Design, Synthesis, and Characterization of Potent and Specific Inhibitors for Dipeptidyl Peptidase II

A thesis

submitted by

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Abstract:

This paper reports on a systemic screen for potent and selective dipeptidyl peptidase (DPP) II inhibitors. The boronic acid derivative boroNorvaline (boroNva) was designated as the P1 position amino acid and a library of amino acids, both natural and synthetic, were coupled with boroNva to form dipeptide inhibitors. The inhibitors were tested against the members of the DPPIV activity and/or structure homolog (DASH) enzymes and the 20S proteasome. It was found that the boroNva based compounds had strong potency for DPPII, especially Methionine (Met)-boroNva (**6**) and Leucine (Leu)-boroNva (**10**), but suffered from poor selectivity. The selectivity for DPPII was improved when α, α carbon di-substituted amino acids were coupled with boroNva. These small molecules can help provide a new route for increased selectivity of boronic acid derivatives, and ultimately facilitate our understanding of DPPII's role in biological systems.

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List of Abbreviations:

DPP ……..	Dipeptidyl Peptidase
DASH ……..	DPP4 activity and/or structure homolog
Xaa ……...	X -Amino Acid
FAP ……..	Fibroblast Activating Protein
PREP ……	Prolyl Endopeptidase
BoroNva ……...	Boro-Norvaline
SI ……..	Selectivity Index
Gly ……	Glycine
Ala ……	Alanine
Etg ……	2-Ethylglycine
Nva ……...	Norvaline
Nle ……	Norleucine
Met …….	Methionine
SeMet ……...	Selenomethionine
Hse (Me) ……..	Homoserine methyl ether
Val ……	Valine
Leu ……...	Leucine
Ile …….	Isoleucine
Tle ……	tert-leucine
Chg ……...	Cyclohexyl-glycine
Cha ……...	Cyclohexyl-alanine
Phe ……	Phenylalanine
Tyr ……..	Tyrosine
Ala(1-naphthyl) …	1-naphthyl-alanine
Trp ……	Tryptophan
His ……	Histidine
Pro ……	Proline
Gly(adamantyl) …	1-adamantyl-glycine
Asn ……..	Asparagine
Gln ……	Glutamine
Ser ……	Serine
Thr ……	Threonine
Lys ……..	Lysine
Arg ……..	Arginine
Dab ……..	Diaminobutyl
Ar …….	2-chlorobenzyl-diaminobutyl
Glu ……	Glutamic Acid
Asp ……...	Aspartic Acid
Aib ……	Aminoisobutyl
Etg(alpha-methyl)	Ethylgycine(alpha-methyl)
Nva(alpha-methyl)	Norvaline(alpha-methyl)
Pro(alpha-methyl)	Proline(alpha-methyl)
Leu(alpha-methyl)	Leucine(alpha-methyl)
Cle ……	Cycloleucine
Hcl ……	Homocycloleucine
Gly(pip) ……	Glycine(piperidine)

Introduction:

1.1 DPPII and the DASH Family

Over the last few decades a growing amount of research has focused on proline specific dipeptidyl peptidases (DPPs) as drug targets. The enzymes involved belong to the DPPIV activity and/or structure homologs (DASH) family, and include DPPIV, DPP8, DPP9, Fibroblast Activating Protein α (FAP), and Prolyl Endopeptidase (PREP)[1]. These enzymes have been shown to cleave dipeptides containing proline at the penultimate position in vitro[1]. DPPIV has been the most extensively researched enzyme out of the DASH family and has led to the drug Sitigliptin, a DPPIV inhibitor used for type II diabetes[10]. Though DPPII is considered part of the DASH family, DPPII has little structurally homology to DPPIV[17], but has been shown to cleave DPPIV substrates. DPPII is most structurally related to Proly Carboxypeptidase (PCP), though their activities are different with the former preferentially cleaving at N-terminal X-Pro dipeptides and the latter cleaving at C-terminal Pro-X proteins[13,24].

1.2 Localization and Function of DPPII:

DPPII is found ubiquitously throughout the body. It is localized in intracellular vesicles and is active in acidic pH[2]. DPPII homodimerizes with the help of a leucine zipper motif, making it unique among serine proteases[4]. It has been shown that DPPII is capable of cleaving brain neuropeptides including substance P, casomorphin and bradykinin[20] though how biologically relevant this may be is in question. DPPII constitutive knockdown is embryonic lethal but viable T-cell specific DPPII knockdown[18] and

neurogenin 3-specfic DPPII knockdown[9] mice have been generated. These studies have shown that DPPII plays an important role in maintaining lymphocyte quiescence[3, 5, 18] and that DPPII is important for preventing hyperinsulemia and maintaining glucose homeostasis[9]. Another study using a pharmacological approach had shown that DPPII inhibition does not lead to apoptosis, necrosis or autophagy in leukocytes. It is important to note that this study did not look at lymphocytes or quiescent cells[16]. From these studies it is clear that potent and selective DPPII inhibitors are essential to pharmacologically elucidate the function of DPPII and distinguish its action from the other DASH enzymes.

1.3 DPPII Inhibitors

There have been various classes of DPPII inhibitors, most notably, boronic acid dipeptide analogues[11, 23], and aminoacyl piperidides[15, 22]. Some of these inhibitors were found indirectly when screening for inhibitors of DPPIV. The dipeptide boronic acid Valine-boroProline (Val-boroPro) was originally found to be a potent DPPIV inhibitor but was also

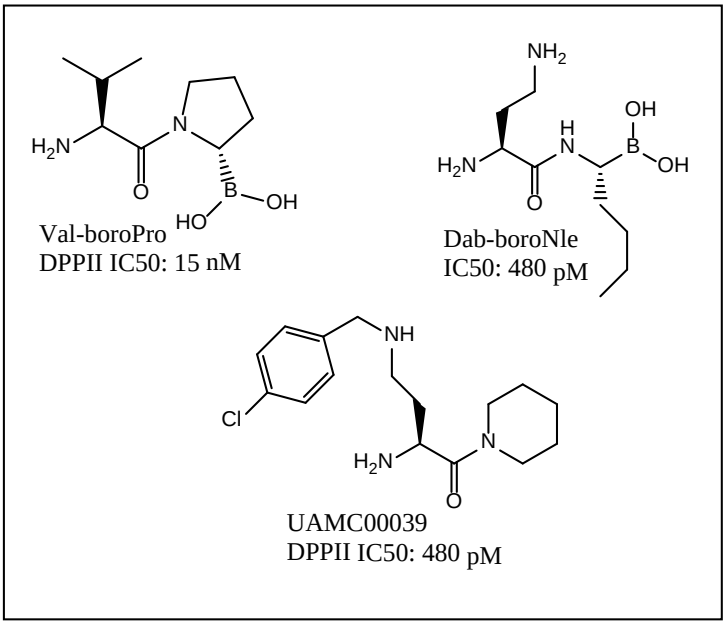

Figure1. Known DPPII Inhibitors. Valine-boroProline (Val-boroPro) is a non-specific DPP inhibitor and has an IC50 of 15 nM[7] for DPPII. Diaminobutyl-boroNorleucine (Dab-boroNle) is a more potent and specific boronic acid analogue then Val-boroPro with an IC50 of 480 pM[22]. UAMC-00039 (N-(4-chlorobenzyl)-4-oxo-4-(1-piperidinyl)-1,3-(S)-butanediamine) is a potent and highly selective DPPII inhibitor with an IC50 of 480 pM[15].

discovered to be a potent inhibitor for DPPII, 8, and 9 as well[6, 7]. Dipeptide boronic acids were further tested to optimize selectivity for DPPII over the other DASH enzymes, and diaminobutyl-boroNorleucine (Dab-Nle) was found to be the most potent and selective inhibitor from that class with an IC50 of 480 pM[23]. Studies with amino-pyrrolidides and piperidides revealed that aminoacyl-piperidides were more specific and potent for DPPII over DPPIV. This study led to the DPPII inhibitor diamobutyl-piperidine (Dab-pip) with an IC50 of 130 nM[22]. Dab-pip was further optimized to N-(4-chlorobenzyl)-4-oxo-4-(1-piperidinyl)-1,3-(S)-butanediamine dihydrochloride (UAMC00039) with an IC50 of 480 pM and very strong selectivity for DPPII[15].

This study focused on the boronic acid analogue boroNorvaline (boroNva) as the P1 peptide and screened various carbon-substituted α-amino acids in the P2 position for inhibition of the DASH enzymes as well as the proteasome to better understand DPPII preferences for boronic acid derivatives. We further enhanced the P2 peptide by having the α carbon di-substituted with two R groups. We found that boroNva inhibitors were more potent for DPPII over the other DASH enzymes and the proteasome, most notably with Met-boroNva (**6**) and Leu-boroNva (**10**), but lacked strong selectivity specifically over DPP8, 9 and the proteasome. Importantly, the di-substituted α-carbon on the P2 peptide increased selectivity for DPPII over DPP8 and 9, as well as the proteasome.

Materials and Methods

2.1 Compound Synthesis:

39 compounds were synthesized (Table 1) and tested. The majority of the compounds were previously synthesized in house and stored by colleagues. Compounds that were not readily available in our own library were synthesized as seen in Figure 2. Briefly, the P2 position amino acid was ordered commercially as the L-enantiomer with tert-butyloxycarbonyl (Boc) protected N-terminus. The coupling reaction was done using 0.1 mMol starting material. First the P2 amino acid was added with 1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate (HATU) and dissolved in 2 mL dimethylformamide (DMF). Then

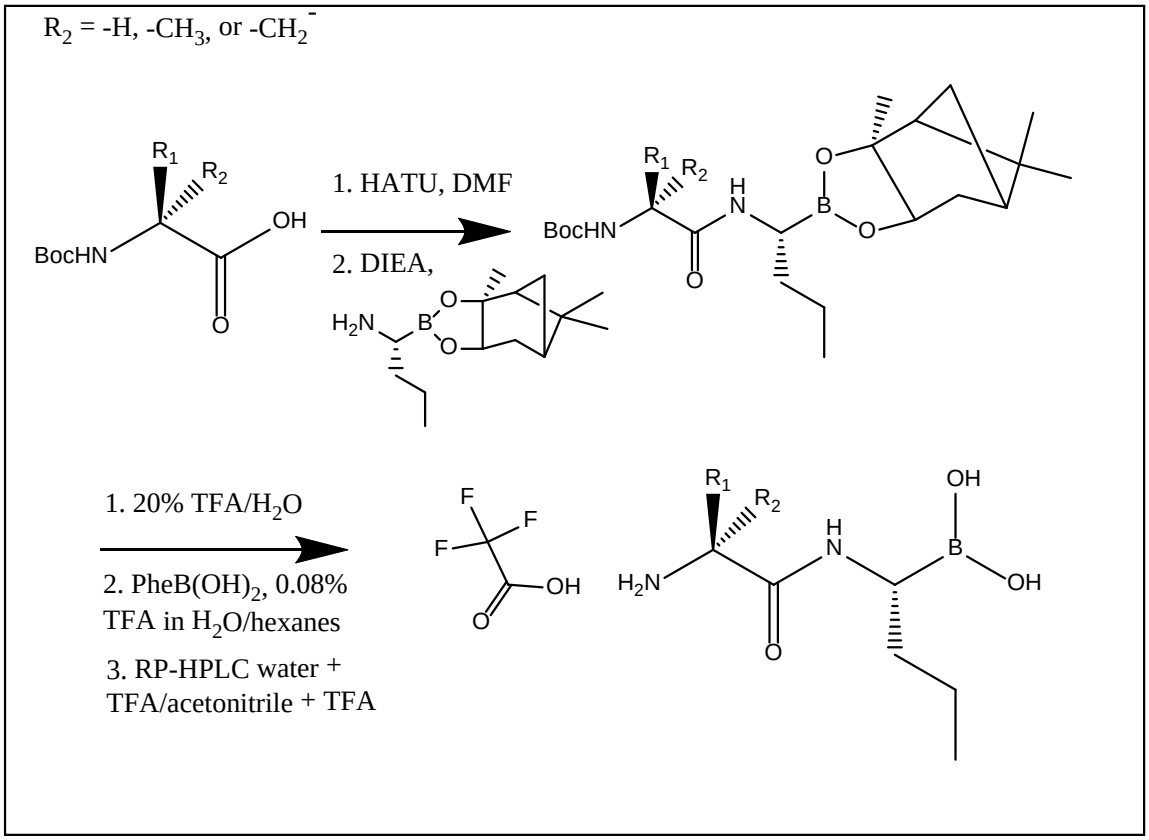

Figure 2. Synthetic Scheme of Xaa-boroNorvaline Compounds.

0.1 mMol L-boroNorvaline (+)-pinanediol ester hydrochloride, synthesized previously in house, was added along with 0.1 mMol N,N-Diisopropylethylamine (DIEA). The coupling reaction product was checked using liquid chromatography–mass spectrometry (LC-MS) with water/0.08% Trifluoroacetic acid (TFA), and acetonitrile/0.08% TFA as the solvents. The solution was then then purified using reversed phase high performance liquid chromatography (RP-HPLC), frozen with acetone and dry ice, and then lypholized. The Boc was removed by adding 2 mL 20% TFA to the fully lypholized product. Afterwards, an equimolar amount of Phenylboronic acid with 0.08% TFA/water was used to remove the pinannediol. This led to the boro-Nva dipeptide final product which was checked with LC-MS and purified by RP-HPLC to produce a TFA salt final product.

2.2 Enzymes and Substrates:

Recombinant Huma Dipeptidyl Peptidase II (3438-SE), Recombinant Human Dipeptidyl Peptidase IV (1180-SE), Dipeptidyl Peptidase 9 (5419-SE), Recombinant Human Fibrogen ActivatingProtein (3715-SE), and Recombinant Human Prolyl Oligopeptidase (4308-SE) were all purchased from R&D Systems. Dipeptidyl Peptidase 8 (BML-SE527) was purchased from Enzo Life Sciences and Human 20S Proteasome was purchased from Boston Biochem. The DPPII substrate KP-AMC (I-1745) was purchased from VWR. The DPPIV, 8 and 9 substrate GP-AMC (I-1225), and the FAP and PREP substrate Z-GP-AMC (I-1145) were purchased from Bachem. The Proteasome substrates used were Suc-LLVY-AMC (S-280), Z-LLE-AMC (S-230) and Boc-LRR-AMC (S-300) were all purchased from Boston Biochem.

2.3 DASH Enzyme Inhibition Assay:

The assays were performed in room temperature in Falcon black 96 well plates with a clear bottom and had a final volume of 210 uL. 100 mM inhibitor stock was prepared by diluting the compound in DMSO. A 1 mM stock in pH2 water was prepared using the 100 mM DMSO stock and allowed to incubate overnight. The 1 mM enzyme stock was diluted in a serial dilution of 1:10 with appropriate enzyme buffer from 1 mM to 100 pM. Enzyme was diluted in buffer and added to the plate for final concentrations of 0.1, 0.4, 1.6, 0.4, 4.2, and 0.4 nM for DPPII, DPPIV, DPP8, DPP9, FAP, and PREP respectively. 20 uL of the serial dilutions of inhibitor were added to the plate after the enzyme, in doing so having final concentrations of inhibitor ranging from 100 uM to 10 pM. The plate was shaken for 10 minutes in room temperature. After 10 minutes, 10 uL of appropriate fluorogenic substrate was added to each well to make a final concentration of 25 uM KP-, GP, or Z-GP-AMC per well. The plate was shaken and allowed to incubate in room temperature for 15 minutes. After 15 minutes, the fluorescence was read in a Molecular Devices SPECTRAmax 340PC384 microtiter plate reader at Ex: 380, Em: 460. The IC50 values were determined by a non-linear regression fit of the data to a sigmoidal dose-response curve using the program Prism. Each experiment was done in triplicate (Figure 3) and the mean IC50 value and the standard error of the mean were presented. The IC50 value is defined as the concentration of inhibitor required to reduce the DPPIV activity to 50% after 10 minute incubation with the enzyme before addition of the substrate.

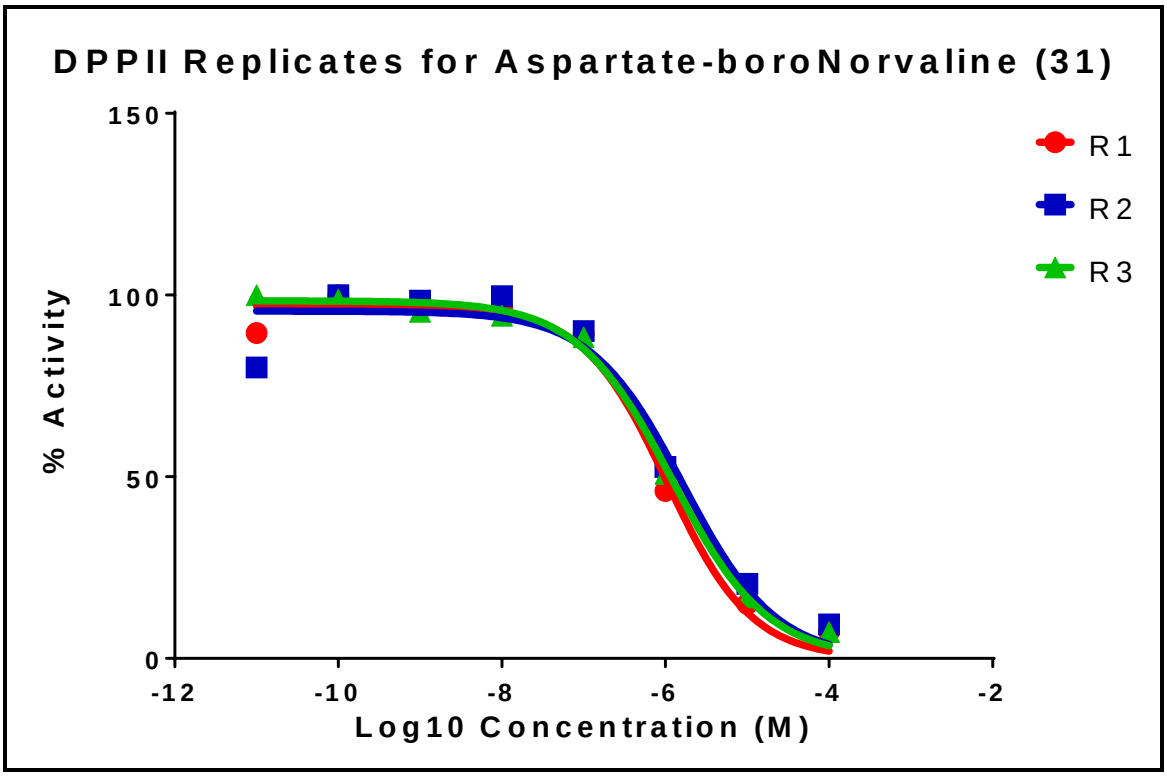

Figure 3. Representative Enzyme Inhibition Graph. Experiments were done in triplicate (denoted R1-R3), and thus produced three curves with three IC50 values in molar concentrations. The mean IC50 from the replicate experiments was presented along with the standard error of the mean.

2.4 Proteasome Inhibition Assay:

Preparation for the serial dilution of inhibitor was the same as the DASH inhibition assay. The proteasome inhibition utilized three different substrates to test for the different activities of the proteasome. The activities include Chymotrypsin-like activity, Caspase-like activity, and Trypsin-like activity, and they were tested using Suc-LLVY-AMC, Z-LLE-AMC, and Boc-LRR-AMC, respectively. The proteasome was diluted in buffer so that a final amount of 0.2 ng proteasome per well for the Chymotrypsin-and Caspase-like activity or 1 ng per well for the Trypsin-like activity was achieved. Before adding the enzyme/buffer stock to the wells, 3% SDS was added to the enzyme stock in a 1:100 dilution and allowed to incubate for 5 minutes. The purpose of the SDS was to activate

the proteasome. After the inhibitor was added to the plate, the plate was shaken and left to incubate for 10 minutes. After 10 minutes, 10 uL of appropriate substrate was added to each well to make a final concentration of 20 uM KP-, GP, or Z-GP-AMC per well. After the substrate was added the, the plate was shaken and left to incubate in room temperature for 30 minutes. The fluorescence was read in a Molecular Devices SPECTRAmax 340PC384 microtiter plate reader at Ex: 380, Em: 460. The IC50 values were determined by a non-linear regression fit of the data to a sigmoidal dose-response curve using the program Prism and the IC50 value was defined the same way as in the DASH enzyme inhibition assay.

Results:

3.1 Alkyl Chains and Branched Alkyl R Groups:

Simple alkyl chains and branched alkyl R groups were synthesized and tested for inhibition of DASH enzyme activity (Table 1 compounds **1-12**). **1** contained no R group and could be used as a baseline. **1** was most potent for DPPII with an IC50 of 134 nM and IC50s of around 90, 16, 25, 2 and over 100 uM for DPPIV, DPP8, DPP9, FAP and PREP respectively. Selectivity indexes (SI) for DPPII vs. the other DASH enzymes were all under 1,000 (Table 2). DPP8, DPP9 and FAP SI were notably low: 126, 192 and 20 for DPP8, 9 and FAP respectively. Compounds **2-5** each had an increase in carbon chain length with **2** having one methyl group as the R group and **5** having a n-butyl group. As carbon chain length increased, potency for DPPII also increased with IC50s of 38, 14, 5.0, and 0.7 nM for **2**, **3**, **4** and **5** respectively. Potency for DPPIV, 8 and 9 also increased with carbon chain elongation, reaching to micro-molar range IC50 for DPPIV and

submicro-molar range for DPP8 and 9. Meanwhile, FAP and PREP activity were unaffected for the most part with IC50s over 100 uM. Interestingly, SI decreased noticeably from that of **1** for DPPII vs DPP8 for **2** and **3** with methyl and ethyl R groups respectively. The SI then increased close to that seen in **1** for **4** and **5** for DPP8. DPPIV SI decreased notably for **3** and **4** from that of **1** and **2**. This SI then rose dramatically for **5** to 6,031 for DPPIV. **5** had the highest potency and selectivity for DPPII over the other DASH enzymes out of the five compounds with simple alkyl chains.

#	Amino Acid	IC50 (nM)					
		DPPII	DPPIV	DPP8	DPP9	FAP	PREP
1	Gly	134 ± 24	90,983 ± 16,675	16,846 ± 6801	25,608 ± 17,313	2,722 ± 1,048	>100,000
2	Ala	38 ± 3.4	34,363 ± 2,564	1,474 ± 93	10,683 ± 2,824	>100,000	29,770 ± 11,127
3	Etg	14 ± 0.3	13,73 ± 102	449 ± 71	1,330 ± 115	>100,000	>100,000
4	Nva	5.0 ± 1.3	1,560 ± 56	759 ± 25	1,064 ± 167	>100,000	>100,000
5	Nle	0.70 ± 0.1	4,220 ± 148	119 ± 3.8	307 ± 19	>100,000	>100,000
6	Met	0.43 ± 0.1	3,701 ± 608	123 ± 33	237 ± 85	>100,000	15,410 ± 918
7	SeMet	0.89 ± 0.3	2,692 ± 443	33 ± 5.8	6,577 ± 6,277	9,655 ± 1,150	2,157 ± 778
8	Hse (Me)	15 ± 2.2	4,123 ± 871	119 ± 22	217 ± 77	>100,000	10,823 ± 1,773
9	Val	5.3 ± 0.3	1,889 ± 69	143 ± 19	307 ± 19	>100,000	>100,000
10	Leu	0.41 ± 0.1	3,915 ± 124	326 ± 19	1,416 ± 73	>100,000	>100,000
11	Ile	4.1 ± 0.5	2,092 ± 137	410 ± 37	1,178 ± 193	>100,000	>100,000
12	Tle	1.4 ± 0.1	659 ± 89	192 ± 18	963 ± 124	>100,000	>100,000
13	Chg	0.53 ± 0.03	388 ± 43	37 ± 4.9	48 ± 4.9	>100,000	38,620 ± 3,582
14	Cha	0.74 ± 0.06	2,188 ± 127	43 ± 8.4	333 ± 2.4	>100,000	>100,000
15	Phe	0.84 ± 0.07	2,895 ± 123	118 ± 9.2	1,986 ± 60	>100,000	>100,000

16	Tyr	2.8 ± 1.05	1,253 ± 69	323 ± 4.7	5,897 ± 1,948	533 ± 234	29,697 ± 23,991
17	Ala(1-naphthyl)	2.2 ± 0.77	3,367 ± 438	170 ± 39	298 ± 43	>100,000	>100,000
18	Trp	0.42 ± 0.11	11,540 ± 441	176 ± 7.9	2,786 ± 583	>100,000	>100,000
19	His	77 ± 13	15,845 ± 3,520	2,202 ± 1,237	9,642 ± 1,735	>100,000	>100,000
20	Pro	33 ± 3.4	15,187 ± 357	1,844 ± 257	2,094 ± 24	>100,000	>100,000
21	Gly(adamantyl)	0.77 ± 0.09	107 ± 5.6	6.1 ± 0.7	5.3 ± 0.2	>100,000	>100,000
22	Asn	14 ± 1.2	1,420 ± 11	647 ± 105	15,50 ± 181	72,200 ± 4,610	>100,000
23	Gln	1.3 ± 0.08	3,063 ± 76	161 ± 22	982 ± 160	>100,000	33,420 ± 12,162
24	Ser	24 ± 6.01	33,523 ± 1867	11,488 ± 5423	4,354 ± 927	>100,000	>100,000
25	Thr	21 ± 2.4	1,156 ± 136	1,937 ± 164	1,100 ± 63	>100,000	>100,000
26	Lys	2.9 ± 0.20	3,044 ± 486	33 ± 1.8	60 ± 13	>100,000	>100,000
27	Arg	3.5 ± 0.21	2,191 ± 580	77 ± 7.2	144 ± 40	13,188 ± 2,548	45,224 ± 39,911
28	Dab	2.9 ± 0.26	28,472 ± 16,501	492 ± 44	465 ± 96	3,198 ± 1,855	11,932 ± 2,029
29	Ar	5.2 ± 0.22	296 ± 32	100 ± 5.0	732 ± 230	>100,000	55,853 ± 5,563
30	Glu	110 ± 14	11,166 ± 704	7,329 ± 452	30,477 ± 7,760	76,627 ± 27,388	>100,000
31	Asp	1,299 ± 177	448 ± 54	49,340 ± 6,171	2,590 ± 354	7,761 ± 1,213	36,103 ± 4,088
32	Aib	20 ± 2.0	78,830 ± 12,365	318 ± 30	12,173 ± 1,404	>100,000	>100,000
33	Etg(alpha-methyl)	7.9 ± 0.53	4,661 ± 945	46,313 ± 2707	2,496 ± 421	>100,000	>100,000
34	Nva(alpha-methyl)	3.8 ± 0.74	23,020 ± 4,145	18,253 ± 3,645	44,865 ± 19,005	>100,000	>100,000
35	Pro(alpha-methyl)	184 ± 1.6	1,861 ± 33	>100,000	2,792 ± 514	>100,000	>100,000
36	Leu(alpha-methyl)	1.4 ± 0.3	1,404 ± 620	2,787 ± 937	45,573 ± 3,958	11,102 ± 2,091	>100,000
37	Cle	9.6 ± 2.8	5,884 ± 50	9,132 ± 1,107	1,249 ± 74	>100,000	>100,000
38	Hcl	1.4 ± 0.1	2,452 ± 180	18,033 ± 4,500	1,272 ± 68	>100,000	>100,000
39	Gly(pip)	15 ± 2.8	48,560 ± 18,868	5,654 ± 2,253	1,014 ± 258	>100,000	27,990 ± 16,287

Table 1. **Xaa-boroNva Inhibition of DASH Enzymes**. 39 compounds were tested for inhibition against DPPII, IV, 8, 9, FAP and PREP activities. Each experiment was done in triplicate and the mean IC50 value and the standard error of the mean are presented.

5 analogues were synthesized in which the 3rd carbon of the n-butyl chain was substituted with sulfur, selenium, or oxygen for **6**, **7** and **8** respectively. **6** and **7** did not change significantly in potency from **5** (Table 1) but **6** had an IC50 of 0.43 nM making it one of the most potent DPPII inhibitors in this study. **8** lost DPPII potency (IC50 = 15 nM) seen in the other n-butyl side chain compounds. Potency for the other DASH enzymes did not significantly change for **6**, **7** and **8** related to those of **5,** with the exception of DPP8 and 9 for **7**. The IC50 of DPP8 decreased down to 33nM while the IC50 for DPP9 increased markedly to 6.6 uM. FAP and PREP activity were also affected by **7**, with IC50s in the low micro-molar range. For **6**, the SI for DPPIV, DPP8 and 9 did not differ greatly from those of **5**. DPPII was 8 and 15 fold more selective than DPP8 and 9 respectively for **8**, a noticeable difference from those of **5** and **6**. **7**, with its decrease potency for DPP9 presented a corresponding increase in SI for DPP9 and decrease for DPP8.

Branched alkyl chains were also synthesized and used for comparison (Table 1, 2 compounds **9-12**). The R groups consisted of isopropyl, isobutyl, sec-butyl, and t-butyl for **9**, **10**, **11**, and **12** respectively. **10** was the most potent DPPII inhibitor found in this study and had the highest selectivity for DPPII out of the alkyl chains and branched alkyl R group inhibitors. DPPII IC50 was 0.41 nM and had SI values over 1,000 for DPPIV, 9, FAP and PREP. DPP8 had an SI of 793. **9**, **11** and **12** had low nano-molar IC50s for DPPII but SI for DPPIV, 8 and 9 did not increase past 520.

Human 20S Proteasome activity was also tested (Table 2, 3). The chymotrypsin-like, caspase-like and trypsin-like activities were tested using different substrates. The chymotrypsin-like activity was affected by increasing chain length, starting at around 1 uM IC50 for **1** and **2** and then decreasing to less than 500 nM for **3-11**. **5** and **7** had the

lowest IC50s of 93 and 65 nM respectively for the chymotrypsin-like activity. **12,** with the tert-butyl R group, had a chymotrypsin-like activity IC50 of 991 nM with a large standard error. The caspase-like activity of the proteasome was generally the same for the compounds **1-12** with IC50s in the low micro-molar range with the exceptions of **5**, **6**, **7**, and **9**. **6** had an IC50 of 618 nM while **5**, **7** and **9** had IC50s above 100 uM. Likewise, the trypsin-like activity of the proteasome remained uniform for the most part ranging in the micro-molar range. **5** and **7** had IC50s of 503 and 491 nM respectively.

3.2 Cyclo-alkanes and Aromatic R Groups:

Amino acids with cyclo-alkanes and aromatic homo/heterocyclic R groups were tested for DASH and Proteasome activity (Tables 1, 2, 3 compounds **13-21**). The compounds in this group had strong potency for DPPII with IC50s in the low nano-molar and subnano-molar range, and largely did not affect FAP or PREP activity with IC50s greater than 100 uM. The exceptions were **19** and **20** with IC50s of 77 and 33 nM respectively for DPPII. The most potent of the group were **13** and **18** with IC50s of 0.53 and 0.42 nM respectively for DPPII. As potent as **13** was for DPPII, it had poor selectivity over DPP8 and 9 specifically with SI of 69 and 90 respectively. **18** also suffered from somewhat poor selectivity with an SI of 423 and 181 for DPP8 and the chymotrypsin-like activity of the proteasome respectively. In general, these compounds inhibited the chymotrypsin-like activity of the proteasome well. Notably, **15**, **16**, and **17** were some of the most potent compounds. Also, **20** had the lowest SI of 2 for the chymotrypsin-like activity of the proteasome. The last compound tested in this group was **21** which had an adamantyl as the R group. Interestingly, it was most potent for DPPII, 8 and 9 with IC50s of 0.77, 6.1

and 5.3 nM respectively. **21** inhibited the chymotrypsin-like activity of the proteasome with an IC50 of 185 nM and micro-molar range for the other two activities.

#	Amino Acid	Proteasome IC50 (nM)		
		Chymotrypsin-like	Caspase-like	Trypsin-like
1	Gly	1,322 ± 416	1,457 ± 142	60,070 ± 3,719
2	Ala	1,056 ± 265	2,374 ± 59	22,930 ± 8,863
3	Etg	203 ± 6.4	1,177 ± 33	5,667 ± 409
4	Nva	433 ± 151	953 ± 47	4,971 ± 1,185
5	Nle	93 ± 4.2	>100,000	503 ± 45
6	Met	210 ± 8.1	618 ± 44	3,057 ± 1,552
7	SeMet	65 ± 6.3	>100,000	491 ± 15
8	Hse (Me)	196 ± 7.9	1,717 ± 25	>100,000
9	Val	336 ± 121	>100,000	3,159 ± 770
10	Leu	142 ± 19	555 ± 29	3,151 ± 243
11	Ile	430 ± 15	1,729 ± 24	2,696 ± 51
12	Tle	991 ± 705	3,201 ± 145	812 ± 260
13	Chg	362 ± 9	1,065 ± 34	1,940 ± 238
14	Cha	187 ± 45	398 ± 21	>100,000
15	Phe	40 ± 8.0	617 ± 23	93,817 ± 26,474
16	Tyr	43 ± 2.2	576 ± 28	1,632 ± 445
17	Ala(1-naphthyl)	86 ± 13	481 ± 27	>100,000
18	Trp	75 ± 1.7	269 ± 5.9	649 ± 310
19	His	2,041 ± 167	6,016 ± 667	2,9113 ± 1,484
20	Pro	53 ± 2.0	201 ± 7.7	1,551 ± 642
21	Gly(adamantyl)	185 ± 13	1,721 ± 34	2,767 ± 911
22	Asn	247 ± 4	748 ± 41	6,674 ± 1,311

23	Gln	98 ± 16	777 ± 4.9	4,705 ± 361
24	Ser	336 ± 40	1,236 ± 22	5,037 ± 620
25	Thr	473 ± 18	2,042 ± 63	13,488 ± 3,167
26	Lys	266 ± 8.4	1,004 ± 14	2,062 ± 159
27	Arg	352 ± 13	652 ± 65	1,369 ± 292
28	Dab	422 ± 45	1,592 ± 44	>100,000
29	Ar	245 ± 25	448 ± 37	1,550 ± 534
30	Glu	738 ± 21	1,457 ± 42	8,080 ± 652
31	Asp	4,737 ± 380	1,618 ± 74	>100,000
32	Aib	1,011 ± 19	1,787 ± 35	40,750 ± 2,046
33	Etg(alpha-methyl)	1,386 ± 39	3,564 ± 202	>100,000
34	Nva(alpha-methyl)	2,480 ± 253	2,497 ± 94	>100,000
35	Pro(alpha-methyl)	165 ± 7	511 ± 55	4,455 ± 860
36	Leu(alpha-methyl)	2,543 ± 528	2,482 ± 74	29,630 ± 1,941
37	Cle	4,926 ± 179	9,472 ± 265	>100,000
38	Hcl	3,892 ± 79	5,539 ± 421	>100,000
39	Gly(pip)	10,733 ± 345	61,647 ± 2,885	>100,000

Table 2. Xaa-boroNva Inhibition of the Proteasome. 39 compounds were tested for inhibition against the chymotrypsin-, caspase-, and trypsin-like activities of the proteasome. Each experiment was done in triplicate and the mean IC50 value and the standard error of the mean are presented.

3.3 Polar and Charged R groups:

Polar and charged R groups were tested against DASH and the Proteasome (Tables 1, 2, 3 compounds **22-31**). 24 and 25 have alcohols as R groups and have good inhibition against DPPII activity (24 and 21 nM). **25** had lower selectivity for DPPII over the other DASH enzymes related to **24**, specifically for DPPIV and DPP8. **24** and **25** had comparable results for proteasome inhibition with the chymotrypsin-like activity having

an IC50 of 336 and 473 nM respectively. The caspase- and trypsin-like activities were both around the low micro-molar range. **22** and **23** were the other polar pair of compounds tested and had amides of different alkyl chain length as the R group. **23** was found to be more potent for DPPII, 8, 9, and PREP than **22** while DPPIV activity was comparably inhibited by both compounds. **23** had an IC50 of 1.3 nM for DPPII while **22** was 14 nM. **22** had a low SI for the chymotrypsin-like activity of the proteasome of 75 while **23** was even lower at 17.

30 and **31** were the carboxylic acid versions of **22** and **23,** respectively, and thus held a negative charge in physiological pH. These compounds were found not to be particularly potent inhibitors of the DASH enzymes nor the proteasome. IC50s were mostly in the low to upper micro-molar for each enzyme with the exception of DPPII for **30** (IC50 110 nM) and DPP8 (IC50 448 nM) for **31**. Interestingly, there was a noticeable difference in IC50s for DPP8 and 9 for **31**.

26-29 had positively charged R groups in physiological pH. DPPII, 8 and 9 favored these compounds and had nano-molar IC50s. With that being said, these compounds had low SI. Both **26** and **28** had primary amines as the R group with **26** having a butyl amine and **28** having an ethyl amine as the R group. They both had comparable IC50s for DPPII of 2.9 nM but **26** had lower IC50s for DPPIV, 8 and 9 than that of **28**. Furthermore, FAP and PREP activity was affected by **28** with IC50s in the low micro-molar range. **29** was a modified version of **28** in which a 2-chlorobenzyl group was attached to the primary amine on the R group. Interestingly, there was no significant change in potency for DPPII but selectivity fell noticeably. **29** had IC50s of 296 and 100 nM for DPPIV and 8 respectively. There was close to a 100-fold decrease in DPPIV IC50 from **28** to **29** and

almost a five-fold decrease for DPP8. FAP and PREP IC50 values rose to over 100 uM and 55 uM respectively for **29**. All the compounds in this group shared similar IC50s ranging from around 250 to 470 nM for the chymotrypsin-like activity of the proteasome. The caspase like- activity IC50s ranged from around 0.45 uM to 1.6 uM while the trypsin-like activities ranged from 1-2 uM. **29** was an exception in which the IC50 for trypsin-like activity was over 100 uM.

#	Amino Acid	Specificity Index							
		DASH Enzymes					Proteasome		
		DPPII	DPPIV	DPP8	DPP9	FAP	Chymotrypsin-like	Caspase-like	Trypsin-like
1	Gly	1	681	126	192	20	10	11	449
2	Ala	1	897	38	279	2,609	28	62	598
3	Etg	1	101	33	98	7,387	15	87	419
4	Nva	1	309	150	211	19,823	86	189	985
5	Nle	1	6,031	170	438	142,925	133	142,925	719
6	Met	1	8,535	284	546	230,610	484	1,426	7,051
7	SeMet	1	3,041	37	7,428	10,904	73	112,939	555
8	Hse (Me)	1	279	8	15	14,548	13	116	6767
9	Val	1	359	27	58	19,015	64	19,015	601
10	Leu	1	9,529	793	3,446	243,408	346	1,350	7,669
11	Ile	1	511	100	288	24,432	105	423	659
12	Tle	1	459	133	670	69,557	689	2,227	565
13	Chg	1	732	69	90	188,383	682	2,007	3,654
14	Cha	1	2,962	58	451	135,410	254	539	584,067
15	Phe	1	3,455	141	2,371	119,360	48	737	111,980
16	Tyr	1	442	114	2,081	188	15	203	576
17	Ala(1-naphthyl)	1	1,524	77	135	45,263	39	218	45,263
18	Trp	1	27,716	423	6,692	24,0173	181	646	1,559
19	His	1	206	29	125	1,298	26	78	378
20	Pro	1	460	56	63	3,027	2	6	47
21	Gly(adamantyl)	1	139	8	7	129,674	240	2,232	3,588
22	Asn	1	100	45	109	5,079	17	53	469
23	Gln	1	2,345	123	752	76,570	75	595	3,602
24	Ser	1	1,370	470	178	4,088	14	51	206
25	Thr	1	55	92	52	4,738	22	97	639
26	Lys	1	1,034	11	20	33,971	90	341	700
27	Arg	1	631	22	41	3,798	101	188	394
28	Dab	1	9,825	170	161	1,104	145	549	34,941

29	Ar	1	57	19	140	19,078	47	85	296
30	Glu	1	101	67	277	696	7	13	73
31	Asp	1	0.3	38	2	6	4	1	77
32	Aib	1	3,861	16	596	4,898	50	88	1,996
33	Etg(alpha-methyl)	1	593	5,891	317	12,720	176	453	12,720
34	Nva(alpha-methyl)	1	6,138	4,867	11,963	26,664	661	666	26,664
35	Pro(alpha-methyl)	1	10	543	15	543	10	3	24
36	Leu(alpha-methyl)	1	969	1,924	31,466	7,666	1756	1,713	20,458
37	Cle	1	612	950	130	10,405	513	986	10,405
38	Hcl	1	1,699	12,494	881	69,284	2,697	3,838	69,284
39	Gly(pip)	1	3,208	373	67	6,605	709	4,072	6,605

Table 3. **Specificity Index (SI) of DASH Enzymes and the Proteasome**. The specificity index was calculated in terms of DPPII and used to quantify how selective a compound is to DPPII vs other enzymes. The mean IC50 of each enzyme was divided by the mean IC50 of DPPII for each compound to attain the SI values.

3.4 Di-substituted α Carbon Amino Acids:

The last group of compounds consisted of P2 position amino acids with di-substituted α carbons (Tables 1, 2 and 3 compounds **32-29**). **32**, **33**, **34,** and **36** all had alkyl chain R groups along with a methyl group on the α carbon, and each of these compounds had a mono-substituted counterparts previously tested (**2**, **3**, **4** and **10** respectively). **32** results were very similar to **2**, with no significant changes in potency or selectivity for all the proteasome and DASH enzymes except for DPP8. DPP8 IC50 was reduced 4 fold. When related to their mono-substituted counterpart (**3** and **4**), **33** and **34** had 100 and 24 fold increase in DPP8 IC50 respectively. **34** had a 44 fold increase in DPP9 IC50 from **4** (44 uM vs 1 uM). DPPII IC50s did not significantly change from their respective mono-substituted versions. DPPIV IC50 increased around 14 times for **34** vs. **4**, while it decreased around 3 fold for **33** vs. **3**. The proteasome IC50s increased around 2 to 3 fold

for **33** and **34** when compared to their mono-substituted counterparts. **34** expressed the best overall selectivity for DPPII vs the other DASH enzymes.

10 was one of the most potent and selective compounds found for the mono-substituted α carbon amino acids, and the α, α di-substituted version (**36**) was synthesized for comparison. **36** lost some of the potency seen in **10**, with an IC50 of 1.4 nM vs 0.41 nM. DPPIV, PREP IC50s remained similar, while DPP8 and 9 IC50s increased 15 and 32 fold respectively. FAP IC50 decreased to low micro-molar. Proteasome IC50s increased around 18-fold, 4-fold and 9-fold for the chymotrypsin-, caspase-, and trypsin-like activity respectively. Altogether, though DPPII potency increased slightly, **36** presented all around increase in DPPII selectivity. **20** was one of the least selective compounds studied and **35** was synthesized to see if the selectivity could be improved with α, α di-substitution. Interestingly, DPPII potency decreased in which the IC50 was 184 nM vs 33 nM for **20**. Furthermore, DPPIV IC 50 decreased from 15 uM to 1.8 uM for **35**. There were 2-3 fold increases in IC50 for the different activities of the proteasome.

37, **38** and **39** all contained tertiary α carbons were the R group was cyclized and connected at the α carbon. They all had low nano-molar IC50s for DPPII with **38** being the most potent at 1.4 nM. **37** had cycloleucine as the R group and expressed decent selectivity for DPPII over the other DASH enzymes and the proteasome. **38** had one more carbon in the ring to form homocycloleucine, which expressed the best overall selectivity for DPPII over the proteasome. The last compound tested was **39** which was similar to **38** except the 4^{th} carbon on the ring from the α carbon was substituted with a nitrogen. The selectivity for DPPII decreased due to DPP8 IC50 decrease from around 5

uM from 18 uM for **39**. The proteasome IC50s for the chymotrypsin and caspase-like activities increased around 2 and 11 fold from **38** to compound **39**.

Discussion and Future Directions:

This study focused on the synthesis and structural activity relationship of 39 boronic acid analogues. The P1 position amino acid was boroNva and the P2 position was experimentally changed using naturally found amino acids as well as synthetic amino acids. It was determined that regardless of what P2 amino acid was used, the boroNva compounds were more potent for DPPII over the other DASH enzymes and the proteasome. **6** and **10** were the most potent inhibitiors for DPPII with IC50s of 0.43 and 0.41 nM respectively. Though potent, the boroNva compounds suffered from poor selectivity due to these compounds also having potency for DPP8, 9 and the proteasome. The selectivity for DPPII was found to increase when the α carbon on the P2 amino acid was di-substituted with either a methyl group or a converging cyclo-alkyl group.

Boronic acids are potent DASH inhibitors, as seen by Val-boroPro, the pan DPP inhibitor[6, 7]. This study highlighted the fact that other boronic acid analogues can be potent but more selective than Val-boroPro. One example of a previously studied boronic acid analogue was boro-norleucine (boroNle). Boro-Nle was tested for DPPII, IV, 8 and 9 inhibition by Shreder et al., and found that increasing the alkyl chain of the P2 amino acid increased DPPII potency where they got an IC50 of 18 nM for DPPII for Nle-boroNle[23]. Similarly, this study found that DPPII potency increases for boroNvas as well with increasing alkyl chains. Nle-boroNva (**5**) had an IC50 of 0.7 nM. When looking at the α, α di-substituted versions of alkyl chain R group amino acids, there was a visible decrease

in DPPII IC50 from **32** to **34**. It would be interesting to see how Nle(α-methyl)-boroNva would affect DPPII activity because it appears P2 α,α di-substitution may not increase DPPII potency or even may slightly decrease potency, but increases DPPII selectivity. This became apparent with Leu-boroNva (**10**) and Leu(α methyl)-boroNva (**36**), in which the IC50 was increased around 3 fold from subnano-molar to nano-molar IC50. On a similar note, the data from this study suggests that neutral R groups produce equal if not slightly better potency for DPPII inhibition versus charged groups like Dab or Lys as mentioned in previous studies[6, 22].

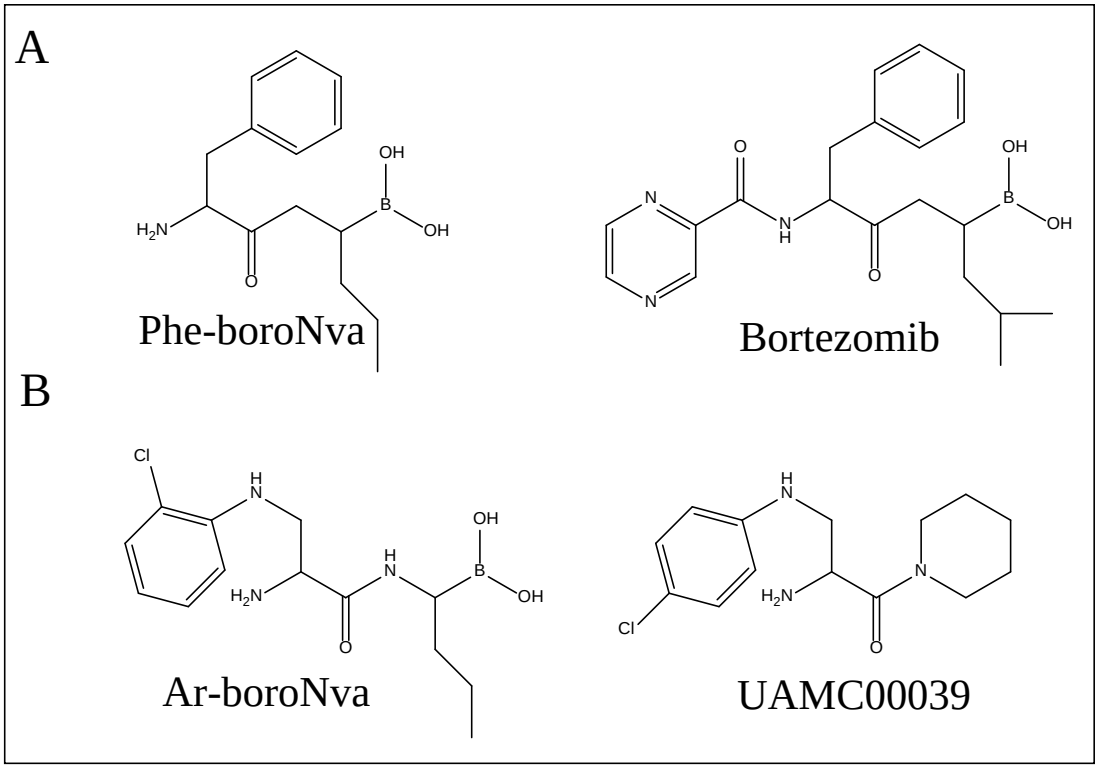

Figure 4. **Phe-boroNva (15) and Ar-boroNva (29) Structural Similarity to Other Well-Known Compounds**. A. Phe-boroNva has similar structure to the proteasome inhibitor bortezomib and may explain the potency Phe-boroNva and related compounds had for inhibiting the proteasome. B. Ar-boroNva shared similar R group structure to the DPPII inhibitor UAMC00039, though Ar-boroNva was not as potent or selective.

One of the weaknesses that became apparent for boroNva compounds was the potency for the proteasome. The IC50s would range in the upper and lower submicro-molar. It is most likely the structure of boroNva that creates this affinity for the proteasome. BoroNva is similar in length to that of boroleucine (boroLeu) with the only difference being that boroLeu has a branched methyl group on the 3rd carbon. This is important because boroLeu is the P1 amino acid position for the drug bortezimib, commercially known as Velcade. Bortezimib's structure is N-Pyrazinylcarbonyl-Phe-boroLeu and inhibits the chymotrypsin-like activity of the 20S proteasome[20]. When specifically looking at **15**, **16** and **17** (phe-, tyr-, ala(1-napthyl)-boroNva), it was clear that these compounds may have partially mimicked bortezimib (Figure 4A), as the IC50s for the chymotrypsin-like activity were 40, 45 and 86 nM respectively. It may be that the boroNva compounds structural similarity to boroLeu is what increases its potency for the proteasome. Studies on the proteasome for boroLeu and or boronic acid analogues with shorter P1 alkyl chains are not known and ValboroPro has been shown, in our lab, to not have any activity against the proteasome. It would be interesting to see if an α carbon di-substituted version of Phe-boroNva (**15**) could significantly reverse the compounds potency for the proteasome.

As mentioned before, UAMC00039 is the most potent and selective DPPII inhibitor available[15] and **29** is a boroNva analogue of UAMC00039 (Figure 4B). **29** was found to have an IC50 of 5.2 nM for DPPII and surprisingly had an IC50 of 296 nM for DPPIV. This is surprising because DPPIIV generally did not tolerate boroNvas well and thus had IC50s in the micro-molar range. **29** has an R group of ethyl amine with a 2-chorobenzyl attached to the amine. This differs slightly from the R group of UAMC00039 in which

the chlorine is on the 4th carbon of the benzene ring forming a 4-chlorobenzyl attached to ethylamine. **28** had an ethylamine with no chlorobenzyl group attached and had an IC50 of around 28 uM for DPPIV. **29** was expected to have similar DPPIV if not a higher IC50 related to **28**. Further experimentation will be needed to identify the change in IC50 for **29**. Not as surprising was the low selectivity of **29** for DPPII over DPP8, 9 and the chymotrypsin-like activity of the proteasome with SI of 19, 140 and 47 respectively. One of the most potent and selective DPP8 and 9 inhibitors right now is 1G244 with IC50s of 14 and 53 nM respectively. From studies with 1G244, it became apparent that DPP8 and 9 can tolerate larger P1 position moieties which made it easier to gain selectivity over DPPIV, which cannot. It appeared that boroNva was large enough to be tolerated more by DPP8 and 9 over DPPIV, and that this partially overlapped with what is tolerable for DPPII. In general, boroNva is still more potent for DPPII over DPP8 and 9, highlighting a difference in the P1 preferences for the enzymes.

Lastly, the data on di-substituted α carbon on the P2 amino acid has shed new light on P2 preferences for the DASH and proteasome enzymes. The α, α di-substitution is more tolerated by DPPII than the other DASH enzymes and the proteasome. This can allow for the discovery of more potent and selective boronic acid derivatives. DPPII structural homology is low to the other DASH enzymes mentioned in this study, and it is thought the α, α di-substituted P2 amino acid increases the rigidity of the compound backbone and this rigidness as well as the extra R group can only be tolerated by DPPII as opposed to the other more structurally related DASH enzymes. This is partially exemplified by one particular study by Coutts et al., who tested Aminoisobutyl (Aib)-boroPro inhibition on DPPIV and found that DPPIV inhibition was greatly reduced from that of Val-

boroPro[7]. This study also tested their compounds against DPPII but data for Aib-boroPro was either not produced or not shown.

The data from this study can help in the creation of potent and selective DPPII inhibitors that could potentially help clear up the somewhat contrasting theories on DPPII's role[16, 18] using pharmacological methods. Future work can focus on synthesizing other di-substituted α carbon amino acids with methyl groups or longer carbon chains. Furthermore, to test whether the DPPII S2 site is responsible for DPPII selectivity seen in these compounds, different boronic acid derivatives can also be tested, including boroProline or boroEthylglycine.

References:

1. Chen, S. J., and W. T. Jiaang. "Current Advances and Therapeutic Potential of Agents Targeting Dipeptidyl Peptidases-IV, -II, 8/9 and Fibroblast ActivatingProtein." Curr Top Med Chem 11.12 (2011): 1447-63. Print.
2. Chiravuri, M., et al. "Vesicular Localization and Characterization of a Novel Post-Proline-Cleaving Aminodipeptidase, Quiescent Cell Proline Dipeptidase." J Immunol 165.10 (2000): 5695-702. Print.
3. Chiravuri, M., and B. T. Huber. "Aminodipeptidase Inhibitor-Induced Cell Death in Quiescent Lymphocytes: A Review." Apoptosis 5.4 (2000): 319-22. Print.
4. Chiravuri, M., et al. "Homodimerization Via a Leucine Zipper Motif Is Required for Enzymatic Activity of Quiescent Cell Proline Dipeptidase." J Biol Chem 275.35 (2000): 26994-9. Print.
5. Chiravuri, M., et al. "A Novel Apoptotic Pathway in Quiescent Lymphocytes Identified by Inhibition of a Post-Proline Cleaving Aminodipeptidase: A Candidate Target Protease, Quiescent Cell Proline Dipeptidase." J Immunol 163.6 (1999): 3092-9. Print.
6. Connolly, B. A., et al. "Dipeptide Boronic Acid Inhibitors of Dipeptidyl Peptidase IV: Determinants of Potency and in Vivo Efficacy and Safety." J Med Chem 51.19 (2008): 6005-13. Print.

7. Coutts, S. J., et al. "Structure-Activity Relationships of Boronic Acid Inhibitors of Dipeptidyl Peptidase Iv. 1. Variation of the P2 Position of Xaa-Boropro Dipeptides." J Med Chem 39.10 (1996): 2087-94. Print.

8. Danilova, O., et al. "Synthesis and Activity of a Potent, Specific Azabicyclo[3.3.0]-Octane-Based Dpp II Inhibitor." Bioorg Med Chem Lett 17.2 (2007): 507-10. Print.

9. Danilova, O. V., et al. "Neurogenin 3-Specific Dipeptidyl Peptidase-2 Deficiency Causes Impaired Glucose Tolerance, Insulin Resistance, and Visceral Obesity." Endocrinology 150.12 (2009): 5240-8. Print.

10. Drucker, D. J., and M. A. Nauck. "The Incretin System: Glucagon-Like Peptide-1 Receptor Agonists and Dipeptidyl Peptidase-4 Inhibitors in Type 2 Diabetes." Lancet 368.9548 (2006): 1696-705. Print.

11. Hu, Y., et al. "Synthesis and Structure-Activity Relationship of N-Alkyl Gly-Boro-Pro Inhibitors of DPP4, FAP, and DPP7." Bioorg Med Chem Lett 15.19 (2005): 4239-42. Print.

12. Jiaang, W. T., et al. "Novel Isoindoline Compounds for Potent and Selective Inhibition of Prolyl Dipeptidase Dpp8." Bioorg Med Chem Lett 15.3 (2005): 687-91. Print.

13. Kozarich, J. W. "S28 Peptidases: Lessons from a Seemingly 'Dysfunctional' Family of Two." BMC Biol 8 (2010): 87. Print.

14. Leiting, B., et al. "Catalytic Properties and Inhibition of Proline-Specific Dipeptidyl Peptidases II, IV and VII." Biochem J 371.Pt 2 (2003): 525-32. Print.

15. Maes, M. B., et al. "In Vivo Effects of a Potent, Selective DPPII Inhibitor: UAMC00039 Is a Possible Tool for the Elucidation of the Physiological Function of DPPII." Adv Exp Med Biol 575 (2006): 73-85. Print.

16. Maes, M. B., et al. "Dipeptidyl Peptidase II and Leukocyte Cell Death." Biochem Pharmacol 72.1 (2006): 70-9. Print.

17. Maes, M. B., S. Scharpé, and I. De Meester. "Dipeptidyl Peptidase II (DPPII), a Review." Clin Chim Acta 380.1-2 (2007): 31-49. Print.

18. Mele, D. A., J. F. Sampson, and B. T. Huber. "Th17 Differentiation Is the Default Program for Dpp2-Deficient T-Cell Differentiation." Eur J Immunol 41.6 (2011): 1583-93. Print.

19. Mentlein, R., and G. Struckhoff. "Purification of Two Dipeptidyl Aminopeptidases II from Rat Brain and Their Action on Proline-Containing Neuropeptides." J Neurochem 52.4 (1989): 1284-93. Print.

20. Mujtaba, Taskeen, and Q. Ping Dou. “Advances in the Understanding of Mechanisms and Therapeutic Use of Bortezomib.” Discovery medicine 12.67 (2011): 471–480. Print.

21. Rosenblum, J. S., and J. W. Kozarich. "Prolyl Peptidases: A Serine Protease Subfamily with High Potential for Drug Discovery." Curr Opin Chem Biol 7.4 (2003): 496-504. Print.

22. Senten, K., et al. "Development of Potent and Selective Dipeptidyl Peptidase II Inhibitors." Bioorg Med Chem Lett 12.20 (2002): 2825-8. Print.

23. Shreder, K. R., et al. "Boro-Norleucine as a P1 Residue for the Design of Selective and Potent Dpp7 Inhibitors." Bioorg Med Chem Lett 15.19 (2005): 4256-60. Print.

24. Soisson, S. M., et al. "Structural Definition and Substrate Specificity of the S28 Protease Family: The Crystal Structure of Human Prolylcarboxypeptidase." BMC Struct Biol 10 (2010): 16. Print.