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Synthesis and PGE_2 Inhibitory Activity of 5,7-Dihydroxyflavones and Their *O*-Methylated Flavone Analogs

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5,7-Dihydroxyflavones and their *O*-methylated flavone analogs were prepared and evaluated their anti-inflammatory activity to decipher the structure-activity relationships. Most of the analogs were achieved from 2,4,6-trihydroxyacetophenone in 4 steps. 5,7-Dihydroxy-4'-methoxyflavone (**4c**) and 7-hydroxy-4',5-dimethoxyflavone(**6c**) were prepared following a different synthetic pathway. Among the synthetic flavones tested, 5-hydroxy-7-methoxyflavone analogs (**3a-3e**) showed moderate inhibitory activities of PGE_2 production from LPS-induced RAW 264.7 cells.

Key words: 5,7-Dihydroxyflavones, *O*-Methylated flavone analogs, Anti-inflammatory activity, Cyclooxygenase-2, PGE_2 production

INTRODUCTION

Although the steroidal and nonsteroidal anti-inflammatory drugs have been used clinically, there is a need for new type of anti-inflammatory drug, especially for chronic disorders including rheumatoid arthritis. Among various mediators of inflammation, recent studies have shown that some inducible enzymes such as cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) are crucial participants for provoking and maintaining the inflammatory response (Needleman and Isakson, 1997; Stichenoth and Frolich, 1998). Therefore, the modulators of the activities and/or the expression of these inducible enzymes may be useful therapeutic agents to control inflammatory disorders.

Flavonoids are natural polyphenol compounds widely distributed in the plant kingdom and possess a variety of biological activities such as anticancer, antiviral, anti-inflammatory, immunomodulatory activities, etc. Although numerous scientific reports have been published concerning the anti-inflammatory activity of naturally occurring flavonoids from many medicinal plants (Gabor, 1986; Lewis, 1989), a few studies were previously tried to establish the structure-activity relationships (SARs). Among the efforts in this area, the anti-inflammatory activities of some flavones and flavonols were evaluated using mouse edema assay (Yasukawa *et al.*, 1989), and we have recently reviewed *in vivo* anti-inflammatory activity of various natural flavonoids (Kim *et al.*, 2000) such as apigenin, baicalein, wogonin, genistein, kampferol, quercetin (their chemical structures are shown in Fig. 1). From these previous investigations, it was demonstrated that the 2,3-double bond on C-ring and the 5,7-dihydroxyl groups on A ring were observed as common structural features of flavonoids showing *in vivo* anti-inflammatory activity (Yasukawa *et al.*, 1989; Kim *et al.*, 2000). From several *in vitro* studies, it is found that the same structural requirements also contribute favorably to inhibitory activity of flavonoids such as apigenin and wogonin against PGE_2 and NO production by COX-2 and iNOS, respectively (Liang *et al.*, 1999; Kim, H.K. *et al.*, 1999; Kim, Y.P. *et al.*, 1999; Chi *et al.*, 2001).

As part of our research directed at the SARs of naturally occurring flavones for the anti-inflammatory activity, we were interested in the effects of the phenol groups on A ring and the substituents on B ring. Although the 5,7-dihydroxylation pattern on A ring was observed as a common structural feature of flavonoids in nature, there was little information concerning the effects of the 5,7-dihydroxyl groups concomitantly with B ring substitution on the anti-inflammatory activity. We, therefore, planned to synthesize serially *O*-methylated analogs of 5,7-dihydroxyflavones to decipher the effects of the hydroxyl groups on A ring. And

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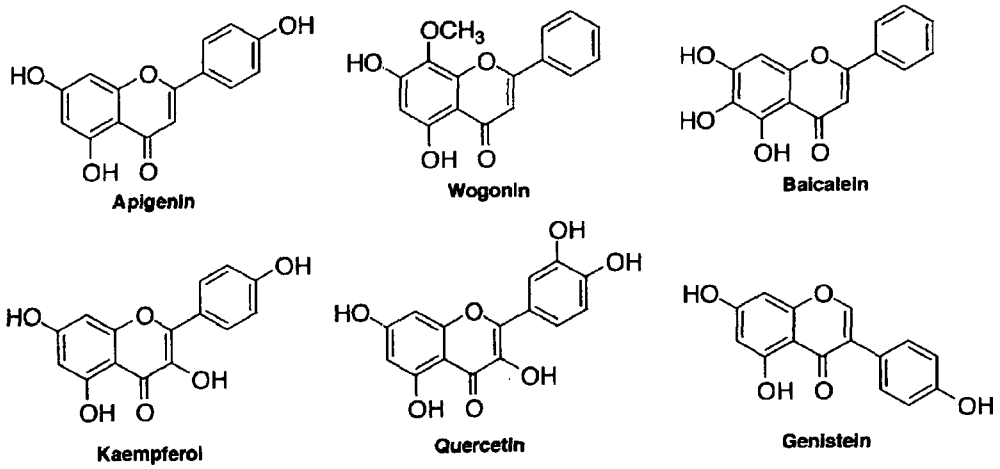


Fig. 1. Structures of some naturally occurring polyhydoxyflavonoids.

we introduced some other substituent group(s) on B ring of flavone structures except the hydroxy group since B ring hydroxy residues are common substituents of many naturally occurring flavonoids. The B ring substituents, 4'-H, 4'-Cl, 4'-Me, 4'-OMe, and 3',4'-Cl_2, were selected following the batchwise Topliss operational scheme where the substituents were grouped by Topliss according to some physical and chemical parameters (Topliss, 1977). Our approach might be able to elucidate the effects of methylated phenol groups on A ring and the substituent(s) on B ring of flavones against the modulatory activity of COX-2 catalysed PGE_2 production. Our synthetic approach can rapidly afford flavone analogs with the structural diversity. Research in this area would lead to better understanding of the structural requirements of flavone analogs for the modulatory activity of COX-2 catalysed PGE_2 production.

We report herein the synthesis and biological evaluation of 5,7-dihydroxyflavone analogs modified at A ring and/or B ring systems. We synthesized 5,7-dimethxoy-flavones, 5-hydroxy-7-methoxyflavones, 7-hydroxy-5-methoxyflavones and 5,7-dihydroxyflavones with the B ring substitution (s).

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 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.

Chemistry

A. Chalcone Formation

Properly protected 2',4',6'-trihydroxyacetophenone (1.05 equiv) and aryldehyde were dissolved in methanol and was added potassium hydroxide (3 equiv) in portions to give a blood-red solution. The reaction mixtures were stirred for 6 hours, during which 4',6'-diprotected-2'-hydroxychalcones precipitated as the potassium salt. The reaction mixtures were poured into cold 1N-HCl solution and was further added c-HCl until the solution became acidic. The resulting precipitate was filtered, washed with water, and crystallized from methanol to give product (**1a-1e**) as crystals. Yield 68-88 %.

B. Flavone Ring Formation

To a solution of the chalcone in DMSO was added a catalytic amount of iodine (0.1 equiv) and the reaction mixture was refluxed for 4-6 h with monitoring by TLC. The solution was cooled to room temperature and poured into saturated aqueous sodium thiosulfate solution and the resulting precipitate was filtered, washed with cold water, and crystallized from dichloromethane-methanol to give product (**2a-2e**) as crystals. Yield 70-85%.

5,7-Dimethoxyflavone (2a)

Yield: 73%, ^{1}H-NMR ($CDCl_3$) δ : 7.75-7.98 (2H, d, J = 8.2 Hz, H2', H6'), 7.49-7.53 (3H, m, H3', H4', H5'), 6.69 (1H, s, H3), 6.59 (1H, s, J = 2.4 Hz, H6), 6.39 (1H, s, J = 2.2 Hz, H8), 3.92-3.97 (6H, s, Ar-OCH_3).

4'-Methyl-5,7-dimethoxyflavone (2b)

Yield: 77%, ^{1}H-NMR ($CDCl_3$) δ : 7.76-7.91 (2H, d, J = 8.2 Hz, H2', H6'), 7.28-7.36 (2H, d, J = 7.8 Hz, H3', H5'), 6.66 (1H, s, H3), 6.57 (1H, s, J = 2.4 Hz, H6), 6.39 (1H, d, J = 2.2 Hz, H8), 3.91-4.0 (6H, s, Ar-OCH_3), 2.43 (3H, s, Ar-CH_3)

4',5,7-Trimethoxyflavone (2c)

Yield: 76%, ^{1}H-NMR ($CDCl_3$) δ : 7.80-7.85 (2H, d, J = 9.0 Hz, H2', H6'), 6.98-7.03 (2H, d, J = 9.0 Hz, H3', H5'), 6.60 (1H, s, H3), 6.56 (1H, s, J = 2.4 Hz, H6), 6.38 (1H, s, J = 2.2 Hz, H8), 3.89-3.96 (9H, s, Ar-OCH_3).

4'-Chloro-5,7-dimethoxyflavone (2d)

Yield: 79%, ^{1}H-NMR ($CDCl_3$) δ : 7.76-7.81 (2H, d, J = 8.6 Hz, H2', H6'), 7.42-7.47 (2H, d, J = 8.6 Hz, H3', H5'), 6.63 (1H, s, H3), 6.53 (1H, s, J = 2.4 Hz, H6), 6.36 (1H, s, J = 2.0 Hz, H8), 3.82-3.86 (6H, s, Ar-OCH_3).

3',4'-Dichloro-5,7-dimethoxyflavone (2e)

Yield: 80%, ^{1}H-NMR ($CDCl_3$) δ : 7.98-8.00 (1H, s, J = 2.2 Hz, H2'), 7.66-7.71 (1H, d, J = 8.6 Hz, J = 2.2 Hz, H6'), 7.55-7.60 (1H, d, J = 8.8 Hz, H5'), 6.67 (1H, s, H3), 6.53 (1H, s, J = 2.4 Hz, H6), 6.36 (1H, d, J = 2.2 Hz, H8), 3.89-3.93 (6H, s, Ar-OCH_3).

C. Removal of Protecting Group(s)

Benzyl protecting group was cleaved in conc-HCl and acetic acid conditions (**6a**, **6b**, **6c** and **6e**). Methoxy group on 5-position of flavones was selectively converted to the corresponding alcohols (**3a-3e**) in $AlCl_3$ and acetonitrile conditions in the presence of 7-methoxy group. 5,7-Dimethoxy groups of flavones were converted to 5,7-dihydroxy groups in BBr_3 and dichloromethane conditions (**4a**, **4b**, **4c** and **4e**). Products were purified by recrystallization in methanol and used for biological evaluation.

5-Hydroxy-7-methoxyflavone (3a)

Yield: 30%, ^{1}H-NMR (DMSO-d_6 + $CDCl_3$) δ : 13.02 (1H, s Ar-OH-5), 8.05-8.09 (2H, dd, J = 9.8 Hz, H2', H6'), 7.56-7.60 (3H, m, H3', H4', H5'), 6.67 (2H, s, H6, H8), 6.47 (1H, s, H3), 3.99 (3H, s, Ar-OCH_3).

4'-Methyl-5-hydroxyflavone (3b)

Yield: 30%, ^{1}H-NMR (DMSO-d_6 + $CDCl_3$) δ : 12.78 (1H, s, Ar-OH-5), 7.77-7.81 (2H, d, J = 8.2 Hz, H2', H6'), 7.31-7.36 (2H, d, 7.8 Hz, H3', H4'), 6.64 (1H, s, H3), 6.49 (1H, s, J = 1.2 Hz, H6), 6.38 (1H, s, H8), 3.89 (3H, s, Ar-OCH_3), 2.45 (3H, s, Ar-CH_3).

4',7-Dimethoxy-5-hydroxyflavone (3c)

Yield: 78%, ^{1}H-NMR (DMSO-d_6 + $CDCl_3$) δ : 12.82 (1H, s, Ar-OH-5), 7.65-7.92 (2H, d, J = 9.0 Hz, H2', H6'), 7.03-7.07 (2H, d, J = 9.0, H3', H4'), 6.62 (1H, s, H3), 6.55 (1H, s, J = 1.8 Hz, H6), 6.32-6.33(1H, s, J = 2.2 Hz, H8). 3.90 (6H, s, Ar-OCH_3).

4'-Chloro-5-hydroxyflavone (3d)

Yield: 86%, ^{1}H-NMR (DMSO-d_6 + $CDCl_3$) δ : 12.75 (1H, s, Ar-OH-5), 8.02-8.06 (2H, d, J = 8.8 Hz, H2', H6'), 7.76-7.81 (2H, d, J = 8.6 Hz, H3', H4'), 7.07 (1H, s, H3), 6.80 (1H, s, J = 2.2 Hz, H6), 6.40 (1H, s, J = 2.0 Hz, H8), 3.87 (3H, s, Ar-OCH_3).

3',4'-Dichloro-5-hydroxyflavone (3e)

^{1}H-NMR (DMSO-d_6 + $CDCl_3$) δ : 1268(1H, s, Ar-OH-5), 8.38 (1H, d, H2'), 8.07-8.12 (1H, d, J = 8.4 Hz, H4'), 7.82-7.87 (1H, d, J = 8.6 Hz, H6'), 7.19 (1H, s, H3), 6.88 (1H, s, H6), 6.39-6.41 (1H, s, J = 2.2 Hz, H8), 3.88 (3H, s, Ar-OCH_3).

5,7-Dihydroxyflavone (4a)

Yield: 87%, ^{1}H-NMR (DMSO-d_6) δ : 12.72 (1H, s Ar-OH-5), 10.09 (1H, s Ar-OH-7), 7.87-7.91 (2H, m, J = 7.4 Hz, J = 3.6 Hz, J = 3.4 Hz, H2', H6'), 7.53-7.55 (3H, m, H3', H4', H5'), 6.64(1H, s, H3), 6.48 (1H, s, H6), 6.34 (1H, s, H8).

4'-Methyl-5,7-dihydroxyflavone (4b)

Yield: 85%, ^{1}H-NMR (DMSO-d_6) δ : 12.87 (1H, s, Ar-OH-5), 10.88 (1H, s Ar-OH-7), 7.95-7.99 (2H, d, J = 8.4 Hz, H2', H6'), 7.36-7.40 (2H, d, 8.0 Hz, H3', H4'), 6.91 (1H, s, H3), 6.50-6.52 (1H, s, J = 1.8 Hz, H6), 6.22 (1H, s, H8), 2.40 (3H, s, Ar-CH_3).

4'-Methoxy-5,7-dihydroxyflavone (4c)

Yield: 78%, ^{1}H-NMR (DMSO-d_6) δ : 12.98 (1H, s, Ar-OH-5), 10.40 (1H, s Ar-OH-7), 7.95-7.92 (2H, d, J = 8.6 Hz, H2', H6'), 6.92-6.96 (2H, d, J = 8. Hz, H3', H4'), 6.86 (1H, s, H3), 6.77 (1H, s, H6), 6.38(1H, s, H8). 3.87 (3H, s, Ar-OCH_3).

4'-Chloro-5,7-dihydroxyflavone (4d)

Yield: 91%, ^{1}H-NMR (DMSO-d_6) δ : 12.85 (1H, s, Ar-OH-5), 11.02 (1H, s Ar-OH-7), 8.15-8.20 (2H, d, J = 8.8 Hz, H2', H6'), 7.70-7.74 (2H, d, J = 8.6 Hz, H3', H4'), 7.09 (1H, s, H3), 6.60 (1H, s, J = 2.2 Hz, H6), 6.30 (1H, s, J = 2.2 Hz, H8).

3',4'-Dichloro-5-hydroxyflavone (4e)

Yield: 92%, ^{1}H-NMR (DMSO-d_6) δ : 12.62 (1H, s, Ar-OH-5), 10.59 (1H, s Ar-OH-7), 8.14 (1H, s, J = 1.8 Hz, H2'), 7.88-7.98 (2H, m, J = 9.2 Hz, J = 8.6 Hz, 2.2 Hz, H5', H6'), 7.67-7.71 (1H, d, J = 8.4 Hz, H6'), 8.85 (1H, s, H3), 6.48 (1H, s, J = 1.8 Hz, H6), 6.24 (1H, s, H8).

5-Methoxy-7-hydroxyflavone (6a)

Yield: 78%, ^{1}H-NMR (DMSO-d_6) δ : 10.29 (1H, s Ar-OH-7), 7.92 (2H, m, H2', H6'), 7.51-7.54 (3H, m, H3', H4', H5'), 6.60 (1H, s, H3), 6.54 (1H, s, J = 2.2 Hz, H6), 6.39 (1H, s, H8), 3.88 (3H, s, Ar-OCH_3).

4'-Methyl-5-methoxy-7-hydroxyflavone (6b)

Yield: 79%, ^{1}H-NMR (DMSO-d_6) δ : 10.38 (1H, s Ar-OH-7), 7.87-7.92 (2H, d, J = 8.2 Hz, H2', H6'), 7.34-7.38 (2H, d, 8.0 Hz, H3', H4'), 6.65 (1H, s, H3), 6.55 (1H, s, J = 2.2 Hz, H6), 6.38 (1H, s, J = 1.8 Hz, H8), 3.40 (3H, s, Ar-OCH_3).

4'-Methoxy-5,7-dihydroxyflavone (6c)

Yield: 75%, ^{1}H-NMR (DMSO-d_6) δ : 10.22 (1H, s Ar-OH-7), 7.87-7.92 (2H, d, J = 8.8 Hz, H2', H6'), 6.89-6.94 (2H,

d, J = 8.6 Hz, H3', H4'), 6.83 (1H, s, H3), 6.59 (1H, s, H6), 6.50 (1H, s, H8). 3.83-3.90 (6H, s, Ar-OCH_3).

4'-Chloro-5-methoxy-7-hydroxyflavone (6d)

Yield: 90%, ^{1}H-NMR (DMSO-d_6) δ : 10.62 (1H, s Ar-OH-7), 8.02-8.06 (2H, d, J = 8.4 Hz, H2', H6'), 7.59-7.63 (2H, d, J = 8.8 Hz, H3', H4'), 6.75 (1H, s, H3), 6.56 (1H, s, J = 2.2 Hz, H6), 6.40 (1H, s, H8), 3.80 (6H, s, Ar-OCH_3).

3',4'-Dichloro-5-hydroxyflavone (6e)

Yield: 89%, ^{1}H-NMR (DMSO-d_6) δ : 8.21 (1H, s, H2'), 7.98-8.03 (2H, d, J = 8.6 Hz, H5', H6'), 7.77-7.82 (2H, d, J = 8.6 Hz, H6'), 6.85 (1H, s, H3), 6.59 (1H, s, H6), 6.40 (1H, s, H8).

Biological Evaluation

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, 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/well). Each synthetic flavone and LPS (1 g/mL) were added and incubated for 24 h. Cell viability was assessed with MTT assay based on the experimental procedures described previously (Parker and Petraitis, 1981). 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.

RESULTS AND DISCUSSION

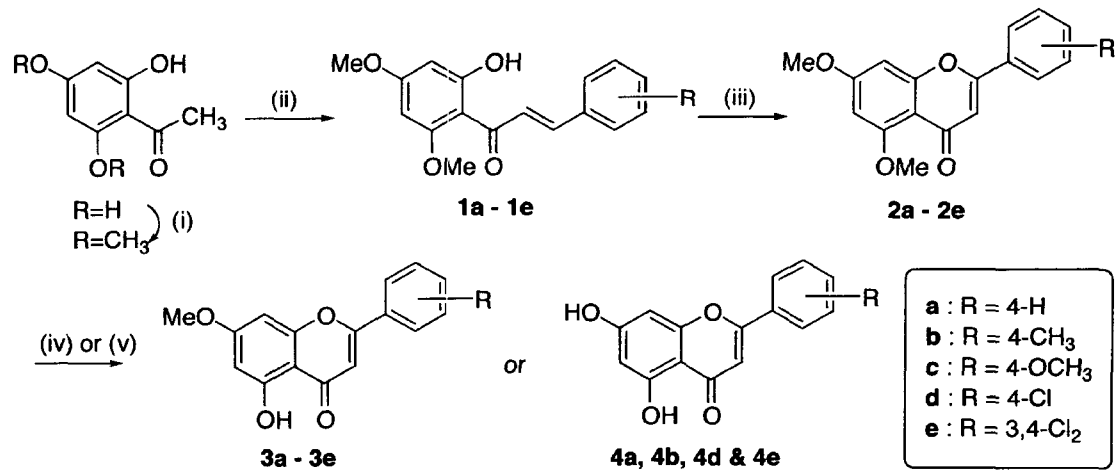
Commercially available 2',4',6'-trihydroxyacetophenone was treated with anhydrous potassium carbonate and dimethyl sulfate (2 equiv) in acetone to give 2'-hydroxy-4',6'-dimethoxyacetophenone in good yield (Nakazawa, 1962). The product was reacted with aryl aldehydes in methanolic KOH solution to afford the corresponding chalcones (**1a-1e**). Treatment of the chalcones with catalytic amount of iodine in dimethyl sulfoxide gave 5,7-dimethoxyflavone analogs with the substituted B ring (**2a-**

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

flavones	% inhibition	flavones	% inhibition	flavones	% inhibition	flavones	% inhibition
2a	8.1*	**3a**	32.5	**4a**	11.6	**6a**	18.0
2b	5.8	**3b**	33.2	**4b**	0.3	**6b**	1.0
2c	5.0	**3c**	21.6	**4c**	3.2	**6c**	6.9
2d	5.2	**3d**	15.0	**4d**	5.8	**6d**	3.4
2e	12.6	**3e**	30.6	**4e**	9.2	**6e**	2.6

1. 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.
2. % PGE2 Production = 100 × (PGE_2 of the treated group - PGE_2 of the basal) / (PGE_2 of LPS treated group - PGE_2 of the basal)
3. NS-398 was used as the reference compound (% inhibition = 98.3; IC_{50} value = 0.1 - 1.0 μM).

*Arithmetic mean of duplicate



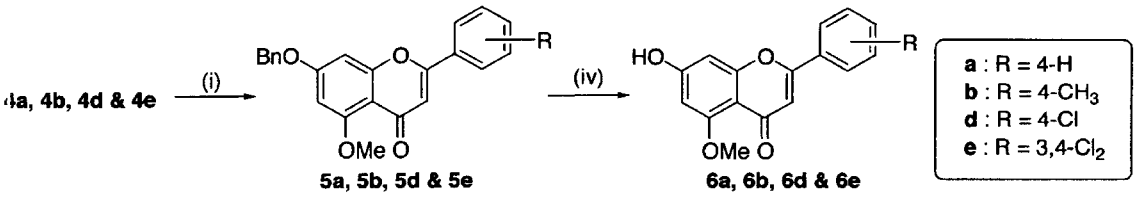
Scheme 1. Synthesis of 5,7-dimethoxy, 5-hydroxy-7methoxy and 5,7-dihydroxyflavone analogs. (i) dimethyl sulfate, K_2CO_3, acetone (ii) aryl aldehydes, KOH, methanol (iii) I_2(cat.), DMSO (iv) $AlCl_3$, CH_2Cl_2 (v) BBr_3, CH_2Cl_2.

2e), respectively (Kosmeder *et al.*, 2000). Reaction of the 5,7-dimethoxyflavones with $AlCl_3$ gave the 5-hydroxy-7-methoxyflavones (**3a-3e**), respectively (Parker and Petraitis, 1981). Reaction of the flavones with boron tribromide in dichloromethane gave the corresponding 5,7-hydroxyflavone analogs (**4a**, **4b**, **4d** and **4e**) as shown in Scheme 1 (Vickery *et al.*, 1979).

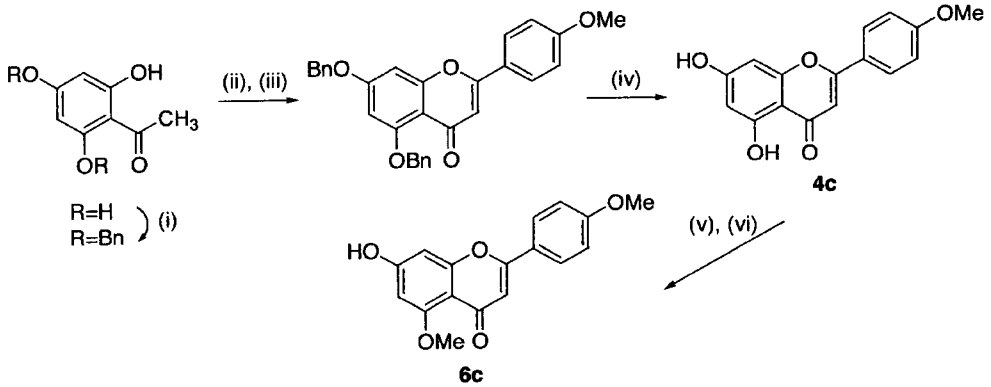
For the synthesis of 7-hydroxy-5-methxoyflavone analogs, the synthesized 5,7-dihydroxyflavones (**4a**, **4b**, **4d** and **4e**) were treated with anhydrous potassium carbonate and benzyl bromide (1 equiv) in *N,N*-dimethylformamide and, after completion of the reactions monitored by TLC, were added dimethyl sulfate (1 equiv) to give the corresponding 7-benzyloxy-5-methoxyflavones (**5a**, **5b**, **5d** and **5e**). The reaction mixtures of the 7-benzyloxy-5-methoxyflavone analogs and c-HCl in glacial acetic acid were refluxed for several hours (Rao *et al.*, 1949) and the mixtures were poured into cold water. Recrystalized of the solid yielded the 7-hydroxy-5-methoxyflavone analogs (**6a**, **6b**, **6d** and **6e**) as shown in Scheme 2.

5,7-Dihydroxy-4'-methoxyflavone (**4c**) and 7-hydroxy-4',5-dimethoxyflavone (**6c**) were synthesized by the following procedures. Treatment of 2,4,6-trihydroxyacetophenone with benzyl bromide (2 equivalents) and K_2CO_3 in *N,N*-dimethylformamide followed by the addition of dimethyl sulfate (1 equivalent) gave 4,6-dibenzyloxy-2-hydroxy-acetophenone. The reaction of the product and anisaldehyde in methanolic KOH followed by the flavone ring formation in iodine/dimethyl sulfoxide conditions gave 5,7-dibenzyloxyflavone. Reaction of the product with c-HCl in glacial acetic acid yielded 5,7-dihydroxy-4-methoxyflavone (**4c**). Selective benzyl protection of 7-phenol group and followed by the methylation of 5-phenol group gave 7-benzyloxy-5,4-dimethoxyflavone. Removal of the benzyl protecting group by refluxing with c-HCl in acetic acid gave 7-hydroxy-4,5-dimethoxyflavone (**6c**) as shown in Scheme 3.

Among the synthetic flavones tested, 5-hydroxy-7-methoxyflavones (**3a-3e**) exhibited moderate inhibitory activities against COX-2 catalyzed PGE_2 production. In contrast, 7-hydroxy-5-methoxyflavones (**2a-2e**), 5,7-dimethoxyflavones (**5a-5e**) and 5,7-dihydroxyflavones (**4a-4e**) showed negligible inhibitory activities. Based on our present results, it may be concluded that 5,7-dihydroxy flavones and their *O*-methylated analogs are not active against COX-2 catalyzed PGE_2 production regardless of the substituent(s) on B ring. In contrast, apigenin, 4',5,7-trihydroxyflavone, and tectorigenin, 4',5,7-trihydroxy-6-methoxyisoflavone, were reported to inhibit the induction of COX-2 (Liang, 1999; Kim YP, 1999). Although these compounds possess a hydroxyl group on B ring, it is not clear whether the hydroxyl group is crucial for bioactivity or not. Many other flavonoids such as wogonin, baicalein and etc. also showed inhibitory activity against the induction of COX-2 whereas these compounds do not have the hydroxyl group on B ring. Further SARs study on A ring and B ring is currently under investigation.



Scheme 2. Synthesis of 7-hydroxy-5-methoxyflavone analogs. (i) BnBr, K_2CO_3, DMF then dimethyl sulfate (ii) c-HCl, AcOH.



Scheme 3. Synthesis of 5,7-dihydroxy-4'-methoxyflavone (**4c**) and 7-hydroxy-4'5-dimethoxyflavone (**6c**). (i) BnBr, K_2CO_3, DMF (ii) anisaldehyde, KOH, methanol (iii) I_2 (cat.), DMSO (iv) c-HCl, AcOH (v) BnBr, K_2CO_3, DMF then dimethyl sulfate (vi) c-HCl, AcOH.

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