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## CYTOTOXIC EUNICELLIN-TYPE DITERPENES FROM THE SOFT CORAL *LITOPHYTON VISCUDIUM*

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**Abstract** – Five new eunicellin-type diterpenes were isolated from the soft coral *Litophyton viscodium*. The structures of the compounds were elucidated mainly on the basis of extensive spectroscopic analysis. Compounds exhibited cytotoxic activity against HL-60 with IC<sub>50</sub> values of 4.2–50  $\mu$ M.

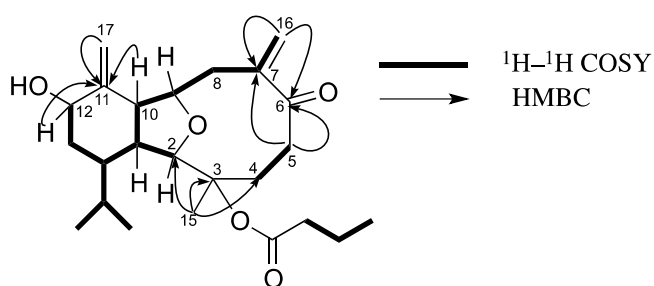
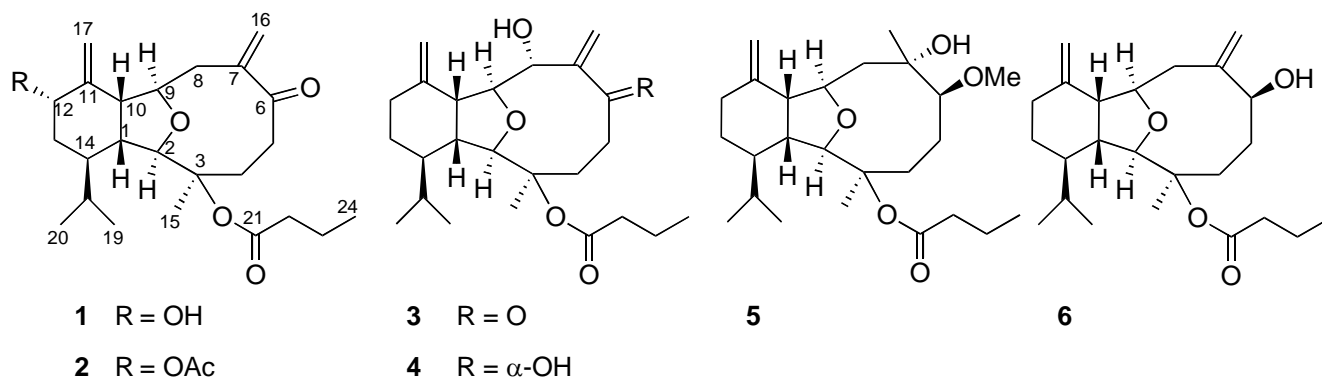
The soft coral *Litophyton* species is known to produce a sesquiterpene,<sup>1</sup> cembranes,<sup>1</sup> eunicellin-based diterpenes,<sup>2-5</sup> and sterols<sup>6</sup> possessing biological activities, such as antiproliferative activity against the cell lines L-929 and K-562, cytotoxicity against HeLa cells,<sup>1</sup> and hemolytic,<sup>2</sup> molluscicidal,<sup>3</sup> repellent,<sup>4</sup> insect growth inhibitory,<sup>5</sup> brine shrimp lethality,<sup>6</sup> and antileukemic<sup>7</sup> activities. As a continuation of our survey on bioactive compounds from marine organisms,<sup>8</sup> the CH<sub>2</sub>Cl<sub>2</sub>-soluble portion (23 g) of a MeOH extract of the soft coral *L. viscodium* (5.3 kg, wet wt.), collected in the area of Otsuki Town area, Kochi Prefecture, Japan, showed moderate cytotoxic activity (IC<sub>50</sub> = 6.9  $\mu$ g/mL) against the proliferation of human promyelocytic leukemia cells (HL-60). Further investigation of this CH<sub>2</sub>Cl<sub>2</sub> fraction has led to the

isolation of five new eunicellin-type diterpenes: **1** (3.7 mg), **2** (2.0 mg), **3** (1.8 mg), **4** (3.7 mg), and **5** (11.7 mg) and a known compound, lithophynin F (**6**)<sup>5(a)</sup> (7.3 mg). The isolates exhibited moderate cytotoxic activity against HL-60 cells. In this paper, we describe the isolation and structure elucidation of these new compounds.

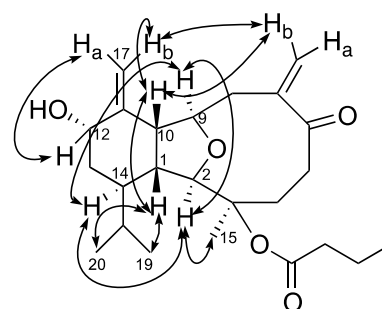
Compound **1** was isolated as an amorphous powder and had a molecular formula of C<sub>24</sub>H<sub>36</sub>O<sub>5</sub>, which was determined by HRFABMS [*m/z* 405.2629, (M + H)<sup>+</sup>, calcd *m/z* (405.2641)]. The IR spectrum showed absorptions characteristic of a hydroxy group at 3426 cm<sup>-1</sup>, an ester carbonyl at 1732 cm<sup>-1</sup>, and a conjugated carbonyl at 1688 cm<sup>-1</sup>. The molecular formula implied seven degrees of unsaturation and the presence of one carbonyl, one ester carbonyl, and four olefinic carbons in the <sup>13</sup>C NMR spectrum (Table 1) indicated that compound **1** had a tricyclic structure. Resonances due to *i*-propyl protons at C-14, methyl protons at C-3, and two terminal methylene protons in the <sup>1</sup>H NMR spectrum (Table 1) were observed along with signals due to two oxygenated methine carbons at δ<sub>C</sub> 90.7 and 80.1 that were correlated to the signals δ 3.77 (s, H-2) and 4.47 (m, H-9), respectively, in an HMQC experiment. This suggested that compound **1** was a eunicellin-based diterpenoid, which is commonly found as a chemical constituent of the *Litophyton* genus.<sup>2-6</sup> The <sup>1</sup>H NMR spectrum further indicated the presence of another oxygenated methine proton (δ 4.45, br s, W<sub>1/2</sub> = 8.4 Hz) and an *n*-butyroxyl group at C-3 (δ 0.95, 1.63, 2.20). The gross structure was elucidated on the basis of <sup>1</sup>H-<sup>1</sup>H COSY and HMBC experiments (Figure 1). HMBC correlations of both H<sub>2</sub>-5 (δ 2.47, 2.73) and H<sub>2</sub>-16 (δ 5.23, 5.39) to C-6 (δ 206.0) and C-7 (δ 147.6) indicated that the carbonyl group was located at C-6. Another terminal methylene and the hydroxy group were determined to be attached to C-11 and C-12, respectively, on the basis of the correlations of H-10 (δ 3.06), H-12 (δ 4.45) and the methylene protons (δ 4.87, 5.12) to C-11 (δ 146.2). Correlations of Me-15 (δ 1.53) to C-2 (δ 90.7), C-3 (δ 84.1), and C-4 (δ 31.9) indicated that C-3 was attached to C-2 and C-4, and the chemical shift of C-3 suggested the presence of the *n*-butyroxyl group at C-3.

The relative stereochemistry was determined on the basis of NOESY analysis (Figure 2). NOESY correlation of H-1 to H-10, Me-19, and Me-20; H-10 to H-16<sub>b</sub> (δ 5.23) and H-17<sub>b</sub> (δ 4.87); H-16<sub>b</sub> to H-17<sub>b</sub>, and H-12 to H-17<sub>a</sub> (δ 5.12) suggested that H-1, H-10, H-12, and the *i*-propyl moiety were on the same face (β) of the ring. The configuration of H-12 was also indicative of a pseudo-equatorial orientation (β) based on the half-height width, W<sub>1/2</sub> = 8.4 Hz, of this proton. H-2 showed NOE's to H-9, H-14, and Me-15, and H-9 gave an NOE to H-14, confirming α-orientations for H-2, H-9, H-14, and Me-15. Thus, compound **1** has the relative structure corresponding to the 6-oxo derivative of lithophynin H.

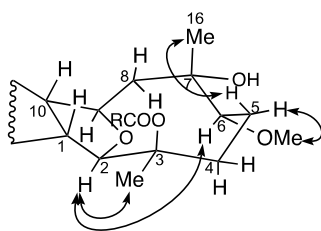
Absorptions of compound **2**, C<sub>26</sub>H<sub>38</sub>O<sub>6</sub>, in the IR spectrum indicated the presence of an ester carbonyl at 1736 cm<sup>-1</sup> and a conjugated carbonyl at 1692 cm<sup>-1</sup>. The <sup>1</sup>H NMR spectrum was similar to that of **1**, except for resonances due to additional acetyl protons (δ 2.00, 3H, s). The position of the acetyl group was determined to be located at C-12 based on the downfield shift of H-12 (δ 5.48, 1H, m) by 1.03 ppm when



**Figure 1.** Selected 2D NMR correlations of **1**



**Figure 2.** Selected NOE correlations of **1**



**Figure 3.** Selected NOE correlations of **5**

compared with that of **1**. The  $\beta$ -configuration of H-12 was supported by the observation of NOE's between H-12 and H-10. Therefore, compound **2** is the 12-*O*-acetyl analogue of **1**.

Compound **3**,  $\text{C}_{24}\text{H}_{36}\text{O}_5$ , was isomeric with **1**, and the presence of a hydroxy group, an ester carbonyl, and a conjugated carbonyl was observed in the IR spectrum similar to the case of **1**. The NMR spectra were essentially similar to those of **1**; however, the chemical shifts corresponding to C-8 ( $\delta$  79.9) and C-12 ( $\delta$  31.3) in the  $^{13}\text{C}$  NMR spectrum were shifted downfield by 43.0 ppm and upfield by 40.2 ppm, respectively when compared with those of **1**. This suggested that the hydroxy group was attached to C-8. The configuration was inferred to be  $\alpha$ -oriented from NOE correlations of H-8 ( $\delta$  4.26) to H-16<sub>b</sub> ( $\delta$  5.44) and H-17<sub>b</sub> ( $\delta$  4.68), the latter of which was also correlated to H-10. The relative configurations of the remaining chiral centers in **3** were deduced from the similarity of their coupling patterns and chemical shifts in the NMR spectra and NOE correlations to those of **1**. Thus, compound **3** has the relative structure corresponding to the keto derivative of lithophynol A.<sup>2</sup>

**Table 1.** NMR Spectroscopic Data for **1–4** in CDCl<sub>3</sub><sup>a</sup>

position	<b>1</b>		<b>2</b>		<b>3</b>		<b>4</b>		<b>5</b>	
	d <sub>c</sub>	d <sub>H</sub> (J in Hz)	d <sub>c</sub>	d <sub>H</sub> (J in Hz)	d <sub>c</sub>	d <sub>H</sub> (J in Hz)	d <sub>c</sub>	d <sub>H</sub> (J in Hz)	d <sub>c</sub>	d <sub>H</sub> (J in Hz)
1	44.6	2.25, m	44.3	2.77, m	45.2	2.23, m	44.4	2.23, m	45.6	2.17, dd (11.0, 7.3)
2	90.7	3.77, s	90.8	3.75, s	91.6	3.75, s	91.3	3.77, s	92.3	3.59, br s
3	84.1		84.1		83.9		84.4		86.4	
4a	31.9	2.73, m	31.4	2.38, 2H, br t (11.7)	31.3	2.31, 2H, m		2.25 <sup>b</sup>	37.0	1.75, m
b		2.47, m						1.86 <sup>b</sup>		2.72, dd (15.5, 8.5)
5a	38.4	2.73, m	38.2	2.73, td (11.3, 2.2)	39.0	2.71 <sup>b</sup>	28.9	2.16 <sup>b</sup>	26.8	1.34, m
b		2.47, m		2.45, br t (11.4)		2.47 <sup>b</sup>		1.57 <sup>b</sup>		1.58, dd (15.5, 8.9)
6	206.0		206.0		208.4		81.4	5.07, dd (11.4, 3.4)	90.7	4.11, d (6.1)
7	147.6		147.5		145.5		148.2		76.0	
8	36.9	2.31, d (14.2)	36.9	3.21, dd (13.3, 5.4)	79.9	4.26, br d (8.0)	77.1	4.22, s	45.1	1.82, m 2H
		3.23, dd (14.2, 5.7)		2.30 <sup>b</sup>						
9	80.1	4.47 m	79.6	4.29, ddd (10.7, 5.8, 2.1)	84.3	4.17, d (11.2)	83.3	4.20, d (10.9)	78.5	4.17, dd (14.6, 7.3)
10	46.9	3.06, dd (10.1, 7.8)	47.3	3.09, dd (10.3, 7.6)	48.6	2.84, dd (11.0, 7.4)	48.1	2.86, dd (10.9, 7.9)	53.9	2.97, t (7.3)
11	146.2		141.4		145.5		145.9		147.6	
12a	71.5	4.45, br s, W <sub>1/2</sub> = 8.4	73.1	5.48, m, W <sub>1/2</sub> = 10.2	31.3	2.12 <sup>b</sup>	31.5	2.13 <sup>b</sup>	31.5	2.04, br t (12.9)
b						2.31, m		2.30, m		2.26, m
13a	31.1	1.90, dt (2.6, 3.4)	29.0	1.96, dt (14.3, 3.1)	25.2	1.77 <sup>b</sup>	25.3	1.77, dd (12.9, 3.1)	24.7	1.02, m
b		2.34, m		1.30, dt (2.5, 13.7)		1.05, m		1.05, ddd (13.0, 13.0, 2.8)		1.71 <sup>b</sup>
14	35.1	1.84, m	36.2	1.69, br t (12.6)	43.1	1.32, m	44.0	1.31, m	44.0	1.27, m
15	22.6	1.53, s	22.6	1.52, s	22.7	1.49, s	22.5	1.59, s	23.2	1.39, s
16a	117.2	5.39, br s	117.5	5.41, br s	119.3	5.52, br s	119.3	5.54, br s	23.6	1.11, s
b		5.23, br s		5.23, br s		5.44, br s		5.37, br s		
17a	116.0	5.12, br s	119.2	5.30, d (1.1)	111.7	4.85, br s	111.6	4.83, br s	109.4	4.68, br s
b		4.87, br s		5.00, br s		4.68, br s		4.67, br s		4.64, br s
18	27.1	1.88, m	27.1	1.87, m	27.7	1.77 <sup>b</sup>	27.5	1.85, m	29.1	1.74 <sup>b</sup>
19	21.7	0.98, d (7.2)	21.6	0.94, d (7.0)	21.9	0.97 d (6.8)	21.9	0.97, d (6.9)	21.9	0.96, d (6.9)
20	15.2	0.77 d (6.8)	15.1	0.76, d (6.8)	15.3	0.76 d (6.7)	15.5	0.75, d (6.8)	15.5	0.78, d (6.8)
21	172.6		172.5		172.6		172.6		172.3	
22	37.4	2.20, t (7.3)	37.3	2.21, m	37.3	2.23 m	37.4	2.14 <sup>b</sup>	37.4	2.30, m, 2.37 m
23	18.5	1.63, hex (7.5)	18.5	1.63, sex (7.5)	18.4	1.63 m	18.5	1.59 <sup>b</sup>	18.4	1.68, m
24	13.6	0.95, t (8.1)	13.6	0.96, t (7.5)	13.7	0.97 t (7.0)	13.6	0.92, t (7.4)	13.7	0.99, t (7.4)
MeOCO			170.0							
MeOCO			21.5	2.00 s						
MeO									56.9	3.34, s

<sup>a</sup>For <sup>1</sup>H, 600 MHz; <sup>13</sup>C, 150 MHz. <sup>b</sup> Overlapped signals. W<sub>1/2</sub>: width at half-height in Herz.

The IR spectrum of compound **4**, C<sub>24</sub>H<sub>38</sub>O<sub>5</sub>, indicated the presence of hydroxy groups at 3366 cm<sup>-1</sup>, an ester carbonyl at 1732 cm<sup>-1</sup>, and olefins at 1645 cm<sup>-1</sup>. However, unlike that observed for **1–3**, absorption due to a conjugated carbonyl was not observed. A resonance due to H-6 (δ 5.07, 1H, dd, *J* = 11.4, 3.4 Hz) in the <sup>1</sup>H NMR spectrum suggested that the carbonyl group in **3** was reduced to a hydroxy group in **4**. The remaining resonances were similar to those of **3**. The configuration of the hydroxy group was found to be the α-oriented based on an NOE correlation between H-6 and H-10. Therefore, the relative structure of **4** is as same as that of the 6-*epi* litophynol A.<sup>2</sup>

The molecular formula of compound **5**, C<sub>25</sub>H<sub>42</sub>O<sub>5</sub>, indicated that the MW of **5** was 16 mass units higher than **4**. The presence of a hydroxy group at 3495 cm<sup>-1</sup> and an ester carbonyl at 1736 cm<sup>-1</sup> was indicated by

the IR spectrum. The  $^1\text{H}$  NMR spectrum was essentially similar to that of **4**, except for the presence of signals extra side chains on the 10-membered ring. Thus, unique resonances due to an additional methoxy group at  $\delta$  3.34 (3H, s) and a methyl group at  $\delta$  1.11 (3H, s) bearing an oxygenated carbon were observed instead of the terminal methylene protons in **4**. The positions of the methoxy and the hydroxy groups were confirmed to be C-6 and C-7, respectively, by interpretation of HMBC correlations between the methoxy protons and C-6 ( $\delta$  90.7) and between the methyl protons and C-7 ( $\delta$  76.0). The relative configuration of chiral carbons on the five-membered ring and six-membered ring was determined by the similarity of the NOESY data for **4** compared with those of **3** and **5**. Regarding the configurations of C-3, C-6, and C-7, NOESY correlations of Me-15 to H-2 and H-6 indicated a  $\beta$ -orientation of the methoxy group, while an  $\alpha$ -orientation of the hydroxy group was suggested from NOESY correlations between the methoxy protons and H-5 $_{exo}$  ( $\delta$  1.58, 1H, dd, 15.5, 8.9 Hz) and between Me-16 and H-5 $_{endo}$  ( $\delta$  1.34, 1H, m), as shown in Figure 3. This meant that C-3, C-6, and C-7 had relative configurations as shown in Figure 5. Therefore, the relative structure of **5** is identical with that of the 6-methyl ether of lithophynol B.<sup>2</sup> However, compound **5** may well be an artifact of the methanol extraction method used, because a hydroxy substituent at C-7 that could form hydrogen bond with the 6-hydroxy would facilitate nucleophilic attack at the 6-position by methanol.

The cytotoxicity of compounds **1–5** and lithophynin F (**6**) in human promyelocytic leukemia cells was determined by an MTT assay. Compounds **1–6** exhibited activity with the following  $\text{IC}_{50}$  values: **1** (20  $\mu\text{M}$ ), **2** (20  $\mu\text{M}$ ), **3** (5.7  $\mu\text{M}$ ), **4** (4.2  $\mu\text{M}$ ), **5** (50  $\mu\text{M}$ ), and **6** (18  $\mu\text{M}$ ). Compounds **1** and **2**, possessing a hydroxy group or acetoxy group at C-12 exhibited moderate cytotoxic activity with an  $\text{IC}_{50} = 20 \mu\text{M}$ , while compounds **3** having an additional hydroxy group at C-8 and its reduced compound **4** showed significant cytotoxic activity with an  $\text{IC}_{50} = 4.2\text{--}5.7 \mu\text{M}$ . The C-6 methoxy and C-7 hydroxy groups apparently reduced the toxicity of compound **5**. Compound **6** with the absence of a hydroxy group at C-8 and the presence of a  $\beta$ -hydroxy group at C-6 displaying less cytotoxic activity with an  $\text{IC}_{50} = 18 \mu\text{M}$  than that of **4**.

## EXPERIMENTAL

Optical rotations were measured at 25 °C on a JASCO DIP-370S polarimeter. UV spectra were measured using a Hitachi U-2001 double-beam spectrophotometer. IR spectra were recorded on a MASCO FT/IR 5300. NMR spectra were recorded with a Bruker AVANCE 600 MHz NMR instrument using  $\text{CDCl}_3$  as a solvent. Chemical shifts are given on a  $\delta$  (ppm) scale ( $^1\text{H}$ , 7.26 ppm;  $^{13}\text{C}$ , 77.0 ppm as the internal standard). MS spectra were obtained with a JEOL JMS XD-303 instrument. VCC separation was performed with silica gel 60H (Merck, 90% < 45  $\mu\text{m}$ ). Column chromatography was carried out on silica gel 60 (Merck, 70–230  $\mu\text{m}$ ). Silica gel 60F (Merck, 0.25 mm thick) was used for TLC. HPLC was

performed using a Waters 501 HPLC pump with a Shodex UV-41 detector. A Develosil ODS-UG-5 C<sub>18</sub> column (10 mm × 250 mm) was used for HPLC.

**Soft Coral Material.** The soft coral *Litophyton viscidium* (collection number 253) was collected in the Otsuki Town area, Kochi Prefecture, Japan (32°44'10"N, 132°43'56"E), on June 15<sup>th</sup> 2004 and was identified by Dr. F. Iwase. A voucher specimen has been deposited at Faculty of Science, Kagoshima University (voucher specimen: 253).

**Extraction and Isolation.** The organism (wet weight: 5.3 kg, dry weight: 748 g) was chopped into small pieces and extracted with MeOH several times. The dried MeOH extract was suspended in H<sub>2</sub>O and extracted with CH<sub>2</sub>Cl<sub>2</sub>. The CH<sub>2</sub>Cl<sub>2</sub> extract (23 g) was subjected to vacuum column chromatography on silica gel. Fractions of 300 mL were collected as follows: 1—3 (CH<sub>2</sub>Cl<sub>2</sub>/*n*-hexane, 4:1), 4—6 (CH<sub>2</sub>Cl<sub>2</sub>), 7—11 (MeOH/CH<sub>2</sub>Cl<sub>2</sub>, 1:49), 12—18 (MeOH/CH<sub>2</sub>Cl<sub>2</sub>, 1:19), 19—21 (MeOH/CH<sub>2</sub>Cl<sub>2</sub>, 1:9), 22—25 (MeOH/CH<sub>2</sub>Cl<sub>2</sub>, 1:4), 26—28 (MeOH). Fractions 15—18 (5.4 g) were subjected to silica gel column chromatography with *n*-hexane/CH<sub>2</sub>Cl<sub>2</sub> (4:1) and then MeOH/CH<sub>2</sub>Cl<sub>2</sub> of increasing polarity. Fractions eluted with MeOH/CH<sub>2</sub>Cl<sub>2</sub> (1:49 to 1:4) were chromatographed on silica gel successively with acetone/CH<sub>2</sub>Cl<sub>2</sub> (3:97 to 1:9) and then MeOH/CH<sub>2</sub>Cl<sub>2</sub> (1:49) followed by reversed-phase HPLC with CH<sub>3</sub>OH/H<sub>2</sub>O (7:3 to 3:2) to yield compounds **(2)** (2.0 mg) and **(3)** (1.8 mg). Compounds **(4)** (3.7 mg), **(5)** (11.7 mg), and **(6)** (7.3 mg) were isolated from the fractions eluted with acetone/CH<sub>2</sub>Cl<sub>2</sub> (1:49 to 1:9) followed by reversed-phase HPLC using MeCN/H<sub>2</sub>O (3:2 to 1:1). Fractions eluted slowly with acetone/CH<sub>2</sub>Cl<sub>2</sub> (1:9) were subjected to reversed-phase HPLC with MeCN/H<sub>2</sub>O (1:1) afforded compound **(1)** (3.7 mg).

**Compound (1):** amorphous powder;  $[\alpha]_D^{25}$  -22.4 (*c* 0.11, MeOH); TLC *R<sub>f</sub>* 0.41 (3:97, MeOH/CH<sub>2</sub>Cl<sub>2</sub>); UV (MeOH)  $\lambda_{\max}$  (log  $\epsilon$ ) 216 (3.41) nm; IR (NaCl)  $\nu_{\max}$  3426, 1732, 1688 cm<sup>-1</sup>; <sup>1</sup>H NMR and <sup>13</sup>C NMR, see Table 1; HRFABMS *m/z* 405.2629 [M + H]<sup>+</sup> (calcd for C<sub>24</sub>H<sub>37</sub>O<sub>5</sub>, 405.2641).

**Compound (2):** amorphous powder;  $[\alpha]_D^{25}$  -9.6 (*c* 0.06, MeOH); TLC *R<sub>f</sub>* 0.36 (3:17, EtOAc/*n*-hexane); UV (MeOH)  $\lambda_{\max}$  (log  $\epsilon$ ) 216 (3.54) nm; IR (NaCl)  $\nu_{\max}$  1736, 1692 cm<sup>-1</sup>; <sup>1</sup>H NMR and <sup>13</sup>C NMR, see Table 1; HRFABMS *m/z* 447.2745 [M + H]<sup>+</sup> (calcd for C<sub>26</sub>H<sub>39</sub>O<sub>6</sub>, 447.2747).

**Compound (3):** amorphous powder;  $[\alpha]_D^{25}$  -5.0 (*c* 0.06, MeOH); TLC *R<sub>f</sub>* 0.25 (3:17, EtOAc/*n*-hexane); UV (MeOH)  $\lambda_{\max}$  (log  $\epsilon$ ) 215 (3.58) nm; IR (NaCl)  $\nu_{\max}$  3451, 1736, 1686 cm<sup>-1</sup>; <sup>1</sup>H NMR and <sup>13</sup>C NMR, see Table 1; HRFABMS *m/z* 405.2647 [M + H]<sup>+</sup> (calcd for C<sub>24</sub>H<sub>37</sub>O<sub>5</sub>, 405.2641).

**Compound (4):** amorphous powder;  $[\alpha]_D^{25}$  +25.0 (*c* 0.06, MeOH); TLC *R<sub>f</sub>* 0.29 (3:97, MeOH/CH<sub>2</sub>Cl<sub>2</sub>); IR (NaCl)  $\nu_{\max}$  3366, 1732, 1645 cm<sup>-1</sup>; <sup>1</sup>H NMR and <sup>13</sup>C NMR, see Table 1; HRFABMS *m/z* 407.2764 [M + H]<sup>+</sup> (calcd for C<sub>24</sub>H<sub>39</sub>O<sub>5</sub>, 407.2797).

**Compound (5):** amorphous powder;  $[\alpha]_D^{25} +33.4$  (*c* 0.35, MeOH); TLC  $R_f$  0.55 (3:97, MeOH/CH<sub>2</sub>Cl<sub>2</sub>); IR (NaCl)  $\nu_{\max}$  3495, 1736, 1645 cm<sup>-1</sup>; <sup>1</sup>H NMR and <sup>13</sup>C NMR, see Table 1; HRFABMS  $m/z$  445.2917 [M + Na]<sup>+</sup> (calcd for C<sub>25</sub>H<sub>42</sub>O<sub>5</sub>Na, 445.2920),  $m/z$  421.2958 [M – H]<sup>+</sup> (calcd for C<sub>25</sub>H<sub>41</sub>O<sub>5</sub>, 421.2954).

**Litophynin F (6):** amorphous powder;  $[\alpha]_D^{25} +12.4$  (*c* 0.28, MeOH); TLC  $R_f$  0.28 (1:99, MeOH/CH<sub>2</sub>Cl<sub>2</sub>);  $\nu_{\max}$  3614, 1734, 1644 cm<sup>-1</sup>; HRFABMS  $m/z$  391.2845 [M + H]<sup>+</sup> (calcd for C<sub>24</sub>H<sub>39</sub>O<sub>4</sub>, 391.2848).

**Cell lines.** Human leukemia HL-60 cells were maintained in RPMI1640 containing 10% fetal calf serum, 2 mM glutamine and the antibiotic/antimycotic solution at 37 °C in a 5 % CO<sub>2</sub> humidified atmosphere.

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