Bioorganic & Medicinal Chemistry Letters 21 (2011) 2155–2159

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Cytotoxic and antioxidant activities of diterpenes and sterols from the Vietnamese soft coral *Lobophytum compactum*

Chau Van Minh [a,*], Phan Van Kiem [a], Nguyen Xuan Nhiem [a,b], Nguyen Xuan Cuong [a], Nguyen Phuong Thao [a], Nguyen Hoai Nam [a], Hoang Le Tuan Anh [a], Do Cong Thung [c], Dinh Thi Thu Thuy [d], Hee-Kyoung Kang [e], Hae-Dong Jang [f], Young Ho Kim [b,*]

[a] *Institute of Marine Biochemistry, Vietnam Academy of Science and Technology (VAST), 18 Hoang Quoc Viet, Caugiay, Hanoi, Viet Nam*
[b] *College of Pharmacy, Chungnam National University, Daejeon 305-764, Republic of Korea*
[c] *Institute of Marine Resources and Environment, VAST, 246 Da Nang street, Haiphong, Viet Nam*
[d] *Institute of Natural Products Chemistry, VAST, 18 Hoang Quoc Viet, Caugiay, Hanoi, Viet Nam*
[e] *School of Medicine, Institute of Medical Sciences, Cheju National University, Jeju 690-756, Republic of Korea*
[f] *Department of Food and Nutrition, Hannam University, Daejeon 305-811, Republic of Korea*

ARTICLE INFO

Article history:
Received 9 November 2010
Revised 31 December 2010
Accepted 17 January 2011
Available online 22 January 2011

Keywords:
Lobophytum compactum
Lobocompactol A
Lobocompactol B
Cytotoxic activity
Oxygen radical absorbance capacity

ABSTRACT

Two new diterpenes, lobocompactols A (**1**) and B (**2**), and five known compounds (**3**–**7**) were isolated from the methanol extract of the soft coral *Lobophytum compactum* using combined chromatographic methods and identified based on NMR and MS data. Each compound was evaluated for cytotoxic activity against A549 (lung) and HL-60 (acute promyelocytic leukemia) human cancer cell lines. Among them, compound **5** exhibited strong cytotoxic activity against the A549 cell line with an IC_{50} of 4.97 ± 0.06 μM. Compounds **3**, **4**, and **7** showed moderate activity with IC_{50} values of 23.03 ± 0.76, 31.13 ± 0.08, and 36.45 ± 0.01 μM, respectively. The cytotoxicity of **5** on the A549 cells was comparable to that of the positive control, mitoxantrone (MX). All compounds exhibited moderate cytotoxicity against the HL-60 cell line, with IC_{50} values ranging from 17.80 ± 1.43 to 59.06 ± 2.31 μM. Their antioxidant activity was also measured using oxygen radical absorbance capacity method, compounds **1** and **2** exhibiting moderate peroxyl radical scavenging activity of 1.4 and 1.3 μM Trolox equivalents, respectively, at a concentration of 5 μM.

Marine organisms are rich in biologically active compounds, many of which have unique structures that are not found in terrestrial organisms.[1] In particular, soft corals have received a great deal of attention from scientists worldwide because of the ease with which samples can be obtained and the diversity of chemical constituents and biological activities. Soft corals comprise a significant group of marine organisms and occur in coral reefs worldwide, with a marked preference for tropical waters at depths between 5 and 30 m. The genera *Sinularia*, *Lobophytum* and *Sarcophyton* are the most prolific. Interestingly, some *Sarcophyton* species contain diterpenes at levels of up to 10% of their dry weight. Such large amounts of these secondary metabolites may be important to the survival of octocorals with regard to defensive, competitive, reproductive, and possibly pheromonal functions. It is conceivable that soft corals, which lack any form of physical defense, are protected from predation by the sheer levels of diterpene toxins in their tissue.[2]

As a part of an ongoing project to catalogue the chemical constituents and biological activities of Vietnamese marine organisms, we report herein the isolation, structural elucidation, and evaluation of *in vitro* cytotoxic and antioxidant activities of four diterpenes including two new compounds, lobocompactols A (**1**) and B (**2**), and three sterols from the methanol extract of the soft coral *Lobophytum compactum* (see Fig. 1).

The specimens of *Lobophytum compactum* were collected in Bay Canh island, Truong Sa archipelago, Khanh Hoa, Viet Nam during January 2010 and deep frozen until used. The sample was identified by one of us, Dr. Do Cong Thung. A voucher of specimen (No. NCCB M-14) was deposited at Institute of Marine Biochemistry and Institute of Marine Resources and Environment, VAST, Viet Nam.

Fresh frozen samples of the soft coral *L. compactum* (30 kg) were finely ground and extracted three times with hot MeOH (50 °C for 3 h each time) and concentrated under reduced pressure to yield the MeOH extract (210 g). This extract was suspended in water (2 L) and partitioned in turn with chloroform (3 × 2 L). The chloroform extract (140 g) was crudely separated on a silica gel chromatographic column (CC) with a gradient elution of ethyl acetate in *n*-hexane from 0% to 100% to yield five fractions (LC1–LC5). Fraction LC1 (47.1 g) was further separated on a silica gel CC eluting

* Corresponding authors. Tel.: +84 4 37917053; fax: +84 4 37917054 (C.V.M.); tel.: +82 42 821 5933; fax: +82 42 823 6566 (Y.H.K.).
E-mail addresses: cvminh@vast.ac.vn (C.V. Minh), yhk@cnu.ac.kr (Y.H. Kim).

0960-894X/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2011.01.072

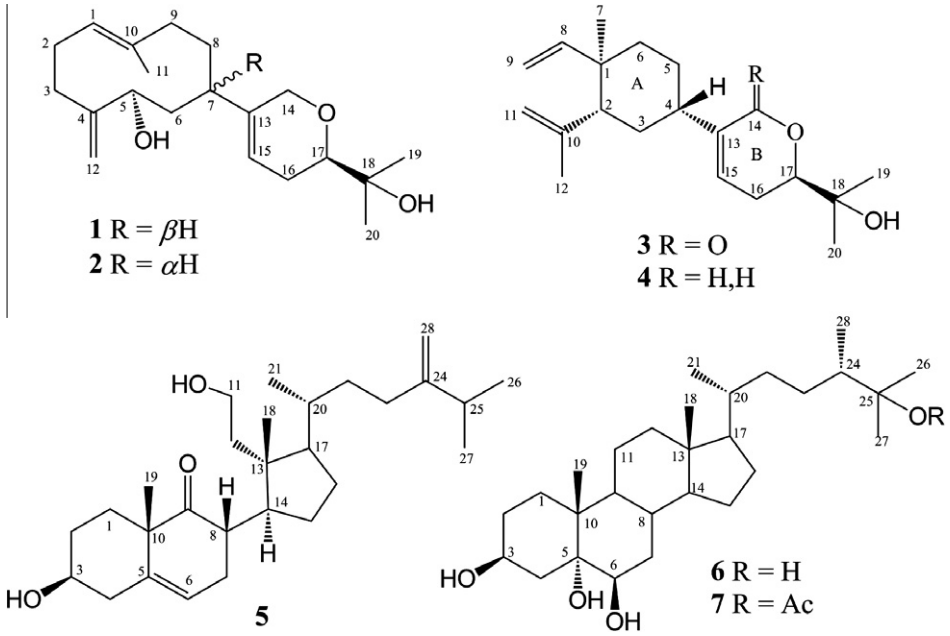

Figure 1. Structures of **1**–**7**.

with *n*-hexane–acetone (30:1, v/v) to obtain nine sub-fractions, LC1A–LC1I. Purification of sub-fraction LC1C (2.5 g) on a silica gel CC with *n*-hexane–ethyl acetate (25:1, v/v) furnished lobatriene (**4**, 200 mg)[3,4] as a colorless oil. Fraction LC2 (32.2 g) was divided into seven sub-fractions, LC2A–LC2G, by a silica gel CC eluted with chloroform–acetone (18:1, v/v). Sub-fraction LC2D (3.5 g) was further separated on an YMC CC using an acetone–water (5:1, v/v) as eluent to give lobocompactol A (**1**, 14 mg),[5] lobocompactol B (**2**, 23 mg),[5] and lobatrienolide (**3**, 38 mg)[6] as colorless oils. Fraction LC4 (13.2 g) was further separated by a silica gel CC eluted with *n*-hexane–acetone (3:1, v/v) to obtain five sub-fractions, LC4A–LC4E. Compounds 3β,11-dihydroxy-24-methylene-9,11-secocholestan-5-en-9-one (**5**, 23 mg),[7] (24*S*)-ergostane-3β,5α,-6β,25-tetraol (**6**, 21 mg),[8] and (24*S*)-ergostane-3β,5α,6β,25-tetraol 25-monoacetate (**7**, 37 mg)[9] were purified as white crystals from sub-fraction LC4C (5 g) following a two-stage separation beginning with a silica gel CC eluted with chloroform–acetone (7:1, v/v), followed by an YMC CC eluted with acetone–water (4:1, v/v).

Table 1
The NMR spectroscopic data of **1** and **2**

Position	**1**		**2**	
	δ_C[a,b]	δ_H[a,c] mult. (*J*, Hz)	δ_C[a,b]	δ_H[a,c] mult. (*J*, Hz)
1	125.4	5.36 d (1.0)	125.4	5.36 br s
2	25.3	2.13 m/2.39 m	24.5	2.23 m/2.40 m
3	25.3	2.13 m	28.8	1.30 m/1.66 m
4	149.1	—	149.2	—
5	75.9	3.88 br d (8.5)	75.5	4.05 br d (3.5)
6	36.5	1.40 m	32.5	1.38 m/1.80 m
7	39.5	1.67 m	37.1	2.12 m
8	31.5	1.42 m	31.9	1.37 m
9	40.2	1.94 m/2.14 m	39.7	1.95 m/2.12 m
10	133.5	—	132.7	—
11	15.7	1.64 s	15.5	1.62 s
12	115.4	5.07 br s/5.10 br s	109.7	5.13 br s/5.28 br s
13	140.5	—	145.1	—
14	67.6	4.12 br d (16.0)	66.9	4.22 br d (16.0)
		4.25 br d (16.0)		4.31 br d (16.0)
15	117.8	5.54 m	117.7	5.66 m
16	25.3	1.93 m (α)	25.1	1.97 m (α)
		2.13 m (β)		2.15 m (β)
17	80.3	3.25 dd (3.0, 11.0)	79.9	3.27 dd (3.0, 11.0)
18	71.7	—	71.6	—
19	26.1	1.21 s	26.2	1.21 s
20	23.8	1.16 s	23.5	1.15 s

All assignments were done by HSQC, HMBC, COSY, and ROESY experiments.
[a] Measured in $CDCl_3$.
[b] 125 MHz.
[c] 500 MHz.

Lobocompactol A (**1**) was isolated as a colorless oil with a molecular formula of $C_{20}H_{32}O_3$ as determined by ion peaks in the ESIMS at *m/z* 303 $[M-H_2O+H]^+$ (positive) and in the Fourier transform ion cyclotron resonance mass spectrum (FTICRMS) at *m/z* 343.22462 $[M+Na]^+$ (calcd for $C_{20}H_{32}O_3Na$, 343.22491). The ^1H NMR spectrum of **1** revealed four olefinic [δ 5.07 (1H, br s), 5.10 (1H, br s), 5.36 (1H, d, *J* = 1.0 Hz), and 5.54 (1H, m)] and four oxygenated protons [δ 3.25 (1H, dd, *J* = 3.0 and 11.0 Hz), 3.88 (1H, br d, *J* = 8.5 Hz), 4.12 (1H, br d, *J* = 16.0 Hz), and 4.25 (1H, br d, *J* = 16.0 Hz)]. Moreover, the proton signals at δ 1.16, 1.21, and 1.64 (each 3H, s) indicated the presence of three tertiary methyl groups. The ^{13}C NMR spectrum indicated 20 carbons including typical signals of three methyl (δ 15.7, 23.8, and 26.1), one oxymethylene (δ 67.6), two oxymethine (δ 75.9 and 80.3), and one oxygenated quaternary carbons (δ 71.7), detected by DEPT experiments. In addition, the presence of one olefinic methylene (δ 115.4), two olefinic methine (δ 117.8 and 125.4), and three quaternary olefinic carbons (δ 133.5, 140.5, and 149.1) indicated three double bonds. All of the protons were assigned to relevant carbons by HSQC correlations (Table 1). Compound **1** was considered to be a bicyclic diterpene by its two remaining degrees of unsaturation

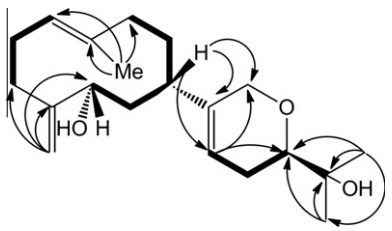

Figure 2. Selected HMBC (→) and ^1H–^1H COSY (—) correlations of **1**.

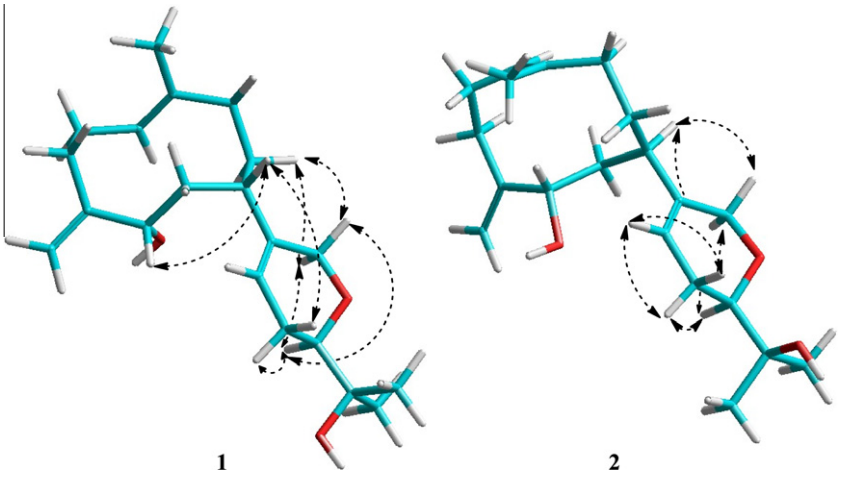

Figure 3. Important ROESY correlations of **1** and **2**.

Table 2
Effects of **1**–**7** on the growth of human cancer cells

Compound	IC_{50}[a] (μM)	
	A549 (Lung)	HL-60 (Leukemia)
1	>100	48.24 ± 1.33
2	>100	37.51 ± 0.38
3	23.03 ± 0.76	24.79 ± 0.77
4	31.13 ± 0.08	33.82 ± 0.27
5	4.97 ± 0.06	17.80 ± 1.43
6	>100	59.06 ± 2.31
7	36.45 ± 0.01	42.76 ± 2.85
MX[b]	7.83 ± 0.04	8.10 ± 0.60

[a] IC_{50} (concentration that inhibits 50% of cell growth). Compounds were tested at a maximum concentration of 100 μM. Data are presented as the mean ± standard deviation (SD) of experiments performed in triplicate.
[b] Mitoxantrone (MX), an anticancer agent, was used as a reference compound.

(five in total from the molecular formula minus three double bonds). The ^1H–^1H COSY experiment of **1** allowed the assignments of the proton–proton correlations for H-1/H_2-2/H_2-3, H-5/H_2-6/H-7/H_2-8/H_2-9, and H-15/H_2-16/H-17. These data together with the HMBC cross peaks between H_3-11 and C-1/C-9/C-10 and between H_2-12 and C-3/C-4/C-5 confirmed the connectivities from C-1 to C-10, indicating a ten-membered ring and the locations of the exo-methylene, olefinic methyl, and hydroxy groups (see Fig. 2).

The configuration of the C-1/C-10 double bond was identified as *trans* based on the ^{13}C NMR chemical shift of C-11 at δ 15.7 (<20 ppm).[10] On the other hand, the chemical shifts of C-3 (δ 25.3), C-4 (δ 149.1), C-5 (δ 75.9), and C-6 (δ 36.5) of **1** suggested hydroxyl group at C-5 to be α-orientation by agreement of them with the corresponding data of the gyrosanol C[11] [δ values for C-3 (25.5), C-4 (149.2), C-5 (76.3), and C-6 (36.8)] and difference from those of structurally similar compounds having a β-orientation of the hydroxyl group at C-5, (1*R*,5*R*,7*S*,9*S*,10*S*)-9-acetoxy-l(10)-epoxy-5-hydroxygermacra-4(15),11(13)-diene[12] [δ values for C-3 (22.9), C-4 (147.3), C-5 (75.6), and C-6 (35.9)]. Moreover, the ROESY correlation between H_β-5 (δ 3.88) and H-7 (δ 1.67) confirmed the same orientation of H-5 and H-7. The ^{13}C NMR chemical shifts from

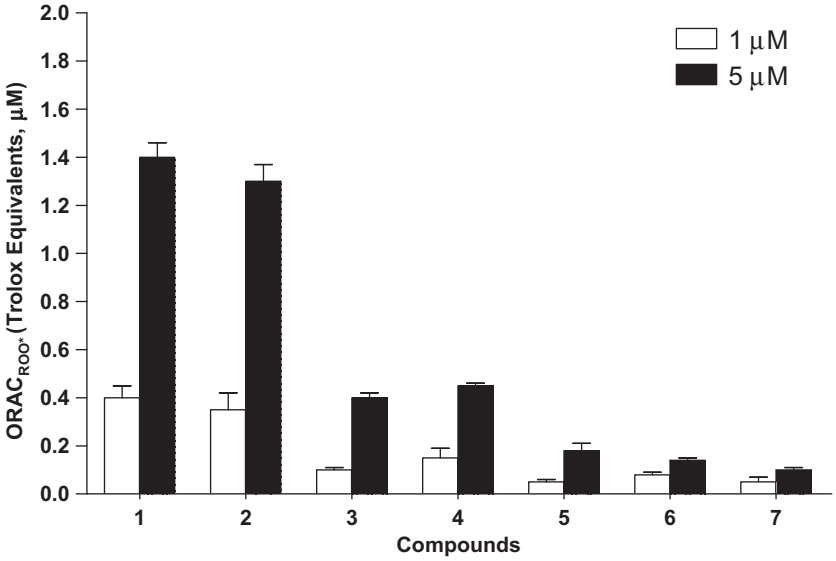

Figure 4. Peroxyl radical-scavenging activity (Trolox equivalent, μM) of **1**–**7**. The ORAC value is calculated by dividing the area under the sample curve by the area under the Trolox curve, with both areas being corrected by subtracting the area under the blank curve. One ORAC unit is assigned as the net area of protection provided by Trolox at a final concentration of 1 μM. The area under the curve of the sample is compared to the area under the curve for Trolox, and the antioxidative value is expressed in micromoles of Trolox equivalent per liter. The results represent the mean ± S.D. of values obtained from three measurements.

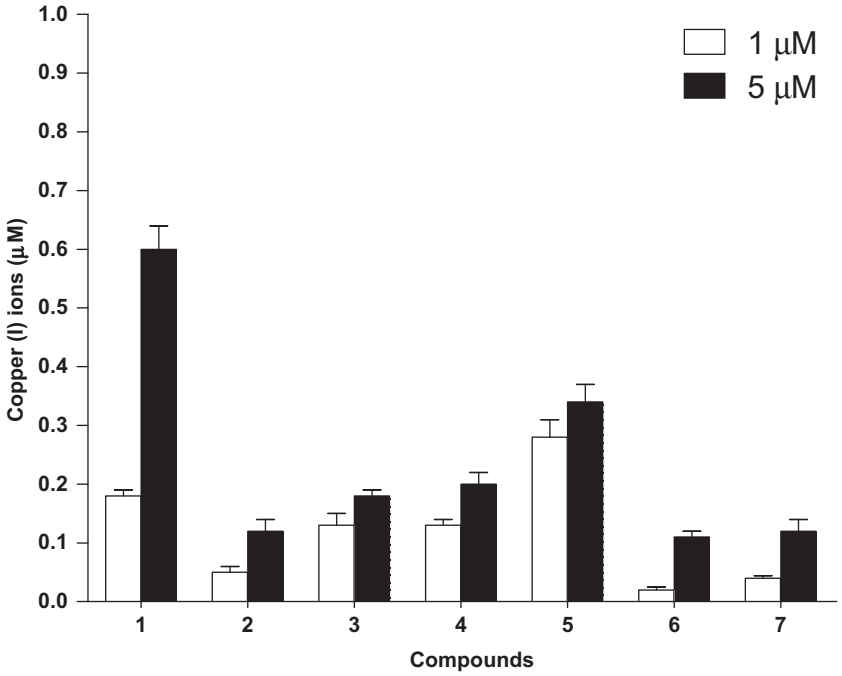

Figure 5. Reduction potential of **1**–**7**. The results represent the mean ± S.D. of values obtained from three measurements.

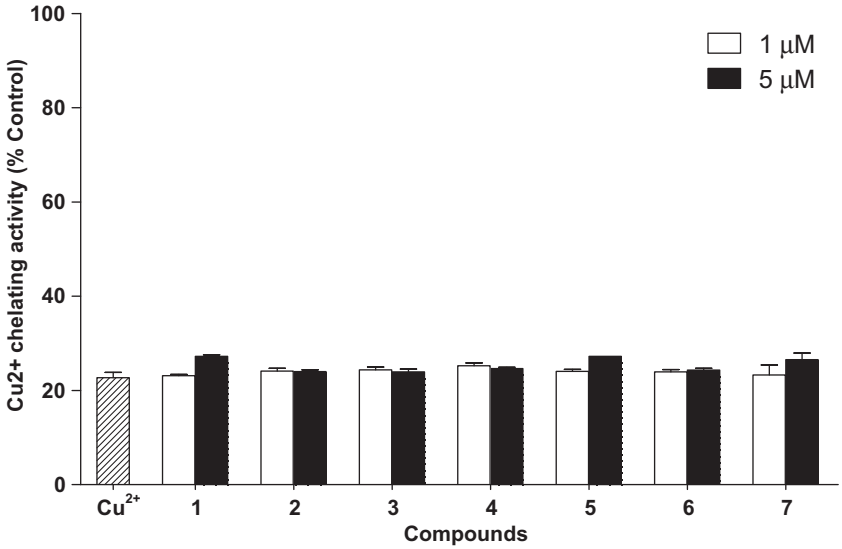

Figure 6. Metal chelating activity of **1**–**7**. The results represent the mean ± S.D. of values obtained from three measurements.

C-13 to C-20 of **1** were identical to those of lobatriene (**4**),[3,4] indicating that these two compounds have the same B ring with a 2-hydroxyisopropyl group at C-17. The configuration at C-17 was determined to be 17*R* by excellent agreement of the ^{1}H and ^{13}C NMR data of lobatriene[4] and further confirmed by ROESY correlations between H-7$_{\beta}$ (δ 1.67) and H$_{\beta}$-16 (δ 2.13), H$_{\alpha}$-16 (δ 1.93) and H$_{\alpha}$-17 (3.25) (see Fig. 3). From all above evidence, the structure of **1** (Fig. 1) was elucidated and the compound was named lobocompactol A.

The molecular formula of lobocompactol B (**2**) was also $C_{20}H_{32}O_3$, as identified by ion peaks in the ESIMS at *m/z* 303 $[M-H_2O+H]^+$ (positive) and FTICRMS at *m/z* 343.22449 $[M+Na]^+$ (calcd for $C_{20}H_{32}O_3Na$, 343.22491). Detailed analyses of the 1D and 2D NMR spectra indicated that **2** has the same planar structure as that of **1**. The chemical shifts of C-3 (δ 28.8), C-4 (δ 149.2), and C-5 (δ 75.5) in the diterpene **2** confirmed hydroxyl group at C-5 to be α-orientation by comparing with the corresponding data of the gyrosanol C[11] [δ values for C-3 (25.5), C-4 (149.2), and C-5 (76.3)] and (1*R*,5*R*,7*S*,9*S*,10*S*)-9-acetoxy-l(10)-epoxy-5-hydroxygermacra-4(15),11(13)-diene[12] [δ values for C-3 (22.9), C-4 (147.3), and C-5 (75.6)]. However, there were some noticeable difference in the ^{13}C NMR chemical shifts, especially C-6 (δ 32.5), C-7 (δ 37.1), and C-13 (δ 145.1), indicating the change of configuration at C-7 in **2** by comparing with that of **1**. This was further confirmed by none ROESY correlations between H$_{\beta}$-5 (δ 4.05) and H-7 (δ 2.12). The configuration at C-17 was also determined to be 17*R* by comparing ^{1}H and ^{13}C NMR data for the B ring of **2** with those of lobatriene[4] and further confirmed by ROESY correlations between H$_{\alpha}$-16 (δ 1.97) and H$_{\alpha}$-17 (δ 3.27) (see Fig. 3). Thus, the structure of **2** was confirmed and named lobocompactol B.

Compounds **1**–**7** were evaluated for cytotoxic activity against A549 (lung) and HL-60 (acute promyelocytic leukemia) human cancer cell lines, after continuous exposure for 72 h (Table 2). Compound **5** exhibited a strong activity against the A549 cell line with an IC_{50} of 4.97 ± 0.06 μM, while **3**, **4**, and **7** exhibited moderate activity with IC_{50} values of 23.03 ± 0.76, 31.13 ± 0.08, and 36.45 ± 0.01 μM, respectively. Compounds **1**, **2**, and **6** were inactive (IC_{50} >100 μM). The cytotoxic activity of **5** on the A549 cells was comparable to that of the positive control, mitoxantrone (MX). All compounds showed moderate cytotoxic activity against the HL-60 cell line with IC_{50} values ranging from 17.80 ± 1.43 to 59.06 ± 2.31 μM.

The antioxidant capacity of compounds **1**–**7** was measured using an oxygen radical absorbance capacity (ORAC) assay (see Fig. 4). Compounds **1** and **2** showed moderate peroxyl radical-scavenging activities of 1.4 and 1.3 μM Trolox equivalents, respectively, at a concentration of 5 μM. The others showed weak or no activity. All of the evaluated compounds exhibited low reducing potentials and weak metal chelating activity (see Figs. 5 and 6).

Acknowledgments

This work was financially supported by Vietnam National Foundation for Science & Technology Development (Project No: 104.01.30.09) and Priority Research Center Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2009-0093815), Republic of Korea. The authors are grateful to Institute of Chemistry, VAST and KBSI for the provision of the spectroscopic instrument.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.01.072.

References and notes

1. Blunt, J. W.; Copp, B. R.; Munro, M. H. G.; Northcote, P. T.; Prinsep, M. R. *Nat. Prod. Rep.* **2010**, *27*, 165.
2. Liang, X. T.; Fang, W. S. *Medicinal Chemistry of Bioactive Natural Products*; John Willey & Son: New Jersey, USA, 2006.
3. Dunlop, R. W.; Wells, R. J. *Aust. J. Chem.* **1979**, *32*, 1345.
4. Kusumi, T.; Hamada, T.; Ishitsuka, M. O.; Ohtani, I.; Kakisawa, H. *J. Org. Chem.* **1992**, *57*, 1033.
5. *Lobocompactol A* (**1**): colorless oil, $[\alpha]_D^{25}$ −15 (*c* 0.5, $CHCl_3$); UV λ_{max} (log ε, $CHCl_3$): 208 (1.4) nm; IR(KBr) ν_{max} 3417 (OH), 2935 (CH), 1705 (C=C), 1077 and 1031 (C–O–C) cm^{-1}; ^1H and ^{13}C NMR are given in Table 1; ESIMS *m*/*z*: 303 $[M-H_2O+H]^+$, 285 $[M-2H_2O+H]^+$; FTICRMS *m*/*z*: 343.22462 $[M+Na]^+$ (calcd for $C_{20}H_{32}O_3Na$, 343.22491). *Lobocompactol B* (**2**): colorless oil, $[\alpha]_D^{25}$ +12 (*c* 0.5, $CHCl_3$); UV λ_{max} (log ε, $CHCl_3$): 208 (1.4) nm; IR(KBr) ν_{max} 3425 (OH), 2931 (CH), 1710 (C=C), 1075 and 1032 (C–O–C) cm^{-1}; ^1H and ^{13}C NMR are given in Table 1; ESIMS *m*/*z*: 303 $[M-H_2O+H]^+$, 285 $[M-2H_2O+H]^+$; FTICRMS *m*/*z*: 343.22449 $[M+Na]^+$ (calcd for $C_{20}H_{32}O_3Na$, 343.22491).
6. Kato, M.; Kosugi, H.; Ichiyanagi, T.; Yamabe, O. *J. Chem. Soc., Perkin Trans. 1* **1999**, *7*, 783.
7. Su, J. H.; Tseng, Y. J.; Huang, H. H.; Ahmed, A. F.; Lu, C. K.; Wu, Y. C.; Sheu, J. H. *J. Nat. Prod.* **2006**, *69*, 850.
8. Vanisree, M.; Subbaraju, G. V.; Bheemasankara, R. C. *J. Asian Nat. Prod. Res.* **2000**, *2*, 87.
9. Raju, B. L.; Subbaraju, G. V.; Reddy, M. C.; Rao, D. V.; Rao, C. B.; Raju, V. S. *J. Nat. Prod.* **1992**, *55*, 904.
10. Konig, G. M.; Wright, A. D. *J. Nat. Prod.* **1998**, *61*, 494.
11. Cheng, S. Y.; Chuang, C. T.; Wang, S. K.; Wen, Z. H.; Chiou, S. F.; Hsu, C. H.; Dai, C. F.; Duh, C. Y. *J. Nat. Prod.* **2010**, *73*, 1184.
12. Vieira, L. M.; Kijjoa, A.; Pereira, J.; Gedris, T. E.; Herz, W. *Phytochemistry* **1997**, *45*, 111.