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Non-proteinogenic amino acids in the pThr-2 position of a pentamer peptide that confer high binding affinity for the polo box domain (PBD) of polo-like kinase 1 (Plk1)

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Abstract

We report herein that incorporating long-chain alkylphenyl-containing non-proteinogenic amino acids in place of His at the pT-2 position of the parent polo-like kinase 1 (Plk1) polo box domain (PBD)-binding pentapeptide, PLHSpT (**1a**) increases affinity. For certain analogues, approximately two orders-of-magnitude improvement in affinity was observed. Although, none of the new analogues was as potent as our previously described peptide **1b**, in which the pT-2 histidine imidazole ring is alkylated at its π nitrogen (N3), our current finding that the isomeric His(N1)-analogue (**1c**) binds with approximately 50-fold less affinity than **1b**, indicates the positional importance of attachment to the His imidazole ring. Our demonstration that a range of modified residues at the pT-2 position can enhance binding affinity, should facilitate the development of minimally-sized Plk1 PBD-binding antagonists.

Keywords

Polo-like kinase; Polo box domain; Non-proteogenic amino acids; Peptide ligands; Signal transduction

Mitosis represents a highly complex and orchestrated process that involves proper functioning of the serine/threonine polo-like kinase 1 (Plk1).¹ Plk1 contains a catalytic domain and C-terminal polo-box domain (PBD) composed of two linked polo-boxes (PB1 and PB2). The PBD serves an important role by directing the Plk1 to specific phosphoserine (pS) and phosphothreonine (pT)-containing sequences.^{2,3} The ability of Plk1 to promote oncogenic transformation^{4–6} has made Plk1 a potential anticancer drug target for both catalytic domain and PBD-directed agents.^{7,8}

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Supplementary Data

Supplementary data (synthetic experimental procedures and analytical data for synthetic products, Plk1 PBD-binding assay protocols and modeling procedures) associated with this article can be found in the Electronic Supplementary Information.

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Peptide-based ligands offer an approach for developing PBD-binding antagonists. One objective in the design of such agents is to maximize binding interactions in the most efficient manner. The Plk1 PBD has been shown to recognize sequences of the general form, [P/F]-[/P]-[]-[T/Q/H/M]-S-[pT/pS]-[P/], where designates a hydrophobic residue.^{9,10} The “S-pT” dipeptide segment represents a critical element that is necessary for high binding affinity. Several crystal structures of the Plk1 PBD in complex with different phosphopeptides have been reported, including MQSpTPL (PDB: 1Q4K),¹¹ PMQSpTPL (PDB: 1UMW),¹⁰ LLCSpTPN (PDB: 3BZI),¹² LHSpTA (PDB: 3FVH),¹³ and PPHSpT (PDB: 3C5L).¹³ These show that the “SpT” motif forms an anchor that is held in place by means of an electrostatic “pincer” arising from the interaction of the negatively-charged phosphoryl group with H538 and K540 present the PB2 unit. The importance of the H538 and K540 residues for phospho-dependent binding has been confirmed by mutational studies.¹⁰ Interactions arising from within this SpT-binding region will likely be critical for high affinity binding of any peptide or peptide mimetic.

The polo-box interacting protein 1 (PBIP1) is a Plk1 substrate that undergoes phosphorylation at T78 to form a Plk1 PBD-binding ligand.¹⁴ We have shown that the PBIP1-derived 5-mer peptide, 74-PLHSpT-78 (**1a**, Figure 1) can serve as a minimal sequence that retains high Plk1 PBD-binding affinity.¹³ Peptide **1a** affords a potentially attractive starting point for developing compact PBD-binding ligands. Abell has demonstrated by using a more extended 9-mer PBIP1 sequence, 71-FDPPLHSpTA-79, that the amino-terminal F71 residue can enter into binding interactions with a newly formed pocket defined by PBD residues V415, Y417, Y421, L478, Y481, F482 and Y485.¹⁵ This pocket is not present or accessible in the crystal structure of the shorter PBD-bound 5-mer peptide (**1a**) (PDB: 3HIK).¹³ However, we recently discovered that structural variants of the parent peptide **1a**, in which the pT-2 histidine imidazole ring is alkylated at its π nitrogen (N3), can afford exceptionally high affinity.¹⁶ This is exemplified by peptide **1b**, which contains a $C_6H_5(CH_2)_8$ -group at this position and which shows an approximate 1000-fold affinity enhancement relative to **1a**.¹⁶ A crystal structure of **1b** bound to the PBD (PDB accession code i3RQ7)¹⁶ demonstrates that binding of the $C_6H_5(CH_2)_8$ -moiety occur in a well-formed hydrophobic channel, which is created by dramatic re-orientation of the Y481 aryl ring. Elements of binding within this channel are reminiscent of what had been reported for the F71 residue of the 9-mer-peptide.¹⁵

Recently, this PBD binding region has been accessed from different locations on peptides.^{17–19} It is noteworthy that for these analogues, the residues that interact with the binding channel originate from positions in the peptide chain further removed from the critical SpT motif than does the alkyl-His residue in peptide **1b**. Therefore, the pT-2 position may be viewed as “privileged” in its ability to access this new binding region, while maintaining close proximity to the signature “S-pT” residues. The current work was undertaken to examine whether modified residues other than His at the pT-2 position of **1a** can also impart high affinity.

Aryl functionality has been shown to bind preferentially in the hydrophobic pocket being targeted by the present work.^{15–19} Accordingly, long chain phenylalkyl groups were introduced by a variety of approaches at the pT-2 position of peptide **1**. These include modifying the side chain nitrogens of Trp (**1d**), Asn (**1e**), Gln (**1f**), ornithine (Orn) (**1g**) and Lys (**1h**) (Figure 1). In addition, the His(N1) alkyl derivative was prepared (**1c**), since only isomeric His(N3) alkyl adducts had been examined previously.^{16,20} The $C_6H_5(CH_2)_8$ -group was employed for His, Trp and Asn residues, while shorter chains were utilized for Gln and Orn [$C_6H_5(CH_2)_7$ -] and Lys [$C_6H_5(CH_2)_5$ -] (Figure 1). The synthesis of peptides **1a** and **1b** has been reported.^{16,20} Synthesis of the remaining peptides was by solid-phase Fmoc-based protocols using acid labile resin (Supporting Information). Introduction of the pT-2-

modified residues bearing long chain phenylalkyl groups was accomplished using appropriate non-coded *N*-Fmoc-protected amino acids **2c** – **2h** (Figure 1), which were prepared as described in the Supporting Information. Completed peptides were cleaved from the resin and purified by HPLC to provide the desired products **1c** – **1h** as white amorphous powders.

All peptides were evaluated for their ability to compete with immobilized PBIP1-derived 13-mer p-T78 peptide for binding to Plk1 PBD in an ELISA-based inhibition assay that employed HEK 293A cell lysates expressing GFP-HA-fused Plk1 (Figure 2).^{13,16} In this assay the control peptide, “PLHST” exhibited negligible affinity, even at the highest concentration tested (300 μ M), confirming the phospho-dependent nature of binding. The low micromolar affinity observed for the parent pT78-derived pentapeptide **1a** (IC_{50} = 16 μ M, Figure 2) is consistent with previous reports.^{16–18} For the all newly prepared peptides, introduction of non-canonical residues bearing long-chain alkylphenyl groups at the pT-2 position conferred additional affinity to the parent peptide **1a**. Modeling studies comparing the crystal structure of PBD-bound **1b** (PDB accession code 3RQ7)¹⁶ with docked and energy minimized structures of the peptides **1c** – **1h**, showed that with the exception of their pT-2 side chains, all other portions of the peptides were nearly unchanged relative to **1b** (Figure 3). In comparison to **1b** (IC_{50} = 5.6 nM),¹⁶ the isomeric His(N1)-analogue (**1c**) bound with approximately 50-fold less affinity (IC_{50} = 0.32 μ M). This indicates the positional importance of attachment to the His imidazole ring. The reasons for the difference in affinities shown by **1b** and **1c** are not clear. Modeling studies show that relative to **1b**, the χ_1 and χ_2 angles of **1c** exhibit slight rotations that result in misalignment of the respective imidazole rings. While the His(N3)-alkyl side chain of **1b** is directed toward the protein surface, the orientation of the His imidazole ring in **1c** directs the (N1)-alkyl side chain slightly away from the protein surface, resulting in a 1.7 Å upward displacement of the first methylene group and displacement of the succeeding three methylene units (Figure 3A).

The Trp adduct (**1d**, IC_{50} = 9.3 μ M) bound with approximately 30-fold less affinity than **1c**. This is in spite of the fact that the pyrrole portion of the indole ring of **1d** aligns closely with the imidazole ring of **1c** and that their respective $C_6H_5(CH_2)_8$ -groups are essentially superimposable (Figure 3B). The loss of affinity by **1d** may result from entropy penalties incurred by the ordering of solvent molecules about the Trp indole phenyl ring, which extends into the solvent and away from the protein (Figure 3B). The Asn-based adduct (**1e**) was shown to bind in nearly superimposable fashion with **1b** (Figure 3C). However, in order to achieve this orientation, its side chain amide bond must assume a highly unfavorable *cis*-conformation. The corresponding energy penalties paid to achieve this conformation could contribute to its approximately 200-fold loss of affinity relative to **1b** (IC_{50} = 1.1 μ M). The Gln adduct (**1f**) exhibited nearly 10-fold higher affinity (IC_{50} = 0.14 μ M) when compared to the Asn adduct (**1e**). This higher affinity may be due in part to the fact that the side chain amide bond of **1f** exists in an energetically-favorable *trans*-orientation (Figure 3D). The higher affinity of **1f** may also be due in part to the formation of a hydrogen bond between its amide carbonyl and the phenolic hydroxyl of Tyr485 (distance 2.3 Å), which is not possible with **1e**.

The ornithine adduct (**1g**, IC_{50} = 0.17 μ M) was found to bind with affinity equal to the Gln adduct (**1f**). This may reflect the fact that similar to **1g**, the side chain amide group of **1f** is in an energetically-favorable *trans*-orientations and that it can also form a hydrogen bond between its amide carbonyl and the phenolic hydroxyl of Tyr485 (distance 2.3 Å) (Figure 3E). Compared with **1f** and **1g**, the Lys adduct (**1h**) binds with approximately 4-fold less affinity (IC_{50} = 0.65 μ M). Although like **1f** and **1g**, the amide bond of **1h** exists in a *trans*-orientation, its ability to hydrogen bond with the phenolic hydroxyl of Tyr485 is

significantly weaker (2.99 Å distance between the amide proton of **1h** and the phenolic oxygen) and this may contribute to its reduced affinity (Figure 3F).

The purpose of the current work was to examine the effects of incorporating long-chain alkylphenyl-containing non-proteinogenic amino acids at the pT-2 position of **1a**. We found that in all cases, peptides modified in this fashion bound with higher affinity than **1a**. For certain analogues, affinity enhancements of approximately two orders-of-magnitude were observed (**1f** and **1g**). Higher affinity was associated with an ability to bind carboxamide functionality in an energetically-favorable *trans*-orientation and to make strong hydrogen bonds with the phenolic hydroxyl of Tyr485. Although, none of the new analogues was as potent as our previously described peptide **1b**, our finding that the isomeric His(N1)-analogue (**1c**) binds with approximately 50-fold less affinity than **1b**, indicates the positional importance of attachment to the His imidazole ring. Our current work demonstrates that a range of modified residues at the pT-2 position can enhance binding. This should facilitate the development of minimally-sized Plk1 PBD-binding antagonists.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1. Structures of peptides and modified *N*-Fmoc-protected amino acids discussed in the text.

Figure 2. ELISA-based Plk1 PBD-binding results (OD: optical density) generated as reported in references 13, 16. A representative graph from three independent experiments is shown.

Figure 3.

In silico docking of peptides **1c** – **1h** (carbons shown in various colors) onto the Plk1 PBD overlaid with the parent peptide **1b** (carbons shown in grey: PBD accession code 3RQ7). Surface is shown as semitransparent electrostatic potential with Tyr485 and key hydrogen bonds indicated.