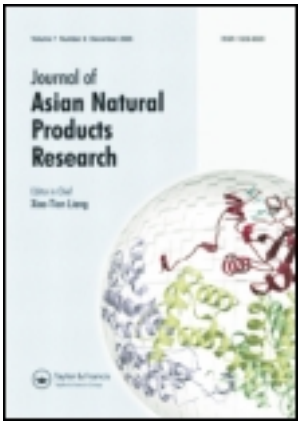


Journal of Asian Natural Products Research

Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/ganp20

A new flavan-3-ol and the anti-inflammatory effect of flavonoids from the fruit peels of *Wisteria floribunda*

Bui Huu Tai [a c], Trinh Nam Trung [a b], Nguyen Xuan Nhiem [a c], Do Thi Ha [a], Chu Van Men [a b], Vu Binh Duong [b], Hoang Van Luong [b], SeokBean Song [a], KiHwan Bae [a] & Young Ho Kim [a]

[a] College of Pharmacy, Chungnam National University, Daejeon, 305-764, Korea

[b] Vietnam Military Medical University, 104 Phung Hung Street, Hadong District, Hanoi, Vietnam

[c] Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet Street, Hanoi, Vietnam

Available online: 10 Oct 2011

To cite this article: Bui Huu Tai, Trinh Nam Trung, Nguyen Xuan Nhiem, Do Thi Ha, Chu Van Men, Vu Binh Duong, Hoang Van Luong, SeokBean Song, KiHwan Bae & Young Ho Kim (2011): A new flavan-3-ol and the anti-inflammatory effect of flavonoids from the fruit peels of *Wisteria floribunda* , Journal of Asian Natural Products Research, 13:11, 1061-1068

To link to this article: http://dx.doi.org/10.1080/10286020.2011.603306

Journal of Asian Natural Products Research
Vol. 13, No. 11, October 2011, 1061–1068

NOTE

A new flavan-3-ol and the anti-inflammatory effect of flavonoids from the fruit peels of *Wisteria floribunda*

Bui Huu Tai[a,c†], Trinh Nam Trung[a,b†], Nguyen Xuan Nhiem[a,c], Do Thi Ha[a], Chu Van Men[a,b], Vu Binh Duong[b], Hoang Van Luong[b], SeokBean Song[a], KiHwan Bae[a] and Young Ho Kim[a]*

[a]*College of Pharmacy, Chungnam National University, Daejeon 305-764, Korea;* [b]*Vietnam Military Medical University, 104 Phung Hung Street, Hadong District, Hanoi, Vietnam;* [c]*Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet Street, Hanoi, Vietnam*

(*Received 22 April 2011; final version received 3 July 2011*)

A new flavan-3-ol, (+)-afzelechin 5-*O*-β-D-glucopyranoside (**2**), together with 13 known flavonoids (**1**, **3**–**14**), was isolated from the fruit peels of *Wisteria floribunda*. Their structures were assigned by detailed interpretation of NMR, MS, and CD spectroscopic data, as well as by comparing with published reports. The *in vitro* anti-inflammatory activity of the isolated compounds (**1**–**14**) was examined. Among them, compounds **3**, **6**, and **9** produced highest inhibitory effects on tumor necrosis factor alpha (TNF-α)-induced nuclear factor kappa-B activation in HepG2 cells with IC_{50} values of 14.1, 16.5, and 11.9 μM, respectively. With the exception of compound **6**, the compounds significantly inhibited the accumulation of pro-inflammatory inducible nitric oxide synthase and cyclooxygenase-2 proteins in TNF-α-stimulated HepG2 cells at a concentration as low as 0.1 μM.

Keywords: COX-2; flavonoids; iNOS; NF-κB; *Wisteria floribunda*

1. Introduction

Persistent nuclear factor kappa-B (NF-κB) activation is found in many chronic inflammatory diseases, including rheumatoid arthritis (RA), asthma, inflammatory bowel disease, ulcerative colitis, and atherosclerosis, and plays a pivotal role in these processes [1,2]. NF-κB controls the expression of the cytokines including interleukin-1 beta (IL-1β) and tumor necrosis factor alpha (TNF-α), which are the essential mediators of chronic inflammation and implicated in leukocytosis, hyperplasia, and tissue breakdown. It has been demonstrated that high levels of nitric oxide and prostaglandins, via the pro-inflammatory enzymes such as inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2), have important roles in tissue response to injury or infectious agents [3]. These inducible enzymes are essential components of the inflammatory response, the ultimate repair of injury, and carcinogenesis [3]. NF-κB regulates both innate and adaptive immune responses. It is activated rapidly in response to a wide range of stimuli including pathogens, stress signals, and pro-inflammatory cytokines, such as TNF and IL-1 [4,5]. Thus, agents that can suppress NF-κB activation

*Corresponding author. Email: yhk@cnu.ac.kr
†These authors contributed equally to this work.

ISSN 1028-6020 print/ISSN 1477-2213 online

http://dx.doi.org/10.1080/10286020.2011.603306
http://www.tandfonline.com

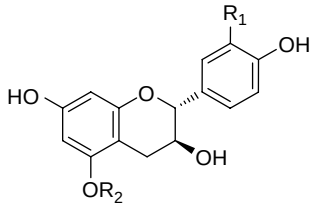
have also the potential to suppress inflammation [6].

Wisteria floribunda (Leguminosae) is a woody liana cultivated widely in Korea, Japan, the USA, and China. Its gall extracts are used as an antitumoral preparation in traditional Oriental medicine [7]. Many Oriental medicine practitioners use *W. floribunda* gall extracts for the treatment of patients suffering from breast cancer, stomach cancer, or RA. Extracts and compounds isolated from the flowers and seeds of *W. floribunda* have also been reported to have antioxidant, anticancer, and immunological activities [7,8]. In our study, to identify anti-inflammatory lead compounds from medicinal plants, we extracted flavonoids from the fruit peels of *W. floribunda* and studied their effects on TNF-α-induced NF-κB activation and their role in the accumulation of pro-inflammatory iNOS and COX-2 proteins.

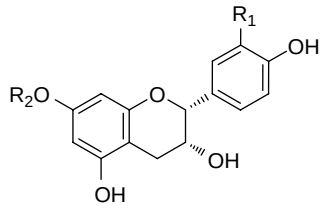
2. Results and discussion

Dried fruit peels of *W. floribunda* were extracted with 95% MeOH and fractionated with *n*-hexane, ethyl acetate (EtOAc), and *n*-butanol. During the screening of plant extracts to identify compounds that have inhibitory effects on TNF-α-induced NF-κB activation in human hepatocellular liver carcinoma cell line (HepG2), the EtOAc extracts of the fruit peels of *W. floribunda* showed significant positive activity. To identify active components, the EtOAc extracts were isolated. The compounds isolated and characterized contained a new flavan-3-ol glycoside (**2**) and 13 known flavonoid compounds (**1**, **3**–**14**) (Figure 1).

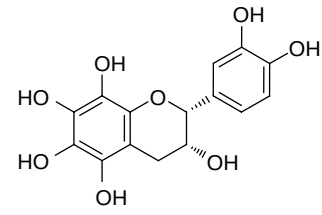
Compound **2** was obtained as light brown solid with m.p. 215–216°C. The UV spectrum showed an absorption maximum at 276 nm, and the IR spectrum exhibited absorption bands at 3392 (OH),



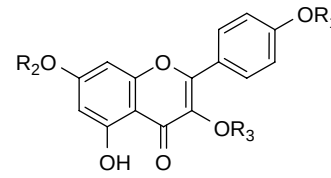
	R_1	R_2
1	H	H
2	H	Glc
3	OH	H



	R_1	R_2
4	H	H
5	H	Glc
6	OH	H



7



	R_1	R_2	R_3
8	H	H	H
9	H	H	Glc
10	H	H	Api(1-6)Glc
11	H	H	Rha(1-6)Glc
12	H	Glc	H
13	H	Glc	Glc
14	Glc	H	Glc

Figure 1. Structures of isolated compounds **1**–**14**.

1614, 1518, 1455 (C═C), 1244, and 1072 (C—O—C) cm^{-1}, which are the characteristics of phenolic compounds. Its positive electrospray ionization (ESI)-MS displayed quasimolecular ions at *m/z* 437 $[M + H]^+$ and *m/z* 459 $[M + Na]^+$, corresponding to a molecular formula of $C_{21}H_{24}O_{10}$, which was further confirmed by a high-resolution ESI-MS (*m/z* 459.1241 $[M + Na]^+$). The ^{13}C NMR and distortionless enhancement by polarization transfer spectral data of **2** showed 21 carbons including 2 methylenes, 13 methines, and 6 quaternary olefinic carbons. Among them, one anomeric carbon at δ_C 102.6, four oxygenated methine carbons at δ_C 71.4, 75.0, 78.2, and 78.3, and one oxygenated methylene carbon at δ_C 62.6 were assigned to the sugar moiety. Fifteen remaining carbons belonged to the aglycone moiety. These data suggested that **2** was a flavonoid glycoside. The presence of three aglycone carbon signals in the upfield at δ_C 83.0, 68.8, and 28.9 suggested that **2** belonged to the class of flavan-3-ols. The ^1H NMR spectrum showed a pair of two-proton doublets at δ_H 7.21 (2H, d, $J = 8.5$ Hz, H-2′,6′) and δ_H 6.79 (2H, d, $J = 8.5$ Hz, H-3′,5′) due to *p*-substituted B-ring. Next, the absolute configuration of **2** was determined by careful examination of its 1D, 2D NMR, and CD spectra. A negatively signed maximum ($\Delta\varepsilon = -9.1$) at 275 nm in the CD spectrum of **2** and, compared with a CD empirical rule on the absolute configuration at C-2 of flavan-3-ols (the *R*-configuration in a negative Cotton curve at 250–300 nm) [9], demonstrated the *R*-configuration at C-2 of **2**. The large coupling constant value of H-2 (δ_H 4.61, d, $J_{2,3} = 8.0$ Hz) was consistent with the 2,3-*trans*-configuration, compared with catechin (2,3-*trans*, H-2, d, $J_{2,3} = 6.7$ Hz) [10] and epicatechin (2,3-*cis*, H-2, s) [10], suggesting the *S*-configuration of C-3. In addition, the 2*R*,3*S*-configuration of **2** was also confirmed by ^{13}C NMR spectrum. The chemical shifts of C-2 (δ_C 83.0), C-3 (δ_C 68.8), and C-4 (δ_C 28.9) were similar to those of 2*R*,3*S*-catechin 5-*O*-β-glucopyranoside (C-2 at δ_C 82.9, C-3 at δ_C 68.7, and C-4 at δ_C 28.4) [11] and different from those of 2*R*,3*R*-epicatechin-5-*O*-β-D-glucopyranoside (C-2 at δ_C 78.2, C-3 at δ_C 64.7, and C-4 at δ_C 28.2) [12]. The chemical shifts of C-5 (δ_C 158.0) and C-7 (δ_C 158.1) when compared with those of catechin 5-*O*-β-glucopyranoside (C-5 at δ_C 158.0, C-7 at δ_C 158.1) and catechin 7-*O*-β-glucopyranoside (C-5 at δ_C 157.5, C-7 δ_C at 158.7) [11] indicated that the sugar moiety was linked to C-5 of aglycone. This was also confirmed by the correlation between the anomeric proton (δ_H 4.84) and C-5 (δ_C 158.0) observed in the HMBC spectrum (Figure 2). Finally, the presence of a D-glucose unit was further confirmed by acid hydrolysis and gas chromatography (GC) analysis, followed by comparing the retention times with authentic samples (see Experimental). Based on the above data, compound **2** was determined as a new flavan-3-ol, (+)-afzelechin 5-*O*-β-D-glucopyranoside.

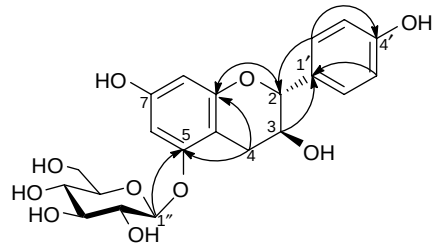


Figure 2. Key HMBC correlations of compound **2**.

The other 13 compounds were characterized as (+)-afzelechin (**1**) [13], (+)-catechin (**3**) [10], (−)-epiafzelechin (**4**) [14], (−)-epiafzelechin 7-*O*-β-D-glucopyranoside (**5**) [15], (−)-epicatechin (**6**) [10], dulcisflavan (**7**) [16], kaempferol (**8**) [17], kaempferol 3-*O*-β-D-glucopyranoside (**9**) [18], kaempferol 3-*O*-β-D-apiofuranosyl(1-6)-β-D-glucopyranoside (**10**) [19], kaempferol 3-*O*-rutinoside (**11**) [20], kaempferol 7-*O*-β-D-glucopyranoside (**12**) [21], kaempferol 3,7-di-*O*-β-D-

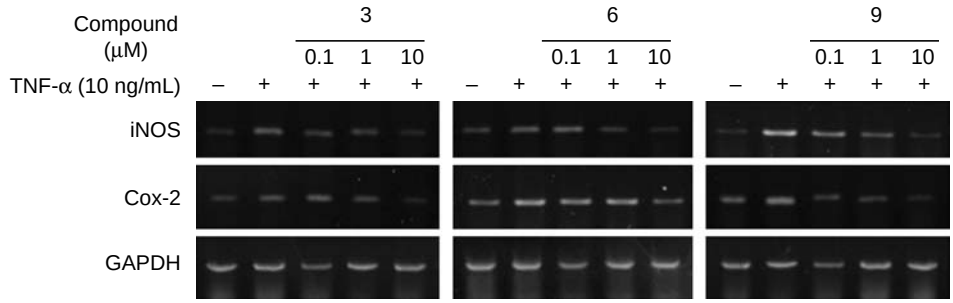


Figure 3. Evaluation of TNF-α-induced iNOS and COX-2 mRNA expression in HepG2 cells.

glucopyranoside (**13**) [22], and kaempferol 3,4′-di-*O*-β-D-glucopyranoside (**14**) [23] (Figure 1). Their structures were established on the basis of spectral and chemical evidence, which were in close agreement with those reported in the literature.

Anti-inflammatory activity of the isolated compounds (**1**–**14**) was examined *in vitro* at concentrations of 0–100 μM. As shown in Table 2, compounds **3**, **6**, and **9** significantly inhibited the activation of NF-κB induced by TNF-α in HepG2 cells. The IC_{50} values were 11.9, 16.5, and 14.1 μM, respectively, in comparison with pyrrolidine dithiocarbamate as a positive control (IC_{50} 4.9 μM). Compounds **1** and **8** exhibited a moderate inhibitory effect. The remaining compounds had weak inhibitory effects. Up to concentration of 100 μM, these compounds did not show cytotoxic effects by 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) assay, suggesting that the anti-inflammatory effects of compounds did not result from cytotoxicity. Compounds **3**, **6**, and **9** were subjected to further assays for investigating their anti-inflammatory effects. The upregulation of pro-inflammatory iNOS and COX-2 proteins in TNF-α-stimulated HepG2 cells was evaluated using reverse transcription polymerase chain reaction (RT-PCR). Results showed that all of the compounds significantly inhibited the accumulation of pro-inflammatory (iNOS and COX-2) proteins in TNF-α-stimulated HepG2 cells using concentrations of 1 and 10 μM. Lower band intensities were observed compared with the stimulus sample. Moreover, compounds **3** and **9** had inhibitory effects at a concentration of 0.1 μM (Figure 3), indicating that (+)-catechin (**3**), (−)-epicatechin (**6**), and kaempferol 3-*O*-β-D-glucopyranoside (**9**) are the most active components. Among them, compound **9**, a major component of the EtOAc extract (10.3% in dried EtOAc extract weight), may cause major inhibitory effects.

3. Experimental

3.1 General experimental procedures

Melting points were determined on an Electrothermal 9100 melting point apparatus. Optical rotations were measured on a JASCO DIP-370 automatic digital polarimeter (JASCO, Tokyo, Japan). UV spectra were acquired in MeOH using a JASCO V-550 UV–VIS spectrometer. IR spectra were recorded on a JASCO 100 IR spectrophotometer. CD spectrum was recorded on a JASCO J-725 spectrometer. The NMR spectra were recorded using a JEOL ECA-600 spectrometer (^{1}H, 600 MHz; ^{13}C, 150 MHz; JEOL, Tokyo, Japan). The ESI mass spectra were recorded on an Agilent 1100 LC-MSD trap spectrometer (Agilent Technologies, Palo Alto, CA, USA), and high-resolution ESI spectra were obtained from a JEOL JMS-HX/HX 110A tandem mass spectrometer (JEOL). GC spectra were

recorded on a Shimadzu-2010 spectrometer (Shimadzu, Kyoto, Japan). Silica gel (70–230, 230–400 mesh, Merck, Whitehouse Station, NJ, USA) and YMC RP-18 resins (75 μm, Fuji Silysia Chemical Ltd, Kasugai, Japan) were used as absorbents in the column chromatography. Thin layer chromatography (TLC) plates (silica gel 60 F254 and RP-18 F254, 0.25 μm, Merck) were purchased from Merck KGaA. Spots were detected under UV radiation (254 and 365 nm) and by spraying the plates with 10% H_2SO_4 followed by heating with a heat gun.

3.2 Plant material

The fruit peels of *W. floribunda* were collected in Daejeon, Korea, in February 2009 and identified by Prof. KiHwan Bae, College of Pharmacy, Chungnam National University. A voucher specimen (CNU 1389–5) is deposited in the College of Pharmacy, Chungnam National University.

3.3 Extraction and isolation

Dried sample (6 kg) was pulverized and extracted with 95% methanol (18 l) under reflux for 3 × 3 h. The extract was concentrated to dryness under reduced pressure. The residue (496 g) was suspended in water and partitioned, sequentially, with *n*-hexane, ethyl acetate (EtOAc), and *n*-butanol (BuOH) to yield *n*-hexane extract (22.5 g), EtOAc extract (116 g), and BuOH extract (210 g), respectively. The EtOAc-soluble extract was fractioned on a silica gel column and eluted with a gradient solvent system of CH_2Cl_2 and CH_3OH (0–100% MeOH in volume) to give six fractions E1–E6. Fraction E1 (2.6 g) was repeatedly chromatographed on a silica gel column eluting with $CHCl_3$–CH_3OH (10/1, v/v) to purify compound **8** (23 mg). The solution was further purified using YMC gel column, eluting with CH_3OH–H_2O (1/3, v/v) to obtain compound **1** (34 mg) and compound **4** (20 mg). Silica gel column chromatography of fraction E3 (11.2 g) eluted with $CHCl_3$–MeOH–H_2O (4/1/0.1, v/v/v) gave four sub-fractions E3A–E3D. Sub-fractions E3A (0.4 g) and E3B (0.3 g) were further purified chromatographically, on silica gel, eluting with $(CH_3)_2CO/CHCl_3/H_2O$ (2.5/1/0.1, v/v/v) to yield compounds **3** (34 mg) and **6** (133 mg). Compounds **5** (34 mg), **7** (50 mg), and **12** (12 mg) were isolated from the sub-fraction E3D (0.8 g) using YMC gel column chromatography and eluted with $(CH_3)_2CO/H_2O$ (1/2.5). Fraction E4 (32.7 g) appeared as precipitates, from which the solvent was then removed and the residue was washed several times with warm methanol yielding compound **9** (12.0 g). The remaining solution was subjected to silica gel and further to YMC gel chromatography to give compound **2** (7 mg). Fraction E5 (1.4 g) was chromatographed on a silica gel column eluting with a solvent system of $CH_2Cl_2/CH_3OH/H_2O$ (4/1/0.1, v/v/v) to obtain compounds **10** (**7** mg) and **11** (6 mg). Finally, compounds **13** (5 mg) and **14** (24 mg) were isolated from fraction E6 (2.5 g) using a silica gel column chromatography and further purified by preparative TLC, eluted with $(CH_3)_2CO/CHCl_3/H_2O$ (5/1/0.2, v/v/v). The purity of isolated compounds was more than 95%, quantified by analytical HPLC.

3.3.1 Compound 2

Light brown solid, m.p. 215–216°C, $C_{21}H_{24}O_{10}$, $[\alpha]_D^{18} = +23.9°$ ($c = 0.45$, MeOH). UV (MeOH), λ_{max} 276, 224 nm. IR (KBr, cm^{-1}) 3392, 1614, 1518, 1455, 1244, and 1072. ^1H NMR (methanol-d_4, 600 MHz) and ^{13}C NMR (methanol-d_4, 150 MHz) spectral data, see Table 1. ESI-MS (positive mode) *m/z*: 459 $[M + Na]^+$, 437 $[M + H]^+$, 275 $[M + H - 162]^+$. HR-ESI-MS (positive mode) *m/z*: 459.1241 $[M + Na]^+$ (calcd for $C_{21}H_{24}O_{10}Na$ 459.1267).

Table 1. The ^{1}H and ^{13}C NMR spectral data for **2** in CD_3OD.

Position	δ_H^a (mult, *J* in Hz)	δ_C^b
2	4.61 (d, 8.0)	83.0
3	3.98 (m)	68.8
4	2.57 (dd, 16.5, 8.5)	28.9
	3.06 (dd, 16.5, 5.5)	
5	–	158.0
6	6.29 (d, 2.2)	98.1
7	–	158.1
8	6.03 (d, 2.2)	96.9
9	–	156.8
10	–	103.5
1′	–	131.4
2′, 6′	7.21 (d, 8.5)	129.7
3′, 5′	6.79 (d, 8.5)	116.2
4′	–	158.5
5-*O*-Glc		
1″	4.84 (d, 7.5)	102.6
2″	3.42[c]	75.0
3″	3.53[c]	78.3
4″	3.53[c]	71.4
5″	3.61[c]	78.2
6″	3.91 d (11.5) 3.73 dd (11.5, 5.0)	62.6

Assignments were done by HMQC, HMBC, and ^{1}H–^{1}H COSY experiments. Measured at [a]600 MHz, [b]150 MHz, [c]overlapped signals.

3.4 Acid hydrolysis of 2

Compound **2** (2.0 mg) was dissolved in 1.0 N HCl (dioxane/H_2O, 1:1, v/v, 1.0 ml) and then heated to 80°C in a water bath for 3 h. The acidic solution was neutralized with silver carbonate, and the solvent was thoroughly driven out under N_2 gas overnight. After extraction with ethyl acetate, the aqueous layer was concentrated to dryness using N_2 gas. The residue was dissolved in 0.1 ml of dry pyridine, and then L-cysteine methyl ester hydrochloride in pyridine (0.06 M, 0.1 ml) was added to the solution. The reaction mixture was heated at 60°C for 2 h, and 0.1 ml of trimethylsilylimidazole solution was added, followed by heating at 60°C for 1.5 h. The dried product was partitioned with *n*-hexane and H_2O (0.1 ml, each), and the organic layer was analyzed by gas liquid chromatography (GLC): column, column SPB-1 (0.25 mm × 30 m); detector, FID; column temperature, 210°C; injector temperature, 270°C; detector temperature, 300°C; and carrier gas, He. The absolute configuration of the monosaccharide was confirmed to be D-glucose by comparing the retention time of the monosaccharide derivative (t_R 14.11 min) with that of authentic sugar derivative samples prepared in the same manner (D-glucose derivative t_R 14.11 min, L-glucose derivative t_R 14.26 min).

3.5 Cell culture

Human hepatocarcinoma HepG2 cells were maintained at 37°C and 5% CO_2 in Dulbecco's Modified Eagle's Medium (Invitrogen, Carlsbad, CA, USA) containing 10% heat-inactivated fetal bovine serum, 100 units/ml penicillin, and 10 μg/ml streptomycin.

3.6 Cytotoxicity assay

An MTS assay (Promega's CellTiter 96® AQueous One Solution cell proliferation Assay) was used to analyze the effect of compounds on cell viability (Table 2). Cells were cultured overnight in a 96-well plate (~1×10^4 cells/well). Cell viability was assessed after exposure to compounds at concentrations of 10, 50, and 100 μM for 24 h. The number of viable cells was assessed by determination of A_{490nm} of the dissolved formazan product after addition of MTS for 30 min as described by the manufacturer (Promega, Madison, WI, USA).

3.7 NFκB-luciferase assay

Cells were seeded at 5×10^5 cells/ml (1.5 ml) in a 6-well plate and grown for 24 h. All cells were transfected using Lipofectamine LTX (Invitrogen) as described by the manufacturer. Luciferase activity was assayed using an LB 953 AutoLumat (EG&G Berthold, Nashua,

Table 2. Inhibition effects of compounds **1**–**14** on TNF-α-induced NF-κB activation in HepG2 cells.

Compounds	IC_{50} (μM)[a]
1	34.0 ± 2.7
2	>100
3	11.9 ± 0.6
4	>100
5	>100
6	16.5 ± 0.6
7	>100
8	27.4 ± 1.3
9	14.1 ± 0.7
10	>100
11	>100
12	>100
13	>100
14	>100
PDTC[b]	**4.9** ± 1.0

[a] IC_{50} values were derived from dose–response curves. Data are means of three independent experiments.
[b] Pyrrolidine dithiocarbamate (purity ~ 99%, Sigma, Seoul, Korea): positive control.

NH, USA) as described previously [24] and was normalized based on the expression of RSV-β-galactosidase. β-Galactosidase activity was assayed colorimetrically. The NFκB-Luc plasmid was kindly provided by Dr Kyoon E. Kim (Chungnam National University, Daejeon, Korea).

3.8 RT-PCR assay

Total RNA was extracted and isolated with Easy-Blue reagent (Intron Biotechnology, Seoul, Korea). Approximately, 2 μg of total RNA was reversely transcribed using Moloney murine leukemia virus reverse transcriptase and oligo-dT primers (Promega) for 1 h at 42°C. PCR for synthetic cDNA was performed using a Taq polymerase pre-mixture (Takara, Shiga, Japan). PCR products were subjected to electrophoresis on 1% agarose gels and stained with ethidium bromide. PCR was conducted with the following primer pairs: iNOS sense 5′-TCATCCGCTATGCTGGCTAC-3′, iNOS antisense 5′-CTCAGGGTCACGGCCATTG-3′, COX-2 sense 5′-GCCCAGCACTTCACGCATCAG-3′, COX-2 antisense 5′-GACCAGGCACCAGACCAAAGACC-3′, GAPDH sense 5′-TGTTGCCATCAATGACCCCTT-3′, and GAPDH antisense 5′-CTCCACGACGTACTCAGCG-3′. The specificity of products generated by each set of primers was examined using gel electrophoresis and further confirmed by melting curve analysis.

Acknowledgements

This work was supported partly by Priority Research Centers Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science, and Technology (2009-0093815), Republic of Korea. We are grateful to the Korean Basic Science Institute for spectroscopic measurements.

References

[1] P.J. Barnes and M. Karin, *New Engl. J. Med.* **336**, 1066 (1997).
[2] C. Jobin and R.B. Sartor, *Am. J. Physiol. Cell Physiol.* **278**, C451 (2000).
[3] J.R. Vane, J.A. Mitchell, I. Appleton, A. Tomlinson, D. Bishop-Bailey, J. Croxtall, and D.A. Willoughby, *Proc. Natl. Acad. Sci. USA* **91**, 2046 (1994).
[4] B.B. Aggarwal, G. Sethi, A. Nair, and H. Ichikawa, *Curr. Signal Transduct. Ther.* **1**, 25 (2006).
[5] D. Melisi and P.J. Chiao, *Exp. Opin. Ther. Targets* **11**, 133 (2007).
[6] M. Karin and F.R. Greten, *Nat. Rev. Immunol.* **5**, 749 (2005).
[7] J.C. Heo, J.Y. Park, J.M. Lee, T.K. Kwon, S.U. Kim, S.K. Chung, and S.H. Lee, *J. Ethnopharmacol.* **102**, 10 (2005).
[8] P.M. Kaladas, R. Goldberg, and R.D. Poretz, *Mol. Immunol.* **20**, 727 (1983).
[9] K. Baba, K. Takeuchi, M. Doi, M. Inoue, and M. Kozawa, *Chem. Pharm. Bull.* **34**, 1540 (1986).
[10] Y. Cai, F.J. Evans, M.F. Roberts, J.D. Phillipson, M.H. Zenk, and Y.Y. Gleba, *Phytochemistry* **30**, 2033 (1991).
[11] T. Raab, D. Barron, F.A. Vera, V. Crespy, M. Oliveira, and G. Williamson, *J. Agric. Food Chem.* **58**, 2138 (2010).

[12] C.B. Cui, Y. Tezuka, T. Kikuchi, H. Nakano, T. Tamaoki, and J.H. Park, *Chem. Pharm. Bull.* **40**, 2035 (1992).
[13] J. Saijyo, Y. Suzuki, Y. Okuno, H. Yamaki, T. Suzuki, and M. Miyazawa, *J. Olea Sci.* **57**, 431 (2008).
[14] K.R. Min, B.Y. Hwang, H.S. Lim, B.S. Kang, G.-J. Oh, J. Lee, S.H. Kang, K.S. Lee, J.S. Ro, and Y. Kim, *Planta Med.* **65**, 460 (1999).
[15] N. Iida, Y. Inatomi, H. Murata, A. Inada, J. Murata, F.A. Lang, N. Matsuura, and T. Nakanishi, *Chem. Biodivers.* **4**, 32 (2007).
[16] S. Deachathai, W. Mahabusarakam, S. Phongpaichit, and W.C. Taylor, *Phytochemistry* **66**, 2368 (2005).
[17] R. Tschesche, T.M. Braun, and W.V. Sassen, *Phytochemistry* **19**, 1825 (1980).
[18] T. Okuyama, K. Hosoyama, Y. Hiraga, G. Kurono, and T. Takemoto, *Chem. Pharm. Bull.* **26**, 3071 (1978).
[19] B. Wu, T. Takahashi, T. Kashiwagi, S.I. Tebayashi, and C.S. Kim, *Chem. Pharm. Bull.* **55**, 815 (2007).
[20] N. Chaurasia and M. Wichtl, *Planta Med.* **53**, 432 (1987).
[21] C.W. Glennie and J.B. Harborne, *Phytochemistry* **10**, 1325 (1971).
[22] D. Strack, J. Heilmann, V. Wray, and H. Dirks, *Phytochemistry* **28**, 2071 (1989).
[23] T. Yoshida, T. Saito, and S. Kadoya, *Chem. Pharm. Bull.* **35**, 97 (1987).
[24] K.K. Kim, K.S. Park, S.B. Song, and K.E. Kim, *Mol. Carcinog.* **49**, 259 (2010).