CHAPTER TWENTY-NINE

Progress in the Development of Non-ATP-Competitive Protein Kinase Inhibitors for Oncology

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Contents

1. INTRODUCTION

Alternative approaches for inhibitor development in targeting sites other than the ATP cleft[1] are increasingly being pursued in the search for new therapeutics targeting protein kinases.[2,3] While approved kinase inhibitor drugs offer benefit in cancer treatment, further advances are required to avoid off-target-related toxicities, effect tumor selective cell killing, and improve survival rates in oncology patients. In addition, lack of target selectivity may be a significant issue in chronic diseases where drugs are administered over a long period. Protein–protein interactions involved in kinase regulation and substrate recognition offer high potential for selectivity and avoid decreased efficacy as a result of competition with high intracellular ATP concentrations. These interfaces, however, are more challenging for inhibitor discovery. We discuss several examples where regulatory and substrate-binding sites have been successfully blocked and therefore offer potential for further drug discovery and development.

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We also discuss the latest developments in methodology for drug discovery that can be applied to the identification of protein–protein interaction inhibitors, including the validation of the REPLACE strategy which has been successfully applied to two inhibitors of protein kinase oncology targets, CDK2/cyclin A and PLK1.[4]

2. INHIBITION OF CYCLIN-DEPENDENT KINASES THROUGH THE CYCLIN GROOVE

The cyclin groove of the cell cycle CDKs is involved in recruitment of substrates prior to phosphorylation and thus provides a target for inhibition of kinase activity in antitumor drug discovery. Certain CDK substrates including the Rb and E2F proteins must undergo cyclin binding before phosphorylation, and therefore, inhibitors of the cyclin groove also block substrate-specific kinase activity. In contrast to CDK inhibitors targeting the ATP-binding site which have generally not fared well in clinical trials, this non-ATP-competitive approach can be used to generate highly selective and cell cycle-specific CDK inhibitors that have reduced inhibition of transcription mediated through CDK7 and 9.[2,5]

CDK2/cyclin A,E and CDK4/cyclin D1 are responsible for regulation of G1 and S phase of the cell cycle and phosphorylate mainly substrates containing the cyclin-binding motif (CBM).[6–8] The cyclin groove interacts with and recruits specific substrates prior to phosphorylation, and endogenous CDK inhibitors such as $p21^{WAF1}$ and $p27^{KIP1}$ block this site in addition to the ATP cleft (Fig. 29.1). Inactivation of CDK inhibitory proteins (i.e., through mutation) can be a transforming event and leads to cells bypassing the G1 checkpoint. CBM peptides that bind to the cyclin groove have been demonstrated to act as potent kinase inhibitors through blocking substrate recruitment and when administered in cell-permeable form have significant antitumor efficacy. Lack of specificity for cell cycle versus transcriptional CDKs is a major potential drawback of ATP-competitive CDK inhibitors, and toxicities arising from inhibition of CDKs 7, 8, and 9 may be the cause of undesirable effects observed with clinically investigated inhibitors. Since only A-, D-, and E-type cyclins possess a functional cyclin groove, it is possible to generate cell cycle-specific CDK inhibitors through non-ATP-competitive approaches.

Extensive structure–activity relationship data on the molecular determinants and binding groups have been obtained for the CBM, and crystallographic analysis of cyclin groove inhibitor (CGI) peptide–cyclin complexes

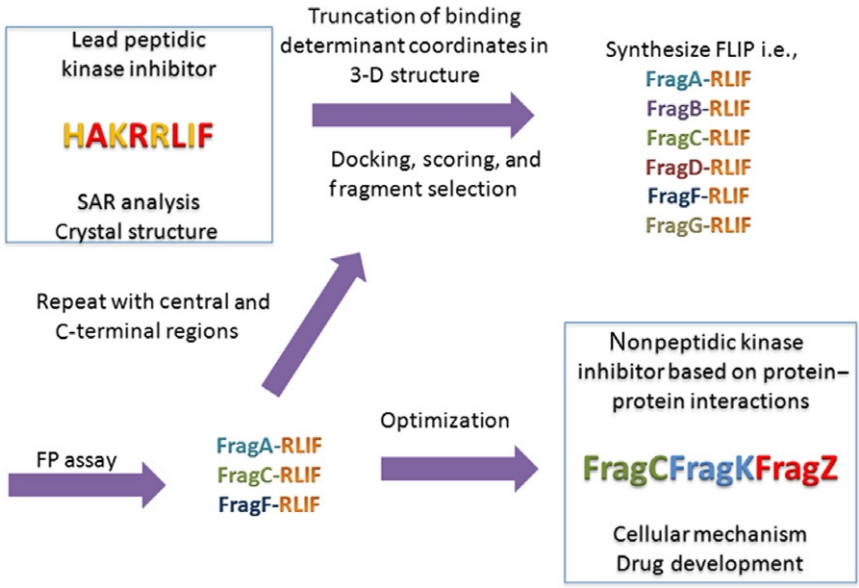


Figure 29.1 Overview of the REPLACE strategy for generation of more drug-like protein–protein interaction inhibitors.

shows that it binds in an extended conformation and that three subsites are important. These are (a) a primary hydrophobic pocket interacting with two lipophilic peptide side chains (typically a Leu and a Phe), (b) a secondary hydrophobic pocket contacting an Ala or Val side chain, and (c) a bridging site providing complementarity with basic residues of the peptide.

In efforts to develop more drug-like CGIs, peptidomimetic approaches have been applied.[9–12] Recently, the SAR of the p21 CBM was explored. After combination of individual substitutions shown to result in potency increases, the largely non-natural amino acid containing sequence, AAURSLNpfF was identified which inhibited CDK2/cyclin A with low nanomolar activity.[12] Truncation studies of the p21 sequence generated pentapeptide CGIs that were scaffolds for further drug development. In this context, 4-fluoro substitution of the C-terminal phenylalanine residue led to enhancement of binding affinity. A 3-chloro substituent on the Phe side chain of the peptide PVKRRLFG[13] also results in similar potency gains.

Another approach to developing compounds that are more pharmaceutically appropriate involved a rigidification strategy through the synthesis of peptides incorporating a cyclic restraint. Cyclization of a lysine side chain to the C-terminal glycine and thus mimicking an intermolecular hydrogen

bond observed in the p27/cyclin A crystal structure (1JSU) led to a decrease in the entropic cost of binding thereby improving *in vitro* potency. Crystal structure of these peptides with cyclin A revealed their binding mode and how this information might be exploited in future design efforts.[14]

In order to develop a general method that could be applied to protein–protein interactions, the REPLACE strategy was conceived and exemplified against CDK2/cyclin A as a target (Fig. 29.1). This method utilizes computational and synthetic chemistry so as to identify fragment alternatives for individual binding determinants.[4] In the case where SAR has previously been established, the peptide is then truncated from the N- or C-terminus in the binding site in the crystal structure. Small drug-like fragments (containing requisite functionality for conjugation to the truncated peptide) are then docked into the cavity previously containing the binding determinant. These fragments are then selected for addition to the truncated peptide sequence after prioritization through scoring functions. In the proof of concept for this approach, a library of 74 fragment ligated inhibitory peptides (FLIPs) was synthesized after *in silico* prediction and tested in a fluorescence polarization-binding assay for activity against CDK2/cyclin A. As a result, 19 hits were obtained with the most active compounds binding with higher affinity relative to the intact pentapeptide.

The discovery of fragment alternatives for the critical N-terminal arginine-binding determinant (p21 peptide HAKRRLIF, structure A, Fig. 29.2) therefore resulted in validation of REPLACE as a strategy. The most successful N-terminal capping group obtained was a phenyltriazole scaffold contacting the secondary hydrophobic subsite occupied by Ala2. Further application of REPLACE to the C-terminal phenylalanine residue (again highly sensitive to replacement) led to the identification of bis-aryl ether capping groups that appropriately mimicked the aromatic side chain in contacting the larger hydrophobic pocket.[4] Crystallographic studies of the FLIPs provided insights for structure-guided design. Overall, the REPLACE strategy was demonstrated to be a viable approach for conversion of peptide inhibitors into more stable and drug-like compounds, and proof of concept was obtained for non-ATP-competitive CDK2 inhibitors through the cyclin groove.

The REPLACE method has certain advantages over conventional approaches of fragment-based design. Potential capping groups are tested while conjugated to truncated peptide sequences, and therefore a successful FLIP recovers binding of the intact native peptide. The peptidic portion acts as an anchor and circumvents the requirement for a highly sensitive detection method in contrast to conventional fragment-based discovery (FBD)

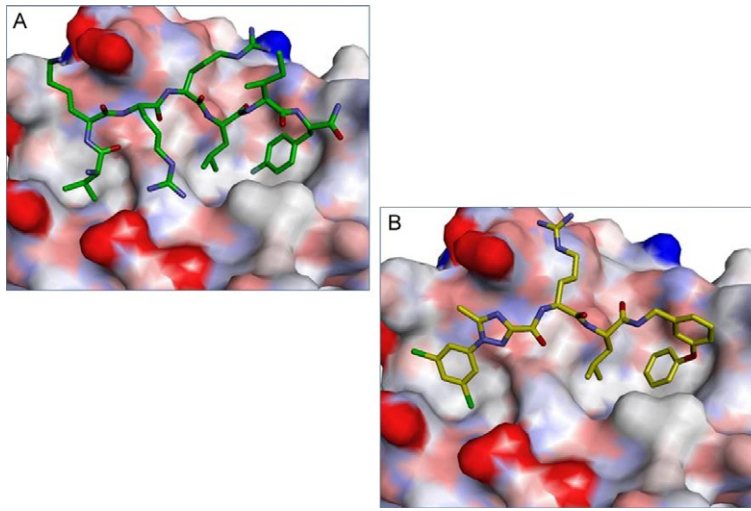
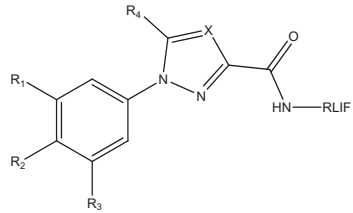


Figure 29.2 REPLACE-mediated conversion of the cyclin groove inhibitor HAKRRLIF (A) into an N- and C-terminally capped dipeptide (B).

which necessitates detection of millimolar binding affinity.[15] An additional requirement of FBD guided by X-ray crystallography is the use of fragments with high levels of solubility. The REPLACE strategy evaluates fragments as peptide hybrids thus providing a solubility handle for the inhibitor. Optimization of the identified fragments is completed while in FLIP context therefore further avoiding the need for expensive and difficult methods for detecting binding.

REPLACE optimization has been pursued through a library of heterocyclic capping groups based on the phenyltriazole system previously identified,[4] which explored the SAR for substitution of the phenyl ring and replacement of the triazole with other 5-membered heterocycles.[16] FLIPs capped with pyrazole, furan, pyrrole, and imidazole rings determined that the phenyl 1,2,4-triazole Ncaps were the most active of the group (Table 29.1). Although weaker in affinity, the pyrazole scaffold provided important SAR information regarding the secondary pocket and specifically that the 3-chloro phenyl analog (**5764**) was shown to be the most potent in the CDK2/cyclin A context. The 4-chlorophenyl derivative in the phenyltriazole context (**5774**) was shown to be the first peptide–small molecule hybrid identified with significant potency on CDK4/cyclin D1 and had increased potency relative to the RRLIF pentapeptide. The greater activity of the phenyltriazole system can be attributed to the increased

Table 29.1 Structure activity of phenylheterocyclic N-terminal partial ligand alternatives



	SCCP ID	R1	R2	R3	R4	X	CDK2/cyclin A IC_{50} (μM)	CDK4/cyclin D1 IC_{50} (μM)
Triazole	5843	H	H	H	H	N	16.2 ± 3	48.7
	5773	Cl	H	Cl	CH_3	N	4 ± 0.6	27
	5774	H	Cl	H	CH_3	N	11.5 ± 3.3	11.3
Pyrazole	5762	H	H	H	CH_3	C	40.3 ± 6.5	53.8
	5763	Cl	H	Cl	CH_3	C	21.8 ± 13.7	100–180
	5764	Cl	H	H	CH_3	C	11.9 ± 2.0	45
	5771	F	H	H	CH_3	C	29.6 ± 12.2	69.6
	5765	H	Cl	H	CH_3	C	33.7 ± 8.1	49

H-bond strength relative to the other heterocycles and a potential bridging water molecule, which is not present in the phenylpyrazole complex.

Validation of fragment alternatives identified as individual capping groups when combined into a single inhibitor is a key aspect of REPLACE since it is possible that conformational changes result after modification of peptide determinants. Optimized N-capping and C-capping groups were combined into individual molecules (structure B, Fig. 29.2) in order to accomplish this and revealed that 3-phenoxybenzylamine had decreased activity when combined with the 3,5-DCPT-Arg-Leu, relative to the peptide context. It was found, however, that addition of halogens onto the phenoxy substituent contacting the primary hydrophobic pocket resulted in increased activity.[17] Either a 3-fluoro or a 4-fluoro substituted bis-aryl ether had enhanced potency, thus replicating the corresponding increase observed in the peptide context.[12] These data indicate that potency lost by combining partial ligand alternatives can be recovered through subsequent optimization.

Another approach to CDK2/cyclin A inhibition through the cyclin groove included combination of fragment-like structures with peptidomimetic design and synthesis. The octapeptide, PVKRRLFG was employed as the starting point, and consistent with cyclin groove interactions, Arg, Leu, and Phe were delineated as critical determinants.[13] The identification of a smaller tetrapeptide lead was optimized through design, and further rigidification of its structure resulted in aminothiazole capping group variants replacing the critical arginine while recovering binding lost through truncation.[13] An optimized CGI was generated with 500-fold increase in activity through reduction of conformational freedom at the C-terminal group using a *trans*-2-arylcyclohexyl combined with the aminothiazole Ncap (compound **1**, Fig. 29.3, 0.021 μM). A proline mimetic was used to replace the second arginine of PVKRRLFG and resulted in peptidomimetic inhibitor with greater drug-likeness as a trade-off for slightly decreased potency (compound **2**, Fig. 29.3, 0.04 μM). In summary, this work led to the generation of a potent noncharged CDK2/cyclin A inhibitor containing fewer rotatable bonds and therefore making it considerably more drug-like.

Although highly potent peptide and small-molecule inhibitors of CDK2/cyclin A substrate recruitment have been generated, much less attention has been focused on the cyclin groove of the CDK4/cyclin D1, a validated anticancer drug target. Structures of the cyclin groove of cyclin D in complex with CGIs, and *in vitro* binding of peptide analogs were examined in detail in order to determine the basis for peptide affinity and selectivity.[18] Cyclin groove comparison of cyclin A2 and D1 resulting from these studies determined that residue substitutions result in significant differences in the acidic region contacting the critical arginine and in the volume of the

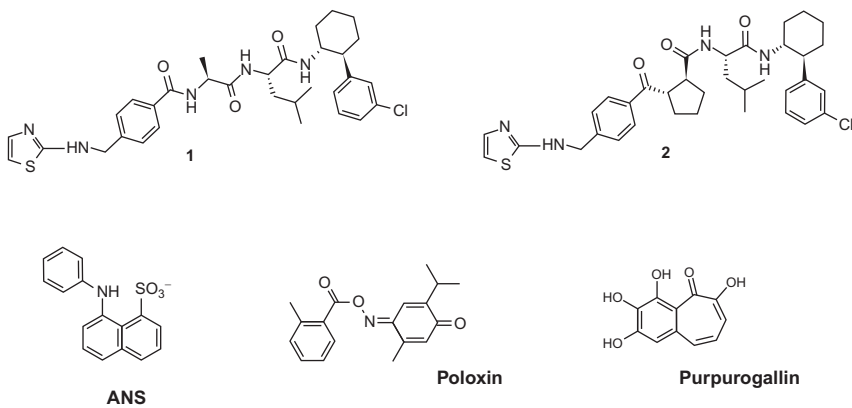


Figure 29.3 Chemical structures of non-ATP-competitive CDK and PLK inhibitors.

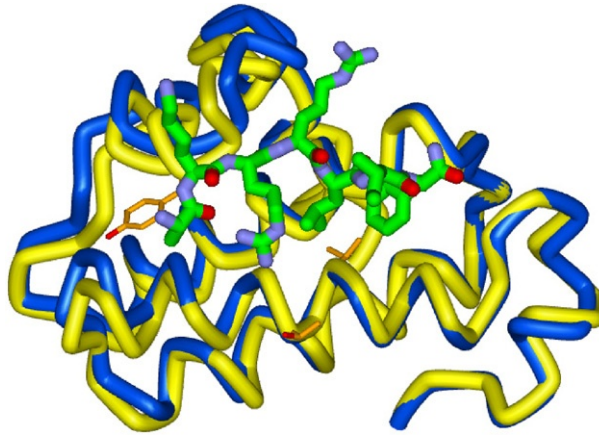


Figure 29.4 The cyclin-binding grooves of cyclin A (blue ribbon) and cyclin D1 (yellow ribbon) are compared through overlay. Unique residues of cyclin D1 shown in orange are Tyr127, Thr62, and Val60 (left to right).

primary and secondary lipophilic pockets (Fig. 29.4). Structural differences were then further investigated through incorporation of non-natural phenylalanine replacements into p21 and p107 octapeptide contexts. Of special note, a 3-thienylalanine substitution was effective and in addition, cyclohexylalanine led to increased binding for cyclin D1 relative to cyclin A2. Subsequently, the interactions of the CDKI, $p27^{KIP1}$, were modeled with cyclin D1 and provided significant insights into the endogenous inhibition of CDK4. Unique features of the CBG of cyclin D1 were observed including an extension to the primary pocket which can potentially be exploited in the design of non-ATP-competitive CDK inhibitors.

As a whole, these studies validate the cyclin grooves of CDK2/cyclin A and CDK4/cyclin D as druggable sites in order to create potent and cell cycle-specific non-ATP-competitive inhibitors and generate considerable insights into how these compounds can be modified through structure-guided design in order to develop chemical biology probes and potential therapeutics based on selective inhibition of CDK4/cyclin D activity.

3. ALTERNATIVE STRATEGIES IN THE DEVELOPMENT OF NON-ATP-COMPETITIVE CDK INHIBITORS

Another strategy for the development of antitumor therapeutics based on CDK inhibition and beyond targeting substrate recruitment sites is to block the association of the cyclin positive regulatory and catalytic subunits and therefore prevent formation of the activated CDK–cyclin complex.

This should theoretically deliver similar results as can be achieved with inhibition of the ATP-binding site and the cyclin groove. Subunits that form protein complexes generally bind too strongly to be competitively dissociated. CDKs and cyclins belong to a class of proteins that exist in both monomeric and heterodimeric form in response to their environment and thus have relatively high K_d values (1 μM to 1 nM).[19] The CDK2–cyclin A complex has a dissociation constant of approximately 50 nM[20] and therefore is in a feasible affinity range for complex dissociation induced by small molecules. Toward this end, an all d-amino acid hexapeptide (NBI1) has been identified that interferes with the formation of the CDK2–cyclin A complex. Inhibition of kinase activity was demonstrated and direct binding to cyclin A was revealed using surface plasmon resonance.[21] In addition, a cell-permeable derivative of NBI1 resulted in apoptotic cell death and antiproliferative effects in tumor cell lines. This study demonstrates the proof of concept that protein–protein interactions regulating CDK activation can be successfully targeted and may be useful in the generation of non-ATP-competitive cell cycle-based cancer therapeutics.

Further studies on the disruption of the CDK–cyclin interaction were undertaken through probing the binding of the fluorophore, 8-anilino-1-naphthalene sulfonate (ANS; Fig. 29.3) with CDK2 and cyclin A using fluorescence spectroscopy and protein crystallography.[22] Results showed that ANS binds to CDK2 with a K_d of 37 μM. Additional experiments revealed that cyclin A was able to displace the fluorescent dye from CDK2 with an EC_{50} value of 0.6 μM. Insights into this novel observation were provided through cocrystallization of monomeric CDK2 with ANS. Other complexes with ATP-competitive inhibitors determined that two ANS molecules bound in a significant cleft extending from the C-helix and were positioned beside each other. Large conformational changes in the catalytic subunit are induced by the binding of ANS and thereby allosterically blocking the binding of CDK2 to cyclin A. The ANS binding pocket is therefore a potentially druggable binding site for inhibitors acting on CDK2/cyclin A through a novel allosteric mechanism.

4. POLO-BOX DOMAIN INHIBITORS OF POLO-LIKE KINASES

The two domains of PLKs include the kinase catalytic region and the polo-box domain (PBD) with the latter being required for subcellular localization and many PLK functions.[23,24] The role of the PBD in mitosis and potential for oncology have been demonstrated through administration of

a permeabilized PB1 fragment to tumor cells which resulted in mitotic arrest, misaligned chromosomes, and multiple centrosomes.[25] Competition between the PB fragment and endogenous PLK1 for binding to substrates is the probable cause for the observed effects.[26–28] A phosphopeptide consensus sequence has been demonstrated to interact with the region containing the two PBs (residues 326–603), and further investigation determined that a number of important mitotic proteins, including CDC25C, contain this motif and avidly bind to the PBD of PLK1.

Several recent crystallographic structures of the human PLK1 PBD in complex with phosphopeptide inhibitors have provided insights into the structural basis for peptide binding which occurs in a shallow groove at the interface of the two PBs. These PBD ligands interact through electrostatic (Gln^2, Ser^3, and Thr^4) and van der Waals contacts (Met^1, Leu^6) and potentially through bridging water molecules.[29–32] Within the PBD, the two PBs were shown to have an almost identical fold despite having relatively low sequence identity.

Insights into PBD inhibitor binding obtained from these crystal structures, in addition to the biological relevance of the PBD, suggest these are druggable interfaces that can be exploited in design of non-ATP-competitive inhibitors (Fig. 29.5). Further rationale for pursing the PBD as a drug target is provided through precedence for similar interfaces found in adaptor proteins that bind to tyrosine kinases.[33] For example, Src-homology (SH2 and SH3) domains recognize phosphopeptide motifs such as pTyr motifs.[34,35]

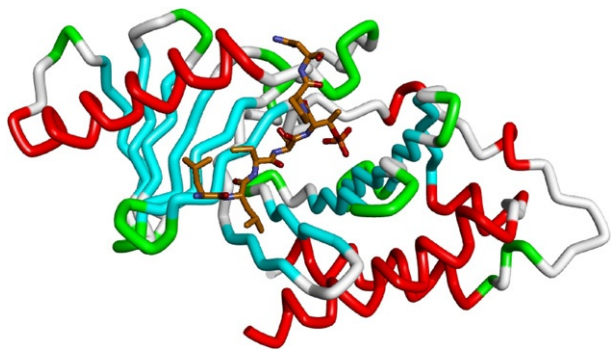


Figure 29.5 Ribbon representation of the polo-box domain showing the CDC25c PBD crystal structure (3BZI) with the peptide bound at the intersection of the two polo boxes. The Leu-Leu-Cys tripeptide replaced by fragment alternatives is on the left side of the figure.

Additional impetus for developing inhibitors of the PBD has come to light in recent studies. Evidence has emerged that PLK3 acts directly or indirectly as a tumor suppressor in that it is activated as a result of DNA damage and its inhibition stimulates proliferation.[36,37] These findings have been coupled with recent data showing that minimized phosphopeptides bind potently and selectively to the PLK1 PBD and not to the PLK2 and 3 phosphopeptide-binding site.[38–42] In contrast to this, many of the reported ATP-competitive PLK inhibitors, although selective for PLKs, bind individual isoforms with similar affinity. As a result of the roles of PLK3 in countering PLK1, isotype selective compounds will be important. Hence, drugs specifically targeting the PLK1 would obviate effects of blocking PLK3 since this may contribute to tumorigenesis in normal cells.

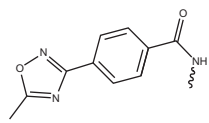
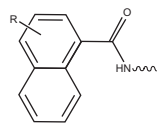
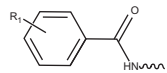
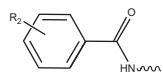
In attempts to discover small-molecule PBD inhibitory compounds, Reindl and colleagues[43] have applied high-throughput screening (HTS) approaches to the PBD although with limited success. A library of 22,461 small molecules was screened in a fluorescence polarization competitive-binding assay with poloxin (Fig. 29.3, IC_{50} of 4.8 ± 1.3 μM), thymoquinone (apparent IC_{50} of 1.14 ± 0.04 μM), and poloxipan[43,44] being identified. Their low activity, limited scope for optimization, and redox potential of these series suggest that better compounds are needed. Although purpurogallin[45] (Fig. 29.3, $IC_{50} = 500$ nM), another compound identified by HTS methods, has improved activity toward the PBD of PLK1, it would require substantial modification in order to improve drug-like properties. The modest success of HTS attempts in the search for PBD inhibitory compounds leads to the conclusion that alternate drug discovery strategies would be expedient with this target.

Further to this, REPLACE, which has been described above and validated for CDK2/cyclin A as a drug target, was applied to the PBD to discover small-molecule alternatives and generate non-ATP-competitive inhibitors that are selective for PLK1. The basis for the application of REPLACE in this context was the CDC25c substrate peptide, LLCS[pT]PNGL which is primed by CDK1 phosphorylation, resulting in high affinity binding to the PBD. Prior to efforts using REPLACE, peptide analogs were synthesized and tested in order to delineate the structure–activity relationship of the CDC25c sequence and to further describe the key binding determinants. Further peptide derivatives were explored based on modification of the sequences from PBIP1 (PLHS[pT]AI) proteins which form complexes with the PLK1 PBD.[38] In the first instance, the activity requirement for the

phosphothreonine residue was investigated in both peptide contexts. Results demonstrated that isosteric Glu replacements bound with weak affinity, thereby providing information that is useful for inhibitor design. An unexpected and serendipitous result was obtained from this study in the observation that acetylation of the N-terminus is crucial for truncated peptides to bind with high affinity to the PBD and thus provided extended SAR data for PBIP1 peptides previously reported.[38] Further examination of the complex structures for the PBD/PBIP1 peptides revealed that the structural basis for this contribution derives from the close proximity of an arginine residue. In the nonacetylated peptide, the basic guanidinium side chain would repel the positively charged N-terminus compared to the acetylated context where H-bonds to the amide are apparent from the crystal structure. Further SAR from alanine replacement of Leu2 of the PBIP1 sequence indicated a threefold loss in binding. Prior to application of REPLACE, it was established that the hydrophobic tripeptide (LLC to generate S[pT]PNGL) of the Cdc25C sequence is a critical determinant as shown through inactivity of the truncated peptide.

Since the peptide SAR studies generated additional information that would be exploited in the design of nonpeptidic inhibitory compounds, the REPLACE strategy was applied to the N-terminal tripeptide using available crystal structures of peptides bound to the PBD. In the first iterations of REPLACE, the three N-terminal residues were excised from the 3-D structure and subsequently 1800 drug-like carboxylate-containing fragments were docked into the vacated pocket of the receptor. While weak partial ligand alternatives were identified initially, subsequent iterations of REPLACE resulted in derivatized benzoic acid-capped peptides (Table 29.2). As shown in this table, with iterations and optimization of REPLACE, compounds progressed in activity from 320 (1G2-S[pT]PNGL) to less than 10 μM (3G2-S[pT]PNGL). This simple scaffold mimics the interactions of the Leu-Leu-Cys tripeptide within one log of the activity of the endogenous Cdc25C peptide and almost equivalent in potency to the truncated PBIP1 peptides, however, with approximately 1/3 the molecular weight. FLIPs displayed a clear structure–activity relationship and activity below 10 μM. A significant aspect of this series is that it provides sufficient potential diversity for optimization through exploiting interactions observed in the peptide complex structures and SAR data provided in the peptide context. In addition, testing of these compounds in a competitive-binding assay for PLK3 determined that PLK1 selectivity was retained. Further optimization through REPLACE, for example, incorporation of an H-bond

Table 29.2 PLK PBD *in vitro* binding and cellular activity for FLIP compounds (Ncap-S[pT]PNGL)

Abbreviation	N-capping group	PLK1 PBD FP IC_{50} (μM)	PLK3 PBD FP IC_{50} (μM)	Apoptosis at 24 h (30 μM)	Aberrant mitoses at 24 h (30 μM)
1G2-S[pT]PNGL		320	ND	ND	ND
2G1-S[pT]PNGL		99	ND	ND	ND
3G1-S[pT]PNGL		16.5	>600	36.2%	47.9%
3G2-S[pT]PNGL		8.6	>600	55.5%	53.8%
Ac-PLH S[pT]A	–	2	>600	34.0%	40.3%

acceptor group to the benzoic acid scaffold in the manner of the critical acetyl group described above could provide enhanced binding affinity. Recent studies of peptide analogs resulted in the serendipitous discovery of a hydrophobic channel that is exploited by lipophilic substituents incorporated on a histidine residue of PBIP sequence.[46] These and more recent compounds resulted in high affinity binding to the PBD.[47] The crystal structures obtained from these experiments suggest that appropriate modification of PBD peptide capping groups would result in similar activity increases, and resulting affinity gains should allow truncation and replacement of the C-terminus through capping groups which mimic the phosphothreonine and C-terminal residues.

As a prelude to the further identification and characterization of nonpeptidic PBD inhibitors, FLIPs and peptides were delivered into tumor cells to determine if phenotypes observed consistent with PLK1 inhibition and specifically through the PBD were obtained.[48] Intracellular

administration was achieved through the use of the QQ transfection reagent, and after treatment of HELA cells, a significant reduction of PLK1 localization to centrosomes was observed with both capped and peptidic compounds. Further investigation determined that aberrant mitoses as visualized by mono and multipolar spindles, abnormal chromosome alignment during metaphase (Table 29.2, 3G1 and 3G2-S[pT]PNGL), and cell death through apoptosis occurred in a dose-dependent fashion. Cellular data obtained for third-generation FLIPs indicate that, in spite of lower affinity, peptide–small molecule hybrids have improved antitumor activity relative to purely peptidic inhibitors (Table 29.2, 3G1 and 3G2-S[pT]PNGL). It is possible that the N-terminal capping group increases cellular half-life through improvements in compound stability toward proteolytic enzymes.[49] As a whole, the data acquired for FLIPs discovered through REPLACE provide additional validation that it is an effective method for producing more drug-like and less peptidic inhibitors of the PBD of PLK1.

5. CONCLUSIONS

Although protein–protein interactions represent potential drug targets in the identification of selective kinase inhibitors, improvements in methodology are required. Two case studies of successful application of the REPLACE strategy are presented in the discovery of nonpeptidic and non-ATP-competitive protein–protein interaction inhibitors of validated oncology targets. More drug-like inhibitors of cyclin-dependent kinase substrate recruitment have been identified through replacement of N- and C-terminal determinants of a potent octapeptide. The PBD provides the opportunity to develop selective PLK1 inhibitors through its phosphopeptide-binding site, and progress is described in the identification of FLIPs with preliminary antitumor activity. In summary, REPLACE has been exemplified against two protein kinase targets and is a viable strategy for the conversion of peptidic inhibitors of protein–protein interactions into more drug-like molecules. In addition, REPLACE has significant advantages over other fragment-based design methods in that it obviates the need for highly soluble capping groups and the availability of sensitive binding detection.

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