Arch Pharm Res Vol 28, No 8, 877-884, 2005

Archives of Pharmacal Research
http://apr.psk.or.kr

Structure and Antiinflammatory Activity Relationships of Wogonin Derivatives

Jinhee Jang, Hyun Pyo Kim, and Haeil Park

College of Pharmacy, Kangwon National University, Chuncheon 200-701, Republic of Korea

(Received June 22, 2005)

A number of wogonin derivatives have been synthesized as congeners of wogonin and evaluated for their inhibitory activities of PGE_2 production. Wogonin derivatives modified at the B ring of wogonin were obtained from 2,4-Dihydroxy-3,6-dimethoxyacetophenone (**1**) *via* several steps. Most wogonin derivatives exhibited much reduced inhibitory activities against COX-2 catalyzed PGE_2 production compared to that of wogonin. Alkylation of 5,7-phenol groups and substitution at the B ring of wogonin generally caused reduction of inhibitory activity.

Key words: Wogonin derivatives, COX-2, Prostaglandin production inhibition, Anti-inflammatory activity

INTRODUCTION

Chronic inflammatory diseases such as rheumatoid arthritis are problematic therapeutic areas to overcome because their long-term therapeutic periods limit the use of therapeutic agents with side effects. Inflammatory process comprises of several aspects provoked by different chemicals/biologicals including proinflammatory enzymes/cytokines, small molecular chemicals such as eicosanoids and tissue degradation enzymes. Among these factors, cyclooxygenase (COX) catalyses the conversion of arachidonic acid to prostaglandins (PGs), key proinflammatory eicosanoids. COX exists in two isoforms. COX-1 is a constitutive enzyme exerting homeostasis function, while COX-2 is an inducible one and known as a major isoform found in the inflammatory lesions (Needleman, 1997).

Flavonoids from plant origin possess anti-inflammatory activity. In addition to the inhibitory activity of some flavonoids against COX-1 and/or COX-2 (Middleton, 2000; Kim, 2004), recent studies have shown that several flavone analogs down-regulate COX-2 expression (Liang, 1999; Kim, 1999; Chi, 2001), suggesting a potential for new class of anti-inflammatory agents.

Wogonin (5,7-dihydroxy-8-methoxyflavone) is one of the major polyhydroxyflavones from *Scutellaria baicalensis* Georgi that possesses a very broad spectrum of biological activities and used as a traditional medicinal plant in Oriental medicine. Wogonin was previously reported to suppress the induction of COX-2 and inducible nitric oxide synthase (Chen, 2001; Chi, 2003; Lee, 2003; Wakabayashi, 1999 & 2000). Chrysin (5,7-dihydroxyflavone) is also a naturally occurring flavone and has a very similar chemical structure with that of wogonin (Fig. 1). In spite of the similarity in their chemical structures, wogonin exhibited much stronger inhibitory activity than chrysin against COX-2 catalyzed prostaglandin production from lipopolysaccharide-treated RAW 264.7 cells (Dao, 2004b).

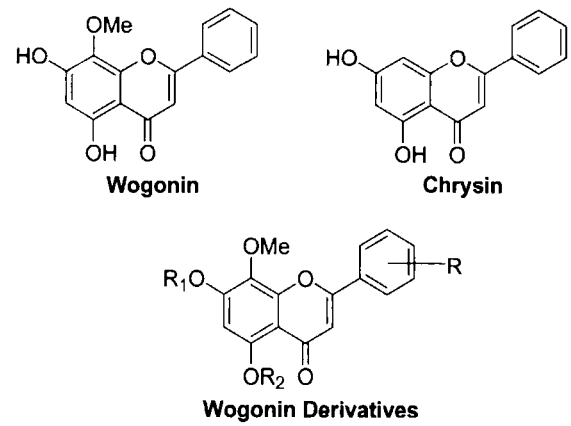


Fig. 1. The chemical structures of wogonin, chrysin and wogonin derivatives

Correspondence to: Haeil Park, College of Pharmacy, Kangwon National University, Chuncheon 200-701, Korea
Tel: 82-33-250-6920, Fax: 82-33-255-7865
E-mail: haeilp@kangwon.ac.kr

From comparison between the chemical structures and anti-inflammatory activities of wogonin and chrysin, we conjectured that the 8-methoxy functional group of wogonin plays a very important role to exhibit strong anti-inflammatory activity. Therefore, it is a part of subject to explore the structural elaboration of wogonin maintaining 8-methoxy group. In this point of view, wogonin derivatives modified at the B ring were synthesized and evaluated for their inhibitory activities against COX-2 catalyzed PGE_2 production from LPS-induced RAW 264.7 cells.

MATERIALS AND METHODS

All chemicals were obtained from commercial suppliers, and used without further purification. All solvents used for reaction were freshly distilled from proper dehydrating agent under nitrogen gas. All solvents used for chromatography were purchased and directly applied without further purification. ^{1}H-NMR spectra were recorded on a Varian Gemini 2000 instrument (200 MHz) spectrometer. Chemical shifts are reported in parts per million (ppm) downfield relative to tetramethylsilane as an internal standard. Peak splitting patterns are abbreviated as m (multiplet), s (singlet), bs (broad singlet), d (doublet), bd (broad doublet), t (triplet), and dd (doublet of doublets). Analytical thin-layer chromatography (TLC) was performed using commercial glass plate with silica gel $60F_{254}$ purchased from Merck. Chromatographic purification was carried out by flash chromatography using Kieselgel 60 (230~400 mesh, Merck).

General synthetic procedures for 4-benzyloxy-2-hydroxy-3,6-dimethoxyacetophenone (2)

To a mixture of **1** in 20 mL of anhydrous acetone, anhydrous potassium carbonate (4.90 mmol) and benzyl bromide (2.45 mmol) added slowly under stirring. The reaction mixture was then put under nitrogen atmosphere and refluxed for 22-23 h. Potassium carbonate was removed by suction filtration, the filtrate was evaporated and then the residues was extracted with dichloromethane. The crude material was re-crystallized with methanol. Yield 97%; ^{1}H-NMR (200 MHz, $CDCl_3$) δ 2.61 (3H, s, acetyl group-CH_3), 3.79, 3.85 (6H, s, s, -OCH_3), 5.25 (2H, s, benzyl group-CH_2), 5.98 (1H, s, C5-H), 7.30-7.43 (5H, m, Ph-H in *O*-benzyl group), 13.83(1H, s, C2-OH).

General synthetic procedures for chalcones (3a-3j)

To a mixture of **2** in 15 mL of methanol, potassium hydroxide (2.0 equiv.) was added very slowly under stirring. To this solution was added aryl aldehyde (1.2 equiv.) and then stirred at 30°C for 5 days. The reaction mixture was cooled with ice water and neutralized with conc-hydrochloric acid at 0°C (ice-bath). The solid was filtered, washed with methanol and dried over under reduced pressure.

1-(4-Benzyloxy-2-hydroxy-3,6-dimethoxyphenyl)-3-phenylpropenone (3a)

Yield 69%; ^{1}H-NMR (200 MHz, $CDCl_3$) δ 3.85, 3.88 (6H, s, s, -OCH_3), 5.26 (2H, s, benzyl group-CH_2), 6.03 (1H, s, C5-H), 7.37-7.44 (7H, m, phenyl group-H & bezene ring-H in *O*-benzyl group), 7.58-7.61 (3H, m, phenyl ring-H), 7.83 (2H, d, J = 3.6Hz, -CH=CH-), 13.93(1H, s, C2-OH).

1-(4-Benzyloxy-2-hydroxy-3,6-dimethoxyphenyl)-3-*p*-tolylpropenone (3b)

Yield 68%; ^{1}H-NMR (200 MHz, $CDCl_3$) δ 2.39(3H, s, -CH_3), 3.84, 3.88 (6H, s, s, -OCH_3), 5.26 (2H, s, benzyl group-CH_2), 6.03 (1H, s, C5-H), 7.21 (2H, t, toluene ring-H), 7.34- 7.52 (7H, m, toluene ring-H & bezene ring-H in *O*-benzyl group), 7.80 (2H, s, -CH=CH-), 13.98 (1H, s, C2-OH).

1-(4-Benzyloxy-2-hydroxy-3,6-dimethoxyphenyl)-3-(4'-methoxyphenyl)propenone (3c)

Yield 49%; ^{1}H-NMR (200 MHz, $CDCl_3$) δ 3.85, 3.86, 3.88 (9H, s, s, s, -OCH_3), 5.26 (2H, s, benzyl group-CH_2), 6.03 (1H, s, C5-H), 6.93 (2H, d, J = 8.8Hz, anisole ring-H), 7.37-7.44 (5H, m, bezene ring-H in *O*-benzyl group), 7.50 (2H, d, J = 8.8 Hz, anisole ring-H), 7.78 (2H, d, J = 2.2 Hz, -CH=CH-), 14.08 (1H, s, C2-OH).

1-(4-Benzyloxy-2-hydroxy-3,6-dimethoxyphenyl)-3-(2',4'-dimethoxyphenyl)propenone (3d)

Yield 58%; ^{1}H-NMR (200 MHz, $CDCl_3$) δ 3.83, 3.86, 3.88, 3.89 (12H, s, s, s, s, -OCH_3), 5.26 (2H, s, benzyl group-CH_2), 6.02 (1H, s, C5-H), 6.47 (1H, s, phenyl group-H), 6.54 (1H, d, J = 5.6 Hz, phenyl group-H), 7.27-7.41 (5H, m, bezene ring-H in *O*-benzyl group), 7.54 (1H, d, J = 8.6Hz, phenyl ring-H), 7.99 (2H, q, -CH=CH-), 14.19 (1H, s, C2-OH).

1-(4-Benzyloxy-2-hydroxy-3,6-dimethoxyphenyl)-3-(3',4'-dimethoxyphenyl)propenone (3e)

Yield 75%; ^{1}H-NMR (200 MHz, $CDCl_3$) δ 3.84, 3.88, 3.93 (12H, s, s, s, -OCH_3), 5.26 (2H, s, benzyl group-CH_2), 6.03 (1H, s, C5-H), 6.90 (1H, d, J = 8.2 Hz, phenyl group-H), 7.13 (1H, s, phenyl group-H), 7.23 (1H, s, phenyl group-H), 7.37-7.44 (5H, m, bezene ring-H in *O*-benzyl group), 7.76(2H, s, -CH=CH-), 14.03(1H, s, C2-OH).

1-(4-Benzyloxy-2-hydroxy-3,6-dimethoxyphenyl)-3-(2',3',4'-trimethoxyphenyl)propenone (3f)

Yield 58%; ^{1}H-NMR (200 MHz, $CDCl_3$) δ 3.84, 3.88, 3.89, 3.91, 3.95 (15H, s, s, s, s, s, -OCH_3), 5.26 (2H, s, benzyl group-CH_2), 6.02 (1H, s, C5-H), 6.72 (1H, d, J = 9.0 Hz,

phenyl group-H), 7.32-7.44 (7H, m, phenyl group & bezene ring-H in *O*-benzyl group), 7.96 (2H, q, -CH=CH-), 14.08 (1H, s, C2-OH).

1-(4-Benzyloxy-2-hydroxy-3,6-dimethoxyphenyl)-3-(3',4',5'-trimethoxyphenyl)propenone (3g)
Yield 70%; ^{1}H-NMR (200 MHz, $CDCl_3$) δ 3.84, 3.88, 3.90, 3.91 (15H, s, s, s, s, $-OCH_3$), 5.27 (2H, s, benzyl group-CH_2), 6.03 (1H, s, C5-H), 6.83 (2H, s, phenyl group-H), 7.38-7.44 (5H, m, bezene ring-H in *O*-benzyl group), 7.73 (2H, s,-CH=CH-), 13.93 (1H, s, C2-OH).

1-(4-Benzyloxy-2-hydroxy-3,6-dimethoxyphenyl)-3-(4'-chlorophenyl)propenone (3h)
Yield 59%; ^{1}H-NMR (200 MHz, $CDCl_3$) δ 3.85, 3.88 (6H, s, $-OCH_3$), 5.24 (2H, s, benzyl group-CH_2), 6.03 (1H, s, C5-H), 7.35-7.44 (7H, m, phenyl group & bezene ring-H in *O*-benzyl group), 7.52 (2H, d, J = 8.4 Hz, phenyl group-H), 7.77 (2H, d, J = 7.4 Hz, -CH=CH-), 13.87(1H, s, C2-OH).

1-(4-Benzyloxy-2-hydroxy-3,6-dimethoxyphenyl)-3-(3',4'-dichlorophenyl)propenone (3i)
Yield 45%; ^{1}H-NMR (200 MHz, $CDCl_3$) δ 3.86, 3.88 (6H, s, s, $-OCH_3$), 5.27 (2H, s, benzyl group-CH_2), 6.03 (1H, s, C5-H), 7.41-7.76 (8H, m, phenyl group & bezene ring-H in *O*-benzyl group), 7.76 (2H, s, -CH=CH-), 13.78 (1H, s, C2-OH).

1-(4-Benzyloxy-2-hydroxy-3,6-dimethoxyphenyl)-3-(4'-benzyloxyphenyl)propenone (3j)
Yield 90%; ^{1}H-NMR (200 MHz, $CDCl_3$) δ 3.8, 3.87 (6H, s, s, $-OCH_3$), 5.12 (2H, s, C4-benzyl group CH_2), 5.26 (2H, s, benzyl group-CH_2), 6.03 (1H, s, C5-H), 6.99 (2H, d, J = 4.4 Hz, phenyl group), 7.36-7.46 (10H, m, bezene ring-H), 7.55 (2H, d, J = 4.4 Hz, benzene ring-H), 7.77 (2H, d, J = 3.0 Hz, -CH=CH-), 14.04 (1H, s, C2-OH).

General synthetic procedures for 7-benzyloxy-5,8-dimethoxyflavone analogs (4a-4j)

A mixture of chalcones **3a-3j** (1.0 equiv.) and iodine (0.1 equiv.) in 5 mL DMSO was heated at 100°C for overnight. After cooling, sodium sulfite solution was added to the reaction mixture for destroy excess iodine. The precipitated solid was filtered, washed with water and re-crystallized with methanol and dichloromethane.

7-Benzyloxy-5,8-dimethoxyflavone (4a)
Yield 77%; ^{1}H-NMR (200 MHz, $CDCl_3$) δ 3.90, 3.99 (6H, s, s, $-OCH_3$), 5.30 (2H, s, benzyl group-CH_2), 6.47 (1H, s, C3-H), 6.70 (1H, s, C6-H), 7.39-7.54 (8H, m, C3',4',5'-H & bezene ring-H in *O*-benzyl group), 7.92-7.97 (2H, m, C2',C6'-H).

7-Benzyloxy-5,8-dimethoxy-4'-methylflavone (4b)
Yield 87%; ^{1}H-NMR (200 MHz, $CDCl_3$) δ 2.44 (3H, s, C4'-CH_3), 3.89, 3.99 (6H, s, s, $-OCH_3$), 5.30 (2H, s, benzyl group-CH_2), 6.46 (1H, s, C3-H), 6.66 (1H, s, C6-H), 7.34-7.46 (7H, m, C3', 5-H & bezene ring-H in *O*-benzyl group), 7.84 (2H, d, J = 8.0Hz, C2',6'-H).

7-Benzyloxy-4',5,8-trimethoxyflavone (4c)
Yield 84%; ^{1}H-NMR (200 MHz, $CDCl_3$) δ 3.89, 3.99 (9H, s, s, $-OCH_3$), 5.29 (2H, s, benzyl group-CH_2), 6.46 (1H, s, C3-H), 6.62 (1H, s, C6-H), 7.03 (2H, d, J = 8.8 Hz, C3',5'-H), 7.27-7.46 (5H, m, bezene ring-H in *O*-benzyl group), 7.90 (2H, d, J = 8.8 Hz, C2',6'-H).

7-Benzyloxy-2',4',5,8-tetramethoxyflavone (4d)
Yield 90%; ^{1}H-NMR (200 MHz, $CDCl_3$) δ 3.89, 3.93, 3.95 (12H, s, s, s, $-OCH_3$), 5.28 (2H, s, benzyl group-CH_2), 6.44 (1H, s, C3-H), 6.56 (1H, s, C6-H), 6.65 (1H, d, J = 10.0 Hz, C3'-H), 7.03 (1H, s, C5'-H), 7.39-7.46 (5H, m, bezene ring-H in *O*-benzyl group), 7.99 (1H, d, J = 8.8 Hz, C6'-H).

7-Benzyloxy-3',4',5,8-tetramethoxyflavone (4e)
Yield 92%; ^{1}H-NMR (200 MHz, $CDCl_3$) δ 3.90, 3.98 (12H, s, s, $-OCH_3$), 5.30 (2H, s, benzyl group-CH_2), 6.47 (1H, s, C3-H), 6.63 (1H, s, C6-H), 7.01 (1H, d, J = 8.4 Hz, C5'-H), 7.39-7.46 (5H, m, bezene ring-H in *O*-benzyl group), 7.59 (2H, d, J = 8.8 Hz, C2',6'-H).

7-Benzyloxy-2',3',4',5,8-pentamethoxyflavone (4f)
Yield 64%; ^{1}H-NMR (200 MHz, $CDCl_3$) δ 3.89, 3.91, 3.94, 3.97 (15H, s, s, s, s, $-OCH_3$), 5.29 (2H, s, benzyl group-CH_2), 6.46 (1H, s, C3-H), 6.82 (1H, d, J = 8.8 Hz, C5'-H), 6.90 (1H, s, C6-H), 7.38-7.46 (5H, m, bezene ring-H in *O*-benzyl group), 7.64 (1H, d, J = 9.0 Hz, C6'-H).

7-Benzyloxy-3',4',5,5',8-pentamethoxyflavone (4g)
Yield 95%; ^{1}H-NMR (200 MHz, $CDCl_3$) δ 3.91, 3.93, 3.96, 3.99 (15H, s, s, s, s, $-OCH_3$), 5.30 (2H, s, benzyl group-CH_2), 6.48 (1H, s, C3-H), 6.65 (1H, s, C6-H), 7.19 (2H, s, C2',6'-H), 7.39-7.46 (5H, m, bezene ring-H in *O*-benzyl group).

7-Benzyloxy-4'-chloro-5,8-dimethoxyflavone (4h)
Yield 63%; ^{1}H-NMR (200 M Hz, $CDCl_3$) δ 3.90, 3.98 (6H, s, s, $-OCH_3$), 5.30 (2H, s, benzyl group-CH_2), 6.48 (1H, s, C3-H), 6.66 (1H, s, C6-H), 7.43-7.52 (6H, m, C3',5'-H & bezene ring-H in *O*-benzyl group), 7.88 (2H, d, J = 8.0 Hz, C2',6'-H).

7-Benzyloxy-3',4'-dichloro-5,8-dimethoxyflavone (4i)
Yield 78%; ^{1}H-NMR (200 MHz, $CDCl_3$) δ 3.90, 3.98 (6H, s, s, $-OCH_3$), 5.30 (2H, s, benzyl group-CH_2), 6.48 (1H, s,

C3-H), 6.64 (1H, s, C6-H), 7.39-7.46 (5H, m, bezene ring-H in *O*-benzyl group), 7.60 (1H, d, *J* = 8.6 Hz, C6'-H), 7.75 (1H, d, *J* = 8.2 Hz, C5'-H), 8.02 (1H, s, C2'-H).

4',7-Dibenzyloxy-5,8-dimethoxyflavone (4j)
Yield 85%; ^{1}H-NMR (200 MHz, $CDCl_3$) δ 3.89, 3.98 (6H, s, s, -OCH_3), 5.16 (1H, s, C3-H), 5.29 (1H, s, C6-H), 7.10 (2H, d, *J* = 9.2 Hz, C3',5'-H), 7.39-7.46 (10H, m, bezene ring-H in *O*-benzyl group), 7.89 (2H, d, *J* = 9.2 Hz, C2',6'-H).

General synthetic procedures for 7-hydroxy-5,8-dimethoxyflavone analogs (5a-5j)

To the mixture of glacial acetic acid 30 mL and conc-hydrochloric acid 10ml was added the compounds **4a-4j** and the reaction mixture was refluxed for 5-6 h. After cooling, the reaction mixture was poured into cold water with vigorous stirring. The precipitated solid was filtered, washed with water, dried and re-crystallized from methanol.

7-Hydroxy-5,8-dimethoxyflavone (5a)
Yield 91%; ^{1}H-NMR (200 MHz, $CDCl_3$ & DMSO-d_6) δ 3.92, 3.97 (6H, s, s, -OCH_3), 6.48 (1H, s, C3-H), 6.67 (1H, s, C6-H), 7.45, 7.54 (3H, s, s, C3',4',5'-H), 7.93 (2H, s, C2',6'-H), 9.79 (1H, s, C7-OH).

7-Hydroxy-5,8-dimethoxy-4'-methylflavone (5b)
Yield 93%; ^{1}H-NMR (200 MHz, $CDCl_3$ & DMSO-d_6) δ 2.45 (3H, s, C4'-CH_3), 3.90, 3.96 (6H, s, s, -OCH_3), 6.48 (1H, s, C3-H), 6.61 (1H, s, C6-H), 7.34 (2H, d, *J* = 6.6 Hz, C3',5'-H), 7.83 (2H, d, *J* = 6.6 Hz, C2',6'-H), 9.99 (1H, s, C7-OH).

7-Hydroxy-4',5,8-trimethoxyflavone (5c)
Yield 91%; ^{1}H-NMR (200 MHz, DMSO-d_6) δ 3.76, 3.85 (9H, s, s, -OCH_3), 6.46 (1H, s, C3-H), 6.67 (1H, s, C6-H), 7.11 (2H, d, *J* = 9.2 Hz, C3',5'-H), 7.98 (2H, d, *J* = 9.2 Hz, C2',6'-H), 10.61 (1H, brs, C7-OH).

7-Hydroxy-2',4',5,8-tetramethoxyflavone (5d)
Yield 97%; ^{1}H-NMR (200 MHz, DMSO-d_6) δ 3.85 (3H, s, -OCH_3), 6.32 (1H, s, C3-H), 7.05 (1H, s, C6-H), 7.69 (2H, d, *J* = 8.8 Hz, C3',5'-H), 8.09 (2H, d, *J* = 8.8 Hz, C2',6'-H), 12.41 (1H, s, C7-OH).

7-Hydroxy-3',4',5,8-tetramethoxyflavone (5e)
Yield 70%; ^{1}H-NMR (200 MHz, $CDCl_3$ & DMSO-d_6) δ 3.92, 3.97, 3.98, 3.99 (12H, s, s, s, s, -OCH_3), 6.48 (1H, s, C3-H), 6.61 (1H, s, C6-H), 7.01 (1H, d, *J* = 8.4 Hz, C5'-H), 7.42 (1H, s, C2'-H), 7.58 (1H, d, *J* = 8.4 Hz, C6'-H).

7-Hydroxy-2',3',4',5,8-pentamethoxyflavone (5f)
Yield 64%; ^{1}H-NMR (200 MHz, $CDCl_3$) δ 3.91, 3.95, 4.00 (15H, s, s, s, -OCH_3), 6.49 (1H, s, C3-H), 6.81 (1H, d, *J* = 9.0 Hz, C5'-H), 6.88 (1H, s, C6-H), 7.56 (1H, d, *J* = 9.0 Hz, C6'-H).

7-Hydroxy-3',4',5,5',8-pentamethoxyflavone (5g)
Yield 52%; ^{1}H-NMR (200 MHz, $CDCl_3$) δ 3.93, 3.96, 3.99, 4.00 (15H, s, s, s, s, -OCH_3), 6.52 (1H, s, C3-H), 6.67 (1H, s, C6-H), 7.15 (2H, s, C2',6'-H).

4'-Chloro-7-hydroxy-5,8-dimethoxyflavone (5h)
Yield 97%; ^{1}H-NMR (200 MHz, $CDCl_3$ & DMSO-d_6) δ 3.90, 3.94 (6H, s, s, -OCH_3), 6.49 (1H, s, C3-H), 6.64 (1H, s, C6-H), 7.52 (2H, d, *J* = 8.6 Hz, C3',5'-H), 7.91 (2H, d, *J* = 8.8 Hz, C2',6'-H).

3',4'-Dichloro-7-hydroxy-5,8-dimethoxyflavone (5i)
Yield 94%; ^{1}H-NMR (200 MHz, $CDCl_3$ & DMSO-d_6) δ 3.95, 4.01 (6H, s, s, -OCH_3), 6.59 (1H, s, C3-H), 6.75 (1H, s, C6-H), 7.72 (1H, d, *J* = 7.2 Hz, C6'-H), 7.91 (1H, d, *J* = 7.4 Hz, C5'-H), 8.14 (1H, s, C2'-H), 10.32 (1H, brs, C7-OH).

4',7-Dihydroxy-5,8-dimethoxyflavone (5j)
Yield 77%; ^{1}H-NMR (200 MHz, CDCl3 & DMSO-d_6) δ 3.92, 3.98 (6H, s, s, -OCH_3), 6.45 (1H, s, C3-H), 6.55 (1H, s, C6-H), 6.97 (2H, d, *J* = 8.4 Hz, C3',5'-H), 7.79 (2H, d, *J* = 8.4 Hz, C2',6'-H), 8.98 (1H, s, C4'-OH), 9.44 (1H, s, C7-OH).

General synthetic procedures for 5,7-dihydroxy-8-methoxyflavone analogs (6a-6j)

To a mixture of **5a-5j** (1.0 equiv.) in 10 mL anhydrous acetonitrile, aluminum chloride (5.0-7.0 equiv.) was added slowly at 0°C (ice-bath) under nitrogen atmosphere and the reaction mixture was refluxed for overnight. 3*N*-Hydrochloric acid was added slowly to the reaction mixture to destroy excess aluminum chloride. This solution was diluted with chloroform, refluxed for further 30-40 minutes. After cooling the reaction mixture, the organic layer was separated, dried over anhydrous magnesium sulfate, filtered and concentrated under reduced pressure. The crude products were purified by recrystallization with methanol.

5,7-Dihydroxy-8-methoxyflavone (6a)
Yield 86%; ^{1}H-NMR (200 MHz, DMSO-d_6) δ 3.86 (3H, s, -OCH_3), 6.32 (1H, s, C3-H), 7.02 (3H, s, C6-H), 7.59-7.64 (3H, m, C3',4',5'-H), 8.06-8.11 (2H, m, C2',6'-H), 10.85 (1H, brs, C7-OH), 12.52 (1H, s, C5-OH).

5,7-Dihydroxy-8-methoxy-4'-methylflavone (6b)
Yield 93%; ^{1}H-NMR (200 MHz, DMSO-d_6) δ 2.40 (3H, s, C4'-CH_3), 3.85 (3H, s, -OCH_3), 6.30 (1H, s, C3-H), 6.96

(1H, s, C6-H), 7.41 (2H, d, J = 6.8 Hz, C3',5'-H), 7.97 (2H, d, J = 6.8 Hz, C2',6'-H), 10.81 (1H, brs, C7-OH), 12.55 (1H, s, C5-OH).

5,7-Dihydroxy-4',8-dimethoxyflavone (6c)
Yield 96%; ^{1}H-NMR (200 MHz, DMSO-d_6) δ 3.84, 3.86 (6H, s, s, -OCH_3), 6.29 (1H, s, C3-H), 6.92 (1H, s, C6-H), 7.16 (2H, d, J = 8.4 Hz, C3',5'-H), 8.04 (2H, d, J = 8.4Hz, C2',6'-H), 10.82 (1H, brs, C7-OH), 12.61 (1H, s, C5-OH).

5,7-Dihydroxy-2',4',8-trimethoxyflavone (6d)
Yield 77%; ^{1}H-NMR (200 MHz, DMSO-d_6) δ 3.81, 3.88, 3.95 (9H, s, s, s, -OCH_3), 6.28 (1H, s, C3-H), 6.81 (3H, d, J = 10.2 Hz, C6,3',5'-H), 7.88 (1H, d, J = 9.2 Hz, C6'-H), 10.87 (1H, brs, C7-OH), 12.21 (1H, s, C5-OH).

5,7-Dihydroxy-3',4',8-trimethoxyflavone (6e)
Yield 92%; ^{1}H-NMR (200 MHz, DMSO-d_6) δ 3.96, 3.98 (12H, s, s, -OCH_3), 6.39 (1H, s, C3-H), 7.11 (1H, s, C5'-H), 7.28 (1H, d, J = 8.6 Hz, C2'-H), 7.68 (1H, s, C6-H), 7.79 (1H, J = 8.6 Hz, C6'-H), 10.86 (1H, brs, C5-OH), 12.70 (1H, s, C5-OH).

5,7-Dihydroxy-2',3',4',8-tetramethoxyflavone (6f)
Yield 93%; ^{1}H-NMR (200 MHz, DMSO-d_6) δ 3.79, 3.89 (12H, s, s, -OCH_3), 6.30 (1H, s, C3-H), 6.72 (1H, s, C6-H), 7.08 (1H, d, J = 9.2 Hz, C5'-H), 7.59 (1H, d, J = 9.0 Hz, C6'-H), 10.81 (1H, brs, C7-OH), 12.55 (1H, s, C5-OH).

5,7-Dihydroxy-3',4',5',8-tetramethoxyflavone (6g)
Yield 91%; ^{1}H-NMR (200 MHz, DMSO-d_6) δ 3.86, 3.97, 4.00 (12H, s, s, s, -OCH_3), 6.39 (1H, s, C3-H), 7.23 (1H, s, C6-H), 7.47 (2H, s, C2',6'-H), 10.90 (1H, brs, C7-OH), 12.61 (1H, s, C5-OH).

4'-Chloro-5,7-dihydroxy-8-methoxyflavone (6h)
Yield 96%; ^{1}H-NMR (200 MHz, DMSO-d_6) δ 3.85 (3H, s, -OCH_3), 6.32 (1H, s, C3-H), 7.05 (1H, s, C6-H), 7.69 (2H, d, J = 8.8 Hz, C3',5'-H), 8.09 (2H, d, J = 8.8 Hz, C2',6'-H), 10.87 (1H, brs, C7-OH), 12.41 (1H, s, C5-OH).

3',4'-Dichloro-5,7-dihydroxy-8-methoxyflavone (6i)
Yield 96%; ^{1}H-NMR (200 MHz, DMSO-d_6) δ 3.85 (3H, s, -OCH_3), 6.32 (1H, s, C3-H), 7.16 (1H, s, C6-H), 7.91 (2H, d, J = 8.6 Hz, C6'-H), 8.04 (2H, d, J = 8.6 Hz, C5'-H), 8.32 (1H, s, C2'-H), 10.91 (1H, brs, C7-OH), 12.42 (1H, s, C5-OH).

4',5,7-Trihydroxy-8-methoxyflavone (6j)
Yield 46%; ^{1}H-NMR (200 MHz, DMSO-d_6) δ 3.84 (3H, s, -OCH_3), 6.28 (1H, s, C3-H), 6.81 (1H, s, C6-H), 6.96 (2H, d, J = 8.8 Hz, C3',5'-H), 7.93 (2H, d, J = 8.8 Hz, C2',6'-H), 10.50 (1H, brs, C7-OH), 12.64 (1H, s, C5-OH).

General synthetic procedures for 5-hydroxy-7,8-dimethoxyflavone analogs (7a-7i)

To the solution of **6a-6j** (1.0 equiv.) and anhydrous potassium carbonate (3.0 equiv.) in anhydrous acetone (30 mL), was added dimethyl sulfate (1.0 equiv.) and then the mixture was refluxed for 6-7 h under nitrogen atmosphere. The reaction mixture was poured into water slowly and was evaporated to remove acetone. The reaction mixture was extracted with chloroform, dried over magnesium sulfate and concentrated under reduced pressure. The crude product was purified by re-crystallization with methanol.

5-Hydroxy-7,8-dimethoxyflavone (7a)
Yield 93%; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 3.95, 3.96 (6H, s, s, C7,8-OCH_3), 6.45 (1H, s, C3-H), 6.68 (1H, s, C6-H), 7.54-7.55 (3H, m, C3',4',5'-H), 7.94-7.97 (2H, m, C2',6'-H), 12.57 (1H, s, C5-OH).

5-Hydroxy-7,8-dimethoxy-4'-methylflavone (7b)
Yield 89%; ^{1}H-NMR (200 MHz, $CDCl_3$) δ 2.45 (3H, s, C4'-CH3), 3.95, 3.96 (6H, s, s, -OCH_3), 6.44 (1H, s, C3-H), 6.65 (1H, s, C6-H), 7.35 (2H, d, J = 8.2 Hz, C3',5'-H), 7.85 (2H, d, J = 8.2 Hz, C2',6'-H), 12.64 (1H, s, C5-OH).

5-Hydroxy-4',7,8-trimethoxyflavone (7c)
Yield 83%; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 3.90, 3.94, 3.95 (9H, s, s, s, -OCH_3), 6.43 (1H, s, C3-H), 6.59 (1H, s, C6-H), 7.04 (2H, d, J = 8.9 Hz, C3',5'-H), 7.91 (2H, d, J = 8.9 Hz, C2',6'-H), 12.66 (1H, s, C5-OH).

5-Hydroxy-2',4',7,8-tetramethoxyflavone (7d)
Yield 85%; ^{1}H-NMR (200 MHz, $CDCl_3$) δ 3.90, 3.92, 3.94 (12H, s, s, s, -OCH_3), 6.42 (1H, s, C3-H), 6.55 (1H, s, C6-H), 6.68 (1H, d, J = 8.8 Hz, C3'-H), 7.05 (1H, s, C5'-H), 8.00 (1H, d, J = 8.8 Hz, C6'-H), 12.79 (1H, s, C5-OH).

5-Hydroxy-3',4',7,8-dimethoxyflavone (7e)
Yield 90%; ^{1}H-NMR (200 MHz, $CDCl_3$) δ 3.95, 3.96, 3.98, 3.99 (12H, s, s, s, s, -OCH_3), 6.43 (1H, s, C3-H), 6.60 (1H, s, C6-H), 6.99 (1H, d, J = 6.0 Hz, C5'-H), 7.44 (1H, s, C2'-H), 7.61 (1H, d, J = 6.2 Hz, C6'-H), 12.63 (1H, s, C5-OH).

5-Hydroxy-2',3',4',7,8-pentamethoxyflavone (7f)
Yield 84%; ^{1}H-NMR (200 MHz, $CDCl_3$) δ 3.91, 3.92, 3.95, 3.98 (15H, s, s, s, s, -OCH_3), 6.43 (1H, s, C3-H), 6.84 (1H, d, J = 9.2 Hz, C5'-H), 6.94 (1H, s, C6-H), 7.66 (1H, d, J = 9.0 Hz, C6'-H), 12.72 (1H, s, C5-OH).

5-Hydroxy-3',4',5',7,8-pentamethoxyflavone (7g)
Yield 87%; ^{1}H-NMR (200 MHz, DMSO-d_6) δ 3.95, 3.97 (15H, s, s, -OCH_3), 6.45 (1H, s, C3-H), 6.63 (1H, s, C6-H), 7.23 (2H, d, J = 16.8 Hz, C2',6'-H), 12.56 (1H, s, C5-OH).

4'-Chloro-5-hydroxy-7,8-dimethoxyflavone (7h)
Yield 85%; ^{1}H-NMR (200 MHz, $CDCl_3$) δ 3.94, 3.96 (6H, s, s, -OCH_3), 6.45 (1H, s, C3-H), 6.65 (1H, s, C6-H), 7.53 (2H, d, *J* = 8.8 Hz, C3',5'-H), 7.89 (2H, d, *J* = 8.8 Hz, C2',6'-H), 12.51 (1H, s, C5-OH).

3',4'-Dichloro-5-hydroxy-7,8-dimethoxyflavone (7i)
Yield 84%; ^{1}H-NMR (200 MHz, $CDCl_3$) δ 3.94, 3.97 (6H, s, s, -OCH_3), 6.46 (1H, s, C3-H), 6.64 (1H, s, C6-H), 7.63 (1H, d, *J* = 8.0 Hz, C6'-H), 7.77 (1H, d, *J* = 10.6 Hz, C5'-H), 8.03 (1H, s, C2'-H), 12.45 (1H, s, C5-OH).

General synthetic procedures for 5-hydroxy-7,8-dimethoxyflavone analogs (8a-8i)

To the solution of **7a-7j** (1.0 equiv.) and anhydrous potassium carbonate (3.0 equiv.) in anhydrous acetone (30 mL), dimethyl sulfate (1.0 equiv.) was added with vigorous stirring. The mixture was refluxed for 6-7 h under nitrogen atmosphere. The reaction mixture was poured into water slowly and was evaporated to remove acetone. The reaction mixture was extracted with chloroform, dried over magnesium sulfate and concentrated under reduced pressure. The crude product was purified by re-crystallization with methanol.

5,7,8-Trimethoxyflavone (8a)
Yield 94%; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 3.97, 4.00, 4.02 (9H, s, s, s, -OCH_3), 6.45 (1H, s, C3-H), 6.70 (1H, s, C6-H), 7.51-7.53 (3H, m, C3',4',5'-H), 7.93-7.96 (2H, m, C2',6'-H).

5,7,8-Trimethoxy-4'-methylflavone (8b)
Yield 87%; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 2.44 (3H, s, C4'-CH_3), 3.95, 3.99, 4.01 (9H, s, s, s, -OCH_3), 6.44 (1H, s, C3-H), 6.66 (1H, s, C6-H), 7.32 (2H, d, *J* = 8.2 Hz, C3',5'-H), 7.83 (2H, d, *J* = 8.2 Hz, C2',6'-H).

4',5,7,8-Tetramethoxyflavone (8c)
Yield 85%; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 3.89, 3.94, 3.99, 4.01 (12H, s, s, s ,s, -OCH_3), 6.43 (1H, s, C3-H), 6.60 (1H, s, C6-H), 7.02 (2H, d, *J* = 8.9 Hz, C3',5'-H), 7.89 (2H, d, *J* = 8.9 Hz, C2',6'-H).

2',4',5,7,8-Pentamethoxyflavone (8d)
Yield 82%; ^{1}H-NMR (200 MHz, $CDCl_3$) δ 3.89, 3.93, 3.99, 4.01 (15H, s, s, s, s, -OCH_3), 6.42 (1H, s, C3-H), 6.55 (1H, s, C6-H), 6.65 (1H, d, *J* = 8.8 Hz, C3'-H), 7.02 (1H, s, C5'-H), 7.98 (1H, d, *J* = 8.8 Hz, C6'-H).

3',4',5,7,8-Pentamethoxyflavone (8e)
Yield 85%; ^{1}H-NMR (200 MHz, $CDCl_3$) δ 3.97, 3.98, 4.00, 4.02 (15H, s, s, s, s, -OCH_3), 6.45 (1H, s, C3-H), 6.63 (1H, s, C6-H), 7.00 (1H, d, *J* = 8.8 Hz, C5'-H), 7.43 (1H, s, C2'-H), 7.60 (1H, d, *J* = 8.6 Hz, C6'-H).

2',3',4',5,7,8-Hexamethoxyflavone (8f)
Yield 88%; ^{1}H-NMR (200 MHz, $CDCl_3$) δ 3.91, 3.92, 3.94, 3.97, 3.99, 4.01 (18H, s, s, s, s, s, s, -OCH_3), 6.44 (1H, s, C3-H), 6.82 (1H, d, *J* = 9.0 Hz, C5'-H), 6.90 (1H, s, C6-H), 7.64 (1H, d, *J* = 8.8 Hz, C6'-H).

3',4',5,5',7,8-Hexamethoxyflavone (8g)
Yield 87%; ^{1}H-NMR (200 MHz, $CDCl_3$) δ 3.93, 3.95, 3.96, 4.00, 4.02 (18H, s, s, s, s, s, -OCH_3), 6.45 (1H, s, C3-H), 6.65 (1H, s, C6-H), 7.19 (2H, s, C2',6'-H).

4'-Chloro-5,7,8-trimethoxyflavone (8h)
Yield 90%; ^{1}H-NMR (200 MHz, $CDCl_3$) δ 3.95, 4.00, 4.02 (9H, s, s, -OCH_3), 6.45 (1H, s, C3-H), 6.66 (1H, s, C6-H), 7.50 (2H, d, *J* = 8.2 Hz, C3',5'-H), 7.88 (2H, d, *J* = 8.4 Hz, C2',6'-H).

3',4'-Dichloro-5,7,8-trimethoxyflavone (8i)
Yield 90%; ^{1}H-NMR (200 MHz, $CDCl_3$) δ 3.96, 4.00, 4.03 (9H, s, s, -OCH_3), 6.46 (1H, s, C3-H), 6.66 (1H, s, C6-H), 7.60 (1H, d, *J* = 8.4 Hz, C6'-H), 7.76 (1H, d, *J* = 8.2 Hz, C5'-H), 8.02 (1H, s, C2'-H).

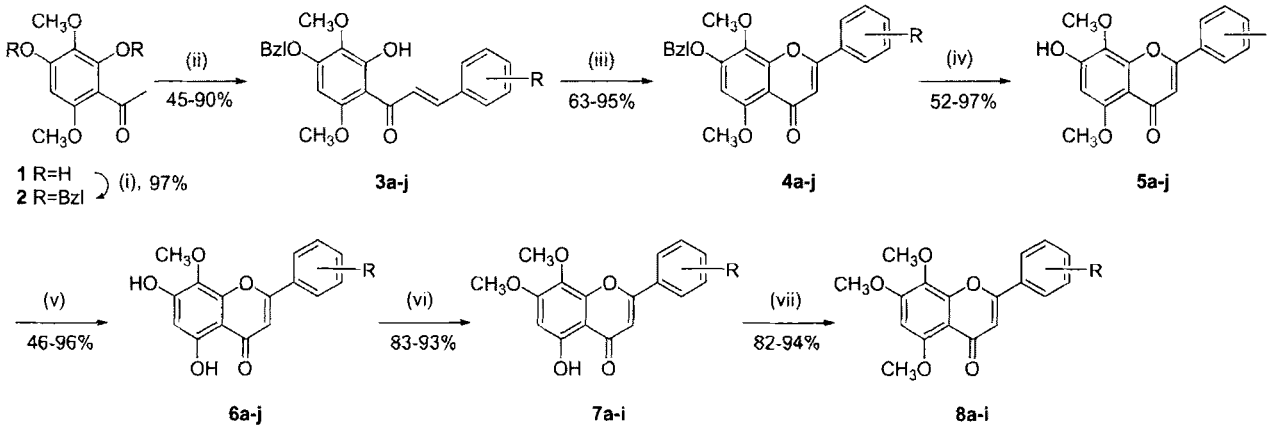
Biological evaluation

The bioassays were performed according to the published procedure (Chi, 2001). RAW 264.7 cells obtained from American Type Culture Collection were cultured with DMEM supplemented with 10% FBS and 1% CO_2 at 37°C and activated with LPS (Lipopolysaccharide, *Escherichia coli* O127:B8). Briefly, cells were plated in 96-well plates (2×10^5 cells/well). Each synthetic flavone was dissolved in dimethyl sulfoxide (DMSO) and LPS (1 μg/mL) were added and incubated for 24 h. Cell viability was assessed with MTT assay based on the experimental procedures described previously (Mossman, 1983). All tested compounds showed no or less than 10% reduction of MTT assay, indicating that they were not significantly cytotoxic to RAW 264.7 cells in the presence or absence of LPS. PGE_2 concentration in the medium was measured using EIA kit for PGE_2 according to the manufacturer's recommendation. All experiments were carried out at least twice and they gave similar results.

RESULTS AND DISCUSSION

2,4-Dihydroxy-3,6-dimethoxyacetophenone (**1**), the starting material for synthesis of wogonin derivatives, was prepared following the procedure in the literature (Tisdale, 2003) from pyrogallol in six steps and 29% overall yield. Wogonin derivatives were prepared from the intermediate **1** in three to seven steps following the procedures de-

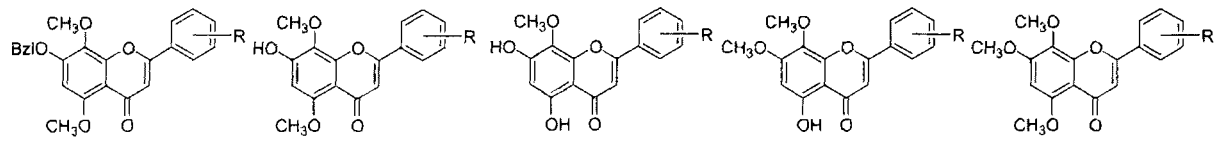
scribed in the previous publication (Dao, 2004a). Reaction of the compound **1** with benzyl bromide (1.1 equiv) and potassium carbonate (2.0 equiv) in anhydrous acetone solution at refluxing condition afforded the compound **2** in 97% yield. Reaction of the compound **2** in methanolic KOH with various aryl aldehydes gave the chalcones (**3a-j**) in 45-90% yields, respectively. Flavone ring formations of the chalcones (**3a-j**) in acetic acid and iodine in acetone gave the flavones (**4a-j**) in 63-95% yield, respectively. Selective deprotection of the benzyl group (52-97%) followed by selective 5-*O*-demethylation with $AlCl_3$ in acetonitrile gave the wogonin derivatives **6a-j** in 46-96% yields, respectively. Selective 7-*O*-methylations of **6a-j** with dimethylsulfate (1.1 equiv) and potassium carbonate (2.0 equiv) in anhydrous acetone solution at refluxing condition afforded the wogonin derivatives **7a-j** in 83-93% yields,



Reagents & reaction conditions: (i) K_2CO_3, benzyl bromide, acetone (ii) aryl aldehydes, KOH, MeOH (iii) I_2, DMSO, 100°C (iv) acetic acid, c-HCl, 80°C (v) $AlCl_3$, CH_3CN, 80°C (vi) & (vii) K_2CO_3, Me_2SO_4 (1 equiv), acetone

Scheme 1. Synthetic pathway for wogonin derivatives

Table I. Inhibition of COX-2 catalyzed PGE_2 production from LPS induced RAW 264.7 cells by wogonin derivatives[1,2]



a: all H, **b**: 4-CH_3, **c**: 4-OCH_3, **d**: 2,4-$(OCH_3)_2$, **e**: 3,4-$(OCH_3)_2$, **f**: 2,3,4-$(OCH_3)_3$, **g**: 3,4,5-$(OCH_3)_3$, **h**: 4-Cl, **i**: 3,4-Cl_2, **j**: 4-OH

Compd No.	% Inhibition[3]	Compd No.	% Inhibition[2]	Compd No.	% Inhibition[2]	Compd No.	% Inhibition[2]	Compd No.	% Inhibition[2]
4a	inactive[4]	**5a**	91	**6a**	99	**7a**	62	**8a**	27
4b	inactive	**5b**	17	**6b**	77	**7b**	16	**8b**	15
4c	inactive	**5c**	34	**6c**	82	**7c**	inactive	**8c**	inactive
4d	inactive	**5d**	inactive	**6d**	22	**7d**	86	**8d**	11
4e	inactive	**5e**	inactive	**6e**	inactive	**7e**	12	**8e**	12
4f	19	**5f**	inactive	**6f**	21	**7f**	19	**8f**	15
4g	inactive	**5g**	33	**6g**	97	**7g**	21	**8g**	65
4h	22	**5h**	16	**6h**	84	**7h**	inactive	**8h**	14
4i	inactive	**5i**	18	**6i**	94	**7i**	inactive	**8i**	inactive
4j(R=OBzl)	25	**5j**	inactive	**6j**	94	Wogonin[5]	99	Norwogonin[6]	inactive

[1]All compounds were treated at 10 μM. Treatment of LPS to raw cells increased PGE_2 production (10 μM) from the basal level of 0.5 μM. [2]All values represented here were arithmetic mean of duplicate. [3]% Inhibition = 100 × [1 – (PGE_2 of LPS with the flavones treated group – PGE_2 of the basal)/(PGE_2 of LPS treated group – PGE_2 of the basal)]. [4]Inactive indicates the % inhibition less than 10%. [5]Wogonin was isolated from plants and used as a reference sample. [6]Nwog indicates norwogonin which is 5,7,8-trihydroxyflavone. The results related with norwogonin will be published to other journal in the near future.

respective. Further 5-*O*-methylation of **7a-j** was carried out in the same reaction conditions for the starting materials from **6a-j** as shown in Scheme 1.

Among the wogonin derivatives tested, only several compounds of 5,7-dihydroxy-8-methoxyflavones (**6a-j**) retained strong inhibitory activity as demonstrated in Table I. Other wogonin derivatives exhibited little to low inhibitory activities against COX-2 catalyzed PGE_2 production. Alkylation of the phenol groups at 5- and/or 7-positions decreased the inhibitory activity regardless of the substituents on the B ring as observed from the results of synthesized wogonin derivatives. Synthetic wogonin (**6a**) showed same inhibitory activity compared to that of naturally isolated wogonin. 5,7,8-Trihydroxyflavone, 8-*O*-demethylated wogonin (norwogonin), was inactive to COX-2 catalyzed PGE_2 production. Our present study indicates that the methoxy group at the 8-position as well as the free phenol groups at the 5- and 7-positions of wogonin seems to play a very important role for the bioactivity. Further SARs study of wogonin with various functional groups at the 8-position is currently under investigation.

ACKNOWLEDGEMENTS

This work was supported by the grant (R05-2002-000856-0) from the Basic Research Program of the Korea Science & Engineering Foundation and also in part supported by the grant (01-PJ2-PG6-01NA01-0003) from Ministry of Health & Welfare, Republic of Korea. The authors would like to thank KNU Institute of Phamacal Research for the use of NMR and bioassay facilities.

REFERENCES

Chen, Y. C., Shen, S. C., Chen, L. G., Lee, T. J., and Yang, L. L., Wogonin, baicalin, and baicalein inhibition of inducible nitric oxide synthase and cyclooxygenase-2 gene expression induced by nitric oxide synthase inhibitors and lipopolysaccharide. *Biochem. Pharmacol.*, 61, 1417-1427 (2001).

Chi, Y. S., Cheon, B. S., and Kim, H. P., Effect of wogonin, a plant flavone from Scutellaria radix, on the suppression of cyclooxygenase-2 and the induction of inducible nitric oxide synthase in lopopolysaccharide-treated RAW 264.7 cells. *Biochem. Pharmacol.*, 61, 1195-1203 (2001).

Chi, Y. S., Lim, H., Park, H., and Kim, H. P., Effects of wogonin, a plant flavone from Scutellaria radix, on skin inflammation: *in vivo* regulation of inflammation-associated gene expression. *Biochem. Pharmacol.*, 66, 1271-1278 (2003).

Dao, T. T., Chi, Y. S., Kim, J., Kim, H. P., Kim, S., and Park, H., Synthesis and inhibitory activity against COX-2 catalyzed prostaglandin production of chrysin derivatives. *Bioorg. Med. Chem. Lett.*, 14, 1165-1167 (2004a).

Dao, T. T., Kim, S. B., Sin, K.-S., Kim, S., Kim, H. P. and Park, H., Synthesis and biological activities of 8-arylflavones. *Arch. Pharm. Res.*, 27, 278-282 (2004b).

Kim, H. P., Son, K. H., Chang, H. W., and Kang, S. S., Anti-inflammatory plant flavonoids and cellular action mechanism. *J. Pharmacol. Sci.*, 96, 229-245 (2004).

Kim, Y. P., Yamada, M., Lim, S. S., Lee, S. H., Ryu, N., Shin, K. H., and Okuchi, K., Inhibition by tectorigenin and tectoridin of prostaglandin E2 production and cyclooxygenase-2 induction in rat peritoneal macrophages. *Biochim. Biophys. Acta*, 1438, 399-407 (1999).

Lee, H., Kim, Y. O., Kim, H., Kim, S. Y., Noh, H. S., Kang, S. S., Cho, G. J., Choi, W. S., and Suk, K., Flavonoid wogonin from medicinal herb is neuroprotective by inhibiting inflammatory activation of microglia. *FASEB J.*, 17, 1943-1944 (2003).

Liang Y.-C., Huang Y.-T., Tsai S.-H., Shiau S.-Y., Chen C.-F., and Lin J.-K., Suppression of inducible cyclooxygenase and inducible nitric oxide synthase by apigenin and related flavonoids in mouse macrophages. *Carcinogenesis,* 20, 1945-1952 (1999).

Middleton, E., Kandaswami, C., and Theoharides, T. C., The effects of plant flavonoids on ammmalian cells: implications for inflammation, heart disease, and cancer. *Pharmacol. Rev.,* 52, 673-751 (2000).

Mossman, T., Rapid colorimetric asay for cellular growth and survival: application to proliferation and cytotoxic assays. *J. Immunol. Methods*, 65, 55-63 (1983).

Needleman, P. and Isakson, P., The discovery and function of COX-2. *J. Rhematol. 24(Suppl. 49)*, 6-8 (1997).

Tisdale, E. J., Vong, B. G., Li, H., Kim, S. H., Chowdhury, C., and Theodorakis, E. A., Total synthesis of *seco*-lateriflorone. *Tetrahedron*, 59, 6873-6887 (2003).

Wakabayashi, I., Inhibitory effects of baicalein and wogonin on lipopolysaccharide-induced nitric oxide production in macrophages. *Pharmacol. Toxicol.*, 84, 288-291 (1999).

Wakabayashi, I. and Yasui, K., Wogonin inhibits inducible prostaglandin E_2 production in marophages. *Eur. J. Pharmacol.*, 406, 477-481 (2000).