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Halichoblelides B and C, potent cytotoxic macrolides from a *Streptomyces* species separated from a marine fish

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ABSTRACT

Halichoblelide B (**1**) and C (**2**), novel macrolides with potent cytotoxicity against tumor cells in culture, have been isolated from a strain of *Streptomyces hygroscopicus* originally derived from the marine fish *Halichoeres bleekeri*, and their absolute stereostructures have been elucidated on the basis of spectroscopic analyses using 1D and 2D NMR techniques and chemical transformations. These compounds exhibited significant cytotoxicity against human cancer cell lines.

Based on the fact that some of the bioactive materials isolated from marine animals have been produced by bacteria, we have focused on new antitumor materials from microorganisms isolated from marine species.[1–3] We previously reported that a cytotoxic compound, halichoblelide A (3), member of elaiophylin,[4,5] was produced by a strain of *Streptomyces hygroscopicus* OUPS-N92 isolated from the marine fish *Halichoeres bleekeri*.[6] Our continuing search for cytotoxic metabolites from this strain led to the isolation of two new 16-membered ring macrolides designated halichoblelides B (**1**)[7] and C (**2**).[8] Both metabolites exhibited significant cytotoxic activity against the murine P388 lymphocytic leukemia cell line, and in addition, appreciable cytotoxicity against a disease-oriented panel of 39 human cancer cell lines. We describe herein the absolute stereostructure and biological activities of **1** and **2**.

The microorganism from *Halichoeres bleekeri* was cultured at 27 °C for 6 weeks in a medium (120 L) containing 0.1% cone steep liquor and 1% dextrin in artificial seawater adjusted to pH 7.5. This culture had more volume and longer incubation than that reported previously.[6] After incubation, the AcOEt extract of the culture filtrate was purified by bioassay-directed fractionation (cytotoxicity against P388 cells) employing stepwise a combination of Sephadex LH-20, silica gel column chromatography, and reverse phased HPLC to afford halichoblelide B (**1**, 10.2 mg) and C (**2**, 8.5 mg) as colorless powders, respectively.

Halichoblelide B (**1**) had the molecular formula $C_{51}H_{84}O_{15}$ as established from the $[M+Na]^+$ peak in high-resolution secondary ion mass spectrometry (HRSIMS). The UV spectrum of **1** exhibited absorption bands characteristic of a conjugated carbonyl group. In addition, the IR spectrum exhibited absorption bands at 3425, 1708, 1643, and 1638 cm^{-1}, characteristic of hydroxy groups, conjugated lactone carbonyls, and double bonds. A close inspection of the ^1H and ^{13}C NMR spectra of **1** (Table 1) using DEPT and ^1H–^{13}C correlation spectroscopy (COSY) revealed the presence of two primary methyls (C-21 and C-21′), nine secondary methyls (C-16–C-19, C-27, and C-16′–C-19′), three oxymethyls (11-OCH_3, 11′-OCH_3, and 13′-OCH_3), five sp^3-hybridized methylenes (C-12, C-20, C-23, C-12′, and C-20′), twenty sp^3-methines (C-6–C-10, C-13–C-15, C-22, C-24–C-26, C-6′–C-10′, and C-13′–C-15′) including eleven oxymethines (C-7, C-9, C-13, C-15, C-24–C-26, C-7′, C-9′, C-13′, and C-15′), and one anomeric methine (C-22), two anomeric quaternary sp^3-carbons (C-11 and C-11′), two 1,3-diene moieties (C-2–C-5 and C-2′–C-5′), two ester carbonyls (C-1 and C-1′), and four hydroxyl groups. The ^1H–^1H COSY analysis of **1** led to five partial structures as shown by bold-faced lines in Figure 1. The geometrical configuration of both the conjugated diene moieties (C-2–C-5 and C-2′–C-5′) was deduced as *trans*-s-*trans* from the coupling constants of the olefinic protons ($J_{2,3} = J_{2',3'} = 15.6$ Hz, $J_{3,4} = J_{3',4'} = 11.6$ Hz, and $J_{4,5} = J_{4',5'} = 15.3$ Hz) and NOEs (H-3/H-5 and H-3′/H-5′). The connection of these five units and the remaining functional groups was determined on the basis of the key HMBC correlations summarized in Figure 1, and the planar structure of **1** was elucidated as 11,11′-dimethylelaiophylin eliminated one sugar unit.

In NOESY experiment, the observed NOEs for unit A closely looked like those for the same unit in halichoblelide A (**3**) (Fig. 2). The vicinal coupling constants from C-6 to C-10 ($J_{6,7} = 10.2$, $J_{7,8} = 1.2$, $J_{8,9} = 9.8$, and $J_{9,10} = 1.8$ Hz) also corresponded to

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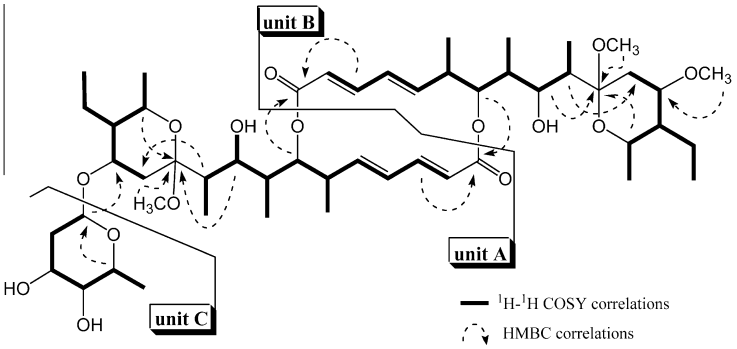


Figure 1. Typical 2D NMR correlations in halichoblelide B (**1**).

Table 1
NMR spectral data for halichoblelide B **(1)** and C **(2)** in $CDC1_3$

1					2				
Position		δ_H[a]	*J* (Hz)	δ_C	Position		δ_H[a]	*J* (Hz)	δ_C
1				169.5 (q)[b]	1				169.5 (q)[b]
2	5.67	d	15.6 (3)	121.3 (t)	2	5.67	d	15.6 (3)	121.3 (t)
3	6.98	dd	15.6 (2), 11.6 (4)	145.1 (t)	3	6.98	dd	15.6 (2), 11.6 (4)	145.1 (t)
4	6.10	dd	15.3 (5), 11.6 (3)	131.7 (t)	4	6.10	dd	15.1 (5), 11.6 (3)	131.8 (t)
5	5.65	dd	15.3 (4), 10.2 (6)	144.5 (t)	5	5.65	dd	15.1 (4), 10.1 (6)	144.5 (t)
6	2.52	tq	10.2 (5, 7), 6.8 (17)	41.3 (t)	6	2.52	tq	10.1 (5, 7), 6.8 (17)	41.3 (t)
7	4.93	dd	10.2 (6), 1.2 (8)	77.9 (t)	7	4.93	dd	10.1 (6), 2.2 (8)	77.9 (t)
8	1.88	dqd	9.8 (9), 7.0 (18), 1.2 (7)	36.6 (t)	8	1.88	dqd	10.3 (9), 7.0 (18), 2.2 (7)	36.6 (t)
9	3.48	br d	9.8 (8)	69.7 (t)	9	3.48	ddd	10.3 (8), 3.8 (9-OH), 1.0 (10)	69.7 (t)
10	2.04	qd	7.1 (19), 1.8 (9)	38.0 (t)	10	2.04	qd	7.1 (19), 1.0 (9)	38.0 (t)
11				103.2 (q)	11				103.2 (q)
12α	2.32	dd	13.6 (12β), 4.5 (13)	34.2 (s)	12α	2.30	dd	13.6 (12β), 4.5 (13)	34.0 (s)
β	1.39	dd	13.6 (12α), 10.6 (13)		β	1.40	dd	13.6 (12α), 10.4 (13)	
13	3.84	td	10.6 (12β, 14), 4.5 (12α)	70.1 (t)	13	3.54	td	10.4 (12β, 14), 4.5 (12α)	73.3 (t)
14	1.21	tt	10.6 (13, 15), 3.8 (20A, 20B)	47.4 (t)	14	1.25	tq	10.4 (13, 15), 7.0 (20)	42.6 (t)
15	3.50	dq	10.6 (14), 6.3 (16)	67.8 (t)	15	3.32	dq	10.4 (14), 6.2 (16)	70.6 (t)
16	1.19	d	6.3 (15)	19.0 (p)	16	1.19	d	6.2 (15)	19.2 (p)
17	1.03	d	6.8 (6)	15.1 (p)	17	1.03	d	6.8 (6)	15.1 (p)
18	0.89	d	7.0 (8)	9.5 (p)	18	0.87	d	7.0 (8)	9.5 (p)
19	0.96	d	7.1 (10)	7.2 (p)	19	0.97	d	7.1 (10)	7.2 (p)
20A	1.44	dqd	13.2 (20B), 7.6 (21), 3.8 (14)	19.4 (s)	20	0.91	d	7.0 (14)	13.4 (p)
B	1.62	dqd	13.2 (20A), 7.6 (21), 3.8 (14)		21				
21	0.85	t	7.6 (20A, 20B)	9.2 (p)	22	5.03	br s		93.0 (t)
22	5.04	t	2.3 (23α, 23β)	93.2 (t)	23α	1.82	dt	12.3 (23β), 2.7 (22, 24)	33.4 (s)
23α	1.79	dt	12.3 (23β), 2.3 (22, 24)	33.4 (s)	β	1.80	ddd	12.3 (23α), 9.8 (24), 2.7 (22)	
β	1.82	ddd	12.3 (23α), 10.8 (24), 2.3 (22)		24	3.96	br d	9.8 (22β)	66.1 (t)
24	3.96	br d	10.8 (23β)	66.1 (t)	25	3.62	br s		71.5 (t)
25	3.61	br s		71.4 (t)	26	3.99	qd	6.8 (27), 1.8 (25)	65.7 (t)
26	3.99	qd	6.9 (27), 1.8 (25)	65.8 (t)	27	1.24	d	6.8 (26)	16.8 (p)
27	1.24	d	6.9 (26)	16.8 (p)	9-OH	3.34	br s		
9-OH	3.32	br s			11-OCH_3	3.05	s		46.6 (p)
11-OCH_3	3.05	s		46.6	23-OH	2.17	br s		
24-OH	2.15	br s			24-OH	1.94	br s		
25-OH	2.04	br s							
1′				169.5 (q)	1′				169.5 (q)
2′	5.67	d	15.6 (3′)	121.3 (t)	2′	5.67	d	15.6 (3′)	121.3 (t)
3′	6.98	dd	15.6 (2′), 11.6 (4′)	145.1 (t)	3′	6.98	dd	15.6 (2′), 11.6 (4′)	145.1 (t)
4′	6.10	dd	15.3 (5′), 11.6 (3′)	131.7 (t)	4′	6.10	dd	15.1 (5′), 11.6 (3′)	131.8 (t)
5′	5.65	dd	15.3 (4′), 10.2 (6′)	144.5 (t)	5′	5.65	dd	15.1 (4′), 10.1 (6′)	144.5 (t)
6′	2.52	tq	10.2 (5′, 7′), 6.8 (17′)	41.3 (t)	6′	2.52	tq	10.1 (5′, 7′), 6.8 (17′)	41.3 (t)
7′	4.95	dd	10.2 (6′), 1.2 (8′)	77.9 (t)	7′	4.95	dd	10.1 (6′), 2.2 (8′)	77.9 (t)
8′	1.91	dqd	9.5 (9), 7.0 (18′), 1.2 (7′)	36.6 (t)	8′	1.91	dqd	10.3 (9′), 7.0 (18′), 2.2 (7′)	36.6 (t)
9′	3.48	br d	9.5 (8′)	69.7 (t)	9′	3.50	ddd	10.3 (8′), 3.8 (9′-OH), 1.0 (10′)	69.7 (t)
10′	2.03	qd	7.1 (19′), 1.8 (9′)	38.0 (t)	10′	2.04	qd	7.1 (19′), 1.0 (9′)	38.0 (t)
11′				103.3 (q)	11′				103.3 (q)
12′α	2.38	dd	13.6 (12′β), 4.5 (13′)	34.2 (s)	12′	2.38	dd	13.6 (12′β), 4.5 (13′)	34.2 (s)
β	1.40	dd	13.6 (12′α), 10.6 (13′)			1.40	dd	13.6 (12′α), 10.4 (13′)	
13′	3.34	td	10.6 (12′β, 14′), 4.5 (12′α)	75.8 (t)	13′	3.34	td	10.4 (12′β, 14′), 4.5 (12′α)	75.9 (t)
14′	1.13	tt	10.6 (13′, 15′), 3.8 (20′A, 20′B)	47.6 (t)	14′	1.13	tt	10.4 (13′, 15′), 3.8 (20′A, 20′B)	48.1 (t)
15′	3.45	dq	10.6 (14′), 6.3 (16′)	68.4 (t)	15′	3.45	dq	10.4 (14′), 6.2 (16′)	68.4 (t)
16′	1.19	d	6.3 (15′)	19.1 (p)	16′	1.19	d	6.2 (15′)	19.1 (p)
17′	1.04	d	6.8 (6′)	15.1 (p)	17′	1.04	d	6.8 (6′)	15.1 (p)
18′	0.90	d	7.0 (8′)	9.5 (p)	18′	0.89	d	7.0 (8′)	9.5 (p)

(*continued on next page*)

Table 1 (*continued*)

	1				2			
Position		δ_H[a]	*J* (Hz)	δ_C	Position		δ_H[a]	*J* (Hz)	δ_C
19′	0.97	d	7.1 (10′)	7.2 (p)	19′	0.97	d	7.1 (10′)	7.2 (p)
20′A	1.46	dqd	13.2 (20′B) 7.6 (21′), 3.8 (14′)	19.4 (s)	20′A	1.46	dqd	13.2 (20′B), 7.6 (21′), 3.8 (14′)	19.4 (s)
B	1.58	dqd	13.2 (20′A) 7.6 (21′), 3.8 (14′)		B	1.59	dqd	13.2 (20′A), 7.6 (21′), 3.8 (14′)	
21′	0.87	t	7.6 (20′A, 20′B)	10.0 (p)	21′	0.87	t	7.6 (20′A, 20′B)	10.1 (p)
9′-OH	3.39	br s			9′-OH	3.36	br s		
11′-OCH_3	3.07	s		46.6 (p)	11′-OCH_3	3.07	s		46.6 (p)
13′-OCH_3	3.33	s		56.3 (p)	13′-OCH_3	3.33	s		56.3 (p)

[a] ^1H chemical shift values (δ ppm from $SiMe_4$) followed by multiplicity and then the coupling constants (*J*/Hz). Figures in parentheses indicate the proton coupling with that position.

[b] Letters, p, s, t, and q, in parentheses indicate respectively primary, secondary, tertiary, and quaternary carbons, assigned by DEPT.

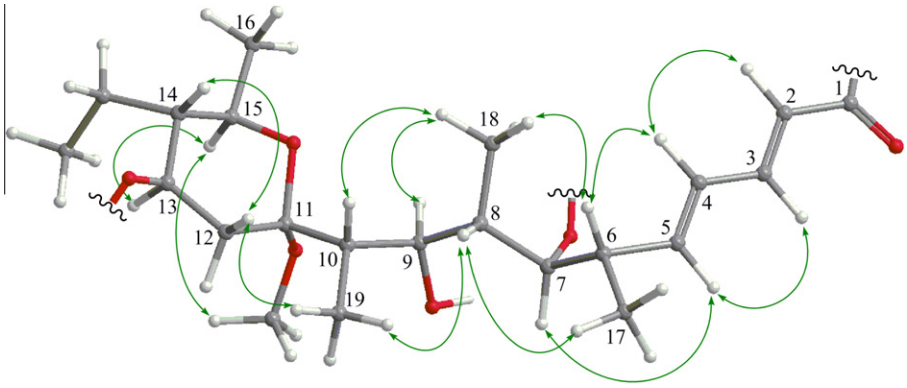
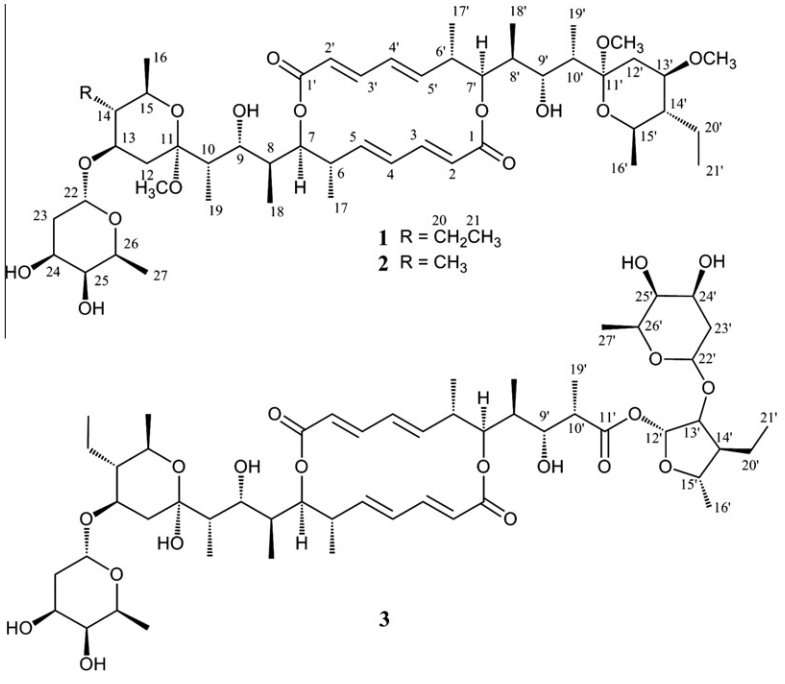
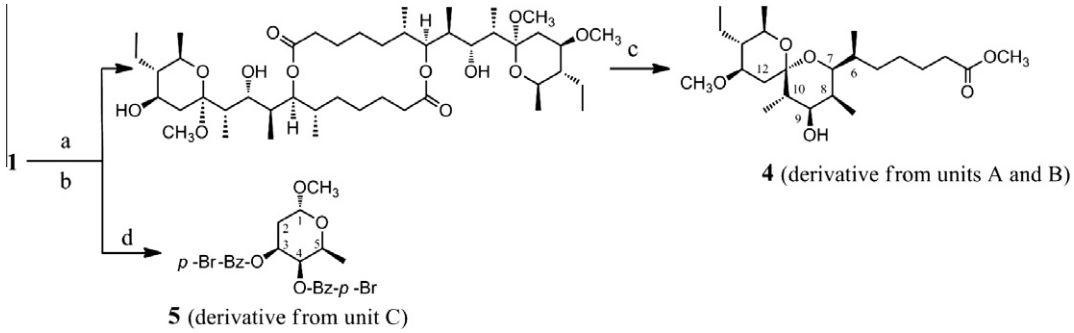


Figure 2. Observed NOEs for unit A in **1**.

those of **3**[6] (Table 1). In addition, the measured coupling constants (Table 1) and NOEs of unit B closely resembled those of unit A. Therefore we deduced that units A and B have the same stereochemistry and conformation as unit A in **3**. We had established the absolute stereostructure of **3** by degradation.[6,9] In the examination, the absolute stereostructure for the degradated product **4** from unit A in **3** had been elucidated by application of the modified Mosher's method.[10] The degradation of **1** gave the product **4**, which was identical with that from **3** for NMR spectral data and the specific rotation (Scheme 1). This evidence demonstrated that units A and B in **1** were the same absolute configuration as unit A in **3**.[6] On the other hand, the derivative **5** from unit C in **1** was also identical with that from **3**.[6] The negative Cotton effect observed in the CD spectrum of the bis-*p*-bromobezoate **5** supported the absolute configuration of **4** as shown in Scheme 1. The absolute configuration of **4** and **5** led us to determine the absolute stereostructures of the three units (A, B, and C), respectively, and consequently the absolute stereostructure for halichoblelide B (**1**).





Scheme 1. Reagents and conditions: (a) Pd-C/H_2, rt; (b) *p*-TsOH/MeOH, rt; (c) *c*-H_2SO_4/MeOH, rt; (d) *p*-BrBzCl/pyridine, rt.

Table 2
Cytotoxicity of halichoblelide B (**1**), C (**2**), and A (**3**) against a panel of 39 human cancer cell lines

Origin of cancer	Cell line	LogGI_{50} (M)[a]		
		1	2	3
Breast	HBC-4	−5.72	−5.70	−5.72
	BSY-1	−5.71	−6.20	−5.78
	HBC-5	−5.71	−6.03	−5.81
	MCF-7	−5.69	−5.73	−5.70
	MDA-MB-231	−5.71	−5.89	−5.63
Central nervous system	U-251	−5.75	−5.84	−5.73
	SF-268	−5.76	−5.65	−5.74
	SF-295	−5.74	−5.78	−5.75
	SF-539	−5.59	−5.79	−5.96
	SNB-75	−5.73	−6.01	−5.95
	SNB-78	−5.63	−5.56	−5.85
Colon	HCC2998	−5.71	−5.95	−5.75
	KM-12	−5.71	−5.82	−5.74
	HT-29	−5.72	−5.80	−7.73
	HCT-15	−5.71	−5.75	−5.75
	HCT-116	−5.73	−5.81	−5.78
Lung	NCI-H23	−5.71	−5.90	−5.68
	NCI-H226	−5.74	−6.18	−5.79
	NCI-H522	−5.78	−6.32	−5.73
	NCI-H460	−5.75	−5.96	−5.71
	A549	−5.75	−6.12	−5.72
	DMS273	−5.76	−6.23	−5.68
	DMS114	−5.75	−6.56	−5.81
Melanoma	LOX-IMVI	−5.69	−5.74	−5.69
Ovary	OVCAR-3	−5.71	−6.43	−5.73
	OVCAR-4	−5.65	−5.73	−5.73
	OVCAR-5	−5.72	−5.77	−5.74
	OVCAR-8	−5.70	−5.80	−5.70
	SK-OV-3	−5.70	−5.61	−5.74
Kidney	RXF-631L	−5.76	−6.52	−5.74
	ACHN	−5.74	−5.73	−5.77
Stomach	St-4	−5.78	−5.85	−5.71
	MKN1	−5.76	−5.81	−5.75
	MKN7	−5.80	−5.88	−5.77
	MKN28	−5.72	−5.69	−5.70
	MKN45	−5.71	−6.26	−5.73
	MKN74	−5.75	−6.31	−5.76
Prostate	DU-145	−5.71	−5.78	−5.75
	PC-3	−5.72	−5.98	−5.72
MG-MID[b]		−5.72	−5.93	−5.75
Delta[c]		0.08	0.63	0.22
Range[d]		0.21	1.00	0.33

[a] Log concentration of compounds for inhibition of cell growth at 50% compared to control.
[b] Mean value of logGI50 over all cell lines tested.
[c] The difference in logGI50 value of the most sensitive cell and MG-MID value.
[d] The difference in logGI50 value of the most sensitive cell and the least sensitive cell.

Halichoblelide C (**2**) had the molecular formula $C_{50}H_{82}O_{15}$ as established by HRSIMS. The general features of its ^1H and ^{13}C NMR spectra (Table 1) closely resembled those of **1** except that the signal for one of the ethyl group in **1** was replaced by that for a methyl group [δ_H 0.91, δ_C 13.44] in **2**. The ^1H–^1H and HMBC correlations implied that the ethyl group at C-14 in **1** was replaced by a methyl group in **2** (vide info). Degradation of **2** also gave the bis-*p*-bromobenzoate **5**. The analysis of coupling constants and NOE signals for **2** suggested that the relative stereochemistry of units A and B in **2** was the same as those in **1**. In addition, the Cotton effect in the CD spectra and the $[\alpha]_D$ value for **2** closely resembled those of **1**.[7,8] The above evidence led us to deduce the absolute stereostructure of halichoblelide C (**2**).

The cancer cell growth inhibitory properties of halichoblelide B (**1**) and C (**2**) were examined using a disease-oriented panel of 39 human cancer cell lines (HCC panel) from the Japanese Foundation for Cancer Research.[11,12] The results of them together with halichoblelide A (**3**) are shown in Table 2. All of these metabolites exhibited significant cytotoxic activity against the 39 human cancer cell lines (Table 2). Halichoblelide C (**2**) also showed appreciable cytotoxic activity against the cell lines (Table 2). As shown in Table 2, the delta and range values of **2** were 0.63, and 1.00, respectively (effective value: delta $\geqslant$ 0.5 as well as range $\geqslant$ 1.0), disclosing that this compound showed selective cytotoxic activity. Furthermore, evaluation of the pattern of differential cytotoxicity using the COMPARE program[12] suggested the mode of action for **2** to be different from that shown by any other anticancer drug developed to date.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2012.03.114.

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7. Halichoblelide B (**1**): colorless powder, mp 143–145 °C, $[\alpha]_D$ −32.2 (*c* 0.19 in EtOH); λ_{max} (EtOH)/nm 253 (logε 4.46); ν_{max} (KBr)/cm^{-1} 3425, 1708, 1643, and 1638 (C=C); HRSIMS *m/z* 959.5691 $[M+Na]^+$ (calcd for $C_{51}H_{84}O_{15}Na$: 959.5697); CD λ (*c* 2.21 × 10^{-4} M in EtOH)/nm 315 ($\Delta\varepsilon$ 0), 280 (+16.54), 263 (0), and 249 (−23.36). ^{1}H and ^{13}C NMR data are listed in Tables 1 and S1 (Supplementary data).
8. Halichoblelide C (**3**): colorless powder, mp 148–150 °C, $[\alpha]_D$ −36.8 (*c* 0.09 in EtOH); λ_{max} (EtOH)/nm 255 (logε 4.37); ν_{max} (KBr)/cm^{-1} 3432, 1711, 1701, 1645, and 1635; HRSIMS *m/z* 945.5532 $[M+Na]^+$ (calcd for $C_{50}H_{82}O_{15}Na$: 945.5532); CD λ (*c* 1.38 × 10^{-4} M in EtOH)/nm 316 ($\Delta\varepsilon$ 0), 280 (+19.22), 262 (0), and 251 (−20.36). ^{1}H and ^{13}C NMR data are listed in Tables 1 and S2 (Supplementary data).
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