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Synthesis and structure–activity relationship of *N*-alkyl Gly-boro-Pro inhibitors of DPP4, FAP, and DPP7

Yi Hu, Lifu Ma, Min Wu, Melissa S. Wong, Bei Li, Sergio Corral, Zhizhou Yu, Tyzoon Nomanbhoy, Senaiet Alemayehu, Stacy R. Fuller, Jonathan S. Rosenblum, Natasha Rozenkrants, Lauro C. Minimo, William C. Ripka, Anna K. Szardenings, John W. Kozarich and Kevin R. Shreder*

ActivX Biosciences, Inc., 11025 N. Torrey Pines Road, La Jolla, CA 92037, USA

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Abstract—The structure–activity relationship of various *N*-alkyl Gly-boro-Pro derivatives against three dipeptidyl peptidases (DPPs) was studied. In a series of *N*-cycloalkyl analogs, DPP4 and fibroblast activation protein-α (FAP) optimally preferred *N*-cycloheptyl whereas DPP7 tolerated even larger cycloalkyl rings. Gly α-carbon derivatization of *N*-cyclohexyl or *N*-(2-adamantyl) Gly-boro-Pro resulted in a significant decrease in potency against all the three DPPs.
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Proline-specific serine hydrolases have become a widely studied enzyme class because of their ability to regulate peptide hormones.[1] The dipeptidyl peptidases (DPPs) are a subclass of this serine protease family that cleaves dipeptides from the amino terminus of proteins and prefers a proline residue at the penultimate and P1 position. Members of this family include DPP4, fibroblast activation protein-α (FAP), and DPP7.

Dipeptides containing proline boronic acid (boro-Pro) in the P1 position with C-substituted α-amino acids in the P2 position have been reported as potent inhibitors of both DPP4 and DPP7.[2] Such compounds hold promise as immunomodulatory agents with applications in both hematology and oncology. For example, Val-boro-Pro has been shown to increase murine cytokine levels in vivo and subsequently stimulate hematopoiesis[3] or augment antibody-mediated cytotoxicity.[4] Most recently, Val-boro-Pro (**18**) was identified as an inhibitor of FAP and shown to attenuate tumor growth in a HT-29 xenograft model.[5,6]

Although boro-Pro inhibitors of DPPs are known, relatively little information exists on the inhibitory activity of such compounds against DPP7 and FAP. We reasoned that a direct comparison of the specificity differences among these three DPPs would be helpful for the development of specific inhibitors for each DPP member. Herein, the SAR against DPP4, FAP, and DPP7 of a structurally distinct class of boro-Pro derived inhibitors with unnatural peptoid structures in the P2 position is described.

N-Alkyl Gly-boro-Pro derivatives were synthesized using two different methods. The majority of the compounds were synthesized as shown in Scheme 1. First, the (+)-pinanediol ester of (*R*)-boro-Pro (**1**)[2,7] was reacted with chloroacetyl chloride under basic conditions to yield the chloroacetamide **2**[8] in near quantitative yield. Nucleophilic displacement of the chloride using a primary amine (e.g., 1-adamantylamine) yielded the pinanediol protected intermediate **3**. The free boronic acid was obtained through acid catalyzed transesterifcation of the pinanediol ester with phenylboronic acid. Purification via ion exchange chromatography (Dowex 50WX2-100) followed by elution using 2% NH_4OH yielded the free base form of compound **11** that was then converted to the HCl salt. For compounds **10**, **14**, **16**, and **17**, a reductive amination strategy was employed (generalized in Scheme 2). Gly-boro-Pro, Ala-boro-Pro, or Val-boro-Pro; a ketone; and $NaBH(OAc)_3$ were stirred overnight in 2% AcOH/dichloroethane (DCE) to

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* Corresponding author. Tel.: +1 8585262517; fax: +1 8585874878; e-mail: kevins@activx.com

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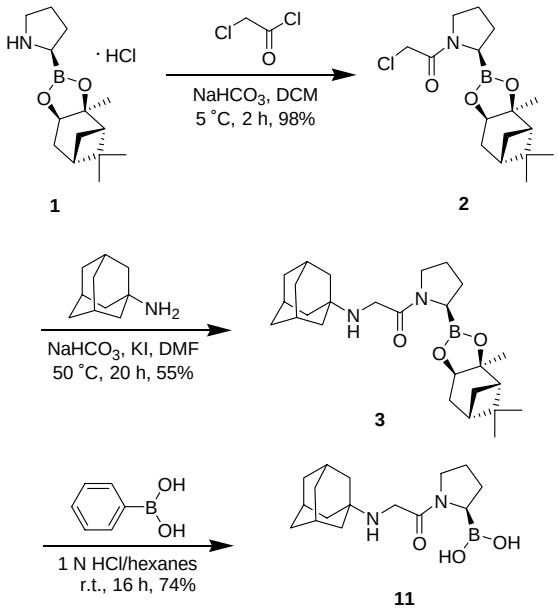
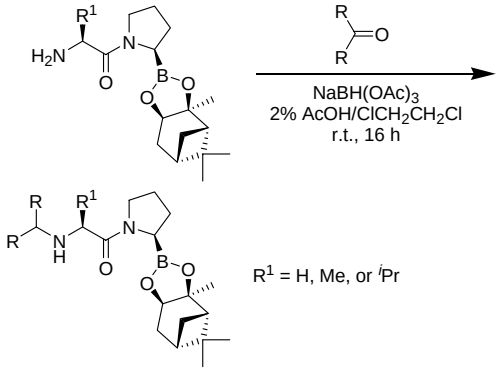


Scheme 1. Synthesis of compound **11** using the α-chloroacetamide intermediate **2**.

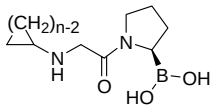


Scheme 2. Reductive amination method used to synthesize the (+)-pinanediol esters of compounds **10**, **14**, **16**, and **17**.

produce the secondary amine. The above-mentioned four inhibitors were generated after subsequent deprotection of the pinanediol esters.

DPP IC_{50} values[9] of a series of *N*-cycloalkyl Gly-boro-Pro inhibitors were determined to understand the relationship between ring size and potency (Table 1). In the case of DPP4 and FAP, the least potent inhibitor was *N*-cyclopropyl-Gly-boro-Pro (**4**). Increasing the ring size increased the potency, but only to a point. *N*-cycloheptyl-Gly-boro-Pro (**8**) was found to be the most potent inhibitor for both enzymes with IC_{50} values of 7.8 and 150 nM, respectively. However, increasing the ring size further resulted in a drop in DPP4 and FAP inhibitory activity. In the case of DPP7, the least potent inhibitor was again the *N*-cyclopropyl derived inhibitor **4** with a DPP7 IC_{50} value of 570 nM. Increasing the ring size increased potency but without a well effect: DPP7 IC_{50} values for compounds **7**–**10** ($n = 6$, 7, 8, and 10) had similar single digit nM potencies. From this series, it would seem that DPP4 and FAP have a *N*-alkyl receiving subsite of limited size that best fits the cycloheptyl ring size. On the other hand, the analogous subsite on DPP7 tolerates ring sizes as large as cyclodecane.

Table 1. DPP4, FAP, and DPP7 inhibition data for compounds **4**–**10**



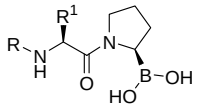
Compound	Ring size (*n*)	IC_{50} (nM)		
		DPP4	FAP	DPP7
4	3	130	30% (33 μM)[a]	570
5	4	75	24000	140
6	5	25	6500	15
7	6	27	650	4.1
8	7	7.8	150	1.8
9	8	27	290	4.0
10	10	58	530	2.2

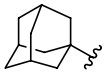
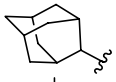
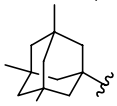
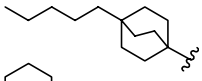
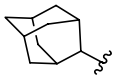
[a] Percent inhibition at the concentration indicated.

The DPP IC_{50} values of Gly-boro-Pro analogs with N-substituted polycyclic groups were also examined (Table 2). *N*-(1-adamantyl)-Gly-boro-Pro (**11**) proved to yield the most potent inhibitor for both DPP4 and FAP with IC_{50} values of 8.0 and 46 nM, respectively. Changing the point of attachment to the secondary carbon on the adamantane scaffold (i.e., inhibitor **12**) resulted in only a modest 2-fold decrease in potency for both the enzymes. Consistent with the hypothesis of a sterically limited *N*-alkyl accepting subsite for DPP4 and FAP, the addition of three methyl groups to the adamantane scaffold (inhibitor **13**) resulted in an inhibitor with reduced potency for these enzymes. By contrast, this bulkier adamantane derivative, with a DPP7 IC_{50} value of 55 nM, was some 8-fold more potent for DPP7 than its unsubstituted *N*-(1-adamantyl) congener **11**. Other N-substituted polycyclic groups yielded even more potent DPP7 inhibitors. The bicyclo[3.3.1]nonane derivative **14** and the 4-pentyl-bicyclo[2.2.2]octane derivative **15** yielded DPP7 IC_{50} values of 8.0 and 1.0 nM, respectively.

Analogs of *N*-cyclohexyl-Gly-boro-Pro (**7**) and *N*-(2-adamantyl)-Gly-boro-Pro (**12**) in which the Gly α-carbon (αC) was substituted with a methyl and isopropyl group, respectively, were studied to investigate the DPP inhibitory activity of dual N- and αC-substituted Gly-boro-Pro derivatives (Table 2). The impact of dual substitution was dramatic. For example, the DPP4 IC_{50} value of the *N*-, αC-disubstituted compound **16** was 16 μM, a 600- and 53,000-fold reduction in DPP4 potency, respectively, when compared to the corresponding 'mono-alkylated' Gly-boro-Pro analogs **7** and **18** (Val-boro-Pro). In addition, no inhibition of the FAP or DPP7 activity could be detected with compound **16**. The decrease in DPP inhibitory activity was also very significant with compound **17** when compared to the inhibitors **12** and **19** (Ala-boro-Pro). Interestingly, analogous dual substituted Gly-cyano-Pro compounds have been shown to be significantly less active against DPP4 when compared to their *N*-alkyl Gly-cyano-Pro congeners.[10]

Table 2. DPP4, FAP, and DPP7 inhibition data for compounds **11**–**19**



Compound	R	R^1	IC_{50} (nM)		
			DPP4	FAP	DPP7
11		H	8.0	46	420
12		H	12	99	120
13		H	560	770	55
14		H	25	180	8.0
15		H	61	270	1.0
16		–^{i}Pr	16,000	ni[a]	ni[a]
17		–Me	1600	37,000	4600
18	H	–^{i}Pr	0.30	11	38
19	H	–Me	0.85	7800	6.3

[a] ni, no inhibition (i.e., no significant inhibition observed at concentrations as high as 33 μM).

In conclusion, a variety of *N*-alkyl Gly-boro-Pro inhibitors were synthesized and their ability to inhibit DPP4, FAP, and DPP7 studied. The size or shape of the alkyl group played a large role in tuning DPP potencies and selectivities. Dual N-, αC-substitution of the Gly-boro-Pro motif was shown to be deleterious. Of note in this study was the similarity in the SAR for this class of compounds against DPP4 and FAP. By contrast, DPP7 exhibited different SAR trends, for example, a tolerance and preference for larger *N*-alkyl groups. Future studies will examine the ability of this new class of compounds to modulate cytokine levels in vivo.

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6. DPP4, FAP, and DPP7 IC_{50} values for Val-boro-Pro (**18**) have previously been determined to be 26, 40, and 15 nM, respectively (see Refs. 2 and 5). Because of the pH dependent cyclization of boro-Pro dipeptides via B–N bond formation, IC_{50} values are dependent on the pH at which a compound is pre-incubated prior to the inhibition assay. For more information, see: Snow, R. J.; Bachovchin, W. W.; Barton, R. W.; Campbell, S. J.; Coutts, S. J.; Freeman, D. M.; Gutheil, W. G.; Kelly, T. A.; Kennedy, C. A.; Krolikowski, D. A.; Leonard, S. F.; Pargellis, C. A.; Tong, L.; Adams, J. *J. Am. Chem. Soc.* **1994**, *116*, 10860.
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8. Compound **2**: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.33 (dd, 1H, J = 2.0 Hz, J = 8.8 Hz), 4.03 (m, 2 H), 3.63 (m, 1H), 3.52 (m, 1H), 3.18 (m, 1H), 2.34 (m, 1H), 2.10 (m, 5H), 1.86 (m, 3H), 1.45 (s, 3H), 1.28 (m, 4H), 0.84 (s, 3H).
9. Enzymatic activities for human DPP4, DPP7, and FAP were determined fluorometrically using Gly-Pro-AMC (100 μM, pH 7.5), Lys-Pro-AMC (100 μM, pH 6.0), and Ala-Pro-AMC (250 μM, pH 8.5), respectively, as substrates. Caco2 cell lysate was used as a source for DPP4. DPP7 and FAP were obtained from the soluble fraction and isolated membranes, respectively, of transfected HEK293 cells. To determine IC_{50} values, test compounds as their HCl salts were dissolved in 50% DMSO/50 mM glycine buffer, pH 2.5 (the final concentration of DMSO in the assays was 1% v/v) and serially diluted into the assay to yield, in the end, 16 different inhibitor concentrations ranging from 33 μM to 0.007 nM. Substrate was then added, and the mixture was incubated at ambient temperature for a fixed period of time (20 min for DPP4/

DPP7, 60 min for FAP). All measurements were carried out in duplicate. Fluorescence intensity versus inhibitor concentration was plotted and fit to the Hill equation to quantify IC_{50} values.

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