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SYNTHESIS AND RADICAL SCAVENGING ACTIVITY OF SUBSTITUTED DIHYDROBENZOFURAN-5-OLS

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Abstract – Antioxidants are important in preventing oxidative stress by scavenging oxygen free radicals. In this study, a series of 2,2-dimethyl-2,3-dihydrobenzofuran-5-ols with amino, methoxy, chloro and nitro groups at the *ortho* position relative to the phenolic OH group were newly synthesized, and their galvinoxyl and hydroxyl radical scavenging activities were measured. Substituted 2,3-dihydrobenzofuran-5-ols showed higher activity than the corresponding 6-chromanols, especially 6-amino-2,2-dimethyl-2,3-dihydrobenzofuran-5-ol, which possessed the highest activity among the tested compounds. The results demonstrated that the planarity and electron-donating capacity of these molecules enhanced their radical scavenging activities, as is known for 6-chromanols.

INTRODUCTION

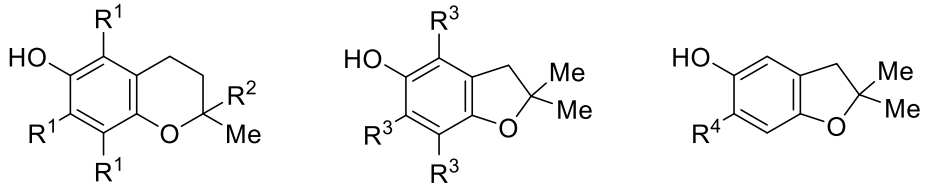
Reactive oxygen species (ROS) are products of normal cellular metabolism under physiological conditions.[1] ROS are usually scavenged by antioxidants; however, overproduction of ROS induced by oxidative stress is associated with chronic diseases such as cancer and cardiovascular, neurodegenerative, and inflammatory diseases.

α-Tocopherol (**1**) has been shown to be the major lipid-soluble chain-breaking antioxidant in the human body and is considered one of the most important free radical scavenging antioxidants in foods and other biological tissues.[2]

We have reported the synthesis of 6-chromanols[3,4] and 6-benzo[*h*]chromanols[5] with substituents (amino, methyl, chloro, and nitro moieties) on the aromatic ring and the evaluation of their radical scavenging

activities, which were shown to be well correlated with the electron-donating ability of the substituent on the benzene ring in 6-chromanols.

2,3-Dihydrobenzofuran-5-ols has been shown to be a good free radical scavenger in various systems.[6] In addition, 2,3-dihydrobenzofuran-5-ols have proven to be effective antioxidants as lipid peroxidation inhibitors,[7] leukotriene biosynthesis inhibitors,[8] and antiproliferative agents.[9] Moreover, 2,3-dihydrobenzofuran-5-ols occur in natural products, and their analogs are thought to have low toxicity.[10] In most previous studies, the only substituents tested were methyl, isopropyl and *tert*-butyl groups at different positions in 2,3-dihydrobenzofuran-5-ol.[11] Therefore, we report the preparation of substituted 2,2-dimethyl-2,3-dihydrobenzofuran-5-ols with nitro, chloro, methyl, methoxy, or amino groups at the 6 position, the evaluation of their radical scavenging activities toward galvinoxyl (G$^{\bullet}$) and hydroxyl radicals ($^{\bullet}$OH) and the discussion of factors promoting the enhancement of radical scavenging capacity (Figure 1).



1: R^1 = Me R^2 = $C_{16}H_{33}$
2: R^1 = Me R^2 = Me
3: R^1 = H R^2 = Me

4: R^3 = Me
5: R^3 = H

6: R^4 = NO_2
7: R^4 = Cl
8: R^4 = Me
9: R^4 = OMe
10: R^4 = NH_2

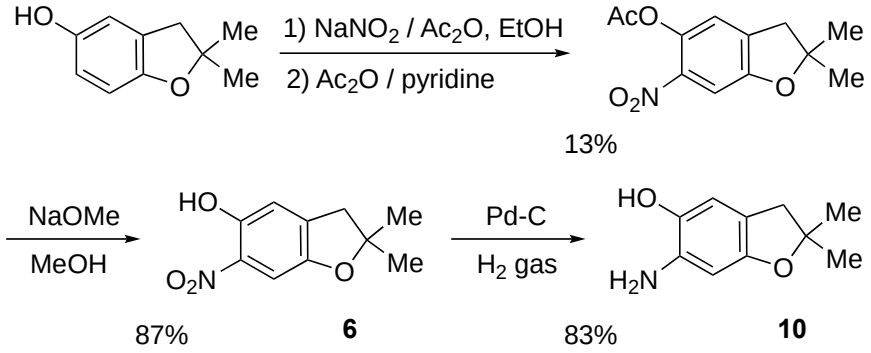
Figure 1. Structure of 6-chromanols and 2,3-dihydrobenzofuran-5-ols

RESULTS AND DISCUSSION

To evaluate the effect of substituents on the radical scavenging activity of 2,3-dihydrobenzofuran-5-ols, **4** and **5** were used as lead compounds. Additionally, 6-membered **2** and **3** were used as controls for **1** since the phytyl side chain in **1** does not contribute to the radical scavenging activity.[12]

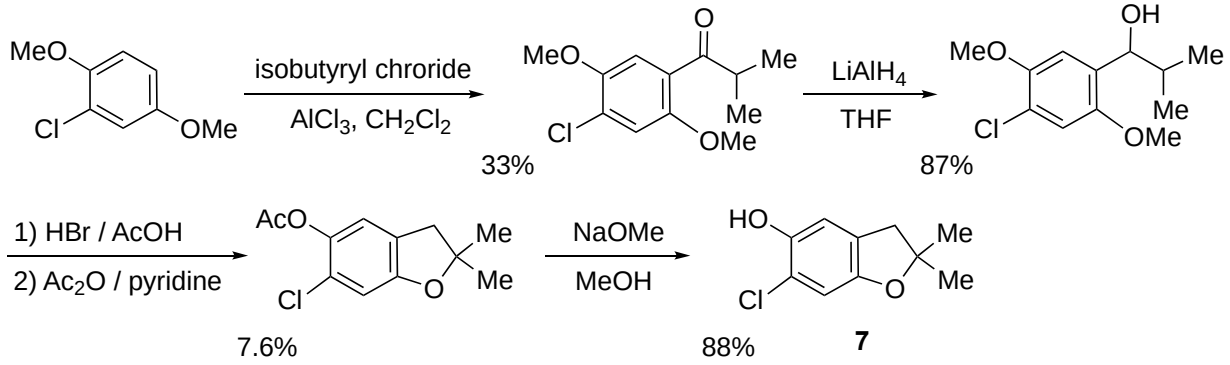
The radical scavenging activity of a series of substituted 6-chromanols was greatly enhanced by introducing electron-donating substituents at the *ortho* position relative to the phenolic OH group.[13] Therefore, we confirmed a similar tendency of the substituent effect in 2,3-dihydrobenzofuran-5-ols. Then, 2,3-dihydrobenzofuran-5-ols substituted with nitro (**6**), chloro (**7**), methyl (**8**), methoxy (**9**) and amino (**10**) groups at the *ortho* position were designed. **6**, **7**, **9**, **10**, acetoxylated **5** (**5-OAc**), **6-OAc**, **7-OAc**, **8-OAc** and **9-OAc** were new compounds.

In the preparation of nitrogen-containing 2,3-dihydrobenzofuran-5-ols (**6**, **10**), non-substituted **5** was nitrated with $NaNO_2$ and Ac_2O, followed by acetylation for purification. **6-OAc** was hydrolyzed by sodium methoxide, and then **6** was reduced by hydrogen gas in the presence of palladium/carbon to give the corresponding amino compound (**10**) (Scheme 1).

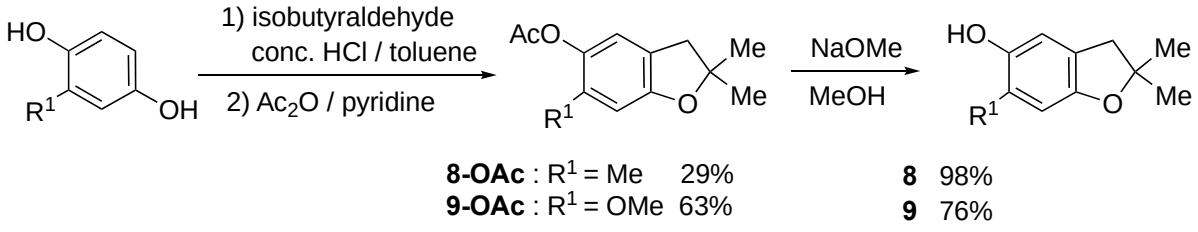


Scheme 1. Synthesis of **6** and **10**

In the preparation of **7**, 1-chloro-2,5-dimethoxybenzene was condensed with isobutyryl chloride in the presence of $AlCl_3$, reduced to the corresponding alcohol by $LiAlH_4$, and then cyclized under acidic conditions (Scheme 2). 2-Methyl or 2-methoxyhydroquinone reacted with isobutyraldehyde under acidic conditions to form **8** or **9**, respectively (Scheme 3). The crude products were acetylated for purification and storage.



Scheme 2. Synthesis of **7**



Scheme 3. Synthesis of **8** and **9**

6-OAc, **7-OAc**, **8-OAc** and **9-OAc** were deprotected using NaOMe and purified on a short silica gel column, and radical scavenging activities were measured immediately. All procedures were carried out under an argon atmosphere.

The abilities of a series of 2,3-dihydrobenzofuran-5-ols to scavenge G$^{\bullet}$ were evaluated in MeCN. G$^{\bullet}$ is a stable and commercially available radical with a strong absorption band at 428 nm.[14] Therefore, rate constant data for radical scavenging of 2,3-dihydrobenzofuran-5-ols can obtain accurately without decomposition of compounds in argon-deaerated organic solvent. G$^{\bullet}$ was presumed to react with phenolic OH in 2,3-dihydrobenzofuran-5-ols via the hydrogen atom transfer mechanism as 6-chromanols.[4] Upon the addition of the test compound to deaerated G$^{\bullet}$ solutions in MeCN, the intensity of the absorption band at 428 nm rapidly decreased. The decay of the absorbance at 428 nm obeyed pseudo-first-order reaction kinetics when the compound concentration was greater than a 10-fold excess compared to the concentration of G$^{\bullet}$. The observed pseudo-first-order rate constant (k_{obs}) was linearly concentration-dependent (Figures S1–S3 in Supporting Information). Second-order rate constant (k) values for the 6-substituted 2,3-dihydrobenzofuran-5-ols and G$^{\bullet}$ reactions were obtained from the slopes of the linear functions of k_{obs} versus the compound concentration (Table 1).

Table 1. Radical scavenging activity of 6-substituted 2,3-dihydrobenzofuran-5-ols toward G$^{\bullet}$ and $^{\bullet}$OH

Compound	Second-order rate constant k (M^{-1} s^{-1}) [a]	Maximum inhibition % [b] (mM) [c]	
1	3,612	10.0	(0.1)
2	2,841	37.9	(3.0)
3	263	66.3	(30.0)
4	5,544	62.2	(4.5)
5	362	79.7	(60.0)
6 (nitro)	ND [d]	28.3	(1.5)
7 (chloro)	303	68.0	(12.0)
8 (methyl)	1,996	66.6	(22.5)
9 (methoxy)	3,688	91.6	(20.0)
10 (amino)	3,178,000	92.5	(1.5)

a: Reaction of G$^{\bullet}$ with 2,3-dihydrobenzofran-5-ols
b: Maximum inhibition (%) of DMPO-OH adduct
c: Concentration of compounds at the maximum inhibition (%)
d: ND: Not detected because the UV absorbance at 428 nm did not decrease.

The G$^{\bullet}$ scavenging activities of **4** and **5** were higher than those of corresponding **2** and **3**, respectively. The data indicated that the 2,3-dihydrobenzofuran-5-ol structure was a more efficient antioxidant than the 6-chromanol structure, as previously reported.[6] Compounds **8**, **9** and **10** showed higher activity than **5**, indicating that the electron-donating substituent enhanced the radical scavenging activity. The G$^{\bullet}$

scavenging activities of the substituted 2,3-dihydrobenzofuran-5-ols were in the order of **10** >> **9** > **8** > **7**, and **6** did not scavenge G•. The G• scavenging activity was completely dependent on the electron-donating capacity of the substituent for the 2,3-dihydrobenzofuran-5-ols, similar to 6-chromanols.

•OH is highly reactive and reacts with biological molecules such as DNA, protein, and lipid compounds, resulting in their chemical modification.[15] Compound **1** exerts antioxidant effects by scavenging lipid peroxyl radicals *in vivo* as well as *in vitro*; however, **1** is not an efficient scavenger of •OH *in vivo*.[16] To investigate the reaction of •OH with these synthesized compounds, the electron spin resonance (ESR) spin-trapping technique was used. The Fenton reaction ($Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + {}^{-}OH + {}^{\bullet}OH$) was the source of •OH, and 5,5-dimethyl-1-pyrroline *N*-oxide (DMPO) was used as the •OH scavenging agent.[17,18] The capacity of the •OH scavenging activity is presented as the inhibition percentage (%) relative to the intensity of the DMPO-OH adduct. The maximum inhibition (%) and the concentration at the maximum inhibition (%) are presented in Table 1 (Figures S4–S6 in the Supporting Information). All of the 2,3-dihydrobenzofuran-5-ols inhibited the formation of the DMPO-OH adducts, indicating that the compounds could scavenge radicals in aqueous solutions. Compound **6** did not exhibit G• scavenging activity; however, **6** reacted with •OH but with low activity. The low •OH scavenging activities of **6** and **2** were caused by their low solubility in phosphate buffer. The maximum inhibitions (%) of **7** and **8** were approximately 70%. Compounds **9** and **10** efficiently inhibited the formation of DMPO-OH at concentrations of 20 mM and 1.5 mM, respectively. Compound **1** has been reported to react directly with radicals to donate H atoms and form phenoxyl radicals.[19] The stability of the phenoxyl radicals is important to achieve high radical scavenging activity.[13,20] In particular, **10** exhibited excellent radical scavenging activities, indicating that the corresponding phenoxyl radical is stable. This stability was achieved because the five-membered ring in the dihydrobenzofuran system provides a better orientation of the lone pair(s) ring oxygen atom for stabilization of the resulting phenoxyl radical than that in 6-chromanols; therefore, the phenoxyl radicals derived from 2,3-dihydrobenzofuran-5-ols were more stabilized by conjugate delocalization.[21,22] The effect of the substituents on the radical scavenging activity was examined, and a lone pair on the nitrogen atom in the amino group was thought to also be donated to the phenoxyl radical. Thus, both the five-membered structure and the electron-donating substituent stabilized the phenoxyl radical and enhanced their radical scavenging activity. Our previous study showed that 5-aminobenzo[*h*]chroman-6-ol, which fused another aromatic ring and introduced an electron-donating amino group into the 6-chromanol structure, possessed high radical scavenging activity.[5] Taken together, these results suggest that the planarity and electron donating capacity of these compounds enhanced their radical scavenging activities.

CONCLUSION

We successfully synthesized a series of 2,3-dihydrobenzofuran-5-ols and showed that the compounds possessed more potent radical scavenging capacity than 6-chromanols. Compounds possessing high radical scavenging activities could contribute to antioxidant therapeutics for rheumatoid arthritis, Alzheimer's disease, Parkinson's disease, cancer, etc.[23]

EXPERIMENTAL

Materials and Methods

Melting points were determined using a Yanaco micromelting point apparatus without correction (Tokyo, Japan). Nuclear magnetic resonance (NMR) spectra were recorded on a JEOL ECZ-600R spectrometer (Tokyo, Japan). Chemical shifts were expressed in ppm and were shifted downfield from the TMS peak. High-resolution mass spectra were collected using a JEOL AccuTOF LC-plus 4G mass spectrometer (Tokyo, Japan). UV-Vis spectrophotometric data were obtained using a Unisoku RSP-2000-03TI spectrophotometer (Osaka, Japan). ESR spectra were recorded on a JEOL JES-X320 (Tokyo, Japan). The 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 (0.040–0.063 mm, Merck).

Materials. Compounds **3** [mp 75.3–76.0 °C (75–76 °C)[24]], **4** [mp 123.0–126.0 °C (122–123 °C)[21]], and **5** [mp 101.0–102.0 °C (98–99 °C)[25]] were prepared according to a previously reported procedure. Aluminum chloride, 2,5-dimethoxybenzene, galvinoxyl, hydrobromic acid, isobutyraldehyde, isobutyryl chloride, methoxyhydroquinone, methylhydroquinone, and sodium methoxide were obtained from Tokyo Kasei Kogyo Co., Ltd. (Tokyo, Japan). Chloroform-*d* (0.03 vol.% TMS) was obtained from Cambridge Isotope Laboratories, Inc. (Tewksbury, MA, USA). MeCN, which was used for spectral measurements, and ethylenediamine-*N*,*N*,*N'*,*N'*-tetraacetic acid (EDTA) disodium salt dihydrate were obtained from Dojindo Laboratories (Kumamoto, Japan). DMPO was purchased from Labotec Co., Ltd. (Tokyo, 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.

Preparation of 2,2-dimethyl-2,3-dihydrobenzofuran-5-yl acetate (**5-OAc**): Compound **5** was prepared according to the method reported by Lars *et al*.[25] The crude extract of **5** (4.18 g) was stirred with acetic anhydride and pyridine at room temperature overnight. The mixture was poured onto crushed ice and extracted with CH_2Cl_2 (15 mL×3). The combined organic phase was washed with 10% aq. $NaHCO_3$ (15 mL×2) and water (15 mL×2). The organic phase was washed with water, dried over anhydrous sodium sulfate, filtered, and evaporated under reduced pressure to afford a reddish oil. The crude product was

purified on a silica gel column (*n*-hexane:AcOEt = 9:1) to give **5-OAc** (1.63 g, 39%) as a tan-yellow oil; ^{1}H NMR (600 MHz, $CDCl_3$): δ 6.86 (*dd*, *J* = 1.4 Hz, 1H, Ar-H), 6.78 (*dd*, *J* = 2.4, 8.6 Hz, 1H, H6), 6.67 (*d*, *J* = 9.0 Hz, 1H, Ar-H), 3.01 (s, 2H, CH_2), 2.26 (s, 3H, Ac), 1.47 (s, 6H, Me); ^{13}C NMR (150 MHz, $CDCl_3$): δ 170.2, 156.6, 143.9, 128.1, 120.7, 118.5, 109.4, 87.3, 43.0, 28.2, 21.1; HRMS (ESI-positive): 207.10352 (calcd for $C_{12}H_{15}O_3$: 207.10157).

Preparation of 2,2-dimethyl-6-nitro-2,3-dihydrobenzofuran-5-yl acetate (**6-OAc**): 2% $NaNO_2$ (2.5 mL, 1.0 eq.) was added to a solution of **5** (164 mg, 1.0 mmol) in EtOH (30 mL) and acetic anhydride (5.0 mL), and stirred for 0.5 h under an argon atmosphere.[18] Then, more 2% $NaNO_2$ (2.5 mL, 1.0 eq.) was added and stirred for an additional 1 h. The mixture was poured onto crushed ice and extracted with CH_2Cl_2 (10 mL×3). The combined organic phase was washed with saturated aq. $NaHCO_3$ (10 mL×2) and water (10 mL×2), dried over anhydrous sodium sulfate, filtered, and evaporated under reduced pressure to afford an orange oil. Pyridine (2.0 mL) and acetic anhydride (1.0 mL) were added to the crude product, and the mixture was stirred at room temperature for 2 h under a nitrogen gas flow. The mixture was poured onto crushed ice and extracted with CH_2Cl_2 (10 mL×3). The combined organic phase was washed with 1 M HCl (10 mL×2), saturated aq. $NaHCO_3$ (10 mL×2), and water (10 mL). The organic phase was dried over anhydrous sodium sulfate and filtered, and the solvent was evaporated under reduced pressure to afford an orange solid. The crude product was purified on a silica gel column (CH_2Cl_2) to give **6-OAc** (32 mg, 13%) as an orange solid. The solid was recrystallized from EtOH and water to give orange needles; mp 87.0–89.0 °C; ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.38 (s, 1H, Ar-H), 6.97 (*d*, *J* = 1.4 Hz, 1H, Ar-H), 3.07 (*d*, *J* = 1.4 Hz, 2H, CH_2), 2.34 (s, 3H, Ac), 1.50 (s, 6H, Me); ^{13}C NMR (150 MHz, $CDCl_3$): δ 169.4, 156.8, 141.2, 137.8, 135.1, 121.4, 105.9, 89.2, 42.7, 28.1, 20.8; HRMS (ESI-positive): 252.08743 (calcd for $C_{12}H_{14}NO_5$: 252.08665).

Preparation of 6-chloro-2,2-dimethyl-2,3-dihydrobenzofuran-5-yl acetate (**7-OAc**): 1-(4-Chloro-2,5-dimethoxyphenyl)-2-methylpropan-1-one: Isobutyryl chloride (2.8 mL, 1.3 eq.) was added to a solution of $AlCl_3$ (2.13 g, 0.8 eq.) in CH_2Cl_2 (40 mL) under a nitrogen gas flow. After a solution of 1-chloro-2,5-dimethoxybenzene (2.8 mL, 0.02 mmol) in CH_2Cl_2 (10 mL) was added for 40 min, the reaction mixture was refluxed for 1 h. The mixture was poured onto crushed ice, 2 M HCl (20 mL) was added, and the mixture was extracted with CH_2Cl_2 (20 mL×2). The combined organic phase was washed with water (20 mL). The organic phase was dried over anhydrous sodium sulfate and filtered, and the solvent was evaporated under reduced pressure to afford an orange oil. The crude product was purified on a silica gel column (*n*-hexane:CH_2Cl_2 = 2:1) to give 1-(4-chloro-2,5-dimethoxyphenyl)-2-methylpropan-1-one (1.59 g, 33%) as a colorless oil; ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.22 (s, 1H, Ar-H), 7.01 (s, 1H, Ar-H), 3.88 (s, 3H, OMe), 3.86 (s, 3H, OMe), 3.52 (sep, *J* = 6.9 Hz, 1H, CH), 1.15 (s, 3H, Me), 1.14 (s, 3H, Me).

1-(4-Chloro-2,5-dimethoxyphenyl)-2-methylpropan-1-ol: A solution of 1-(4-chloro-2,5-dimethoxyphenyl)-2-methylpropan-1-one (2.1 g, 3.4 mmol) in THF (10 mL) was added dropwise to a solution of $LiAlH_4$ (81 mg, 1.0 eq.) in 5 mL of THF for 30 min under a nitrogen gas flow and the mixture was stirred for 30 min at room temperature. Then, more $LiAlH_4$ (81 mg, 1.0 eq.) was added and stirred for an additional 1 h. The mixture was poured onto crushed ice, 2 M HCl (20 mL) was added, and the mixture was extracted with CH_2Cl_2 (20 mL×3). The combined organic phase was dried over anhydrous sodium sulfate and filtered, and the solvent was evaporated under reduced pressure to afford a pale, yellow oil. The crude product was purified on a silica gel column ($CHCl_3$) to give 1-(4-chloro-2,5-dimethoxyphenyl)-2-methylpropan-1-ol (1.80 g, 87%) as a light yellow oil; ^{1}H NMR (600 MHz, $CDCl_3$): δ 6.94 (s, 1H, Ar-H), 6.89 (s, 1H, Ar-H), 4.56 (t, *J* = 6.6 Hz, 1H C<u>H</u>OH), 3.86 (s, 3H, OMe), 3.78 (s, 3H, OMe), 2.33 (d, *J* = 6.2 Hz, 1H, OH), 2.03 (sep, *J* = 6.2 Hz, 1H, CH), 1.00 (d, *J* = 6.9 Hz, 3H, Me), 0.83 (d, *J* = 6.9 Hz, 3H, Me); ^{13}C NMR (150 MHz, $CDCl_3$): δ 150.7, 149.1, 131.4, 120.9, 113.2, 112.5, 75.6, 56.9, 56.0, 34.2, 19.4, 18.0; HRMS (DART-positive): 245.09425 (calcd for $C_{12}H_{18}O_3Cl$: 245.09390).

6-Chloro-2,2-dimethyl-2,3-dihydrobenzofuran-5-yl acetate (**7-OAc**): A solution of 47% hydrobromic acid (1.0 mL) was added to a solution of 1-(4-chloro-2,5-dimethoxyphenyl)-2-methylpropan-1-ol (200 mg, 0.83 mmol) in AcOH (10 mL). The mixture was refluxed for 1.5 h under a nitrogen gas flow, poured onto crushed ice, and extracted with CH_2Cl_2 (20 mL×3). The combined organic phase was washed with saturated aq. $NaHCO_3$ (20 mL×2) and water (20 mL). The organic phase was dried over anhydrous sodium sulfate and filtered, and the solvent was evaporated under reduced pressure to afford a brown oil. Pyridine (2 mL) and acetic anhydride (2 mL) were added to the crude product, and the mixture was stirred at room temperature for 2 h under a nitrogen gas flow. The mixture was poured onto crushed ice and extracted with (20 mL×3). The combined organic phase was washed with saturated aq. $NaHCO_3$ (20 mL×2), water (20 mL), 1 M HCl (20 mL×2), and water (20 mL) successively. The organic phase was dried over anhydrous sodium sulfate and filtered, and the solvent was evaporated under reduced pressure to afford a brown oil. The oil was purified on a silica gel column (*n*-hexane:CH_2Cl_2= 1:1) to give **7-OAc** as a white solid. The solid was recrystallized from EtOH and water to obtain white needles (15 mg, 7.6%); mp 85.0–86.5 °C; ^{1}H NMR (150 MHz, $CDCl_3$): δ 6.88 (s, 1H, Ar-H), 6.77 (s, 1H, Ar-H), 2.99 (s, 2H, CH_2), 2.32 (s, 3H, Ac), 1.46 (s, 6H, Me); ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.2, 157.2, 140.0, 126.9, 125.5, 119.8, 110.7, 88.4, 42.6, 28.2, 20.6; HRMS (ESI-positive): 241.06401 (calcd for $C_{12}H_{14}O_3Cl$: 241.06260).

Preparation of 2,2,6-trimethyl-2,3-dihydrobenzofuran-5-yl acetate (**8-OAc**): Isobutyraldehyde (0.27 mL, 1.3 eq.) and conc. HCl (2.4 mL) were added to a solution of methylhydroquinone (271 mg, 2.17 mmol) in toluene (2.4 mL). The mixture was refluxed overnight under a nitrogen gas flow. The mixture was poured onto crushed ice and extracted with CH_2Cl_2 (15 mL×3). The combined organic phase was washed with

water, dried over anhydrous sodium sulfate, filtered, and evaporated under reduced pressure to afford a reddish oil. The crude product was dissolved in pyridine (2 mL), acetic anhydride (2.0 mL, 20 eq.) was added, and the mixture was stirred for 30 min at room temperature under an argon gas atmosphere. The mixture was poured onto crushed ice and extracted with CH_2Cl_2 (15 mL×3). The combined organic phase was washed with 10% aq. $NaHCO_3$ (10 mL×3) and water (10 mL×2), dried over anhydrous sodium sulfate, filtered, and evaporated under reduced pressure to afford a yellow oil. The crude product was purified on a silica gel column (*n*-hexane:CH_2Cl_2 = 3: 7) twice to give **8-OAc** (140 mg, 29%) as white needles; mp 45.3–45.6 °C; ^{1}H NMR (600 MHz, $CDCl_3$): δ 6.77 (s, 1H, Ar-H), 6.56 (s, 1H, Ar-H), 2.97 (s, 2H, CH_2), 2.29 (s, 3H, Ac), 2.10 (s, 3H, Ar-Me), 1.46 (s, 6H, Me); ^{13}C NMR (150 MHz, $CDCl_3$): δ 169.9, 156.7, 142.4, 129.4, 125.4, 118.5, 111.1, 87.2, 42.8, 28.2, 20.8, 16.4; HRMS (ESI-positive): 221.11946 (calcd for $C_{13}H_{17}O_3$: 221.11722).

Preparation of 6-methoxy-2,2-dimethyl-2,3-dihydrobenzofuran-5-yl acetate (**9-OAc**): Isobutyraldehyde (0.27 mL, 1.3 eq.) and conc. HCl (2.4 mL) were added to a solution of methoxyhydroquinone (304 mg, 2.17 mmol) in toluene (2.4 mL). The mixture was refluxed for 6 h under a nitrogen gas flow. The mixture was poured onto crushed ice and extracted with CH_2Cl_2 (15 mL×3). The combined organic phase was washed with water (15 mL×2), dried over anhydrous sodium sulfate, filtered, and evaporated under reduced pressure to afford a brown oil (630 mg). The crude product was dissolved in pyridine (2.0 mL), acetic anhydride (2.0 mL, 20 eq.) was added, and the mixture was stirred overnight at room temperature under an argon gas atmosphere. The mixture was poured onto crushed ice and appeared as a light pink solid. The solid was collected by suction filtration and washed with water. The solid was recrystallized from EtOH and water to give **9-OAc** (324 mg, 63%) as white needles; mp 97.8–98.3 °C; ^{1}H NMR (600 MHz, $CDCl_3$): δ 6.78 (s, 1H, Ar-H), 6.41 (s, 1H, Ar-H), 3.76 (s, 3H, OMe), 2.95 (s, 2H, CH_2), 2.29 (s, 3H, Ac), 1.46 (s, 6H, Me); ^{13}C NMR (150 MHz, $CDCl_3$): δ 169.8, 157.3, 151.0, 132.8, 118.9, 117.8, 95.1, 87.9, 56.0, 42.5, 28.3, 20.7; HRMS (ESI-positive): 237.11360 (calcd for $C_{13}H_{17}O_4$: 237.11214).

Hydrolysis of acetylated compounds (**5-OAc**, **6-OAc**, **7-OAc**, **8-OAc**, **9-OAc**): The acetylated compounds were hydrolyzed immediately before measuring radical scavenging activities. A solution of 0.1 M sodium methoxide (2.0 eq.) was added to a solution of each acetylated compounds (ca. 10 mg) in distilled MeOH (0.8 mL) under an argon atmosphere. The reaction mixture was stirred at room temperature under an argon atmosphere until the starting material was no longer visible on TLC. The reaction mixture was neutralized by the addition of 0.1 M hydrochloric acid and extracted four times with CH_2Cl_2. The combined organic phase was dried over anhydrous sodium sulfate and filtered, and the solvent was evaporated to afford a

crude product. The crude product was immediately purified on a short silica gel column to give the desired product as the single compound. The yields exceeded approximately 70% for all compounds.

2,2-Dimethyl-2,3-dihydrobenzofuran-5-ol (**5**): white needles; yield 89%; ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.66 (s, 1H, Ar-H), 6.57 (s, 1H, Ar-H), 4.34 (s, 1H, OH), 2.96 (s, 2H, CH_2), 1.46 (s, 6H, Me).

2,2-Dimethyl-6-nitro-2,3-dihydrobenzofuran-5-ol (**6**): orange needles; yield 87%; mp 85.0–86.0 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 10.61 (s, 1H, OH), 7.34 (s, 1H, Ar-H), 6.94 (*dd*, *J* = 1.4 Hz, Ar-H), 3.04 (*d*, *J* = 1.8 Hz, 2H, CH_2), 1.48 (s, 6H, Me); ^{13}C NMR (150 MHz, $CDCl_3$) δ 152.3, 151.0, 140.9, 132.5, 115.8, 102.7, 88.1, 43.2, 27.9; HRMS (ESI-negative) 208.06343 (calcd for $C_{10}H_{10}NO_4$: 208.06153).

6-Chloro-2,2-dimethyl-2,3-dihydrobenzofuran-5-ol (**7**): colorless oil; yield 88%; ^{1}H NMR (600 MHz, $CDCl_3$): δ 6.81 (s, 1H, Ar-H), 6.68 (S, 1H, Ar-H), 5.10 (s, 1H, OH), 2.95 (s, 2H, CH_2), 1.45 (s, 6H, Me); ^{13}C NMR (150 MHz, $CDCl_3$): δ 152.9, 145.1, 127.7, 117.9, 112.7, 109.2, 87.5, 42.9, 28.0; HRMS (ESI-negative): 197.03792 (calcd for $C_{10}H_{10}O_2Cl$: 197.03748).

2,2,6-Trimethyl-2,3-dihydrobenzofuran-5-ol (**8**): white solid; yield 98%; mp 98.8–99.3; ^{1}H NMR (600 MHz, $CDCl_3$): δ 6.61 (s, 1H, Ar-H), 6.50 (s, 1H, Ar-H), 4.32 (s, 1H, OH), 2.93 (s, 2H, CH_2), 2.19 (s, 3H, Ac), 1.44 (s, 6H, Me).

6-Methoxy-2,2-dimethyl-2,3-dihydrobenzofuran-5-ol (**9**): white solid; yield 76%; mp 84.7–85.1 °C; ^{1}H NMR (600 MHz, $CDCl_3$): δ 6.72 (s, 1H, Ar-H), 6.37 (s, 1H, Ar-H), 5.15 (s, 1H, OH), 3.83 (s, 3H, OMe), 2.93 (s, 2H, CH_2), 1.45 (s, 6H, Me); ^{13}C NMR (150 MHz, $CDCl_3$): δ 152.1, 146.1, 139.2, 117.8, 110.9, 94.2, 86.9, 56.2, 43.0, 28.1; HRMS (ESI-negative): 193.08574 (calcd for $C_{11}H_{13}O_3$: 193.08702).

Preparation of 6-amino-2,2-dimethyl-2,3-dihydrobenzofuran-5-ol (**10**): Pd-C (5%, 4.7 mg) was added to a solution of **6** (10.1 mg, 48.3 μmol) in distilled MeOH (0.8 mL) under hydrogen gas and stirred for 30 min at room temperature. The mixture was filtered through a syringe filter. The filtrate was dried by flushing N_2 gas to afford a tan yellow solid. The crude product was immediately purified on a short silica gel column (10% MeOH-CH_2Cl_2) to give **10**: white solid; yield 83%; ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.24 (s, 1H, OH), 6.44 (s, 1H, Ar-H), 5.99 (s, 1H, Ar-H), 4.33 (s, 2H, NH_2), 2.76 (s, 2H, CH_2), 1.32 (s, 6H, Me); ^{13}C NMR (150 MHz, DMSO-d_6): δ 151.8, 137.3, 136.0, 112.6, 111.4, 96.1, 85.3, 42.4, 27.9; HRMS (ESI-negative): 178.08623 (calcd for $C_{10}H_{12}NO_2$: 178.08735).

Kinetic measurements

The reaction kinetics were determined by measuring the disappearance of absorbance at 428 nm under pseudo-first-order conditions at 25 °C using a stopped-flow technique on a UNISOKU RSP-2000-03TI spectrophotometer. 6-Substituted 2,3-dihydrobenzofuran-5-ols (with the following final concentrations: 0–0.25 mM for **1**, **2**, **4** and **9**; 0–0.85 mM for **3**; 0–5.0 mM for **5**; 0–0.5 mM for **7** and **8**; and 0–0.085 mM for

10) and G$^{\bullet}$ (5.0 μM) were dissolved in argon deaerated with MeCN. Two syringes were loaded with 2 mL of each compound and G$^{\bullet}$ solution. The pneumatic drive accessory initiated mixing after the initiation of data acquisition by the spectrophotometer at 300–600 nm and time intervals (120 ms for **1**–**9**, 0.5 ms for **10**). Half of the starting concentration of G$^{\bullet}$ solution and antioxidant was used after mixing. The radical scavenging rates were determined by monitoring the changes in absorbance due to G$^{\bullet}$ 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. To avoid the influence of minor absorption from the G$^{\bullet}$ reduction products at this wavelength, only the first G$^{\bullet}$ absorption decay values were used in kinetics analyses. The k_{obs} values were determined using the least-squares method and were observed to be compound concentration-dependent. k values for the reactions between the compounds and G$^{\bullet}$ 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 triplicate experiments.

ESR measurements

ESR spectra were collected on a JEOL JES-X320 instrument with the following settings: microwave frequency, 9.418 GHz; magnetic field, 336 mT; microwave power, 10.0 mW; modulation frequency, 9.42 GHz; modulation width, 0.05 mT; sweep width, ±5 mT; sweep time, 30 s; receiver gain, 200; time constant, 0.03 s; and temperature, 298 K. $FeSO_4$, H_2O_2, and DMPO were dissolved in argon-deaerated 0.1 M sodium phosphate buffer (pH 7.4). The test compounds were diluted in argon-deaerated MeCN. Oxygen was removed by flushing with argon. Test compounds (33 μL of each), 0.1 M sodium phosphate buffer (pH 7.4, 157 μL) and $FeSO_4$ (0.1 μmol per 10 μL) were added to a test tube containing DMPO (9 μmol per 10 μL), H_2O_2 (0.1 μmol per 10 μL) was added, and the mixture was vortexed for 5 s at room temperature and then transferred to a 10 × 50 mm quartz flat cell. MeCN (33 μL) was used instead of the compound solution for the blank. The ESR measurements were started 2 min after preparing each reaction mixture. The capacity of the $^{\bullet}$OH scavenging activity at each antioxidant concentration is presented as a relative intensity determined by calculating the ratio of the peak height of the ESR signal due to the $^{\bullet}$OH adduct of DMPO (DMPO–OH) to the peak height of the Mn^{2+} marker. The $^{\bullet}$OH scavenging activity in the presence of antioxidants is expressed as the percentage of $^{\bullet}$OH scavenging activity [% = (R-Rs)/R×100], where Rs is the DMPO-OH adduct intensity in the presence of antioxidant and R is the DMPO-OH adduct intensity in the absence of antioxidants. The data were collected in at least triplicate experiments.

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