Inhibitors of Osteoclast Formation from Rhizomes of *Cibotium barometz*

Nguyen Xuan Cuong,[†] Chau Van Minh,[†] Phan Van Kiem,[†] Hoang Thanh Huong,[†] Ninh Khac Ban,[‡] Nguyen Xuan Nhiem,[†,§] Nguyen Huu Tung,[†,§] Ji-Won Jung,[⊥] Hyun-Ju Kim,[⊥] Shin-Yoon Kim,[⊥] Jeong Ah Kim,[§] and Young Ho Kim*[,§]

Institute of Natural Products Chemistry, Vietnam Academy of Science and Technology (VAST), 18 Hoang Quoc Viet, Caugiay, Hanoi, Vietnam, Institute of Ecology and Biological Resources, VAST, 18 Hoang Quoc Viet, Caugiay, Hanoi, Vietnam, College of Pharmacy, Chungnam National University, Daejeon 305-764, Korea, and Skeletal Diseases Genome Research Center, School of Medicine, Kyungpook National University, Daegu 700-412, Korea

Received July 14, 2009

Eight compounds (**1**−**8**) were isolated from a methanol extract of *Cibotium barometz* rhizomes including two new furan derivatives, cibotiumbarosides A (**1**) and B (**2**), and a new glycoglycerolipid, cibotiglycerol (**4**). Their structures were elucidated by chemical and spectroscopic methods. Compounds **2**−**5** each showed inhibition of osteoclast formation with no affect on BMM cell viability.

Osteoporosis, a major worldwide health problem, affects 4−6 million women and 1−2 million men in the United States. More people than ever have decreased bone mass, which, in addition to other risk factors, can be a major therapeutic challenge. Fractures, the most important consequence of osteoporosis, are associated with enormous costs and substantial morbidity and mortality. The prevention and treatment of this disease are, therefore, of paramount importance.[1] Bone deposition and bone resorption are ongoing dynamic processes. Normally, a balance exists between the two. An imbalance can have serious consequences; when bone mass is not constant, bone renewal is compromised. Osteopetrosis potentially impairs bone marrow function. If osteoclast production is increased or if osteoblast production is decreased, bone mass is decreased: Osteoporosis results in increased susceptibility to bone fractures.[2] Thus, a substance that decreases bone resorption, increases bone deposition, or both is important for treatment of osteoporosis. There are several reports demonstrating improvement in clinical association with the use of traditional medicines in the treatment of fractures.[3] Nevertheless, despite encouraging preliminary reports, basic science and clinical reports justifying the clinical application of specific traditional medicines are not well established.

Many plant-derived substances have been used as drugs for the treatment of various diseases since ancient times, and traditional oriental therapies are rich in phytotherapeutic regimens. These medications typically have fewer side effects and are more suitable for long-term use as compared to chemically synthesized medications. The genus *Cibotium* Kaulf. (Thyrsopteridaceae) comprises about 10 species, mainly occurring in tropical and subtropical regions of Asia and America. Of these, *Cibotium barometz* (L.) J. Sm. is abundant in China, NE India, Malaysia, Myanmar, Indonesia, Thailand, Vietnam, and Japan. In Vietnam, the plant is widely distributed in mountainous areas at altitudes ranging from 500 to 700 m. The rhizomes are used in Vietnamese folk medicine to treat rheumatism, limb-ache, lumbago, neuralgia, and pollakiuria in the elderly. It is also used against sciatica, enuresis, and body-ache in pregnant women.[4,5]

In line with this laboratory's ongoing investigations into constituents of Vietnamese medicinal plants possessing inhibitory effects on osteoclast formation, this paper addresses the isolation, structural elucidation, and evaluation of antiosteoclastogenic activity of two new furan derivatives, cibotiumbarosides A (**1**) and B (**2**), and a new glycoglycerolipid, cibotiglycerol (**4**), together with five known compounds, from a methanol extract of *C. barometz* rhizomes. Compounds **2**−**5** showed inhibition of osteoclast formation with no cytotoxic effect on osteoclast precursors.

Results and Discussion

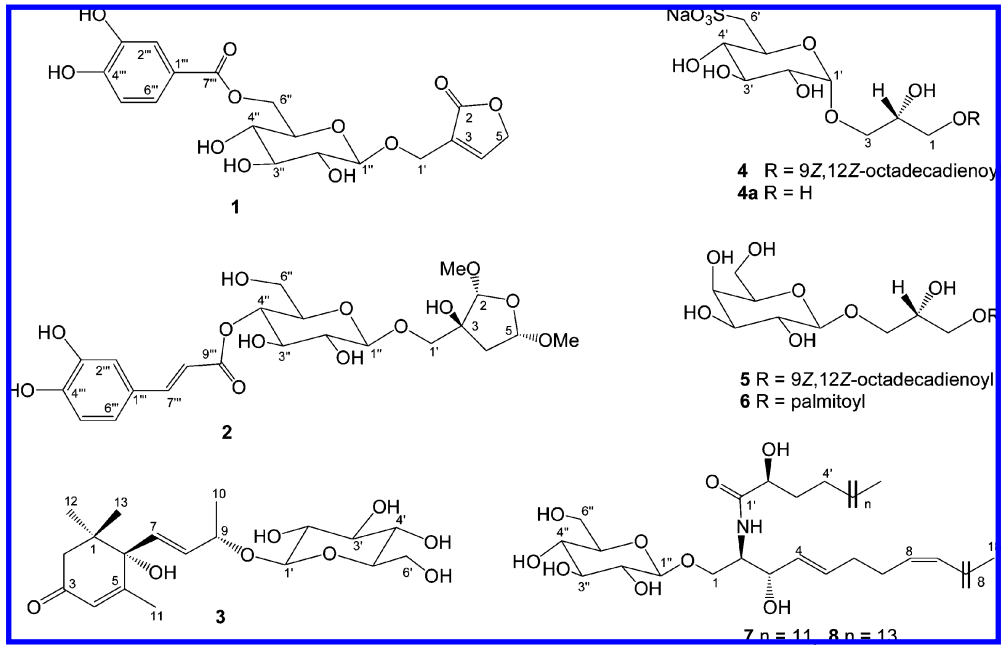
A methanol extract of rhizomes of the fern *C. barometz* yielded three new compounds (**1**, **2**, and **4**). Compound **1** was obtained as a colorless oil having the molecular formula $C_{18}H_{20}O_{11}$, as indicated by the HRTOFMS peak at *m*/*z* 411.0910 [M − H]$^-$ (calcd for $C_{18}H_{19}O_{11}$, 411.0927). The ^{1}H and ^{13}C NMR spectra were similar to those of 3-[(β-D-glucopyranosyloxy)methyl]-2(5*H*)-furanone,[6] except for additional signals of a protocatechuoyl group at δ_C 168.2 (C), 151.9 (C), 146.3 (C), 123.9 (CH), 122.6 (C), 117.1 (CH), and 116.1 (CH)/δ_H 6.81 (1H, d, J = 8.0 Hz), 7.44 (1H, dd, J = 8.0, 2.0 Hz), and 7.45 (1H, d, J = 2.0 Hz).[7] The downfield shifted signal of C-6″ (δ_C 64.7) suggested that the acyl moiety was attached to C-6″, and this was supported by HMBC cross-peaks from protons H-6″ (δ_H 4.58 and 4.41) to the carbonyl carbon C-7‴ at δ_C 168.2. In the HMBC spectrum, the anomeric proton (H-1″, δ_H 4.43) correlated with C-1′ (δ_C 64.1), confirming attachment of the glucose moiety at C-1′ (Figure 1). Moreover, the configuration of the glucopyranosyl moiety of **1** was identified by acid hydrolysis (see Experimental Section). Thus, the structure of **1** was elucidated as 3-[(6-*O*-protocatechuoyl-β-D-glucopyranosyloxy)methyl]-2(5*H*)-furanone, a new compound named cibotiumbaroside A.

The molecular formula of **2** was suggested to be $C_{22}H_{30}O_{13}$ by the ESIMS peaks at *m*/*z* 507 [M − H_2O + Na]$^+$ and 483 [M − H_2O − H]$^-$, and this was confirmed by the HRTOFMS peak at *m*/*z* 483.1501 [M − H_2O − H]$^-$ (calcd for $C_{22}H_{27}O_{12}$, 483.1503). In the ^{1}H NMR spectrum, the appearance of three ABX-type aromatic protons at δ_H 6.81 (d, J = 8.0 Hz), 6.98 (dd, J = 8.0, 2.0 Hz), and 7.08 (d, J = 8.0 Hz) and two *trans* olefinic protons at δ_H 6.32 and 6.72 (each d, J = 16.0 Hz) suggested a caffeoyl moiety. The anomeric proton signal at δ_H 4.38 (d, J = 8.0 Hz) suggested a β-glycosidic linkage. The ^{1}H−^{1}H COSY experiment allowed assignments of the sugar proton correlations as shown in Figure 1. The above evidence, together with the spin-coupling pattern of the sugar proton signals ($J_{1''-2''}$ = 8.0 Hz, $J_{2''-3''}$ = 9.0 Hz, and $J_{3''-4''}$ = 9.0 Hz) indicated a β-D-glucopyranosyl unit. Glucose was confirmed by acid hydrolysis (see Experimental Section). In addition, two OCH_3 groups were identified by signals at δ_H 3.39 and 3.42. The ^{13}C NMR spectrum of **2** revealed 22 carbon signals, including two OCH_3 groups, a caffeoyl moiety, a glucopyranosyl unit, and a five-carbon aglycone. Assignments of the NMR data of **2** were confirmed by HSQC, HMBC, and ^{1}H−^{1}H COSY experiments. Esterification of the caffeoyl group at C-4″ of the glucose

* To whom correspondence should be addressed. Tel: 82-42-821-5933. E-mail: yhk@cnu.ac.kr.
[†] Institute of Natural Products Chemistry, VAST.
[‡] Institute of Ecology and Biological Resources, VAST.
[§] Chungnam National University.
[⊥] Kyungpook National University.

10.1021/np9004097 CCC: $40.75
Published on Web 09/09/2009

Chart 1



unit was defined by the HMBC correlation between H-4″ (δ_H 4.98) and the carbonyl carbon C-9‴ (δ_C 168.3). The aglycone portion was comprised of two dioxymethines, one oxygenated quaternary, one oxymethylene, and one methylene carbon at δ_C 110.4, 108.0, 84.0, 70.6, and 39.8, respectively. The above data implied the 3-hydroxymethyl-2,3,5-trihydroxytetrahydrofuran aglycone structure (Figure 1). Furthermore, the signals at δ_H 3.39 and 3.42 had HMBC correlations with C-2 (δ_C 110.40) and C-5 (δ_C 107.98), respectively, indicating that OCH_3 groups were attached to C-2 and C-5 of the 3-hydroxymethyl-2,3,5-trihydroxytetrahydrofuran. Assignment of the relative configuration at C-2, C-3, and C-5 was achieved with the aid of NOESY data. An NOE correlation was observed between H-2 (δ_H 4.82) and H-5 (δ_H 5.24), suggesting a 2,5-*cis*-dimethoxy configuration. However, no NOE correlations between H-1′ (δ_H 3.89 and 3.99) and H-2 (δ_H 4.82)/H-5 (δ_H 5.24) were observed, suggesting that the two methoxy groups and the hydroxy group were on opposite sides of the tetrahydrofuran ring. Consequently, compound **2** was 3-[(4-*O*-caffeoyl-*β*-D-glucopyranosyloxy)methyl]-2,5-dimethoxy-3-hydroxytetrahydrofuran, and it was named cibotiumbaroside B.

Compound **4** was isolated as a pale yellow wax. The molecular formula, $C_{27}H_{47}NaO_{11}S$, was determined by the ESIMS peaks at

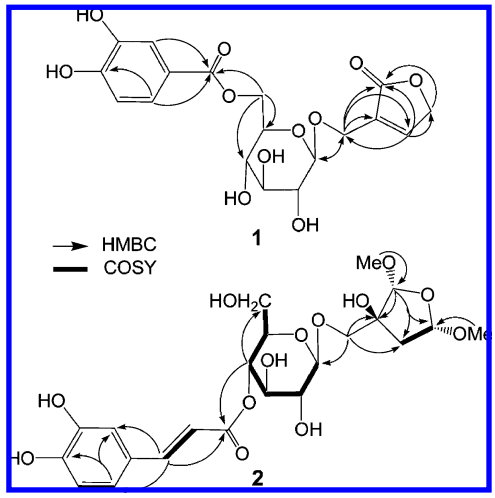


Figure 1. Key HMBC and $^1H-^1H$ COSY correlations of **1** and **2**.

m/z 625 $[M + Na]^+$ and 603 $[M + H]^+$ and the HRTOFMS at *m/z* 625.2633 $[M + Na]^+$ (calcd for $C_{27}H_{47}Na_2O_{11}S$, 625.2634). The ^1H and ^{13}C NMR spectra indicated sugar and long-chain unsaturated fatty acid ester moieties, suggesting a glycoglycerolipid. These data were similar to those of the sodium salt of 1-*O*-myristoyl-3-*O*-(6-sulfo-α-D-quinovopyranosyl)glycerol,[8] except for the signals of the fatty acid moiety. The ^1H NMR spectrum exhibited signals typical of a doubly allylic methylene group at δ_H 2.80 (2H, t, $J = 6.5$ Hz). A small coupling constant of the anomeric proton (H-1′, δ_H 4.80, d, $J = 4.0$ Hz) indicated *cis-axial-equatorial* relationship between H-1′ and H-2′ of the sugar ($J = 7.5$ Hz for *trans*-di-*axial* situation as in case of **5**). This evidence and the spin-coupling pattern of other sugar proton signals ($J_{2'-3'} = 9.0$ Hz and $J_{3'-4'} = 9.0$ Hz) indicated the 6-sulfo-α-D-quinovopyranosyl unit. Two double bonds in the fatty acid moiety were indicated by olefinic carbon signals at δ_C 130.93, 130.89, 129.07, and 129.06. Alkaline hydrolysis of **4** with NaOMe−MeOH afforded (6-sulfo-α-D-quinovopyranosyl)-glycerol (**4a**)[8−10] and methyl 9*Z*,12*Z*-octadecadienoate (identified by GC-MS). The double bonds were defined as *cis* on the basis of the ^{13}C chemical shifts (δ_C 26.5 and 28.1) of the adjacent carbons.[11] The anomeric proton at δ_H 4.80 (H-1′) exhibited HMBC correlations with the glycerol carbon at δ_C 70.6 (C-3), indicating attachment of the sulfo-quinovose moiety at this carbon. The fatty acid attached to C-1″ was determined by HMBC correlations between H-1 (δ_H 4.12 and 4.22) and the carbonyl carbon C-1″ (δ_C 175.6). The usual *S* configuration of *sn*-2 in the glycerol portion was determined by comparing the specific rotation of **4a**, $[\alpha]_D^{25}$ +40 (*c* 1.00, H_2O), with that previously reported.[9,10] Thus, **4** was identified as the sodium salt of (2*S*)-1-*O*-(9*Z*,12*Z*-octadecadienoyl)-3-*O*-(6-sulfo-α-D-quinovopyranosyl)glycerol, a new compound named cibotiglycerol.

The known compounds were characterized as corchoionoside C (**3**),[12,13] (2*S*)-3-*O*-(9*Z*,12*Z*-octadecadienoyl)glyceryl-*β*-D-galacto-pyranoside (**5**),[14] (2*S*)-1-*O*-palmitoyl-3-*O*-*β*-D-galactopyranosyl-*sn*-glycerol (**6**),[15] soya-cerebroside II (**7**),[16] and 1-*O*-*β*-D-glucopyranosyl-(2*S*,3*R*,4*E*,8*Z*)-2-[(2*R*-hydroxyoctadecanoyl)amido]-4,8-octadecadiene-1,3-diol (**8**)[11] by comparison of their NMR spectroscopic data with the literature values. These compounds, however, were isolated from *C. barometz* for the first time.

To investigate the inhibitory effects of the isolated compounds on osteoclast development, BMMs were cultured with different concentrations of tested compounds in the presence of RANKL and M-CSF, and stained cells for tartrate-resistant acid phosphatase

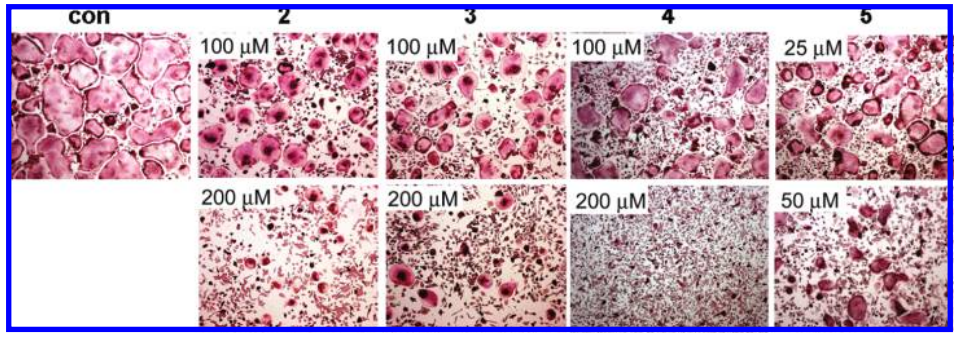


Figure 2. Effect of **2**−**5** on osteoclast development. BMMs were cultured with 20 ng/mL of M-CSF and 100 ng/mL of RANKL. After 4 days, cells were stained for TRAP activity. *$p < 0.001$ versus DMSO-treated cells for control.

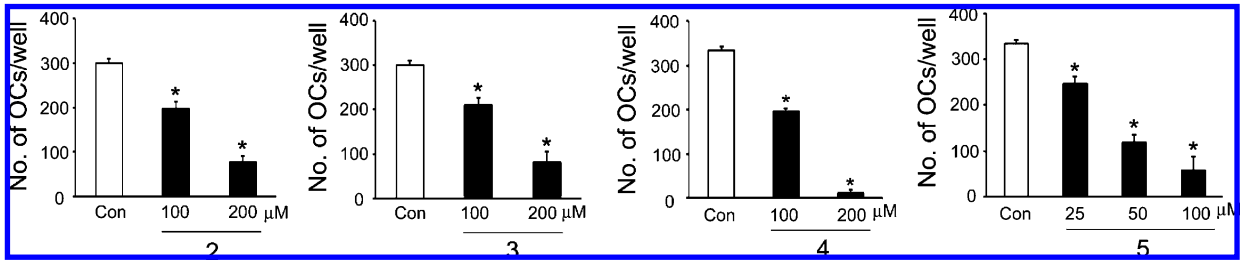


Figure 3. Statistical analysis of the number of TRAP-positive multinucleated cells/well at day 4. *$p < 0.001$ versus DMSO-treated cells for control.

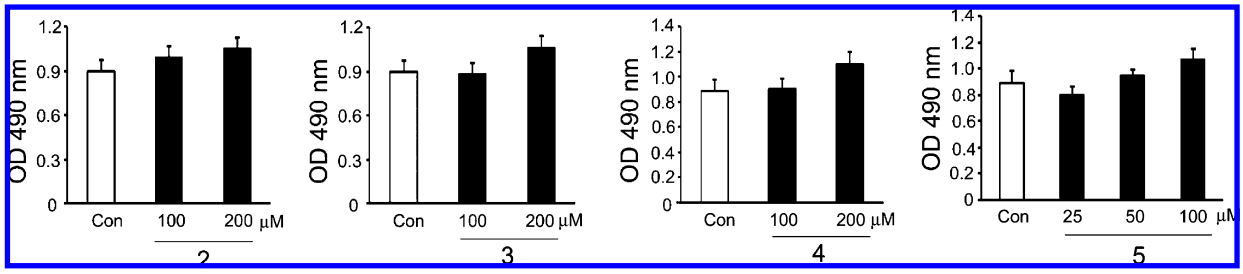


Figure 4. Cell viability was measured with **2**−**5** as described in the Experimental Section. *$p < 0.001$ versus DMSO-treated cells for control.

(TRAP) after 4 days. Compounds **2**−**4** suppressed osteoclast formation in a dose-dependent manner and inhibited up to 73, 75, and 97%, respectively, at a 200 μM concentration, as revealed by the decreased number of TRAP-positive multinuclear osteoclasts (Figures 2 and 3). In addition, compound **5** inhibited osteoclastogenesis dose dependently to 65 and 83% at 50 and 100 μM concentrations, respectively. These results could be regarded as the inhibitory effects of compounds on cell viability. To assess this issue, the cytotoxicity of each compound upon osteoclast precursors was examined. None of them showed any cytotoxic effects at the concentrations used in this study (Figure 4). In particular, these results suggest that compounds **2**−**5** have antiosteoclastogenic activities without any effect on cell viability.

Osteoclasts are multinucleated cells generated by monocyte/macrophage precursors. Regulation of osteoclast formation is considered to be an effective therapeutic approach to treat bone-related diseases. In this study, the inhibitory effects of compounds **2**−**5** on the generation of osteoclast from primary bone marrow-derived macrophages were demonstrated. Although further studies are required to confirm efficacy *in vivo*, it is the contention of the authors that these compounds could be used in the development of therapeutic targets for osteoporosis.

Experimental Section

General Experimental Procedures. Optical rotations were determined on a Jasco DIP-370 digital polarimeter. Electrospray ionization (ESI) mass spectra were obtained using an Agilent 1200 LC-MSD Trap spectrometer. HRTOF mass spectra were obtained using a JEOL JMS-T100LC spectrometer. The ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectra were recorded on a Bruker AM500 FT-NMR spectrometer, and TMS was used as an internal standard. GC was performed on a Shidmazu-2010 instrument. Column chromatography (CC) was performed on silica gel (Kieselgel 60, 70−230 and 230−400 mesh, Merck) and YMC RP-18 resins.

Plant Material. Rhizomes of *C. barometz* were collected at Bachma National Botanical Park, Hue, Vietnam, during May 2008, and were identified by one of us (N.K.B.). An authentic sample (No. KC10-14) was deposited in the herbarium of the Institute of Natural Products Chemistry, VAST, Vietnam.

Extraction and Isolation. The dried rhizomes of *C. barometz* (5.0 kg) were powdered and extracted with MeOH (3 × 10 L) to give the methanol extract (300 g), which was then suspended in water (5 L) and extracted in turn with *n*-hexane and ethyl acetate (each 3 × 5 L), giving corresponding extracts, *n*-hexane (H, 50 g) and ethyl acetate (E, 15 g), and a water layer. The water layer was passed through a Dianion HP-20 column using stepwise elution with MeOH−H_2O (0:10, 2:10, 5:10, and 10:0) to give four fractions, W1−W4. Fraction W2 (5 g) was separated by silica gel CC eluting with acetone−$CHCl_3$−H_2O (3:1:0.1) to obtain four subfractions, W2A−W2D. Subfraction W2B (1 g) was further separated by silica gel CC using CH_2Cl_2−*n*-hexane−MeOH (3:2:1) as eluent to give four smaller fractions, W2B1−W2B4. Compound **1** (100 mg) was isolated from fraction W2B1 (300 mg) by silica gel CC eluting with acetone−$CHCl_3$−H_2O (2:1:0.1). In a similar procedure, compound **3** (30 mg) was purified from fraction W2B3 (200 mg). The ethyl acetate extract (E, 15 g) was separated using silica gel CC eluting with $CHCl_3$−MeOH (8:1) to give

Table 1. NMR Spectroscopic Data (500 MHz, CD_3OD) for **1** and **2**

	1		**2**	
position	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
aglycone				
2	175.4		110.4	4.82, s
3	131.5		84.0	
4	150.9	7.63, q (1.5, 1.5, 1.5)	39.8	2.62, dd (14.0, 5.5)
				2.15, dd (14.0, 3.5)
5	72.7	4.82, q (1.5, 1.5, 1.5)	108.0	5.24, dd (5.5, 3.5)
1′	64.1	4.49, dq (13.5, 1.5, 1.5, 1.5), 4.41[a]	70.6	3.89, d (12.5)
				3.99, d (12.5)
2-OMe			55.7	3.39, s
5-OMe			55.9	3.42, s
glucose				
1″	104.5	4.43, d (8.0)	99.8	4.38, d (8.0)
2″	75.1	3.28, dd (9.0, 8.0)	75.7	3.28, dd (9.0, 8.0)
3″	77.9	3.43[a]	72.8	3.77, t (9.0)
4″	71.7	3.45[a]	72.5	4.98, t (9.0)
5″	75.6	3.60, m	77.8	3.69, m
6″	64.7	4.58, dd (12.0, 2.0), 4.41[a]	62.2	3.58, dd (12.0, 6.0)
				3.66, dd (12.0, 2.0)
		protocatechuoyl		caffeoyl
1‴	122.6		127.6	
2‴	117.1	7.45, d (2.0)	115.3	7.08, d (2.0)
3‴	146.3		146.8	
4‴	151.9		149.7	
5‴	116.1	6.81, d (8.0)	116.5	6.81, d (8.0)
6‴	123.9	7.44, dd (8.0, 2.0)	123.1	6.98, dd (8.0, 2.0)
7‴	168.2		147.8	7.62, d (15.5)
8‴			114.6	6.32, d (15.5)
9‴			168.3	

[a] Overlapped signals; assignments were made by HSQC, HMBC, and $^1H-^1H$ COSY experiments.

eight fractions, E1−E8. Fraction E3 (2 g) afforded compound **2** (25 mg), after subjecting it to silica gel CC eluting with *n*-hexane−acetone (1.5:1) followed by YMC RP-18 CC with acetone−H_2O (5:1). Fraction E6 (5 g) was separated into five subfractions, E6A−E6E, by silica gel CC eluting with $CHCl_3$−*n*-hexane−MeOH (6:1:1). Compounds **7** (40 mg) and **8** (35 mg) were obtained from subfraction E6B after subjecting it to silica gel CC with acetone−CH_2Cl_2−H_2O (1.4:1:0.7) followed by YMC RP-18 CC with MeOH−H_2O (15:1). Subfraction E6D (1.5 g) was treated in the same manner as E6B to furnish compounds **5** (35 mg) and **6** (20 mg). Fractions E7 (4 g) and E8 (5 g) were combined and then separated into four subfractions, E7A−E7D, by silica gel CC eluting with $CHCl_3$−MeOH−H_2O (3:1:0.1). Compound **4** (25 mg) was purified from subfraction E7D (1.9 g) using YMC RP-18 CC eluting with MeOH−H_2O (2.5:1).

Cibotiumbaroside A (1): colorless oil; $[\alpha]^{25}_D$ −25 (*c* 1.00, MeOH); IR (KBr) ν_{max} 3412 (OH), 2958 (CH), 1738 (C=O), 1652 (aromatic ring), 1081 (C−O−C) cm^{-1}; UV (MeOH) λ_{max} (log ε) 210 (3.70), 265 (3.24), 300 (3.09); ^1H and ^{13}C NMR are given in Table 1; ESIMS *m/z* 435 $[M + Na]^+$, 411 $[M - H]^-$; HRTOFMS *m/z* 411.0910 $[M - H]^-$ (calcd for $C_{18}H_{19}O_{11}$, 411.0927).

Cibotiumbaroside B (2): pale yellow wax; $[\alpha]^{25}_D$ −35 (*c* 1.00, MeOH); IR (KBr) ν_{max} 3424 (OH), 2936 (CH), 1701 (C=O), 1631 (aromatic ring), 1041 (C−O−C) cm^{-1}; UV (MeOH) λ_{max} (log ε) 220 (3.65), 245 (3.53), 292 (3.68), 323 (3.74); ^1H and ^{13}C NMR see Table 1; ESIMS *m/z* 507 $[M - H_2O + Na]^+$, 483 $[M - H_2O - H]^-$; HRTOFMS *m/z* 483.1501 $[M - H_2O - H]^-$ (calcd for $C_{22}H_{27}O_{12}$, 483.1503).

Corchoionoside C (3): amorphous, white powder; $[\alpha]^{25}_D$ +25 (*c* 0.50, MeOH); ESIMS *m/z* 409 $[M + Na]^+$.

Cibotiglycerol (4): pale yellow wax; $[\alpha]^{25}_D$ +45 (*c* 1.00, MeOH); IR (KBr) ν_{max} 3448 (OH), 2932 (CH), 1728 (C=O), 1177 and 1034 (sulfonate) cm^{-1}; ^1H NMR (CD_3OD, 500 MHz) δ for glycerol moiety 4.22 (1H, dd, J = 12.0, 7.0 Hz, H_a-1), 4.12 (1H, dd, J = 12.0, 2.0 Hz, H_b-1), 4.11 (1H, m, H-2), 4.08 (1H, dd, J = 11.0, 5.0 Hz, H_a-3), 3.42 (1H, dd, J = 11.0, 6.0 Hz, H_b-3), for sugar moiety 4.80 (1H, d, J = 4.0 Hz, H-1′), 4.11 (1H, m, H-5′), 3.67 (1H, t, J = 9.0 Hz, H-3′), 3.43 (1H, dd, J = 9.0, 4.0 Hz, H-2′), 3.37 (1H, dd, J = 14.5, 2.0 Hz, H_a-6′), 3.11 (1H, t, J = 9.0 Hz, H-4′), 2.94 (1H, dd, J = 14.5, 9.5 Hz, H_b-6′), for fatty acid 5.36 (4H, m, H-9″, 10″, 12″, 13″), 2.80 (2H, t, J = 6.5 Hz, H-11″), 2.39 (2H, t, J =7.5 Hz, H-2″), 2.08 (4H, dd, J = 13.5, 6.5 Hz, H-8″, 14″), 1.64 (2H, m, H-3″), 1.35 (14H, m, H-4″−7″, H-15″−17″), 0.93 (3H, t, J = 7.0 Hz, H-18″); ^{13}C NMR (CD_3OD, 125 MHz) δ for glycerol moiety 70.6 (CH_2, C-3), 69.8 (CH, C-2), 66.5 (CH_2, C-1), for sugar moiety 100.2 (CH, C-1′), 75.1 (CH, C-3′), 74.9 (CH, C-4′), 73.7 (CH, C-2′), 69.8 (CH, C-5′), 54.2 (CH_2, C-6′), for fatty acid moiety 175.6 (C, C-1″), 129.06, 129.07, 130.89, 130.93 (CH, C-9″, 10″, 12″, 13″), 34.9 (CH_2, C-2″), 32.6 (CH_2, C-16″), 30.19, 30.22, 30.3, 30.4, 30.7 (CH_2, C-4″−7″, C-15″), 28.1 (CH_2, C-8″, 14″), 26.5 (CH_2, C-11″), 26.0 (CH_2, C-3″), 23.6 (CH_2, C-17″), 14.4 (CH_3, C-18″); ESIMS *m/z* 625 $[M + Na]^+$, 603 $[M + H]^+$; HRTOFMS *m/z* 625.2633 $[M + Na]^+$ (calcd for $C_{27}H_{47}Na_2O_{11}S$, 625.2634).

(2*S*)-1-*O*-(9*Z*,12*Z*-Octadecadienoyl)-3-*O*-β-D-galactopyranosylglycerol (5): colorless oil; $[\alpha]^{25}_D$ −25 (*c* 1.00, MeOH); ESIMS *m/z* 539.1 $[M + Na]^+$, 515 $[M - H]^-$.

(2*S*)-1-*O*-Palmitoyl-3-*O*-β-D-galactopyranosyl-*sn*-glycerol (6): amorphous, white powder; $[\alpha]^{25}_D$ +10 (*c* 1.00, MeOH); ESIMS *m/z* 515 $[M + Na]^+$, 475 $[M - H_2O + H]^+$, 331 $[M -$ galactose $+ H]^+$.

Soya-cerebroside II (7): amorphous, white powder; $[\alpha]^{25}_D$ +13 (*c* 1.00, MeOH); ESIMS *m/z* 736 $[M + Na]^+$, 714 $[M + H]^+$.

1-*O*-β-D-Glucopyranosyl-(2*S*,3*R*,4*E*,8*Z*)-2-[(2*R*-hydroxyoctadecanoyl)-amido]-4,8-octadecadiene-1,3-diol (8): amorphous, white powder; $[\alpha]^{25}_D$ +15 (*c* 1.00, MeOH); ESIMS *m/z* 764 $[M + Na]^+$, 742 $[M + H]^+$.

Acid Hydrolysis of 1 and 2. Compounds **1** and **2** (2 mg, each) were dissolved in 1 N HCl (dioxane−H_2O, 1:1, 1 mL) and then heated to 80 °C in a water bath for 3 h. The acidic solution was neutralized with silver carbonate and the solvent thoroughly driven out under N_2 gas overnight. After extraction with $CHCl_3$, the aqueous layer was concentrated to dryness using N_2 gas. The residue was dissolved in 0.1 mL of dry pyridine, and then L-cysteine methyl ester hydrochloride in pyridine (0.06 M, 0.1 mL) was added to the solution. The reaction mixture was heated at 60 °C for 2 h, and 0.1 mL of trimethylsilylimidazole was added, followed by heating at 60 °C for 1.5 h. The dried product was partitioned with *n*-hexane and H_2O (0.1 mL each), and the organic layer was analyzed by gas liquid chromatography (GC): column SPB-1 (0.25 mm × 30 m); detector FID, column temp 210 °C, injector temp 270 °C, detector temp 300 °C, carrier gas He (2 mL/min). Under these conditions, standard sugars gave peaks at t_R (min) 8.55 and 9.25 for D- and L-glucose. A peak at t_R (min) 8.55 corresponding to D-glucose was observed for both compounds **1** and **2**.

Alkaline Hydrolysis and GC Analysis of 4. Compound **4** (5 mg) was dissolved in dry MeOH (1 mL) and treated with 5% NaOMe−MeOH (0.5 mL) at room temperature for 10 min. The reaction mixture was neutralized with Dowex 50 W×8, and the resin was removed by filtration. The filtrate was extracted with *n*-hexane to yield a fatty acid methyl ester. This was analyzed by gas liquid chromatography (GC): column DB-5 (30m × 0.25 mm); detector FID, oven temp 50 °C (2 min) → 15 °C/min → 300 °C (2 min); detector temp 230 °C, carrier He, flow rate 1 mL/min. The retention time of methyl 9*Z*,12*Z*-octadecadienoate was 14.01 min, which was identical to the authentic standard methyl 9*Z*,12*Z*-octadecadienoate. Removal of solvent from the MeOH layer under reduced pressure gave a residue, which was purified by silica gel CC ($CHCl_3$−MeOH−H_2O, 6:4:1) to furnish (6-sulfo-α-D-quinovopyranosyl)glycerol (**4a**).

Osteoclast Culture. Primary bone marrow-derived macrophages (BMMs) were prepared as described previously.[17] Whole bone marrow cells were extracted from femora and tibia of 6−8 week old mice and cultured in α-MEM containing 10% FBS and M-CSF (100 ng/mL). Cells were incubated at 37 °C for 3 days and then lifted with 1× trypsin/EDTA in PBS. To generate osteoclasts, the cells were cultured in α-MEM containing 10% FBS with recombinant M-CSF (20 ng/mL) and RANKL (100 ng/mL). Media were exchanged every 2 days. Osteoclasts were stained for tartrate-resistant acid phosphatase (TRAP) activity after 4 days.

MTS Assay. BMMs were plated on 96-well plates. Cells were incubated with the indicated dose of the isolated compounds in the presence of recombinant M-CSF (20 ng/mL) and RANKL (100 ng/mL). After incubation for 72 h at 37 °C, the percentage of cell survival was determined with the MTS/CellTiter 96 Aqueous Assay (Promega, Madison, WI).

Acknowledgment. This work was partially supported by the Vietnam National Project (No. KC10.20/06-10) and the Korea Foundation for International Cooperation of Science & Technology (KICOS) through

a grant provided by the Korean Ministry of Science & Technology (MOST) in Korea (No. K2072100000208B010000210). The authors are grateful to Mr. D. V. Luong, Institute of Chemistry, VAST and KBSI, for recording the NMR and mass spectra.

Supporting Information Available: 1D and 2D NMR and ESI mass spectra for the new compounds **1**, **2**, and **4**. This material is available free of charge via the Internet at http://pubs.acs.org.

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NP9004097