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Structural Alterations of Wogonin Significantly Reduce the Inhibitory Activity against COX-2 Catalyzed PGE_2 Production from LPS-Induced RAW 264.7 Cells

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Abstract − Structural alterations of wogonin were conducted to observe the role of each functional group at the A-ring of wogonin on the inhibitory activities against COX-2 catalyzed PGE_2 production from LPS-induced RAW 264.7 cells. Serial deletion of the functional groups at the A-ring of wogonin exhibited low to comparable bioactivity. The present study validated that all the functional groups at the A-ring of wogonin are important factors to exhibit the optimum inhibitory activities.

Keywords: Structural alterations, Wogonin, PGE_2 production, COX-2, Anti-inflammatory activity

INTRODUCTION

Flavonoids are naturally occurring polyphenolic compounds which possess a wide range of biological activities such as anti-inflammatory, anti-tumor and anti-oxidant (Read, 1995; Harborne and Williams, 2000). They are ubiquitously present in plant kingdom and also integral components abundant in our daily common diet. The various flavonoid of plant origin have been reported to possess the inhibitory activity on cyclooxygenase and lipoxygenase (Wakabayashi and Yasui, 2000; Chen *et al*., 2001; Chi *et al*., 2001). Many flavonoids derivatives have been reported previously to inhibit nitrogen oxide (NO) production by suppressing inducible nitric oxide synthase (*i*NOS) expression (Wakabayashi, 1999; Chen *et al*., 2001; Chi *et al*., 2001, 2003; Kim *et al*., 2001; Lee *et al*., 2003).

Scutellariae radix, the dried root of *Scutellaria baicalensis* Georgi is a well known traditional Chinese medicinal herb used since ancient time to treat many diseases in oriental countries. According to Chinese herbology, *Scutellariae radix* has been used in prescriptions for "heat-removing". Its flavor is bitter and produces a "cooling" sensation. Wogonin, (5,7-dihydroxy-8-methoxyflavone) is a major bioactive constituent which was isolated from *Scutellaria radix*. Wogonin was previously reported to suppress the induction of COX-2 and inucible nitric oxide *synthase* (Chen *et al*., 2001; Chi *et al*., 2003; Lee *et al*., 2003; Wakabayashi, 1999, 2000). It was already reported that the functional group replacement at the 8-position of wogonin from methoxy to aryl group caused loss of inhibitory activity (Dao *et al*., 2004b). Similarly, the Wogonin derivatives modified at the B-ring of Wogonin exhibited much reduced inhibitory activities against COX-2 catalyzed PGE_2 production compared to that of wogonin (Jang *et al*., 2005). In spite of the structural similarity between chrysin and wogonin, wogonin exhibited much stronger inhibitory activity than chrysin against COX-2 catalyzed PGE_2 production from LPS-treated RAW 264.7 cells (Dao *et al*., 2004a) Therefore, these precedent results led us to carry out the structure alteration at the A-ring of wogonin to observe the effect of each substituent. In this study, several flavones were designed (Fig. 1) with serial deletion of substituents at the A-ring of wogonin. The synthesized flavones were evaluated for their inhibitory activities against COX-2 catalyzed PGE_2 production from LPS-treated RAW 264.7 cells.

MATERIALS AND METHODS

Chemicals

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

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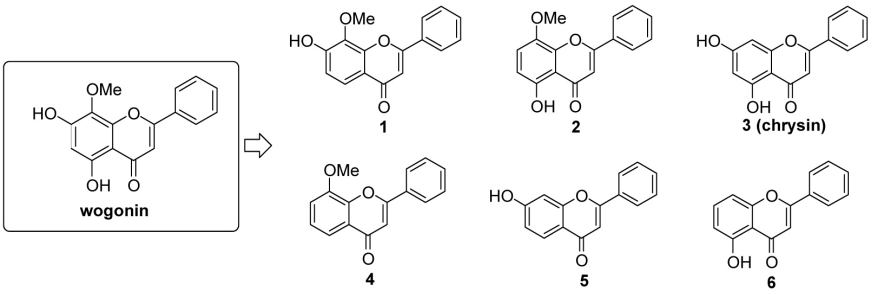

Fig. 1. Chemical structures of tested wogonin analogs.

purification. 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).

Flavones for the inhibitory activity tests were prepared following the procedure as described in the precedent literatures (Dao *et al*., 2004a; Jang *et al*., 2005). Thus, commercially available desired hydroxyacetopheones were selectively protected and were reacted with appropriately substituted aryl aldehydes to yield the corresponding chalcones. Each chalcone was transformed to the corresponding flavones in iodine-DMSO conditions as good yields and the subsequent deprotection procedure (Huang *et al.,* 2003; Gao *et al.,* 2004) provided the desired flavones for tests, respectively. Chrysin (3) was purchased from Sigma-Aldrich Korea and used for the bioassay without further purification. Wogonin for test was previously synthesized by us (Jang *et al*., 2005) and fully characterized.

7-Hydroxy-8-methoxyflavone (1)

mp 229-232°C, ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.03-8.05 (dd, *J*=2.3 Hz, 7.9 Hz, 2H, H2', H6'), 7.69-7.72 (d, 1H, *J*=8.8 Hz, H5), 7.50-7.57 (m, 3H, H3', H4', H5'), 7.06-7.09 (d, 1H, *J*=8.8 Hz, H6), 6.78 (s, 1H, H3), 4.02 (s, 3H, OMe). ^{13}C-NMR (100 MHz, $CDCl_3$): δ 179.8 (C-4), 164.3 (C-2), 151.7 (C-7), 146.2 (C-9), 134.9 (C-8), 131.9 (C-1'), 131.9 (C-4'), 129.2 (C-3', C-5'), 126.8 (C-2', C-6'), 118.4 (C-5), 115.9 (C-10), 109.7 (C-6), 106.3 (C-3), 56.6 (C-OMe).

5-Hydroxy-8-methoxyflavone (2)

mp 188-191°C, ^{1}H-NMR (400 MHz, $CDCl_3$): δ 11.96 (s, 1H, 5-OH), 7.96-7.98 (dd, 2H, *J*=1.9 Hz, 8.1 Hz, H2', H6'), 7.51-7.57 (m, 3H, H3', H4', H5'), 7.18-7.20 (d, 1H, *J*=8.9 Hz, H7), 6.76-6.77 (d, 2H, *J*=8.9 Hz, H3, H6), 3.97 (s, 3H, OMe). ^{13}C-NMR (100 MHz, $CDCl_3$): δ 183.5 (C-4), 164.4 (C-2), 153.4 (C-9), 146.0 (C-5), 140.5 (C-8), 132.0 (C-1'), 131.2 (C-4'), 129.2 (C-3', C-5'), 126.5 (C-2', C-6'), 118.8 (C-7), 111.5 (C-3), 109.9 (C-10), 106.0 (C-6), 57.3 (C-OMe).

8-Methoxyflavone (4)

mp 108-112°C, ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.88-7.90 (dd, *J*=2.3 Hz, 7.3 Hz, 2H, H2', H6'), 7.55-7.59 (dd, 1H, *J*=8.2 Hz, 8.4 Hz, H6), 7.48-7.51 (m, 3H, H3', H4', H5'), 7.12-7.14 (d, 1H, *J*=8.4 Hz, H5), 6.81-6.83 (d, 1H, *J*=8.2 Hz, H7), 6.75 (s, 1H, H3), 4.00 (s, 3H, OMe). ^{13}C-NMR (100 MHz, $CDCl_3$): δ 178.8 (C-4), 161.5 (C-2), 160.2 (C-8), 158.7 (C-9), 134.2 (C-1'), 131.8 (C-4'), 129.4 (C-3', C-5'), 126.5 (C-2', C-6', C-10), 115.0 (C-6), 110.6 (C-7), 109.5 (C-5), 106.8 (C-3), 56.9 (C-OMe).

7-Hydroxyflavone (5)

mp 239-243°C, ^{1}H-NMR (400 MHz, DMSO-d_6): δ 10.84 (s, 1H, OH), 8.05-8.07 (dd, *J*=2.1 Hz, 7.9 Hz, 2H, H2', H6'), 7.88-7.90 (d, 1H, *J*=8.8 Hz, H5), 7.54-7.61 (m, 3H, H3', H4', H5'), 7.00-7.01 (d, 1H, *J*=2.3 Hz, H8), 6.92-6.94 (dd, 1H, *J*=2.3 Hz, 8.8 Hz, H6), 6.90 (s, 1H, H3). ^{13}C-NMR (100 MHz, DMSO-d_6): δ 177.3 (C-4), 163.6 (C-7), 162.8 (C-2), 158.4 (C-9), 132.4 (C-1'), 132.2 (C-5), 130.0 (C-3', C-5'), 127.4 (C-4'), 127.0 (C-2', C-6'), 117.0 (C-10), 116.0 (C-6), 107.5 (C-3), 103.4 (C-8).

5-Hydroxyflavone (6)

mp 150-153°C, ^{1}H-NMR (400 MHz, $CDCl_3$): δ 12.58 (s, 1H, OH), 7.90-7.92 (dd, *J*=2.6 Hz, 8.1 Hz, 2H, H2', H6'), 7.51-7.57 (m, 4H, H7, H3', H4',H5'), 6.99-7.01 (d, 1H, *J*=8.4 Hz, H8), 6.80-6.83 (d, 1H, *J*=8.4 Hz, H6), 6.74 (s, 1H, H3). ^{13}C-NMR (100 MHz, $CDCl_3$): δ 183.6 (C-4), 164.6 (C-2), 160.8 (C-5), 156.4 (C-9), 135.4 (C-7), 132.1 (C-1'), 131.2 (C-4'), 129.1 (C-3', C-5'), 126.4 (C-2', C-6'), 111.5 (C-6), 110.9 (C-10), 107.1 (C-8), 106.1 (C-3).

Inhibition of COX-2 catalyzed PGE_2 production from LPS-induced RAW 264.7 cells by synthetic flavones

Inhibition of COX-2 catalyzed PGE_2 production from LPS-induced RAW 264.7 cells by synthetic flavones was determined according to the published procedure (Chi *et al*., 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. Briefly, cells were plated in 96-well plates (2×10^5 cells/wells). Each synthetic flavone and LPS (1 g/ml) were added and incubated for 24 hrs. Cell viability was assessed with MTT assay based on the experimental procedures described previously. PGE_2 concentration in the medium was measured using EIA kit for PGE_2 according to the manufacture's recommendation. All experiments were carried out at least twice and they gave similar results.

RESULTS AND DISCUSSION

This study is aimed to find the structural alterations and their effect on the extent of inhibitory activity in comparison to parent compound-wogonin. In order to obtain analogs for the goal, wogonin was altered structurally by serial deletion of functional groups. Thus, the two groups of analogs were obtained. In the first group, three analogs (analog 1, 2 and 3) were obtained by deleting one functional group serially. Similarly in the other group, three analogs (analog 4, 5 and 6) were obtained by deleting two functional groups at each time. The inhibitory activities of synthetic flavones on COX-2 catalyzed PGE_2 production from LPS-induced RAW 264.7 cells were estimated and the results are shown in Table I.

As shown in Table I, the analog 1, which was obtained by deleting 5-hydroxyl group, almost retained the inhibitory activity and comparable to the reference compound. Deletion of 7-hydroxyl group provided the analog 2, which showed much reduced inhibitory activity than the analog 1. The analog 3 (chrysin), which possess two hydroxyl groups and lacks 8-methoxy group, exhibited stronger inhibitory activity than the analog 2 but weaker than the analog 1. These observations showed that the inhibitory activity is affected to somewhat extent by deleting one functional group at each time in moderate extent.

Removal of two hydroxy groups almost completely reduced the inhibitory activity as observed from the analog 4. Removal of 8-methoxy and 5-hydroxyl group provided somewhat moderate inhibitory activity as shown in the analog 5. Removal of 8-methoxy and 7-hydroxyl groups (analog 6) resulted in as equally reduced the inhibitory activity as the analog 5.

As we observed from Table I, all the analogs tested showed moderate to significantly reduced inhibitory activities. Especially, the analog 4, lack of the hydroxyl group at the A-ring, totally lose its inhibitory activity.

In conclusion, this study is meaningful to observe the important role of each functional group and establish the relationship of structural alterations of wogonin on the inhibitory activity against COX-2 catalyzed PGE_2 production.

Table I. Inhibition of COX-2 catalyzed PGE_2 production from LPS-treated RAW 264.7 cells by wogonin analogs

Wogonin analogs	1	2	3	4	5	6	Wogonin
% inhibition	92.6	55.2	74.0	2.6	64.0	65.0	99.9

All compounds were treated at 10 μM. Treatment of LPS to RAW cells increased PGE_2 production (10.0 μM) from the basal level of 0.5 μM. % 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)]. Wogonin was used as the reference compound.

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