibiting molecules [60]. In a study the scientists held 'low intratumoral drug levels' responsible for the observed decrease in the efficacy of the PLK-1 inhibitor BI2536 with reference to progressive hepatocellular carcinoma [61]. In a notable study, Sakkiah et al. employed a combination of in silico (this included: molecular modeling, docking and virtual screening) and in vitro approach to identify fresh inhibitors of the PBD of PLK-1 [36]. They performed an extensive virtual screening of a drug-like database. The database comprised of 159,757 drug like structures. The screening unraveled 9327 drug like structures, having a max-fit-score of 3 or more. These selected structures were further subjected to molecular docking and interaction analyses. Hence, a shortlist of 93 drug structures was generated. These drug candidates displayed significant interactions with important amino acid residues found within the PLK-1-PBD active crevice. Finally, these 93 drug candidates were tested in vitro to assess their inhibition efficacy against the PBD of PLK-1. In vitro tests revealed that many of these candidates did possess inhibitory activity for the said site. A lead drug candidate, designated as 'Chemistry_28272' was suggested as the most efficient inhibitor of PBD of PLK-1. In a 2017 article, the authors have stated that many inhibitor-candidates aimed at targeting PLK-1-PBD finally turn out as alkylators of protein and are non-specific [62]. Accordingly, the authors suggested that the biologists involved in 'small molecule inhibitor design and discovery targeting PBD of PLK-1' should be very careful in the light of above mentioned fact. Fig. 6 shows molecular interaction of important small molecule inhibitors and peptidomimetics with Polo Box Domain of PLK-1 (Fig. 6). Molecular modeling and docking was used to generate interaction poses for Purpurogallin and (-)-Epigallocatechin by Autodock 4.2 using our 'Click by Click Protocol' [63]. As a trend of interactions, it can be safely stated that the amino acid residues Trp414, H538 and K540 are consistently observed in the binding poses. These residues are highlighted (Fig. 6).

5. An outline of strategy for drug design targeting the 'Y'-shaped pocket of PLK-1-PBD

Previously, we have reported critical binding interactions for varied targets which included neuroenzymes like acetylcholinesterase [64,65], and also cancer targets like PLK-1 [2], MMP-2 and MMP-9 [66]. Recently, Park et al. have suggested targeting the 'Y'-shaped pocket of PLK-1-PBD [67]. The authors have encouraged design of ligands that could simultaneously bind to two or more modules of the 'Y' pocket [67]. The 'Y' pocket consists of 3 binding modules: (i) p-Thr/p-Ser-binding module (ii) Pro-binding module (iii) hydrophobic channel. Herein, a drug design strategy is described that should be subsequently validated in wet lab (Figs. 7 and 8). It is clearly stated that the described strategy is largely built upon the core idea of Park et al. which is sincerely acknowledged [67]. GLIDE is one among the many reliable

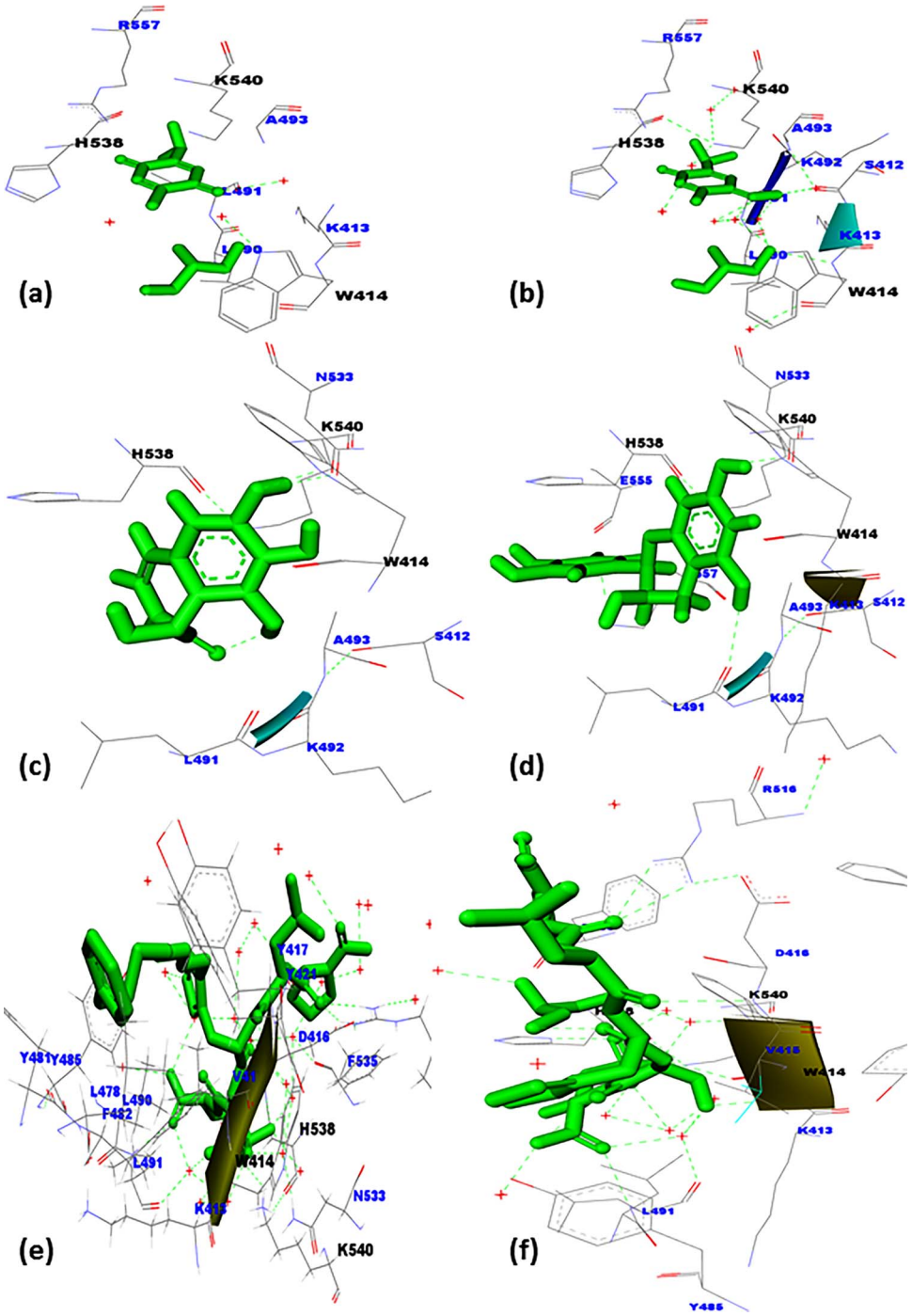

Fig. 6. Molecular interaction of small molecule inhibitors and peptidomimetics with Polo Box Domain of PLK-1: (a) Thymoquinone (b) Poloxime (c) Purpurogallin (d) (-)-Epigallocatechin (e) 4j (f) PLHSpT. Molecular modeling and docking was used to generate interaction poses for (c) and (d).

programs and is frequently cited [68]. SP and XP stand for 'Standard Precision' and 'Extra Precision', respectively, which are the names of two modes built in GLIDE software. HTVS stands for high throughput virtual screening, which represents rough screening of millions of compounds stored in the database to perform an *initial dig in* function related to drug search. ΔG and Ki stand for free energy of binding and inhibition constant for a docked complex, respectively. Δ ASA stands for 'change in accessible surface area' of amino acid residues prior and post docking. ADMET is an abbreviation which refers to properties of any putative drug molecule, namely Absorption, Distribution, Metabolism, Excretion and Toxicity, respectively. The schematic workflow of computational strategies for design of multi-modular inhibitors targeting the Y-pocket of PLK-1-PBD consists of the following steps, (1) *Pharmacophore modeling* based on the holistic tri-modular geometrical constraints of the Y-pocket (2) *Ligand optimization* for simultaneous interaction with a minima of two binding modules (3) *Library design* (4) *Library screening* by large scale molecular docking experiments using high throughput, standard precision and extra precision modes of GLIDE (5) *Calculation of Relevant Parameters* including ΔG, Ki, Δ ASA and ADMET (6) *Molecular Dynamics Simulations* to reveal promising drug candidate(s).

6. Conclusion

Cancer continues to be a disease of deep research thrust. It is understandable that screening and/or designing small-molecule inhibitors against the PLK-1-PBD binding site is really challenging and very limited success has been achieved in this direction so far. Recently,

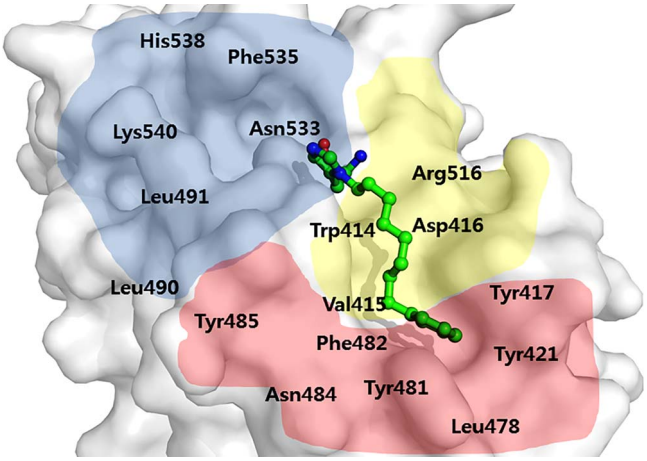

Fig. 7. The X-ray co-crystal structure of PLK-1 Polo Box Domain in complex with PL-2 (PDB ID: 4RCP). Binding module 1 (*p*-Thr/p-Ser) is highlighted in blue, Binding module 2 (Pro-binding module) is highlighted in yellow while binding module 3 (hydrophobic channel) is highlighted in red. The inhibitor in this complex is shown in ball and stick form.

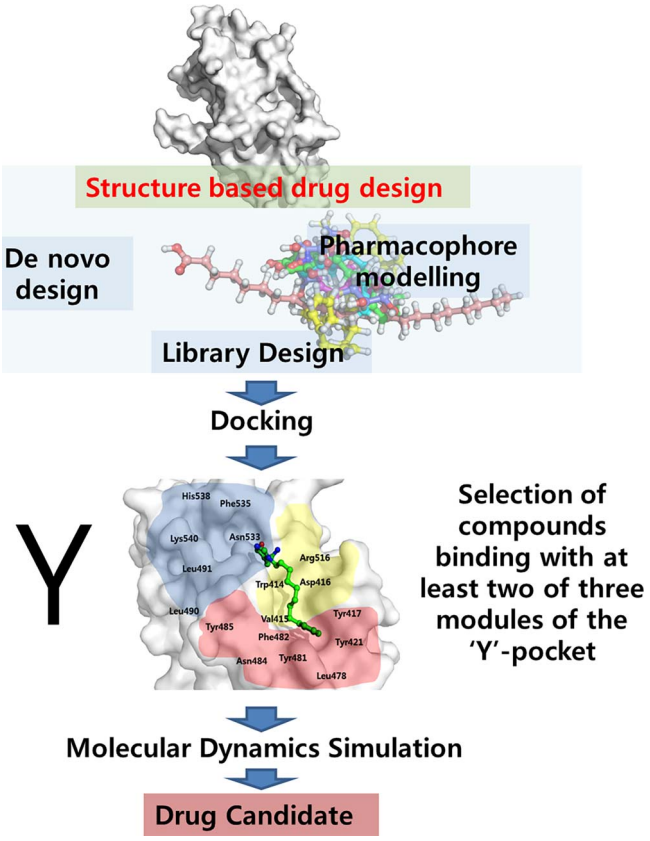

Fig. 8. A general outline of computational drug design targeting the 'Y'-shaped pocket of PLK-1-PBD.

eminent scientists have proposed targeting the 'Y'-shaped pocket of PLK-1-PBD and encouraged design of ligands that should be able to concurrently bind to two or more modules of the 'Y' pocket. Hence, it is suggested that during molecular interaction analyses, particular focus should be kept on the moiety in each ligand/drug candidate which directly interacts with the amino acid residue(s) that belong(s) to one of the three binding modules which together create this Y-shaped cavity. This obviously includes (but it is not limited to) the 'shallow cleft'-forming residues i.e. **Trp414, H538 and K540**, as significance of these binding residues has been consistently highlighted by many studies. It was our intent that the present article should match the technical expectations of cancer drug-design experts besides having a flavor of simplicity so as to be acceptable to a wider scientific readership. Herein, we have tried our level best to prepare a concise albeit duly updated article for PLK-1 as it continues to be a hot target in anti-cancer drug research.

Conflict of interest

None to declare.

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