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Structure and Activity of Largazole, a Potent Antiproliferative Agent from the Floridian Marine Cyanobacterium *Symploca* sp.

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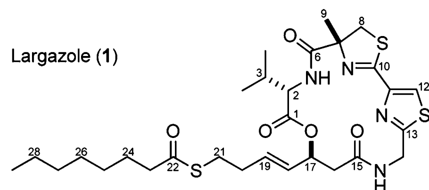
The identification of new pharmacophores is of paramount biomedical importance and natural products have recently been regaining attention for this endeavor.[1] This renaissance is closely tied to the successful exploitation of the marine environment which harbors unmatched biodiversity that is presumably concomitant with chemical diversity.[2] In particular, marine cyanobacteria are prolific producers of bioactive secondary metabolites,[3] many of which are modified peptides or peptide−polyketide hybrids with promising antitumor activities, such as dolastatin 10,[4] curacin A,[5] and apratoxin A.[6] As a result of our ongoing investigations to identify new drug leads from cyanobacteria in Florida, we report here the structure determination and preliminary biological characterization of a marine cyanobacterial metabolite with novel chemical scaffold and nanomolar antiproliferative activity from a cyanobacterium of the genus *Symploca*. *Symploca* species have scarcely been investigated compared to the more prevalent *Lyngbya* spp., yet a Palauan *Symploca* sp. previously yielded the clinical trial compound dolastatin 10,[4] prompting us to target this genus.

A sample of *Symploca* sp. was collected from Key Largo, Florida Keys, and extracted with organic solvents. The resulting cytotoxic crude extract was subjected to bioassay-guided fractionation by solvent partition, silica gel chromatography, and reversed-phase HPLC to yield largazole (**1**) as a colorless, amorphous solid {$[\alpha]^{20}_{D}$ +22 (*c* 0.1, MeOH)}.



^{1}H and ^{13}C NMR data coupled with a $[M + H]^+$ peak at *m/z* 623.2397 in the HRESI/APCIMS of **1** suggested a molecular formula of $C_{29}H_{42}N_4O_5S_3$ (Δ +0.1 mmu, Δ +0.16 ppm). The ^{1}H NMR spectrum exhibited two signals characteristic for secondary amides ($\delta_{2\text{-NH}}$ 7.15, $\delta_{14\text{-NH}}$ 6.45). Further two-dimensional NMR analysis in $CDCl_3$ using COSY, HSQC, and HMBC data indicated that these exchangeable protons belong to valine and modified glycine residues, respectively (Table 1 and Supporting Information). The putative glycine carbonyl ($\delta_{C\text{-}13}$ 167.9) was part of a 2,4-disubstituted thiazole unit as evidenced by HMBCs from the only aromatic methine ($\delta_{H\text{-}12}$ 7.76, $\delta_{C\text{-}12}$ 124.2) to C-13 and to another quaternary sp^2 carbon, C-11 (δ_C 147.4). Furthermore, HMBCs from a methyl singlet ($\delta_{H\text{-}9}$ 1.87) to carbonyl C-6 (δ_C 173.5), quaternary carbon C-7 (δ_C 84.4), and methylene carbon C-8 (δ_C 43.3), combined with an HMBC from H-8a (δ_H 4.04) to C-10 (δ_C 164.6) suggested the presence of a 2-substituted thiazoline-4-methyl-4-carboxylic acid unit (C-6 to C-10). The only other HMBC to C-10 was from the thiazole proton H-12, indicating that C-10 bore the thiazole substituent. The methyl thiazoline carboxylate and the amino terminus of the valine residue were unambiguously connected via an amide linkage based on HMBC data (Table 1). The remaining signals in the ^{1}H NMR spectrum belonged to two spin systems, as concluded from COSY analysis (Supporting Information). One of the units was a 7-substituted 3-hydroxyhept-4-enoic acid moiety (C-15 to C-21) with *E*-geometry of the double bond based on a large coupling constant for $^3J_{H\text{-}18,H\text{-}19}$ of 15.6 Hz, consistent with NOESY cross-peaks between H-18 and H$_2$-20. This unit was attached to the amino terminus of the glycine-derived unit as shown by HMBCs from 14-NH and H-14a/b to C-15 as well as ROESY cross-peaks between 14-NH and H-16a and H-16b. The last unit

Table 1. NMR Spectral Data for Largazole (**1**) in $CDCl_3$ (600 MHz)

C/H no.	δ_H (*J* in Hz)	δ_C, mult.	HMBC[a,b]
1		168.9, qC	
2	4.61, dd (9.2, 3.3)	57.7, CH	1, 3, 4, 5, 6
3	2.10, m	34.2, CH	1,[c] 2[c]
4	0.68, d (7.2)	18.9, CH$_3$	2, 3, 5
5	0.50, d (7.2)	16.6, CH$_3$	2, 3, 4
2-NH	7.15, d (9.2)		1, 6[c]
6		173.5, qC	
7		84.4, qC	
8a	4.04, d (−11.4)	43.3, CH$_2$	6, 7, 10
8b	3.27, d (−11.4)		6, 7, 9
9	1.87, br s	24.2, CH$_3$	6, 7, 8
10		164.6, qC	
11		147.4, qC	
12	7.76, s	124.2, CH	10,[c] 11, 13
13		167.9, qC	
14a	5.29, dd (−17.4, 9.6)	41.1, CH	13, 15
14b	4.27, dd (−17.4, 2.5)		13, 15
14-NH	6.45, dd (9.6, 2.5)		15[c]
15		169.4, qC	
16a	2.86, dd (−16.5, 10.5)	40.5, CH$_2$	15, 17, 18
16b	2.68, dd (−16.5, 1.8)		15
17	5.66, ddd (10.5, 7.2, 1.8)	72.0, CH	
18	5.51, dd (15.6, 7.2)	128.4, CH	17, 20
19	5.82, dt (15.6, 7.2)	132.7, CH	17, 20
20	2.31, br q (7.2) (2H)	32.3, CH$_2$	18, 19, 21
21	2.90, t (7.2) (2H)	27.9, CH$_2$	19, 20, 22
22		199.4, qC	
23	2.52, t (7.5) (2H)	44.1, CH$_2$	22, 24, 25
24	1.64, m (2H)	25.6, CH$_2$	22, 23, 25/26
25	1.29, m (2H)	28.9, CH$_2$	26
26	1.25, m (2H)	28.9, CH$_2$	25, 27
27	1.26, m (2H)	31.6, CH$_2$	
28	1.28, m (2H)	22.6, CH$_2$	
29	0.87, br t (6.9)	14.0, CH$_3$	27, 28

[a] Protons showing HMBC correlations to the indicated carbon. [b] Optimized for ^nJ = 7 Hz if not indicated otherwise. [c] Optimized for ^nJ = 3.5 Hz.

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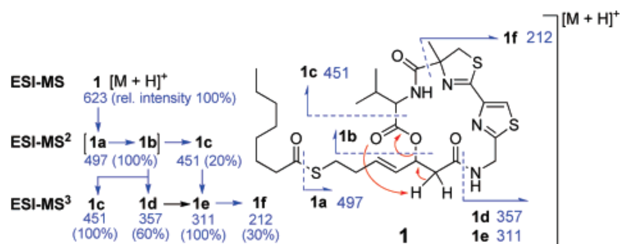

Figure 1. MS^n fragmentation pattern for largazole (**1**).

Scheme 1. Degradation Strategy to Liberate Chiral Subunits

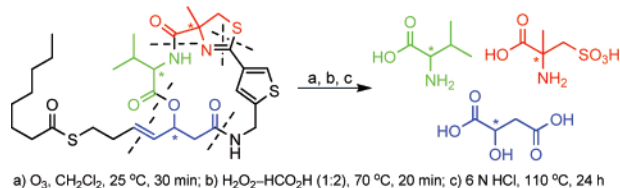

Table 2. Growth-Inhibitory Activity (GI_{50}) of Natural Product Drugs

	MDA-MB-231	NMuMG	U2OS	NIH3T3
Largazole (**1**)	7.7 nM	122 nM	55 nM	480 nM
Paclitaxel	7.0 nM	5.9 nM	12 nM	6.4 nM
Actinomycin D	0.5 nM	0.3 nM	0.8 nM	0.4 nM
Doxorubicin	310 nM	63 nM	220 nM	47 nM

was an *n*-octanoyl group (C-22 to C-29) which was connected with C-21 based on HMBC from H_2-21 to C-22. The low-field chemical shift for C-22 (δ_C 199.4) coupled with the fact that one sulfur atom remained yet to be assigned was strong evidence for a thioester functionality. Finally, to account for the molecular formula requirements and for the low-field chemical shift of H-17 (δ_H 5.66) suggestive of an acyloxy substituent, C-17 had to be ester-linked to the carboxyl terminus of valine. This was further supported by a weak NOE between H-17 and H_3-5 (δ_H 0.50), leading to the cyclic planar structure shown for **1**. MS^n analysis (Figure 1, Scheme S1, Supporting Information) is consistent with the proposed structure.

To assign the absolute configuration of the three chiral centers, our strategy was to generate optically active fragments, for which enantiomeric standards are readily available (Scheme 1). Specifically, ozonolysis followed by oxidative workup and acid hydrolysis generated 2-methylcysteic acid, valine, and malic acid. The product mixture was subjected to chiral HPLC analysis, comparing retention times with those of authentic standards. This analysis identified L-valine, (*R*)-2-methylcysteic acid, and L-malic acid, establishing the absolute configuration of **1** as 2*S*,7*R*,17*S*.

Largazole (**1**) potently inhibited the growth of highly invasive transformed human mammary epithelial cells (MDA-MB-231) in a dose-dependent manner (GI_{50} 7.7 nM) and induced cytotoxicity at higher concentrations (LC_{50} 117 nM). In contrast, nontransformed murine mammary epithelial cells (NMuMG) were less susceptible to compound **1** (GI_{50} 122 nM, LC_{50} 272 nM). We have not observed this remarkable selectivity for other validated antitumor natural products tested in parallel (Table 2). Similarly, the selectivity of **1** for transformed fibroblastic osteosarcoma U2OS cells (GI_{50} 55 nM, LC_{50} 94 nM) over nontransformed fibroblasts NIH3T3 (GI_{50} 480 nM, LC_{50} >8 μM) was unmatched by other natural product drugs tested (Table 2). The differential growth-inhibitory activity between transformed and nontransformed cells suggests that cancer cells are preferentially targeted by **1**. The growth of cancer cell lines derived from colon (HT29) and neuroblastoma (IMR-32) was also strongly inhibited by **1** (GI_{50}/LC_{50} 12 nM/22 nM; 16 nM/22 nM).

Largazole (**1**) possesses a dense combination of unusual structural features, including a substituted 4-methylthiazoline linearly fused to a thiazole as in didehydromirabazole,[7] a member of the group of terrestrial cyanobacterial cytotoxins from *Scytonema mirabile* with solid tumor selectivity.[8] Another remarkable structural element is the thioester moiety; thioester-containing secondary metabolites have been reported to be produced by sponges,[9] eukaryotic algae,[10] and bacteria,[11] but not by cyanobacteria. The 3-hydroxy-7-mercaptohept-4-enoic acid unit in **1** is unprecedented in natural products. Most significantly, the potent biological activity and selectivity for cancer cells warrants further investigation as to the mode of action, cancer chemotherapeutic potential, and biosynthesis of largazole (**1**).

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Supporting Information Available: Experimental section and physical data for **1**; Scheme S1; NMR spectra of **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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