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Synthesis and structure–activity relationship of *N*-acyl-Gly-, *N*-acyl-Sar- and N-blocked-boroPro inhibitors of FAP, DPP4, and POP

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Abstract—The structure–activity relationship of various *N*-acyl-Gly-, *N*-acyl-Sar-, and N-blocked-boroPro derivatives against three prolyl peptidases was explored. Several *N*-acyl-Gly- and N-blocked-boroPro compounds showed low nanomolar inhibitory activity against fibroblast activation protein (FAP) and prolyl oligopeptidase (POP) and selectivity against dipeptidyl peptidase-4 (DPP4). *N*-Acyl-Sar-boroPro analogs retained selectivity against DPP4 and potent POP inhibitory activity but displayed decreased FAP inhibitory activity.

The prolyl peptidases dipeptidyl peptidase-4 (DPP4), prolyl oligopeptidase (POP), and fibroblast activation protein (FAP) cleave bioactive peptides preferentially after proline residues and represent promising therapeutic targets for diabetes, cognitive disorders, and cancer, respectively.[1] Although these serine proteases share a preference for proline at the P_1 position of substrates, they display distinct activities. DPP4 displays only dipeptidyl peptidase (DPP) activity that removes P_2-Pro_1-dipeptides from the N-terminus of substrates, whereas, POP acts solely as a proline-specific endopeptidase (Fig. 1). FAP displays both activities; however, FAP endopeptidase activity is limited to substrates containing a Gly-Pro motif (Fig. 1).[2,3]

The unique activity of prolyl peptidases has been exploited for inhibitor development as outlined in Figure 1. For example, potent inhibition of DPP4 and FAP has been achieved with aminoacyl-proline boronic acids (boroPro)[4] and inhibitors such as Val-boroPro (PT-100) stimulate hematopoiesis[5] and demonstrate anti-tumor activity.[6,7] Although aminoacyl-boroPros with free N-termini are poor POP inhibitors,[4] they non-selectively inhibit several proline-specific DPPs besides DPP4 and FAP such as DPP7, DPP8, and DPP9. More recently, *N*-alkyl-Gly-boroPro inhibitors have been developed and these compounds inhibit

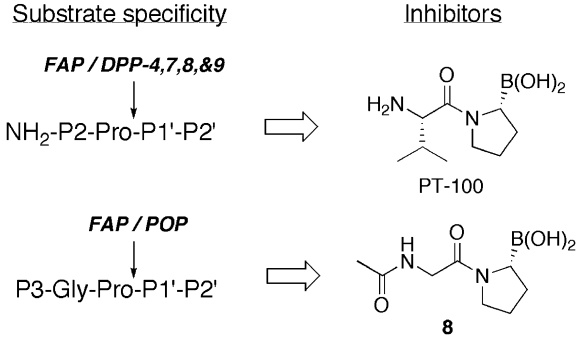

Figure 1. Substrates and boroPro-inhibitors of prolyl peptidases. Note that FAP has both dipeptidyl peptidase activity and endopeptidase activity, whereas, DPPs-4, -7, -8, and -9 display only dipeptidyl peptidase activity and POP only endopeptidase substrates (left panel). Val-boroPro (PT-100) and Ac-Gly-boroPro (**8**) are examples of dipeptidyl peptidase and endopeptidase inhibitors, respectively.

Keywords: Prolyl peptidase; Dipeptidyl peptidase; BoroPro; FAP; DPP4; POP.
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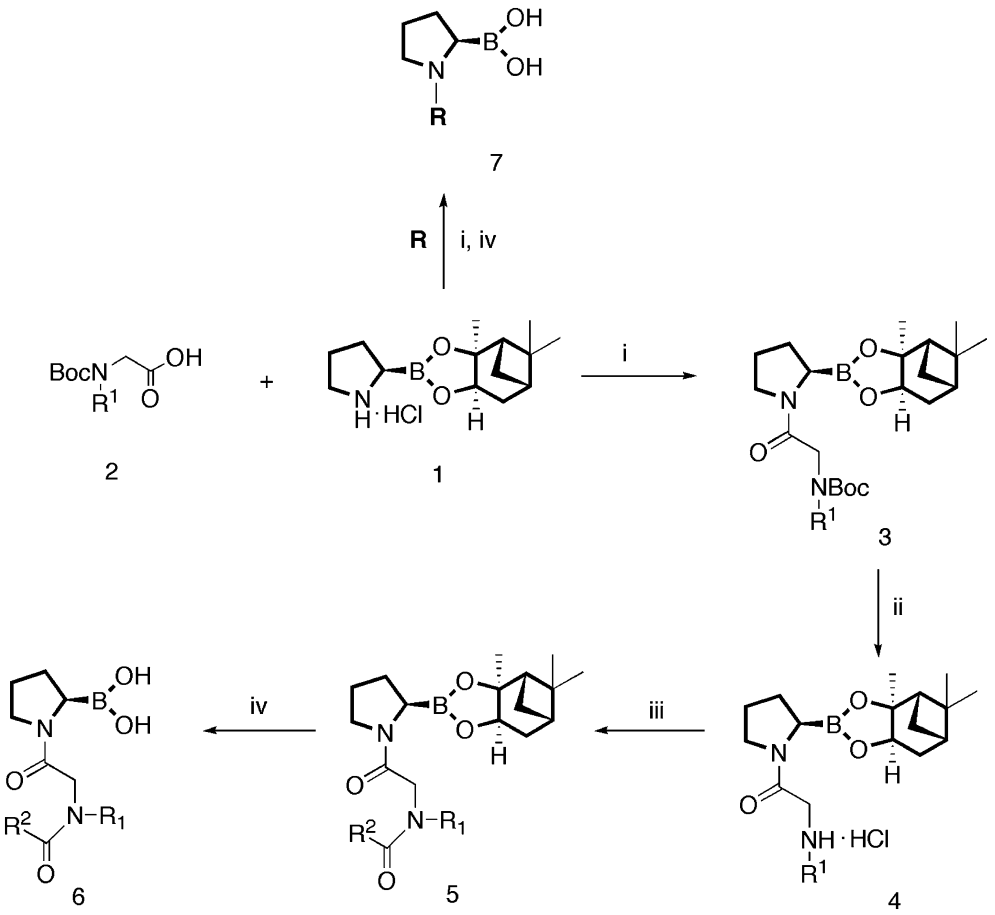
DPP4, FAP, and DPP7; however, their reactivity with POP is unknown.[8]

Based on FAP's preference for Gly-Pro-based endopeptidase substrates, we recently synthesized Ac-Gly-boroPro (Fig. 1) and tested its reactivity against prolyl peptidases.[2] This compound preferentially inhibited FAP versus other prolyl peptidases, showing marked selectivity against DPP8 and DPP9, but only modest selectivity against DPP4 and POP. To expand the structure–activity relationship (SAR) and further optimize inhibitor selectivity for FAP, we created a novel series of *N*-acyl-Gly-, *N*-acyl-Sar (sarcosine)-, and N-blocked-boroPros. We report here on their synthesis and inhibitory activity against FAP, DPP4, and POP.

The amino boronic ester **1** (Scheme 1) was prepared as previously described.[4] Either Boc-glycine-OH or Boc-sarcosine-OH was coupled to **1** in the presence of 1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide hydrochloride (EDC) to generate the fully protected dipeptides **3**. The Boc group was removed with HCl to produce the unprotected amines **4**. Acyl or aryl acids R^2 were coupled using the same conditions as for the Boc amino acids. Deprotection of the boronic ester was then effected by transesterification of the pinanediol with phenylboronic acid in a biphasic MTBE (methyl-*tert*-butyl ether)–water mixture.[9] Pinanediol phenylborate was recovered from the organic phase and the *N*-acyl-Gly or the *N*-acyl-Sar-boroPros **6** (Table 1) were isolated from the aqueous phase by reverse phase HPLC. Directly N-blocked-boroPros **7** (Table 2) were prepared by acylation of **1** using the previously described coupling conditions followed by removal of the pinanediol.

Inhibition constants (K_i)[10] for FAP, DPP4, and POP were determined for a series of acyl-Gly-boroPros to explore the SAR of the N-blocking group (Table 1). Acyl-Gly-boroPros containing alkyl-R^2 groups (compounds **8**–**10**) were nanomolar inhibitors of FAP and POP, but less effective inhibitors of DPP4. With FAP, **8** was the most potent inhibitor, whereas, **9** was the most potent POP inhibitor. Although a decrease in FAP potency was observed for compound **10**, selectivity against DPP4 relative to FAP increased throughout the series. Cyclopentyl and cyclohexyl analogs (**11** and **12**) were similar to compound **9**, showing slight increases in potency for FAP and POP, while increasing overall selectivity against DPP4. Benzoyl-Gly-boroPros (**13**–**15**) also inhibited FAP and POP preferentially, and the di-chloro-aryl compounds (**14** and **15**) were among the most potent FAP/POP inhibitors in this series. Together, these data show that the acyl-blocking group



Scheme 1. Reagents (i) HOBt, EDC, CH_2Cl_2, DIPEA; (ii) HCl, dioxane; (iii) HOBt, EDC, CH_2Cl_2, DIPEA; (iv) H_2O, MTBE, $PhB(OH)_2$.