http://dx.doi.org/10.5012/bkcs.2013.34.5.1407

NF-κB Inhibition and PPAR Activation by Phenolic Compounds from *Hypericum perforatum* L. Adventitious Root

Wei Li,[†] Yan Ding,[†,§] Tran Hong Quang,[†] Nguyen Thi Thanh Ngan,[†] Ya Nan Sun,[†] Xi Tao Yan,[†] Seo Young Yang,[†] Chun Whan Choi,[†] Eun Jung Lee,[‡] Kee Yoeup Paek,[‡] and Young Ho Kim[†,*]

[†]*College of Pharmacy, Chungnam National University, Daejeon 305-764, Korea.* [*]*E-mail: yhk@cnu.ac.kr*
[‡]*Research Center for the Development of Advanced Horticultural Technology, Chungbuk National University, Cheongju 361-763, Korea*
[§]*School of Food Science and Technology, Dalian Polytechnic University, Dalian, Liaoning, 116034, P. R. China*
Received December 27, 2012, Accepted February 9, 2013

A new compound, perforaphenonoside A (**1**), along with 11 known compounds (**2-12**) were isolated from a methanol extract of adventitious roots of *Hypericum perforatum*. Their chemical structures were elucidated using chemical and physical methods as well as comparison of NMR and mass spectral data with previously reported data. Their inhibition of NF-κB and activation of PPAR was measured in HepG2 cells using a luciferase reporter system. Among the compounds **3**, **6**, **7** and **12** inhibited NF-κB activation stimulated by TNFα in a dose-dependent manner, with IC_{50} values ranging from 0.85 to 8.10 μM. Moreover, compounds **1-3**, **7**, **11** and **12** activated the transcriptional activity of PPARs in a dose-dependent manner, with EC_{50} values ranging from 7.3 to 58.7 μM. The transactivational effects of compounds **1-3**, **7**, **11** and **12** were evaluated on three individual PPAR subtypes. Among them, compound **2** activated PPARα transcriptional activity, with 153.97% stimulation at 10 μM, while compounds **1**, **2** and **11** exhibited transcriptional activity of PPARγ, with stimulation from 124.76% to 126.91% at 10 μM.

Key Words : *Hypericum perforatum*, Hypericaceae, NF-κB-luciferase assay, PPRE-luciferase assay, PPAR transactivational activity

Introduction

Hypericum perforatum L., commonly known as St. John's wort (SJW), is a spontaneous perennial herbaceous plant, belonging to the family Hypericaceae found in Europe, Asia, Northern Africa, and North America.[1] It is used as a phytotherapeutic agent to treat moderate forms of depression,[2] and has gained international popularity for its treatment of depression and as a dietary supplement in phytomedicine.[3] It also has other broad pharmacological activities, such as antiviral and anti-inflammatory properties.[4,5] Presently, field-grown plant material is generally used, but the quality of these products may be greatly affected by various environmental conditions, fungi, bacteria, viruses, and insects, which can result in heavy loss of yield and alter the medicinal content of plants.[6] Therefore, cell or organ cultures have emerged as valuable routes for biosynthesizing phytochemicals.[7] The main constituents of *H. perforatum* are flavonoids, naphthodianthrones, phloroglucinols, and xanthones.[8] Hyperforin and adhyperforin have been shown to contribute to the antidepressant activity of *H. perforatum* by inhibiting the re-uptake of a number of neurotransmitters.[9,10] Xanthones are one of the main constituents of *H. perforatum*, they have a wide range of biological and pharmacological properties, such as monoamine oxidase inhibition, and antioxidant, antimicrobial, antifungal, cytotoxic, and hepatoprotective activities.[11,12] Although, *H. perforatum* has been reported in a wide variety of metabolites, few chemical investigations of the adventitious roots of *H. perforatum* have been reported. In this report, a new compound (**1**) and 11 known compounds (**2-12**) were isolated from a methanol extract of adventitious roots of *H. perforatum*.

Nuclear factor kappa B (NF-κB) represents a family of Rel domain-containing proteins including RelA, RelB, c-Rel, NF-κB1, and NF-κB2. Activation of NF-κB has been linked to multiple pathophysiological conditions such as cancer, arthritis, asthma, inflammatory bowel disease, and other inflammatory conditions.[13] NF-κB activation by various stimuli including the inflammatory cytokines such as tumor necrosis factor alpha (TNF-α) and interleukin-1 (IL-1), T-cell activation signals, growth factors, and stress inducers cause transcription at κB sites that are involved in a number of diseases, such as inflammatory disorders and cancer.[13,14] In the present study, the effects of compounds **1-12** on TNFα-induced NF-κB transcriptional activity in human hepatocarcinoma (HepG2) cells were evaluated using an NF-κB-luciferase assay.

Peroxisome proliferator-activated receptor (PPAR) is a member of the nuclear receptor superfamily of ligand-dependent transcription factors, and is predominantly expressed in adipose tissue, adrenal glands, and the spleen.[15,16] Three isoforms, PPARα, PPARγ, and PPARβ(δ) have been identified. PPARs regulate the expression of genes involved in the regulation of glucose, lipid, and cholesterol metabolism by binding to specific peroxisome proliferator response elements (PPREs) in the enhancer sites of regulated genes.[17-20]

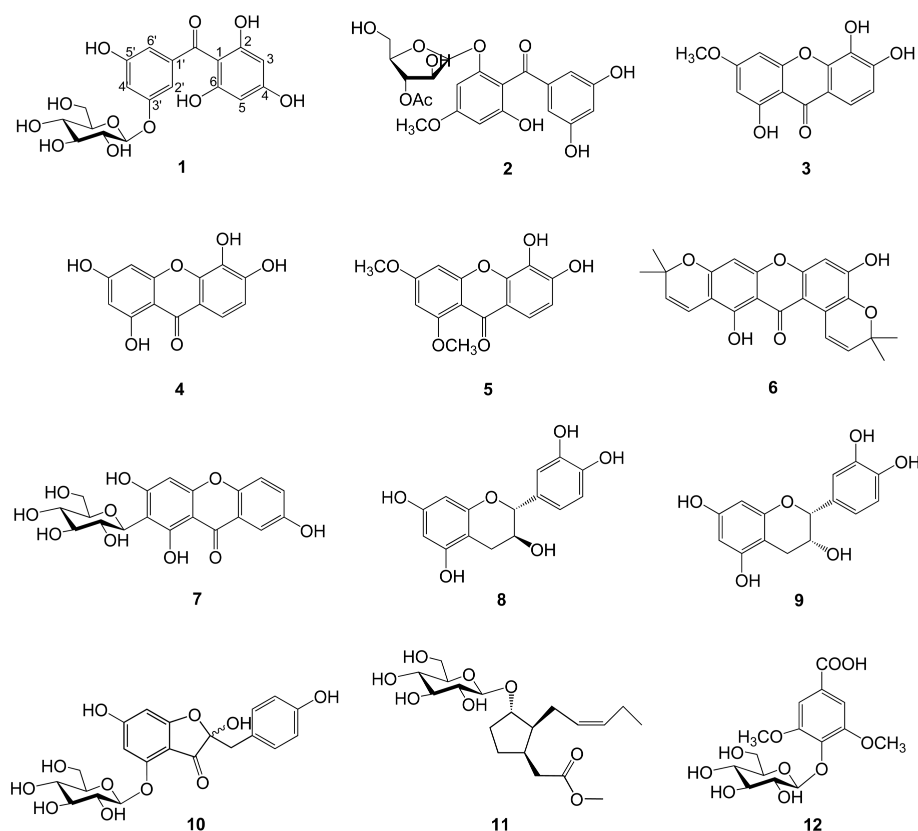

Figure 1. Structure of compounds **1-12** from *H. perforatum*.

Accordingly, compounds that modulate the PPARs functions are attractive for the treatment of type 2 diabetes, obesity, metabolic syndromes, inflammation, and cardiovascular disease.[21]

A MeOH extract (460.0 g) of *H. perforatum* was suspended in water and partitioned with EtOAc and *n*-BuOH. These fractions were subjected to various separation procedures and 12 compounds were isolated (Figure 1). Structures of known compounds (**2**-**12**) were elucidated by comparing spectroscopic data to published values and identified as acetylannulatophenonoside (**2**),[22] 1,5,6-trihydroxy-3-methoxyxanthone (**3**),[23] 1,3,5,6-tetrahydroxyxanthone (**4**),[24] ferrxanthone (**5**),[25] brasilixanthone B (**6**),[26] neolancerin (**7**),[27] (+)-catechin (**8**),[28] (−)-epicatechin (**9**),[29] hovetrichoside C (**10**),[30] methyl 3-*O*-β-D-glucopyranosylcucurbate (**11**),[31] and glucosyringic acid (**12**).[32] Of these, compounds **2**, **5**-**7**, **10**, and **11** were isolated from this plant for the first time. In addition, these compounds were screened for their *anti*-inflammatory effects. Inhibitory effects of the compounds on NF-κB activation were also evaluated, as well as their trans-activational activity against PPRE and members of the PPAR family (PPARα, PPARγ, and PPARβ/δ).

Compound **1** was isolated as pale yellow needles. Its molecular formula was established as $C_{19}H_{20}O_{11}$ by a pseudo-molecular ion peak of high-resolution electrospray ionization mass spectrometry (HR-ESI-MS) at *m/z* 459.0665 $[M+Cl]^-$ (calcd for $C_{19}H_{20}ClO_{11}$: 459.0694). The infrared (IR) absorption bands at 3315 and 1632 cm^{-1} were consistent with the presence of a hydroxyl group and a carbonyl group, respectively. The ^1H- and ^{13}C-NMR spectra (Table 1)

Table 1. The NMR spectroscopic data of compound **1**

Position	δ_C [a,b]	δ_H [a,c] (*J* in Hz)
1	104.8	
2	162.1	
3	94.5	5.84 (1H, br s)
4	164.3	
5	94.5	5.84 (1H, br s)
6	162.1	
1'	143.2	
2'	109.0	6.70 (1H, t, 2.0)
3'	158.2	
4'	106.7	6.67 (1H, t, 2.0)
5'	157.7	
6'	107.8	6.82 (1H, t, 2.0)
1''	101.0	4.87 (1H, d, 7.4)
2''	73.5	3.38 (1H, m)
3''	76.6	3.43 (1H, m)
4''	69.8	3.43 (1H, m)
5''	76.7	3.38 (1H, m)
6''	61.0	3.70 (1H, dd, 4.8, 12.0)
		3.85 (1H, dd, 2.0, 12.0)
C=O	198.6	

Assignments were done by HMQC, HMBC and ^1H-^1H COSY experiments; [a]Measured in methanol-d_4. [b]150 MHz. [c]600 MHz

suggested that compound **1** related to a benzophenone derivative. The ^{1}H-NMR spectrum showed a singlet at δ_H 5.84, belonging to the H-3 and H-5 of an A-ring, and three triplet signals at δ_H 6.67, 6.70 and 6.82, belonging to H-4', H-2' and H-6' of a phloroglucinol ring (B-ring) with J = 2.0 Hz, suggesting the meta position. The ^{13}C-NMR spectrum showed a characteristic carbonyl group at δ_C 198.6; δ_C 94.5 (C-3, C-5), 104.8 (C-1), 162.1 (C-2, C-6) and 164.3 (C-4) at A-ring; δ_C 106.7 (C-4'), 107.8 (C-6'), 109.0 (C-2'), 143.2 (C-1'), 157.7 (C-5') and 158.2 (C-3') at a B-ring (Table 1). Both ^{1}H- and ^{13}C-NMR signal patterns suggested that compound **1** is a 2,3',4,5',6-pentahydroxybenzophenone derivative.[33] ^{1}H- and ^{13}C-NMR also showed D-glucopyranosyl group signals at δ_C 61.0 (C-6"), 69.8 (C-4"), 73.5 (C-2"), 76.6 (C-3"), 76.7 (C-5"), and 101.0 (C-1"); δ_H 3.38 (H-2", H-5"), 3.43 (H-3", H-4"), 3.70, 3.85 (H-6"), and 4.87 (H-1"), and the relatively large coupling constant (J = 7.4 Hz) of the doublet signal at H-1" suggested its β-configuration. D-Glucopyranose was analyzed by gas chromatography. The retention time of the monosaccharide derivative was t_R 14.11 min, which was confirmed by comparison with those of authentic standards. The heteronuclear multiple bond correlation (HMBC) spectrum revealed a key correlation signal between 4.87 (H-1") and 158.2 (C-3') suggesting that the D-glucopyranosyl group was attached to C-3' of a B-ring (Figure 2). Thus, compound **1** was identified as 2,4,5',6-tetrahydroxybenzophenone-3'-*O*-β-D-glucopyranoside, and termed perforaphenonoside A.

To investigate the cellular toxicity of the 12 isolated compounds, various concentrations of the compounds were applied to HepG2 cells for 24 h, after which cell viability was measured by the MTT [3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide] colorimetric method of the compounds displayed any cellular toxicity at 10.0 μM (data not shown). Therefore, the compounds were used in subsequent experiments at alternate concentrations (< 10 μM). The results demonstrated the inhibitory effects of the

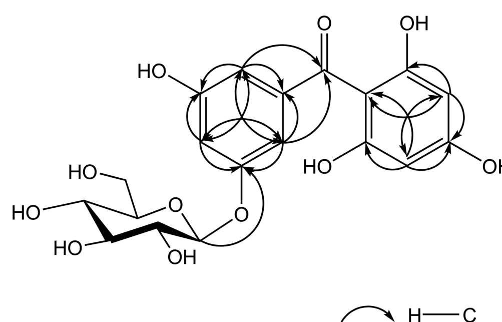

Figure 2. HMBC correlations of compound **1**.

tested compounds on NF-κB transcriptional activation in HepG2 cells stimulated with TNFα (Figure 3). Compounds **3**, **6**, **7** and **12** exhibited a significant effect on the inhibition of NF-κB activation in a dose-dependent manner, with IC_{50} values from 0.85 to 8.10 μM (Table 2). Compound **7** was the most effective and was more potent than the positive control, sulfasalazine (IC_{50} = 0.9 μM). However, other compounds were inactive in this system. Next, we evaluated the effects of compounds **1-12** on PPAR activity using a nuclear transcription PPRE cell-reporter system. The PPAR-responsive luciferase reporter construct, used carries a copy of the firefly luciferase gene under the control of a minimal CMV pro-

Table 2. Inhibitory effects of compounds **1-12** on the TNFα-induced NF-κB transcriptional activity

Compound	IC_{50} (μM)
3	5.50 ± 2.62
6	8.10 ± 0.35
7	0.85 ± 0.07
12	0.93 ± 0.18
Sufasalazine	0.9 ± 0.1

The values are mean ± SD (n = 3). Compounds **1**, **2**, **4**, **5**, and **8-11** were inactive at tested concentrations (IC_{50} >10 μM).

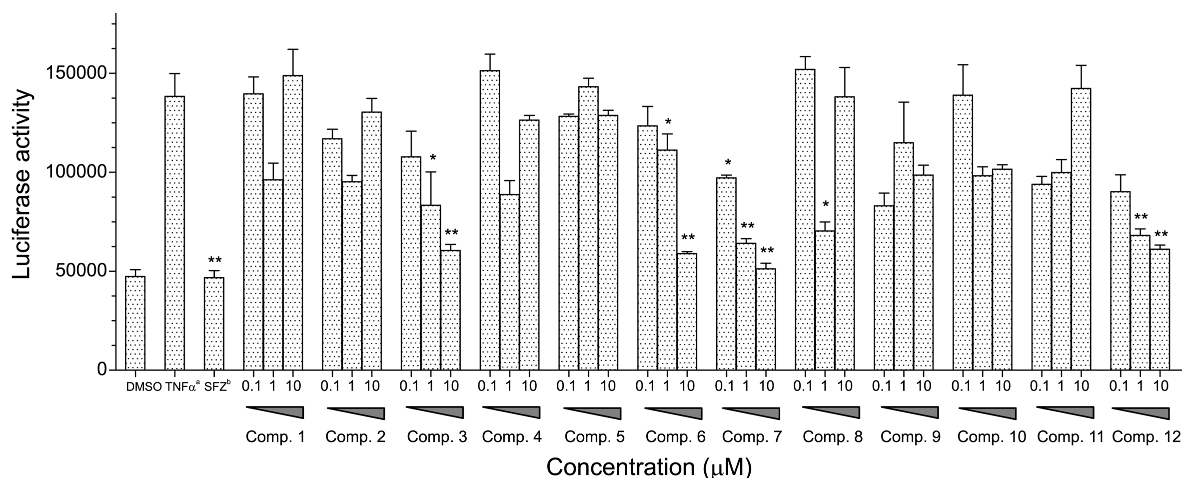

Figure 3. Effects of compounds **1-12** on the TNFα-induced NF-κB luciferase reporter activity in HepG2 cells. The values are means ± SDs (n = 3). [a]Stimulated with TNFα. [b]Stimulated with TNFα in the presence of **1-12** (0.1, 1, and 10 μM) and sulfasalazine. SFZ: sulfasalazine, positive control (10 μM). Statistical significance is indicated as *($p < 0.05$) and **($p < 0.01$) as determined by Dunnett's multiple comparison test.

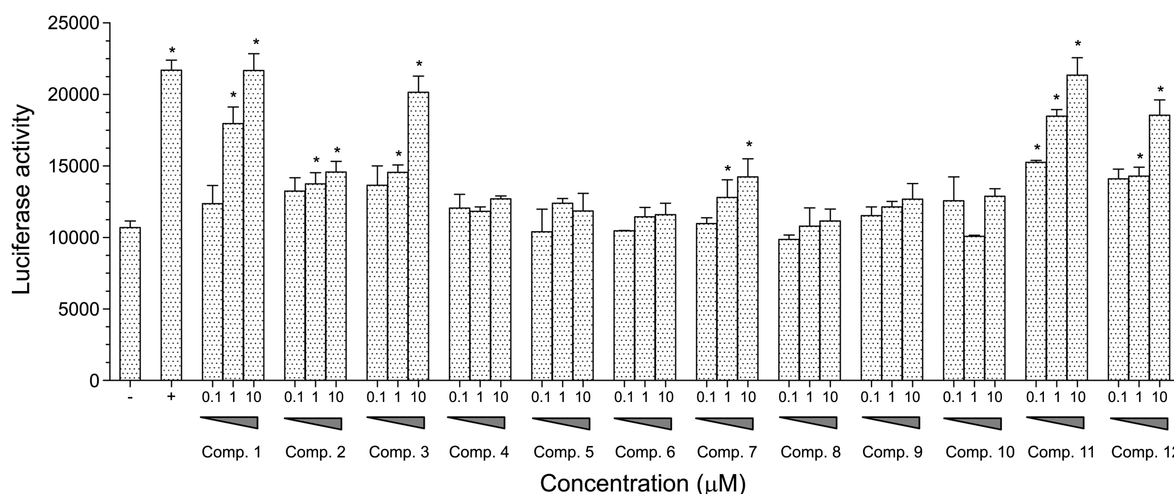

Figure 4. PPARs transactivational activity of compounds **1-12** in HepG2 cells. (–) Vehicle group; (+) positive control (1 μM): benzafibrate. All values represent the means ± S.E.M. (n = 3). $P < 0.05$ versus control.

Table 3. PPARs transactivational activities of compounds **1-12**

Compound	EC_{50} (μM)
1	7.9 ± 0.8
2	58.7 ± 7.2
3	17.9 ± 1.8
4	> 60[a]
5	> 60
6	> 60
7	57.8 ± 6.9
8	> 60
9	> 60
10	> 60
11	7.3 ± 0.7
12	27.0 ± 1.9
Sufasalazine	1.05 ± 0.15

EC_{50}: the concentration of a tested compound that gave 50% of the maximal reporter activity. [a]A compound was considered inactive with EC50 > 60 μM. The values are mean ± SD (n = 3).

moter and tandem repeats of a PPRE sequence. Activated PPAR binds to the PPRE and activates transcription of the luciferase reporter gene. Sulfasalazine was used as a positive control. HepG2 cells were co-transfected with the PPRE luciferase reporter and PPAR expression plasmids (Figure 4). Compounds **1** and **11** significantly activated the transcriptional activity of PPARs in a dose-dependent manner, with EC_{50} values of 7.9 and 7.3 μM, respectively. Compounds **2**, **3**, **7** and **12** displayed moderate activity, with EC_{50} values ranging from 17.9 to 58.7 μM (Table 3), whereas compounds **4-6** and **8-10** were not active at the tested concentrations. To determine how the compounds influence the response to inflammatory stimuli, the PPAR transactivational effects of the isolated compounds were further examined on individual PPAR subtypes, including PPARα, PPARγ, and PPARβ(δ) (Figures 5-7). Among the compounds tested, compound **2** exhibited dose-dependent PPARα transactivational activity, with a stimulation of 153.97% while compounds **1**, **2** and **11** moderately activated PPARγ transcriptional activity, with stimulations from 124.76% to 126.91% (Table 4). However,

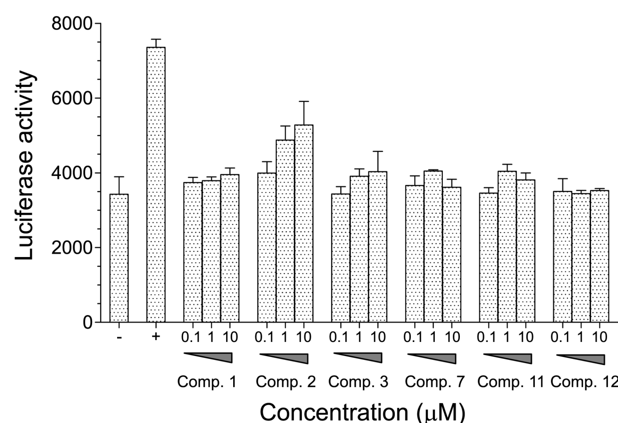

Figure 5. PPARα transactivational activity of compounds Compounds **1-3**, **7**, **11** and **12** in HepG2 cells. (–) Vehicle group; (+) positive control (1 μM): ciprofibrate. All values represent the means ± S.E.M. (n = 3). $P < 0.05$ versus control.

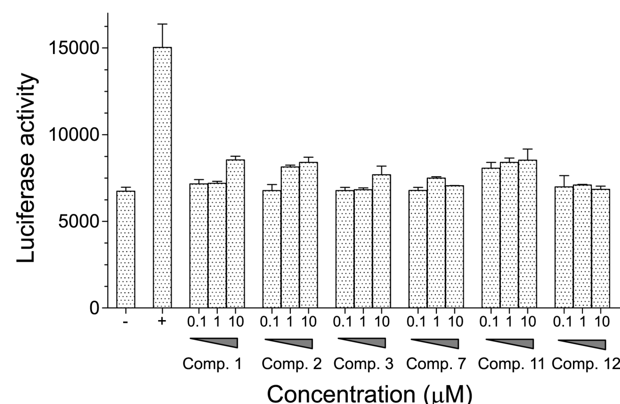

Figure 6. PPARγ transactivational activity of compounds Compounds **1-3**, **7**, **11** and **12** in HepG2 cells. (–) Vehicle group; (+) positive control (1 μM): troglitazone. All values represent the means ± S.E.M. (n = 3). $P < 0.05$ versus control.

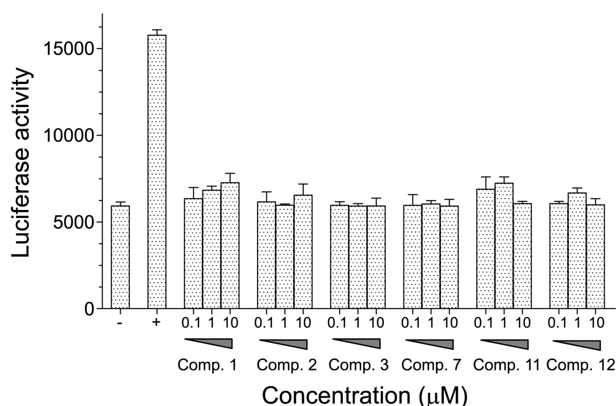

Figure 7. PPARβ(δ) transactivational activity of compounds Compounds **1**-**3**, **7**, **11** and **12** in HepG2 cells. (−) Vehicle group; (+) positive control (1 μM): L-165041. All values represent the means ± S.E.M. (n = 3). $P < 0.05$ versus control.

compounds **3**, **7** and **12** did not activate any PPAR subtypes. In addition, compounds **1** and **11** significantly activated the transcriptional activity of PPARs, but only moderately activated PPARγ transcriptional activity. Therefore, it appears that compounds **1** and **11** may influence another subtype of PPARs. As previous work, NF-κB inhibitory of xanthone derivatives was reported.[34] However, to the best of our knowledge, this is the first report of PPAR activation effects of the constituents from adventitious roots of *H. perforatum.* In conclusion, isolated compounds from root cultures of *H. perforatum* exhibited significant *anti*-inflammatory effects. These results provide scientific support for the use of *H. perforatum* root cultures in the prevention of inflammatory diseases.

Experimental

General Procedures. Melting points were determined using an Electrothermal IA-9200 system. Optical rotations were determined using a Jasco DIP-370 automatic polarimeter. The FT-IR spectra were measured using a Jasco Report-100 infrared spectrometer. GC was carried out on a Shimadzu-2010 spectrometer: detector, FID; detection temperature, 300 °C; column, SPB-1 (0.25 mm i.d. × 30 m); column temperature, 230 °C; carrier gas, He (2 mL/min) injection temperature, 250 °C; injection volume, 0.5 μL; The NMR spectra were recorded using a JEOL ECA 600 spectrometer (^{1}H, 600 MHz; ^{13}C, 125 MHz), EI-MS mass spectra were obtained using A Hewlett Packard HP 5985B spectrometer, and High-resolution electrospray ionization mass spectra (HR-ESI-MS) were obtained using an Agilent 6530 Accurate-Mass Q-TOF LC/MS system. Column chromatography was performed using a silica gel (Kieselgel 60, 70-230, and 230-400 mesh, Merck, Darmstadt, Germany), YMC RP-18 resins, and thin layer chromatography (TLC) was performed using pre-coated silica-gel 60 F_{254} and RP-18 $F_{254}S$ plates (both 0.25 mm, Merck, Darmstadt, Germany).

Plant Material. Adventitious roots (2.0 kg) of *H. perforatum* was kindly supplied from Research Center for the Development of Advanced Horticultural Technology, Chungbuk National University, Korea in 2010.[35] A voucher specimen (CNU11101) was deposited at the Herbarium of College

Table 4. PPARα, γ, and β(δ) transactivational activities of compounds **1-3**, **7**, **11** and **12**

Compound	Concentration (μM)	Stimulation (%)		
		Gal4/PPARα-LBD	Gal4/PPAR γ-LBD	Gal4/PPAR β(δ)-LBD
1	0.1	109.06 ± 1.25	106.31 ± 2.96	107.12 ± 4.25
	1	110.59 ± 1.38	106.89 ± 2.12	115.23 ± 1.68
	10	115.20 ± 3.84	126.91 ± 1.23	122.52 ± 3.30
2	0.1	116.44 ± 2.33	100.57 ± 1.11	103.94 ± 4.73
	1	142.13 ± 2.84	120.86 ± 2.32	100.71 ± 2.35
	10	153.97 ± 1.92	124.76 ± 3.79	110.43 ± 1.64
3	0.1	100.16 ± 1.25	100.62 ± 2.48	100.59 ± 1.50
	1	114.01 ± 1.43	101.31 ± 1.17	99.75 ± 1.38
	10	117.59 ± 2.35	114.22 ± 2.99	99.88 ± 1.02
7	0.1	106.68 ± 1.78	100.72 ± 1.39	100.47 ± 1.39
	1	118.13 ± 3.28	111.31 ± 3.79	101.86 ± 3.79
	10	105.38 ± 1.32	104.70 ± 1.35	99.74 ± 1.35
11	0.1	100.79 ± 2.02	119.78 ± 1.19	111.42 ± 2.58
	1	117.79 ± 1.47	124.83 ± 1.26	116.13 ± 1.09
	10	111.17 ± 1.39	126.66 ± 1.93	122.06 ± 1.25
12	0.1	102.20 ± 1.46	103.77 ± 2.53	102.28 ± 2.53
	1	100.42 ± 2.51	105.33 ± 0.89	112.53 ± 0.89
	10	102.77 ± 1.28	101.66 ± 1.27	101.05 ± 1.27
Ciprofibrate	1	214.57 ± 1.57		
Troglitazone	1		223.27 ± 2.33	
L-165041	1			266.04 ± 3.01

of Pharmacy, Chungnam National University, Korea.

Extraction and Isolation. Adventitious roots of *H. perforatum* (1.8 kg) were extracted with MeOH under reflux for 5 h (3 L × 3 times) to yield 460 g of extract. This extract was suspended in water and partitioned with EtOAc to yield 70.0 g EtOAc extract and 390.0 g water extract. The water extract was partitioned with *n*-BuOH to yield 112.0 g *n*-BuOH extract. The EtOAc extract was subjected to silica gel (5 × 30 cm) column chromatography with a gradient of CH_2Cl_2–MeOH (1:0 → 50:1 → 20:1 → 10:1 → 1:1; 1 L for each step) to give 5 fractions (1A–1E). Fraction 1A was separated using YMC column (2 × 80 cm) with a MeOH–H_2O (1:4) elution solvent to yield compound **2** (22.0 mg). Fraction 1B was separated using YMC column (1 × 80 cm) with a MeOH–H_2O (1:3.5) elution solvent to yield compounds **8** (30.0 mg) and **9** (550.0 mg). Fraction 1C was separated using YMC column (2 × 80 cm) with a MeOH–H_2O (0.4:1 → 1.5:1) elution solvent to yield compounds **3** (52.0 mg), and **4** (70.0 mg). The *n*-BuOH extract was subjected to silica gel column chromatography with a $CHCl_3$–MeOH–H_2O (10:1:0.1 → 7:1:0.1 → 4:1:0.1 → 2:1:0.1 → 1:1:0.2; 2 L for each step) elution solvent to give 5 fractions (2A–2E). Fraction 2B was separated using YMC column (2 × 80 cm) with acetone–H_2O (0.4:1 → 1:1) elution solvent to yield compounds **10** (28.0 mg), **11** (11.0 mg) and **12** (18.0 mg). Fraction 2C was separated using YMC column (1 × 80 cm) with acetone–H_2O (0.3:1 → 0.5:1 → 1:1) elution solvent to yield compounds **5** (105.0 mg) and **6** (24.0 mg). Fraction 2D was separated using YMC column (1 × 80 cm) with a MeOH–H_2O (0.38:1) elution solvent to give compound **7** (170.0 mg). Fraction 2E was separated using a silica gel column chromatography with a $CHCl_3$–MeOH (8:1) elution solvent to yield compound **1** (12.0 mg).

Perforaphenonoside A (1): Yellow amorphous powder; $[\alpha]_D^{28}$ = −133.0 (*c* 0.8, MeOH); IR (KBr): ν_{max} 3315, 2359, 2341, 1632, 1598, 1443, 1391, 1334, 1168, 1131, 1069, 1019, 823, 668 cm^{-1}; HR-ESI-MS: *m/z* 459.0665 $[M+Cl]^-$ (calcd for $C_{19}H_{20}ClO_{11}$: 459.0694). ^1H (methanol-d_4, 600 MHz) and ^{13}C NMR data (methanol-d_4, 150 MHz), see Table 1.

General Acid Hydrolysis. A solution of compound **1** (5 mg) in 3 mL 10% HCl (dioxane-H_2O, 1:1) was heated at 90 °C under reflux for 3 h. The residue was partitioned between EtOAc and H_2O to yield aglycone and sugar, respectively. The aqueous layer was evaporated until dry to yield a residue. The residue was dissolved in anhydrous pyridine (200 μL) and mixed with a pyridine solution of 0.1 M L-cysteine methyl ester hydrochloride (200 μL). After warming to 60 °C for 1 h, trimethylsilylimidazole solution was added, and the reaction solution was warmed to 60 °C for 1 h. The mixture was evaporated in *vacuo* to yield a dry product, that was partitioned between EtOAc and H_2O. The water layer was filtered and analyzed by gas chromatography. Retention time of the monosaccharide derivative, D-glucopyranose (t_R, 14.11 min), was confirmed by comparison with those of authentic standards.

Cell Culture and Reagents. Human hepatocarcinoma HepG2 cells were maintained in Dulbecco's modified Eagles' medium (DMEM) (Invitrogen, Carlsbad, CA) containing 10% heat-inactivated fetal bovine serum (FBS), 100 units/mL penicillin, and 10 μg/mL streptomycin at 37 °C and 5% CO_2.

NF-κB-Luciferase Assay. The luciferase vector was first transfected into HepG2 cells. After a limited amount of time, the cells were lysed, and luciferin, the substrate of luciferase, was introduced into the cellular extract along with Mg^{2+} and an excess of ATP. Under these conditions, luciferase enzymes expressed by the reporter vector could catalyze the oxidative carboxylation of luciferin. Cells were seeded at 1.5×10^5 cells per well in 12-well plates and grown for 24 h. All cells were transfected using WelFect M Gold (WelGENE Inc.), as guided by the manufacturer. The luciferase activity was assayed using an LB 953 Autolumat (EG&G Berthold, Nashua, NH).[36] The transfected HepG2 cells were pretreated for 1 h with either vehicle (DMSO) and compounds, followed by 1 h of treatment with 10 ng/mL TNFα. Unstimulated HepG2 cells were used as a negative control (−). Cells were then harvested, and the luciferase activity was assayed.

PPRE-Luciferase Assay. Human hepatoma cells (HepG2) were seeded at 1.5×10^5 cells per well in 12-well plates and grown for 24 h before transfection. An optimized amount of DNA plasmid (0.5 μg of PPRE-Luc and 0.2 μg of PPAR-inpCMV) was diluted in 100 μL of DMEM. All cells were transfected with the plasmid mixture using WelFect M Gold (WelGENE Inc.) as described by the manufacturer. After 30 min of incubation at room temperature, the DNA plasmid solution (100 μL) was introduced and mixed gently with cells. After 24 h of transfection, the medium was changed to TOM (Transfection Optimized Medium, Invitrogen) containing 0.1 mM NEAA, 0.5% charcoal-stripped FBS, and the individual compounds (test group), dimethyl sulfoxide (vehicle group), or benzafibrate (positive control group). The cells were then cultured for 20 h. Next, the cells were washed with PBS and harvested with 1× passive lysis buffer (200 μL). The intensity of emitted luminescence was determined using an LB 953 Autolumat (EG&G Berthold, Bad Wildbad, Germany).[17,18]

PPAR Subtype Specific Transactivational Assay. Human hepatoma cells (HepG2) were seeded at 1.5×10^5 cells per well in 12-well plates and grown for 24 h before transfection. Cells were transfected separately with one pGal4-PPAR subfamily vector [pFAGal4-PPARα-LBD, pFA-Gal4-PPARγ-LBD, or pFA-Gal4-PPARβ(δ)-LBD expression plasmids], together with pFR-Luc using the WelFect M Gold transfection reagent (WelGENE Inc.), as described by the manufacturer. After 24 h of transfection, the medium was changed to TOM (Invitrogen) containing 0.1 mM NEAA, 0.5% charcoal-stripped FBS, and each compound (test group), dimethyl sulfoxide (vehicle group), ciprofibrate (positive control group for PPARα), troglitazone (positive control group for PPARγ), or L-165041 [positive control group for PPARβ(δ)]. The cells were then cultured for 20 h, after which the cells were washed with PBS and harvested with 1× passive lysis buffer (200 μL). The intensity of emitted luminescence was determined using a Centro LB 960 micro-

plate luminometer (EG&G Berthold) by measuring light emission for 5 s.[37]

Statistical Analysis. All data represent the mean ± standard deviation (SD) of at least three independent experiments performed in triplicate. Statistical significance is indicated as *(p <0.05) as determined by one-way analysis of variance followed by Dunnett's multiple comparison test.

Supporting Information. IR, HR-ESI-MS, ^{1}H, ^{13}C NMR, HMQC, HMBC and COSY spectrum of compound **1**, ^{1}H and ^{13}C NMR data of compounds **2-12** are available as Supporting Information.

Acknowledgments. This study was supported by the Priority Research Center Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2009-0093815), Republic of Korea.

References

1. Robson, N. K. B. *Cambridge University Press: London* **1968**, *2*, 261.
2. Butterweck, V. *CNS Drugs* **2003**, *17*, 539.
3. Miller, A. L. *Altern. Med. Rev.* **1998**, *3*, 18.
4. Hu, Z. P.; Yang, X. X.; Chan, S. Y.; Xu, A. L.; Duan, W.; Zhu, Y. Z.; Sheu, F. S.; Boelsterli, U. A.; Chan, E.; Zhang, Q.; Wang, J. C.; Ee, P. L.; Koh, H. L.; Huang, M.; Zhou, S. F. *Toxicol. Appl. Pharmacol*. **2006**, *216*, 225.
5. Birt, D. F.; Widrlechner, M. P.; Hammer, K. D.; Hillwig, M. L.; Wei, J.; Kraus, G. A.; Murphy, P. A.; McCoy, J.; Wurtele, E. S.; Neighbors, J. D.; Wiemer, D. F.; Maury, W. J.; Price, J. P. *Pharm. Biol.* **2009**, *47*, 774.
6. Conceição, L. F. R.; Ferreres, F.; Tavares, R. M.; Dias, A. C. P. *Phytochemistry* **2006**, *67*, 149.
7. Jeong, J. A.; Wu, C. H.; Murthy, H. N.; Hahn, E. J.; Paek, K. Y. *Biotechnol Bioprocess Eng.* **2009**, *14*, 91.
8. Nahrstedt, A.; Butterweck, V. *J. Nat. Prod.* **2010**, *28*, 1015.
9. Müller, W. E. *Pharmacol. Res.* **2003**, *47*, 101.
10. Medina, M. A.; Martínez-Poveda, B.; Amores-Sánchez, M. I.; Quesada, A. R. *Life Sc*. **2006**, *79*, 105.
11. Fotie, J.; Bohle, D. S. *Anti-Infective Agents Med. Chem.* **2006**, *5*, 15.
12. Tocci, N.; Ferrari, F.; Santamaria, A. R.; Valletta, A.; Rovardi, I.; Pasqua, G. *Nat. Prod. Res.* **2010**, *24*, 286.
13. Baldwin, A. S. *J. Clin. Invest.* **2001**, *107*, 3.
14. Pande, V.; Ramos, M. J. *Curr. Med. Chem.* **2005**, *12*, 357.
15. Moraes, L. A.; Piqueras, L.; Bishop-Bailey, D. *Pharmacol. Ther.* **2006**, *110*, 371.
16. Sharma, A. M.; Staels, B. *J. Clin. Endocrinol. Metab.* **2007**, *92*, 386.
17. Berger, J.; Moller, D. E. *Annu. Rev. Med.* **2002**, *53*, 409.
18. Balint, B. L.; Nagy, L. *Endocr. Metab. Immune Disord. Drug Targets* **2006**, *6*, 33.
19. Barish, G. D.; Narkar, V. A.; Evans, R. M. *J. Clin. Invest.* **2006**, *116*, 590.
20. Haluzik, M. M.; Haluzik, M. *Physiol. Res.* **2006**, *55*, 115.
21. Kuroda, M.; Mimaki, Y.; Honda, S.; Tanaka, H.; Yokota, S.; Mae, T. *Bioorg. Med. Chem*. **2010**, *18*, 962.
22. Nedialkov, P. T.; Kitanov, G. M. *Phytochemistry* **2002**, *59*, 867.
23. Terreaux, C.; Maillard, M.; Gupta, M. P.; Hostettmann, K. *Phytochemistry* **1995**, *40*, 1791.
24. Abou-Shoer, M.; Suwanborirux, K.; Habib, A. A. M.; Chang, C. J.; Cassady, J. M. *Phytochemistry* **1993**, *34*, 1413.
25. Walia, S.; Mukerjee, S. K. *Phytochemistry* **1984**, *23*, 1816.
26. Marques, V. L.; De Oliveira, F. M.; Conserva, L. M.; Brito, R. G.; Guilhon, G. M. *Phytochemistry* **2000**, *55*, 815.
27. Schaufelberger, D.; Hostettmann, K. *Planta Med.* **1988**, *54*, 219.
28. Lin, W. H.; Deng, Z. W.; Lei, H. M.; Fu, H. Z.; Li, J. *J. Asian Nat. Prod. Res.* **2002**, *4*, 287.
29. Bajaj, K. L.; Anan, T.; Tsushida, T.; Ikegaya, K. *Agric. Biol. Chem.* **1987**, *51*, 1767.
30. Yoshikawa, K.; Eiko, K.; Mimura, N.; Kondo, Y.; Arihara, S. *J. Nat. Prod.* **1998**, *61*, 786.
31. Fukui, H.; Koshimizu, K.; Yamazaki, Y.; Usuda, S. *Agric. Biol. Chem.* **1977**, *41*, 189.
32. Klick, S.; Herrmann, K. *Phytochemistry* **1988**, *27*, 2177.
33. Jiang, H. Z.; Quan, X. F.; Tian, W. X.; Hu, J. M.; Wang, P. C.; Huang, S. Z.; Cheng, Z. Q.; Liang, W. J.; Zhou, J.; Ma, X. F.; Zhao, Y. X. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 6045.
34. Weinstein, D. S.; Chen, P.; Dhar, T. G. M.; Duan, J.; Gong, H.; Jiang, B.; Yang, B. V.; Doweyko, A. M. U.S. Pat. *Appl. Publ*. **2009**, US 20090075995 A1 20090319.
35. Cui, X. H.; Murthy, H. N.; Jin, Y. X.; Yim, Y. H.; Kim, J. Y.; Paek, K. Y. *Bioresour. Technol.* **2011**, *102*, 10072.
36. Kim, K. K.; Park, K. S.; Song, S. B.; Kim, K. E. *Mol. Carcinog.* **2010**, *49*, 259.
37. Shearer, B. G.; Billin, A. N. *Biochim. Biophys. Acta, Mol. Cell. Biol. Lipids* **2007**, *1771*, 1082.