New Cembranoid Diterpenes from the Vietnamese Soft Coral *Sarcophyton mililatensis* Stimulate Osteoblastic Differentiation in MC3T3-E1 Cells

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Chemical investigation of the Vietnamese soft coral *Sarcophyton mililatensis* resulted in the isolation of four new cembranoid diterpenes, namely (−)-7β-hydroxy-8α-methoxydeepoxysarcophytoxide (1), (+)-7β,8β-dihydroxydeepoxysarcophytoxide (2), (−)-17-hydroxysarcophytonin A (3) and sarcophytol V (4), along with two known compounds, (+)-sarcophine (5) and sarcophytoxide (6). The NMR spectral data of the new compounds were completely assigned by using a combination of 2D NMR experiments including ^{1}H–^{1}H COSY, HSQC, HMBC, and ROESY. To investigate the bioactivities of compounds, which act on bone metabolism, we studied the effects of compounds on the function of osteoblastic MC3T3-E1 cells. Compound 1 caused a significant elevation of alkaline phosphatase activity, collagen content, and nodules mineralization compared to those of the control ($p<0.05$). These results suggest that newly isolated compound 1 has a direct stimulatory effect on bone formation *in vitro* and may contribute to the prevention for osteoporosis.

Key words *Sarcophyton mililatensis*; soft coral; cembranoid diterpene; osteoblast

Soft corals are marine invertebrates possessing a vast range of terpenoid metabolites. These terpenes, mainly cembranoids, represent the main chemical defense tools of animal against its natural predators. Cembranoids, which have 14-membered macrolic skeleton, are well known to exhibit a wide range of biological activities including neuroprotective, antimicrobial, Ca-antagonistic, antiinflammatory and antitumor properties.[1—3] The antitumor effect of cembranes is, however, one of the most important activities of this class of natural products.[1—3] Among soft corals, *Sarcophyton* species is one of the most abundant coral reef animals with a high cembranoid content. In the course of our ongoing studies on marine natural products, we have investigated on the *S. mililatensis* and found four new cembranoid diterpenes as (−)-7β-hydroxy-8α-methoxydeepoxysarcophytoxide (**1**), (+)-7β,8β-dihydroxydeepoxysarcophytoxide (**2**), (−)-17-hydroxysarcophytonin A (**3**) and sarcophytol V (**4**), along with two known compounds (+)-sarcophine (**5**) and sarcophytoxide (**6**). Their structures were established on the basis of NMR and MS spectral experiments.

Osteoporosis is characterized by a reduced bone mass, which results in increased bone fragility and fracture risk. Many osteoporotic patients have already lost a substantial amount of bone; therefore, a method of increasing bone mass by stimulating new bone formation is required. Osteoblasts are the active mature bone cells that synthesize the organic matrix and regulate its mineralization. To investigate whether compounds isolated from *S. mililatensis* could stimulate the function of osteoblasts, alkaline phosphatase (ALP) activity, collagen content, and calcium deposition were assessed in the pre-osteoblastic target cell line, MC3T3-E1, which has been a well-characterized as an *in vitro* model for osteoblast differentiation.

Results and Discussions

Combined chromatographic methods led to the isolation of four new cembranoid diterpenes **1**—**4** from the methanolic extract of the soft coral *S. mililatensis*. Compound **1** was obtained as a colorless oil with $[\alpha]_D^{25}$ −31° (c=0.50, $CHCl_3$). The molecular formula of compound **1**, $C_{21}H_{34}O_3$, was determined at m/z 334.2515 (Calcd for $C_{21}H_{34}O_3$, 334.2509) by HR-EI-MS. The ^{1}H- and ^{13}C-NMR spectral data (Table 1) indicated the presence of sarcophytoxide skeleton (one of the typical skeletons of *Sarcophyton* species),[4,5] which contains four tertiary methyl (δ 10.6, 16.1, 16.5, 18.5), six methylene (δ 21.6, 24.4, 29.0, 32.0, 38.2, 38.6), one methoxyl (δ 48.9), four oxygenated (δ 74.0, 78.4, 79.6, 85.8), and four olefinic carbons (δ 124.5, 126.4, 136.5, 142.6). All the protons were assigned to relevant carbons by an HSQC experiment. The ^{1}H–^{1}H COSY experiment allowed to assign the proton–proton correlations of H-2/H-3, H_2-5/H_2-6/H-7, H_2-9/H_2-10/H-11 and H_2-13/H_2-14. These data together with the HMBC cross peaks between H-2/C-1, H_3-18/C-3, H_3-18/C-4, H_3-18/C-5, H_3-19/C-7, H_3-19/C-8, H_3-19/C-9, H_3-20/C-11, H_3-20/C-12, H_3-20/C-13 and H_2-13/C-1 confirmed the connectivities from C-1 to C-14 of the 14-membered ring. The position of the C-17 methyl group was confirmed by the HMBC correlations from H_3-17 to C-1, C-15 and C-16. The HMBC

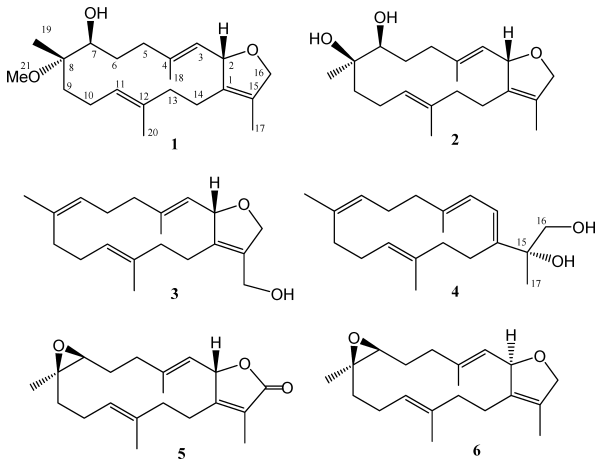

Fig. 1. Structures of Compounds **1**—**6**

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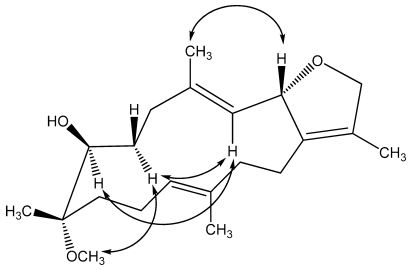

Fig. 2. Probable Conformation and Selected ROESY Correlations of **1**

cross peak from H_3-21 to C-8 identified the attached position of the methoxyl group. The relative stereochemistry of **1** was determined by comparison of ^{1}H- and ^{13}C-NMR spectral data of **1** with those of cembranoids (+)-sarcophytoxide, (−)-7β-hydroxy-8α-methoxydeepoxysarcophine,[4] sarcophytonin C,[6] and further confirmed by the ROESY experiment. The H_3-18 showed ROESY correlation with H-2 indicating the β-position of H-2, and the *trans*-configuration of the C-3/C-4 double bond.[4] The ROEs cross peaks between H-3/H-7, H-3/H-6 (δ 1.77), and H-6 (δ 1.77)/H_3-21 clearly revealed that H-3, H-6 (δ 1.77) and H_3-21 are located at the α-side of **1**.[4] The configurations of C-3/C-4 and C-11/C-12 double bonds were both defined as *trans* on the basis of the ^{13}C chemical shifts of C-18 and C-20 (<20 ppm).[7] Based on all the above analysis, the structure of **1** was established for the new natural product (−)-7β-hydroxy-8α-methoxydeepoxysarcophytoxide.

The ^{1}H- and ^{13}C-NMR spectral data of **2** are nearly identical to those of **1**, except for the absence of the methoxyl group. These data together with the HR-EI-MS (Found *m*/*z*: 320.2359; Calcd for $C_{20}H_{32}O_3$: 320.2352) and the 2D NMR (HSQC, COSY, HMBC and ROESY) spectral data of **2**, confirmed that the structure of **2** is similar to **1**. The stereochemistry of the hydroxyl group at C-8 was clearly identified as β-orientation by comparison of the NMR spectral data of **1** and **2** with those of both 7α,8β-dihydroxydeepoxysarcophine and 7β,8α-dihydroxydeepoxysarcophine.[8] In the ^{1}H-NMR spectrum of **2**, the chemical shift for the C-19 methyl group is sensitive to the stereochemistry at C-8, and the value of δ 1.20 for H_3-19 is identical with the value of δ 1.18 reported for the 8β-hydroxyl isomer (*vs.* δ 1.54 for the 8α-hydroxyl isomer). Further more, the characteristic differences between 8α- and 8β-hydroxyl isomers were also recognized in the ^{13}C-NMR spectrum, especially at C-8 and C-19, and those values for these carbons at δ 75.4 and 24.6 were in good agreement with those of δ 75.4 and 24.2 found for the 8β-hydroxyl isomer (*vs.* δ 78.0 and 26.4 for the 8α-hydroxyl isomer).[8] The ROEs cross peaks between H-3/H-7 and H-7/H_3-19 revealed that H-7 and H_3-19 are both α-oriented. Thus the structure of **2** was determined to be a new compound (+)-7β,8β-dihydroxydeepoxysarcophytoxide.

Compound **3** was isolated as colorless oil with a negative optical rotation ($[\alpha]_D^{25}$ −97° in $CHCl_3$). The HR-EI-MS result (Found: *m*/*z* 302.2251; Calcd for $C_{20}H_{30}O_2$: 302.2246) indicated the molecular formula as $C_{20}H_{30}O_2$. Its ^{1}H- and ^{13}C-NMR (Table 1) spectra indicated the presence of three tertiary methyl, eight methylene, four methine and five quaternary carbons. In these, two oxymethylene groups were evident at δ_C 75.7 (C-16) and 56.8 (C-17), one oxymethine group at δ_C 84.3 (C-2); one fully-substituted double bond at δ_C 138.2 (C-1)/130.9 (C-15), and three tri-substituted double bonds at δ_C 124.8 (C-3)/141.0 (C-4), 125.5 (C-7)/133.3 (C-8) and 124.2 (C-11)/134.9 (C-12); and three tertiary methyl groups were evident at δ_C 15.0 (C-18), 15.1 (C-19) and 15.6 (C-20). By means of an HSQC experiment, all protons were assigned to relevant carbons. The NMR data of **3** are similar to those of sarcophytonin A[9] except for the additional oxymethylene group [δ_C 56.8 (C-17)] in **3** instead of the methyl group in this compound. All the NMR spectral data of **3** were assigned by comparison with those of sarcophytonin A[9] and further confirmed by the HSQC, ^{1}H–^{1}H COSY, and HMBC experiments (Table 1). The additional oxymethylene group was defined to connect to C-17 by the HMBC correlations from its protons (δ 4.24/4.28) to C-1 (δ 138.2), C-15 (δ 130.9), and C-16 (δ 75.7). On the basis of ^{13}C chemical shifts of C-18, C-19 and C-20 (<20 ppm),[7] similar to **1** and **2**, the configurations of C-3/C-4, C-7/C-8 and C-11/C-12 double bonds were also all defined as *trans*. The ROEs cross peak was observed between H_3-18 and H-2 clearly indicated the β-position of H-2 and H-18, and the *trans*-configuration of the C-3/C-4 double bond.[4] Thus, the structure of **3** was deduced for the new natural product (−)-17-hydroxysarcophytonin A.

Compound **4** was also isolated as colorless oil. The molecular formula was determined to be $C_{20}H_{32}O_2$ by HR-EI-MS (Found: *m*/*z* 304.2397 $[M]^+$, Calcd for $C_{20}H_{32}O_2$: 304.2402). The NMR spectral data of **4** were very similar to those of 15-hydroxycembra-1,3,7,11-tetraene.[5] The easily visible changes were the additional presence of an oxymethylene group (at δ 69.3) and the absence of one methyl group in **4** comparing with 15-hydroxycembra-1,3,7,11-tetraene. All the NMR spectral data of **4** were assigned by comparison with those of 15-hydroxycembra-1,3,7,11-tetraene[5] and confirmed by the HSQC, ^{1}H–^{1}H COSY and HMBC experiments (Table 1). The additional oxymethylene group was defined to connected to C-16 by the HMBC cross peaks from its protons (δ 3.43, 3.63) to C-1 (δ 143.0)/C-15 (δ 76.4)/C-17 (δ 24.6). The stereochemistry of the hydroxyl group at C-15 was identified to be α-orientation by the good agreement of the NMR spectral data of **4** with those of the corresponding part in the related compounds in the series.[10] From all the above data, the structure of **4** was established as a new natural product 1*E*,3*E*,7*E*,11*E*-cembratetraene-15α,16-diol, which was named as sarcophytol V.

Compounds **5** and **6** were identified as (+)-sarcophine[4] and sarcophytoxide,[5] respectively, by comparison of their mass and NMR spectral data with the literature values. These compounds were isolated for the first time from *S. mililatensis*. Interestingly, sarcophytoxide was isolated at an extremely high proportion from this species.

In the present study, we evaluated the differentiation-inducing activities of compounds **1**—**6** on MC3T3-E1 cells by assessing for intracellular ALP activity, collagen content, and calcium deposition. The effect of compounds **1**—**6** on osteoblast differentiation was first assessed by measuring the ALP activity, one of the major osteoblast differentiation markers. As shown in Table 2, compounds **1**—**6** significantly increased the ALP activity at concentrations of 0.3 and 3 μM ($p<0.05$). Since these compounds significantly increased ALP activity in osteoblastic MC3T3-E1 cells, we further in-

Table 1. The NMR Spectral Data of Compounds **1**—**4**

No.	**1**			**2**			**3**			**4**		
	δ_C [a,b]	δ_H [a,c] mul. (*J* in Hz)	HMBC (H→C)	δ_C [a,b]	δ_H [a,c] mul. (*J* in Hz)	HMBC (H→C)	δ_C [a,b]	δ_H [a,c] mul. (*J* in Hz)	HMBC (H→C)	δ_C [a,b]	δ_H [a,c] mul. (*J* in Hz)	HMBC (H→C)
1	133.9	—		133.9	—		138.2	—		143.0	—	
2	85.8	5.38 d (11.0)	1, 3	85.1	5.40 d (11.0)	1, 3	84.3	5.56 d (11.0)	1, 3	121.5	6.39 d (11.0)	1, 3, 14, 15
3	124.5	5.22 d (11.0)	5, 18	125.1	5.24 d (11.0)	1, 2, 5, 18	124.8	5.10 d (11.0)	5, 18	120.5	5.91 d (11.0)	1, 2, 5, 18
4	142.6	—		141.7	—		141.0	—		138.6	—	
5	38.6	2.22 m/2.18 m		36.3	2.24 m	6, 18	39.1	1.91 m/2.12 m		38.8	2.18 m	3, 4
6	29.0	1.77 m/1.55 m		29.8	1.80 m/1.61 m		24.7	2.09 m/2.38 m	7	24.8	2.22 m	4, 7, 8
7	74.0	3.38 t (7.0)	5, 6, 8, 19	74.4	3.43 t (7.0)		125.5	4.85 t (7.0)		125.5	4.99 t (7.0)	5, 6, 8, 9, 19
8	79.6	—		75.4	—		133.3	—		134.1	—	
9	32.0	1.70 m/1.65 m	10	38.3	1.77 m/1.67 m	10	40.2	2.18 m/2.31 m	10	39.0	2.10 m	
10	21.6	2.15 m/1.99 m		22.8	2.21 m/2.11 m	9, 11	23.4	2.06 m		24.4	2.15 m	8, 9, 11, 12
11	126.4	5.36 t (7.0)	10, 13, 20	125.2	5.20 t (7.0)	13	124.2	5.01 t (7.0)	13, 20	125.5	4.99 t (7.0)	10, 13, 20
12	136.5	—		136.6	—		134.9	—		134.6	—	
13	38.2	2.29 m/2.08 m	1, 11, 12, 14	37.5	2.17 m/2.05 m	11, 12, 14, 20	37.4	1.95 m		41.2	2.14 m	1, 11, 12, 14, 20
14	24.4	2.39 m/1.64 m	12	23.8	2.20 m/1.93 m	1, 2, 13, 15	25.9	1.93 m/2.45 m	1, 2, 13	26.4	2.30 t (7.0)	1, 2, 12, 13, 15
15	128.0	—		128.2	—		130.9	—		76.4	—	
16	78.4	4.45 d (11.0) 4.56 dd (11.0, 4.0)		78.4	4.44 d (11.0) 4.53 dd (11.0, 4.0)	1, 15	75.7	4.66 dd (11.0, 3.0) 4.75 dd (11.0, 4.0)	1, 15	69.3	3.43 d (11.0) 3.63 d (11.0)	1, 15, 17
17	10.6	1.69 s	1, 15, 16	10.5	1.67 s	1, 15, 16	56.8	4.24 d (11.0) 4.28 d (11.0)	1, 15, 16	24.6	1.31 s	1, 15, 16
18	16.5	1.81 s	3, 4, 5	17.6	1.84 s	3, 4, 5	15.0	1.70 s	3, 4, 5	17.8	1.77 s	3, 4, 5
19	18.5	1.09 s	7, 8, 9	24.6	1.20 s	7, 8, 9	15.1	1.58 s	7, 8, 9	15.6	1.54 s	7, 8, 9
20	16.1	1.67 s	11, 12, 13	16.0	1.64 s	11, 12, 13	15.6	1.59 s	11, 12, 13	16.0	1.62 s	11, 12, 13
21	48.9	3.19 s	8									

a) Recorded in $CDCl_3$, *b*) at 125 MHz, *c*) at 500 MHz.

Table 2. Effect of Compounds **1**—**6** on the Differentiation of MC3T3-E1 Cells

Compounds	μM	Collagen content (%)	ALP activity (%)	Mineralization (%)
1	0.3	108.2±1.860*	127.8±1.392*	110.6±2.470*
	3	101.9±2.680	125.1±2.760*	106.7±0.403*
	30	91.48±3.231	115.5±6.288	105.1±2.619
2	0.3	109.5±1.898*	128.9±2.660*	100.7±1.684
	3	106.8±4.880	139.5±2.237*	104.0±1.277
	30	99.79±4.012	123.4±1.814*	105.4±2.868
3	0.3	101.0±3.154	131.0±6.834*	97.11±1.485
	3	102.5±1.832	127.2±1.292*	101.7±2.556
	30	111.4±1.208*	130.8±3.774*	107.9±3.767
4	0.3	102.7±0.137	127.0±2.449*	96.77±1.650
	3	101.0±1.938	121.0±2.237*	97.72±1.546
	30	110.4±1.440*	113.2±2.973*	101.6±0.990
5	0.3	103.5±0.836	133.3±2.091*	96.17±1.540
	3	109.7±2.000*	130.2±6.923*	99.87±0.941
	30	104.0±1.716	121.9±1.062*	99.66±1.515
6	0.3	103.2±3.421	127.8±2.995*	96.10±2.443
	3	106.9±1.636*	125.1±3.296*	97.78±3.635
	30	109.1±1.734*	117.4±2.995*	102.8±1.296
17β-Estradiol	0.01	113.2±1.499*	106.8±2.190*	106.3±1.376*

Data shown are mean±S.E.M., expressed as a percentage of control. The control values for collagen content, ALP activity, and mineralization were 26.37±0.410 μg, 1.122±0.017 Unit/10^6 cells, and 0.496±0.004 OD, respectively. $*p<0.05$ *vs.* control.

vestigated the effect of compounds on collagen synthesis using Sirius Red-based colorimetric assay. As the results, compounds **1**—**6** increased collagen synthesis significantly. Next, we examined the effects of compounds **1**—**6** on mineralization, another important process in differentiation, by measuring the calcium deposition by Alizarin Red staining. Compound **1** showed significant stimulatory effect on mineralization. 17β-Estradiol used as positive control significantly increased ALP activity, collagen content, and mineralization at 0.01 μM. In bone formation, osteoblasts are key cell in bone matrix formation and calcification. Osteogenesis starts with osteoblasts producing and secreting type I collagen, which makes about 90% of the organic bone matrix, or the osteoid. Osteoblasts also become high in alkaline phosphatase, a phosphate-splitting enzyme. Alkaline phosphatase is released into the osteoid to initiate the deposit of minerals. After mineralization, the complete bone becomes hard and rigid with necessary mechanical properties to withstand the external forces to support the body and protect the internal organs.[11] Our study demonstrates that compound **1** can increase ALP activity, collagen synthesis and calcium deposition in osteoblastic MC3T3-E1 cells *in vitro*. These studies suggest that compound **1** may be able to stimulate osteoblastic bone formation and play an important role in bone remodeling.

Experimental

General Optical rotations were determined on a JASCO DIP-1000 KUY polarimeter. All NMR spectra (^{1}H, ^{13}C, DEPT, HSQC, HMBC, COSY and ROESY) were recorded on a Bruker AM500 FT-NMR spectrometer (500 MHz for ^{1}H and 125 MHz for ^{13}C), and chemical shifts (δ) are reported in ppm using tetramethylsilane (TMS) as an internal standard. The ESI-MS was obtained on an AGILENT 1200 SERIES LC-MSD Trap spectrometer. HR-EI-MS were recorded on a JEOL JMS-DX 303 mass spectrometer. Column chromatography (CC) was performed on silica gel 230—400 mesh (0.040—0.063 mm, Merck) or YMC RP-18 resins (30—50 μm, Fuji Silysia Chemical Ltd.). Thin layer chromatography (TLC) was performed on DC-Alufolien 60 F_{254} (Merck 1.05715) or RP_{18} F_{254s} (Merck) plates. Spots were visualized by spraying 10% H_2SO_4 aqueous and heating for 5 min.

Animal Material The specimens of *S. mililatensis* were collected at 30—45 m depth in Baycanh Island, Condao district, Baria-Vungtau province, Vietnam during May, 2007 and deep frozen until used. The scientific name was identified by Dr. Do Cong Thung, Institute of Marine Resources and Environment, Vietnamese Academy of Science and Technology, Vietnam. A voucher of specimen (No. 20070506) was deposited at Institute of Natural Products Chemistry and Institute of Marine Resources and Environment, VAST, Hanoi, Vietnam.

Extraction and Isolation Fresh frozen samples of the soft coral (20 kg) were well grinded and extracted with hot MeOH three times (50 °C for 3 h each time) and then concentrated under reduced pressure to give MeOH extract (200 g). This extract was suspended in water and partitioned in turn with chloroform and *n*-buthanol. The chloroform extract (150 g) was dissolved in methanol and extracted with *n*-hexane to give *n*-hexane (100 g) and methanol (50 g) soluble extracts. The former was crudely separated on normal-phase silica gel column (Φ=10 cm, L=50 cm) gradient concentration of acetone in *n*-hexane from 0 to 100% to give 9 sub-fractions (H1—H9). The sub-fraction H-2 (70 g) was separated on reversed phase silica gel column (Φ=6 cm, L=50 cm) eluted with MeOH/H_2O (4 : 1) to give **5** (0.5 g) and **6** (50 g) as colorless oil. Normal-phase silica gel column chromatography (Φ=2.5 cm, L=40 cm, eluted with *n*-hexane–acetone, 7 : 1) of the sub-fraction H-5 (5 g) yielded 10 smaller fractions (H-5A to H-5J). Compounds **3** (15 mg) and **4** (20 mg) as colorless oil were obtained from the fraction H-5C (200 mg) by using normal-phase silica gel column chromatography (Φ=1 cm, L=20 cm, eluted with *n*-hexane–chloroform–MeOH, 10 : 1 : 0.5). Finally, reversed phase silica gel column chromatography (Φ=5 cm, L=50 cm, eluted with MeOH–H_2O, 4 : 1) of the fraction H-5F (150 mg) gave **1** (40 mg) and **2** (9 mg) as colorless oil.

(−)-7β-Hydroxy-8α-methoxydeepoxysarcophytoxide (**1**): Colorless oil; $[\alpha]_D^{25}$ −31° (c=0.50, $CHCl_3$); positive ESI-MS *m/z*: 357 $[M+Na]^+$, 317 $[M-H_2O+H]^+$, 303 $[M-MeOH+H]^+$, 285 $[M-MeOH-H_2O+H]^+$; HR-EI-MS *m/z*: 334.2515 $[M]^+$ (Calcd for $C_{21}H_{34}O_3$: 334.2509); ^{1}H-NMR (500 MHz, $CDCl_3$) and ^{13}C-NMR (125 MHz, $CDCl_3$): see Table 1.

(+)-7β,8β-Dihydroxydeepoxysarcophytoxide (**2**): Colorless oil; $[\alpha]_D^{25}$ +105° (c=0.50, $CHCl_3$); positive ESI-MS *m/z*: 343 $[M+Na]^+$, 303 $[M-H_2O+H]^+$; HR-EI-MS *m/z*: 320.2359 $[M]^+$ (Calcd for $C_{20}H_{32}O_3$: 320.2352); ^{1}H-NMR (500 MHz, $CDCl_3$) and ^{13}C-NMR (125 MHz, $CDCl_3$): see Table 1.

(−)-17-Hydroxysarcophytonin A (**3**): Colorless oil; $[\alpha]_D^{25}$ −97° (c=1.00, $CDCl_3$); positive ESI-MS *m/z*: 325 $[M+Na]^+$, 303 $[M+H]^+$, 285 $[M-H_2O+H]^+$; HR-EI-MS *m/z*: 302.2251 $[M]^+$ (Calcd for $C_{20}H_{30}O_2$: 302.2246); ^{1}H-NMR (500 MHz, $CDCl_3$) and ^{13}C-NMR (125 MHz, $CDCl_3$): see Table 1.

Sarcophyton V (**4**): Colorless oil; $[\alpha]_D^{25}$ −45° (c=1.00, $CDCl_3$); positive ESI-MS *m/z*: 327 $[M+Na]^+$, 287 $[M-H_2O+H]^+$, 269 $[M-2H_2O+H]^+$; HR-EI-MS *m/z*: 304.2397 $[M]^+$ (Calcd for $C_{20}H_{32}O_2$: 304.2402); ^{1}H-NMR (500 MHz, $CDCl_3$) and ^{13}C-NMR (125 MHz, $CDCl_3$): see Table 1.

(+)-Sarcophine (**5**): $[\alpha]_D^{25}$ +95° (c=1.00, $CHCl_3$); positive ESI-MS *m/z*:

339 $[M+Na]^+$, 317 $[M+H]^+$, 299 $[M-H_2O+H]^+$.

Sarcophytoxide (**6**): $[\alpha]_D^{25}$ +65° (c=1.00, $CHCl_3$); positive ESI-MS m/z: 325 $[M+Na]^+$, 303 $[M+H]^+$, 285 $[M-H_2O+H]^+$.

Cell Culture MC3T3-E1 cells (RCB1126, an osteoblast-like cell line from C57BL/6 mouse calvaria) were obtained from the RIKEN Cell Bank (Tsukuba, Japan), and cultured at 37 °C in a 5% CO_2 atmosphere using α-modified minimal essential medium (α-MEM; GIBCO). Unless otherwise specified, the medium contained 10% heat-inactivated fetal bovine serum (FBS), 100 U/ml penicillin and 100 μg/ml streptomycin.

Cell Growth MTT test was used to determine the growth of MC3T3-E1 cells. Cells were seeded at a density of 5×10^3 cells per well in 48-well plates. Cell viability was determined 2 d after compounds treatment using a colorimetric assay based on the uptake of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide salt (MTT) by viable cells. In brief, the MTT solution (0.5 mg/ml) was added to the cells and incubated at 37 °C to allow cleavage of the tetrazolium ring by mitochondrial dehydrogenases and formation of blue formazan crystals. After 2 h, the residual MTT was carefully removed and the crystals were dissolved by incubation with DMSO for 20 min. The plates were shaken for 5 min and the absorbance at 570 nm was measured by spectrophotometry.

Alkaline Phosphatase Activity The cells were treated, at 90% confluence, with culture medium containing 10 mM β-glycerophosphate and 50 μg/ml ascorbic acid, to initiate *in vitro* mineralization. The medium was changed every 2—3 d. After 6 d, the cells were cultured with medium containing 0.3% bovine serum albumin (BSA) and compounds isolated individually for 3 d. On harvesting, the medium was removed and the cell monolayer gently washed twice with phosphate buffered saline (PBS). The cells were lysed with 0.2% Triton X-100, with the lysate centrifuged at 14000×***g*** for 5 min. The clear supernatant was used to measure the ALP activity, which was determined using an ALP activity assay kit (Asan Co., Korea).

Measurement of Collagen Content Collagen content was quantified by Sirius Red-based colorimetric assay. Cultured osteoblasts were washed with PBS, followed by fixation with Bouin's fluid for 1 h. After fixation, the fluid was removed and the culture dishes were washed by immersion in running tap water for 15 min. The culture dishes were air dried and stained by Sirius Red dye reagent for 1 h under mild shaking on a shaker. Thereafter, the solution was removed and the cultures were washed with 0.01 N HCl to remove non-bound dye. The stained material was dissolved in 0.1 N NaOH and absorbance was measured at 550 nm against 0.1 N NaOH as a blank.

Calcium Deposition Assay The cells were treated, at 90% confluence, with culture medium containing 10 mM β-glycerophosphate and 50 μg/ml ascorbic acid, to initiate *in vitro* mineralization. After 10 d, the cells were cultured with medium containing 0.3% BSA and isolated compounds individually for 3 d. On harvesting, the cells were fixed with 70% ethanol for 1 h, and then stained with 40 mM Alizarin Red S for 10 min with gentle shaking. To quantify the bound dye, the stain was solubilized with 10% cetylpyridinum chloride by shaking for 15 min, while shielding from light. The absorbance of the solubilized stain was measured at 561 nm.

Statistics The results are expressed as the mean±S.E.M. Statistical analysis was performed using a one-way ANOVA ($p<0.05$) with the SAS statistical software.

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