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Enhancing polo-like kinase 1 selectivity of polo-box domain-binding peptides

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ABSTRACT

An important goal in the development of polo-like kinase 1 (Plk1) polo-box domain (PBD) binding inhibitors is selectivity for Plk1 relative to Plk2 and Plk3. In our current work we show that Plk1 PBD selectivity can be significantly enhanced by modulating interactions within a previously discovered "cryptic pocket" and a more recently identified proximal "auxiliary pocket."

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1. Introduction

Members of the polo family of serine/threonine kinases (polo-like kinases; Plks 1–5) play important roles in mammalian cellular physiology.[1] Overexpression of the cell cycle regulatory Plk1 occurs in a number of cancers, where it is associated with a poor prognosis and Plk1 is recognized as a potentially promising anticancer target.[2] In addition to their amino-terminal catalytic kinase domains (KD) (not present in Plk5[3]), the Plks are characterized by C-terminal polo-box domains (PBDs), which mediate protein-protein interactions (PPIs) by recognizing and binding to phosphoserine (pSer) and phosphothreonine (pThr)-containing sequences.[4] Development of Plk1 inhibitors is made complicated by a number of factors. Among these is the fact that Plks 2 and 3 may function as tumor suppressors, thereby making Plk1-selectivity an important objective.[5] Issues of collateral cytotoxicity have arisen for KD-directed inhibitors, potentially due to similarities among the KDs of Plk1, 2 and 3 as well as high homology among protein kinases in general. Because both the KD and PBD are essential for proper Plk function,[6] PBD-binding antagonists offer a potentially attractive alternative to KD-directed inhibitors for down-regulating Plk function.[7] Such agents may offer advantages in terms of selectivity, since PBDs are unique to the Plks and the domains exhibit differences in their binding preferences for phosphoepitope sequences.[8,9] Additionally, the results of blocking PBD-binding may not be the same as inhibiting kinase activity, opening the possibility of qualitatively different therapeutic profiles or synergism with KD inhibitors.[10] Accordingly, significant effort has been devoted to develop Plk1 PBD-binding antagonists.[2,7]

Competitive PBD-binding inhibitors have been approached using peptides or peptide mimetics, which are based on cognate recognition sequences. Starting from the polo-box interacting protein 1 (PBIP) pT78-derived peptide PLHSpT (**1**),[11] we have previously reported that appending alkylphenyl groups from different positions on the peptide, including the His N(π)-position [the sequence having a $(CH_2)_8$Ph group appended is designated as PLH*SpT (**2a**)],[12] the 4-position on the Pro residue (**3**)[13] and an amino-terminal *N*-alkyl Gly residue (**4**),[14] can result in up to one thousand-fold enhancement in Plk1 PBD-binding affinity (Fig. 1). We found in each case that the alkylphenyl groups bind within a "cryptic binding pocket" on the PBD surface, which is defined by PBD residues Y417, Y421, Y481, L478, F482 and Y485 and revealed by rotation of the Y481 side chain (Fig. 2). Abell has also shown that the same cryptic pocket can be accessed by the phenyl ring of the terminal Phe residue in the longer PBIP1 pT78-derived sequence FDPPLHSpTA[15] and by the phenyl ring of 3-phenylpropylamide on the Pro residue of **1**.[16] This group has also shown that higher affinity can be realized by replacing the terminal Phe residue in the FDPPLHSpTA sequence with a residue that contains a more lipophilic 2-benzothiophene ring (**5**), which is consistent with the highly lipophilic nature of the cryptic pocket (Fig. 1).[17]

Recently, we examined access to the cryptic pocket from the His N(π)-position in peptide **1** using an oxime-based post-solid phase peptide diversification strategy[18] and found that replacing the phenyl ring with motifs having two aryl rings arranged in an angular

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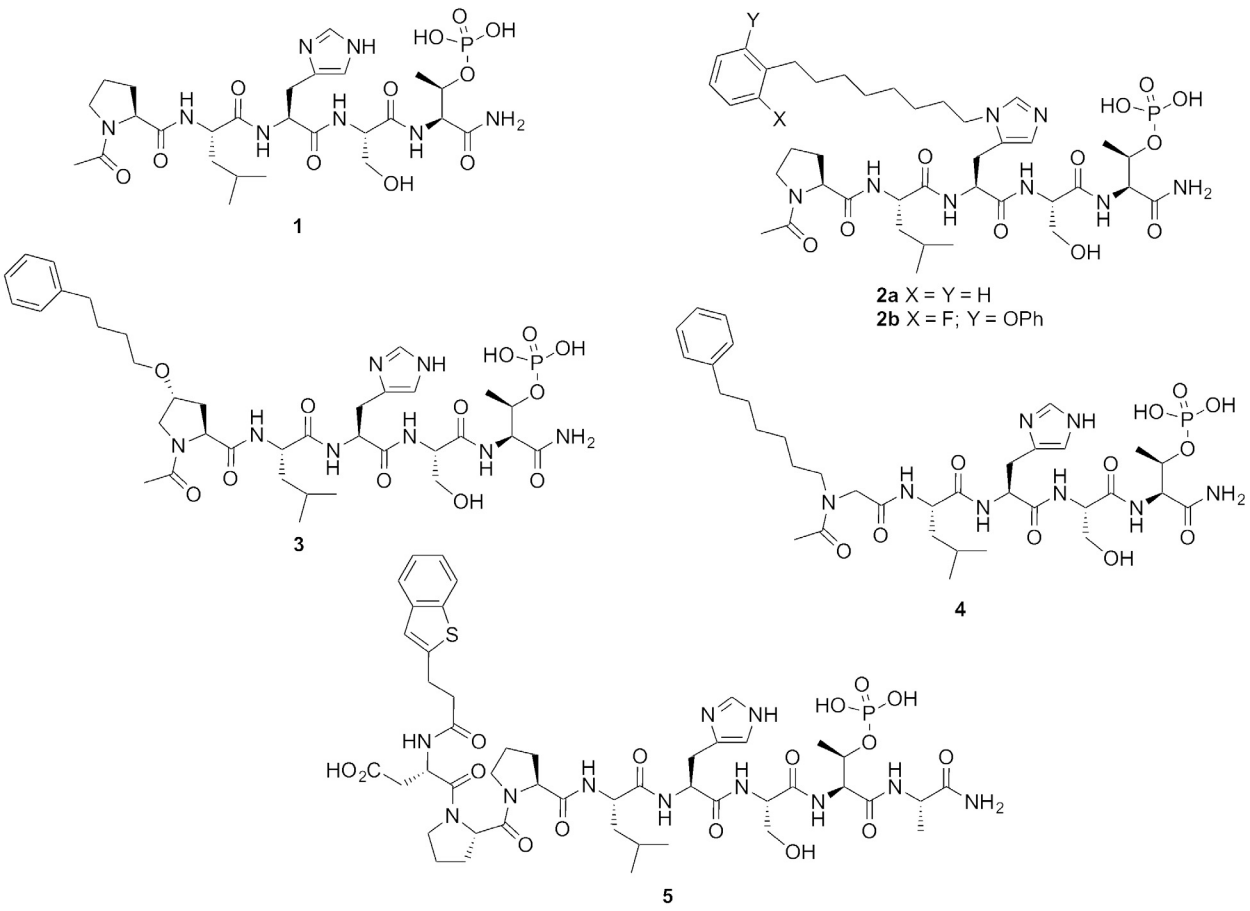

Fig. 1. Structures of peptides discussed in the text.

orientation, can result in several fold greater Plk1 PBD-binding affinity relative to the parent phenyl ring.[19] We hypothesized that enhanced affinity may result from interactions within an auxiliary region that is contiguous with the canonical cryptic pocket. Yet, beyond the above, very little has been reported to exploit binding within the cryptic pocket to greater advantage. Therefore, as we report herein, we used the simple tethered phenyl ring of peptide **2a** and our recently disclosed 2-fluoro-6-phenyloxylphenyl peptide **2b** as starting points for the design of peptides **6a** and **6b**, which include hydroxyls at different locations. Our thought was that this added functionality could potentially afford interactions with hydroxyl or carbonyl groups of residues Y421 and Y481 or other residues proximal to the Plk1 PBD cryptic pocket. The benzyl protected precursors **7a** and **7b** also provided the opportunity to extend aromatic interactions in this region. Our ultimate goal was to more fully explore what could be achieved by altering known binding motifs, particularly effects related to Plk1, 2 and 3 selectivity.

2. Results and discussion

2.1. Chemistry

Peptides **6**(**a**, **b**) and **7**(**a**, **b**) were obtained by Fmoc-based solid-phase peptide synthesis using reagents **18**(**a**–**d**), which were prepared as shown in Scheme 1.[20] The synthesis began with the *t*-butyl and benzyl-protected alcohols **9**(**a**, **b**), which were synthesized by protecting the free hydroxyl of the commercially available alcohol 2-(4-bomophenyl)ethan-1-ol (**8**). Coupling of the aryl bromides **9**(**a**, **b**) and oct-7-yn-1-ol,[21] followed by hydrogenation catalyzed by Pd·C (for **10a**) or Wilkinson's catalyst (for **10b**) afforded alcohols **11**(**a**, **b**) having *t*-butyl and benzyl protecting groups, respectively. Separately, SNAr reaction of 2,6-difluorbenzaldehyde (**12**) with 3-(*tert*-butoxy)phenol or 3-(benzyloxy)phenol gave the 2-fluoro-6-phenoxybenzaldehydes **13**(**a**, **b**), respectively.[22] Reduction of the aldehyde functionality in **13(a, b)** and replacement of the hydroxyls in the resulting **14(a, b)** with bromide gave the corresponding benzyl bromides **15(a, b)**. Coupling of **15(a, b)** with hept-6-yn-1-ol or ((hept-6-yn-1-yloxy)methyl)benzene afforded the alkynes **16(a, b)**. Hydrogenation catalyzed by Pd·C or Wilkinson's catalyst afforded the alcohols **17(a, b)**. Finally, reaction of alcohols **11(a, b)** and **17(a, b)** with either the 2,4-dimethoxylbenzyl ester or the benzyl ester of N^{α}-Fmoc-His using our previously reported methodology afforded the key orthogonally protected His N(π)-alkylated analogs **18(a–d)**.[19,20] Employing these reagents, we prepared peptides **19(a–d)** on NovaSyn TGR resin. Cleavage of the peptides from the resin using a cocktail solution of TFA/H_2O/TIS (95/2.5/2.5) and purification by HPLC gave the desired peptide products **6(a, b)** and **7(a, b)** (Scheme 1).

2.2. Biological evaluation

2.2.1. PBD-binding activity in full-length Plk1

In order to evaluate PBD-binding affinities, we employed an ELISA assay, which measures the ability of the synthetic peptides to compete with an immobilized phosphopeptide "PMQSpTPLN"