Bioorganic & Medicinal Chemistry Letters 22 (2012) 700–705

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

4′-Bromo-5,6,7-trimethoxyflavone represses lipopolysaccharide-induced iNOS and COX-2 expressions by suppressing the NF-κB signaling pathway in RAW 264.7 macrophages

Dong Han Kim [a,†], Chang Hyeon Yun [a,†], Min Hwan Kim [a], Ch. Naveen Kumar [a], Bo Hee Yun [a], Ji-Sun Shin [a,b], Hyo Jin An [a], Young Hun Lee [a], Yong Don Yun [a], Hong-Kun Rim [a,b], Min-Sang Yoo [a,b], Kyung-Tae Lee [a,b,*], Yong Sup Lee [a,*]

[a] Department of Life and Nanopharmaceutical Science & Department of Pharmaceutical Science, College of Pharmacy, Kyung Hee University, Seoul 130 701, Republic of Korea
[b] Department of Biomedical Science, College of Medical Science, Kyung Hee University, Republic of Korea

ARTICLE INFO

Article history:
Received 12 August 2011
Revised 10 October 2011
Accepted 12 October 2011
Available online 31 October 2011

Keywords:
5,6,7-Trimethoxyflavone
Oroxylin A
iNOS
COX-2
NF-κB
Anti-inflammatory

ABSTRACT

The regulations of the NO and PGE_2 productions are research topics of interest in the field of anti-inflammatory drug development. In the present study, 5,6,7-trimethoxy- and 5,6,7-trihydroxyflavones **3a–3g** were synthesized from cinnamic acid derivatives. In particular, 4′-bromo-5,6,7-trimethoxyflavone (**3b**) most potently inhibited the productions of NO and PGE_2 in LPS-treated RAW 264.7 cells (IC_{50} = 14.22 ± 1.25 and 10.98 ± 6.25 μM, respectively), and these inhibitory effects were more potent than those of oroxylin A or baicalein. Consistent with these findings, **3b** concentration-dependently reduced the LPS-induced expressions of iNOS and COX-2 at the protein and mRNA levels. In addition, the release of TNF-α, IL-6, and IL-1β and the mRNA expressions of these cytokines were reduced by **3b** in a concentration-dependent manner. Furthermore, **3b** attenuated the LPS-induced transcriptional activities of NF-κB and this was accompanied by parallel reductions in the degradation and phosphorylation of IκB-α, and consequently by a decrease in the nuclear translocation of the p65 subunit of NF-κB. Taken together, these results suggest that suppressions of the expressions of iNOS, COX-2, TNF-α, IL-6, and IL-1β via NF-κB inactivation are responsible for the anti-inflammatory effects of **3b**.

Inflammation, characterized by redness, swelling, pain, and a sensation of heat, is one of the most important components of the host defense mechanisms against invading pathogens. However, inflammation may also aid microbial pathogeneses because the inflammatory responses elicited by invading microorganisms can result in considerable host damage, make nutrients available, and provide access to host tissues.[1]

In the inflammatory state, activated immune cells secrete excessive amounts of NO, PGE_2, and pro-inflammatory cytokines. NO and PGs are pleiotypic mediators produced at inflammatory sites by iNOS and COX-2, respectively. The overproduction of NO is mainly caused by iNOS, which is up-regulated in macrophages by cytokines and/or bacterial LPS.[2,3] Furthermore, despite its beneficial role in host defense, sustained NO production can be deleterious to the host, for example, NO has been implicated in the pathogeneses of various inflammatory diseases, such as, arthritis, asthma, multiple sclerosis, inflammatory bowel disease, and atherosclerosis.[1,2,4] On the other hand, elevated COX-2 expression causes the excessive production of inflammatory PGs. In particular, PGE_2 is a major COX-2 product at inflammatory sites, where it contributes to increases in local blood flow, edema formation, and pain sensitization.[5]

The pathogenesis of inflammation is highly complex process in cells of the immune system, which in turn is regulated by cytokine networks and a plethora of pro-inflammatory genes. Furthermore, the release of pro-inflammatory cytokines by macrophages importantly regulates inflammatory responses, and macrophages are the major cellular source of TNF-α, IL-1, and IL-6, which in turn participate in the mediation of acute phase responses to injury.[6] Accordingly, the regulations of NO, PGE_2, and cytokines, such as IL-1β, IL-6, and TNF-α are considered important targets by those trying to develop anti-inflammatory agents.[7–9]

Scutellaria baicalensis Georgi (Huang Qui) is an important medicinal herb, which is often used to treat inflammatory diseases, hepatitis, tumors, and diarrhea in China, Korea, Taiwan, and Japan.[10,11] Furthermore, this plant has been reported to contain a large number of flavonoids. Oroxylin A (**1**, 5,7-dihydroxy-6-methoxyflavone) is the major component of Scutellariae Radix, and has been shown to possess diverse pharmacological activities, which include anti-cancer, hepatoprotective, anti-allergic, and

* Corresponding authors. Tel.: +82 2 961 0370; fax: +82 2 966 3885.
E-mail addresses: ktlee@khu.ac.kr (K.-T. Lee), kyslee@khu.ac.kr (Y.S. Lee).
† These authors contributed equally to this paper.

0960-894X/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2011.10.067

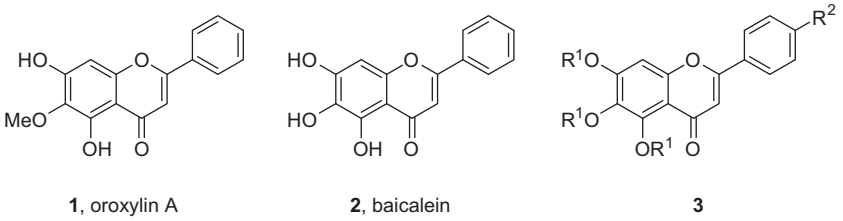
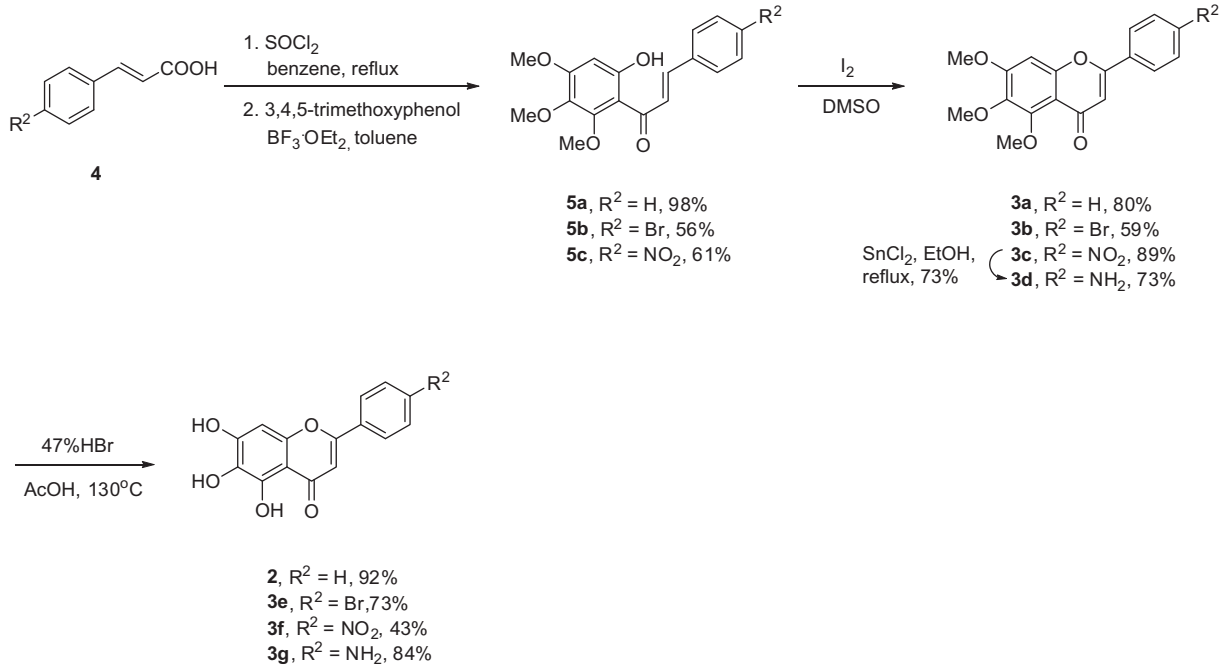
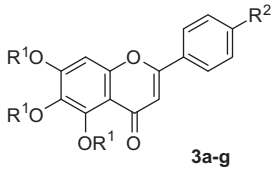


Figure 1. Chemical structures of oroxylin A (**1**), baicalein (**2**) and 5,6,7-trimethoxyflavones (**3**).



Scheme 1. Synthetic route to 5,6,7-trimethoxy- and 5,6,7-trihydroxyflavones **3a**–**3g**.

Table 1
The cytotoxicities and inhibitory effects of oroxylin A, baicalein, and compounds **3a**–**3g** on the LPS-induced productions of NO and PGE_2



3a-g

Compounds	R^1	R^2	Cell viability, IC_{50} (μM)	% Inhibition at 25 μM[a]	
				NO production	PGE_2 production
3a	CH_3	H	>100	12.10 ± 0.93	42.19 ± 3.83
3b	CH_3	Br	69.88 ± 5.32	89.63 ± 7.32 (14.22 ± l.25)[b]	89.61 ± 6.25 (10.98 ± 6.25)[b]
3c	CH_3	NO_2	>100	17.41 + 1.53	84.41 ± 5.32
3d	CH_3	NH_2	>100	23.10 + 1.26	69.54 ± 4.95
3e	H	Br	57.11 + 1.91	27.03 + 2.06	29.87 ± 2.32
3f	H	NO_2	>100	7.65 ± 0.61	29.87 ± 2.01
3g	H	NH_2	94.13 ± 6.71	6.98 ± 0.48	83.11 ± 5.99
1 (Oroxylin A)			16.73 ± 1.35	12.44 + 1.02	—[c]
2 (Baicalein)	H	H	>100	11.13 + 0.88	60.87 ± 3.91

[a] Values represent means ± SDs of three independent experiments.
[b] Numbers in parenthesis are IC_{50} values in μM.
[c] No inhibition at less than the cytotoxic concentration.

anti-inflammatory effects.[12–14] It has also been reported to inhibit the LPS-induced expressions of iNOS and COX-2 by suppressing NF-κB activation.[15] However, during our efforts to identify new anti-inflammatory agents by examining their inhibitory effects

on the productions of NO and PGE_2, we realized that structural modification of oroxylin A was probably needed to increase its anti-inflammatory effect. In fact, in a previous study involving structural modifications of oroxylin A, it was observed that acylation of its 7-hydroxyl group significantly enhanced its antibacterial activity.[16] Methylations of the hydroxyl groups of oroxylin A (**1**) or baicalein (**2**) to produce 5,6,7-trimethoxyflavone, were also found to enhance the anticancer,[17] anti-aggregatory,[18] and anti-inflammatory activities[19] of the parent compound. However, although the inhibitory effects of 5,6,7-trimethoxyflavones derivatives were screened with respect to LPS-induced NO production, and arachidonic acid and collagen-induced aggregation, the underlying mechanism involved was not elucidated. Accordingly, we synthesized various baicalein and oroxylin A derivatives with the aim to producing adducts that more potently inhibit the LPS-induced productions of inflammatory mediators in RAW 264.7 cells. In addition, we also attempted to identify the mechanisms underlying their anti-inflammatory effects. Structural modifications were carried out by methylating the 5- and 7-hydroxyl groups or by demethylating the 6-*O*-methyl group on the A-ring, and by introducing substituents on the B-ring of oroxylin A (Fig. 1).

Oroxylin A derivatives were synthesized as shown in Scheme 1 using a modification of previously reported procedures.[18,20] Cinnamic acid derivatives **4** were transformed to cinnamoyl chlorides by reaction with thionyl chloride in benzene and coupled with 3,4,5-trimethoxyphenol in the presence of $BF_3{\cdot}Et_2O$ to afford the corresponding chalcones **5a**–**c**. Intramolecular oxidative cyclization of these chalcones with iodine in DMSO then provided the 5,6,7-trimethoxyflavones **3a**–**c**. The 4′-nitro group in **3c** was reduced using tin(II) chloride to give the 4′-amino-5,6,7-trimethoxyflavone (**3d**). The methyls of the methoxy groups on the A-ring of the trimethoxyflavones **3a**–**d** were removed by treating them with 47% HBr in glacial acetic acid to provide 5,6,7-trihydroxyflavones, baicalein (**2**) and **3e**–**g**.[21]

Initially, the synthesized flavones **3a**–**3g** were screened for their inhibitory effects on the LPS-induced productions of NO and PGE_2 in RAW 264.7 cells. The cytotoxic effects and inhibitory activities of the oroxylin A derivatives **3a**–**3g** on the productions of NO and PGE_2 are listed in Table 1; data on oroxylin A (**1**) and baicalein (**2**) were included for comparison purposes. In most cases, the 5,6,7-trimethoxyflavones (**3a**–**3d**) and 5,6,7-trihydroxyflavones (**3e**–**3g**) showed much less cytotoxicity (IC_{50} > 50 μM) than the parent compound oroxylin A (**1**, IC_{50} = 16.73 μM). In our assay systems, oroxylin A

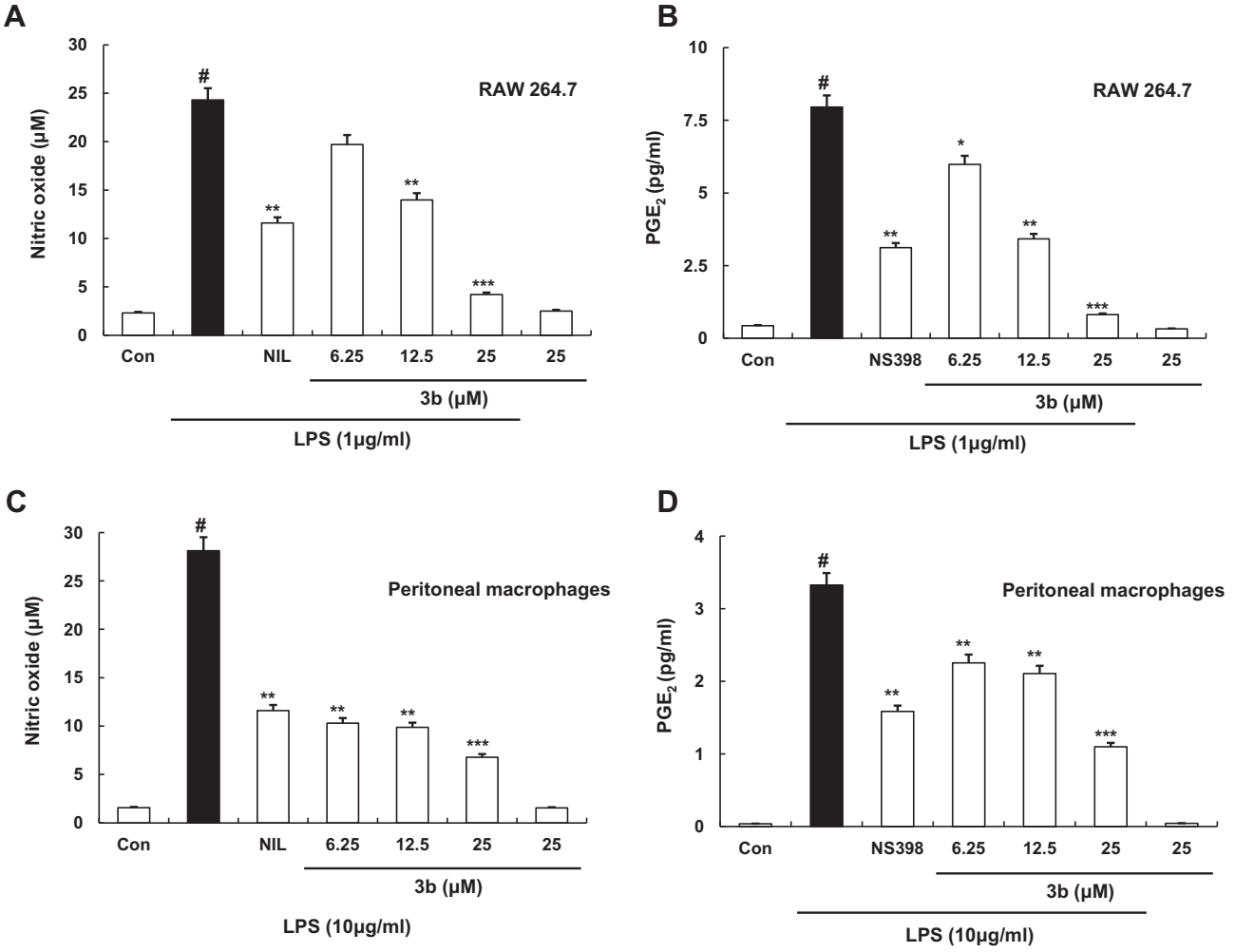


Figure 2. Effects of **3b** on LPS-induced NO and PGE_2 production in RAW 264.7 macrophages. (A) Cells were pretreated with different concentrations (6.25, 12.5 or 25 μM) of **3b** for 1 h, then with LPS (1 μg/ml), and incubated for 24 h. NO levels in culture media were determined using Griess assays. L-*N*6-(1-Iminoethyl) lysine (L-NIL, 10 μM) was used as a positive NO production inhibitor. (B) Samples were treated as described in the legend of panel A. *N*-[2-(cyclohexyloxy)-4-nitrophenyl]-methanesulfonamide (NS-398, 10 μM) was used as a positive PGE_2 production inhibitor. Level of PGE_2 in culture media was quantified using EIA kits. (C) Peritoneal macrophages isolated from C57BL/6 mice were treated as described in the legend of panel A. Values represent the means ± SDs of three independent experiments. $^{\#}p < 0.05$ as compared with the control cells, and $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ as compared with the LPS-only treated cells. ANOVA and Dunnett's post-hoc test were used to determine the significances of differences.

(**1**) exhibited no noticeable effect on NO or PGE_2 production under its cytotoxic concentration and baicalein inhibited only the production of PGE_2 by 60.87% at 25 μM concentration. On the other hand, compounds **3b**–**3d** and **3g** more potently inhibited PGE_2 production (by 69.54–89.61%) than baiclein at the same concentration. In particular, 4′-bromo-5,6,7-trimethoxyflavone (**3b**) potently inhibited the productions of NO and PGE_2, and these inhibitory effects (IC_{50} = 14.22 ± 1.25, and 10.98 ± 6.25 μM, respectively) were greater than those of oroxylin A (**1**) and baicalein (**2**). As shown in Figure 2A and B, **3b** significantly and concentration-dependently inhibited LPS-induced NO and PGE_2 production in RAW 264.7 cells. The synthesized compounds **3a**–**3g** did not affect RAW 264.7 cell viability in either the presence or absence of LPS at concentrations up to 50 μM for 24 h, indicating that modification or substitution of the A- or B-rings of oroxylin A may reduce cytotoxicity and enhance inhibition of NO and PGE_2 production in LPS-induced RAW 264.7 cells. Accordingly, we sought to identify the mechanisms responsible for the anti-inhibitory effect of **3b** in RAW 264.7 cells.

To determine whether **3b** has inhibitory effects on the LPS-induced productions of NO and PGE_2 in vivo, we examined the effects of **3b** on LPS-induced NO and PGE_2 production in peritoneal macrophages isolated from thioglycolate-injected C57BL/6 mice. **3b** was found to inhibit the LPS-induced productions of NO and PGE_2 concentration-dependently in these cells (Fig. 2C and D). We then investigated whether the inhibitory effects of **3b** on the production of NO and PGE_2 are related to changes in the expressional levels of iNOS and COX-2. In unstimulated RAW 264.7 cells, iNOS and COX-2 protein levels were undetectable, but after treatment with LPS (1 μg/ml), they were markedly upregulated. Furthermore, **3b** significantly and concentration-dependently inhibited these expressions (Fig. 3A). Because changes in iNOS and COX-2 levels could reflect altered protein synthesis or degradation, qRT-PCR was performed to determine whether **3b** suppresses LPS-induced iNOS and COX-2 expression via a pre-translational mechanism. After treating RAW 264.7 macrophages with **3b** (6.25, 12.5, or 25 μM) and LPS (1 μg/ml) for 4 h, iNOS and COX-2 mRNA levels were elevated about five and seven-fold, respectively. Furthermore, **3b** significantly suppressed the gene expressions of iNOS and COX-2 (Fig. 3B). These results indicate that the inhibition of iNOS and COX-2 expressions by **3b** occurs in parallel with inhibition of the productions of NO and

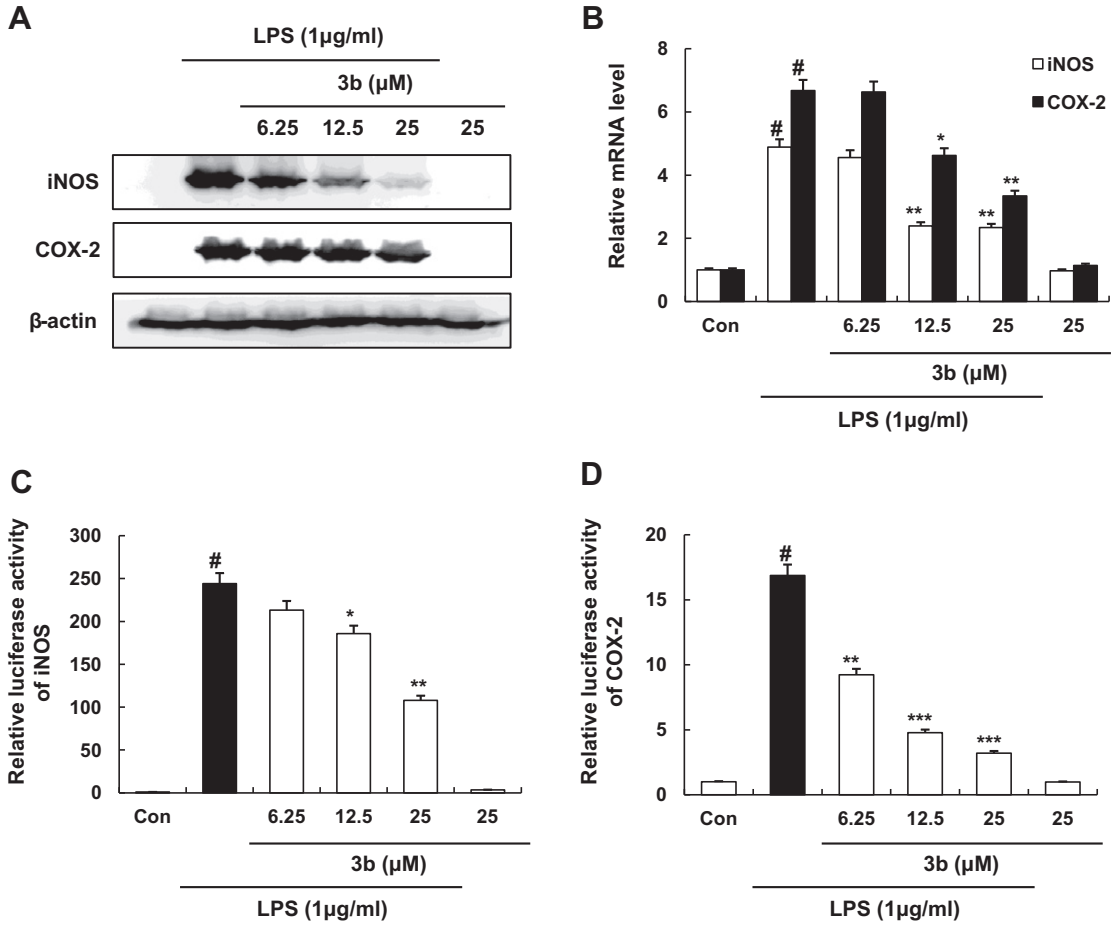


Figure 3. Effects of **3b** on LPS-induced iNOS and COX-2 protein and mRNA expressions, and promoter activity in RAW 264.7 macrophages. (A) Cells were pretreated with different concentrations (6.25, 12.5, or 25 μM) of **3b** for 1 h, then treated with LPS (1 μg/ml), and incubated for 24 h. Control (Con) values were obtained in the absence of LPS and **3b**. Total cellular proteins (30 μg) were resolved by SDS–PAGE, transferred to nitrocellulose, and detected using specific antibodies for iNOS and COX-2. The data shown are representative of three independent experiments, and the immunoblot is representative of three separate experiments. (B) Total RNA was prepared for qRT-PCR analysis of iNOS and COX-2 from cells stimulated with LPS (1 μg/ml) with/without **3b** (6.25, 12.5, or 25 μM) for 4 h. Steady-state mRNA levels of iNOS, COX-2 and β-actin were determined by qRT-PCR using the Takara thermal cycler dice® (Takara Bio Inc., Japan). The results were expressed as the ratio of optimal density to β-actin. Values represent the means ± SDs of three independent experiments. $^{\#}p < 0.05$ as compared with the control cells, and $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ as compared with the LPS-only treated cells. ANOVA and Dunnett's post-hoc test were used to determine the significances of differences. (C) Cells were transiently co-transfected with piNOS-luc reporter or pCOX-2-luc reporter with pRL-TK-luc either not pretreated (Con) or pretreated with different concentrations (6.25, 12.5, or 25 μM) of **3b** for 1 h. Cells were then harvested and luciferase activities were determined using a Promega luciferase assay system and a luminometer. Values represent the means ± SDs of three independent experiments. $^{\#}p < 0.05$ as compared with the control cells, and $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ as compared with the LPS-only treated cells. ANOVA and Dunnett's post-hoc test were used to determine the significances of differences.

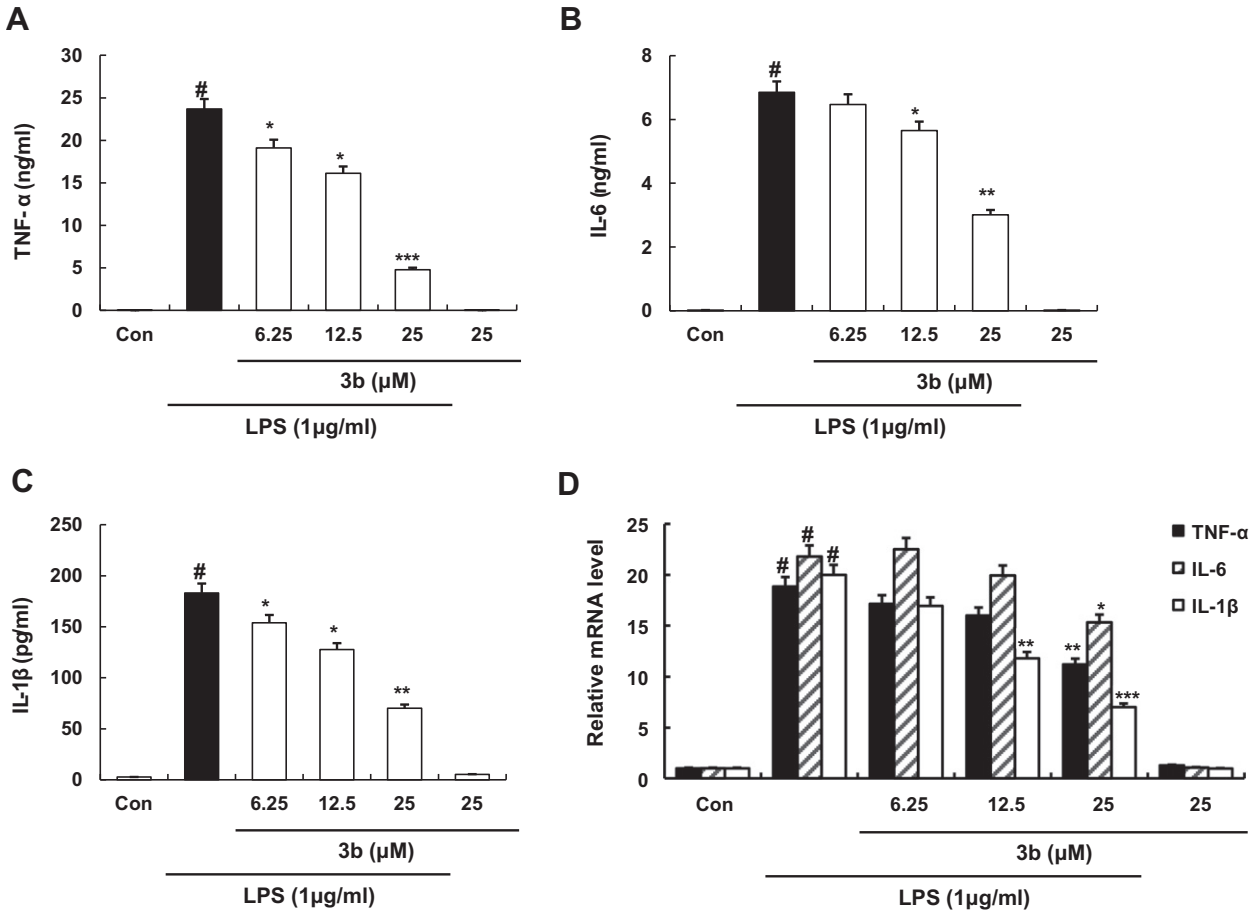


Figure 4. Effects of **3b** on LPS-induced TNF-α, IL-6, and IL-1β production and gene expressions. (A–C) Cells were pretreated with different concentrations (6.25, 12.5, or 25 μM) of **3b** for 1 h, then with LPS (1 μg/ml), and incubated for 24 h. The levels of TNF-α, IL-6 and IL-1β in macrophage culture medium were measured by EIA kits according to the manufacturer's instructions (R&D Systems). Values represent the means ± SDs of three independent experiments. $^{\#}p < 0.05$ as compared with the control cells, and $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ as compared with the LPS-only treated cells. ANOVA and Dunnett's post-hoc test were used to determine the significances of differences. (D) Total RNA was prepared for qRT-PCR analysis of iNOS and COX-2 from cells stimulated with LPS (1 μg/ml) with/without **3b** (6.25, 12.5, or 25 μM) for 4 h. Steady-state mRNA levels of TNF-α, IL-6, IL-1β and β-actin were determined by qRT-PCR using the Takara thermal cycler dice® (Takara Bio Inc., Japan). The results were expressed as the ratio of optimal density to β-actin. Values represent the means ± SDs of three independent experiments. $^{\#}p < 0.05$ as compared with the control cells, and $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ as compared with the LPS-only treated cells. ANOVA and Dunnett's post-hoc test were used to determine the significances of differences.

PGE_2 (Fig. 2A and B). In addition, **3b** dose-dependently inhibited LPS-induced iNOS and COX-2 promoter luciferase activity (Fig. 3C and D).

In the inflammatory state, macrophages release pro-inflammatory cytokines like TNF-α, IL-6, and IL-1β.[22] We examined the productions and expressions of these cytokines by ELISA and qRT-PCR, respectively, and found that pre-treatment with **3b** considerably reduced LPS-induced TNF-α, IL-6, and IL-1β release (Fig. 4A–C) and their mRNA expressions (Fig. 4D). These findings suggest that the decreased protein levels of these cytokines are responsible for the reduced transcription level.

Because the activation of NF-κB is critical for LPS-induced expressions of iNOS, COX-2, TNF-α, IL-6, and IL-1β,[23] we examined the effect of **3b** using an LPS-stimulated NF-κB-dependent reporter gene assay. Analysis of reporter gene expression using pNF-κB-luc demonstrated that **3b** concentration-dependently inhibited NF-κB-dependent luciferase activity (Fig. 5A). In general, NF-κB is inactive in the cytosol because it is bound by IκB and becomes active after IκB is phosphorylated and degraded, which enables the subsequent nuclear translocation of NF-κB.[24,25] Thus, we explored whether **3b** could inhibit the LPS-stimulated phosphorylation and degradation of IκB-α in RAW 264.7 cells by Western blotting. Figure 5B shows that the LPS-induced IκB-α degradation and phosphorylation was significantly blocked by **3b**. Accordingly, we investigated whether **3b** prevents the translocation of the subunit of NF-κB (p65) to the nucleus, and found that pretreatment with **3b** dose-dependently attenuated LPS-induced p65 translocation to the nucleus (Fig. 5A). These findings show that **3b** prevents NF-κB-activation by inhibition the phosphorylation and degradation of IκB-α. However, the enhancing expression of IκB-α band after only **3b** treatment is remained for further studies.

Summarizing, we synthesized 5,6,7-trimethoxy- and 5,6,7-trihydroxyflavones **3a**–**3g** from cinnamic acid derivatives to enhance inhibitory effects on LPS-induced NO and PGE_2 production versus oroxylin A and baicalein in RAW 264.7 cells. Of the compounds synthesized, 4′-bromo-5,6,7-trimethoxyflavone (**3b**) was found to significantly inhibit the productions of NO and PGE_2 production by LPS. Consistent with these results, **3b** inhibited the LPS-stimulated expressions of iNOS, COX-2, TNF-α, IL-6, and IL-1β and induced attendant reductions in the productions of NO, PGE_2, TNF-α, IL-6, and IL-1β in RAW 264.7 cells. Furthermore, our molecular data suggest that **3b** inhibits these anti-inflammatory modulators by suppressing NF-κB activation via the inhibition of IκB-α phosphorylation and degradation. Accordingly, our findings provide a partial description of the mechanism underlying the anti-inflammatory effects of **3b**.

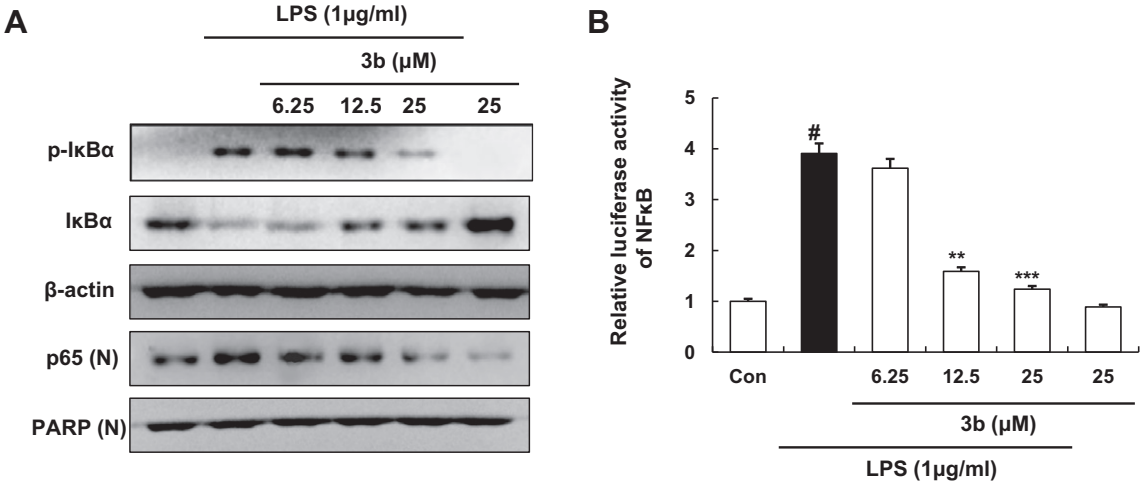


Figure 5. Effects of **3b** on the NF-κB promoter activity, phosphorylation and degradation of IκBα, and p65 nuclear translocation in RAW 264.7 macrophages. (A) Cells were transiently co-transfected with pNF-κB-luc reporter and pRL-TK-luc and either not pretreated or pretreated with different concentrations (6.25, 12.5, or 25 μM) of **3b** for 1 h. LPS (1 μg/ml) was then added, and cells were further incubated for 1 h. Cells were collected and luciferase activities were determined using a Promega luciferase assay system and a luminometer. Values represent the means ± SDs of three independent experiments. $^{\#}p < 0.05$ as compared with the control cells, and $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ as compared with the LPS-only treated cells. ANOVA and Dunnett's post-hoc test were used to determine the significances of differences. (B) Cells were pretreated with different concentrations (6.25, 12.5, or 25 μM) of **3b** for 1 h, then with LPS (1 μg/ml), and incubated for 15 min or 1 h. Cytosol and nuclear extracts were then prepared for the Western blotting by using a specific p-IκBα, IκBα and NF-κB p65 specific antibodies. A representative immunoblot of three separate experiments is shown. PARP and β-actin were used as internal controls. The data shown are representative of three independent experiments, and the immunoblot is representative of three separate experiments.

Acknowledgments

This study was partly supported by the Korea Healthcare Technology R&D Project, Ministry for Health, Welfare & Family Affairs (#A090487) and the Seoul Research and Business Development Program (#10524), Republic of Korea.

References and notes

1. Oberyszyn, T. M. *Front Biosci.* **2007**, *12*, 2993.
2. Nathan, C.; Xie, Q. W. *Cell* **1994**, *78*, 915.
3. Liu, R. H.; Hotchkiss, J. H. *Mutat. Res.* **1995**, *339*, 73.
4. Pan, M. H.; Lai, C. S.; Wang, Y. J.; Ho, C. T. *Biochem. Pharmacol.* **2006**, *72*, 1293.
5. Hinz, B.; Brune, K. *J. Pharmacol. Exp. Ther.* **2002**, *300*, 367.
6. Bendtzen, K. *Immunol. Lett.* **1988**, *19*, 183.
7. Mercurio, F.; Zhu, H.; Murray, B. W.; Shevchenko, A.; Bennett, B. L.; Li, J.; Young, D. B.; Barbosa, M.; Mann, M.; Manning, A.; Rao, A. *Science* **1997**, *278*, 860.
8. Szabó, C. *New Horiz.* **1995**, *3*, 2.
9. MacMicking, J.; Xie, Q. W.; Nathan, C. *Ann. Rev. Immunol.* **1997**, *15*, 323.
10. Bochoráková, H.; Paulová, H.; Slanina, J.; Musil, P.; Táborská, E. *Phytother. Res.* **2003**, *17*, 640.
11. Aung, H. H.; Dey, L.; Mehendale, S.; Xie, J. T.; Wu, J. A.; Yuan, C. S. *Cancer Chemother. Pharmacol.* **2003**, *52*, 453.
12. Shih, H. C.; Hsu, C. S.; Yang, L. L. *J. Biomed. Sci.* **2009**, *16*, 27.
13. Zhao, L.; Chen, Z.; Wang, J.; Yang, L.; Zhao, Q.; Qi, Q.; Mu, R.; You, Q. D.; Guo, Q. L. *Cancer Chemother. Pharmacol.* **2010**, *65*, 481.
14. Kim, D. H.; Jeon, S. J.; Son, K. H.; Jung, J. W.; Lee, S.; Yoon, B. H.; Lee, J. J.; Cho, Y. W.; Cheong, J. H.; Ko, K. H.; Ryu, J. H. *Neurobiol. Learn. Mem.* **2007**, *87*, 536.
15. Chen, Y.; Yang, L.; Lee, T. J. *Biochem. Pharmacol.* **2000**, *59*, 1445.
16. Suresh Babu, K.; Hari Babu, T.; Srinivas, P. V.; Sastry, B. S.; Hara Kishore, K.; Murty, U. S.; Madhusudana Rao, J. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 3953.
17. Lee, Y.; Yeo, H.; Liu, S. H.; Jiang, Z.; Savizky, R. M.; Austin, D. J.; Cheng, Y. C. *J. Med. Chem.* **2004**, *47*, 5555.
18. Liao, H. L.; Hu, M. K. *Chem. Pharm. Bull.* **2004**, *52*, 1162.
19. Huang, W. H.; Lee, A. R.; Chien, P. Y.; Chou, T. C. *J. Pharm. Pharmacol.* **2005**, *57*, 219.
20. Huang, W. H.; Chien, P. Y.; Yang, C. H.; Lee, A. R. *Chem. Pharm. Bull.* **2003**, *51*, 339.
21. *Spectral data of* ***3b***: ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.07 (2H, d, J = 8.8 Hz), 7.83 (2H, d, J = 8.8 Hz), 7.29 (1H, s), 6.92 (1H, s), 4.01 (3H, s), 3.86 (3H, s), 3.83 (3H, s); ^{13}C NMR (100 MHz, DMSO-d_6) δ 175.5, 159.0, 157.6, 153.9, 151.5, 139.8, 132.0 (2C), 130.1, 127.9 (2C), 125.1, 112.1, 107.8, 97.3, 61.8, 60.9, 56.4; HR-DART-MS m/z 391.0078 (calcd for $C_{18}H_{16}BrO_5$ $[M+H]^+$, 391.0181).
22. Riedemann, N. C.; Guo, R. F.; Ward, P. A. *Nat. Med.* **2003**, *9*, 517.
23. Baeuerle, P. A.; Henkel, T. *Ann. Rev. Immunol.* **1994**, *12*, 141.
24. Hla, T.; Ristimäki, A.; Appleby, S.; Barriocanal, J. G. *Ann. N. Y. Acad. Sci.* **1993**, *696*, 197.
25. Mestre, J. R.; Chan, G.; Zhang, F.; Yang, E. K.; Sacks, P. G.; Boyle, J. O.; Shah, J. P.; Edelstein, D.; Subbaramaiah, K.; Dannenberg, A. J. *Ann. N. Y. Acad. Sci.* **1999**, *889*, 62.