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Boro-norleucine as a P1 residue for the design of selective and potent DPP7 inhibitors

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Abstract—Dipeptide-based inhibitors with C-substituted (alkyl or aminoalkyl) α-amino acids in the P2 position and boro-norleucine (boro-Nle) in the P1 position were synthesized. Relative to boro-proline, boro-Nle as a P1 residue was shown able to significantly dial out DPP4, FAP, DPP8, and DPP9 activity. Dab-boro-Nle (**4g**) proved to be the most selective and potent DPP7 inhibitor with a DPP7 IC_{50} value of 480 pM.

Proline-specific serine hydrolases have become a widely studied enzyme class due to growing evidence that they play an important role in various metabolic functions. The dipeptidyl peptidases (DPPs) are a subclass of the serine protease family that cleaves dipeptides from the amino terminus of proteins and prefers a proline residue in the penultimate position. Members of the DPP family include DPP4, DPP7, fibroblast activation protein α (FAP), DPP8, and DPP9.[1]

DPP7 is a 58 kDa glycoprotein with an enzymatic function similar to DPP4 but only distantly related by sequence homology.[2] Huber and co-workers have reported that inhibition of DPP7 activity induces apoptosis in quiescent lymphocytes.[3] As part of an effort to identify the substrate specificity of DPP7, Leiting, et al. synthesized a positional scanning library of fluorescent dipeptide substrates to identify preferred P1 and P2 residues.[4] Not surprisingly, it was found that DPP7 has an almost absolute specificity for proline in the P1 position. The second most preferred P1 residue for DPP7 was determined to be norleucine (Nle). Furthermore, DPP7 was shown to have a preference in order at the P2 position for Lys, Nle, Met, Ala, and Pro. In contrast, Pro and Ala, in that order, were found to be preferred P1 residues for DPP4. In addition, the DPP4 S2 site was found tolerant of a wide variety of P2 residues. This study indicated that there was a distinction between the S_1 and the S_2 subsites of these DPPs which, in turn, could be exploited to synthesize selective DPP7 inhibitors.

Inhibitors of DPP7 have been discovered as the result of both indirect and targeted efforts. As part of counter-screening efforts during the design of DPP4 inhibitors, some researchers have identified sub-μM DPP7 inhibitors based on boro-proline (boro-Pro) dipeptides,[5] substituted cycloalkylglycines,[6] homophenylalanine,[7] substituted piperazines,[8] and fluoropyrrolidine amides.[9] DPP7 active inhibitors based on thioxo amides of pyrrolidides and thiazolidides,[10] phosphonates,[11] and boro-Pro[12] have also been discovered while profiling these compounds against multiple prolylpeptidase family members. Most deliberately, Augustyns and co-workers designed DPP7 inhibitors taking advantage of the P2 preference of this enzyme for cationic sidechains. (*S*)-2,4-diaminobutanoic acid (Dab) was found to be a potent and selective DPP7 active P2 residue. For example, 1-[Dab]-piperidine was shown to have a DPP7 IC_{50} value of 130 nM and a more than 7500-fold selectivity over DPP4.[13,14] Further optimization of this P2 residue has yielded γ-amino-arylalkyl-substituted Dab analogs with sub-nM potency for DPP7 and a more than million-fold selectivity versus DPP4.[15]

Keywords: Dipeptidyl peptidase; Boro-norleucine; DPP4; FAP; DPP7; DPP8; DPP9.
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Herein, we report the synthesis and DPP inhibitory activity (DPP4, DPP7, FAP, DPP8, and DPP9) of a novel series of dipeptide-based inhibitors with C-substituted α-amino acids in the P2 position and boro-norleucine (boro-Nle) in the P1 position.

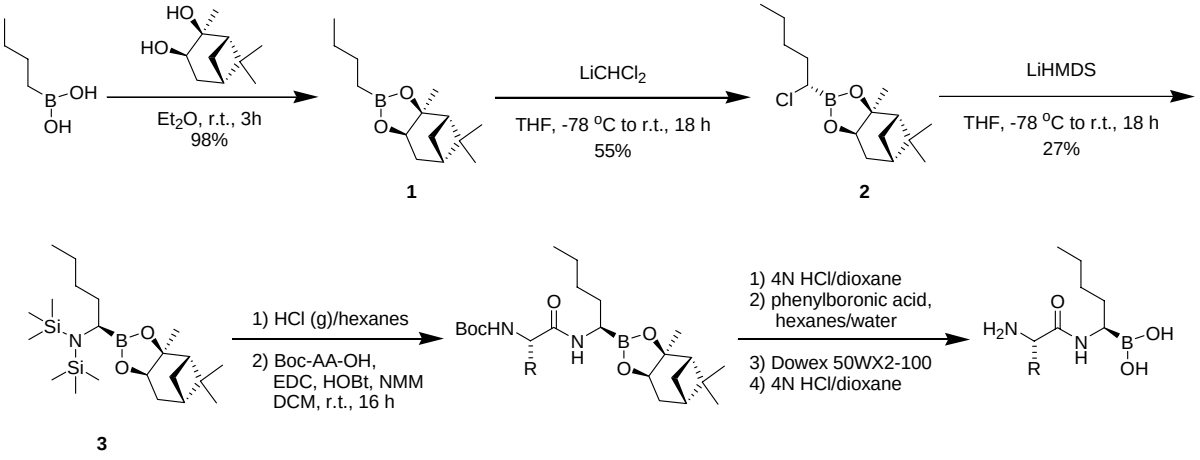
Boro-Nle inhibitors were prepared as shown in Scheme 1 using methodology developed by Matteson for the synthesis of chiral amino boronic acids.[16] The commercially available butyl boronic acid was converted to the boronic ester **1** by stirring with (+)-pinanediol in diethyl ether. Homologation with (dichloromethyl)lithium and catalytic zinc chloride yielded the α-chloro boronic pinanediol ester **2** in 55% yield.[17] Nucleophilic displacement of the chlorine atom by lithium hexamethyldisilazane gave the bis(trimethylsilyl)-protected amine **3** that was purified using silica gel chromatography (0–10% EtOAc/hexanes) with an isolated yield of 27%. Treatment of compound **3** with dry HCl saturated hexanes at 0 °C afforded the hydrochloride salt, which was taken on without further purification. The reaction of this salt with various Boc-protected amino acids under standard peptide coupling conditions generated the corresponding Boc/pinanediol protected inhibitors. After removal of the Boc group with 4 N HCl/ dioxane, the pinanediol ester was deprotected through transesterification with phenylboronic acid using a biphasic hexane/water system.[18] The aqueous layer containing the free boronic acid was loaded onto a Dowex 50-WX2-100 ion-exchange column, washed with water, and the product was eluted with 2% ammonium hydroxide. The resulting free base form was converted to the HCl salt using 4 N HCl/dioxane.

Two classes of boro-Nle-based inhibitors were synthesized; those with alkyl (**4a**–**4e** and **5**) and aminoalkyl (**4f**–**4i**) C-substituted α-amino acids in the P2 position (see Table 1). IC_{50} values for these compounds were determined against five members of the DPP family: DPP7, DPP4, FAP, DPP8, and DPP9.[19] Among the straight chain alkyl variants (**4a**–**4d**), Nle-boro-Nle (**4d**) was the most potent inhibitor for both DPP7 and DPP4 with IC_{50} values of 18 and 370 nM, respectively. The activities of the two other types of alkyl P2 residues, Val and Pro, were also examined. The P2 β-alkyl branched Val-boro-Nle (**4e**) could be compared to its straight chain congener **4c**. In both the cases, the inhibitory activity against DPP7 was similar, but there was a 21-fold reduction in DPP4 potency for the former compound. When the cyclic P2 containing Pro-boro-Nle (**5**) was examined, activity against DPP7 was diminished 10-fold compared to its 'ring-opened' congener **4c**. For Pro-boro-Nle (**5**), no inhibition of DPP4 was observed.

When compared to their straight chain alkyl analogs, P2 aminoalkyl derived boro-Nle inhibitors (**4f**–**4i**) were more potent inhibitors of DPP7. Compounds **4f**, **4h**, and **4i** were found to have similar single digit nM potencies against DPP7 with IC_{50} values of 8.2, 4.7, and 3.7 nM, respectively. Of all the boro-Nle-based inhibitors, the aminoethyl P2 containing Dab-boro-Nle (**4g**) was the most potent against DPP7 with an IC_{50} value of 480 pM. Interestingly, the DPP7/4 selectivity was found to be >68,000, making Dab-boro-Nle (**4g**) the most selective DPP7 inhibitor between these two DPPs.

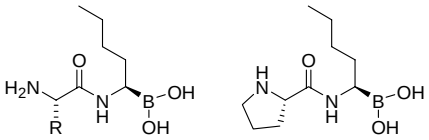
The FAP, DPP8, and DPP9 inhibitory activities for all of the boro-Nle inhibitors were also examined. No significant inhibition of FAP activity was observed for any of the inhibitors. For DPP8, the alkyl series yielded two inhibitors (**4b** and **4e**) with sub-μM DPP8 potencies of 400 and 190 nM, respectively. Both compounds are structurally related (*S*)-ethylglycine derivatives. With a DPP8 IC_{50} value of 350 nM, Lys-boro-Nle (**4i**) was identified as the most potent DPP8 inhibitor in the aminoalkyl series. For DPP9, potency increased as length increased in both the straight chain alkyl and the aminoalkyl series. Among the inhibitors with quantifiable values, Dab-boro-Nle (**4g**) was the most selective DPP7 inhibitor when contrasted against DPP8 and DPP9. For this inhibitor, DPP7/8 and DPP7/9 selectivity factors of 9000 and 1700 were noted, respectively. In summary for the latter of the three DPPs, no P2 residue was found to deliver a compound that inhibited FAP activity. In the case of DPP8 and DPP9, the choice of P2 residue played a critical role in determining potency.

To determine the impact on selectivity of boro-Nle as a P1 residue versus boro-Pro, boro-Pro congeners of



Scheme 1. Synthesis of boro-Nle inhibitors.

Table 1. DPP4, FAP, and DPP7 inhibition data for compounds **4a–i** and **5**



4a-i **5**

Compound	R	IC_{50}s (nM)[a]				
		DPP7	DPP4	FAP	DPP8	DPP9
4a	CH_3	220	ni	ni	6000	40% (20 μM)[b]
4b	CH_2CH_3	49	32,000	ni	400	220
4c	$CH_2CH_2CH_3$	71	810	ni	5200	160
4d	$CH_2CH_2CH_2CH_3$	18	370	ni	1000	65
4e	$CH(CH_3)_2$	40	17,000	ni	190	510
4f	CH_2NH_2	8.2	2000	ni	ni	4300
4g	$CH_2CH_2NH_2$	0.48	40% (33 μM)[b]	ni	4300	800
4h	$CH_2CH_2CH_2NH_2$	4.7	1200	ni	2600	400
4i	$CH_2CH_2CH_2CH_2NH_2$	3.7	ni	ni	350	280
5	—	890	ni	ni	ni	35% (20 μM)[b]

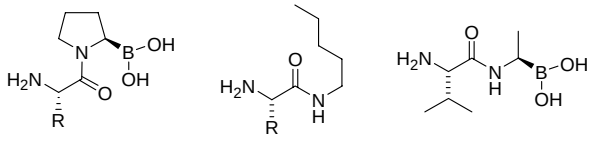
[a] ni = no inhibition (i.e., no significant inhibition observed at concentrations as high as 33 μM (DPP7, DPP4, and FAP) or 20 μM (DPP8 and DPP9)).
[b] Percent inhibition at the concentration indicated.

inhibitors **4d** and **4g** (Table 2) were synthesized from the (+)-pinanediol ester of (*R*)-boro-Pro and the Boc protected versions of Nle or Dab according to literature procedure.[5] The resulting Nle-boro-Pro (**6a**) and Dab-boro-Pro (**6b**) were potent inhibitors of DPP7 with IC_{50} values of 2.0 and 0.72 nM, respectively.[20] Both compounds were also potent inhibitors of DPP4, which resulted in only modest DPP7/4 selectivity factors (2.2 and 21, respectively). By comparison, Nle-boro-Nle (**4d**) and Dab-boro-Nle (**4g**) yielded DPP7/DPP4 selectivity factors of 21 and >68,000. Similar trends were seen for FAP, DPP8, and DPP9; derivatization with boro-Pro resulted in significantly more potent inhibitors for these three DPPs versus derivatization with boro-Nle. In summary, the use of boro-Nle over boro-Pro as a P1 residue can greatly increase DPP7 selectivity.

Since potent DPP7 inhibitors without an electrophilic functionality were known (e.g., 1-[Dab]-piperidine), we chose to examine the role that the boronic acid played in DPP potency. The *n*-pentyl amides of Nle (**7a**) and Dab (**7b**) were synthesized from *n*-pentylamine and the Boc protected versions of these amino acids using the coupling and Boc deprotection conditions outlined in Scheme 1. These compounds were designed as controls lacking the boronic acid moiety for direct comparison to Nle-boro-Nle (**4d**) and Dab-boro-Nle (**4g**). The DPP7 IC_{50} values for the compounds **7a** and **7b** were 5500 and 2600 nM, respectively. These values represent a 300- and 5400-fold reduction in DPP7 potency relative to their boronic acid containing congeners **4d** and **4g**. In addition, compounds **7a** and **7b** did not significantly inhibit the activity of any other DPP. Thus, the presence of the boronic acid contributes significantly to the DPP potency of inhibitors **4d** and **4g**.

Because alanine has been previously identified as an acceptable P1 residue for DPP4 substrates,[21] we specu-

Table 2. DPP4, FAP, and DPP7 inhibition data for compounds **6a** through **8**.



6a-c **7a-b** **8**

Compound	R	IC_{50}s (nM)[a]				
		DPP7	DPP4	FAP	DPP8	DPP9
6a	$CH_2CH_2CH_2CH_3$	2.0	4.5	4100	30	4.6
6b	$CH_2CH_2NH_2$	0.72	15	6800	95	6.7
6c	$CH(CH_3)_2$	38	0.30	11	15	2
7a	$CH_2CH_2CH_2CH_3$	5500	35% (33 μM)[b]	ni	ni	ni
7b	$CH_2CH_2NH_2$	2600	ni	ni	ni	ni
8	—	160	0.89	22,000	670	3200

[a] ni = no inhibition (i.e., no significant inhibition observed at concentrations as high as 33 μM (DPP7, DPP4, and FAP) or 20 μM (DPP8 and DPP9)).
[b] Percent inhibition at the concentration indicated.

lated that a shortening of the alkyl chain length of boro-Nle to the corresponding boro-alanine (boro-Ala) would improve DPP4 potency. Val-boro-Ala (**8**) was synthesized from methylboronic acid in an analogous fashion to the boro-Nle synthesis described earlier. In agreement with the proposed influence of the P1 chain length, Val-boro-Ala (**8**) is a potent inhibitor of DPP4 with a DPP4 IC_{50} value of 0.89 nM. In contrast, the potency of Val-boro-Nle (**4e**) against DPP4 was some 19,000-fold less. For both the compounds **4e** and **8**, the DPP7 potency differed by a factor of 4 with DPP7 IC_{50} values of 40 and 160 nM, respectively.[22] Thus, a shorter P1 chain length can significantly increase DPP4 potency but may have a less profound effect on DPP7 potency.

The selectivity profiles of both these compounds were also compared to Val-boro-Pro (**6c**). For DPP7, IC_{50} values for all three compounds did not differ by a factor of more than 4. For the other DPPs, when compared to compounds **4e** and **8**, Val-boro-Pro (**6c**) was found to be the most potent inhibitor and, in some cases, by several orders of magnitude. Using the P2 residue Val as an example, it is clear that DPP selectivity and potency can sometimes best be modulated by choosing amino boronic acids other than boro-Pro for the P1 position.

In conclusion, boro-Nle is a novel P1 residue for the design of DPP7 selective dipeptide inhibitors. When compared to their boro-Pro congeners, Nle-boro-Nle (**4d**) and Dab-boro-Nle (**4g**) were shown to be significantly more selective for DPP7. Factors such as the choice of P2 residue, the presence of the boronic acid, and the P1 alkyl chain length all play a role in the DPP7 selectivity and potency of boro-Nle-based inhibitors. The most DPP7 selective inhibitor found in this study was Dab-boro-Nle (**4g**). As such, this compound holds promise as a tool that can be used to investigate DPP7 biology. This research sets the stage for the design of other DPP selective dipeptides inhibitors which incorporate amino boronic acids based on P1 residues other than the canonical proline. Reports along these lines will be presented in due course.

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FP-TAMRA (1 μM final concentration) was then added. After an incubation time of 30 min the mixture was quenched with loading buffer. All measurements were carried out in duplicate. Unreacted probe was separated from probe-labeled DPP8 or DPP9 using SDS–PAGE and the fluorescent signal of each TAMRA-tagged enzyme was measured using a flatbed gel scanner. Samples containing enzyme alone were considered 100% active and test compound treated samples were expressed as a percentage of remaining activity. Percent remaining activity versus inhibitor concentration was plotted and fit to the Hill equation to quantify IC_{50} values.

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