Oploxynes A and B, Polyacetylenes from the Stems of *Oplopanax elatus*

Min Cheol Yang,[†,§] Hak Cheol Kwon,[†,§] Young-Joo Kim,[†] Kang Ro Lee,[‡] and Hyun Ok Yang*[,†]

Natural Products Research Center, Korea Institute of Science and Technology, Gangneung, Gangwon-do 210-340, Republic of Korea, and Natural Products Laboratory, College of Pharmacy, Sungkyunkwan University, Suwon 440-746, Republic of Korea

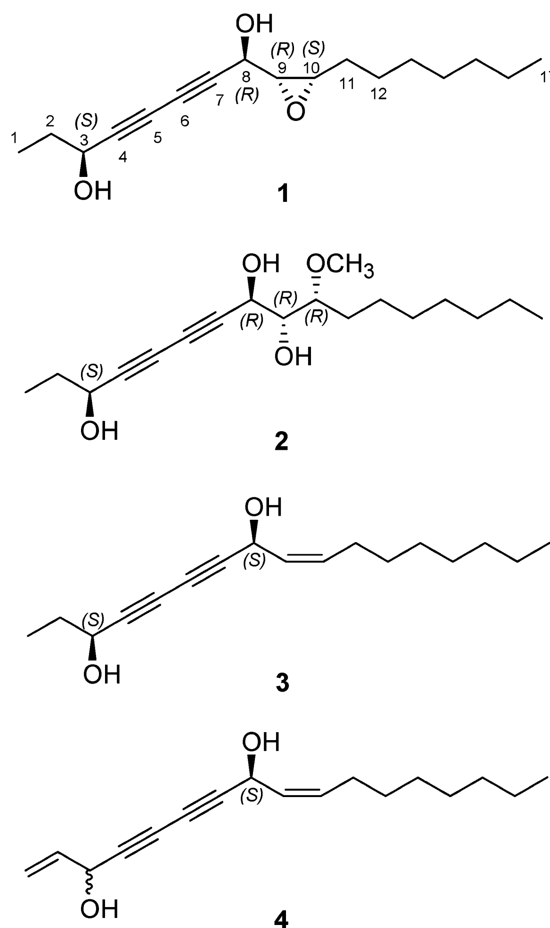
Received October 6, 2009

Two new polyacetylenes, oploxynes A (**1**) and B (**2**), and the known oplopandiol (**3**) and falcarindiol (**4**) were isolated from the stem of *Oplopanax elatus*. The structures of compounds **1** and **2** were determined to be 9,10-epoxyheptadeca-4,6-diyne-3,8-diol and 10-methoxyheptadeca-4,6-diyne-3,8,9-triol, respectively, on the basis of their UV, MS, and NMR data. The absolute configurations of these compounds were determined using the modified Mosher's method and acetonide formation. Oploxyne A (**1**), oplopandiol (**3**), and falcarindiol (**4**) inhibited the formation of nitric oxide (NO) and prostaglandin E_2 (PGE_2) in lipopolysaccharide (LPS)-induced murine macrophage RAW 267.7 cells.

Plants of the genus *Oplopanax* (Araliaceae) contain various bioactive secondary metabolites, such as lignans,[1] saponins,[2,3] sesquiterpenes,[4,5] polyacetylenes,[6,7] and anthraquinones.[8] In particular, *O. horridus*, commonly known as Devil's Club, is used for the treatment of diabetes, rheumatism, tuberculosis, colds, headaches, and lung hemorrhages. Polyacetylenes are major constituents of Devil's Club, and these compounds exhibit significant antimycobacterial and antifungal activity.[7]

Oplopanax elatus NAKAI has been used in Korean and Chinese traditional medicine for anti-inflammatory and analgesic purposes.[9,10] Saponins,[2,3] an anthraquinone,[8] an antipsoriasis lignin,[1] and the antibacterial essential oil[11,12] have been identified from this plant. However, neither the active principles in the traditional medicine nor the polyacetylenes in this plant have been investigated. Because of the ethnopharmacological prescription of this plant for inflammation, we examined the inhibitors produced in the stem on the formation of nitric oxide (NO) and prostaglandin E_2 (PGE_2) in lipopolysaccharide (LPS)-induced murine macrophage RAW 267.7 cells. During this survey, we isolated two new diynes, oploxynes A (**1**) and B (**2**), together with two known diynes, oplopandiol (**3**)[7] and falcarindiol (**4**),[13] from the CH_2Cl_2 extract of the stem of *O. elatus*.

The polyacetylenes, including conjugated diynes, possess a variety of biological activities, particularly antitumor, anti-inflammatory, antimicrobial, and antiviral activities. For example, pentadeca-(8*Z*,13*Z*)-dien-11-yn-2-one (from the roots of *Echinacea pallida*) exhibited anticancer activity against MIA PaCa-2 and COLO320 cells.[14] (3*Z*,5*E*,11*E*)-Tridecatriene-7,9-diynyl-1-*O*-(*E*)-ferulate (from the rhizomes of *Atractylodes lancea*) showed strong inhibition of 5-LOX and COX-1 activities.[15] Octadeca-1,9-diene-4,6-diyne-3,8,18-triol and 18-acetoxyoctadeca-1,9-diene-4,6-diyne-3,8-diol (from *Angelica gigas*) downregulated expression of the inducible form of nitric oxide synthase.[16] 13(*E*),17-Octadecadiene-9,11-diynoic acid (from *Mitrephora celebica*) demonstrated significant activity against methicillin-resistant *Staphylococcus aureus* and *Mycobacterium smegmatis*.[17] α-Terthienyl was extremely toxic against murine cytomegalovirus (CMV) and Sindbis virus.[18] However, the anti-inflammatory effects of acyclic, conjugated diynes lacking double bonds have not been evaluated.



Results and Discussion

The stems of *O. elatus* were collected in Gangwon Province (Korea) in September 2006. The air-dried stems (1.0 kg) of *O. elatus* were extracted using CH_2Cl_2 at room temperature. The extract (25.0 g) was subjected to a diversity of chromatographic purification steps to afford oploxynes A (**1**) and B (**2**), together with two known polyacetylenes, oplopandiol (**3**) and falcarindiol (**4**).

Compound **1** was obtained as an optically active colorless oil ($[\alpha]_D$ +123.4). The molecular formula was assigned as $C_{17}H_{26}O_3$ by HRFABMS (obsd $[M + Na]^+$ at *m/z* 301.1775; calcd 301.1780). The IR spectrum of **1** displayed absorption bands at 3384, 2253, and 2146 cm^{-1}, indicating the presence of hydroxy and triple-bond functionalities, while UV absorption bands at 231, 243, and 257 nm suggested a diyne moiety.[19] In addition, the ^{13}C NMR signals at δ_C 81.0, 68.7, 70.3, and 77.4 were attributable to a conjugated diyne.[20,21] The characteristic features of the ^1H NMR spectrum of **1** included the presence of four oxymethine protons at δ_H 4.40 (1H,

* To whom correspondence should be addressed. Tel: 82-33-650-3501. Fax: 82-33-650-3529. E-mail: hoyang@kist.re.kr.
† Korea Institute of Science and Technology.
‡ Sungkyunkwan University.
§ These authors contributed equally to this work.

10.1021/np900628j © 2010 American Chemical Society and American Society of Pharmacognosy
Published on Web 04/13/2010

br dt, $J = 6.0, 6.0$ Hz), 4.36 (1H, br dd, $J = 7.5, 3.5$ Hz), 3.16 (1H, dd, $J = 7.5, 4.0$ Hz), and 3.07 (1H, br ddd, $J = 7.0, 5.5, 4.0$ Hz) and two exchangeable protons at δ_H 2.19 (1H, br d, $J = 6.0$ Hz) and 2.50 (1H, br d, $J = 3.5$ Hz). The ^{1}H−^{1}H COSY NMR spectrum showed that these two exchangeable protons correlated with protons at δ_H 4.40 and 4.36. Also, correlations between δ_H 4.36, 3.16, and 3.07 were observed in the ^{1}H−^{1}H COSY NMR spectrum of **1**. The HSQC spectrum showed that the four oxymethine proton signals correlated with ^{13}C NMR signals at δ_C 64.0, 60.7, 58.0, and 58.1, respectively. In addition, the ^{1}H NMR spectrum of **1** displayed signals attributable to two methyl protons [δ_H 1.03 (3H, t, $J = 7.5$ Hz) and 0.89 (3H, t, $J = 7.0$ Hz)] and seven aliphatic methylenes [δ_H 1.76, 1.64, 1.51, 1.35 (all are 2H, m), and 1.29 (6H, m)]. The ^{13}C NMR and HSQC spectra of **1** allowed assignment of all protons to their respective carbons. The ^{1}H and ^{13}C NMR spectra of **1** were similar to those of a known heptadeca-1-ene-9,10-epoxy-4,6-diyne-3,8-diol.[20,21] The key difference between **1** and heptadeca-1-ene-9,10-epoxy-4,6-diyne-3,8-diol was the presence of a methyl group [δ_H 1.03 and δ_C 9.3 (C-1)] in **1**, instead of the terminal olefinic methylene group in heptadeca-1-ene-9,10-epoxy-4,6-diyne-3,8-diol.

The relative configuration of the epoxide ring in **1** was assigned by the analysis of coupling constants in its ^{1}H NMR spectrum and ^{1}H−^{1}H NOE correlations. H-8 and H-9 showed consistent NOESY correlations with H_2-11 and H-10, respectively, while there was no NOESY correlation between H-8 and H-10 in the 2D NOESY spectrum of **1**. These NOE data and the coupling constant (4.0 Hz) between H-9 and H-10 supported the *cis* configuration for the epoxide.[22]

The absolute configurations of C-3, C-8, C-9, and C-10 of compound **1** were assigned on the basis of chiral derivatization. Treatment of **1** with (*R*)-(−)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride [(*R*)-MTPA-Cl] and DMAP in pyridine yielded a mixture of 3,8-bis-(*S*)-Mosher ester (**5a**) and 3,9-bis-(*S*)-Mosher ester (**5b**) of 10-chloroheptadeca-4,6-diyne-3,8,9-triol. Similar treatment of **1** with (*S*)-(+)-MTPA-Cl afforded a mixture of 3,8-bis-(*R*)-Mosher ester (**6a**) and 3,9-bis-(*R*)-Mosher ester (**6b**) of 10-chloroheptadeca-4,6-diyne-3,8,9-triol. Analysis of the ^{1}H NMR chemical shift differences ($\Delta\delta_{S-R}$)[23] (Figure 1) of the mixtures allowed the assignment of the absolute configuration of C-3, C-8, and C-9 of **1** as *S*, *R*, and *R*, respectively. The absolute configuration of C-10 was thus assigned as *S*.

Compound **2** was isolated as an optically active, colorless oil ([α]$_D$ +11.7) and gave the molecular formula $C_{18}H_{31}O_4$, based on HRCIMS data (obsd [M + Na]$^+$ at *m/z* 311.2222). The IR and UV spectra of **2** were similar to those of **1**, suggesting the presence of hydroxy and conjugated diyne functionalities. As with compound **1**, comprehensive analysis of the 2D NMR (^{1}H−^{1}H COSY, HSQC, HMBC, and NOESY) data of **2** allowed assignment of proton and carbon signals. The ^{1}H NMR spectrum of **2**, including HMBC and ^{1}H−^{1}H COSY data, showed signals attributable to three exchangeable hydroxy groups (δ_H 1.85, 2.68, and 3.41) attached to C-3 (δ_C 64.3), C-9 (δ_C 73.1), and C-8 (δ_C 65.7), and a methoxy group (δ_H 3.44) attached to C-10 (δ_C 81.7). Furthermore, the C-9 and C-10 signals were observed at δ_C 73.1 and 81.7, respectively, while the chemical shifts of C-9 and C-10 in the ^{13}C NMR spectrum of **1** were δ_C 58.0 and 58.1, respectively. These data indicated that compound **2** contains hydroxy and methoxy functionalities at C-9 and C-10, respectively, in place of the epoxide in **1**. The chemical shifts of C-8, C-9, and C-10 were similar to those of the reported 10-methoxyheptadec-16-ene-4,6-diyne-8,9-diol, ciryneol B.[22] The C-8, C-9, and C-10 resonances in ciryneol B were observed at δ_C 65.3, 73.5, and 81.3.[22] However, the chemical shifts of C-8−C-10 carbons in heptadec-16-ene-4,6-diyne-8,9,10-triol were at δ_C 65.5, 75.0, and 71.3, respectively.

The relative configurations of C-3, C-8, C-9, and C-10 of oploxyne B (**2**) were again assigned by chiral derivatization and chemical transformation. Treatment of **2** with 2,2-dimethoxypropane and pyridinium *p*-toluenesulfonate in acetone afforded the acetonide **7**,[24] which resulted from the acetal formation involving the C-8 and C-9 hydroxy groups. The NOE correlation between H-8 (δ_H 4.71) and H-9 (δ_H 4.05) in the NOESY spectra of **7** revealed a 2,3-*syn*-isopropylidene moiety. The acetonide methyl groups showed chemical shifts at δ_H 1.57 and 1.37, which further supported the assignment of a *syn* relationship between H-8 and H-9.[25] The relative configuration of C-10 in **2** was deduced from the ^{1}H coupling constants and the NOESY data of compound **7** (Figure 2). The coupling constant between H-9 and H-10 of **7** was 8.5 Hz

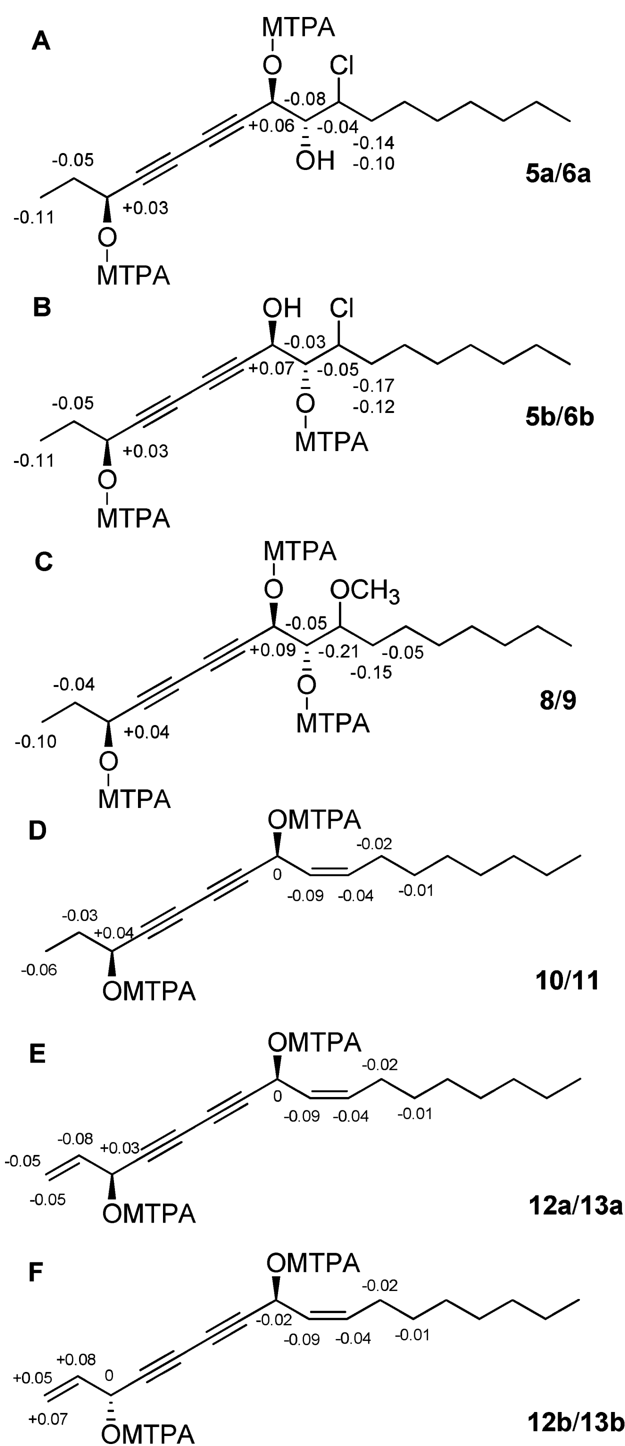


Figure 1. $\Delta\delta_{S-R}$ values for (A) the bis-Mosher esters **5a/6a**, (B) the bis-Mosher esters **5b/6b**, (C) the tris-Mosher esters **8/9**, (D) the bis-Mosher esters **10/11**, (E) the bis-Mosher esters **12a/13a**, and (F) the bis-Mosher esters **12b/13b**, in pyridine-d_5.

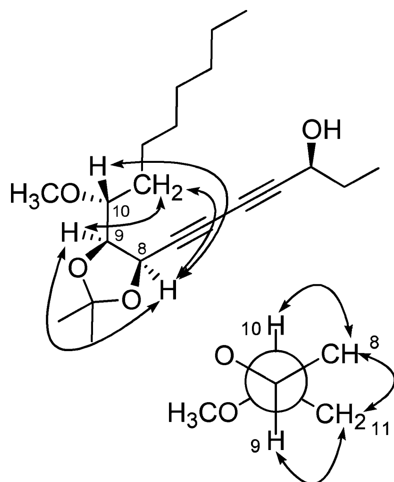


Figure 2. Key NOE correlations in the NOESY spectra of the acetonide **7** in $CDCl_3$.

and suggested an *anti* configuration.[26] The NOESY correlations were in support of this assignment. In the 2D NOESY spectrum of **7**, H-9 and H-10 showed consistent correlations with H_2-11 and H-8, respectively. However, there was no apparent correlation between H-9 and H-10 in its 1D NOE spectrum (Figure 2). The coupling constant and NOE data indicated that H-9 and H-10 in **7** were in the *anti* configuration. The correlation between H-8 and H_2-11 was also observed in the 2D NOESY spectrum of **7**. These NOESY data, in combination with the large $^3J_{H-9,H-10}$ value, allowed us to assign the relative configurations at C-9 and C-10 in **7** as *S** and *R**.

The CD spectra of both **1** and **2** showed negative Cotton effects at 202 nm (Figures S4 and S14). These data suggested that the absolute configurations of carbinol carbons adjacent to triple bonds of **2** were identical with those of **1**. The absolute configurations of C-3, C-8, and C-9 of **2** were determined by application of the modified Mosher method.[27] Separate treatment of **2** in pyridine with (*R*)-MTPA-Cl and (*S*)-MTPA-Cl yielded the tris-(*S*)- (**8**) and tris-(*R*)-Mosher esters (**9**), respectively. Analysis of the ^{1}H chemical shift differences ($\Delta\delta_{S-R}$) between **8** and **9** (Figure 1) revealed negative $\Delta\delta_{S-R}$ values for H-1 and H-2 of the C-3 ester, negative values for H-10−H-12, and a positive value for H-8 of the C-9 ester. Furthermore, a negative $\Delta\delta_{S-R}$ value for H-9 of the C-8 ester was observed, while the H-3 signals showed a positive difference. These data allowed the absolute configurations at C-3, C-8, and C-9 of **2** to be assigned as *S*, *R*, and *R*, respectively.[23,27] The absolute configuration of C-10 was determined to be *R* on the basis of the relative configuration of C-9 and C-8 in compound **7**.

The NMR data of compound **3** were in accordance with published data.[7] Those authors assigned H-9 and C-9 at δ_H 5.6 (1H, ddt, J = 10.8, 7.3, 1.5 Hz) and δ_C 134.6, respectively, while the resonances at δ_H 5.5 (1H, ddt, J = 10.8, 8.3, 1.5 Hz) and δ_C 127.8 were assigned to H-10 and C-10, respectively. However, analysis of the 2D NMR data of **3** allowed us to reassign H-9 and H-10 at δ_H 5.47 (1H, ddt, J = 11.0, 8.5, 1.0 Hz, H-9) and 5.55 (1H, dtd, J = 11.0, 8.0, 1.0 Hz, H-10). The chemical shifts of C-9 and C-10 should accordingly be changed to δ_C 127.8 and 134.0, respectively.

The absolute configurations of C-3 and C-4 in oplopandiol (**3**) were defined by the analysis of $\Delta\delta_{S-R}$ values for the two fluorine signals in the ^{19}F NMR spectra of the (*S*)- and (*R*)-MTPA esters. Because the assignment of absolute configuration using only ^{19}F NMR data may not always be feasible, we prepared MTPA esters of **3** in order to obtain ^{1}H NMR data for the (*S*)-MTPA (**10**) and (*R*)-MTPA (**11**) esters. The modified Mosher ester analysis resulted in negative $\Delta\delta_{S-R}$ values for H_3-1, H_2-2, H-9, H-10, and H_2-11, while a positive value was obtained for H-3 (Figure 1). These data allowed us to assign *S* configuration at both C-3 and C-8, which confirmed the previously described configurations of oplopandiol.[7]

Although the chemical and physical properties of falcarindiol (**4**) have been described,[7,28,29] NMR and optical rotation data related to the absolute configuration at C-3 and C-8 are inconclusive. Review of reported physical data of (3*S*,8*S*)- and (3*R*,8*S*)-falcarindiols revealed that it is hard to distinguish the isomers by comparison of specific rotation and NMR data (Tables S1, S2, and S3). This uncertainty of the stereochemical assignments for falcarindiol (**4**) prompted us to examine CD spectra and prepare its Mosher esters. The CD spectra of both **3** and **4** exhibited positive Cotton effects at 202 nm (Figures S21 and S28). These data suggested that the absolute configuration of chiral carbons flanked by an acetylene and an olefin of **4** is identical with those of **3**. Analysis of ^{1}H NMR chemical shift differences between the (*S*)-(**12a**) and (*R*)-(**13a**) Mosher esters revealed that the absolute configurations of C-3 and C-8 are both *S*. However, small proton signals, which were readily distinguishable from the proton signals of **12a** or **13a** by an approximately 30% smaller integral, were also observed in the ^{1}H NMR spectra of the Mosher esters (Figures S56 and S57). The $\Delta\delta_{S-R}$ values calculated from these resonances were positive for H_2-1 and H-2 and negative for H-8 (Figure 1). These data indicate that the Mosher esters of **4** were composed of a mixture of (3*S*,8*S*)- and (3*R*,8*S*)-isomers in a ca. 3.3:1 ratio. However, it was not possible to determine whether the (3*R*,8*S*)-isomer was a naturally occurring metabolite or an artifact of the isolation or the reaction procedure.

Because *O. elatus* is commonly used for the treatment of inflammation in Korean traditional medicine, the potential anti-inflammatory properties of compounds **1**−**4** were investigated by using RAW264.7 macrophage cells, which can produce NO and PGE_2 upon stimulation with LPS (Gram-negative bacterial lipopolysaccharide). Compounds **1**, **3**, and **4** showed inhibition of NO and PGE_2 production with an IC_{50} range of 1.28 to 3.08 μM (Table S4 and Table 2). Compound **4** showed significant inhibitory effects on NO and PGE_2 production with IC_{50} values of 1.28 and 1.54 μM, respectively, while **4** was cytotoxic to the tested cells with an IC_{50} value of 3.77 μM. However compounds **1**, **2**, and **3** showed no cytotoxicity against the tested cells. Furthermore, both the production of NO and the quantity of PGE_2 secreted decreased to an approximately basal level in RAW264.7 cells exposed to 8 μM of **1** or **3** for 24 h (Table 2 and Table S4).

Experimental Section

General Experimental Procedures. Optical rotations were measured using a 35 polarimeter (PerkinElmer Co., USA). CD spectra were measured on a JASCO J-715 polarimeter (JASCO Co., Japan). UV spectra were recorded on a Lambda 35 UV/vis spectrophotometer (PerkinElmer Co., USA), and FT-IR spectra were recorded on a Bruker IFS-66/S instrument (Bruker Co., Germany). The NMR spectra were obtained on a Varian Unity Plus 500 MHz NMR system (Varian Co., USA). Low-resolution ESIMS data were obtained either on an Agilent 1100 HPLC/MS system (Agilent Co., USA) at Kangnung-Wonju National University or on a Varian 320-MS (Varian Co., USA) mass spectrometer. The HRFABMS and HRCIMS were measured on JMS-AX505WA (JEOL Co., Japan) and JMS-600W (JEOL Co., Japan) mass spectrometers at the National Center for Inter-University Facilities of Seoul National University, Seoul, Korea. The analytical HPLC data were obtained on a Waters analytical HPLC system that included a 1525 binary HPLC pump, a Waters 717 plus autosampler, and a Waters 2996 PDA (Waters Co., USA). Preparative HPLC was performed using a Gilson 321 HPLC system (Gilson Co., France), with a UV detector (254 nm) and either a Luna C_{18}(2) (10 × 250 mm, 10 μm and 21 × 250 mm, 15 μm, Phenomenex Co., USA), a Luna C_8(2) (10 × 250 mm, 10 μm, Phenomenex Co., USA), a Luna silica (10 × 250 mm, 10 μm, Phenomenex Co., USA), or a Waters Delta Pak C_{18} (30 × 300 mm, 15 μm, Waters Co., Tokyo, Japan) column. Open column chromatography was performed using silica gel (particle size 70−230 mesh, Silicycle Co., Canada). TLC was performed on silica gel 60 F_{254} and RP-18 F_{254s} (Merck Co., Germany).

Table 1. NMR Data of Compounds **1** and **2**

	1		**2**	
position	δ_H mult (J (Hz))[a]	δ_C[b]	δ_H mult (J (Hz))[a]	δ_C[b]
1	1.03 t (7.5)	9.3 CH_3	1.04 t (7.5)	9.5 CH_3
2	1.76 m	30.6 CH_2	1.77 m	30.8 CH_2
3	4.40 br dt (6.0, 6.0)	64.0 CH	4.40 dt (6.0, 6.0)	64.3 CH
4		81.0 C		80.7 C
5		68.7 C		69.0 C
6		70.3 C		70.7 C
7		77.4 C		77.8 C
8	4.36 br dd (7.5, 3.5)	60.7 CH	4.53 dd (9.0, 4.5)	65.7 CH
9	3.16 dd (7.5, 4.0)	58.0 CH	3.66 ddd (8.5, 4.5, 3.5)	73.1 CH
10	3.07 br ddd (7.0, 5.5, 4.0)	58.1 CH	3.61 ddd (7.5, 5.0, 3.5)	81.7 CH
11	1.64 m	27.5 CH_2	1.64 m	29.4[c] CH_2
12	1.51 m	26.5 CH_2		25.2 CH_2
13	1.35 m	29.4 CH_2	1.31 m	30.0 CH_2
14	1.29 m	29.2 CH_2	1.31 m	29.3[c] CH_2
15	1.29 m	31.8 CH_2	1.30 m	32.0 CH_2
16	1.29 m	22.6 CH_2	1.31 m	22.9 CH_2
17	0.89 t (7.0)	14.1 CH_3	0.90 t (7.0)	14.3 CH_3
3-OH	2.19 br d (6.0)		1.85 d (6.0)	
8-OH	2.50 br d (3.5)		3.41 d (9.0)	
9-OH			2.68 d (8.5)	
OCH_3			3.44 s	57.5 CH_3

[a] 500 MHz, $CDCl_3$ (7.27 ppm). [b] 125 MHz, $CDCl_3$ (77.0 ppm). [c] Exchangeable.

Table 2. IC_{50} Values of NO, PGE_2, and Cytotoxicity

compound	NO inhibition (IC_{50}, μM)	PGE_2 inhibition (IC_{50}, μM)	cytotoxicity (IC_{50}, μM)
1	1.98 ± 0.28	3.08 ± 0.37	>40
2	>40	>40	>40
3	2.72 ± 0.10	2.99 ± 0.12	>40
4	1.28 ± 0.14	1.54 ± 0.01	3.77

Plant Material. The stems of *O. elatus* were collected in Gangwon Province (Korea) in September 2006. The botanical identification was made by Prof. Kyu Song Lee at Kangnung-Wonju National University. A voucher specimen (KIST NP-2006-10-001) was deposited at the herbarium, KIST Gangneung Institute.

Extraction and Isolation. The air-dried stems (1.0 kg) of *O. elatus* were extracted with CH_2Cl_2 (3 × 10 L) at room temperature. The CH_2Cl_2 extract (25.0 g) was subjected to silica gel (300 g) column chromatography, eluting with a step gradient of *n*-hexane−CH_2Cl_2 (10:1, 5:1, and 2:1) and CH_2Cl_2−MeOH (20:1, 10:1, 5:1, 2:1, and 1:1) to afford eight fractions, R1−R8 (each 1 L). The R3 fraction (2.95 g), which eluted with *n*-hexane−CH_2Cl_2 (2:1), was then fractionated by preparative HPLC using a Delta Pak C_{18} column (300 × 30 mm, 15 μm) and a CH_3CN−H_2O gradient solvent system (60 to 100% for 60 min, 15 mL/min) to obtain nine subfractions, R31−R39. Subfraction R36 (453 mg) was further purified by repeated HPLC using a silica gel column (Luna, silica, 250 × 10 mm, 10 μm) with *n*-hexane−EtOAc (3:1) isocratic solvent (4 mL/min) to afford compound **3** (300 mg). Subfraction R35 (498 mg) was purified by repeated reversed-phase (RP) HPLC using a Luna C_{18} (2) column (250 × 10 mm, 10 μm) and 75% MeOH (4 mL/min) to afford compound **4** (100 mg), as well as two subfractions, R351 and R352. Compound **1** (23 mg) was obtained from subfraction R351 (25 mg) by C_{18} RP HPLC in the same manner as was used to purify compound **4**. Fraction R33 (9.5 mg) was purified by repeated HPLC with a Luna C_{18} (2) column (250 × 10 mm, 10 μm) with 75% MeOH, followed by a Luna silica column (250 × 10 mm, 10 μm) with *n*-hexane−EtOAc (2:1) to afford compound **2** (1.5 mg).

Oploxyne A (1): colorless oil; $[\alpha]^{25}_D$ +123.4 (*c* 0.4, MeOH); UV (MeOH) λ_{max} (log ε) 257 (2.34), 243 (2.55), 231 (2.56) nm; CD (MeOH) λ_{max} ($\Delta\varepsilon$) 202 (−7.28) nm; IR (neat) ν_{max} 3384 (OH), 2253 (C≡C), 2146 (C≡C) cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; HRFABMS *m/z* 301.1775 [M + Na]$^+$ (calcd for $C_{17}H_{26}O_3Na$, 301.1780).

Oploxyne B (2): colorless oil; $[\alpha]^{25}_D$ +11.7 (*c* 0.06, MeOH); UV (MeOH) λ_{max} (log ε) 257 (2.42), 243 (2.60), 231 (2.62) nm; CD (MeOH) λ_{max} ($\Delta\varepsilon$) 202 (−7.64) nm; IR (neat) ν_{max} 3383 (OH), 2257 (C≡C), 2149 (C≡C) cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; HRCIMS *m/z* 311.2222 [M + Na]$^+$ (calcd for $C_{18}H_{31}O_4Na$, 311.2222).

Preparation of (*S*)- and (*R*)-MTPA Esters (5a/b, 6a/b, 8, 9, 10, 11, 12a/b, and 13a/b).[23] Oploxyne A (**1**) (1 mg) was dissolved in 1 mL of pyridine, and DMAP (1 mg) and (*R*)-MTPA-Cl (10 μL) were added. The mixture was stirred for 3 h at room temperature, and then two drops of H_2O were added. The solution was concentrated under reduced pressure, and the residue was subjected to silica gel chromatography (normal phase Sep-Pak (1 g)) using *n*-hexane−EtOAc (10:1) to afford a mixture (0.5 mg) of two chlorinated bis-(*S*)-Mosher esters, **5a** and **5b**. In a similar fashion, a solution of **1** in pyridine was treated with DMAP (1 mg) and (*S*)-MTPA-Cl (10 μL) to afford a mixture (0.5 mg) of two chlorinated bis-(*R*)-Mosher esters, **6a** and **6b**.

In a similar manner, separate treatment of **2** in pyridine with (*R*)-MTPA-Cl and (*S*)-MTPA-Cl yielded tris-(*S*)- (**8**) and tris-(*R*)- (**9**) Mosher esters, respectively. Each of these compounds was purified by reversed-phase HPLC using a Lunar C_8 (2) column (250 × 10 mm, 10 μm) with a CH_3CN−H_2O gradient solvent system (10 to 100% for 30 min, 4 mL/min).

Compound **3** (6.0 mg) was divided into two portions, and each was dissolved in 700 μL of 99.5% pyridine-d_5 in a 5 mm NMR tube. DMAP (1 mg) was added to both NMR tubes. (*R*)-MTPA-Cl (10 μL) and (*S*)-MTPA-Cl (10 μL) were then added into each NMR tube, respectively. After 1 h, the ^1H NMR spectra for (*S*)-Mosher ester **10** and (*R*)-Mosher ester **11** were recorded (see Supporting Information for NMR data).

Compound **4** (3 mg) was divided into two portions, and each was treated with pyridine-d_5, DMAP, (*R*)-MTPA-Cl, and (*S*)-MTPA-Cl in separate 5 mm NMR tubes as above. The ^1H NMR spectra for the (*S*)-Mosher ester **12a/b** and (*R*)-Mosher ester **13a/b** were recorded (see Supporting Information for NMR data).

Bis-(*S*)-MTPA esters (5a and 5b). 5a: ^1H NMR ($CDCl_3$, 500 MHz,) δ 0.89 (3H, t, J = 7.0 Hz, H_3-17), 0.94 (3H, t, J = 7.5 Hz, H_3-1), 1.26 (8H, m, H_2-13−16), 1.36 (2H, m, H_2-12), 1.65 (1H, m, H_2-11a), 1.79 (1H, m, H_2-11b), 1.86 (2H, m, H_2-2), 2.23 (1H, d, J = 9.0 Hz, 9-OH), 3.57−3.63 (6H, s, OCH_3), 3.60 (1H, m,[a] H-10), 3.87 (1H, br dd, J = 9.0, 9.0 Hz, H-9), 5.56 (1H, br t, J = 6.0 Hz, H-3), 5.62 (1H, d, J = 9.0 Hz, H-8), 7.42 (5H, m, aromatic H), 7.53 (3H, m, aromatic H), 7.62 (2H, m, aromatic H). [a]The multiplicity could not be interpreted because of peak overlap. **5b**: ^1H NMR ($CDCl_3$, 500 MHz,) δ 0.89 (3H, t, J = 7.0 Hz, H_3-17), 0.94 (3H, t, J = 7.5 Hz, H_3-1), 1.26 (8H, m, H_2-13−16), 1.36 (2H, m, H_2-12), 1.51 (1H, m, H_2-11a), 1.59 (1H, m, H_2-11b), 1.86 (2H, m, H_2-2), 2.49 (1H, d, J = 6.5 Hz, 8-OH), 3.57−3.63 (6H, s, OCH_3), 4.85 (1H, dd, J = 7.0, 6.5 Hz, H-8), 4.28 (1H, br ddd, J = 8.5, 5.0, 3.0 Hz, H-10), 5.35 (1H, dd, J = 7.0, 3.0 Hz, H-9), 5.56 (1H, br t, J = 6.0 Hz, H_2-3), 7.42 (5H, m, aromatic H), 7.53 (3H, m, aromatic H), 7.62 (2H, m, aromatic H); ESIMS *m/z* 769 [M + Na]$^+$, 771 [M + 2 + Na]$^+$.

Bis-(*R*)-MTPA esters (6a and 6b). 6a: ^1H NMR ($CDCl_3$, 500 MHz,) δ 0.89 (3H, t, J = 7.0 Hz, H_3-17), 1.05 (3H, t, J = 7.5 Hz, H_3-1), 1.27 (8H, m, H_2-13−16), 1.44 (2H, m, H_2-12), 1.79 (1H, m, H_2-11a), 1.89

(1H, m, H_2-11b), 1.91 (2H, m, H_2-2), 2.30 (1H, d, J = 8.5 Hz, 9-OH), 3.55−3.57 (6H, s, OCH_3), 3.95 (1H, ddd, J = 8.5, 8.5, 2.0 Hz, H-9), 4.00 (1H, br ddd, J = 8.5, 5.0, 2.0 Hz, H-10), 5.53 (1H, t, J = 6.5 Hz, H-3), 5.56 (1H, d, J = 8.0 Hz, H-8), 7.43 (5H, m, aromatic H), 7.52 (3H, m, aromatic H), 7.62 (2H, m, aromatic H). **6b**: ^{1}H NMR ($CDCl_3$, 500 MHz,) δ 0.89 (3H, t, J = 7.0 Hz, H_3-17), 1.05 (3H, t, J = 7.5 Hz, H_3-1), 1.27 (8H, m, H_2-13−16), 1.44 (2H, m, H_2-12), 1.68 (1H, m, H_2-11a), 1.71 (1H, m, H_2-11b), 1.91 (2H, m, H-2), 2.33 (1H, d, J = 6.5 Hz, 8-OH), 3.55−3.57 (6H, s, OCH_3), 4.33 (1H, br ddd, J = 8.5, 5.5, 3.0 Hz, H-10), 4.78 (1H, dd, J = 7.0, 6.5 Hz, H-8), 5.38 (1H, dd, J = 7.0, 3.0 Hz, H-9), 5.53 (1H, t, J = 6.5 Hz, H-3), 7.43 (5H, m, aromatic H), 7.52 (3H, m, aromatic H), 7.62 (2H, m, aromatic H); ESIMS m/z 769 [M + Na]$^+$, 771 [M + 2 + Na]$^+$.

Tris-(*S*)-MTPA ester (8): ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.89 (3H, t, J = 7.0 Hz, H_3-17), 0.94 (3H, t, J = 7.5 Hz, H_3-1), 1.26 (8H, m, H_2-13−16), 1.31 (1H, m, H_2-12), 1.40 (2H, m, H_2-11), 1.86 (2H, m, H_2-2), 3.19 (1H, ddd, J = 8.0, 5.0, 5.0 Hz, H-10), 3.51, 3.52, 3.54, and 3.56 (each 3H, s, OCH_3), 5.40 (1H, dd, J = 5.0, 5.0 Hz, H-9), 5.56 (1H, t, J = 6.5 Hz, H-3), 5.91 (1H, d, J = 5.0 Hz, H-8), 7.45−7.63 (15H, m, aromatic H); ESIMS m/z 981 [M + Na]$^+$.

Tris-(*R*)-MTPA ester (9): ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.89 (3H, t, J = 7.0 Hz, H_3-17), 1.04 (3H, t, J = 7.5 Hz, H_3-1), 1.26 (8H, m, H_2-13−16), 1.36 (1H, m, H_2-12), 1.55 (1H, m, H_2-11), 1.90 (2H, m, H_2-2), 3.30, 3.40, 3.54, and 3.55 (each 3H, s, OCH_3), 3.40 (1H, m, H-10), 5.45 (1H, dd, J = 6.0, 4.5 Hz, H-9), 5.52 (1H, t, J = 6.5 Hz, H-3), 5.82 (1H, d, J = 4.5 Hz, H-8), 7.36−7.54 (15H, m, aromatic H); ESIMS m/z 981 [M + Na]$^+$.

Formation of Acetonide 7.[24] Oploxyne B (**2**) (0.3 mg) was dissolved in 2,2-dimethoxypropane (1 mL) and MeOH (0.2 mL), and pyridinium *p*-toluenesulfonate (2 mg) was added. The reaction was allowed to stir for 6 h in an ice bath. The reaction mixture was quenched with 1% aqueous $NaHCO_3$, and the aqueous phase was extracted three times with CH_2Cl_2. The CH_2Cl_2 was removed under reduced pressure, and the residue was purified by silica gel column chromatography using CH_2Cl_2−MeOH (10:1) to provide the acetonide **7** (0.2 mg).

Acetonide 7: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.89 (3H, t, J = 6.5 Hz, H_3-17), 1.04 (3H, t, J = 7.5 Hz, H_3-1), 1.31 (10H, m, H_2-13−17), 1.37 (3H, s, acetonide Me), 1.57 (3H, s, acetonide Me), 1.44 (2H, m, H_2-11), 1.52 (2H, m, H_2-12), 1.78 (2H, m, H_2-2), 1.80 (1H, d, J = 6.0 Hz, 3-OH), 3.50 (1H, br ddd, J = 9.0, 8.5, 1.5 Hz, H-10), 3.51 (3H, s, OCH_3), 4.05 (1H, dd, J = 8.5, 5.5 Hz, H-9), 4.41 (1H, td, J = 6.0, 6.0 Hz, H-3), 4.71 (1H, d, J = 5.5 Hz, H-8); ESIMS m/z 373 [M + Na]$^+$.

Measurement of Nitric Oxide Production.[30,31] NO production was assayed by the Griess reaction, which measures an accumulation of nitrite in the supernatant of spent cell culture media. Briefly, RAW264.7 cells were seeded in a 96-well plate (1×10^5 cells/well) and incubated for 6 h to attach onto the plate. After 6 h, the attached cells were treated with phenol red-free medium containing 1 μg/mL of *E. coli* LPS (strain 055:B5) in the presence or absence of compound **1**−**4** at a final volume of 180 μL/well, and the plate was incubated for 20−24 h. Each compound stock was serially diluted, ranging from 40 to 0.2 μM. The supernatant was collected, mixed with an equal volume (100 μL) of Griess reagent [1% sulfanilamide, 5% H_3PO_4, 0.1% *N*-(1-naphthyl)-ethylenediamine], and incubated at room temperature for 5−10 min. Absorbance was measured in a microplate reader at λ = 540 nm. $NaNO_2$ was used to generate a standard reference curve of nitrite concentration. The control was exposed to phenol red-free medium containing 0.5% (v/v) DMSO. These experiments were done in triplicate wells.

Measurement of PGE_2 Production.[30,31] The PGE_2 production from endogenous arachidonic acid was measured using the PGE_2 Parameter Assay kit (R&D Systems, Minneapolis, MN) according to the manufacturer's protocol.

Acknowledgment. This study was supported by the Korea Institute of Science and Technology institutional program, grant numbers 2Z03100 and 2Z03270. We thank the Seoul National University National Center for Inter-University Research Facilities (NCIRF) for use of the HRMS, and the Center for Scientific Instruments at Kangnung-Wonju National University for use of the LC/MS and the FT-IR spectrometer.

Supporting Information Available: Physical and spectral data sets (1D and 2D NMR, ESIMS, UV/vis, CD, IR, HRMS, etc.) for compounds **1**−**4**, ^{1}H NMR, ^{1}H−^{1}H COSY, and ESIMS data for **5**−**9**, ^{1}H NMR spectra for **10**−**13**, and the previously reported optical rotation values and NMR data for falcarindiol. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Dou, D.-Q.; Hu, X.-Y.; Zhao, Y.-R.; Kang, T.-G.; Liu, F.-Y.; Kuang, H.-X.; Smith, D. C. *Nat. Prod. Res.* **2009**, *23*, 334–342.
(2) Wang, G.-S.; Yang, X.-H.; Xu, J.-D. *Acta. Pharm. Sin.* **2004**, *39*, 354–358.
(3) Qin, Z.; Hu, X. *Zhongcaoyao* **1989**, *20*, 447–448.
(4) Minato, H.; Ishikawa, M. *J. Chem. Soc. (C)* **1967**, 423–427.
(5) Takeda, K.; Minato, H.; Ishikawa, M. *Tetrahedron* **1966**, *22*, 219–225.
(6) Xu, L.; Wu, X. H.; Zheng, G. R.; Cai, J. C. *Chin. Chem. Lett.* **2000**, *11*, 213–216.
(7) Kobaisy, M.; Abramowski, Z.; Lermer, L.; Saxena, G.; Hancock, R. E. W.; Towers, G. H. N.; Doxsee, D.; Stokes, R. W. *J. Nat. Prod.* **1997**, *60*, 1210–1213.
(8) Xu, S.; Liang, H. *Zhongcaoyao* **1998**, *29*, 222–223.
(9) Song, J. T. *Korean Resources Plants*; KeoBuk Co.: Seoul, 1986; p 2524.
(10) Kim, J. H.; Eom, S.; Lee, H. S.; Kim, J. K.; Yu, C. Y.; Kwon, Y. S.; Lee, J. K.; Kim, M. J. *Korean J. Med. Crop Sci.* **2007**, *15*, 112–119.
(11) Garneau, F.-X.; Collin, G.; Gagnon, H.; Jean, F.-I.; Strobl, H.; Pichette, A. *Flavour Fragr. J.* **2006**, *21*, 792–794.
(12) Zhang, H.; Wu, G. *J. Chin. Pharm. Sci.* **1996**, *5*, 112.
(13) Bohlmann, F.; Bornowski, H. *Chem. Ber.* **1961**, *94*, 3189–3192.
(14) Pellati, F.; Calo, S.; Benvenuti, S.; Adinolfi, B.; Nieri, P.; Melegari, M. *Phytochemistry* **2006**, *67*, 1359–1364.
(15) Resch, M.; Heilmann, J.; Steigel, A.; Bauer, R. *Planta Med.* **2001**, *67*, 437–442.
(16) Choi, Y. E.; Ahn, H.; Ryu, J.-H. *Biol. Pharm. Bull.* **2000**, *23*, 884–886.
(17) Zgoda, J. R.; Freyer, A. J.; Killmer, L. B.; Porter, J. R. *J. Nat. Prod.* **2001**, *64*, 1348–1349.
(18) Hudson, J. B.; Graham, E. A.; Chan, G.; Finlayson, A. J.; Towers, G. H. N. *Planta Med.* **1986**, 453–457.
(19) Hu, C. Q.; Chang, J. J.; Lee, K. H. *J. Nat. Prod.* **1990**, *53*, 932–935.
(20) Appendino, G.; Pollastro, F.; Verotta, L.; Ballero, M.; Romano, A.; Wyrembek, P.; Szczuraszek, K.; Mozrzymas, J. W.; Taglialatela-Scafati, O. *J. Nat. Prod.* **2009**, *72*, 962–965.
(21) Fujimoto, Y.; Satoh, M.; Takeuchi, N.; Kirisawa, M. *Chem. Pharm. Bull.* **1991**, *39*, 521–523.
(22) Takaishi, Y.; Okuyama, T.; Masuda, A.; Nakano, K.; Murakami, K.; Tomimatsu, T. *Phytochemistry* **1990**, *29*, 3849–3852.
(23) Ohtani, I.; Kusumi, T.; Kashman, Y.; Kakisawa, H. *J. Am. Chem. Soc.* **1991**, *113*, 4092–4096.
(24) Rychnovsky, S. D.; Rogers, B. N.; Richardson, T. I. *Acc. Chem. Res.* **1998**, *31*, 9–17.
(25) (a) Takaishi, Y.; Okuyama, T.; Nakano, K.; Murakami, K.; Tomimatsu, T. *Phytochemistry* **1991**, *30*, 2321–2324. (b) El Ashry, E. S. H. *J. Chem. Soc., Chem. Commun.* **1986**, 1024–1026.
(26) Matsumori, N.; Kaneno, D.; Murata, M.; Nakamura, H.; Tachibana, K. *J. Org. Chem.* **1999**, *64*, 866–876.
(27) Freire, F.; Seco, J. M.; Quiñoa, E.; Riguera, R. *J. Org. Chem.* **2005**, *70*, 3778–3790.
(28) (a) Bernart, M. W.; Cardellina, J. H., II; Balaschak, M. S.; Alexander, M. R.; Shoemaker, R. H.; Boyd, M. R. *J. Nat. Prod.* **2005**, *59*, 748–753. (b) Yamazoe, S.; Hasegawa, K.; Suenaga, K.; Shigemori, H. *Nat. Prod. Commun.* **2006**, *1*, 87–94. (c) Jeong, G.-S.; Kwon, O.-K.; Park, B.-Y.; Oh, S.-R.; Ahn, K.-S.; Chang, M.-J.; Oh, W. K.; Kim, J.-C.; Min, B.-S.; Kim, Y.-C.; Lee, H.-K. *Biol. Pharm. Bull.* **2007**, *30*, 1340–1343. (d) Dang, N. H.; Zhang, X. F.; Zheng, M. S.; Son, K. H.; Chang, H. W.; Kim, H. P.; Bae, K. H.; Kang, S. S. *Arch. Pharm. Res.* **2005**, *28*, 28–33. (e) Czepa, A.; Hofmann, T. *J. Agric. Food. Chem.* **2003**, *51*, 3865–3873.
(29) (a) Lemmich, E. *Phytochemistry* **1981**, *20*, 1419–1420. (b) Zheng, G.; Lu, W.; Cai, J. *J. Nat. Prod.* **1999**, *62*, 626–628. (c) Matsuura, H.; Saxena, G.; Farmer, S. W.; Hancock, R. E.; Towers, G. H. *Planta Med.* **1996**, *62*, 256–259. (d) Ratnayaka, A. S.; Hemscheidt, T. *Org. Lett.* **2002**, *4*, 4667–4669. (e) Lechner, D.; Stavri, M.; Oluwatuyi, M.; Pereda-Miranda, R.; Gibbons, S. *Phytochemistry* **2004**, *65*, 331–335. (f) Sabitha, G.; Bhaskar, V.; Reddy, C. S.; Yadav, J. S. *Synthesis* **2008**, 115–121. (g) Seger, C.; Godejohann, M.; Spraul, M.; Stuppner, H.; Hadaacek, F. *J. Chromatogr. A* **2006**, *1136*, 82–88.
(30) Gomes, A.; Fernandes, E.; Silva, A. M. S.; Pinto, D. C. G. A.; Santos, C. M. M.; Cavaleiro, J. A. S.; Lima, J. L. F. C. *Biochem. Pharmacol.* **2009**, *78*, 171–177.
(31) Cho, W.; Park, S.-J.; Shin, J.-S.; Noh, Y.-S.; Cho, E.-J.; Nam, J.-H.; Lee, K.-T. *Biomol. Ther.* **2008**, *16*, 385–393.

NP900628J