Bioorganic & Medicinal Chemistry 16 (2008) 7795–7803

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

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

Inhibitory effect of the alkyl side chain of caffeic acid analogues on lipopolysaccharide-induced nitric oxide production in RAW264.7 macrophages

Koji Uwai [a], Yuu Osanai [b,*], Takuma Imaizumi [a], Syu-ichi Kanno [b], Mitsuhiro Takeshita [a], Masaaki Ishikawa [b]

[a] Laboratory of Pharmaceutics, Tohoku Pharmaceutical University, 4-4-1 Komatsushima, Aoba-ku, Sendai 981-8558, Japan
[b] Laboratory of Clinical Pharmacotherapeutics, Tohoku Pharmaceutical University, 4-4-1 Komatsushima, Aoba-ku, Sendai 981-8558, Japan

ARTICLE INFO

Article history:
Received 16 May 2008
Revised 28 June 2008
Accepted 1 July 2008
Available online 8 July 2008

Keywords:
Caffeic acid
Nitric oxide
Cytotoxicity
QSAR

ABSTRACT

Caffeic acid esters, one of the components of propolis, are known to show a variety of biological effects such as anti-tumor, anti-oxidant, and anti-inflammatory activities. Although, the anti-inflammatory activities of caffeic acid esters have been studied by analyzing their structure, the detailed mechanisms of their activities remain unclear. Thus, in this study, we examined the function of the ester functional group and the alkyl side chain (alcoholic part) and transformed caffeic acid to several derivatives. The inhibitory effect of these derivatives on NO production in murine macrophage RAW264.7 cells was dependent on the length and size of the alkyl moiety, and undecyl caffeate was the most potent inhibitor of NO production. In addition, individual experiments using undecanol, caffeic acid, undecanol plus caffeic acid, and undecyl caffeate showed that the connection between caffeic acid and the alkyl chain is critical for activity. Amide and ketone derivatives showed that not only the ester functional group but also the amide and ketone functional groups exhibit an inhibitory effect on NO production.

1. Introduction

Nitric oxide (NO) is an important intracellular and intercellular signaling molecule involved in the regulation of diverse physiological mechanisms in immunological systems.[1] NO reacts with superoxide ($O_2^{\cdot -}$) to produce a peroxynitrite ion, which induces excess inflammatory activity and can lead to sepsis, ulcerative colitis, arthritis, systemic lupus erythematosus (SLE), and Sjögren's syndrome (SS).[2–4]

Propolis is an anti-bacterial agent produced by the European honey-bee (*Apis mellifera*) that has been used as a folk medicine and a restorative. Recently, with the growing the importance of a healthy lifestyle, propolis is becoming popular as a health-food due to its anti-cancer, anti-oxidant, anti-mycotic, and anti-inflammatory effects.[5] Caffeic acid esters are a component of propolis and are known to show various biological activities[6] such as anti-oxidant[7] and anti-inflammatory[8] effects. Although the anti-inflammatory activities of caffeic acid esters have been studied using derivatives,[2] the detailed mechanism of these activities are still unclear. In addition, Celli et al. reported that the ester connection in the structure of caffeic acid derivatives is severed by hydrolysis by choline esterase.[9] This result suggests that either the aromatic ring or the alcoholic part of the structure is involved in the activity of caffeic acid esters.[10]

Thus, in this study we prepared several caffeic acid ester derivatives with various lengths and forms of the alcoholic alkyl side chains, and their effects on NO production in murine macrophage RAW264.7 cells were investigated. To clarify the role of the ester functional group, individual assays using caffeic acid, alcohol, caffeic acid + alcohol, and ester were investigated, and amide and ketone derivatives were prepared and their inhibitory effects on NO production were also examined.

2. Results and discussion

Caffeic acid phenethyl ester and its derivatives, the typical compounds contained in propolis, were reported to show an inhibitory effect on NO production.[11] Thus, caffeic acid, which is the basal form of caffeic acid phenethyl ester, was selected as a lead compound and several caffeic acid analogues were synthesized, and their NO inhibition activities were investigated.

2.1. Synthesis of caffeic acid analogues

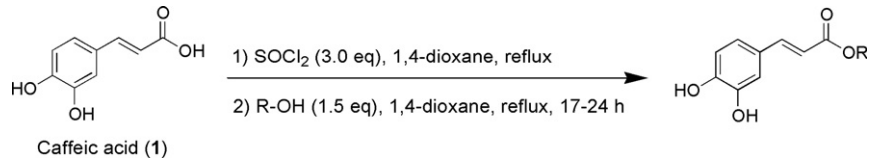
Caffeic acid esters were synthesized by preparing acid chlorides of caffeic acid followed by alkoxy-dehalogenation (Scheme 1).[12]

N-Undecylcaffeamide (**23**) was synthesized using PyBOP as a coupling agent (Scheme 2).[13,14]

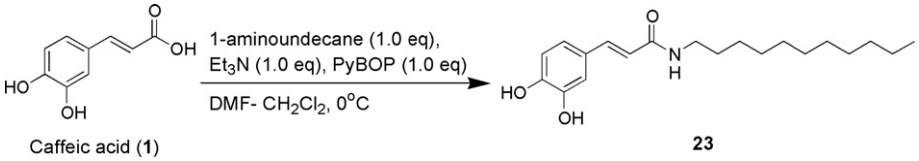
Ketone (**24**) was synthesized by treatment of Weinreb's amide with Grignard reagent (Scheme 3).

* Corresponding author. Tel.: +81 22 727 0112; fax: +81 22 275 2013.
E-mail address: 006021@is.tohoku-pharm.ac.jp (Y. Osanai).

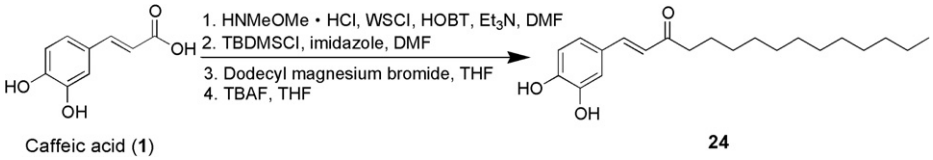
0968-0896/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2008.07.006



Scheme 1. Syntheses of caffeic acid esters. R = CH_3 (**2**), C_2H_5 (**3**), C_3H_7 (**4**), C_4H_9 (**5**), C_6H_{13} (**6**), C_7H_{15} (**7**), C_8H_{17} (**8**), C_9H_{19} (**9**), $C_{10}H_{21}$ (**10**), $C_{11}H_{23}$ (**11**), $C_{12}H_{25}$ (**12**), $C_{14}H_{27}$ (**13**), $C_{16}H_{33}$ (**14**), $C_{18}H_{37}$ (**15**), $CH(CH_3)_2$ (**16**), $C_2H_3(CH_3)_2$ (**17**), *cyclo*-Hexyl (**18**), Benzyl (**19**), Prenyl (**20**), Geranyl (**21**), Farnesyl (**22**).



Scheme 2. Synthesis of *N*-undecylcaffeamide (**23**).



Scheme 3. Synthesis of ketone (**24**).

2.2. Biological studies

Firstly, the cytotoxic effect of caffeic acid derivatives in mice macrophage RAW264.7 cells was measured by MTT methods.[15,16] After 24 h of treatment with caffeic acid derivatives (0.005–1000 μM), the survival rates of RAW264.7 cells were measured, and their EC_{50} values were calculated (Table 1).

The effect of the length of the alkyl side chain on cytotoxicity in murine RAW264.7 cell was clearly apparent. When the alkyl chain was C_0–C_{11}, the EC_{50} values of the caffeic acid esters decreased with the increasing length of the alkyl chain. In contrast, for esters with C_6–C_{18} alkyl chains, their EC_{50} values were almost constant. Undecyl (**11**) and dodecyl (**12**) caffeate were most potent cytotoxic compounds from the result of Table 1. Isopropyl ester (**16**), with the same alkyl chain length as ethyl ester (**3**) and the same carbon number as propyl ester (**4**), showed a similar EC_{50} value to propyl ester (**4**). A similar result was obtained in the case of isobutyl ester (**17**). Cyclohexyl (**18**) and benzyl (**19**) esters, which possess relatively bulky moieties, showed low cytotoxicity. These results suggested that the balance between lipophilicity and the size of the alcoholic moieties affected the cytotoxicity levels. However, prenyl (**20**), geranyl (**21**), and farnesyl (**22**) esters, which were anticipated

Table 1
EC_{50} values of the caffeic acid esters decreased with increasing length of the alkyl chain on mice macrophage RAW264.7 cells. The EC_{50} values were determined using 3–5 observations. (N = 3–5)

Compound	R	EC_{50}		*C*log*P*	Ratio
		Cytotoxicity (μM)	NO Inhibition (μM)		
1	H	3406.000 ± 714	165.295 ± 16.05	1.06	20.61
2	CH_3	367.500 ± 133	3.199 ± 0.27	1.55	114.88
3	C_2H_5	121.700 ± 17.16	3.183 ± 0.27	2.08	38.23
4	C_3H_7	26.420 ± 4.38	0.440 ± 0.08	2.61	60.04
5	C_4H_9	7.419 ± 0.93	0.240 ± 0.06	3.14	30.91
6	C_6H_{13}	2.677 ± 0.96	0.340 ± 0.05	4.19	7.87
7	C_7H_{15}	4.594 ± 1.12	0.236 ± 0.02	4.72	19.4
8	C_8H_{17}	1.588 ± 0.22	0.060 ± 0.01	5.25	26.47
9	C_9H_{19}	1.658 ± 0.02	0.052 ± 0.02	5.78	31.88
10	$C_{10}H_{21}$	1.542 ± 0.18	0.045 ± 0.09	6.31	35.29
11	$C_{11}H_{23}$	1.188 ± 0.06	0.018 ± 0.02	6.84	66.00
12	$C_{12}H_{25}$	1.000 ± 0.08	0.556 ± 0.05	7.37	1.80
13	$C_{14}H_{29}$	1.256 ± 0.07	0.292 ± 0.03	8.43	4.30
14	$C_{16}H_{33}$	3.200 ± 0.36	0.713 ± 0.13	9.48	4.48
15	$C_{18}H_{37}$	2.671 ± 0.29	0.573 ± 0.04	10.54	4.66
16	$(CH_3)_2CH$	42.200 ± 5.97	0.302 ± 0.03	2.39	139.74
17	$C_2H_3(CH_3)_2$	13.000 ± 1.81	0.303 ± 0.08	3.01	42.90
18	*cyclo*-Hexyl	28.700 ± 3.88	1.655 ± 0.36	3.58	17.34
19	Benzyl	38.800 ± 3.57	0.347 ± 0.04	3.38	111.82
20	Prenyl	30.000 ± 1.61	0.578 ± 0.04	2.08	51.90
21	Geranyl	3.054 ± 0.16	0.223 ± 0.01	3.14	13.70
22	Farnesyl	2.658 ± 0.16	0.258 ± 0.03	4.19	10.30
	Phenethyl	4.518 ± 0.04	0.193 ± 0.04	3.65	23.41

to be lipophilic and cell membrane-philic relative to their corresponding straight chain esters, showed EC_{50} values 2 to 6 times higher than butyl (**5**), octyl (**8**), and dodecyl (**12**) esters, respectively. These phenomena could be explained by the relationship between *C*log*P* and cytotoxicity. The relationship between *C*log*P* and cytotoxicity showed a biphasic correlation, as shown in Figure 1. This suggests that the cytotoxic effects of caffeic acid esters increased depending on their hydrophobicity to their optimal *C*log*P* value of 4.36 and over, as indicated by a constant value[17] (Fig. 1).

NO inhibition assay was demonstrated under the concentration, which not show any cytotoxic effect (data did not shown). Thus, on the basis of these values, we investigated the effects of caffeic acid derivatives on LPS-induced NO production in RAW264.7 cells. NO production was evaluated by a colorimetric assay for NO^- accumulation in the cell culture.[18,19] The background level of NO production was determined from RAW264.7 cells after 24 h without LPS stimulation. RAW264.7 cells treated with caffeic acid derivatives but without LPS stimulation produced NO levels similar to the background conditions (4.4 ± 0.1 μM). After 24 h of LPS (1 μg/ml) treatment, the NO level in the RAW264.7 cell culture increased to 21.3 ± 5.6 μM. Caffeic acid esters were supplied for 1 h before LPS treatment, and following further cultivation for 24 h the production of NO was inhibited in a manner depending on the length of the alkyl side chain ($0 \leqslant n \leqslant 11$). The EC_{50} values showed a minimum when $n = 11$, and undecyl caffeate (**11**) was the most potent compound tested in our study (Table 1). The EC_{50} values of NO inhibition by caffeic acid derivatives that possess C_1–C_8 alkyl groups decreased depending on the length of alkyl side chain. On the other hand, further increases in the chain length of the alkyl part increased the EC_{50} values. Esters that possessed branched alkyl side chains showed very complicated tendencies. Isopropyl ester (**16**), isobutyl (**17**) and farnesyl ester (**22**) showed higher activities than the corresponding ethyl (**3**), propyl (**4**), and dodecyl (**12**) esters. On the other hand, prenyl (**20**) and geranyl (**21**) esters showed lower activities than the corresponding butyl (**5**) and octyl (**8**) esters. Isobutyl (**17**) ester showed almost same activity as propyl ester (**4**). Cyclohexyl (**18**) and benzyl (**19**) esters, which possess bulky moieties, showed relatively high activities compared to their cytotoxicity values. These results suggested that optimal levels of the lipophilicity of the compounds and/or the size of the alkyl side chain exist with regard to the inhibition of NO production.

Caffeic acid esters were mainly divided into three groups according to the their *C*log*P* values: Group 1, the range of *C*log*P* was 1.06–4.72 for compounds **1**–**7** and **17**–**22**; group 2, *C*log*P* was 5.25–6.84 for compounds **8**–**11**; and group 3, *C*log*P* was 7.37–10.54 for compounds **12**–**15**. In comparing these groups, group 2 (R: C_8H_{17}, C_9H_{19}, $C_{10}H_{21}$, and $C_{11}H_{23}$) showed stronger NO inhibition activity than the other two groups, suggesting that the range 5.24–6.84 was the optimal *P* value for caffeic acid derivatives to inhibit NO production (Fig. 2).

Caffeic acid esters were constructed with caffeic acid and alcohols. Our results suggested the *C*log*P* value of the caffeic acid derivatives affected the inhibitory effect on NO production. Most previous reports investigated only caffeic acid esters, but some reports suggested that the ester groups of caffeic acid esters are labile in biological fluids.[10,13] From these previous reports, it was unclear if the inhibitory effect on NO production was exhibited by the alcohol alone, caffeic acid alone, or concerted effect of the alcohol and caffeic acid. As described above, undecyl (**11**) and dodecyl (**12**) caffeate were most potent cytotoxic compounds. However, the ratio (EC_{50} of cytotoxicity/EC_{50} of NO inhibition) of undecyl caffeate (**11**, 66.00) was 35-fold against that of dodecyl caffeate (**12**, 1.80). This result suggested that undecyl caffeate (**11**) has a possibility to show the inhibitory effect on NO production without any cytotoxic effect. As shown in Figure 3, at 1 μM, undecyl caffeate (**11**) did exhibit significant inhibitory effect on NO production compared to control, but did not show significant cytotoxic effect. Thus, we used maximum concentrations of undecyl caffeate (**11**) 1 μM for the subsequent experiments. Thus, caffeic acid, undecalol, caffeic acid + undecanol, and undecyl caffeate (**11**) were applied to murine RAW264.7 cells and the production of NO was investigated (Fig. 3c). Although when LPS alone was supplied, NO production increased significantly relative to the background, caffeic acid (2.46 ± 0.29 μM), undecanol (3.98 ± 1.19 μM), and caffeic acid + undecanol (1.82 ± 0.40 μM), did not show any significant difference in NO production and the value was similar to the background level. When caffeic acid, undecanol, undecyl caffeate (**11**), and caffeic acid + undecanol were applied for 1 h before LPS treatment, the production of NO after 24 h was not inhibited in the case of pretreatment with caffeic acid (16.7 ± 0.92 μM), undecanol (17.2 ± 0.84 μM), or caffeic acid + undecanol (12.7 ± 1.59 μM), while a significant difference was observed in the case of pre-treatment with undecyl caffeate (7.87 ± 1.45 μM, $n = 3$, $P < 0.01$) (**11**). Cytotoxicity was not observed in any of these treatments. These results indicated that the connection between caffeic acid and the alcohol was critical for the inhibition of NO production (Fig. 3).

Because these results suggested the importance of the connection between caffeic acid and the alcohol and some reports sug-

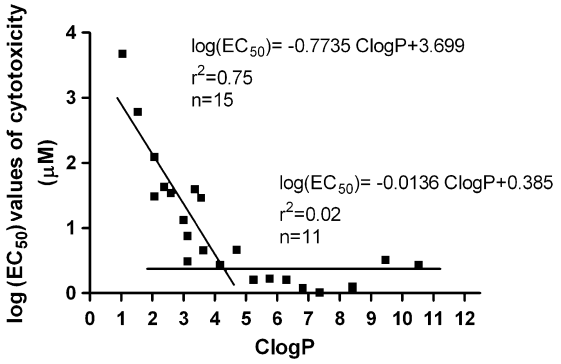


Figure 1. Relationship between *C*log*P* and cytotoxicity in RAW264.7 cells. Cells were incubated with caffeic acid esters for 24 h. EC_{50} values were defined as the concentration that showed a 50% decrease in survival. *C*log*P* values were calculated using Bio-Loom software. The linear line was calculated by the least-squares method. The values of the intersection was *C*log*P* = 4.36 and the EC_{50} value of cytotoxicity = 2.11 μM.

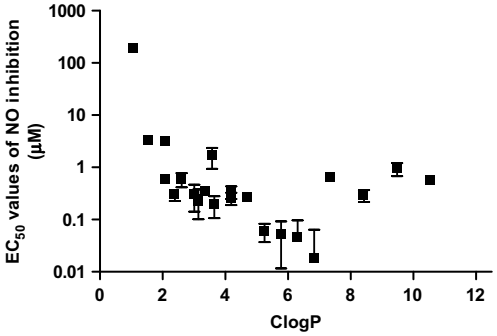


Figure 2. Effect of *C*log*P* of the caffeic acid esters on the LPS-induced NO production of RAW264.7 cells. Cells were incubated with LPS (1 μg/mL) in the presence or absence caffeic acid ester for 24 h. Caffeic acid esters were added 1 h before incubation with LPS. EC_{50} values were defined as the concentration that showed a 50% decrease in NO production.

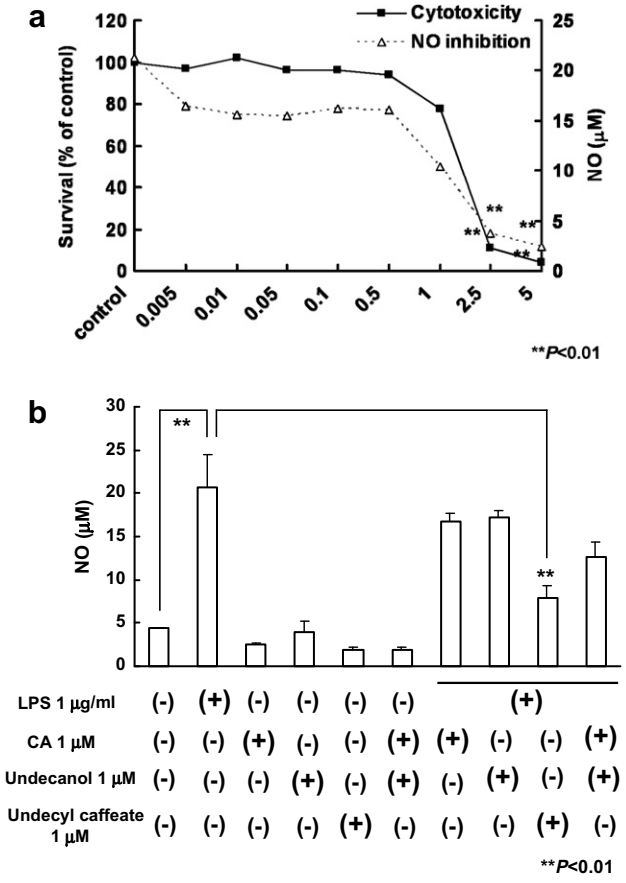


Figure 3. Effect of undecyl caffeate (**11**)-induced cytotoxicity or inhibitory effect of undecyl caffeate (**11**) on LPS-induced production of nitrite in mice RAW264.7 macrophages. (a) Cells were incubated with undecyl caffeate for 24 h and then cytotoxicity was estimated by MTT assay and cells were incubated for with LPS 1 μg/ml in the presence or absence of undecyl caffeate (**11**). (b) Inhibitory effect of caffeic acid, undecanol, caffeic acid + undecanol, and undecyl caffeate (**11**) on LPS-induced NO production in RAW264.7 cells. RAW264.7 cells were incubated for 24 h with LPS (1 μg/mL) in the presence or absence of caffeic acid (1 μM), undecanol (1 μM), or undecyl caffeate (**11**, 1 μM). Accumulated NO in the culture medium was determined by Griess reaction. The values are means ± SEM ($n = 5$). $^{**}P < 0.01$.

gested that the ester functional groups of caffeic acid esters were labile in biological fluids, the requirement for the ester functional groups was evaluated: that is, the inhibitory effect on NO production by amide (**23**) and ketone (**24**) derivatives was investigated. When caffeamide (**23**) was applied for 1 h before LPS treatment, the production of NO after 24 hrs increased slightly compared to the case of pre-treatment with undecyl caffeate (**11**) (Table 2), and a similar result was obtained in the case of the ketone derivative (**24**).

These results suggested that ester was not hydrolyzed in the cells, and although there was little effect of ester functional group on the inhibitory effect on NO production, the effect of the ester functional group was less important than anticipated. However, Zhu et al.[20] suggested that in caco-2 cells, the drug permeability was increased by increasing their log*P*. Therefore, the lipophilicity of the lipophilic moiety was much more important for the inhibitory effect on NO production. And alkyl side chain seems to play a role for transporting the caffeic acid into cell nuclear.

Table 2
Inhibitory effect of C_{11}-ester (**11**), amide (**23**), and ketone (**24**) derivatives on LPS-induced nitrite production

Compound	EC_{50}	
	Cytotoxicity (μM)	NO Inhibition (μM)
Ester (**11**)	1.324 (0.799–2.194)	0.01335 (0.006–0.031)
Amide (**23**)	1.940 (1.188–3.168)	0.08151 (0.037–0.180)
Ketone (**24**)	1.473 (0.867–2.503)	0.06079 (0.041–0.091)

3. Conclusion

In this study, we investigated the function of the ester group and the alkyl side chain (alcoholic part) of caffeic acid derivatives. Their inhibitory effect on NO production in murine macrophage RAW264.7 cells was dependent on the length and size of the alkyl side chain, and undecyl caffeate was most potent inhibitor of NO production. In addition, individual experiments with undecanol, caffeic acid, undecanol + caffeic acid, and undecyl caffeate showed that the connection of caffeic acid and the alcohol moiety is critical for the activity. Inhibition assays with amide and ketone derivatives suggested that the ester was not hydrolyzed by esterases, so the lipophilicity of the lipophilic moiety and/or the molecular shape is critical for the activity.

The present study suggested part of the mechanism concerning the NO inhibition activity of caffeic acid derivatives, and these compounds may be applicable for the treatment of NO-mediated diseases. Further studies are now underway in our laboratory, including a search for more effective analogues and further elucidation of the NO-inhibitory mechanism of action of these compounds.

4. Materials and methods

4.1. Experimental materials

Lipopolysaccharide (LPS; *Escherichia coli* O55:B5) and other reagents were supplied by either Nacalai Tesque (Kyoto, Japan), Sigma (St. Louis, MO, USA), or Wako Pure Chemical Industries, Ltd (Osaka, Japan) and were of the highest grade available. All cell culture reagents were obtained from Invitrogen Corp. (Carlsbad, CA, USA).

4.2. Instrumental

^{1}H and ^{13}C NMR spectra were measured using JEOL JNM-EX 270 (270 MHz) and JEOL JNM-AL 400 (400 MHz) spectrometers. Chemical shifts (δ) were reported as ppm, and coupling constants were given in Hz. Multiplicities were indicated as follows: s (singlet), d (doublet), dd (doublet of doublets), t (triplet), q (quartet), quint (quintet), and m (multiplet). IR spectra were obtained on a Perkin-Elmer Spectrum One spectrophotometer. Mass spectra were recorded on JEOL JMS DX-303 and JMA-DA 5000 spectrometers.

4.3. Cell culture

Murine macrophage RAW264.7 cells were obtained from the American Type Culture Collection (#TIB-71, Manassas, VA, USA). The cells were grown in RPMI-1640 medium supplemented with 100 U/mL penicillin, 100 μg/mL streptomycin, and 10% heat-inactivated fetal bovine serum at 37 °C in a humidified 5% CO_2–95% air incubator under standard conditions. The RAW264.7 cells were cultured at 4×10^5 cells/mL in 100 μL aliquots in separate wells of 96-well plates for the measurement of NO concentrations.[15]

4.4. Cytotoxicity assay

Cytotoxicity was assessed by a colorimetric MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay.

Following treatment with caffeic acid derivatives and LPS, 10 μL of MTT (5 mg/ml in saline) was added to each well. Then, the sample was incubated for 90 min at 37 °C. After aspiration of the supernatant, the cells were lysed and solubilized by the addition of 100 μL of 0.04 N HCl containing isopropanol. The absorbance of each well was determined at 590 nm using an Inter-med model NJ-2300 Microplate Reader. Survival (%) was calculated relative to the control.[16]

4.5. Measurement of nitrite concentrations

To measure the concentration of NO produced in RAW264.7 macrophage cells, unless otherwise specified, the stable conversion product of NO, nitrite ($NO_2^{\cdot-}$), was measured. NO production was determined by measuring the accumulation of nitrite in the culture medium supernatant using Griess reagent. Briefly, 50 μL of the supernatant from each sample was mixed with an equal volume of Griess reagent (1:1 v/v of 1% sulfanilamide and 0.1% naphthylethylenediamine dihydrochloride in 5% H_3PO_4) in 96-well plates and incubated for 10 min at room temperature. Nitrite levels were determined colorimetrically at 540 nm using an Inter-med model NJ-2300 Microplate Reader and a standard curve of sodium nitrite.[16,18]

4.6. Calculation of log*P*

The calculation of the octanol–water partition coefficient log*P* (*C*log*P*) was made using Bio-Loom software (BioByte Corp., CA, USA).[17]

4.7. Statistical analysis

Statistical analysis of the results was performed with a one-way analysis of variance (ANOVA) followed by Scheff's *F* test by rank test. A *p*-value of less than 0.05 was considered significant.

4.8. Synthesis of caffeic acid analogues

4.8.1. General procedure for the synthesis of caffeic acid esters

Thionyl chloride (1.5 equiv) was added dropwise to a stirred solution of caffeic acid in 1,4-dioxane and stirred under reflux for 1 h. *n*-Hexanol (1.5 equiv) was added over 1 h, and the reaction mixture was stirred for 17 h. The reaction mixture was then concentrated in vacuo, and the residue was purified by column chromatography on silica gel (*n*-hexane–AcOEt) to give the caffeic acid ester.

4.8.2. Methyl 1-(3′,4′-dihydroxyphenyl)propenate (methyl caffeate) (2)

Yield: 82%. Mp: 166–167 °C (recrystallized from Et_2O, colorless scale). FT-IR ν_{max} (KBr): 3478, 3106, 2954, 1679, 1607, 1536. ^{1}H NMR (400 MHz, acetone-d_6) δ: 8.32 (2H, br s), 7.53 (1H, d, J = 16.1 Hz), 7.16 (1H, d, J = 2.0 Hz), 7.03 (1H, dd, J = 2.0, 7.8 Hz), 6.86 (1H, d, J = 7.8 Hz), 6.28 (1H, d, J = 16.1 Hz), 3.70 (3H, s). ^{13}C NMR (100 MHz, acetone-d_6) δ: 167.9, 148.6, 146.2, 145.7, 127.5, 122.5, 116.3, 115.3, 115.1, 51.5. MS *m*/*z*: 194 (M^+), 163 (100%), 134, 117, 89, 77. High-resolution MS calcd for $C_{10}H_{10}O_4$ (M^+): 194.0579. Found: 194.0573. Anal. calcd for $C_{10}H_{10}O_4$: 61.85% C, 5.19% H. Found: 61.79% C, 5.10% H. All spectral data agreed with previously reported data.[21]

4.8.3. Ethyl 1-(3′,4′-dihydroxyphenyl)propenate (ethyl caffeate) (3)

Yield: 82%. Mp: 149–151 °C (recrystallized from Et_2O, colorless scale). FT-IR ν_{max} (KBr): 3438, 3190, 2983, 1659, 1609, 1533. ^{1}H NMR (400 MHz, acetone-d_6) δ: 8.29 (2H, br s), 7.53 (1H, d, J = 16.1 Hz), 7.16 (1H, d, J = 2.0 Hz), 7.03 (1H, dd, J = 2.0, 8.3 Hz), 6.87 (1H, d, J = 7.8 Hz), 6.27 (1H, d, J = 16.1 Hz), 4.17 (2H, q, J = 7.3 Hz), 1.25 (3H, t, J = 7.3 Hz). ^{13}C NMR (100 MHz, acetone-d_6) δ: 167.4, 148.6, 146.2, 145.5, 127.6, 122.5, 116.3, 115.7, 115.2, 60.5, 14.6. MS *m*/*z*: 208 (M^+), 180, 163 (100%), 134, 89, 77. High-resolution MS calcd for $C_{11}H_{12}O_4$ (M^+): 208.0736. Found: 208.0735. Anal. calcd for $C_{11}H_{12}O_4$: 63.45% C, 5.81% H. Found: 63.46% C, 5.84% H. All spectral data agreed with previously reported data.[21]

4.8.4. Propyl 1-(3′,4′-dihydroxyphenyl)propenate (propyl caffeate) (4)

Yield: 86%. Mp: 129–131 °C (recrystallized from Et_2O, colorless scale). FT-IR ν_{max} (KBr): 3462, 3080, 2970, 2897, 1666, 1605, 1535. ^{1}H NMR (400 MHz, acetone-d_6) δ: 8.33 (2H, br s), 7.54 (1H, d, J = 16.1 Hz), 7.16 (1H, d, J = 2.0 Hz), 7.03 (1H, dd, J = 2.0, 7.8 Hz), 6.87 (1H, d, J = 7.8 Hz), 6.28 (1H, d, J = 16.1 Hz), 4.09 (2H, t, J = 6.8 Hz), 1.71–1.62 (2H, m), 0.94 (3H, t, J = 7.3 Hz). ^{13}C NMR (100 MHz, acetone-d_6) δ: 167.5, 148.6, 146.2, 145.5, 127.6, 122.5, 116.3, 115.7, 115.2, 66.1, 22.8, 10.6. MS *m*/*z*: 222 (M^+), 208, 193, 180, 163 (100%), 134, 89, 77. High-resolution MS calcd for $C_{12}H_{14}O_4$ (M^+): 222.0892. Found: 222.0899. Anal. calcd for $C_{12}H_{14}O_4$: 64.85% C, 6.35% H. Found: 64.81% C, 6.42% H. All spectral data agreed with previously reported data.[22,23]

4.8.5. Butyl 1-(3′,4′-dihydroxyphenyl)propenate (butyl caffeate) (5)

Yield: 86%. Mp: 109–111 °C (recrystallized from Et_2O, colorless needles). FT-IR ν_{max} (KBr): 3490, 3342, 2954, 2929, 2870, 1684, 1639, 1605, 1537. ^{1}H NMR (400 MHz, acetone-d_6) δ: 8.27 (2H, br s), 7.54 (1H, d, J = 16.1 Hz), 7.16 (1H, d, J = 2.0 Hz), 7.03 (1H, dd, J = 2.0, 8.3 Hz), 6.86 (1H, d, J = 8.3 Hz), 6.28 (1H, d, J = 16.1 Hz), 4.13 (1H, t, J = 6.3 Hz), 1.68–1.58 (m, 2H), 1.46–1.34 (m, 2H), 0.92 (3H, t, J = 7.8 Hz). ^{13}C NMR (100 MHz, acetone-d_6) δ: 167.5, 148.6, 146.2, 145.5, 127.6, 122.4, 116.3, 115.7, 115.2, 64.4, 31.6, 19.8, 14.0. MS *m*/*z*: 236 (M^+), 219, 208, 193, 180 (100%), 163, 136, 89, 77. High-resolution MS calcd for $C_{13}H_{16}O_4$ (M^+): 236.1049. Found: 236.1053. Anal. calcd for $C_{13}H_{16}O_4$: 66.09% C, 6.83% H. Found: 66.04% C, 6.84% H. All spectral data agreed with previously reported data.[21]

4.8.6. Hexyl 1-(3′,4′-dihydroxyphenyl)propenate (*n*-hexyl caffeate) (6)

Yield: 55%. Mp: 127–128 °C (recrystallized from $CHCl_3$, colorless needles). FT-IR ν_{max} (KBr): 3497, 3340, 2959, 2935, 2857, 1688, 1640, 1605, 1536. ^{1}H NMR (270 MHz, acetone-d_6) δ: 8.42 (2H, br s), 7.53 (1H, d, J = 16.0 Hz), 7.16 (1H, d, J = 2.1 Hz), 7.02 (1H, dd, J = 2.1, 8.1 Hz), 6.86 (1H, d, J = 8.1 Hz), 6.27 (1H, d, J = 16.0 Hz), 4.13 (2H, t, J = 6.6 Hz), 1.65 (2H, quint, J = 6.6 Hz), 1.45–1.24 (6H, m), 0.87 (3H, t, J = 6.6 Hz). ^{13}C NMR (67.8 MHz, acetone-d_6) δ: 167.5, 148.7, 146.2, 145.5, 127.5, 122.4, 116.3, 115.6, 115.0, 64.7, 32.1, 29.4, 26.3, 23.1, 14.2. MS *m*/*z*: 264, 247, 236, 208, 180 (100%), 163, 77. High-resolution MS calcd for $C_{15}H_{20}O_4$ (M^+):264.1362. Found: 264.1342. Anal. calcd for $C_{15}H_{20}O_4$: 68.16% C, 7.64% H. Found: 67.98% C, 7.80% H. All spectral data agreed with previously reported data.[21]

4.8.7. Heptyl 1-(3′,4′-dihydroxyphenyl)propenate (*n*-heptyl caffeate) (7)

Yield: 58%. Mp: 107–108 °C (recrystallized from *n*-hexane–Et_2O, colorless needles). FT-IR ν_{max} (KBr) 3491, 3337, 2856, 1688, 1639, 1696, 1536. ^{1}H NMR (270 MHz, acetone-d_6) δ: 8.42 (1H, s), 8.13 (1H, s), 7.52 (1H, d, J = 16.0 Hz), 7.15 (1H, d, J = 2.3 Hz), 7.04 (1H, dd, J = 2.3, 8.4 Hz), 6.86 (1H, d, J = 8.4 Hz), 6.27 (1H, d, J = 16.0 Hz), 4.13 (2H, t, J = 6.6 Hz), 1.66 (2H, quint, J = 6.6 Hz), 1.46–1.24 (8H, m), 0.88 (3H, t, J = 7.1 Hz). ^{13}C NMR (67.8 MHz ace-

tone-d_6) δ: 167.4, 148.7, 146.3, 145.5, 127.7, 122.4, 116.4, 115.8, 115.2, 64.6, 34.5, 29.7, 29.6, 26.7, 23.2, 14.3. MS m/z: 278 (M^+), 261, 250, 235, 221, 207, 193, 180 (100%), 163, 117, 77. High-resolution MS calcd for $C_{16}H_{22}O_4$ (M^+): 278.1519. Found: 278.1530. Anal. calcd for $C_{16}H_{22}O_4$: 69.04% C, 7.97% H. Found: 69.12% C, 8.26% H. All spectral data agreed with previously reported data.[24]

4.8.8. Octyl 1-(3′,4′-dihydroxyphenyl)propenate (*n*-octyl caffeate) (8)

Yield: 66%. Mp: 110–111 °C (recrystallized from Et_2O, colorless needles). FT-IR ν_{max} (KBr): 3488, 3319, 2921, 2856, 1679, 1606, 1532. ^{1}H NMR (270 MHz, acetone-d_6) δ: 8.41 (1H, s), 8.13 (1H, s), 7.54 (1H, d, J = 16.0 Hz), 7.15 (1H, d, J = 2.0 Hz), 7.03 (1H, dd, J = 2.0, 8.1 Hz), 6.86 (1H, d, J = 8.1 Hz), 6.28 (1H, d, J = 16.0 Hz), 4.13 (2H, t, J = 6.7 Hz), 1.66 (2H, quint, J = 6.7 Hz), 1.45–1.14 (10H, m), 0.86 (3H, t, J = 6.9 Hz). ^{13}C NMR (67.8 MHz acetone-d_6) δ: 167.4, 148.6, 146.2, 145.5, 127.6, 122.42, 116.3, 115.7, 115.1, 64.7, 32.5, 29.9, 29.9, 29.5, 26.6, 23.2, 14.3. MS m/z: 292 (M^+), 193, 180 (100%), 163, 117. High-resolution MS calcd for $C_{17}H_{24}O_4$ (M^+): 292.1675. Found: 292.1683. Anal. calcd for $C_{17}H_{24}O_4$: 69.8% C, 8.27% H. Found: 69.7% C, 8.45% H. All spectral data agreed with previously reported data.[21,25]

4.8.9. Nonyl 1-(3′,4′-dihydroxy phenyl)propenate (*n*-nonyl caffeate) (9)

Yield: 68%. Mp: 105–106 °C (recrystallized from *n*-hexane–Et_2O, colorless needles). FT-IR ν_{max} (KBr): 3489, 3331, 2960, 2923, 2848, 1686, 1604, 1536. ^{1}H NMR (270 MHz, acetone-d_6) δ: 8.42 (1H, s), 8.13 (1H, s), 7.53 (1H, d, J = 16.0 Hz), 7.15 (1H, d, J = 2.0 Hz), 7.04 (1H, dd, J = 2.0, 8.2 Hz), 6.86 (1H, d, J = 8.2 Hz), 6.27 (1H, d, J = 16.0 Hz), 4.13 (2H, t, J = 6.6Hz), 1.67 (2H, quint, J = 6.6 Hz), 1.46–1.19 (12H, m), 0.87 (3H, t, J = 6.9 Hz). ^{13}C NMR (67.8 MHz acetone-d_6) δ: 167.4, 148.7, 146.3, 145.5, 127.7, 122.4, 116.4, 115.8, 115.2, 64.6, 32.6, 30.2, 30.0, 30.0, 26.7, 23.3, 14.3. MS m/z: 306 (M^+), 278, 249, 235, 221, 207, 193, 180 (100%), 163, 136, 117. High-resolution MS calcd for $C_{18}H_{26}O_4$ (M^+): 306.1832. Found: 306.1839. Anal. calcd for $C_{18}H_{26}O_4$: 70.56% C, 8.55% H. Found: 70.53% C, 8.67% H. All spectral data agreed with previously reported data.[26]

4.8.10. Decanyl 1-(3′,4′-dihydroxyphenyl)propenate (*n*-decanyl caffeate) (10)

Yield: 89%. Mp: 111–112 °C (recrystallized from *n*-hexane–Et_2O, colorless needles). FT-IR ν_{max}(KBr): 3485, 3313, 1684, 1606, 1532, 1474, 1317, 1246, 1179, 1110, 975, 861, ^{1}H NMR (270 MHz, $CDCl_3$) δ: 7.59 (1H, d, J = 16.0 Hz), 7.08 (1H, d, J = 1.8 Hz), 7.02 (1H, dd, J = 1.8, 8.1 Hz), 6.87 (1H, d, J = 8.1 Hz), 6.27 (1H, d, J = 16.0 Hz), 5.39 (1H, S), 5.25 (1H, S), 4.18 (2H, t, J = 6.8 Hz), 1.69 (2H, quint, J = 6.8 Hz), 1.46–1.14 (14H, m), 0.88 (3H, t, J = 6.6 Hz). ^{13}C NMR (67.8 MHz, $CDCl_3$) δ: 168.0, 146.2, 144.8, 143.7, 127.6, 122.4, 115.8, 115.5, 114.5, 77.2, 64.9, 31.9, 29.5, 29.3, 29.3, 28.7, 26.0, 22.7, 14.1. MS m/z: 320 (M^+), 264, 221, 180 (100%), 163, 136, 117. High-resolution MS calcd for $C_{19}H_{28}O_4$ (M^+): 320.1988. Found: 320.1979. Anal. calcd for $C_{19}H_{28}O_4$: 71.2% C, 8.81% H. Found: 71.2% C, 9.04% H. All spectral data agreed with previously reported data.[22,27]

4.8.11. Undecyl 1-(3′,4′-dihydroxy phenyl)propenate (*n*-undecyl caffeate) (11)

Yield: 23%. Mp: 109–110.5 °C (recrystallized from Et_2O, colorless needles). FT-IR ν_{max} (KBr): 3488, 3333, 2959, 2918, 2850, 1687, 1604, 1536. ^{1}H NMR (400 MHz, CD_3OD) δ: 7.52 (1H, d, J = 16.0 Hz), 7.03 (1H, d, J = 2.0 Hz), 6.93 (1H, dd, J = 2.0, 7.8 Hz), 6.76 (1H, d, J = 7.8 Hz), 6.24 (1H, d, J = 16.0 Hz), 4.15 (2H, t, J = 6.8 Hz), 1.68 (2H, quint, J = 6.8 Hz), 1.45–1.18 (16H, m), 0.88 (3H, t, J = 6.8 Hz). ^{13}C NMR (100 MHz, CD_3OD) δ: 169.4, 149.5, 146.8, 146.8, 127.7, 122.9, 116.5, 115.2, 115.1, 65.6, 33.1, 30.7, 30.7, 30.6, 30.5, 30.4, 29.8, 27.1, 23.7, 14.4. MS m/z: 334 (M^+), 292, 278, 236, 193, 180 (100%), 163, 136. High-resolution MS calcd for $C_{20}H_{30}O_4$ (M^+): 334.2145. Found: 334.2137. Anal. calcd for $C_{20}H_{30}O_4$: 71.82% C, 9.04% H. Found: 71.63% C, 9.20% H.

4.8.12. Dodecyl 1-(3′,4′-dihydroxyphenyl)propenate (*n*-dodecyl caffeate) (12)

Yield: 44%. Mp: 105–106 °C (recrystallized from Et_2O, colorless needles). FT-IR ν_{max} (KBr): 3482, 3313, 2956, 2921, 2850, 1685, 1606, 1532. ^{1}H NMR (270 MHz, $CDCl_3$) δ: 7.57 (1H, d, J = 16.0 Hz), 7.08 (1H, d, J = 2.0 Hz), 7.02 (1H, dd, J = 2.0, 8.2 Hz), 6.87 (1H, d, J = 8.2 Hz), 6.27 (1H, d, J = 16.0 Hz), 4.18 (2H, t, J = 6.7 Hz), 1.69 (2H, quint, J = 6.7 Hz), 1.45–1.18 (18H, m), 0.88 (3H, t, J = 6.9 Hz). ^{13}C NMR (67.8 MHz, DMSO-d_6) δ: 166.9, 148.7, 146.0, 145.3, 125.9, 121.6, 116.0, 115.0, 114.3, 64.0, 31.8, 30.9, 29.5, 29.5, 29.4, 29.2, 29.2, 28.7, 25.9, 22.5, 14.2. MS m/z: 348 (M^+), 320, 235, 221, 193, 180 (100%), 163, 136. High-resolution MS calcd for $C_{21}H_{32}O_4$ (M^+): 348.2302. Found: 348.2306. Anal. calcd for $C_{21}H_{32}O_4$: 72.38% C, 9.26% H. Found: 72.43% C, 9.52% H. All spectral data agreed with previously reported data.[22]

4.8.13. Tetradecyl 1-(3′,4′-dihydroxyphenyl)propenate (*n*-tetradecyl caffeate) (13)

Yield: 35%. Mp: 108–109 °C (recrystallized from $CHCl_3$–toluene, colorless needles). FT-IR ν_{max}(KBr): 3480, 3310, 2956, 2918, 2849, 1686, 1604, 1532. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.41 (2H, br s), 7.45 (1H, d, J = 16.0 Hz), 7.02 (1H, d, J = 2.0 Hz), 6.96 (1H, dd, J = 2.0, 8.3 Hz), 6.74 (1H, d, J = 8.3 Hz), 6.22 (1H, d, J = 16.0 Hz), 4.07 (2H, t, J = 6.8 Hz), 1.59 (2H, quint, J = 6.8 Hz), 1.37–1.13 (22H, m), 0.82 (3H, t, J = 6.3 Hz). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 166.6, 148.5, 145.7, 145.1, 125.6, 121.3, 115.8, 114.9, 114.1, 63.8, 31.4, 29.3–29.2 (C × 4), 29.1, 29.1, 28.9, 28.8, 28.4, 25.6, 22.2, 14.0. MS m/z: 376 (M^+), 348, 334, 269, 193, 180 (100%), 163, 136. High-resolution MS calcd for $C_{23}H_{36}O_4$ (M^+): 376.2615. Found: 376.2614. Anal. calcd for $C_{23}H_{36}O_4$: 70.56% C, 8.55% H. Found: 73.51% C, 9.93% H. All spectral data agreed with previously reported data.[22]

4.8.14. Hexadecyl 1-(3′,4′-dihydroxyphenyl)propenate (*n*-hexadecyl caffeate) (14)

Yield: 47%. Mp: 110–111 °C (recrystallized from Et_2O, colorless needles). FT-IR ν_{max} (KBr): 3480, 3308, 2956, 2916, 2849, 1690, 1607, 1532. ^{1}H NMR (400 MHz, CD_3OD) δ: 7.5 21H, d, J = 16.1 Hz, 7.0 31H, d, J = 2.0 Hz), 6.93 (1H, dd, J = 2.0, 7.8Hz), 6.77 (1H, d, J = 7.8 Hz), 6.25 (1H, d, J = 16.1 Hz), 4.16 (2H, t, J = 6.3 Hz), 1.69 (2H, quint), 1.44–1.15 (26H, m), 0.89 (3H, t, J = 7.3 Hz). ^{13}C NMR (100 MHz, CD_3OD) δ: 169.4, 149.6, 146.8, 146.8, 127.7, 122.9, 116.5, 115.2, 115.1, 65.6, 33.1, 30.8–30.7 (C×6), 30.6, 30.6, 30.5, 30.3, 29.8, 27.1, 23.7, 14.4. MS m/z: 404 (M^+), 376, 291, 239, 180 (100%), 163, 136. High-resolution MS calcd for $C_{25}H_{40}O_4$ (M^+): 404.2928. Found: 404.2906. Anal. calcd for $C_{25}H_{40}O_4$: 74.22% C, 9.97% H. Found: 74.32% C, 10.42% H. All spectral data agreed with previously reported data.[22,23]

4.8.15. Octadecyl 1-(3′,4′-dihydroxyphenyl)propenate (*n*-octadecyl caffeate) (15)

Yield: 38%. Mp: 110–112 °C (recrystallized from $CHCl_3$, colorless needles). FT-IR ν_{max} (KBr): 3481, 3312, 2926, 2920, 2851, 1684, 1607, 1532. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.57 (1H, s), 9.11 (1H, s), 7.45 (1H, d, J = 15.6 Hz), 7.03 (1H, d, J = 2.0 Hz), 6.98 (1H, dd, J = 2.0, 8.3 Hz), 6.74 (1H, d, J = 8.3 Hz), 6.24 (1H, d, J = 15.6 Hz), 4.08 (2H, t, J = Hz), 1.60 (2H, quint), 1.34–1.10 (30H, m), 0.84 (3H, t, J = 6.3 Hz). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 166.5, 148.3, 145.5, 144.9, 125.5, 121.5, 121.3, 115.7, 114.8, 114.0, 63.6, 31.2, 28.9–28.8 (C×10), 28.6, 28.6, 28.2, 25.4, 22.0,

13.9. MS *m/z*: 432 (M^+), 404, 309, 250, 207, 180 (100%), 136. High-resolution MS calcd for $C_{27}H_{44}O_4$ (M^+): 432.3241. Found: 432.3246. Anal. calcd for $C_{27}H_{44}O_4$: 74.96% C, 10.25% H. Found: 74.96% C, 10.54% H. All spectral data agreed with previously reported data.[23]

4.8.16. 1-Methylethyl 1-(3′,4′-dihydroxyphenyl)propenate (isopropyl caffeate) (16)

Yield: 82%. Mp: 147.5–149 °C (recrystallized from Et_2O, colorless needles). FT-IR ν_{max} (KBr): 3467, 3311, 2985, 2968, 1680, 1633, 1601, 1536. ^{1}H NMR (400 MHz, acetone-d_6) δ: 8.26 (2H, br s), 7.52 (1H, d, J = 16.1 Hz), 7.15 (1H, d, J = 2.0 Hz), 7.03 (1H, dd, J = 2.0, 8.3 Hz), 6.87 (1H, d, J = 8.3 Hz), 6.25 (1H, d, J = 16.1 Hz), 5.04 (1H, sep, J = 6.3 Hz), 2.49 (6H, d, J = 6.3 Hz). ^{13}C NMR (100 MHz, acetone-d_6) δ: 167.0, 148.5, 146.1, 145.3, 127.6, 122.4, 116.3, 116.2, 115.1, 67.7, 22.1. MS *m/z*: 222 (M^+), 207, 180, 163 (100%), 136, 89, 77. High-resolution MS calcd for $C_{12}H_{14}O_4$ (M^+): 222.0892. Found: 222.0887. Anal. calcd for $C_{12}H_{14}O_4$: 64.85% C, 6.35% H. Found: 64.90% C, 6.39% H. All spectral data agreed with previously reported data.[21]

4.8.17. 1-Methylpropyl 1-(3′,4′-dihydroxyphenyl)propenate (*sec*-butyl caffeate) (17)

Yield: 88%. Mp: 128–130 °C (recrystallized from Et_2O, colorless powder). FT-IR ν_{max} (KBr): 3466, 3082, 2972, 2942, 1665, 1624, 1606, 1534. ^{1}H NMR (400 MHz, acetone-d_6) δ: 8.24 (2H, br s), 7.53 (1H, d, J = 16.1 Hz), 7.15 (1H, d, J = 2.0 Hz), 7.03 (1H, dd, J = 2.0, 7.8 Hz), 6.86 (1H, d, J = 7.8 Hz), 6.26 (1H, d, J = 16.1 Hz), 4.89 (1H, sext, J = 6.3 Hz), 1.69–1.51 (m, 2H), 1.21 (3H, d, J = 5.9 Hz), 0.90 (3H, t, J = 7.8 Hz). ^{13}C NMR (67.8 MHz, acetone-d_6) δ: 167.1, 148.5, 146.2, 145.3, 127.7, 122.4, 116.3, 116.2, 115.2, 72.2, 29.5, 19.8, 10.0. MS *m/z*: 236 (M^+), 222, 207, 191, 180, 163 (100%), 136, 89, 77. High-resolution MS calcd for $C_{13}H_{16}O_4$ (M^+): 236.1049. Found: 236.1064. Anal. calcd for $C_{13}H_{16}O_4$: 66.09% C, 6.83% H. Found: 65.87% C, 6.89% H. All spectral data agreed with previously reported data.[23]

4.8.18. Cyclohexyl 1-(3′,4′-dihydroxyphenyl)propenate (cyclohexyl caffeate) (18)

Yield: 3.3%. Mp: 152–154 °C (recrystallized from $CHCl_3$, colorless powder). FT-IR ν_{max} (KBr): 3448, 3276, 2940, 2861, 1686, 1635, 1602, 1528. ^{1}H NMR (270 MHz, $CDCl_3$) δ: 7.56 (1H, d, J = 16.0 Hz), 7.09 (1H, d, J = 2.0 Hz), 7.02 (1H, dd, J = 2.0, 8.2 Hz), 6.87 (1H, d, J = 8.2 Hz), 6.27 (1H, d, J = 16.0 Hz), 5.52 (2H, d, J = 7.6 Hz), 4.94–4.81 (1H, m), 1.98–1.85 (2H, m), 1.86–1.67 (2H, m), 1.52–1.17 (5H, m). ^{13}C NMR (67.8 MHz, $CDCl_3$) δ: 167.2, 146.1, 144.4, 143.7, 127.7, 122.3, 116.4, 115.5, 114.4, 83.9, 72.9, 31.7, 25.4, 23.79. MS *m/z*: 262 (M^+), 180 (100%), 163, 136, 117, 77. High-resolution MS calcd for $C_{15}H_{18}O_4$ (M^+): 262.1205. Found: 262.1186. Anal. calcd for $C_{15}H_{18}O_4$: 68.68% C, 6.92% H. Found: 68.45% C, 7.11% H. All spectral data agreed with previously reported data.[28]

4.8.19. Phenylmethyl 1-(3′,4′-dihydroxyphenyl)propenate (benzyl caffeate) (19)

Yield: 29%. Mp: 153–155 °C (recrystallized from $CHCl_3$, colorless needles). FT-IR ν_{max} (KBr): 3480, 3328, 1694, 1638, 1605, 1536. ^{1}H NMR (270 MHz, acetone-d_6) δ: 8.31 (2H, br s), 7.58 (1H, d, J = 15.8 Hz), 7.45–7.28 (5H, m), 7.17 (1H, d, J = 2.1 Hz), 7.07, 7.06 (1H, dd, J = 2.1, 8.1 Hz), 6.86 (1H, d, J = 8.1 Hz), 6.34 (1H, d, J = 16.0 Hz), 5.21 (2H, s). ^{13}C NMR (67.8 MHz, acetone-d_6) δ: 167.3, 148.8, 146.2, 146.1, 137.7, 129.3, 128.9, 128.8, 127.6, 122.7, 116.4, 115.4, 115.3, 66.3. MS *m/z*: 270 (M^+, 100%), 179, 163, 136, 117. High-resolution MS calcd for $C_{16}H_{14}O_4$ (M^+): 270.0892. Found: 270.0878. Anal. calcd for $C_{16}H_{14}O_4$: 71.1% C, 5.22% H. Found: 71.12% C, 5.22% H. All spectral data agreed with previously reported data.[21]

4.8.20. 3-Methylbut-2-enyl 1-(3′,4′-dihydroxyphenyl)propenate (prenyl caffeate) (20)

Yield: 23%. Mp: 131–132 °C (recrystallized from toluene, colorless needles). FT-IR ν_{max} (KBr): 3481, 3319, 2932, 1681, 1636, 1603, 1536. ^{1}H NMR (400 MHz, acetone-d_6) δ: 8.30 (2H, br s), 7.53 (1H, d, J = 15.6 Hz), 7.15 (1H, d, J = 2.0 Hz), 7.03 (1H, dd, J = 2.0, 8.3 Hz), 6.86 (1H, d, J = 8.3 Hz), 6.27 (1H, d, J = 16.1 Hz), 5.39–5.35 (1H, m), 4.64 (2H, d, J = 7.3 Hz), 1.73 (3H, s), 1.72 (3H, s). ^{13}C NMR (100 MHz, acetone-d_6) δ: 167.4, 148.7, 146.3, 145.6, 138.9, 127.7, 122.5, 120.2, 116.4, 115.8, 115.2, 61.4, 25.8, 18.0. MS *m/z*: 248 (M^+), 233, 219, 203, 180 (100%). High-resolution MS calcd for $C_{14}H_{16}O_4$ (M^+): 248.1049. Found: 248.1057. Anal. calcd for $C_{14}H_{16}O_4$: 67.73% C, 6.50% H. Found: 67.44% C, 6.52% H. All spectral data agreed with previously reported data.[29]

4.8.21. 3,7-Dimethyloct-2,6-dienyl 1-(3′,4′-dihydroxyphenyl)-propenate (geranyl caffeate) (21)

Yield: 14%. Mp: 103.5–104 °C (recrystallized from toluene, colorless cotton). FT-IR ν_{max} (KBr): 3483, 3291, 2972, 2928, 2848, 1677, 1629 1601, 1535. ^{1}H NMR (400 MHz, acetone-d_6) δ: 8.23 (2H, br s), 7.48 (1H, d, J = 16.1 Hz), 7.10 (1H, d, J = 2.0 Hz), 6.97 (1H, dd, J = 2.0, 7.8 Hz), 6.80 (1H, d, J = 7.8 Hz), 6.21 (1H, d, J = 16.1 Hz), 5.35–5.31 (1H, m), 5.05–5.02 (1H, m), 4.61 (2H, d, J = 6.8 Hz), 2.06–1.98 (m, 4H), 1.67 (3H, s), 1.58 (3H, s), 1.52 (3H, s). ^{13}C NMR (100 MHz, acetone-d_6) δ: 167.5, 148.8, 146.4, 145.7, 142.3, 132.2, 127.8, 124.8, 122.6, 120.1, 116.5, 115.9, 115.3, 61.5, 40.3, 27.1, 26.0, 17.9, 16.6. MS *m/z*: 316 (M^+), 247, 233, 203, 180(100%), 163. High-resolution MS calcd for $C_{19}H_{24}O_4$ (M^+): 316.1375. Found: 316.1685. Anal. calcd for $C_{19}H_{24}O_4$: 72.13% C, 7.65% H. Found: 72.19% C, 7.77% H. All spectral data agreed with previously reported data.[30]

4.8.22. 3,7,11-Trimethyldodec-2,6,10-trienyl 1-(3′,4′-dihydroxyphenyl)propenate (farnesyl caffeate) (22)

Yield: 11% (amorphous solid). FT-IR ν_{max} (KBr): 3485, 3320, 2967, 2926, 1680, 1634 1602, 1535. ^{1}H NMR (400 MHz, acetone-d_6) δ: 8.21 (2H, br s), 7.45 (1H, d, J = 16.1 Hz), 7.06 (1H, d, J = 2.0 Hz), 6.94 (1H, dd, J = 2.0, 8.3 Hz), 6.78 (1H, d, J = 8.3 Hz), 6.19 (1H, d, J = 16.1 Hz), 5.36–5.29 (1H, m), 5.08–4.97 (2H, m), 4.59 (2H, d, J = 6.8 Hz), 2.10–1.86 (m, 8H), 1.66 (3H, s), 1.58 (3H, s), 1.55 (3H, s), 1.51 (3H, s). ^{13}C NMR (100 MHz, acetone-d_6) δ: 167.5, 148.8, 146.4, 145.7, 142.3, 136.1, 131.8, 127.8, 125.0, 124.8, 122.7, 120.3, 116.5, 115.9, 115.4, 61.5, 40.6, 40.0, 27.6, 27.0, 26.0, 17.9, 16.7, 16.3. MS *m/z*: 384 (M^+), 315, 247, 233, 204, 180(100%), 163. High-resolution MS calcd for $C_{24}H_{32}O_4$ (M^+): 384.2300. Found: 384.2299. Anal. calcd for $C_{24}H_{32}O_4$: 74.97% C, 8.39% H. Found: 74.76% C, 8.47% H. All spectral data agreed with previously reported data.[29]

4.8.23. Undecyl 1-(3′,4′-dihydroxyphenyl)propen amide (23)

A mixture of caffeic acid (1.73 g, 10 mmol) and Et_3N (1.3 mL, 10 mmol) in DMF (19.2 mL) was added to 1-aminoundecane (2.1 mL, 10 mmol) and a solution of PyBOP (5.0 g, 10 mmol) in 20 mL of CH_2Cl_2 at 0 °C. The mixture was stirred at 0 °C for 30 min and warmed to room temperature. After 2 h, the reaction mixture was concentrated in vacuo, and the residue was diluted with H_2O and extracted with AcOEt. The extract was successively washed with 1 N HCL, water, a saturated solution of aqueous $NaHCO_3$, and H_2O, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (*n*-hexane–AcOEt (1:1 v/v) to give a title compound (1.8 g, 55%). Part of the product was recrystallized from Et_2O to give colorless needles. Mp: 138–139 °C. FT-IR ν_{max} (KBr): 3478, 3377, 3192, 2953, 2919, 2850, 1649, 1591, 1544, 1466, 1441. ^{1}H NMR (400 MHz, acetone-d_6) δ: 8.22 (2H, br s), 7.34 (1H, d, J = 15.6 Hz), 7.05 (1H, d, J = 2.0 Hz), 6.91 (1H, dd, J = 2.0, 8.3 Hz), 6.82 (1H, d, J = 8.3 Hz),

6.42 (1H, d, J = 15.6 Hz), 3.28 (2H, q, J = 5.9 Hz), 1.52 (2H, quint, J = 6.8 Hz), 1.39–1.19 (16H, m), 0.86 (3H, t, J = 6.8 Hz). ^{13}C NMR (100 MHz, acetone-d_6) δ: 166.4, 147.7, 146.2, 140.3, 128.5, 121.4, 119.9, 116.3, 114.8, 39.9, 32.6, 30.5, 30.3, 30.1, 30.0, 27.7, 23.3, 14.5. MS m/z: 333 (M^+), 304, 276, 262, 248, 220, 206, 193, 163 (100%), 145, 135, 117, 89. High-resolution MS calcd for $C_{20}H_{31}NO_3$ (M^+): 333.2305. Found: 333.2305. Anal. calcd for $C_{20}H_{31}NO_3$: 72.09% C, 9.38% H, 4.2% N. Found: 71.80% C, 9.62% H, 4.04% N.

4.8.24. Synthesis of ketone (24)

4.8.24.1. *N*-Methoxy-*N*-methyl-3-(3′,4′-dihydroxyphenyl)propenamide. A mixture of caffeic acid (3.0 g, 16.7 mmol), HOBt (2.3 g, 16.7 mmol), and WSC HCl (3.2 g, 16.7 mmol) in DMF (59 mL) was stirred at 0 °C. *N*-Methyl-*O*-methylhydroxylamine hydrochloride (1.6 g, 16.7 mmol) was added to the reaction mixture followed by Et_3N (4.7 mL) and stirring was continued for 22 h. Then the reaction mixture was concentrated in vacuo, and the residue was partitioned between AcOEt and a saturated solution of $NaHCO_3$. Successively, the organic layer was washed with H_2O and brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (*n*-hexane–AcOEt (2:3 and 1:4 v/v)) to give a title compound (1.4 g, 38%). Part of the product was recrystallized from Et_2O to give yellowish needles. Mp: 175–178 °C. FT-IR ν_{max} (KBr): 3469, 2938, 2894, 1643, 1602, 1460, 982, 883, 847, 748. ^{1}H NMR (400 MHz, acetone-d_6) δ: 8.2 9 (2H, br s), 7.50 (1H, d, J = 16.0 Hz), 7.16 (1H, d, J = 2.0 Hz), 7.02 (1H, dd, J = 2.0, 8.3 Hz), 6.91 (1H, d, J = 16.0 Hz), 6.85 (1H, d, J = 8.3 Hz) 3.78 (3H, s), 3.21 (3H, s). ^{13}C NMR (100 MHz, acetone-d_6) δ: 167.8, 148.2, 146.2, 143.8, 128.4, 122.2, 116.3, 115.1, 114.0, 62.1, 32.6. MS m/z: 223 (M^+), 189, 163 (100%), 135, 77. High-resolution MS calcd for $C_{11}H_{13}NO_4$ (M^+): 223.0845. Found: 223.0838. Anal. calcd for $C_{11}H_{13}NO_4$: 59.25% C, 5.88% H, 6.28% N. Found: 59.13% C, 5.87% H, 6.22% N.

4.8.24.2. *N*-Methoxy-*N*-methyl-3-(3′,4′-di-tert-butyldimethylsiloxyphenyl)propenamide. A mixture of amide (0.5 g, 2.24 mmol), TBDMSCl (0.81 g, 5.38 mmol), and imidazole (0.76 g, 11.2 mmol) in DMF (1.0 mL) was stirred at 0 °C for 20 h. The reaction mixture was concentrated in vacuo, and the residue was partitioned between EtOAc and a saturated solution of aqueous NH_4Cl. The organic layer was washed with a saturated solution of aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (*n*-hexane–AcOEt (8:2 v/v) to give a title compound (0.96 g, 95%) as colorless oil. FT-IR ν_{max} (KBr): 2932, 2897, 2859, 1660, 1596, 1254. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.61 (1H, s, J = 15.6 Hz), 7.09 (1H, dd, J = 2.4, 8.3 Hz), 7.03 (1H, d, J = 2.4 Hz), 6.89–6.80 (2H, m), 3.75 (3H, s), 3.30 (3H, s), 1.00 (9H, s), 0.99 (9H, s), 0.26–0.20 (12H, m). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 167.1, 148.7, 146.8, 143.0, 128.7, 121.3, 121.0, 113.5, 61.5, 32.3, 25.8, 25.7, 18.3, −4.26, −4.28. MS m/z: 451 (M^+), 436, 391 (100%), 364, 306, 277, 219. High-resolution MS calcd for $C_{23}H_{41}NO_4Si_2$ (M^+): 451.2547. Found: 451.2572. Anal. calcd for $C_{23}H_{41}NO_4Si_2$: 61.22% C, 9.169% H, 3.10% N. Found: 60.52% C, 9.32% H, 3.08% N.

4.8.24.3. 1-(3′,4′-tert-butyldimethylsiloxyphenyl)pentadec-1-en-3-one. Dodecyl magnesium bromide (5.54 mL) was added dropwise at 0 °C to a stirred solution of amide (0.5 g, 1.11 mmol) in anhydrous Et_2O (7.12 mL). The mixture was stirred at 0 °C for 9 h and quenched with a saturated solution of aqueous NH_4Cl. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (*n*-hexane–AcOEt) (95:5 v/v) to give a title compound (178 mg, 29%) as a colorless oil. FT-IR ν_{max} (KBr): 2928, 2857, 1691, 1595, 1510, 1472, 1362, 1255. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.43 (1H, d, J = 16.1 Hz), 7.07–7.01 (2H, m), 6.83 (1H, d, J = 7.1 Hz), 6.55 (1H, d, J = 16.1 Hz), 2.64 (2H, t, J = 7.8 Hz), 1.67 (2H, quint), 1.00 (9H,s), 0.99 (9H, s), 0.88 (3H, t, J = 6.8 Hz), 0.22 (6H, s), 0.22 (6H, s). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 200.8, 149.6, 147.3, 142.4, 128.2, 124.4, 122.4, 121.2, 120.6, 77.2, 40.7, 31.9, 29.7, 29.6, 29.5, 29.4, 29.3, 25.9, 25.9, 24.5, 22.7, 18.5, 18.4, 14.1, −4.07, −4.12. MS m/z: 560, 504, 503 (100%), 447, 406, 391, 351, 277, 261, 219, 179. High-resolution MS calcd for $C_{33}H_{60}NO_3Si_2$ (M^+): 560.4083. Found: 560.4083. Anal. calcd for $C_{33}H_{60}NO_3Si_2$: 70.73% C, 10.79% H. Found: 70.84% C, 11.13% H.

4.8.24.4. 1-(3′,4′-Dihydroxyphenyl)pentadec-1-en-3-one (24). A mixture of silyl ether (58.7 mg, 0.105 mmol) and TBAF (0.12 mL, 0.42 mmol) in THF (0.6 mL) was stirred at 0 °C for 30 min. The reaction mixture was concentrated in vacuo. The residue was diluted with H_2O and extracted with AcOEt. The organic layer was washed with H_2O and brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (*n*-hexane–AcOEt) (6:4 v/v) to give a title compound (33 mg, 95%). A part of the product was recrystallized from MeOH to give yellowish needles. Mp: 108–109 °C. FT-IR ν_{max} (KBr): 3479, 2956, 2925, 2850, 1646, 1608, 1514, 1467, 1377. ^{1}H NMR (400 MHz, CD_3OD) δ: 7.41 (1H, d, J = 16.0 Hz), 6.98 (1H, d, J = 1.9 Hz), 6.89 (1H, dd, J = 1.9, 7.7 Hz), 6.69 (1H, d, J = 7.7 Hz), 6.49 (1H, d, J = 16.0 Hz), 2.56 (2H, t, J = 7.7 Hz), 1.53 (2H, quint), 1.30–1.08 (18H, m), 0.79 (3H, t, J = 6.8Hz). ^{13}C NMR (100 MHz, CD_3OD) δ: 207.6, 153.8, 150.8, 149.4, 131.7, 127.8, 127.3, 120.4, 119.2, 45.1, 37.0, 34.7, 34.6, 34.6, 34.5, 34.5, 34.4, 34.3, 29.7, 27.6, 18.3. MS m/z: 332 (M^+), 315, 289, 247, 220, 179, 163 (100%), 123, 89, 77. High-resolution MS calcd for $C_{21}H_{32}O_3$ (M^+): 332.2353. Found: 332.2339. Anal. calcd for $C_{21}H_{32}O_3$: 75.9% C, 9.7% H. Found: 75.79% C, 10.01% H.

Acknowledgments

This work was supported by a Grant-in-Aid from the High Technology Research Program from the Ministry of Education, Culture, Sports, Science, and Technology of Japan.

References

1. Aktan, F. *Life Sci* **2004**, *75*, 639.
2. Nagaoka, T.; Banskota, A. H.; Tezuka, Y.; Midorikawa, K.; Matsushige, K.; Kadota, S. *Biol. Pharm. Bull.* **2003**, *26*, 487.
3. Lechner, M.; Lirk, P.; Rieder, J. *Semin. Cancer Biol.* **2005**, *15*, 277.
4. Clancy, R. M.; Amin, A. R.; Abramson, S. B. *Arthritis Rheum.* **1998**, *41*, 1141.
5. Banskota, A. H.; Tezuka, Y.; Kadota, S. *Phytother. Res.* **2001**, *15*, 561.
6. Banskota, A. H.; Tezuka, Y.; Midorikawa, K.; Matushige, K.; Kadota, S. *J. Nat Prod.* **2000**, *63*, 1277.
7. Burdock, G. A. *Food Chem. Toxicol.* **1998**, *36*, 347.
8. Banskota, A. H.; Nagaoka, T.; Sumioka, L. Y.; Tezuka, Y.; Awale, S.; Midorikawa, K.; Matsushige, K.; Kadota, S. *J. Ethnopharmacol.* **2002**, *80*, 67.
9. Celli, N.; Mariani, B.; Dragani, L. K.; Murzilli, S.; Rossi, C.; Rotilio, D. *J. Chromatogr. B* **2004**, *810*, 129.
10. Celli, N.; Dragani, L. K.; Murzilli, S.; Pagliani, T.; Poggi, A. *J. Agric. Food Chem.* **2007**, *55*, 3398.
11. Shin, K. M.; Kim, I. T.; Park, Y. M.; Ha, J.; Choi, J. W.; Park, H. J.; Lee, Y. S.; Lee, K. T. *Biochem. Pharmacol.* **2004**, *68*, 2327.
12. Lee, Y. J.; Liao, P. H.; Chen, W. K.; Yang, C. Y. *Cancer Lett.* **2000**, *153*, 51.
13. Rajan, P.; Vedernikova, I.; Cos, P.; Berghe, D. V.; Augustyns, K.; Haemers, A. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 215.
14. Alley, M. C.; Scudiero, D. A.; Monks, A.; Hursey, M. L.; Czerwinski, M. J.; Boyd, M. R. *Cancer Res.* **1988**, *48*, 589.
15. Kanno, S.; Ishikawa, M.; Takayanagi, M.; Takayanagi, Y.; Sasaki, K. *Biol. Pharm. Bull.* **2000**, *23*, 446.
16. Kanno, S.; Shouji, A.; Hirata, R.; Asou, K.; Ishikawa, M. *Life Sci.* **2004**, *75*, 353.
17. Defraiteur, C.; Lemaire, C.; Luxen, A.; Plenevaux, A. *Nucl. Med. Biol.* **2006**, *33*, 667.
18. Green, L. C.; Wagner, D. A.; Glogowski, J.; Skipper, P. L.; Wishnok, J. S.; Tannenbaum, S. R. *Anal. Biochem.* **1982**, *126*, 131.
19. Kanno, S.; Shouji, A.; Tomizawa, A.; Hiura, T.; Osanai, Y.; Ujibe, M.; Obara, Y.; Nakahata, N.; Ishikawa, M. *Life Sci.* **2006**, *78*, 673.

20. Zhu, C.; Jiang, L.; Chen, T.-M.; Hwang, K.-K. *Eur. J. Med. Chem.* **2002**, *37*, 399.
21. Etzenhouser, B.; Hansch, C.; Kapur, S.; Selassie, C. D. *Bioorg. Med. Chem.* **2001**, *9*, 199.
22. Nagaoka, T.; Banskota, A. H.; Tezuka, Y.; Saiki, I.; Kadota, S. *Bioorg. Med. Chem.* **2002**, *10*, 3351.
23. Jayaprakasam, B.; Vanisree, M.; Zhang, Y.; Dewitt, D. L.; Nair, M. G. *J. Agric. Food Chem.* **2006**, *54*, 5375.
24. Weixiao, H.; Chunnian, X.; Zhongyu Y. Chinese Patent Faming Zhuanli Shenqing Gongkai Shuomingshu 1,730,460A, **2006**.
25. Batovska, D. I.; Kishimoto, T.; Bankova, V. S.; Kamenarska, Z. G.; Ubukata, M. *Molecules* **2005**, *10*, 552.
26. Sugiura, M.; Naito, Y.; Yamaura, Y.; Fukaya, C.; Yokoyama, K. *Chem. Pharm. Bull.* **1989**, *37*, 1039.
27. Sarker, S. D.; Shaheen, E. M.; Eynon, E.; Nahar, L. *Chem. Nat. Comp.* **2005**, *41*, 293.
28. Saito, S.; Kurakane, S.; Seki, M.; Takai, E.; Kasai, T.; Kawabata, J. *Bioorg. Med. Chem.* **2005**, *13*, 4191.
29. Kartal, M.; Yildiz, S.; Kaya, S.; Kurucu, S.; Topcu, G. *J. Ethnopharmacol.* **2003**, *86*, 69.
30. Suzuki, T.; Nishimaki-Mogami, T.; Kawai, H.; Kobayashi, T.; Shinozaki, Y.; Sato, Y.; Hashimoto, T.; Asakawa, Y.; Inoue, K.; Ohno, Y.; Hayakawa, T.; Kawanishi, T. *Phytomedicine* **2006**, *13*, 401.