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Structure–activity relationship of boronic acid derivatives of tyropeptin: Proteasome inhibitors

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ABSTRACT

The structure–activity relationship of the boronic acid derivatives of tyropeptin, a proteasome inhibitor, was studied. Based on the structure of a previously reported boronate analog of tyropeptin (**2**), 41 derivatives, which have varying substructure at the N-terminal acyl moiety and P2 position, were synthesized. Among them, 3-phenoxyphenylacetamide **6** and 3-fluoro picolinamide **22** displayed the most potent inhibitory activity toward chymotryptic activity of proteasome and cytotoxicity, respectively. The replacement of the isopropyl group in the P2 side chain to H or Me had negligible effects on the biological activities examined in this study.

Proteasome, a multicatalytic threonine protease, is responsible for ubiquitin-dependent nonlysosomal proteolysis.[1] This enzyme has three distinct active sites that are individually responsible for the chymotrypsin-like, caspase-like, and trypsin-like proteolytic activities.[2] Among these, the chymotrypsin-like activity is of greatest interest, and much research in medicinal chemistry has been focused on it.[3,4]

Elevated levels of the proteasome have been implicated in many diseases including cancer. In fact, it has been reported that the anti-cancer activity of proteasome inhibitors is due to inhibition of the transcriptional factor NF-κB;[5,6] stabilization of p21, p27, and p53;[7,8] and suppression of the unfolded protein response (UPR).[9] Indeed, proteasome inhibitors have been recognized as promising candidates for anti-cancer agent,[10,11] since the US Food and Drug Administration approved the first clinical use of a compound from this class, bortezomib **3** (also referred to as PS-341, Velcade®), for the treatment of multiple myeloma.

Previously, we reported the isolation and structural determination of the novel proteasome inhibitors tyropeptins A (**1**) produced by *Kitasatospora sp.* MK993-dF2,[12,13] and structure–activity relationship (SAR) studies of tyropeptin derivatives.[14,15] In these studies, tyropeptin-boronic acid derivatives (**2** as a representative) were found to exhibit enhanced inhibitory activity against chymotrypsin-like activity of human proteasome when compared to tyropeptin A (Fig. 1).

Encouraged by these results, we conducted further SAR studies of tyropeptin-boronic acid derivatives. In the present study, the effect of acyl moiety located at the N-terminus on the proteasome-inhibitory activity and cytotoxicity against RPMI8226 cells derived from multiple myeloma was investigated. Proteasome-inhibitory activities were determined using purified human erythrocyte-derived 20S proteasome (Enzo Life Sciences. Plymouth Meeting, PA) as previously described.[13]

Scheme 1 summarizes the procedure for synthesizing the tyropeptin-boronic acid derivatives used in this study. According to the method reported previously,[15] 41 analogs of **2** that have a variety of acyl groups at the N-terminus were prepared using WSC·HCl as a coupling reagent with corresponding carboxylic acids and the peptide boronate **4**.[16]

Table 1 shows the inhibitory activity toward proteasome and the cytotoxicity of tyropeptin-boronic acid derivatives synthesized for this study. The almost identical biological activities were observed for the previously reported 1-naphtylacetyl derivative **2** and its regioisomer **5**. The most potent inhibitor of chymotrypsin-like activity was analog **6**, which has a 3-phenoxyphenylacetyl group at the N-terminal acyl moiety; almost ninefold more potent than bortezomib **3** (IC_{50}: 0.0041 for **6** and 0.039 μM for **3**). Unfortunately these compounds showed weak antitumor activity,[17] which prompted us to use different acyl groups. Instead, we chose

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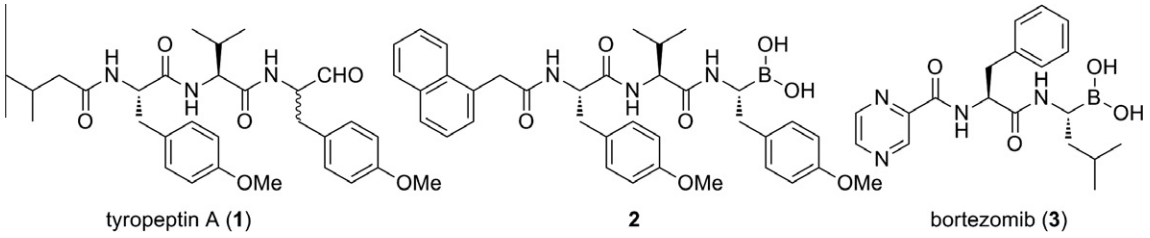
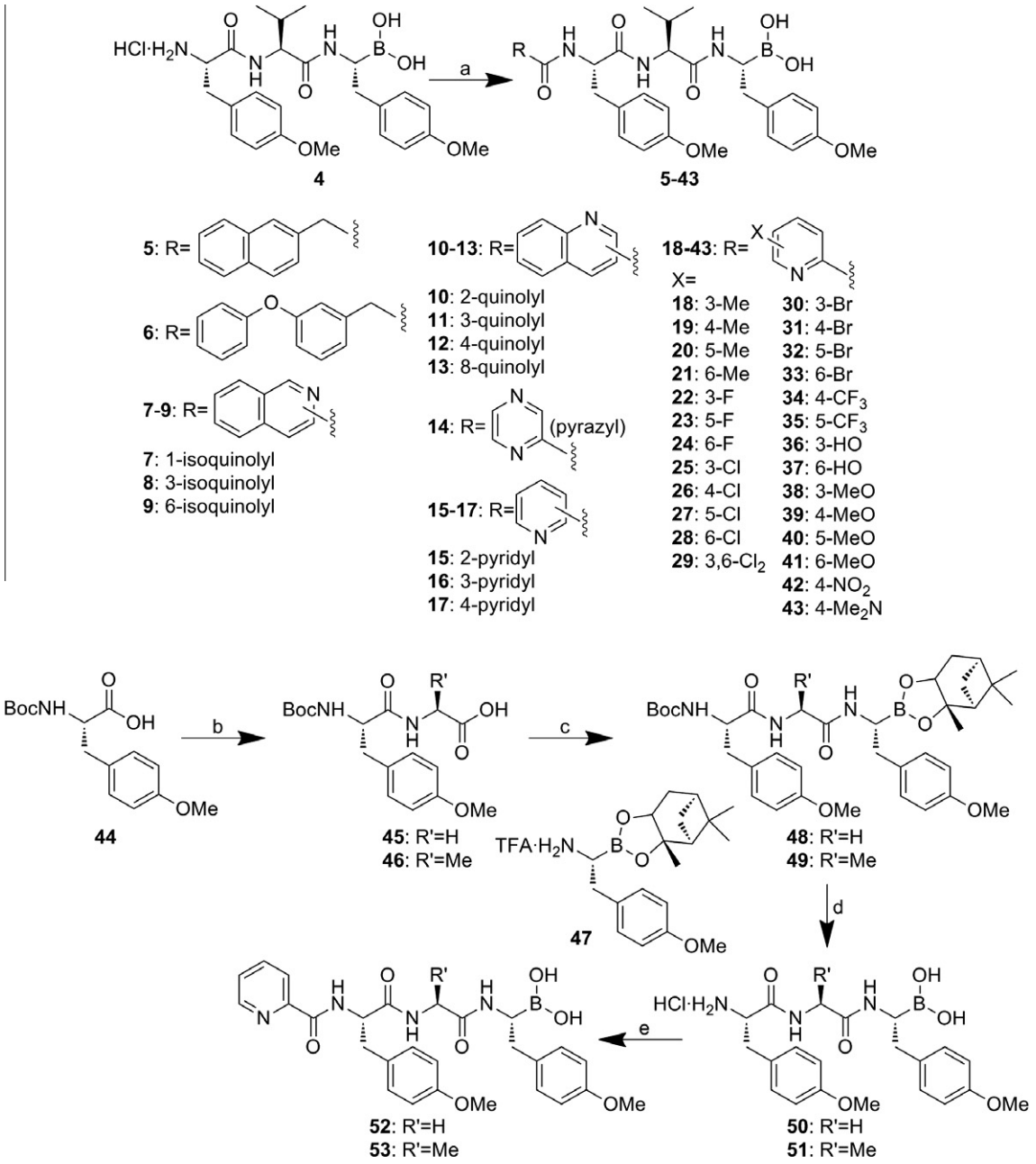


Figure 1. Structure of tyropeptin A (**1**), a tyropeptin-boronic acid derivative (**2**), and bortezomib (**3**).



Scheme 1. Reagents and conditions: (a) carboxylic acid, WSC·HCl, HOBt, iPr$_2$NEt, CH_2Cl_2; (b) (i) H-Gly-OBn or H-Ala-OBn, WSC·HCl, HOBt, iPr$_2$NEt, CH_2Cl_2; (ii) H_2, Pd/C, MeOH; (c) **47**, WSC·HCl, HOBt, iPr$_2$NEt, CH_2Cl_2; (d) (i) TFA, $CHCl_3$; (ii) iBuB(OH)$_2$, 1 M HCl, hexane; (e) 2-picolinic acid, WSC·HCl, HOBt, iPr$_2$NEt, CH_2Cl_2.

various N-heteroaromatic rings because bortezomib **3** has a pyrazine carboxamide moiety.

First, amide derivatives of commercially available carboxylic acids having quinoline, isoquinoline, pyrazine, and pyridine nuclei

Table 1
Biological activities of tyropeptin-boronic acid derivatives, and bortezomib (IC_{50}: μM)

Compounds	Chymotrypsin-like activity	Caspase-like activity	Trypsin-like activity	Cytotoxicity (RPMI8226)
5	0.022	39	12	0.17
6	0.0041	29	1.1	0.19
7	0.041	19	10	0.034
8	0.059	11	9	0.093
9	0.38	>40	>40	0.26
10	0.10	16	5.4	0.073
11	0.056	32	10	0.054
12	0.049	24	8.6	0.049
13	0.093	16	18	0.056
14	0.24	33	19	0.017
15	0.23	23	40	0.013
16	0.50	>40	>40	0.87
17	2.3	>40	>40	0.87
18	0.085	>40	20	0.014
19	0.14	30	20	0.014
20	0.12	30	14	0.014
21	0.088	30	17	0.014
22	0.14	25	24	0.0049
23	0.081	30	14	0.019
24	0.11	27	20	0.015
25	0.083	20	20	0.0097
26	0.088	21	15	0.039
27	0.083	16	15	0.039
28	0.10	23	10	0.029
29	0.053	26	13	0.014
30	0.061	20	17	0.013
31	0.095	17	14	0.047
32	0.093	24	14	0.046
33	0.092	27	14	0.044
34	0.15	25	20	0.053
35	0.11	28	19	0.047
36	0.39	29	>40	0.052
37	0.24	>40	11	0.34
38	0.13	26	31	0.041
39	0.087	20	16	0.013
40	0.094	21	14	0.013
41	0.059	35	21	0.051
42	0.19	34	19	0.048
43	0.11	21	15	0.044
52	0.26	34	>40	0.024
53	0.11	>40	>40	0.057
2	0.019	39	>40	0.028
3	0.039	0.75	>40	0.0088

without any substituents were prepared (**7**–**17**). Except for compound **10**, the quinoline and isoquinoline derivatives inhibited the chymotrypsin-like activity of proteasome more effectively than the pyrazine and pyridine congeners. It is noteworthy that inhibition of proteasome did not necessarily correlate with the cytotoxicity. Indeed, the most potent cytotoxicity, comparable to that of bortezomib, was observed for picolinic acid amide **15**, albeit a modest inhibitory activity against proteasome. Because the analog **15** displayed an antitumor activity in a preliminary experiment,[17] further SAR studies were performed starting with this analog to clarify the effects of substituents on the pyridine ring. To this end, various picolinic groups installed with one (or two in the case of 3,6-dichloroderivative **29**) functional group were introduced at the N-terminus (**18**–**43**): Me, F, Cl, Br, CF_3, OH, OMe, NO_2, or NMe_2 derivatives.

In most cases, when tested against the chymotrypsin-like activity of proteasome, analogs with additional substituents showed IC_{50} values lower than that of **15** (0.23 μM) except for hydroxylated derivatives **36** and **37** (IC_{50}: 0.39 and 0.24 μM, respectively). In particular, the 3,6-Cl_2 (**29**), 3-Br (**30**), and 6-Me (**41**) derivatives showed comparable potency (IC_{50}: 0.053, 0.061, and 0.059 μM, respectively) to that of bortezomib **3**.

Substantial loss of cytotoxicity toward RPMI8226 was not observed for the compounds of this class. Notably, 3-F derivative **22** displayed one of the most potent cytotoxicities against RPMI8226 among the tyropeptin-related compounds synthesized in our laboratory (IC_{50}: 0.0049 μM). Here again, the potency of the inhibitory activity toward proteasome and cytotoxicity did not coincide with each other. In fact, **22** showed only a moderate activity toward proteasome (IC_{50}: 0.14 μM).

Other than the deleterious effect of an OH group on the inhibition of chymotryptic activity, no obvious relationship was observed between the biological activities examined in this study and the structure of the pyridyl moiety.

In addition, a preliminary study to evaluate the effect of the P2 side chain was conducted. Based on the structure of **15**, two analogs, in which the P2 valine was replaced with either glycine (**52**) or alanine (**53**), were prepared using a procedure that was analogous to the synthesis of the above-mentioned tyropeptin derivatives. As a result, removal of all or part of the P2 side chain of **15** did not influence the biological activity tested in this study.[16]

In summary, boronic acid derivatives of tyropeptin were synthesized and tested for proteasome-inhibitory activity and cytotoxicity against RPMI8226 in this study. The most potent compounds found were 3-phenoxyphenylaceamide **6** (for proteasome-inhibitory activity) and 3-fluoropicolinamide **22** (for cytotoxicity). The structural change in P2 did not affect the in vitro activities tested in this study. In order to clarify whether the structural change of P2 side chain can alter the physicochemical properties of analogs without affecting the biological activities, a SAR study on this moiety is currently under way. Moreover, full details of the antitumor activities of these compounds will be also reported in due course.

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16. All the new compounds showed satisfactory analytical data. The representatives are listed below: **6**: a white powder: mp 102–105 °C; $[\alpha]_D^{20}$ −36.2 (*c* 0.150, $CHCl_3$); IR (KBr) ν_{max} 3294, 1643, 1512, 1250, 1034 cm^{-1}; ^1H NMR (CD_3OD, 600 MHz); δ 7.32 (2H, m), 7.20 (1H, t, *J* = 7.9 Hz), 7.12 (2H, d, *J* = 8.6 Hz), 7.08 (1H, m), 7.06 (2H, d, *J* = 8.6 Hz), 6.94 (2H, d, *J* = 7.9 Hz), 6.87–6.80 (5H, m), 6.75 (2H, d, *J* = 8.6 Hz), 4.62 (1H, m), 4.31 (1H, d, *J* = 7.6 Hz), 3.73 (3H, s), 3.71 (3H, s), 3.40 (2H, d, *J* = 14.4 Hz), 3.02 (1H, dd, *J* = 14.1, 5.1 Hz), 2.82–2.74 (2H, m), 2.51 (1H, dd, *J* = 14.1, 10.0 Hz), 2.07 (1H, m), 0.95–0.92 (6H, m); ^{13}C NMR (CD_3OD, 150 MHz); δ 177.8, 173.8, 173.5, 160.0, 159.7, 158.8,

158.7, 138.6, 133.8, 131.3, 130.91, 130.88, 130.0, 125.1, 124.4, 120.7, 119.9, 118.2, 115.0, 114.9, 56.5, 55.8, 55.70, 55.65, 43.4, 37.8, 37.3, 31.9, 19.4, 18.8; HRMS (ESI-Orbitrap) m/z calcd for $C_{38}H_{44}BN_3NaO_8^+$ [(M+Na)$^+$] 704.3114, found: 704.3096. Compound **15**: a white powder: mp 123–125 °C; $[\alpha]_D^{20}$ −48.0 (c 0.260, $CHCl_3$); IR (KBr) ν_{max} 3305, 1658, 1512, 1246, 1176, 1034 cm^{-1}; ^{1}H NMR (CD_3OD, 600 MHz); δ 8.60 (1H, m), 8.00 (1H, m), 7.92 (1H, dt, J = 7.7, 1.8 Hz), 7.53 (1H, m), 7.16–7.13 (4H, m), 6.85 (2H, d, J = 8.7 Hz), 6.78 (2H, d, J = 8.7 Hz), 4.36 (1H, d, J = 8.0 Hz), 3.74 (3H, s), 3.71 (3H, s), 3.16 (1H, dd, J = 13.9, 5.2 Hz), 2.98 (1H, dd, J = 13.9, 8.5 Hz), 2.90 (1H, m), 2.84 (1H, dd, J = 14.2, 5.5 Hz), 2.53 (1H, dd, J = 14.2, 10.0 Hz), 2.13 (1H, m), 1.00 (3H, d, J = 6.2 Hz), 0.99 (3H, d, J = 6.2 Hz); ^{13}C NMR (CD_3OD, 150 MHz); δ 177.6, 173.6, 166.1, 160.1, 159.7, 150.4, 149.8, 138.8, 133.8, 131.4, 130.8, 129.7, 128.0, 123.1, 115.0, 114.9, 56.6, 55.9, 55.7, 55.6, 38.4, 37.2, 31.9, 19.4, 18.9; HRMS (ESI-TOF) m/z calcd for $C_{30}H_{37}BN_4NaO_7^+$ [(M+Na)$^+$] 599.2648, found: 599.2639. Compound **22**: a white powder: mp 126–128 °C; $[\alpha]_D^{20}$ −39.8 (c 0.215, $CHCl_3$); IR (KBr) ν_{max} 3305, 1658, 1512, 1246, 1176, 1034 cm^{-1}; ^{1}H NMR (CD_3OD, 600 MHz); δ 8.60 (1H, m), 7.74–7.70 (1H, m), 7.64–7.61 (1H, m), 7.17 (2H, d, J = 8.6 Hz), 7.09 (2H, d, J = 8.6 Hz), 6.83 (2H, d, J = 8.6 Hz), 6.71 (2H, d, J = 8.6 Hz), 4.36 (1H, d, J = 7.2 Hz), 3.74 (3H, s), 3.72 (3H, s), 3.10–3.03 (2H, m), 2.84 (1H, dd, J = 8.9, 6.2 Hz), 2.76 (1H, dd, J = 14.2, 6.2 Hz), 2.54 (1H, dd, J = 14.2, 8.9 Hz), 2.07 (1H, m), 2.13 (1H, m), 0.84 (3H, d, J = 6.5 Hz), 0.81 (3H, d, J = 6.5 Hz); ^{13}C NMR (CD_3OD, 150 MHz); δ 178.0, 173.7, 164.3 (d, J = 5.7 Hz), 160.5 (d, J = 268.6 Hz), 160.3, 159.6, 145.9 (d, J = 5.7 Hz), 138.6 (d, J = 5.7 Hz), 133.9, 131.5, 130.9, 130.1 (d, J = 5.7 Hz), 129.6, 127.5 (d, J = 20.1 Hz), 115.1, 114.9, 56.9, 56.2, 55.71, 55.69, 38.7, 37.0, 31.1, 19.4, 18.6; HRMS (ESI-Orbitrap) m/z calcd for $C_{30}H_{36}BFN_4NaO_7^+$ [(M+Na)$^+$] 617.2553, found: 617.2568.

17. Manuscript in preparation.