Hydroxylated Hydrocinnamides as Hypocholesterolemic Agents

Sangku Lee,* Chul-Ho Lee, Eungsoo Kim, Sang-Hun Jung,† and Hyeong Kyu Lee*

*Natural Medicine Research Center, KRIBB, Daejeon 305-806, Korea. *E-mail: sangku@kribb.re.kr*
*†College of Pharmacy, Chungnam National University, Daejeon 305-764, Korea. *E-mail: hykylee@kribb.re.kr*
Received July 6, 2007

Several hydroxylated cinnamic acid derivatives were prepared from the corresponding acids and amino acid residues, and their hypocholesterolemic activities were evaluated in high cholesterol-fed mice. The presence of the double bond in hydroxylated cinnamide derivatives decreases cholesterol-lowering activities and the number of free phenolic hydroxy groups affect greatly the activities. 3,4-Dihydroxy hydrocinnamides obtained from amino acid derivatives containing a hydrophobic side chain such as alanine, valine, phenylalanine, and isoleucine exhibited potent cholesterol-lowering activities.

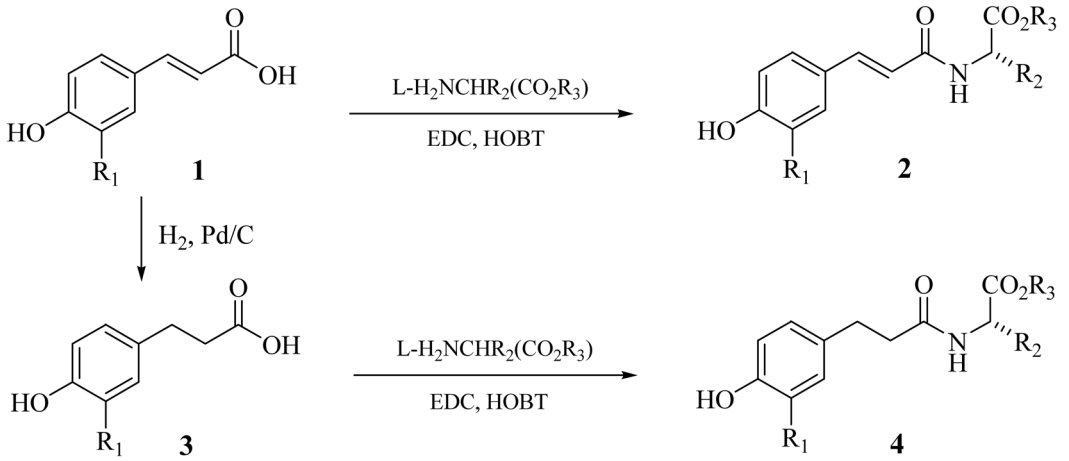
Key Words : Cinnamic acid, Hydrocinnamide, Amino acid, Hypocholesterolemic activity

Introduction

Cinnamic acids and their derivatives have exhibited a wide range of biological activities, including antioxidative effect[1] of low-density lipoprotein (LDL), peroxyl radical scavenging effect,[2] hepatoprotective effect,[3] anti-inflammatory effect,[4] antimutagenic effect,[5] and inhibitory effect of HIV-1 integrase.[6] It was reported that alkoxycinnamates, in which phenolic hydroxy groups were substituted with an alkyl chain, showed hypocholesterolemic activities and the moderate length (C_{12}-C_{16}) of the alkoxy substituents were important for activity.[7] In the course of exploring a new hypocholesterolemic agent, we synthesized free phenolic cinnamic acid derivatives, in which phenolic hydroxy groups were unsubstituted with an alkyl chain.[8] We have been interested in free phenolic cinnamides and hydrocinnamides substituted at the carboxylic acid carbon with amino acid residues due to a variety of biological activities of amino acids. Herein we describe the preparation of 4-hydroxy cinnamides **2**, 4-hydroxy hydrocinnamides **4a**, and 3,4-dihydroxy hydrocinnamides **4b** from the corresponding acids and several natural amino acid derivatives. We also have evaluated hypocholesterolemic effectiveness of these compounds in high cholesterol-fed mice.

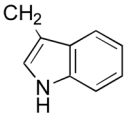
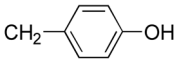
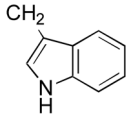
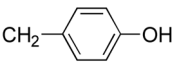
4-Hydroxy and 3,4-dihydroxy hydrocinnamic acids **3** were obtained respectively by reduction of the double bond of the corresponding cinnamic acids **1** utilizing hydrogen (50 psi) and 5% Pd/C in a quantitative yield (Scheme 1). 4-Hydroxy cinnamides **2**, 4-hydroxy hydrocinnamides **4a**, and 3,4-dihydroxy hydrocinnamides **4b** were prepared by condensation of the corresponding acids with several amino acid residues using 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC), 1-hydroxybenzotriazole (HOBT), and triethylamine in methylene chloride in 74-92% yields. All L-amino acid esters were obtained from Aldrich Chemical Co., except L-tryptophan ethyl ester and L-tyrosine ethyl ester. They were prepared from the corresponding amino acids using thionyl chloride[9] in ethanol or Fischer esterification.

The hypocholesterolemic effect of the synthesized hydroxylated cinnamide derivatives was evaluated by measuring plasma total cholesterols in high cholesterol-fed C57BL/6J mice. Table 1 shows the total cholesterol levels in the mice after feeding a high cholesterol diet supplemented with



Scheme 1

Table 1. Effects of the synthesized hydroxylated cinnamide derivatives on plasma total cholesterols in high cholesterol-fed mice

Compound	R_1	R_2	R_3	Total cholesterol[a] (mg/dL)
control				222 ± 30
lovastatin				182 ± 25
2aa	H	$CH_2C_6H_5$	CH_3	207 ± 15
2ab	H	H	C_2H_5	208 ± 14
4aa	H	CH_3	CH_3	195 ± 22
4ab	H	$CH(CH_3)_2$	CH_3	198 ± 17
4ac	H	$CH_2C_6H_5$	CH_3	198 ± 20
4ad	H	$CH_2CH_2SCH_3$	CH_3	199 ± 14
4ae	H		CH_3	208 ± 16
4af	H	H	C_2H_5	202 ± 14
4ag	H		CH_3	220 ± 18
4ah	H	$CH_2CH_2CO_2C_2H_5$	C_2H_5	214 ± 11
4ba	OH	CH_3	CH_3	190 ± 13
4bb	OH	$CH(CH_3)_2$	CH_3	192 ± 10
4bc	OH	$CH_2C_6H_5$	CH_3	190 ± 18
4bd	OH	$CH_2CH_2SCH_3$	C_2H_5	196 ± 15
4be	OH		C_2H_5	198 ± 20
4bf	OH	H	C_2H_5	198 ± 9
4bg	OH		C_2H_5	216 ± 12
4bh	OH	$CH_2CH_2CO_2C_2H_5$	C_2H_5	212 ± 15
4bi	OH	CH_2CO_2 $CH_2C_6H_5$	$CH_2C_6H_5$	210 ± 16
4bj	OH	$CH(CH_3)C_2H_5$	C_2H_5	182 ± 16

[a]Mean ± SD; all values are significantly different ($p < 0.05$) from the control group.

0.05% (wt/wt in diet) of the test compounds for 10 days. As compared to the control group, the hydroxylated cinnamide derivatives, **2** and **4**, significantly lowered plasma cholesterol levels ($p < 0.05$). The extent of decreasing plasma total cholesterol levels was greater in 4-hydroxy hydrocinnamides **4a** than 4-hydroxy cinnamides **2** (**2aa** *vs.* **4ac** and **2ab** *vs.* **4af**). The presence of a double bond in the structure of 4-hydroxy cinnamides **2** decreased the efficiency of hypocholesterolemic activity. In general, 3,4-dihydroxy hydrocinnamides **4b** were more effective than 4-hydroxy hydrocinnamides **4a** in lowering cholesterol levels (**4aa** *vs.* **4ba**, **4ab** *vs.* **4bb**, **4ac** *vs.* **4bc**, **4ad** *vs.* **4bd**, **4ae** *vs.* **4be**, **4af** *vs.* **4bf**, **4ag** *vs.* **4bg**, and **4ah** *vs.* **4bh**). The number of free phenolic hydroxy groups affects greatly the activity. Regarding the effect of the substituent R_2 of the hydroxylated hydrocinnamides **4b** on hypocholesterolemic activities, hydrophobic groups (**4ba**, **4bb**, **4bc**, **4bj**) enhanced the hypocholesterolemic activities. 3,4-Dihydroxy hydrocinnamides derived from amino acid residues containing a hydrophobic side chain such as alanine, valine, phenylalanine, and isoleucine showed potent hypocholesterolemic activities in comparision with the derivatives obtained from tyrosine, aspartic acid, and glutamic acid. Among the tested compounds, compound **4bj** exhibited the strongest cholesterol-lowering effects, as potent as lovatatin.

In conclusion, a series of hydroxylated cinnamide derivatives were prepared from the corresponding acids and amino acid residues, and examined for hypocholesterolemic activity in high cholesterol-fed mice. The presence of a double bond in 4-hydroxy cinnamic acid derivatives decreases the activity. Also, the number of free phenolic hydroxy groups greatly affects cholesterol-lowering activities. 3,4-Dihydroxy hydrocinnamides **4b** obtained from amino acid esters containing a hydrophobic side chain exhibited potent cholesterol-lowering effects.

Experimental Section

All reactions were conducted under a nitrogen atmosphere using oven-dried glassware unless otherwise noted. Analytical TLC was done on 0.25 mm E. Merck precoated silica gel 60 F_{254} plates. Visualization was accomplished with UV light at 254 nm and with phosphomolybdic acid, ninhydrin, or anisaldehyde stain. Flash chromatography was performed on silica gel 60 (E. Merck 9385, 230-400 mesh). Yields refer to chromatographically and spectroscopically pure compounds unless otherwise stated. Melting points are uncorrected unless otherwise noted. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Unity 300 or Varian Inova 400 spectrometer at ambient temperature. Chemical shifts are reported in ppm relative to tetramethylsilane/$CHCl_3$.

3,4-Dihydroxy hydrocinnamic acid (L-aspartic acid dibenzyl ester) amide (4bi). Typical Procedure. To a solution of 3,4-dihydroxy hydrocinnamic acid (3.0 g, 16.5 mmol), L-aspartic acid dibenzyl ester *p*-toluenesulfonate (8.0 g, 16.5 mmol), and 1-hydroxybenzotriazole (2.3 g, 17.0 mmol) in *N,N*-dimethylformamide (50 mL) at 0 °C was added triethylamine (6.9 mL, 49.5 mmol). After 10 min, 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (3.2 g, 16.7 mmol) was added at 0 °C and the resultant reaction mixture was stirred for 18 h at room temperature. The mixture was poured into water (200 mL), extracted with ethyl acetate (4 × 200 mL), washed with water (2 × 100 mL) and brine (100 mL), dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by flash chromatography on silica gel (1:1 hexane-EtOAc) to afford 6.0 g (76%) of 3,4-dihydroxy hydrocinnamic acid (L-aspartic acid dibenzyl ester) amide as a pale yellow oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36-7.24 (m, 10H), 6.73 (d, J = 8.0 Hz, 1H), 6.66 (d, J = 2.0 Hz, 1H), 6.55 (dd, J = 8.0, 2.0 Hz, 1H), 6.49 (d, J = 7.6 Hz, 1H), 5.10 (s, 2H), 5.05 (d, J = 12.0 Hz, 1H), 5.00 (d, J = 12.0 Hz, 1H), 4.87 (m, 1H), 3.01 (dd, J = 16.8, 4.4 Hz, 1H), 2.83-2.77 (m, 3H), 2.43 (m, 2H); ^{13}C NMR (100

MHz, $CDCl_3$) δ 172.9, 171.0, 170.6, 143.8, 142.8, 135.4, 135.1, 132.9, 128.9, 128.8, 128.7, 128.6, 128.5, 120.6, 115.6, 115.4, 67.9, 67.2, 48.8; EIMS *m/z* 477 (M^+).

4-Hydroxy cinnamic acid (L-phenylalanine methyl ester) amide (2aa). Compound **2aa** was prepared from 4-hydroxy cinnamic acid and L-phenylalanine methyl ester hydrochloride as described for **4bi** in 86% yield as a white solid: mp 153-154 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.53 (d, *J* = 15.2 Hz, 1H), 7.31 (d, *J* = 8.8 Hz, 2H), 7.27 (m, 3H), 7.11 (m, 2H), 6.82 (d, *J* = 8.8 Hz, 2H), 6.20 (d, *J* = 15.2 Hz, 1H), 6.16 (s, 1H), 5.02 (dd, *J* = 13.6, 6.0 Hz, 1H), 3.74 (s, 3H), 3.22 (dd, *J* = 14.0, 6.0 Hz, 1H), 3.16 (dd, *J* = 14.0, 6.0 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.3, 166.2, 158.1, 142.1, 135.7, 129.7, 129.2, 128.6, 127.2, 126.7, 116.7, 115.9, 53.4, 52.4, 37.8; EIMS *m/z* 325 (M^+).

4-Hydroxy cinnamic acid (glycine ethyl ester) amide (2ab). Compound **2ab** was prepared from 4-hydroxy cinnamic acid and glycine ethyl ester hydrochloride as described for **4bi** in 84% yield as a white solid: mp 171-172 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.86 (s, 1H), 8.38 (t, *J* = 6.0 Hz, 1H), 7.41 (d, *J* = 8.4 Hz, 2H), 7.35 (d, *J* = 15.6 Hz, 1H), 6.79 (d, *J* = 8.7 Hz, 2H), 6.48 (d, *J* = 15.9 Hz, 1H), 4.10 (q, *J* = 7.2 Hz, 2H), 3.93 (d, *J* = 6.0 Hz, 2H), 1.19 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 169.9, 165.8, 158.9, 139.4, 129.2, 125.6, 117.8, 115.7, 60.3, 45.2, 14.0.

4-Hydroxy hydrocinnamic acid (L-alanine methyl ester) amide (4aa). Compound **4aa** was prepared from 4-hydroxy hydrocinnamic acid and L-alanine methyl ester hydrochloride as described for **4bi** in 82% yield as a white solid: mp 78-79 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.12 (s, 1H), 8.23 (d, *J* = 6.6 Hz, 1H), 6.98 (d, *J* = 8.7 Hz, 2H), 6.65 (d, *J* = 8.7 Hz, 2H), 4.25 (m, 1H), 3.60 (s, 3H), 2.68 (t, *J* = 7.5 Hz, 2H), 2.33 (t, *J* = 7.5 Hz, 2H), 1.23 (d, *J* = 7.2 Hz, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 173.1, 171.4, 155.3, 131.2, 128.9, 114.9, 51.6, 47.4, 36.9, 30.0, 16.9.

4-Hydroxy hydrocinnamic acid (L-valine methyl ester) amide (4ab). Compound **4ab** was prepared from 4-hydroxy hydrocinnamic acid and L-valine methyl ester hydrochloride as described for **4bi** in 88% yield as a pale yellow oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.02 (d, *J* = 8.0 Hz, 2H), 6.75 (d, *J* = 8.0 Hz, 2H), 6.61 (brs, 1H), 6.01 (brd, *J* = 8.4 Hz, 1H), 4.53 (dd, *J* = 8.8, 5.2 Hz, 1H), 3.71 (s, 3H), 2.88 (t, *J* = 7.6 Hz, 2H), 2.52 (td, *J* = 7.6, 3.6 Hz, 2H), 2.07 (m, 1H), 0.84 (d, *J* = 6.8 Hz, 3H), 0.82 (d, *J* = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.7, 172.6, 154.6, 131.8, 129.3, 115.4, 57.0, 52.1, 38.4, 31.1, 30.7, 18.7, 17.7.

4-Hydroxy hydrocinnamic acid (L-phenylalanine methyl ester) amide (4ac). Compound **4ac** was prepared from 4-hydroxy hydrocinnamic acid and L-phenylalanine methyl ester hydrochloride as described for **4bi** in 80% yield as a pale yellow oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.21 (m, 3H), 6.98 (d, *J* = 8.4 Hz, 2H), 6.93 (m, 2H), 6.74 (d, *J* = 8.4 Hz, 2H), 5.99 (d, *J* = 7.6 Hz, 1H), 4.87 (m, 1H), 3.68 (s, 3H), 3.04 (d, *J* = 5.6 Hz, 2H), 2.83 (m, 2H), 2.43 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.2, 171.9, 154.6, 135.5, 131.8, 129.3, 129.1, 128.5, 127.1, 115.4, 53.0, 52.3, 38.3, 37.7, 30.5.

4-Hydroxy hydrocinnamic acid (L-methionine methyl ester) amide (4ad). Compound **4ad** was prepared from 4-hydroxy hydrocinnamic acid and L-methionine methyl ester hydrochloride as described for **4bi** in 92% yield as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.02 (d, *J* = 8.4 Hz, 2H), 6.75 (d, *J* = 8.4 Hz, 2H), 6.67 (s, 1H), 6.25 (d, *J* = 8.0 Hz, 1H), 4.69 (dd, *J* = 12.4, 7.2 Hz, 1H), 3.73 (s, 3H), 2.88 (m, 2H), 2.50 (m, 2H), 2.35 (m, 2H), 2.09 (m, 1H), 2.04 (s, 3H), 1.90 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.5, 172.4, 154.6, 131.8, 129.3, 115.4, 52.5, 51.4, 38.4, 31.4, 30.6, 29.7, 15.3.

4-Hydroxy hydrocinnamic acid (L-tryptophan methyl ester) amide (4ae). Compound **4ae** was prepared from 4-hydroxy hydrocinnamic acid and L-tryptophan methyl ester hydrochloride as described for **4bi** in 89% yield as a white solid: mp 57-58 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.48 (s, 1H), 7.40 (s, 1H), 7.39 (d, *J* = 8.0 Hz, 1H), 7.24 (d, *J* = 8.0 Hz, 1H), 7.11 (t, *J* = 7.6 Hz, 1H), 7.03 (t, *J* = 7.6 Hz, 1H), 6.85 (d, *J* = 8.0 Hz, 2H), 6.66 (d, *J* = 8.0 Hz, 2H), 6.56 (m, 1H), 6.15 (d, *J* = 7.6 Hz, 1H), 4.85 (dd, *J* = 13.2, 5.6 Hz, 1H), 3.58 (s, 3H), 3.17 (d, *J* = 5.2 Hz, 2H), 2.73 (m, 2H), 2.32 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.8, 172.4, 154.6, 136.0, 131.7, 129.3, 127.3, 123.0, 122.0, 119.5, 118.2, 115.4, 111.3, 109.2, 52.9, 52.4, 38.2, 30.4, 27.3.

4-Hydroxy hydrocinnamic acid (glycine ethyl ester) amide (4af). Compound **4af** was prepared from 4-hydroxy hydrocinnamic acid and glycine ethyl ester hydrochloride as described for **4bi** in 92% yield as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.99 (d, *J* = 8.8 Hz, 2H), 6.74 (d, *J* = 8.8 Hz, 2H), 6.71 (s, 1H), 6.14 (brs, 1H), 4.19 (q, *J* = 6.8 Hz, 2H), 3.99 (d, *J* = 5.2 Hz, 2H), 2.87 (t, *J* = 8.0 Hz, 2H), 2.50 (t, *J* = 8.0 Hz, 2H), 1.26 (t, *J* = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.9, 170.0, 154.6, 131.9, 129.2, 115.4, 61.6, 41.4, 38.2, 30.5, 14.0.

4-Hydroxy hydrocinnamic acid (L-tyrosine methyl ester) amide (4ag). Compound **4ag** was prepared from 4-hydroxy hydrocinnamic acid and L-tyrosine methyl ester hydrochloride as described for **4bi** in 83% yield as a colorless oil: ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.17 (brs, 2H), 8.23 (d, *J* = 7.8 Hz, 1H), 6.95 (m, 4H), 6.65 (m, 4H), 4.38 (m, 1H), 3.57 (s, 3H), 2.88 (dd, *J* = 13.5, 6.0 Hz, 1H), 2.75 (dd, *J* = 13.5, 9.0 Hz, 1H), 2.63 (m, 2H), 2.31 (m, 2H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 172.2, 171.6, 155.9, 155.4, 131.2, 130.0, 129.9, 128.9, 127.2, 115.0, 53.8, 51.6, 37.0, 36.0, 30.1.

4-Hydroxy hydrocinnamic acid (L-glutamic acid di-ethyl ester) amide (4ah). Compound **4ah** was prepared from 4-hydroxy hydrocinnamic acid and L-glutamic acid diethyl ester hydrochloride as described for **4bi** in 90% yield as a pale yellow oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.02 (d, *J* = 8.4 Hz, 2H), 6.74 (d, *J* = 8.4 Hz, 2H), 6.23 (d, *J* = 7.6 Hz, 1H), 4.58 (td, *J* = 7.6, 5.2 Hz, 1H), 4.22-4.09 (m, 4H), 2.87 (m, 2H), 2.46 (m, 2H), 2.27 (m, 2H), 2.14 (m, 1H), 1.91 (m, 1H), 1.25 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.0, 172.4, 171.9, 154.5, 132.0, 129.3, 115.4, 61.7, 60.8, 51.6, 38.4, 30.6, 30.1, 27.2, 14.1, 14.0.

3,4-Dihydroxy hydrocinnamic acid (L-alanine methyl

ester) amide (4ba). Compound **4ba** was prepared from 3,4-dihydroxy hydrocinnamic acid and L-alanine methyl ester hydrochloride as described for **4bi** in 80% yield as a white solid: mp 98-99 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.69 (s, 1H), 8.61 (s, 1H), 8.23 (d, J = 7.2 Hz, 1H), 6.58 (d, J = 8.0 Hz, 1H), 6.54 (d, J = 1.6 Hz, 1H), 6.40 (dd, J = 8.0, 1.6 Hz, 1H), 4.22 (quint, J = 7.2 Hz, 1H), 3.58 (s, 3H), 2.59 (m, 2H), 2.28 (m, 2H), 1.22 (d, J = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 173.9, 172.2, 145.6, 143.9, 132.7, 119.3, 116.3, 116.0, 52.4, 48.1, 37.7, 31.0, 17.6; EIMS *m/z* 267 (M^+).

3,4-Dihydroxy hydrocinnamic acid (L-valine methyl ester) amide (4bb). Compound **4bb** was prepared from 3,4-dihydroxy hydrocinnamic acid and L-valine methyl ester hydrochloride as described for **4bi** in 81% yield as a pale yellow oil: ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.63 (br s, 2H), 8.09 (d, J = 8.1 Hz, 1H), 6.61 (d, J = 7.8 Hz, 1H), 6.58 (d, J = 2.4 Hz, 1H), 6.43 (dd, J = 7.8, 2.4 Hz, 1H), 4.17 (m, 1H), 3.61 (s, 3H), 2.63 (m, 2H), 2.40 (m, 2H), 2.00 (m, 1H), 0.85 (d, J = 5.7 Hz, 3H), 0.82 (d, J = 5.4 Hz, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 172.2, 172.1, 145.0, 143.3, 132.1, 118.7, 115.7, 115.4, 57.4, 51.5, 37.0, 30.6, 29.9, 18.9, 18.2.

3,4-Dihydroxy hydrocinnamic acid (L-phenylalanine methyl ester) amide (4bc). Compound **4bc** was prepared from 3,4-dihydroxy hydrocinnamic acid and L-phenylalanine methyl ester hydrochloride as described for **4bi** in 84% yield as a pale yellow oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.21 (m, 3H), 6.92 (dd, J = 8.4, 2.4 Hz, 2H), 6.74 (d, J = 8.0 Hz, 1H), 6.68 (d, J = 1.6 Hz, 1H), 6.51 (dd, J = 8.0, 2.0 Hz, 1H), 6.18 (d, J = 7.6 Hz, 1H), 4.83 (dd, J = 13.6, 6.0 Hz, 1H), 3.66 (s, 3H), 3.01 (dd, J = 6.0, 2.4 Hz, 2H), 2.76 (t, J = 8.0 Hz, 2H), 2.41 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.9, 171.9, 144.1, 142.8, 135.4, 132.4, 129.2, 129.1, 128.6, 128.5, 127.1, 120.1, 115.3, 115.2, 53.2, 52.3, 38.2, 37.7, 30.7.

3,4-Dihydroxy hydrocinnamic acid (L-methionine ethyl ester) amide (4bd). Compound **4bd** was prepared from 3,4-dihydroxy hydrocinnamic acid and L-methionine ethyl ester hydrochloride as described for **4bi** in 84% yield as a colorless oil: ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.64 (s, 2H), 8.20 (d, J = 7.5 Hz, 1H), 6.60 (d, J = 7.8 Hz, 1H), 6.57 (d, J = 1.8 Hz, 1H), 6.42 (dd, J = 7.8, 1.8 Hz, 1H), 4.34 (m, 1H), 4.07 (q, J = 7.2 Hz, 2H), 2.62 (m, 2H), 2.42 (m, 2H), 2.33 (m, 2H), 2.01 (s, 3H), 1.87 (m, 2H), 1.17 (t, J = 7.2 Hz, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 171.9, 171.8, 144.9, 143.2, 131.9, 118.6, 115.6, 115.3, 60.4, 50.9, 37.0, 30.5, 30.4, 29.5, 14.5, 13.9.

3,4-Dihydroxy hydrocinnamic acid (L-tryptophan ethyl ester) amide (4be). Compound **4be** was prepared from 4-hydroxy hydrocinnamic acid and L-tryptophan ethyl ester as described for **4bi** in 80% yield as a yellow oil: ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.83 (s, 1H), 8.70 (s, 1H), 8.60 (s, 1H), 8.26 (d, J = 7.6 Hz, 1H), 7.50 (d, J = 7.6 Hz, 1H), 7.34 (d, J = 8.0 Hz, 1H), 7.13 (d, J = 1.6 Hz, 1H), 7.07 (t, J = 7.6 Hz, 1H), 6.99 (t, J = 7.6 Hz, 1H), 6.60 (m, 2H), 6.41 (d, J = 8.0 Hz, 1H), 4.50 (m, 1H), 4.01 (q, J = 6.8 Hz, 2H), 3.13 (dd, J = 14.4, 6.4 Hz, 1H), 3.03 (dd, J = 14.4, 8.0 Hz, 1H), 2.58 (dd, J = 8.8, 6.0 Hz, 2H), 2.32 (t, J = 8.8 Hz, 2H), 1.07 (t, J = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.7, 172.3, 145.6, 143.9, 136.7, 132.7, 127.8, 124.3, 121.6, 119.3, 119.0, 118.7, 116.3, 116.1, 112.0, 110.2, 61.0, 53.8, 37.8, 31.1, 27.8, 14.5.

3,4-Dihydroxy hydrocinnamic acid (glycine ethyl ester) amide (4bf). Compound **4bf** was prepared from 3,4-dihydroxy hydrocinnamic acid and glycine ethyl ester hydrochloride as described for **4bi** in 80% yield as a pale yellow oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.19 (br s, 2H), 6.72 (d, J = 8.1 Hz, 1H), 6.65 (d, J = 2.1 Hz, 1H), 6.50 (m, 2H), 4.14 (q, J = 7.2 Hz, 2H), 3.95 (d, J = 5.4 Hz, 2H), 2.75 (t, J = 7.5 Hz, 2H), 2.44 (t, J = 7.5 Hz, 2H), 1.21 (t, J = 7.2 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.8, 170.0, 144.0, 142.7, 132.6, 120.1, 115.3, 61.7, 41.4, 37.9, 30.7, 13.9.

3,4-Dihydroxy hydrocinnamic acid (L-tyrosine ethyl ester) amide (4bg). Compound **4bg** was prepared from 3,4-dihydroxy hydrocinnamic acid and L-tyrosine ethyl ester as described for **4bi** in 74% yield as a yellow oil: ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.20 (s, 1H), 8.69 (s, 1H), 8.60 (s, 1H), 8.21 (d, J = 7.6 Hz, 1H), 6.94 (m, 2H), 6.63 (m, 2H), 6.58 (d, J = 8.0 Hz, 1H), 6.54 (d, J = 2.4 Hz, 1H), 6.37 (dd, J = 8.0, 2.4 Hz, 1H), 4.32 (m, 1H), 4.00 (q, J = 6.8 Hz, 2H), 2.83 (dd, J = 14.0, 6.0 Hz, 1H), 2.74 (dd, J = 14.0, 8.8 Hz, 1H), 2.53 (m, 2H), 2.27 (m, 2H), 1.08 (t, J = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.4, 172.3, 156.6, 145.6, 143.9, 132.6, 130.7, 127.8, 119.3, 116.2, 116.0, 115.6, 61.0, 54.6, 37.8, 36.8, 31.1, 14.7.

3,4-Dihydroxy hydrocinnamic acid (L-glutamic acid diethyl ester) amide (4bh). Compound **4bh** was prepared from 3,4-dihydroxy hydrocinnamic acid and L-glutamic acid diethyl ester hydrochloride as described for **4bi** in 86% yield as a pale yellow oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.76 (d, J = 8.1 Hz, 1H), 6.69 (d, J = 2.4 Hz, 1H), 6.55 (dd, J = 7.8, 1.8 Hz, 1H), 6.45 (d, J = 7.8 Hz, 1H), 4.57 (m, 1H), 4.12 (m, 4H), 2.81 (m, 2H), 2.46 (m, 2H), 2.27 (m, 2H), 2.12 (m, 1H), 1.90 (m, 1H), 1.25 (t, J = 7.2 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.3, 173.1, 171.8, 143.9, 142.9, 132.6, 120.2, 115.4, 115.2, 61.8, 61.0, 60.4, 51.7, 38.3, 30.8, 30.2, 27.2, 14.0.

3,4-Dihydroxy hydrocinnamic acid (L-isoleucine ethyl ester) amide (4bj). Compound **4bj** was prepared from 3,4-dihydroxy hydrocinnamic acid and L-isoleucine ethyl ester hydrochloride as described for **4bi** in 84% yield as a pale yellow oil: ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.66 (s, 1H), 8.61 (s, 1H), 8.05 (d, J = 8.0 Hz, 1H), 6.57 (d, J = 7.6 Hz, 1H), 6.54 (d, J = 2.0 Hz, 1H), 6.40 (dd, J = 8.0, 2.0 Hz, 1H), 4.15 (m, 1H), 4.06 (m, 2H), 2.58 (t, J = 7.6 Hz, 2H), 2.35 (m, 2H), 1.70 (m, 1H), 1.31 (m, 1H), 1.15 (t, J = 7.2 Hz, 3H), 1.10 (m, 1H), 0.79 (m, 6H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.5, 172.3, 145.6, 143.9, 132.6, 119.3, 116.3, 115.9, 79.8, 60.8, 56.9, 37.6, 36.9, 31.2, 25.4, 16.0, 11.7.

Evaluation of hypocholesterolemic activity. The hypocholesterolemic effects of the synthesized compounds were investigated in male C57BL/6J mice maintained at Korea Research Institute of Bioscience and Biotechnology

(Taejeon, Korea). The mice were housed in a room with controlled temperature (22 ± 2 °C), relative humidity (55 ± 5%), and lighting (alternating 12 h cycle of light and dark). At 8 weeks of age, six animals were randomly assigned to a group, and fed a high cholesterol diet (CRF-1 supplemented with 15% fat, 1.25% cholesterol, and 0.5% Na-cholate, Oriental Yeast Co. Ltd., Japan) without additional supplement (control), or a high cholesterol diet supplemented with 0.05% (wt/wt in diet) of the test compounds (experimental group). The diet and water were given *ad libitum*. After treating the test compounds for 10 days, the mice were anesthetized with ethyl ether, and the blood was obtained from the retro-orbital sinus using a heparinized capillary tube. Then, the blood was centrifuged at 8,000 × g for 10 min, and the plasma was collected. The concentration of plasma total cholesterol was measured with an automatic blood chemical analyzer (CIBA Corning, OH, USA). To evaluate statistical significance between control and experimental groups, student's *t*-test was performed, and a p value of < 0.05 was considered to be statistically significant.

Acknowledgments. Mass spectroscopic data was obtained from the Korea Basic Science Institute, Daejeon.

References

1. Moon, J.-H.; Terao, J. *J. Agric. Food Chem*. **1998**, *46*, 5062.
2. Castelluccio, C.; Bolwell, G. P.; Gerrish, C.; Rice-Evans, C. A. *Biochem. J*. **1996**, *316*, 691.
3. Perez-Alvarez, V.; Bobadilla, R. A.; Muriel, P. *J. Appl. Toxicol*. **2001**, *21*, 527.
4. Sudina, G. F.; Mirzoeva, O. K.; Pushkareva, M. A.; Korshunova, G. A.; Sumbutya, N. V.; Varfolomeev, S. D. *FEBS Lett*. **1993**, *329*, 21.
5. Namiki, M. *Crit. Rev. Food Sci. Nutr*. **1990**, *29*, 273.
6. Burke, T. R., Jr.; Fesen, M. R.; Mazumder, A.; Wang, J.; Carothers, A. M.; Grunberger, D.; Driscoll, J.; Kohn, K.; Pommier, Y. *J. Med. Chem*. **1995**, *38*, 4171.
7. Watanabe, T.; Hayashi, K.; Yoshimatsu, S.; Sakai, K. *J. Med. Chem*. **1980**, *23*, 50.
8. Lee, S.; Lee, C.-H.; Oh, J.-H.; Kim, E. E.; Choi, Y.-K.; Kim, E.-H.; Lee, W. S.; Bok, S.-H.; Jeong, T.-S. *Bioorg. Med. Chem. Lett*. **2003**, *13*, 2681.
9. Patel, R. P.; Price, S. *J. Org. Chem*. **1965**, *30*, 3575.