Bioorganic & Medicinal Chemistry 20 (2012) 4049–4055

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Chlorine atom substitution influences radical scavenging activity of 6-chromanol

Keiko Inami [a,b,*], Yuko Iizuka [a], Miyuki Furukawa [a], Ikuo Nakanishi [c], Kei Ohkubo [d], Kiyoshi Fukuhara [e], Shunichi Fukuzumi [d,f], Masataka Mochizuki [a,b]

[a] Kyoritsu University of Pharmacy, Shibako-en 1-5-30, Minato-ku, Tokyo 105-8512, Japan
[b] Faculty of Pharmaceutical Sciences, Tokyo University of Science, 2641 Yamazaki, Noda-shi, Chiba 278-8510, Japan
[c] Radio-Redox-Response Research Team, Advanced Particle Radiation Biology Research Program, Research Center for Charged Particle Therapy, National Institute of Radiological Sciences (NIRS), Inage-ku, Chiba 263-8555, Japan
[d] Department of Material and Life Science, Graduate School of Engineering, Osaka University, ALCA, Japan Science and Technology Agency, Suita, Osaka 565-0871, Japan
[e] Division of Organic Chemistry, National Institutes of Health Sciences, Setagaya-ku, Tokyo 158-8501, Japan
[f] Department of Bioinspired Science, Ewha Womans University, Seoul 120-750, Republic of Korea

ARTICLE INFO

Article history:
Received 7 April 2012
Revised 4 May 2012
Accepted 5 May 2012
Available online 12 May 2012

Keywords:
Chlorine
Antioxidant
Chromanol
Electron-donating resonance effect
Electron-withdrawing inductive effect

ABSTRACT

Synthetic 6-chromanol derivatives were prepared with several chlorine substitutions, which conferred both electron-withdrawing inductive effects and electron-donating resonance effects. A trichlorinated compound (**2**), a dichlorinated compound (**3**), and three monochlorinated compounds (**4**, **5**, and **6**) were synthesized; compounds **2**, **3**, and **6** were novel. The antioxidant activities of the compounds, evaluated in terms of their capacities to scavenge galvinoxyl radical, were associated with the number and positioning of chlorine atoms in the aromatic ring of 6-chromanol. The activity of compound **1** (2,2-dimethyl-6-chromanol) was slightly higher than the activities of compounds **2** (2,2-dimethyl-5,7-dichloro-6-chromanol) or **3** (2,2-dimethyl-5,7,8-trichloro-6-chromanol), in which the chlorine atoms were *ortho* to the phenolic hydroxyl group of 6-chromanol. The scavenging activity of compound **3** was slightly higher than that of **2**, which contained an additional chlorine substituted in the 8 position. The activities of polychlorinated compounds **2** and **3** were higher than the activities of any of the monochlorinated compounds (**4**–**6**). Compound **6**, in which a chlorine was substituted in the 8 position, exhibited the lowest activity. Substitution of a chlorine atom *meta* to the hydroxyl group of 6-chromanol (compounds **2** and **6**) decreased galvinoxyl radical scavenging activity, owing to the electron-withdrawing inductive effect of chlorine. Positioning the chloro group *ortho* to the hydroxyl group (compounds **4** and **5**) retained antioxidant activity because the intermediate radical was stabilized by the electron-donating resonance effect of chlorine in spite of the electron-withdrawing inductive effect of chlorine. Antioxidant activities of the synthesized compounds were evaluated for correlations with the O–H bond dissociation energies (BDEs) and the ionization potentials. The BDEs correlated with the second-order rate constants (k) in the reaction between galvinoxyl radical and the chlorinated 6-chromanol derivatives in acetonitrile. This indicated that the antioxidant mechanism of the synthesized compounds consisted of a one-step hydrogen atom transfer from the phenolic OH group rather than an electron transfer followed by a proton transfer. The synthesized compounds also exhibited hydroxyl radical scavenging capacities in aqueous solution.

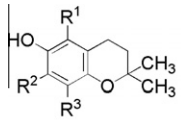
1. Introduction

Antioxidants are of great scientific interest because of their involvement in important biological processes such as the etiologies of cancers, cardiovascular diseases, and inflammation.[1] Phenols and vitamin E derivatives are regarded as particularly significant antioxidants.[2–7] The radical scavenging activity of the antioxidants is essential in the prevention of oxidative stress-related diseases.[8–13]

2,2-Dimethyl-6-chromanol (**1**) is the essential structure of vitamin E. This compound and its derivatives inhibit lipid peroxidation and lipoxygenase activity.[14,15] Most lipoxygenase inhibitors used as anti-inflammatory agents function as antioxidants or free radical scavengers, and their inhibitory activities are related to their lipophilicities.[16,17] The radical scavenging activity of vitamin E increases with the substitution of electron-donating moieties into its chromanol phenyl ring. In addition, the introduction of a halogen atom into any molecule increases its lipophilicity.

* Corresponding author. Tel./fax: +81 4 7121 3641.
E-mail address: inami@rs.noda.tus.ac.jp (K. Inami).

0968-0896/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.bmc.2012.05.008



1	R^1 = H	R^2 = H	R^3 = H
2	R^1 = Cl	R^2 = Cl	R^3 = Cl
3	R^1 = Cl	R^2 = Cl	R^3 = H
4	R^1 = Cl	R^2 = H	R^3 = H
5	R^1 = H	R^2 = Cl	R^3 = H
6	R^1 = H	R^2 = H	R^3 = Cl

Figure 1. Structures of 6-chromanol (2,2-dimethyl-6-chromanol) and synthesized chlorinated 6-chromanols.

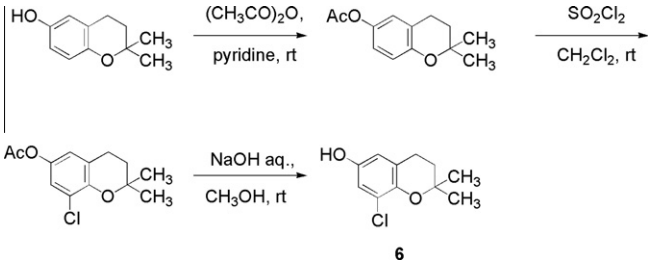
Previous studies have described the synthesis of 2,2-dimethyl-6-chromanols substituted with halogen atoms at the 5 or 7 positions;[18–21] however, those reports did not focus on the radical scavenging activities of the prepared compounds. We reasoned that the introduction of one or more chlorine atoms into the phenyl ring of 2,2-dimethyl-6-chromanol (compound **1**) would retard hydrogen atom abstraction due to the electron-withdrawing inductive effect of chlorine. Whereas chlorine substitution would predict to stabilize the intermediate radical due to its electron-donating resonance effect, thereby enhancing antioxidative activity. We synthesized five derivatives of compound **1** (compounds **2**–**6**) with chlorine atoms inserted at several positions in its chroman ring, and we evaluated the radical scavenging activities of the compounds in an aprotic solvent and in aqueous solution (Fig. 1).

2. Results & discussion

2.1. Chemistry

Compound **1** has the basic structure of 2,2-dimethyl-6-chromanol. Compound **2** carries three chlorine atoms at positions 5, 7, and 8 on the aromatic ring; compound **3** carries two chlorine atoms at positions 5 and 7. Compounds **4**, **5**, and **6** are substituted with a chlorine atom at positions 5, 7, and 8, respectively, on the chroman ring of compound **1**.

Chlorinated 6-chromanols **2**, **3**, **4**, and **5** were synthesized according to the methods of Nilsson et al.[22] (Scheme 1). Briefly, compounds **2**, **3**, **4**, and **5** were prepared by condensation of the corresponding chlorohydroquinones with 2-methyl-3-buten-2-ol. Reaction mixtures were refluxed in formic acid under a nitrogen atmosphere and were extracted with CH_2Cl_2. Extracts were washed with water, dried over anhydrous Na_2SO_4, filtered, and evaporated to yield oils, which then were dissolved in methanol and concentrated hydrochloric acid. Mixtures were refluxed for 1 h to induce compound cyclization and were cooled to room temperature. The reaction mixture was evaporated, extracted with CH_2Cl_2, and then crude product was acetylated with acetic anhydride in pyridine and was purified. Following acetylation of compound **1**, compound **6** was synthesized by chlorination with sulfuryl chloride (Scheme 2). All acetylated 6-chromanols carrying chlorine atom(s) were hydrolyzed with 1 M aqueous sodium hydroxide prior to measuring radical scavenging activity.



Scheme 2. Synthesis of chlorinated chromanol **6**.

The substitution of chlorine atom(s) into 6-chromanol increased the acidity of the synthesized compounds. The p*K*a of each compound was measured as the change in UV–visible absorption spectra in aqueous buffer solution. Results were as follows: Compound **1**, p*K*a 10.3; compound **2**, 7.1; compound **3**, 7.7; compound **4**, 8.6; compound **5**, 9.1; compound **6**, 9.8. The measured acidities were consistent with the number and positioning of chlorine atom(s) substituted into 6-chromanol.

The lipophilicities of the compounds were calculated as the *n*-octanol-water partition coefficients (log*P*) calculated using ChemDraw. Log*P* values increased significantly with the number of chlorine group(s) carried by the synthesized compounds (Table 1). Increased lipophilicities have been reported to influence aqueous-phase radical scavenging properties and chain-breaking antioxidant activities in biological membranes.[17,23] The presence of such compounds precludes the initial reaction between aqueous radicals and biological membranes.[24] In addition, compound lipophilicity is necessary for anti-inflammatory activity, as it affects distribution, bioavailability, metabolism, and excretion of the antioxidants.[17,23]

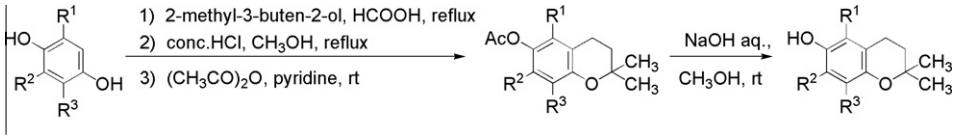
2.2. Galvinoxyl radical scavenging activity of chlorinated 6-chromanols

The abilities of the halogenated 6-chromanols to scavenge galvinoxyl radical were evaluated in acetonitrile. Galvinoxyl radical is a reactive oxygen species with a strong absorption band at

Table 1
Second-order rate constant (k), BDE, IP, and log*P* of 6-chromanol derivatives

Compound	k^a (M^{-1} s^{-1})	BDE (kcal/mol)	IP (eV)	LogP^b
1	98 ± 0.002	395.2	6.83	2.45
2	54 ± 0.002	396.2	7.30	4.12
3	67 ± 0.001	395.7	7.22	3.57
4	37 ± 0.001	397.0	7.04	3.01
5	42 ± 0.001	397.0	7.04	3.01
6	21 ± 0.001	396.3	7.01	3.01

[a] Standard error of regression line for each concentration.
[b] Log*P* calculated using ChemDraw ver.8.0 (CambridgeSoft, Cambridge, USA)



2	R^1 = Cl	R^2 = Cl	R^3 = Cl
3	R^1 = Cl	R^2 = Cl	R^3 = H
4	R^1 = Cl	R^2 = H	R^3 = H
5	R^1 = H	R^2 = Cl	R^3 = H

Scheme 1. Synthesis of chlorinated chromanols **2**–**5**.

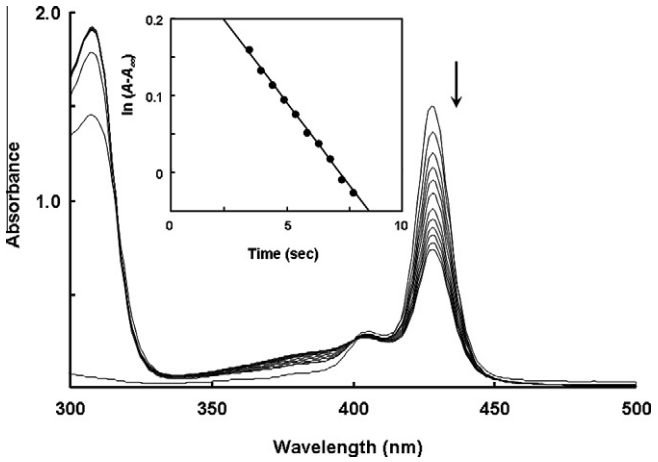


Figure 2. Spectral changes in the reaction of compound **1** (4.2×10^{-4} M) with galvinoxyl radical (10×10^{-6} M) in deaerated acetonitrile at 298 K. (Inset) First-order function describing the change in absorbance at 428 nm.

428 nm.[25–28] Upon addition of compound **1** to a deaerated solution of galvinoxyl radical in acetonitrile, the absorption band at 428 nm decreased precipitously (Fig. 2). The decay in absorbance at 428 nm obeyed pseudo-first-order kinetics when the concentration of compound **1** was held at a >10-fold excess of galvinoxyl radical concentration (Fig. 2 inset). The observed pseudo-first-order rate constant (k_{obs}) was dependent on the concentration of compound **1**, as demonstrated by the linear correlation between k_{obs} and the concentration of compound **1**. The second-order rate constants (k) for the reaction between the halogenated 6-chromanols and galvinoxyl radical were obtained from the slopes of the linear functions of k_{obs} versus the compound concentrations (Fig. 3, Table 1).

The chlorinated 6-chromanols were ordered by radical scavenging activity as follows: $1 \geq 3 \geq 2 > 5 = 4 >> 6$ (Fig. 3, Table 1). Compound **3** exhibited slightly weaker activity than non-halogenated compound **1**, and halogenated compound **2** exhibited less activity than **3**. Monochlorinated compounds **4**, **5**, and **6** displayed lower activities than compounds **1**, **2**, or **3**. The monochlorinated 6-chromanols with chloro groups positioned *ortho* to the hydroxyl group (**4** and **5**), showed higher activities than compound **6**, which carried a chloro group in *meta*. In both polychlorinated and monochlorinated 6-chromanols, the introduction of a chlorine atom *meta* to the hydroxyl group in the chromanol ring was associated

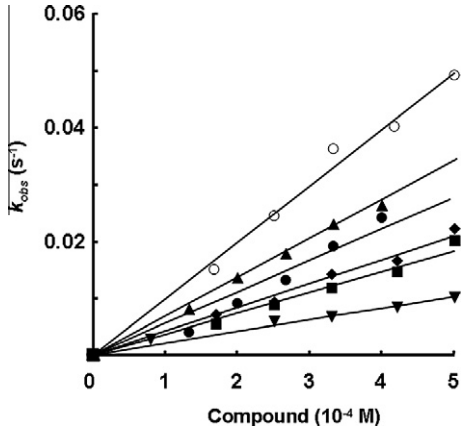


Figure 3. Plot of the pseudo-first-order rate constant (k_{obs}) versus the concentration of compound **1** (○), **2** (●), **3** (▲), **4** (■), **5** (♦), **6** (▼).

with a decreased second-order rate constant in the reaction with galvinoxyl radical.

Halogen substitution confers aromatic rings with an electron-withdrawing inductive effect and an electron-donating resonance effect. The k values of all synthesized chlorinated chromanols were lower than that of compound **1**. The data indicated that, in the context of 6-chromanol, chlorine atom(s) withdraw electrons via the inductive effect more strongly than they donate electrons by resonance. The inductive effect depends on the distance of substituent from the halogen atom, whereas the resonance effect influences atoms positioned *ortho* or *para* to the hydroxyl group. Compound **6** carried a chlorine in the *meta* position; therefore, the halogen exerted only an electron-withdrawing effect, and this compound displayed the weakest relative scavenging activity. Compounds **4** and **5** had higher activities than **6** despite of having chlorines positioned where they would exert greater electron-withdrawing effects.

Scavenging activity increased with the number of chlorine atoms substituted into the 6-chromanol (compounds **2** and **3**), indicating that chlorine's electron-donating resonance effect conferred stability to the intermediate radical. In contrast, the chloro group *meta* to the hydroxyl group (compound **6**) reduced scavenging activity by destabilizing the intermediate radical via chlorine's electron-withdrawing inductive effect. Therefore, stability of the intermediate radical was a key factor in radical scavenging activity.

To elucidate the antioxidant mechanisms of the chlorinated 6-chromanols, the rate constants k for compounds **1**–**6** were determined and compared with the O–H bond dissociation enthalpies (BDEs) and the ionization potentials (IPs). BDEs and IPs were calculated according to density functional theory (see Section 4). Phenolic compounds scavenge oxyl radicals via two primary mechanisms: one-step hydrogen atom transfer (HAT; Fig. 4A) or electron transfer followed by proton transfer (ET–PT; Fig. 4B).[29] One-step HAT involves hydrogen atom abstraction from a phenolic hydroxyl group to form a phenoxyl radical. By this mechanism, O–H bond dissociation is homolytic, and the reaction is driven by the BDE. Lower BDEs are associated with higher O–H bond dissociation reactivities. In contrast to HAT, ET–PT involves electron loss followed by exothermic proton loss and radical cation formation. ET–PT is driven by the IP.

Antioxidant capacity was inferred from galvinoxyl radical scavenging activity because galvinoxyl radical is stable at room temperature and is commercially available. Table 1 lists the values of k, BDEs, and IPs for the synthesized 6-chromanols. The $\log k$ values of the halogenated 6-chromanols correlated linearly with the BDEs ($R = 0.98$) except in the case of compound **6** (Fig. 5). In contrast, the k values did not correlate with the IPs for any of the compounds. These results suggest that the synthesized compounds scavenge galvinoxyl radical via one-step HAT in acetonitrile.[30–32]

Several reports have calculated the BDEs of chlorinated phenols;[33–35] however, the BDEs of chlorinated 6-chromanols are not well characterized. Other studies have reported correlations between BDEs and radical scavenging activities of paracetamols or hydroxycinnamic acids carrying halogen atom(s) positioned *ortho* to hydroxyl groups.[36,37] Our findings support these results.

No correlation was observed between the $\log k$ value and the BDE for compound **6**, suggesting that the electron-withdrawing effect of chlorine positioned *meta* to the hydroxyl group was larger than the calculated BDE of this compound and induced disadvantageous hydrogen abstraction from the hydroxyl group.

2.3. Hydroxyl radical scavenging activity

Antioxidants must display radical scavenging activity in aqueous solution, as the hydroxyl radical is an extremely reactive and short-lived species.[38] We used electron spin resonance (ESR)[39] to

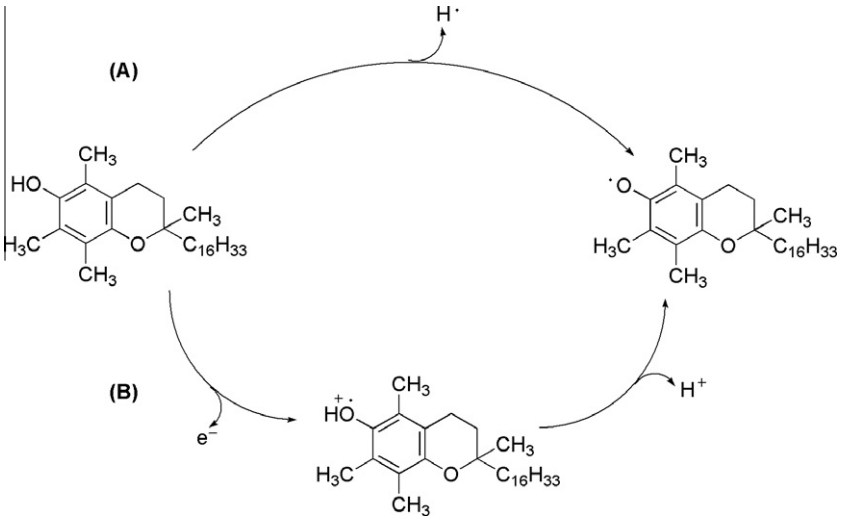


Figure 4. Mechanisms of HAT (A) and ET–PT (B).

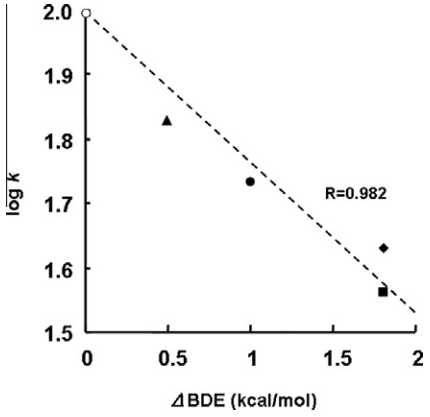


Figure 5. Log *k* versus ⊿ BDE for compound 1 (○), 2 (●), 3 (▲), 4 (■), 5 (♦). ⊿ BDE (kcal/mol) = BDE - 395.2 (BDE of compound 1).

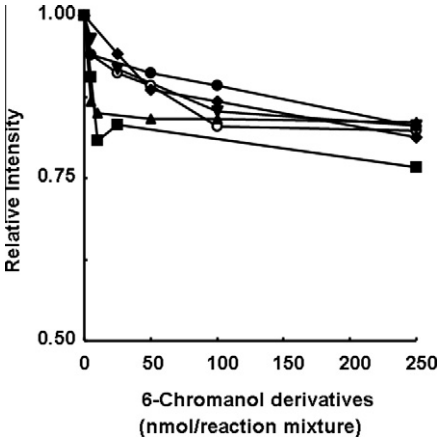


Figure 6. Hydroxyl radical scavenging activities of compound **1** (○), 2 (●), 3 (▲), 4 (■), 5 (♦), 6 (▼) determined by ESR spectroscopy.

measure the capacity of the antioxidants to compete with the radical trapping agent, 5,5-dimethyl-1-pyrroline *N*-oxide (DMPO), in scavenging hydroxyl radical generated by the Fenton reaction. Hydroxyl radical induces a characteristic four-line (1:2:2:1, a^N = 14.8 G) DMPO–OH signal. For concentration of each compound, the relative hydroxyl radical scavenging activity was expressed as the ratio of the peak height of the DMPO–OH adduct to the peak height of the Mn^{2+} internal standard. The DMPO–OH adduct decreased in the presence of each synthesized compound (Fig. 6).

Each of the chlorinated 6-chromanols inhibited the formation of the DMPO–OH adduct in a dose-dependent manner and exhibited scavenging activity in aqueous solution similar to compound **1**. The low solubility of the chlorinated 6-chromanols in aqueous solution made the measurement at higher concentrations impossible. The synthesized 6-chromanols all functioned as hydroxyl radical scavengers.

3. Conclusion

The radical scavenging activities of the synthesized 6-chromanol compounds were influenced by the number and positioning of substituted chlorine(s). The presence of two chlorine atoms *ortho* to the phenolic hydroxyl group (compound **3**) retained radical scavenging activity through weak electron-donating resonance effects in spite of electron-withdrawing inductive effect. However, a chlorine atom positioned *meta* to the hydroxyl group (compound **6**) decreased radical scavenging activity via the electron-withdrawing inductive effect. The lipophilicity (log*P*) of the chlorinated 6-chromanols increased. It is important for antioxidants and anti-inflammatory agents to increase the lipophilicity with having the radical scavenging activity. Our results support the continued investigation of substituted 6-chromanols as candidates for anti-inflammatory agents based on their antioxidant activity.

4. Experimental methods

4.1. Reagents

2,6-Dichlorophenol, 2-methyl-3-buten-2-ol, and 2,3,6-trichlorophenol were obtained from Tokyo Kasei Kogyo Co., Ltd (Tokyo, Japan). Chlorohydroquinone was purchased from Kanto Chemical Co., Inc. (Tokyo, Japan). DMPO was acquired from Labotec Co., Ltd (Hiroshima, Japan). Galvinoxyl free radical was obtained commercially from Sigma–Aldrich (St. Louis, USA). Acetonitrile, used for spectral measurements, was obtained from Dojindo Laboratories

(Kumamoto, Japan). Other reagents were purchased from Wako Pure Chemical Industries (Osaka, Japan). All reagents and solvents used were the best quality commercially available and were not further purified unless otherwise noted. Reaction progressions were monitored using thin-layer chromatography (TLC) on silica gel 60 F_{254} (0.25 mm, Merck). Column chromatography was performed on silica gel 60 (0.063–0.200 mm, Merck). Melting points were determined using a Yanagimoto micro melting-point apparatus without correction. Infrared spectra were recorded on a Nihon Bunkou V-560 spectrophotometer (Tokyo, Japan). ^{1}H NMR spectra were recorded with a JEOL JNM A500 spectrometer (Tokyo, Japan). Chemical shifts were expressed in terms of ppm downfield shifted from tetramethylsilane (s = singlet, d = doublet, t = triplet, m = multiplet, br = broad peak). Mass spectra were determined using a Shimadzu GCMSQP5050A spectrometer (Kyoto, Japan). High resolution mass spectra were collected using a JEOL JMS700 mass spectrometer. ESR spectra were obtained using a JEOL X-band spectrometer (JES-RE1X) under nonsaturating microwave power conditions. Compound **1** was synthesized as described previously (mp: 74.2–75.2 °C).[22]

4.2. Synthesis of 6-acetoxy-2,2-dimethylchroman substituted with chlorine atom

2,3,6-Trichlorohydroquinone or 2,6-dichlorohydroquinone were synthesized from the corresponding polychlorophenols using PbO_2 with $HClO_4$ in acetic acid,[40] followed by reduction of the polychloro-*p*-benzoquinone ether solution with aqueous $Na_2S_2O_4$ in a separatory funnel.

A solution of the corresponding chlorohydroquinone in distilled formic acid was refluxed for 1 h under a nitrogen atmosphere. 2-Methyl-3-buten-2-ol (4.8 equiv for compound **2**; 1.3 equiv for **3**, **4** and **5**) in distilled tetrahydrofuran was added dropwise over 2–4 h, and the solution was refluxed overnight under a nitrogen atmosphere. The reaction mixture was poured into crushed ice and extracted twice with CH_2Cl_2. The combined organic phase was serially washed with water, saturated $NaHCO_3$ aqueous solution, and water. The organic phase was dried over Na_2SO_4, filtered, and evaporated to yield a brown oil, which was dissolved in methanol, combined with concentrated HCl, and refluxed for 1 h. The methanol was evaporated to yield crude product, extracted with CH_2Cl_2, washed with water, dried over Na_2SO_4, filtered, and evaporated. The crude product was fractionated by silica gel column chromatography. A fraction was dissolved in excesses of pyridine and acetic anhydride and stirred at room temperature for 5 h. Water was added to the reaction mixture, and samples were extracted three times with diethyl ether following neutralization with saturated Na_2CO_3 aqueous solution. The combined organic phase was serially washed with water, 5% HCl, water, saturated $NaHCO_3$ aqueous solution, and water. It subsequently was dried over Na_2SO_4, filtered, and evaporated to give an oil. The residue was purified by silica gel column chromatography.

4.3. Acetylated compound 2

White solid; yield 6.0%; mp 103–105 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.38 (6H, s, gem-C*H*$_3$), 1.86 (2H, t, *J* = 6.7 Hz, H-3), 2.39 (3H, s, Ar-OCOC*H*$_3$), 2.79 (2H, t, *J* = 6.7 Hz, Ar-C*H*$_2$–); HR-MS 321.9915 (calcd for $C_{13}H_{13}Cl_3O_3$: 321.9932).

4.4. Acetylated compound 3

White solid; yield 7.2%; mp 99–102 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.32 (6H, s, gem-C*H*$_3$), 1.81 (2H, t, *J* = 6.7 Hz, H-3), 2.37 (3H, s, Ar-OCOC*H*$_3$), 2.74 (2H, t, *J* = 6.7 Hz, Ar-C*H*$_2$–), 6.83 (1H, s, Ar-*H*); HR-MS: 288.0281 (calcd for $C_{13}H_{14}Cl_2O_3$: 288.0321).

4.5. Acetylated compound 4

Brown oil; yield 3.5%; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.32 (6H, s, gem-C*H*$_3$), 1.82 (2H, t, *J* = 6.7 Hz, H-3), 2.33 (3H, s, –COC*H*$_3$), 2.78 (2H, t, *J* = 6.7 Hz, Ar-C*H*$_2$–), 6.71 (1H, d, *J* = 9.2 Hz, Ar-*H*), 6.87 (1H, d, *J* = 9.3 Hz, Ar-*H*); HR-MS 254.0709 (calcd for $C_{13}H_{15}ClO_3$: 254.0710).

4.6. Acetylated compound 5

Pale yellow oil; yield 7.1%; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.31 (6H, s, gem-C*H*$_3$), 1.77 (2H, t, *J* = 6.7 Hz, H-3), 2.31 (3H, s), 2.72 (2H, t, *J* = 6.7 Hz, Ar-C*H*$_2$–), 6.81 (1H, s, Ar-*H*), 6.85 (1H, s, Ar-*H*); HR-MS 254.0715 (calcd for $C_{13}H_{15}ClO_3$: 254.0710).

4.7. Synthesis of 6-acetoxy-8-chloro-2,2-dimethylchroman (acetylated compound 6)

Acetylated compound **1** was prepared with acetic anhydride and pyridine. To a solution of compound **1** (1.0 g, 5.6 mmol) in pyridine (5 mL), acetic anhydride (5 mL) was slowly added with stirring under an argon atmosphere at room temperature. After 4 h, the reaction mixture was poured into crushed ice, filtered, and a white solid was isolated (yield 95.6%, mp 71.5–74.0 °C). To a solution of acetylated 1 (500 mg, 2.3 mmol) in CH_2Cl_2 (20 mL), SO_2Cl_2 (0.92 mL, 11.4 mmol) was added dropwise, and the solution was stirred for 5 min at room temperature under a nitrogen atmosphere. Water was added to the reaction mixture, and the mixture was extracted three times with CH_2Cl_2. The combined organic phase was serially washed with water, saturated $NaHCO_3$ solution, and water, and then was dried over Na_2SO_4, filtered, and evaporated, yielding 652 mg of yellow oil. The crude product was purified by silica gel column chromatography to yield 277 mg of pale yellow needles. Yield 48%; mp 47–49 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.37 (6H, s, gem-C*H*$_3$), 1.81 (2H, t, *J* = 6.7 Hz, H-3), 2.26 (3H, s, Ar-OCOC*H*$_3$), 2.78 (2H, t, *J* = 7.0 Hz, Ar-C*H*$_2$–), 6.72 (1H, d, *J* = 2.4 Hz, Ar-*H*), 6.94 (1H, d, *J* = 3.1 Hz, Ar-*H*); HR-MS 254.0677 (calcd for $C_{13}H_{15}ClO_3$: 254.0710).

4.8. Hydrolysis of the acetylated 6-chromanol derivatives

The acetylated compounds were hydrolyzed immediately before measuring galvinoxyl radical scavenging activities. To a solution of the acetylated compound in acetonitrile, 1 M NaOH was added, and the solution was stirred at room temperature under an argon atmosphere until the starting material was no longer visible by TLC. Acetylated compounds **2** and **3** were stirred for 4 h. Acetylated **4**, **5**, and **6** were stirred for 2 h. The reaction mixture was neutralized with 5% aqueous HCl and extracted three times with diethyl ether. The combined organic phase was washed with water and dried over Na_2SO_4. Samples were filtered and evaporated to yield crude products, which were then purified by column chromatography. The yields exceeded 80% for all compounds.

Compound **2**: White solid; mp 83–86 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.36 (6H, s, gem-C*H*$_3$), 1.85 (2H, t, *J* = 7.0 Hz, H-3), 2.77 (2H, t, *J* = 7.0 Hz, Ar-C*H*$_2$–), 5.51 (1H, s, Ar-O*H*); HR-MS 279.9825 (calcd for $C_{11}H_{11}Cl_3O_2$: 279.9825).

Compound **3**: Colorless oil; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.30 (6H, s, gem-C*H*$_3$), 1.81 (2H, t, *J* = 7.0 Hz, H-3), 2.72 (2H, t, *J* = 6.7 Hz, Ar-C*H*$_2$–), 6.77 (1H, s, Ar-*H*); HR-MS 246.0216 (calcd for $C_{11}H_{12}Cl_2O_2$: 246.0214).

Compound **4**: White solid; mp 68–71 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.31 (6H, s, gem-C*H*$_3$), 1.81 (2H, t, *J* = 6.7 Hz, H-3), 2.75 (2H, t, *J* = 7.0 Hz, Ar-C*H*$_2$–), 5.10 (1H, s, Ar-O*H*), 6.65 (1H, d, *J* = 8.6 Hz, Ar-*H*), 6.81 (1H, d, *J* = 8.5 Hz, Ar-*H*); HR-MS 212.0583 (calcd for $C_{11}H_{13}ClO_2$: 212.0605).

Compound **5**: Pale yellow oil; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.30 (6H, s, gem-CH_3), 1.77 (2H, t, J = 6.7 Hz, H-3), 2.71 (2H, t, J = 6.7 Hz, Ar-CH_2–), 5.04 (1H, s, Ar-OH), 6.72 (1H, s, Ar-H), 6.76 (1H, s, Ar-H); EI-MS: 212 (M^+), 214 ($[M+2]^+$).

Compound **6**: White solid; mp 34–37 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.35 (6H, s, gem-CH_3), 1.79 (2H, t, J = 6.7 Hz, H-3), 2.74 (2H, t, J = 6.7 Hz, Ar-CH_2–), 4.43 (1H, s, Ar-OH), 6.48 (1H, d, J = 3.6 Hz, Ar-H), 6.73 (1H, d, J = 3.1 Hz, Ar-H); HR-MS 212.0604 (calcd for $C_{11}H_{13}ClO_2$: 212.0605).

4.9. Kinetics

Kinetics were determined by measuring the disappearance of absorbance at 428 nm under pseudo-first-order conditions at 25 °C. An aliquot of chlorinated chromanols (final concentrations: 0.17, 0.25, 0.33, 0.42, 0.50 μM for compounds **1**, **4**–**6** and 0.13, 0.20, 0.27, 0.33, 0.40 μM for compounds **2**, **3**) in deaerated acetonitrile was added to a quartz cuvette (10 mm id) containing galvinoxyl radical (final concentration 10.0 μM) in deaerated acetonitrile (3 mL). UV–vis spectral changes following this reaction were monitored using a Hewlett-Packard 8453 photo diode array spectrophotometer (Hanover, USA). Radical scavenging rates were determined by monitoring the absorbance change at 428 nm due to the decrease in galvinoxyl radical. Pseudo-first-order rate constants were determined by the method of least-squares. Pseudo-first-order rate plots of $\ln(A–A_\infty)$ versus time, where A and A_∞ refer to the absorbance at the reaction time and the final absorbance, respectively, were linear until three or more half-lives. First-order rate constants were obtained from the slopes of plots of k_{obs} versus various concentrations of the compounds. All experiments were conducted in triplicate.

4.10. Hydroxyl radical scavenging activity

Hydroxyl radical scavenging capacity of the chlorinated chromanols was determined by ESR, which was based on the competition between the trapping agent (DMPO) and the chlorinated chromanols. Hydroxyl radical was generated by the Fenton reaction. Acetonitrile was used to dissolve each chlorinated chromanol. Reaction mixtures contained 10 μL of 10 mM freshly prepared $FeSO_4$, 10 μL of 200 mM DMPO, 10 μL of 10 mM H_2O_2, 180 μL of 0.1 M sodium phosphate buffer (pH 7.4), and 10 μL of chlorinated chromanols in acetonitrile solution (5, 10, 20, 50, 100, 250, and 500 nmol). Pure acetonitrile was used for the blank. The mixture was transferred into a quartz cell and was incubated at room temperature. ESR measurements were initiated at room temperature 1 min after preparing each reaction mixture. The magnitude of modulation was chosen to optimize the resolution and the signal-to-noise (S/N) ratio of the observed spectra. The spectrometer settings were: magnetic field 335.5 ± 5.0 mT, microwave power 8.0 mW, modulation frequency 9.42 GHz, modulation width 0.079 mT, sweep time 2.0 min, response time 0.1 s, receiver gain 50–100.

4.11. p*K*a measurement

An aliquot of chlorinated chromanols ($1.3–2.5 \times 10^{-4}$ M) in deaerated water was added to a quartz cuvette (10 mm id), and either 2 M HCl or 2 M NaOH aqueous solution, were added by syringe. Sigmoid curves were obtained by monitoring the absorbance changes at the following wavelengths for each compound: **1**, 312 nm; **2**, 313 nm; **3**, 321 nm; **4**, 313 nm; **5**, 319 nm; **6**, 315 nm (y axis) as a function of pH (x axis) (data not shown). The p*K*a was obtained from the 50% value in the sigmoid curve, subtracting the minimum absorbance from the maximum absorbance for each compound.

4.12. Theoretical calculations

Density functional theory calculations were performed using an 8 CPU workstation (PQS, Quantum Cube QS8-2400C-064). Geometry optimizations were carried out using the RB3LYP/6-31G(d) basis set for the neutral forms of chromanols and the UB3LYP/6-31G(d) basis set for the radical anion and phenoxyl radicals forms, as implemented in the Gaussian 03 program, revision C.02 (Wallingford, USA). The energies of the radical anion and phenoxyl radical were obtained from the single point energy calculations in the ROB3LYP/6-31G(d) basis set using the geometry-optimized structures. IP values were calculated from the energy differences between the neutral and radical anion forms. Relative BDE values were determined from the energy difference between the neutral and phenoxyl radical forms, where the values are normalized by the BDE of compound **1**.

Acknowledgements

This work was partially supported by Grant-in-Aid (Nos. 23590064, 23750014 and 20108010) from the Ministry of Education, Culture, Sports, Science and Technology, Japan, the Global COE (center of excellence) program "Global Education and Research Center for Bio-Environmental Chemistry" of Osaka University from MEXT, Japan, and NRF/MEST of Korea through WCU (R31-2008-000-10010-0) and GRL (2010-00353) programs.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2012.05.008.

References and notes

1. Halliwell, B.; Gutteridge, J. M. C. *Free Radicals in Biology and Medicine*, 4th ed.; Oxford University Press: Oxford, UK, 2006.
2. Foti, M. C. *J. Pharm. Pharmacol.* **2007**, *59*, 1673.
3. Burton, G. W.; Ingold, K. U. *Acc. Chem. Res.* **1986**, *19*, 194.
4. Niki, E.; Noguchi, N. *Acc. Chem. Res.* **2004**, *37*, 45.
5. Burton, G. W.; Doba, T.; Gabe, E. J.; Hughes, L.; Lee, F. L.; Prasad, L.; Ingold, K. U. *J. Am. Chem. Soc.* **1985**, *107*, 7053.
6. Mukai, K.; Kageyama, Y.; Ishida, T.; Fukuda, K. *J. Org. Chem.* **1989**, *54*, 552.
7. Tafazoli, S.; Wright, J. S.; O'Brien, P. J. *Chem. Res. Toxicol.* **2005**, *18*, 1567.
8. Cuzzocrea, S.; Riley, D. P.; Caputi, A. P.; Salvemini, D. *Pharmacol. Rev.* **2001**, *53*, 135.
9. Fresco, P.; Borges, F.; Diniz, C.; Marques, M. P. M. *Med. Res. Rev.* **2006**, *26*, 747.
10. Kładna, A.; Aboul-Enein, H. Y.; Kruk, I.; Lichszteld, K.; Michalska, T. *Biopolymers* **2006**, *82*, 99.
11. Grammas, P.; Hamdheydari, L.; Benaksas, E. J.; Mou, S.; Pye, Q. N.; Wechter, W. J.; Floyd, R. A.; Stewart, C.; Hensley, K. *Biochem. Biophys. Res. Commun.* **2004**, *319*, 1047.
12. Koufaki, M.; Detsi, A.; Theodorou, E.; Kiziridi, C.; Calogeropoulou, T.; Vassilopoulos, A.; Kourounakis, A. P.; Rekka, E.; Kourounakis, P. N.; Gaitanaki, C.; Papazafiri, P. *Bioorg. Med. Chem.* **2004**, *12*, 4835.
13. Neuzil, J.; Tomasetti, M.; Zhao, Y.; Dong, L.; Birringer, M.; Wang, X.; Low, P.; Wu, K.; Salvatore, B. A.; Ralph, S. J. *Mol. Pharmacol.* **2007**, *71*, 1185.
14. Cichewicz, R. H.; Kenyon, V. A.; Whitman, S.; Morales, N. M.; Arguello, J. F.; Holman, T. R.; Crews, P. *J. Am. Chem. Soc.* **2004**, *126*, 14910.
15. Casas, J.; Gorchs, G.; Sánchez-Baeza, F.; Teixidor, P.; Messeguer, A. *J. Agric. Food Chem.* **1992**, *40*, 585.
16. Pontiki, E.; Hadjipavlou-Litina, D. *Med. Chem.* **2006**, *2*, 251.
17. Pontiki, E.; Hadjipavlou-Litina, D.; Litinas, K.; Nicolotti, O.; Carotti, A. *Eur. J. Med. Chem.* **2011**, *46*, 191.
18. Koufaki, M.; Theodorou, E.; Galaris, D.; Nousis, L.; Katsanou, E. S.; Alexis, M. N. *J. Med. Chem.* **2006**, *49*, 300.
19. Prado, S.; Toum, V.; Saint-Joanis, B.; Michel, S.; Koch, M.; Cole, S. T.; Tillequin, F.; Janin, Y. L. *Synthesis* **2007**, *10*, 1566.
20. Mori, K.; Yamamura, S.; Nishiyama, S. *Tetrahedron* **2001**, *57*, 5533.
21. Takizawa, Y.; Tateishi, A.; Sugiyama, J.; Yoshida, H.; Yoshihara, N. *J. Chem. Soc., Chem. Commun.* **1991**, 104.
22. Nilsson, J. L. G.; Sievertsson, H.; Selander, H. *Acta Chem. Scand.* **1968**, *22*, 3160.
23. Pontiki, E.; Hadjipavlou-Litina, D. *Bioorg. Med. Chem.* **2007**, *15*, 5819.
24. Justino, G. C.; Rodrigues, M.; Florêncio, M. H.; Mira, L. *J. Mass Spectrom.* **2009**, *44*, 1459.

25. Gotoh, N.; Shimizu, K.; Komuro, E.; Tsuchiya, J.; Noguchi, N.; Niki, E. *Biochim. Biophys. Acta* **1992**, *1128*, 147.
26. Nakanishi, I.; Fukuhara, K.; Shimada, T.; Ohkubo, K.; Iizuka, Y.; Inami, K.; Mochizuki, M.; Urano, S.; Itoh, S.; Miyata, N.; Fukuzumi, S. *J. Chem. Soc. Perkin Trans. 2* **2002**, 1520.
27. Cevallos-Casals, B. A.; Cisneros-Zevallos, L. *J. Agric. Food Chem.* **2003**, *51*, 3313.
28. Nakanishi, I.; Kawashima, T.; Ohkubo, K.; Kanazawa, H.; Inami, K.; Mochizuki, M.; Fukuhara, K.; Okuda, H.; Ozawa, T.; Itoh, S.; Fukuzumi, S.; Ikota, N. *Org. Biomol. Chem.* **2005**, *3*, 626.
29. Anouar, E.; Calliste, C. A.; Košinová, P.; Meo, F. D.; Duroux, J. L.; Champavier, Y.; Marakchi, K.; Trouillas, P. *J. Phys. Chem. A* **2009**, *113*, 13881.
30. Wright, J. S.; Johnson, E. R.; DiLabio, G. A. *J. Am. Chem. Soc.* **2001**, *123*, 1173.
31. Wright, J. S.; Carpenter, D. J.; McKay, D. J.; Ingold, K. U. *J. Am. Chem. Soc.* **1997**, *119*, 4245.
32. Hussain, H. H.; Babic, G.; Durst, T.; Wright, J. S.; Flueraru, M.; Chichirau, A.; Chepelev, L. L. *J. Org. Chem.* **2003**, *68*, 7023.
33. Bosque, R.; Sales, J. *J. Chem. Inf. Comput. Sci.* **2003**, *43*, 637.
34. Korth, H.; de Heer, M. I.; Mulder, P. *J. Phys. Chem. A* **2002**, *106*, 8779.
35. Diniz, J. E. M.; Borges, R. S.; Alves, C. N. *J. Mol. Struct. (Theochem)* **2004**, *673*, 93.
36. Gasper, A.; Garrido, E. M.; Esteves, M.; Quezada, E.; Milhazes, N.; Garrido, J.; Borges, F. *Eur. J. Med. Chem.* **2009**, *44*, 2092.
37. Bordwell, F. G.; Cheng, J. *J. Am. Chem. Soc.* **1991**, *113*, 1736.
38. Huang, D.; Ou, B.; Prior, R. L. *J. Agric. Food Chem.* **2005**, *53*, 1841.
39. Buettner, G. R. *Free Radic. Biol. Med.* **1987**, *3*, 259.
40. Omura, K. *Synthesis* **1998**, 1145.