

## Free Radical Scavenging and Antioxidative Activity of Caffeic Acid Amide and Ester Analogues: Structure–Activity Relationship

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The structure–activity relationships of synthetic caffeic acid amide and ester analogues as potential antioxidants and free radical scavengers have been investigated. The 2,2-diphenyl-1-picrylhydrazyl radical (DPPH•) scavenging activity of the test compounds was *N-trans*-caffeoyl-L-cysteine methyl ester (**5**) > *N-trans*-caffeoyldopamine (**4**) > *N-trans*-caffeoyltyramine (**3**) > *N-trans*-caffeoyl- $\beta$ -phenethylamine (**2**) > Trolox C (**8**) > caffeic acid phenethyl ester (**1**) > caffeic acid (**6**) > ferulic acid (**7**). This established that the radical scavenging activity of the compounds increased with increasing numbers of hydroxyl groups or catechol moieties and also with the presence of other hydrogen-donating groups (–NH, –SH). The antioxidative activity of the compounds was also investigated in an emulsified linoleic acid oxidation system accelerated by 2,2'-azobis(2-amidinopropane) dihydrochloride. The order was **1** > **2** > **4** > **3**  $\geq$  **5** > **6** > **8** > **7**. Therefore, in the emulsion system, the antioxidative activity of the test compounds depends not only on the hydroxyl groups or catechol rings but also on the partition coefficient (log *P*) or hydrophobicity of the compounds. This supports the concept that hydrophobic antioxidants tend to exhibit better antioxidative activity in an emulsion system.

**KEYWORDS:** Antioxidants; antioxidative activity; free radical scavenging activity; caffeic acid analogues; ferulic acid; partition coefficient; structure–activity relationship

## INTRODUCTION

Caffeic acid and its analogues are widely distributed in the plant kingdom and are found in coffee beans, olives, propolis, fruits, and vegetables. They are usually found as various simple derivatives including amides, esters, sugar esters, and glycosides, or in rather more complex forms such as rosmarinic acid (dimer), lithospermic acid (trimer), verbascoside (heterosidic ester and glycoside of dihydroxyphenylethanol and caffeic acid), and the flavonoid-linked derivatives (*1*).

The physiological functionality of caffeic acid and its analogues has attracted much attention and has been studied by many research groups in recent years. The compounds are known to have antibacterial (*2*), antiviral (*3*), antiinflammatory (*4*), antiatherosclerotic (*5*), antioxidative (*6*, *7*), antiproliferative (*8*), immunostimulatory (*9*), and neuroprotective properties (*10*). These properties are associated with either their properties as antioxidants and enzyme inhibitors or their binding activity with specific receptors. Caffeic acid and its analogues are potential natural antioxidants with multiple mechanisms involving free radical scavenging, metal ion chelation, and inhibitory actions

on specific enzymes that induce free radical and lipid hydroperoxide formation (*11*, *12*). Therefore, their antioxidative actions could prevent oxidative rancidity in foods and oxidative damages in vivo, relating to diseases such as cancer, diabetes, and cardiovascular, Alzheimer's, and Parkinson's diseases.

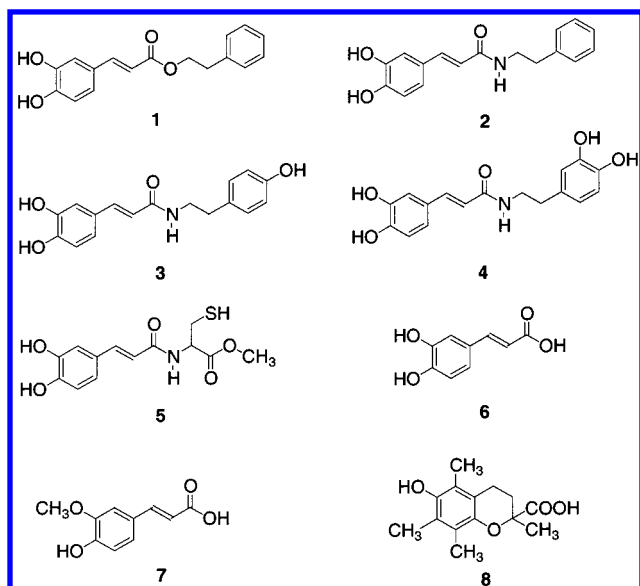
The structural feature responsible for the antioxidative and free radical scavenging activity of caffeic acid is the *ortho*-dihydroxyl functionality in the catechol ring. The presence of the electron-donating hydroxyl group at the *ortho*-position also lowers the O–H bond dissociation enthalpy and increases the rate of H-atom transfer to peroxy radicals (*13*). The unsaturated 2,3-double bond of the side chain also maximizes the stabilization of the phenolic radical (*14*). The antioxidative activity of caffeic acid analogues, however, depends on several other factors such as the electron-donating and withdrawing substituents on the catechol ring, the number of hydroxyl groups or catechol moieties, the involvement of other H-donating groups (–NH, –SH), the chemical stability, and the hydrophobicity or partition coefficient (log *P*) of the compounds.

To compare the antiradical and antioxidative activity of caffeic acid amide and ester analogues, and to better understand the effects of the physicochemical parameters mentioned above, we synthesized a series of caffeic acid amide and ester analogues and conducted a structure–activity relationship study of them (**Figure 1**). Furthermore, caffeic acid amide analogues have not

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**Figure 1.** Structures of caffeic acid analogues and reference compounds. 1, Caffeic acid phenethyl ester; 2, *N-trans*-caffeoyl- $\beta$ -phenethylamine; 3, *N-trans*-caffeoyltyramine; 4, *N-trans*-caffeoyldopamine; 5, *N-trans*-caffeoyl-L-cysteine methyl ester; 6, caffeic acid; 7, ferulic acid; 8, Trolox C.

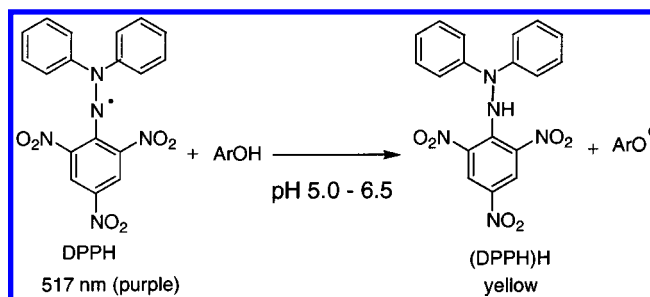
been studied to any extent. Their efficiency as radical scavengers was evaluated by their activity toward a stable free radical, 2,2-diphenyl-1-picrylhydrazyl (DPPH) (15, 16). Their potency as antioxidants was evaluated using a Tween-emulsified linoleic acid oxidation system induced by 2,2'-azobis(2-amidinopropane) dihydrochloride (17, 18) and measured by the ferric thiocyanate assay (19, 20). Trolox C, and caffeic and ferulic acids served as reference compounds (Figure 1).

## MATERIALS AND METHODS

**Chemicals.** Caffeic acid, ferulic acid, Trolox C, (2-bromoethyl)-benzene,  $\beta$ -phenethylamine, tyramine, 3-hydroxytyramine hydrochloride (dopamine hydrochloride), L-cysteine methyl ester hydrochloride, hexamethylphosphoramide, 2,2-diphenyl-1-picrylhydrazyl (DPPH $\cdot$ ), 2,2'-azobis(2-amidinopropane) dihydrochloride, linoleic acid, polyoxyethylenesorbitan monolaurate (Tween 20), and ferrous chloride tetrahydrate were purchased from Aldrich-Sigma Chemical Co. Sodium dihydrogen phosphate monohydrate, anhydrous sodium hydrogen phosphate, and ammonium thiocyanate were purchased from Fischer Scientific, Inc. All other reagents and solvents were of analytical, spectrometric, or HPLC grade.

**Apparatus.** Synthesized compounds were purified on a silica gel H (32–63 mesh) (Selecto Scientific) column and identified by TLC, UV, NMR, and X-ray diffraction analysis. Melting points were determined on a Fisher-Johns melting apparatus and are uncorrected. Thin-layer chromatography (TLC) was performed on precoated silica gel F<sub>254</sub> plates (Merck) using a 254-nm UV lamp (model UVG-54) or/and iodine vapor to visualize the compounds. IR spectra were recorded on a Perkin-Elmer 683 infrared spectrophotometer using Nujol as mulling agent or performed neat; only the most significant absorption bands are reported ( $\nu_{\max}$ ,  $\text{cm}^{-1}$ ).  $^1\text{H}$  NMR data were acquired at room temperature on a Varian VXR-400S operating at 400 MHz. Acetone- $d_6$  was used as solvent; chemical shifts are expressed in  $\delta$  (parts per million) values relative to tetramethylsilane (TMS) as internal reference; coupling constants ( $J$ ) are given in Hertz. A Spectronic Genesys 8 UV/VIS spectrophotometer was used in the DPPH $\cdot$  and ferric thiocyanate assays.

**Synthesis of Caffeic Acid Phenethyl Ester.** The compound was synthesized by base-catalyzed alkylation of caffeic acid salt with (2-bromoethyl)benzene in hexamethylphosphoramide (21). Recrystallization of the product from ether/*n*-hexane gave the final product as a pale-yellow powder; mp 124.5–126  $^{\circ}\text{C}$ , yield ca. 70%.



**Figure 2.** Radical scavenging action of antioxidants (ArOH).

### General Synthetic Procedure for Caffeic Acid Amide Analogues.

The amides were synthesized from caffeic acid and the corresponding amines (either in the free base or hydrochloride form) using benzo-triazol-1-yloxy-tris(dimethylamino)phosphonium hexafluorophosphate (BOP) as coupling reagent (22, 23). Briefly, 10 mmol of caffeic acid was dissolved in 20 mL of dimethylformamide and 1.4–2.8 mL (10–20 mmol) of triethylamine. The solution was cooled in an ice–water bath and 10 mmol of the amine was added followed by 10 mmol of BOP dissolved in 20 mL of dichloromethane. The reaction mixture was stirred at 0  $^{\circ}\text{C}$  for 30 min and then stirred at room temperature for 2 h. Dichloromethane was removed under reduced pressure, and the residual solution was diluted with 150 mL of water. The crude product was then extracted with ethyl acetate, washed successively with 1 N HCl, water, 1 M  $\text{NaHCO}_3$ , and water, then dried over  $\text{MgSO}_4$ , and the solvent was evaporated. The residue was chromatographed on a silica gel column using a mixture of ethyl acetate/*n*-hexane (1:1 or 2:1) as eluents. Yields were between 45 and 76%.

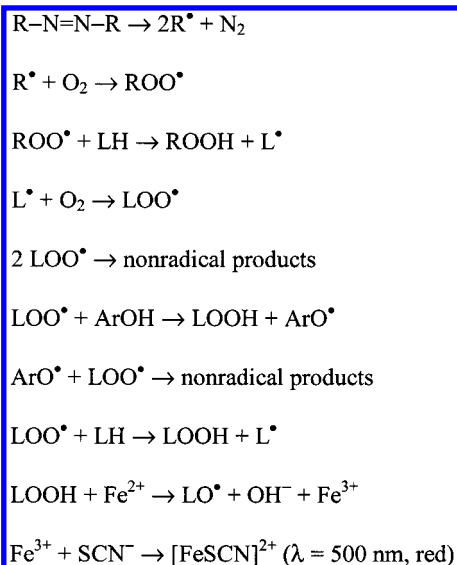
***N-trans*-Caffeoylphenethylamine.** Mp 138–140  $^{\circ}\text{C}$ ; yield 76%. IR  $\nu_{\max}$  ( $\text{cm}^{-1}$ ): 3490–3300, 3100–3000, 1660, 1625–1600, 1515, 1200.  $^1\text{H}$  NMR:  $\delta$  2.83 (t,  $J$  = 7.8, 2H,  $\text{CH}_2$ -Ar), 3.51 (dd,  $J$  = 5.9, 2H, -NH- $\text{CH}_2$ ), 6.43 (d,  $J$  = 15.6, 1H, -CH=CH-), 7.41 (d,  $J$  = 15.6, 1H, -CH=CH-), caffeic acid ring: 6.81 (d,  $J$  = 8.3, 1H, H-5), 6.9 (dd,  $J$  = 2.0,  $J$  = 7.81, 1H, H-6), 7.06 (d,  $J$  = 2.0, 1H, H-2), phenethylamine ring:  $\delta$  7.2 2–7.26 (set of signals, 5H, H-2', H-6', H-3', H-5', H-4'), phenolic hydroxyl groups: 8.3 (br, 2H, 2  $\times$  OH), amide N-H group: 7.3 (s, 1H, N-H).

***N-trans*-Caffeoyltyramine.** Mp 206–208  $^{\circ}\text{C}$ ; yield 45%. IR  $\nu_{\max}$  ( $\text{cm}^{-1}$ ): 3490–3200, 3100–3000, 1655, 1625–1600, 1515, 1210.  $^1\text{H}$  NMR:  $\delta$  2.72 (t,  $J$  = 7.3, 2H,  $\text{CH}_2$ -Ar), 3.47 (dd,  $J$  = 5.8, 2H, -NH- $\text{CH}_2$ ), 6.43 (d,  $J$  = 15.6, 1H, -CH=CH-), 7.40 (d,  $J$  = 15.6, 1H, -CH=CH-), caffeic acid ring: 6.72 (d,  $J$  = 2.9, 1H, H-2), 6.81 (d,  $J$  = 8.3, 1H, H-5), 6.89 (dd,  $J$  = 2.9,  $J$  = 8.0, 1H, H-6), tyramine ring: 7.02 (d,  $J$  = 8.3, 2H, H-2', H-6'), 7.04 (d,  $J$  = 8.3, 2H, H-3', H-5'), phenolic hydroxyl groups: 8.2 (br, 3H, 3  $\times$  OH), amide N-H group: 7.35 (s, 1H, N-H).

***N-trans*-Caffeoyldopamine.** Mp 171–173  $^{\circ}\text{C}$ ; yield 57%. IR  $\nu_{\max}$  ( $\text{cm}^{-1}$ ): 3490–3200, 3100–3000, 1650, 1625–1540, 1500, 1200.  $^1\text{H}$  NMR:  $\delta$  2.67 (t,  $J$  = 7.3, 2H,  $\text{CH}_2$ -Ar), 3.47 (dd,  $J$  = 6.3, 2H, -NH- $\text{CH}_2$ ), 6.43 (d,  $J$  = 15.6, 1H, -CH=CH-), 7.4 (d,  $J$  = 15.6, 1H, -CH=CH-), caffeic acid ring: 6.8 (d,  $J$  = 8.3, 1H, H-5), 6.9 (dd,  $J$  = 2.9,  $J$  = 8.0, 1H, H-6), 7.05 (d,  $J$  = 2.0, 1H, H-2), dopamine ring: 6.54 (dd,  $J$  = 1.95,  $J$  = 7.8, 1H, H-6'), 6.7 (d,  $J$  = 7.8, 1H, H-5'), 6.72 (s, 1H, H-2'), phenolic hydroxyl groups: 8.1 (br, 4H, 4  $\times$  OH), amide N-H group: 7.32 (s, 1H, N-H).

***N-trans*-Caffeoyl-L-cysteine methyl ester.** Mp 155–157  $^{\circ}\text{C}$ ; yield 45%. IR  $\nu_{\max}$  ( $\text{cm}^{-1}$ ): 3500–3200, 3100–3000, 2555, 1725, 1650, 1640–1580, 1500, 1200, 1100.  $^1\text{H}$  NMR: 1.94 (t,  $J$  = 8.0, 1H, -SH), 2.99 (t,  $J$  = 6.3, 2H, -CH<sub>2</sub>-SH), 3.7 (s, 3H, -O-CH<sub>3</sub>), 4.82 (m, 1H, -CH (attached to -CH<sub>2</sub>-SH, -COOCH<sub>3</sub>, and -NH-CO-)), 6.6 (d,  $J$  = 15.6, 1H, -CH=CH-), 7.44 (d,  $J$  = 15.6, 1H, -CH=CH-), caffeic acid ring: 6.83 (d,  $J$  = 8.3, 1H, H-5), 6.94 (dd,  $J$  = 1.9,  $J$  = 6.3, 1H, H-6), 7.08 (d,  $J$  = 1.9, 1H, H-2), phenolic hydroxyl groups: 8.2 (br s, 2H, 2  $\times$  OH), amide N-H group: 7.55 (d,  $J$  = 7.3, 1H, N-H).

**Determination of Radical Scavenging Activity.** 2,2-Diphenyl-1-picrylhydrazyl (DPPH $\cdot$ ) was used as a stable radical (Figure 2). DPPH $\cdot$  in ethanol (500  $\mu\text{M}$ , 2 mL) was added to 2 mL of the test compounds at different concentrations in ethanol. The final concentrations of the



**Figure 3.** Inhibitory effects of antioxidants on linoleic acid peroxidation induced by 2,2'-azobis(2-amidinopropane) dihydrochloride and measured by the ferric thiocyanate assay.  $\text{R-N=N-R}$  is the 2,2'-azobis(2-amidinopropane) dihydrochloride;  $\text{R} = -(\text{CH}_3)_2\text{C}(\text{NH}_2) = \text{NH}\cdot\text{HCl}$ ;  $\text{LH} =$  linoleic acid;  $\text{LOOH} =$  linoleic acid hydroperoxide;  $\text{ArOH} =$  antioxidants.

test compounds in the reaction mixtures were 12.5, 25, 37.5, and 50  $\mu\text{M}$ . Each mixture was then shaken vigorously and held for 30 min at room temperature and in the dark. The decrease in absorbance of DPPH $\cdot$  at 517 nm was measured. Ethanol was used as a blank solution. DPPH $\cdot$  solution (2 mL) in ethanol (2 mL) served as the control. Special care was taken to minimize the loss of free radical activity of DPPH $\cdot$  stock solution as recommended by Blois (15). All tests were performed in triplicate. The radical scavenging activity of the samples (antioxidants) was expressed in terms of  $\text{IC}_{50}$  (concentration in  $\mu\text{M}$  required for a 50% decrease in absorbance of DPPH $\cdot$  radical) and as % Inhibition of DPPH $\cdot$  absorbance (% Inhibition =  $[(A_{\text{control}} - A_{\text{test}})/A_{\text{control}}] \times 100$ ), where  $A_{\text{control}}$  is the absorbance of the control (DPPH $\cdot$  solution without test sample) and  $A_{\text{test}}$  is the absorbance of the test sample (DPPH $\cdot$  solution plus antioxidant). A plot of absorbance vs concentration was made to establish the standard curve and to calculate  $\text{IC}_{50}$ . Caffeic acid, ferulic acid, and Trolox C were used as reference compounds.

**Determination of Antioxidative Activity.** The antioxidative activity was evaluated by using 2,2'-azobis(2-amidinopropane) dihydrochloride (AAPH)-induced lipid peroxidation of a Tween-emulsified linoleic acid system and measured by the ferric thiocyanate assay (Figure 3). Briefly, 0.2 mL of distilled water, 0.5 mL of 0.2 M phosphate buffer, pH 7.0 (prepared from stock solutions of  $\text{NaH}_2\text{PO}_4$  and  $\text{NaHPO}_4$ , 0.2 M each), and 0.5 mL of 0.25% Tween-20 (in buffer solution) were mixed with 0.5 mL of 2.5% (w/v) linoleic acid in ethanol. The mixture was then stirred for 1 min. The peroxidation was initiated by the addition of AAPH solution (0.1 M, 50  $\mu\text{L}$ ). The ethanolic solution of antioxidant (100  $\mu\text{M}$ , 0.5 mL) was then added, and the reaction was carried out at 37  $^\circ\text{C}$  for 675 min in the dark. The degree of inhibition of oxidation was measured by the ferric thiocyanate method for each interval of 75, 150, 225, 300, and 375 min. To 0.1 mL of peroxidation reaction mixture at each interval, 0.1 mL of 30% ammonium thiocyanate and 0.1 mL of  $2 \times 10^{-2}$  M freshly prepared  $\text{FeCl}_2$  (in 3.5% aqueous HCl) were added. Precisely 3 min after addition, the absorbance of the red complex  $[\text{Fe}(\text{SCN})]^{2+}$  (24) was measured at 500 nm. The control for the assay was prepared in the same manner by mixing all of the chemicals and reagents except the test compound. Caffeic acid, ferulic acid, and Trolox C served as reference compounds. All tests were performed in triplicate.

**Determination of Partition Coefficient (Log P).** The logarithm of the partition coefficient for *n*-octanol/water was computed using CS ChemPropPro software, an add-on program to ChemDraw Ultra (CambridgeSoft). The program performs the calculations in three different methods using least-squares analysis. The differences between

the three methods are based on the number of atomic contributions, the number of molecules used in the database, and the types of atoms in the molecules. The correlation coefficients between the calculated and the observed values of the molecules used in these calculations were in the range of 0.926–0.931. The average log *P* values ( $\pm$  SD) of our test compounds were as follows: **1** ( $3.493 \pm 0.127$ ), **2** ( $2.797 \pm 0.175$ ), **3** ( $2.447 \pm 0.232$ ), **4** ( $2.093 \pm 0.295$ ), **5** ( $0.787 \pm 0.081$ ), **6** ( $1.303 \pm 0.240$ ), **7** ( $1.580 \pm 0.144$ ), and **8** ( $3.200 \pm 0.026$ ).

**Statistical Analysis.** Correlation and regression analyses of the radical scavenging activity versus the concentrations of antioxidants were carried out using the regression program in StatView (SAS Institute Inc., Cary, NC).  $\text{IC}_{50}$  values were obtained from the slope equations ( $Y = a + bX$ ). Multiple comparisons were by one-way analysis of variance (ANOVA), followed by Fisher's PLSD test using the same software package. Statistical significance was accepted if the null hypothesis was rejected at the  $P < 0.05$  level. The data in Figure 4 were plotted using SigmaPlot 2000 (SPSS Science, Chicago, IL).

## RESULTS AND DISCUSSION

**Free Radical Scavenging Activity.** DPPH $\cdot$  assay is the simplest method to measure the ability of antioxidants to intercept free radicals. The scavenging effects of caffeic acid amide and ester analogues are shown in Table 1. Their scavenging activity of DPPH $\cdot$  radicals decreased in the following order: **5** > **4** > **3** > **2** > **8** > **1** > **6** > **7** with all values significantly different at  $P < 0.05$ . This sequence indicates that the DPPH $\cdot$  radical scavenging activity of the test compounds is due to their hydrogen-donating ability. Increasing the number of hydroxyl or catechol groups, as seen from the sequence **4** > **3** > **2** > **1** > **6** > **7**, increases radical scavenging activity. The presence of other H-donating groups (sulfhydryl, amide) in the molecule also accelerates this activity. Compound **5** with an  $-\text{SH}$  group as an additional H-donating group in the molecule is the most active of the test compounds. The difference in radical scavenging activity between **2** and **1** (with **2** being more active) may be due to the larger effect of the amide bond on the stability and radical scavenging activity of the molecule than the ester bond.

**Antioxidative Activity.** The in vitro model using AAPH-induced lipid peroxidation of Tween-emulsified linoleic acid is a common method used to measure the antioxidative activity of synthetic and natural antioxidants. In this model assay, the oxidation is carried out under conditions relatively similar to the in vivo system, and the oxidation rate is proportional to the concentration of AAPH. The alkylperoxyl radicals produced from AAPH, which later on cause lipid peroxidation, are very similar to the radicals formed in biological systems. Factors such as compositional and interfacial phenomena relating to solubility and hydrophobicity of components, and partitioning properties of antioxidants between lipid and aqueous phases, can also be included in this assay. The high initial velocity of the reaction in this assay also makes it easy to estimate the extent to which oxidation is delayed in the presence of antioxidants (18).

The inhibitory effects on lipid peroxidation or the antioxidative activity of the compounds are shown in Figure 4. We defined the antioxidative activity of the compounds as the ability to delay lipid peroxidation, i.e., the time required to reach maximum absorbance of 4. The stronger the antioxidant the longer the period of time to reach this maximal absorbance. The inhibitory activity of the compounds in decreasing order was **1** > **2** > **4** > **3**  $\geq$  **5** > **6** > **8** > **7** with all values significantly different at  $P < 0.05$  except **3** and **5**. Caffeic acid amide and ester analogues all exhibited stronger activity than Trolox C, caffeic, and ferulic acid. Compounds **1** and **2** were highly active compounds. The results show that the antioxidative

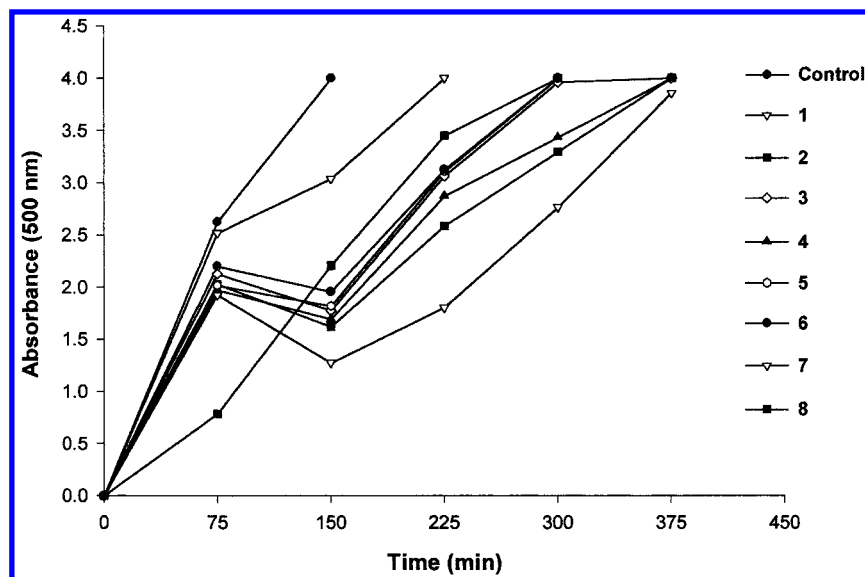


Figure 4. Antioxidative activity of the compounds on 2,2'-azobis(2-amidinopropane) dihydrochloride-induced lipid peroxidation of a Tween-emulsified linoleic acid system, measured by the ferric thiocyanate assay.

Table 1. Scavenging Activity of Antioxidants for DPPH• Radical<sup>a</sup>; Data Are Shown as IC<sub>50</sub> (μM)<sup>b</sup> and % Inhibition at 50 μM of Antioxidants<sup>c</sup>

compound	IC <sub>50</sub> (μM)	inhibition, % (± SD)
1	51.80	47.81 ± 0.15 <sup>a</sup>
2	43.07	55.63 ± 0.49 <sup>b</sup>
3	41.62	58.89 ± 2.16 <sup>c</sup>
4	24.22	91.33 ± 0.59 <sup>d</sup>
5	23.36	94.02 ± 0.32 <sup>e</sup>
6	54.31	44.37 ± 0.09 <sup>f</sup>
7	57.21	26.01 ± 0.62 <sup>g</sup>
8	46.39	53.58 ± 0.32 <sup>h</sup>

<sup>a</sup> The final concentration of DPPH ethanolic solution was  $2.5 \times 10^{-4}$  M. <sup>b</sup> The IC<sub>50</sub> (μM) values were calculated from the slope equations of the dose-response curves ( $r^2 = 0.946 - 0.992$ ). <sup>c</sup> Values are means ± standard deviation (SD) of three determinations. Values with different superscripts are significantly different ( $P < 0.05$ ).

activity in the emulsion system depends not only on the number of hydroxyl groups or catechol rings but also on the solubility, hydrophobicity (or partition coefficient,  $\log P$ ), and stability of the compounds. These results confirm the paradoxical behavior of antioxidants in lipid emulsions. On the basis of  $\log P$  values and the results shown in Figure 4, it is apparent that within the series of caffeic acid amide and ester analogues, the highly active compounds 1 and 2 have high  $\log P$  values. However, when comparing two compounds of comparable lipophilicity, the structural factors predominate in determining the activity of a specific compound. For example, compound 4, although having lower  $\log P$  value but with an additional hydroxyl group in the structure, exhibited stronger antioxidative activity than 3. The same explanation holds for 6 compared to 7. The fact that 5 does not have the highest potential activity in the emulsion system, as it does in the radical scavenging assay, may be due to (a) its low affinity for partitioning between the lipid and aqueous phases (lower value of  $\log P$ ), (b) its ability to form the hydroperoxide-adducts, or (c) the rapid oxidation of the sulfhydryl (–SH) group to the disulfide (–S–S–) group (25, 26). Trolox C shows stronger activity at the initial stage of peroxidation, but then it rapidly loses activity. This may be explained by its high partitioning affinity (high value of  $\log P$ ) in the emulsion system and by its ability to undergo one-electron oxidation to its corresponding phenoxyl radical which is then

converted, through subsequent reactions, to a ketodiene intermediate and finally to the stable Trolox C quinone (27, 28). In addition, Trolox C has only one hydroxyl group.

In conclusion, our results clearly show that the synthetic amide and ester analogues of caffeic acid exhibit stronger antioxidative activity in the two assays studied, as compared to that of the parent compounds caffeic and ferulic acids, and to the synthetic antioxidant Trolox C. The free radical scavenging activity of the compounds increased significantly with the numbers of hydroxyl groups or catechol moieties and also with the presence of other hydrogen-donating groups (–SH and –NH–CO) in the molecule. Compounds 5 and 4 are the most active compounds in the DPPH radical scavenging assay. The antioxidative activity of the compounds in the AAPH-induced lipid peroxidation of linoleic acid emulsion system, however, depends not only on the hydroxyl groups or catechol rings but also on the partition coefficient ( $\log P$ ) or hydrophobicity of the compounds. It is clear that hydrophobic antioxidants tend to exhibit better antioxidative activity in this system. Compounds 1 and 2 are the most active compounds in this emulsion system.

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