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SYNTHESIS AND RADICAL SCAVENGING ACTIVITIES OF TOCOPHEROL ANALOGS CONTAINING HETEROCYCLIC RINGS

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Abstract – Antioxidants play an essential role in preventing oxidative stress. In this study, we synthesized novel tocopherol analogs with heterocyclic rings such as quinoline (**5**), indole (**6**), and benzimidazole (**7**), and evaluated their radical scavenging activities. The results showed that **6** has excellent radical scavenging activity. The data suggested that radical scavenging activity was enhanced in compounds containing heterocyclic rings with sufficient π-electrons and decreased in compounds with heterocyclic rings deficient π-electrons.

INTRODUCTION

Reactive oxygen species (ROS) are responsible for homeostasis, including bactericidal activity and signal transduction.[1,2] Overexpressed ROS can cause cancer, atherosclerosis, and neurodegenerative diseases. In addition, ROS are known to promote amyotrophic lateral sclerosis (ALS) symptoms.[3]

α-Tocopherol (**1**), the most potent antioxidant form of vitamin E, protects biotissues against oxidative stress. Compound **1** is composed of 6-chromanol, which is essential for radical scavenging activity, and a long carbon chain phytyl group. In previous studies, we synthesized 6-chromanols[4,5] and 6-benzo[*h*]chromanols[6] with substituents (such as amino, methoxy, methyl, and nitro) on the aromatic ring and assessed their radical scavenging activities. Expanding the aromatic ring improved antioxidant activity, but we found that 6-benzo[*h*]chromanols are unstable and thus difficult to use as drugs. However, introduction of a nitrogen-containing substituent at position 8 of 6-chromanols resulted in both high antioxidant activity and stability. The previous work also showed that nitrogen- and oxygen-containing groups worked as electron-donating groups even though the substituent was placed at *meta* position relative to the phenolic OH group, and nitrogen-containing substituents were more effective than oxygen-containing substituents because of their lower electronegativity.[5] In this study, we designed and synthesized various compounds with heterocyclic structures bearing an expanded aromatic ring and a

nitrogen atom at position 8 of the chromanol skeleton and compared their antioxidant activities. Furthermore, to investigate the effect of nitrogen atoms in the aromatic ring on radical scavenging activity, we prepared compounds with quinoline (**5**), indole (**6**), and benzimidazole (**7**) skeletons and evaluated their radical scavenging activities for galvinoxyl (G•) and hydroxyl radical (•OH) (Figure 1).

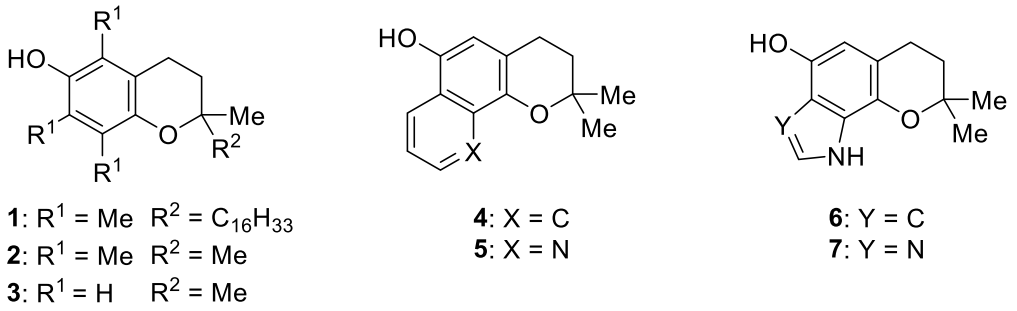
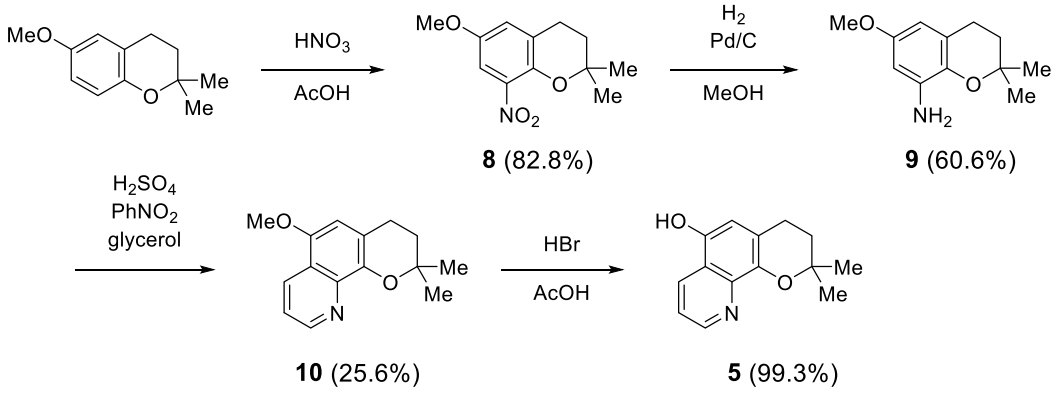


Figure 1. Structures of tocopherol analogs containing heterocyclic rings investigated in this study

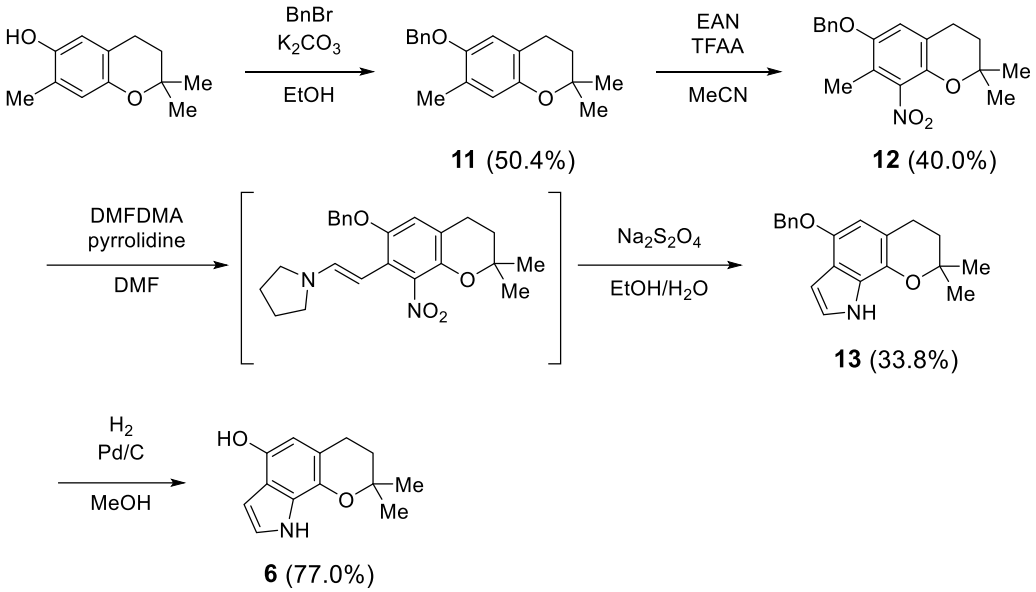
RESULTS AND DISCUSSION

2,2,5,7,8-Pentamethyl-6-chromanol (**2**), in which the phytyl group is converted to a methyl group, is often used as a model compound for **1**,[7] as the phytyl group is not necessarily required for radical scavenging activity. 2,2-Dimethyl-6-chromanol (**3**) was used as a control for the 6-chromanol skeleton and compared with 2,2-dimethyl-3,4-dihydro-2*H*-benzo[*h*]chroman-6-ol (**4**) to determine the effect of introducing a nitrogen atom in the aromatic ring on radical scavenging activity. Compounds **5**, **6**, and **7** are novel compounds, as are the synthetic intermediates.



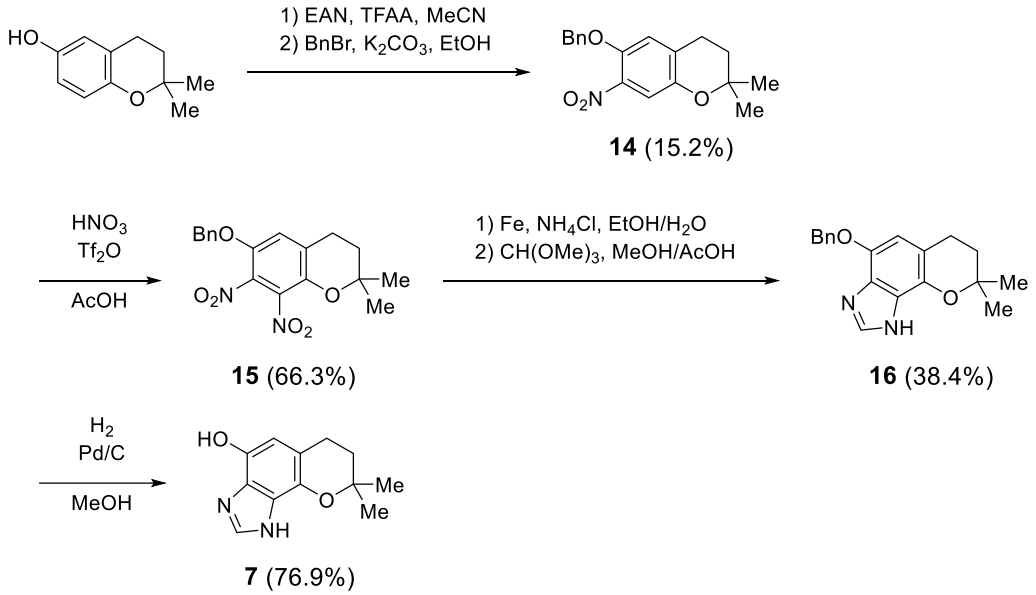
Scheme 1. Synthesis of **5**

6-Methoxy-2,2-dimethylchroman, a known compound, was nitrated with nitric acid and the nitro group was reduced by catalytic reduction. The amino group introduced in chroman **9** was cyclized to form a quinoline skeleton with glycerol using sulfuric acid, then **10** was demethylated by hydrobromic acid to afford **5** (Scheme 1).



Scheme 2. Synthesis of **6**

In the preparation of **6**, 2,2,7-trimethylchroman-6-ol was protected with a benzyl group and nitrated with ethylammonium nitrate (EAN) and trifluoroacetic anhydride (TFAA), followed by purification on silica gel column to afford **12**. Compound **12** was converted to enamine by *N*,*N*-dimethylformamide dimethyl acetal (DMFDMA) and pyrrolidine, then reduced to build an indole ring to give **13** (Scheme 2).



Scheme 3. Synthesis of **7**

To synthesize the benzimidazole skeleton, 2,2-dimethyl-6-chromanol was nitrated by EAN and TFAA at positions 5 or 7 of chromanol, protected with a benzyl group for purification to isolate **14**, followed by a second nitration with concd HNO_3 and trifluoromethanesulfonic anhydride (Tf_2O). Then, **15** was reduced to phenylenediamine and condensed with trimethyl orthoformate to form the benzimidazole skeleton.

The benzylated compounds (**13** and **16**) were deprotected by catalytic hydrogenation and purified on a short silica gel column prior to measuring G• and •OH scavenging activity.

The G• scavenging activities of the synthesized compounds were determined in MeCN. G• is a stable and commercially available oxygen radical with an absorption maximum at 428 nm,[8] allowing accurate determination of the reaction rate between tocopherol analogs and radicals. G• decreases according to pseudo-first-order reaction kinetics when the concentration of the scavenging compound is more than 10 times the concentration of G•. The observed pseudo-first-order rate constant (k_{obs}) is concentration-dependent. The second-order rate constant (k) was determined from the slope of the line obtained from the concentration of the compound and k_{obs} (Table 1).

Table 1. Radical scavenging activities of tocopherol analogs containing heterocyclic rings toward G• and •OH

Compound	Second-order rate constant k ($\times 10^3$ M^{-1} s^{-1}) [a]	Maximum inhibition % [b] (mM) [c]
1	3.61	10.0 (0.1)
2	2.84	37.9 (3.0)
3	0.263	66.3 (30.0)
4 (naphthalene)	13.2	36.8 (3.0)
5 (quinoline)	6.52	29.5 (3.0)
6 (indole)	246	91.8 (3.0)
7 (benzimidazole)	12.9	15.5 (3.0)

a: Reaction of G• with heterocyclic tocopherol analogs
b: Maximum inhibition (%) of DMPO-OH adduct
c: Concentration of the compound providing maximum inhibition

The G• scavenging activities of the measured compounds followed the order **6**>>**4**≒**7**>**5**>**1**≒**2**>**3**. The G• scavenging activity of **5** was lower than that of **4**, that of **7** was similar to **4**, whereas **6** exhibited significantly increased activity compared to **4**. These data indicate that nitrogen atoms on the aromatic rings may or may not decrease radical scavenging activity, depending on the π-electron density of the aromatic ring.

•OH, the most reactive ROS of biological importance, reacts with biomolecules such as lipids, proteins, and DNA to cause oxidative damage.[9] The antioxidant activity of **1** is due to its scavenging of lipid peroxyl radicals *in vitro* and *in vivo*, but is ineffective at scavenging •OH *in vivo*.[10] Electron spin resonance (ESR) spin-trapping was used to evaluate the •OH scavenging activities of the synthesized compounds.[11] 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-trapping agent.[12] The capacity of •OH scavenging activity was evaluated from the intensity of the DMPO-OH adduct as percent inhibition (%). The maximum inhibition values and the concentrations are shown in Table 1 (see also Supporting Information). Compound **1** was previously reported to exhibit no *in vivo* •OH or alkoxyl radical scavenging activity,[13] consistent with the results of the present study. Compounds **2** and **4** showed maximum inhibitions of only approximately 10-40% due to their low solubility in phosphate buffer at pH 7.4. On the other hand, **3** showed high activity (maximum inhibition of 66.3%) due to its high solubility in phosphate buffer. Compound **6** effectively inhibited the formation of the DMPO-OH adduct at 3.0 mM. The reaction of tocopherol analogs with radicals generates phenoxyl radicals via hydrogen atom transfer (HAT)[14] and the stabilization of phenoxyl radicals improves radical scavenging activity.[15] Our results suggest that sufficient π-electrons in the indole ring compared to other heterocyclic and aromatic rings stabilize the phenoxyl radical, increasing radical scavenging activity. Furthermore, imidazole ring generally has amine (-NH-) and aza (-N=) nitrogen atoms. Amine nitrogen atom behaves similarly to pyrrole and aza nitrogen atom behaves similarly to pyridine.[16] Thus, compound **7** reduced G• scavenging activity due to the electron-withdrawing aza nitrogen atom. Compounds **5** and **7** showed maximum inhibition of 29.5% and 15.5%, respectively. Since there is a possibility that the inhibition rate was affected by p*K*a of the compounds due to their basicity, the p*K*a of each compound was measured using UV-vis spectroscopy and calculated according to the complete basis set (CBS) -4M and Hartree–Fock methods[17] (Table 2).

Table 2. p*K*a values of tocopherol analogs containing heterocyclic rings

	2	**3**	**4**	**5**	**6**	**7**
exp. p*K*a	12.0	10.4	11.6	8.3 [b] 9.8 [c]	9.8	9.5 [b] 10.4 [c]
calcd p*K*a [a]	13.3	8.72	11.7	8.67 [b] 12.1 [c]	13.1	12.9 [b] 10.8 [c]

a: p*K*a calculations were performed using CBS-4M for the gas phase and HF/6-31G(d) with solvation model based on density (SMD) for the aqueous phase.
b: p*K*a of conjugated acid.
c: p*K*a of phenolic hydroxyl group.

The experimental and calculated values were in close agreement and showed that the calculated and experimental p*K*a value of conjugated acid for **7** was higher than that for **5**. Therefore, since **7** is more basic than **5**, the ratio of conjugated acid in **7** is higher than in **5** at pH 7.4. The conjugated acid of **7** exhibited low •OH scavenging activity because of the deficit of π-electrons in phenoxyl radicals, and thus

the radical scavenging activity at pH 6.4, 7.4, and 8.4 was measured using stable **5** (Table 3). 2,2-Diphenyl-1-picrylhydrazyl (DPPH) was used as the source of radicals because galvinoxyl is unstable in aqueous solution.

Table 3. Radical scavenging activity of **5** toward DPPH in aqueous solution and MeCN

	MeCN	pH 6.4	pH 7.4	pH 8.4
k ($\times 10^3$ M^{-1} s^{-1})	1.04	144	679	901

The results indicated that the DPPH scavenging activity increased in aqueous solution compared to in acetonitrile, and that activity increased with increasing pH. Importantly, high activity was obtained at pH 7.4, which is close to *in vivo* conditions. The DPPH scavenging activity is enhanced because an amount of quinolinium form decreased at a higher pH, as •OH scavenging activity. The mechanism of radical scavenging in aqueous solution involves not only HAT but also sequential proton loss electron transfer.[18]

In summary, we synthesized novel tocopherol analogs containing heterocyclic rings and measured their radical scavenging activities. The reactivity of compound **6** with radicals is about 65 times higher than that of α-tocopherol (**1**). In addition, radical scavenging activity in aqueous solution decreased with the quinolilium form. It is assumed that radical scavenging activity is enhanced in π-sufficient heterocycle systems and reduced in π-deficient heterocycle systems. The design and synthesis of compounds with high antioxidant activity may contribute to the prevention and treatment of various diseases caused by ROS.

EXPERIMENTAL

Materials and Methods

Melting points were determined using Yanako micro-melting point apparatus without correction (Tokyo, Japan). NMR spectra were recorded on a JEOL ECZ-600R spectrometer (Tokyo, Japan). Chemical shifts were expressed in ppm, and downfield shifted from the TMS peak. High-resolution mass spectra were collected using a JEOL AccuTOF LC-plus 4G mass spectrometer (Tokyo, Japan). UV-Vis spectrophotometry data were obtained using a Unisoku RSP-2000 spectrophotometer (Osaka, Japan) for kinetics measurements and a Agilent 8453 (Hanover, USA) for p*K*a measurements. ESR spectra were recorded on a JEOL JES-X320 (Tokyo, Japan). The reaction progress 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. Compound **3** [mp 75.3-76.0 °C (75-76 °C)[19]], compound **4** (colorless oil),[6] 6-hydroxy-2,2,7-trimethylchroman [mp 84.5-85.6 °C (87-88 °C)[19]], ethylammonium nitrate (colorless

oil)[20] were prepared according to the previously reported procedure. The galvinoxyl free radical, *N*,*N*-dimethylformamide dimethyl acetal (DMFDMA), trifluoromethanesulfonic anhydride (Tf_2O), and hydrobromic acid were obtained from Tokyo Kasei Kogyo Co., Ltd. (Tokyo, Japan). The iron powder was purchased from Kanto Chemical Co., Inc. (Tokyo, Japan). Chloroform-*d* (0.03 vol.% TMS), dimethyl sulfoxide-d_6, and methanol-d_4 (0.05 vol.% TMS) were obtained from Cambridge Isotope Laboratories, Inc. (Tewksbury, MA, USA). DMPO was purchased from Labotec Co., Ltd. (Tokyo, Japan). MeCN was used for spectral measurements, and other reagents were purchased from FUJIFILM Wako Pure Chemical Industries (Osaka, Japan). All the reagents used were of the best commercially available quality and were not further purified unless otherwise noted.

Synthesis of 6-methoxy-2,2-dimethyl-8-nitrochroman (**8**)

Concd HNO_3 (0.71 mL, 1.1 equiv) was added to a solution of 6-methoxy-2,2-dimethylchroman (2.82 g, 14.7 mmol) in AcOH (40 mL). The reaction mixture was stirred for 10 min at room temperature, then poured onto crushed ice and extracted with CH_2Cl_2 (20 mL×3). The combined organic phase was washed with 5% aq. $NaHCO_3$ (20 mL×4) and water (20 mL), 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:AcOEt = 9:1) to give **8** (2.88 g, 82.8%) as a yellow oil; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.20 (d, *J* = 2.8 Hz, 1H, Ar-H), 6.87 (d, *J* = 3.4 Hz, 1H, Ar-H), 3.78 (s, 3H, OMe), 2.82 (t, *J* = 6.5 Hz, 2H, CH_2), 1.86 (t, *J* = 6.9 Hz, 2H, CH_2), 1.37 (s, 6H, Me); ^{13}C NMR (151 MHz, $CDCl_3$) δ 151.3, 142.6, 139.2, 125.3, 120.7, 107.7, 76.1, 55.9, 32.1, 26.7, 22.8; HRMS (ESI-positive) 238.10907 (calcd for $C_{12}H_{16}NO_4$ 238.10738).

Synthesis of 8-amino-6-methoxy-2,2-dimethylchroman (**9**)

To **8** (921.5 mg, 3.88 mmol) dissolved in MeOH (20 mL) was added Pd/C (90.1 mg). The reaction mixture was stirred for 3 h under a room temperature hydrogen atmosphere. The mixture was filtered through Celite®, and the filtrate was evaporated under reduced pressure to afford a tan yellow oil. The crude product was purified on a silica gel column (CH_2Cl_2:MeOH = 99:1) to give a white solid. The solid was recrystallized with EtOH and water to afford white prisms (488.2 mg, 60.6%); mp 69.7-70.8 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.18 (d, *J* = 2.8 Hz, 1H, Ar-H), 6.03 (d, *J* = 2.8 Hz, 1H, Ar-H), 3.75 (s, 2H, NH_2), 3.71 (s, 3H, OMe), 2.70 (t, *J* = 6.9 Hz, 2H, CH_2), 1.78 (t, *J* = 6.5 Hz, 2H, CH_2), 1.32 (s, 6H, Me); ^{13}C NMR (151 MHz, $CDCl_3$) δ 153.0, 136.5, 136.0, 120.7, 102.4, 99.9, 73.9, 55.5, 33.1, 26.9, 22.8; HRMS (ESI-positive) 208.13432 (calcd for $C_{12}H_{18}NO_2$ 208.13321).

Synthesis of 6-methoxy-2,2-dimethyl-3,4-dihydro-2*H*-pyrano[3,2-*h*]quinoline (**10**)

A solution of **9** (483.7 mg, 2.33 mmol) and nitrobenzene (0.29 mL, 1.2 equiv) in glycerol (3 mL) was heated at 150 °C and concd H_2SO_4 (0.19 mL, 1.5 equiv) was added. The reaction mixture was stirred for 30 min and diluted with water (10 mL), then Et_2O (20 mL) was added and the mixture was extracted with

4 M HCl (10 mL×2). The combined 4 M HCl phase was neutralized with 10 M NaOH (15 mL), then the mixture was extracted with CH_2Cl_2 (10 mL×3) and washed with water (10 mL). The organic phase was dried over anhydrous sodium sulfate, filtered, and evaporated under reduced pressure to afford a black oil. The crude product was purified on a silica gel column (*n*-hexane:AcOEt = 1:2) to give a pale yellow oil. The oil was recrystallized with EtOH and water to afford white prisms (145.2 mg, 25.6%); mp 110.9-111.8 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.94 (dd, *J* = 4.5, 1.7 Hz, 1H, Ar-H), 8.48 (dd, *J* = 8.3, 2.1 Hz, 1H, Ar-H), 7.34 (dd, *J* = 8.3, 4.1 Hz, 1H, Ar-H), 6.56 (s, 1H, Ar-H), 3.94 (s, 3H, OMe), 2.91 (t, *J* = 6.9 Hz, 2H, CH_2), 1.93 (t, *J* = 6.9 Hz, 2H, CH_2), 1.50 (s, 6H, Me); ^{13}C NMR (151 MHz, $CDCl_3$) δ 149.8, 147.5, 143.1, 140.9, 130.6, 120.6, 120.0, 118.0, 105.9, 74.7, 55.8, 33.0, 26.8, 23.9; HRMS (ESI-positive) 244.13387 (calcd for $C_{15}H_{18}NO_2$ 244.13321).

Synthesis of 2,2-dimethyl-3,4-dihydro-2*H*-pyrano[3,2-*h*]quinolin-6-ol (**5**)

10 (270.2 mg, 1.11 mmol) was dissolved in 47% hydrobromic acid (3 mL) and refluxed for 4.5 h under a nitrogen atmosphere. The reaction mixture was neutralized with 5% aq. $NaHCO_3$ (40 mL) and the precipitated solid was filtered. The solid was washed with water and dried to afford a yellow solid (252.8 mg, 99.3%); ^{1}H NMR (600 MHz, CD_3OD) δ 9.08 (d, *J* = 8.3 Hz, 1H, Ar-H), 8.87 (d, *J* = 4.8 Hz, 1H, Ar-H), 7.77 (dd, *J* = 8.3, 4.8 Hz, 1H, Ar-H), 6.87 (s, 1H, Ar-H), 2.95 (t, *J* = 6.5 Hz, 2H, CH_2), 1.96 (t, *J* = 6.9 Hz, 2H, CH_2), 1.43 (s, 6H, Ar-H); ^{13}C NMR (151 MHz, CD_3OD) δ 147.5, 145.5, 141.0, 138.6, 133.1, 126.9, 122.3, 120.7, 112.8, 77.4, 33.5, 26.8, 24.2; HRMS (ESI-positive) 230.11705 (calcd for $C_{14}H_{16}NO_2$).

Synthesis of 6-benzyloxy-2,2,7-trimethylchroman (**11**)

2,2,7-Trimethyl-6-chromanol (224.6 mg, 1.16 mmol) was dissolved in DMF (5 mL) and potassium carbonate (484 mg, 3.0 equiv) and benzyl bromide (0.21 mL, 1.5 equiv) were added. The reaction mixture was stirred overnight at room temperature and diluted with water (20 mL), then extracted with Et_2O (10 mL×3). The combined organic phase was washed with water (10 mL) and dried over anhydrous sodium sulfate, and filtered and evaporated under reduced pressure to afford a yellow solid. The crude product was purified on a silica gel column (*n*-hexane:AcOEt = 19:1) to give a white solid. The solid was recrystallized with EtOH and water to afford white prisms (166.3 mg, 50.4%); mp 82.2-83.0 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.44-7.31 (m, 5H, Bn), 6.61 (s, 1H, Ar-H), 6.59 (s, 1H, AR-H), 4.98 (s, 2H, OCH_2), 2.71 (t, *J* = 6.5 Hz, 2H, CH_2), 2.21 (s, 3H, Me), 1.76 (t, *J* = 6.9 Hz, 2H, CH_2), 1.31 (s, 6H, Me); ^{13}C NMR (151 MHz, $CDCl_3$) δ 150.5, 147.6, 137.9, 128.4, 127.6, 127.2, 126.6, 119.2, 118.0, 112.7, 73.6, 70.8, 33.0, 26.8, 22.6, 16.1; HRMS (ESI-positive) 283.17178 (calcd for $C_{19}H_{23}O_2$ 283.16926).

Synthesis of 6-benzyloxy-2,2,7-trimethyl-8-nitrochroman (**12**)

A solution of **11** (304.7 mg, 1.08 mmol) and ethylammonium nitrate (0.11 mL, 1.1 equiv) in MeCN (10 mL) at 0 °C was added to trifluoroacetic anhydride (0.17 mL, 1.1 equiv). The reaction mixture was

stirred for 30 min under an argon atmosphere at 0 °C. The mixture was diluted with water (30 mL) and extracted with CH_2Cl_2 (10 mL×3). The combined organic phase was washed with 5% aq. $NaHCO_3$ (10 mL×2) and water (10 mL), then dried over anhydrous sodium sulfate, filtered, and evaporated under reduced pressure to afford a brown oil. The crude product was purified on a silica gel column (*n*-hexane:CH_2Cl_2 = 1:1) to give a yellow solid. The solid was recrystallized with EtOH and water to afford yellow needles (141.4 mg, 40.0%); mp 138.0-138.8 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.41-7.32 (m, 5H, Bn), 6.70 (s, 1H, Ar-H), 5.01 (s, 2H, OCH_2), 2.74 (t, *J* = 6.9 Hz, 2H, CH_2), 2.15 (s, 3H, Me), 1.82 (t, *J* = 6.5 Hz, 2H, CH_2), 1.30 (s, 6H, Me); ^{13}C NMR (151 MHz, $CDCl_3$) δ 149.3, 142.4, 139.4, 136.9, 128.6, 128.0, 127.2, 120.3, 118.4, 114.3, 75.5, 71.3, 32.4, 26.5, 22.4, 10.6; HRMS (ESI-positive) 328.15233 (calcd for $C_{19}H_{22}NO_4$ 328.15433).

Synthesis of 6-benzyloxy-2,2-dimethyl-2,3,4,9-tetrahydropyrano[3,2-*g*]indole (**13**)

12 (334.3 mg, 1.02 mmol) was dissolved in DMF (8 mL) and heated to 120 °C. To this solution were added pyrrolidine (0.42 mL, 5.0 equiv) and *N*,*N*-dimethylformamide dimethyl acetal (0.68 mL, 5.0 equiv), then stirred for 3 h under a nitrogen atmosphere at 120 °C. To the mixture were then added pyrrolidine (0.27 mL, 2.0 equiv) and *N*,*N*-dimethylformamide dimethyl acetal (0.17 mL, 2.0 equiv), followed by stirring for 17.5 h. The reaction mixture was diluted with water (30 mL) and extracted with CH_2Cl_2 (20 mL×3). The combined organic phase was washed with water (10 mL×3) and dried over anhydrous sodium sulfate. The mixture was filtered and evaporated under reduced pressure to afford a reddish-black solid. The solid was washed with MeOH, and the precipitated solid was filtered to give a red solid.

The solid was dissolved in EtOH (15 mL), then sodium dithionite (616 mg, 5.0 equiv) in H_2O (10 mL) was added. The mixture was refluxed for 1 h and diluted with water (20 mL). The solution was extracted with AcOEt (20 mL×3) and washed with water (10 mL). The organic phase was dried over anhydrous sodium sulfate, filtered, and evaporated under reduced pressure to afford a brown oil. The crude product was purified on a silica gel column (*n*-hexane:AcOEt = 4:1) to give a colorless oil (106.1 mg, 33.8%); ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.29 (s, 1H, NH), 7.50-7.29 (m, 5H, Bn), 7.02 (dd, *J* = 3.0 Hz, 1H, Ar-H), 6.62 (dd, *J* = 3.6 Hz, 1H, Ar-H), 6.27 (s, 1H, Ar-H), 5.14 (s, 2H, OCH_2), 2.80 (t, *J* = 6.5 Hz, 2H, CH_2), 1.85 (t, *J* = 6.9 Hz, 2H, CH_2), 1.36 (s, 6H, Me); ^{13}C NMR (151 MHz, $CDCl_3$) δ 145.8, 138.1, 134.8, 128.4, 127.7, 127.5, 127.3, 121.7, 118.9, 111.6, 102.3, 100.4, 74.1, 70.5, 33.5, 26.8, 22.6; HRMS (ESI-positive) 308.16327 (calcd for $C_{20}H_{22}NO_2$ 308.16451).

Synthesis of 6-benzyloxy-2,2-dimethyl-7-nitrochroman (**14**)

2,2-Dimethyl-6-chromanol **3** (769.5 mg, 4.32 mmol) was dissolved in MeCN (50 mL) and ethylammonium nitrate (0.39 mL, 1.0 equiv) was added. The solution was cooled below 0 °C, then trifluoroacetic anhydride (0.61 mL, 1.0 equiv) was added. The reaction mixture was stirred for 15 min at 0 °C under an argon atmosphere, diluted with water (50 mL), and then evaporated. The residue was

extracted with CH_2Cl_2 (20 mL×3) and washed with water (20 mL). The organic phase was dried over anhydrous sodium sulfate, filtered, and evaporated under reduced pressure to give a brown oil. The crude product was purified twice on a silica gel column (*n*-hexane:AcOEt = 4:1) to obtain an orange oil (360.2 mg, 37.4%, comprising 2,2-dimethyl-5-nitrochroman-6-ol and 2,2-dimethyl-7-nitrochroman-6-ol).

To a solution of 2,2-dimethyl-5-nitrochroman-6-ol and 2,2-dimethyl-7-nitrochroman-6-ol mixture (360.2 mg, 1.61 mmol) in EtOH (10 mL) were added potassium carbonate (669 mg, 3.0 equiv) and benzyl bromide (0.23 mL, 1.5 equiv), and stirred for 15 h at room temperature. The reaction mixture was diluted with water (30 mL) and extracted with CH_2Cl_2 (10 mL×4). The combined organic phase was washed with water (10 mL×2), dried over anhydrous sodium sulfate and filtered, then evaporated under reduced pressure to give a yellow oil. The crude product was purified on a silica gel column (*n*-hexane:AcOEt = 4:1) to afford a yellow oil. The oil was recrystallized with EtOH and water to afford yellow needles (76.6 mg, 15.2%); mp 97.8-98.8 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.46-7.32 (m, 6H, Bn, Ar-H), 6.81 (s, 1H, Ar-H), 5.13 (s, 2H, OCH_2), 2.78 (t, *J* = 6.9 Hz, 2H, CH_2), 1.81 (t, *J* = 6.9 Hz, 2H, CH_2), 1.32 (s, 6H, Me); ^{13}C NMR (151 MHz, $CDCl_3$) δ 147.9, 145.2, 139.3, 136.2, 128.6, 128.1, 127.9, 127.2, 116.9, 114.2, 74.8, 72.3, 32.1, 26.7, 22.9; HRMS (DART-positive) 314.13497 (calcd for $C_{18}H_{20}NO_4$).

Synthesis of 6-benzyloxy-2,2-dimethyl-7,8-dinitrochroman (**15**)

14 (109.1 mg, 0.35 mmol) was dissolved in AcOH (5 mL), and concd HNO_3 (0.044 mL, 2.0 equiv) and trifluoromethanesulfonic anhydride (0.11 mL, 2.0 equiv) were added. The reaction mixture was stirred for 10 min under an argon atmosphere at room temperature. The mixture was poured onto crushed ice, and the precipitated solid was filtered to afford a yellow solid. The solid was recrystallized with EtOH to afford yellow prisms (82.7 mg, 66.3%); mp 157.5-158.4 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.39-7.34 (m, 5H, Bn), 6.95 (s, 1H, Ar-H), 5.14 (s, 2H, OCH_2), 2.82 (t, *J* = 6.9 Hz, 2H, CH_2), 1.87 (t, *J* = 6.9 Hz, 2H, CH_2), 1.35 (s, 6H, Me); ^{13}C NMR (151 MHz, $CDCl_3$) δ 143.1, 141.0, 135.1, 128.8, 128.5, 128.3, 127.2, 118.4, 77.3, 72.7, 31.7, 26.5, 23.0; HRMS (DART-positive) 359.12185 (calcd for $C_{18}H_{19}N_2O_6$ 359.12376).

Synthesis of 4-benzyloxy-8,8-dimethyl-1,6,7,8-tetrahydrochromeno[7,8-*d*]imidazole (**16**)

15 (93.3 mg, 0.26 mmol) was dissolved in EtOH (12 mL), then iron powder (145 mg, 10 equiv) and ammonium chloride (139 mg, 10 equiv) in H_2O (3 mL) were added. The reaction mixture was refluxed for 2 h under a nitrogen atmosphere. The mixture was filtered, and the filtrate was diluted with 5% aq. $NaHCO_3$ (30 mL). The solution was extracted with CH_2Cl_2 (10 mL×3) and washed with water (10 mL). The organic phase was dried over anhydrous sodium sulfate, filtered, and evaporated under reduced pressure to give a brown oil.

The oil was dissolved in MeOH-AcOH (9:1, 10 mL) and trimethyl orthoformate (0.039 mL, 1.5 equiv) was added. The reaction mixture was refluxed for 30 min under a nitrogen atmosphere, then the mixture

was diluted with water (30 mL) and extracted with CH_2Cl_2 (10 mL×3). The combined organic phase was washed with 5% aq. $NaHCO_3$ (10 mL×2) and water (10 mL), dried over anhydrous sodium sulfate, filtered, and evaporated under reduced pressure to give a brown oil. The crude product was purified on a silica gel column (CH_2Cl_2:MeOH = 19:1) to afford a yellow oil. The oil was recrystallized with EtOH and water to afford white prisms (30.8 mg, 38.4%); mp 182.0-183.2 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.83 (s, 1H, Ar-H), 7.47-7.30 (m, 5H, Bn), 6.47 (s, 1H, Ar-H), 5.20 (s, 2H, OCH_2), 2.81 (t, *J* = 6.5 Hz, 2H, CH_2), 1.86 (t, *J* = 6.5 Hz, 2H, CH_2), 1.37 (s, 6H, Me); ^{13}C NMR (151 MHz, $CDCl_3$) δ 138.6, 137.4, 128.5, 127.9, 127.8, 112.6, 106.2, 74.4, 71.2, 33.3, 26.7, 22.7; HRMS (ESI-positive) 309.16025 (calcd for $C_{19}H_{21}N_2O_2$ 309.15975).

Catalytic hydrogenation of benzylated compounds (**13** and **16**)

The benzylated compounds were deprotected immediately before measuring radical scavenging activity. To the benzylated compounds (ca. 30-45 μmol) dissolved in MeOH (0.8 mL) was added Pd/C (ca. 3.0 mg). The reaction solution was stirred at room temperature under a hydrogen atmosphere until the disappearance of the starting material was confirmed by TLC. The mixture was filtered through a syringe filter, and the filtrate was dried by flushing argon gas to afford a yellow oil. The oil was purified on a silica gel column (CH_2Cl_2:MeOH = 19:1 for **6**, CH_2Cl_2:MeOH = 9:1 for **7**) to give the desired product as a single compound. The yields exceeded approximately 70% for all compounds. Though ^{1}H NMR spectroscopy, it was determined that the test compounds did not contain any impurities.

2,2-Dimethyl-2,3,4,9-tetrahydropyrano[3,2-*g*]indol-6-ol (**6**); colorless oil; ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.31 (s, 1H, NH), 7.06 (dd, *J* = 2.8 Hz, 1H, Ar-H), 6.50 (dd, *J* = 2.8 Hz, 1H, Ar-H), 6.23 (s, 1H, Ar-H), 4.55 (s, 1H, OH), 2.79 (t, *J* = 6.9 Hz, 2H, CH_2), 1.85 (t, *J* = 6.9 Hz, 2H, CH_2), 1.36 (s, 6H, Me); ^{13}C NMR (151 MHz, $CDCl_3$) δ 141.6, 134.4, 122.0, 117.5, 112.2, 104.4, 99.1, 74.2, 33.5, 26.8, 22.3; HRMS (ESI-positive) 218.11996 (calcd for $C_{13}H_{16}NO_2$ 218.11756).

8,8-Dimethyl-1,6,7,8-tetrahydrochromeno[7,8-*d*]imidazol-4-ol (**7**); white solid; ^{1}H NMR (600 MHz, CD_3OD) δ 7.93 (s, 1H, Ar-H), 6.35 (s, 1H, Ar-H), 2.79 (t, *J* = 6.5 Hz, 2H, CH_2), 1.86 (t, *J* = 6.5 Hz, 2H, CH_2), 1.37 (s, 6H, Me); ^{13}C NMR (151 MHz, CD_3OD) δ 140.6, 140.0, 136.5, 114.6, 108.6, 75.2, 34.4, 27.0, 23.5; HRMS (ESI-positive) 219.11422 (calcd for $C_{12}H_{15}N_2O_2$ 219.11280).

Kinetics measurements in MeCN

The reaction kinetics were measured from the decrease in absorbance of galvinoxyl at 428 nm by pseudo-first-order reaction at 25 °C using a stopped-flow technique and an UNISOKU RSP-2000 spectrometer. Tocopherol analogs (final concentrations: 0-0.25 mM for **1**, **2**, **5**, **6**, and **7**; 0-0.85 mM for **3**; 0-0.125 mM for **4**) and G• (final concentration 2.5 μM) were dissolved in argon-deaerated MeCN. Two syringes were charged with 2 mL (one with a test compound, the other with G•). The two solutions were mixed in a pneumatically driven system, and the absorbance at 428 nm was measured at time intervals

(1 ms for **7**; 100 ms for the other compounds).

The reaction rate was determined by monitoring the decrease in absorbance of galvinoxyl at 428 nm. The pseudo-first-order rate constants (k_{obs}) were obtained from the slope of the plot of $\ln(A\text{-}A_\infty)$ versus time at the initial reaction, where A and A_∞ refer to the absorbance obtained at a given time and the final absorbance, respectively. The pseudo-first-order reaction rate constants were determined by the least-squares method. k_{obs} values were measured for 5 doses to obtain the second-order reaction constants. Second-order reaction constants for the compounds and galvinoxyl were obtained from the slope of the linear line of the plot of k_{obs} versus the concentration of the compound.

Kinetics measurement in aqueous solution

The reaction kinetics were measured from the decrease in absorbance of DPPH at 525 nm by pseudo-first-order reaction at 25 °C. **5** (final concentrations: 0-0.45 mM), and DPPH (final concentration 25 μM) were dissolved in argon-deaerated buffer-40% MeCN. 0.1 M Tris-HCl buffer was used for the pH 8.4 experiments, and 0.1 M sodium phosphate buffer for the pH 6.4 and 7.4 experiments. Two syringes were charged with 2 mL (one with a test compound, the other with DPPH). The two solutions were mixed in a pneumatically driven system, and the absorbance at 525 nm was measured at time intervals (50 μs).

The reaction rate was determined by monitoring the decrease in absorbance of DPPH at 525 nm. Pseudo-first-order and second-order rate constants were calculated using the same method as for the measurements in MeCN.

ESR measurements

ESR spectra were recorded on a JEOL JES-X320 instrument with the following settings: microwave frequency, 9.42 GHz; magnetic field, 336 mT; sweep width, ±5 mT; microwave power, 10 mW; modulation frequency, 9.42 GHz; sweep time, 30 s; receiver gain, 200; time constant, 0.03 s; and temperature, room temperature. $FeSO_4$•$7H_2O$, H_2O_2, and DMPO were dissolved in argon-deaerated distilled water. The test samples were dissolved in argon-deaerated MeCN. The test samples (each 33 μL), 0.1 M sodium phosphate buffer (157 μL), and 10 mM $FeSO_4$•$7H_2O$ (10 μL) were added to a test tube containing 0.9 M DMPO (10 μL) and 10 mM H_2O_2 (10 μL), then the mixture was vortexed for 5 s at room temperature and transferred to a 10 × 50 mm flatted cell. ESR measurements were started 2 minutes after each solution was prepared. Each compound was measured for 5 doses, with the concentration just before precipitation or at which the adduct of •OH with DMPO (DMPO-OH) disappeared as the maximum concentration. The •OH radical scavenging activity at each concentration was calculated from the peak intensity of the DMPO-OH relative to the peak intensity of the Mn^{2+} marker. The •OH scavenging activity with added antioxidants is expressed as a percentage [$\% = (R\text{-}R_s)/R \times 100$], where R is the DMPO-OH adduct intensity in the absence of antioxidants, and R_s is the DMPO-OH adduct intensity in the presence of antioxidant.

p*Ka* measurements

An aliquot of tocopherol analogs (8.3-16.7×10^{-3} mg/mL) in argon-deaerated water was added to a quartz cell (width 1 cm). After deaeration with argon gas, either 0.1-1 M HCl or 0.1-1 M NaOH was added using a syringe, and the pH was recorded. A sigmoid curve was obtained from the change in absorbance for each of the following compounds: **2**, 307 nm; **3**, 312 nm; **4**; 256 nm; **5**, 255 nm for conjugated acid, 264 nm for phenoxide anion; **6**, 222 nm; **7**, 215 nm for conjugated acid and phenoxide anion (y-axis) as a function of pH (x-axis). The p*K*a was obtained from the intermediate value between the maximum and minimum absorbance in the sigmoid curve for each compound.

Theoretical calculations

The p*K*a calculations used a previously reported methodology.[17] All calculations were performed using the Gaussian 16 program package.[21] The geometry of each compound, anion, and conjugated acid for the gas phase was optimized by the B3LYP/6-31G(d) basis set. Gibbs free energy (G_{gas}) was calculated using the CBS-4M method at 298.15 K for a 1 atm standard state. For aqueous phase geometry, each species optimization and calculated solvation energy (ΔG_s) was obtained using the HF/6-31G(d) basis set with solvation model based on density (SMD). The calculated p*K*a values were determined according to the following equation.

$$\mathrm{p}K\mathrm{a} = \left\{G_{gas}(anion) - G_{gas}(acid) + \Delta G_s(anion) - \Delta G_s(acid) - 269.9\right\}/1.3644$$

REFERENCES

1. X. Zhao and K. Drlica, *Curr. Opin. Microbiol*., 2014, **21**, 1.
2. K. Chen, M. T. Kirber, H. Xiao, Y. Yang, and J. F. Keaney Jr., *J. Cell Biol.*, 2008, **181**, 1129.
3. Y. Xiao, C. Karam, J. Yi, L. Zhang, X. Li, D. Yoon, H. Wang, K. Dhakal, P. Ramlow, T. Yu, Z. Mo, J. Ma, and J. Zhou, *Pharmacol. Res.*, 2018, **138**, 25.
4. K. Inami, M. Suzuki, A. Shimizu, M. Furukawa, M. Morita, and M. Mochizuki, *RSC Adv.*, 2014, **4**, 43882.
5. K. Inami, Y. Okayama, M. Suzuki, and M. Mochizuki, *Heterocycles*, 2018, **96**, 1910.
6. Y. Okayama, M. Harada, M. Morita, M. Mochizuki, and K. Inami, *Heterocycles*, 2017, **94**, 865.
7. G. W. Burton and K. U. Ingold, *J. Am. Chem. Soc.*, 1981, **103**, 6472.
8. Z. Chen, Q. Liu, Z. Zhao, B. Bai, Z. Sun, L. Cai, Y. Fu, Y. Ma, Q. Wang, and G. Xi, *RSC Adv.*, 2021, **11**, 34456.
9. M. Ozyurek, B. Bektasoglu, K. Guclu, and R. Apak, *Anal. Chim. Acta*, 2008, **616**, 196.
10. E. Niki, *Free Radic. Biol. Med.*, 2014, **66**, 3.
11. D. Huang, B. Ou, and R. L. Prior, *J. Agric. Food Chem.*, 2005, **53**, 1841.
12. J. Jiang, J. F. Bank, and C. P. Scholes, *J. Am. Chem. Soc.*, 1993, **115**, 4742.

13. S. B. Nimse and D. Palb, *RSC Adv.*, 2015, **5**, 27986.
14. I. Nakanishi, K. Fukuhara, T. Shimada, K. Ohkubo, Y. Iizuka, K. Inami, M. Mochizuki, S. Urano, S. Itoh, N. Miyata, and S. Fukuzumi, *J. Chem. Soc., Perkin Trans. 2*, 2002, 1520.
15. G. W. Burton, T. Doha, E. J. Gabe, L. Hughes, F. L. Lee, L. Prasad, and K. U. Ingold, *J. Am. Chem. Soc.*, 1985, **107**, 7053.
16. A. Richaud, N. Barba-Behrens, and F. Mendez, *Org. Lett.*, 2011, **13**, 972.
17. M. D. Liptak, K. C. Gross, P. G. Seybold, S. Feldgus, and G. C. Shields, *J. Am. Chem. Soc.*, 2002, **124**, 6421.
18. M. Musialik and G. Litwinienko, *Org. Lett.*, 2005, **7**, 4951.
19. J. L. G. Nilsson, H. Sievertsson, and H. Selander, *Acta Chem. Scand.*, 1968, **22**, 3160.
20. G. Aridoss and K. K. Laali, *Eur. J. Org. Chem.*, 2011, 2827.
21. Gaussian 16, Revision A.03, M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, G. Scalmani, V. Barone, G. A. Petersson, H. Nakatsuji, X. Li, M. Caricato, A. V. Marenich, J. Bloino, B. G. Janesko, R. Gomperts, B. Mennucci, H. P. Hratchian, J. V. Ortiz, A. F. Izmaylov, J. L. Sonnenberg, D. Williams-Young, F. Ding, F. Lipparini, F. Egidi, J. Goings, B. Peng, A. Petrone, T. Henderson, D. Ranasinghe, V. G. Zakrzewski, J. Gao, N. Rega, G. Zheng, W. Liang, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, K. Throssell, J. A. Montgomery, Jr., J. E. Peralta, F. Ogliaro, M. J. Bearpark, J. J. Heyd, E. N. Brothers, K. N. Kudin, V. N. Staroverov, T. A. Keith, R. Kobayashi, J. Normand, K. Raghavachari, A. P. Rendell, J. C. Burant, S. S. Iyengar, J. Tomasi, M. Cossi, J. M. Millam, M. Klene, C. Adamo, R. Cammi, J. W. Ochterski, R. L. Martin, K. Morokuma, O. Farkas, J. B. Foresman, and D. J. Fox, Gaussian, Inc., Wallingford CT, 2016.