JOURNAL OF NATURAL PRODUCTS

Note

pubs.acs.org/jnp

Polyhydroxylated Macrolides from *Seimatosporium discosioides* and Their Effects on the Activation of Peroxisome Proliferator-Activated Receptor Gamma

Nguyen Tuan Hiep,[†] Yun-hyeok Choi,[†] Nahyun Kim,[†] Seong Su Hong,[†,‡] Seung-Beom Hong,[§] Bang Yeon Hwang,[⊥] Hak-Ju Lee,[∥] Sung-Joon Lee,[†] Dae Sik Jang,*[,#] and Dongho Lee*[,†]

[†]School of Life Sciences and Biotechnology, Korea University, Seoul 136-713, Republic of Korea

[‡]Natural Products Research Institute, Gyeonggi Institute of Science & Technology Promotion, Suwon 443-270, Republic of Korea

[§]Korean Agricultural Culture Collection, National Academy of Agricultural Science, Suwon 441-707, Korea

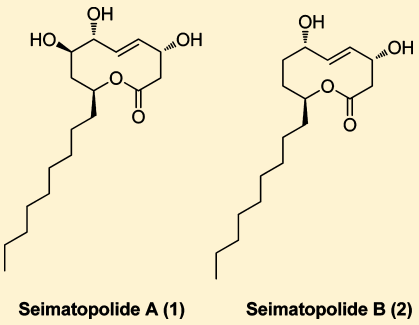
[⊥]College of Pharmacy, Chungbuk National University, Cheongju 361-763, Republic of Korea

[∥]Korea Forest Research Institute, Seoul 130-712, Korea

[#]College of Pharmacy, Kyung Hee University, Seoul 130-701, Korea

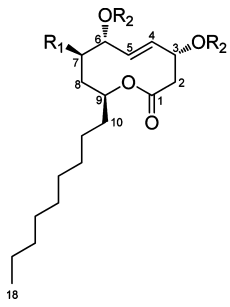
Supporting Information

ABSTRACT: Two new polyhydroxylated macrolides, seimatopolides A (**1**) and B (**2**), were isolated from an EtOAc extract of *Seimatosporium discosioides* culture medium. The structures of the new compounds were established on the basis of spectroscopic analysis, including 1D and 2D NMR, and their absolute configurations were determined using the modified Mosher's method. Seimatopolides A (**1**) and B (**2**) activated peroxisome proliferator-activated receptor (PPAR)-γ with EC_{50} values of 1.15 and 11.05 μM, respectively. The expression of PPAR-γ target genes in HepG2 hepatocytes was significantly altered; in particular, expression of the gluconeogenic genes glucose-6-phosphatase (G6Pase) and phosphoenolpyruvate carboxykinase (PEPCK) was reduced upon stimulation with **1**, supporting the proposal that compound **1** is both a PPAR-γ agonist and a possible therapeutic candidate for treatment of diabetes.



The peroxisome proliferator-activated receptors (PPARs) are ligand-activated transcription factors belonging to the superfamily of nuclear hormone receptors,[1] whose activity is regulated by direct binding of steroid and thyroid hormones, vitamins, lipid metabolites, and xenobiotics.[2] Three PPAR subtypes, PPAR-α, PPAR-γ, and PPAR-δ, have been identified, and all have well-described functions as regulators of lipid and lipoprotein metabolism,[3,4] glucose and fatty acid homeostasis,[5] and cellular differentiation, and also appear to control the inflammatory response.[6,7] PPAR-γ, the most studied PPAR, has therapeutic potential as a target in the treatment of type 2 diabetes, inflammatory disease, and certain cancers.[8]

In our search for biologically active agents of natural origin, the culture broth of *Seimatosporium discosioides* exhibited significant activity in a reporter gene assay for activation of peroxisome proliferator-activated receptor γ (PPAR-γ).[9] The investigation of an EtOAc extract of the fungal culture medium led to the isolation of two new polyhydroxylated macrolides, namely, seimatopolides A (**1**) and B (**2**), along with three known compounds, monosporascone,[10] arthrinone,[11] and 3a,9a-deoxy-3a-hydroxy-1-dehydroxyarthrinone.[11] Polyhydroxylated macrolides, such as members of the decarestrictine family,[12] microcarpalide,[13] pinolidoxin,[14] achaetolide,[15] and herbarumins I–II,[16] have been isolated from fungal sources and have received special attention for several years, owing to their interesting biological effects, including inhibition of cholesterol biosynthesis[17−20] and microfilament formation,[13] antimalarial and antibacterial activity,[21,22] and phytotoxicity.[14] In addition, synthetic and biosynthetic studies of polyhydroxylated macrolides have been reported by several groups.[12,14−16,20,22] We describe herein the isolation, structural characterization, and biological evaluation of seimatopolides A (**1**) and B (**2**).



1 R_1 = OH, R_2 = H
1a R_1 = O(*S*)-MTPA, R_2= (*S*)-MTPA
1b R_1 = O(*R*)-MTPA, R_2= (*R*)-MTPA
2 R_1 = H, R_2 = H
2a R_1 = H, R_2= (*S*)-MTPA
2b R_1 = H, R_2= (*R*)-MTPA

Received: December 8, 2011
Published: March 16, 2012

Seimatopolide A (**1**) was isolated as a white, amorphous solid, and the HRESIMS spectrum showed the molecular formula as $C_{18}H_{32}O_5$ with 3 degrees of unsaturation. The IR spectrum showed absorption bands at 1701 and 3389 cm^{-1}, indicating the presence of a lactone carbonyl group and one or more hydroxyl groups, respectively. The ^{13}C NMR and HSQC spectra showed 18 carbon signals: one lactone carbonyl carbon at δ_C 170.6 (C-1); two olefinic methine carbons at δ_C 136.6 (C-4) and 128.4 (C-5); four oxymethine carbons at δ_C 67.6 (C-3), 79.9 (C-6), 77.4 (C-7), and 73.7 (C-9); 10 methylene carbons at δ_C 44.9 (C-2), 42.4 (C-8), 37.7 (C-10), 25.8 (C-11), 29.9 (C-12), 30.1 (C-13 and C-14), 30.2 (C-15), 32.4 (C-16), and 23.3 (C-17); and one methyl carbon at δ_C 14.6 (C-18). The ^1H NMR spectrum of **1** showed signals representing two olefinic protons at δ_H 6.14 and 6.46; four oxymethine protons at δ_H 4.96, 4.35, 3.95, and 5.14; two methylene groups at δ_H 2.85, 2.73, 2.31, and 2.27; one methyl group at δ_H 0.86; and signals for a long aliphatic chain at δ_H 1.20−1.68, suggesting the presence of a nonyl unit, which was substantiated by the ^{13}C NMR and mass spectra. The gross structure of **1** was further established by 2D NMR studies, particularly $^1H-^1H$ COSY and HMBC (Figure 1). In the COSY spectrum, two olefinic

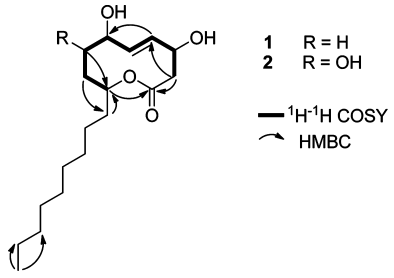


Figure 1. Selected $^1H-^1H$ COSY and HMBC correlations for **1** and **2**.

protons at δ_H 6.14 (H-4) and 6.46 (H-5) were coupled with oxymethine protons at δ_H 4.96 (H-3) and 4.35 (H-6), respectively. Furthermore, the serial correlations between H-2a/H-3, H-2b/H-3, H-4/H-5, H-6/H-7, H-7/H-8a, H-7/H-8b, H-8a/H-9, and H-8b/H-9 contributed to elucidation of the skeleton of **1** from C-2 to C-9. In addition, the HMBC correlations of δ_H 5.14 (H-9), 2.85 (H-2a), and 2.73 (H-2b) with the lactone carbonyl carbon at δ_C 170.6 (C-1) established this compound as a 10-membered macrolactone, with one double bond (C-4/C-5) and three hydroxy groups (C-3, C-6, and C-7). The results obtained from the ^1H and ^{13}C NMR and mass spectra indicated that compound **1** contains one nonyl unit. The position of this group at C-9 was determined unambiguously by HMBC correlations H-8a/C-10, H-8b/C-10, H-10a/C-9, H-10b/C-9, H-10a/C-8, and H-10b/C-8. Elucidation of the planar structure of **1** was thus achieved.[15]

The relative conformation of **1** was determined by analyzing ^3J coupling constants with the aid of NOE correlations obtained from a NOESY experiment. The coupling constant between the two olefinic protons H-4 and H-5 was 15.5 Hz, indicating the *trans* configuration of the double bond. In addition, the large coupling constants $J_{H\text{-}5/H\text{-}6}$ (9.5 Hz), $J_{H\text{-}6/H\text{-}7}$ (9.0 Hz), $J_{H\text{-}7/H\text{-}8b}$ (8.5 Hz), and $J_{H\text{-}8b/H\text{-}9}$ (7.0 Hz) indicated *anti* orientations for H-5/H-6, H-6/H-7, H-7/H-8b, and H-8b/H-9,[23] respectively, which indicated the stereochemical relationships in the C-3−C-9 moiety (Figure 2). Interestingly, H-8a appeared as a doublet ($J_{H\text{-}8a/H\text{-}8b}$ = 15.5 Hz), coupled only with H-8b, which suggests perpendicular relationships for H-7/H-8a and H-8a/H-9.[24]

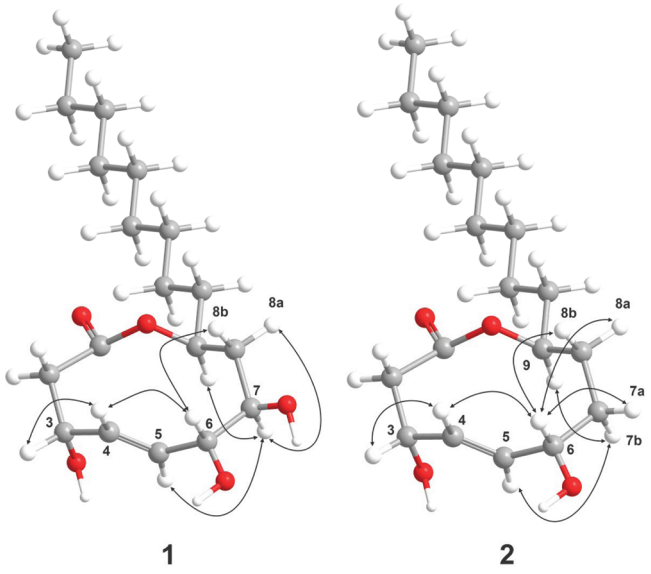


Figure 2. Key NOE correlations for **1** and **2**.

Furthermore, a *gauche* orientation between H-3 and H-4 was inferred on the basis of a small coupling constant, $J_{H\text{-}3/H\text{-}4}$ (3.0 Hz).[23] On the other hand, the NOESY spectrum showed correlation peaks for H-3/H-4, H-4/H-6, and H-6/H-8b, as well as H-5/H-7, H-7/H-8a, and H-7/H-9, which confirmed the relative stereochemistry of the C-3−C-9 moiety (Figure 2). These results revealed the relative conformation of **1**, as depicted in Figure 2.

The absolute configuration of **1** was determined by application of the modified Mosher's method.[25] The (*S*)- and (*R*)-MTPA esters of **1** (**1a** and **1b**) were prepared using (*R*)- and (*S*)-MTPA chloride, respectively. The chemical shift difference between the ^1H NMR spectra of **1a** and **1b** was determined as shown in Table 2. From this, the chiralities for the C-3 and C-7 positions were found to be 3*R* and 7*R*, respectively. Taking the data discussed above into account, the absolute stereochemical configuration of **1** was assigned as 3*R*, 6*R*, 7*R*, and 9*S*.

Seimatopolide B (**2**) was isolated as a white, amorphous solid, with a molecular formula of $C_{18}H_{32}O_4$, as deduced by HRESIMS spectrum. The ^1H and ^{13}C NMR spectra of **2** exhibited strong similarities to those of seimatopolide A (**1**) (Table 1). However, preliminary inspection of the ^1H NMR spectrum of **2** revealed the absence of an oxymethine proton at δ_H 3.95, observed in **1**, and the presence of a methylene group (δ_H 2.30 and 2.00). Moreover, comparison of the ^{13}C NMR data for compounds **1** and **2** indicated that the oxymethine carbon of **1** at C-7 (δ_C 77.4) was replaced by a methylene carbon (δ_C 38.5) in **2**. Thus, seimatopolide B (**2**) was elucidated as a 7-dehydroxyl analogue of seimatopolide A (**1**). The relative conformation of **2** was determined in a similar manner to that of **1**. The ^3J coupling constants $J_{H\text{-}4/H\text{-}5}$ (16.0 Hz) and $J_{H\text{-}3/H\text{-}4}$ (3.0 Hz) indicated a *trans* configuration of the double bond (H-4/H-5) and a *gauche* orientation for H-3/H-4, respectively.[23] Furthermore, $J_{H\text{-}5/H\text{-}6}$ (8.5 Hz) and $J_{H\text{-}6/H\text{-}7b}$ (7.5 Hz) indicated an *anti* orientation for H-5/H-6 and H-6/H-7b, respectively.[23] In addition, the NOE correlations between H-3/H-4, H-4/H-6, H-6/H-7a, H-6/H-8a, and H-6/H-8b, as well as H-5/H-7b and H-7b/H-9, suggested that the relative stereochemistry at positions C-3, C-6, and C-9 of **2** is analogous to that at the same positions of **1** (Figure 2). The absolute

dx.doi.org/10.1021/np200955z | *J. Nat. Prod.* 2012, 75, 784−788

Table 1. NMR Spectroscopic Data (500 MHz, Pyridine d_5) for Seimatopolides A (1) and B (2)

	seimatopolide A (**1**)				seimatopolide B (**2**)			
position	δ_C, type		δ_H (J in Hz)	HMBC[a]	δ_C, type		δ_H (J in Hz)	HMBC[a]
1	170.6	C			170.5	C		
2	44.9	CH_2	2.85, dd (3.5, 12.0)	1, 3, 4	45.7	CH_2	2.89, dd (3.0, 11.5)	1, 3, 4
			2.73, dd (3.5, 11.5)	1, 3			2.72, dd (3.0, 11.5)	1, 3
3	67.6	CH	4.96, bd (2.5)	1	67.8	CH	4.96, m	
4	136.6	CH	6.14, dd (3.0, 15.5)	3, 6	133.4	CH	5.98, dd (3.0, 16.0)	3, 6
5	128.4	CH	6.46, ddd (1.5, 9.5, 16.0)	3	133.4	CH	6.56, dd (8.5, 16.0)	3
6	79.9	CH	4.35, dd (9.0, 9.0)	4, 5, 7, 8	74.9	CH	4.62, dd (7.5, 7.5)	
7	77.4	CH	3.95, ddd (1.5, 8.5, 8.5)	6, 9	38.5	CH_2	2.30, m	5, 6, 9
							2.00, m	5, 6, 9
8	42.4	CH_2	2.31, d (15.5)	6, 7, 10	31.0	CH_2	2.00, m	6, 9
			2.27, m	6, 7, 9, 10			1.72, m	6, 9
9	73.7	CH	5.14, ddd (7.0, 7.0, 13.5)	1, 7, 8, 10, 11	76.5	CH	5.06 ddd (7.0, 7.0, 13.0)	1, 10, 11
10	37.7	CH_2	1.68, m	8, 9, 11	36.3	CH_2	1.62, m	8, 9, 11
			1.60, m	8, 9, 11			1.51, m	8, 11
11	25.8	CH_2	1.37, m		26.0	CH_2	1.22, m	
12	29.9[b]	CH_2	1.20, m		29.9[b]	CH_2	1.22, m	
13	30.1[b]	CH_2	1.20, m		30.1[b]	CH_2	1.22, m	
14	30.1[b]	CH_2	1.20, m		30.2[b]	CH_2	1.22, m	
15	30.2[b]	CH_2	1.20, m		30.2[b]	CH_2	1.22, m	
16	32.4	CH_2	1.20, m		32.4	CH_2	1.22, m	
17	23.3	CH_2	1.20, m		23.2	CH_2	1.22, m	
18	14.6	CH_3	0.86, dd (7.0, 7.0)	16, 17	14.6	CH_3	0.86, dd (7.0, 7.0)	16, 17

[a]HMBC correlations, optimized for 8 Hz. [b]Interchangeable within the same column.

Table 2. ^{1}H NMR Chemical Shift Differences ($\Delta\delta$) for the MTPA Esters of 1 and 2

	$\Delta\delta^a$	
position	**1a/1b**	**2a/2b**
2	−0.110	−0.055
	−0.071	−0.031
3	−0.021	−0.011
4	0.002	0.025
5	−0.088	0.062
6	−0.109	0.011
7	−0.055	0.110
		0.099
8	−0.201	0.099
	−0.182	0.051
9	−0.112	0.001
10	−0.048	0.006
	−0.047	0.006

[a]$\Delta\delta = \delta_S - \delta_R$ in ppm.

configuration of seimatopolide B (**2**) was also determined by application of the modified Mosher's method.[25] The chemical shift difference between the (*S*)-MTPA ester (**2a**) and the (*R*)-MTPA ester (**2b**) of **2** demonstrated the absolute configurations at C-3 and C-6 to be *R* and *S*, respectively (Table 2). Therefore, the absolute configuration of **2** was assigned as 3*R*, 6*S*, and 9*S*.

The PPAR transactivation abilities of the compounds isolated, including seimatopolides A (**1**) and B (**2**), monosporascone, arthrinone, and 3a,9a-deoxy-3a-hydroxy-1-dehydroxyarthrinone, were evaluated using a reporter gene assay in CHO cells,[26] which were cotransfected with a PPAR-γ expression vector and a peroxisome proliferator response element (PPRE)-driven luciferase reporter gene construct. Compounds **1** and **2**, at concentrations up to 20 μM, did not activate PPAR-α or PPAR-β. However, seimatopolide A (**1**) activated PPAR-γ with an EC_{50} value (concentration of compound required to induce 50% of the maximum luciferase activity) of 1.15 μM, while seimatopolide B (**2**) showed activity, with an EC_{50} value of 11.05 μM. Under the same experimental conditions, the EC_{50} of troglitazone, a PPAR-γ agonist used as a positive control, was 0.44 μM. Monosporascone, arthrinone, and 3a,9a-deoxy-3a-hydroxy-1-dehydroxyarthrinone did not show PPAR transactivation ability.

Quantitative real-time PCR was used to verify the altered expression of the PPAR-γ target genes, including PPAR-γ, stearoyl CoA desaturase-1 (SDC-1), glucose-6-phosphatase (G6Pase), and phosphoenolpyruvate carboxykinase (PEPCK), in HepG2 hepatocytes when stimulated by **1** (Figure 3). The expression of *PPAR-γ* was dose dependently up-regulated, with a maximum increase of 2.0-fold. Compound **1** also reduced the expression of two gluconeogenic genes, G6Pase and PEPCK, to an extent comparable with the effect of troglitazone; however, the expression of SCD-1 was unaltered. These results suggest that compound **1** is a possible candidate for an antidiabetic drug.[27]

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured on a JASCO P-2000 polarimeter, using a 10 cm microcell. IR spectra were obtained using a Varian 640-IR spectrometer. NMR spectra were obtained using a Varian 500 MHz NMR spectrometer with tetramethylsilane (TMS) as an internal standard, and chemical shifts are expressed as δ values. ESIMS was performed on a Waters Q-TOF micromass spectrometer. Column chromatography was performed using silica gel (Kieselgel 60, 70−230 and 230−400 mesh, Merck) and Diaion HP-20 resin (Supelco, Bellefonte, PA, USA), and thin layer chromatography was performed using precoated silica gel 60 F254 plates (0.25 mm, Merck). Preparative HPLC was conducted using the Varian Prostar 210 system.

dx.doi.org/10.1021/np200955z | J. Nat. Prod. 2012, 75, 784−788

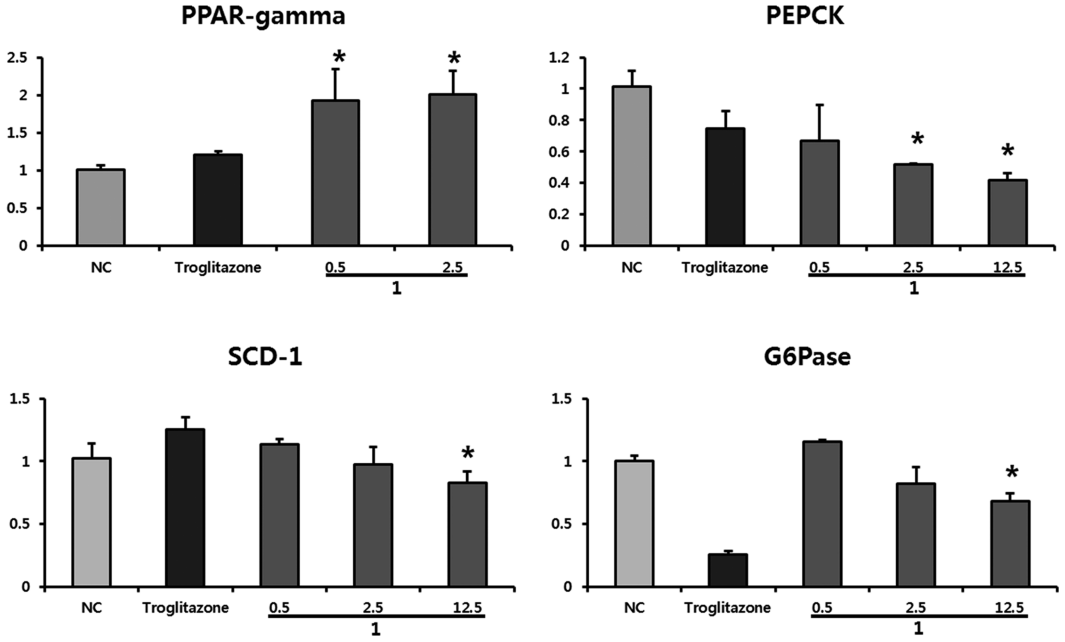


Figure 3. Expression of PPAR-γ target genes in HepG2 hepatocytes. Cells were stimulated with either troglitazone or compound **1** at various concentrations (μM), and the expression of target genes was assessed by real-time PCR. NC, nontreated control; PEPCK, phosphoenolpyruvate carboxykinase; SCD-1, stearoyl CoA desaturase-1; G6 Pase, glucose-6-phosphatase; *p < 0.05 compared with NC.

Fungus Cultivation. *S. discosioides* (Ellis & Everhart) Shoemaker (KACC 42490) was obtained from the Korean Agricultural Culture Collection (http://www.genebank.go.kr), Suwon, Korea, and was cultivated in 6 L (300 mL × 20 × 1 L) baffled flasks of potato dextrose broth medium (PDB; Difco, Detroit, MI, USA), containing 4.0 g/L potato starch and 20.0 g/L dextrose. Each flask was seeded with a 1 × 1 cm piece of fully grown fungal mycelium on potato dextrose agar (PDA; Difco, Detroit, MI, USA), and the flasks were incubated on a rotary shaker (165 rpm) for 15 days at 28 °C. The culture broth (6 L) was then filtered to harvest the fungal cells.

Extraction and Isolation. The fungal cells were extracted with MeOH (3 × 2 L), and these extracts were filtered through a fritted funnel *in vacuo* to remove insolubles. The volume of the filtrate was reduced *in vacuo* and then partitioned between EtOAc and H_2O to yield the organic extract (5.2 g). The EtOAc-soluble extract was fractionated on a Diaion HP-20 column, using stepwise elution with MeOH–H_2O (0:10, 2:10, 4:10, 6:10, 8:10, and 10:0) to give eight fractions, F1–F8. Fraction F6 (1.4 g) was precipitated with MeOH to obtain two subfractions, F6A and F6B. Subfraction F6B (97.7 mg) was purified by HPLC (YMC J'sphere ODS-H80, 4 μm, 250 × 20 mm i.d., 70–100% MeOH in H_2O, flow rate 8.0 mL/min), to afford compounds **1** (52.1 mg), **2** (20.5 mg), and monosporascone (11.3 mg). Fraction F4 was separated into 10 subfractions, F4A–F4J, by silica gel column chromatography with gradient elution ($CHCl_3$–MeOH, 98:2 to 50:50). Subfraction F4B was separated by RP_{18} HPLC (YMC J'sphere ODS-H80, 4 μm, 250 × 20 mm i.d., 20% MeOH in H_2O, flow rate 8.0 mL/min) to afford arthrinone (14.1 mg) and 3a,9a-deoxy-3a-hydroxy-1-dehydroxyarthrinone (4.0 mg).

Seimatopolide A: white, amorphous solid; $[\alpha]^{26}_D$ −188.3 (*c* 0.05, MeOH); IR (ATR) ν_{max} 3389, 2913, 2848, 2359, 1701 cm^{-1}; ^{13}C and ^{1}H NMR (500 MHz, pyridine-d_5), see Table 1; ESIMS (negative) *m/z* 373.2 $[M + HCOO]^-$, 701.4 $[2\,M + HCOO]^-$; ESIMS (positive) *m/z* 293.1 $[M + H - 2H_2O]^+$, 352.2 $[M + H + Na]^+$, 657.3 $[2\,M + H]^+$; HRESIMS *m/z* 373.2219 $[M + HCOO]^-$ (calcd for $C_{19}H_{33}O_7$, 373.2226).

Seimatopolide B: white, amorphous solid; $[\alpha]^{26}_D$ −125.4 (*c* 0.03, MeOH); IR (ATR) ν_{max} 3242, 2914, 2848, 2359, 1721 cm^{-1}; ^{13}C and ^{1}H NMR (500 MHz, pyridine-d_5), see Table 1; ESIMS (negative) *m/z* 357.2 $[M + HCOO]^-$; ESIMS (positive) *m/z* 280.2 $[M + H - H_2O - CH_3]^+$, 336.1 $[M + H + Na]^+$, 607.3 $[2\,M + H - H_2O]^+$; HRESIMS *m/z* 357.2263 $[M + HCOO]^-$ (calcd for $C_{19}H_{33}O_6$, 357.2277).

Preparation of (*S*)- and (*R*)-MTPA Ester Derivatives of Seimatopolides A and B. One milliliter of a solution of pure compound **1** (3.8 mg) in pyridine-d_5 was divided into two parts, which were then transferred to clean NMR tubes. DMAP and (*S*)-MTPACl (20 μL) or (*R*)-MTPACl (20 μL) were added successively to the NMR tube under a N_2 gas stream, and the tube was carefully shaken to mix the sample, DMAP, and MTPA chloride evenly. The NMR tubes were heated at 40 °C for 5 h to afford the MTPA esters of **1** (**1a** and **1b**). These esters were then purified using silica gel column chromatography with $CHCl_3$. The same procedure was used to prepare the MTPA esters of compound **2** (**2a** and **2b**).

*(S)-**1a**:* ^{1}H NMR (pyridine-d_5, 500 MHz) δ 3.005 (dd, *J* = 3.0, 13.0 Hz, H-2a), 2.946 (dd, *J* = 4.0, 13.0 Hz, H-2b), 6.215 (d, *J* = 3.0 Hz, H-3), 6.684 (dd, *J* = 4.0, 15.5 Hz, H-4), 5.925 (dd, *J* = 10.0, 16.0 Hz, H-5), 6.039 (t, *J* = 9.5 Hz, H-6), 5.748 (t, *J* = 10.5 Hz, H-7), 1.998 (d, *J* = 16.0 Hz, H-8a), 2.282 (ddd, *J* = 7.0, 9.5, 16.0 Hz, H-8b), 5.159 (dd, *J* = 7.0, 13.5 Hz, H-9), 1.656 (m, H-10a), 1.556 (m, H-10b); HRESIMS *m/z* 994.3592 $[M + H_2O]^+$ (calcd for $C_{48}H_{55}O_{12}F_9$, 994.3350).

*(R)-**1b**:* ^{1}H NMR (pyridine-d_5, 500 MHz) δ 3.115 (dd, *J* = 3.0, 13.0 Hz, H-2a), 3.017 (dd, *J* = 4.0, 13.0 Hz, H-2b), 6.236 (d, *J* = 3.5 Hz, H-3), 6.682 (dd, *J* = 4.0, 16.0 Hz, H-4), 6.013 (dd, *J* = 10.0, 16.5 Hz, H-5), 6.148 (t, *J* = 9.5 Hz, H-6), 5.803 (t, *J* = 10.0 Hz, H-7), 2.180 (d, *J* = 16.0 Hz, H-8a), 2.483 (ddd, *J* = 7.0, 9.5, 16.5 Hz, H-8b), 5.279 (dd, *J* = 7.0, 13.5 Hz, H-9), 1.704 (m, H-10a), 1.603 (m, H-10b); HRESIMS *m/z* 994.3570 $[M + H_2O]^+$ (calcd for $C_{48}H_{55}O_{12}F_9$, 994.3350).

*(S)-**2a**:* ^{1}H NMR (pyridine-d_5, 500 MHz) δ 3.000 (1H, dd, *J* = 3.0, 13.0 Hz, H-2a), 2.980 (1H, dd, *J* = 4.0, 13.0 Hz, H-2b), 6.149 (1H, d, *J* = 3.0 Hz, H-3), 6.239 (1H, dd, *J* = 3.0, 16.0 Hz, H-4), 5.938 (1H, dd, *J* = 8.5, 16.5 Hz, H-5), 5.776 (1H, t, *J* = 8.5 Hz, H-6), 2.110 (1H, m, H-7a), 1.857 (1H, m, H-7b), 1.857 (1H, m, H-8a), 1.638 (1H, m, H-8b), 4.874 (1H, dd, *J* = 6.5, 13.0 Hz, H-9), 1.524 (1H, m, H-10a), 1.414 (1H, m, H-10b); HRESIMS *m/z* 762.3256 $[M + H_2O]^+$ (calcd for $C_{38}H_{48}O_9F_6$, 762.3203).

*(R)-**2b**:* ^{1}H NMR (pyridine-d_5, 500 MHz) δ 3.055 (1H, dd, *J* = 3.0, 13.0 Hz, H-2a), 2.921 (1H, dd, *J* = 4.0, 13.0 Hz, H-2b), 6.160 (1H, d, *J* = 3.0 Hz, H-3), 6.214 (1H, dd, *J* = 3.0, 15.5 Hz, H-4), 5.876 (1H, dd, *J* = 8.5, 16.5 Hz, H-5), 5.765 (1H, t, *J* = 7.5 Hz, H-6), 2.000 (1H, m, H-7a), 1.758 (1H, m, H-7b), 1.758 (1H, m, H-8a), 1.587 (1H, m, H-8b), 4.873 (1H, dd, *J* = 6.5, 13.0 Hz, H-9), 1.518 (1H, m, H-10a), 1.408 (1H, m, H-10b); HRESIMS *m/z* 762.3175 $[M + H_2O]^+$ (calcd for $C_{38}H_{48}O_9F_6$, 762.3203).

dx.doi.org/10.1021/np200955z | *J. Nat. Prod.* 2012, 75, 784–788

Bioassay for Activation of PPAR-γ. CHO cells were seeded in 24-well plates at 1 × 105 cells per well. The following day, the cells were cotransfected with the reporter vector pGL3-PPRE3-TK-luc (which contains the firefly luciferase gene under control of a PPRE) and expression vectors encoding full-length human PPAR-γ and β-galactosidase, using Hilymax transfection reagent (Dojindo Laboratories, Rockville, MD, USA). The medium was removed after 4 h of incubation and replaced with high-glucose DMEM. After 18 h, the cells were treated with the isolated compounds (at 0, 5, 20, 40, 60, 80, or 100 μM) or troglitazone (10 μM) for 24 h and then lysed in firefly luciferase lysis buffer (Biotium, Inc., Hayward, CA, USA). The luciferase activity in the cell lysate was measured using a firefly luciferase assay kit (Biotium), according to the manufacturer's protocol. β-Galactosidase activity was determined using the β-galactosidase enzyme assay system (Promega, Madison, WI, USA). To normalize the results for transfection efficiency, luciferase activity is expressed relative to the β-galactosidase activity in the same lysate.

RNA Isolation and RT-PCR. Total RNA was isolated from control or treated cells using an RNAiso Plus kit (Takara Bio Inc., Otsu, Japan). For cDNA synthesis, 2 μg of total RNA was reverse-transcribed with oligo(dT) and PrimerScript reverse transcriptase (Takara Bio Inc.) in a 20 μL reaction volume. Human gene-specific primers for PCR were designed using the nucleotide BLAST tool provided by the National Center for Biotechnology Information (NCBI). PCR was performed using these primers and iQ SYBR Green Supermix reagent (Bio-Rad Laboratories, Inc.), on an iQ5 iCycler system (Bio-Rad Laboratories, Inc.). The PCR conditions consisted of an initial denaturation step (95 °C for 3 min) followed by 60 cycles of 95 °C for 10 s, 60 °C for 15 s, and 72 °C for 20 s. To determine primer specificity, a 71-cycle melting curve analysis was carried out, beginning at 55 °C and increasing by 0.5 °C every 10 s. Gene expression levels were normalized to the corresponding value for the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene and analyzed using iQ5 System software (version 2).

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

MS and ^{1}H and ^{13}C NMR spectra of **1** and **2** are available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Tel: +82-2-961-0719. E-mail: dsjang@khu.ac.kr (D.S.J.). Tel: +82-2-3290-3017. E-mail: dongholee@korea.ac.kr (D.L.).

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This study was supported by Forest Science & Technology Project (No. S120910L130140, Korea Forest Service, Republic of Korea) and Agenda Project (No. 20100501030008, Rural Development Administration, Republic of Korea).

■ REFERENCES

(1) Michalik, L.; Auwerx, J.; Berger, J. P.; Chatterjee, V. K.; Glass, C. K.; Gonzalez, F. J.; Grimaldi, P. A.; Kadowaki, T.; Lazar, M. A.; O'Rahilly, S.; Palmer, C. N.; Plutzky, J.; Reddy, J. K.; Spiegelman, B. M.; Staels, B.; Wahli, W. *Pharmacol. Rev.* **2006**, *58*, 726−741.

(2) Chawla, A.; Repa, J. J.; Evans, R. M.; Mangelsdorf, D. J. *Science* **2001**, *294*, 1866−1870.

(3) Oliver, W. R. Jr.; Shenk, J. L.; Snaith, M. R.; Russell, C. S.; Plunket, K. D.; Bodkin, N. L.; Lewis, M. C.; Winegar, D. A.; Sznaidman, M. L.; Lambert, M. H.; Xu, H. E.; Sternbach, D. D.; Kliewer, S. A.; Hansen, B. C.; Willson, T. M. *Proc. Natl. Acad. Sci. U. S. A.* **2001**, *98*, 5306−5311.

(4) Vosper, H.; Patel, L.; Graham, T. L.; Khoudoli, G. A.; Hill, A.; Macphee, C. H.; Pinto, I.; Smith, S. A.; Suckling, K. E.; Wolf, C. R.; Palmer, C. N. *J. Biol. Chem.* **2001**, *276*, 44258−44265.

(5) Willson, T. M.; Brown, P. J.; Sternbach, D. D.; Henke, B. R. *J. Med. Chem.* **2000**, *43*, 527−550.

(6) Rosen, E. D.; Spiegelman, B. M. *J. Biol. Chem.* **2001**, *276*, 37731−37734.

(7) Li, A. C.; Binder, C. J.; Gutierrez, A.; Brown, K. K.; Plotkin, C. R.; Pattison, J. W.; Valledor, A. F.; Davis, R. A.; Willson, T. M.; Witztum, J. L.; Palinski, W.; Glass, C. K. *J. Clin. Invest.* **2004**, *114*, 1564−1576.

(8) Murphy, G. J.; Holder, J. C. *Trends Pharmacol. Sci.* **2000**, *21*, 469−474.

(9) Lee, D.; Lee, J. H.; Cai, X. F.; Shin, J. C.; Lee, K.; Hong, Y. S.; Lee, J. J. *J. Antibiot.* **2005**, *58*, 615−620.

(10) Stipanovic, R. D.; Zhang, J.; Bruton, B. D.; Wheeler, M. H. *J. Agric. Food. Chem.* **2004**, *52*, 4109−4112.

(11) Whyte, A. C.; Gloer, K. B.; Gloer, J. B.; Koster, B.; Malloch, D. *Can. J. Chem.* **1997**, *75*, 768−772.

(12) Victor, M. M.; Riatto, V. B.; Pilli, R. A. *Tetrahedron* **2008**, *64*, 2279−2300.

(13) Ratnayake, A. S.; Yoshida, W. Y.; Mooberry, S. L.; Hemscheidt, T. *Org. Lett.* **2001**, *3*, 3479−3481.

(14) Furstner, A.; Radkowski, K. *Chem. Commun.* **2001**, 671−672.

(15) Bodo, B.; Molho, L.; Davoust, D.; Molho, D. *Phytochemistry* **1983**, *22*, 447−451.

(16) Furstner, A.; Radkowski, K.; Wirtz, C.; Goddard, R.; Lehmann, C. W.; Mynott, R. *J. Am. Chem. Soc.* **2002**, *124*, 7061−7069.

(17) Gohrt, A.; Zeeck, A.; Hutter, K.; Kirsch, R.; Kluge, H.; Thiericke, R. *J. Antibiot.* **1992**, *45*, 66−73.

(18) Grabley, S.; Granzer, E.; Hutter, K.; Ludwig, D.; Mayer, M.; Thiericke, R.; Till, G.; Wink, J.; Philipps, S.; Zeeck, A. *J. Antibiot.* **1992**, *45*, 56−65.

(19) Grabley, S.; Hammann, P.; Hutter, K.; Kirsch, R.; Kluge, H.; Thiericke, R.; Mayer, M.; Zeeck, A. *J. Antibiot.* **1992**, *45*, 1176−1181.

(20) Mayer, M.; Thiericke, R. *J. Antibiot.* **1993**, *46*, 1372−1380.

(21) Rukachaisirikul, V.; Pramjit, S.; Pakawatchai, C.; Isaka, M.; Supothina, S. *J. Nat. Prod.* **2004**, *67*, 1953−1955.

(22) Drager, G.; Kirschning, A.; Thiericke, R.; Zerlin, M. *Nat. Prod. Rep.* **1996**, *13*, 365−375.

(23) Matsumori, N.; Kaneno, D.; Murata, M.; Nakamura, H.; Tachibana, K. *J. Org. Chem.* **1999**, *64*, 866−876.

(24) Takada, N.; Tayone, W. C.; Shindo, S.; Murakami, T.; Hashimoto, M.; Tanaka, K. *Tetrahedron* **2009**, *65*, 7464−7467.

(25) Ohtani, I.; Kusumi, T.; Kashman, Y.; Kakisawa, H. *J. Am. Chem. Soc.* **1991**, *113*, 4092−4096.

(26) Jia, Y.; Bhuiyan, M. J.; Jun, H. J.; Lee, J. H.; Hoang, M. H.; Lee, H. J.; Kim, N.; Lee, D.; Hwang, K. Y.; Hwang, B. Y.; Choi, D. W.; Lee, S. J. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 5876−5880.

(27) Barthel, A.; Schmoll, D. *Am. J. Physiol. Endocrinol. Metab.* **2003**, *285*, E685−E692.