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1-HYDROXYETHYLHALENAQUINONE: A NEW PROTEASOME INHIBITOR FROM THE MARINE SPONGE *XESTOSPONGIA* SP.

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Abstract – A new halenaquinone derivative, 1-hydroxyethylhalenaquinone (**1**), was isolated from the marine sponge *Xestospongia* sp. as a proteasome inhibitor together with three known compounds, halenaquinone (**2**) and 3-ketoadociaquinones A (**3**) and B (**4**). 1-Hydroxyethylhalenaquinone (**1**) was the first halenaquinone derivative containing an alkyl group at the keto-furan C-1 position. Compounds **1** and **2** inhibited the chymotrypsin-like activity of the proteasome with IC_{50} values of 0.19 and 0.63 μM, respectively, whereas **3** or **4**, each containing a thiomorpholine 1,1-dioxide moiety, scarcely inhibited its activity, even at a concentration of 5 μM.

Halenaquinone (**2**, Figure 1)[1] is a pentacyclic compound that has been isolated from marine sponges. This compound and its derivatives are known to exhibit antimicrobial, antifungal, cytotoxic, and antimalarial activities and also inhibit various enzymes, such as v-Src tyrosine kinase,[2] phosphoinositide 3-kinase (PI3K),[3] Cdc25B phosphatase,[4] RAD51 (homologous-paring activity),[5] phospholipase A_2,[6] and farnesyltransferase.[6] In our search for biologically active natural products, we found that the extract of the marine sponge *Xestospongia* sp. inhibited proteasome activity. We here described the bioassay-guided isolation of a new halenaquinone derivative, 1-hydroxyethylhalenaquinone (**1**, Figure 1), and **2** as proteasome inhibitors.

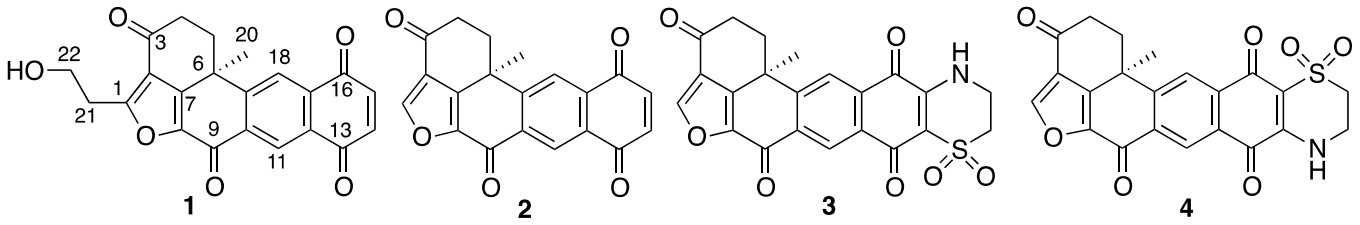

Figure 1. Structures of **1**-**4**

The sponge collected in Likpan, North Sulawesi, Indonesia, was immediately soaked in EtOH. The EtOH extract was partitioned between EtOAc and H_2O. The EtOAc-soluble fraction was purified to afford **1**, **2**, and 3-ketoadociaquinones A (**3**) and B (**4**)[7] (Figure 1). Compound **1** was obtained as a brown amorphous solid, and HRFABMS established its molecular formula as $C_{22}H_{16}O_6$. 2D NMR spectra including COSY, HSQC, and HMBC (Table 1 and Figure 2) indicated that **1** was a congener of **2**, except for the presence of a hydroxyethyl group [δ_H 3.39 (2H, m, H_2-21), 4.07 (2H, m, H_2-22); δ_C 32.5 (C-21), 60.2 (C-22)] and quaternary carbon [δ_C 165.6 (C-1)] together with the absence of the sp_2 methine [δ_H 8.25 (1H, s, H-1); δ_C 149.0 (C-1)] detected in **2**. HMBC correlations from H_2-21 to C-2 (δ_C 118.5) and from H_2-22 to C-1 showed that the hydroxyethyl group in **1** was attached to C-1. The CD spectrum of **1** was similar to that of **2**, which indicated that **1** and **2** had the same absolute configuration, 6*S* (Figure 3). Although various halenaquinone derivatives have been isolated from marine sponges to date, **1** was the first containing an alkyl group at the keto-furan C-1 position.

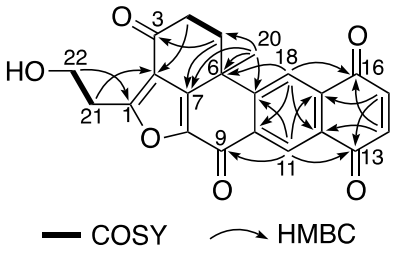

Figure 2. COSY and key HMBC correlations of **1**

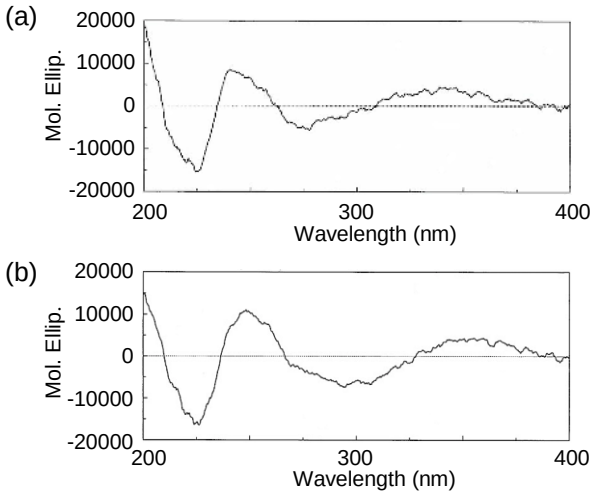

Figure 3. CD spectra of **1** (a) and **2** (b) in MeCN

Since the synthetic proteasome inhibitor, bortezomib (Velcade™), was approved for the treatment of relapsed multiple myeloma,[8] drugs targeting the proteasome and the upstream ubiquitin system have been anticipated as excellent anticancer agents.[9] Therefore, we have been searching for inhibitors of the ubiquitin−proteasome system from natural sources.[10] In this study, **1** and **2** were identified as inhibitors of

the chymotrypsin-like activity of the proteasome with IC_{50} values of 0.19 and 0.63 μM, respectively, while **3** and **4** scarcely inhibited its activity, even at a concentration of 5 μM. Compounds **3** and **4** contained a thiomorpholine 1,1-dioxide unit that was attached to the quinone moiety. Structurally diverse proteasome inhibitors have been identified and many form covalent bonds with the active-site threonine residue of the proteasome.[11] The structure-activity relationships among **1**-**4** suggested that the C-14 and/or C-15 positions of the quinone in **1** and **2** may undergo Michael-type 1,4-addition of the hydroxy group of the catalytic threonine residue in a manner similar to syringolin A,[12] a proteasome inhibitor composed of a 12-membered lactam with an α,β-unsaturated vinyl ketone.

Table 1. ^{1}H and ^{13}C NMR data for **1** ($CDCl_3$)[a]

Position	δ_C	δ_H (*J* in Hz)	HMBC
1	165.6		
2	118.5		
3	192.4		
4	36.4	2.82 m	2, 3, 5
		3.00 ddd 19.2, 13.3, 5.6	3, 5, 6, 7
5	33.3	2.82 m	3, 6, 7, 8, 20
		2.26 td 13.3, 4.8	4, 6, 19, 20
6	36.8		
7	148.8		
8	143.0		
9	169.6		
10	137.4		
11	127.3	9.04 s	1, 9, 13, 17
12	130.8		
13	183.5		
14	139.4	7.07 d 10.4	12, 16
15	138.7	7.04 d 10.4	13, 14, 17
16	184.4		
17	133.7		
18	123.6	8.23 s	6, 9, 10, 12, 16, 17
19	153.9		
20	30.7	1.66 s	5, 6, 7, 19
21	32.5	3.39 m	1, 2
22	60.2	4.07 m	1, 21

[a] ^{1}H and ^{13}C NMR spectra were measured at 500 and 125 MHz, respectively.

EXPERIMENTAL

General Experimental Procedures.

Optical rotation was measured on a JASCO DIP-1000 polarimeter in DMSO. UV spectrum was measured on a JASCO V-550 spectrophotometer in MeCN. CD spectrum was measured on a JASCO J-820 spectropolarimeter in MeCN. IR spectrum was measured on a JEOL JIR-6500W spectrophotometer.

NMR spectra were recorded on a Bruker Avance 500 spectrometer in $CDCl_3$. Chemical shifts were referenced to residual solvent peaks (δ_H 7.24 and δ_C 77.0 for $CDCl_3$). Mass spectrum was measured on a JEOL JMS-700 MStation mass spectrometer.

Biological Material.

The marine sponge was collected at a depth of 10 m in Likpan, Indonesia, in December 2006 and immediately soaked in EtOH. The sponge was identified as *Xestospongia* sp. A voucher specimen (RMNH POR. 8674) has been deposited in the Naturalis Biodiversity Center, The Netherlands.

Extraction and Isolation.

The marine sponge (400 g, wet weight) was extracted with EtOH. The concentrated aqueous solution was subsequently extracted with EtOAc. The EtOAc fraction (3.9 g) was subjected to silica gel column chromatography with $CHCl_3$/MeOH (95:5) (fraction A) and $CHCl_3$/MeOH (80:20) (fraction B). Fraction A (1.5 g) was purified by silica gel column chromatography with hexane/EtOAc (60:40) (fraction A1, 39.8 mg) and $CHCl_3$/MeOH (80:20) (fraction A2, 471.4 mg). Fraction A1 was identified as halenaquinone (**2**) based on the ^1H and ^{13}C NMR spectra and FAB mass spectrum. Fraction A2 was purified by silica gel column chromatography with $CHCl_3$/MeOH (98:2) to afford fractions A2-1 (86.3 mg) and A2-2 (104.0 mg), which were further purified by silica gel HPLC with a gradient elution from $CHCl_3$ to $CHCl_3$/MeOH (98:2) to afford 1-hydroxyethylhalenaquinone (**1**, 3.2 mg) and 3-ketoadociaquinone A (**3**, 10.8 mg), respectively. Fraction B (299.4 mg) was purified by Sephadex LH-20 with CH_2Cl_2/MeOH/H_2O (6:4:1) and Diol HPLC with a gradient elution from hexane/CH_2Cl_2/MeOH (100:95:5) to CH_2Cl_2/MeOH (95:5) to afford 3-ketoadociaquinone B (**4**, 4.4 mg).

Measurement of Proteasome Inhibitory Activity.

The chymotrypsin-like activity of the proteasome was measured with a rat liver proteasome preparation using the fluorogenic substrate, Suc-Leu-Leu-Val-Tyr-MCA (Peptide Institute). The proteasome in a mixture (100 μL) that contained 50 mM Tris-HCl, pH 7.8, 1 mM dithiothreitol, 5 mM EDTA, and 0.02% SDS was pre-incubated with test compounds at various concentrations at 30 ^oC for 10 min. Then, the substrate (10 μM) was added to the mixture and the mixture was further incubated at 30 ^oC for 6 h. The reaction was stopped by adding 100 μL of 10% SDS and the fluorescence intensity owing to 7-amino-4-methylcoumarin (AMC) was measured (excitation, 360 nm; emission, 450 nm). The value of IC_{50}, the concentration required for 50% inhibition of proteasome inhibitory activity, was calculated from the data of duplicate measurements.

1-Hydroxyethylhalenaquinone (1): A brown amorphous solid; $[\alpha]_D^{20}$ -11.5° (*c* 0.13, DMSO); UV (MeCN) λ_{max} (log ε) 292 (3.92), 260 (5.01), 214 (4.16) nm; CD (MeCN), see Figure 3. IR (film) ν_{max} 3423, 2926, 1672, 1322 cm^{-1}; HRFABMS *m/z* 377.1029 $[M+H]^+$ (calcd for $C_{22}H_{17}O_6$, 377.1025); ^1H and ^{13}C NMR data ($CDCl_3$), see Table 1.

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REFERENCES

1. D. M. Roll, P. J. Scheuer, G. K. Matsumoto, and J. Clardy, *J. Am. Chem. Soc.*, 1983, **105**, 6177.
2. R. H. Lee, D. L. Slate, R. Moretti, K. A. Alvi, and P. Crews, *Biochem. Biophys. Res. Commun.*, 1992, **184**, 765.
3. H. Fujiwara, K. Matsunaga, M. Saito, S. Hagiya, K. Furukawa, H. Nakamura, and Y. Ohizumi, *Eur. J. Pharmacol.*, 2001, **413**, 37.
4. S. Cao, C. Foster, M. Brisson, J. S. Lazo, and D. G. Kingston, *Bioorg. Med. Chem.*, 2005, **13**, 999.
5. M. Takaku, T. Kainuma, T. Ishida-Takaku, S. Ishigami, H. Suzuki, S. Tashiro, R. W. van Soest, Y. Nakao, and H. Kurumizaka, *Genes Cells*, 2011, **16**, 427.
6. A. Longeon, B. R. Copp, M. Roué, J. Dubois, A. Valentin, S. Petek, C. Debitus, and M.-L. Bourguet-Kondracki, *Bioorg. Med. Chem.*, 2010, **18**, 6006.
7. F. J. Schmitz and S. J. Bloor, *J. Org. Chem.*, 1988, **53**, 3922.
8. J. Adams, *Drug Discov. Today*, 2003, **8**, 307.
9. (a) L. Bedford, J. Lowe, L. R. Dick, R. J. Mayer, and J. E. Brownell, *Nat. Rev. Drug Disc.*, 2011, **10**, 29; (b) P. Cohen and M. Tcherpakov, *Cell*, 2010, **143**, 686.
10. (a) E1 inhibitor: R. Yamanokuchi, K. Imada, M. Miyazaki, H. Kato, T. Watanabe, M. Fujimuro, Y. Saeki, S. Yoshinaga, H. Terasawa, N. Iwasaki, H. Rotinsulu, F. Losung, R. E. P. Mangindaan, M. Namikoshi, N. J. de Voogd, H. Yokosawa, and S. Tsukamoto, *Bioorg. Med. Chem.*, 2012, **20**, 4437; (b) E2 inhibitor: S. Ushiyama, H. Umaoka, H. Kato, Y. Suwa, H. Morioka, H. Rotinsulu, F. Losung, R. E. P. Mangindaan, N. J. de Voogd, H. Yokosawa, and S. Tsukamoto, *J. Nat. Prod.*, 2012, **75**, 1495; (c) E3 inhibitor: Y. Nakamura, H. Kato, T. Nishikawa, N. Iwasaki, Y. Suwa, H. Rotinsulu, F. Losung, W. Maarisit, R. E. P. Mangindaan, H. Morioka, H. Yokosawa, and S. Tsukamoto, *Org. Lett.*, 2013, **15**, 322; (d) Deubiquitinating enzyme inhibitor: M. Yamaguchi, M. Miyazaki, M. P. Kodrasov, H. Rotinsulu, F. Losung, R. E. P. Mangindaan, N. J. de Voogd, H. Yokosawa, B. Nicholson, and S. Tsukamoto, *Bioorg. Med. Chem. Lett.*, 2013, **23**, 3884; (e) Proteasome inhibitor: S. Tsukamoto, R. Yamanokuchi, M. Yoshitomi, K. Sato, T. Ikeda, H. Rotinsulu, R. E. P. Mangindaan, N. J. de Voogd, R. W. M. van Soest, and H. Yokosawa, *Bioorg. Med. Chem. Lett.*, 2010, **20**, 3341.
11. A. F. Kisselev, W. A. van der Linden, and H. S. Overkleeft, *Chem. Biol.*, 2012, **19**, 99.
12. M. Groll, B. Schellenberg, A. S. Bachmann, C. R. Archer, R. Huber, T. K. Powell, S. Lindow, M.

Kaiser, and R. Dudler, *Nature*, 2008, **452**, 755.