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Synthesis of Ochnaflavone and Its Inhibitory Activity on PGE_2 Production

Sung Soo Kim,[†] Van Anh Vo,[†] and Haeil Park[*]

College of Pharmacy and [†]Medicine of Kangwon National University, Chuncheon 200-701, Korea
[*]E-mail: haeilp@kangwon.ac.kr
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Ochnaflavone, a naturally occurring biflavonoid composed of two units of apigenin (5,7,4'-trihydroxyflavone) joined *via* a C-O-C linkage, was first synthesized and evaluated its inhibitory activity on PGE_2 production. Total synthesis was accomplished through modified Ullmann diaryl ether formation as a key step. Coupling reactions of 4'-halogenoflavones and 3'-hydroxy-5,7,4'-trimethoxyflavone were explored in diverse reaction conditions. The reaction of 4'-fluoro-5,7-dimethoxyflavone (**2c**) and 3'-hydroxy-5,7,4'-trimethoxyflavone (**2d**) in *N,N*-dimethylacetamide gave the coupled compound **3** in 58% yield. Synthetic ochnaflavone strongly inhibited PGE_2 production (IC_{50} = 1.08 μM) from LPS-activated RAW 264.7 cells, which was due to reduced expression of COX-2. On the contrary, the inhibition mechanism of wogonin was somewhat different from that of ochnaflavone although wogonin, a natural occurring anti-inflammatory flavonoid, showed strong inhibitory activity of PGE_2 production (IC_{50} = 0.52 μM), and seems to be COX-2 enzyme inhibition. Our concise total synthesis of ochnaflavone enable us to provide sufficient quantities of material for advanced biological studies as well as to efficiently prepare derivatives for structure-activity relationship study.

Key Words : Ochnaflavone, Apigenin dimer, Anti-inflammatory activity, Ullmann diaryl ether formation, COX-2 expression

Introduction

Biflavonoids belong to flavonoid family and they are dimers of flavonoids which are connected by a C-C or a C-O-C bond such as amentoflavone, robustaflavone, ochnaflavone, hinokiflavone and so forth (Figure 1). Although a wealth of biflavonoids have been discovered from various plant species, their biological and pharmacological data are limited. Previously, certain biflavonoids were reported to inhibit phosphodiesterase,[1] lens aldose reductase,[2] mast cell histamine release[3] and to show anticancer activity.[4] Recently, some C-C and C-O-C biflavonoids were synthesized and their anti-microbial[5] and anti-inflammatory[6,7] activities were demonstrated.

Ochnaflavone (Figure 1), a naturally occurring biflavonoid, is first isolated from *Ochna squarrosa* Linn. (Ochnaceae)[8] and later in the medicinal plant *Lonicera japonica* (Caprifoliaceae).[9] Several biological activities mediated by ochnaflavone include the inhibition of PLA_2 in rat platelet, lymphocyte proliferation, and arachidonate release from rat peritoneal macrophage.[10,11] The anti-artherogenic activity was also revealed with the correlation of inhibition of human vascular smooth muscle cell proliferation induced by TNF-α *via* regulation of cell cycle related proteins, ERK and MMP-9.[12,13] Recent study also exhibited the inhibitory activity on CCl_4-induced PE degradation in rat liver microsome[14] and anti-inflammatory effects with the down regulation of inducible nitric oxide, COX-2 and 5-lipoxygenase through

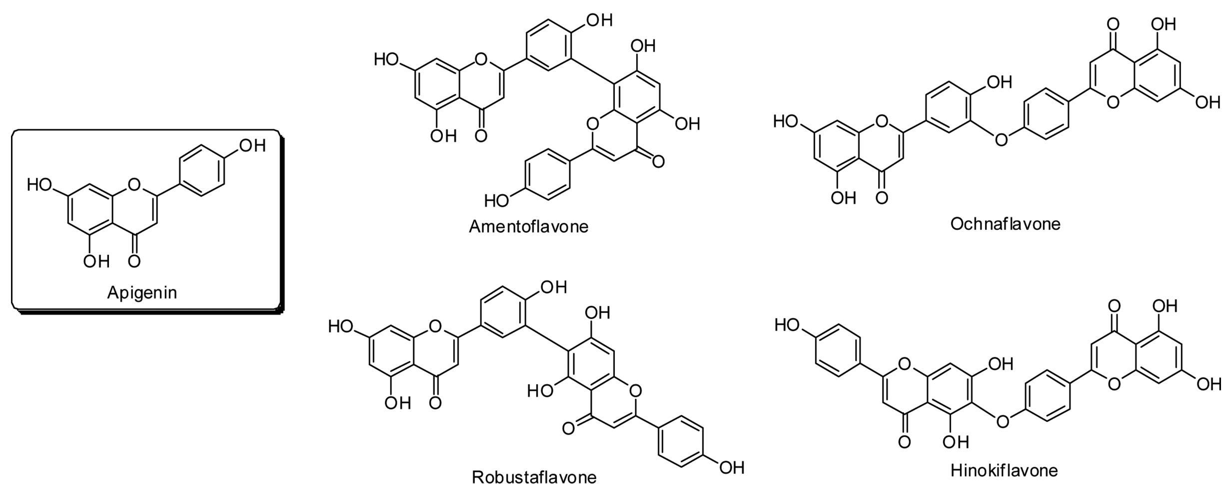


Figure 1. Structures of biologically important biflavonoids composed of two units of apigenin.

the effects on NF-κB and ERK pathway.[9,12,13] These results demonstrate that ochnaflavone has a dual COX-2/5-lipoxygenase inhibitory activity. In addition, this compound strongly inhibited degranulation reaction in a dose dependent manner, with an IC_{50} value of 3.01 μM. Therefore, this compound might provide a basis for novel anti-inflammatory drugs. Furthermore, ochnaflavone and its analogs were reported to possess anti-HIV-1 RT[15] and neuroprotective effects.[16] Thus, ochnaflavone is a promising lead to develop a new anti-inflammatory drug.

Ochnaflavone is a biflavonoid composed of two units of apigenin (5,7,4'-trihydroxyflavone) joined *via* a [3'-4'''] C-O-C linkage as shown in Figure 1. As described above, ochnaflavone has been proved as a potential lead compound for diverse diseases, however, further pharmacological and clinical studies have been delayed because of its limited supply due to tedious multiple separation and purification processes from plant. Interestingly, only a synthetic procedure for penta-*O*-methylochnaflavone was reported to identify the chemical structure of ochnaflavone isolated from plant.[17] Penta-*O*-methylochnaflavone was synthesized through a bichalcone by Baker-Venkataraman rearrangement of the corresponding diphenyl ether dialdehyde (or its chemical equivalents) followed by flavones ring formation.[18] However, the previous method suffered from some drawbacks such as low chemical yield and limited structural diversity.

Our present method applied Ullmann diaryl ether formation between two different flavone intermediates as a key reaction. This method overcomes the drawbacks of the previous one. The reactivity of the coupling reactions between 4'-halogenoflavones and 3'-hydroxy-5,7,4'-trimethoxyflavone was largely dependent on the species of halogen atoms and reaction conditions. Our present method can be applied to prepare diverse ochnaflavone analogs with different flavone units for SAR study.

Experimental

Synthesis (General). 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 3000 instrument (300 MHz) and a Bruker DPX 400 (400 MHz) spectrometers. 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), dd (doublet of doublets) and ddd (doublet of double doublet). ^{13}C-NMR spectra were recorded on a Bruker DPX 400 (100 MHz) spectrometer, fully decoupled and chemical shifts are reported in parts per million (ppm) downfield relative to tetramethylsilane as an internal standard. Melting points were recorded on a Fisher-Johns microscopic scale melting point apparatus. Mass spectra were recorded on a Autospec M363 and MALDI-TOM MS (Voyager DE STR). 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).

General Procedure for the Synthesis of Chalcones 1a-1d. To a mixture of 2-hydroxy-4,6-dimethoxyacetophenone (1.0 equiv) and KOH (4 equiv) in MeOH was added the appropriate aryl aldehyde (1.0 equiv). The resulting mixture was stirred overnight at room temperature then an excess volume of HCl (3%) was added to form a yellow solid that was filtered, washed with water and methanol to give the corresponding product (**1a-1d**).

2-Hydroxy-4,6-dimethoxy-4'-bromochalcone (1a): Yellow solid; Yield 79%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 14.23 (s, 1H, 2'-OH); 7.85-7.93 (d, 1H, J=15.4 Hz, H_β); 7.81 (d, 1H, J = 15.4 Hz, H_α); 7.53-7.57 (d, 2H, J = 8.2 Hz, H2 and H6); 7.44-7.48 (d, 2H, J = 8.2 Hz, H3 and H5); 6.11-6.12 (ds, 1H, J = 2.4 Hz, H5'); 5.97-5.98 (ds, 1H, J = 2.4 Hz, H3'); 3.92, 3.84 (s, 6H, $2xOCH_3$).

2-Hydroxy-4,6-dimethoxy-4'-chlorochalcone (1b): Yellow solid; Yield 76%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 12.85 (s, 1H, 5-OH); 11.02 (s, 1H, 7-OH); 8.15-8.20 (d, 1H, J = 8.8 Hz, H2' and H6'); 7.70-7.74 (d, 2H, J = 8.6 Hz, H3' and H4'); 7.09 (s, 1H, H3); 6.60 (s, 1H, J = 2.2 Hz, H6); 6.30 (s, 1H, J = 2.2 Hz, H8); 14.04 (s, 1H, 2'-OH), 6.05 (ds, 1H, J = 2.2Hz, H5'); 5.92 (ds, 1H, J = 2.2Hz, H3'); 3.87-3.82 (d, 6H, $2xArOCH_3$), 2.61 (s, 3H, $COCH_3$).

2-Hydroxy-4,6-dimethoxy-4'-fluorochalcone (1c): Yellow solid; Yield 70%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 14.25 (s, 1H, OH), 7.81-7.86 (d, J = 15.6 Hz, 1H, H_α), 7.72-7.78 (d, J = 15.6 Hz, 1H, H_β), 7.57-7.62 (m, 2H, H-2, H-6), 7.07-7.13 (t, J = 8.7 Hz, 2H, H-3, H-5), 6.12 (d, J = 2.0 Hz, 1H, H-5'), 5.98 (d, J = 2.0 Hz, 1H, H-3'), 3.92 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3).

2'-Dihydroxy-4,4',6'-trimethoxychalcone (1d): Yellow solid; Yield 87%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 14.42 (s, 1H, OH-2'), 7.77-7.82 (d, J = 15.5 Hz, 1H, H_β), 7.70-7.76 (d, J = 15.5 Hz, 1H, H_α), 7.26-7.27 (d, J = 1.9 Hz, 1H, H-2), 7.09-7.13 (dd, J = 1.9 Hz, 8.3 Hz, 1H, H-6), 6.86-6.89 (d, J = 8.3 Hz, 1H, H-5), 6.11-6.12 (d, J = 2.3 Hz, 1H, H-5'), 5.96-5.97 (d, J = 2.3 Hz, 1H, H-3'), 5.68 (s, 1H, OH-3), 3.95 (s, 3H, OCH_3), 3.92 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3).

General Procedure for the Synthesis of Flavones 2a-2d. To a solution of chalcones **1a-1d** (1.0 equiv) in DMSO was added I_2 (1.0 equiv) slowly. The reaction mixture was stirred at 100 °C for overnight, cooled to room temperature and followed by adding an excess volume of saturated $Na_2S_2O_3$ solution. The precipitate was filtered, washed with water and methanol to give the corresponding product as a white solid.

5,7-Dimethoxy-4'-bromoflavone (2a): Yield 75%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.71-7.75 (d, 2H, J = 8.2 Hz, H3' and H5'); 6.89 (s, 1H, H3); 6.42 (s, 1H, H6); 6.16 (s, 1H, H8).

5,7-Dimethoxy-4'-chloroflavone (2b): Yield 82%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 12.85 (s, 1H, 5-OH); 11.02 (s,

1H, 7-OH); 8.15-8.20 (d, 1H, J = 8.8 Hz, H2' and H6'); 7.70-7.74 (d, 2H, J = 8.6 Hz, H3' and H4'); 7.09 (s, 1H, H3); 6.60 (s, 1H, J = 2.2 Hz, H6); 6.30 (s, 1H, J = 2.2 Hz, H8).

5,7-Dimethoxy-4'-fluoroflavone (2c): Yield 80%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.84-7.89 (m, 2H, H-2', H-6'), 7.16-7.22 (m, 2H, H-3', H-5'), 6.61 (s, 1H, H-3), 6.55-6.56 (d, J = 2.2 Hz, 1H, H-8), 6.38-6.39 (d, J = 2.2 Hz, 1H, H-6), 3.96 (s, 3H, OCH_3), 3.92 (s, 3H, OCH_3).

3'-Hydroxy-5,7,4'-trimethoxyflavone (2d): Yield 86%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.39 (s, 1H, OH), 7.48-7.51 (dd, J = 2.2 Hz, 8.5 Hz, 1H, H-6'), 7.39-7.41 (d, J = 2.2 Hz, 1H, H-2'), 7.06-7.09 (d, J = 8.6 Hz, 1H, H-5'), 6.81 (d, J = 2.3 Hz, 1H, H-8), 6.55 (s, 1H, H-3), 6.50-6.51 (d, J = 2.3 Hz, 1H, H-6), 3.90 (s, 3H, OCH_3), 3.86 (s, 3H, OCH_3), 3.83 (s, 3H, OCH_3).

General Procedure for the Ullmann Diaryl Ether Formation. To a round bottom flask containing compound **2d** (1.0 equiv), K_2CO_3 (1.0 equiv) under N_2 atmosphere was added *N,N*-dimethylacetamide and dry toluene then vigorously stirred at 140 °C for 4 h. After adding compounds **2a-2c** (1.0 equiv), the reaction mixture was heated at 160 °C for 12h then cooled to room temperature. The reaction mixture was extracted with CH_2Cl_2 (3 times), washed with brine, dried over anhydrous $MgSO_4$, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography ($CHCl_3$-MeOH = 200:1) to afford the title compound.

Penta-*O*-methylochnaflavone (3). White solid; mp 160-162 °C; Yield 15% (from **2a**)/8% (from **2b**)/58% (from **2c**); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.82-7.85 (m, 2H, H-2''', H-6'''), 7.73-7.75 (dd, J = 2.2 Hz, 8.6 Hz, 1H, H-6'), 7.62-7.63 (d, J = 2.3 Hz, 1H, H-5'), 7.12-7.15 (d, J = 8.7 Hz, 1H, H-2'), 7.02-7.05 (m, 2H, H-3''', H-5'''), 6.61 (s, 1H, H-3''), 6.57 (s, 1H, H-3), 6.56 (d, J = 2.2 Hz, 1H, H-8''), 6.53 (d, J = 2.2 Hz, 1H, H-8), 6.36-6.37 (t, J = 2.9 Hz, 2H, H-6, H-6''), 3.95 (s, 3H, OCH_3), 3.94 (s, 3H, OCH_3), 3.91 (s, 3H, OCH_3), 3.90 (s, 3H, OCH_3), 3.89 (s, 3H, OCH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.99 (C-4''), 177.85 (C-4), 164.43 (C-7''), 164.39 (C-7), 161.28 (C-2, C-2''), 160.72 (C-5''), 160.66 (C-5), 160.23 (C-4'''), 160.14 (C-9''), 159.96 (C-9), 154.46 (C-4'), 144.21 (C-3'), 128.15 (C-2''', C-6'''), 126.18 (C-1'''), 125.15 (C-6'), 124.40 (C-1'), 120.15 (C-3''', C-5'''), 117.08 (C-2'), 113.38 (C-5'), 109.59 (C-10''), 109.54 (C-10), 108.60 (C-3, C-3''), 96.59 (C-6''), 96.53 (C-6), 93.21 (C-8, C-8''), 56.84 (7-OCH_3, 7''-OCH_3), 56.57 (4'-OCH_3), 56.18 (5''-OCH_3), 56.16 (5-OCH_3); HRMS for $C_{35}H_{28}O_{10}$ (MALDI-TOF, alpha-cyano-4-hydroxycinnamic acid as matrix): calculated $[M+H]^+$ 609.1689, observed $[M+H]^+$ 609.1652.

General Procedure for Demethylation. Penta-*O*-methylochnaflavone **3** (1.0 equiv) was dissolved in dry CH_2Cl_2 and 1 M BBr_3 solution in CH_2Cl_2 (15 equiv) was added slowly at ice bath. The reaction mixture was stirred for 30 min at room temperature then refluxed for 1 day. The reaction mixture was added MeOH and stirred for 5h. The precipitate was filtered, washed with CH_2Cl_2 and then recrystallized from CH_2Cl_2/MeOH to afford the title product.

Ochnaflavone (4). Yellow solid; mp 235-240 °C; Yield 86%; ^{1}H NMR (400 MHz, DMSO) δ 12.87 (s, 2H, 2xOH), 10.85 (s, 1H, OH), 10.81 (s, 1H, OH), 10.72 (s, 1H, OH), 8.03-8.05 (d, J = 8.8 Hz, 2H, H-2''', H-6'''), 7.89-7.91 (m, 2H, H-6', H-5'), 7.16-7.18 (d, J = 9.2 Hz, 1H, H-2'), 7.02-7.04 (d, J = 8.8 Hz, 2H, H-3''', H-5'''), 6.86 (s, 1H, H-3''), 6.85 (s, 1H, H-3), 6.49-6.50 (d, J = 2.0 Hz, 2H, H-8, H-8''), 6.19-6.20 (d, J = 2.0 Hz, 1H, H-6''), 6.19-6.19 (d, J = 2.0 Hz, 1H, H-6); ^{13}C NMR (100 MHz, DMSO) δ 182.63 (C-4''), 182.60 (C-4), 165.15 (C-7''), 165.06 (C-7), 163.88 (C-2''), 163.51 (C-2), 162.33 (C-5''), 162.28 (C-5), 161.67 (C-4'''), 158.22 (C-9''), 158.16 (C-9), 154.15 (C-4'), 142.46 (C-3'), 129.28 (C-2''', C-6'''), 126.16 (C-1'''), 125.24 (C-6'), 123.21 (C-1'), 122.11 (C-3''', C-5'''), 118.77 (C-2'), 116.94 (C-5'), 104.90 (C-10''), 104.68 (C-10), 104.62 (C-3''), 104.50 (C-3), 99.81 (C-6''), 99.75 (C-6), 94.96 (C-8''), 94.90 (C-8); HRMS for $C_{30}H_{18}O_{10}$ (MALDI-TOF, alpha-cyano-4-hydroxycinnamic acid as matrix): calculated $[M+H]^+$ 539.0907, observed $[M+H]^+$ 539.1248.

Reagents and Cell Culture. Bacterial LPS from *Escherichia coli* serotype 055:B5 was purchased from Sigma-Aldrich (St. Louis, MO, USA). RAW 264.7 cells were grown in Dulbecco's modified Eagle's medium (DMEM; Gibco BRL, Grand Island, NY, USA) containing 10% fetal bovine serum (FBS; Gibco BRL), 100 U/mL penicillin, and 100 mg/mL streptomycin (Gibco BRL) at 37 °C in 5% CO_2.

PGE_2 Assay. RAW 264.7 cells were treated with the indicated chemical agent for 1 h before the addition of LPS (200 ng/mL). After 24 h incubation, the level of PGE_2 in culture media was quantified using mono-clonal anti-PGE_2 antibody according to the manufacture's instruction (R&D).

Western Blot Analysis. RAW 264.7 cells were pretreated with the indicated chemical for 1 h and stimulated with LPS. Cells were washed with PBS and lysed with lysis buffer [50 mM Tris–HCl, pH 8.0, 150 mM NaCl, 0.02% sodium azide, 0.1% sodium dodecyl sulfate (SDS), 1% NP-40, 0.5% sodium deoxycholate, and 1 mM phenyl methylsulfonyl fluoride]. Samples from these cell lysates were denaturated and resolve on 10% SDS–polyacrylamide gels and transferred to Hyond PVDF membrane (Amersham Biosciences, Piscataway, NJ, USA). Blots were blocked by incubation with 5% skim milk in TBST for 1 h at room temperature. Specific

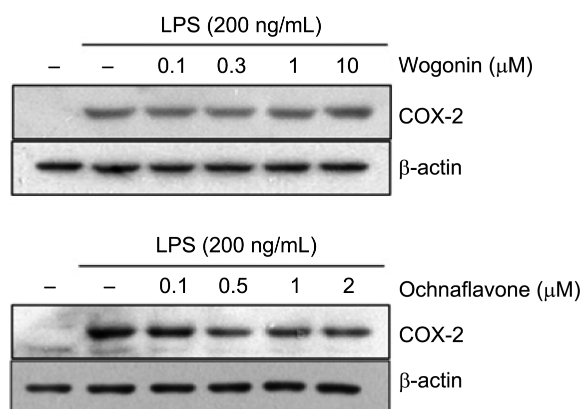


Figure 2. Ochnaflavone inhibited expression of COX-2 protein in LPS-stimulated RAW 264.7 cells.

antibodies COX-2, IκB-α (1:1,000; Santa Cruz Biotechnology Inc), were diluted in 5% skim milk. After thoroughly washing with TBST, horseradish peroxidase-conjugated secondary antibodies were applied. The blots were developed by the enhanced chemiluminescence detection (Amersham Biosciences).

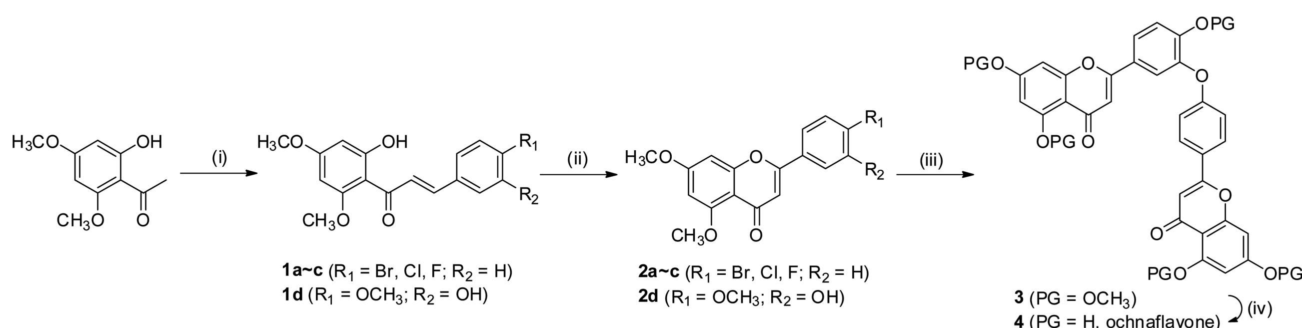
Results and Discussion

Synthesis. Ochnaflavone was prepared following the procedures and conditions as shown in Scheme 1. Each flavone unit was synthesized through known reaction procedure and conditions.[17,18] Reactions of 2'-hydroxy-4',6'-dimethoxyacetophenone and 4-halogenobenzaldehydes in Claisen-Schmidt reaction conditions gave the corresponding chalcones **1a-1c**. Subsequent flavone ring formation with I_2-DMSO yielded halogenoflavones **2a-2c** in good yields. The other flavone unit, 3'-hydroxy-4,6,4'-trimethoxyflavone (**2d**), was synthesized following the same procedure and conditions except using 3-hydroxy-4-methoxybenzaldehyde. Ullmann modified diaryl ether coupling reactions of **2d** with different halogenoflavones (**2a-2c**), bases, ligands and conditions gave analog of apigenin dimer **3** in 8-58% yields. Reaction conditions for Ullmann coupling reactions were summarized in Table 1. On the while, coupling reactions between 4'-hydroxy-5,7-dimethoxyflavone and 3'-bromo-5,7,4'-trimethoxyflavone were not successful in various reaction conditions. Demethylation of **3** with BBr_3 in DCM gave ochnaflavone (**4**) as yellow solid in 86% yield. The structure of ochnaflavone (**4**) was fully characterized by instrumental analysis and by comparing with reported data (Mp).[19]

Biological Evaluations. Inhibition of COX-2 catalyzed PGE_2 production from LPS-activated RAW 264.7 cells by ochnaflavone (**4**) and wogonin (reference compound) was determined according to the published procedure.[20,21] 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 (200 ng/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 manufacturer's recommendation. For nitrite measurement, different concentrations of ochnaflavone (**4**) and wogonin (reference compound) were treated 1 hour prior to LPS treatment for 18 hr. At the end of treatment, one hundred microliters of each supernatant was mixed with the same volume of Griess reagent (0.1% naphthylethylenediamine dihydrochloride and 1% sulfanilamide in 5% phosphoric acid) in a 96-well microtiter plate. Absorbance of the mixture at 540 nm was measured on an ELISA plate reader. Sodium nitrite was used as standard to calculate nitrite concentrations. All experiments were carried out at least three times and they gave similar results. The inhibitory activities of ochnaflavone (**4**) and wogonin (reference compound) on COX-2 catalyzed PGE_2 production from LPS-activated RAW 264.7 cells were estimated. Ochnaflavone exhibited quite strong inhibitory activity (IC_{50} = 1.08 μM) on COX-2 catalyzed PGE_2 production from LPS-activated RAW 264.7 cells. Wogonin, a naturally occurring anti-inlfammatory flavone, was used as a reference compound and showed strong inhibitory activity (IC_{50} = 0.52 μM). In order to clarify the action mechanism of ochnaflavone, COX-2 expression level was analysed by western blotting. Ochnaflavone clearly inhibited COX-2 expression in dose-dependent manner. In contrast to ochnaflavone, wogonin did not inhibited COX-2 expression (Figure 2). These above data suggest that ochanflavone inhibit PEG_2 production on protein expression level, but wogonin inhibit

Table 1. Reagents and conditions for Ullmann diaryl ether coupling reactions

Entry	X	Reagents & conditions	Yield
1	Br	CuI, TBAB, K_3PO_4, DMF, 100 °C, 24 h	NR
2	Cl	CuI, TBAB, K_3PO_4, DMF, 100 °C, 24 h	NR
3	Br	K_2CO_3, *N,N*-DMA, toluene, 160 °C, 16 h	NR
4	Cl	K_2CO_3, *N,N*-DMA, toluene, 160 °C, 16 h	NR
5	Br	CuI, Cs_2CO_3, *N,N*-DMG, dioxane, reflux, 24 h	15%
6	Cl	CuI, Cs_2CO_3, *N,N*-DMG, dioxane, reflux, 24 h	8%
7	F	CuI, Cs_2CO_3, *N,N*-DMG, dioxane, reflux, 24 h	28%
8	F	K_2CO_3, *N,N*-DMA, toluene, 160 °C, 16 h	58%



Scheme 1. Total synthesis of ochnaflavone (**4**).
Reagents and conditions: (i) 4-halogenobenzaldehyde, KOH, MeOH, rt, overnight, 70-87% (ii) I_2, DMSO, 100 °C, overnight, 75-86% (iii) Ullmann modified coupling reaction, 8-58% (iv) BBr_3, CH_2Cl_2, 0-60 °C, 1 day, 86%.

PGE_2 production on enzyme activity level.

Conclusion

In conclusion, we established an efficient total synthesis of ochnaflavone *via* modified Ullmann diaryl ether formation of two flavone units. The reactivity of coupling reactions was largely dependent on the species of halogen atoms and reaction conditions. Our concise total synthesis of ochnaflavone could prepare unsymmetrical C-O-C bioflavonoids with structural diversity, overcome the limited supply problem of ochnaflavone for advanced pharmacological and clinical studies against diverse therapeutic areas and also could be used efficiently prepare derivatives for SAR study. Strong inhibitory activities of ochnaflavone against production of inflammation mediator, PGE_2, imply its potential as a lead compound to discover a new anti-inflammatory agent. In addition, our data suggested that ochanflavone inhibit PEG_2 production on protein expression level, but wogonin inhibit PGE_2 production on enzyme activity level. Further pharmacological study related with anti-inflammatory activity will be conducted using sufficient quantities of ochnaflavone prepared by our synthetic pathway. SAR study of ochnaflavone analogs with different flavone units will be conducted and reported in a near future.

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References and Notes

1. Ruckstuhl, M.; Beretz, A.; Anton, R.; Landry, Y. *Biochem. Pharmacol.* **1979**, *28*, 535.
2. Iwu, M. M.; Igboko, O. A.; Okunji, C. O.; Tempesta, M. S. *J. Pharm. Pharmacol.* **1990**, *42*, 290.
3. Amella, M.; Bronner, C.; Briancon, F.; Hagg, M.; Anton, R.; Landry, Y. *Planta Med.* **1985**, *51*, 16.
4. Sun, C.-M.; Syu, W.-J.; Huang, Y.-T.; Chen, C.-C.; Ou, J.-C. *J. Nat. Prod.* **1997**, *60*, 382.
5. Lin, Y.-M.; Flavin, M. T.; Cassidy, C. S.; Mar, A.; Chen, F.-C. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2101.
6. Chen, J.; Chang, H. W.; Kim, H. P.; Park, H. *BioOrg. Med. Chem. Lett.* **2006**, *16*, 2373.
7. Che, H.; Park, B. K.; Lim, H.; Kim, H. P.; Chang, H. W; Jeong, J.-H.; Park, H. *BioOrg. Med. Chem. Lett.* **2009**, *19*, 74.
8. Okigawa, M.; Kawano, N. *Tetrahed. Lett.* **1973**, *22*, 2003.
9. Son, K. H.; Park, J. O.; Chung, K. C.; Chang, H. W.; Kim, H. P.; Kim, J. S.; Kang, S. S. *Arch. Pharm. Res.* **1992**, *15*, 365.
10. Chang, H. W.; Baek, S. H.; Chung, K. W.; Son, K. H.; Kim, H. P.; Kang, S. S. *Biochem. Biophys. Res. Comm.* **1994**, *205*, 843.
11. Lee, S. J.; Son, K. H.; Chang, H. W.; Kang, S. S.; Kim, H. P. *Arch. Pharm. Res*. **1997**, *20*, 533.
12. Suh, S. J.; Jin, U. H.; Kim, S. H.; Chang, H. W.; Son, J. K.; Lee, S. H.; Son, K. H.; Kim, C. H. *J. Cell. Biochem.* **2006**, *99*, 1298.
13. Suh, S. J.; Chung, T. W.; Son, M. J.; Kim, S. H.; Moon, T. C.; Son, K. H.; Kim, H. P.; Chang, H. W.; Kim, C. H. *Arch. Biochem. Biophy.* **2010**, *493*, 249.
14. Moon, T. C.; Hwang, H. S.; Quan, Z.; Son, K. H.; Kim, C. H.; Kim, H. P.; Kang, S. S.; Son, J. K.; Chang, H. W. *Biol. Pharm. Bull.* **2006**, *29*, 2359.
15. Reutrakul, V.; Ningnuek, N.; Pohmakotr M.; Yoosook, C.; Napaswad, C.; Kasisit, J.; Santisuk, T.; Tuchinda, P. *Planta Med.* **2007**, *73*, 683.
16. Kang, S. S.; Lee, J. Y.; Choi, Y. K.; Song, S. S.; Kim, J. S.; Jeon, S. J.; Han, Y. N.; Son, K. H.; Han, B. H. *BioOrg. Med. Chem. Lett.* **2005**, *15*, 3588.
17. Park, H.; Tran, T. D.; Kim, H.-P. *Eur. J. Med. Chem.* **2005**, *40*, 943.
18. Pham, T.-A.; Che, H.; Phan, P.-T.; Lee, J.-W.; Kim, S.-S.; Park, H. *BioOrg. Med. Chem. Lett.* **2012**, *22*, 2534.
19. Okigawa, M.; Kawano, N.; Aquil, M.; Rahman, W. *J.C.S. Perkin I* **1976**, 580.
20. Tran, T. D.; Chi, Y. S.; Kim, J.; Kim, H.-P.; Kim, S.; Park, H. *Arch. Pharm. Res.* **2003**, *26*, 345.
21. Tran, T. D.; Truong, N. T.; Park, H. *Arch. Pharm. Res.* **2005**, *28*, 652.