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Synthesis and PGE_2 Inhibitory Activity of Vinylated and Allylated Chrysin Analogues

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Vinylated and allylated chrysin analogues were prepared as congeners of prenylated flavonoids and evaluated their anti-inflammatory activity. 8-Substituted chrysin analogues were prepared from 2'-hydroxy-3'-iodo-4',6'-dimethoxyacetophenone in 3 steps. 3-Allylated chrysin analogues were prepared from chrysin in 3 steps. Synthesized chrysin analogues (**4**, **5** and **8**) showed moderate inhibitory activities of PGE_2 production from LPS-induced RAW 264.7 cells.

Key words: Prenylated flavonoids, Allylated chrysin analogues, Vinylated chrysin analogues, Anti-inflammatory activity, Cyclooxygenase-2, PGE_2 production, Stille reaction

INTRODUCTION

Flavonoids are a broad class of polyphenolic compounds which possess various pharmacological properties including anti-oxidant, anti-cancer, anti-viral, and anti-inflammatory properties (Gabor, 1986; Kim, 1999; Kim, 2000; Lewis, 1989; Needleman, 1997; Strichtenoth, 1998; Yasukawa, 1989). Among the flavonoids, prenylated flavonoids are the unique and minor class of flavonoids distributed mainly among several plant families such as Leguminosa and Moraceae. Previous investigations concerning some prenylated flavonoids such as morusin, kuwanon C, and Papyriflavonol A (Fig. 1) demonstrated their anti-inflammatory potential *in vitro* and *in vivo* (Cheon 2000; Reddy, 1991).

Prenylated flavonoids are found in nature as minor secondary metabolites and they possess chemical entities having an isoprenyl (3,3,-dimethylallyl), a geranyl, 1,1-dimethylallyl, and/or a lavandulyl moieties in their structures. As part of our research directed at the SARs of naturally occurring flavones for the anti-inflammatory activity, we were interested in the substituent effects of hydrophobic groups such as methyl, isopropyl, benzyl, vinyl allyl and isoprenyl groups for the modulatory activity of COX-2 catalysed PGE_2 production. We, therefore, prepared vinylated and allylated flavonoids as congeners of prenylated flavonoids since those flavonoids were easy to be prepared and seemed to have similar hydrophobicity compared with prenylated flavonoids. We report herein the synthesis and the inhibitory activity of COX-2 catalysed PGE_2 production of vinylated and allylated chrysin analogues.

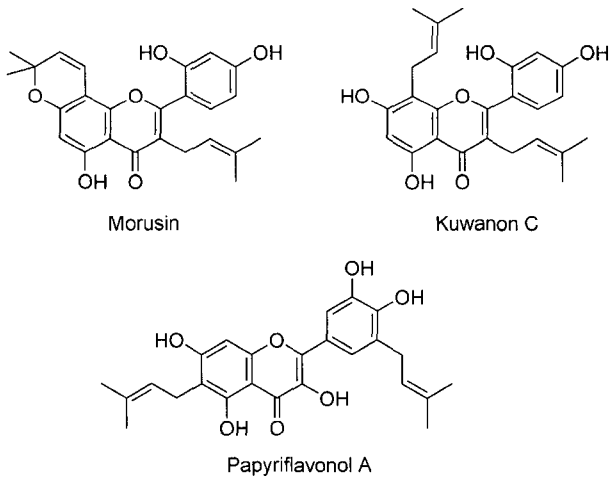

Fig. 1. Structures of some naturally occurring prenylated flavonoids

MATERIALS AND METHODS

Chemicals

All chemicals were purchased from commercial suppliers, and used without further purification. All solvents used for reaction were freshly distilled from proper dehydrating agent

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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). ^{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).

Chemistry

Synthesis of 8-iodo-5,7-dimethoxyflavone (3)

2-Hydroxy-3-iodo-4,6-dimethoxyacetophenone (**1**, 1.05 equiv) prepared from 2,4,6-trihydroxyacetophenone in two steps (Nakazawa, 1962) and benzaldehyde were dissolved in methanol and was added potassium hydroxide (3 equiv) in portions to give a blood-red solution. The reaction mixture was stirred for 4 h, during which 2-hydroxy-3-iodo-4,6-dimethoxychalcone (**2**) precipitated as the potassium salt. The reaction mixture was poured into cold 1 N 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 (**2**) as crystals. Yield 84%.

To a solution of the chalcone (**2**) in DMSO was added a catalytic amount of iodine (0.1 equiv) and the reaction mixture was refluxed for 7 h. The reaction 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 (**3**) as crystals. Yield 75%.

2-Hydroxy-3-iodo-4,6-dimethoxychalcone (2)

^{1}H-NMR ($CDCl_3$) δ: 12.75 (s, 1H, OH), 6.28 (s, 1H, H_5), 3.95-3.86 (d, 6 H, -ArOCH_3), 2.95 (s, 3H, -$COCH_3$).

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

^{1}H-NMR ($CDCl_3$) δ: 8.07-8.05 (d, 2H, $H_{2',6'}$), 7.55-7.52 (m, 3H, $H_{3'4'5'}$), 6.75 (s, 1H, H_3), 6.45 (s, 1H, H_6), 4.04-3.97 (d, 6H, ArOCH_3).

Synthesis of 5,7-dimethoxy-8-vinylflavone (4) and 8-allyl-5,7-dimethoxyflavone (5)

8-Iodo-5,7-dimethoxyflavone (**3**, 1.0 equiv) and $Pd(PPh_3)_4$ (0.02 equiv) were added to a flask equipped with a reflux condenser and a septum inlet. The flask was flushed with nitrogen and charged with anhydrous DMF. Vinyltributyltin or allyltributyltin (1.1 equiv) was added by means of a hypodermic syringe through the septum inlet. The mixture was stirred at 100°C, monitoring by TLC with solvent system of $CHCl_3$-MeOH (20:1). After completion of the reaction, reaction mixture was cooled to room temperature, and filtered through cellite, then were added cold water and dichloromethane and shaked successfully. The organic layer was washed with saturated NaCl solution and dried over anhydrous $MgSO_4$ and filtered and concentrated under reduced pressure. The recrystallization of the residue with CH_2Cl_2 yielded the title compounds both as pale yellow solids. Yield 68% (**4**), 58% (**5**).

5,7-Dimethoxy-8-vinylflavone (4)

^{1}H-NMR ($CDCl_3$) δ: 7.91-7.86 (q, 2H, H_2-H_6), 7.53-7.50 (t, 3H, $H_{3'}$-$H_{4'}$-$H_{5'}$), 7.12-6.97 (q, 1 H, -ArCH=), 6.69 (s, 1H, H_6), 6.45 (s, 1H, H_8), 6.12-6.02 (q, 1H, =CH_2 trans), 5.65-5.57 (q, 2H, =CH_2 cis), 4.03-4.00 (d, 6H, OCH_3). ^{13}C-NMR (DMSO-d_6) δ: 175.99 (C-4), 161.66 (C-2), 159.84 (C-7), 159.76 (C-5), 155.48 (C-10), 131.29 (C-1"), 129.14 (C-1'), 125.97 (C-3',4',5'), 125.70 (C-2',6'), 114.90 (C-3"), 108.27 (C-9), 106.47 (C-8), 92.85 (C-3), 92.74 (C-6), 56.20 (2 C-OMe).

8-Allyl-5,7-dimethoxyflavone (5)

^{1}H-NMR ($CDCl_3$) δ: 7.88-7.86 (q, 2H, H_2-H_6), 7.53-7.50 (t, 3H, $H_{3'}$-$H_{4'}$-$H_{5'}$), 6.68 (s, 1H, H_6), 6.45 (s, 1H, H_8), 6.04-5.60 (m, 1H, Ar-C=CH), 5.08-4.99 (t, 2H, Ar-CH_2=C), 4.02-3.97 (d, 6H, OCH_3), 3.66-3.63 (d, 2H, =CH_2). ^{13}C-NMR (DMSO-d_6) δ: 176.09 (C-4), 161.09 (C-7), 159.55 (C-5), 159.22 (C-2), 155.76 (C-10), 135.95 (C-1"), 131.27 (C-2"), 129.19 (C-3',4',5'), 125.91 (C-2',6'), 114.90 (C-3"), 107.89 (C-3), 106.80 (C-8), 92.78 (C-9), 92.70 (C-6), 56.23 (C-OMe), 56.10 (C-OMe), 26.57 (C-1").

Synthesis of 3-allyl-5,7-dimethoxyflavone (8)

To a solution of chrysin (1.0 equiv) in acetone were added dimethyl sulfate (2.5 equiv) and K_2CO_3 (8 equiv) and the reaction mixture was refluxed for 4 h. After removing potassium carbonate, the reaction solution was added to a breaker containing 100 mL of water. Precipitated solid was filtered off, washed with cold water, dried and recrystallized from methanol to yield 5,7-dimethoxyflavone (**6**) as a colorless solid (90% yield).

A solution of 5,7-dimethoxyflavone (**6**, 1.0 equiv) and NBS (1.1 equiv) in $CHCl_3$ was heat to reflux for 4 h. The resulting mixture was washed with saturated $NaHCO_3$, brine and dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was crystallized from methanol-dichloromethane to yield 3-

bromo-5,7-dimethoxyflavone (**7**) as a pale yellow solid (91%).

The titled compound was produced by the same procedure described in the synthesis of 8-allyl-5,7-dimethoxyflavone (**5**) but replacing 3-bromo-5,7-dimethoxyflavone (**7**) for 8-iodo-5,7-dimethoxyflavone (**3**). Yield 64%.

5,7-Dimethoxyflavone (6)

^{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).

3-Bromo-5,7-dimethoxyflavone (7)

^{1}H-NMR ($CDCl_3$) δ: 8.00 (d, 2H, $H_{2'}$-$H_{6'}$), 7.54-7.52 (t, 3H, $H_{3'}$-$H_{4'}$-$H_{5'}$), 6.73 (s, 1H, H_6), 6.47 (s, 1H, H_8), 4.05-4.03 (d, 6H, OCH_3).

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

^{1}H-NMR ($CDCl_3$) δ: 7.88-7.86 (q, 2H, H_2-H_6), 7.53-7.50 (t, 3H, $H_{3'}$-$H_{4'}$-$H_{5'}$), 6.68 (s, 1H, H_6), 6.45 (s, 1H, H_8), 6.04-5.60 (m, 1H, Ar-C=CH), 5.09-45.00 (t, 2H, Ar-CH_2=C), 4.02-3.97 (d, 6H), 3.65-3.63 (d, 2H). ^{13}C-NMR (DMSO-d_6) δ: 176.17 (C-4), 161.09 (C-7), 159.53 (C-5), 159.22 (C-2), 155.76 (C-10), 135.93 (C-1"), 131.27 (C-2"), 129.19 (C-3',4',5'), 125.88 (C-2',6'), 114.92 (C-3"), 107.89 (C-3), 107.78 (C-9), 106.78 (C-8), 92.81 (C-6), 56.25 (C-OMe), 56.12 (C-OMe), 26.59 (C-1").

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. PGE_2 concentration in the medium was measured using EIA kit for PGE_2 according to the manufacturers 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 I.

Table I. Inhibition of COX-2 catalyzed PGE_2 production from LPS-induced RAW 264.7 cells by allylated and vinylated chrysin analogues

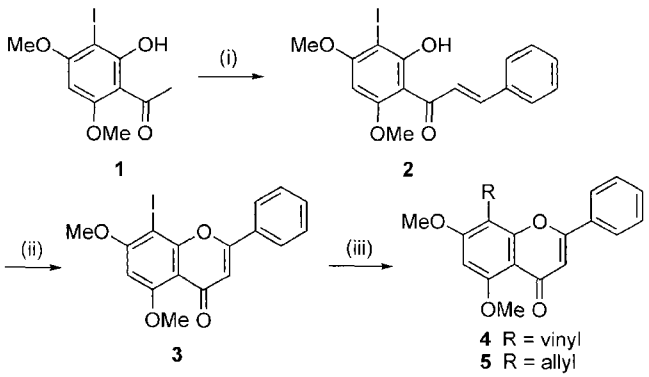
Chrysin analogues	Compound **4**	Compound **5**	Compound **8**
% inhibition	54.4	48.6	43.7

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. % inhibition = 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).

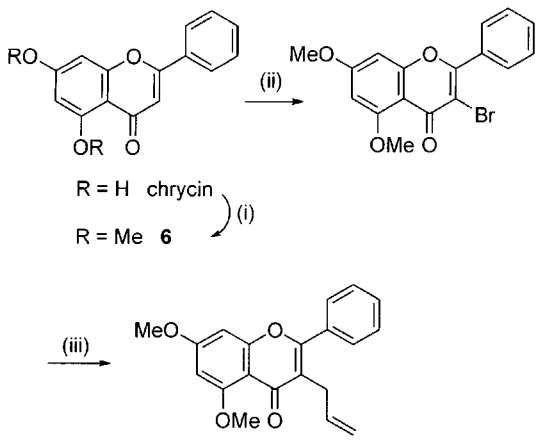
RESULTS AND DISCUSSION

8-Iodo-5,7-dimethoxyflavone (**1**) was prepared from commercially available 2',4',6'-trihydroxyacetophenone in two steps following the known procedure (Nakazawa, 1962). Reaction of the compound **1** with benzaldehyde in methanolic KOH solution afforded the corresponding chalcone (**2**). Treatment of the chalcone with catalytic amount of iodine in dimethyl sulfoxide gave 8-iodo-5,7-dimethoxyflavone (**3**, Kosmeder *et al.*, 2000). Stille reaction conditions were applied to introduce vinyl and allyl groups to the flavone ring system (Stille, 1986). Reaction of the iodoflavone (**3**) and vinylbutyltin or allylbutyltin in the presence of catalytic amount of $Pd(PPh_3)_4$ in anhydrous DMF gave 5,7-dimethoxy-8-vinylflavone (**4**) and 8-allyl-5,7-dimethoxyflavone (**5**) in moderate yields as shown in Scheme 1.

For the synthesis of 3-allyl-5,7-dimethxoyflavone (**8**), commercially available chrysin (5,7-dihydroxyflavones) was treated with anhydrous potassium carbonate and dimethylsulfate in acetone to afford 5,7-dimethoxyflavones (**6**). Reaction of 5,7-dimethoxyflavone (**6**) and *N*-bromosuccinimide (NBS) in $CHCl_3$ (10 mL) gave the crude product and the residue was crystallized from methanol-chloromethane to yield the title compound as a pale yellow solid. Recrystallized of the solid yielded the 3-bromo-5,7-dimethoxyflavone (**7**). Reaction of the bromoflavone (**7**) and allylbutyltin in the presence of catalytic amount of $Pd(PPh_3)_4$ in anhydrous DMF gave 3-allyl-5,7-dimethoxyflavone (**8**) as shown in Scheme 2.



Scheme 1. Synthesis of 8-allyl-5,7-dimethoxy (5) and 5,7-dihydroxy-8-vinylflavone (4). (i) aryl aldehydes, KOH, methanol (ii) I_2 (cat.), DMSO (iii) vinyltributyltin or allyltributyltin, $Pd(PPh_3)_4$.



Scheme 2. Synthesis of 5,7-dihydroxy-3-vinylflavone (8). (i) dimethyl sulfate, K_2CO_3, DMF (ii) NBS, $CHCl_3$ (iii) allyltributyltin, $Pd(PPh_3)_4$.

Synthesized 5,7-dimethoxyflavones with the allyl or the vinyl groups as the congener of the isoprenyl group (**4**, **5** and **8**) exhibited moderate inhibitory activities against COX-2 catalyzed PGE_2 production. It is not clear from these data wether the hydrophobic groups influence to the bioactivity or not. Further substituent effect of hydrophobic groups on A ring and B ring is currently under investigation.

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