Proceedings of the 36th European Peptide Symposium
Michal Lebl (Editor)
European Peptide Society, 2022 *https://doi.org/10.17952/36EPS.2022.295*

Fluorinated Peptide Approach for the Inhibition of Rotamase

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Introduction

Peptidyl-Prolyl isomerase NIMA-interacting-1 (Pin1) is a small two-domain protein member of the Peptidyl-Prolyl *cis-trans* Isomerases (PPIases) which catalyses the *cis-trans* isomerisation of Xaa-Proline amide ω-bonds in proteins [1]. Pin1 is structurally made up of two different domains, WW and the catalytic PPIase, connected by a flexible linker loop region [2]. Pin1 differs from all others 30 PPIases through its unique substrate specificity for phosphorylated Serine/Threonine-Proline (pSer/Thr-Pro) peptide bonds. Pin1 interacts with conformation-specific Pro-directed phosphatases and kinases to control common targets' stability, subcellular localization, and activity [3]. Pin1 is frequently overexpressed and/or overactivated in different types of cancer, and elevated Pin1 overexpression correlates with poor clinical prognosis [4]. Polymorphisms that under-express Pin1 are linked with reduced tumour risk [5], and the depletion of Pin1 significantly inhibits tumorigenesis in mice models [6]. However, Pin1 is not essential for cellular viability [7]. Therefore, the development of potent inhibitors of Pin1 is an attractive topic for cancer therapy. Both small molecules and peptides have shown Pin1's inhibition and cancer suppression ability in multiple studies [8]. Nevertheless, despite decades of research, developing selective and potent Pin1 inhibitors remains challenging. Compared to small-molecules, peptides display generally more selectivity towards the target but suffer of chemical and/or metabolic instability as well as poor cell-permeability [9]. The incorporation of fluorine into biomolecules has gained a considerable interest due to its ability to modulate properties of pharmaceutical compounds [10]. In this context, we decided to design and synthesize fluorinated peptidic Pin1 inhibitors. This way, we could access inhibitors with high selectivity, potency and enhanced biological profiles.

Our group is interested in the synthesis of enantiopure fluorinated amino acids and their incorporation into peptides in order to tune their properties. The aim of this project is to rationally design fluorinated peptide ligands to access Pin1 inhibitors with enhanced biological profile. Previously, we demonstrated that the introduction of CF_3-pseudoproline can lower the rotational barrier of the *cis-trans* peptide bond [11]. Moreover, the CF_2-phosphonate moiety is known to be a stable bioisostere of phosphate group [12]. Therefore, we decided to introduce these two moieties into a peptide scaffold to access potent Pin1 inhibitors, stabilizing the transition state conformation and/or improving their metabolic stability (Figure 1). Our peptide scaffold is based on previously reported Pin1 ligands that comprised the minimal peptide backbone length (three residues) and exploited the Pin1 preference for *C*-terminal aromatic amino acid and *N*-terminal aromatic moiety [13].

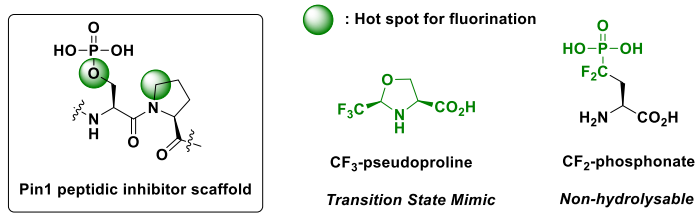


Fig. 1. Modification of the Pin1 inhibitor's scaffolds.

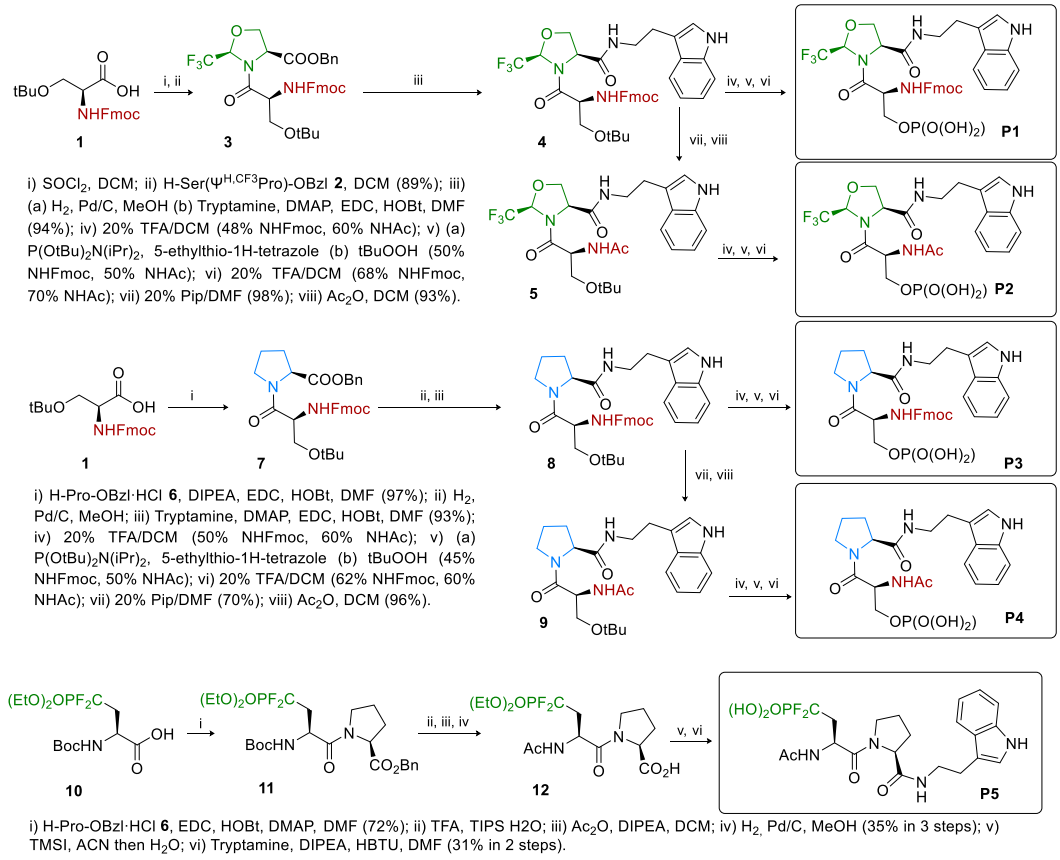
Results and Discussion

Synthesis of Pin1 ligands

Two "transition state mimic" Pin1 ligands **P1** and **P2** have been synthesized in solution (Scheme 1). The first step involves the coupling reaction between the Fmoc-L-Ser(O*t*Bu)-OH **1** and Ser($\Psi^{CF3,H}$Pro)-OBn **2**. Because of the lack of nucleophilicity of the amino group and the steric bulkiness of the vicinal CF_3-group of the pseudoproline, the usual coupling reagent are not effective.

Therefore, the *N*-coupling reaction has been achieved using the most electrophilic Fmoc-protected serine acyl chloride to give the dipeptide **3** in very good yield. Then, debenzylation under hydrogen atmosphere in the presence of Pd/C catalyst, and the subsequent reaction with tryptamine under standard conditions provided compound **4**. Deprotection of the *t*Bu group (TFA/DCM) and phosphorylation of the resulting alcohol gave the desired peptide **P1**. The acetylated analogue **P2** were synthesized from compound **4**. The sequence Fmoc-deprotection/acetylation afforded compound **5** in good yield. Finally, peptide **P2** was obtained following the similar *t*Bu deprotection/phosphorylation sequence used for **P1**.

In order to assess the effect of the trifluoromethylated pseudoproline in terms of affinity for Pin1, we synthesized the non-fluorinated analogues replacing the pseudoproline by the proline residue. Peptides **P3** and **P4** were prepared started from Fmoc-L-Ser(O*t*Bu) **1** and L-Pro-OBn **6** following the same pathway than **P1** and **P2** with the exception of the conditions of the first step (HOBt, EDC, DIPEA, DMF) (Scheme 1, intermediates **7**, **8**, and **9**).



*Scheme. 1. Synthesis of **P1-P5** Pin1 Ligands.*

Table 1. Pin1 ligands Kd values and trans/cis ratio of the pSer-Pro amide bond.

Peptide	Kd_{WW} *(μM)*	Kd_{PPIase} *(μM)*	*trans/cis ratio*[a] *(D_2O - 298 K)*
P1	7 ± 4	41 ± 9	0:100
P2	> 1 mM	490 ± 50	0:100
P3	26 ± 6	438 ± 80	82:18
P4	32 ± 6	> 1 mM	70:30
P5	275 ± 55	430 ± 123	73:27

A "non-hydrolysable" Pin1 ligand **P5** has also been prepared (Scheme 1). The synthesis of the phospho-serine bioisostere **10**, namely the Boc-protected L-2-amino-4-(phosphono)-4,4-difluoro-butanoic acid, was performed by adapting a procedure reported in the literature [14]. Coupling reaction between compound **10** and proline benzyl ester **6** gave the corresponding dipeptide **11** in good yield. Removal of the Boc protecting group (TFA/TIPS/H_2O) followed by acetylation (Ac_2O, DIPEA, DMC) and benzyl deprotection (H_2, Pd/C) provided carboxylic acid **12**. Finally, deprotection of the ethyl groups of the difluorophosphonate moiety and the coupling with tryptamine (HBTU, DIPEA, DMF) gave peptide **P5**.

NMR affinity constant (Kd) evaluation

The affinity constants were calculated by NMR, using the experimental CSP (Chemical Shift Perturbation) upon ligand addition. Pin1 titrations were performed for each ligand by recording a series of ^{1}H-^{15}N HSQC (500 MHz, 298 K). The *Kd* values of **P1**-**P5** for each Pin1 domain (WW/PPIase) were determined by fitting the shape of the titration curve (CSP vs. concentration of ligand) (Table 1) [15]. The binding preference of our peptides towards Pin1 domains is correlated to the *trans*/*cis* conformation of the pSer-Pro amide bond, in agreement with the literature [16]. In most of the cases, *cis* amide bond shows a preference for the catalytic domain (PPiase) while *trans* amide bond targets the WW domain. The incorporation of the trifluoromethylated pseudoproline into the peptide scaffold (**P1** and **P2**) strongly favors the *cis* amide bond while the use of the difluorophosphonate **P5** does not affect the *trans*/*cis* ratio compared to the non-fluorinated phosphate analogue **P4**. The *trans*/*cis* conformations of Pin1 ligands **P1**-**P5** were determined by NMR (Table 1). 2D ^{1}H NMR NOESY experiments allowed the assignment of the pSer-Pro amide bond conformation while the *trans*/*cis* ratio was determined by ^{1}H, ^{31}P and ^{19}F NMR experiments.

Acknowledgments

This work is supported by ANR (Agence Nationale de la Recherche): ANR-18-CE07-0032.

References

1. Gestwicki, J.E., et al. *J. Med. Chem.* **59**, 9622-9644 (2016), https://doi.org/10.1021/acs.jmedchem.6b00411
2. Matena, A., et al. *Biol. Chem.* **399**, 101-125 (2018), https://doi.org/10.1515/hsz-2017-0137
3. Pawson, T., Scott, J. *Trends Biochem. Sci.* **30**, 286-290 (2005), https://doi.org/10.1016/j.tibs.2005.04.013
4. Pu, W., Zheng, Y., Peng, Y. *Front. Cell Dev. Biol.* **8**, 168 (2020), https://doi.org/10.3389/fcell.2020.00168
5. Li, Q., et al. *PLoS ONE* **8**, e68148 (2013), https://doi.org/10.1371/journal.pone.0068148
6. Girardini, J.E., et al. *Cancer Cell.* **20**, 79-91 (2011), https://doi.org/10.1016/j.ccr.2011.06.004
7. Nabet, B., et al. *Nat. Chem. Biol.* **14**, 431-441 (2018), https://doi.org/10.1038/s41589-018-0021-8
8. Asano T., et al. *Curr Med Chem.* **27**, 3314-3329 (2020), https://doi.org/10.2174/0929867325666181105120911
9. Potter, A., et al. *Bioorg. Med. Chem.* **23**, 4283-4291 (2013), https://doi.org/10.1016/j.bmcl.2013.05.088
10. Inoue, M., et al. *ACS Omega* **5**, 10633-10640 (2020), https://doi.org/10.1021/acsomega.0c00830
11. Feytens, D., et al. *J. Phys. Chem. B.* **116**, 4069-4079 (2012), https://doi.org/10.1021/jp300284u
12. Elliott, T.S., et al. *Med. Chem. Commun.* **3**, 735-751(2012), https://doi.org/10.1039/C2MD20079A
13. Xu, G.G., et al. *Biochemistry* **50**, 9545-9550 (2011), https://doi.org/10.1021/bi201055c
14. Berkowitz, D.B., et al. *J. Org. Chem.* **61**, 4666-4675 (1996), https://doi.org/10.1021/jo9604752
15. Williamson, M.P. *Prog. Nucl. Magn. Reson. Spectrosc.* **73**, 1-16 (2013), doi.org/10.1016/j.pnmrs.2013.02.001
16. Wang, X. *Structure* **23**, 2224-2233 (2015), https://doi.org/10.1016/j.str.2015.08.019