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Structurally Novel Highly Potent Proteasome Inhibitors Created by the Structure-Based Hybridization of Nonpeptidic Belactosin Derivatives and Peptide Boronates

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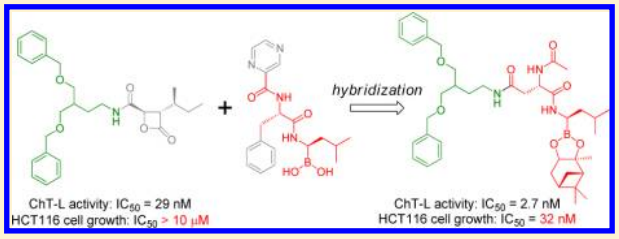
Supporting Information

ABSTRACT: We previously developed highly potent proteasome inhibitor **1** (IC_{50} = 5.7 nM) and its nonpeptide derivative **2** (IC_{50} = 29 nM) by systematic structure–activity relationship studies of the peptidic natural product belactosin A and subsequent rational topology-based scaffold hopping, respectively. Their cell growth inhibitory activities, however, were only moderate (IC_{50} = 1.8 μM (**1**) and >10 μM (**2**)). We therefore planned to replace the unstable β-lactone warhead with a more stable boronic acid warhead. Importantly, belactosin derivatives bind mainly to the proteasome binding site, which is different from that occupied by known peptide boronate proteasome inhibitors such as bortezomib, suggesting that their hybridization might lead to the development of novel potent inhibitors. Here we describe design, synthesis, and biological activities of the newly developed potent hybrid proteasome inhibitors. Interestingly, these hybrids, unlike bortezomib, were highly selective for proteasomes and have long residence times despite having the same boronic acid warhead.



INTRODUCTION

The ubiquitin–proteasome system is the major pathway for systematic degradation of intracellular proteins[1] and is involved in many physiologically important cellular processes, including signal transduction,[2] the immune response,[3] the unfolded protein response,[4] and cell cycle progression.[5] The 20S proteasome, the catalytic core particle of proteasome, is an attractive target molecule for anticancer and antiautoimmune drugs because proteasome inhibition causes cell cycle arrest and induces apoptosis.[6] In fact, bortezomib (Figure 1), a dipeptide boronic acid proteasome inhibitor, is a currently available prescription drug against various cancer types and represents one of the best known blockbuster drugs.[7]

The eukaryotic 20S proteasome contains three active β subunits β1, β2, and β5, which are responsible for the caspase-like (C-L), trypsin-like (T-L), and chymotrypsin-like (ChT-L) activities, respectively,[8] whereas vertebrates possess an even more elaborate version of proteasomes[9] that harbor three different types of the 20S proteasome: constitutive proteasomes, immunoproteasomes, and thymoproteasomes. Each of these proteasome types has its own set of active sites:[10] the constitutive proteasome incorporates subunits β1c, β2c, and β5c, the immunoproteasome incorporates subunits β1i (LMP2), β2i (MECL-1), and β5i (LMP7), and the thymoproteasome incorporates subunits β1i, β2i, and β5t. The mechanism of peptide bond cleavage follows a universal principle among all active sites, which is nucleophilic attack of the *N*-terminal Thr hydroxyl group, but it is the singularity of each substrate binding channel that determines the chemical nature of the specificity (S) pockets and accommodates the ligand's side chains (P sites) with respect to their amino acid progression. Interestingly, translation of the immunoproteasome is induced by interferon-γ to be primarily expressed in hematopoietic cells,[11] whereas constitutive proteasome is ubiquitously expressed in all kinds of cells. Thus, blocking either the constitutive proteasome or immunoproteasome has a major impact on specific biological potencies.[3,6a]

Belactosin A is a naturally occurring tripeptide metabolite produced by *Streptomyces* sp., that comprises L-alanine, 3-(*trans*-2-aminocyclopropyl)-L-alanine, and a chiral carboxy-β-lactone moiety (Figure 1)[12] and inhibits proteasome ChT-L activity[13] by acylating the active site Thr residue via its strained β-lactone-opening, as confirmed by X-ray crystallographic analysis of belactosin derivatives in complex with proteasome.[14] Importantly, belactosin A and its derivatives are the only known proteasome inhibitors that bind to the primed substrate binding site of the proteasome as well as the nonprimed binding site[14,15] so that it is an attractive lead compound for developing

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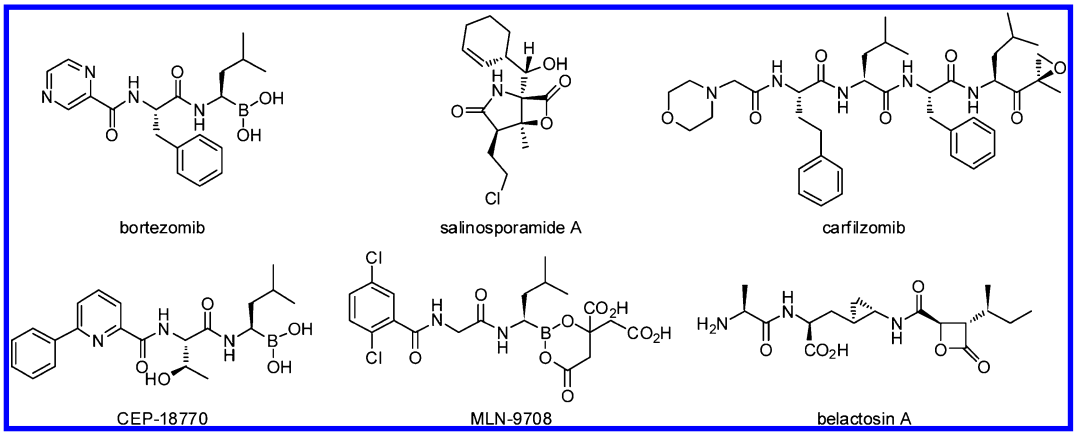

Figure 1. Known proteasome inhibitors.

proteasome inhibitors that are unique from the currently known inhibitors.

We performed systematic structure−activity relationship (SAR) studies of belactosin A to develop a highly potent proteasome inhibtor **1**.[14c,16] On the basis of the results of our SAR and the binding mode studies,[17] we identified a remarkably simplified nonpeptide inhibitor **2** by the topology-based scaffold hopping of **1**.[18] Although **2** had remarkably high proteasome inhibitory activity (IC_{50} = 29 nM), its cell growth inhibitory effect was insignificant (IC_{50} > 10 μM), perhaps due to the instability of the β-lactone warhead under biological condition.[19] To develop stabilized analogues of belactosin A, we previously designed and synthesized a series of belactosin analogues in which the β-lactone moiety was replaced with a β-lactam[17] or more sterically hindered β-lactones[19] as a warhead, but this kind of modification significantly impaired proteasome inhibitory activity (Figure 2).[20] Therefore, we planned to develop more potent cell growth inhibitors by replacing the β-lactone warhead of nonpeptide inhibitor **2** with another chemotype of stable warhead.

Peptide boronates are the most valuable class of proteasome inhibitors as represented by the clinically useful drug bortezomib and drug candidates CEP-18770[21] and MLN-9708[22] (Figure 1), which are currently undergoing clinical trials. This class of inhibitors binds only to the nonprimed binding site of proteasome[23] and exhibits remarkable cell growth inhibitory effects, in which the boronic acid moiety functions as a very effective warhead.[21,22,24] Therefore, we thought that replacing the β-lactone moiety of the nonpeptide belactosin A derivatives with a boronic acid warhead might allow us to identify the structurally novel proteasome inhibitors with potent cell growth inhibitory effects.

On the basis of the above-mentioned results and considerations, we designed and synthesized a series of hybrids of the nonpeptide belactosin A derivatives and peptide boronates and investigated various biological properties of these compounds such as their inhibitory effects on constitutive 20S proteasome subunits and immunoproteasome, cell growth inhibitory effects, and the reversibility of their proteasome inhibition. The most potent compound **4a** identified in the present study has inhibitory effect on ChT-L activity that is as strong as or even stronger than that of bortezomib, and the inhibitory effects against proteolytic enzymes are highly selective to proteasome. Interestingly, **4a** has a long residence time compared to bortezomib despite having the same boronic acid warhead as bortezomib. Here we report our results in detail.

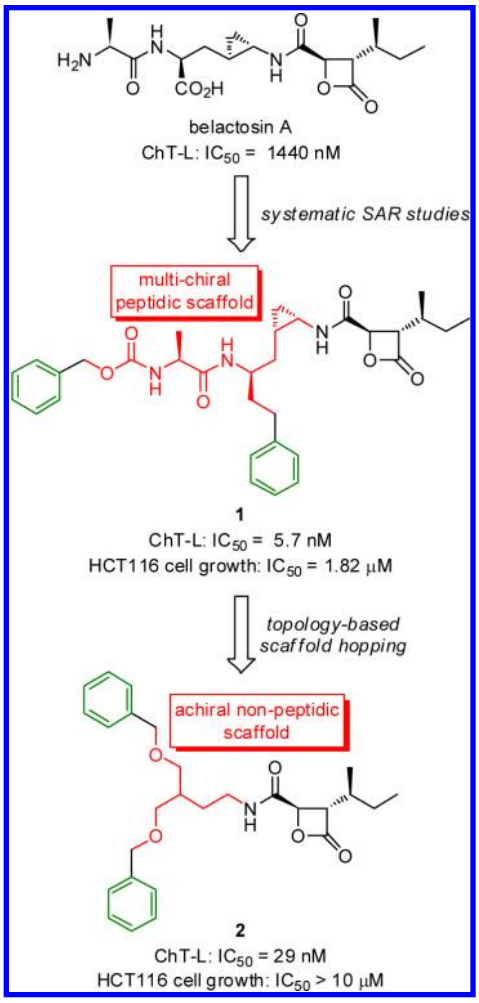

Figure 2. Belactosin A derivatives previously developed by us.

RESULTS AND DISCUSSION

Design of Compounds. The two X-ray crystal structures of a proteasome in complex with bortezomib[23] or belactosin A derivative **1** are superimposed in Figure 3,[14c] which clearly shows that bortezomib binds to the nonprimed binding site with its P2 side chain oriented toward the vacant primed binding site and that **1** binds mainly to the primed binding site.

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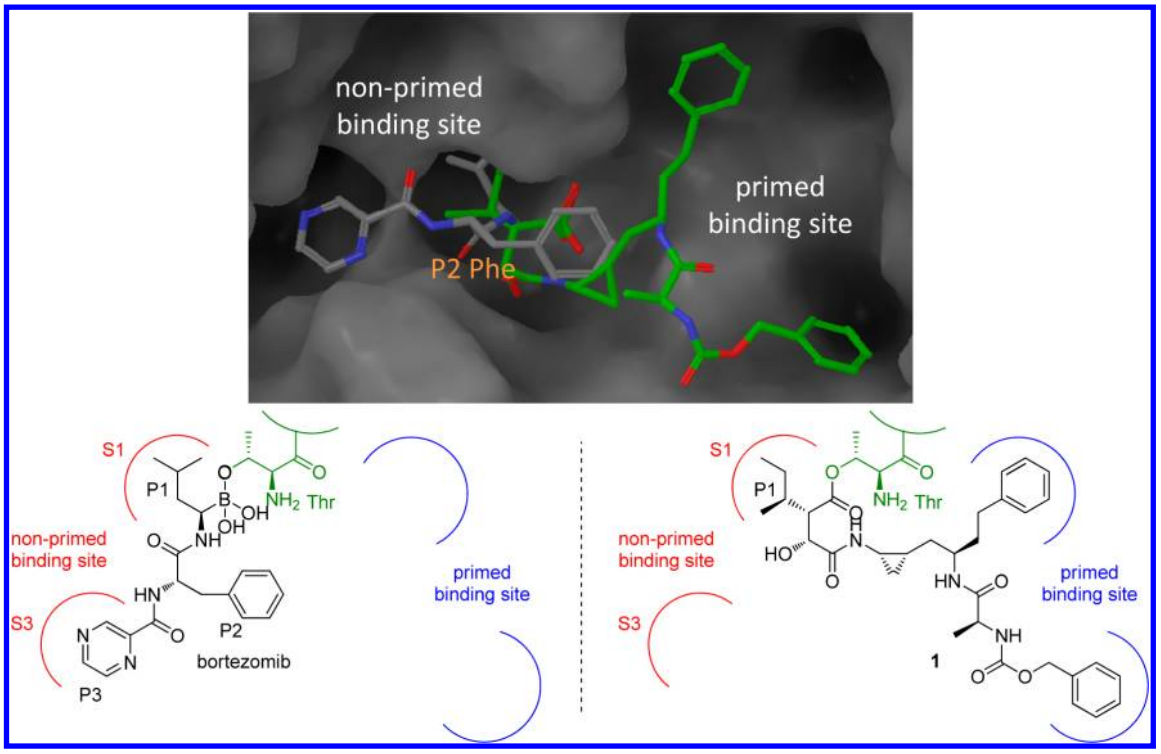

Figure 3. Superposition of the X-ray crystal structures of bortezomib[23] (gray tube; PDB code 2F16) and belactosin A derivative **1**[14c] (green tube; PDB code 4J70) in complex with proteasome.

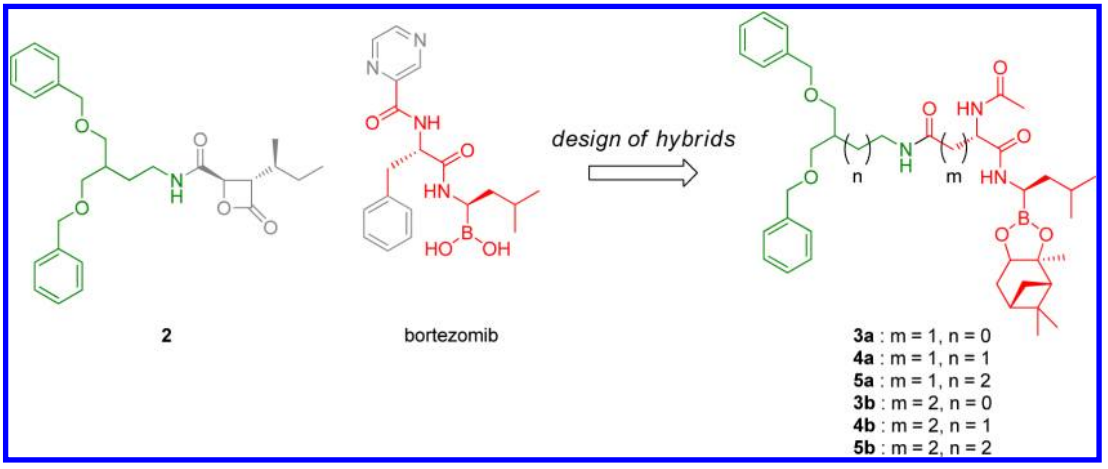

Figure 4. Design of hybrid inhibitors of nonpeptide belactosin A derivatives and peptide boronates.

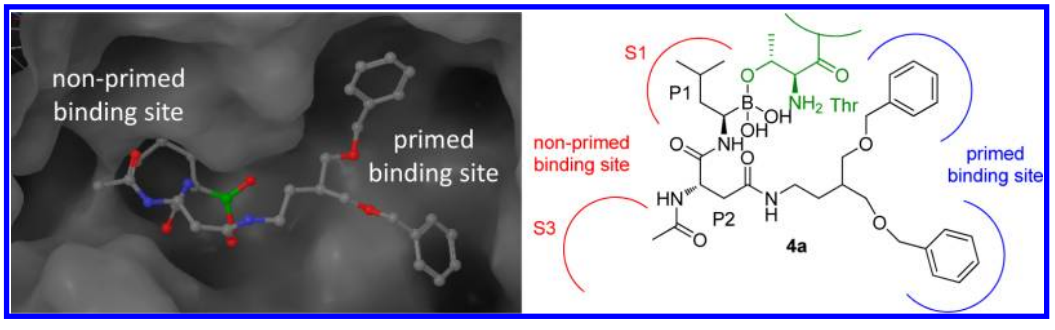

Figure 5. Predicted binding mode of **4a** by docking simulation.

Therefore, we assumed that elongation of the P2 side chain of bortezomib toward the primed binding site would allow us to develop novel hybrid inhibitors of peptide boronates and belactosin A derivatives. As mentioned above, we previously developed nonpeptide derivative **2** by topology-based scaffold hopping of **1**,[18] and therefore we decided to take advantage of **2**

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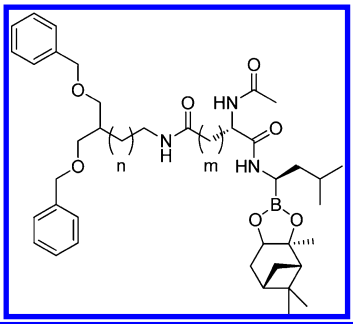
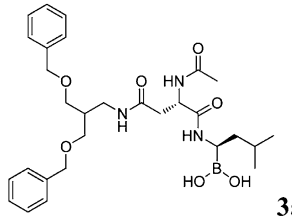
Table 1. Inhibitory Effects of Compounds 3a−5a, 3b−5b, and Bortezomib on Human 20S Proteasome and HCT116 Cell Growth



compound number	m	n	IC_{50} [nM][a]			
			ChT-L activity	C-L activity	T-L activity	HCT116 cell growth
3a		0	3.9 ± 2.2	140 ± 11	1100 ± 130	45.4
4a	1 (Asp)	1	2.7 ± 1.0	150 ± 24	2700 ± 330	31.8
5a		2	4.1 ± 1.1	120 ± 23	2700 ± 720	43.0
3b		0	5.1 ± 1.8	75 ± 8.7	2400 ± 880	63.6
4b	2 (Glu)	1	5.7 ± 3.6	76 ± 6.0	2500 ± 1700	110.3
5b		2	4.1 ± 2.8	46 ± 3.9	3000 ± 610	67.2
 **3a'**			7.7 ± 2.2			79.6
bortezomib			7.0 ± 0.5	120 ± 10	2000 ± 790	5.0

[a]Based on three experiments.

for designing hybrid compounds to reduce the number of peptide bonds. Thus, we designed hybrid compounds **3a−5a** and **3b−5b**, in which the moiety derived from the nonpeptidic scaffold of **2** with two aromatic rings was ligated with the P2 side chain via an amide linkage (Figure 4).[25,26] To search for the precise three-dimensional positioning of the two aromatic rings and the boronic acid warhead for effective proteasome binding in these hybrid compounds, we designed compounds with Asp (m = 1) or Glu (m = 2) P2 residues and different lengths of alkyl linkers (n = 0−2).

To justify the molecular design described above, we performed docking simulation of the designed hybrids **3a−5a** and **3b−5b** by following the previously described docking procedure.[17] As an example, the predicted binding mode of **4a** is shown in Figure 5. In the simulated binding mode, the moiety originating from the nonpeptidic belactosin A derivative is accommodated in the primed binding site and the moiety originating from the bortezomib is accommodated in the nonprimed binding site, respectively, as we expected. We therefore synthesized these newly designed hybrid molecules and evaluated their biological effects.

SAR Analysis. The inhibitory effects of synthesized compounds **3a−5a** and **3b−5b** and bortezomib on the ChT-L, C-L, and T-L activities of human 20S proteasomes were measured using the chromophoric substrates Suc-LLVY-AMC, Ac-*n*LP*n*LD-AMC, and Ac-RLR-AMC, respectively, and the results are summarized in Table 1. All of these compounds strongly inhibited proteasome ChT-L activity, with IC_{50} values ranging from 2.7 to 5.7 nM, which are lower than that of bortezomib (IC_{50} = 7.0 nM). The P2 Asp-type (m = 1) compounds **3a−5a** showed subunit selectivity similar to that of bortezomib, with IC_{50} values ranging from 120 to 150 nM for C-L and 1100 to 2700 nM for T-L activities. The inhibitory potency of the P2 Glu-type (m = 2) compounds **3b−5b** on T-L activity (IC_{50} = 2400−3000 nM) was similar to that of the Asp-type compounds, but these compounds were more potent inhibitors of C-L activity (IC_{50} = 46−76 nM) than the Asp-type compounds, suggesting that Glu was more favored than Asp as the P2 residue to be accommodated in the binding site of the β1 subunit. In these compounds, the linker length (n = 0−2) had only a small impact on the proteasome inhibitory activities, suggesting that the flexible nature of the linker moieties permits the insertion of the two aromatic groups into primed binding site regardless of their length.

We also evaluated cell growth inhibitory effects of the compounds on HCT116 cells. As summarized in Table 1, all of these compounds effectively inhibited cell growth. Interestingly, the P2 Asp-type compounds **3a−5a** were more potent cell growth inhibitors (IC_{50} = 32−45 nM) than the P2 Glu-type compounds **3b−5b** (IC_{50} = 64−110 nM).

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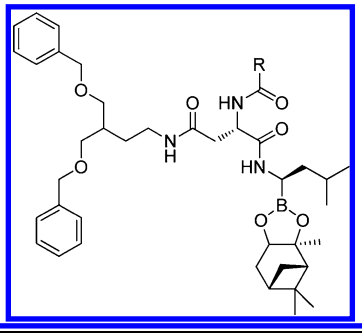
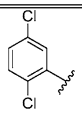
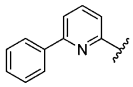
Table 2. Inhibitory Effects of Compounds 6a−9a on Human 20S Proteasome and HCT116 Cell Growth



compound number	R	IC_{50} [nM][a]			
		ChT-L activity	C-L activity	T-L activity	HCT116 cell growth
6a		5.9 ± 0.5	250 ± 140	> 10000	140
7a		7.8 ± 2.6	130 ± 6.2	2700 ± 380	60.0
8a		7.1 ± 4.2	210 ± 73	3300 ± 600	45.0
9a		7.5 ± 0.9	1000 ± 890	> 10000	260
4a		2.7 ± 1.0	150 ± 24	2700 ± 330	31.8
bortezomib		7.0 ± 0.5	120 ± 10	2000 ± 790	5.0

[a]Based on three experiments.

Furthermore, we evaluated proteasome and cell growth inhibitory activities of **3a**′, which is a boronic acid congener of **3a**. As shown in Table 1, the inhibitory activities of **3a**′ (ChT-L activity, IC_{50} = 9.1 nM; HCT116 cell growth, IC_{50} = 80 nM) were comparable to those of **3a**, suggesting that the pinanediol ester works as a promoiety and does not have significant effect on the activities, in accord with the results reported previously.[26]

On the basis of the above-mentioned results, we next investigated the SAR at the P3 site with compounds **6a**−**9a**, and the results are summarized in Table 2. The inhibitory potency on ChT-L activity was not improved by the P3 modification, however, introduction of large substituents effectively improved selectivity for ChT-L activity. Particularly, compound **9a** with the biaryl (3-phenyl-pyridin-2-yl) P3 substituent had very weak inhibitory potency on C-L activity (IC_{50} = 1000 nM) despite its remarkable inhibitory potency on ChT-L activity (IC_{50} = 7.5 nM). Because CEP-18770, with the same biaryl P3 substituent as **9a**, strongly inhibits C-L activity with an IC_{50} value of around 70 nM,[21] the binding to the primed site in these hybrid compounds might differentiate recognition of the P3 substituents.

Finally, we investigated the impact of the moiety derived from nonpeptidic belactosin A derivatives ligated into the P2 side chain on their biological activities. For this purpose, we evaluated the biological activities of compounds **10a** and **11a**, in which the moiety was entirely removed (**10a**) or one of the aromatic rings was removed (**11a**), and the results are summarized in Table 3. Compared with **10a** without the primed site-binding moiety, compounds **11a** with one aromatic ring and **4a** with two aromatic rings exhibited 10- and 20-times greater inhibitory effects on ChT-L activity, respectively, demonstrating that the moiety ligated into the P2 side chain largely contributes to increase their inhibitory potency.

Compound **10a** had almost no effect on cell growth (IC_{50} = 2500 nM), which likely reflects its lowered proteasome inhibitory activity and membrane permeability. Although **11a** had proteasome inhibitory effects comparable or even higher than those of **7a** and **8a**, it had only moderate cell growth inhibitory effects (IC_{50} = 130 nM), which might be due to differences in the membrane permeability, biological stability, and/or binding reversibility, as described below.

Thus, we rationally developed a novel class of proteasome inhibitors with hybrid structures of nonpeptidic belactosin A derivatives and peptide boronates, having proteasome inhibitory effects that were comparable or even higher than those of bortezomib. In addition, these compounds effectively inhibited HCT116 cell growth.

Inhibitory Effects on β5i of Immunoproteasome. The therapeutic benefits of selective inhibition of immunoprotea-

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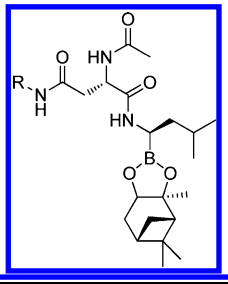
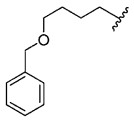
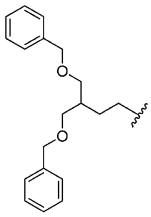
Table 3. Inhibitory Effects of Compounds 10a and 11a on Human 20S Proteasome and HCT116 Cell Growth



compound number	R	IC_{50} [nM][a] ChT-L activity	C-L activity	T-L activity	HCT116 cell growth
10a		54 ± 3.8	500 ± 64	> 10000	2500
11a		4.8 ± 1.0	170 ± 114	2170 ± 150	130
4a		2.7 ± 1.0	150 ± 24	2700 ± 330	31.8

[a]Based on three experiments.

somes were recently demonstrated, especially for autoimmune diseases.[3] On the other hand, inhibition of both constitutive proteasomes and immunoproteasomes is desirable for potent cell growth inhibitory activity.[27] Thus, we investigated the inhibitory activity of the newly identified hybrid inhibitors **4a** and **11a** on β5i of immunoproteasomes. The results are shown in Figure 6, compared with the results of bortezomib, PR-957, and lactacystin, which were shown to be nonselective, β5i selective, and β5 selective inhibitors, respectively, inconsistent with the previous report.[3,6a,28] The compound **4a** and **11a** were shown to be slightly selective for β5i compared to the nonselective inhibitor bortezomib, while they have the same boronic acid warhead. The structures originating from the nonpeptidic belactosin derivatives might differentiate the selectivity between β5 and β5i subunits.

Inhibitory Effects on Other Proteases. Proteasome inhibitors with a boronic acid moiety as the active warhead generally suffer from their promiscuous inhibitory activity on various serine proteases because of the high reactivity of the warhead with hydroxyl groups.[29] In fact, peripheral neuropathy due to bortezomib treatment, which is the major dose-limiting toxicity of bortezomib, is thought to be caused by its off-target activity on serine proteases.[29b] Thus, the inhibitory activity of the newly identified hybrid inhibitors **4a** and **11a** on cathepsin A and cathepsin G (serine proteases) and cathepsin B (cysteine proteas) were examined. As summarized in Table 4, bortezomib showed inhibitory activity on cathepsin A and cathepsin G, while carfilzomib, with an epoxyketone warhead, did not inhibit these serine proteases as reported previously by Arastu-Kapur et al.[29b] The carfilzomib, however, inhibited cathepsin B,

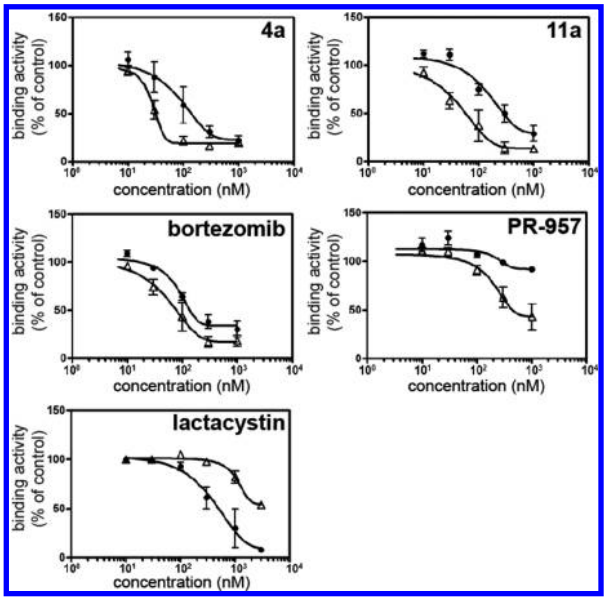


Figure 6. The binding activity of **4a** and **11a** to β5 and β5i subunits investigated by active-site ELISA.[3] The activity to β5i (Δ) subunits of immunoproteasome and β5 (●) subunits of the constitutive proteasome were normalized to values derived from DMSO-treated controls. The known proteasome inhibitors, bortezomib, PR-957, and lactacystin, were used as controls. The IC_{50} values of these compounds were as follows: **4a**, IC_{50} = 140 nM (β5), 31 nM (β5i); **11a**, IC_{50} = 280 nM (β5), 59 nM (β5i); bortezomib, IC_{50} = 140 nM (β5), 78 nM (β5i); PR-957, IC_{50} > 1.0 μM (β5), 450 nM (β5i); lactacystin, IC_{50} = 500 nM (β5), > 3.0 μM (β5i).

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Table 4. Inhibitory Effects of 4a, 11a, Bortezomib, and Carfilzomib on Cathepsin A, B, and G

compd	IC_{50} [μM][a]		
	cathepsin A	cathepsin B	cathepsin G
4a	>30	>30	>30
11a	>30	>30	>30
bortezomib	9.2	>30	3.1
carfilzomib	>30	11	>30

[a]Based on three experiments.

probably because of the high reactivity of its epoxyketone warhead with the thiol group. In contrast, compounds **4a** and **11a** exhibited no inhibitory activity on any of these proteases. Their large P2 moiety comprised of the nonpeptidic scaffold originating from the belactosin derivative might effectively improve the selectivity for proteasome. Thus, these hybrid inhibitors seem to be promising candidates for the development of anticancer drugs without off-target toxicities due to the inhibition of proteases other than proteasome.

Mechanism of Cell Growth Inhibition. To clarify the mechanism of cell growth inhibition, we examined the cell cycle distribution of HCT116 cells treated with compounds **4a** and **11a**. As shown in Figure 7, **4a** and **11a** inhibited cell cycle progression at the G2/M stage, similar to bortezomib and the belactosin derivative **1**.[14c]

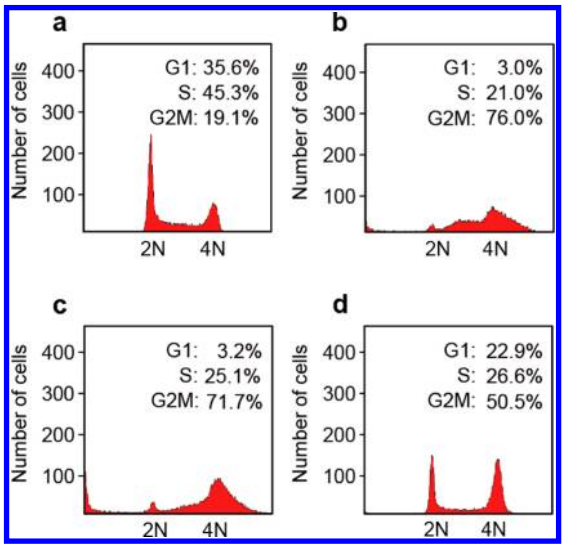

Figure 7. Cell cycle profiles of HCT116 cells treated with DMSO (a), 100 nM of bortezomib (b), **4a** (c), or **11a** (d). After 24 h, the cells were harvested and stained with propidium iodide. The DNA content of each sample was analyzed by flow cytometry.

Furthermore, we investigated the proteasome inhibitory effects of these compounds in a cellular system by immunoblot analysis. Figure 8 shows the clear accumulation of a tumor suppressor gene product p53, which is a well-known proteasome target,[30] in HCT116 cells treated with **4a** and **11a**. In addition, cleavage of poly(ADP-ribose) polymerase (PARP), a prominent marker of apoptosis,[31] was observed in the treated cells, indicating activation of the apoptotic pathway in the cells.

These results suggest that proteasome inhibition by the newly identified hybrid inhibitors **4a** or **11a** induced G2/M cell

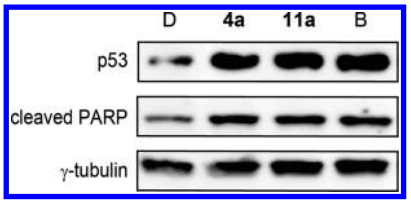

Figure 8. Immunoblot analysis of p53 and cleaved PARP in HCT116 cells treated with DMSO (D), **4a**, **11a**, or bortezomib (B).

cycle arrest and caused an accumulation of p53, resulting in HCT116 cell apoptosis similar to bortezomib.

Reversibility of Proteasome Inhibition. Although peptide boronate proteasome inhibitors bind covalently to proteasome, the reaction between the boronic acid warhead and the proteasome Thr hydroxyl group is reversible. The dissociation half-life of two peptide boronates, MLN9708 and bortezomib, from β5 is reported as 18 and 110 min, respectively.[22] Because these two inhibitors have the same boronic acid warhead, the difference between their dissociation half-lives seems to arise from their structures other than the warhead.

To investigate the impact of the moiety originating from the nonpeptidic belactosin A derivatives on the dissociation half-life, we preincubated proteasome with **4a** or **11a**, and the ChT-L activity was measured before and after dialysis, in comparison with bortezomib and carfilzomib as controls for a reversible and an irreversible inhibitor, respectively.[22,28,32] As shown in Figure 9, the inhibitory activity of compound **11a** and bortezomib on

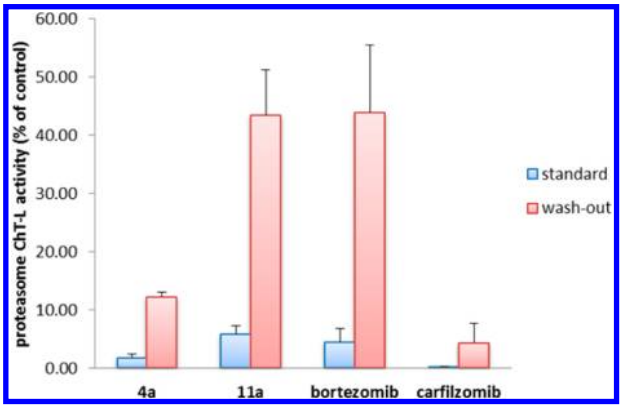

Figure 9. ChT-L activity of the human 20S proteasome treated with **4a**, **11a**, bortezomib, or carfilzomib before and after wash-out.

the ChT-L activity was significantly attenuated by wash-out, while compound **4a** retained strong inhibitory activity even after wash-out like carfilzomib, which has an epoxyketone warhead. These findings suggest that the interaction of the two aromatic groups in **4a** effectively prolongs its residence time, whereas the interaction by the one aromatic group in **11a** does not. Because the drug-target residence time is an important factor for in vivo efficacy and target selectivity of ligands,[33] **4a** might be an attractive lead for developing anticancer drugs with prolonged in vivo efficacy and reduced off-target toxicity.

CHEMISTRY

Synthesis of target compounds **3a**–**11a** and **3b**–**5b** is shown in Scheme 1. The pinanediol ester of leucine boronic acid **12**[34] was condensed with Boc-Asp(OBn)-OH or Boc-Glu(OBn)-OH by the mixed anhydride method to yield **13a** or **13b**,

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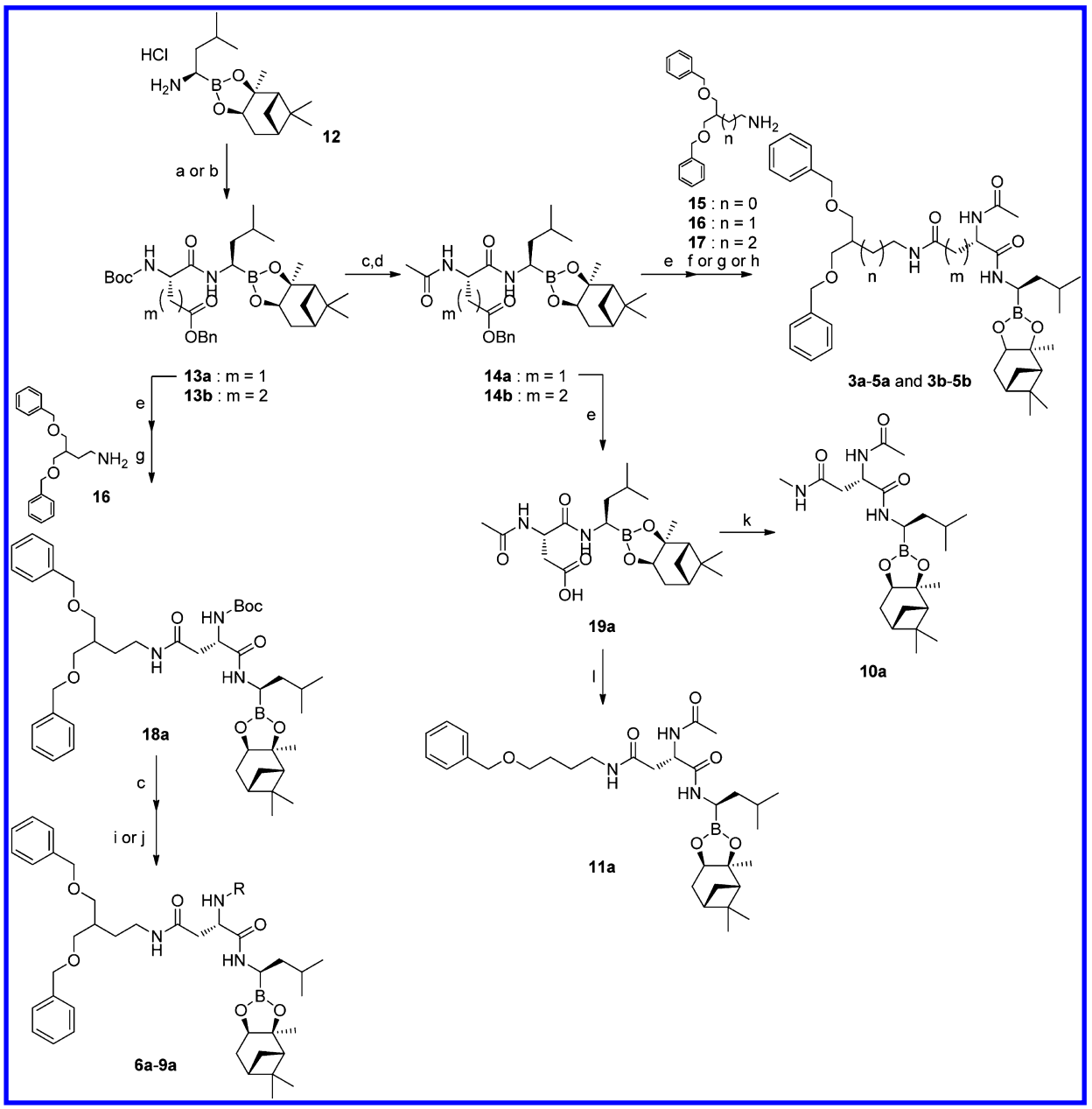
Scheme 1. Synthesis of Target Compounds 3a−11a and 3b−5b[a]



[a]Reagents and conditions: (a) Boc-Asp(OBn)-OH, PivCl, Et_3N, CH_2Cl_2, 0 °C to rt; (b) Boc-Glu(OBn)-OH, PivCl, Et_3N, CH_2Cl_2, 0 °C to rt; (c) TFA, CH_2Cl_2, 0 °C to rt; (d) Ac_2O, Et_3N, CH_2Cl_2, 0 °C, 68% for **14a** (3 steps from **12**), 84% for **14b** (3 steps from **12**); (e) Pd/C, H_2, THF; (f) **15**, EDC, HOAt, DIEA, CH_2Cl_2, −18 °C to rt, 68% for **3a** (2 steps from **14a**), 78% for **3b** (2 steps from **14b**); (g) **16**, EDC, HOAt, DIEA, CH_2Cl_2, −18 °C to rt, 77% for **4a** (2 steps from **14a**), 84% for **4b** (2 steps from **14b**); (h) **17**, EDC, HOAt, DIEA, CH_2Cl_2, −18 °C to rt, 71% for **5a** (2 steps from **14a**), 75% for **5b** (2 steps from **14b**); (i) R-Cl, Et_3N, CH_2Cl_2, 0 °C, 49% for **6a** (R = 2,5-dichlorobenzoyl, 5 steps from **12**), 53% for **7a** (R = benzoyl, 5 steps from **12**); (j) R-OH, PivCl, Et_3N, CH_2Cl_2, 0 °C to rt, 47% for **8a** (R = pyrazine-2-carbonyl, 5 steps from **12**), 54% for **9a** (R = 6-phenylpyridine-2-carbonyl, 5 steps from **12**); (k) CH_3NH_2·HCl, EDC, HOAt, DIEA, CH_2Cl_2, 0 °C to rt, 2 steps 18% from **14a**; (l) 4-(benzyloxy)butan-1-amine·HCl, EDC, HOAt, DIEA, CH_2Cl_2, −18 °C to rt, 2 steps 76% from **14a**.

respectively. The Boc group of **13a** and **13b** was removed by treatment with TFA/CH_2Cl_2 and following acetylation of the resulting amino group with Ac_2O afforded **14a** and **14b**, respectively. The Bn group of **14a** and **14b** was removed by hydrogenolysis, and subsequent condensation with **15**−**17** gave target compounds **3a**−**5a** and **3b**−**5b**, respectively.

Target compounds **6a**−**11a** were synthesized similarly. The Bn group of **13a** was removed, and subsequent condensation with **16** afforded **18a**. The Boc group of **18a** was removed, and subsequent condensation with corresponding carboxylic acids or acyl chlorides gave target compounds **6a**−**9a**. The Bn group of **14a** was removed, and following condensation with CH_3NH_2 or 4-(benzyloxy)butan-1-amine gave target compounds **10a** or **11a**, respectively.

All of these target compounds were purified by reverse-phase column chromatography because pinanediol esters of these peptide boronic acids are unstable under usual silica gel column chromatography conditions.[35]

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CONCLUSIONS

We successfully developed a highly potent novel chemotype of proteasome inhibitors using a rational hybridization strategy with nonpeptide belactosin derivatives and peptide boronates. These new inhibitors exhibited high cell-growth inhibitory activity due to their proteasome inhibitory activity. These inhibitors have the same boronic acid active warhead as bortezomib, but unlike bortezomib, the hybrids are highly selective for proteasomes without inhibiting serine proteases and have long residence times compared to bortezomib. Thus, these newly identified proteasome inhibitors are promising candidates for the development of anticancer drugs without the off-target toxicities that are problematic in bortezomib treatment.

ASSOCIATED CONTENT

Supporting Information

Experimental details of synthesis, biological evaluations, computational simulations, and combustion analysis data for target compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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ABBREVIATIONS USED

AMC, aminomethylcoumarin; Boc, *t*-butoxycarbonyl; C-L, caspase-like; ChT-L, chymotrypsin-like; DIEA, diisopropylethylamine; EDC, 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide; HOAt, 1-hydroxy-7-azabenzotriazole; LMP2, large multifunctional peptidase 2; LMP7, large multifunctional peptidase 7; MECL-1, multicatalytic endopeptidase complex-like 1; Piv, pivaloyl; Suc, succinyl; T-L, trypsin-like

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