Pergamon

SCIENCE DIRECT®

Bioorganic & Medicinal Chemistry 11 (2003) 3807–3813

BIOORGANIC & MEDICINAL CHEMISTRY

Synthesis of Amide Compounds of Ferulic Acid, and Their Stimulatory Effects on Insulin Secretion In Vitro

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Received 1 March 2003; accepted 23 April 2003

Abstract—We prepared amide compounds which were derived from ferulic acid using various amines, and investigated their stimulatory effects on insulin secretion using rat pancreatic RIN-5F cells. Most of these compounds exhibited significant promotion of the insulin-release at a concentration of 10 μM and in particular, the amides having *n*-butyl, *n*-pentyl, pyrrolidine, and piperidine groups showed high activity.
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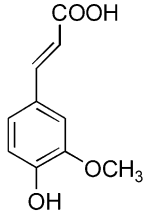
Introduction

Ferulic acid **1** is a phenolic compound and some authors have recently developed a mass production method of **1** from rice bran.[1,2] Interest in biological activities of **1** and the synthetic compounds derived from **1** has intensified in recent years. For example, alkyl ferulates **2** and **3** have an anti-carcinogenic potential and in particular, **3** was more effective cancer chemopreventive agents than **1**.[3] A ferulic acid derivative in which geranyl group is attached to the phenolic hydroxyl group of ethyl ferulate exhibits colon anti-carcinogenesis in rodent models.[2–5] A novel polyphenol **6** which was composed of two naturally occurring products, ferulic and gallic acids, was found to have higher activity as an anti-carcinogen than the original phytochemicals.[6] The compounds **7–9** consisting of ferulic acid and *myo*-inositol are also novel polyphenols that have potential of cancer chemopreventive agents.[7–9] Biological activities of natural occurring compounds related to ferulic acid also have been investigated. For example, caffeic acid **4** and chlorogenic acid **5** are natural products from green coffee beans and have various bioactivities.[10] The compound **5** is the ester compound consisting of (−)-quinic acid and **4**, and inhibits glucose-6-phosphatase to regulate hepatic glucose production.[11,12] Recently, some authors have found that the blood glucose level in STZ-induced diabetic mice was reduced by administration of **1**.[13] This finding prompted us to investigate the activity of ferulic acid and its related compounds on their stimulatory effects on insulin secretion in vitro. We focused some amide compounds of **1** for an assay system by the following reasons: (1) Some of the amides were isolated from plants[14,15] but their biological activities were not investigated. (2) Common antidiabetic agents such as sulfonylureas contains the bond-structure of amide.



Ferulic acid **1**

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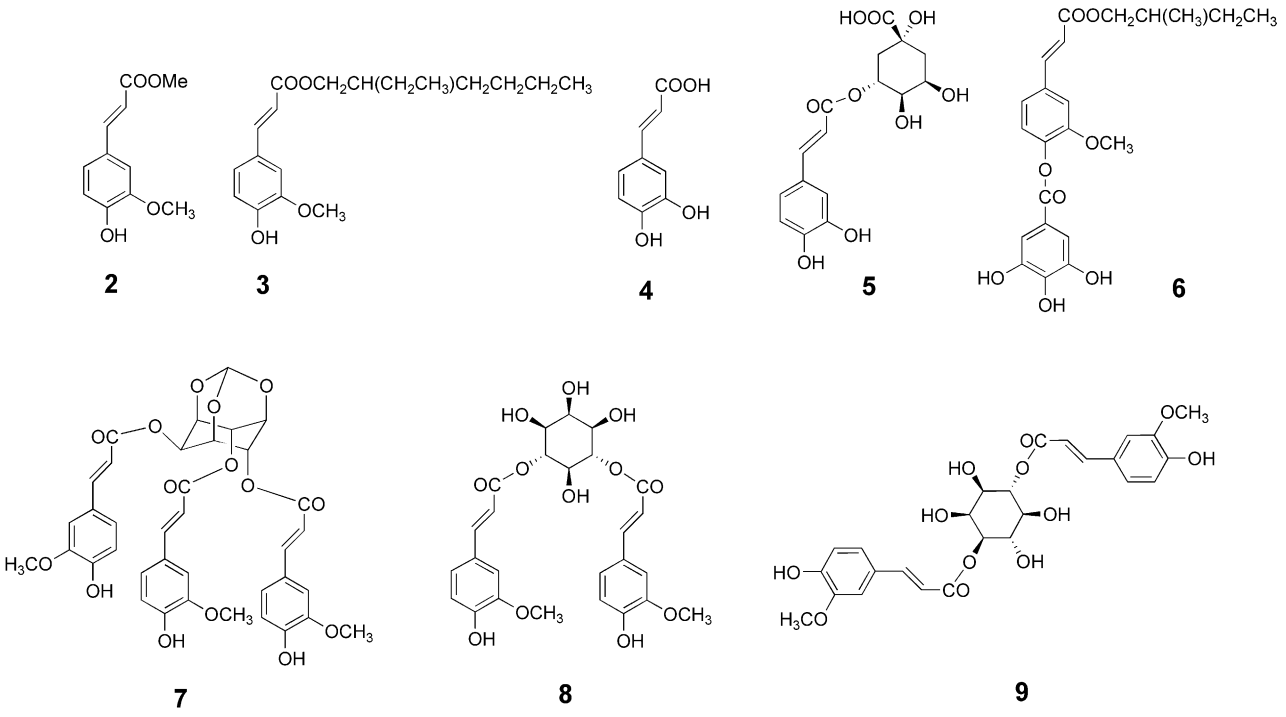
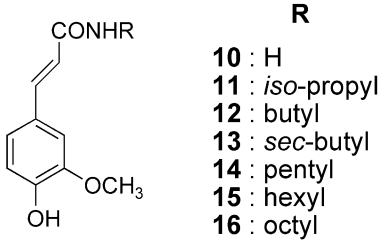
doi:10.1016/S0968-0896(03)00280-3

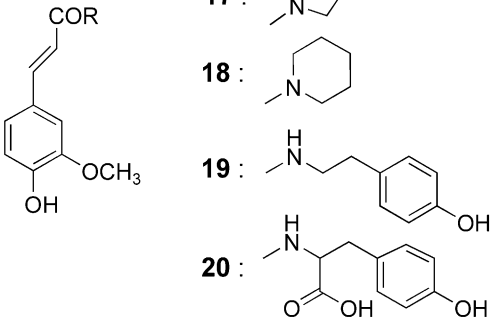


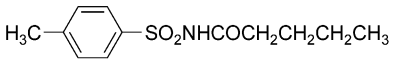
In this paper, we describe the synthesis of the amide compounds of **1** and a preliminary experiment to investigate the mechanism of antidiabetic properties by insulin secretion in rat pancreatic RIN-5F cells in vitro incubations using **1** and related compounds including the amide compounds.



R

10 : H
11 : *iso*-propyl
12 : butyl
13 : *sec*-butyl
14 : pentyl
15 : hexyl
16 : octyl





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The number of people afflicted by diabetes mellitus is estimated to be more than 200 million in the world. Most of the patients are suffering from non-insulin-dependent diabetes mellitus (NIDDM, type 2 diabetes).[16] Studies on ingredients of plants in the treatment of diabetes have been undertaken and served effective dietary adjuncts such as Eucalyptus globules.[17] On the other hand, common antidiabetic medicines such as sulfonylureas to the treatment of NIDDM patients are prescribed carefully with many restrictions due to various side-effects. Therefore, studies on biological activities of **1** and their derivatives might serve a new therapeutic approach as safe hypoglycemic agents which are synthetic compounds based on natural sources.

Results and Discussion

Chemistry

Various types of ferulic acid derivatives **2**–**9** are chosen to examine the activity on insulin secretion. The amide compounds of ferulic acid were synthesized by condensation reactions. The reaction was performed by the reaction of *O*-acetyl feruloyl chloride with various amines, followed by deacetylation. *O*-Acetyl feruloyl chloride was prepared by the method reported previously.[6] The condensation was performed in dichloromethane in the presence of an alkylamine at room temperature, and then the removal of acetyl groups was carried out using sodium methoxide or hydrazine monohydrate at room temperature to yield corresponding amide compounds **10**–**17**, and **19**. The amides **18** and **20** were prepared alternative methods described in details in the experimental section. The amides **18** and **19** are natural compounds which are isolated from the fruit of white pepper.[14,15] We used the compounds **1**–**20**

to assess their antidiabetic potential. Tolbutamide **21** that is one of common sulfonylurea antidiabetic agents was also tested for comparing with the compounds prepared.

Biological activity

For ferulic acid **1** and their related compounds **2**–**10**, we investigated their stimulatory effects on insulin secretion in vitro in order to estimate their antidiabetic potential. The rat insulin enzyme-immunoassay system was available commercially for the evaluation of the activities in vitro. The activities are estimated from concentrations of insulin which are released from pancreatic RIN-5F cells by adding the compounds. The results are shown in Figure 1. The control experiment without a test compound was carried out in parallel. As shown in Figure 1A, the compounds **1**, **5**, **7**–**9**, and the amide compound **10** increased the amounts of insulin secretion at a concentration of 100 μM comparing with control experiment. On the other hand, alkyl ferulates (**2** and **3**), **4**, and **6** showed no stimulatory effect. The compounds **1** and **10** showed a marked promotion effect of the insulin secretion at the concentration of 10 μM as shown in Figure 1B. Therefore, we then examined the activities for the amide compounds carrying various alkyl chains and residues. Tolbutamide **21** was also tested for comparing with the compounds prepared. The results are shown in Figure 2. All of the amide compounds tested showed significant differences of the activation between test compounds and the control experiment at the both concentration of 100 μM and 10 μM. In particular, the amides, **12**, **13**, **14**, **17**, and **18,** exhibited 6–8 times insulin secretion, compared with the control experiment. The structural feature of these amides is an alkyl chain having 4 or 5 carbons. The other amides, **10**, **11**, **15**, and **16**, in which have shorter or longer alkyl chains, showed moderate activity (3–5 times compared to the control). The amides, **19** and **20**, consisted of tyramine and tyrosine, respectively, showed also moderate activity (3–5 times compared to the control). To make clear the activities of selected compounds including **1** and **21**, dose-response curves were shown in Figure 3. All of compounds tested showed dose-dependent activities. In particular, the amide compounds showed higher activities than **1**, but relatively lower than **21**. However, the activities of the amides seem to be comparable to that of **21** due to no significant differences between the amides tested and **21** as shown in Figure 2.

Cell viability in cell culture was determined for selected test compounds by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The results are shown in Figure 4. All compounds tested have no notable cytotoxicity (cell viability of 89–98%). It is known that an insulin secretion from pancreatic beta cells is stimulated by sulfonylureas.[18] The mechanisms of insulin releasing-action of these drugs have been

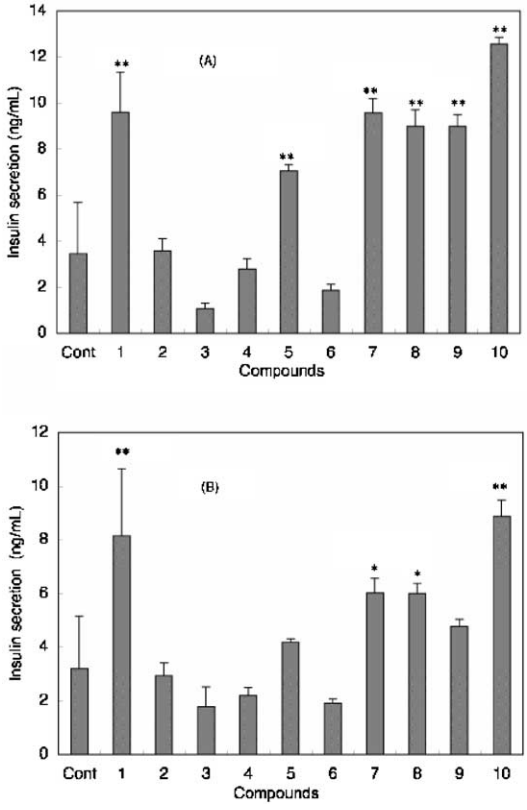

Figure 1. Stimulatory effect of ferulic acid and related compounds on insulin secretion in vitro incubating of RIN-5F cells. Test compounds; 100 μM (A): 10 μM (B). Values are means ± SD. Significant differences compared with control group; $*p < 0.05$ and $**p \not< 0.01$.

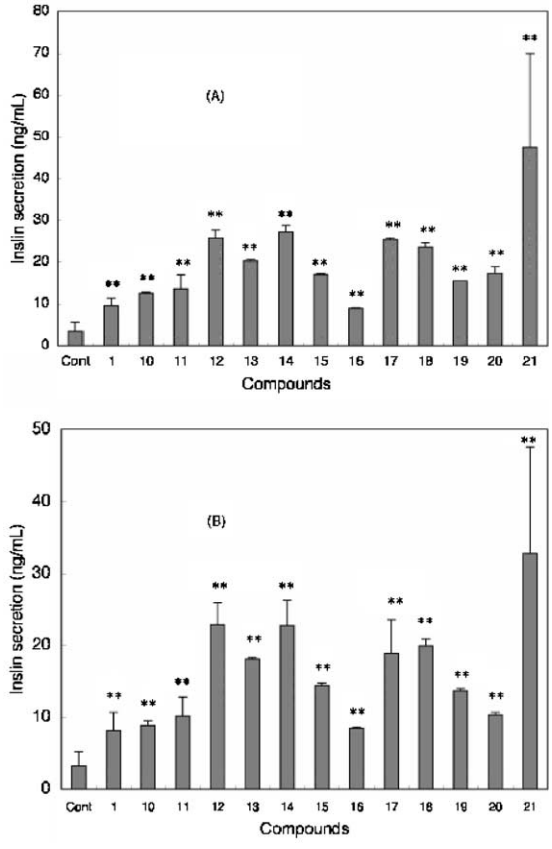

Figure 2. Stimulatory effect of ferulic acid and the amides on insulin secretion in vitro incubating of RIN-5F cells. Test compounds; 100 μM (A): 10 μM (B). Values are means ± SD. Significant differences compared with control group; $**p < 0.01$.

demonstrated. Sulfonylureas such as tolbutamide bind to sulfonylurea receptors, resulting in closure of ATP sensitive K^+ channels in the beta cell plasma membrane to cause membrane depolarization, calcium influx, and insulin secretion.[18] In addition to these effects, sulfonylureas act by direct interaction with the secretory machinery.[18,19] It was found that the amide compounds we tested stimulated insulin secretion from pancreatic RIN-5F cells. The mechanisms of these compounds are not clear. However, the similarity of alkylamide groups of the ferulic acid derivatives to that of tolbutamide suggests similar mechanisms of the stimulatory effects in cells. Various types of sulfonylureas as antidiabetic agents are known and they have only a bond-structure of sulfonylurea in common. Therefore, the antidiabetic activity related to the binding ability toward the sulfonylurea receptors depends on the other groups connecting with the sulfonylurea. In the case of the amides, important structural factors seem to be the presence of an appropriate alkyl group and feruloyl group connected with amide. Ferulic acid from rice bran is useful for a food additive as an antioxidant. The amides such as **18** and **19** are natural occurring compounds and the other amides are made up of ferulic acid and simple amines. Therefore, these compounds would be expected to be safe hypoglycemic agents with few side effects in the future diabetes therapy.

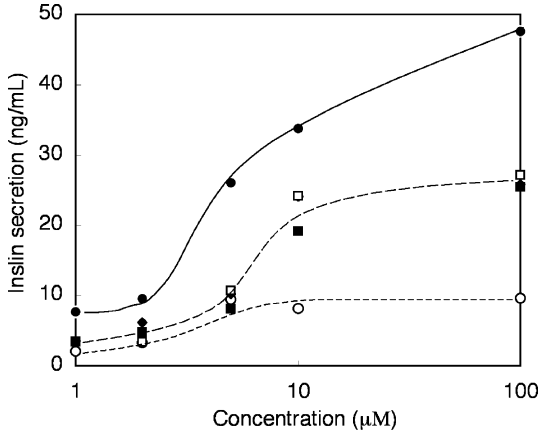

Figure 3. Dose–response curves for insulin secretion on rat RIN-F5 cells. Test compounds: **1** (○), **12** (◆), **14** (□), **17** (■), **21** (●). Plots are shown in mean values.

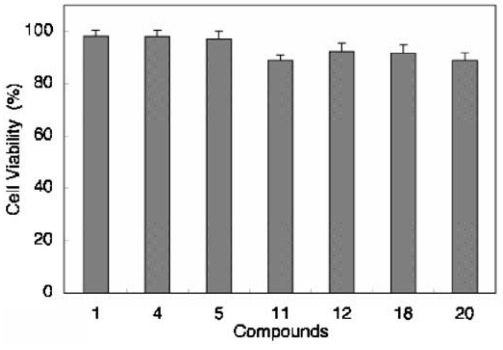

Figure 4. Cell viability of ferulic acid and the amide compounds. Test compounds: 100 μM. Values are means±SD.

Conclusion

The amide compounds of ferulic acid were prepared. Most of these compounds exhibited their stimulatory abilities on insulin secretion in rat pancreatic RIN-5F cells. In particular, the amides, **12**, **14**, **17**, and **18** could be the most promising antidiabete agents among the compounds evaluated in the present study. Mass production method of ferulic acid from rice bran was developed in recent years[1,2] and the production was already on a commercial basis. Therefore, it is valuable to obtain effective biological active agents as derivatives from natural compounds for practical use. The present study represents a potential of the amide compounds as insulin-secretion stimulatory agents in the first stage of the assay system. This finding might provide new and safe orally active agents based on natural occurring compounds for the treatment of diabetes in the future therapy.

Experimental

General

Ferulic acid and alkyl ferulates were supplied by Tsuno Rice Fine Chemicals Co., Ltd. (Wakayama, Japan). Chlorogenic acid was purchased from Aldrich Chemical Co. Ltd. Anhydrous CH_2Cl_2 was purchased from Kanto Chemical Co., Inc. (Tokyo, Japan) and used without further purification. Other solvents and reagents were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan), and used without further purification. The *O*-acetyl feruloyl chloride was prepared by the literature method.[6] Melting points were determined by a Yanaco micro melting point apparatus and are uncorrected. Elemental analyses were performed on a Perkin–Elmer 2400II. ^1H and ^{13}C NMR spectra were recorded on a Varian Unity-plus 400 spectrometer using a residual solvent as an internal standard. FT-IR spectra were obtained on a Shimadzu FT-IR 8200D spectrometer using a diffuse reflectance cell. ESI-TOF MS spectra were measured using PE Biosystems Mariner spectrometer.

Chemicals and cells for bioassay

Rat insulin enzymeimmunoassays (EIA) system (PRN 2567, 96 wells) was obtained from Amersham Pharmacia Biotech UK limited (Buckinghamshire, England). The RIN-5F cell line from the RIN-m rat islet cell line was purchased from ATCC the Global Bioresource Center. RPMI 1640 media, fetal bovine serum (FBS), and Penicillin-Streptomycin were purchased from Gibco BRL (NY, USA). Other chemicals were purchased from Wako Pure Chemical Industries, Ltd., unless specified otherwise.

Assay of insulin secretion activity

RIN-5F cells which were clones derived from rat pancreatic beta cells were used to evaluate insulin secretion activity. The cells at a concentration of 2.0×10^5 of the

cells/well in 24-well plates were seeded in RPMI 1640 medium (supplemented with 10% FBS) containing sodium hydrogen carbonate (2 g/L), streptomycin (100 μg/mL), and peniciline (100 units/mL) at 37 °C under 5% CO_2 atmosphere. After the incubation for 72 h, the medium in the each well was exchanged 1 mL of the fresh medium and the cells were incubated for another 24 h.

The medium in the wells was removed and the cells were washed with the fresh medium (supplemented with 1% FBS). A 360 μL of the medium and 40 μL of the test compound (10 and 100 μM) were pipetted into the all wells. After incubation at 37 °C under a 5% CO_2 for 3 h, aliquots in all wells were withdrawn and centrifuged to separate the cells. The concentration of insulin in the mediums was determined by EIA system. The activity was evaluated by an increase of the concentration of insulin-release comparing with the control experiment without test compounds. Each experiment was done in triplicate, and the results are presented as means ± SD. Group of data were compared using Student's *t*-test.

MTT assay

Cell viability in cell culture was determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. After treatment using selected ferulic acid derivatives for 24 h, the cells were further incubated in a medium containing 0.5 mg/mL of MTT for 1 h the MTT formazan produced by living cells was dissolved in dimethyl sulfoxide and absorbance at 570 nm was measured on a microplate reader (Spectra & Rainbow Readers, TECAN).

Procedure of synthesis of the amide compounds

Feruloylamide (10). To a solution of *O*-acetyl feruloyl chloride (25 g) in CH_2Cl_2 (250 mL) was added dropwise 15 mL of 28% aqueous ammonium solution in an ice-bath. The mixture was stirred for 30 min at room temperature to produce precipitates. The precipitates were filtrated and dissolved in MeOH (400 mL). A 46 g of 28% sodium methoxide in MeOH was added dropwise to the mixture in an ice-bath. After additional stirring at room temperature for 15 min, the mixture was concentrated under reduced pressure, and water (100 mL) was added. The solution was neutralized with 10% aqueous sulfuric acid and extracted with ethyl acetate. The organic layer was washed with water and dried over $MgSO_4$. After filtration and removal of the solvent, the product was recrystallized from EtOH to afford 13.1 g (69%) of prisms: mp = 151–153 °C; IR (KBr) ν = 3458, 3344, 1657, 1580, 1512 cm^{-1}; ^1H NMR (DMSO-d_6) δ = 3.79 (s, 3H, OCH_3), 6.41 (d, 1H, J = 15.6 Hz, = CH), 6.77 (d, 1H, J = 8.0 Hz, ArH), 6.96 (brs, 1H, NH), 6.97 (dd, 1H, J = 1.6, 8.0 Hz, ArH), 7.11 (d, 1H, J = 1.6 Hz, ArH), 7.30 (d, 1H, J = 15.6 Hz, = CH), 7.39 (brs, 1H, NH), 9.42 (s, 1H, OH); ^{13}C NMR (DMSO-d_6) δ = 55.7, 110.9, 115.8, 119.2, 121.9, 126.5, 139.8, 148.0, 148.5, 167.4. MS (ESI-TOF) calcd for $[C_{10}H_{12}NO_3]^+$ 194.08, Found 194.07 $[M+H]^+$. Anal. calcd for $C_{10}H_{11}NO_3$: C, 62.17; H, 5.74; N, 7.25; Found: C, 61.95; H, 5.74; N, 7.22.

***N*-Isopropylferuloylamide (11).** To a solution of *O*-acetyl feruloyl chloride (20 g) and triethylamine (11.9 g) in anhydrous CH_2Cl_2 (200 mL) was added dropwise 7.0 g of *iso*-propylamine in an ice-bath. The mixture was stirred for 15 min at room temperature. Acetic acid was added to the mixture to produce precipitates. The precipitates were filtered and dissolved in MeOH (400 mL). A 36.7 g of 28% sodium methoxide in MeOH was added dropwise to the solution in an ice-bath. After additional stirring at room temperature for 15 min, the mixture was neutralized with 10% aqueous sulfuric acid, concentrated, and extracted with ethyl acetate. The organic layer was washed with water and dried over $MgSO_4$. After filtration and removal of the solvent, the product was recrystallized from EtOH to afford 13.1 g (71%) of prisms: mp = 186–188 °C; IR (KBr) ν = 3329, 1655, 1518 cm^{-1}; ^1H NMR (DMSO-d_6) δ = 1.04 (d, 6H, J = 6.6 Hz, CH_3), 3.86 (s, 3H, OCH_3), 3.86–3.92 (m, 1H, CH), 6.36 (d, 1H, J = 15.7 Hz, NH), 6.73 (d, 1H, J = 8.1 Hz, ArH), 6.92 (dd, 1H, J = 8.2, 1.8 Hz, ArH), 7.05 (d, 1H, J = 2.0 Hz, ArH), 7.24 (d, 1H, J = 15.8 Hz, = CH), 7.76 (d, 1H, J = 8.0 Hz, NH), 9.35 (bs, 1H, OH); ^{13}C NMR (DMSO-d_6) δ = 22.7, 40.5, 55.7, 110.8, 115.8, 119.6, 121.6, 126.7, 138.8, 148.0, 148.3, 164.5. MS (ESI-TOF) calcd for $[C_{13}H_{18}NO_3]^+$ 236.13, Found 236.09 $[M+H]^+$. Anal. calcd for $C_{13}H_{17}NO_3$: C, 66.36; H, 7.28; N, 5.95. Found: C, 66.17; H, 7.30; N, 5.92.

***N*-Butylferuloylamide (12).** Using the procedure for **11**. The product was recrystallized from EtOH to afford 0.66 g (67%) of prisms: mp = 126–128 °C; IR (KBr) ν = 3315, 2963, 2872, 1653, 1587, 1518 cm^{-1}; ^1H NMR (DMSO-d_6) δ = 0.87 (t, 3H, J = 7.2 Hz, CH_3), 1.25–1.34 (m, 2H, CH_2), 1.38–1.45 (m, 2H, CH_2), 3.12–3.16 (m, 2H, CH_2), 3.79 (s, 3H, OCH_3), 6.42 (d, 1H, J = 15.7 Hz, = CH), 6.77 (d, 1H, J = 8.2 Hz, ArH), 6.96 (dd, 1H, J = 8.2, 1.9 Hz, ArH), 7.10 (d, 1H, J = 1.8 Hz, ArH), 7.28 (d, 1H, J = 15.8 Hz, = CH), 7.91 (t, 1H, J = 4.9 Hz, NH), 9.41(bs, 1H, OH); ^{13}C NMR (DMSO-d_6) δ = 13.8, 19.8, 31.5, 38.4, 55.7, 110.9, 115.8, 119.3, 121.6, 126.6, 138.9, 148.0, 148.4, 165.4. MS (ESI-TOF) calcd for $[C_{14}H_{20}NO_3]^+$ 250.14, found 250.10 $[M+H]^+$, Anal. calcd for $C_{14}H_{19}NO_3 \cdot H_2O$: C, 62.90; H, 7.92; N, 5.24. Found: C, 62.86; H, 7.96; N, 5.25.

***N*-*s*-Butylferuloylamide (13).** To a solution of 0.5 g of *O*-acetyl feruloyl chloride in anhydrous CH_2Cl_2 (20 mL) was added 0.32 g of *s*-butylamine. The mixture was stirred for 2 h at room temperature under nitrogen. The reaction mixture was washed with water and the organic portion was dried over $MgSO_4$. After filtration, the solution was concentrated and hexane was added to afford crystals (0.5 g). To a solution of 0.2 g of the crystals in 10 mL acetonitrile was added hydrazine monohydrate (38 mg). The mixture was stirred for 2 h at room temperature. The organic portion was extracted with ethyl acetate, washed with water and saturated aqueous NaCl, and dried over $MgSO_4$. After the solvent was evaporated, the product was purified by SiO_2 column using $CHCl_3$/MeOH (2/1) to afford 0.12 g (73%) of solids: mp = 130–132 °C; IR (KBr) ν = 3344, 2970, 2835, 1655, 1587, 1518 cm^{-1}; ^1H NMR ($CDCl_3$) δ = 0.92 (t, 3H, J = 7.5 Hz, CH_3), 1.16 (d, 3H, J = 6.6 Hz, CH_3),

1.47–1.54 (m, 2H, CH_2), 3.88 (s, 3H, OCH_3), 4.00–4.07 (m, 1H, CH), 5.39 (d, 1H, $J=8.1$ Hz, NH), 5.91 (s, 1H, OH), 6.21 (d, 1H, $J=15.6$ Hz, =CH), 6.87 (d, 1H, $J=8.2$ Hz, ArH), 6.96 (d, 1H, $J=1.8$ Hz, ArH), 7.02 (dd, 1H, $J=8.2$, 1.8 Hz, ArH), 7.50 (d, 1H, $J=15.6$ Hz, =CH); ^{13}C NMR ($CDCl_3$) δ = 10.3, 20.5, 29.8, 46.7, 55.9, 109.6, 114.7, 118.6, 122.0, 127.4, 140.7, 146.7, 147.3, 165.5. MS (ESI-TOF) calcd for $[C_{14}H_{20}NO_3]^+$ 250.14, Found 250.13 $[M+H]^+$. Anal. calcd for $C_{14}H_{19}NO_3$: C, 67.45; H, 7.68; N, 5.62. Found: C, 67.08; H, 7.79; N, 5.53.

***N*-Pentylferuloylamide (14).** Following a method similar to that for **13**. SiO_2 column using $CHCl_3$/MeOH (2/1) afford 0.13 g (76%) of solids: mp = 73–76 °C; IR (KBr) ν = 3261, 2934, 2866, 1655, 1597, 1518 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ = 0.86 (t, 3H, $J=6.8$ Hz, CH_3), 1.24–1.31 (m, 4H, CH_2), 1.39–1.46 (m, 2H, CH_2), 3.11–3.14 (m, 2H, CH_2), 3.79 (s, 3H, OCH_3), 6.42 (d, 1H, $J=15.8$ Hz, =CH), 6.77 (d, 1H, $J=8.2$ Hz, ArH), 6.96 (dd, 1H, $J=8.2$, 1.8 Hz, ArH), 7.10 (d, 1H, $J=1.8$ Hz, ArH), 7.29 (d, 1H, $J=15.6$ Hz, =CH), 7.91 (t, 1H, $J=5.5$ Hz, NH), 9.41 (brs, 1H, OH); ^{13}C NMR (DMSO-d_6) δ = 14.1, 22.1, 28,9, 29.1, 38.8, 55.7, 110.8, 115.8, 119.3, 121.6, 126.6, 138.9, 148.0, 148.4, 165.4. MS (ESI-TOF) calcd for $[C_{15}H_{22}NO_3]^+$ 264.16, found 264.15 $[M+H]^+$, Anal. calcd for $C_{15}H_{21}NO_3$: C, 68.42; H, 8.04; N, 5.32. Found: C, 68.03; H, 8.08; N, 5.36.

***N*-Hexylferuloylamide (15).** Following a method similar to that for **13**. SiO_2 column using $CHCl_3$/MeOH (20/1) to afford 0.18 g (94%) of oil: IR (KBr) ν = 3283, 2930, 2860, 1655, 1591, 1516 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ = 0.87 (t, 3H, $J=6.9$ Hz, CH_3), 1.27–1.38 (m, 6H, CH_2), 1.50–1.57 (m, 2H, CH_2), 3.33–3.38 (m, 2H, CH_2), 3.90 (s, 3H, OCH_3), 5.52 (t, 2H, $J=6.8$ Hz, NH), 5.79 (s, 1H, OH), 6.21 (d, 1H, $J=15.6$ Hz, =CH), 6.88 (d, 1H, $J=8.2$ Hz, ArH), 6.97 (d, 1H, $J=1.8$ Hz, ArH), 7.04 (dd, 1H, $J=8.24$, 1.8 Hz, ArH), 7.51 (d, 1H, $J=15.6$ Hz, =CH); ^{13}C NMR ($CDCl_3$) δ = 14.0, 22.5, 26.6, 29.7, 31.5, 39.8, 55.9, 109.6, 114.7, 118.3, 121.9, 127.4, 140.8, 146.7, 147.3, 166.1. MS (ESI-TOF) calcd for $[C_{16}H_{24}NO_3]^+$ 278.18, found 278.17 $[M+H]^+$, Anal. calcd for $C_{16}H_{23}NO_3$: C, 69.29; H, 8.36; N, 5.05. Found: C, 69.10; H, 8.60; N, 4.85.

***N*-Octylferuloylamide (16).** Following a method similar to that for **13**. SiO_2 column using $CHCl_3$/MeOH (20/1) to afford 0.12 g (68%) of oil: IR (KBr) ν = 3281, 2928, 2856, 1657, 1591, 1516 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ = 0.86 (t, 3H, $J=6.9$ Hz, CH_3), 1.25–1.35 (m, 10H, CH_2), 1.50–1.57 (m, 2H, CH_2), 3.33–3.38 (m, 2H, CH_2), 3.89 (s, 3H, OCH_3), 5.58 (t, 1H, $J=5.8$ Hz, NH), 5.87 (s, 1H, OH), 6.22 (d, 1H, $J=15.6$ Hz, =CH), 6.87 (d, 1H, $J=8.1$ Hz, ArH), 6.96 (d, 1H, $J=1.8$ Hz, ArH), 7.03 (dd, 1H, $J=8.2$, 1.8 Hz, ArH), 7.51 (d, 1H, $J=15.4$ Hz, =CH); ^{13}C NMR ($CDCl_3$) δ = 14.1, 22.6, 27.0, 29.2, 29.3, 29.7, 31.8, 39.8, 55.9, 109.6,114.7, 118.4, 122.0, 127.4, 140.8, 146.7, 147.3, 166.1. MS (ESI-TOF) calcd for $[C_{18}H_{28}NO_3]^+$ 306.21, found 306.20 $[M+H]^+$, Anal. calcd for $C_{18}H_{27}NO_3$: C, 70.79; H, 8.91; N, 4.59. Found: C, 70.42; H, 8.96; N, 4.64.

***N*-Feruloyl pyrrolidine (17).** Following a method similar to that for **13**. Recrystallized from $CHCl_3$/Hexane to afford 0.08 g (60%) of solids: mp = 170–172 °C; IR (KBr) ν = 3431, 2963, 2874, 2787, 1641, 1601, 1564 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ = 1.76–1.82 (m, 2H, CH_2), 1.87–1.93 (m, 2H, CH_2), 3.32–3.39 (m, 2H, CH_2), 3.59–3.63 (m, 2H, CH_2), 3.37 (t, 2H, $J=6.9$ Hz, CH_2), 3.61 (t, 2H, $J=6.9$ Hz, CH_2), 3.81 (s, 3H, OCH_3), 6.76 (d, 1H, $J=15.4$ Hz, =CH), 6.76 (d, 1H, $J=8.2$ Hz, ArH), 7.07 (dd, 1H, $J=8.2$, 1.8 Hz, ArH), 7.26 (d, 1H, $J=2.0$ Hz, ArH), 7.36 (d, 1H, $J=15.4$ Hz, =CH), 9.42 (brs, 1H, OH); ^{13}C NMR (DMSO-d_6) δ = 24.1, 25.8, 45.8, 46.2, 55.9, 111.5, 115.7, 116.7, 122.4, 126.8, 141.0, 148.0, 148.6, 164.1. MS (ESI-TOF) calcd for $[C_{14}H_{18}NO_3]^+$ 248.13, Found 248.12 $[M+H]^+$, Anal. calcd for $C_{14}H_{17}NO_3 \cdot 1/2H_2O$: C, 65.61; H, 7.09; N, 5.46. Found: C, 65.27; H, 6.99; N, 5.55.

***N*-Feruloyl piperidine (18).** To a solution of ferulic acid (0.39 g) in anhydrous DMF/CH_2Cl_2 (0.5/3 mL) was added triethylamine (0.61 mL) at room temperature. *Iso*-butylchloroformate (0.57 mL) was added dropwise at −15 °C under nitrogen. After stirring for 30 min, piperidine (1.0 mL) was added dropwise and the temperature rose room temperature in 30 min. After additional stirring for 1 h, 10% citric acid aqueous solution (20 mL) was added to reach pH 2. The organic portion was extracted with ethyl acetate (50 mL ×2) and the organic layer was washed with 10% citric acid aq solution, water, and saturated aqueous NaCl, followed by drying over $MgSO_4$. After filtration and removal of the solvent under reduced pressure, the product was purified by SiO_2 column using hexane/ethyl acetate (1/0–5/1) to afford 0.61 g (91%) of white powder: mp = 132–135 °C; (134–135 °C[14]). ^{1}H NMR ($CDCl_3$) δ = 1.56–1.68 (m, 6H, CH_2), 3.56–3.63 (m, 4H, 2×NCH_2), 3.90 (s, 3H, CH_3O), 6.72 (d, 1H, $J=15.2$ Hz, =CH–), 6.89 (d, 1H, $J=8.4$ Hz, ArH), 6.96 (d, 1H, $J=2.0$ Hz, ArH), 7.06 (dd, 1H, $J=8.4$, 1.6 Hz, ArH), 7.56 (d, 1H, $J=15.2$ Hz,-CH=), ^{13}C NMR ($CDCl_3$) δ = 24.6, 26.2, 55.9, 109.9, 114.7, 115.0, 121.7, 128.0, 142.5, 146.7, 147.2, 165.6. MS (ESI-TOF) calcd for $[C_{15}H_{20}NO_3]^+$ 262.14, Found 262.10 $[M+H]^+$. Anal. calcd for $C_{15}H_{19}NO_3$: C, 68.94; H, 7.33; N, 5.36. Found: C, 68.66; H, 7.27; N, 5.57.

***N*-Feruloyl tyramine (19).** Following a method similar to that for **13**. SiO_2 column using $CHCl_3$/MeOH (20/1) to afford 0.17 g (94%) of solids: mp = 68–74 °C; IR (KBr) ν = 3300, 2939, 2848, 1655, 1591, 1516 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ = 2.63 (t, 2H, $J=7.3$ Hz, CH_2), 3.29–3.32 (m, 2H, CH_2), 3.79 (s, 3H, OCH_3), 6.41 (d, 1H, $J=15.8$ Hz, =CH), 6.66 (d, 1H, $J=8.4$ Hz, ArH), 6.77 (dd, 1H, $J=8.1$ Hz, ArH), 6.96 (dd, 2H, $J=8.3$, 1.7 Hz, ArH), 6.99 (d, $J=8.4$ Hz, ArH), 7.09 (d, 1H, $J=1.7$ Hz, ArH), 7.29 (d, 1H, $J=15.6$ Hz, =CH), 7.97 (t, 1H, $J=5.6$ Hz, NH), 9.17 (brs, 1H, OH), 9.40 (brs, 1H, OH); ^{13}C NMR (DMSO-d_6) δ = 34.6, 40.8, 55.7, 110.9 115.3, 115.8, 119.2, 121.7, 126.6, 129.6, 129.7, 139.0, 148.0, 148.4, 155.8, 165.5. MS (ESI-TOF) calcd for $[C_{18}H_{20}NO_3]^+$ 314.14, Found 314.13 $[M+H]^+$, Anal. calcd for $C_{18}H_{19}NO_4 \cdot 2/3H_2O$: C, 66.44; H, 6.30; N, 4.30. Found: C, 66.58; H, 6.00; N, 4.61.

***N*-Feruloyl tyrosine (20).** To a solution of (4-*tert*-butoxycarbonyloxy-3-methoxyphenyl)-2-propenate (0.29 g), 4-*tert*-butoxycarbonyl-tyrosine-tert-butyl ester (0.34 g), and dimetylaminopyridine (12 mg) in anhydrous CH_2Cl_2 (2 mL) was added EDC·HCl (0.38 g) at room temperature. After stirring for 6 h, solvent was evaporated and the product was purified by SiO_2 column using hexane/ethyl acetate (10/1–4/1) to afford 0.53 g of white powder. A 61 mg of the powder was dissolved in 1 mL of acetic acid and allowed to stand for 24 h at room temperature. Water (3 mL) was added to the solution and the solvent was evaporated under reduced pressure. Ether was added and allowed to stand in refrigerator to produce precipitates. The precipitates were filtrated and washed with a small amount of ethyl acetate to afford 28 mg of white powder (78%). mp = 207–211 °C; IR (KBr) ν = 3140, 2940, 2850, 1720, 1632, 1593, 1508 cm^{-1}; ^1H NMR (DMSO-d_6) δ = 2.84–2.90 (m, 1H, CH), 3.14 (dd, 2H, J = 14.5, 4.4 Hz, CH_2), 3.82 (s, 3H, OCH_3), 6.69 (d, 1H, J = 15.9 Hz, = CH), 6.81 (d, 1H, J = 8.2 Hz, ArH), 7.08 (d, 2H, J = 8.6 Hz, ArH), 7.19 (dd, 1H, J = 8.2, 1.9 Hz, ArH), 7.31 (d, 2H, J = 8.6 Hz, ArH), 7.40 (d, 1H, J = 8.6 Hz, ArH), 7.72 (d, 1H, J = 15.7 Hz, = CH); ^{13}C NMR (DMSO-d_6) δ = 36.9, 56.1, 56.4, 112.1, 114.1, 116.2, 122.2, 124.3, 126.1, 131.0, 135.6, 147.6, 148.6, 149.9, 150.5, 166.0, 169.8. MS (ESI-TOF) calcd for $[C_{19}H_{20}NO_3]^+$ 358.13, found 358.05 $[M+H]^+$. Anal. calcd for $C_{19}H_{19}NO_6 \cdot 1.5H_2O$: C, 59.37; H, 5.77; N, 3.64. Found: C, 59.04; H, 5.52; N, 4.02.

Acknowledgements

We wish to thank Prof. S. Nanjyo and Dr. H. Sasaki of Wakayama Medical University for helpful discussion.

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