Tetradehydrohalicyclamine B, a new proteasome inhibitor from the marine sponge *Acanthostrongylophora ingens*

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ABSTRACT

A new halicyclamine derivative, tetradehydrohalicyclamine B (**1**), was isolated from the marine sponge *Acanthostrongylophora ingens,* along with halicyclamine B (**2**) as proteasome inhibitors. Compound **1** is the second example found to have a pyridinium ring in the halicyclamine family. Although the relative configuration of **2** was previously determined by X-ray crystallographic analysis, here we determined the absolute configuration of **2** by ECD experiment. Compounds **1** and **2** inhibited the constitutive proteasome as well as the immunoproteasome. The inhibitory activities of **2** were 4- to 10-fold more potent than those of **1**.

Keywords: proteasome inhibitor, constitutive proteasome, immunoproteasome, halicyclamine, *Acanthostrongylophora ingens*

Natural products play a significant role in the discovery and development of drug entities.[1] Among the secondary metabolites from marine sponges, 3-alkylpiperidine alkaloids show a variety of structures including monomeric 3-alkylpyridines (theonelladine A[2]), dimeric (cyclostellettamine A[3]) or oligomeric pyridinium macrocycles (halitoxin[4]), and condensed bis-3-alkylpiperidines of the halicyclamine class (halicyclamine A[5]) and manzamine class (manzamine A[6]). More than 20 halicyclamine derivatives have been isolated so far, showing a wide range of biological activities including cytotoxic,[7,8] antifungal,[8] antibacterial,[8] anti-tuberculosis,[9] and CK1δ/ε inhibitory[10] activities. In the search for the bioactive metabolites from marine invertebrates, we found that the EtOH extract of the marine sponge *Acanthostrongylophora ingens*[11] (600 g, wet weight) showed strong cytotoxicity, with a survival ratio of 1% at 50 μg/mL. Bioassay-guided purification furnished a new metabolite, tetradehydrohalicyclamine B (**1**), along with the known halicyclamine B (**2**) (Fig. 1). We here report the structure determination and biological activity of the new compound.

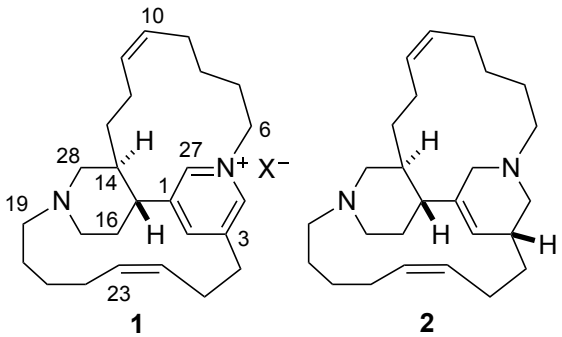

Fig. 1. Structures of **1** and **2**.

The sponge extract was partitioned between EtOAc and H_2O[12] and the fractions showed 1 and 2% survival ratios, respectively, at 50 μg/mL. The ^{1}H NMR spectrum readily showed the main metabolite in the EtOAc fraction to be manzamine A.[6] The H_2O fraction was extracted with *n*-BuOH, and the *n*-BuOH fraction with a survival ratio of 1% (50 μg/mL) was fractionated and afforded a new compound **1** (4.1 mg) along with **2** (873 mg).

Compound **1** has the molecular formula of $C_{26}H_{39}N_2$, having one H_3 unit less than **2**. The ^{1}H NMR spectrum of **1** showed four olefinic signals (δ_H 5.16 (H-10), 5.26 (H-24), 5.28 (H-11), and 5.45 (H-23)) (Table 1) and methylene and methine signals in δ_H 1.1–3.0, which was similar to that of **2** except for the presence of three singlet low-field signals (δ_H 8.42 (H-2), 8.84 (H-4), and 8.89 (H-27)) and mutually-coupled methylene signals (δ_H 4.95 and 5.05 (H_2-6)) along with the absence of an olefinic signal at δ_H 5.8 (br s, H-2) observed in **2**. Interpretation of 2D NMR spectra of **1**, including COSY, HSQC, and HMBC, indicated the presence of a 1,3,4-trisubstituted piperidine ring and two aliphatic chains that were identical to those of **2** (Fig. 2). Detailed analysis of HMBC correlations showed that **1** contained a 1,3,5-trisubstituted pyridinium ring (δ_C 148.0 (C-1); δ_H 8.42/δ_C 143.0 (C-2); δ_C 143.3 (C-3); δ_H 8.84/δ_C 143.1 (C-4); δ_H 8.89/δ_C 140.7(C-27)) instead of a 1,2,3,6-tetrahydropyridine ring in **2**. It is noteworthy that **1** is the second halicyclamine derivative found to contain the 1,3,5-trisubstituted pyridinium ring,[7] although more than 20 derivatives have been reported so far. Although the relative configuration of **2** was determined by X-ray study,[13] the absolute configuration has remained undetermined. We calculated the ECD spectrum of **2** to determine the absolute configuration in the same

manner as previously reported.[14] Briefly, the ECD calculation was performed at the BHandHLYP/TZVP level and the wavelength was corrected by +20 nm to match the experimental and calculated UV maximum at 195 nm. The experimental ECD spectrum of **2** was similar to the calculated spectrum of 3*R*,14*S*,15*R*-**2** (Fig. 3), and therefore, the biosynthetic relationship indicates that **1** may contain the 14*S*,15*R*-configuration. The absolute configuration of chloromethylhalicyclamine B has also been determined by ECD experiment as 3*R*,14*S*,15*R*.[10]

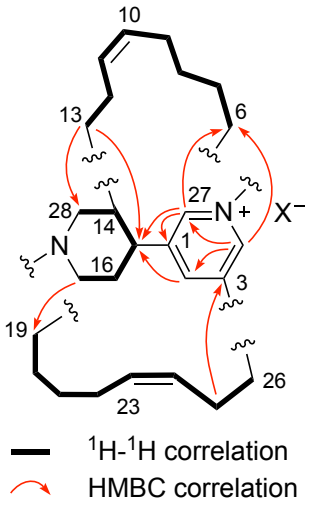

Fig. 2. ^{1}H-^{1}H and HMBC correlations of **1**.

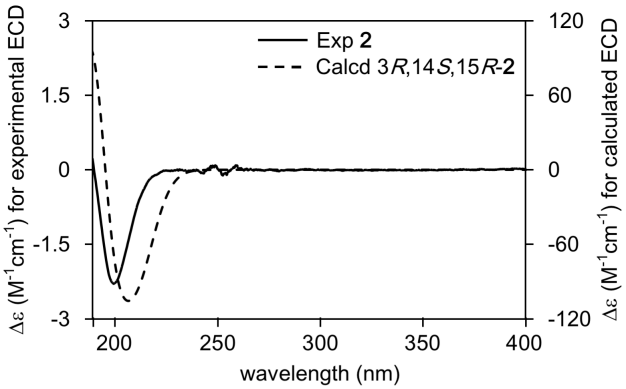

Fig. 3. Experimental ECD spectrum (MeCN) of **2** and calculated ECD spectrum of 3*R*,14*S*,15*R*-**2** with BHandHLYP/TZVP level.

Table 1

NMR data[a] for **1** in $CDCl_3$.

No.	δ_C	δ_H, mult (*J* in Hz)	HMBC[b]	No.	δ_C	δ_H, mult (*J* in Hz)	HMBC[b]
1	148.0			17	49.8	2.58, m	
2	143.0	8.42, s	15, 26			3.07, m	
3	143.3			19	53.4	2.36, m	
4	143.1	8.84, s	2, 6, 26, 27			2.63, m	
6	59.6	4.95, m		20	25.0	1.87, m	
		5.05, m				2.44, m	
7	25.6	2.07, m		21	32.9	1.13, m	
8	28.9	1.32, m				1.47, m	
		2.09, m		22	25.2	1.89, m	
9	25.3	1.88, m				2.10, m	
		2.46, m		23	132.2	5.45, m (10.7)	
10	129.5	5.16, m (10.3)		24	127.3	5.26, m (10.7)	
11	129.8	5.28, m (10.3)		25	25.2	2.42, m	
12	25.4	1.72, m				2.68, m	3
13	33.1	1.18, m	15, 28	26	31.2	2.98, m	24
		1.44, m		27	140.7	8.89, s	1, 6, 15
14	40.2	1.62, m		28	53.6	1.92, m	
15	49.7	3.02, m	2, 27			2.68, m	
16	40.5	2.04, m	1				
		2.54, m					

[a] ^1H NMR: 600 MHz, ^{13}C NMR: 150 MHz.

[b] HMBC correlations were from proton(s) stated to the indicated carbon(s).

The cytotoxic activities of **1** and **2** were tested by HeLa cells.[15] Compound **2** showed cytotoxicity with an IC_{50} value of 12 μM, whereas **1** did not show cytotoxicity even at 50 μM (Table 2). Further, we evaluated the biological activities using our in-house screening system and found that **1** and **2** inhibited the proteasome.[15] The IC_{50} values of **2** for the

chymotrypsin-like (Ch-L), trypsin-like (T-L), and caspase-like (C-L) activities of the constitutive proteasome were 0.42, 6.3, and 0.48 μM, respectively, while those of **1** were less potent (Table 2). These potencies were similar to those against the immunoproteasome (Table 2), which indicates that **1** and **2** are not selective inhibitors for either the constitutive proteasome or the immunoproteasome. These results show that the cytotoxicity and inhibition of the proteasome of **2** are 4- to 10-fold more potent than those of **1**, which indicates that the presence of the pyridinium ring in **1** may be involved in the decrease of these activities. Following the US Food and Drug Administration (FDA) approval of the proteasome inhibitors, e.g., bortezomib (Velcade),[17] calfilzomib (Kyprolis),[18] and ixazomib (Ninlaro),[19] proteasome inhibitors are recognized as effective anti-cancer agents. The constitutive proteasome is expressed ubiquitously and plays a role in non-lysosomal intracellular protein degradation. In contrast, the immunoproteasome is mostly found in immune cells.[20] While the proteasome inhibitors, currently used for therapeutic interventions, inhibit both proteasomes, selective inhibitors of the immunoproteasome are needed and anticipated for the treatment of autoimmune disorders.[21,22] So far, preparation from the epoxyketone analogues has afforded selective inhibitors for the constitutive proteasome, PR-825[23] and PR-893 (CPSI),[24] and for the immunoproteasome, PR-924 (IPSI)[24] and PR-957.[23] A search for selective inhibitors for the immunoproteasome with new scaffolds from natural sources is now underway in our laboratory.

Table 2

Biological activities (IC_{50}, μM) of **1** and **2**.

Compd	Cytotoxicity	Constitutive proteasome			Immuno-proteasome		
		CT-L	T-L	C-L	CT-L	T-L	C-L
1	a	1.8	b	4.7	4.1	b	3.1
2	12	0.42	6.3	0.48	0.63	8.0	0.44

[a] Not cytotoxic even at 50 μM. [b] No inhibition even at 15 μM.

Acknowledgements

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11. Animal material: The marine sponge (600 g, wet weight) was collected at a depth of 10 m at Bajotalawaan in North Sulawesi, Indonesia, in December 2006, and immediately soaked in EtOH. The sponge was identified as *Acanthostrongylophora ingens*. A voucher specimen (06M115, RMNH POR. 3992) has been deposited in the Naturalis Biodiversity Center, The Netherlands.

12. Extraction and isolation: The sponge was extracted with EtOH. After evaporation, the residual aqueous solution was extracted with EtOAc and then *n*-BuOH. The *n*-BuOH fraction (4.6 g) was subjected to Diaion HP-20 column chromatography with H_2O and MeOH. The fraction (1.7 g) eluted with MeOH was fractionated by Sephadex LH-20 column chromatography with CH_2Cl_2/MeOH (35:65) followed by NH_2 HPLC (Inertsil NH_2) with CH_2Cl_2 to afford **1** (4.1 mg) and **2** (873 mg). Tetradehydrohalicyclamine B (**1**): yellow, amorphous solid; $[\alpha]^{D}_{21}$ +55 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 272 nm (3.0); ECD (MeCN) λ_{max} (Δε) 317 (-0.20), 272 (0.32), 229 (-0.33), 206 (0.84), 194 (-2.98) nm; IR (MeOH) ν_{max} 3376, 2924, 2854, 1635, 1464 cm^{-1}; NMR data, see Table 1; HRESITOFMS *m/z* 379.3115 $[M]^+$ (calcd for $C_{26}H_{39}N_2^+$, 379.3108).

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of the constitutive proteasome, respectively. For the assay of the C-L activity of the immunoproteasome, Ac-Pro-Ala-Leu-MCA (S-310, Boston Biochem, Inc.) was used instead of Z-Leu-Leu-Glu-MCA. The human 20S proteasome from erythrocytes (S-360) and the human 20S immunoproteasome (S-370) were purchased from Boston Biochem, Inc.

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