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Chemical Components from the Vietnamese Soft Coral *Lobophytum* sp.

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Chromatographic separation resulted in the identification of one new squalene derivative, named lobophytene (**1**), three cembranoid diterpenes (**2**-**4**), and two sterols (**5** and **6**) from the Vietnamese marine soft coral *Lobophytum* sp. Their structures were identified on the basis of extensive spectroscopic data and comparison of those with reported data. Compounds **1** and **2** showed significant cytotoxic activities against lung (A549) and colon (HT-29) cell lines with IC_{50} values of 8.2 and 5.6 μM for **1**; 5.1 and 1.8 μM for **2**, respectively.

Key words: Soft coral, *Lobophytum* sp., Lobophytene, Cytotoxic activity

INTRODUCTION

Previous chemical investigation on *Lobophytum* species resulted in the identification of various cembranoid diterpenes and several steroids, glycolipids, and alkaloids; some of which showed cytotoxic and anti-inflammatory activities (Uchio et al., 1981; Chang et al., 2000; Radhika et al., 2004; Muralidhar et al., 2005; Chao et al., 2007; Fattorusso et al., 2008; Cheng et al., 2009). In continuation of our search for bioactive products from Vietnamese marine organisms (Dang et al., 2007; Minh et al., 2007a, 2007b, 2008; Cuong et al., 2008; Tung et al., 2009), the present study on the soft coral *Lobophytum* sp. led to the isolation of one new squalene-type triterpene, named lobophytene (**1**), and five known compounds (**2**-**6**). Here, this paper deals with their structural elucidation and cytotoxic activity evaluation.

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MATERIALS AND METHODS

General procedures

Melting points were obtained with an Electrothermal 9100 melting point apparatus (Electrothermal Ltd.). Optical rotation was measured on a DIP-360 digital polarimeter (JASCO). NMR spectra were recorded on a DRX 500 (Bruker) and an ECA 400 NMR (JEOL) spectrometers. High resolusion (HR) Fourier-transform (FT) ion cyclotron resonance (ICR) mass spectrometry (MS) spectra were obtained using a Variant 910 FT-ICR mass spectrometer (Varian). ESI-MS data were obtained on an 1100 LC-MSD Trap spectrometer (Agilent).

Column chromatography (CC) was performed on silica gel (70-230 and 230-400 mesh; Merck) and YMC C-18 resins (30-50 μm; Fuji Silysia Chemical Ltd.). Thin layer chromatography analyses were run on Kieselgel 60 F_{254} (Merck 1.05715) and RP-18 F_{254s} (Merck) plates; spots were visualized by spraying with 10% aqueous H_2SO_4, followed by heating.

Animal material

The marine soft coral *Lobophytum* sp. (2.0 kg, wet weight) was collected at Conco island, Quang Tri province in March, 2007, and was kept frozen (-20°C)

until used; it was identified by Dr. Do Cong Thung, Institute of Marine Resources and Environment, Vietnam Academy of Science and Technology (VAST). The specimen vouchers of the *Lobophytum* sp. were deposited at the Institute of Natural Products Chemistry and the Institute of Marine Resources and Environment, VAST.

Extraction and isolation

The frozen sample of *Lobophytum* sp. was exhaustively refluxed in MeOH (4 L × 3), and the combined MeOH extract was concentrated *in vacuo* to give a black gum residue (53 g). Then, the residue was suspended in H_2O (0.5 L), and partitioned with EtOAc (0.5 L × 3). Next, the EtOAc-soluble portion (9.5 g) was subjected to a silica gel column eluted stepwise with *n*-hexane-EtOAc (20:1-0:1, v/v) and EtOAc-MeOH (10:1-0:1) to give nine fractions (fr. 1.1-fr. 1.9). Thereafter, fr. 1.2 (1.1 g) was repeatedly chromatographed using a silica gel column eluted with *n*-hexane-EtOAc (15:1), followed by a reversed-phased (RP) column with acetone-MeOH-H_2O (2:5:1) to afford **1** (3.5 mg) and **2** (70.0 mg). Fr. 1.3 (1.3 g) was further chromatographed using a RP column with acetone-MeOH-H_2O (1:5:2), followed by a silica gel column with *n*-hexane-EtOAc (8:1) to afford **4** (7.5 mg) and **6** (14.0 mg). Fr. 1.4 (600 mg) was subjected to a silica gel column with *n*-hexane-EtOAc (4:1), followed by a RP column with acetone-MeOH-H_2O (1:5:2) to yield **3** (25.0 mg). Finally, repeated column chromatography of fr. 1.7 (800 mg) using silica gel ($CHCl_3$-MeOH, 10:1) and reversed-phase C-18 YMC (acetone-MeOH-H_2O, 1:5:2) resulted in the isolation of **5** (12.5 mg).

Lobophytene (1)

Colorless gum, $[\alpha]_D^{20}$ +8° (*c* 0.16, $CHCl_3$); IR (neat) ν_{max} 3458, 2945, 1655, 1639 cm^1; ^1H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz): see Table I; ESI-MS (positive) *m/z* 429 $[M+H]^+$; HR-FT-ICR-MS (positive) *m/z* 429.3985 $[M+H]^+$ (Calcd. for $C_{30}H_{53}O$, 429.4096).

(1*S*,2*S*,3*E*,7*E*,11*E*)-3,7,11,15-Cembratetraen-17,2-olide (2)

White powder, $[\alpha]_D^{20}$ +28° (*c* 0.36, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz) δ 6.20 (1H, d, *J* = 3.2 Hz, H-16a), 5.48 (1H, d, *J* = 3.2 Hz, H-16b), 5.37 (1H, dd, *J* = 10.0, 7.6 Hz, H-2), 4.96 (1H, d, *J* = 10.0 Hz, H-3), 4.94 (1H, br d, *J* = 6.0 Hz, H-11), 4.89 (1H, t, *J* = 7.2 Hz), 3.01 (1H, m, H-1), 1.63, 1.54, 1.53 (each 3H, s, H-18, 20, 19); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 43.4 (C-1), 78.1 (C-2), 123.8 (C-3), 142.3 (C-4), 39.6 (C-5), 24.4 (C-6), 125.4 (C-7), 133.5 (C-8), 39.9 (C-9), 23.4 (C-10), 120.3 (C-11), 133.5 (C-12), 36.3 (C-13), 27.0 (C-14), 138.9 (C-15), 120.0 (C-16), 170.7 (C-17), 15.7 (C-18), 15.1 (C-19), 15.2 (C-20); and ESI-MS (positive) *m/z* 301 $[M+H]^+$.

Lobohedleolide (3)

White powder, $[\alpha]_D^{20}$ +18° (*c* 0.32, $CHCl_3$); ^1H NMR ($CDCl_3$, 500 MHz) δ 6.29 (1H, d, *J* = 3.5 Hz, H-16a), 5.76 (1H, dd, *J* = 8.5, 4.0 Hz, H-7), 5.55 (1H, d, *J* = 3.5 Hz, H-16b), 5.45 (1H, dd, *J* = 10.0, 8.0 Hz, H-2), 5.06 (1H, d, *J* = 10.0 Hz, H-3), 4.96 (1H, br d, *J* = 8.0 Hz, H-11), 3.13 (1H, m, H-1), 1.74 and 1.55 (each 3H, s, H-18 and 20); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 43.2 (C-1), 78.1 (C-2), 120.8 (C-3), 142.2 (C-4), 40.0 (C-5), 25.2 (C-6), 148.5 (C-7), 129.0 (C-8), 36.3 (C-9), 26.9 (C-10), 123.1 (C-11), 135.5 (C-12), 36.3 (C-13), 27.2 (C-14), 138.9 (C-15), 121.0 (C-16), 170.8 (C-17), 15.5 (C-18), 172.5 (C-19), 16.3 (C-20); and ESI-MS (positive) *m/z* 331 $[M+H]^+$.

Lobocrassolide (4)

White powder, $[\alpha]_D^{20}$ +27° (*c* 0.37, $CHCl_3$); ^1H NMR ($CDCl_3$, 500 MHz) δ 6.26 (1H, d, *J* = 3.5 Hz, H-16a), 5.52 (1H, d, *J* = 3.5 Hz, H-16b), 5.42 (1H, dd, *J* = 10.0, 8.0 Hz, H-2), 5.08 (1H, dd, *J* = 9.0, 3.5 Hz, H-7), 5.03 (1H, d, *J* = 10.0 Hz, H-3), 4.95 (1H, t, *J* = 7.0 Hz, H-11), 4.58 (2H, s, H-19), 3.08 (1H, m, H-1), 2.07 (3H, s, $COC\underline{H}_3$), 1.69 and 1.58 (each 3H, s, H-18 and 20); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 43.5 (C-1), 78.2 (C-2), 120.8 (C-3), 142.0 (C-4), 39.8 (C-5), 24.2 (C-6), 132.4 (C-7), 132.5 (C-8), 36.3 (C-9), 24.4 (C-10), 123.8 (C-11), 134.5 (C-12), 36.3 (C-13), 27.3 (C-14), 139.0 (C-15), 120.8 (C-16), 170.8 (C-17), 15.3 (C-18), 61.9 (C-19), 16.1 (C-20), 171.3 ($\underline{C}OCH_3$), 21.2 ($CO\underline{C}H_3$); and ESI-MS (positive) *m/z* 359 $[M+H]^+$.

24-Methylcholestane-3β,5α,6β,25-tetrol 25-monoacetate (5)

White powder, $[\alpha]_D^{20}$ -17° (*c* 0.30, CH_3OH); ^1H NMR (CD_3OD, 400 MHz) δ 4.00 (1H, m), 3.45 (1H, br d, *J* = 3.2 Hz, H-6), 1.94 (3H, s, $COC\underline{H}_3$), 1.39 (3H, s, H-27), 1.37 (3H, s, H-26), 1.15 (3H, s, H-19), 0.95 (3H, d, *J* = 6.8 Hz, H-21), 0.88 (3H, d, *J* = 6.8 Hz, H-28), and 0.71 (3H, s, H-18); ^{13}C NMR (CD_3OD, 100 MHz) δ 31.7 (C-1), 33.5 (C-2), 68.3 (C-3), 43.3 (C-4), 75.8 (C-5), 76.8 (C-6), 40.0 (C-7), 31.6 (C-8), 46.6 (C-9), 39.3 (C-10), 22.3 (C-11), 41.4 (C-12), 43.9 (C-13), 57.3 (C-14), 25.2 (C-15), 28.7 (C-16), 57.4 (C-17), 12.6 (C-18), 17.3 (C-19), 35.3 (C-20), 19.5 (C-21), 37.5 (C-22), 29.2 (C-23), 41.5 (C-24), 87.4 (C-25), 23.2 (C-26), 23.8 (C-27), 14.9 (C-28), 172.4 ($\underline{C}OCH_3$), 22.4 ($CO\underline{C}H_3$); and ESI-MS (positive) *m/z* 493 $[M+H]^+$.

Pakisterol A (6)

White powder, $[\alpha]_D^{20}$ -13° (*c* 0.08, CH_3OH); ^1H NMR (pyridine-d_5, 400 MHz) δ 5.36 (1H, t, *J* = 5.2 Hz, H-6), 5.22 (1H, dd, *J* = 12.0, 5.2 Hz, H-11), 5.06 (1H, d, *J* = 7.2 Hz, H-1), 5.05 (1H, d, *J* = 12.0 Hz, H-12), 4.00 (1H, m), 1.01 (3H, d, *J* = 6.4 Hz, H-21), 0.95 (3H, s, H-19), 0.90 (3H, d, *J* = 6.4 Hz, H-26), 0.89 (3H, t, *J* = 7.2 Hz, H-29), 0.88 (3H, d, *J* = 6.8 Hz, H-27), 0.67 (3H, s, H-18); ^{13}C NMR (pyridine-d_5, 100 MHz) δ 37.5 (C-1), 32.1 (C-2), 78.4 (C-3), 39.9 (C-4), 140.9 (C-5), 121.9 (C-6), 24.5 (C-7), 50.3 (C-8), 51.4 (C-9), 36.9 (C-10), 129.4 (C-11), 138.8 (C-12), 42.4 (C-13), 56.8 (C-14), 24.5 (C-15), 28.6 (C-16), 56.2 (C-17), 12.2 (C-18), 19.4 (C-19), 36.4 (C-20), 19.0 (C-21), 39.3 (C-22), 26.4 (C-23), 46.0 (C-24), 29.5 (C-25), 20.0 (C-26), 19.0 (C-27), 23.4 (C-28), 12.0 (C-29), 102.5 (C-1), 75.2 (C-2), 78.1 (C-3), 71.6 (C-4), 78.4 (C-5), 62.7 (C-6); and ESI-MS (positive) *m/z* 575 $[M+H]^+$.

Cytotoxic assay

Cell growth inhibition by different samples was analyzed using colorimetric MTT [3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl-tetrazolium bromide] assay (Mosmann, 1983). Briefly, cells were seeded into 96-well plates at a density of $1.5\sim3 \times 10^5$ cells/well. The cells were then treated with 1, 10, 30, 50, and 100 μM of compounds. Mitoxantrone (MX) (Sigma-Aldrich) was used as the positive control. After 3 days, the cells were treated with 50 μL MTT (2 mg/mL). Plates were incubated at 37°C for 4 h, the media was carefully aspirated. 150 μL Dimethylsulfoxide (DMSO) was then added to each well to dissolve the formazan crystals. The plates were read immediately at 540 nm on a microplate reader (Amersham Pharmacia Biotech.). All the experiments were performed at least three times in triplicate and the mean absorbance values were calculated.

RESULTS AND DISCUSSION

The sample *Lobophytum* sp. was extracted with MeOH, then the MeOH residue was partitioned between EtOAc and H_2O. The EtOAc-soluble portion was chromatographed using silica gel and C-18 YMC resin columns to afford **1**-**6** (Fig. 1).

Compound **1**, obtained as a colorless gum, has the

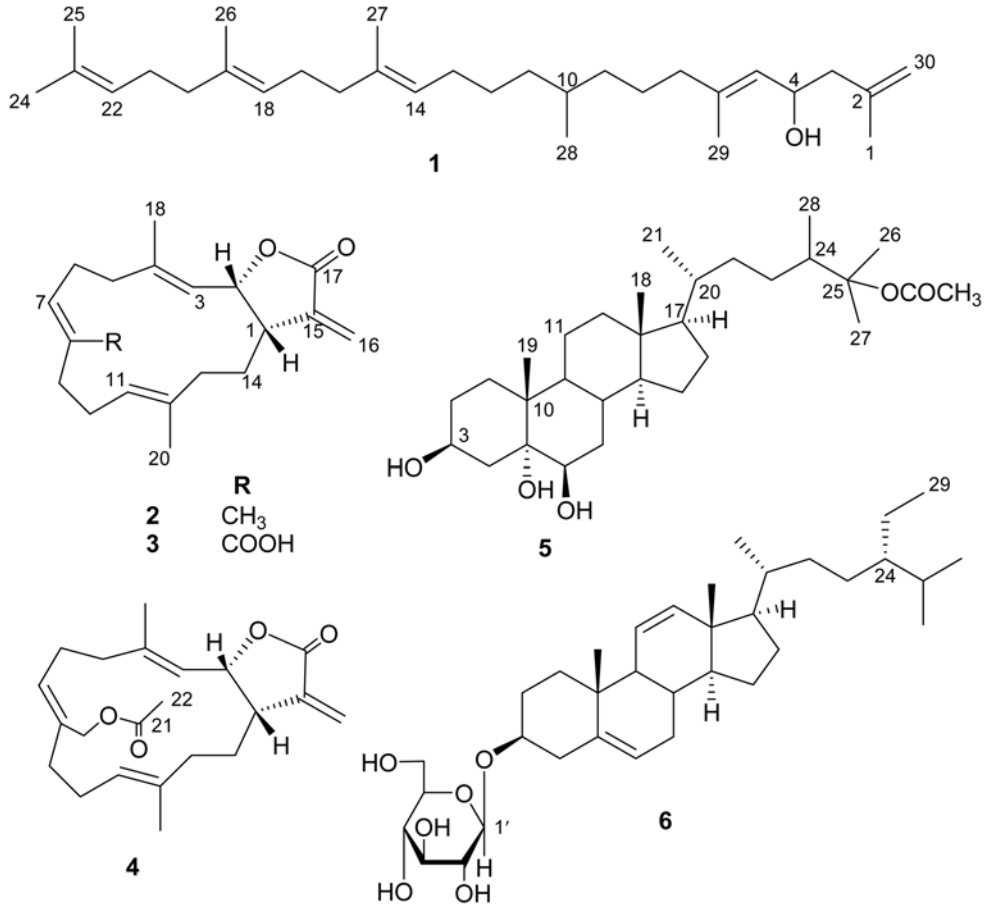

Fig. 1. Structures of **1**-**6**

molecular formula $C_{30}H_{50}O$ as deduced by HR-FT-ICR-MS measurement. The ^{1}H NMR spectrum (Table I) of **1** exhibited signals of six olefinic protons accounting for two geminal protons of the >C=CH_2 group [δ 4.81 and 4.97 (1H each, br s, H-30)] and four vinylic protons [δ 4.88 (1H, m, H-18), 5.00 (1H, m, H-5), 5.07 (1H, m, H-22), and 5.40 (1H, br d, J = 8.0 Hz, H-14)]; an oxymethine proton at δ 4.44 (1H, br d, J = 8.0 Hz, H-4); five vinylic methyls at δ 1.54 (3H, s, H-29), 1.59 (6H, s, H-25, 26), 1.61 (3H, s, H-27), and 1.80 (3H, s, H-24); and another methyl at δ 1.58 (3H, d, J = 10 Hz, H-28). The ^{13}C NMR spectrum (Table I) of **1** showed signals of thirty carbons including ten olefinic carbons at δ 147.1 (C-2), 136.1 (C-15), 134.2 (C-6), 134.0 (C-19), 133.0 (C-23), 127.8 (C-14), 126.1 (C-18), 125.0 (C-5), 121.1 (C-22), 111.6 (C-30), and an oxymethine carbon at δ 70.1 (C-4). In addition, it was possible to assign carbon signals with corresponding proton signals using the HMQC spectrum, and the partial structures of **1** were confirmed from the HMBC, COSY, and ROESY spectra. Furthermore, the partial structure in part of C-12~C-27 was well in consistent with that of (6*S*, 7*S*)-squalene-6,7-epoxide (Napoli et al., 1982). The hydroxy group at C-4 was assured by HMBC correlations of H-4 at δ 4.44 (1H, br d, J = 8.0 Hz, H-4) with C-2 at δ 147.1 and C-6 at δ 134.2. The configuration of double bonds at C-5, 14, and C-18 was proposed all as *E* form based on its ROESY spectrum, in which no correlations of H-5/H-29, H-14/H-27, and H-18/H-26 were observed. Moreover, this indication was putatively assigned on the basis of the general biosynthesis of squalene derivatives, which were further in process to form various triterpene and steroid skeletons (Abe et al., 1993; Haralampidis et al., 2002; Vincken et al., 2007). Consequently, the structure of **1** was elucidated as 6,10,15,19,23-pentamethyltetracosa-2(30),5*E*,14*E*,18*E*,22-pentaen-4-ol, a new squalene derivative, namely lobophytene.

Table I. NMR data for **1**

Position	δ_C[a,b]	δ_H[a,c]	HMBC
1	23.3	1.82 s	C-2, 3, 30
2	147.1		
3	50.5	2.16-2.25[d]	
4	70.1	4.44 br d (8.0)	C-2, 5, 6
5	125.0	5.00 m	C-7, 29
6	134.2		
7	40.0	2.12 m 2.26 m	C-5, 29
8	21.6	1.77 m 2.07 m	
9	38.8	2.05 m 2.15 m	
10	33.7	2.14 m	
11	39.3	2.01 m 2.14 m	
12	25.4	2.13 m 2.37 m	
13	24.9	2.07 m 2.30 m	
14	127.8	5.40 d (8.0)	C-12
15	136.1		
16	39.9	2.01 m 2.14 m	
17	24.0	2.10-2.15[d]	
18	126.1	4.88 m	
19	134.0		
20	40.0	2.10-2.30[d]	
21	24.3	2.06 m 2.20 m	
22	121.1	5.07 m	
23	133.0		
24	23.0	1.80 s	C-22, 23
25	15.7	1.59 s	C-22, 23
26	15.0	1.59 s	C-18, 20
27	15.5	1.61 s	C-14, 16
28	19.1	1.58 d (10.0)	C-9, 10, 11
29	15.4	1.54 s	C-5, 6
30	111.6	4.81 br s 4.97 br s	C-2, 3

[a]Measured in $CDCl_3$, [b]125 MHz, [c]500 MHz, [d]overlapped signal. Assignments were confirmed by COSY, HMQC, HMBC, and ROESY experiments

Compounds **2**-**6** were identified as (1*S*,2*S*,3*E*,7*E*,11*E*)-3,7,11,15-cembratetraen-17,2-olide (**2**) (Uchio et al., 1982), lobohedleolide (**3**) (Uchio et al., 1981), lobocrassolide (**4**) (Chang et al., 2000), 24-methylcholestane-3β,5α,6β,25-tetrol 25-monoacetate (**5**) (Yamada et al., 1980), and pakisterol A (**6**) (Hussain et al., 2008) on the basis of physicochemical properties, spectroscopic (NMR, MS) data, and comparison of those with reported data in the literature. To the best of our knowledge, pakisterol A (**6**) was isolated for the first

Table II. Cytotoxicity data of compounds **1**-**6**

Compound	IC_{50} (μM)	
	A-549 (Lung)	HT-29 (Colon)
1	8.2	5.6
2	5.1	1.8
3	42.6	35.5
4	31.4	22.0
5	36.9	3.7
6	29.3	23.8
Mitoxantrone[a]	6.1	6.5

[a]Positive control

time from *Lobophytum* species.

Cytotoxic activities of all the isolated compounds were tested against two human cancer cell lines, lung (A549) and colon (HT-29), using the MTT assay. As the results (Table II), lobophytene (**1**) and **2**, a cembranoid diterpene, showed significantly cytotoxic activity against both cell lines with IC_{50} values of 8.2 and 5.6 μM for **1**; 5.1 and 1.8 μM for **2**, respectively. The activities of **1** and **2** were comparable to that of the positive control, mitoxantrone. Compound **5** showed strong effect on HT-29 cells with an IC_{50} value of 3.7 μM, but weak effect on A549 cells with IC_{50} value of 36.9 μM; whereas the others displayed weak activity against both the cancer cell lines. In this regard, there have been some squalene derivatives and cembranoid diterpenes exhibiting anticancer properties (Morita et al., 1993; Norte et al., 1997; Chang et al., 2000; Radhika et al., 2004). In addition, some components from *Lobophytum* species were also found to have cytotoxic activity (Chang et al., 2000; Radhika et al., 2004). In conclusion, the present study suggests contributions for anticancer evidence of *Lobophytum* sp. Especially, one new cytotoxic squalene derivative, lobophytene (**1**), was investigated.

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REFERENCES

Abe, I., Rohmer, M., and Prestwich, G. C., Enzymatic cyclization of squalene and oxidosqualene to sterols and triterpenes. *Chem. Rev.*, 93, 2189-2206 (1993).

Chang, Y. D., Shang, K. W., Bao, T. H., and Chang, F. D., Cytotoxic cembrenolide diterpenes from the Formosan soft coral *Lobophytum crassum*. *J. Nat. Prod.*, 63, 884-885 (2000).

Chao, C. H., Huang, H. C., Wu, Y. C., Lu, C. K., Dai, C. F., and Sheu, J. H., Glycolipids from the Formosan soft coral *Lobophytum crassum*. *Chem. Pharm. Bull.*, 55, 1720-1723 (2007).

Cheng, S. Y., Wen, Z. H., Wang, S. K., Chiou, S. F., Hsu, C. H., Dai, C. F., and Duh, C. Y., Anti-inflammatory cembranolides from the soft coral *Lobophytum durum*. *Bioorg. Med. Chem.*, 17, 3763-3769 (2009).

Cuong, N. X., Tuan, T. A., Kiem, P. V., Minh, C. V., Choi, E. M., and Kim, Y. H., New cembranoid diterpenes from the Vietnamese soft coral *Sarcophyton mililatensis* stimulate osteoblastic differentiation in MC3T3-E1 cells. *Chem. Pharm. Bull.*, 56, 988-992 (2008).

Dang, N. H., Thanh, N. V., Kiem, P. V., Huong, L. M., Minh, C. V., and Kim, Y. H., Two new triterpene glycosides from the Vietnamese sea cucumber *Holothuria scabra*. *Arch. Pharm. Res.*, 30, 1387-1391 (2007).

Fattorusso, E., Romano, A., Taglialatela-Scafati, O., Achmad, M. J., Bavestrello, G., and Cerrano, C., Lobozoanthamine, a new zoanthamine-type alkaloid from the Indonesian soft coral *Lobophytum* sp. *Tetrahedron Lett.*, 49, 2189-2192 (2008).

Haralampidis, K., Trojanowska, M., and Osbourn, A., Biosynthesis of triterpenoid saponins in plants. *Adv. Biochem. Eng.*, 75, 31-49 (2002).

Hussain, M., Zahra, D. N., Hussain, S. M. S., Ahmed, E., Ahmad, I., Malik, A., and Ahmed, Z., Structure determination of new steroids from *Abutilon pakistanicum* by NMR techniques. *Magn. Reson. Chem.*, 46, 274-277 (2008).

Minh, C. V., Cuong, N. X., Tuan, T. A., Choi, E. M., Kim, Y. H., and Kiem, P. V., A new 9,11-secosterol from the Vietnamese Sea soft coral *Sarcophyton mililatensis* increases the function of osteoblastic MC3T3-E1 cells. *Nat. Prod. Commun.*, 2, 1095-1100 (2007a).

Minh, C. V., Kiem, P. V., and Cuong, N. X., New 9,11-secosterol glycoside and ceramide from a soft coral *Cladiella* sp. living in Vietnamese sea. *Asean J. Sci. Tech. Develop.*, 24, 275-283 (2007b).

Minh, C. V., Kiem, P. V., Tuan, T. A., and Cuong, N. X., Two new brominated fatty acids from the sponge *Xestospongia testudinaria* living in Vietnamese sea. *Adv. Nat. Sci.*, 9, 157-161 (2008).

Morita, H., Kishi, E., Takeya, K., Itokawa, H., and Iitaka, Y., Squalene derivatives from *Eurycoma longifolia*. *Phytochemistry*, 34, 765-771 (1993).

Mosmann, T., Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *J. Immunol. Methods*, 65, 55-63 (1983).

Muralidhar, P., Kumar, M. M., Krishna, N., Rao, C. B., Rao, D. V., New sphingolipids and a sterol from a *Lobophytum* species of the Indian ocean. *Chem. Pharm. Bull.*, 53, 168-171 (2005).

Napoli, L. D., Fattorusso, E., Magno, S., and Mayol, L., Three squalene derivatives from *Caulerpa prolifera*. *Phytochemistry*, 21, 782-784 (1982).

Norte, M., Fernandez, J. J., Souto, M. L., Gavin, J. A., and Garcia-Gravalos, M. D., Thyrsenols A and B, two unusual polyether squalene derivatives. *Tetrahedron*, 53, 3173-3178 (1997).

Radhika, P., Rao, V. L., and Laatsch, H., Chemical constituents of a marine soft coral of the genus *Lobophytum*. *Chem. Pharm. Bull.*, 52, 1345-1348 (2004).

Tung, N. H., Minh, C. V., Ha, T. T., Kiem, P. V., Huong, H. T., Dat, N. T., Nhiem, N. X., Tai, B. H., Hyun, J. H., Kang, H. K., and Kim, Y. H., C_{29} sterols with a cyclopropane ring at C-25 and 26 from the Vietnamese marine sponge *Ianthella* sp. and their anticancer properties. *Bioorg. Med. Chem. Lett.*, 19, 4584-4588 (2009).

Uchio, Y., Toyota, J., Nozaki, H., Nakayama, M., Nishizono, Y., and Hase, T., Lobohedleolide and (7Z)-lobohedleolide, new cembranolides from the soft coral *Lobophytum hedleyi* Whitelegge. *Tetrahedron Lett.*, 22, 4089-4092 (1981).

Uchio, Y., Eguchi, S., Nakayama, M., and Hase, T., The isolation of two simple γ-lactonic cembranolides from the soft coral *Sinularia mayi*. *Chem. Lett.*, 3, 277-278 (1982).

Vincken, J. P., Heng, L., De Groot, A., and Gruppen, H., Saponins, classification and occurrence in the plant kingdom. *Phytochemistry*, 68, 275-297 (2007).

Yamada, Y., Suzuki, S., Iguchi, K., Kikuchi, H., Tsukitani, Y., Horiai, H., and Nakanishi, H., Studies on marine natural products. II. New polyhydroxylated sterols from the soft coral *Lobophytum pauciflorum* (Ehrenberg). *Chem. Pharm. Bull.*, 28, 473-478 (1980).