Arch Pharm Res Vol 34, No 8, 1277-1282, 2011
DOI 10.1007/s12272-011-0806-8

Archives of Pharmacal Research
www.springer.com/12272

Biological Evaluation of Isoegomaketone Isolated from *Perilla frutescens* and its Synthetic Derivatives as Anti-inflammatory Agents

Yong Dae Park[1], Chang Hyun Jin[1], Dae Seong Choi[1], Myung-Woo Byun[2], and Il Yun Jeong[1]

[1]*Advanced Radiation Technology Institute, Korea Atomic Energy Research Institute, Jeongup 580-185, Korea and* [2]*Department of Culinary Nutrition, Woosong University, Daejeon 300-718, Korea*

(Received Januray 3, 2011/Revised March 22, 2011/Accepted April 4, 2011)

The anti-inflammatory activities of a prepared isoegomaketone **3a** and its derivatives **3b-3f** were evaluated in RAW 264.7 cells. Among these, the compound **3d** was displayed the most potent inhibitory activities against production of nitric oxide, monocyte chemoattractant protein-1 and interleukin-6. Based on these results, the abilities of compounds **3a**-**3f** to modulate NF-κB and AP-1-mediated gene transcription using a luciferase reporter assay were investigated. The transcriptional activities of NF-κB and AP-1 decreased when pretreated with **3a**-**3f**. Interestingly, at 10 μM, compound **3d** markedly suppressed the lipopolysaccharide-induced NF-κB and activator protein-1 DNA binding activities. Some preliminary structure-activity relationships were proposed that may provide a direction for further study.

Key words: Isoegomaketone, Anti-inflammatory activity, Synthesis, *Perilla frutescens*

INTRODUCTION

Inflammation is a natural biological response to injury or infection in the human body. Unregulated inflammation results in the cause of a large number of diseases including rheumatoid arthritis, obesity, cardiovascular diseases, neurodegenerative diseases, diabetes, and cancer. Much progress has been made in the delineation of cell-signalling pathways that the inflammation initiates a cascade of events that result in the overproduction of certain inflammation-associated genes and pro-inflammatory cytokines (O'Neill, 2006). Two inflammation-associated genes, nuclear factor-κ-binding (NF-κB) and activator protein-1 (AP-1), control the production of the cytokines and inflammatory mediators elevated in inflammatory diseases (Manning and Mercurio, 1997; Kristof et al., 2001). NF-κB plays a pivotal role in the expression of various inducible target genes related to immune and inflammatory responses. These genes include tumor necrosis factor-α, interleukin-6 (IL-6), monocyte chemoattractant protein-1 (MCP-1), inducible nitric oxide synthase, cyclooxygenase-2, and intercellular adhesion molecule-1 (Aderem et al., 2000). NF-κB is located in the cytoplasm of unstimulated cells in complex with IκBα, which causes it to remain in its inactive form. Extracellular stimuli, such as lipopolysaccharide (LPS), activate NF-κB by inducing serine phosphorylation of IκBα, which leads to its ubiquitination and degradation. AP-1 is a transcription factor which is a homo- or heterodimeric protein composed of Fos family (c-Fos, Fos-B, Fra-1 and Fra-2) and Jun subfamily (c-Jun, Jun-B and Jun-D) proteins (Shaulian and Karin, 2002).

Inhibition of inflammatory cytokine and mediator production serves as a key mechanism in the control of inflammation. A number of anti-inflammatory drugs that suppress single inflammatory mediator production, such as IL-1, tumor necrosis factor-α, or nitric oxide (NO) have already entered clinical trials for the treatment of inflammatory disorder (Reinhart and Karzai, 2001). Drugs that suppress the expression of these inflammatory mediator productions have, therefore, attracted significant interest as potential therapeutics for the treatment of inflammatory diseases.

While searching for small molecule inhibitors of inflammatory mediator production in mouse macrophages stimulated with LPS, we found that *Perilla frutescens*

Correspondence to: Il Yun Jeong, Advanced Radiation Technology Institute, Korea Atomic Energy Research Institute, Jeongup 580-185, Korea
Tel: 82-63-570-3150, Fax: 82-63-570-3159
E-mail: iyjeong@kaeri.re.kr

methanol extract inhibited NO production. The investigation of *P. frutescens* extract led to the isolation of isoegomaketone (Park et al., 2010), which is an essential oil component of *P. frutescens*. A previous study from our laboratory demonstrated that the inhibition mechanisms of LPS-induced NO production is distinct, in which isoegomaketone inhibited the activation of NF-κB and AP-1 pathways (Jin et al., 2010).

Chemical modification of isoegomaketone (**3a**), focusing on the aromatic heterocyclic ring, was carried out to improve the suppressive effects on the production of NO, MCP-1, and IL-6. In this report, we describe the synthesis, structure-activity relationships, and biological activities of the novel series of isoegomaketone derivatives.

MATERIALS AND METHODS

General experimental procedures

^{1}H-NMR and ^{13}C-NMR were measured downfield relative to tetramethylsilane in $CDCl_3$ unless otherwise noted (value in ppm); coupling constants J are reported in hertz, and conducted on a JNM-ECA 500 spectrometer (Jeol). IR spectra (KBr) were recorded with a Bruker-Vector22 instrument (Bruker). High resonance mass spectra (HRMS) were recorded on JMS-700 (Jeol) and measured at Gyeongsang National University (Chinju). The stationary phases used for column chromatography (Silica gel 60, 70-230 mesh), and TLC plates (Silical-gel 60 F_{254}) were purchased form Merck KGaA. Spots were detected under UV radiation. All the reagent-grade chemicals were purchased from the Sigma-Aldrich.

General procedure for synthesis of 2-substituted-(*E*)-3-(dimethylamino)but-2-en-1-one derivatives 2a-f

A mixture of 20 mmol of acetylated 5-numbered aromatic heterocyclic compounds **1a-1f** and 25 mL of *N*,*N*-dimethylformamide dimethyl acetal was heated under reflux for 12 h. Standard workup procedures were follows. The mixture was evaporated *in vacuo* and the residue was partitioned between the ethyl acetate and water. The organic extracts were combined and washed in the indicated order using the following aqueous solutions: water, 5% aqueous sodium carbonate ($NaHCO_3$) solution, and saturated sodium chloride (NaCl) solution. Extracts were dried over anhydrous magnesium sulfate ($MgSO_4$), filtered, and evaporated *in vacuo*. Chromatographic separations were carried out on silica gel using chloroform/methanol 9:1 to give compound **2a-2f**.

General procedure for synthesis of isoegomaketone (3a) and its derivatives 3b-f

To 10 mmol of the *trans*-enamino ketone **2a-2f** in 100 mL of dry tetrahydrofuran under nitrogen was added 18 mL of 0.7 M isopropyllithium reagent in pentane. After stirring at −40°C for 30 min, the solution was stirred to room temperature for 30 min and 5 mL of water was added. The solvent was evaporated *in vacuo*, and the residue was extracted with dichloromethane. The organic layer was washed with saturated sodium chloride solution, dried over $MgSO_4$, and evaporated under reduced pressure. The obtained residue was purified by chromatography on silica gel using n-hexane/ethyl acetate 9:1 to give the compounds **3a-f**.

(*E*)-3-(dimethylamino)-1-(furan-3-yl)prop-2-en-1-one (2a)

Yield 57%; IR (ν_{max}, cm^{-1}, KBr): 1640 (C=O); HRMS calcd for $C_9H_{11}NO_2$: 165.0789. Found: 165.0792; ^{1}H-NMR (500 MHz, $CDCl_3$, δ, ppm): 2.89 (3H, s), 3.12 (3H, s), 5.40 (1H, d, J = 12.6 Hz), 6.76 (1H, d, J = 1.7 Hz), 7.40 (1H, d, J = 1.1 Hz), 7.75 (1H, d, J = 12.6 Hz), 7.93 (1H, d, J = 1.7 Hz); ^{13}C-NMR (125 MHz, $CDCl_3$, δ, ppm): 36.9, 43.9, 93.1, 109.2, 129.2, 143.3, 144.7, 153.2, 182.7.

(*E*)-3-(dimethylamino)-1-(2,5-dimethylfuran-3-yl) prop-2-en-1-one (2b)

Yield 68%; IR (ν_{max}, cm^{-1}, KBr): 1641 (C=O); HRMS calcd for $C_{11}H_{15}NO_2$: 193.1128. Found: 193.1102; ^{1}H-NMR (500 MHz, $CDCl_3$, δ, ppm): 2.24 (3H, s), 2.55 (3H, s), 2.90 (3H, s), 3.12 (3H, s), 5.35 (1H, d, J = 12.6 Hz), 6.16 (1H, s), 7.67 (1H, d, J = 12.6 Hz); ^{13}C-NMR (125 MHz, $CDCl_3$, δ, ppm): 13.2, 14.1, 36.7, 43.8, 94.3, 105.8, 122.9, 149.0, 152.8, 154.6, 184.8.

(*E*)-3-(dimethylamino)-1-(furan-2-yl)prop-2-en-1-one (2c)

Yield 61%; IR (ν_{max}, cm^{-1}, KBr): 1637 (C=O); HRMS calcd for $C_9H_{11}NO_2$: 165.0789. Found: 165.0793; ^{1}H-NMR (500 MHz, $CDCl_3$, δ, ppm): 2.91 (3H, s), 3.13 (3H, s), 5.67 (1H, d, J = 12.6 Hz), 6.47 (1H, m), 7.06 (1H, m), 7.48 (1H, m), 7.78 (1H, d, J = 12.6 Hz); ^{13}C-NMR (125 MHz, $CDCl_3$, δ, ppm): 37.2, 44.9, 91.4, 111.7, 113.2, 144.1, 153.4, 154.7, 177.4.

(*E*)-3-(dimethylamino)-1-(5-methylfuran-2-yl)prop-2-en-1-one (2d)

Yield 67%; IR (ν_{max}, cm^{-1}, KBr): 1644 (C=O); HRMS calcd for $C_{10}H_{13}NO_2$: 179.0946. Found: 179.0943; ^{1}H-NMR (500 MHz, $CDCl_3$, δ, ppm): 2.37 (3H, s), 2.92 (3H, s), 3.12 (3H, s), 5.62 (1H, d, J = 12.6 Hz), 6.09

(1H, d, J = 3.4 Hz), 6.96 (1H, d, J = 3.4 Hz), 7.76 (1H, d, J = 12.6 Hz); ^{13}C-NMR (125 MHz, $CDCl_3$, δ, ppm): 13.9, 36.8, 43.9, 91.3, 108.3, 114.9, 153.1, 153.3, 154.9, 177.3.

(*E*)-3-(dimethylamino)-1-(thiophen-3-yl)prop-2-en-1-one (2e)

Yield 56%; IR (ν_{max}, cm^{-1}, KBr): 1641 (C=O); HRMS calcd for $C_9H_{11}NOS$: 181.0561. Found: 181.0563; ^{1}H-NMR (500 MHz, $CDCl_3$, δ, ppm): 2.91 (3H, s), 3.13 (3H, s), 5.58 (1H, d, J = 12.6 Hz), 7.27 (1H, dd, J = 3.1, 5.1 Hz), 7.53 (1H, dd, J = 1.1, 4.8 Hz), 7.77 (1H, d, J = 12.6 Hz), 7.90 (1H, dd, J = 1.1, 2.9 Hz); ^{13}C-NMR (125 MHz, $CDCl_3$, δ, ppm): 36.7, 43.8, 92.9, 125.4, 127.3, 128.4, 144.9, 153.9, 182.9.

(*E*)-3-(dimethylamino)-1-(1-methyl-1H-pyrrol-3-yl) prop-2-en-1-one (2f)

Yield 49%; IR (ν_{max}, cm^{-1}, KBr): 1644 (C=O); HRMS calcd for $C_{10}H_{14}N_2O$: 178.1106. Found: 178.1104; ^{1}H-NMR (500 MHz, $CDCl_3$, δ, ppm): 2.96 (6H, s), 3.66 (3H, s), 5.48 (1H, d, J = 12.6 Hz), 6.54 (2H, m), 7.21 (1H, s), 7.69 (1H, d, J = 12.6 Hz); ^{13}C-NMR (125 MHz, $CDCl_3$, δ, ppm): 36.3, 36.9, 92.9, 108.6, 122.2, 124.7, 127.4, 152.1, 184.2.

(*E*)-1-(furan-3-yl)-4-methylpent-2-en-1-one (3a)

Yield 38%; IR (ν_{max}, cm^{-1}, KBr): 1668 (C=O); HRMS calcd for $C_{10}H_{12}O_2$: 164.0837. Found: 164.0841; ^{1}H-NMR (500 MHz, $CDCl_3$, δ, ppm): 1.12 (6H, d, J = 6.9 Hz), 2.53 (1H, m), 6.49 (1H, dd, J = 1.4, 15.4 Hz), 6.83 (1H, m), 7.03 (1H, dd, J = 6.9, 15.4 Hz), 7.45 (1H, m), 8.05 (1H, m); ^{13}C-NMR (125 MHz, $CDCl_3$, δ, ppm): 21.3, 31.2, 109.1, 123.9, 128.1, 144.1, 147.1, 154.5, 184.8.

(*E*)-1-(2,5-dimethylfuran-3-yl)-4-methylpent-2-en-1-one (3b)

Yield 62%; IR (ν_{max}, cm^{-1}, KBr): 1668 (C=O); HRMS calcd for $C_{12}H_{16}O_2$: 192.1150. Found: 192.1149; ^{1}H-NMR (500 MHz, $CDCl_3$, δ, ppm): 1.10 (6H, d, J = 6.6 Hz), 2.26 (3H, s), 2.50 (1H, m), 2.55 (3H, s), 6.23 (1H, s), 6.47 (1H, dd, J = 1.4, 15.4 Hz), 6.94 (1H, dd, J = 6.6, 15.4 Hz); ^{13}C-NMR (125 MHz, $CDCl_3$, δ, ppm): 13.1, 14.3, 21.4, 31.1, 105.7, 122.1, 124.9, 149.8, 153.6, 157.5, 186.7.

(*E*)-1-(furan-2-yl)-4-methylpent-2-en-1-one (3c)

Yield 31%; IR (ν_{max}, cm^{-1}, KBr): 1664 (C=O); HRMS calcd for $C_{10}H_{12}O_2$: 164.0837. Found: 164.0836; ^{1}H-NMR (500 MHz, $CDCl_3$, δ, ppm): 1.12 (6H, d, J = 6.9 Hz), 2.56 (1H, m), 6.55 (1H, m), 6.75 (1H, dd, J = 1.4, 15.4 Hz), 7.13 (1H, dd, J = 6.9, 15.4 Hz), 7.25 (1H, m), 7.62 (1H, m); ^{13}C-NMR (125 MHz, $CDCl_3$, δ, ppm): 21.1, 31.2, 112.1, 117.3, 121.5, 146.3, 153.2, 154.9, 178.3.

(*E*)-4-methyl-1-(5-methylfuran-2-yl)pent-2-en-1-one (3d)

Yield 73%; IR (ν_{max}, cm^{-1}, KBr): 1659 (C=O); HRMS calcd for $C_{11}H_{14}O_2$: 178.0993. Found: 179.0994; ^{1}H-NMR (500 MHz, $CDCl_3$, δ, ppm): 0.96 (6H, d, J = 6.9 Hz), 2.25 (3H, s), 2.38 (1H, m), 6.03 (1H, m), 6.54 (1H, dd, J = 1.4, 15.4 Hz), 6.92 (1H, dd, J = 6.9, 15.4 Hz), 7.02 (1H, m); ^{13}C-NMR (125 MHz, $CDCl_3$, δ, ppm): 13.7, 21.1, 30.9, 108.8, 119.2, 121.8, 151.8, 153.9, 157.6, 177.4.

(*E*)-4-methyl-1-(thiophen-3-yl)pent-2-en-1-one (3e)

Yield 28%; IR (ν_{max}, cm^{-1}, KBr): 1657 (C=O); HRMS calcd for $C_{10}H_{12}OS$: 180.0608. Found: 180.0605; ^{1}H-NMR (500 MHz, $CDCl_3$, δ, ppm): 1.11 (6H, d, J = 6.8 Hz), 2.53 (1H, m), 6.69 (1H, dd, J = 1.4, 15.5 Hz), 7.03 (1H, dd, J = 6.8, 15.5 Hz), 7.32 (1H, m), 7.57 (1H, m), 8.05 (1H, m); ^{13}C-NMR (125 MHz, $CDCl_3$, δ, ppm): 21.4, 31.4, 123.6, 126.2, 127.4, 131.9, 142.8, 155.2, 184.6.

(*E*)-4-methyl-1-(1-methyl-1H-pyrrol-3-yl)pent-2-en-1-one (3f)

Yield 34%; IR (ν_{max}, cm^{-1}, KBr): 1660 (C=O); HRMS calcd for $C_{11}H_{15}NO$: 177.1153. Found: 177.1155; ^{1}H-NMR (500 MHz, $CDCl_3$, δ, ppm): 1.07 (6H, d, J = 6.8 Hz), 2.49 (1H, m), 3.67 (3H, s), 6.54 (1H, dd, J = 1.4, 15.5 Hz), 6.56 (1H, m), 6.62 (1H, m), 6.94 (1H, dd, J = 6.8, 15.5 Hz), 7.26 (1H, m); ^{13}C-NMR (125 MHz, $CDCl_3$, δ, ppm): 21.4, 31.1, 36.5, 109.6, 123.3, 124.1, 126.2, 126.9, 152.2, 185.2.

Cytotoxicity assay

To measure the cell viability, an EZ-Cytox cell viability assay kit (DAEIL lab) was used. The cells were cultured in a 96-well plate (5.0×10^4 cell/well) at 37°C, 5% CO_2 for 24 h and subsequently treated with various concentrations of the derivatives **3a-3f** for 24 h. After the incubation period, 10 μL of the kit solution was added into each well and incubated for 4 h. The index of cell viability was determined by measuring the OD of formazan production at 480 nm using an enzyme-linked immunosorbent assay (ELISA) plate reader at an absorbance of 480 nm. The reference wavelength was 650 nm.

NO measurement

RAW 264.7 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), penicillin (100 U/mL), and

streptomycin (100 μg/mL), and held at 37°C with 5% CO_2. The cells were cultured in a 96-well plate at a concentration of 2.0×10^4 cell/well for 24 h. Cells were then treated with various concentrations of the derivatives **3a-3f** for 2 h. Then, cells were treated with LPS (1 μg/mL) for 18 h. The cell supernatants were collected at the end of the culture and tested for nitrite, as a measurement of NO production. Equal volumes of the Griess reagent (100 μL; Sigma-Aldrich) were added to each cell supernatant (100 μL), and the absorbance was measured at 570 nm. The concentration of nitrite (μM) was calculated from a standard curve drawn with a known concentration of sodium nitrite dissolved in DMEM. The results are presented as the mean ± S.D. from 3 separate experiments, with 4 replicates each.

Measurement of MCP-1 and IL-6 by ELISA

Cells cultured at a concentration of 2.0×10^5 cell/mL were incubated for 24 h and then the derivatives **3a-3f** were added for 2 h. Then, LPS (1 μg/mL) was added for 4 h. To determine the resultant cytokines, the supernatants were harvested and the levels of IL-6 and MCP-1 were measured by using an ELISA kit (R&D systems). The results are presented as the mean ± S.D. from 3 separate experiments, with 4 replicates each.

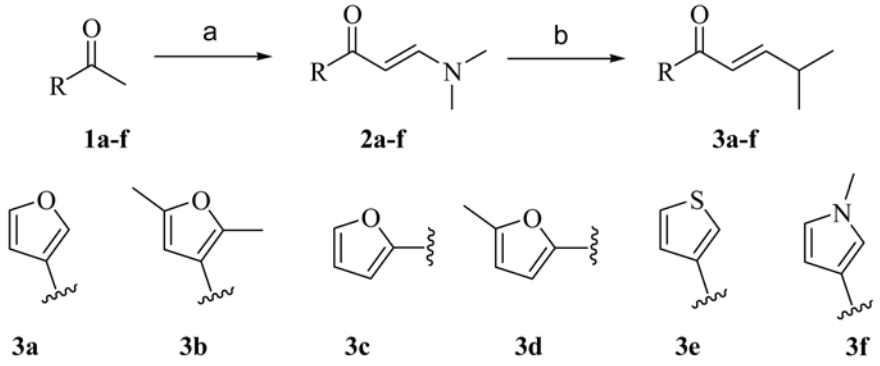
Luciferase assay

The cells were cultured in a 6-well dish for 24 h at a concentration of 4×10^5 cell/mL. Vectors were transfected into the cell using Lipofectamine 2000 according to the manufacturer's instructions. The pNF-κB-*Luc* (Stratagene) and pAP-1-*Luc* vectors (Panomics) were transfected into cells at concentration of 5 μg per well. The pRL-TK vector (Promega) was transfected into cells at a concentration of 1 μg per well. After the transfection, compounds **3a-3f** were added at a 10 μM concentration for 2 h and the cells were treated with LPS at a concentration of 1 μg/mL for 24 h. Luciferase activity was measured with the Dual-luciferase Reporter Assay System (Promega).

RESULTS AND DISCUSSION

Isoegomaketone **3a** and its derivatives **3b-f** were synthesized according to the method shown in Scheme 1. The conversion of ketones to α,β-unsaturated ketones was performed as previously reported method (Abdulla and Fuhr, 1978). The appropriate commercially available acetylated aromatic heterocyclic compounds **1a-f** and *N,N*-dimethylformamide dimethyl acetal were heated under reflux for 12 h to produce compounds **2a-f**. The isoegomaketone derivatives **3a-f** were obtained by isopropylation using an isopropyllithium reagent. The chemical structures of these compounds were determined using several spectroscopic analyses including IR, ^{1}H-NMR, ^{13}C-NMR and mass spectrometry.

The pro-inflammatory cytokine, IL-6, as well as pro-inflammatory mediators, NO and MCP-1, play important roles in the inflammatory process (Heinrich et al., 2003; Melgarejo et al., 2009). Therefore, the compounds were evaluated for their suppressive activity against NO, MCP-1, and IL-6 production using a murine macrophage cell line, RAW 264.7, induced by LPS. We initially examined the cytotoxicity. After treatment with compounds **3a-3f**, RAW 264.7 cell viability was determined using an MTT assay. Compounds **3a-3f** did not significantly affect cell viability in the 0-10 μM range (Fig. 1). As shown in Table I, isoegomaketone **3a** showed moderate potency with an IC_{50} value of 6.9 and 6.6 μM against NO and MCP-1, respectively. Introduction of a methyl group at the 2,5-positions of the furan ring **3b** resulted in a two-fold increase in potency in suppression of NO production, but only slightly improved inhibitory activity toward MCP-1 production (IC_{50} 5.1 μM). Compound **3c**, which con-



Scheme 1. Synthesis of derivatives **3a-3f**. Reagents and conditions: (a) $(CH_3)_2NCH(OCH_3)_2$, reflux; (b) *i*-PrLi, THF, -40.

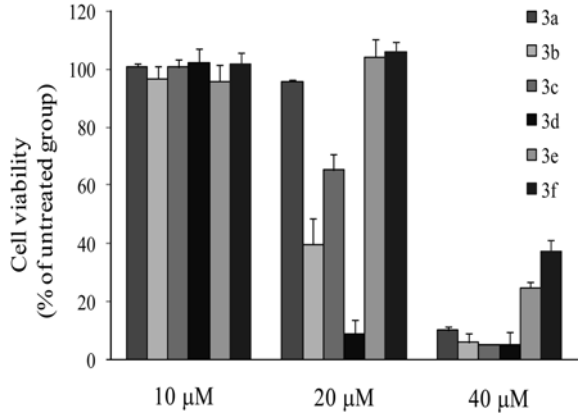


Fig. 1. Cytotoxicity of derivatives **3a-3f** in RAW 264.7 cells. RAW 264.7 cells were incubated with the derivatives **3a-3f** for 24 h, and cell viability was determined by an MTT assay. The absorbance of control cells was taken as 100%. The results are expressed as the mean ± S.D. of values obtained from triplicate cultures. Data shown are representative of three independent experiments with similar results.

Table I. Inhibitory activities (IC_{50}) of derivatives **3a-3f** against LPS-induced NO, MCP-1 and IL-6 production

Compound	IC_{50} (μM) [a]		
	NO	MCP-1	IL-6
3a	6.9 ± 0.21	6.6 ± 0.45	13.3 ± 1.14
3b	3.8 ± 0.31	5.1 ± 0.18	14.2 ± 1.21
3c	5.5 ± 0.47	4.1 ± 0.12	> 15
3d	1.9 ± 0.18	2.0 ± 0.34	9.4 ± 0.84
3e	8.2 ± 1.21	14.8 ± 1.14	> 20
3f	8.4 ± 0.84	12.6 ± 0.78	16.2 ± 0.89

[a] IC_{50} values are calculated from means ± S.D. of three experiments.

tains an α,β-unsaturated carbonyl side chain substitution at the 2-position of furan ring, was equipotent to compound **3a**. Introduction of a methyl group at the 5-position of compound **3d**, however, resulted in a three-fold improvement in inhibitory activities toward NO and MCP-1 production, with IC_{50} values of 1.9 and 2.0 μM respectively. These data suggest that hydrophobic substituents at the 2- and 5-position of furan ring are favorable for its inhibitory effects. The thiophene-, and pyrrole-substituted analogues **3e-f** at the 3-position of the compounds showed slightly lower activities. In the case of cytokine IL-6, inhibitory activity was not significantly improved in these derivatives, but compound **3d** showed slightly higher activity compared to compound **3a**, with an IC_{50} value of 9.4 μM.

Next, attention was focused on the ability of the compounds **3a-3f** to modulate NF-κB and AP-1-mediated gene transcription using a luciferase reporter assay in RAW 264.7 cells. For the purpose of this

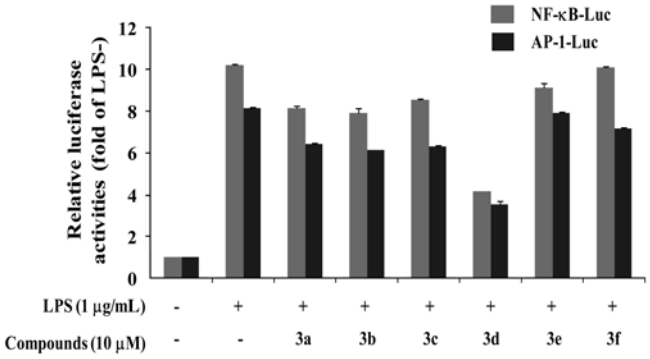


Fig. 2. Effect of derivatives **3a-3f** on the NF-κB and AP-1 pathway. After transfection of pNF-κB-*Luc* or pAP-1-*Luc* vectors, the pRL-TK vector was transfected into cells. After transfection, derivatives **3a-3f** were added at a 10 μM for 2 h and the cells were treated with LPS for 24 h.

study, we hypothesized that isoegomaketone derivatives **3a-3f** would inhibit the expression of these genes through the suppression of NF-κB or AP-1 activation. NF-κB plays an important role in many aspects of human inflammatory diseases (Diaz-Guerra et al., 1996). As indicated in Fig. 2, LPS treatment caused a significant increase in NF-κB and AP-1 DNA binding activities. These binding activities were decreased by pretreatment with **3a-3f**. In particular, compound **3d** markedly suppressed the LPS-induced NF-κB and AP-1 DNA binding activities at 10 μM. These results are strongly suggest that the inhibitory effect of isoegomaketone derivatives on LPS-stimulated pro-inflammatory cytokines and mediator production might be regulated by both NF-κB and AP-1 transcriptional activation.

In this study we have described the anti-inflammatory activities of isoegomaketone derivatives. To investigate the mode of action of derivatives **3a-3f** as anti-inflammatory agents, the effect on LPS-induced inflammatory responses was investigated in a RAW 264.7 mouse macrophage cell line. Among all the compounds prepared, **3d** exhibited the most potent inhibitory effect on the production of NO, MCP-1, and IL-6. These IC_{50} values were approximately 3-fold stronger than isoegomaketone. Furthermore, **3d** showed significant suppression of NF-κB and AP-1 DNA binding activities. The α,β-unsaturated ketone position of the isoegomaketone is most likely the pharmacophore of this class of compounds since naturally occurring perillaketone which is C-C double bond reduced compound of isoegomaketone completely lost inhibitory effect on NO production (Jin et al., 2010).

In conclusion, we have discovered a novel series of isoegomaketone derivatives. A representative compound **3d** of the series showed significant anti-in-

flammatory activities, indicating that isoegomaketone modified compounds might have therapeutic value for inflammatory disorders.

ACKNOWLEDGEMENTS

This research was supported by Agricultural R&D Promotion Center of the Ministry for Food, Agriculture, Forestry and Fisheries, Korea.

REFERENCES

Abdulla, R. F. and Fuhr, K. H., An efficient conversion of ketones to α,β-unsaturated ketones. *J. Org. Chem*., 43, 4248-4250 (1978).

Aderem, A. and Ulevitch, R. J., Toll-like receptors in the induction of the innate immune response. *Nature,* 406, 782-787 (2000).

Diaz-Guerra, M. J., Velasco, M., Martin-Sanz, P., and Bosca, L., Evidence for common mechanisms in the transcriptional control of type II nitric oxide synthase in isolated hepatocytes. Requirement of NF-kappaB activation after stimulation with bacterial cell wall products and phorbol esters. *J. Biol. Chem.,* 271, 30114-30120 (1996).

Heinrich, P. C., Behrmann, I., Haan, S., Hermanns, H. M., Muller-Newen, G., and Schaper, F., Principles of interleukin (IL)-6-type cytokine signalling and its regulation. *Biochem. J.,* 374, 1-20 (2003).

Jin, C. H., Lee, H. J., Park, Y. D., Choi, D. S., Kim, D. S., Kang, S. Y., Seo, K. I., and Jeong, I. Y., Isoegomaketone inhibits lipopolysaccharide-induced nitric oxide production in RAW 264.7 macrophages through the heme oxygenase-1 induction and inhibition of the interferon-beta-STAT-1 pathway. *J. Agric. Food Chem.,* 58, 860-867 (2010).

Kristof, A. S., Marks-Konczalik, J., and Moss, J., Mitogen-activated protein kinases mediate activator protein-1-dependent human inducible nitric-oxide synthase promoter activation. *J. Biol. Chem.,* 276, 8445-8452 (2001).

Manning, A. M. and Mercurio, F., Transcription inhibitors in inflammation. *Expert Opin. Investig. Drugs,* 6, 555-567 (1997).

Melgarejo, E., Medina, M. A., Sanchez-Jimenez, F., and Urdiales, J. L., Monocyte chemoattractant protein-1: a key mediator in inflammatory processes. *Int. J. Biochem. Cell Biol.,* 41, 998-1001 (2009).

O'Neill, L. A., Targeting signal transduction as a strategy to treat inflammatory diseases. *Nat. Rev. Drug Discov.,* 5, 549-563 (2006).

Park, Y. D., Lee, Y. M., Kang, M. A., Lee, H. J., Jin, C. H., Choi, D. S., Kim, D. S., Kang, S. Y., Kim, W. G., and Jeong, I. Y., Phytochemical profiles and *in vitro* anti-inflammatory properties of *Perilla frutescens* cv. Chookyoupjaso mutants induced by mutagenesis with γ-ray. *Food Sci. Biotechnol*., 19, 305-311 (2010).

Reinhart, K. and Karzai, W., Anti-tumor necrosis factor therapy in sepsis: update on clinical trials and lessons learned. *Crit. Care Med.,* 29, S121-S125 (2001).

Shaulian, E. and Karin, M., AP-1 as a regulator of cell life and death. *Nat. Cell Biol.,* 4, E131-E136 (2002).