Synthesis of Dihydroxylated Chalcone Derivatives with Diverse Substitution Patterns and Their Radical Scavenging Ability toward DPPH Free Radicals

Beom-Tae Kim,[‡] Kwang-Joong O, Jae-Chul Chun,[§] and Ki-Jun Hwang[†,*]

Research Center of Bioactive Materials, [†]Department of Chemistry, College of Natural Science, [‡]The Center for Healthcare Technology Development, [§]Division of Applied Biotechnology, College of Agriculture and Life Science, Chonbuk National University, Chonju 561-756, Korea. [*]E-mail: kijun@chonbuk.ac.kr
Received March 16, 2008

A series of dihydroxylated chalcone derivatives with diverse substitution patterns on a phenyl ring B and the *para*-substituents on a phenyl ring A were prepared, and their radical scavenging activities were evaluated by simple DPPH test to determine quantitative structure-activity relationship in these series of compounds. The chalcone compounds with the *ortho*- (*i.e.* 2',3'- and 3',4'-) and *para*- (*i.e.* 2,5'-) substitution patterns show an excellent antioxidant activities (80-90% of control at the concentration of 50 μM) which are comparable to those of ascorbic acid and α-tocopherol as positive reference materials. On the contrary, the compounds with *meta*- (*i.e.* 2',4'-, 3',5'-) substitution pattern demonstrate very dramatic decrease in activities which are around 25% of the control even at the concentration of 200 μM (IC_{50} > 200 μM). These dramatic differences could be interpreted in terms of the ease formation of fairly stable semiquinone radicals from the *ortho*- and *para*-substituted chalcone molecules through facilitating electron delocalization. Our results indicate that the substitution patterns of two hydroxyl groups on ring B are very important structural factors for their radical scavenging activity enhancement. Meanwhile, the substituents at *para*-position of the phenyl ring A of chalcones have no influence on the activity.

Key Words : Chalcones, Antioxidant, DPPH free radical, Structure-activity relationship, Semiquinone radical

Introduction

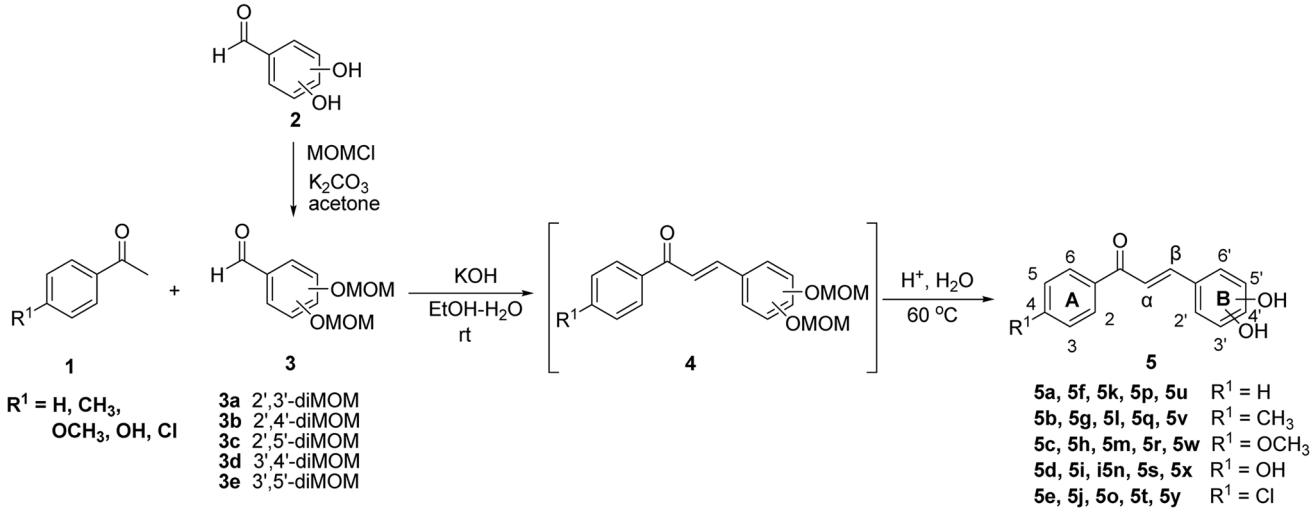
Chalcones (1,3-diaryl-2-propen-1-ones) constitute an important class of natural products belonging to the flavonoid family, which have been reported to possess a wide spectrum of biological activities, including antibacterial, antifungal, anti-inflammatory, antimicrobial, antitumour, insect antifeedant and antimutagenic.[1] Additionally, some of chalcone derivatives have been found to inhibit several important enzymes in cellular systems, such as xanthine oxidase and protein tyrosine kinase.[2] The evidences that these biological activities would be closely correlated to their antioxidant potential have been emerged in recent reports.[3] The antioxidant properties of chalcones are known to be influenced to a great extent by the two aryl structures, *i.e.* the substituents on two aryl rings of chalcone molecule and their substitution patterns. Especially, the hydroxyl substituent is one of the key groups to enhance greatly the antioxidant activity of chalcone mainly due to its easy conversion to phenoxy radicals through the hydrogen atom transfer mechanism. This phenoxy radical formation may be central to the antioxidant properties which are assessed primarily as radical scavenging potential of phenolic chalcones.[4] In fact, the hydroxyl substituent is widespread among chalcones from natural sources.[5] Thereby, a number of structurally diverse chalcones including phenolic chalcones have been prepared and evaluated for their activities. Although some sporadic structure-activity relationships (SAR) of some phenolic chalcone derivatives have been reported in the recent literature,[6] it is fairly rare to find the *systematic* study on the relationships between structural variations and antioxidant activity of structurally distinguishable polyphenolic chalcone derivatives.

So, we were determined to investigate systematic SAR of structurally-diverse chalcone compounds with two hydroxyl groups on benzaldehyde-originated aryl ring, in which two hydroxyl groups are diversely oriented each other. Herein, we report synthetic details of the dihydroxylated chalcone derivatives having diverse substitution patterns on a phenyl nucleus (Scheme 1), and their antioxidant activities. The activity results were analyzed to determine the quantitative structure-activity relationships. The antioxidant activity of the chalcones was measured by standard free radical scavenging assay (DPPH test) as a primary tool.

Experimental Section

Chemistry. Melting points were recorded on Electrothermal melting point apparatus and were uncorrected. ^{1}H- and ^{13}C-NMR spectra were recorded on Jeol 400 MHz or 600 MHz spectrometer. Chemical shifts are shown in δ values (ppm) with tetramethylsilane (TMS) as internal standard. All chemicals were purchased from Sigma-Aldrich Co., U.S.A., and all solvents for column chromatography were of reagent grade, and were purchased from commercial sources.

General synthetic procedure for the preparation of di-MOM-benzaldehyds (2a-e, Scheme 1). A solution of appropriate dihydroxybenzaldehyde (21.71 mmol) and K_2CO_3 (217.20 mmol) in acetone (100 mL) was cooled to 0



Scheme 1. General procedure for the formation of chalcones **5**.

°C under Ar atmosphere, and then methoxymethyl chloride (MOM-Cl, 93.65 mmol) was added dropwise. The resulting mixture was stirred at room temperature for 6-10 hours. The reaction mixture was diluted with water (100 mL) and extracted with ethyl acetate (50 mL × 3). The combined organic layer was washed with water and brine, dried over anhydrous $MgSO_4$ and evaporated to dryness to yield crude MOM-protected benzaldehyde which was purified by silica gel column chromatography to give analytically pure compounds (90-99%). **3a**: ^{1}H-NMR (400 MHz, $CDCl_3$); δ 10.46 (s, 1H, -CHO), 7.51 (dd, 1H, *J* = 2.0 Hz, *J* = 7.6 Hz, H-6'), 7.49 (dd, 1H, *J* = 2.0 Hz, *J* = 7.6 Hz, H-4'), 7.16 (t, 1H, *J* = 7.6 Hz, H-5'), 5.25 (s, 2H, -OCH_2O-), 5.23 (s, 2H, OCH_2O-), 3.57 (s, 3H, -OCH_3), 3.51 (s, 3H, -OCH_3); ^{13}C-NMR (100 MHz, $CDCl_3$); δ 190.1, 150.0, 149.8, 130.6, 124.5, 122.2, 120.9, 99.6, 95.2 (-O-CH_2-O-), 93.5 (-O-CH_2-O-), 57.8, 56.3. **3b**: mp 51-52 °C; ^{1}H-NMR (400 MHz, $CDCl_3$); δ 9.87 (s, 1H, -CHO), 7.68 (d, 1H, *J* = 1.8 Hz, H-2'), 7.50 (d,d, 1H, *J* = 1.8 Hz, *J* = 8.0 Hz, H-6'), 7.28 (d, 1H, *J* = 8.0 Hz, H-5'), 5.33 (s, 2H, -OCH_2O-), 5.30 (s, 2H, -OCH_2O-), 3.53 (s, 3H, -OCH_3), 3.53 (s, 3H, -OCH_3); ^{13}C-NMR, (150 MHz, $CDCl_3$); δ 190.83 (-CHO), 152.54, 147.38, 131.06, 126.32, 115.79, 115.27, 95.29, 94.90, 56.49, 56.36. **3c**: ^{1}H-NMR (400 MHz, $CDCl_3$); δ 10.45 (s, 1H, -CHO), 7.49 (d, 1H, *J* = 2.8 Hz, H-6'), 7.24 (dd, 1H, *J* = 2.8 Hz, *J* = 9.2 Hz, H-4'), 7.18 (d, 1H, *J* = 9.2, H-3'), 5.25 (s, 2H, -OCH_2O-), 5.15 (s, 2H, -OCH_2O-), 3.52 (s, 3H, -OCH_3), 3.48 (s, 3H, -OCH_3); ^{13}C-NMR (100 MHz, $CDCl_3$); δ 189.3, 154.9, 151.9, 126.0, 124.7, 116.8, 114.5, 95.1, 94.8, 56.3, 55.9. **3d**: mp 51-52 °C; ^{1}H-NMR, (400 MHz, $CDCl_3$) 9.87 (s, 1H, -CHO), 7.68 (d, 1H, *J* = 1.8 Hz, H-2'), 7.50 (dd, 1H, *J* = 1.8 Hz, 7.8 Hz, H-6'), 7.28 (d, 1H, *J* = 8.4 Hz, H-5'), 5.33 (s, 2H, -OCH_2O-), 5.30 (s, 2H, -OCH_2O-), 3.53 (s, 3H, -OCH_3), 3.53 (s, 3H, -OCH_3); ^{13}C-NMR (150 MHz, $CDCl_3$) δ 190.83 (-CHO), 152.54, 147.38, 131.06, 126.32, 115.79, 115.27, 95.29, 94.90, 56.49, 56.36. **3e**: ^{1}H-NMR (400 MHz, $CDCl_3$); δ 9.90 (s, 1H, -CHO), 7.21 (s, 2H, H-2', H-6'), 6.97 (s, 1H, H-4'), 5.21 (s, 4H, -OCH_2O-), 3.48 (s, 6H, -OCH_3); ^{13}C-NMR (100 MHz, $CDCl_3$); δ 191.5 (-CHO), 158.6, 138.4, 111.0, 110.3, 94.4 (-OCH_2O- ×2), 56.0.

General synthetic procedure for the preparation of dihydroxychalcones (5a-y, Scheme 1). To a solution of appropriate MOM-protected benzaldehyde (0.9 mmol) and acetophenone (1.0 mmol) in ethanol (10 mL), 5% sodium aqueous sodium hydroxide (1.1 mmol, 0.5 mL) was added, and the reaction mixture was stirred at room temperature for 1-2 hours. After checking the completion of the condensation reaction, judged from simple tlc analysis, 10% HCl (1 mL) was added and the mixture was further stirred for further 30 min at 60 °C to deprotect the MOM groups. And then the whole mixture was diluted with water (20 mL) and its pH was adjusted to 5 with 1N aqueous NaOH solution. The aqueous solution was extracted with EtOAc (20 mL × 3). The combined organic layer was washed with water (20 mL × 2) and brine (20 mL), dried over anhydrous $MgSO_4$, and evaporated to give a crude solid. The desired product was purified by column chromatography eluted with hexane-ethyl acetate co-solvent to afford a solid (**5a-y**, Table 1). **5a**: ^{1}H-NMR (400 MHz, DMSO-d_6): δ 8.07-8.02 (3H, *J* = 15.6 Hz, H-β, H-2), 7.78 (d, 1H, *J* = 15.6 Hz, H-α), 7.66 (t, *J* = 7.2 Hz, H-4), 7.57 (d, 2H, *J* = 7.2 Hz, H-3), 7.31 (d, 1H, *J* = 7.2 Hz, H-6'), 6.86 (d, 1H, *J* = 7.2 Hz, H-4'), 6.71 (t, 1H, *J* = 7.2 Hz, H-5'); ^{13}C-NMR (100 MHz, DMSO-d_6): δ 189.5, 145.9, 145.6, 139.8, 137.9, 132.8, 128.7, 128.2, 121.9, 120.8, 119.1, 118.6, 117.1. **5b**: ^{1}H-NMR (400 MHz, DMSO-d_6): δ 8.09 (d, 2H, *J* = 8.8 Hz, H-2), 8.04 (d, 1H, *J* = 15.6 Hz, H-β), 7.79 (d, 1H, *J* = 15.6 Hz, H-α), 7.32 (d, 1H, *J* = 8.4 Hz, H-6'), 7.07 (d, 2H, *J* = 8.8 Hz, H-3), 6.85 (d, 1H, *J* = 8.4 Hz, H-4'), 6.71 (t, 1H, *J* = 8.4 Hz, H-5'), 3.85 (s, 3H, -OCH_3); ^{13}C-NMR (100 MHz, DMSO-d_6): δ 187.6, 162.9, 145.7, 145.6, 138.9, 130.7, 130.6, 122.1, 120.7, 119.1, 118.5, 116.9, 113.9, 55.5. **5c**: ^{1}H-NMR (400 MHz, DMSO-d_6): δ 8.05 (d, 1H, *J* = 15.6 Hz, H-β), 7.99 (d, 2H, *J* = 8.0 Hz, H-2), 7.77 (d, 1H, *J* = 15.6 Hz, H-α), 7.36 (d, 2H, *J* = 8.0 Hz, H-3), 7.32 (d, 1H, *J* = 8.4 Hz, H-6'), 6.86 (d, 1H, *J* = 8.4 Hz, H-4'), 6.71 (t, 1H, *J* = 8.4 Hz, H-5'), 2.38 (s, 3H, -CH_3); ^{13}C-NMR (100 MHz, DMSO-d_6): δ 188.9, 145.8, 145.6, 143.1, 139.4, 135.3, 129.2, 128.3, 122.0, 120.9, 119.1, 118.6, 117.0, 21.1. **5d**: ^{1}H-NMR (400 MHz, DMSO-d_6): δ 8.01 (d, 1H, *J* = 15.6

Hz, H-β), 7.99 (d, 2H, *J* = 8.4 Hz, H-2), 7.76 (d, 1H, *J* = 15.6 Hz, H-α), 7.29 (d, 1H, *J* = 7.6 Hz, H-6'), 6.89 (d, 2H, *J* = 8.4 Hz, H-3), 6.84 (d, 1H, *J* = 7.6 Hz, H-4'), 6.70 (t, 1H, *J* = 7.6 Hz, H-5'); ^{13}C-NMR (100 MHz, DMSO-d_6): δ 187.4, 161.8, 145.6, 138.4, 130.8, 129.4, 122.2, 120.8, 119.0, 118.5, 116.8, 115.2. **5e**: ^{1}H-NMR (400 MHz, DMSO-d_6): δ 9.63 (s, 1H, 2'C-OH), 9.13 (s, 1H, 3'C-OH), 8.04 (d, 2H, *J* = 8.0 Hz, H-2), 8.03 (d, 1H, *J* = 15.04 Hz, H-β), 7.72 (d, 1H, *J* = 15.0 Hz, H-α), 7.57 (d, 2H, *J* = 8.0 Hz, H-3), 7.28 (d, 1H, *J* = 7.6 Hz, H-6'), 6.81 (d, 1H, *J* = 7.6 Hz, H-4'), 6.65 (t, 1H, *J* = 7.6 Hz, H-5'); ^{13}C-NMR (100 MHz, DMSO-d_6): δ 188.3, 146.0, 145.6, 140.2, 137.7, 136.5, 130.2, 128.8, 121.8, 120.4, 119.1, 118.6, 117.2. **5f**: ^{1}H-NMR (400 MHz, DMSO-d_6): δ 10.99 (s, 1H, 2'C-OH), 9.96 (s, 1H, 4'C-OH), 8.04 (d, 2H, *J* = 7.2 Hz, H-2), 7.99 (d, 1H, *J* = 15.6 Hz, H-β), 7.68 (d, 1H, *J* = 8.8 Hz, H-6'), 7.64 (d, 1H, *J* = 15.6 Hz, H-α), 7.61 (t, 1H, *J* = 4.4 Hz, H-4), 7.55 (t, 2H, *J* = 8.0 Hz, H-3), 6.38 (d, 1H, *J* = 2.0 Hz, H-3'), 6.32 (d, 1H, *J* = 2.0 Hz, *J* = 8.8 Hz, H-5'); ^{13}C-(100 MHz, DMSO-d_6): δ 189.2, 161.4, 159.1, 144.0, 140.2, 138.4, 132.4, 130.3, 128.6, 128.0, 121.2, 117.1, 113.3, 107.9, 102.4. **5g**: ^{1}H-NMR (400 MHz, DMSO-d_6): δ 10.13 (s, 1H, 2'C-OH), 9.91 (s, 1H, 4'C-OH), 8.06 (d, 2H, *J* = 8.8 Hz, H-2), 7.95 (d, 1H, *J* = 15.6 Hz, H-β), 7.68 (d, 1H, *J* = 8.4 Hz, H-6'), 7.65 (d, 1H, *J* = 15.6 Hz, H-α), 7.06 (d, 2H, *J* = 8.8 Hz, H-3), 6.37 (d, 1H, *J* = 2.0 Hz, H-3'), 6.31 (dd, 1H, *J* = 2.0 Hz, *J* = 8.4 Hz, H-5'), 3.84 (s, 3H, -OCH_3); ^{13}C-NMR (100 MHz, DMSO-d_6): δ 187.5, 162.7, 161.2, 158.9, 139.2, 131.1, 130.4, 130.1, 117.0, 113.8, 113.4, 107.8, 102.4, 55.4. **5h**: ^{1}H-NMR (400 MHz, DMSO-d_6): δ 10.16 (s, 1H, 2'C-OH), 9.93 (s, 1H, 4'C-OH), 7.97 (d, 1H, *J* = 15.6 Hz, H-β), 7.59 (d, 2H, *J* = 8.4 Hz, H-2), 7.67 (d, 1H, *J* = 8.0 Hz, H-6'), 7.63 (d, 1H, *J* = 15.6 Hz, H-α), 7.34 (d, 2H, *J* = 8.4 Hz, H-3), 6.38 (d, 1H, *J* = 2.0 Hz, H-3'), 6.32 (dd, 1H, *J* = 2.0 Hz, *J* = 8.0 Hz, H-5'), 2.37 (s, 3H, -CH_3); ^{13}C-NMR (100 MHz, DMSO-d_6): δ 188.6, 161.3, 159.0, 142.7, 139.7, 135.8, 130.2, 129.1, 128.2, 117.1, 113.3, 107.9, 102.4, 59.7, 21.1. **5i**: ^{1}H-NMR (400 MHz, DMSO-d_6): δ 10.30 (s, 1H, 4C-OH), 10.10 (s, 1H, 2'C-OH), 9.90 (s, 1H, 4'C-OH), 7.96 (d, 2H, *J* = 8.4 Hz, H-2), 7.92 (d, 1H, *J* = 15.6 Hz, H-β), 7.65 (d, 1H, *J* = 8.8 Hz, H-6'), 7.62 (d, 1H, *J* = 15.6 Hz, H-α), 6.87 (d, 2H, *J* = 8.4 Hz, H-3), 6.36 (d, 1H, *J* = 2.0 Hz, H-3'), 6.31 (dd, 1H, *J* = 2.0 Hz, *J* = 8.8 Hz, H-5'); ^{13}C-NMR (100 MHz, DMSO-d_6): δ 187.4, 161.6, 161.0, 158.8, 138.9, 130.6, 130.1, 129.8, 117.1, 115.2, 113.5, 107.8, 102.5. **5j**: ^{1}H-NMR (400 MHz, DMSO-d_6): δ 10.22 (s, 1H, 2'C-OH), 9.99 (s, 1H, 4'C-OH), 8.07 (d, 2H, *J* = 8.4 Hz, H-2), 8.00 (d, 1H, *J* = 15.2 Hz, H-β), 7.70 (d, 1H, *J* = 8.4 Hz, H-6'), 7.63 (d, 1H, *J* = 15.2 Hz, H-α), 7.60 (d, 2H, *J* = 8.4 Hz, H-3), 6.37 (d, 1H, *J* = 2.0 Hz, H-3'), 6.32 (dd, 1H, *J* = 2.0 Hz, *J* = 8.4 Hz, H-5'); ^{13}C-NMR (100 MHz, DMSO-d_6): δ 188.0, 161.6, 159.2, 140.6, 137.3, 137.0, 130.0, 128.7, 116.6, 113.2, 108.0, 102.4. **5k**: ^{1}H-NMR (400 MHz, DMSO-d_6): δ 9.58 (s, 1H, 2'C-OH), 8.90 (s, 1H, 5'C-OH), 8.07 (d, 2H, *J* = 6.8 Hz, H-2), 7.98 (d, 1H, *J* = 15.6 Hz, H-β), 7.71 (d, 1H, *J* = 15.6 Hz, H-α), 7.57 (t, 2H, *J* = 7.6Hz, H-3), 7.51 (t, 1H, *J* = 7.6 Hz, H-4), 7.18 (s, 1H, H-4'), 6.75 (s, 2H, H-3',6'); ^{13}C-NMR (100 MHz, DMSO-d_6): δ 189.4, 150.2, 149.9, 139.7, 137.9, 132.9, 128.6, 128.2, 121.5, 120.6, 119.7, 116.9, 113.4. **5l**: ^{1}H-NMR (400 MHz, DMSO-d_6): δ 9.57 (s, 1H, 2'C-OH), 8.93 (s, 1H, 5'C-OH), 8.15 (d, 2H, *J* = 7.6 Hz, H-2), 8.02 (d, 1H, *J* = 14.8 Hz, H-β), 7.78 (d, 1H, *J* = 14.8 Hz, H-α), 7.23 (s, 1H, H-4'), 7.14 (d, 2H, *J* = 7.6 Hz, H-3), 6.80 (s, 2H, H-3',6'), 3.92 (s, 3H, -OCH_3); ^{13}C-NMR (100 MHz, DMSO-d_6): δ 187.6, 162.9, 150.1, 149.9, 138.7, 130.7, 130.5, 121.7, 120.5, 119.4, 116.8, 113.9, 113.3, 55.5. **5m**: ^{1}H-NMR (400 MHz, DMSO-d_6): δ 9.54 (s, 1H, 2'C-OH), 8.88 (s, 1H, 5'C-OH), 7.98 (d, 2H, *J* = 8.4 Hz, H-2), 7.97 (d, 1H, *J* = 14.8 Hz, H-β), 7.70 (d, 1H, *J* = 14.8 Hz, H-α), 7.36 (d, 2H, *J* = 8.4 Hz, H-3), 7.17 (s, 1H, H-4'), 6.74 (s, 2H, H-3',6'), 2.37 (s, 3H, -CH_3); ^{13}C-NMR (100 MHz, DMSO-d_6): δ 188.8, 150.2, 149.9, 143.1, 139.3, 135.4, 129.2, 128.3, 121.6, 120.6, 119.6, 116.9, 113.3, 21.1. **5n**: ^{1}H-NMR (400 MHz, DMSO-d_6): δ 10.33 (s, 1H, 4C-OH), 9.47 (s, 1H, 2'C-OH), 8.84 (s, 1H, 5'C-OH), 7.98 (d, 2H, *J* = 8.4 Hz, H-2), 7.92 (d, 1H, *J* = 15.6 Hz, H-β), 7.68 (d, 1H, *J* = 16.4 Hz, H-α), 7.14 (s, 1H, H-4'), 6.88 (d, 2H, *J* = 8.0 Hz, H-3), 6.72 (s, 2H, H-3',6'); ^{13}C-NMR (100 MHz, DMSO-d_6): δ 187.4, 161.8, 150.0, 149.9, 138.3, 130.8, 129.4, 121.8, 120.6, 119.2, 116.8, 115.2, 113.3. **5o**: ^{1}H-NMR (400 MHz, DMSO-d_6): δ 9.58 (s, 1H, 2'C-OH), 8.89 (s, 1H, 5'C-OH), 8.10 (d, 2H, *J* = 8.8 Hz, H-2'), 8.00 (d, 1H, *J* = 15.2 Hz, H-β), 7.71 (d, 1H, *J*=16.4 Hz, H-α), 7.62 (d, 2H, *J* = 7.6 Hz, H-3), 7.19 (s, 1H, H-4'), 6.74 (s, 2H, H-3',6'); ^{13}C-NMR (100 MHz, DMSO-d_6): δ 188.2, 150.3, 149.9, 140.1, 137.7, 136.5, 130.1, 128.8, 121.4, 120.2, 119.9, 116.9, 113.4. **5p**: ^{1}H-NMR (600 MHz, DMSO-d_6): δ 8.90 (d, t, 2H, *J* = 7.2 Hz, *J* = 1.2 Hz, H-2), 7.65 (t, t, 1H, *J* = 7.8 Hz, *J* = 1.2 Hz, H-4), 7.61 (d, 1H, *J* = 15.6, H-β), 7.59 (d, 1H, *J* =15.6, H-α), 7.56 (t, t, 2H, *J* = 1.8 Hz, 7.8 Hz, H-3), 7.24 (d, 1H, *J* = 2.4 Hz, H-1'), 7.18 (dd, 1H, *J* = 2.4 Hz, 7.8 Hz, H-6'), 6.80 (d, 1H, *J* = 7.8 Hz, H-5'); ^{13}C-NMR (150 MHz $CDCl_3$): δ 190.67 (-CO), 147.57, 145.47, 144.89, 138.55, 132.34, 128.40, 128.25, 127.20, 122.29, 119.16, 115.51, 114.60. **5q**: ^{1}H-NMR (600 MHz, DMSO-d_6): δ 8.11 (d, 2H, *J* = 8.4 Hz, H-2), 7.61 (d, 1H, *J* = 15Hz, H-β), 7.56 (d, 1H, *J* = 15 Hz, H-α), 7.23 (d, 1H, *J* = 1.2 Hz, H-2'), 7.16 (d, d, 1H, *J* = 1.2 Hz, 8.4 Hz, H-6'), 7.06 (d, 2H, *J* = 8.4 Hz, H-3), 6.80 (d, 1H, *J* = 8.4 Hz, H-5'), 3.84 (s, 3H, -OCH_3); ^{13}C-NMR (150 MHz, DMSO-d_6): δ 187.26 (-CO), 162.97, 148.57, 145.57, 144.07, 130.82, 130.60, 126.33, 121.96, 118.35, 115.71, 115.48, 113.91, 55.49 (-OCH_3). **5r**: ^{1}H-NMR (600 MHz, DMSO-d_6): δ 8.00 (d, 2H, *J* = 8.4 Hz, H-2), 7.60 (d, 1H, *J* = 15.6 Hz, H-β), 7.57 (d, 1H, *J* = 15.6 Hz, H-α), 7.35 (d, 2H, *J* = 7.8 Hz, H-3), 7.24 (d, 1H, *J* = 2.4 Hz, H-2'), 7.17 (d, d, 1H, *J* = 2.4 Hz, 8.4 Hz, H-6'), 6.80 (d, 1H, *J* = 8.4 Hz, H-5'), 2.38 (s, 3H, CH_3); ^{13}C-NMR (150 MHz $CDCl_3$): δ 188.42 (-CO), 145.58, 144.54, 143.07, 135.47, 129.25, 129.17, 128.40, 128.01, 122.07, 118.40, 115.72, 115.48, 21.12 (Ar-CH_3). **5s**: ^{1}H-NMR (600 MHz, DMSO-d_6): δ 8.00 (d, 2H, *J* = 8.4 Hz, H-2), 7.58 (d, 1H, *J* = 15.2 Hz, H-β), 7.52 (d, 1H, *J* = 15.2 Hz, H-α), 7.21 (d, 1H, *J* = 1.6 Hz, H-2'), 7.14 (d, d, 1H, *J* = 1.6 Hz, 8.0 Hz H-6'), 6.87 (d, 2H, *J* = 8.4 Hz, H-3), 6.79 (d, 1H, *J* = 8.4 Hz, H-5'); ^{13}C-NMR (150 MHz, DMSO-d_6): δ 186.98 (-CO), 161.77, 148.32, 145.49, 143.51, 130.78, 129.45, 126.40, 121.71,

118.41, 115.66, 115.33, 115.22. **5t**: ^{1}H-NMR (600 MHz, DMSO-d_6): δ 8.12 (d, 2H, *J* = 9 Hz, H-2), 7.61-7.59 (m, 4H, H-β, H-α, H-3), 7.26 (d, 1H, *J* = 1.8 Hz, H-2'), 7.19 (d, d, 1H, *J* = 1.8 Hz, 7.8 Hz, H-6'), 6.80 (d, 1H, *J* = 7.8 Hz, H-5'); ^{13}C-NMR (150 MHz, DMSO-d_6): δ 187.81 (-CO), 148.90, 145.59, 145.44, 137.65, 136.65, 130.20, 128.77, 126.15, 122.36, 118.04, 115.71, 115.62. **5u**: ^{1}H-NMR (400 MHz, DMSO-d_6): δ 9.48 (s, 2H, -OH), 8.11 (d, 2H, *J* = 8.0 Hz, H-2), 7.71 (d, 1H, *J* = 15.6 Hz, H-β), 7.67 (t, 1H, *J* = 7.2 Hz, H-4), 7.57 (t, 2H, *J* = 8.0 Hz, H-3), 7.54 (d, 1H, *J* = 15.6 Hz, H-α), 6.67 (d, 2H, *J* = 1.6 Hz, H-2',6'), 6.34 (t, 1H, *J* = 1.6 Hz, H-4'); ^{13}C-NMR (100 MHz, DMSO-d_6): δ 189.2, 158.6, 144.6, 137.6, 136.2, 133.0, 128.7, 128.4, 121.6, 106.8, 105.1, 59.7. **5v**: ^{1}H-NMR (400 MHz, DMSO-d_6): δ 9.54 (s, 2H, -OH), 8.22 (d, 2H, *J* = 8.8 Hz, H-2), 7.81 (d, 1H, *J* = 15.6 Hz, H-β), 7.60 (d, 1H, *J* = 15.6 Hz, H-α), 7.17 (d, 2H, *J* = 8.8 Hz, H-3), 6.76 (d, 2H, *J* = 2.0 Hz, H-2',6'), 6.44 (t, 1H, *J* = 2.0 Hz, H-4'), 3.95 (s, 3H, -OCH_3); ^{13}C-NMR (100 MHz, DMSO-d_6): δ 187.4, 163.1, 158.6, 143.7, 136.4, 130.8, 130.4, 121.6, 114.0, 106.7, 104.9, 55.5. **5w**: ^{1}H-NMR (400 MHz, DMSO-d_6): δ 9.46 (s, 2H, -OH), 8.02 (d, 2H, *J* = 8.4 Hz, H-2), 7.70 (d, 1H, *J* = 15.6 Hz, H-β), 7.51 (d, 1H, *J* = 15.6 Hz, H-α), 7.36 (d, 2H, *J* = 8.4 Hz, H-3), 6.66 (d, 2H, *J* = 2.0 Hz, H-2',6'), 6.34 (t, 1H, *J* = 2.0 Hz, H-4'), 2.38 (s, 3H, -CH_3); ^{13}C-NMR (100 MHz, DMSO-d_6): δ 188.6, 158.6, 144.2, 143.5, 136.3, 135.1, 129.3, 128.5, 121.6, 106.8, 105.0, 21.1. **5x**: ^{1}H-NMR (400 MHz, DMSO-d_6): δ 9.43 (s, 2H, 5-OH), 8.02 (d, 2H, *J* = 8.8 Hz, H-2), 7.67 (d, 1H, *J* = 15.6 Hz, H-β), 7.47 (d, 1H, *J* = 15.6 Hz, H-α), 6.88 (d, 2H, *J* = 8.8 Hz, H-3), 6.64 (d, 2H, *J* = 2.0 Hz, H-2',6'), 6.32 (t, 1H, *J* = 2.0 Hz, H-4'); ^{13}C-NMR (100 MHz, DMSO-d_6): δ 187.2, 162.1, 158.6, 143.3, 136.5, 131.0, 129.1, 121.7, 115.3, 106.6, 104.8. **5y**: ^{1}H-NMR (400 MHz, DMSO-d_6): δ 9.48 (s, 2H, -OH), 8.14 (d, 2H, *J* = 8.4 Hz, H-2), 7.71 (d, 1H, *J* = 15.6 Hz, H-β), 7.62 (d, 2H, *J* = 8.4 Hz, H-3), 7.55 (d, 1H, *J* = 15.6 Hz, H-α), 6.68 (d, 2H, *J* = 2.0 Hz, H-2',6'), 6.35 (t, 1H, *J* = 2.0 Hz, H-4'); ^{13}C-NMR (100 MHz, DMSO-d_6): δ 188.1, 158.6, 145.1, 138.0, 136.2, 130.3, 128.8, 121.3, 106.9, 105.2.

DPPH radical scavenging assay. The DPPH assay was based on the reported methods.[7] Briefly, The ethanolic sample solution of 100 μL at several concentrations was added to 100 μL of 100 μM of 1,1-diphenyl-2picryl-hydrazyl (DPPH) solution in ethanol in 96 well plates. The mixed solution was incubated at room temperature for 30 min. The absorbance of reaction mixture was read at 517 nm using a microplate reader (VERSA max, Molecular device, CA, U.S.A.) and the remaining DPPH was calculated. The free radical scavenging activity was expressed as follow:

$$\text{DPPH scavenging activity (\%)} = \left(\frac{Ac - As}{Ac - Ab}\right) \times 100 ,$$

where *Ac* was the absorbance of the control, As was the the sample and *Ab* was the blank (EtOH). Each sample was assayed at five concentrations (10, 20, 50, 100, and 200 μM) and four wells for each concentration. All experiments were carried out in triplicate. The IC_{50} values were defined as the concentration that could scavenge 50% DPPH free radical. Ascorbic acid and α-tocopherol were used as positive control.

Statistical analysis. Determination of all samples was carried out in triplicate for DPPA assays. All results were calculated as mean standard deviation (S.D.).

Results and Discussion

The chalcone derivatives were prepared through base-catalyzed Claisen-Schmidt condensation of MOM-protected benzaldehydes (**3**) with *para*-substituted acetophenones followed by acid-catalyzed hydrolysis (Scheme 1). In the reactions, the MOM-protected benzaldehydes were used instead of non-protected dihydroxylated ones, because the procedure with the free dihydroxylated benzaldehydes required long reaction time (more than one day) and relatively high temperature (above 60 ^{o}C) which resulted in poor yields with unknown degraded products. Among several other protecting groups including TBDMS, the MOM group was proven to be the best choice. The MOM-protected benzaldehydes which were readily prepared by treating the corresponding benzaldehydes with MOMCl in basic condition (K_2CO_3/acetone), were successfully converted to the chalcone derivatives **4** with diverse substitution patterns of two hydroxyl groups on benzaldehyde-origin ring B. Now the hydroxyl-protected chalcones **4** were deprotected in situ by acid hydrolysis to provide the desired final chalcone derivatives **5** in good yields (> 70%) with some exception (Table 1). The relatively low yields for some products containing *para*-hydroxyl acetophenone were considered probably due to the phenoxide ion formation from the acetophenone in the presence of strong and excess amount of base. The double bond geometry of all chalcones was determined as *E* from the characteristic coupling constants between α and β protons, 15-16 Hz.

With the synthetic chalcone compounds in hand, the antioxidant activity was measured by DPPH free radical scavenging assay as a primary tool to investigate systematic structure-activity relationship. The structures of the synthetic compounds and their inhibitory activity (IC_{50}) values are listed in Table 1. The statistically analyzed results of scavenging activity of DPPH radicals of each compound are shown in Figure 1. These results revealed that the DPPH radical scavenging activities of the compounds increase in concentration-dependent manner and reach a plateau at the concentration of 50 μM in most cases.

The most significant finding is that the remarkable differences in radical scavenging activities are exhibited depending on the substitution patterns of two hydroxyl groups on B ring of the chalcones. Three groups of chalcone compounds (2',3'-dihydroxyl: **5a-e**, 2,5'-dihydroxyl: **5k-o**, 3',4'-dihydroxyl: **5p-t**) show an excellent antioxidant activities (80-90% of control at the concentration of 50 μM) which are comparable to those of ascorbic acid and α-tocopherol as positive reference materials. On the contrary, two groups (2',4'-dihydroxyl: **5f-j**, 3',5'-dihydroxyl: **5u-y**) demonstrate very dramatic decrease in activities which are around 25% of the control even at the concentration of 200 μM (IC_{50}: > 200

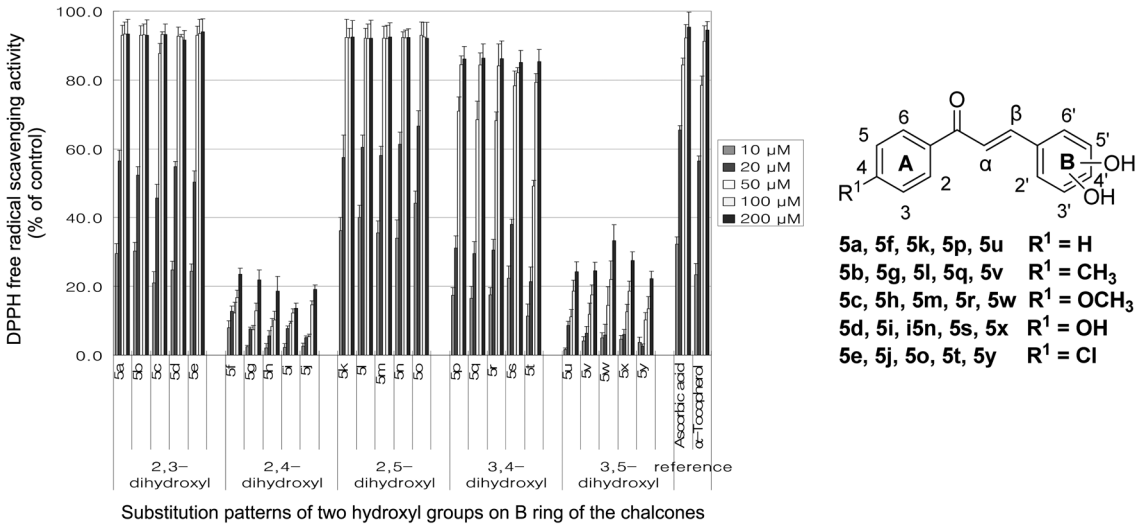


Figure 1. Rates of scavenging DPPH radicals of dihydroxylated chalcone compounds **5a-y**.

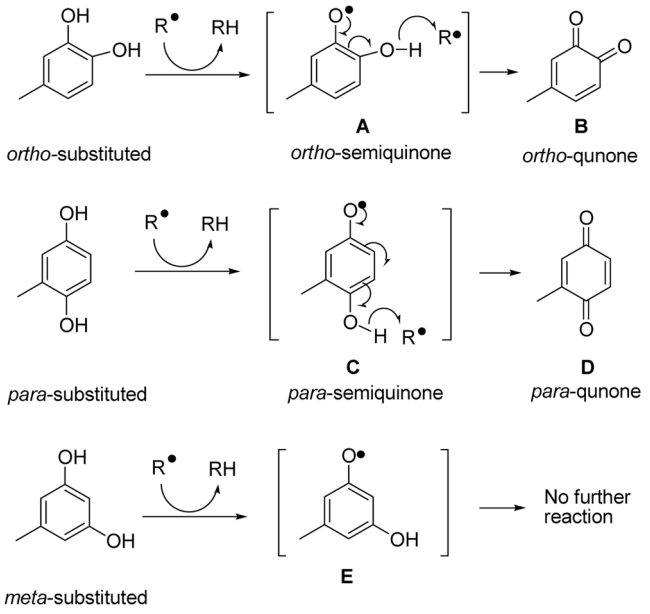


Figure 2. Proposed rationale for strong activity of *ortho*- and *para*-dihydroxylated chalcones *vs meta*- dihydroxylated ones.

μM). Namely, the *ortho*- (*i.e.* 2',3'- and 3',4'-) and *para*- (*i.e.* 2,5'-) substitution patterns show much stronger antioxidant potentials than *meta*- (*i.e.* 2',4'-, 3',5'-) substitution pattern. These dramatic differences could be interpreted in terms of the capability of the hydroxyl groups of chalcones to react with radicals and the subsequent formation of the stable chalcone adducts (Figure 2). When the chalcone molecules react with the radicals, they are readily converted to the phenoxy radicals due to the high reactivity of phenolic hydroxyl groups of chalcones.[4,8] Since the *ortho*- (*i.e.* catechol structure) and *para*-dihydroxylated benzene ring system are generally known to be very efficient systems to delocalize electrons, but not for *meta*-dihydroxylated system, the phenoxy radicals occurring at the *ortho*- (*i.e.* catechol structure) or *para*-dihydroxylated benzene ring system are much more readily converted to a fairly stable semiquinone radicals (compound A and C of Figure 2) and

Table 1. Structures, yields, melting points (mp) and IC_{50} values of synthetic chalcones **5**

Compound	R^1 on ring A	Substituents on ring B	Yield (%)[a]	mp (°C)	IC_{50}[b] (μg/mL)
5a	H	2,3-diOH	77	125	23
5b	CH_3	2,3-diOH	86	140	14
5c	OCH_3	2,3-diOH	86	145	30
5d	OH	2,3-diOH	38	173	26
5e	Cl	2,3-diOH	58	123	27
5f	H	2,4-diOH	71	104	>200
5g	CH_3	2,4-diOH	22	119	>200
5h	OCH_3	2,4-diOH	58	120	>200
5i	OH	2,4-diOH	25	95	>200
5j	Cl	2,4-diOH	35	107	>200
5k	H	2,5-diOH	81	135	19
5l	CH_3	2,5-diOH	64	145	16
5m	OCH_3	2,5-diOH	82	142	20
5n	OH	2,5-diOH	53	148	19
5o	Cl	2,5-diOH	89	128	11
5p	H	3,4-diOH	83	198-200	38
5q	CH_3	3,4-diOH	91	198	40
5r	OCH_3	3,4-diOH	79	184-186	39
5s	OH	3,4-diOH	59	201	34
5t	Cl	3,4-diOH	58	208-210	68
5u	H	3,5-diOH	83	151	>200
5v	CH_3	3,5-diOH	85	172	>200
5w	OCH_3	3,5-diOH	56	144	>200
5x	OH	3,5-diOH	56[c]	175	>200
5y	Cl	3,5-diOH	52	150	>200
Ascobic acid[d]					21
α-tocopherol[d]					29

[a]Isolated yields. [b]The IC_{50} values were the 50% inhibition concentration and were calculated from regression lines using three different concentrations in triplicate experiments. [c]4-MOM-acetophenone was used. [d]The compounds were used as positive control.

subsequent quinone structures (compound B and D of Figure 2) than *meta*- system (compound E of Figure 2). The central role of catechol structure in the enhancement of antioxidant activity has been reported for other classes of polyphenolic antioxidants.[9] A precedent study reported that a 3',4'-catechol structure in the B ring of flavonoid strongly enhances the antioxidant activity such as lipid peroxidation inhibition.[10] Among three active groups mentioned above, the *para*-substituted group exhibited better free radical scavenging activities than *ortho*- substituted system. The IC_{50} values of 2,5'-dihydroxyl chalcones (*para*-disubstituted) are stronger about three times to those of 3',4'-dihydroxyl chalcones (*ortho*-disubstituted) (Table 1). Another observation to be worth to mention here is that the variation of the substituents at *para*-position of ring A make no distinctive differences in activities. This observation indicates that the electronic effects of *para*-substituents of the phenyl ring A do not affect the radical scavenging activity, and therefore are unlikely to contribute to variation of antioxidant activities.

In summary, we carefully designed and prepared a series of dihydroxylated chalcone derivatives with diverse substitution patterns on a phenyl ring B and the *para*-substituents on a ring A to investigate QSAR of chalcone derivatives in more systematic manner. Their evaluation of antioxidant activities was measured by standard DPPH free radical scavenging activity assay, and the structure-activity relationships were analyzed. Our SAR analysis clearly shows that the *ortho*- and *para*-substituted chalcones show much stronger antioxidant potentials than *meta*-substituted chalcones, indicating the substitution patterns of two hydroxyl groups are very important structural factors for their radical scavenging activity enhancement. Meanwhile, the substituents at *para*-position of the ring A of chalcones have no influence on the activity.

Acknowledgements. This work was supported from Korean Research Foundation Grant (KRF-2004-005-F00015) endowed to Research Center of Bioactive Materials (RCBM) of Chonbuk National University in 2007.

References

1. (a) Dimmock, J. R.; Elias, D. W.; Beazely, M. A.; Kandepu, N. M. *Curr. Med. Chem.* **1999**, *6*, 1125. (b) Go, M. L.; Wu, X.; Liu, X. L. *Curr. Med. Chem.* **2005**, *12*, 483. (c) Nowakowska, Z. *Eur. J. Med. Chem.* **2007**, *42*, 125.
2. (a) Sogawa, S.; Nihro, Y.; Ueda, H.; Miki, T.; Matsumoto, H.; Satoh, T. *Biol. Pharm. Bull.* **1994**, *17*, 251. (b) Nerya, O.; Musa, R.; Khatib, S.; Tamir, S.; Vaya, J. *Phytochemistry* **2004**, *65*, 1389.
3. (a) Anto, R. J.; Sukumaran, K.; Kuttan, G.; Rao, M. N. A.; Subbaraju, V.; Kuttan, R. *Cancer Lett.* **1995**, *97*, 33. (b) Calliste, C. A.; Le Bail, J. C.; Trouillas, P.; Pouget, C.; Habrioux, G.; Chulia, A. J.; Duroux, J. L. *Anticancer Res.* **2001**, *21*, 3949. (c) Tapiero, H.; Tew, K. D.; Ba, N.; Mathé, G. *Biomed. Pharm. Ther.* **2002**, *56*, 200.
4. Rezk, B. M.; Haenen, G. R. M. M.; van der Vijgh, W. F. F.; Bast, A. *Biochem. Biophys. Res. Commun.* **2002**, *295*, 9.
5. De Vincenzo, R.; Ferlini, C.; Distefano, M.; Gaggini, C.; Riva, A.; Bombardelli, E.; Morazzoni, P.; Valenti, P.; Belluti, F.; Ranelletti, F. O.; Mancuso, S.; Scambia, G. *Cancer Chemotheraphy and Pharmacology* **2000**, *46*, 305.
6. (a) Cai, Y.-Z.; Sun, M.; Xing, J.; Luo, Q.; Corke, H. *Life Sciences* **2006**, *78*, 2872. (b) Liu, M.; Wilairat, P.; Go, M.-L. *J. Med. Chem.* **2001**, *44*, 4443.
7. Ko, F. N.; Liao, C. H.; Kuo, Y. H.; Lin, Y. L. *Biochim. Biophys. Acta* **1995**, *1258*, 145.
8. (a) Cao, G.; Sofic, E.; Prior, R. L. *Free Radic. Biol. Med.* **1997**, *22*, 749. (b) Burda, S.; Oleszek, W. *J. Agric. Food Chem.* **2001**, *49*, 2774.
9. (a) Arora, A.; Nair, M. G.; Strasburg, G. M. *Free Radic. Biol. Med.* **1998**, *24*, 1355. (b) Pietta, P.-G. *J. Nat. Prod.* **2000**, *63*, 1035.
10. Dugas Jr., A. J.; Castaneda-Acosta, J.; Bonin, G. C.; Price, K. L.; Fischer, N. H.; Winston, G. W. *J. Nat. Prod.* **2000**, *63*, 327.