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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

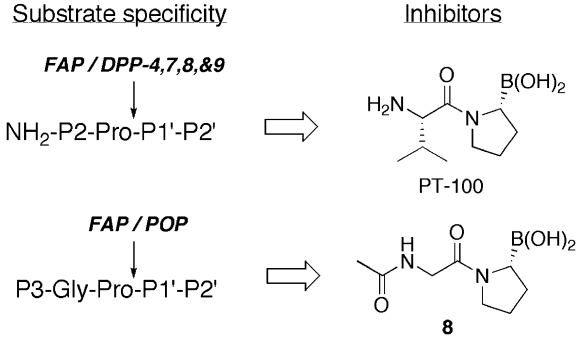

Figure 1. Substrates and boroPro-inhibitors of prolyl peptidases. Note that FAP has both dipeptidyl peptidase activity and endopeptidase activity, whereas, DPPs-4, -7, -8, and -9 display only dipeptidyl peptidase activity and POP only endopeptidase substrates (left panel). Val-boroPro (PT-100) and Ac-Gly-boroPro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

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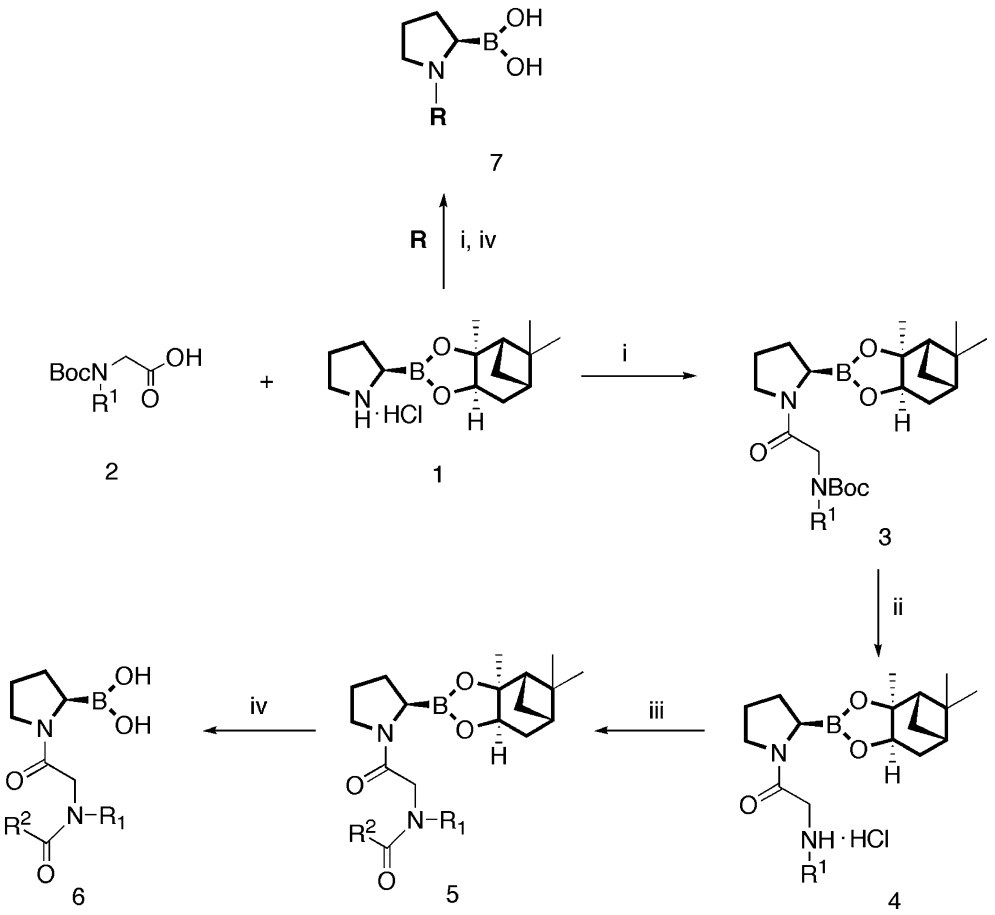
DPP4, FAP, and DPP7; however, their reactivity with POP is unknown.[8]

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.

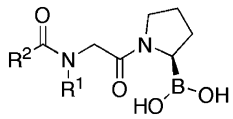
The amino boronic ester **1** (Scheme 1) was prepared as previously described.[4] Either Boc-glycine-OH or Boc-sarcosine-OH was coupled to **1** in the presence of 1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide hydrochloride (EDC) to generate the fully protected dipeptides **3**. The Boc group was removed 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.

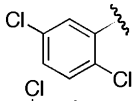
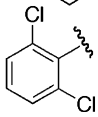
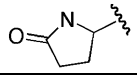
Inhibition constants (K_i)[10] for FAP, DPP4, and POP were determined for a series of acyl-Gly-boroPros to explore the SAR of the N-blocking group (Table 1). Acyl-Gly-boroPros containing alkyl-R^2 groups (compounds **8**–**10**) were nanomolar inhibitors of FAP and POP, but less effective inhibitors 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, $PhB(OH)_2$.

Table 1. FAP, DPP4, and POP inhibition data for compounds **8**–**19**



Compound	R^2	R^1	K_i (nM)		
			FAP	DPP4	POP
8	Me	H	23[a]	377[a]	211[a]
9		H	51	4300	4.5
10		H	751	608,000	191
11		H	20	9080	2.3
12		H	14	7391	2.7
13		H	142	38,400	25
14		H	29	9500	2.2
15		H	12	6871	4.4
16	Me	Me	161	4600	53
17		Me	265	19,500	13
18		Me	191	11,300	7.4
19		Me	146	68	101

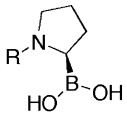
[a] Values from Ref. 2.

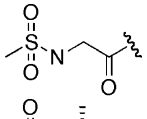
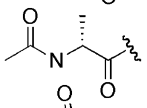
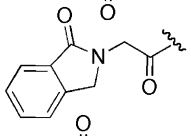
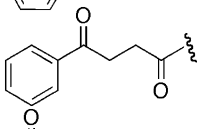
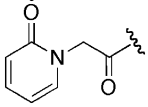
consistently confers significant selectivity against DPP4 and that the nature of this group can modulate the potency of FAP and POP inhibition.

Sarcosyl analogs (**16**–**18**) of selected acyl-Gly-boroPros were studied to examine the inhibitory activity of dual N-substitution (Table 1). With FAP, the sarcosyl analogs showed decreased potency relative to the acyl-Gly-boroPros; however, these analogs showed equivalent or increased potency with POP. As with the acyl-Gly-boroPros, the sarcosyl analogs were generally poor DPP4 inhibitors. One exception was compound **19**, which preferentially inhibited DPP4 relative to FAP and POP. These data indicate that the hydrophobic nature of the previously mentioned inhibitors may be important for FAP and POP selectivity.

We next synthesized compounds to explore the importance of the terminal amide carbonyl and NH (Table 2). Compound **20**, in which a mesyl-group replaces the acetyl group of the parental compound (**8**), shows decreased potency against FAP, DPP4, and POP. Decreased potency was also observed when the P_2 Gly of the parental compound was replaced with D-Ala (**21**). FAP and POP showed 12- to 15-fold increases in K_i values with **21** and DPP4 demonstrated an even greater drop in potency with little inhibition observed at 500 μM inhibitor. Finally, compounds **22**–**24** were synthesized to examine the contribution of the amide NH to inhibition. Cyclic amide (**22**) and ketone (**23**) compounds from this group showed the most significant inhibitory activity against FAP and POP, indicating that the amide NH is not required for these enzymes. By

Table 2. FAP, DPP4, and POP inhibition data for compounds **20**–**24**



Compound	R	K_i (nM)		
		FAP	DPP4	POP
20		246	1430	1359
21		350	17% (500 μM)[a]	2705
22		7.5	22,700	2.8
23		94	451,000	1.7
24		2900	182,000	353

[a] Percent inhibition at the concentration indicated.

contrast, compound **24**, which maintains the planarity of compound **22**, but is more compact and has a less electrophilic oxygen, was a relatively poor inhibitor of FAP and POP. Compounds **22**–**24** showed marked selectivity against DPP4 as observed with the previous series of compounds.

To identify potential structural differences that underlie the observed SAR, we modeled binding of compound **22** to FAP, DPP4, and POP. As shown in Figure 2, the active site residues of FAP and DPP4 are nearly identical and highly similar to POP, particularly with regard to the position of the catalytic serine, which likely forms a covalent adduct with the inhibitor's boronic acid moiety and the S_1 subsite, which accommodates the inhibitor's P_1-pyrrolidine ring. As well, each protease contains a conserved arginine (e.g., FAP R123) and tyrosine (e.g., FAP Y541) that may hydrogen bond with the carbonyl oxygens of the inhibitor's P_2 residue and indole ring, respectively. Therefore, structural differences distal to these interactions must underlie the observed SAR. In this context, POP Cys 255, Trp 595, and other hydrophobic residues provide a non-polar environment for the inhibitor's indole ring, whereas, the di-Glu repeat at the base of the FAP and DPP4 binding pockets provides a relatively more polar environment. This potentially explains the higher affinity binding observed with POP and compound **22**, but does not account for its inhibition of FAP and selectivity against DPP4. FAP inhibition may involve hydrogen bonding of Glu 203

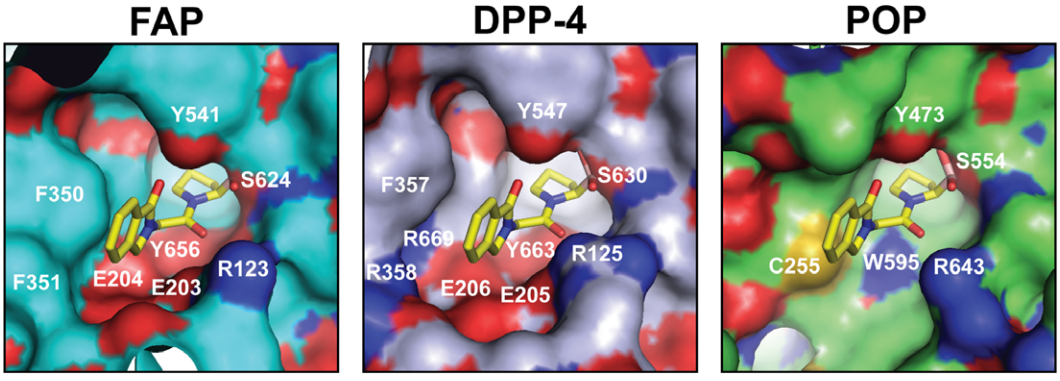


Figure 2. Inhibitor binding models. Compound **22** was docked into the active site of FAP (pdb 1z68)[11], DPP4 (pdb 2ajd),[12] and POP (pdb 1e8m)[13] using Pymol (http://www.pymol.org). Inhibitor carbon atoms are colored yellow, whilst those of FAP are cyan, DPP4 gray, and POP green. Oxygen atoms are red, nitrogens blue, borons pink, and sulfurs yellow.

and Glu 204 to the amide nitrogen of the inhibitor's indole ring, which may also interact with Phe 350 and Phe 351. Although DPP4 Glu 205 and Glu 206 could similarly bind the inhibitor, the polar nature of Arg 358 and Arg 669 may disfavor binding of the indole ring. Additionally, an amino acid difference more removed from the active site (eg. FAP Ala657 = DPP4 Asp663; not shown) imparts significant differences in FAP and DPP4 substrate specificity[11] and thus may influence inhibitor selectivity as well. Future mutagenesis and crystallography studies will test these possibilities.

In conclusion, various *N*-acyl-Gly-, *N*-acyl-Sar-, and N-blocked-boroPros were synthesized and tested as inhibitors of FAP, DPP4, and POP. Several of the *N*-acyl-Gly- and N-blocked-boroPros showed low nanomolar inhibitory activity against FAP and POP, and marked selectivity against DPP4, suggesting that they will be useful tools for the study of FAP and POP biology. *N*-Acyl-Sar-boroPro analogs retained selectivity against DPP4 and potent POP inhibitory activity but displayed decreased FAP inhibitory activity. The results presented here provide a framework for future studies aimed at developing selective inhibitors for FAP and POP.

Acknowledgment

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References and notes

1. Rosenblum, J. S.; Kozarich, J. W. *Curr. Opin. Chem. Biol.* **2003**, *7*, 496.
2. Edosada, C. Y.; Quan, C.; Wiesmann, C.; Tran, T.; Sutherlin, D.; Reynolds, M.; Elliott, J. M.; Raab, H.; Fairbrother, W.; Wolf, B. B. *J. Biol. Chem.* **2006**, *281*, 7437.
3. Edosada, C. Y.; Quan, C.; Tran, T.; Pham, V.; Wiesmann, C.; Fairbrother, W.; Wolf, B. B. *FEBS Lett.* **2006**, *580*, 1581.
4. Coutts, S. J.; Kelly, T. A.; Snow, R. J.; Kennedy, C. A.; Barton, R. W.; Adams, J.; Krolikowski, D. A.; Freeman, D. M.; Campbell, S. J.; Ksiazek, J. F.; Bachovchin, W. W. *J. Med. Chem.* **1996**, *39*, 2087.
5. Jones, B.; Adams, S.; Miller, G. T.; Jesson, M. I.; Watanabe, T.; Wallner, B. P. *Blood* **2003**, *102*, 1641.
6. Adams, S.; Miller, G. T.; Jesson, M. I.; Watanabe, T.; Jones, B.; Wallner, B. P. *Cancer Res.* **2004**, *64*, 5471.
7. Cheng, J. D.; Valianou, M.; Canutescu, A. A.; Jaffe, E. K.; Lee, H. O.; Wang, H.; Lai, J. H.; Bachovchin, W. W.; Weiner, L. M. *Mol. Cancer Ther.* **2005**, *4*, 351.
8. Hu, Y.; Ma, L.; Wu, M.; Wong, M. S.; Li, B.; Corral, S.; Yu, Z.; Nomanbhoy, T.; Alemayehu, S.; Fuller, S. R.; Rosenblum, J. S.; Rozenkrants, N.; Minimo, L. C.; Ripka, W. C.; Szardenings, A. K.; Kozarich, J. W.; Shreder, K. R. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 4239.
9. Coutts, S. J.; Adams, J.; Krolikowski, D. A.; Snow, R. J. *Tetrahedron Lett.* **1994**, *35*.
10. Recombinant FAP, DPP4, and POP were expressed and purified as described previously (Ref. 2). Inhibitor stocks were made in DMSO and assayed at various concentrations up to 0.5 mM. Final DMSO levels in reactions were less than 1% v/v to avoid inhibition of proteases by excess DMSO. K_i values for protease inhibition were determined by the method of progress curves (Ref. 2). Briefly, proteases were added to a mixture of inhibitor and substrate (Gly-Pro-AFC for FAP and DPP4; zGP-AMC for POP) in 50 mM Tris–HCl, 100 mM NaCl, pH 7.4, at 23 °C. Dithiothreitol was included in POP reactions at final concentration of 1 mM. Protease activity was monitored with time by following release of AFC (FAP, DPP4) or AMC (POP) fluorophores. Data were plotted as $v_0/v_i - 1$ versus [I], where v_0 is the rate of substrate hydrolysis in the absence of inhibitor, v_i is the steady-state rate of substrate hydrolysis in the presence of inhibitor, and [I] is the concentration of inhibitor. Plots of $v_0/v_i - 1$ versus [I] were linear and the apparent inhibition constant (K_{app}) was determined from the reciprocal of the slope. The true equilibrium inhibition constant (K_i) was calculated from the relationship: $K_i = K_{app}/(1 + [S]/K_m)$, where [S] is the concentration of substrate used in the assay and K_m is the Michaelis constant for substrate hydrolysis. The presented values represent the average of at least three independent experiments with standard errors less than 10%.
11. Aertgeerts, K.; Levin, A.; Shi, L.; Snell, G. P.; Jennings, A.; Prasad, G. S.; Zhang, Y.; Kraus, M. L.; Salakian, S.; Sridhar, V.; Wijnands, R.; Tennant, M. G. *J. Biol. Chem.* **2005**, *280*, 19441.
12. Engel, M.; Hoffmann, T.; Manhart, S.; Heiser, U.; Chambre, S.; Huber, R.; Demuth, H.; Bode, W. *J. Mol. Biol.* **2006**, *355*, 768.
13. Fulop, V.; Bocskei, Z.; Polgar, L. *Cell* **1998**, *94*, 161.