Synthesis and Antioxidant Activity of 3-Methoxyflavones

Ho Yoo,[†] Seung Hwan Kim,[†,‡] Jiyong Lee,[†] Hyoung Ja Kim,[†] Seon Hee Seo,[†] Bong Young Chung,[‡] Changbae Jin,[†] and Yong Sup Lee[§,*]

[†]*Life Sciences Division, Korea Institute of Science & Technology, P.O. Box 131, Seoul 130-650, Korea*
[‡]*Department of Chemistry, Korea University, Seoul 136-701, Korea*
[§]*Kyung Hee East-West Pharmaceutical Research Institute and Department of Pharmaceutical Science, College of Pharmacy, Kyung Hee University, Seoul 130-701, Korea. *E-mail: kyslee@khu.ac.kr*
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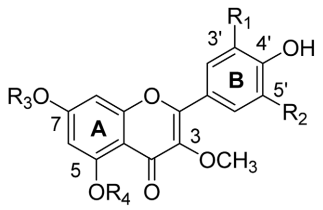
It is becoming increasingly apparent that the overproduction of reactive oxygen species may overwhelm the protective antioxidative defense mechanisms resulting in oxidative tissue injury.[1] Reactive oxygen species have been implicated in several different diseases including ischemia, inflammation and cancer.[2] Fortunately, plants contain a wide variety of free radical scavenging molecules such as flavonoids, anthocyanins, carotenoids, dietary glutathione, vitamins and endogenous metabolites. Such natural products are rich in antioxidant activities.[3] Recently, important biological property of natural flavonoids was suggested mainly due to their antioxidant activity elicited by scavenging oxygen radicals and inhibiting peroxidation.[4] Also, the antioxidant activity of the flavonoids varies considerably depending on the backbone structures and functional groups.[5]

In the course of searching for neuroprotective agents, we recently identified quercetin 3-*O*-methyl ether (**1b**, R_1, R_3, R_4 = H, R_2 = OH) as a potent antioxidant from *Opuntia ficus-indica* var. *saboten*.[6] Quercetin 3-*O*-methyl ether also exhibited potent neuroprotective effects on the oxidative injuries to neuronal cells.[7] For the purpose of the development of neuroprotective agents for therapeutic use, we needed to modify the structure or substitute suitable groups in the structure of **1b** to improve physicochemical properties or enhance antioxidant activities. Therefore, we synthesized a series of 3-methoxyflavones (**1c-g**) and examined their antioxidant activities to elucidate a suitable position for modification (Figure 1). To investigate briefly the influence of substituents of 3-methoxyflavones on antioxidant activity, we methylated the C-5 or C-7 hydroxyl group at A-ring of the chromone backbone or introduced mono-, di-, and trihydroxyl groups at B-ring.



3-Methoxyflavones (**1a ~ f**)
R_1, R_2 = H or OH; R_3, R_4 = H or CH_3

Figure 1

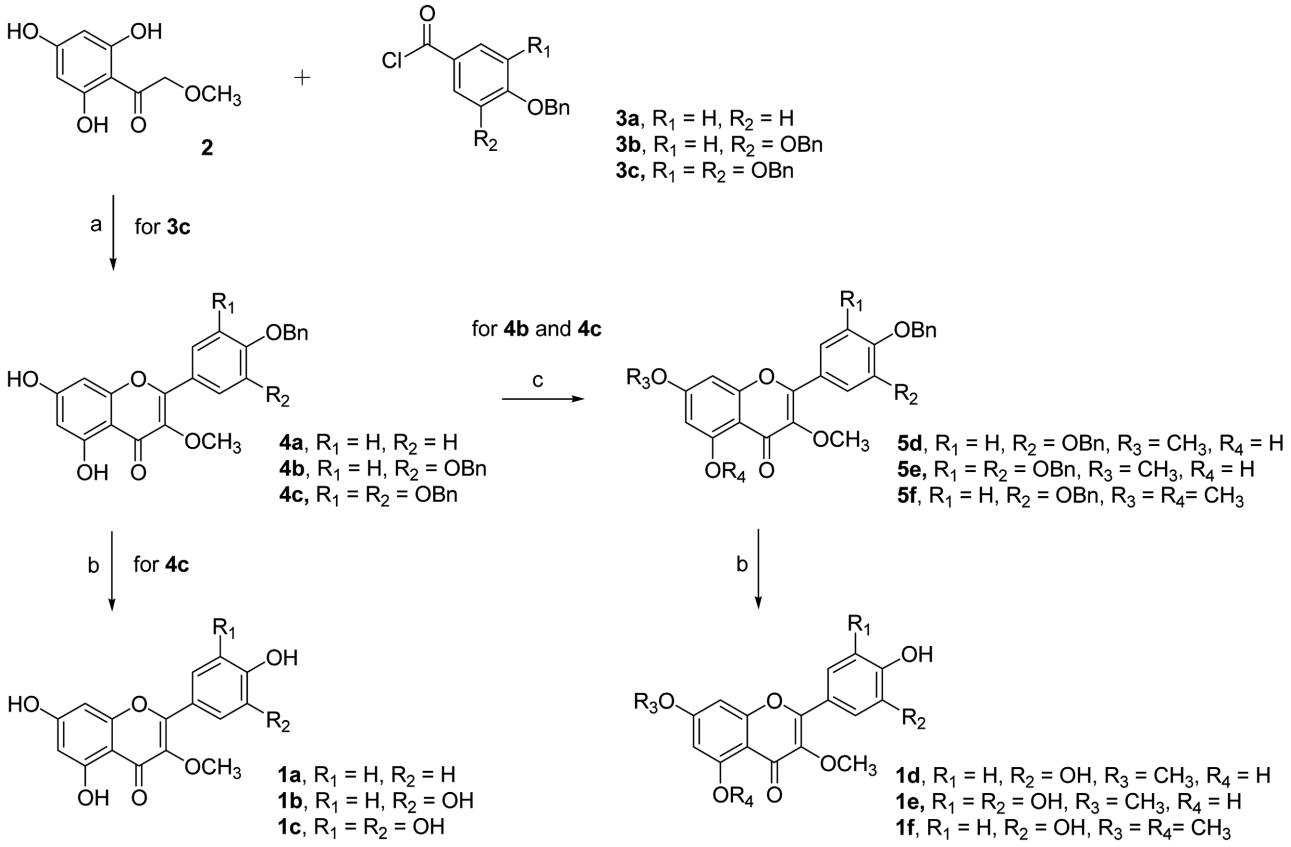
Results and Discussion

Chemistry. Kaempferol 3-*O*-methyl ether (**1a**) and quercetin 3-*O*-methyl ether (**1b**) were obtained from the ethyl acetate fraction of the stems of *Opuntia ficus-indica* var. *saboten* as previously reported by us.[6] The other 3-*O*-methyl ether derivatives (**1c-1f**) were synthesized as illustrated in Scheme 1. *O*-Benzyl-protected flavone **4c** was prepared in 76% yield by coupling acetophenone **2** with *O*-benzyl-protected benzoyl chloride **3c** followed by *in situ* cyclization of the resulting ester to form chromone ring in the presence of tetrabutylammonium hydrogen sulfate (TBAHS) under a basic condition.[8] Deprotection of benzyl group in **4c** using $Pd(OH)_2$/C and cyclohexene afforded myricetin 3-*O*-methyl ether (**1c**) in 66% yield.

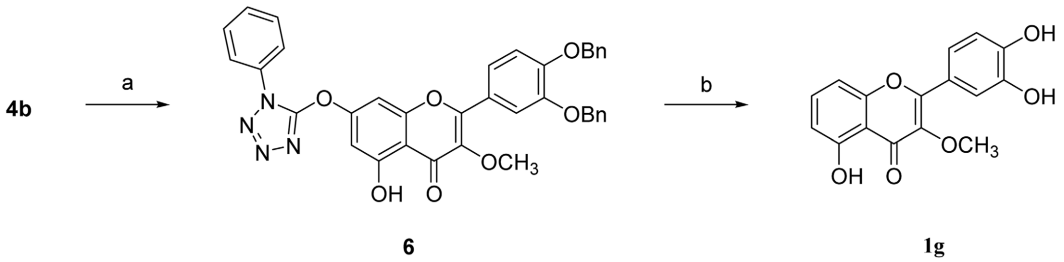
Selective mono-methylation at the C-7 position of benzyl-protected 3-methoxyflavones **4b** and **4c** were performed using dimethyl sulfate and potassium carbonate in acetone at room temperature to provide **5d** and **5e** in 94% and 79% yields, respectively.[9] Di-methylation of **4b** was also achieved at reflux temperature to obtain **5f** in 91% yield. The benzyl groups in **5d-f** were removed using again $Pd(OH)_2$ and cyclohexene to yield **1d-f** in 72-87% yields.

To further investigate the influence of the C-7 position on the antioxidant activity, we synthesized compound **1g**, which has no C-7 hydroxyl group in quercetin 3-*O*-methyl ether as shown in Scheme 2. The C-7 hydroxyl group of **4b** was selectively converted into the tetrazolyl ether **6** with 5-chloro-1-phenyltetrazole, which was then reduced with formic acid and palladium on charcoal to lead **1g**.[10]

Biological Activity. Table 1 summarizes the results of the antioxidant activities obtained using three different bioassay systems; 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging,[11] superoxide anion radical scavenging[12] and lipid peroxidation inhibition activity assays.[13] Vitamin C and trolox were used for comparisons of activities in our assay



Scheme 1. *Reagents and conditions*: (a) i. K_2CO_3, TBAHS, toluene, ii. reflux; (b) $Pd(OH)_2/C$, EtOH/cyclohexene: 1/1, reflux; (c) K_2CO_3, dimethyl sulfate, acetone.



Scheme 2. *Reagents and conditions*: (a) potassium *tert*-butoxide, 5-chloro-1-phenyltetrazole; (b) HCOOH, Pd/C, benzene, EtOH, H_2O.

systems as hydrophilic and lipophilic antioxidants, respectively.

Almost 3-methoxyflavones except for **1a** exhibited potent antioxidant activities in three different assay systems. The DPPH radical scavenging activities of synthesized compounds (**1c-g**) were comparable to the activity of parent compound, quercetin 3-*O*-methyl ether (**1b**) and vitamin C. On the other hand, lipid peroxidation inhibition activities of the compounds were much more potent than that of trolox. The antioxidant activity data of **1a-c** indicate that at least two hydroxyl groups are required in the B-ring of flavones for antioxidant activities. On methylation of the C-5 and/or C-7 hydroxyl groups in the A-ring (**1d-f**), the antioxidant activities were not much affected. However, when the C-7 hydroxyl group was removed from **1b** as in **1g**, superoxide anion radical scavenging activity was reduced about 5-fold, while DPPH radical scavenging and lipid peroxidation inhibition activities were retained.

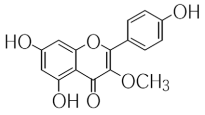
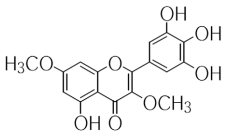
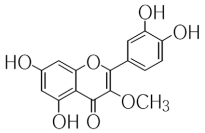
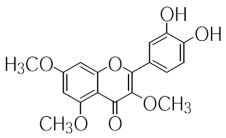
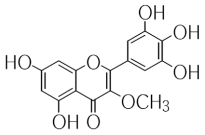
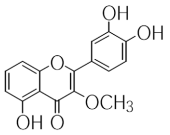
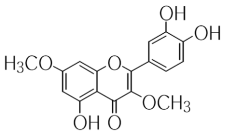
In conclusion, a series of 3-methoxyflavones (**1a-g**) were prepared and evaluated for the antioxidant activities. On methylation of the C-5 and/or C-7 hydroxyl groups in 3-methoxyflavones, the antioxidant activities were retained, while removal of the C-7 hydroxyl group diminished superoxide anion radical scavenging activity. Therefore, the substitution of the functional group at the C-5 or C-7 position seems desired in the design of new antioxidative 3-methoxyflavones with improved physicochemical properties.

Experimental Section

Chemistry.

General: ^1H and ^{13}C NMR spectra were recorded on a

Table 1. Antioxidant activities of 3-methoxyflavones (**1a-g**)

Entry	Structure	IC_{50} (μg/mL)[a]			Entry	Structure	IC_{50} (μg/mL)[a]		
		DPPH[b]	Superoxide anion[c]	Lipid peroxidation[d]			DPPH[b]	Superoxide anion[c]	Lipid peroxidation[d]
1a		> 50	> 50	> 50	**1e**		5.48 ± 0.63	7.10 ± 1.74	3.78 ± 0.74
1b		3.76 ± 0.22	4.77 ± 0.14	4.41 ± 1.06	**1f**		4.20 ± 0.49	4.78 ± 0.48	6.84 ± 0.75
1c		4.91 ± 0.48	5.07 ± 0.68	4.33 ± 0.14	**1g**		4.99 ± 0.87	24.17 ± 1.70	8.27 ± 0.46
1d		4.12 ± 0.51	6.22 ± 0.83	4.84 ± 0.31	**Vitamin C**		5.07 ± 0.73	> 50	>5 0
					Trolox		NT[e]	> 50	26.4 ± 1.26

[a]IC_{50} values with standard deviation are at least from three independent experiments. [b]DPPH radical scavenging activity. [c]Superoxide anion radical scavenging activity generated in the xanthine/xanthine oxidase system. [d]Iron-dependent lipid peroxidation inhibition activity using rat liver homogenate. [e]Not tested.

Gemini Varian-300 (300 and 75 MHz, respectively). Analytical thin layer chromatographies (TLC) were carried out by precoated silica gel (E. Merck Kiesegel 60F_{254} layer thickness 0.25 mm). Flash column chromatographies were performed with Merck Kiesegel 60 Art 9385 (230–400 mesh). All solvents used were purified according to standard procedures. Compounds **1a** and **1b** were obtained from the ethyl acetate fraction of the stems of *Opuntia ficus-indica* var. *saboten* as previously reported.[6] Compounds **4b** was prepared by the known procedure.[7]

3',4',5'-Tribenzyloxy-5,7-dihydroxy-3-methoxyflavone (4c). To a solution of 2',4',6'-trihydroxy-2-methoxyacetophenone (**2**, 1.0 g, 5.4 mmol), TBAHS (1.8 g, 5.4 mmol) and K_2CO_3 (1.4 g, 10.8 mmol) in toluene (70 mL) was added 3,4,5-tribenzyloxybenzoyl chloride (**3c**, 5.0 g, 11.0 mmol) portionwise at 0 °C and the solution was heated at 90 °C for 12 h. The mixture was cooled and diluted with H_2O and then extracted with CH_2Cl_2. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated. The residue was purified by flash column chromatography (CH_2Cl_2/MeOH = 98 : 2) to afford **4c** (2.4 g, 76%) as a solid. ^{1}H NMR (DMSO-d_6) δ 12.54 (1H, br s, *OH*), 7.49-7.26 (17H, m, *H2'*, *H6'*, OCH_2Ph), 6.49 (1H, s, *H8*), 6.22 (1H, s, *H6*), 5.23 (4H, s, CH_2Ph), 5.08 (2H, s, -CH_2Ph), 3.66 (3H, s, OCH_3).

3',4'-Dibenzyloxy-3,7-dimethoxy-5-hydroxyflavone (5d). To a solution of compound **4b** (0.2 g, 0.4 mmol) in acetone (30 mL) was added K_2CO_3 (2.0 g, 14.5 mmol) and dimethyl sulfate (40 mL, 0.42 mmol). The reaction mixture was stirred for 24 h at room temperature and the mixture was filtered through Celite. The filtrate was concentrated, diluted with water and extracted with EtOAc. The organic layer was dried over $MgSO_4$, concentrated, and purified by flash column chromatography (EtOAc/*n*-hexane = 1 : 5) to afford **5d** (0.19 g, 94%) as a solid. ^{1}H NMR ($CDCl_3$) δ 12.61 (1H, br s, *OH*), 7.75 (1H, d, *J* = 2.0 Hz, *H2'*), 7.66 (1H, dd, *J* = 2.0, 8.6 Hz, *H6'*), 7.49-7.33 (10H, m, OCH_2Ph), 7.02 (1H, d, *J* = 8.6 Hz, *H5'*), 6.37 (1H, d, *J* = 2.1 Hz, *H8*), 6.33 (1H, d, *J* = 2.1 Hz, *H6*), 5.26 (2H, s, OCH_2Ph), 5.24 (2H, s, OCH_2Ph), 3.86 (3H, s, OCH_3), 3.70 (3H, s, OCH_3).

3',4',5'-Tribenzyloxy-3,7-dimethoxy-5-hydroxyflavone (5e). To a solution of compound **4c** (0.2 g, 0.33 mmol) in acetone (30 mL) was added K_2CO_3 (2.0 g, 14.5 mmol) and dimethyl sulfate (31 mL, 0.33 mmol). The reaction mixture was stirred for 24 h at room temperature and the mixture was filtered through Celite. The filtrate was concentrated, diluted with water, and extracted with EtOAc. The organic layer was dried over $MgSO_4$, concentrated, and purified by flash column chromatography (EtOAc/*n*-hexane = 1 : 5) to afford **5e** (0.16 g, 79%) as a solid. ^{1}H NMR ($CDCl_3$) δ 12.55 (1H, s, OH), 7.46-7.25 (17H, m, *H2'*, *H6'*, OCH_2Ph), 6.36 (1H, d, *J* = 2.2 Hz, *H8*), 6.35 (1H, d, *J* = 2.2 Hz, *H6*), 5.19 (6H, s, OCH_2Ph), 3.88 (3H, s, OCH_3), 3.66 (3H, s, OCH_3).

3',4'-Dibenzyloxy-3,5,7-trimethoxyflavone (5f). To a solution of compound **4b** (0.2 g, 0.4 mmol) in acetone (30 mL) was added K_2CO_3 (3.0 g, 21.7 mmol) and dimethyl sulfate (71 mL, 0.74 mmol). The reaction mixture was refluxed for 2 h and the mixture was filtered through Celite. The filtrate was concentrated, diluted with water and

extracted with EtOAc. The organic layer was dried over $MgSO_4$, concentrated, and purified by flash column chromatography (EtOAc/*n*-hexane = 3 : 1) to afford **3e** (0.19 g, 91%) as a solid. ^{1}H NMR ($CDCl_3$) δ 7.79 (1H, d, J = 2.1 Hz, *H2'*), 7.66 (1H, dd, J = 2.1, 8.6 Hz, *H6'*), 7.52-7.34 (10H, m, OCH_2Ph), 7.02 (1H, d, J = 8.6 Hz, *H5'*), 6.42 (1H, d, J = 2.1 Hz, *H8*), 6.31 (1H, d, J = 2.1 Hz, *H6*), 5.26 (4H, s, OCH_2Ph), 3.94 (3H, s, OCH_3), 3.88 (3H, s, OCH_3), 3.76 (3H, s, OCH_3).

3',4',5-Trihydroxy-3-methoxyflavone (1c). To a solution of **4c** (150 mg, 0.25 mmol) in mixture of solvents (ethanol/cyclohexene = 5 : 1, 6 mL) was added excess $Pd(OH)_2$/C (25 mg) and heated at 60-70 °C for 1 h. The mixture was filtered through Celite and concentrated. The residue was purified by flash column chromatography (CH_2Cl_2/MeOH = 15 : 1) to afford **1c** (55 mg, 66%) as a solid. ^{1}H NMR (DMSO-d_6) δ 12.82 (1H, br s, *OH*), 7.23 (2H, s, *H2'*, *H6'*), 6.48 (1H, d, J = 1.9 Hz, *H8*), 6.29 (1H, d, J = 1.9 Hz, *H6*), 3.79 (3H, s, OCH_3).

3',4',5-Trihydroxy-3,7-dimethoxyflavone (1d). By using the similar procedure for **1c**, compound **1d** was obtained from **5d** (120 mg, 0.24 mmol) and $Pd(OH)_2$/C (24 mg) as a solid in 97% yield (75 mg). ^{1}H NMR (DMSO-d_6) δ 7.65 (1H, d, J = 1.4 Hz, *H2'*), 7.55 (1H, dd, J = 1.4, 8.4 Hz, *H6'*), 6.97 (1H, d, J = 8.4 Hz, *H5'*), 6.77 (1H, d, J = 1.9 Hz, *H8*), 6.43 (1H, d, J = 1.9 Hz, *H6*), 3.93 (3H, s, OCH_3), 3.86 (3H, s, OCH_3).

3',4',5',5-Tetrahydroxy-3,7-dimethoxyflavone (1e). By using the similar procedure for **1c**, compound **1e** was obtained from **5e** (112 mg, 0.18 mmol) and $Pd(OH)_2$/C (12 mg) as a solid in 72% yield (45 mg). ^{1}H NMR (DMSO-d_6) δ 12.70 (1H, br s, *OH*), 7.16 (2H, s, *H2'*, *H6'*), 6.65 (1H, br s, *H8*), 6.36 (1H, br s, *H6*), 3.85 (3H, s, OCH_3), 3.78 (3H, s, OCH_3).

3',4'-Dihydroxy-3,5,7-trimethoxyflavone (1f). By using the similar procedure for **1c**, compound **1f** was obtained from **5f** (125 mg, 0.24 mmol) and $Pd(OH)_2$/C (26 mg) as a solid in 87% yield (71 mg). ^{1}H NMR (DMSO-d_6) δ 7.53 (1H, br s, *H2'*), 7.41 (1H, d, J = 7.4 Hz, *H6'*), 6.87 (1H, d, J = 7.4 Hz, *H5'*), 6.72 (1H, s, *H8*), 6.45 (1H, s, *H6*), 3.86 (3H, s, OCH_3), 3.82 (3H, s, OCH_3), 3.70 (3H, s, OCH_3).

3',4'-Dibenzyloxy-3-methoxy-7-[(1-phenyl-tetrazol-5-yl)oxy]flavone (6). To a solution of **4b** (100 mg, 0.20 mmol) in dry DMF (1.5 mL) was added potassium *tert*-butoxide (41 mg, 0.35 mmol) at room temperature. When the base was dissolved, 5-chloro-1-phenyltetrazole (58 mg, 0.35 mmol) in dry DMF (1 mL) was added to the solution. The mixture was stirred at room temperature for 10 h and poured into ice water. The mixture was extracted with EtOAc and the organic layer was washed with brine, dried over $MgSO_4$, and concentrated. The residue was purified by flash column chromatography (EtOAc/*n*-hexane = 1 : 2) to afford **6** (72 mg, 56%) as a solid. ^{1}H NMR ($CDCl_3$) δ 7.82-7.36 (7H, m, tetrazole-*Ph*, *H2'*, *H6'*), 7.27 (1H, d, J = 1.5 Hz, *H8*), 7.08 (1H, d, J = 8.7 Hz, *H5'*), 6.81 (1H, d, J =1.5 Hz, *H6*), 5.32 (2H, s, OCH_2Ph), 5.31 (2H, s, OCH_2Ph), 3.77 (3H, s, OCH_3).

3',4',5-Trihydroxy-3-methoxyflavone (1g). To a vigorously stirred solution of **6** (66 mg, 0.10 mmol) in benzene (2.3 mL), H_2O (2.5 mL), and EtOH (4.6 mL) was added Pd/C (10%, 80 mg). HCOOH (2 mL) was then added and the mixture was refluxed at 100 °C for 3 h. The solution was cooled, filtered through Celite, and concentrated. The residue was purified by flash column chromatography (CH_2Cl_2/MeOH = 15 : 1) to afford **1g** (8 mg, 26%) as a solid. ^{1}H NMR (CD_3OD) δ 7.70 (1H, d, J = 2.4 Hz, *H2'*), 7.63 (1H, dd, J = 2.4, 8.1 Hz, *H6'*), 7.60 (1H, t, J = 8.1 Hz, *H7*), 7.06 (1H, dd, J = 1.2, 8.4 Hz, *H8*), 6.94 (1H, d, J = 8.1 Hz, *H5'*), 6.77 (1H, dd, J = 1.2, 8.4 Hz, *H6*), 6.77 (3H, s, OCH_3).

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