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Review Article

Recent Progress in Development of Polo Like Kinase 1 Inhibitors: Efforts So Far

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Abstract

Polo-like kinase 1(Plk1) plays an essential role in inhibiting cell proliferation and comes under the family of serine/threonine-protein kinase, which is a particular target for cancer therapy. In some clinical studies, Plk1 has been identified as a target for cancer. Currently, so many scientists are developing the Plk1 inhibitors, and they are thinking about working on them. A recent strategy for Plk1 inhibition is the development of small-molecule inhibitors, which will inhibit the Plk1 through the ATP-binding site of the Plk1. Now new generation Plk1 inhibitors being tested clinically, which are targeting the polo box domain. This review highlights the recent progress made in the development of Plk1 inhibitors as anticancer agents.

Introduction

Polo-like kinases (Plks) belong to the family of serine/threonine protein kinases and play a prominent role in regulating the cell cycle by regulating initiation, maintenance and completion of the mitosis phase.[1] The polo-like kinase was identified firstly in 1988 with a chain of 577 amino acids from the genetic screens of Drosophila larval neuroblast.[2] Five types of Plks have been identified in the human species to date, i.e., Plk1, Plk2, Plk3, Plk4 and Plk5 and Plk1 is the most studied member from the Plks family due to its regulatory role in the cell division and genomic stability.[3] It is a specific target for cancer therapy because its high expression in the cell's proliferation is responsible for tumorigenesis.[4] A decrease in Plk1 expression activity results in inhibition of cell proliferation of cancer cell lines and xenografts.[5-7] Plk2 plays its role in centriole duplication during the G1 to S transition of the cell cycle and plays a vital role in DNA damage checkpoint.[8-10] In contrast, Plk3 is important in S-phase entry and depletion arrest in the process of cell proliferation.[11] The remaining Plk4 is required to maintain cell viability, and Plk5 has been implicated in the G1 phase of cell cycle arrest and subsequent apoptosis.[12,13] Polo-like kinases consist of C-terminal, known as polo box domain(PBD) and N-terminal, a kinase domain(KD).[14] The PDB is helpful for protein interactions, and the kinase domain plays an essential role in forming a binding pocket that is essential for the kinases.[15,16]

Polo-like kinases play a promising role in regulating the cell cycle; therefore, Plks inhibition is the best strategy for preventing tumorigenesis. However, Plk1 shows its action in regulating various cell cycle steps like centrosome maturation, mitotic entry, cytokinesis, mitotic exit, chromatin segregation, and spindle assembly.[17] In the last few years, some small molecule inhibitors targeting the kinase domain are undergoing clinical trials.[18] Kinase domain is the specific target for attachment and which is helpful in the inhibition of enzyme.[19]

The current review provides an update of various Plk1 inhibitors reported in recent years and their structure-activity relationship (SAR), underlining important structural attributes required to design specific Plk1 inhibitors and guide medicinal chemists towards them.

Plk1 Inhibitors

Protein kinases have grabbed much attention in the last several years as potential targets in treating cancer.[20,21] In an attempt to understand the molecular mechanism that forms structural requirements for inhibition of Plk1 action, the kinase domain of the complex resembled the previously determined isolated KD structures and consists of residues (18-116) forming an N-terminal lobe and C terminal lobe formed by residues (295-311). These both lobes are connected by the short hinge region formed by the residues 117-123. The kinase domain contact area comprises C-terminal 295-311 residues, alpha-helix from the C-terminal lobe with hinge area, and an N-terminal beta-strand. In contrast, PBD has two polo boxes, namely, PB1 and PB2, connected by two connecting linkers. One

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is the short linker L1, formed by residues 392-402, and the other is secondary linker L2, having residues 479-490. These two linkers, L1 and L2, include the KD binding domain of PBD (Figure 1).

Currently, more than 51 kinase inhibitors are approved for the management of cancer.[22] Some Plk1 inhibitors which are mentioned in Table 1. However, designing selective kinase inhibitors is challenging as most of the kinases share the structural similarity in their ATP binding cleft, which targets most kinase inhibitors. Developing competitive ATP inhibitors has been explored so far. However, a series of peptide-based inhibitors targeting PBD of Plk1 has also been reported to display high target specificity.[23]

In 2015, Chen *et al.* designed, synthesized, and evaluated novel benzimidazole and indole-based molecules as potential Plk1 PBD inhibitors. The study focused on the logical designing of Plk1 PBD inhibitors in terms of the substrate-binding site of PDB. In their study, the authors performed the screening of an in-house database followed by molecular docking to screen CJ-032 as a lead. Furthermore, they modified the structure with different substitutions, synthesized and performed *in vitro*

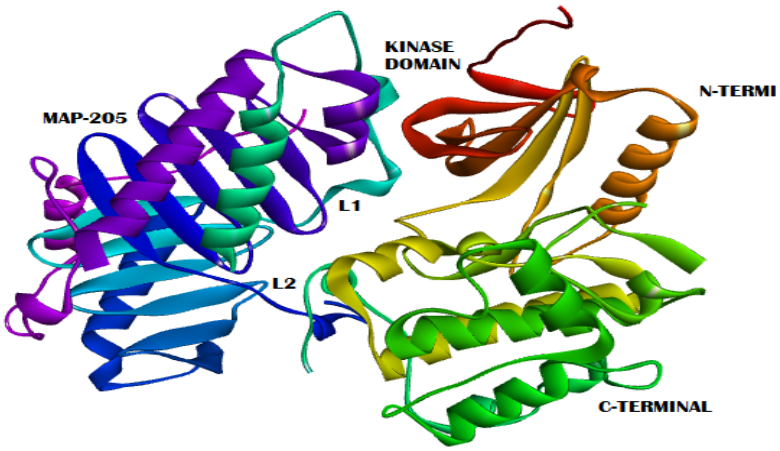


Figure 1. Structure of Plk1 showing N-lobe (orange), C-lobe (green) and kinase domain (red) joined by linkers, L1 and L2 to PBD (PDB ID 2J5F) (purple).

Table 1. PLK-1 kinase domain-targeted inhibitors.

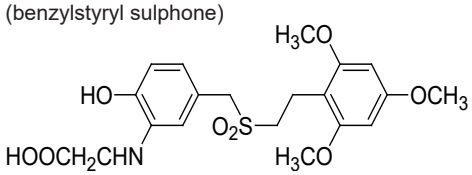
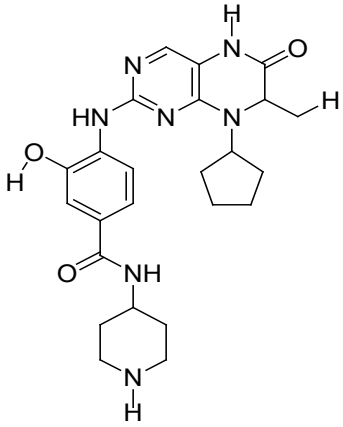
Structure	Mechanism of action	IC_{50} values for PLK1	Selectivity
Rigosertib (ON 01910.Na) (benzylstyryl sulphone) 	A-non-ATP competitive Plk1 inhibitor; Affects microtubule dynamics.[24-27]	9-10 nM	Also inhibits PDGFR,ABL, FLT1, CDK-2, PLK-2,Src, and Fyn. Efficacious both as a single agent and in combination with cytotoxic drugs in xenograft models.
Dihydropteridinone derivative (BI 2536) 	ATP-competitive inhibitor.[24,28-30]	0.83 nM	(i) Exhibited 1,000-fold selectivity against a wide panel of tyrosine and serine /threonine kinases (ii) PLK2b IC_{50} = 3.5 nM (iii) PLK3c IC_{50} = 9.0 nM (iv) EC50 = 2–25 nM

Table 1 Continued.

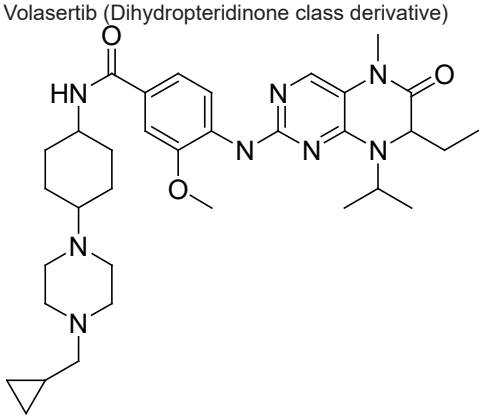
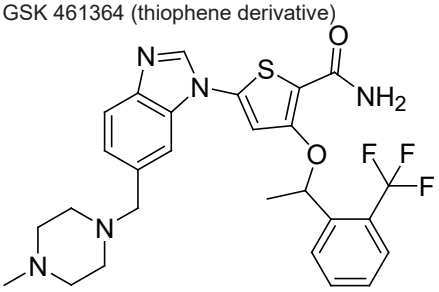
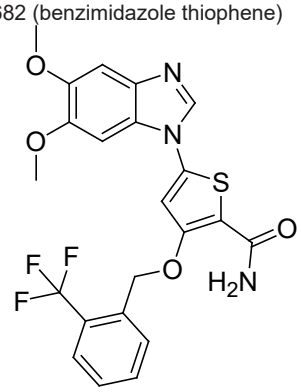
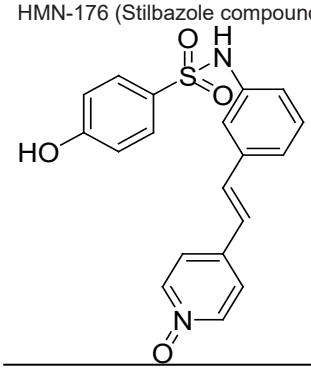
BI 6727 Volasertib (Dihydropteridinone class derivative) 	ATP-competitive kinase inhibitor.[31,32]	0.87 nM	(i) No inhibitory activity against a wide panel of more than 50 protein kinases (ii) PLK2 IC_{50} = 5 nM (iii) PLK3 IC_{50} = 56 nM (iv) EC50 = 11–37 nM
GSK 461364 (thiophene derivative) 	ATP-competitive inhibitor. [33-37]	2 nM	(i) No inhibitory activity against a wide panel of more than 50 protein kinases (ii) PLK2 IC_{50} = 5 nM (iii) PLK3 IC_{50} = 56 nM (iv) EC50 = 11–37 nM
GW 843682 (benzimidazole thiophene) 	ATP-competitive inhibitor.[33-37]	2.2 nM	
HMN-176 (Stilbazole compound) 	ATP-competitive Inhibitor.[38-40]	118 nM	Shows potent antitumor activity in gastric, breast, and lung human tumor xenografts and so forth. Better activity compared to known drugs such as cisplatin, doxorubicin, vincristine, and tegafur-uracil Inhibits the expression of NF-Y and induces the cell cycle arrest

Table 1 Continued.

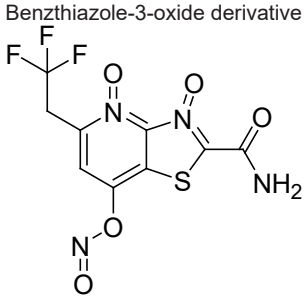
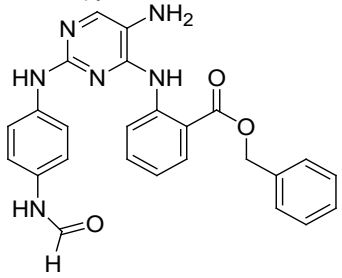
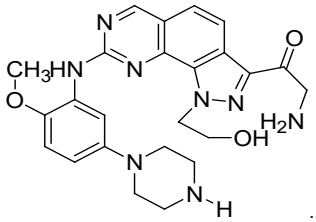
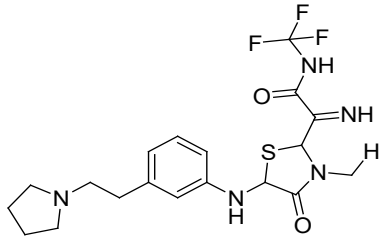
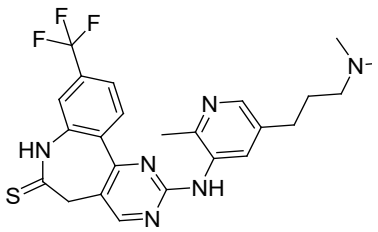
Cyclapolin 1 Benzthiazole-3-oxide derivative 	Non-competitive with respect to ATP.[41]	20 nM	Inhibits PLK1; other family members were not determined Inhibits C terminal Src kinase; IC_{50}~100 μM Cell cycle may also be affected in G1/S
DAP-81 Diaminopyrimidine Derivative 	Predicted to target the nucleotide pocket.[42]	0.9 nM	Destabilized kinetochore microtubules. Dose-dependent reduction of CDC25C phosphorylation in cells and recapitulation of key aspects of the loss-of-function phenotype for PLK1
NMS-P937 Pyrazolequinazoline 	ATP-competitive Inhibitor.[43-45]	20 nM	More than 100 cell lines and 200 protein kinases have been tested Shows prolonged M phase and induce apoptosis Active in Xenograft tumor model IC_{50} < 100 nm on solid tumor
ZK-Thiazolidinone TAL 	ATP-competitive Inhibitor.[46]	19 ± 12 nM	Induced arrest in prometaphase-like arrest and finally cytokinesis failure and multinucleation IC_{50} = 0.2–1.3 μM on human and mouse tumor cell lines
MLN0905 (benzolactam derivative) 	ATP-competitive inhibitor.[47]	2 nM	Mitotic arrest on tumor growth inhibition

Table 1 Continued.

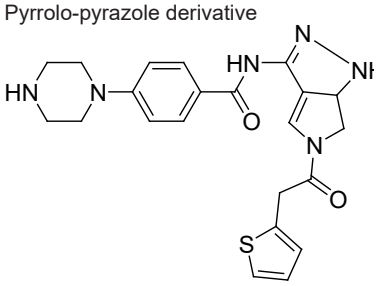
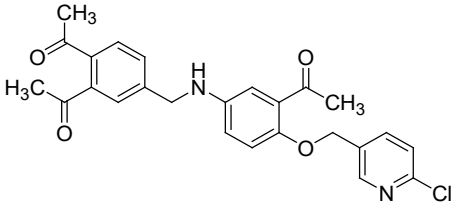
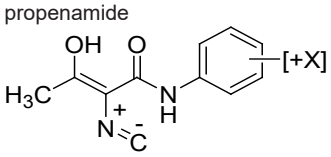
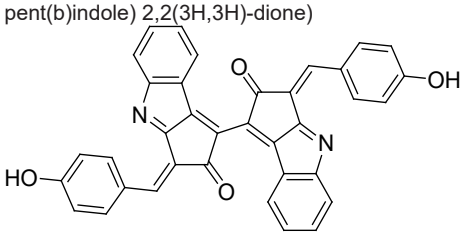
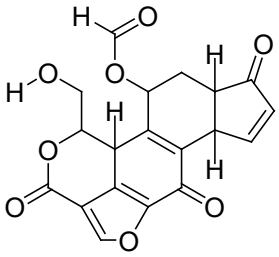
PHA-680626 Pyrrolo-pyrazole derivative 	ND	0.53 nM	PLK-2 (IC_{50} = 0.07μM) PLK-3 (IC_{50} = 1.61 μM) Weaker inhibition was detected on few kinases.[48]
SBE13 Vanillin derivative 	Non ATP- competitive inhibitor.[49]	12–39 μM (EC_{50})	Shows 1000-fold selectivity within the PLK family
LFM-A13 α-cyano-β-hydroxy-β-methyl-*N*-(2,5-dibromophenyl)-propenamide 		Plx1 32.5 μM Using GST-CDC 25 as a substrate	PLK-3 IC_{50} = 61 μM. Also inhibits human BTK with an IC_{50} of 17.2 ± 0.81 μM The activity is 3–15 fold greater against a panel of protein kinases.[50-52]
Scytonemin Subunit derived from tryptophan and Phenylpropanoid isolated from many strains of cyanobacteria. (3,3-Bis((4- hydroxyphenyl)methylene)-(1,1- bicyclo-pent(b)indole) 2,2(3H,3H)-dione) 	ATP-competitive Inhibitor.[53,54]	2.0 ± 1 μM	Also inhibits the transcriptional factor MYT1 CDK-1, Chk-1, and PKC. Does not directly inhibit PLK1 up to 3-4 μM
Wortmannin Steroidal furanoids, originally isolated from Penicillium wortmannii 	ATP-competitive Inhibitor.[55-57]	24 nM	Also inhibits the other member of PLK family and interacts with similar binding affinity Inhibits the PI3K

Table 1 Conttinued.

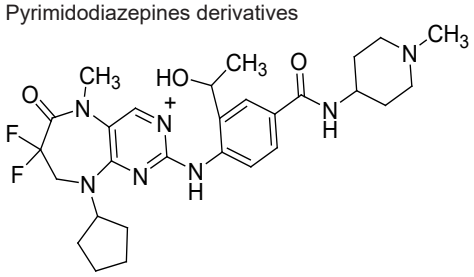
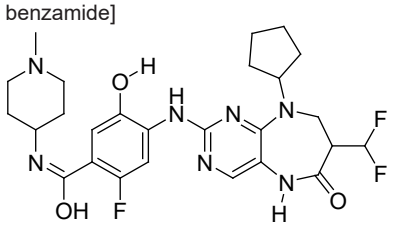
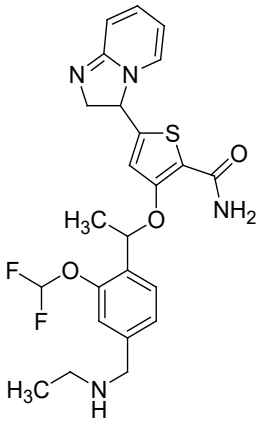
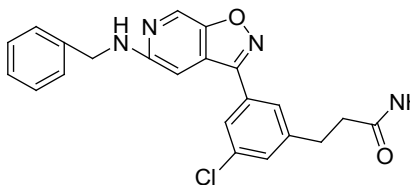
RO3280 Pyrimidodiazepines derivatives 	ATP-competitive Inhibitor.[58]	0.09 nM	318 wild type and mutants protein kinases tested More than 85% protein kinases inhibits at 1mM
TAK-960 [4-[(9-cyclopentyl-7,7-difluoro-5-methyl-6-oxo-6,7,8,9 tetrahydro-5H pyrimido[4,5-b][1,4]diazepin-2-yl)amino]-2-fluoro-5-methoxy-N-(1-methylpiperidin-4-yl) benzamide] 	ATP-competitive Inhibitor.[59]	0.8 nM	No inhibitory activity against 282 protein kinases Anti-tumor activity against *TP53, KRAS, MDR* mutated cell lines Monopolar spindle and G2/M phase arrest
Compound 36 Imidazopyridine derivative 	ATP-competitive Inhibitor.[60,61]	9.8 nM	No inhibitory activity against 212 protein kinases at 1 μM. Tolerated toxicity Observed against WBC
Compound 15 2-Aminoisoxazopyridine 	ATP-competitive Inhibitor.[62]	0.051 μM	Treated cells showed monopolar phenotype and mitotic arrest in colorectal carcinoma cell lines

Table 1 Continued.

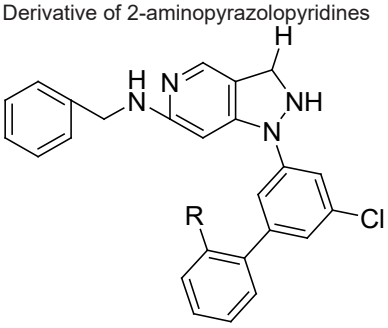
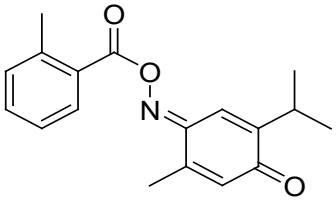
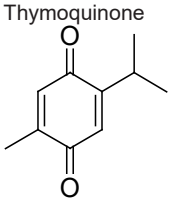
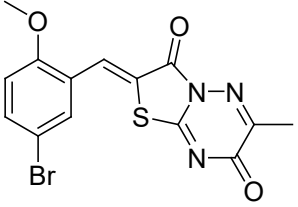
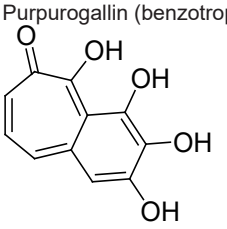
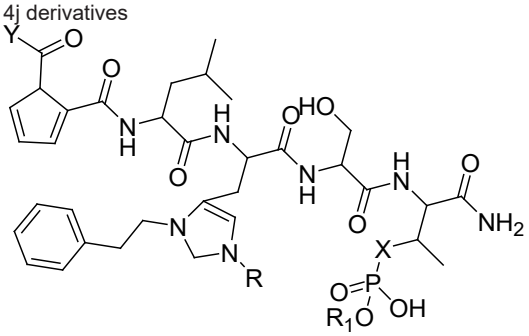
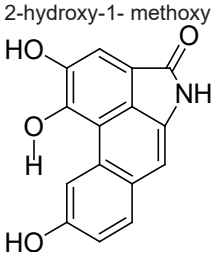
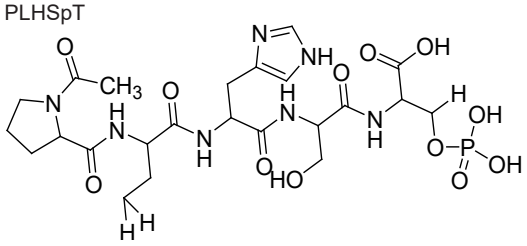
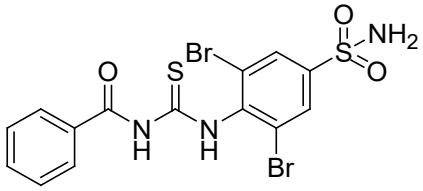
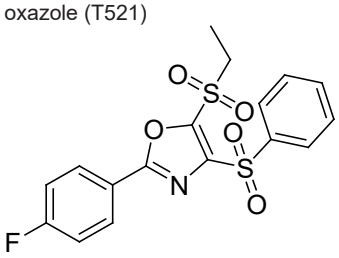
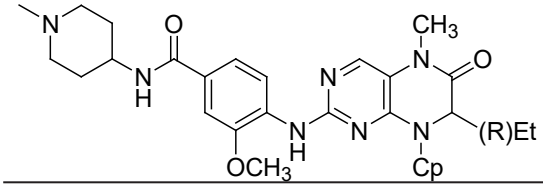
Compound 38 Derivative of 2-aminopyrazolopyridines 	ATP-competitive Inhibitor.[63]	0.042 μM	HCT116 colorectal cancer cell lines showed G2/M arrest and induced apoptosis
Poloxin (thymoquinone derivative) 	PBD site Targeting.[64]	4.8 ± 1.3 μM	Interferes with PLK1-PBD functions *in vitro* and *in vivo*
Thymoquinone 	PBD site Targeting.[64]	1.14 ± 0.04 mM	Inhibits PBD dependent binding and subcellular localization
Poloxipan 	PBD site Targeting.[65]	3.2 ± 0.3 mM	Pan inhibitor of Plk1–3 PBDs
Purpurogallin (benzotropolone- containing compound) 	PBD site Targeting.[66]	< 0.3 mM	Inhibits PBD-dependent binding in vitro and in vivo
4j derivatives 	PBD site Targeting.[67,68]	3 nM (320 mM)	Inhibits PBD-dependent binding in vitro

Table 1 Continued.

Aristolactam AIIIa CCRIS 2996, Aristolactam-aii, Dibenz(cd,f)indol- 4(5H)-one, 2-hydroxy-1- methoxy 	PBD site Targeting.[69]	10 μM	Inhibits kinase and PBD domain with different inhibitory concentration
MAGPMQSpTPLGAKK (Optimal phosphopeptide sequence)	PBD site Targeting.[70-73]	5 μM	Polo Boxtide is recognized by pincer grips like pocket PB1 and PB2
LLCSpTPNG and LLCSTPNG Cdc25C-P & Cdc25C (Optimal phosphopeptide derived from Cdc25C protein)	PBD site Targeting.[74]	1.8 μM	LLCSpTPNG is recognized by the trp414 residue of PB1
PLHSpT 	PBD site Targeting.[75,76]	0.445 μM	The side-chain of N-terminal Pro docked into a surrounding core of hydrophobic amino acid Trp414, Phe535, Arg516 residue
4-(3-Benzoyl-thioureido)-3,5-dibromo-benzene sulfonamide 	Most selective and potent Plk1 PBD inhibitor.[29]	39.8 ± 3.5 μM	Inhibits PBD-dependent binding in vitro.
5-(Ethylsulfonyl)-2-(4-fluorophenyl)-4-(phenylsulfonyl) oxazole (T521) 	T521 PBD Plk1 inhibitor.[31]	NA	
BI-2536 	Dual Plk1 Kinase–BRD4 Bromo domain Inhibitor.[77]	BRD4: 56 ± 9 And Plk1: 0.22 ± 0.01	It is shown to induce mitotic arrest and apoptosis in bone marrow precursors from treated patients.

growth inhibition assay. Compound **1** shows potent Plk1 inhibition activity having IC_{50} was 6.83±0.52μM and high sevenfold higher selectivity against some kinases like Plk2 (IC_{50}=14.72±4.07μM) and Plk3 (IC_{50} ≥ 50μM). The SAR studies revealed that Plk1 inhibition is greatly affected by the phenyl ring's electron density (Figure 2). The authors also found the mechanism of action, which shows that compound 1 was an ATP-independent and substrate-dependent Plk1 inhibitor.[76]

Liu *et al.*[77] in 2015, reported the synthesis and biological evaluation of indole-3-carboxylic acids for a small molecular non-ATP-competitive inhibition of Plk1, as shown in Figure 3. They have calculated the inhibitory activity of Plk1 and *in vitro* cell lines growth inhibition of eleven indole-3-carboxylic acid derivatives using thymoquinone, a naturally occurring product reported as a non-ATP inhibitor of the Plk1 as a control. Compound **2** (IC_{50}=0.41±0.09μM) and **3** (IC_{50}=0.13±0.02μM) showed significantly more promising Plk1 inhibitory activity compared with thymoquinone. SAR investigation from biological data revealed that the carbon chain's length between the indole nucleus and acetic acid greatly influences the Plk1 inhibition. The compounds are substituted by 4-methyl piperazine and morpholine as the side branch exhibits the same activity as that of thymoquinone and lead CJ054. When the compound was substituted for piperidine (3) and pyrrolidine (4) as a side chain, it emerged as the most potent chemical in the series. Compound 3 was also investigated for selection of other similar kinases (Plk2 and Plk3) and demonstrated the excellent function of the Plk1 inhibition specificity towards Plk2 (IC_{50}>50μM) and Plk3 (IC_{50} >50 μM).

In 2015, Scharow *et al.*[78] reported their studies on poloxin analogs as Plk1 PBD inhibitors. Poloxin induces mitotic arrest due to chromosome impairment in Plk1 localization leads to apoptosis, thereby treating tumor cells. Authors focused on synthesizing the polo-box domain of Plk1 inhibitors for good action and specificity. All the compounds tested against the polo-box domain of Plk1-Plk3 were evaluated by the fluorescence polarization assay method. Compound **4** displayed the most significant Plk1 PBD (IC_{50}=0.31±0.02 μM) inhibitory activity and 7-fold and 59-fold specificity with the polo-box domain of Plk2 (IC_{50}=2.32±0.44 μM) and Plk3 (IC_{50}=18.3±1.8 μM). The SAR investigations suggested that the aromatic ring's substitutions significantly affect the Plk1 inhibitory activity by tailoring the ester group's interaction. The presence of an electron-withdrawing group or change in the ortho-substituted methyl substituent increases the action (Figure 4). Simultaneously, the aromatic ring's replacement with aliphatic residue displayed similar potency, showing that the aromatic nucleus is not essential for biological action. Also, the ester group's replacement with acyl hydrazone abolished the activity due to loss of protein acylation, thereby suggesting acylation of protein for the poloxin mechanism of action and its analogs.

In 2016, Long *et al.*[79] studied newly synthesized heteroaryl styryl sulfone analogs as mechanistic mimetic of rigosertib. This synthesized benzyl styryl sulfone is under clinical trials for Myelodysplastic syndrome (MDS) treatment and

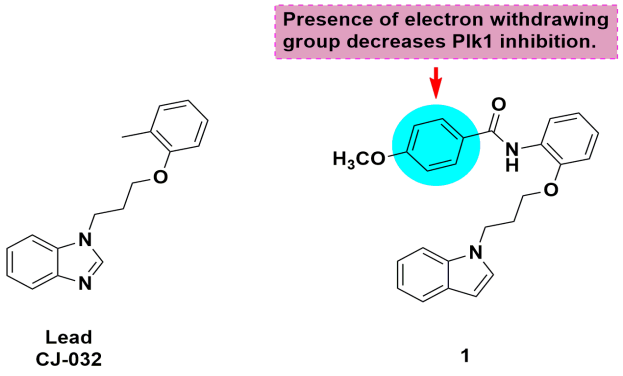


Figure 2. Non-ATP competitive benzimidazole lead CJ-032 and its indole derivatives as Plk1 PBD inhibitors.

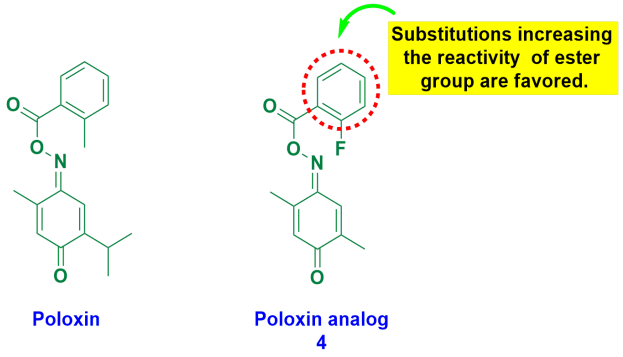
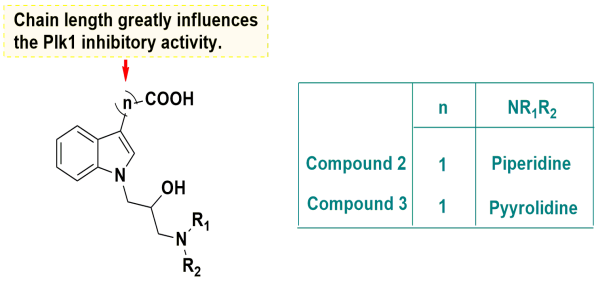


Figure 4. Poloxin and its derivative as Plk1 PBD inhibitor.



	n	NR_1R_2
Compound 2	1	Piperidine
Compound 3	1	Pyyrolidine

Figure 3. Indole-3-carboxylic acids derivatives reported as Plk1 PBD inhibitors.

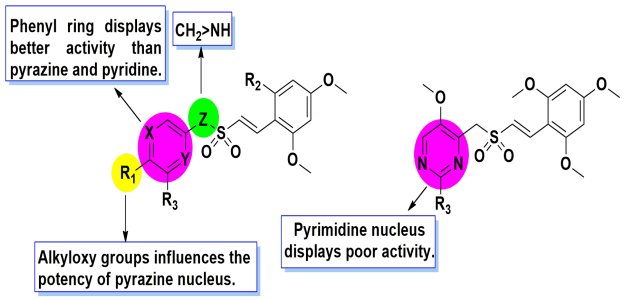


Figure 5. Rigosertib analogs reported as potential anti-cancer agents.

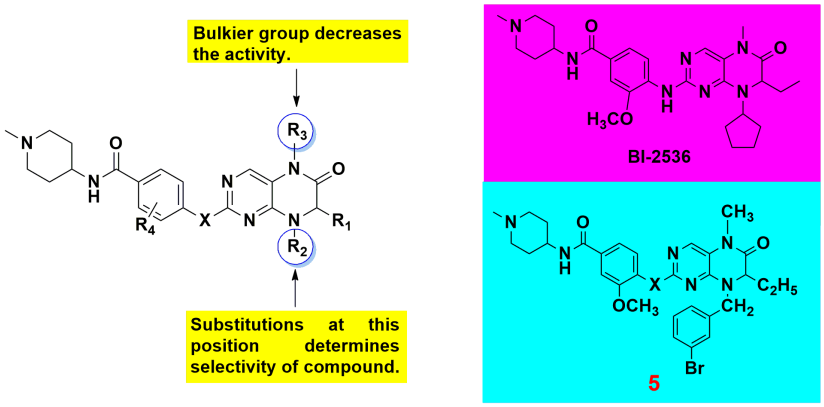


Figure 6. Dual Plk1/BRD4 inhibitors identified for management of cancer.

other cancers. Rigosertib targets Plk1, controlling the cell cycle progression, which helps the cyclin B1 activation and CDC25C phosphatase ATP dependent system. They synthesized novel styryl sulfonyl analogs bearing some nitro-heteroaryl systems. Compounds bearing simple phenyl rings displayed the most promising results by inhibiting CDC25C and demonstrated growth inhibition in various tumor cell lines in the range of 0.01-0.35 μM. It was hypothesized that the Plk1 inhibition modulates CHK2 and p53, which eventually results in a decrease of CDC25C expression. The SAR studies revealed that the compounds bearing pyrazine nucleus as heterocyclic core displayed promising activity than the pyrimidine derivatives, suggesting that the number and position of heteroaryl nitrogen atom significantly affects the antiproliferative activities (Figure 5).

In the same year, Chen *et al.*[80] reported the synthesis and biological evaluation of Plk1 inhibitor, BI-2536 analogs, as dual Plk1 kinase/BRD4 bromodomain inhibitors (Figure 6). The BRD proteins are known to be 'epigenetic readers' and can identify histones of ε-N-acetylated lysines. Due to their crucial role in transcriptional gene regulation involved in tumor enlargement, they have an attractive druggable target for cancer management. Since BRD4 and Plk1 actively participate in mitosis, dual inhibition of both these targets by a single molecule may be a novel strategy to treat cancer. Their study reflects the compound **5** is the most active inhibitor of BRD4, having Ki = 8.7 nM, and equally potent Plk1 inhibitors (Ki = 5.80 nM). The SAR investigations suggested that the replacement of the NH group of pyrimidine with oxygen atom abolishes the Plk1 inhibitory activity by losing critical hydrogen bonding.

In the same year, Yun *et al.*[81] also published the study in which they synthesized and evaluated acyl thiourea derivatives as Plk1 PBD inhibitors. They are screened an in-house database which resulted in N-((4-sulfamoylphenyl) carbamothioyl)acetamide as a starting lead displaying an excellent binding affinity to the Plk1 PBD *in vitro*. They synthesized series of acyl thiourea derivatives and also studied their binding affinities against Plk1 PBD. A series of twenty-four compounds were synthesized by keeping acyl thiourea as a core fragment, and various substitutions were done at positions R1 and R2. R1 position of the derivative was replaced with different aromatic, aliphatic or alicyclic hydrocarbons, while various bioisosteric groups of sulfamoylphenyl group explored the R2 position. Their study reported compound 6 as the most selective and potent Plk1 PBD inhibitor having an IC_{50} value of 39.8±3.5 μM (Figure 7). The lead compound's binding affinities were compared to synthesized compounds that revealed that methyl, ethyl and phenyl groups were well tolerated at position R1. In contrast, substitutions with cyclopropyl and benzyl deteriorated the binding affinities. At the same time, halogen substitutions at position R2 improved binding affinity by occupying the empty pocket in the active site of the Plk1 PBD.

In 2016, Scharow *et al.*[82] reported the synthesis and evaluation of bifunctional Plk1 inhibitors, comprising both Plk1 ATP-competitive ligand and the Plk1 PBD inhibitors. The ATP-competitive inhibitor of Plk1 in clinical trials, BI2536, was incorporated to design both peptidic (Ac-PLHSpT based) and non-peptidic (Poloxin 2 based) Plk1 PBD inhibitors (Figure 8). The compound shown in Figure 9 was found to be the most potent compound displaying IC_{50} of 0.054±0.004 μM for Plk1 PBD and also an IC_{50} of 0.038±0.002 μM against Plk1. It also showed an excellent specificity profile for Plk1 (Ki=0.012±0.001 μM) over other two kinases, Plk2 (Ki=5.5±0.7 μM) and Plk3 (Ki=3.7±0.2 μM). This study provided an excellent opportunity to design Plk1 inhibitors targeting both functional and enzymatic domains to overcome the specificity of ATP-competitive kinase inhibitors.

Chen *et al.*[83] also reported 5-(ethylsulfonyl)-2-(4-fluorophenyl)-4-(phenylsulfonyl) oxazole (T521) as PBD

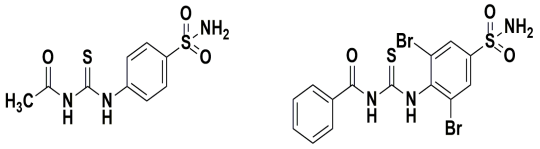


Figure 7. Acylthiourea derivatives as Plk1 PBD inhibitors.

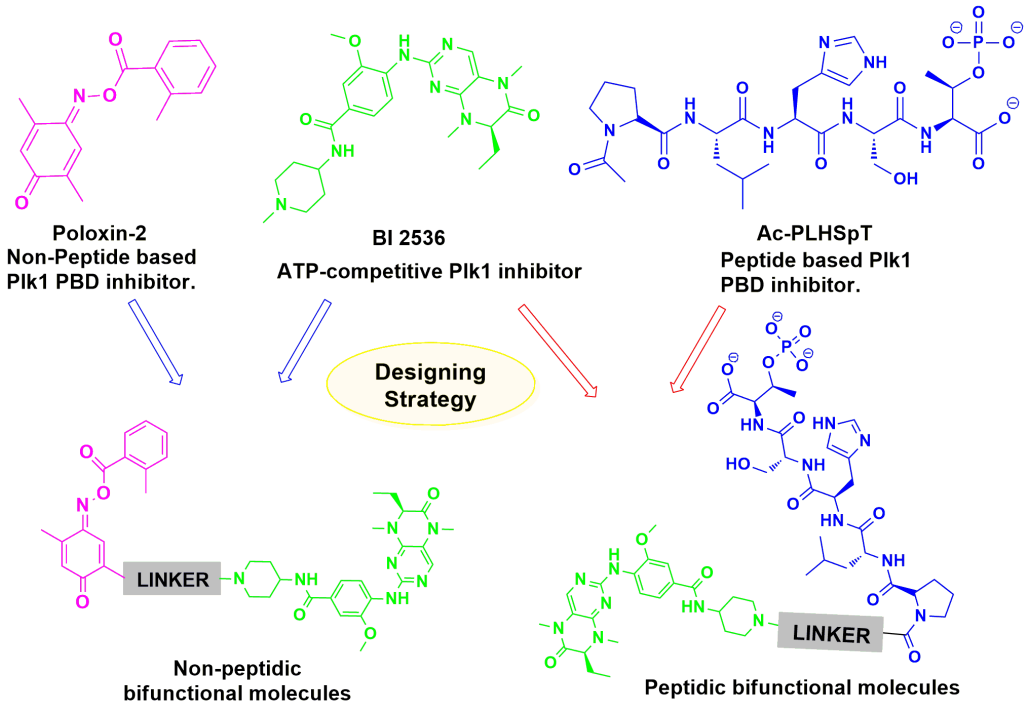


Figure 8. Designing strategy employed to synthesize bifunctional Plk1 inhibitors.

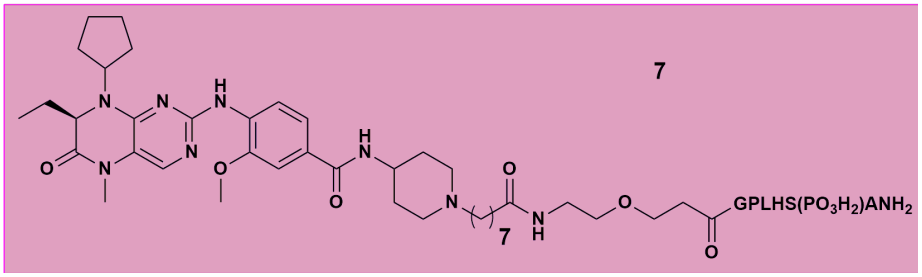


Figure 9. Most potent and selective bifunctional inhibitor of Plk1.

inhibitor of Plk1 that selectively blocks the function of PDB in Plk1. Their study utilized fluorescence polarization assay to screen the library of 20,000 molecules and reported T521 as a specific inhibitor of PBD Plk1 (Figure 10). The inhibitor covalently binds to the lysine residues in the active site of the PBD Plk1, thereby resulting in a significant change in the Plk1 secondary structure. The inhibition assay of cell proliferation studies was also carried out, which demonstrated that T521 hampers the binding of Plk1 to a checkpoint protein of spindle assembly, Bub1 *in vivo*. The compound was also found to suppress the growth of A549 cells by using xenograft mice models and showed marked mitotic defects when treated with HeLa cells.

Later in the year 2017, Pan *et al.*[84] published their study in which they reported Plk1/EEF2K (polo-like kinase 1/ eukaryotic elongation factor 2 kinase) dual inhibition for the management of breast cancer. Their research created pharmacophore models for EEF2K and Plk and used them as a screening tool to retrieve EEF2K/Plk1 dual inhibitors. The ten derivatives hits were then subjected to *in vitro* studies; five derivatives displayed EEF2K and Plk1 inhibition. By analyzing the binding mode of the top

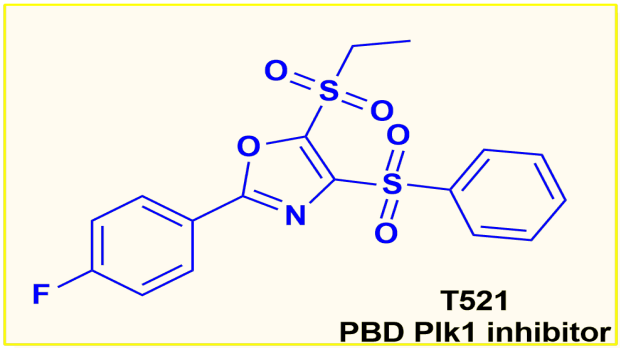


Figure 10. Structure of T521 reported as specific PBD Plk1 inhibitor.

hits, various derivatives were synthesized and evaluated. Compound substituted R1 position with ethyl group, R2 with trifluoromethyl group and R3 position with chlorine substitution, displayed the most potent novel Plk1/EEF2K dual inhibition IC_{50} values of 0.085 and 0.762 μM respectively. In contrast, compounds containing R1 position substituted with cyclopropyl group or R3 position naphthalene group displayed poor EEF2K inhibition. SAR

analysis revealed that hydrophobic groups are less tolerated at position R3 (Figure 11).

Later in 2017, Nogawa and his research group isolated trachyspic acid 19-butyl ester from the fungus RKGSF2684 and reported it as Plk1 PBD dependent inhibitor (Figure 12). The isolated compound's inhibitory potential was determined by performing in vitro studies using purpurogallin, a potent Plk1 PBD inhibitor as a positive control. The compound displayed the activity with an IC_{50} of 102 µM, similar to purpurogallin (114 µM). Thus, the study suggests that microbial fraction library can also be utilized to develop more potent compounds for cancer management of cancer.[85]

Sun *et al.*[86] also reported a study in which they utilized an *in-silico* modification technique to identify potent Plk1 PBD inhibitors. They employed docking studies and *in vitro* primary screening in combination to identify novel leads as Plk1 PBD inhibitors. The retrieved lead was derivatized, synthesized and evaluated using various bioassays. They synthesized twenty-two nitroimidazole oxime-based derivatives and evaluated them for antiproliferative activity *in-vitro*. The SAR analysis revealed that niacin substitution at the R position resulted in better activity than benzoic acid derivatives. The unsubstituted niacin ring (IC_{50}=0.01 µg/ml) and isonicotinic ring (IC_{50}=0.02 µg/ml) displayed better activity than the compounds substituted with niacin ring bearing chloro-group (IC_{50}=0.71-1.28 µg/ml) against four cancer cell lines (human). The synthesized compounds were also evaluated to affirm their Plk1 inhibitory activity using thymoquinone as a positive control. Compound **11** emerged as the most potent compound with Plk1 PBD inhibitory activity in the range of 0.002±0.02 µg/ml. The compound also displayed better potency than poloxin against the MGC803 cancer cell line (Figure 13).

To study the binding mode of the synthesized compounds, the most potent **compound 11** was docked in the active site of polo-box domain Plk1 (PDB code:4HCO). The molecular docking results revealed that the compound occupied the active site by forming three hydrogen bonds with Lys540 and Arg 557, one Pi-cation bond with Lys540, and one Pi-Pi bond with Trp 414. Docking simulations showed that the nitroimidazole ring displays good binding with the Lys540-His538 pincer clinches in the electrostatic binding region (EBR) and 6-methoxypyridine substitution accurately occupies hydrophobic motif (HM) region similar to the previously reported thymoquinone, as Plk1 PBD inhibitor. **Compound 11** also displays some Van der Waals interactions that further contribute to binding affinity.

Lin *et al.*[87] published a study in which they utilized the Fmoc-solid-phase peptide synthesis (SPPS) strategy to synthesize nine phosphopeptides with non-natural amino acids as Plk1 PBD inhibitors. The synthesized derivatives were subjected to their Plk1 PBD inhibitory potential by determining the binding affinity using fluorescence

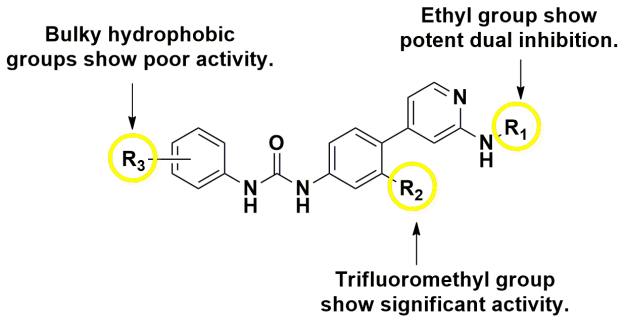


Figure 11. SAR analysis of novel PLK1/EEF2K dual inhibitors.

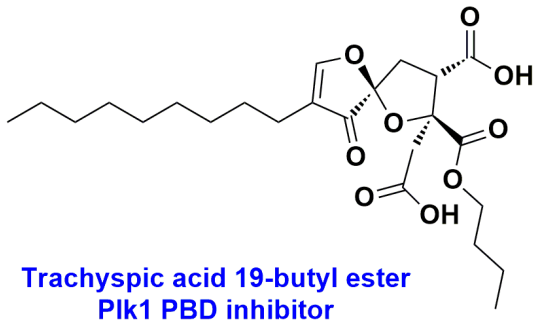


Figure 12. Structure of ester derivative of trachyspic acid reported as Plk1 PBD inhibitor

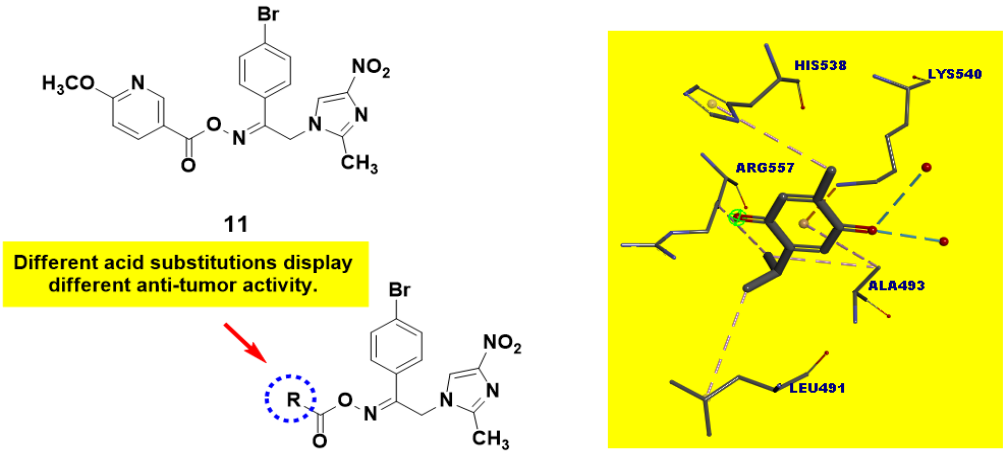


Figure 13. SAR profile of nitroimadazoleoximes as Plk1 PBD inhibitors and the stereo view of binding mode of thymoquinone in the active site of the Plk1 PBD.

polarization (FP) assay. Phosphopeptide, Ac-QTF(3,4-Cl) DPPLHSpTAIYANNH2**(12)** displayed the most significant inhibition of Plk1 PBD (IC_{50} of 38.99 nM) and were highly selective. The compound showed 600 times more selectivity over Plk3 and no binding affinity to Plk2. *In vitro* biological assay was also carried out for the phosphopeptide **12,** which revealed that compound stop the cell cycle progression by inhibiting G2/M phase transit, depending on concentration and time. In addition to this, cell membrane studies were also performed, which revealed that phosphopeptide **12** apoptosis induced by inhibition which is time and attention. According to Western blot analysis, studies demonstrated that phosphopeptide **12** increases protein cleavage of PARP caspase-3, thereby arresting G2/M phase transition of the cell cycle by controlling CyclinB1-CDK1. Also, phosphopeptide **12** penetrates the cell membranes and enters into the cytoplasm and overcomes the shortcomings of peptide-Plk1 inhibitors that cannot penetrate cell membrane.

Later the same year, Liu *et al.*[88] employed a structure-based drug design strategy to design and synthesize promising and specific multipurpose polo-like kinase 1 (Plk1)/ bromodomain 4 (BRD4) inhibitors. The extra-terminal domain (BET) and bromodomain proteins family play an essential role in recognizing acetylated lysines (KAc) on chromatin. They are the target of therapeutic value in cancer treatment inflammation and viral infectious diseases. The simultaneous inhibition of both BRD4 bromodomain and Plk1 by one molecule is a good strategy for inhibiting Plk1, and BRD4 was applied in his work. The essential structural features were extracted from the BI-2535 crystal structures with Plk1 and BRD4-BD1 were removed, followed by synthesis and evaluation of various BI-2536 analogs. It was observed cyclopentyl group and methylated amide group of BI-2536 are required for the maintenance of the bromodomain binding activity, whereas substitution of bulky groups on the asymmetrical ethyl group on the dihydropteridine nucleus resulted in compounds with potent BRD4/Plk1 inhibitory activity. Compound 13 was reported as the most active compound displaying the most potent BRD4/Plk1 dual inhibitory activity (BRD4-BD1 IC_{50} = 28 nM and Plk1 IC_{50} = 40 nM) (Figure 14).

In 2018, Hymel *et al.*[89] reported a series of peptide macrocycles that mimic the phosphopeptide binding site of Plk1 PBD. In their work, they utilized previously reported PLH*SpT (**14**) is a highly attractive ligand for Plk1 PBD as starting material and derivatized C-terminal macrocyclization of 14 employing N(π), N(bis-alkylated histidine residues as ring joint. The resulting compounds were evaluated by performing biochemical assays and displayed high target affinity and improved selectivity for Plk1-PBD. **Compound 15** emerged as an active Plk1-PBD inhibitor and with an IC_{50} of 45 nM. The crystal structure of compound 15 bound to Plk1-PBD was also solved at 1.45 Å resolution. From the binding mode of compound 15, it was observed that the ring-closing methylene chain aided the C terminal carboxamide to acquire a favorable trans amide configuration, also assisted in retaining significant H bond interactions with the basic amide of Leu491. The polymethylene chain was involved in making hydrophobic interactions with the sidechain of Leu490, which were not observed in the case of the basic nucleus compound (Figure 15).

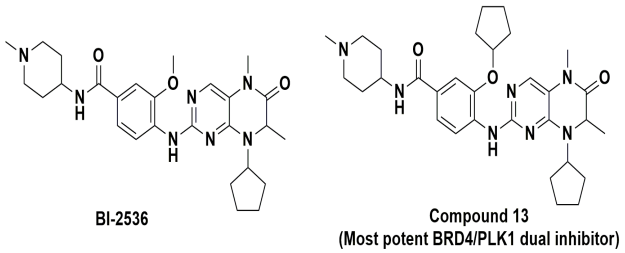


Figure 14. Most potent structural analogue of BI-2536 reported as a potent BRD4/PLK1 dual inhibitor.

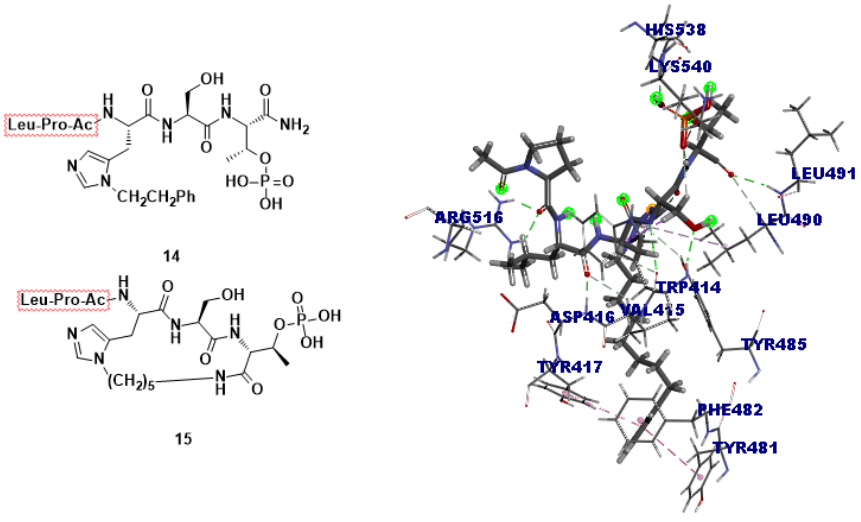


Figure 15. Most potent macrocyclic peptide (15), analogue of 14 reported as Plk1-PBD inhibitors and its binding mode in the active site (PDB: 3RQ7)

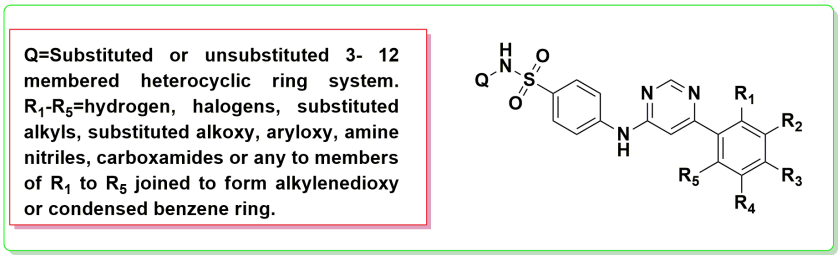


Figure 16. General structure of patented 4-pyrimidinyl amino-benzenesulfonamide derivatives as Plk1. inhibtors.

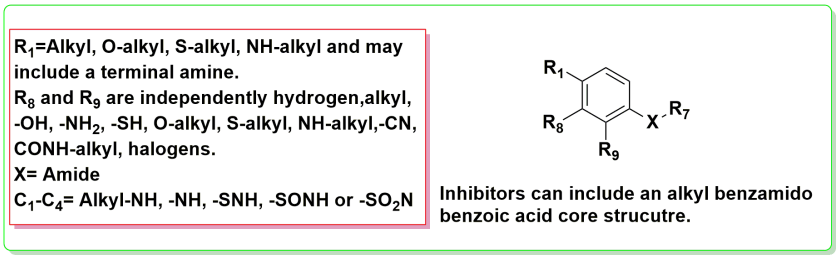


Figure 17. General structure of Plk1-PBD inhibitors.

In 2018, Liu and his research group synthesized and designed pyrrole-imidazole polyamide-Hoechst conjugate, PIP3 DNA sequence targeted Plk1 promoter. It is also responsible for specifically inhibiting cell cycle regulation which inhibits Plk1 expression and consequently retard cancer cell growth. The treatment of cancerous cells with PIP3 resulted in some mitotic misfunctions, which causes apoptosis of cells, while it will not show any effect on a normal cell by PIP3 treatment. The compound displayed the great antitumor effective activity of xenografts along with less toxicity. Hence, this PIP-Hoechst conjugate can serve as an essential lead to further develop more specific and highly selective Plk1 inhibitors.[90]

Plk1 inhibitors patents

In 2014, Vichemchemie Kutato Kft. filed a patent relating to the invention of 4-pyrimidinylamino-benzenesulfonamide derivatives as Plk1 inhibitors (Figure 16). The inhibitors were evaluated by performing *in vitro, in vivo* and genotoxicity studies. The inhibitors have also been identified as new drug candidates for the treatment of resistant tuberculosis.[91]

In 2015, McInnes *et al.*[41] filed two patents reporting fragment-specific inhibitors are selective against PBD. They developed fragment-specific Plk1 inhibitors, including non-peptide fragmentation that terminal of the inhibitor, the non-peptide fragment shows similar SAR of a peptide fragment selective for the polo-box domain of a Plk1peptide inhibitor. The invention was related to designing non-peptidic PBD inhibitors and the method of development of SAR for peptide fragment inhibitors and then developing non-peptide fragment alternatives for portions of the peptide inhibitors. In this work, the development of protein-protein interaction inhibitors has been applied to create fragment alternatives for portions of existing known peptide inhibitors; in this fragment alteration approach, binding key determinants are identified for further treatment by understanding the peptide SAR.

In 2017, McInnes *et al.*[52] filed another patent relating to non-peptide small-molecule inhibitors having the atomic mass of 1000 Daltons or less as selective for polo-like kinase proteins. The molecules were reported to target the PBD of Plk1. The inhibitors synthesized are specific for Plk1 are also much less likely to affect the activity of the Plk3 tumor suppressor, as certain of the Plk1 PBD domain inhibitors have minimal activity against Plk3. The inhibitors can be effectively displayed antitumor activity against cancer cells and can be effectively used against cancer cells that acquire resistance to ATP-based inhibitors. It was also disclosed that inhibitors could combine competitive ATP inhibitors as a synergistic means to target Plk1 clinically. Moreover, by targeting non-catalytic functions, Plk1 can be less likely to obtain resistance to the inhibitors. The general structure inhibitor is shown in Figure 17.

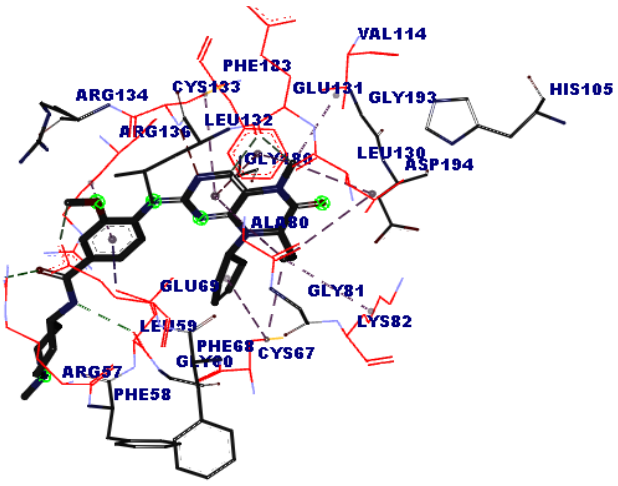


Figure 18. Stereo view of BI 2536 showing the binding mode bound to the active site. (PDB: 3RQ7).

Thus, by studying the binding orientation of different inhibitors of Plk1 reported so far, it can be observed that various amino acid residues other than those in the active site can be explicitly targeted to ensure the specificity of the newly designed derivatives. The binding mode of BI 2536, a selective Plk1 inhibitor reported so far, which is currently in clinical trials, revealed that the hinge region composed of Leu132 and Arg 136 are specific for Plk1 (Figure 18). Thus, designing molecules that will exploit this hinge region of Plk1 will result in more potent and highly selective inhibitors of Plk1 with less off-target effects.

Conclusion

Plk1 is a specific and selective target for the treatment of cancer, as its overexpression promotes tumorigenesis and plays a vital role in regulating the cell cycle, which acts as a specific oncogene for cancer development. Several Plk1 inhibitors have been reported during the past years, and many have been clinically investigated. The Plk1 contains two domains, and they are the highly conserved kinase domain at N-terminal and the PBD containing two polo boxes. So far, the classical ATP-binding site of Plk1 has been investigated to develop Plk1 inhibitors, but they suffer from the problem of selectivity. However, recently, various researchers have exploited the conserved PBD of Plk1 to report more novel and highly specific Plk1 inhibitors that inhibit the tumor suppression activity of Plk2. The efforts should be laid towards improving ATP-competitive inhibitors' specificity and developing new clinically applicable PBD inhibitors by investigating the active site residues of the PBD-binding domain. Understanding the binding mode and molecular mechanism of how the inhibitors interact with the active site of the Plk1 will provide novel insights in developing particular Plk1 inhibitors for future research activities.

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Author Contributions

RLS: Conception or design of the work and revising it critically for important intellectual content. JBW: Language editing and reviewing. RDU: Drafting of the manuscript. GDB: Drafting and revision of the manuscript. All authors have read and agreed to the published version of the manuscript.

Conflict of Interest

Authors declare no conflict of interest.

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