HETEROCYCLES, Vol. 94, No. 5, 2017, pp. 865 - 878. © 2017 The Japan Institute of Heterocyclic Chemistry
Received, 13th February, 2017, Accepted, 11th April, 2017, Published online, 25th April, 2017
DOI: 10.3987/COM-17-13671

SYNTHESIS AND RADICAL SCAVENGING ACTIVITY OF SUBSTITUTED BENZO[*h*]CHROMANOLS

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Abstract – Benzo[*h*]chromanols, which possess a tocopherol moiety, have been reported to exhibit potent antioxidant activity. Several benzo[*h*]chromanols with various substituents (nitro, chloro, bromo, methyl, or amino groups at the position *ortho* to the phenolic OH group) were synthesized, and the second-order rate constants (*k*) of their reaction with the galvinoxyl radical were determined. The introduction of electron-withdrawing bromo, chloro and nitro groups decreased the activity, and the activity correlated well with the substituent effect. *ortho*-Aminobenzo[*h*]chromanol showed the highest radical scavenging activity among the compounds synthesized.

INTRODUCTION

α-Tocopherol (**1**) is a well-known lipophilic antioxidant and is thought to prevent oxidative damage, such as cancer- and ischemia/reperfusion-related tissue damage.[1,2] Numerous tocopherol analogues have been synthesized and assayed for their antioxidant and physiological activities.[3,4] The antioxidant activities of **1** are attributed to the radical scavenging ability of the phenolic hydroxyl groups, producing the corresponding chromanoxyl radical.[5] Many studies have reported a correlation between the bond dissociation enthalpies (BDEs) of the OH group of phenolic antioxidants and the radical scavenging activity.[6,7]

2,2,5,7,8-Pentamethyl-6-chromanol (**2**) is a tocopherol analogue in which the phytyl chain is replaced by a methyl group.[8] **2** was reported to have more potent radical scavenging activity toward iron-induced LPO in rat brain homogenates than **1**.[9] **2** was also highly effective at preventing carbon tetrachloride-induced hepatotoxicity *in vivo*.[10] We have reported the preparation of a series of substituted 6-chromanol

analogues and the structure–activity relationship for the radical scavenging activity.[11-13] Benzo[*h*]chromanol (**4**), which consists of an aromatic ring fused to 6-chromanol (Figure 1), has a basic structure derived from a product of the enzymatic reduction of vitamin K_1 *in vivo*.[14] Benzo[*h*]chromanols are expected to be an extremely effective radical scavenger in various systems;[3,14-16] however, no studies have been conducted using the analogues, except methyl-substituted compound (**8**). Herein, we describe the preparation of a series of 5-substituted-6-benzo[*h*]chromanols with a methyl group replacing the phytyl side chain. Additionally, to determine the contribution of a fused benzene ring and a substituent to the radical scavenging activity of 6-benzo[*h*]chromanols, the radical scavenging activities of these derivatives were evaluated in terms of the rate constants (*k*) of their reactions with the galvinoxyl radical (G•).

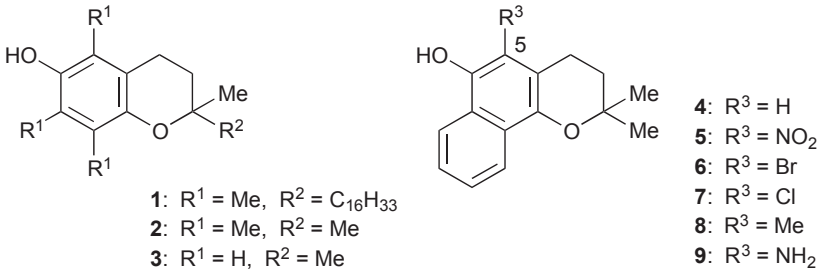


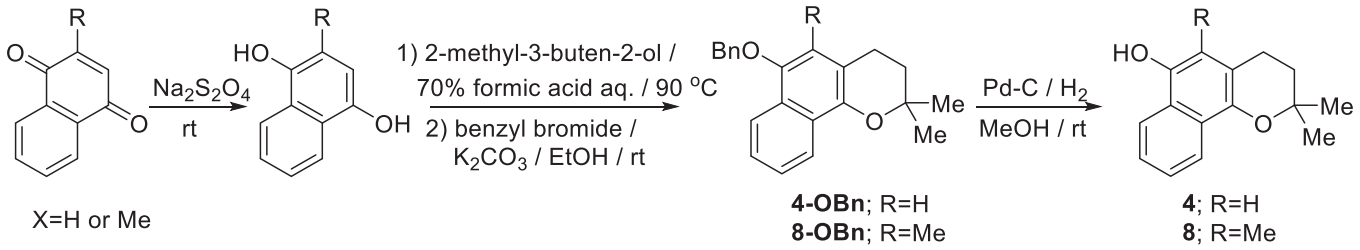
Figure 1. Structures of 6-chromanols and benzo[*h*]chromanols

RESULTS AND DISCUSSION

The radical scavenging activity of simple phenols is well correlated with the substituent effect, and the Hammett-Brown constant (σ^+) has been reported to be a good parameter for the correlation with the radical scavenging activity with the assumption of $\sigma^+_{ortho} = \sigma^+_{para}$.[11,18,19] Electron-donating groups increased the antioxidant activity, so the σ^+ values of all substituents must be as negative as possible. Nitro (0.79), bromo (0.15) and chloro (0.11) groups are normally electron withdrawing, whereas methyl (-0.31) and amino (-1.3) groups are electron donating.[20] The number in parenthesis is the σ^+ value for each substituent. The radical scavenging activity of a series of substituted 6-chromanols was greatly enhanced by introducing electron-donating substituents at the position *ortho* to the phenolic OH group.[11] Then, 5-substituted benzo[*h*]chromanols containing an *ortho* substituent were designed. The selected substituents were based on their ability to accept (NO_2, Br and Cl) or donate (CH_3 and NH_2) electrons, and the substituents were introduced *ortho* to the phenolic OH group on benzo[*h*]chromanol (Figure 1). The benzo[*h*]chromanols, including nitro (**5**), bromo (**6**), chloro (**7**) and amino (**9**) compounds, benzyloxylated **4** (**4-OBn**), **5-OBn** and **8-OBn**, and acetylated **6** (**6-OAc**) and **7-OAc**, were newly synthesized.

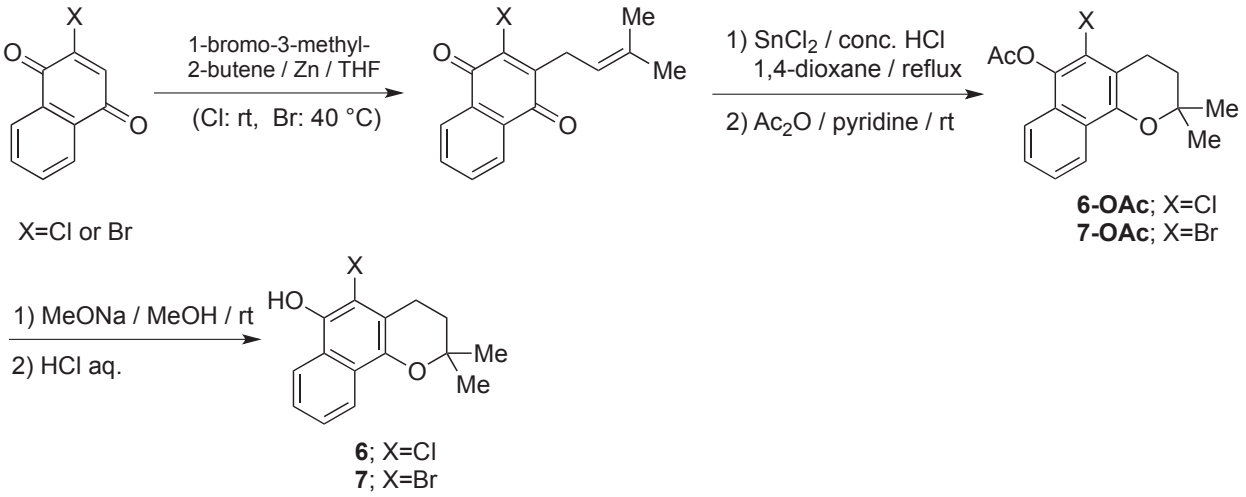
In the preparation of **4** and **8**, the corresponding hydrobenzoquinone was condensed with

2-methyl-3-buten-2-ol and then cyclized under acidic conditions. The crude products were benzyloxylated in the presence of K_2CO_3 for purification and storage (Scheme 1).



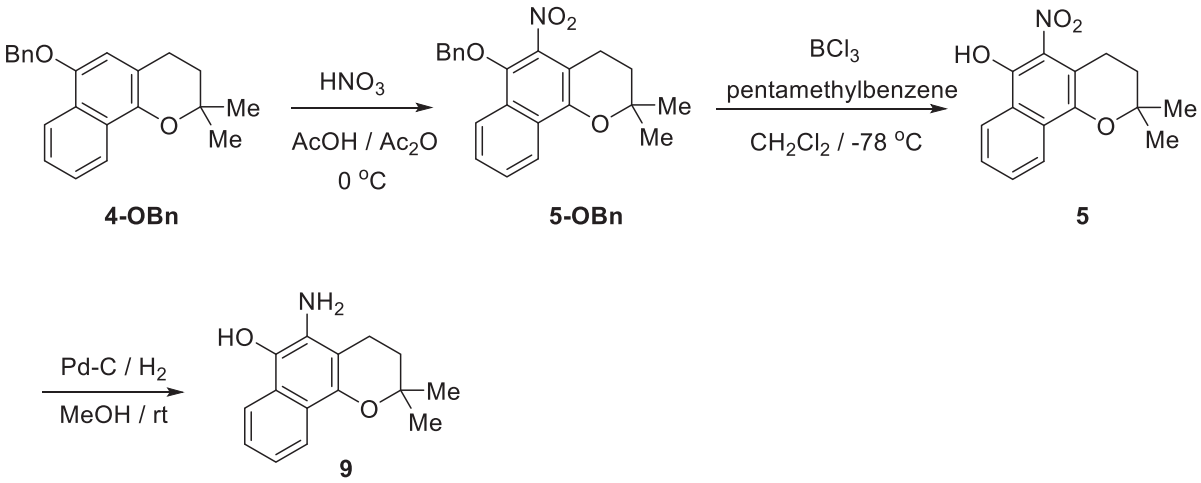
Scheme 1. Synthesis of **4** and **8**

For the halogen-substituted benzo[*h*]chromanols (**6**, **7**), 2-halogenated quinone was condensed with 1-bromo-3-methyl-2-butene in the presence of a Lewis acid, and the corresponding lapachol was isolated. The lapachol was cyclized under acidic conditions, and the crude product was acetylated for purification and storage (Scheme 2).



Scheme 2. Synthesis of **6** and **7**

In the preparation of nitrogen-containing benzo[*h*]chromanols (**5**, **9**), non-substituted benzo[*h*]chromanol **4-OBn** was nitrated with HNO_3 to give **5-OBn**. **5** was synthesized from the reaction of **5-OBn** and BCl_3. **5** was reduced by hydrogen gas in the presence of palladium/carbon to give the corresponding amino compound (**9**) (Scheme 3).



Scheme 3. Synthesis of **5** and **9**

The compounds (**4-OBn**, **5-OBn**, **8-OBn**, **6-OAc** and **7-OAc**) were deprotected and purified on a short silica gel column, and the galvinoxyl (G$^{\bullet}$) scavenging activity was measured immediately. All procedures were carried out under an argon atmosphere. The structure of **9** could not be confirmed by NMR because of its high instability, but the structures was assigned by HRMS.

The antioxidant activity of these benzo[*h*]chromanols was determined as a function of their G$^{\bullet}$ scavenging ability.[21,22] G$^{\bullet}$ is a reactive oxygen species with a strong absorption band at 428 nm. The absorbance at 428 nm decreased during the reaction of G$^{\bullet}$ with the compounds in equimolar concentrations.[23,24] The plot of the absorbance $(A_0–A)$ / $(A–A_\infty)$ versus time yielded a straight line, indicating that the reaction was second order. In addition, the second-order rate constants (k) for the reactions were calculated from the slopes of the linear functions by dividing by the initial concentration (Table 1).

Table 1. Second-order rate constants (k) of the reactions between the benzo[*h*]chromanols and G$^{\bullet}$

Compound	k (M^{-1} s^{-1})
1	13,040
2	12,040
3	160
4	34,600
5	11,840
6	21,480
7	20,800
8	38,200
9	267,880

The 5-substituted benzo[*h*]chromanols were ranked based on their G• scavenging activity as follows: **9** > **8** > **4** > **7** = **6** > **1** = **2** > **5**. The activity of **1** was similar to that of **2**, indicating that the phytyl side chain was not necessary for the radical scavenging activity.

Comparison of the substituent effect in 6-benzochromanols on the radical scavenging activity revealed that the radical scavenging capacity followed a similar order as the electron-donating capacity based on the Hammett constant (Figure 2). A plot of log ($k/k_{\text{compound 4}}$) and σ showed a negative linear slope with good correlation (r^2 = 0.97), indicating that benzo[*h*]chromanols with electron-donating substituent increased G• scavenging activity. The electron-deficient phenoxyl radical would be stabilized by the electron-donating effect from the substituents.[11,12] Amino substitution at the position *ortho* to the phenolic OH group remarkably enhanced the radical scavenging activity, as in 6-chromanol.[11] The electron-deficient phenoxyl radical has been reported to be largely stabilized by hydrogen donors such as OH and NH_2 at the *ortho* position through the formation of an intramolecular hydrogen bond.[11,25-27] The high reactivity of catechols and *o*-aminophenols is explained by both the electron-donating substituent effect and the hydrogen-donating effect.[28]

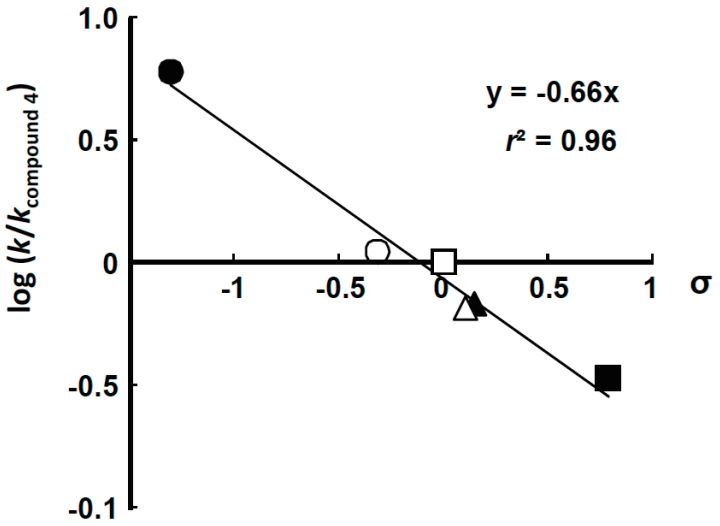


Figure 2. log ($k/k_{\text{compound 4}}$) versus calculated σ value plots. **4** (□), **5** (■), **6** (△), **7** (▲), **8** (○), **9** (●). The $k_{\text{compound 4}}$ is presented as k (34,600 M^{-1} s^{-1}) for compound **4**.

As a comparison of two molecular structures, 6-benzochromanol and 6-chromanol, the rate of the reaction of non-substituted 6-benzochromanol **4** was 200-fold higher than that of non-substituted 6-chromanol **3**. The incorporation of a second fused aromatic ring caused a remarkable increase in the G• scavenging activity. The second aromatic ring held in benzo[*h*]chromanol would contribute to the delocalization and stabilization of unpaired electron in the phenoxyl radical so that the enhanced antioxidant activity is observed.

Moreover, the influence of the incorporation of an electron-donating substituent into the chromanol and benzo[*h*]chromanol structures on the enhancement of the radical scavenging activity was investigated. Amino-substituted **9** showed 7-fold higher activity than that of **4**, whereas the corresponding 6-chromanol (5-amino-2,2-dimethyl-6-chromanol, 487,800 M^{-1} s^{-1}) had 3,000-fold higher activity than that of **3**. These data indicated that the introduction of an electron-donating group such as an amino group into the benzo[*h*]chromanol structure slightly increased the radical scavenging activity.

Interestingly, compounds **9** (20), **8** (3.0), **4** (2.7), **7** (1.6) and **6** (1.6) had higher activity than **1**. The number in parentheses indicates the ratio of the radical scavenging activity of the compound to the activity of **1**. Overall, the additional fused aromatic ring enhances the antioxidant activity of the molecule, but the substituents do not appear to have a large influence on the radical scavenging activity of 6-benzochromanol. Compounds possessing a high radical scavenging activity are expected to be candidates for anti-inflammatory agents and radioprotective agents.[29-31]

EXPERIMENTAL

Materials and Methods

The galvinoxyl radical, 2-methyl-3-buten-2-ol, 2-methyl-1,4-naphthoquinone, sodium methoxide and zinc powder were obtained from Tokyo Kasei Kogyo Co., Ltd. (Tokyo, Japan). Cyclohexane was purchased from Kanto Chemical Co., Inc. (Tokyo, Japan). 2-Bromo-1,4-naphthoquinone was obtained from Sigma-Aldrich (St. Louis, MO, USA). Chloroform-*d* (0.03 vol% TMS) and dimethyl sulfoxide-d_6 were purchased from Cambridge Isotope Laboratories, Inc. (Tewksbury, MA, USA). 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 used were of the best commercially available quality and were not further purified unless otherwise noted.

The reaction progression was monitored using thin-layer chromatography (TLC) on silica gel 60 F_{254} (0.25 mm, Merck, Darmstadt, Germany). Column chromatography was performed using silica gel 60 (0.040–0.063 mm, Merck) or aluminium oxide 90 active basic (0.063–0.200 mm, Merck). Melting points were determined using a Yanaco micro-melting point apparatus without correction (Tokyo, Japan). NMR spectra were recorded on a JEOL JNM-LA400 spectrometer (Tokyo, Japan). Chemical shifts were expressed in ppm, downfield shifted from the TMS peak. High-resolution mass spectra were collected using a JEOL JMS-SX102A mass spectrometer (Tokyo, Japan). UV-Vis spectrophotometry data were obtained using a Hewlett-Packard 8453 photodiode array spectrophotometer (Hanover, USA) equipped with an Applied Photophysics RX2000 stopped-flow device (Leatherhead, UK). **3** [75.3–76.0 °C (75–76 °C)[32]], **4-OAc** [66.0–66.1 °C (66.9–67.1 °C)[33]], 2-bromo-1,4-naphthoquinone [127.0–127.5 °C (130–132 °C)[34]], 2-chlorolapachol [75.0–76.0 °C (81–83 °C)[35]], 2-chloro-1,4-naphthoquinone

[107.5–108.0 °C (116 °C)[36]], 2-methyl-1,4-naphthalenediol [167.0–167.5 °C (164–167 °C)[37]] and 1,4-naphthalenediol [194.0–194.5 °C (187–189 °C)[38]] were prepared according to a previously reported procedure. During the preparation of the benzo[*h*]chromanols, all of the procedures were carried out while with flushing argon gas.

Synthesis of **4**

1,4-Dihydroxynaphthalene (1.50 g, 9.37 mmol) and ascorbic acid (375 mg) were dissolved in 70% formic acid (50 mL). After the addition of 2-methyl-3-buten-2-ol (1.0 mL, 1.0 eq.) for 10 min at 90 °C under nitrogen gas flow, the mixture was then stirred for 3.5 h. The reaction mixture was extracted with *n*-hexane (30 mL×6). The combined organic phase was washed with saturated aq. sodium bicarbonate (50 mL) and water (10 mL), dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure to afford a dark brown oil. The oil was dissolved in EtOH (10 mL), and potassium carbonate (1.94 g, 1.5 eq.) and benzyl bromide (1.1 mL, 1.0 eq.) were added, and the solution was then stirred vigorously under an argon atmosphere overnight. The mixture was poured into crushed ice and extracted with CH_2Cl_2 (25 mL×3). The combined organic phase was dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure to afford a brown oil. The crude product was purified by column chromatography [silica gel, *n*-hexane:Et_2O (95:5) and aluminum oxide (containing 5% water), *n*-hexane:CH_2Cl_2 (10:1)] to afford a tan yellow oil (1.56 g). The oil was recrystallized from EtOH and water to yield the single compound **4-OBn** (660 mg).

4-OBn (10 mg, 0.032 mmol) was dissolved in MeOH (2.5 mL), and 5% palladium/carbon (20 mg) was added. The mixture was stirred at room temperature under a hydrogen atmosphere for 20 min. The mixture was filtered through Celite with suction, and the filter cake was washed with CH_2Cl_2. The filtrate was concentrated under reduced pressure to afford a tan yellow oil. The crude product was immediately purified on a short silica gel column (2% MeOH/CH_2Cl_2) to yield the single compound **4** (3.6 mg).

6-Benzyloxy-2,2-dimethyl-3,4-dihydro-2*H*-benzo[*h*]chromene (4-OBn): white needle crystals; yield: 22%; mp 85–86 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.21 (m, 2H), 7.42 (m, 7H), 6.59 (s, 1H), 5.17 (s, 2H), 2.84 (t, *J*= 6.8 Hz, 2H), 1.89 (t, *J*= 6.6 Hz, 2H), 1.40 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 147.6, 142.7, 137.6, 128.5, 127.7, 127.3, 126.5, 125.6, 125.5, 124.9, 121.8, 121.3, 113.3, 107.0, 73.9, 70.5, 33.0, 26.8, 23.3; HRMS (FAB-positive): 318.1690 (calcd for $C_{22}H_{22}O_2$ 318.1620).

2,2-Dimethyl-3,4-dihydro-2*H*-benzo[*h*]chromen-6-ol (4): colorless oil; yield: 50%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.17–8.00 (m, 2H), 7.44 (m, 2H), 6.55 (s, 1H), 4.70 (s, 1H), 2.81 (t, *J*= 6.6 Hz, 2H), 1.88 (t, *J*= 6.8 Hz, 2H), 1.40 (s, 6H).

Synthesis of **5**

4-OBn (259 mg, 0.71 mmol) was dissolved in a mixture of acetic acid (10 mL) and acetic anhydride (10 mL), and a solution of 70% nitric acid in acetic anhydride (1.25 mL, 6.5 eq.) was added at 0 °C over 15 min. After the addition, the reaction mixture was immediately poured into crushed ice, and the mixture was extracted with CH_2Cl_2 (20 mL×3). The combined organic phase was washed with saturated aq. sodium bicarbonate (40 mL) and water (20 mL). The organic phase was dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure to afford an orange oil. The crude product was purified twice on silica gel columns [*n*-hexane:CH_2Cl_2 (5:3)] to afford the desired product as a yellow oil (87 mg). The oil was recrystallized from EtOH and water to yield the single compound **5-OBn** (56 mg).

5-OBn (6.98 mg, 0.019 mmol) and pentamethylbenzene (8.54 mg, 3.0 eq.) were dissolved in CH_2Cl_2 (15 mL), and a solution of 1 M boron trichloride in CH_2Cl_2 (0.3 mL, 16 eq.) was added at -78 °C under an argon atmosphere.[39] After the addition, the mixture was quenched with water (10 mL) and the CH_2Cl_2 phase was separated. The organic phase was dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure to yield an orange oil. The crude product was purified on a silica gel column [*n*-hexane:CH_2Cl_2 (5:3)] to give the single compound **5** (4.4 mg).

6-Benzyloxy-2,2-dimethyl-5-nitro-3,4-dihydro-2*H*-benzo[*h*]chromene (5-OBn): yellow needle crystals; yield: 34%; mp 78–79 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.26 (m, 1H), 8.09 (m, 1H), 7.60-7.30 (m, 7H), 5.14 (s, 2H), 2.80 (t, *J*= 6.6 Hz, 2H), 1.92 (t, *J*= 6.8 Hz, 2H), 1.46 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 146.3, 142.8, 139.4, 136.5, 128.6, 128.5, 128.2, 127.4, 127.3, 127.0, 126.9, 122.8, 122.5, 106.0, 78.5, 75.2, 31.8, 26.7, 18.6; HRMS (FAB-positive): 363.1469 (calcd for $C_{22}H_{21}NO_4$ 363.1471).

5-Nitro-2,2-dimethyl-3,4-dihydro-2*H*-benzo[*h*]chromen-6-ol (5): orange solid; yield: 84%; mp 93–94 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.0 (s, 1H), 8.44 (d, *J*= 8.3 Hz, 1H), 8.21 (d, *J*= 8.6 Hz, 1H), 7.68 (m, 2H), 3.12 (t, *J*= 6.8 Hz, 2H), 1.85 (t, *J*= 6.8 Hz, 2H), 1.44 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 150.9, 142.8, 131.0, 129.9, 129.6, 127.0, 124.8, 124.0, 122.1, 107.1, 74.1, 32.7, 26.4, 22.0; HRMS (FAB-positive): 273.1000 (calcd for $C_{15}H_{15}NO_4$ 273.1001).

Synthesis of **6**

2-Bromo-1,4-naphthoquinone (5.0 g, 0.02 mol) was dissolved in THF (50 mL), and zinc powder (2.1 g, 1.6 eq.) was added.[40] After the addition of 1-bromo-3-methyl-2-butene (3.5 mL, 1.5 eq.) at 40 °C for 35 min, the mixture was stirred for 21 h under nitrogen gas flow. After cooling the mixture to room temperature, 0.1 M hydrochloric acid was added, and the mixture was filtered. The filtrate was extracted with *n*-hexane (40 mL×5). The combined organic phase was washed with water (20 mL×2), dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure to afford a black solid. The crude product was purified twice on silica gel columns [cyclohexane:CH_2Cl_2 (1:1) and cyclohexane:

CH_2Cl_2 (3:1)] to afford the desired product as the single compound (236 mg). The yellow solid was recrystallized from *n*-hexane to yield the single compound 2-bromolapachol (99 mg).

2-Bromolapachol (100 mg, 0.33 mmol) was dissolved in 1,4-dioxane (25 mL), and anhydrous tin chloride (188 mg, 3.0 eq.) and conc. hydrochloric acid (0.24 mL) were added. After the mixture was refluxed under nitrogen gas flow for 3 h, the mixture was extracted with *n*-hexane (30 mL×3). The combined organic phase was washed with water (30 mL), dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure to afford a yellow oil. The crude product was dissolved in pyridine (2.0 mL), acetic anhydride (0.12 mL, 4.0 eq.) was added, and the mixture was stirred for 2.4 h at room temperature under an argon atmosphere. The reaction mixture was poured into crushed ice, and extracted with CH_2Cl_2 (20 mL×3). The combined organic phase was washed with 1 M hydrochloric acid (15 mL), saturated aq. sodium bicarbonate (10 mL), and water. The organic phase was dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure to afford a yellow oil. The crude product was purified on a silica gel column [*n*-hexane:CH_2Cl_2 (3:2)] to yield a white solid (58 mg). The solid was recrystallized from EtOH and water to yield the single compound **6-OAc** (21 mg).

6-OAc (10.1 mg, 0.03 mmol) was dissolved in MeOH (0.2 mL), and a 40 mM sodium methoxide in MeOH (0.79 mL, 1.1 eq.) was added. The mixture was stirred at room temperature under an argon atmosphere. After 10 min, more of the 40 mM sodium methoxide in MeOH (0.08 mL, 0.15 eq.) was added, and the mixture was stirred for 10 min. The reaction mixture was neutralized with 30 mM hydrochloric acid (1.6 mL), and extracted with CH_2Cl_2 (1 mL×5). The combined organic phase was concentrated under reduced pressure to afford a yellow oil. The crude product was immediately purified on a short silica gel column [$CHCl_3$:*n*-hexane (3:5)] to give the single compound **6** (8.6 mg).

2-Bromolapachol: yellow needle crystals; yield: 4%; mp 88.5–89.0 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.13 (m, 2H), 7.74 (m, 2H), 5.11 (m, 1H), 3.58 (d, *J*= 7.1 Hz, 2H), 1.84 (s, 3H), 1.71 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 181.7, 178.0, 150.5, 138.6, 135.6, 134.1, 133.8, 131.6, 131.2, 127.5, 127.1, 117.3, 31.0, 25.8, 18.4; HRMS (EI): 304.0098 and 306.0075 (calcd for $C_{15}H_{13}{}^{79}BrO_2$ 304.0099).

6-Acetoxy-5-bromo-2,2-dimethyl-3,4-dihydro-2*H*-benzo[*h*]chromene (6-OAc): white needle crystals; yield: 50%; mp 100–101 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.21 (m, 1H), 7.67 (m, 1H), 7.48 (m, 2H), 2.88 (t, *J*= 6.6 Hz, 2H), 2.49 (s, 3H), 1.92 (t, *J*= 6.6 Hz, 2H), 1.43 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.9, 147.9, 137.1, 127.0, 126.6, 125.7, 125.2, 122.3, 120.7, 117.0, 114.2, 74.8, 32.8, 26.6, 24.3, 20.7; HRMS (EI): 348.0363 (calcd for $C_{17}H_{17}{}^{79}BrO_3$ 348.0361).

5-Bromo-2,2-dimethyl-3,4-dihydro-2*H*-benzo[*h*]chromen-6-ol (6): colorless oil; yield: 85%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.15 (m, 2H, Ar-H), 7.48 (m, 2H, Ar-H), 2.83 (t, *J*= 6.8 Hz, 2H, CH_2), 1.91 (t, *J*= 6.8 Hz, 2H, CH_2), 1.40 (s, 6H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 143.3, 141.5, 125.9, 125.8, 125.3, 123.2, 122.0, 121.7, 113.1, 107.4, 74.0, 33.0, 26.5, 24.3; HRMS (FAB-negative): [M^+-1] 305.0175 (calcd

for $C_{15}H_{15}{}^{79}BrO_2$ 306.02554).

Synthesis of **7**

2-Chloro-1,4-naphthoquinone (3.0 g, 0.02 mol) was dissolved in THF (40 mL), and zinc powder (2.1 g, 1.6 eq.) was added.[40] After the addition of 1-bromo-3-methyl-2-butene (2.3 mL, 1.5 eq.) under an argon atmosphere at room temperature for 15 h, the mixture was heated to 50 °C for 1.25 h and then refluxed for 4 h under nitrogen gas flow. The reaction mixture was cooled to room temperature and poured into crushed ice, 1 M hydrochloric acid (40 mL) was added, and the mixture was then filtered. The filtrate was extracted with *n*-hexane (40 mL×3). The combined organic phase was washed with water (30 mL), dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure to afford a mixture of a yellow solid and yellow oil. The crude product was purified twice on silica gel columns [cyclohexane: CH_2Cl_2 (1:1) and cyclohexane:CH_2Cl_2 (3:1)] to give the single 2-chlorolapachol (286 mg).

2-Chlorolapachol (100 mg, 0.38 mmol) was dissolved in 1,4-dioxane (30 mL), and anhydrous tin chloride (216 mg, 3.0 eq.) and conc. hydrochloric acid (0.28 mL) were added. After the mixture was refluxed for 1.5 h under nitrogen gas flow, the reaction mixture was extracted with *n*-hexane (30 mL×3). The combined organic phase was washed with water (30 mL), dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure to afford a yellow oil. The crude product was dissolved in pyridine (2.0 mL), and acetic anhydride (0.14 mL, 4.0 eq.) was added. The mixture was stirred for 2.5 h at room temperature under an argon atmosphere. The reaction mixture was poured into crushed ice, extracted with CH_2Cl_2 (20 mL×3). The combined organic phase was washed with 1 M hydrochloric acid (15 mL), saturated aq. sodium bicarbonate (15 mL), and water (20 mL). The organic phase was dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure to afford a yellow oil. The crude product was purified on a silica gel column [*n*-hexane:CH_2Cl_2 (3:2)] to yield a white solid (44 mg). The solid was recrystallized from EtOH and water to give the single compound **7-OAc** (18 mg).

7-OAc (5.0 mg, 0.02 mmol) was dissolved in MeOH (0.2 mL), and a 30 mM sodium methoxide in MeOH (0.73 mL, 1.1 eq.) was added. After 15 min, more of the 30 mM sodium methoxide in MeOH (0.07 mL, 0.1 eq.) was added, and the mixture was stirred at room temperature for 20 min under an argon atmosphere. The reaction mixture was neutralized with 30 mM hydrochloric acid (0.8 mL) and extracted with CH_2Cl_2 (1 mL×4). The combined organic phase was concentrated under reduced pressure to afford a purple oil. The crude product was immediately purified on a short silica gel column [$CHCl_3$:*n*-hexane (3:5)] to give the single compound **7** (5.5 mg).

6-Acetoxy-5-chloro-2,2-dimethyl-3,4-dihydro-2*H*-benzo[*h*]chromene (7-OAc): white needle crystals; yield: 38%; mp 106–107 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.20 (dd, *J*= 0.79, 8.0 Hz, 1H), 7.67 (dd, *J*= 2.0, 8.0 Hz, 1H), 7.45 (m, 2H), 2.90 (t, *J*= 6.8 Hz, 2H), 2.49 (s, 3H), 1.92 (t, *J*= 6.8 Hz, 2H), 1.43 (s, 6H);

^{13}C NMR (100 MHz, $CDCl_3$) δ 168.9, 147.9, 135.6, 127.0, 126.5, 125.6, 124.7, 124.6, 122.2, 120.5, 113.0, 74.8, 32.4, 26.7, 21.4, 20.6; HRMS (EI): 304.0863 (calcd for $C_{17}H_{17}ClO_3$ 304.0866).

5-Chloro-2,2-dimethyl-3,4-dihydro-2*H*-benzo[*h*]chromen-6-ol (7): colorless oil; yield: 90%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.13 (m, 2H), 7.47 (m, 2H), 2.86 (t, *J*= 6.8 Hz), 1.92 (t, *J*= 6.8 Hz, 2H), 1.40 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 143.2, 140.3, 125.8, 125.7, 124.9, 123.3, 121.7, 121.6, 114.4, 112.1, 74.0, 32.6, 26.5, 21.5; HRMS (FAB-negative): [M^+-1] 261.0681 (calcd for $C_{15}H_{14}{}^{35}ClO_2$ 262.07606).

Synthesis of **8**

A solution of sodium dithionite (11.0 g, 64 mmol) in water (100 mL) was shaken with a solution of 2-methyl-1,4-naphthoquinone (1.00 g, 5.8 mmol) in EtOAc (60 mL) in a separatory funnel until the yellow color in the organic phase disappeared.[41] The organic phase was separated, and the aqueous phase was extracted with EtOAc (20 mL). The combined organic phase was dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure to give the single compound 2-methyl-1,4-naphthalenediol as a purple solid (1.02 g).

2-Methyl-1,4-naphthalenediol (750 mg, 4.3 mmol) and ascorbic acid (185 mg) were dissolved in 70% formic acid (25 mL). 2-Methyl-3-buten-2-ol (0.5 mL, 1.0 eq.) was added over 60 min at 90 °C under nitrogen gas flow, and the mixture was stirred for 1 h. After cooling the reaction mixture to room temperature, the mixture was extracted with *n*-hexane (30 mL×3). The combined organic phase was washed with saturated aq. sodium carbonate (30 mL×2) and water, dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure to afford a brown oil (823 mg). The crude product was dissolved in EtOH (10 mL), and potassium carbonate (894 mg, 1.5 eq.) and benzyl bromide (0.51 mL, 1.0 eq.) were added. The mixture was stirred overnight under an argon atmosphere. The mixture was poured into crushed ice and extracted with *n*-hexane (20 mL×4). The combined organic phase was dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure to afford an orange oil (1.08 g). The crude product was purified on a silica gel column [*n*-hexane:CH_2Cl_2 (3:1)] to give the single compound **8-OBn** (130 mg).

8-OBn (22.9 mg, 0.069 mmol) was dissolved in MeOH (3.0 mL), and 5% palladium/carbon (49 mg) was added. The mixture was stirred at room temperature under a hydrogen atmosphere for 15 min. The mixture was filtered through Celite with suction, and the filter cake was washed with CH_2Cl_2. The filtrate was concentrated under reduced pressure to afford a yellow oil. The crude product was immediately purified on a short silica gel column (2% MeOH/CH_2Cl_2) to give the single compound **7** (11 mg).

6-Benzyloxy-2,2,5-trimethyl-3,4-dihydro-2*H*-benzo[*h*]chromene (8-OBn): tan yellow oil; yield: 9%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.21 (m, 1H), 8.03 (m, 1H), 7.57 (m, 2H), 7.41 (m, 5H), 4.93 (s, 2H), 2.75 (t, *J*= 6.6 Hz, 2H), 2.33 (s, 3H), 1.92 (t, *J*= 6.6 Hz, 2H), 1.41 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ

145.2, 144.9, 137.9, 128.5, 127.9, 127.8, 127.2, 126.0, 125.6, 125.1, 124.4, 121.9, 121.4, 114.5, 75.6, 73.4, 32.9, 26.7, 21.1, 12.3; HRMS (FAB-positive): 332.1774 (calcd for $C_{23}H_{24}O_2$ 332.1776).

2,2,5-Trimethyl-3,4-dihydro-2*H*-benzo[*h*]chromen-6-ol (8): white solid; yield: 67%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.17 (d, *J*= 7.8 Hz, 1H), 7.99 (d, *J*= 7.6 Hz, 1H), 7.42 (m, 2H), 4.61 (s, 1H), 2.76 (t, *J*= 6.8 Hz, 2H), 2.30 (s, 3H), 1.92 (t, *J*= 6.8 Hz, 2H), 1.39 (s, 6H).

Synthesis of **9**

5 (1.4 mg, 5.0 μmol) was dissolved in MeOH (0.8 mL), and 5% palladium/carbon (4.5 mg) was added. The mixture was stirred at room temperature under a hydrogen atmosphere. After 20 min, the reaction mixture was filtered through a syringe filter (0.22 μm, hydrophobic PTFE). The filtrate was adjusted to a total volume of 5 mL (final concentration of 1 mM) and immediately used in the measurement of the galvinoxyl radical scavenging activity.

^{1}H and ^{13}C NMR data could not be obtained owing to the compound's high instability in air. HRMS (FAB-positive): 243.1255 (calcd for $C_{15}H_{17}NO_2$ 243.1259).

Kinetics measurements[23,24]

Benzo[*h*]chromanols and G$^\bullet$ in equimolar concentrations (final concentration of 5.0 μM) were dissolved in MeCN that had been deaerated with argon. One syringe was loaded with 2 mL of a solution of each benzo[*h*]chromanol, and another syringe was loaded with the G$^\bullet$ solution. The pneumatic drive accessory initiated mixing after the initiation of data acquisition by the spectrophotometer at 300–600 nm. Half of the starting amount of the G$^\bullet$ solution and antioxidant had been used after mixing. The radical scavenging rates were determined by monitoring the changes in absorbance due to the galvinoxyl radical at 428 nm at 25 °C. Second-order rate plots of the absorbance $(A_0–A)$ / $(A–A_\infty)$ versus time, where A, A_0 and A_∞ refer to the absorbance at a given time, the initial absorbance and the final absorbance, respectively. To avoid the influence of the minor absorption from the G$^\bullet$ reduction products at this wavelength, only the first G$^\bullet$ absorption decay values were used in the kinetics analyses. The slope of the line was determined using the least-squares method. The second-order rate constants (k) for the reactions were calculated from the slopes of the linear functions by dividing by the initial concentration. The data were collected in at least two different experimental sessions. The radical scavenging activity of the compounds was evaluated immediately after they were deprotected.

ACKNOWLEDGMENTS

This work was supported by JSPS KAKENHI Grant Number JP26460734.

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