New Inhibitor against Nuclear Factor of Activated T Cells Transcription from *Ribes fasciculatum* var. *chinense*

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Received August 12, 2004; accepted October 12, 2004

Two new compounds were isolated from the stem and twigs of *Ribes fasciculatum* var. *chinense* and their structures were identified to be *threo*-(7*S*,8*R*)-1-(4-hydroxyphenyl)-2-[4-(*E*)-propenylphenoxy]-propan-1-ol (1), and 5,4′-dihydroxy-7-methoxyflavone-3-*O*-[α-L-rhamnopyranosyl(1→3)-*O*-α-L-rhamnopyranosyl(1→6)-*O*-β-D-glucopyranoside] (2). With nine other known components, they were tested on inhibitory activity against nuclear factor of activated T cells (NFAT) transcription factor. Compound 1 showed a potent inhibitory activity (IC_{50}=15.6 μM), while compounds 4, 5 and 9 showed moderate inhibitory activity (IC_{50} 22.4, 24.5 and 25.7 μM, respectively).

Key words *Ribes fasciculatum* var. *chinense*; Saxifragaceae; NFAT transcription factor

Transcription factor called "nuclear factor of activated T cells" (NFAT) is a cytoplasmic protein and activated by stimulation of cell surface receptors coupled to Ca^{2+} mobilization. The Ca^{2+} activated phosphatase, calcineurin, dephosphorylates this NFAT proteins, thereby promoting their translocation into the nucleus and subsequent activation.[1] However, excessive activation of NFAT provokes immunopathological reactions including autoimmunity, transplant rejection and inflammation.[2] Therefore, modulation of NFAT transcription factor should be useful in the therapy of immune diseases.

Ribes fasciculatum var. *chinense* MAX. (Saxifragaceae) grows widely in Korea, Japan, China and other countries, but its chemical constituents and biological activities have not been studied previously. In the biological pre-screening program, the MeOH extract of *R. fasciculatum* var. *chinense* showed significantly inhibitory activity for the NFAT transcription factor. Because of this property, the plant was investigated on the chemical components with NFAT transcription inhibitory activity. The work produced two new compounds (**1**, **2**) as well as nine known compounds: butan-2-*O*-β-D-glucopyranoside (**3**),[3] catechin (**4**),[4] (±)-gallocatechin (**5**),[5] sarmentosin (**6**),[6] quercitrin (**7**),[7] glucosyl *p*-coumarate ester (**8**),[8] octadecanyl 3-(4-hydroxy-3-methoxy-phenyl)-acrylate ester (**9**),[9,10] 1,2-dimethoxy-4-(1-*cis*-propenyl)-benzene (**10**)[11] and lupenyl acetate (**11**).[12]

Results and Discussion

Compound **1** was obtained as yellow oil and the positive FAB-MS showed the $[M+H]^+$ peak at *m*/*z* 285. The HR-FAB-MS revealed the molecular formula $C_{18}H_{21}O_3$ base on the peak at *m*/*z* 285.1387. The ^{13}C-NMR spectrum of **1** revealed totally eighteen carbon atoms structure with two *para*-substituted benzene rings. Two tertiary carbon signals appeared at δ 79.5 and 78.1 indicating that each one bore an oxygen atom. In the ^{1}H-NMR spectrum, the aromatic proton produced the broad peaks at δ 7.31 (2H, dd, *J*=2.1, 6.6 Hz, H-2, 6), 7.27 (2H, dd, *J*=2.1, 6.6 Hz, H-2′, 6′), 6.90 (2H, dd, *J*=2.1, 6.6 Hz, H-3, 5) and 6.83 (2H, dd, *J*=2.1, 6.6 Hz, H-3′, 5′). The 1-(*E*)-propenyl moiety was observed with the large coupling constant (*J*=15.6 Hz) between double bond protons. These characteristics suggested that compound **1** has the 8-*O*-4′ neolignan skeleton.[13—15] By comparing the spectral data with reference, structure of **1** was similar to that of *erythro*-1-(4-hydroxyphenyl)-2-[4-(*E*)-propenylphenoxy]-propan-1-ol isolated from *Krameria cystisodes*.[15] The coupling constant between protons H-7 and H-8 of the *erythro* compound was 4.0 Hz while that of **1** was much larger (*J*=7.8 Hz) confirming that **1** was *threo* enantiomer.[16] The Mosher's method[13] experiments were carried out to determine the absolute configuration at C-7. In the ^{1}H-NMR spectrum of (*S*)-MTPA ester (**1a**), proton signals assigned to H-8, H-9 were observed in higher field than those of the (*R*)-MTPA ester (**1b**), while signals due to H-3, H-5 in **1a** were shifted to slightly lower field than those of **1b**. Therefore, the absolute configuration at C-7 was concluded to be 7*S*. The nuclear Overhauser effect correlations were observed between protons H-3, 5 and H-8, and not between protons H-7 and H-8 in the NOESY spectrum of **1**. By the Newman projection, protons H-8 and H-3, 5 made an angle of 60° and protons H-7 and H-8 established an angle of 180° due to their *threo* position.[17] Therefore, C-8 position was concluded as 8*R*. Consequently, compound **1** was determined as *threo*-(7*S*,8*R*)-1-(4-hydroxyphenyl)-2-[4-(*E*)-propenylphenoxy]-propan-1-ol.

Compound **2** was obtained as yellow amorphous powder and the HR-FAB-MS spectrum showed the $[M+H]^+$ peak at *m*/*z* 755.2399 corresponding to the molecular formula $C_{34}H_{43}O_{19}$. The ^{13}C-NMR spectrum revealed fifteen carbon signals of flavone, a methoxy group and characteristic signals for sugars. The ^{1}H-NMR spectrum showed two broad doublets at δ 6.90 and 8.13 (each 2H, d, *J*=9.0 Hz) indicating the presence of hydroxy group at C-4′ of B-ring, two other doublets at δ 6.36 and 6.61 (each 1H, d, *J*=1.8 Hz) suggested two substituted oxygen moieties at C-5 and C-7.[18] Three anomeric carbon signals at δ 104.5, 102.9 and 100.9 and two methyl signals at δ 16.9 confirmed one glucopyranoside and two rhamnopyranosides in the structure of **2**. The large coupling constant (*J*=7.8 Hz) of the anomeric proton at δ 5.09 was due to the β-glucopyranosyl unit[19,20] and the small coupling constant (*J*=1.2 Hz) of two other anomeric protons at δ 4.94 and 4.52 indicated the α-rhamnopyranosyl units.[21] The

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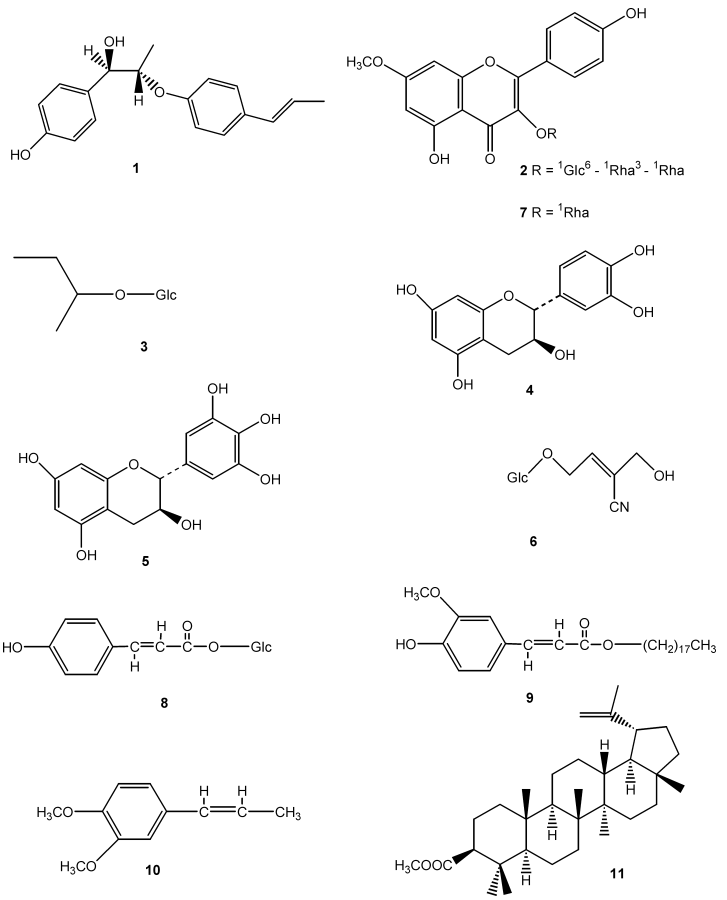

Fig. 1. Compounds Isolated from *R. fasciculatum* var. *chinense*

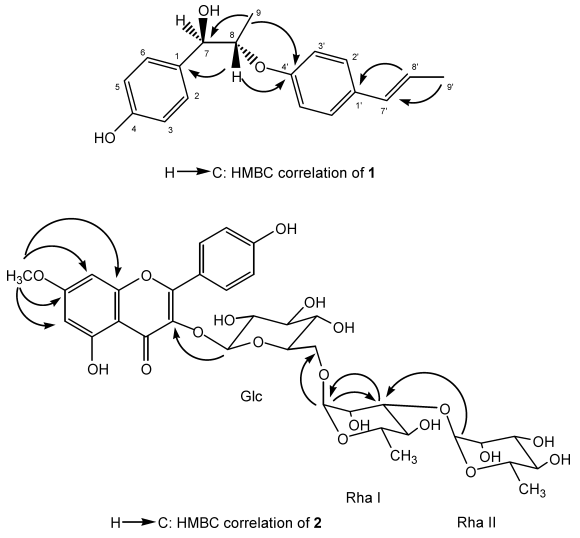

Fig. 2. HMBC Correlation

heteronuclear multiple bond correlations (HMBC) experiment showed the long-range coupling between the methoxy protons (δ 3.90) and the carbon atom C-7 (δ 166.4), which meant that the methoxy group was attached to the C-7. Therefore, the aglycone of **2** was confirmed as rhamnocitrin skeleton.[18] Moreover, the HMBC experiment revealed the correlation between C-3 (δ 134.8) of the aglycone and H-1 (δ 5.09) of the glucopyranosyl unit; between C-6 (δ 66.5) of glucopyranosyl unit and H-1 (δ 4.52) of one rhamnosyl unit. The doublet of doublets at δ 3.59 (1H, dd, J=3.0, 8.5 Hz) was due to axial proton H-3 of the inner rhamnopyranosyl unit (Rha I) and the three-bond strong correlation between two rhamnose anomeric protons (δ 4.94, 4.52) and the C-3 of the Rha I at δ 78.5 indicating that the terminal rhamnose (Rha II) was linked to the C-3 of the Rha I.[22] Acid hydrolysis of **2** afforded D-glucose and L-rhamnose by comparing with authentic sugars.[19] From these data, compound **2** was elucidated to be 5,4′-dihydroxy-7-methoxyflavone-3-*O*-[α-L-rhamnopyranosyl(1→3)-*O*-α-L-rhamnopyranosyl(1→6)-*O*-β-D-glucopyranoside].

Table 1. ^{1}H- and ^{13}C-NMR Data of **1**

No.	^{1}H-NMR	^{13}C-NMR
C_1		132.0
$C_{2,6}$	7.31 (2H, dd, J=2.1, 6.6 Hz)	129.0
$C_{3,5}$	6.90 (2H, dd, J=2.1, 6.6 Hz)	115.7
C_4		156.0
C_7	4.65 (1H, d, J=7.8 Hz)	79.5
C_8	4.37 (1H, dq, J=7.8, 6.0 Hz)	78.1
C_9	1.11 (3H, d, J=6.0 Hz)	16.1
$C_{1'}$		132.4
$C_{2',6'}$	7.27 (2H, dd, J=2.1, 6.6 Hz)	127.3
$C_{3',5'}$	6.83 (2H, dd, J=2.1, 6.6 Hz)	116.8
$C_{4'}$		156.9
$C_{7'}$	6.36 ((1H, dd, J=1.5, 15.6 Hz)	130.6
$C_{8'}$	6.10 (1H, m)	124.4
$C_{9'}$	1.88 (3H, dd, J=1.5, 6.6 Hz)	18.7

Eleven isolated components were examined on inhibitory activity against NFAT transcription factor. Of these, compound **1** showed the strongest inhibition with IC_{50} value of 15.6 μM and compounds **4**, **5** and **9** showed moderate inhibitory activity with IC_{50} value of 22.4, 24.5 and 25.7 μM, respectively (see Table 3).

Table 2. ^{1}H- and ^{13}C-NMR Data of **2**

No.	^{1}H-NMR	^{13}C-NMR
Aglycone		
C_2		157.4
C_3		134.8
C_4		178.6
C_5		161.2
C_6	6.36 (1H, d, J=1.8 Hz)	98.6
C_7		166.4
C_8	6.61 (1H, d, J=1.8 Hz)	92.3
C_9		158.8
C_{10}		105.4
$C_{1'}$		121.1
$C_{2'}$	6.90 (1H, d, J=9.0 Hz)	131.5
$C_{3'}$	8.13 (1H, d, J=9.0 Hz)	115.3
$C_{4'}$		161.3
$C_{5'}$	8.13 (1H, d, J=9.0 Hz)	115.3
$C_{6'}$	6.90 (1H, d, J=9.0 Hz)	131.5
OCH_3	3.90 (3H, br s)	55.5
Glu		
C_1	5.09 (1H, d, J=7.8 Hz)	104.5
C_2	3.80 (1H, m)	72.0
C_3	3.66 (1H, m)	77.0
C_4	3.72 (1H, m)	71.1
C_5	3.56 (1H, m)	76.9
C_6	3.73, 3.42 (each 1H, m)	66.5
Rha I		
C_1	4.52 (1H, d, J=1.2 Hz)	100.9
C_2	3.68 (1H, m)	70.8
C_3	3.59 (1H, dd, J=9.2, 3.0 Hz)	78.5
C_4	3.41 (1H, m)	74.3
C_5	3.55 (1H, m)	72.1
C_6	1.18 (3H, d, J=6.0 Hz)	16.9
Rha II		
C_1	4.94 (1H, d, J=1.2 Hz)	102.9
C_2	3.78 (1H, m)	69.2
C_3	3.93 (1H, m)	71.2
C_4	3.34 (1H, m)	73.0
C_5	3.67 (1H, m)	68.9
C_6	1.13 (3H, d, J=6.0 Hz)	16.9

Experimental

The melting points were measured by Yanagimoto micro hot-stage melting point apparatus and were uncorrected. The IR spectra were made on Jasco 100 IR spectrophotometer. ^{1}H- and ^{13}C-NMR spectra were obtained from a Bruker DRX-300 and 600-NMR spectrometer and from a Jeol AL400 spectrometer. FAB-MS and HR-FAB-MS spectra were measured on JMS-HX/HX 110A tandem mass spectrometer. Thin layer chromatography was performed on pre-coated TLC plate silicagel 60F_{254} (Merck). Column chromatographic materials were Si gel 60 (230—400 mesh), Sephadex LH-20, YMC-gel (ODS-A, 12 nm, S-150 μm). The activity against NFAT transcription factor was measured on ELISA reader (Molecular Devices, Sunnyvale, U.S.A.). GC analysis was performed on HP 5890 Series II (Hewlett Packard, U.S.A.).

Plant Material The stem and twigs of *R. fasciculatum* var. *chinense* was collected in Ocheon, Chungbuk (Korea) in August 2002 and identified by Prof. Young Ho Kim. The voucher specimen (CNU02001) was deposited at the herbarium in College of Pharmacy, Chungnam National University, Korea.

Extraction and Isolation The dried stem and twigs (1.6 kg) of *R. fasciculatum* var. *chinense* were powdered and extracted with hot MeOH and the extract (43.0 g) was suspended in water (1.5 l) and then partitioned successively in hexane, ethyl acetate and buthanol (each 500 ml×3 times). The ethyl acetate residue (5.0 g) was subjected on silica gel column with gradient solvent dichloromethane–methanol (10 : 1 to 1 : 1 v/v) to give eight subfractions (subfr. 8-A—H). Compound **1** (5.6 mg) was separated from subfr. 8-B by silica gel column using hexane–ethyl acetate (5 : 1). Reverse phase column chromatography was performed on methanol–water (6 : 1) for the subfr. 8-F and subfr. 8-G, eluting compound **4** (101.6 mg) and **5** (98.7 mg), respectively.

Nine subfractions (subfr. 17-A—J) were provided from the butanol residue (25.0 g). Compounds **2** (9.6 mg) and **3** (9.8 mg) were eluted from subfr. 17-I by using silica gel column eluted with chloroform–methanol–ethanol (6 : 4 : 1). Compound **6** (21.5 mg) and **7** (10.8 mg) were isolated from subfr. 17-E and subfr. 17-G by reverse phase column chromatography using methanol–water (1 : 1) solvent system. The subfr. 17-C was subjected on silica gel column using chloroform–methanol (7 : 1) to give compound **8** (51.5 mg).

From the hexane residue (9.0 g), repeated silica gel column chromatography was carried out with solvent hexane–ethyl acetate (10 : 1) to afford compound **9** (18.7 mg), **10** (37.0 mg) and **11** (10.5 mg).

Threo-1-(4-hydroxyphenyl)-2-[4-(*E*)-propenylphenoxy]-propan-1-ol (**1**): Yellow oil; $[\alpha]_D^{20}$ +24.99° (c=0.5, $CHCl_3$). UV λ_{max} ($CHCl_3$) nm (log ε): 258 (1.05). IR (KBr) ν (cm^{-1}): 3410 (OH), 1520, 1590 (C=C ring), 1215 (C–O). Positive FAB-MS m/z: 285 $[M+H]^+$. HR-FAB-MS m/z: 285.1387 (Calcd for $C_{18}H_{21}O_3$ 285.1382). ^{1}H-NMR (300 MHz, $CDCl_3$) and ^{13}C-NMR (75 MHz, $CDCl_3$): see Table 1.

(*S*)-MTPA Ester of 1 (1a) The mixture of **1** (1.5 mg) in 1 ml CH_2Cl_2, (*S*)-MTPA (10 mg), EDC·HCl (8 mg) and 4-DMAP (3 mg) was stirred at room temperature for 8 h. The reaction solution was then poured into cooled water and the CH_2Cl_2 fraction was extracted and then subjected on silica PTLC, solvent hexane–ethyl acetate 3 : 1 to give **1a** (0.8 mg). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.310 (2H, dd, J=2.1, 6.6 Hz, H-2, 6), 7.220 (2H, m, H-2′, 6′), 7.071 (2H, d, J=8.4 Hz, H-3, 5), 6.785 (2H, d, J=8.4 Hz, H-3′, 5′), 6.266 (1H, d, J=14.4 Hz, H-7′), 6.035 (1H, m, H-8′), 4.651 (1H, d,

Table 3. Inhibitory Activity against NFAT Transcription of Components Isolated from *R. fasciculatum* var. *chinense*

Compound	IC_{50} (μM)[a]
Threo-(7*S*,8*R*)-1-(4-hydroxyphenyl)-2-[4-(*E*)-propenylphenoxy]-propan-1-ol (**1**)	15.6±0.35
5,4′-Dihydroxy-7-methoxyflavone-3-*O*-[α-L-rhamnopyranosyl(1→3)-*O*-α-L-rhamnopyranosyl(1→6)-*O*-β-D-glucopyranoside] (**2**)	>100
Butan-2-*O*-β-D-glucopyranoside (**3**)	>100
Catechin (**4**)	22.4±0.50
Gallocatechin (**5**)	24.5±0.85
Sarmentosin (**6**)	>100
Quercitrin (**7**)	>100
Glucosyl *p*-coumarate ester (**8**)	>100
Octadecanyl 3-(4-hydroxy-3-methoxy-phenyl)-acrylate ester (**9**)	25.7±1.73
1,2-Dimethoxy-4-(1-*cis*-propenyl)-benzene (**10**)	>100
Lupenyl acetate (**11**)	>100
Cyclosporin A[b]	0.29±0.006

a) Values of IC_{50} are presented as mean±S.E. of three experiments. *b*) Cyclosporin A[27,28] was used as a positive control.

J=6.8 Hz, H-7), 4.301 (1H, m, H-8), 1.788 (3H, d, J=6.4 Hz, H-9′), 1.055 (3H, d, J=6.4 Hz, H-9).

(*R*)-MTPA Ester of 1 (1b) Repeat the same procedure above with (*R*)-MTPA yielding **1b** (0.8 mg). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.310 (2H, dd, J=2.1, 6.6 Hz, H-2, 6), 7.220 (2H, m, H-2′, 6′), 7.070 (2H, d, J=8.4 Hz, H-3, 5), 6.787 (2H, d, J=8.8 Hz, H-3′, 5′), 6.267 (1H, d, J=14.4 Hz, H-7′), 6.041 (1H, m, H-8′), 4.651 (1H, d, J=6.8 Hz, H-7), 4.302 (1H, m, H-8), 1.790 (3H, d, J=6.8 Hz, H-9′), 1.059 (3H, d, J=6.0 Hz, H-9).

5,4′-Dihydroxy-7-methoxyflavone-3-*O*-[α-L-rhamnopyranosyl(1→3)-*O*-α-L-rhamnopyranosyl(1→6)-*O*-β-D-glucopyranoside] (**2**): Yellow powder, mp 210—212 °C. $[\alpha]_D^{20}$ −41.99° (c=0.5, MeOH). UV λ_{max} (MeOH) nm (log ε): 265 (0.49), 349 (0.49). IR (KBr) ν (cm^{-1}): 3350 (OH), 1625 (C=O), 1495, 1420 (C=C ring), 1240 (C–O). FAB-MS m/z: 753 [M−H]$^-$ 607 [M−H−rha]$^-$, 461 [M−H−2rha]$^-$ 283 [M−glu−2rha]$^-$ HR-FAB-MS m/z: 755.2399 [M+H]$^-$(Calcd 755.2401 for $C_{34}H_{43}O_{19}$). ^{1}H-NMR (300 MHz, CD_3OD). ^{13}C-NMR (75 MHz, CD_3OD): see Table 2.

Acid Hydrolysis Compound **2** (1.5 mg) was treated with 1 N HCl (2 ml) at 95 °C for 1 h. After cooling the reaction mixture was neutralized by NaOH and then extracted with EtOAc. The water layer was dried and dissolved in pyridine (50 μl) and stirred with L-cysteine methyl ester (3 mg) at 60 °C for 2 h. After cooling and drying under N_2 flow, the residue was treated with *N*-(trimethylsilyl)imidazone (40 μl) at 60 °C for 1 h. The reaction solution was dried by N_2 and partitioned in water and hexane, the supernatant was then analyzed by GC on column SPB-1 (30 m×0.53 mm, 0.5 μm film), column temperature 230 °C, carrier gas N_2. D-Glucopyranoside (26.6 min) and L-rhamnopyranoside (24.9 min) was detected.

Butan-2-*O*-β-D-glucopyranoside (**3**): White gum; $[\alpha]_D^{20}$ −89.49° (c=0.3, $CHCl_3$). IR (KBr) ν (cm^{-1}): 3420 (OH), 1210 (C–O). Positive FAB-MS m/z: 237 [M+H]$^+$.

Catechin (**4**): Pale-yellow powder; mp 95—98 °C. $[\alpha]_D^{20}$ +17.5° (c=0.3, $CHCl_3$). IR (KBr) ν (cm^{-1}): 3400 (OH), 1650 (C=O), 1510, 1420 (C=C ring), 1210 (C–O). Positive FAB-MS m/z: 291 [M+H]$^+$.

(±)-Gallocatechin (**5**): Yellow powder, mp 188—190 °C. $[\alpha]_D^{20}$ −80° (c=0.5, $CHCl_3$). IR (KBr) ν (cm^{-1}): 3400 (OH), 1650 (C=O), 1500, 1420 (C=C ring), 1215 (C–O). Positive FAB-MS m/z: 307 [M+H]$^+$.

Sarmentosin (**6**): White gum, $[\alpha]_D^{20}$ +39.99° (c=0.5, $CHCl_3$). IR (KBr) ν (cm^{-1}): 3410 (OH), 1120 (C–O). Positive FAB-MS m/z: 276 [M+H]$^+$.

Quercitrin (**7**): Yellow powder, mp 178—180 °C. $[\alpha]_D^{20}$ +147.5° (c=0.5, MeOH). IR (KBr) ν (cm^{-1}): 3450 (OH), 1700 (C=O), 1520, 1450 (C=C ring), 1180 (C–O). Positive FAB-MS m/z: 449 [M+H]$^+$.

Glucosyl *p*-coumarate Ester (**8**): White crystal, mp 248—251 °C. $[\alpha]_D^{20}$ +21.5° (c=0.5, $CHCl_3$). IR (KBr) ν (cm^{-1}): 3350 (OH), 1650 (C=O), 1500, 1490 and 1410 (C=C ring). Positive FAB-MS m/z: 349 [M+H]$^+$.

Octadecanyl 3-(4-hydroxy-3-methoxy-phenyl)-acrylate Ester (**9**): White powder, mp 86—89 °C. IR (KBr) ν (cm^{-1}): 1650 (C=O), 1510, 1410 (C=C ring), 1100 (C–O). Positive FAB-MS m/z: 447 [M+H]$^+$.

1,2-Dimethoxy-4-(1-*cis*-propenyl)-benzene (**10**): White oil. IR (KBr) ν (cm^{-1}): 1520, 1400 (C=C ring), 1150 (C–O). Positive FAB-MS m/z: 179 [M+H]$^+$.

Lupenyl acetate (**11**): White powder, mp 214—116 °C. $[\alpha]_D^{20}$ −0.90° (c=0.5, $CHCl_3$). IR (KBr) ν (cm^{-1}): 1650 (C=O), 1450 (C=C), 1150 (C–O). Positive FAB-MS m/z: 491 [M+H]$^+$.

Selection of NFAT Dependent Reporter Cell Line The sense and antisense oligonucleotides which containing the NFAT binding site were synthesized, annealed and ligated to get the repeated NFAT binding site. The digested DNA fragment was transfected into T Jurkat cell and then the growing clones resistant to G418 (0.8 mg/ml) were selected.

Preparation of Buffers and Reagents RPMI 1640 without phenol red (11835-030, Gibco. BRL) was mixed with 0.5% fetal bovine serum and 1% penicillin–streptomycin. Phobol 12-myristate 13-acetate (25 ng/ml) and ionomycin (0.5 μM) as a stimulator were dissolved in DMSO. *p*-Nitrophenylphosphate (120 mM) as a substrate was dissolved with secreted alkaline phosphatase (SEAP) buffer (1 M diethanolamine, 0.5 mM $MgCl_2$, 10 mM homoarginine).

Preparation of Cells and Samples The selected Jurkat T cell line was maintained in RPMI 1640 supplemented with 10% fetal bovine serum and 1% penicillin–streptomycin. The harvested cells were resuspended in RPMI 1640 without phenol red. Each sample was dissolved in DMSO and diluted in RPMI 1640 without phenol red.

Determination of Inhibitory Activity against NFAT Transcription Factor Inhibitory activity on NFAT transcription factor was determined by the modified SEAP assay[23] as described previously.[24,25] For assay, 100 μl of cells (1×10^4 cells/well) were incubated with 50 μl of sample and 50 μl of stimulator at 37 °C for 18 h. The reaction mixture was centrifuged and 100 μl of supernatant were heated at 65 °C for 1 h. The heated sample was incubated with 50 μl of SEAP buffer and 50 μl of substrate at 37 °C for 4 h. After incubation of the reaction mixture, optical density was measured at 405 nm. Inhibitory activity on NFAT transcription factor was expressed as percent inhibition of the control and statistical significance was evaluated by Students's t-test. The positive control was used with cyclosporin A, which blocks the phosphatase activity of calcineurin, thus preventing the subsequent dephosphorylation and translocation of NFAT to the nucleus.[26] Cell viability was determined by using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) cell proliferation Kit (1465007, Roche).

Acknowledgments This work was supported by a grant of the Korea Health 21 R&D Project, Ministry of Health and Welfare, Republic of Korea (00-PJ22-PG1-CD02-0018). We are grateful to KBSI for performing the NMR and MS spectral measurements.

References

1) Winter G., Harris W. J., *Immunol. Today*, **14**, 243—246 (1993).
2) Abbas A. K., Lichtman A. H., Pober J. S., "Cellular and Molecular Immunology," W.B. Saunders Company, Philadelphia, 1997, pp. 315—338.
3) Cable J., Nocke H., *Australian J. Chem.*, **28**, 2737—2739 (1975).
4) Nahrstedt A., Proksch P., Conn E. E., *Phytochemistry*, **26**, 1546—1548 (1987).
5) Someya S., Yoshiki Y., Okubo K., *Food Chem.*, **79**, 351—354 (2002).
6) Nishida R., Rothschild M., Mummery R., *Phytochemistry*, **36**, 37—38 (1994).
7) Kosuge T., Ishida H., Satoh T., *Chem. Pharm. Bull.*, **33**, 206—209 (1985).
8) Baderschneider B., Winterhalter P., *J. Agric. Food Chem.*, **49**, 2788—2798 (2001).
9) Kang S. S., Son K. H., "Structure Determination of Natural Products," Seoul National University Publisher, Seoul, 2000, pp. 427—443.
10) Lu Y., Foo L. Y., *Food Chem.*, **80**, 71—76 (2003).
11) Patra A., Mitra A. K., *J. Nat. Prod.*, **44**, 668—669 (1981).
12) Talapatra S. K., Shrestha K. M., Pal M. K., Batrax A., Talapatra B., *Phytochemistry*, **28**, 3437—3442 (1989).
13) Braga A. C.-H., Zacchino S., Badano H., Sierra M. G., Ruvenda E. A., *Phytochemistry*, **23**, 2025—2028 (1984).
14) Cavalcante S. H., Yoshida M., Gottlieb O. R., *Phytochemistry*, **24**, 1051—1055 (1985).
15) Hada S., Hattory M., Tezuka Y., Kikuchi T., Namba T., *Phytochemistry*, **27**, 563—568 (1988).
16) Achenbach H., Grob J., Dominguez X. A., Cano G., Star J. V., Brussolo L. D.-C., Munoz G., Salgado F., Lopez L., *Phytochemistry*, **26**, 1159—1166 (1987).
17) Yoshikawa M., Shimada H., Saka M., Yoshizumi S., Yamahara J., Matsuda H., *Chem. Pharm. Bull.*, **45**, 464—469 (1997).
18) Barbera O., Sanz J. F., Parareda J. S., Marco J. A., *Phytochemistry*, **25**, 2361—2365 (1986).
19) Kanchanapoom T., Kasai R., Yamasaki K., *Phytochemistry*, **59**, 557—563 (2002).
20) Jia Z., Koike K., Nikaido T., *Major J. Nat. Prod.*, **61**, 1368—1373 (1998).
21) Nabil H.-E. S., Wojcinska M., Krystyna D. K., Matlawaska I., Williams J., Mabry O., *Phytochemistry*, **60**, 835—838 (2002).
22) Satake T., Hori K., Kamiya K., Saiki Y., Fujimoto Y., Kimura Y., Maksut C., Mekin T., *Chem. Pharm. Bull.*, **41**, 1743—1745 (1993).
23) Yang T. T., Sinai P., Kitts P. A., Kain S. R., *Biotechniques*, **23**, 1110—1114 (1997).
24) Lee I. S., Lee H.-K., Dat N. T., Lee M. S., Kim J. W., Na D. S., Kim Y. H., *Planta Med.*, **69**, 63—64 (2003).
25) Dat N. T., Lee I. S., Cai X. F., Shen Q., Kim Y. H., *Biol. Pharm. Bull.*, **27**, 426—428 (2004).
26) Jain J., Loh C., Rao A., *Curr. Opin. Immunol.*, **7**, 333—342 (1995).
27) Kung L., Halloran P. F., "Therapeutic Immunosuppression," ed. by Thomson A. W., Kluwer Academic Publishers, Netherlands, 2001, pp. 1—30.
28) Beata P., Agnieszka L., Grazyna M., Bozena K., *Neurochem. Int.*, **38**, 409—415 (2001).