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RSC Advances, Regular article

Substituent Effect on the Radical Scavenging Activity of 6-Chromanol Derivatives

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Abstract

Several 6-chromanol derivatives with various substituents (one or two amino, acetylamino, chloro or nitro substituents at the 5-, 7-, 8- or 5,7- positions on the phenyl ring of 2,2-dimethyl-6-chromanol) were synthesized, and their second order rate constants (k) for a reaction that demonstrates radical scavenging activity (reaction with the galvinoxyl radical) were determined. Three monoacetylamino compounds, 8-nitro compound, and 5,7-diamino, 5,7-diacetylamino, and 5,7-dinitro compounds were newly synthesized. Log k was plotted against the Hammett sigma (σ_m) or Taft sigma (σ^*) constants for the compounds containing each of the four substituents to obtain their reaction constants (ρ) from the slopes. The σ plots representing radical scavenging activity showed a linear correlation with negative ρ values for all compounds with substituted positions. The results indicate that the electron-donating effect of the amino and acetylamino groups on the chroman ring enhanced radical scavenging activity, whereas the electron-withdrawing effect of the chloro and nitro groups decreased this activity. Furthermore, the magnitude of ρ for the substituted compounds increased in the following order with respect to the substitution position: *meta*-substituted (-3.71 for **6a-d**), *ortho*-monosubstituted (-0.86 for **4a-d**, -0.87 for **5a-d**), and *ortho*-disubstituted (-0.47 for **7a-d**). The greater ρ magnitudes for the *meta*-substituted compound indicated that the radical scavenging reactions were more sensitive to inductive substituent effects than for the *ortho*-substituent compounds. The ρ values for *ortho*-mono- and *ortho*-disubstituted compounds were smaller than that for the

meta-substituted compound, despite the fact that the k values for the *ortho*-substituted compounds were higher than those for the *meta*-substituted compounds. Thus, electron-donating groups in *ortho*-substituted 6-chromanols accelerate the reaction rate through resonance stabilization in addition to the inductive substituent effect.

Introduction

α -Tocopherol (**1**) is involved in immune function, cell signaling, gene expression regulation, and other metabolic processes.^{1,2} In addition to its physiological activities, α -tocopherol is an antioxidant and protects cells from damage caused by reactive oxygen species. Thus, α -tocopherol is thought to serve in preventing oxidative damage, such as that in cancer and ischemia/reperfusion tissue damage.^{3,4} The antioxidant activities of **1** are attributed to the radical scavenging function of their phenolic hydroxyl groups.⁵⁻⁷ A large number of tocopherol derivatives have been synthesized and assayed for their antioxidant and physiological activities.⁸⁻¹¹

The chemical properties, including the antioxidant activity, of phenols, have been widely studied and correlated with antioxidant activity that has radical scavenging activity,¹²⁻¹⁴ O-H bond dissociation enthalpy (BDE),¹⁴⁻¹⁷ and ionization enthalpy¹⁸. Despite the large number of reports focused on the effect of substituents on the relationship between radical scavenging activity and calculated BDE in simple phenols,¹⁹⁻²⁶ few reports have examined the effect of substituents in the chroman ring on radical scavenging activity²⁴⁻²⁷. The aim of this study was to understand the structure-activity relationships of 6-chromanol derivatives in radical scavenging reactions. A series of 6-chromanol derivatives with substituents *ortho* or *meta* to the phenolic group was synthesized. The radical scavenging activities of these derivatives were then investigated based on the rate constants (*k*) of their reactions with the galvinoxyl radical (G \cdot), and then compared with substituent sensitivity

for each positions, which obtained from the correlation of $\log k$ and Hammett sigma (σ_m) or Taft sigma (σ^*) constants.

Experimental methods

Material and Methods

2-Methyl-3-buten-2-ol was obtained from Tokyo Kasei Kogyo Co., Ltd. (Tokyo, Japan). The galvinoxyl radical was purchased from Kanto Chemical Co., Inc. (Tokyo, Japan). Acetonitrile, which was used for spectral measurements, was obtained from Dojindo Laboratories (Kumamoto, Japan). Other reagents were purchased from Wako Pure Chemical Industries (Osaka, Japan). All of the reagents and solvents used were of the best commercially available quality and were not further purified unless otherwise noted.

Reaction progression was monitored using thin-layer chromatography (TLC) on silica gel 60 F₂₅₄ (0.25 mm, Merck, Darmstadt, Germany). Column chromatography was performed using silica gel 60 (230–400 mesh, Merck). Melting points were determined using a Yanaco micro-melting point apparatus without correction. NMR spectra were recorded with a JEOL JNM-LA400 spectrometer (Tokyo, Japan). Chemical shifts were expressed in terms of ppm downfield shifted from TMS. High-resolution mass spectra were collected using a JEOL JMS-SX102A mass spectrometer. FT-IR spectra were recorded with a Perkin Elmer Spectrum 100 (Waltham, USA). UV-Vis spectrophotometry was obtained using a Hewlett-Packard 8453 photodiode array spectrophotometer (Hanover, USA) equipped with an Applied Photophysics RX 2000 stopped-flow device (Leatherhead, UK).

2,2-Dimethyl-6-chromanol (**3**) was synthesized using the method described by Hansen et al. (mp: 75.3–76.0°C).²⁸ Amino (**4a**, **5a**, **6a**), chloro (**4c**, **5c**, **6c**, **7c**), and nitro (**4d**, **5d**) 6-chromanols and

6-acetoxy-2,2-dimethyl-8-nitrochroman (mp: 56.5–57.0°C) and 6-acetoxy-8-amino-2,2-dimethylchroman (mp: 86.5–87.5°C) were synthesized as reported previously.^{29,30}

Syntheses of 6-acetoxyacetylaminochromans (**4b-OAc**, **5b-OAc**)

4d or **5d** was dissolved in methanol, followed by the addition of an excess of acetic anhydride and palladium/carbon (5%). The reaction mixture was stirred overnight at room temperature under a hydrogen atmosphere to produce the following two distinct spots via TLC: acetyl amino (**4b**, **5b**) and acetylated acetyl amino compounds (**4b-OAc**, **5b-OAc**). The reaction mixture was filtered through celite, and the filter cake was washed with CH₂Cl₂. Water was added to the filtrate, and the organic layer was extracted twice with CH₂Cl₂. The combined organic layer was washed with saturated NaHCO₃ and water and dried over anhydrous sodium sulfate, filtered, and then the solvent was evaporated under reduced pressure. The crude product was dissolved in pyridine, followed by the addition of an excess of acetic anhydride. The reaction mixture was stirred at room temperature for 2 h to produce a single spot by TLC. The reaction mixture was poured onto crushed ice and extracted twice with CH₂Cl₂. The combined organic layer was washed with saturated NaHCO₃, water, 1 M HCl, and water, then dried over anhydrous sodium sulfate, filtered, and the solvent evaporated under reduced pressure. The crude product was purified on a silica gel column to afford the single desired product.

4b-OAc; white needle-like crystals (recrystallized from ether and hexane); yield 40%; mp 121.0–123.0 °C; ¹H NMR (400 MHz, CDCl₃) δ 6.85 (d, *J* = 9.0

Hz, 1H), 6.75 (d, $J=9.0$ Hz, 1H), 6.66 (br, 1H), 2.64 (t, $J=6.8$ Hz, 2H), 2.29 (s, 3H), 2.15 (s, 3H), 1.76 (t, $J=6.8$ Hz, 2H), 1.33 (s, 6H); ^{13}C NMR (100 MHz, CDCl_3) δ 169.8, 168.2, 152.4, 139.1, 127.3, 120.6, 120.3, 116.9, 74.2, 32.0, 26.7, 23.3, 20.9, 19.5; IR (cm^{-1} , neat) 3266, 2932, 1766, 1660; HRMS (FAB) 278.1394 (calcd. for $\text{C}_{15}\text{H}_{19}\text{NO}_4$ 277.1314).

5b-OAc; white needle-like crystals (recrystallized from ether and hexane); yield 27%; mp 147.5–150.0 °C; ^1H NMR (400 MHz, CDCl_3) δ 7.51 (s, 1H), 6.99 (br, 1H), 6.80 (s, 1H), 2.71 (t, $J=6.6$ Hz, 2H), 2.32 (s, 3H), 2.14 (s, 3H), 1.77 (t, $J=6.6$ Hz, 2H), 1.31 (s, 6H); ^{13}C NMR (100 MHz, CDCl_3) δ 169.3, 167.8, 151.9, 133.9, 128.3, 121.9, 117.4, 111.7, 74.4, 32.5, 26.9, 24.5, 22.2, 21.0; IR (cm^{-1} , neat) 3315, 2976, 1752, 1669; HRMS (FAB) 278.1392 (calcd. for $\text{C}_{15}\text{H}_{19}\text{NO}_4$ 277.1314).

Synthesis of 6-acetoxy-8-acetylaminochroman (6b-OAc)

6-Acetoxy-8-amino-2,2-dimethylchroman (39.7 mg, 0.17 mmol) was dissolved in pyridine (0.5 mL), followed by the addition of an excess of acetic anhydride (1 mL). The reaction mixture was stirred at room temperature for 3 h. The reaction mixture was then poured onto crushed ice, and the precipitate was filtered via suction to afford the desired product.

6b-OAc; white needle-like crystals (recrystallized from ethanol and H_2O); yield 40%; mp 186.0–187.5 °C; ^1H NMR (400 MHz, CDCl_3) δ 8.00 (d, $J=2.7$ Hz, 1H) 7.72 (br, 1H), 6.54 (d, $J=2.9$ Hz, 1H), 2.75 (t, $J=6.8$ Hz, 2H), 2.26 (s, 3H), 2.19 (s, 3H), 1.81 (t, $J=6.8$ Hz, 2H), 1.36 (s, 6H); ^{13}C NMR (100 MHz, CDCl_3) δ 170.2, 168.0, 143.0, 139.6, 127.8, 120.5, 116.2, 111.0, 75.7, 32.6, 27.0,

25.0, 22.4, 21.1; IR (cm⁻¹, neat) 3339, 1744, 1675; HRMS (FAB) 278.1394 (calcd. for C₁₅H₁₉NO₄ 277.1314).

Syntheses of acetylamino-6-chromanols via acetylated compound hydrolyses

The purified acetylated compounds were dissolved in methanol and treated with 1 M sodium methoxide. After the disappearance of the starting material disappeared was observed by TLC, the reaction mixture was neutralized with 0.5 M HCl and extracted twice with CH₂Cl₂. The combined organic layer was washed with water, dried over anhydrous sodium sulfate and filtered. Following concentration under reduced pressure, the product was purified on a short silica gel column, even when a single compound was obtained.

4b; tan yellow solid; yield 96%; mp 184.0–186.0 °C (decomposition); ¹H NMR (400 MHz, CDCl₃) δ 7.51 (s, 1H), 7.12 (br, 1H), 6.85 (d, *J* = 9.0 Hz, 1H), 6.67 (d, *J* = 8.8 Hz, 1H), 2.59 (t, *J* = 6.8 Hz, 2H), 2.28 (s, 3H), 1.81 (t, *J* = 6.8 Hz, 2H), 1.30 (s, 6H); ¹³C NMR (100 MHz, CDCl₃) δ 170.3, 147.8, 143.2, 123.5, 119.1, 116.8, 113.3, 73.1, 32.1, 26.4, 23.6, 19.4; IR (cm⁻¹, neat) 3267, 2974, 2929, 1639; HRMS (FAB) 236.1209 (calcd. for C₁₃H₁₇NO₃ 235.1208).

5b; tan yellow solid; yield 97%; mp 205.0–206.5 °C (decomposition); ¹H NMR (400 MHz, CDCl₃) δ 8.02 (s, 1H), 7.48 (br, 1H), 6.72 (s, 1H), 6.41 (s, 1H), 2.71 (t, *J* = 6.3 Hz, 2H), 2.23 (s, 3H), 1.76 (t, *J* = 6.8 Hz, 2H), 1.29 (s, 6H); ¹³C NMR (100 MHz, CDCl₃) δ 170.3, 147.5, 141.8, 124.4, 120.2, 119.8, 110.0, 74.1, 32.7, 26.7, 23.7, 22.1; IR (cm⁻¹, neat) 3415, 2970, 2929, 1655, 1608; HRMS (FAB) 235.1287 (calcd. for C₁₃H₁₇NO₃ 235.1208).

6b; tan yellow solid; yield 99%; mp 193.0–194.5 °C (decomposition); ¹H NMR

(400 MHz, CDCl₃) δ 8.62 (s, 1H), 8.24 (d, J = 2.9 Hz, 1H), 7.87 (br, 1H), 6.37 (d, J = 2.9 Hz, 1H), 2.72 (t, J = 6.7 Hz, 2H), 2.24 (s, 3H), 1.78 (t, J = 6.7 Hz, 2H), 1.33 (s, 6H); ¹³C NMR (100 MHz, CDCl₃) δ 169.2, 150.0, 135.2, 126.8, 121.0, 110.1, 105.5, 74.9, 32.9, 26.8, 24.9, 22.4; IR (cm⁻¹, neat) 3560, 2978, 2933, 1637; HRMS (FAB) 235.1203 (calcd. for C₁₃H₁₇NO₃ 235.1208).

Synthesis of 2,2-dimethyl-8-nitro-6-chromanol (6d)

6-Acetoxy-2,2-dimethyl-8-nitrochroman (27 mg, 0.1 mmol) was dissolved in methanol (1 mL). Sodium methoxide (0.2 mL) was then added to the solution, and this mixture was stirred for 0.5 h at room temperature. The reaction mixture was acidified with 1 M HCl and extracted three times with CH₂Cl₂. The combined organic phase was washed with 5% NaHCO₃ and H₂O, dried over Na₂SO₄, filtered, and evaporated to give an orange oil. The crude product was purified using silica gel column chromatography (silica gel 60, 4% methanol-chloroform) to afford a yellow solid.

6d; yellow solid; yield 101%; mp 88.0–91.0 °C; ¹H NMR (400 MHz, CDCl₃) δ 7.17 (d, J = 3.2 Hz, 1H), 6.83 (d, J = 3.2 Hz, 1H), 2.80 (t, J = 6.8 Hz, 2H), 1.85 (t, J = 6.8 Hz, 2H), 1.36 (s, 6 H); ¹³C NMR (100 MHz, CDCl₃) δ 147.1, 142.5, 139.0, 125.5, 121.3, 110.1, 76.1, 32.0, 26.6, 22.7; IR (cm⁻¹, neat) 3539, 3413, 2975, 1524, 1358; HRMS (EI) 223.0842 (calcd. for C₁₁H₁₃NO₄ 223.0845).

Synthesis of 2,2-dimethyl-5,7-dinitro-6-chromanol (7d)

2,2-Dimethyl-6-chromanol (592 mg, 3.33 mmol) was dissolved in acetic acid (40 mL) and cooled to below 15 °C while stirring. Nitric acid (422 μ L, 2 eq.)

was then added to the solution, and the mixture was stirred below 15 °C for 10 min. The reaction mixture was poured onto crushed ice and extracted three times with CH₂Cl₂. The combined organic layer was extracted three times with 10% Na₂CO₃. The aqueous layer was acidified to pH 3 upon concentrated HCl addition, at which time, a precipitate appeared. The solid was filtered via suction to afford a yellow solid.

7d; orange prism crystals (recrystallized from carbon tetrachloride); yield 46%; mp 168.0–168.1 °C; ¹H NMR (400 MHz, CDCl₃) δ 10.3 (s, 1H), 7.69 (s, 1H), 2.80 (t, *J* = 6.8 Hz, 2H), 1.86 (t, *J* = 6.8 Hz, 2H), 1.36 (s, 6H); ¹³C NMR (100 MHz, CDCl₃) δ 146.6, 141.0, 140.3, 132.9, 125.2, 114.0, 75.7, 30.8, 26.5, 19.3; IR (cm⁻¹, neat) 3123, 2980, 1530, 1369; HRMS (EI) 268.0695 (calcd. for C₁₁H₁₂N₂O₆ 268.0695).

Synthesis of 5,7-diamino-2,2-dimethyl-6-chromanol (**7a**)

7d (20 mg, 0.07 mmol) was first dissolved in methanol (5 mL), and 5% palladium/carbon (19 mg) was then added to the solution. The reaction mixture was stirred under hydrogen gas for 30 min at room temperature. The reaction mixture was filtered through celite, and the filter cake was washed with deaerated CH₂Cl₂ under nitrogen gas. The filtrate was blown dry with argon to afford a red solid.

7a; red solid; yield 97%; ¹H NMR (400 MHz, CDCl₃) δ 7.36 (s), 2.55 (br), 2.32 (br), 2.19 (br), 1.81 (br), 1.37 (br), 1.30 (s); HRMS (FAB) 209.1288 (calcd for C₁₁H₁₆N₂O₂ 208.1212). ¹³C NMR data could not be obtained owing to the compound's high instability in air.

Synthesis of 5,7-diacetylamino-2,2-dimethyl-6-chromanol (7b)

7d (55 mg, 0.21 mmol) was dissolved in methanol (10 mL), followed by the addition of an excess of acetic anhydride (4.2 mL) and 5% palladium/carbon (26 mg). The reaction mixture was stirred under a hydrogen atmosphere at room temperature for 9.5 h. The reaction mixture was filtered through celite, and the filter cake was washed with CH₂Cl₂. The filtrate was acidified to pH 3 upon 1 M HCl addition and extracted three times with CH₂Cl₂. The combined organic layer was washed with water, dried over anhydrous sodium sulfate, filtered, and the solvent was evaporated under reduced pressure. The residues were termed **7b** and acetoxyated **7b** (**7b-OAc**). The mixture was then dissolved in methanol (2 mL), and 0.5 M NaOMe (0.2 mL) was added. The mixture was stirred for 10 min at room temperature under an argon atmosphere. The mixture was neutralized by the addition of 1 M HCl and extracted then three times with CH₂Cl₂. The combined organic layer was washed with water, dried over anhydrous sodium sulfate, filtered, and the solvent was evaporated under reduced pressure. The crude product was purified on a silica gel column (ethyl acetate:CHCl₃ = 1:1) to afford the desired single product.

7b-OAc; ¹H-NMR (400 MHz, CDCl₃) δ 6.79 (s, 1H), 2.56 (t, 2H, *J* = 6.1 Hz), 2.30 (s, 3H), 2.14 (s, 6H), 1.75 (t, 2H, *J* = 6.7 Hz), 1.31 (s, 6 H).

7b; white prism crystals (recrystallized from ethanol); yield 64 %; mp 224.0–224.2 °C; ¹H NMR (400 MHz, CDCl₃) δ 8.20 (s, 1H), 8.03 (br, 1H), 7.48 (s, 1 H), 2.55 (t, *J* = 6.7 Hz, 2H), 2.29 (s, 3H), 2.19 (s, 3H), 1.77 (t, *J* = 6.7 Hz,

2H), 1.27 (s, 6H); ^{13}C NMR (100 MHz, CDCl_3) δ 170.6, 168.8, 147.5, 133.2, 127.9, 123.3, 109.2, 107.9, 73.3, 32.1, 26.4, 24.6, 23.5, 19.0; IR (cm^{-1} , neat) 3259, 2974, 2929, 1639; HRMS (FAB) 293.1501 (calcd. for $\text{C}_{15}\text{H}_{20}\text{N}_2\text{O}_4$ 292.1423).

Kinetics measurements

The kinetics were determined by measuring the disappearance of absorbance at 428 nm under pseudo-first-order conditions at 25 °C.³¹ Substituted 6-chromanols (with the following final concentrations: 0–100 μM for compounds **4a**, **5a**, and **7a**; 0–300 μM for compounds **4b**, **4c**, **5b**, **6a**, **6b**, **7b**, and **7c**; 0–600 μM for compounds **4d**, **5d**, **6d**, and **7d**; and 0–1000 μM for compounds **5c** and **6c**) and G^\cdot (final concentration of 5.0 μM) were dissolved in argon deaerated with acetonitrile. The two syringes were loaded with 2 mL of each compound and G^\cdot solution. The pneumatic drive accessory initiated mixing after the initiation of data acquisition by the spectrophotometer at 300–600 nm and 0.5 s intervals. Half of the starting concentration of G^\cdot solution and antioxidant was used after mixing.

The radical scavenging rates were determined by monitoring the changes in absorbance due to the galvinoxyl radical at 428 nm. Pseudo-first-order rate plots of $\ln(A-A_\infty)$ versus time, where A and A_∞ refer to the absorbance at a given time and the final absorbance, respectively, were linear until three or more half-lives. The A_∞ used was the absorbance 10 min after each reaction started. To avoid the influence of minor absorption from the G^\cdot reduction products at this wavelength, only the first G^\cdot absorption decay values were

used in kinetics analyses. Pseudo-first-order rate constants were determined using the least-squares method. The observed pseudo-first-order rate constant (k_{obs}) was compound concentration-dependent. The second-order rate constants (k) for the reactions between the compounds and G^{\cdot} were obtained from the slopes of the linear functions of k_{obs} versus various compound concentrations under pseudo-first order reaction conditions. The data were collected in at least three different experimental sessions.

Results

Chemistry

The lead compound optimized in this manuscript is 2,2-dimethyl-6-chromanol (**3**). Compounds **1** and **2** are considered to be derivatives of **3**. The substituents were chosen based on their ability to donate electrons (NH₂ and NHCOCH₃) or accept electrons (Cl and NO₂). Compounds **4a–d** and **5a–d** possess a substituent at position 5 or 7, respectively, which are *ortho* to the phenolic OH group. Compounds **6a–d** have a substituent at position **8**, which is *meta* to the phenolic OH group. Compounds **7a–d** have two substituted groups, both in *ortho* positions (Figure 1).

(Figure 1)

Three monoacetylamino compounds (**4b**, **5b**, **6b**), a nitro compound (**6d**), and 5,7-diamino, 5,7-diacetylamino, and 5,7-dinitro compounds (**7a**, **7b**, **7d**) were newly synthesized.

The nitro compounds were prepared by nitration, reduced by hydrogen gas in the presence of palladium/carbon to the corresponding amino compound. The acetylamino compounds were prepared using simple catalytic reduction of the corresponding nitro compounds (**4d**, **5d**, **6d**, **7d**) in the presence of acetic anhydride to obtain the acetoxy acetylaminochromans for storage. The

acetoxy compounds were hydrolyzed with alkaline reagents immediately before use (Scheme 1–3).

(Scheme 1)

(Scheme 2)

(Scheme 3)

Galvinoxyl radical scavenging activity of the substituted 6-chromanols

The ability of a series of 6-chromanols to scavenge $G\cdot$ was evaluated in acetonitrile. $G\cdot$ is a reactive oxygen species with a strong absorption band at 428 nm.³¹ Upon addition of a compound to deaerated $G\cdot$ solutions in acetonitrile, the absorption band at 428 nm rapidly decreased. The absorbance decay at 428 nm obeyed pseudo-first-order reaction kinetics when the compound concentration was at more than a 10-fold excess compared to the $G\cdot$ concentration. The observed pseudo-first-order rate constant (k_{obs}) was compound concentration-dependent in a linear manner. Second-order rate constants (k) for the reactions between 6-chromanols and $G\cdot$ were obtained from the slopes of the linear functions of k_{obs} versus compound concentration (Table 1).

(Table 1)

The aromatic ring substituent effects on G^{\cdot} -scavenging activity showed that the amino 6-chromanols exhibited the highest radical scavenging activity, followed by acetylamino, chloro, and finally nitro 6-chromanols at any of the substituent positions. In terms of substituent position, the *ortho*-disubstituted or *ortho*-monosubstituted compounds demonstrated higher radical scavenging activities, whereas *meta*-substituted compounds were less efficient.

The data also showed that the electron-donating substituents (NH_2 and $NHCOCH_3$) led to an accelerated G^{\cdot} -scavenging reaction rate, whereas the electron-withdrawing substituents (Cl and NO_2) led to a decreased reaction rate.

Correlation of radical scavenging activity with Hammett and Taft substituent constants for 6-chromanol derivatives

The Hammett sigma constant is one of the most widely used means for studying and interpreting organic reactions and their mechanisms. The Hammett sigma constant (σ_m) for the position *meta* to the phenolic OH group, which has been shown to interact with radicals, was used.³²⁻³⁴ The Taft sigma constant (σ^*) describes the electronic effect, which includes a substituent's steric effect. In this case, the σ^* for substituents in positions *ortho* to the phenolic OH group were applied.³⁴

To identify the electronic effects of all of the substituents in a given compound in each reaction with the series of 6-chromanols and G^{\cdot} , the sum

of the sigma constants was plotted against $\log k$ for the radical scavenging activity at each substituent position. The reaction constants (ρ) were then obtained from the slope values. For *meta*-substituted 6-chromanols, a linear relationship between $\log k$ and σ was observed ($\rho_{meta} = -3.71$, $r^2 = 0.91$). The *ortho*-monosubstituted 6-chromanols ($\rho_{5-ortho} = -0.86$, $r^2 = 0.89$; $\rho_{7-ortho} = -0.87$, $r^2 = 0.89$) and the 5,7-disubstituted 6-chromanols ($\rho_{5,7-diortho} = -0.47$, $r^2 = 0.90$) also exhibited linear relationships between their σ and $G\cdot$ scavenging activities. In all cases, σ versus $G\cdot$ scavenging activity showed negative linear slopes with good correlation.

The magnitude of ρ was compared among substituent positions.^{32,33} The ρ of *meta*-substituted 6-chromanols (3.71) was the greatest, indicating that the $G\cdot$ scavenging reaction was more sensitive to the inductive substituents. The $\rho_{5-ortho}$ (0.86) and $\rho_{7-ortho}$ (0.87) values were similar in magnitude, suggesting that a substituent at either the 5- or the 7- *ortho* position has the same effect on the radical scavenging reaction. The $\rho_{5,7-diortho}$ (0.47) showed a smaller value.

(Figure 2)

Discussion

A series of 6-chromanol derivatives containing amino, acetylamino, chloro, or nitro groups were synthesized, and the effects of the substituents and their position on $G\cdot$ scavenging activity were investigated.

Phenolic compounds scavenge oxyl radicals via the following two primary mechanisms: one-step hydrogen atom transfer (HAT), and electron transfer followed by proton transfer (ET-PT).³⁴ One-step HAT involves the abstraction of a hydrogen atom from the phenolic hydroxyl group to form a phenoxyl radical. By this mechanism, the O–H bond dissociation is homolytic, and the reaction is driven by BDE. In contrast to HAT, ET-PT involves the loss of an electron followed by the exothermic loss of a proton and formation of a radical cation. ET-PT is driven by IP.³⁴

We report that BDE of chlorinated 6-chromanols (**4c**, **5c**, **6c**, **7c**) correlate with $\log k$ in $G\cdot$ scavenging reactions, indicating that the hydrogen atom transfer mechanism is the rate-determining step in these reactions.^{35–38} Moreover, high radical scavenging activity of aminochromanols (**4a**, **5a**, **6a**) related with the high stability of the corresponding phenoxyl radicals.³⁰

At any substituent position, all of the 6-chromanols showed $G\cdot$ scavenging activity, with increasing k values for substituents in the following order: amino, acetylamino, chloro, and then nitro groups. The data indicated that 6-chromanols with electron-donating substituents significantly increased $G\cdot$ scavenging activity. Furthermore, the introduction of an electron-donating substituent at a position *ortho* to the phenolic OH group in the chroman ring has been reported to significantly enhance $G\cdot$ scavenging activity. These

data agree well with our previous report.³⁰

Many studies have reported that the hydrogen atom abstraction rates for a series of *meta*- or *para*-substituted phenols linearly correlate with the BDE or Hammett sigma constant (σ).^{22,23,25,27,39} In this study, the sums of σ_m and σ^* were used to investigate the relationship between the effects of substituents in 6-chromanols and the radical scavenging activity of the compounds. σ was plotted against $\log k$. For many reactions, a series of $\log k$ values is linearly related to the respective σ values. By contrast, a number of reactions have been shown to deviate from this linearity.^{40,41} Nonlinear Hammett plots are due to a variation in transition state or reaction mechanism for a series of compounds with different substituents. In this study, nonlinear relationships between the σ value and $\log k$ are possible. However, calculated BDEs in phenols are linearly related to σ values.^{22,23,25, 27,39}

The linear slope of σ value versus $\log k$ for each substituent position was obtained. The reaction constants for the $G\cdot$ scavenging reactions were extrapolated from the slopes (ρ).^{32,33} The ρ value magnitudes reveal the susceptibility of a reaction to substituent effects. The ρ_{meta} value was the greatest, indicating that the radical scavenging reaction was highly sensitive to the inductive substituent effect at the *meta* position. Even though the *ortho*-substituted 6-chromanols demonstrated high radical scavenging activity, the ρ value magnitudes were low. The magnitude of ρ depends on several factors, including temperature, solvent, and transition state stability.⁴¹⁻⁴⁴ The 5- and 7-aminochromanoxyl radicals have been detected by ESR,³⁰ indicating that their intermediates are highly stable at room

temperature (half-life of 43 min). These data agreed with our results and explained that the low magnitude of ρ was caused by the stability of the intermediate radical. A relatively small ρ value reflects high 6-chromanol derivative reactivity. Therefore, the radical reactions of the *ortho*-monosubstituted and disubstituted 6-chromanols are largely dependent on the stabilities of the intermediates to increase radical scavenging activity.

There is a possibility that radical scavengers can be developed as anti-inflammatory agents and disease chemopreventive agents.⁴⁵⁾ As our 6-chromanols showed positive radical scavenging activities, a protecting group will be introduced into a phenolic hydroxyl group and/or amino groups, and then their cytotoxicity will be evaluated.

Conclusion

For a series of substituted 6-chromanols, amino-6-chromanols substituted at *ortho* positions demonstrated the highest radical scavenging activities. Additionally, Hammett or Taft sigma constants can be used to predict the radical scavenging activities of substituted 6-chromanols to design new antioxidants based on chromanols.

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List of Schemes, Figures and Table

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Figure legends

Fig. 1 Structures of α -tocopherol and its derivatives.

Fig. 2 $\log k/k_3$ versus calculated σ value plots.

(A) 5-substituted 6-chromanols: **4a** (●), **4b** (▲), **4c** (■), **4d** (◆); (B)

7-substituted 6-chromanols: **5a** (●), **5b** (▲), **5c** (■), **5d** (◆); (C) 8-substituted

6-chromanols: **6a** (●), **6b** (▲), **6c** (■), **6d** (◆); (D) 5,7-disubstituted

6-chromanols: **7a** (●), **7b** (▲), **7c** (■), **7d** (◆).

k_3 is presented as k ($340.3 \text{ M}^{-1} \text{ s}^{-1}$) for compound **3**.

The σ_m and the σ^* value are quoted from ref. 34.

Scheme 1. Synthesis of 5- or 7-substituted 6-chromanols

Reagents and conditions: (A) (i) 5% Pd-C, H_2 gas, Ac_2O , rt, (ii) Ac_2O , pyridine, rt; (B) 1 M NaOMe, MeOH, rt.

Scheme 2. Synthesis of 8-substituted 6-chromanols

Reagents and conditions: (A) 5% Pd-C, H_2 gas, rt; (B) 1 M NaOMe, MeOH, rt.

Scheme 3. Synthesis of 5- and 7-disubstituted 6-chromanols

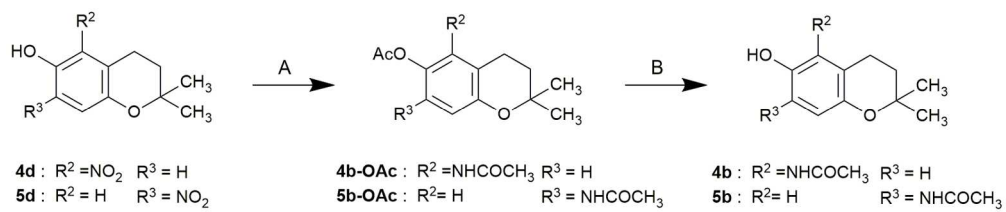
Reagents and conditions: (A) HNO₃, AcOH, 15 °C; (B) 5% Pd-C, H₂ gas, rt; (C)

(i) 5% Pd-C, H₂ gas, Ac₂O, rt, (ii) 0.5 M NaOMe, MeOH, rt.

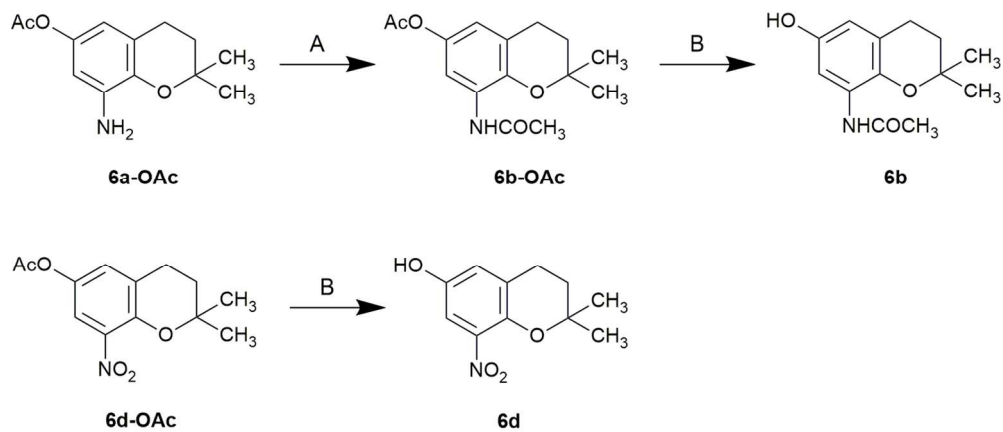
Table 1. The second-order rate constants (k) of the substituted 6-chromanol derivatives with the galvinoxyl radical

Compound	k (M ⁻¹ s ⁻¹) ^a	Compound	k (M ⁻¹ s ⁻¹) ^a
4a	50840 ± 0.09	6a	23970 ± 0.25
4b	831.0 ± 0.003	6b	396.8 ± 0.002
4c	100.3 ± 0.001	6c	45.2 ± 0.003
4d	31.2 ± 0.001	6d	23.3 ± 0.001
5a	48610 ± 0.02	7a	61300 ± 0.26
5b	914.1 ± 0.009	7b	859.4 ± 0.004
5c	100.5 ± 0.001	7c	181.7 ± 0.001
5d	33.2 ± 0.001	7d	30.1 ± 0.000

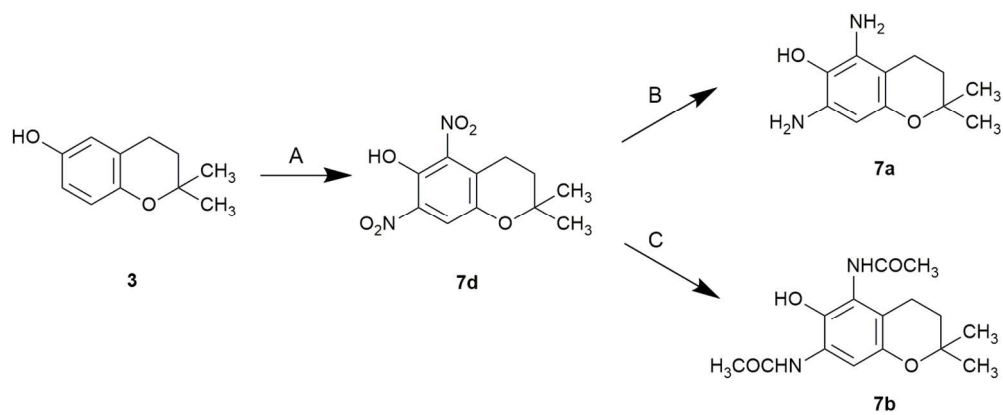
^a Standard error of the regression line for each concentration



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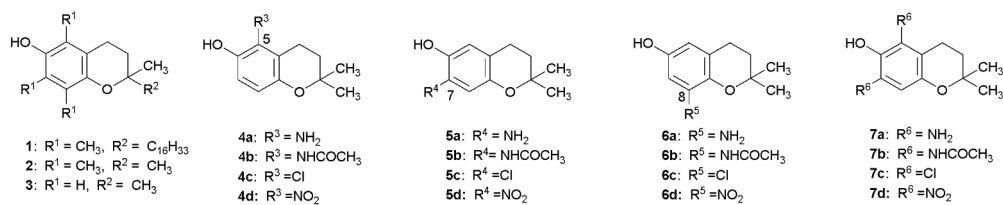


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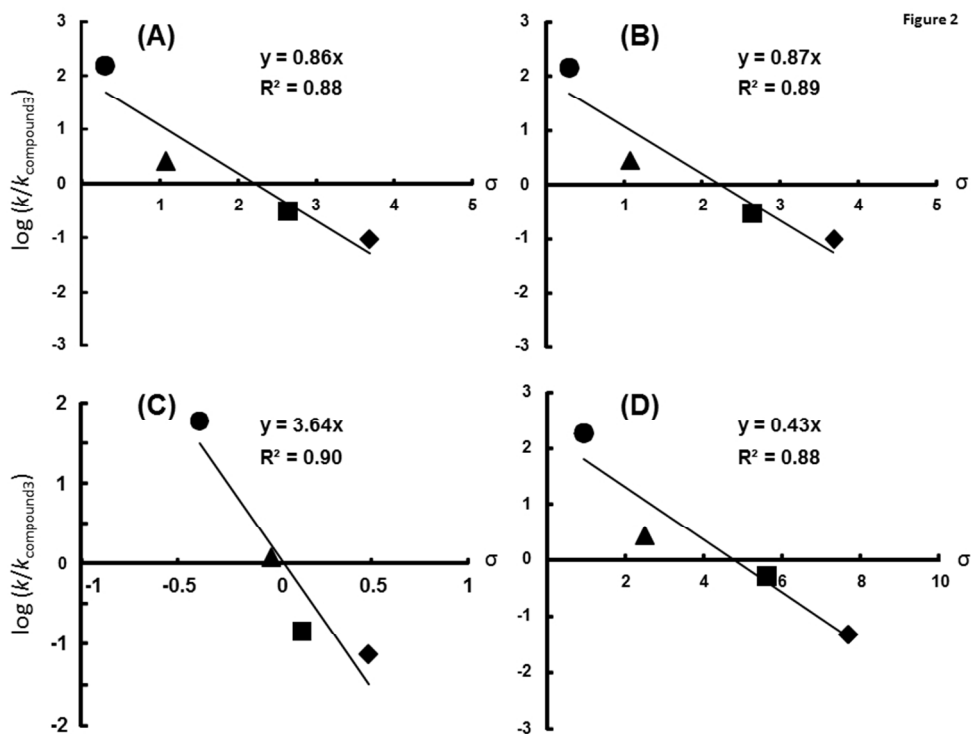


128x53mm (300 x 300 DPI)

Figure 1



Structures of α -tocopherol and its derivatives.
 182x63mm (300 x 300 DPI)



$\log k/k_3$ versus calculated σ value plots.

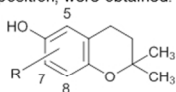
(A) 5-substituted 6-chromanols: 4a (●), 4b (▲), 4c (■), 4d (◆); (B) 7-substituted 6-chromanols: 5a (●), 5b (▲), 5c (■), 5d (◆); (C) 8-substituted 6-chromanols: 6a (●), 6b (▲), 6c (■), 6d (◆); (D) 5,7-disubstituted 6-chromanols: 7a (●), 7b (▲), 7c (■), 7d (◆).

k_3 is presented as k ($340.3 \text{ M}^{-1} \text{ s}^{-1}$) for compound 3.

The σ_m and the σ^* value are quoted from ref. 31.

254x190mm (96 x 96 DPI)

A series of 6-chromanol derivatives with one or two amino, acetylamino, chloro or nitro group at 5, 7, 8 or 5,7- position(s) on the phenyl ring of 2,2-dimethyl-6-chromanol were synthesized, and determined their second order rate constants (k) of the reaction with galvinoxyl radical. The reaction constants from the slope, which plotted with the $\log k$ versus the Hammett sigma or Taft sigma constants for the four substituents at each position, were obtained.



The order of k ;
R; $\text{NH}_2 > \text{NHCOCH}_3 > \text{Cl} > \text{NO}_2$ -substituted 6-chromanol
R positions; 5,7- > 5- > 7- > 8-substituted 6-chromanol

254x190mm (96 x 96 DPI)