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Discovery of Carboranes as Inducers of 20S Proteasome Activity

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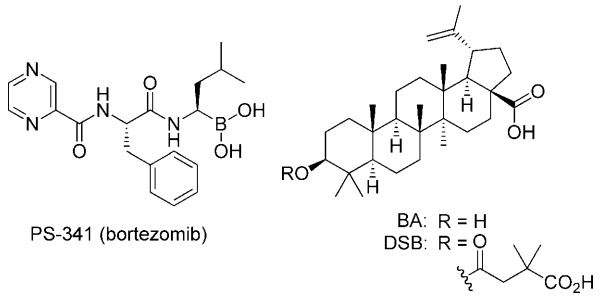
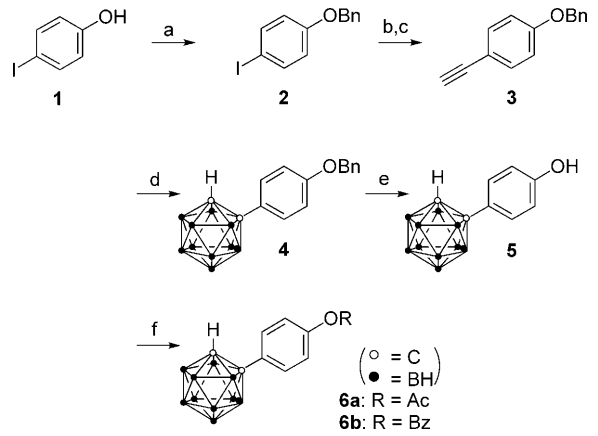
In eukaryotes, proteasomes play a critical role in the degradation of most intracellular proteins.[1–4] Degradation by ubiquitin-proteasome pathway controls many fundamental cellular functions. These include cell differentiation, cell-cycle regulation, signal transduction pathways, antigen processing in immune responses, stress signaling, inflammatory responses, and apoptosis. The first step in the pathway is activation of ubiquitin-activating enzyme (E1), followed by ubiquitin transfer to an ubiquitin-conjugating enzyme (E2). The second step is transfer of ubiquitin from E2 to the substrate by ubiquitin ligase (E3). The polyubiquitinated protein is then degraded by a 26S proteasome.[5] The 26S proteasome comprises of a 20S proteasome cylinder capped by a regulatory 19S complex at each extremity. The 20S proteasome has three major proteolytic activities: caspase-like (β1), trypsin-like (β2), and chymotrypsin-like (β5).[6] The 19S complex (PA700) activates proteasome degradation of ubiquitin-conjugated proteins. In addition to PA700, 20S proteasome can separately interact with other regulatory complexes such as PA28[7] or PA200.[8] Both complexes can activate the 20S proteasome using peptide substrates.

Many natural and synthetic inhibitors of the proteasome have been developed, such as peptide aldehydes, peptide epoxyketones, peptide vinyl sulfones, lactacystins, and peptide boronates.[9–11] The peptide boronate PS-341 (bortezomib), an inhibitor of the chymotrypsin-like activity of 20S proteasome, has been approved for the treatment of multiple myeloma.[12–14] Small-molecule proteasome activators have been developed to a lesser extent. Several biological lipids, including sodium dodecyl sulfate (SDS),[15] fatty acids,[16] ceramide sulfates,[17] lysophosphatidylinositol,[18] and cardiolipin[19] have been observed to activate the 20S proteasome. In general, proteasomal degradation of synthetic substrates by these molecules is more variable but not remarkable. Dramatic activation of proteasome by peptide-based activators has been investigated.[20] Recently, betulinic acid (BA) has attracted attention for its anti-HIV-1 and antitumor activities.[21] BA and its dimethylsuccinyl derivative (DSB) were found to be preferential activators of the β5 and β1 activities of the 20S proteasome, respectively.[22]

Carboranes (dicarba-*closo*-dodecaboranes; $C_2B_{10}H_{12}$) exhibit remarkable thermal stability, and their icosahedral geometry and exceptional hydrophobic property may allow their use as a hydrophobic pharmacophore in biologically active molecules that interact hydrophobically with target proteins.[23–25] In this study, we discovered that *ortho*-carborane derivatives have the potential to induce the β1, β2, and β5 activities of the 20S proteasome in the absence of PA28.



The synthesis of *ortho*-carboranyl phenol[23] and its ester derivatives is shown in Scheme 1. Benzylation was performed by treating 4-iodophenol (**1**) with benzyl bromide in the presence of K_2CO_3. The resulting benzyl ether **2** underwent the Sonogashira coupling reaction with ethynyltrimethylsilane in the presence of catalysts, $PdCl_2(PPh_3)_2$ and CuI. The reaction was performed in a sealed vial under microwave-irradiated (120 °C). The reaction reached completion within 25 min to give the corresponding coupling product in 98% yield. The TMS group was removed by treatment with LiOH in aqueous THF solution to afford alkyne **3** in quantitative yield. The reaction of alkyne **3** with decaborane proceeded in the presence of acetonitrile (5 equiv) in toluene at reflux to give the corresponding carborane **4** in 25% yield. The benzyl group was removed by hydro-



Scheme 1. *Reagents and conditions*: a) BnBr, K_2CO_3, DMF, RT, 95%; b) ethynyltrimethylsilane, $PdCl_2(PPh_3)_2$, CuI, DIEA, microwave (120 °C), 98%; c) LiOH·H_2O, THF/H_2O (1:1), >99%; d) $B_{10}H_{14}$, CH_3CN, toluene, reflux, 25%; e) H_2, Pd/C, CH_3OH, >99%; f) Ac_2O or BzCl, DMAP, Py, CH_2Cl_2, **6a**: 97%, **6b**: 33%.

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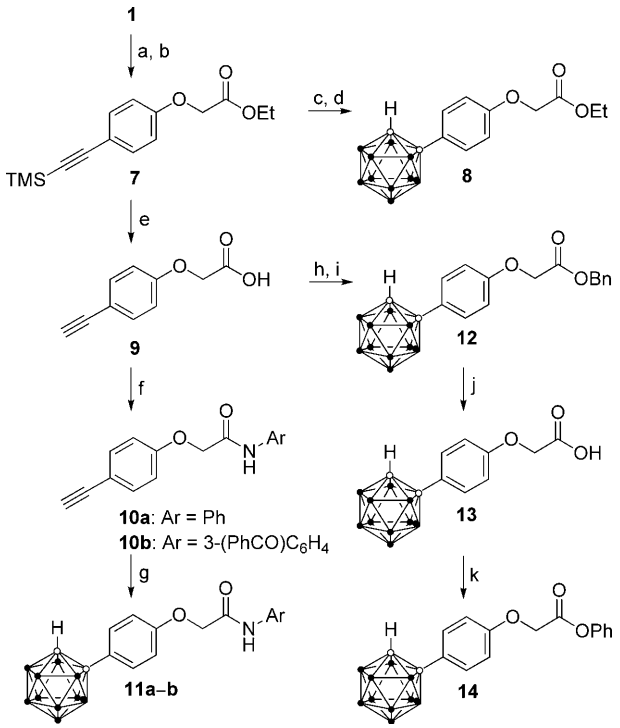
genolysis to afford **5** in quantitative yield. Acetate **6a** and benzoate **6b** were obtained from **5** in 97% and 33% yields, respectively.

The synthesis of *ortho*-carboranyl phenoxyacetic acid **13** and its derivatives **8**, **11a**–**b**, **12**, and **14** is shown in Scheme 2. The reaction of 4-iodophenol (**1**) with ethyl chloroacetate proceeded quantitatively by treatment with K_2CO_3 in DMF. The resulting ester underwent the Sonogashira coupling reaction with ethynyltrimethylsilane in the presence of $PdCl_2(PPh_3)_2$ and CuI under microwave irradiation to give **7** in 97% yield. Compound **7** was treated with tetrabutylammonium fluoride to remove the TMS group. The resulting acetylene was reacted with decaborane, under the same conditions used to synthesize carborane **4**, to give the corresponding carborane **8** in 48% yield. When compound **7** was treated with LiOH in aqueous THF solution, deprotection of both TMS and ester groups occurred to afford carboxylic acid **9** in quantitative yield. Carboxylic acid **9** was reacted with aniline or 3-benzoylaniline to give the anilides **10a** and **10b**, respectively, which were then treated with decaborane under the established conditions to give the corresponding carboranes **11a** and **11b** in 61% and 36% yields, respectively. The protection of carboxylic acid **9** with benzyl chloride in the presence of sodium bicarbonate followed by decaborane coupling gave the carborane benzyl ester **12**, which was treated under hydrogenation conditions to give carboxylic acid **13**. The phenoxy ester derivative **14** was then synthesized from carboxylic acid **13** using *N*,*N*′-dicyclohexylcarbodiimide (DCC) coupling with phenol in 23% yield.



Scheme 2. *Reagents and conditions*: a) $ClCH_2CO_2Et$, K_2CO_3, DMF, RT, >99%; b) ethynyltrimethylsilane, $PdCl_2(PPh_3)_2$, CuI, Et_3N, microwave (120 °C), 91%; c) TBAF, THF, RT, 60%; d) $B_{10}H_{14}$, CH_3CN, toluene, reflux, 48%; e) $LiOH{\cdot}H_2O$, THF/H_2O (1:1), >99%; f) aniline or 3-aminobenzophenone, EDCI, HOBt, DIPEA, DMF, **10a**: quant; **10b**: 70%; g) $B_{10}H_{14}$, CH_3CN, toluene, reflux, **11a**: 61%, **11b**: 36%; h) BnCl, Na_2CO_3, DMF, 97%; i) $B_{10}H_{14}$, CH_3CN, toluene, reflux, 49%; j) H_2, Pd/C, CH_3OH, 45%; k) phenol, DCC, DMAP, CH_2Cl_2, 23%.

The effects of the prepared carboranes **5**, **6a**–**b**, **8**, **11**–**14**, *ortho*-carborane and BA on the caspase-like (β1), trypsin-like (β2) and chymotrypsin-like (β5) activities of the 20S proteasome were evaluated using fluorogenic substrates Z-LLE-AMC, Boc-LRR-AMC, and Suc-LLVY-AMC, respectively. PA28 was used as a positive control for comparison. The results are summarized in Table 1. Although *ortho*-carborane, carboranylphenol **5**, and its acetate **6a** did not affect β1, β2, and β5 activities of the 20S proteasome at 10 μM, significant induction of β5 activity was observed for compound **6b** (358%) along with slight induction of β1 (176%) and β2 (160%) activities. Conversely, compounds **11a** and **11b** significantly induced β1 (350%) and β2 (373%) activities, respectively.

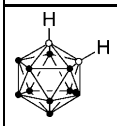
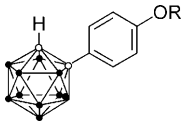
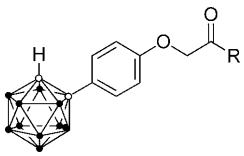
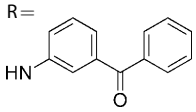
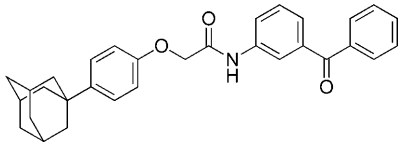
The profiles of these compounds toward the β5 activity of the 20S proteasome and HeLa cell growth inhibition were different. Compound **11a** weakly induced β2 (205%) and inhibited β5 (87.6%) activities at 10 μM without inhibiting cell growth ($GI_{50} \geq 100$ μM), while compound **11b** significantly inhibited β5 activity (4.5%) at the same concentration, and its GI_{50} value was 20.3 μM. The phenoxy ester derivative **14** showed weak biological activity compared to compound **6b**, and significant induction of β1 and β2 activities was not observed.

Furthermore, we tested the adamantine derivative **15**, which is considered to be a mimic of the carborane derivative **11b**. Compound **15** also induced β1 (149%) and β5 (192%) activities of the 20S proteasome without affecting β2 (114%) activity at 10 μM. Under the assay conditions used here, BA showed significant, but nonselective, induction of β1 (526%), β2 (206%), and β5 (128%) activities. This is in contrast to the selective activation of β5 was reported by Chen and co-workers.[22] These differing results may be caused by variations in the assay conditions. Because the detailed assay conditions were not described in their report, the current assay was carried out based on the protocol reported by Asai and co-workers.[26] Regarding the 20S proteasome activities induced by PA28, the results described here are consistent with reported results.[22]

We next investigated the dose- and time-dependent nature of the effects of compounds **11a** and **11b** on proteasome activity. As shown in Figure 1, compound **11a** increased β1 and β2 activities and slightly inhibited β5 activity of the 20S proteasome at 10 and 30 μM (Figure 1). For compound **11b**, β1 and β2 activities were increased and β5 activity was significantly inhibited at 10 μM (Figure 2).

According to the results obtained in this study, both carboranylphenoxy and phenyl moieties are important structural features for activation of the 20S proteasome. The ester derivatives **6b** and **14** showed selective induction of β5, whereas the amide derivatives **11a** and **11b** preferentially activated β1 and β2, respectively. The adamantine derivative **15** only weakly induced the β1 and β5 activities of the 20S proteasome, sug-

Table 1. The effect of carboranes **5**, **6**, **8**, **11**–**14** and adamantine **15** on caspase-like (β1), trypsin-like (β2), and chymotrypsin-like (β5) activities of the 20S proteasome[a] and growth inhibition of HeLa cells.[b]

Compound[c]			β1 [%]	β2 [%]	β5 [%]	GI_{50} [μM]
	*o*-carborane		138±6.4	94.0±1.8	103±7.8	>100
	R=H	**5**	102±5.6	76.0±0.8	124±1.9	17.1±3.7
	R=Ac	**6a**	116±18.6	95.2±0.1	106±4.4	>100
	R=COPh	**6b**	176±1.8	160±2.5	358±57.9	21.9±1.4
	R=OEt	**8**	106±12.7	73.7±1.2	91.5±0.8	56.2±1.9
	R=OCH_2Ph	**12**	67.1±20.9	81.1±0.5	133±41.4	23.4±0.1
	R=OH	**13**	111±7.3	102±7.0	124±7.2	>100
	R=OPh	**14**	97.1±17.3	70.0±3.8	145±8.5	85.7±12.1
	R=NHPh	**11a**	350±28.4	205±26.5	87.6±3.5	>100
	R= 	**11b**	181±7.7	373±49.8	4.5±1.0	20.3±0.8
		**15**	149±15.5	114±11.8	192±15.8	>100
PA28			526±4.3	206±2.7	128±6.0	N.D.
Betulinic acid			609.5±24.0	522.8±24.9	545.2±39.6	15.9±0.4

[a] Human 20S proteasome activities were measured using specific fluorophore-tagged peptides (Z-LLE-AMC for β1; Boc-LRR-AMC for β2;, Suc-LLVY-AMC for β5). [b] HeLa cells were incubated for 72 h with various concentrations (100 nM–100 μM) of compounds, and viable cells were determined by MTT assay. The drug concentration required to inhibit cell growth by 50% (GI_{50}) was determined from semi-logarithmic dose–response plots, and results represent the mean ±SD of triplicate samples. [c] Proteasome activities were measured with compounds (10 μM), PA28 (500 ng), or betulinic acid (10 μM).

gesting that a carborane framework is more suitable for induction of the 20S proteasome activities.

In conclusion, carborane derivatives **6b**, **11a**, and **11b** were found to be potent inducers of β5, β1, and β2 activities of the 20S proteasome, respectively. The carborane framework is essential for induction of the 20S proteasome activities. Since small-molecule proteasome activators have not been developed, the current compounds represent potential chemical probes for the investigation of proteasome-dependent degradation pathways.

Experimental Section

Synthesis

The experimental procedure for the synthesis of compound **11a** from 4-iodophenol (**1**) is reported below. Spectral data of all tested compounds **5**, **6a**–**b**, **8**, **12**–**14**, and **15** are described in the Supporting Information.

Ethyl 2-(4-iodophenoxy)acetate: Ethyl chloroacetate (0.54 mL, 5.1 mmol) was added to a mixture of **1** (1.1 g, 5 mmol) and K_2CO_3 (2.1 g, 15 mmol) in DMF. The mixture was stirred at RT overnight, and the reaction was quenched with H_2O. The mixture was extracted with EtOAc, washed with brine, dried over anhyd $MgSO_4$, filtered and concentrated in vacuo. The residue was purified by column chromatography (silica gel; hexane/EtOAc, 10:1) to give the title compound as a white solid (1.47 g, 4.8 mmol, 96%): ^{1}H NMR (400 MHz, $CDCl_3$): δ=7.57 (2H, d, J=8.8 Hz), 6.69 (2H, d, J=9.2 Hz), 4.59 (2H, s), 4.27 (2H, q, J=7.2 Hz), 1.29 ppm (3H, t, J=7.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ= 168.3, 157.6, 138.2, 116.9, 83.9, 65.2, 61.3, 14.1 ppm; MS (ESI+): m/z 328.9 $[M+Na]^+$.

Ethyl 2-(4-(2-(trimethylsilyl)ethynyl)phenoxy)acetate (7): Trimethylacetylene (3.4 mL, 2.4 mmol) was added to a solution of 2-(4-iodophenoxy)acetate (8 mmol), $PdCl_2(PPh_3)_2$ (110 mg, 0.16 mmol), CuI (76 mg, 0.4 mmol), and Et_3N (8.1 mL, 78 mmol) in DMF under Ar. The mixture was heated by microwave irradiation to 120°C for 25 min. The solvent was removed in vacuo, and the residue was dissolved in EtOAc. The mixture was washed with aq HCl (2N), neutralized with saturated aq $NaHCO_3$, washed with brine, dried over anhyd $MgSO_4$, filtered and concentrated in vacuo. Purification by column chromatography (silica gel; 100% hexane) gave **7** (2.02 g, 7.32 mmol, 91%): ^{1}H NMR (400 MHz, $CDCl_3$): δ=7.40 (2H, d, J=9.2 Hz), 6.82 (2H, d, J=8.8 Hz), 4.61 (2H, s), 4.27 (2H, q, J=7.2 Hz), 1.29 (3H, t, J=7.2 Hz), 0.23 ppm (9H, s); ^{13}C NMR (72 MHz, $CDCl_3$): δ=168.5, 157.8, 133.5, 116.4, 114.5, 104.7, 92.9, 65.3, 61.4, 14.1 ppm; MS (ESI+): m/z 299.0 $[M+Na]^+$, 315.0 $[M+K]^+$.

2-(4-Ethynylphenoxy) acetic acid (9): A solution of **7** (1.98 g, 7.2 mmol) in THF/H_2O (1:1; 40 mL) was treated with LiOH·H_2O (1.21 g, 28.8 mmol) at RT overnight. After neutralization of the reaction with aq HCl (1N), the mixture was extracted with EtOAc, washed with brine, dried over anhyd $MgSO_4$, filtered and concentrated in vacuo. Purification by column chromatography (silica gel; 100% EtOAc) gave **9** (379 mg, 1.8 mol, quant): ^{1}H NMR (400 MHz, $CDCl_3$): δ=7.45 (2H, d, J=8.8 Hz), 6.87 (2H, d, J=8.8 Hz), 4.69 (2H, s), 3.01 ppm (1H, s); ^{13}C NMR (72 MHz, CD_3OD): δ=172.3, 159.7, 134.4, 116.6, 115.7, 84.1, 77.3, 65.7 ppm; MS (ESI−): m/z 175.9 $[M-Na]^-$.

2-(4-Ethynylphenoxy)-*N*-phenylacetamide (10a): A DMF solution of **9** (100 mg, 0.57 mmol), aniline (0.08 mL, 0.85 mmol), EDCI (164 mg, 0.85 mmol), HOBt (131 mg, 0.86 mmol), and DIPEA (0.146 mL, 8.56 mmol) was stirred at RT overnight. The reaction was quenched with H_2O, extracted with EtOAc, washed with brine, dried over anhyd $MgSO_4$, filtered and concentrated in vacuo. Purification by column chromatography (silica gel; hexane/EtOAc, 3:1) gave **10a** (143 mg, 0.57 mmol, quant) as a colored solid: ^{1}H NMR (400 MHz, $CDCl_3$): δ=8.21 (1H, br s), 7.57 (2H, d, J=7.6 Hz), 7.48 (2H, d, J=8.8 Hz), 7.35 (2H, t, J=7.6, 8.4), 7.16 (1H, t, J=7.6, 7.6), 6.94 (2H, d, J=6.8), 4.60 (2H, s), 3.04 ppm (1H, s); ^{13}C NMR

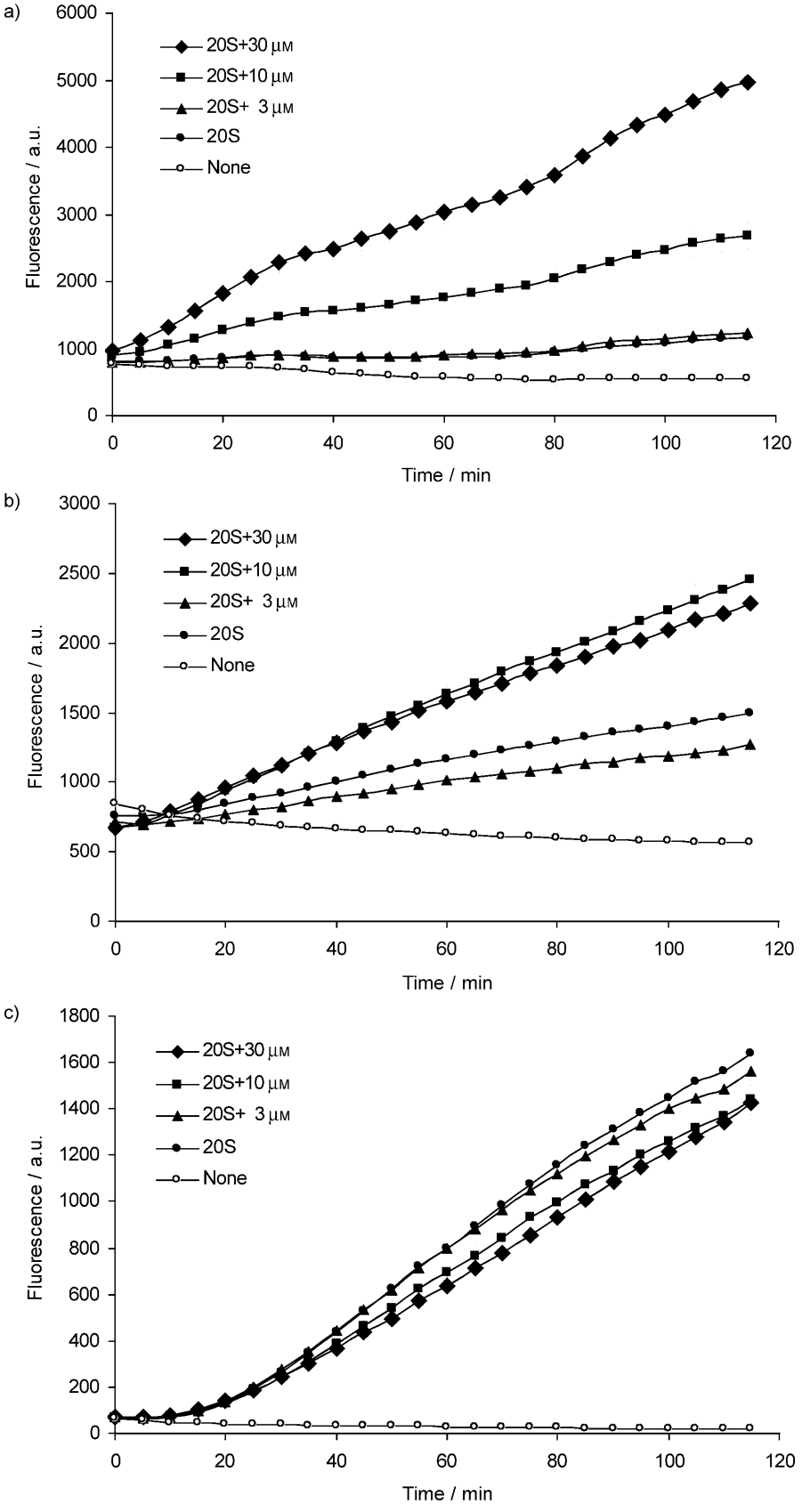


Figure 1. Effects of compound **11a** on a) trypsin-like activity, b) caspase-like activity, and c) chymotrypsin-like activity of the 20S proteasome. The fluorescence of specific fluorophore-tagged peptides (Z-LLE-AMC for β1; Boc-LRR-AMC for β2; Suc-LLVY-AMC for β5) in the absence of 20S proteasome were measured as a control (none, ○). Florescence: excitation, 365 nm; detection, 460 nm.

(100 MHz, $CDCl_3$): $\delta = 165.7$, 157.1, 136.6, 133.9, 129.1, 125.0, 120.1, 116.2, 114.8, 82.9, 76.7, 67.5 ppm; MS (ESI+): *m/z* 328.9 $[M+Na]^+$.

2-(4-Carboranylphenoxy)-*N*-phenylacetamide (11a): CH_3CN (0.17 mL, 3.3 mmol) was added to a mixture of **10a** (140 mg, 0.57 mmol) and decaborane (77 mg, 0.63 mmol) in toluene under Ar. The mixture was refluxed overnight. After the solvent was removed in vacuo, the residue was purified by column chromatography (silica gel; hexane/EtOAc, 50:1) to give **11a** as a white solid (130 mg, 0.35 mmol, 61%): $R_f = 0.27$ (*n*-hexane/EtOAc, 3:1); mp: 139–140 °C; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 8.13$ (1H, br s), 7.57 (2H, d, $J = 8.8$ Hz), 7.49 (2H, d, $J = 6.8$ Hz), 7.37 (2H, t, $J = 8.4$, 8.4), 7.17 (1H, t, $J = 8.8$, 7.2), 6.94 (2H, d, $J = 7.2$), 4.61 (2H, s), 3.89 ppm (1H, br s); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 165.4$, 158.0, 136.6, 129.6, 129.1, 127.5, 125.1, 120.2, 114.9, 76.1, 67.6, 60.7 ppm; IR (KBr): 3749.4, 3672.2, 3649.1, 3390.6, 3336.6, 3062.7, 2920.0, 2592.2, 1685.7, 1600.8, 1535.2, 1512.1, 1446.5, 1361.7, 1296.1, 1253.6, 1188.1, 1076.2, 1026.1, 906.5, 883.3, 837.0 cm^{-1}; HRMS (ESI−): *m/z* $[M-H]^-$ calcd for $C_{16}H_{23}B_{10}NO_2$: 368.2654, found: 368.2653.

Biology

Cytotoxicity assays: The human cervical carcinoma cell line, HeLa, was used for the cell growth assays. Cells (5×10^3 cells per well of a 96-well plate) were incubated at 37 °C for 72 h in 100 μL RPMI-1640 medium containing various concentrations of test compounds. After incubation, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (10 μL, 5 mg mL^{-1} in PBS) was added to each well and cells were further incubated at 37 °C for 4 h. After removal of the medium, DMSO (100 μL) was added and absorbance at 595 nm was determined using a 96-well plate reader.

Activity of the 20S proteasome: The three peptidase activities (β1, β2, and β5) of the 20S proteasome were assayed with specific fluorophore-tagged peptides (Z-LLE-AMC

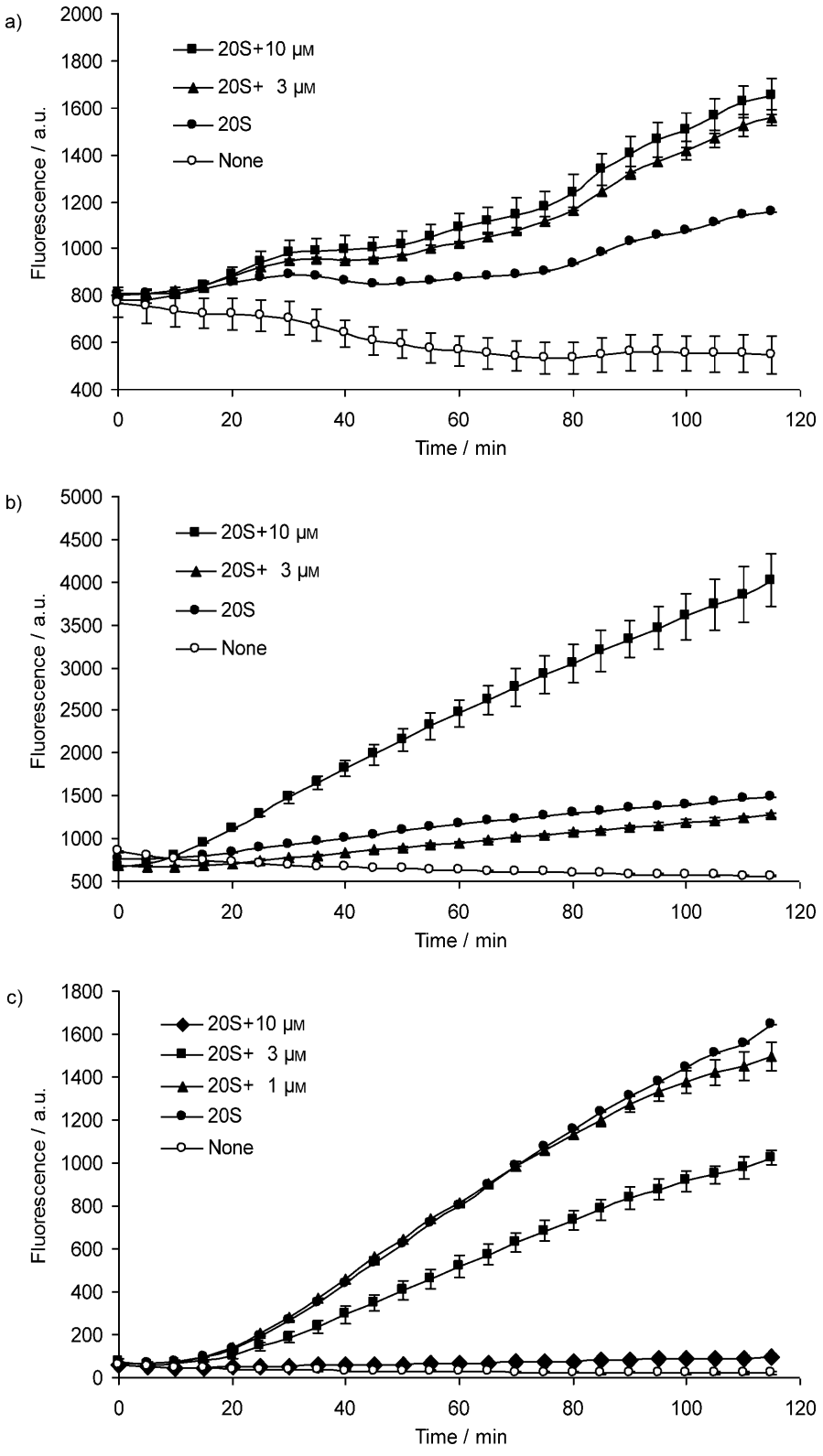


Figure 2. Effects of compound **11 b** on a) trypsin-like activity, b) caspase-like activity, and c) chymotrypsin-like activity of the 20S proteasome. The fluorescence of specific fluorophore-tagged peptides (Z-LLE-AMC for β1; Boc-LRR-AMC for β2; Suc-LLVY-AMC for β5) in the absence of 20S proteasome were measured as a control (none, ○). Florescence: excitation, 365 nm; detection, 460 nm.

for β1; Boc-LRR-AMC for β2; Suc-LLVY-AMC for β5). Briefly, compounds were mixed with 50 ng of the 20S proteasome isolated from human erythrocytes in assay buffer (20 mM Tris (pH 8.0), 0.5 mM EDTA, and 0.035% SDS). Hydrolysis was initiated by the addition of 70 μM of substrate. After incubation for 2 h at 37 °C, the reaction was quenched with stop buffer (20 mM Tris (pH 8.9) and 0.5% SDS). Fluorescence of AMC cleaved from substrates by each enzymatic activity was measured (excitation, 365 nm; detection, 460 nm). Proteasome–activator protein complex PA28, composed of two homologous subunits: PA28α and PA28β (Boston Biochem, Inc., Boston, USA), was used as a control experiment.

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Keywords: 20S proteasome · activators · caspase-like activity · drug discovery · trypsin-like activity

[1] K. L. Rock, C. Gramm, L. Rothstein, K. Clark, R. Stein, L. Dick, D. Hwang, A. L. Goldberg, *Cell* **1994**, *78*, 761–771.
[2] A. Hershko, A. Ciechanover, *Annu. Rev. Biochem.* **1998**, *67*, 425–479.
[3] G. Nalepa, M. Rolfe, J. W. Harper, *Nat. Rev. Drug Discovery* **2006**, *5*, 596–613.
[4] C. M. Pickart, *Cell* **2004**, *116*, 181–190.
[5] A. Ciechanover, *Angew. Chem.* **2005**, *117*, 6095–6119; *Angew. Chem. Int. Ed.* **2005**, *44*, 5944–5967.
[6] M. Groll, C. R. Berkers, H. L. Ploegh, H. Ovaa, *Structure* **2006**, *14*, 451–456.
[7] M. Chu-Ping, C. A. Slaughter, G. N. DeMartino, *J. Biol. Chem.* **1992**, *267*, 10515–10523.
[8] V. Ustrell, L. Hoffman, G. Pratt, M. Rechsteiner, *EMBO J.* **2002**, *21*, 3516–3525.
[9] L. Borissenko, M. Groll, *Chem. Rev.* **2007**, *107*, 687–717.

[10] T. Watanabe, I. Momose, M. Abe, H. Abe, R. Sawa, Y. Umezawa, D. Ikeda, Y. Takahashi, Y. Akamatsu, *Bioorg. Med. Chem. Lett.* **2009**, *19*, 2343 – 2345.
[11] H. Nakamura, M. Watanabe, H. S. Ban, W. Nabeyama, A. Asai, *Bioorg. Med. Chem. Lett.* **2009**, *19*, 3220 – 3224.
[12] J. Adams, M. Behnke, S. Chen, A. A. Cruickshank, L. R. Dick, L. Grenier, J. M. Klunder, Y.-T. Ma, L. Plamondon, R. L. Stein, *Bioorg. Med. Chem. Lett.* **1998**, *8*, 333 – 338.
[13] R. Z. Orlowski, E. L. Zeger, *Expet. Opin. Investig. Drugs* **2006**, *15*, 117 – 130.
[14] R. C. Kane, P. F. Bross, A. T. Farrell, R. Pazdur, *Oncologist* **2003**, *8*, 508 – 513.
[15] N. Orlowski, S. Wilk, *Biochem. Biophys. Res. Commun.* **1981**, *101*, 814 – 822.
[16] B. Dahlmann, M. Rutschmann, L. Kuehn, H. Reinauer, *Biochem. J.* **1985**, *228*, 171 – 177.
[17] I. Ohkubo, S. Gasa, C. Namikawa, A. Makita, M. Sasaki, *Biochem. Biophys. Res. Commun.* **1991**, *174*, 1133 – 1140.
[18] K. Matsumura, K. Aketa, *Development* **1991**, *33*, 259 – 266.
[19] I. Ruiz de Mena, E. Mahillo, J. Arribas, J. G. Castaño, *Biochem. J.* **1993**, *296*, 93 – 97.
[20] S. Wilk, W.-E. Chen, *Mol. Biol. Rep.* **1997**, *24*, 119 – 124.
[21] S. Fulda, *Mol. Nutr. Food Res.* **2009**, *53*, 140 – 146.
[22] L. Huang, P. Ho, C.-H. Chen, *FEBS Lett.* **2007**, *581*, 4955 – 4959.
[23] Y. Endo, T. Iijima, Y. Yamakoshi, M. Yamaguchi, H. Fukasawa, K. Shudo, *J. Med. Chem.* **1999**, *42*, 1501 – 1504.
[24] Y. Endo, T. Iijima, Y. Yamakoshi, H. Fukasawa, C. Miyaura, M. Inada, A. Kubo, A. Itai, *Chem. Biol.* **2001**, *8*, 341 – 355.
[25] R. L. Julius, O. K. Farha, J. Chiang, L. J. Perry, M. F. Hawthorne, *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 4808 – 4813.
[26] A. Asai, T. Tsujita, S. V. Sharma, Y. Yamashita, S. Akinaga, M. Funakoshi, H. Kobayashi, T. Mizukami, *Biochem. Pharmacol.* **2004**, *67*, 227 – 234.

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