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Strongylophorines, meroditerpenoids from the marine sponge *Petrosia corticata*, function as proteasome inhibitors



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ABSTRACT

Two new strongylophorine derivatives, along with six known strongylophorines, were isolated from the marine sponge *Petrosia corticata* as proteasome inhibitors. Of these, a hemiacetal mixture of strongylophorines-13/-14 was the strongest inhibitor of the proteasome with an IC_{50} of 2.1 μ M.

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Velcade® (bortezomib)¹ was approved, in 2003, by FDA as the first anticancer agent that inhibited the proteasome. The proteasome has since become an emerging oncology target,^{2–4} and another proteasome inhibitor, Kyprolis® (carfilzomib),⁵ was approved, in 2012, as the second anticancer agent. Several proteasome inhibitors have been developed by screening chemical libraries containing synthetic and natural small molecules and by chemical modifications of lead compounds. As an alternative approach, we have been searching for proteasome inhibitors from natural sources in order to obtain inhibitors with new carbon frameworks.⁶ We herein isolated two new meroditerpenoids, 26-O-methylstrongylophorine-16 (**1**) and 26-O-ethylstrongylophorine-16 (**2**), along with six known congeners (**3–8**) (Fig. 1) and measured their proteasome inhibitory activities.

The EtOAc soluble fraction of the EtOH extract of the marine sponge *Petrosia corticata*⁷ significantly inhibited the proteasome⁸ (98% inhibition at 50 μ g/mL). Purification from the EtOAc-soluble fraction by repeated column chromatography and HPLC⁹ afforded two new meroditerpenoids (**1** and **2**) along with six known strongylophorines-2 (**3**), -3 (**4**), -4 (**5**), -8 (**6**), -13/-14 (**7**), and -22 (**8**) (Fig. 1).¹⁰

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The FABMS of **1**¹¹ showed an ion peak at m/z 425 $[M-H]^-$ and its molecular formula was indicated to be $C_{27}H_{38}O_4$. The ¹H NMR spectrum of **1** in CDCl₃ (Table 1) showed three singlet methyl signals at δ 0.855, 0.864, and 1.12, four downfield signals at δ 3.24 (d, J = 11.0 Hz, H-24), 3.29 (3H, s, H₃-27), 4.05 (d, J = 11.0 Hz, H-24), and 4.14 (s, H-26), and signals due to a 1,2,4-trisubstituted phenyl group at δ 6.53 (d, J = 1.0 Hz, H-21), 6.55 (dd, J = 8.3, 1.0 Hz, H-19), and 6.58 (d, J = 8.3 Hz, H-18). An analysis of 2D NMR spectra clearly indicated that **1** was a congener of strongylophorines-15/-16 (**9**) (Fig. 1).¹² HMBC correlations from H₃-27 (δ_H 3.29) to C-26 (δ_C 106.0) and from H-26 (δ_H 4.14) to C-3 (δ_C 40.4), C-5 (δ_C 48.5), and C-24 (δ_C 62.1) (Fig. 2(a)) suggested **1** to be a 26-O-methyl derivative of strongylophorine-15 or -16. The relative configuration of C-26 was determined by NOE correlations between H-26 (δ 4.14) and H-2 (δ 1.49) (Fig. 2(b)). On the basis of the biogenetic correlation with **4**, the absolute configuration of which has already been determined,^{10a} the absolute configuration was secured to be 26S, the same as strongylophorine-16.^{10d} Thus, **1** was determined to be 26-O-methylstrongylophorine-16. The molecular formula $C_{28}H_{40}O_4$ was established for **2**¹³ by HRESIMS, with an additional CH₂ unit to **1**. The ¹H and ¹³C NMR spectra of **2** (Table 1) were similar to those of **1** and readily indicated that the methoxy group in **1** was replaced with an ethoxy group (δ_H 1.17 (3H, s, H₃-28)/ δ_C 15.40 (C-28) and δ_H 3.32 and 3.66 (H₂-27)/ δ_C 62.6 (C-27)) in **2**. The 2D

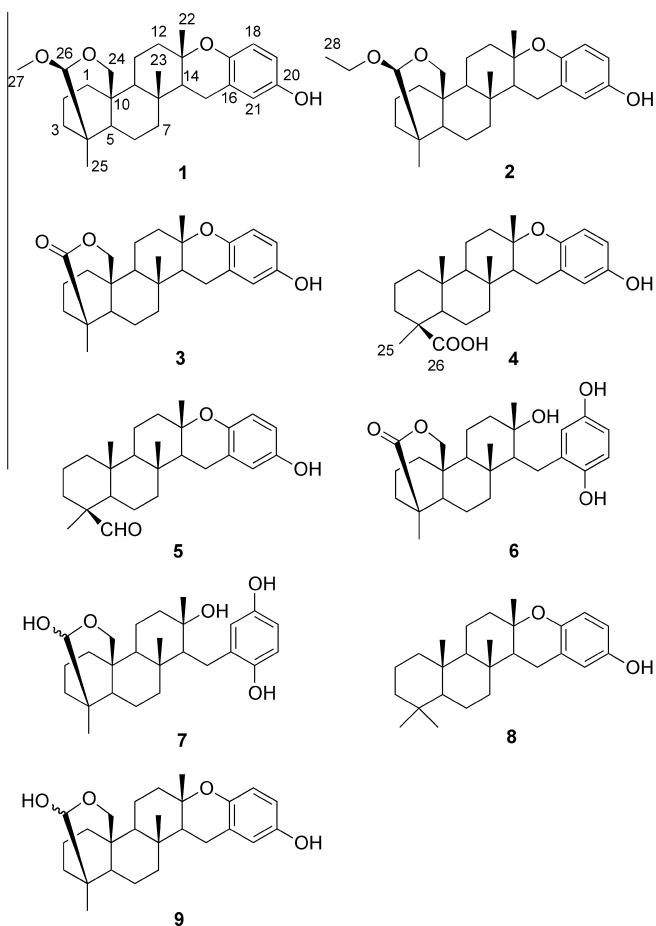


Figure 1. Structures of 1–9.

NMR spectra including NOESY¹³ clearly showed that **2** was 26-O-ethylstrongylophorine-16. In order to determine whether **1** and **2** were artifacts produced during extraction and isolation, compound **9**, a mixture of strongylophorines-15/-16,¹² was semi-synthesized from **3**¹⁴ and was kept in MeOH or EtOH in the presence of silica gel at room temperature. However, **9** did not convert to **1** nor **2** after one week, indicating that **1** and **2** were not artifacts.

The inhibitory activities of **1**–**8**, together with that of **9**, against the chymotrypsin-like activity of the proteasome were tested⁸ and their IC₅₀ values together with their structures were shown in Figure 3. Compounds containing acetal, that is, **1** (IC₅₀, 6.6 μM) and **2** (9.3 μM), exhibited more potent inhibitory activity than that containing lactone, that is, **3** (100 μM<). A comparison among the acetals (**1** and **2**) and their hemiacetals (**9**) revealed that the inhibitory activities (3.6 μM) of hemiacetals were more potent than those of acetals. On the other hand, compounds containing hydroquinone, that is, **6** (8.4 μM) and **7** (2.1 μM), showed more potent inhibitory activities than those containing their dehydrated compounds, that is, **3** and **9**, respectively. Compounds **4** (9.5 μM), **5** (19 μM), and **8** (100 μM<) contained a carboxylic acid, aldehyde, and methyl group, respectively, bound to a common skeleton, and their inhibitory activities decreased in this order. The most potent proteasome inhibitor tested in this study was compound **7**, which contains hemiacetal and hydroquinone moieties. Although 25 strongylophorine derivatives have been isolated and their various biological activities, that is, ichthyotoxic activity,^{10a} antimicrobial activity,^{10b} cytotoxicity,^{10c} and inhibitory activity against the maturation of starfish oocytes,^{10d} have been reported

Table 1
NMR data (CDCl₃) for **1** (400 MHz) and **2** (500 MHz)

No.	1			2		
	δ _C	δ _H , mult., J in Hz	HMBC ^a	δ _C	δ _H , mult., J in Hz	HMBC ^a
1	40.1	1.08 m 2.22 m		40.25	1.04 m 2.20 m	2, 9, 10 2, 9, 10, 24
2	22.2	1.49 m 2.25 m		22.3	1.49 m 2.17 m	
3	40.4	1.24 m 1.34 m		40.31	1.30 m 1.50 m	4, 26 5, 26
4	36.7			36.6		
5	48.5	1.00 m		48.5	0.99 dd, 13.2, 3.5	1, 3, 4, 10, 24, 26
6	19.6	1.56 m 2.04 m	5	19.6	1.56 m 2.12 m	5 5
7	38.8	0.87 m 1.72 m		38.8	0.86 m 1.72 br d, 12.9	
8	36.3			36.3		
9	57.1	0.93 m	11, 23, 24	57.1	0.93 br d, 12.3	1, 8, 11, 12, 23, 24
10	37.1			37.1		
11	18.7	1.24 m 1.84 m		18.7	1.23 m 1.83 br d, 13.6	
12	41.5	1.59 m 2.02 m		41.5	1.59 m 2.02 br d, 12.6	11, 22
13	76.7			76.3		
14	52.3	1.57 m	8, 15, 22, 23	52.2	1.58 m	15, 22, 23
15	22.5	2.54 m	14, 16	22.5	2.54 m	8, 13, 14, 16, 17, 21
16	123.1			123.0		
17	147.0			147.0		
18	117.5	6.58 d, 8.3	16, 20	117.5	6.59 d, 8.9	16, 17, 20
19	114.2	6.55 dd, 8.3, 1.0	17, 21	114.2	6.54 dd, 8.9, 1.0	17, 20, 21
20	148.6			148.5		
21	115.7	6.53 d, 1.0	15, 17, 19	115.7	6.53 d, 1.0	15, 17, 19, 20
22	20.7	1.12 s	12, 13, 14	20.3	1.12 s	12, 13, 14
23	15.5	0.864 s	7, 8, 9, 14	15.43	0.86 s	7, 8, 9, 14
24	62.1	3.24 d, 11.0 4.05 d, 11.0	1, 5, 10 1	62.2	3.22 d, 11.2 4.07 d, 11.2	1, 5, 10, 26 1, 5, 10
25	23.1	0.855 s	3, 4, 5, 26	23.2	0.85 s	3, 4, 5, 26
26	106.0	4.14 s	3, 5, 24, 27	104.3	4.25 s	3, 5, 24, 25, 27
27	55.0	3.29 s	26	62.6	3.32 m 3.66 m	26, 28 26, 28
28				15.40	1.17 s	27

^a HMBC correlations were from protons started for the indicated carbons.

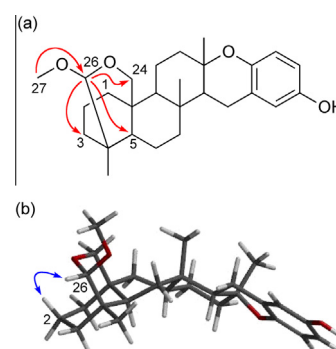


Figure 2. Key HMBC correlations (red arrows) (a) and a key NOE correlation (blue arrows) for the energy-minimized conformation of **1** calculated by Spartan'14 (Wavefunction, Inc.) (b).

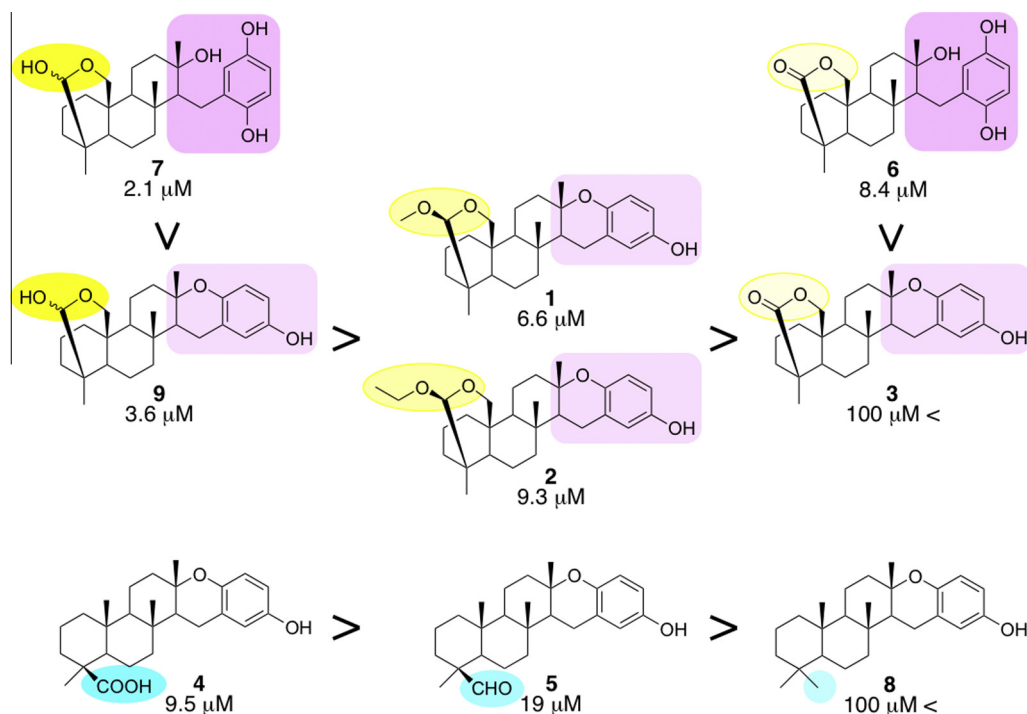


Figure 3. Structure–activity relationships of **1–9** with their IC_{50} values for the inhibition of the proteasome.

to date, this is the first study on the inhibitory activities of strongylophorine derivatives against the proteasome.

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- The marine sponge, *Petrosia corticata*, was collected at a depth of 10 m in North Sulawesi, Indonesia, in December 2007 and immediately soaked in EtOH. A voucher specimen (RMNH POR 8523) of the sponge has been deposited in the Naturalis Biodiversity Centre, The Netherlands.
- The human 20S proteasome from erythrocytes was purchased from Boston Biochem, Inc. (E-360). The fluorogenic substrate Suc-Leu-Leu-Val-Tyr-MCA (Peptide Institute, Inc.) was used as a substrate for the chymotrypsin-like activity of the proteasome. The proteasome in a reaction mixture (100 μ L) that contained 50 mM Tris-HCl, pH 7.8, 1 mM dithiothreitol, 5 mM EDTA, and 0.02% SDS was preincubated with test compounds at various concentrations at 30 $^{\circ}$ C for 10 min. The substrate (10 μ M) was then added to the mixture, which was further incubated at 30 $^{\circ}$ C for 6 h. The intensity of fluorescence owing to 7-amino-4-methylcoumarin (AMC) was measured (excitation, 360 nm; emission, 450 nm). The value of IC_{50} , the concentration required for the 50% inhibition of proteasome activity, was calculated from the data of duplicate measurements.
- The marine sponge (124 g, wet weight) was extracted with EtOH, and the extract was then partitioned between EtOAc and water. The EtOAc-soluble fraction (2.8 g) was subjected to silica gel column chromatography ($CHCl_3$ /MeOH (9:1)). Fraction A was purified by silica gel column chromatography (hexane/EtOAc) to afford fractions A1 (hexane/EtOAc (4:1), 194 mg), A2 (hexane/EtOAc (2:1), 86.2 mg), A3 (hexane/EtOAc (2:1), 410 mg), A4 (hexane/EtOAc (1:1), 220 mg), and A5 (hexane/EtOAc (1:2), 188 mg). The fraction A4 was identified as strongylophorine-8 (**6**). Fraction A1 was fractionated by gel filtration HPLC (Asahipak GS-310P, CH_2Cl_2 /MeOH/ H_2O (6:4:1)), NH_2 silica gel column chromatography (hexane/EtOAc (2:1)), and finally Diol HPLC (hexane/EtOAc (7.5:1)) to afford 26-O-methylstrongylophorine-16 (**1**, 4.4 mg), 26-O-ethylstrongylophorine-16 (**2**, 3.5 mg), and strongylophorine-22 (**8**, 49.0 mg). Fractions A2 and A3 were fractionated by Diol column chromatography (hexane/ CH_2Cl_2 (1:2) for fraction A2 and hexane/EtOAc (1:1) for fraction A3) to afford strongylophorine-4 (**5**, 17.0 mg) and strongylophorine-3 (**4**, 250 mg), respectively. Fraction A5 was fractionated by gel filtration HPLC (Asahipak GS-310P, CH_2Cl_2 /MeOH (1:1)) followed by ODS HPLC (MeOH/ H_2O (8:2)) to afford strongylophorine-2 (**3**, 3.2 mg) and strongylophorine-4 (**5**, 1.8 mg). Fraction B was purified by silica gel column chromatography ($CHCl_3$ /MeOH (15:1)), NH_2 silica gel column chromatography (CH_2Cl_2 /MeOH (95:5)), and Diol HPLC (CH_2Cl_2 / CH_3CN (95:5)) to afford a mixture of interchangeable strongylophorines-13/-14 (**7**, 4.8 mg).
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- 1**: $[\alpha]_D^{25} +31$ (c 0.50, CH_2Cl_2 /MeOH/ H_2O (6:4:1)); UV (CH_2Cl_2 /MeOH/ H_2O (6:4:1)) λ_{max} (log ϵ) 210 (4.5), 298 (3.7) nm; IR (film) ν_{max} 3387, 2928, 2851, 1493, 1231, 1045 cm^{-1} ; NMR data ($CDCl_3$), see Table 1; HRFABMS m/z 425.2660 $[M-H]^+$ (Calcd for $C_{27}H_{37}O_4$: 425.2692).
- A similar situation to strongylophorines-13 (26R) and -14 (26S) ^{10d} occurred with strongylophorines-15 (26R) and -16 (26S), which were also isolated as a mixture (**9**) of interchangeable hemiacetal isomers. ^{10d}
- 2**: $[\alpha]_D^{25} +18$ (c 0.50, CH_2Cl_2 /MeOH/ H_2O (6:4:1)); UV (CH_2Cl_2 /MeOH/ H_2O (6:4:1)) λ_{max} (log ϵ) 208 (4.2), 298 (3.4) nm; IR (film) ν_{max} 3373, 2926, 2851, 1493, 1449, 1231, 1107, 1041 cm^{-1} ; NMR data ($CDCl_3$), see Table 1; key NOESY

- correlations: H-24 (δ_{H} 3.22)/H₃-23 (δ_{H} 0.86) and H-1 (δ_{H} 2.20); H-26 (δ_{H} 4.25)/H-3 (δ_{H} 1.50) and H₂-27 (δ_{H} 3.32); HRFABMS m/z 439.2851 [$\text{M}-\text{H}$][−] (Calcd for C₂₈H₃₉O₄: 439.2848).
14. DIBAL-H (8.5 mL, 0.048 mmol) was added to a solution of **3** (10 mg, 0.024 mmol) in toluene (0.2 mL) and the mixture was stirred at −78 °C for 1.5 h. Rochelle salt was added to this mixture, and the resulting mixture was stirred at room temperature. After 30 min, the mixture was partitioned

between CH₂Cl₂ and H₂O. The CH₂Cl₂ layer was subjected to Diol HPLC with CH₂Cl₂/CH₃CN (99:1) to afford a mixture (**9**, 2.3 mg) of strongylophorines-15/-16. **9**: ¹H NMR (500 MHz, pyridine-*d*₅), strongylophorine-15: δ 0.75 (3H, s, H₃-23), 1.03 (3H, s, H₃-25), 1.165 (3H, s, H₃-22), 3.67 (d, J = 10.0 Hz, H₂-24), 4.20 (d, J = 10.0 Hz, H₂-24), 5.20 (br s, H-26); strongylophorine-16: δ 0.86 (3H, s, H₃-23), 1.10 (3H, s, H₃-25), 1.159 (3H, s, H₃-22), 3.42 (d, J = 11.0 Hz, H₂-24), 4.61 (d, J = 11.0 Hz, H₂-24), 5.08 (br s, H-26); FABMS m/z 411 [$\text{M}-\text{H}$][−].