Arch Pharm Res Vol 27, No 3, 278-282, 2004

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

Synthesis and Biological Activities of 8-Arylflavones

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(Received January 15, 2004)

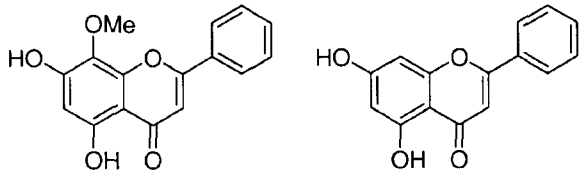
A number of 8-arylflavones have been synthesized as congeners of wogonin and evaluated for their inhibitory activities of PGE_2 production. 8-Arylflavones were obtained from commercially available chrysin *via* two different synthetic pathways. Most 8-arylflavones exhibited much reduced inhibitory activities against COX-2 catalyzed PGE_2 production compared to that of wogonin. Functional group replacement at the 8-position of wogonin from methoxy to aryl group caused loss of inhibitory activity. Our present results imply that the functional group at the 8-position of flavones seems to play very important roles for bioactivity.

Key words: 8-Arylflavones, COX-2, Prostaglandin production inhibition, Anti-inflammatory activity, Suzuki reaction, Carbon-carbon coupling reaction

INTRODUCTION

Flavonoids are natural polyphenol compounds of plant origin and exhibit various biological activities such as anti-inflammatory, anti-oxidant, and anti-tumor activities (Read, 1995; Harborne, 2000). It has been previously reported that various plant flavonoids possess the inhibitory activity on cyclooxygenase/lipoxygenase (Wakabayashi, 2000; Chi, 2001; Chen, 2001). Some flavonoids, such as flavone derivatives, have been reported previously to inhibit nitrogen oxide (NO) production by suppressing inducible nitric oxide synthase (iNOS) expression (Wakabayashi, 1999; Chi, 2001 & 2003; Chen, 2001; Kim, 2001; Lee, 2003). And these properties of flavonoids might contribute to their anti-inflammatory activity both *in vitro* and *in vivo*. Wogonin (5,7-dihydroxy-8-methoxyflavone), a flavonoid isolated from the root of a medicinal herb *Scutellaria baicalensis* Georgi, has been shown to possess various anti-inflammatory activities (Wakabayashi, 1999 & 2000; Shieh, 2000; Chi, 2001 & 2003; Chen, 2001; Kim, 2001; Lee, 2003). These results led us to investigate the structural requirement of wogonin for anti-inflammatory activity.

Base on the results from the structure-activity relationships of wogonin and some other naturally occurring flavonoids such as chrysin (Fig. 1), we conjectured that the methoxy functional group at 8-position of wogonin plays important roles to possess strong anti-inflammatory activity since wogonin exhibited much stronger inhibitory activity than chrysin against COX-2 catalyzed prostaglandin production from lipopolysaccharide-treated RAW 264.7 cells. In order to improve the biological activities of wogonin, we started to prepare wogonin derivatives modified 8-methoxy group of the A ring by various functional groups. In this work, we report the synthesis of 8-aryl substituted wogonin derivatives and their inhibitory activities of PGE_2 production from lipopolysaccharide-treated RAW 264.7 cells.



Wogonin **Chrysin**

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

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

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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). ^{13}C-NMR spectra were recorded on a Varian Gemini 2000 instrument (50 MHz) spectrometer, fully decoupled and chemical shifts are reported in parts per million (ppm) downfield relative to tetramethylsilane as an internal standard. 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).

5,7-Dimethoxyflavone (2)

Chrysin (7.5 mmol), K_2CO_3 (30 mmol) and dimethyl sulfate (15 mmol) in 20 mL of acetone were refluxed for 4 h, monitoring by TLC with solvent system of chloroform-methanol (20:1). After removing potassium carbonate, the reaction solution was poured into a beaker containing 100 mL of water. Solid was filtered, washed with water, dried and crystallized from methanol to yield colorless solid with overall yield 90%. ^{1}H-NMR (200 MHz, $CDCl_3$): δ 7.86-7.91 (d, 2H, H2', H6'), 7.49-7.53 (m, 3H, H3', H4', H5'), 6.70 (s, 1H, H8), 6.59 (S, 1H, H6), 6.4 (s, 1H, H8), 3.97 (s, 3H, OCH_3), 3.92 (s, 3H, OCH_3).

8-Iodo-5,7-dimethoxyflavone (3)

A mixture of iodine (20 mmole), 5,7-dimethoxyflavone (**2**, 20 mmole) in 20 mL of dry acetone and 20 mL of glacial acetic acid was stirred in ice bath for 30 minutes and was slowly added 2 g of 65% HNO_3 acid in 10 mL of acetic acid. The product was precipitated during the addition. The reaction mixture was stirred for two hours and filtered. The solid was washed with 10% $Na_2S_2O_4$ solution, cold methanol and water. 8-Iodo-5,7-dimethoxy-flavone (**3**) was precipitated as pale yellow solid with overall yield 75%. ^{1}H-NMR (200 MHz, $CDCl_3$): δ 8.05-8.09 (d, 2H, H2', H6'), 7.52-7.55 (m, 3H, H3', H4', H5'), 6.75 (s, 1H, H6), 6.45 (s, 1H, H3), 4.04 (s, 6H, 2xOCH_3).

General procedure for carbon-carbon coupling reaction (Suzuki reaction)

To a solution of 8-iodo-5,7-dimethoxyflavone (**3**, 5 mmol) and areneboronic acid derivatives (6 mmol) in 20 mL of dry dimethoxyethane was added Pd $(PPh_3)_4$ (0.2 mmol). The resulting mixture was degassed and stirred at ambient temperature for 20 minutes before adding saturated aqueous Na_2CO_3 solution (10 mL). The mixture was degassed again and then stirred under nitrogen gas for 1 h. The areneboronic acid (10 mmol) then added, and the reaction mixture was heated at 80 °C for 4 hrs. After cooling to room temperature, the mixture was diluted with dichloromethane (40 mL) and water (20 mL), the organic phase was separated, washed with water and dried over magnesium sulfate, filtered and evaporated in reduced pressure. The residue was purified by flash column to obtain 5,7-dimethoxy-8-arylflavones (**4a-4e**).

5,7-Dimethoxy-8-phenylflavone (4a)

^{1}H-NMR (200 MHz, $CDCl_3$): δ 8.04-8.08 (d, 2H, H2', H6'), 7.50-7.54 (m, 5H, phenyl), 7.16-7.20 (m, 3H, H3', H4', H5'), 6.74 (s, 1H, H6), 6.45 (s, 1H, H3), 4.03 (s, 6H, 2xOCH_3); Yield 68%; mp 167-169 °C.

5,7-Dimethoxy-8-(3',4'-dimethoxyphenyl)flavone (4b)

^{1}H-NMR (200 MHz, $CDCl_3$): δ 7.35-7.659 (m, 5H, H2', H3', H4', H5', H6'), 7.0 (s, 1H, C2"), 6.74-6.98 (d, 2H, H5", H6"), 6.72 (s, 1H, H6), 6.53 (s, 1H, H3), 4.07 (s, 3H, OCH_3), 3.98 (s, 3H, OCH_3), 3.92 (s, 3H, OCH_3), 3.86 (s, 3H, OCH_3); Yield 71%; mp 201-204 °C.

5,7-Dimethoxy-8-(3',4',5'-trimethoxyphenyl)flavone (4c)

^{1}H-NMR (200 MHz, $CDCl_3$): δ 7.35-7.59 (m, 5H, H2', H3', H4', H5', H6)', 6.72 (s, 1H, H6), 6.65 (s, 2H, H2", H6"), 6.53 (s, 1H, H3), 4.07 (s, 3H, OCH_3), 3.96 (s, 3H, OCH_3), 3.94 (s, 3H, OCH_3), 3.85 (s, 6H, 2xOCH_3); Yield 70%; mp 245-247 °C.

8-3-(Formylphenyl)-5,7-dimethoxyflavone (4d)

^{1}H-NMR (200 MHz, $CDCl_3$): δ 10.1 (s, 1H, CHO), 7.30-8.0 (m, 9H, H2', H3', H4', H5', H6', H2", H4", H5", H6"), 6.70 (s, 1H, H3), 6.54 (s, 1H, H6), 4.08 (s, 3H, OCH_3), 3.91 (s, 3H, OCH_3); Yield 75%; mp 219-222 °C.

8-(4-Formylphenyl)-5,7-dimethoxyflavone (4e)

^{1}H-NMR (200 MHz, $CDCl_3$): δ 10.13 (s, 1H, CHO), 8.00-8.04 (d, 2H, J = 8 Hz, J = 2 Hz, H2', H6'), 7.16-7.63 (m, 7H, H3', H4', H5', H2", H3", H5", H6"), 6.70 (s, 1H, H3), 6.54 (S, 1H, H6), 4.09 (s, 3H, OCH_3), 3.92 (s, 3H, OCH_3) ; Yield 80%; mp 244-246 °C.

7-Benzyloxy-5-methoxyflavone (5)

Selective benzyl protection at the phenol at 7-position was carried out with chrysin, K_2CO_3 and benzyl chloride (1 equiv.) in acetone. Further methyl protection of the reaction intermediate with dimethyl sulfate (1.1 equiv.) yielded the title product. Yield 72%. ^{1}H-NMR (200 MHz, $CDCl_3$): δ 7.85-7.90 (d, 2H, H2', H6'), 7.40-7.53 (m, 8H, H3', H4', H5', phenyl), 6.70 (s, 1H, H6), 6.66 (S, 1H, H3), 6.47 (s, 1H, H3), 5.17 (s, 2H, -CH_2), 3.96 (s, 3H, OCH_3).

7-Benzyloxy-8-iodo-5-methoxyflavone (6)

Iodination of the compound **5** was followed the procedure for the compound **3**. ^{1}H-NMR (200 MHz, $CDCl_3$): δ 8.04-8.09 (d, 2H, H2', H6'), 7.40-7.55 (m, 8H, H3', H4', H5', phenyl), 6.73 (s, 1H, H6), 6.47 (S, 1H, H3), 5.32 (s, 2H, -CH_2), 3.96 (s, 3H, OCH_3).

7-Benzyloxy-5-methoxy-8-phenylflavone (7)

Aryl substitution of the compound **6** was followed the procedure for the compounds **4a-4e**. ^{1}H-NMR (200 MHz, $CDCl_3$): 7.29-7.53 (m, 10H, H2', H3', H4', H5', H6', H2", H3", H4", H5", H6"), 6.70 (s, 1H, H6), 6.55 (s, 1H, H3), 5.20 (s, 2H, -CH_2), 3.97 (s, 3H, OCH_3); Yield 68%; mp 188-191 °C.

7-Hydroxy-5-methoxy-8-phenylflavone (8)

The compound **7** was reacted in acetic acid with c-HCl at refluxing condition. When the reaction was completed, the mixture was diluted with dichloromethane and washed three times with water. The organic layer was dried over $MgSO_4$ and concentrated under the reduced pressure. The residue was crystallized from chloroform-methanol to obtain the titled compound. ^{1}H-NMR (200 MHz, $CDCl_3$ + DMSO-d_6): δ 9.88 (s, 1H, OH), 7.38-7.55 (m, 10H, H2', H3', H4', H5', H6', H2", H3", H4", H5", H6"), 6.64 (s, 2H, H3, H6), 3.97 (s, 3H, OCH_3); Yield 65%; mp 296-298 °C (decompose).

5,7-Dihydroxy-8-phenylflavone (9)

To a solution of **8** in 20 mL of dry chloroform was added BBr_3 (1.0 M in $CHCl_2$, 24 mmole), and the reaction mixture was stirred at refluxing condition for overnight. The reaction mixture was cooled to room temperature and was added methanol to decompose the excess BBr_3. After evaporation in vacuum, the residue was dissolved in aqueous 1 N NaOH solution and washed two times with ethyl acetate. The solution was cooled to 0 °C and acidified to pH = 3 by using 3 M HCl solution. The precipitated solid was filtered and recrystallized from methanol to get the titled compound. ^{1}H-NMR (200 MHz, DMSO-d_6): δ 13.03 (bs, 1H, OH), 10.95 (s, 1H, OH), 7.41-8.10 (m, 10H, H2', H3', H4', H5', H6', phenyl), 7.14 (s, 1H, H6), 6.45 (s, 1H, H3); Yield 58%; mp 194-196 °C.

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. The inhibitory activities of synthetic flavones on COX-2 catalyzed PGE_2 production from LPS-induced RAW 264.7 cells were estimated and shown in Table I.

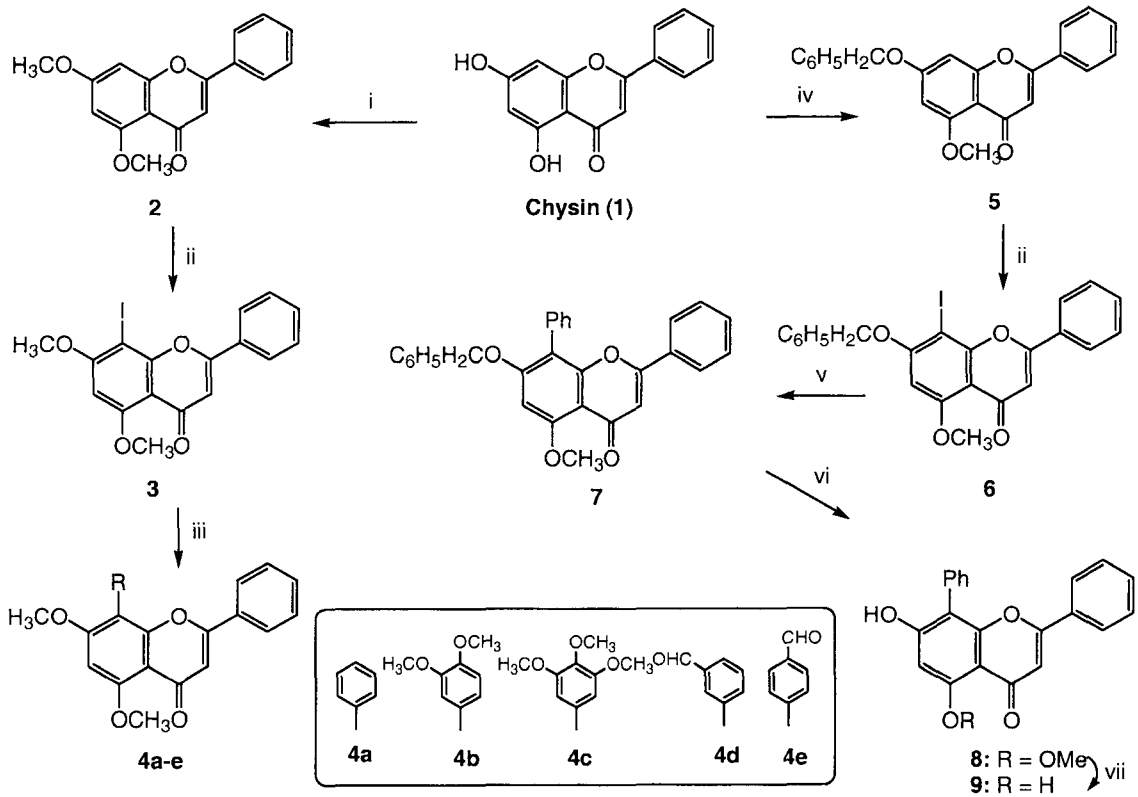
Table I. Inhibition of COX-2 catalyzed PGE_2 production from LPS induced RAW 264.7 cells by 8-arylflavones

Flavones[a]	% Inhibition of PGE_2 production[b,c]
1 (chrysin)	11.12
4a	inactive
4b	inactive
4c	inactive
4d	14.63
4e	inactive
7	33.21
8	25.23
9	45.07
wogonin	99.27
NS-398[d]	100.44

[a]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.
[b]% 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)].
[c]All values represented here were arithmetic mean of duplicate.
[d]NS-398, *N*-(2-cyclohexyloxy)-4-nitrophenylmethanesulfonamide, was used as the reference compound for inhibition of PGE_2 production test.

RESULTS AND DISCUSSION

8-Iodo-5,7-dimethoxyflavone (**3**), a key intermediate for synthesis of 8-arylflavones, was prepared from commercially available chrysin (**1**) in two steps. Reaction of chrysin with dimethyl sulfate (2.2 equiv) and potassium carbonate in anhydrous acetone solution at refluxing condition afforded 5,7-dimethoxyflavone (**2**). Reaction of the compound **2** in acetic acid and iodine in acetone gave 8-iodo-5,7-dimethoxyflavone (**3**, de Rossi *et al.*, 1986). Suzuki reaction conditions were applied to introduce aryl groups to the flavone ring system (Suzuki, 1999). Carbon-carbon coupling reaction of the iodoflavone (**3**) and areneboronic acid in the presence of catalytic amount of $Pd(PPh_3)_4$ in anhydrous DMF gave 8-aryl-5,7-dimethoxyflavone (**4a-e**) as shown in Scheme 1. For synthesis of 5,7-dihydroxy-8-arylflavones, we followed a different synthetic pathway. Reaction of chrysin with benzyl bromide



Scheme 1. Synthetic procedure for 8-arylflavones from chrysin. i: Dimethyl sulfate (2 equiv.) K_2CO_3, anhydrous acetone, reflux. ii: Iodine in acetone, flavone in acetic acid, 0°C. iii: Areneboronic acid, DMF, K_2CO_3, $Pd(PPh_3)_4$, 80°C. iv: (a) Benzyl chloride (1 equiv.), K_2CO_3, acetone, reflux. (b) Dimethyl sulfate (1.1 equiv.) reflux. v: Benzeneboronic acid, DMF, K_2CO_3, $Pd(PPh_3)_4$, 80°C. vi: *c*-HCl, acetic acid, 80°C. vii: BBr_3, chloroform, reflux.

(1.1 equiv) and potassium carbonate in anhydrous acetone solution at refluxing condition and followed by the reaction with dimethyl sulfate afforded 7-benzyloxy-5-methoxyflavone (**5**). Reaction of the compound **5** in acetic acid and iodine in acetone gave 7-benzyloxy-8-iodo-5-methoxyflavone (**6**). Phenyl group introduction in Suzuki reaction conditions gave 7-benzyloxy-5-methoxy-8-phenylflavone (**7**). 7-Hydroxy-5-methoxy-8-phenylflavone (**8**) was prepared by reaction of the compound **7** in c-HCl solution (Dao, 2004). Reaction of the compound **8** with BBr_3 in chloroform solution gave 5,7-dihydroxy-8-phenylflavone (**9**) as shown in Scheme 1. Selective deprotection of 5-methoxy group in the presence of multiple methoxy groups was possible with $AlCl_3$. This synthetic pathway was also applied for synthesis of 5,7-dihydroxy analogs of the compound **4b** and **4c**.

As shown in Table I, most 8-arylflavones exhibited little to low inhibitory activities against COX-2 catalyzed PGE_2 production. Aryl substitution instead of the methoxy group at the 8-position of wogonin caused loss of the inhibitory activity. Methylation of the phenol groups at 5- and 7-positions also decreased the inhibitory activity as we observed from the results of **4a** and **9**. Our present study indicates that the methoxy group at the 8-position of wogonin seems to play very important roles 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.

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