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Synthesis and inhibitory activity against COX-2 catalyzed prostaglandin production of chrysin derivatives

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Abstract—A series of chrysin derivatives were prepared and evaluated for their inhibitory activities of cyclooxygenase-2 catalyzed prostaglandin production. Chrysin derivatives were prepared from 2-hydroxyacetophenone, 2,4-dihydroxyacetophenone and 2,6-dihydroxyacetophenone in 2 to 4 steps, respectively. Methxoylated chrysin derivatives were converted to the corresponding hydroxylated chrysin derivatives by the reaction with BBr_3 in good yields. The inhibitory activity of the chrysin derivatives against prostaglandin production from lipopolysaccharide-treated RAW 264.7 cells was measured. We found that chrysin derivatives with 3′,4′-dichloro substituents (**5e**, **6e** and **7e**) exhibited good inhibitory activity of prostaglandin production.

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) catalyzes the conversion of arachidonic acid to prostaglandins (PGs), a key proinflammatory eicosanoid. COX exists in two isoforms. COX-1 is a constitutive enzyme processing homeostasis function, while COX-2 is an inducible one and known as a major isoform found in the inflammatory lesions.[1] Recently developed COX-2 inhibitors show promising results in clinical use. Therefore, a search for COX-2 inhibitors or modulators may be important to develop new anti-inflammatory agents.

Flavonoids from plant origin possess anti-inflammatory activity. In addition to the inhibitory activity of some flavonoids against COX-1 and/or COX-2,[2,3] recent studies have shown that several flavone analogues such as apigenin, wogonin and tectorigenin (Fig. 1) down-regulate COX-2 expression,[4–6] suggesting a potential for new class of anti-inflammatory agents. Chrysin (Fig. 1), a natural flavonoid widely distributed in plants, has been reported to have various biological activities such as anti-oxidant,[7] anti-anxiolytic,[8] and anti-cancer[9] effects. Furthermore chrysin has been reported to possess anti-inflammatory effects.[10] In order to improve the anti-inflammatory activity of chrysin, 25 derivatives of chrysin with serial deletion and methylation of the phenol

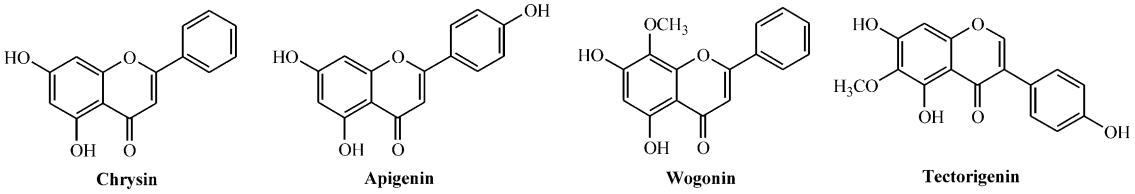

Figure 1. Structures of some naturally occurring polyhydroxyflavonoids.

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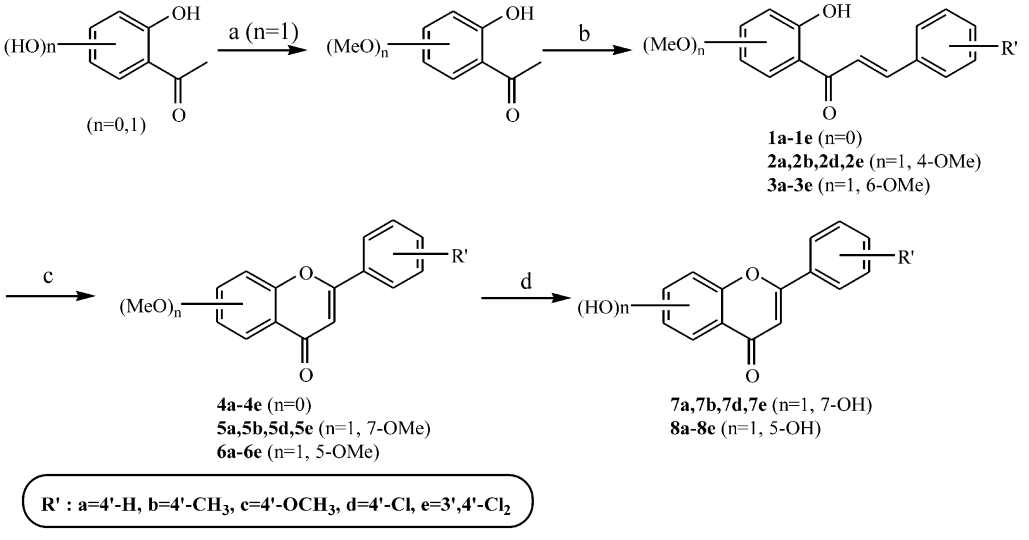
groups on the A ring and/or substitution of the B ring were prepared. Herein, we described the synthesis of chrysin derivatives and their inhibitory activities against COX-2 catalyzed PGE_2 production from LPS-induced RAW 264.7 cells.

Commercially available 2,4- and 2,6-dihydroxyacetophenones were treated with anhydrous potassium carbonate and dimethyl sulfate (1 equiv) in acetone to give 4-methoxy and 6-methoxy-2-hydroxyacetophenone in good yields, respectively.[11] These compounds were reacted with aryl aldehydes in methanolic KOH solution to afford the corresponding chalcones (**2a**, **b**, **d**, **e**, and **3a**–**e**). Treatment of the chalcones with catalytic amount of iodine in dimethyl sulfoxide gave 5-methoxyflavones (**6a**–**e**) and 7-methoxyflavones (**5a**, **b**, **d**, and **e**).[12] Reaction of 2-hydroxyacetophenone with aryl aldehydes followed by the flavone ring formation in same conditions gave the flavone analogues (**4a**–**e**) without any phenol group on A ring. Reaction of the methoxyflavones with BBr_3 in dichloromethane gave 5-hydroxyflavones (**8a**, **b**, **d** and **e**) and 7-hydroxyflavones (**7a**, **b**, **d** and **e**), respectively.[13] Reaction of the 4′,5-dimethoxyflavone (**6c**) with $AlCl_3$ gave the 5-hydroxyflavones (**8c**).[14] The synthetic procedure and reaction conditions are shown in Scheme 1. For the synthesis of 7-hydroxy-4′-methoxyflavone (**7c**), we protected the 4-hydroxyl group of 2,4-dihydroxyacetophenone with benzyl group in the standard conditions.[11] Reaction of 4-benzyloxy-2-hydroxyacetophenone in methanolic KOH yielded the chalcone (**2c**). The chalcone was converted to 7-benzyloxy-4′-methoxyflavone (**5c**) and the removal of the protecting group gave the compound (**7c**).

The bioassays were performed according to the published procedure.[6] 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.[15] 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. Therefore, the inhibition of PGE_2 production by flavone derivatives might be not associated with their cytotoxicity. 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 the results are shown in Table 1.

Most chrysin derivatives showed better biological activities than chrysin against COX-2 catalyzed PGE_2 production from LPS-induced RAW 264.7 cells. Among the chrysin derivatives tested, 7-methoxyflavone analogues (**5a**–**e**) generally showed potent inhibitory activities regardless of the substituent on B ring, whereas other series of synthetic flavones mostly exhibited moderate to little inhibitory activities as demonstrated in Table 1. Also flavones with 3′,4′-dichloro substituents on B ring (**6e** and **7e**) exhibited strong inhibitory activities. Flavones without any substituent on A ring showed moderate to little inhibitory activities. 5-Hydroxyflavones were inactive regardless of B ring substituent. Further bioassays at lower concentrations were performed for the active analogues (**4a**, **5d**, **e**, **6e** and **7e**) which exhibited more than 90% inhibition of PGE_2 production at 10 μM concentration and the results were shown in Table 2. The compounds (**4a**, **5d**, **e**, **6e** and **7e**) were proved to possess more potent inhibitory activities ($IC_{50}=0.1$–0.5 μM) than wogonin ($IC_{50}=1.08$ μM), a plant-originated flavone with anti-inflammatory activity. Thus, we found that the 3′,4′-dichloro-7-methoxy-



Scheme 1. Synthesis of flavone analogues: (a) dimethyl sulfate, K_2CO_3, acetone, reflux, >90%; (b) aryl aldehydes, KOH, MeOH, rt, 70–90%; (c) I_2, DMSO, heat, 60–70%; (d) BBr_3, CH_2Cl_2, 0 °C to rt, 80–85%; for **8c**, $AlCl_3$, CH_2Cl_2, rt, 83%.

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

Compd	% Inhibition of PGE_2 production[b]
4a	92.2[d]
4b	69.0[d]
4c	38.9[d]
4d	45.6[d]
4e	46.2[d]
5a	36.2[d]
5b	87.2[d]
5c	84.2[d]
5d	92.1[d]
5e	96.7[d]
6a	28.0[d]
6b	29.9[d]
6c	33.0[d]
6d	4.9[d]
6e	98.8
7a	70.8
7b	19.9
7c	9.8
7d	8.9
7e	97.4
8a	45.0
8b	72.4
8c	84.5
8d	50.4
8e	0.0
NS-398[c]	98.3
Chrysin	11.0
Wogonin	98.8

[a] All compounds were treated at 10 μM. Treatment of LPS to RAW cells increased PGE_2 production (10.0 nM) from the basal level of 0.5 nM.

[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] NS-398, *N*-(2-cyclohexyloxy)-4-nitrophenylmethanesulfonamide, was used as the reference compound.

[d] All values represented here were arithmetic mean of duplicate.

Table 2. IC_{50} values of the chrysin derivatives on COX-2

Compd	**4a**	**5d**	**5e**	**6e**	**7e**	NS-398	Wogonin
IC_{50} (μM)	0.5	0.5	0.1–0.5	0.1–0.5	0.1–0.5	0.05	1.08

flavone (**5e**), 3′,4′-dichloro-5-methoxyflavone (**6e**) and 3′,4′-dichloro-7-hydroxyflavone (**7e**) exhibited strong inhibitory activities on COX-2 catalyzed PGE_2 production from LPS-induced RAW 264.7 cells. These results may imply that the 3′,4′-dichloro substituents contribute to the bioactivity regardless of the substitution patterns of the hydroxy or the methoxy group on the A ring except 5-hydroxyflavones.

In summary, we prepared chrysin derivatives modified on A and B rings and evaluated their inhibitory activities on COX-2 catalyzed PGE_2 production from LPS-induced RAW 264.7 cells. We found that some chrysin derivatives (**5e**, **6e** and **7e**) possessed strong inhibitory activities. Our results are not enough to identify the structural requirement of chrysin for better biological activity at this moment, however, 3′,4′-dichloro substituents on the B ring of flavones enhance biological activity based on this experiment. Further SARs study on the A ring and the B ring of chrysin is currently under investigation.

Acknowledgements

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References and notes

1. Needleman, P.; Isakson, P. *J. Rhematol.* **1997**, *24* (Suppl. 49), 6.
2. Middleton, E.; Kandaswami, C. In *The Flavonoids: Advances in Research Since 1986*; Harborne, J. B., Ed.; London, 1994; 619.
3. Chi, Y. S.; Jong, H. G.; Son, K. H.; Chang, H. W.; Kang, S. S.; Kim, H. P. *Biochem. Pharmacol.* **2001**, *62*, 1185.
4. Liang, Y.-C.; Huang, Y.-T.; Tsai, S.-H.; Shiau, S.-Y.; Chen, C.-F.; Lin, J.-K. *Carcinogenesis* **1999**, *20*, 1945.
5. Kim, Y. P.; Yamada, M.; Lim, S. S.; Lee, S. H.; Ryu, N.; Shin, K. H.; Okuchi, K. *Biochim. Biophys. Acta* **1999**, *1438*, 399.
6. Chi, Y. S.; Cheon, B. S.; Kim, H. P. *Biochem. Pharmacol.* **2001**, *61*, 1195.
7. Chan, E. C. H.; Patchareewan, P.; Owen, L. W. *J. Cardiovasc. Pharmacol.* **2000**, *35*, 326.
8. Zanoli, P.; Aallone, R.; Baraldi, M. *Filtoterapia* **2000**, *71*, 117.
9. Leu, Y. L.; Ho, D. K.; Cassady, J. M.; Cook, V. M.; Baird, W. M. *J. Nat. Prod.* **1992**, *55*, 357.
10. Fishkin, R. J.; Winslow, J. T. *Psychopharmacology (Berl)* **1997**, *132*, 335.
11. Nakazawa, K. *Chem. Pharm. Bull.* **1962**, *10*, 1032.
12. Kosmeder, J. W.; Moriarty, R. M.; Pezzuto, J. M.; Song, L.L.; Moon, R. C. US Patent 6,046,231, 2000.
13. Parker, K. A.; Petraitis, J. J. *Tetrahedron Lett.* **1981**, *22*, 397.
14. Vickery, E. H.; Pahler, L. F.; Eisenbraun, E. J. *J. Org. Chem.* **1979**, *44*, 4444.
15. Mossman, T. *J. Immunol. Methods* **1983**, *65*, 55.