Structure–Activity Relationships of *N*-(3,5-Dimethoxy-4-*n*-octyloxycinnamoyl)-*N′*-(3,4-dimethylphenyl)piperazine and Analogues as Inhibitors of Acyl-CoA: Cholesterol *O*-Acyltransferase

Kenji Ohishi,* Ritsuo Aiyama, Hiroshi Hatano, Yasuto Yoshida, Yasue Wada, Wakae Yokoi, Haruji Sawada, Tsunekazu Watanabe, and Teruo Yokokura

Yakult Central Institute for Microbiological Research, 1796 Yaho, Kunitachi, Tokyo 186–8650, Japan.
Received January 10, 2001; accepted March 23, 2001

A novel series of acyl-CoA: cholesterol *O*-acyltransferase (ACAT) inhibitors were synthesized from a lead compound, 1-(4-hydroxy-3-methoxyphenyl)-7-phenylhept-1-en-3-one (1, Yakuchinone B) through a modification of three regions (A, B, C) in the molecule. In this study, the compounds prepared were tested for *in vitro* inhibitory activity on microsomal ACAT from the liver of rats and for *in vivo* hypocholesterolemic activity in rats given a high cholesterol diet. *N*-(3,5-Dimethoxy-4-*n*-octyloxycinnamoyl)-*N′*-(3,4-dimethylphenyl)piperazine (45), which belongs to the amide compounds, has finally been discovered. Compound 45 inhibited rat hepatic ACAT in a more striking manner than CI-976, an amide compound ACAT inhibitor, and it exhibited a high level of hypocholesterolemic activity *in vivo*. Since 45 strongly inhibited both microsomal ACAT prepared from HepG2 (a cell line derived from human hepatocarcinoma) and Caco2 (a cell line derived from human colon adenocarcinoma), there is speculation that 45 might have the ability to inhibit ACAT in both the human intestine and liver independent of the difference in the distribution of ACAT isozymes. On the other hand, 45 did not induce adrenotoxicity in subacute toxicity studies in rats. These results suggest that it has promise for development as a new therapeutic agent for hypercholesterolemia and atherosclerosis.

Key words acyl-CoA: cholesterol *O*-acyltransferase inhibitor; Yakuchinone B; amide compound; hypocholesterolemic activity; structure–activity relationship study

Since the pioneering results of the Framingham study disclosed in 1971,[1] hypercholesterolemia has been recognized as a major risk factor for the development of coronary heart disease (CHD).[2,3] Agents controlling total plasma cholesterol levels are expected to serve as an effective therapeutic method for atherosclerosis,[4] since lowering plasma cholesterol levels has been proven to reduce mortality from myocardial infarction.[5] Acyl-CoA: cholesterol *O*-acyltransferase (ACAT, EC 2.3.1.26)[6,7] is an intracellular enzyme responsible for catalyzing the esterification of free cholesterol with fatty acyl-CoA to produce cholesteryl esters. This enzyme plays important roles in the absorption of dietary cholesterol from the small intestine, the secretion of very low-density lipoprotein (VLDL) from the liver, and the accumulation of cholesteryl esters in atherosclerotic lesions. Inhibition of ACAT should reduce the absorption of cholesterol, lower plasma cholesterol levels,[8—12] and should arrest the progression and promote the regression of atherosclerotic plaque.[13,14] Therefore, ACAT inhibitors are a prime objective in the development of new therapeutic agents for hypercholesterolemia and atherosclerosis.[15]

On this basis, metabolites were screened from about three thousand fungi and numerous plant components that produce an ACAT inhibitor. Of these, it was found that 1-(4-hydroxy-3-methoxyphenyl)-7-phenylhept-1-en-3-one (**1**, Yakuchinone B), which is a component of the seeds of *Alpinia oxyphylla* Miquel (*Zingiberaceae*),[16] has ACAT inhibitory activity.

In order to obtain ACAT inhibitors that exhibit a high level of hypocholesterolemic activity *in vivo*, the structure–activity relationships of three structural moieties of Yakuchinone B (**1**) as the lead compound (Fig. 1) were examined. The compounds prepared were tested for the ability to inhibit the microsomal ACAT from rat liver and to suppress the elevation of plasma cholesterol levels in rats given a high cholesterol diet.

The present paper will describe the structure–activity relationships and biological activities of these novel ACAT inhibitors.

Chemistry Diarylheptanoids (**1**—**7**) were synthesized by condensation of 1-phenyl-5-hexanone with corresponding benzaldehydes according to the previously described method.[17]

As shown in Chart 1, phenylalkylamides (**11**, **13**—**15**) or anilides (**12**, **17**, **18**) were prepared by alkylation of **8**, followed by amidation with appropriate phenylalkylamine or aniline by 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) in $CHCl_3$.

Phenylpiperamides (**16**, **19**—**46**) can be synthesized from **8** *via* intermediates **9a**, **b** or **10a**, **b** (Chart 1). In the case of the modification of region C in the molecule (Fig. 1), phenylpiperamides (**16**, **30**—**41**) were prepared by alkylation of **8** with corresponding halide, followed by amidation with appropriate phenylpiperazine. With modification of region A in the molecule (Fig. 1), phenylpiperamides (**19**—**29**, **42**—**46**) were prepared by amidation of **8** with corresponding phenylpiperazine, followed by alkylation with appropriate halide.

Biology The ability to inhibit hepatic ACAT *in vitro* was measured by incubating [1-^{14}C]oleoyl-CoA with microsome fractions prepared from the liver of Sprague–Dawley rats given a normal diet. ACAT inhibitory activity was expressed by IC_{50}.

In vivo hypocholesterolemic activity was assessed in these rats by admixing a test compound into their diet at a concentration of 0.005 or 0.1% for four days. For the next three days the rats were fed a high cholesterol diet containing a test

* To whom correspondence should be addressed. e-mail: kenji-ooishi@yakult.co.jp

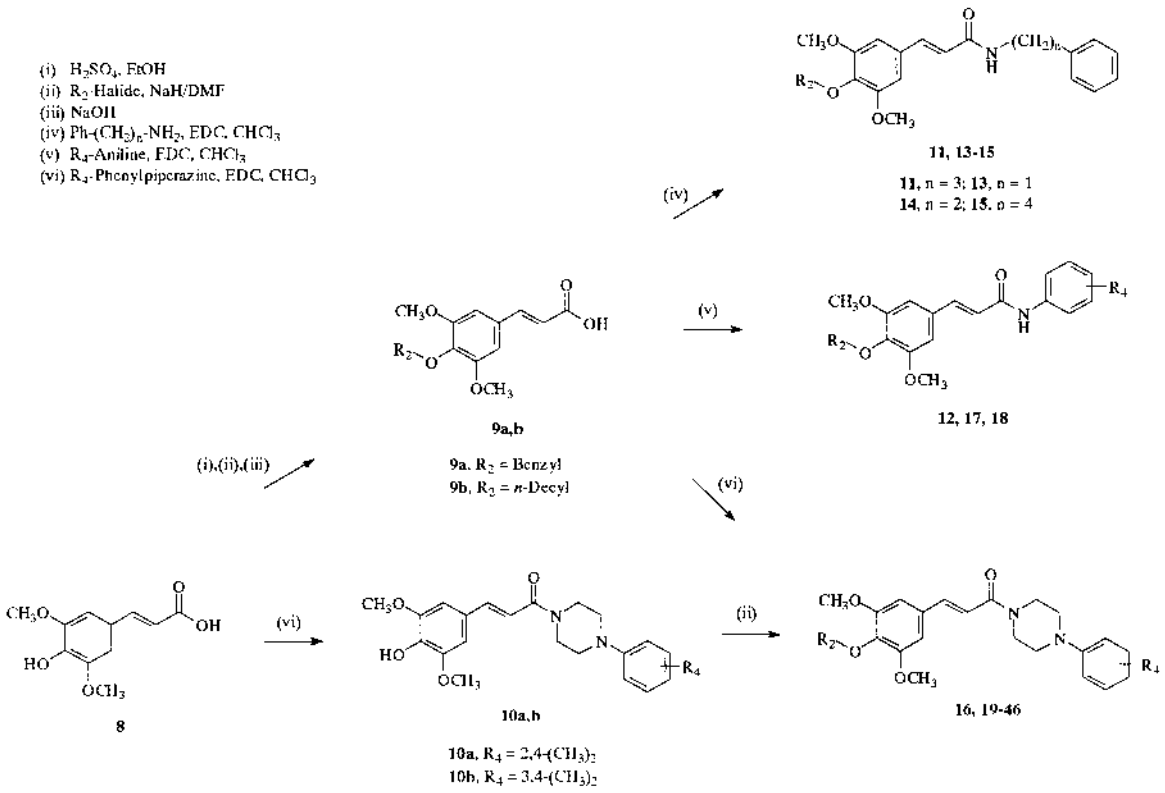


Chart 1. Preparation of Target Molecules **11**—**46**

compound at the same concentration ($n=8$). For this investigation the dosages of each compound calculated from the body weight and the food consumption during the study period were about 5 and 70—80 mg/kg/d for each concentration. Cholesterol lowering effect expressed as an inhibitory rate (%) was calculated as follows: cholesterol lowering effect (%)$=[(B-A)/(B-C)]\times 100$ (A, B, and C represent plasma total cholesterol levels in the drug-treated, control, and normal groups, respectively).

Results and Discussion

To obtain ACAT inhibitors that exhibit a high level of hypocholesterolemic activity *in vivo*, the structure–activity relationships for three structural regions (Fig. 1) of Yakuchinone B (**1**) were examined as a lead compound.

The Structure–Activity Relationships for Region A The biological data for Yakuchinone B (**1**) and its derivatives (**2**—**7**), substituted on the A-phenyl ring, has been shown in Table 1. Compound **1** inhibited rat hepatic ACAT with an IC_{50} value of 20.6 μM. When an acetyl residue was introduced at R_2 of **1** (**2**), ACAT inhibitory activity increased by 2.1 times. Compound **3**, which had a benzyl residue at R_2, showed higher activity than **2**. However, compound **4**, which had a benzoyl residue at R_2, exhibited only a weak ACAT inhibitory activity. The ACAT inhibitory activity of **5**, which did not have a methoxy residue like that at R_1 of **3**, was remarkably lower than that of **3**. Compound **6**, which had an ethoxy residue instead of a methoxy residue of **3** at R_1, exhibited almost the same activity as **3**. Compound **7**, which had another methoxy residue like that at R_3 of **3**, provided greater inhibition of ACAT than **3**.

These data indicate that compound **7** with methoxy residues at R_1 and R_3 and a benzyl residue at R_2 in the A-phenyl ring exhibited excellent ACAT inhibitory activity. On the other hand, compounds **1**—**7** were not able to suppress a rise in the plasma total cholesterol levels when the derivative was mixed in the diet at a concentration of 0.1% and administered at a dose of 70—80 mg/kg/d to rats given a high cholesterol diet.

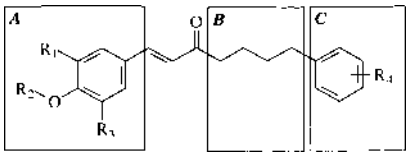
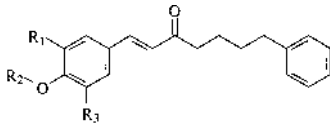


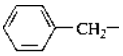
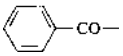
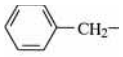
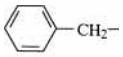
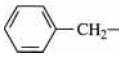
Fig. 1. Three Structural Regions Examined in Structure–Activity Relationship Studies

The reason for the weak hypocholesterolemic activity *in vivo* for these compounds is not clear, but may be related to poor efficacy for ACAT inhibition and/or to low bioavailability. Next, we synthesized the derivatives of compound **7** and examined the ACAT inhibitory activity *in vitro* and hypocholesterolemic activity *in vivo.*

The Structure–Activity Relationships of Regions B and C The amide compounds CI-976[8,18,19] and FR129169[20] (Fig. 2) are excellent ACAT inhibitors. Therefore, introduction of the amino units into region B of **7** was investigated in order to further increase ACAT inhibitory activity. As shown in Table 2, introduction of a *n*-propylamino-linker in region B (**11**) increased ACAT inhibitory activity by 2 times, as we expected. Additionally, derivative **12** in which an amino-linker or **16** in which a tertiary amine of a piperazino-linker was introduced in region B had higher inhibitory activity of

Table 1. Effect of Region A Phenyl Substituent on Biological Activities



No.	R_1	R_2	R_3	ACAT inhibition IC_{50} (nM)[a]	Cholesterol lowering (% reduction)[b]
1	CH_3O–	H	H	20600	5
2	CH_3O–	CH_3CO–	H	9800	2
3	CH_3O–		H	2700	6
4	CH_3O–		H	38000	−2
5	H		H	21600	5
6	CH_3CH_2O–		H	3500	3
7	CH_3O–		CH_3O–	1700	2

a) *In vitro* ACAT inhibition was measured using hepatic microsomes isolated from rats fed a normal diet. Each determination was performed in triplicate. *b*) Sprague–Dawley rats were fed a diet containing each compound at a concentration of 0.1% (70—80 mg/kg/d).

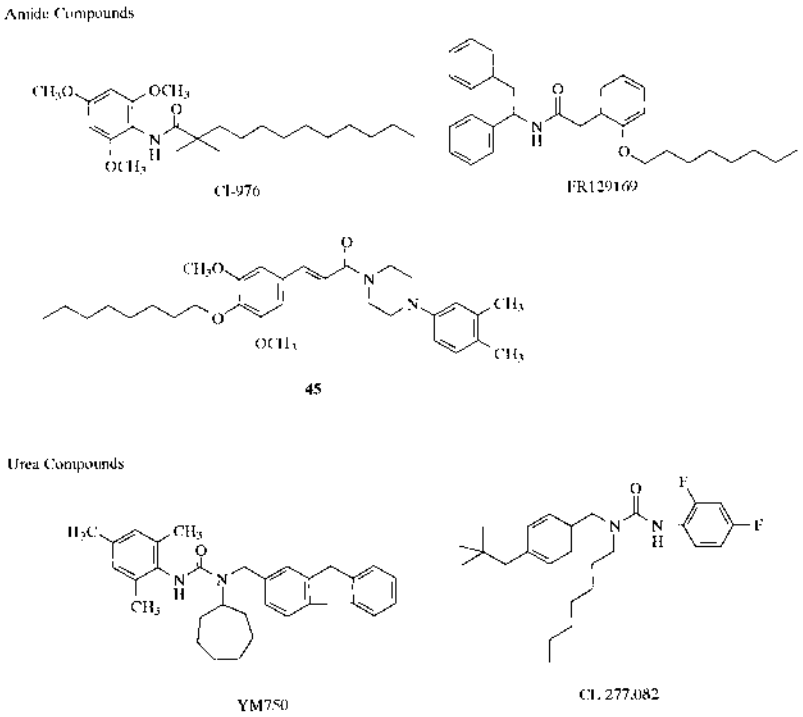
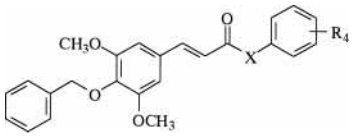


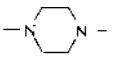
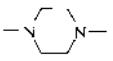
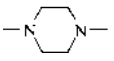
Fig. 2. ACAT Inhibitors

rat hepatic ACAT than **11**. ACAT inhibitory activity decreased in the following order: amino (**12**)>piperazino (**16**)>*n*-propylamino (**11**)≒*n*-butylamino (**15**)≫ethylamino (**14**)=*n*-butyl (**7**)≫methylamino (**13**).

Further, ACAT inhibitory activity markedly increased with the introduction of methyl residues to the C-phenyl ring of **12** and **16** (**17**—**20**). In particular, **19**, which had a piperazino-linker in region B and methyl residues at positions 2 and 4 in the C-phenyl ring, significantly inhibited the elevation of plasma total cholesterol levels in rats given a high cholesterol diet when administered as a dietary admixture at a concentration of 0.1%. On the other hand, **17**, which had methyl residues at positions 2 and 4 in the C-phenyl ring like **19** but which had an amino-linker in region B, did not exhibit hypocholesterolemic activity. Moreover, **20**, which was a structural isomer of **19** with a similar ACAT inhibitory pro-

Table 2. Effect of X-Linker and Region C Phenyl Substituent on Biological Activities



No.	X	R_4	ACAT inhibition IC_{50} (nM)[a]	Cholesterol lowering (% reduction)[b]
7	$-(CH_2)_4-$	H	1700	2
11	$-NH(CH_2)_3-$	H	840	9
12	–NH–	H	260	7
13	$-NHCH_2-$	H	3000	−4
14	$-NH(CH_2)_2-$	H	1700	2
15	$-NH(CH_2)_4-$	H	900	−11
16		H	550	3
17		2,4-$(CH_3)_2$	52	−4
18		3,4-$(CH_3)_2$	47	3
19		2,4-$(CH_3)_2$	62	63**
20		3,4-$(CH_3)_2$	60	7

a, *b*) See corresponding footnotes of Table 1. Significantly different from drug-untreated control using unpaired, two-tailed Student's *t*-test, $^{**}p<0.01$.

file *in vitro*, did not exhibit hypocholesterolemic activity.

The Structure–Activity Relationships of 19 and Its Derivatives Derivatives of **19** were synthesized with substitution in the A- and C-phenyl rings as their ability to inhibit microsomal ACAT from the liver of rats and hypocholesterolemic activity *in vivo* was investigated. In addition, the concentration of the derivatives added to the diet was set at 0.005% to select derivatives that were more potent than **19**. For this investigation, the dosage of each derivative was about 5 mg/kg/d. Biological data for **19** and its derivatives **21**—**46** is shown in Table 3.

Although **19** inhibited the elevation in plasma total cholesterol levels when administered as a dietary admixture at a concentration of 0.1% (Table 2), it did not exhibit hypocholesterolemic activity when administered at a concentration of 0.005% (Table 3).

In respect to derivatives of **19** with substitution in the A-phenyl ring, conversion of benzyl residue of **19** into a 3-phenylpropyl (**21**) or phenoxyethyl (**22**) residue decreased ACAT inhibitory activities *in vitro*, and these derivatives did not produce significant hypocholesterolemic activity. Next, benzyl residue of **19** was converted into a geranyl, farnecyl or *n*-alkyl residue, since it seemed that the ACAT inhibitory activity might increase by the introduction of a long-chain aliphatic moiety with a structure similar to an acyl residue of an acyl-CoA which was one substrate for ACAT.

When benzyl residue of **19** was converted into a geranyl (**23**) or farnecyl (**24**) residue, inhibitory activity on hepatic ACAT increased in a striking manner as we expected. However, these compounds did not exhibit hypocholesterolemic activity. Conversion of benzyl residue of **19** into an *n*-heptyl (**26**), *n*-octyl (**27**), *n*-nonyl (**28**), or *n*-decyl (**29**) residue but not an *n*-hexyl (**25**) residue significantly increased ACAT inhibitory activity.

In addition, the hypocholesterolemic activity of *n*-alkyl derivatives increased according to the length of the alkyl chain, and *n*-octyl (**27**), *n*-nonyl (**28**), and *n*-decyl (**29**) derivatives produced significant hypocholesterolemic activity when administered as a dietary admixture at a concentration of 0.005%. Although **23** and **24** inhibited ACAT in a more striking fashion than **29**, these derivatives did not exhibit hypocholesterolemic activity. Based on these results, it is assumed that the elevation in this activity with the introduction of a long *n*-alkyl residue is related not only to an increase in their specific activity with respect to ACAT but also to other factors such as their increased bioavailability.

On the other hand, **30**, which had an *n*-decyl residue in the A-phenyl ring but no residue in the C-phenyl ring, did not lower plasma cholesterol level. This result suggests that appropriate substituents in the C-phenyl ring are needed to exhibit hypocholesterolemic activity.

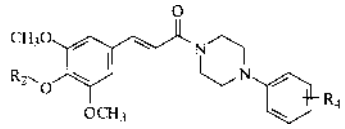
The structure–activity relationship studies of the derivatives with substituents in the C-phenyl ring, which had an *n*-decyl residue at R_2 in the A-phenyl ring (**29**—**42**), indicate the following. Results for monomethyl-substituted compounds (**31**—**33**) demonstrated that a 4-methyl residue was very important in exhibiting a high level of hypocholesterolemic activity *in vivo*, while a 2-methyl or 3-methyl residue was not.

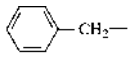
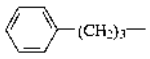
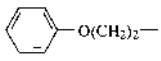
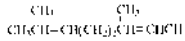
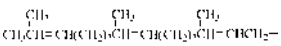
With respect to a 4-substituent in the C-phenyl ring, methyl (**33**), *tert*-butyl (**34**), methoxy (**35**), and fluorine (**36**) analogs had a higher level of ACAT inhibitory activity than a nitro analog (**37**). Therefore, the electrical nature of the substituents did not clearly correlate with their ACAT inhibitory activity. On the other hand, methyl (**33**), *tert*-butyl (**34**), or methoxy (**35**) analogs, which were electron-donating groups, had a higher level of hypocholesterolemic activity *in vivo* than those with fluorine (**36**) or nitro (**37**), which were electron-withdrawing groups. These results indicated that although the electrical properties of C-phenyl substituents did not affect specific activity with respect to ACAT, the effectiveness of lowering plasma cholesterol level *in vivo* was largely influenced by the electrical properties of those substituents. The supposition is that the introduction of electron-donating groups in the C-phenyl ring at position 4 influenced their pharmacokinetics, such as absorption, distribution, and clearance, as with the introduction of a long-chain alkyl residue at R_2 in the A-phenyl ring, so *in vivo* hypocholesterolemic activity increased. Factors determining the potency of ACAT inhibitors of *in vivo* hypocholesterolemic activity are being analyzed through the comparison of the pharmacokinetics of the ACAT inhibitors that were synthesized. These results will be reported later.

With respect to dimethyl-substituted compounds (**29**, **38**—**42**), a 3,4-dimethyl-substituted derivative (**42**) had more of an effect in lowering cholesterol level than a 4-methyl substituted derivative (**33**) or a 2,4-dimethyl substituted derivative (**29**).

With respect to A-phenyl substituted derivatives that had

Table 3. Effect of Regions A and C Phenyl Substituent of **19** on Biological Activities



No.	R_2	R_4	ACAT inhibition IC_{50} (nM)[a)]	Cholesterol lowering (% reduction)[b)]
19		2,4-$(CH_3)_2$	62	2
21		2,4-$(CH_3)_2$	100	17
22		2,4-$(CH_3)_2$	790	20
23		2,4-$(CH_3)_2$	6.4	2
24		2,4-$(CH_3)_2$	4.5	2
25	*n*-C_6H_{13}–	2,4-$(CH_3)_2$	79	12
26	*n*-C_7H_{15}–	2,4-$(CH_3)_2$	15	25
27	*n*-C_8H_{17}–	2,4-$(CH_3)_2$	15	38**
28	*n*-C_9H_{19}–	2,4-$(CH_3)_2$	19	49**
29	*n*-$C_{10}H_{21}$–	2,4-$(CH_3)_2$	22	65***
30	*n*-$C_{10}H_{21}$–	H	52	2
31	*n*-$C_{10}H_{21}$–	2-CH_3	18	27
32	*n*-$C_{10}H_{21}$–	3-CH_3	38	2
33	*n*-$C_{10}H_{21}$–	4-CH_3	23	55***
34	*n*-$C_{10}H_{21}$–	4-$C(CH_3)_3$	35	28*
35	*n*-$C_{10}H_{21}$–	4-OCH_3	37	50**
36	*n*-$C_{10}H_{21}$–	4-F	47	10
37	*n*-$C_{10}H_{21}$–	4-NO_2	180	−2
38	*n*-$C_{10}H_{21}$–	2,3-$(CH_3)_2$	24	23
39	*n*-$C_{10}H_{21}$–	2,5-$(CH_3)_2$	316	31*
40	*n*-$C_{10}H_{21}$–	2,6-$(CH_3)_2$	650	−3
41	*n*-$C_{10}H_{21}$–	3,5-$(CH_3)_2$	93	20
42	*n*-$C_{10}H_{21}$–	3,4-$(CH_3)_2$	26	70**
43	*n*-C_6H_{13}–	3,4-$(CH_3)_2$	54	20
44	*n*-C_7H_{15}–	3,4-$(CH_3)_2$	38	46**
45	*n*-C_8H_{17}–	3,4-$(CH_3)_2$	11	79***
46	*n*-C_9H_{19}–	3,4-$(CH_3)_2$	20	59***

a) See corresponding footnotes of Table 1. *b*) Sprague-Dawley rats were fed a diet containing each compound at a concentration of 0.005% (about 5 mg/kg/d). Significantly different from drug-untreated control using unpaired, two-tailed Student's *t*-test, $*p<0.05$, $**p<0.01$, $***p<0.001$.

two methyl residues in the C-phenyl ring at positions 3 and 4 (**42**—**46**), *n*-heptyl (**44**), *n*-octyl (**45**), *n*-nonyl (**46**), and *n*-decyl (**42**) derivatives produced significant hypocholesterolemic activity. For these derivatives, unlike those with a 2,4-dimethyl residue in the C-phenyl ring (**25**—**29**), the drug efficacy did not increase according to the length of the *n*-alkyl chain at R_2 in the A-phenyl ring, and the *n*-octyl derivative (**45**) had the highest level of hypocholesterolemic activity.

From the structure–activity relationship studies on three structural regions using Yakuchinone B (**1**) as a lead compound, **45**, *N*-(3,5-dimethoxy-4-*n*-octyloxycinnamoyl)-*N*′-(3,4-dimethylphenyl)piperazine (Fig. 2) was finally synthesized.

Biological Activities of 45 As shown in Table 4, **45** more strongly inhibited rat hepatic ACAT than did other ACAT inhibitors (an amide compound CI-976 and a urea compound YM750[21)]) and had a level of hypocholesterolemic activity *in vivo* that was as high as that with CI-976 and YM750.

A number of potent ACAT inhibitors that exhibit excellent hypocholesterolemic activity in various species have been synthesized for the purpose of pharmaceutical development,[22—24)] and some of these have demonstrated highly satisfactory results in model animals. Clinical development of ACAT inhibitors, however, has not been successful,[25,26)] be-

cause of poor clinical results and/or the induction of toxicity in the adrenal glands of several animal species.[27—38] Recently, ACAT has been classified into two isozymes, ACAT-1 and ACAT-2, and research suggests that they differ in terms of their distribution in the body.[39—43] Two reasons are believed to be involved in why the numerous ACAT inhibitors that have been developed thus far have not been clinically successful.

One is the relationship between adrenotoxicity and the inhibition of ACAT-1, which was found ubiquitously throughout the body including macrophages and adrenal glands. Tanaka *et al.* reported that since a compound with a high level of macrophage ACAT (ACAT-1) inhibitory activity induced adrenal toxicity, there might be a positive correlation between specific activity with respect to ACAT-1 and adrenotoxicity.[37] On the other hand, Dominick *et al.*[29] and Sliskovic *et al.*[33] reported that the adrenotoxicity observed with ACAT inhibitors was likely to be independent of ACAT inhibition. Thus, it is still unclear as to whether or not adrenotoxicity is related to specific activity for ACAT-1 inhibition. As shown in Table 4, **45** strongly inhibited microsomal ACAT from HepG2, in which ACAT-1 was predominantly expressed.[42] On the other hand, in subacute toxicity studies of **45** using rats, which abundantly expressed ACAT-1 in adrenal glands,[43] no adrenotoxicity was observed when it was orally administered *via* a stomach tube once a day to the animals at a dose of 500 mg/kg/d for four weeks (data not shown). Reindel *et al.* reported that in 2 weeks of oral toxicity studies on PD 132301-2, an urea compound ACAT inhibitor, principal toxicity occurred in adrenals and that the susceptibility for adrenal toxicity of this compound differed among species: rank order of the toxicity (minimal toxic dose, mg/kg) was dogs (6)>guinea pigs (30)>rabbits (30)>monkeys (50)>rats (1000)>hamsters (no lesions at 1000 mg/kg).[38] From this report, it suggests that subacute toxicity of compound **45** prefers to assess in other species, such as dogs and guinea pigs, because susceptibility for adrenal toxicity of this compound also seems different between species like that of PD 132301-2.

Another reason why numerous ACAT inhibitors did not provide clinical success seems to relate to the large differences in the distribution of ACAT-1 and ACAT-2 in the liver and intestines with respect to humans and rodents. In the liver and intestines of mice, ACAT-2 is predominantly expressed[41] and produces ACAT activity. Additionally, in the liver of rats, it seems that ACAT-2 is predominantly expressed, whereas the expression of ACAT-1 is detected in the intestines.[43]

On the other hand, research has shown that ACAT-1 is predominantly expressed in the human liver and that both ACAT-1 and ACAT-2 play a major role in human intestines.[42] Based on these reports, one can speculate that with selective inhibitors for isozymes, the results in model animals such as mice and rats might be quite different from those of clinical studies in humans. As shown in Table 4, **45** strongly inhibited microsomal ACAT prepared from HepG2 (human hepatocarcinoma), in which ACAT-1 isozyme is expressed predominantly as in human liver tissue.[42] On the other hand, **45** also strongly inhibited microsomal ACAT prepared from differentiating Caco2 (human colon adenocarcinoma) with properties like intestinal epithelial cells, in which both ACAT-1 and ACAT-2 are expressed to the same extent as in human intestinal tissue.[42] Because the IC_{50} values for the inhibition of microsomal ACAT derived from the two cell lines were the same, it is assumed that **45** might have sufficient ability to inhibit ACAT in both the human intestines and liver regardless of the pattern of expression of ACAT isozymes.

Table 4. Biological Activities of **45**

No.	ACAT inhibitory activity IC_{50} (nM)[a]			Hypocholesterolemic activity ED_{50} (mg/kg/d)[b]
	Rat Liver	HepG2	Caco2	
45	11	88	63	2.4
CI-976	98	112	94	5.1
YM750	55	32	18	4.2

a) *In vitro* ACAT inhibition was measured using microsomes isolated from rat livers, HepG2, or Caco2. Each determination was performed in triplicate. *b*) Hypocholesterolemic activity expressed as an effective dose to reduce plasma total cholesterol levels by 50% of the control value ($n=8$).

Although there remains much to explore through clinical studies of **45** as to whether the inhibition of intestinal ACAT or of hepatic ACAT plays a greater role in the treatment of hypercholesterolemia in humans, the results mentioned suggest that **45** might be an effective therapeutic agent for this condition.

Conclusion

A novel series of amide compounds were synthesized to find a new ACAT inhibitor, which exhibited a high level of hypocholesterolemic activity *in vivo*, using Yakuchinone B (**1**) as a starting material. Among the compounds synthesized, *N*-(3,5-dimethoxy-4-*n*-octyloxycinnamoyl)-*N'*-(3,4-dimethylphenyl)piperazine (**45**) strongly inhibited rat hepatic ACAT to a level higher than that with CI-976 and YM750, and it exhibited a high level of *in vivo* hypocholesterolemic activity equivalent to that of CI-976 and YM750. Moreover, since **45** strongly inhibited the microsomal ACAT prepared from human cell lines (Caco2, HepG2), there is speculation that it might have the ability to inhibit ACAT in both the human intestines and the liver. Thus, **45** has fulfilled expectations for its use as a new therapeutic drug for hypercholesterolemia and atherosclerosis.

Experimental

General Procedures All melting points (mp) were determined on an Ishii micromelting point apparatus. IR spectra were recorded on a Shimadzu IR435 spectrophotometer as KBr disks. ^{1}H-NMR spectra were measured with a JEOL JNM-A400 (400 MHz) instrument. Chemical shifts were reported in δ units from tetramethylsilane as the internal standard. Coupling constants (J) were reported in hertz. Mass spectra were obtained with a Hitachi M-80B mass spectrometer using electron impact (EI) for ionization, and data were exhibited as m/z. Elemental analyses were carried out on a CHN Coder MT-3 (Yanagimoto MFG. Co., Ltd., Kyoto, Japan). The reagents and solvents used were obtained from commercial suppliers without further purification. Column chromatography was performed on silica gel (Merck; particle size 0.063—0.200 mm). Reaction progress was checked by TLC analysis on silica gel coated glass plates (Kieselgel 60 F_{254}, thickness of 0.25 mm). Visualization was with UV light (254 nm) or iodine.

Yakuchinone B (**1**),[17] CI-976[18] and YM750[21] were synthesized according to the previously described method in our laboratories. 4-*tert*-Butylphenylpiperazine, 2,6-dimethylphenylpiperazine, 3,4-dimethylphenylpiperazine, and 3,5-dimethylphenylpiperazine were prepared from bis(2-chloroethyl)-*N*-(ethoxycarbonyl)amine and corresponding anilines according to the procedure described by Mishani *et al.*[44]

1-(4-Acetyloxy-3-methoxyphenyl)-7-phenylhept-1-en-3-one (2) Ten milliliters of 10% KOH aq. solution was added to the 1-phenyl-5-hexanone (2.64 g, 15.0 mmol) in EtOH (100 ml) and stirred at room temperature for an hour. Then 4-acetyloxy-3-methoxybenzaldehyde (2.91 g, 15.0 mmol) was added to the reaction mixture and stirred overnight at room temperature. The resulting precipitate was collected by filtration and the precipitate was recrystallized from $CHCl_3$–EtOH to give **2** as white crystals (3.15 g, yield 59.6%). mp 74—76 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.65—1.78 (4H, m), 2.32 (3H, s), 2.62—2.72 (4H, m), 3.87 (3H, s), 6.66 (1H, d, J=16.4 Hz), 7.03—7.35 (8H, m), 7.48 (1H, d, J=16.4 Hz). IR (KBr) cm^{-1}: 1763, 1653, 1227. MS *m/z*: 352 (M^+), 309, 118. *Anal.* Calcd for $C_{22}H_{24}O_4$: C, 74.98; H, 6.86. Found: C, 75.02; H, 6.85.

The following compounds **3**—**7** were prepared in a manner similar to that described for **2** from 1-phenyl-5-hexanone and the appropriate benzaldehyde.

1-(4-Benzyloxy-3-methoxyphenyl)-7-phenylhept-1-en-3-one (**3**): Yield 78.0%. mp 84—87 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.60—1.76 (4H, m), 2.62 (4H, m), 3.86 (3H, s), 5.13 (2H, s), 6.58 (1H, d, J=16.1 Hz), 6.83 (1H, d, J=8.3 Hz), 7.01 (1H, dd, J=8.3, 2.0 Hz), 7.05 (1H, d, J=2.0 Hz), 7.10—7.42 (10H, m), 7.45 (1H, d, J=16.1 Hz). IR (KBr) cm^{-1}: 1639, 1267. MS *m/z*: 400 (M^+), 309, 118, 92. *Anal.* Calcd for $C_{27}H_{28}O_3$: C, 80.97; H, 7.05. Found: C, 81.35; H, 7.07.

1-(4-Benzoyloxy-3-methoxyphenyl)-7-phenylhept-1-en-3-one (**4**): Yield 68.3%. mp 112—114 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.70—1.73 (4H, m), 2.63—2.72 (4H, m), 3.85 (3H, s), 6.69 (1H, d, J=16.1 Hz), 7.15—7.21 (6H, m), 7.25—7.30 (2H, m), 7.48—7.55 (3H, m), 7.52—7.65 (1H, m), 8.21 (2H, d, J=8.3 Hz). IR (KBr) cm^{-1}: 1734, 1655, 1261. MS *m/z*: 414 (M^+), 309, 118, 92. *Anal.* Calcd for $C_{27}H_{26}O_4$: C, 78.24; H, 6.32. Found: C, 77.89; H, 6.31.

1-(4-Benzyloxyphenyl)-7-phenylhept-1-en-3-one (**5**): Yield 72.5%. mp 99—100 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.60—1.76 (4H, m), 2.65 (4H, m), 5.09 (2H, s), 5.14 (2H, m), 6.61 (1H, d, J=16.1 Hz), 6.98 (1H, d, J=8.8 Hz), 7.05—7.52 (10H, m), 7.45 (1H, d, J=16.1 Hz), 7.83 (1H, d, J=8.8 Hz). IR (KBr) cm^{-1}: 2928, 1686, 1248. MS *m/z*: 370 (M^+), 309, 118, 92. *Anal.* Calcd for $C_{26}H_{26}O_2$: C, 84.29; H, 7.07. Found: C, 84.59; H, 6.96.

1-(4-Benzyloxy-3-ethoxyphenyl)-7-phenylhept-1-en-3-one (**6**): Yield 61.8%. mp 110—112 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.48 (3H, t, J=7.1 Hz), 1.60—1.76 (4H, m), 2.62—2.68 (4H, m), 4.14 (2H, q, J=7.1 Hz), 5.19 (2H, s), 6.58 (1H, d, J=16.1 Hz), 6.88 (1H, d, J=8.3 Hz), 7.04 (1H, dd, J=8.3, 2.2 Hz), 7.09 (1H, d, J=2.2 Hz), 7.15—7.20 (3H, m), 7.25—7.48 (8H, m). IR (KBr) cm^{-1}: 2939, 1655, 1269. MS *m/z*: 414 (M^+), 353, 118, 92. *Anal.* Calcd for $C_{28}H_{30}O_3$: C, 81.13; H, 7.29. Found: C, 80.88; H, 7.35.

1-(4-Benzyloxy-3,5-dimethoxyphenyl)-7-phenylhept-1-en-3-one (**7**): Yield 76.7%. mp 93—95 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.64—1.79 (4H, m), 2.63—2.71 (4H, m), 3.85 (6H, s), 5.05 (2H, s), 6.62 (1H, d, J=16.1 Hz), 6.75 (2H, s), 7.15—7.20 (3H, m), 7.25—7.37 (5H, m), 7.42—7.49 (3H, m). IR (KBr) cm^{-1}: 2937, 1693, 1265. MS *m/z*: 430 (M^+), 369, 118, 92. *Anal.* Calcd for $C_{28}H_{30}O_4$: C, 78.11; H, 7.02. Found: C, 77.90; H, 7.11.

4-Benzyloxy-3,5-dimethoxycinnamic Acid (9a) 3,5-Dimethoxy-4-hydroxycinnamic acid **8** (124.8 g, 556.6 mmol) was dissolved in EtOH (1.5 l) and H_2SO_4 (50 ml) was added dropwise to this mixture under an ice-cold condition and then refluxed at 100 °C for 5.5 h. After cooling to room temperature, the reaction mixture was concentrated and poured into water (4.5 l) and the resulting precipitate was collected by filtration and dried *in vacuo* to give ethyl 3,5-dimethoxy-4-hydroxycinnamate (131.4 g, yield 93.6%). Ethyl 3,5-dimethoxy-4-hydroxycinnamate (30.00 g, 118.9 mmol) was dissolved in *N*,*N*-dimethylformamide (DMF) (500 ml) and then sodium hydride (10.88 g of 60% in oil, 272.2 mmol) was added very carefully to this solution. Benzyl chloride (18.05 g, 142.2 mmol) was added to this mixture portionwise and the mixture was stirred at room temperature for 96 h. The reaction mixture was poured into water (2 l) and the resulting precipitate was collected by filtration. The precipitate was purified by silica gel column chromatography ($CHCl_3$) and dried *in vacuo* to give ethyl 4-benzyloxy-3,5-dimethoxycinnamate as white crystals (40.12 g, yield 99.3%). 1 N aq. NaOH (65 ml, 65 mmol) was added to a solution of ethyl 4-benzyloxy-3,5-dimethoxycinnamate (1.97 g, 5.8 mmol) in ethanol (100 ml). The mixture was stirred for 7 d at room temperature. Then, the reaction mixture was acidified with 1 N aq. HCl and was concentrated. This solution was then poured into water (2 l). The resulting precipitate was collected by filtration, washed with water, and dried to give **9a** as white crystals (1.63 g, yield 89.5%). mp 110—112 °C. ^{1}H-NMR ($CDCl_3$) δ: 3.85 (6H, s), 5.06 (2H, s), 6.36 (1H, d, J=15.9 Hz), 6.76 (2H, s), 7.25—7.48 (5H, m), 7.70 (1H, d, J=15.9 Hz). IR (KBr) cm^{-1}: 3049, 1626, 1281. MS *m/z*: 314 (M^+), 223, 164.

4-*n*-Decyloxy-3,5-dimethoxycinnamic Acid (9b) Ethyl 3,5-dimethoxy-4-hydroxycinnamate (8.77 g, 34.8 mmol) was dissolved in DMF (500 ml) and sodium hydride (1.81 g, 45.2 mmol) was then very carefully added to this solution. 1-Bromodecane (10.00 g, 45.2 mmol) was added to this mixture portionwise and the mixture was stirred at room temperature for 96 h. The reaction mixture was poured into water (5 l) and was adjusted at pH 3 with 1 N aq. HCl. Ethyl 4-*n*-decyloxy-3,5-dimethoxycinnamate was extracted with $CHCl_3$ (2 l) and the extracts were dried with sodium sulfate anhydrous. The solvent was removed *in vacuo* to give ethyl 4-*n*-decyloxy-3,5-dimethoxycinnamate as a clear oil which was used for the next reaction without purification. To the solution of ethyl 4-*n*-decyloxy-3,5-dimethoxycinnamate in EtOH (250 ml) was added 1 N aq. NaOH (65 ml, 65 mmol) followed by stirring for 4 d at room temperature. Then, 1 N aq. HCl was added to the reaction mixture and concentrated. This solution was then poured into water (2 l). The resulting precipitate was collected by filtration, washed with water, and dried to give **9b** as pale yellow crystals (4.44 g, yield 35.0%). mp 79—81 °C. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (3H, t, J=7.0 Hz), 1.27—1.44 (14H, m), 1.75 (2H, m), 3.88 (6H, s), 4.01 (2H, t, J=6.8 Hz), 6.36 (1H, d, J=15.9 Hz), 6.78 (2H, s), 7.71 (1H, d, J=15.9 Hz). IR (KBr) cm^{-1}: 2919, 1683, 1281.

***N*-(3,5-Dimethoxy-4-hydroxycinnamoyl)-*N'*-(2,4-dimethylphenyl)-piperazine (10a)** EDC (8.55 g, 44.6 mmol) and 1-(2,4-dimethylphenyl)-piperazine (8.49 g, 44.6 mmol) were added to a solution of **8** (10.00 g, 44.6 mmol) in $CHCl_3$ (600 ml) and stirred overnight at room temperature. The reaction mixture was directly purified by silica gel column chromatography ($CHCl_3$) and recrystallized from EtOH to give **10a** as pale yellow crystals (5.06 g, yield 28.6%). mp 213—215 °C. ^{1}H-NMR ($CDCl_3$) δ: 2.20 (3H, s), 2.24 (3H, s), 2.79 (4H, s), 3.32 (4H, s), 3.80 (6H, s), 6.90—7.01 (5H, m), 7.13 (1H, d, J=15.4 Hz), 7.44 (1H, d, J=15.4 Hz). IR (KBr) cm^{-1}: 3110, 1636, 1233. MS *m/z*: 397 (M^+), 208, 161, 133, 91.

Compound **10b** was prepared in a manner similar to that described for **10a** from **8** and 1-(3,4-dimethylphenyl)piperazine.

N-(3,5-Dimethoxy-4-hydroxycinnamoyl)-*N'*-(3,4-dimethlphenyl)piperazine (**10b**): Yield 46.0%. mp 215—217 °C. ^{1}H-NMR ($CDCl_3$) δ: 2.20 (3H, s), 2.24 (3H, s), 3.17 (4H, s), 3.88 (4H, br s), 3.94 (6H, s), 5.74 (1H, s), 6.70—6.77 (5H, m), 7.05 (1H, d, J=8.0 Hz), 7.63 (1H, d, J=15.4 Hz). IR (KBr) cm^{-1}: 3200, 1638, 1192. MS *m/z*: 397 (M^+), 208, 161, 134.

***N*-(3-Phenyl)propyl-(2*E*)-3-(4-benzyloxy-3,5-dimethoxyphenyl)-2-propenamide (11)** EDC (12.20 g, 63.6 mmol) and 1-amino-3-phenylpropane (12.90 g, 95.4 mmol) were added to a solution of **9a** (10.00 g, 31.8 mmol) in $CHCl_3$ (320 ml). The mixture was stirred overnight at room temperature. The reaction mixture was washed with 1 N aq. HCl and saturated NaCl solution, and then dried with sodium sulfate anhydrous. The mixture was purified by silica gel column chromatography ($CHCl_3$) and recrystallized from EtOH to give **11** as white crystals (0.50 g, yield 3.6%). mp 115—116 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.89 (2H, m), 2.68 (2H, t, J=7.6 Hz), 3.41 (2H, q, J=6.6 Hz), 3.81 (6H, s), 5.03 (2H, s), 5.72 (1H, br t, J=5.4 Hz), 6.26 (1H, d, J=15.4 Hz), 6.69 (2H, s), 7.15—7.52 (11H, m). IR (KBr) cm^{-1}: 3276, 1648, 1238. MS *m/z*: 431 (M^+), 340, 207, 91. *Anal.* Calcd for $C_{27}H_{29}NO_4$: C, 75.15; H, 6.77; N, 3.25. Found: C, 75.43; H, 6.80; N, 3.25.

The following compounds **12**—**15**, **17**, **18** were prepared in a manner similar to that described for **11** from **9a** and the appropriate aniline or phenylalkylamine.

N-Phenyl-(2*E*)-3-(4-benzyloxy-3,5-dimethoxyphenyl)-2-propenamide (**12**): Yield 71.5%. mp 109—110 °C. ^{1}H-NMR ($CDCl_3$) δ: 3.64 (6H, s), 5.00 (2H, s), 6.59 (2H, s), 6.66 (1H, d, J=15.4 Hz), 7.06 (1H, t, J=7.1 Hz), 7.22—7.32 (5H, m), 7.40—7.72 (5H, m), 8.75 (1H, s). IR (KBr) cm^{-1}: 3247, 1654, 1232. MS *m/z*: 389 (M^+), 280, 132, 91. *Anal.* Calcd for $C_{24}H_{23}NO_4$: C, 74.02; H, 5.95; N, 3.60. Found: C, 74.20; H, 5.94; N, 3.44.

N-Benzyl-(2*E*)-3-(4-benzyloxy-3,5-dimethoxyphenyl)-2-propenamide (**13**): Yield 15.1%. mp 147—148 °C. ^{1}H-NMR ($CDCl_3$) δ: 3.80 (6H, s), 4.54 (2H, d, J=5.6 Hz), 5.02 (2H, s), 6.08 (1H, br t, J=5.8 Hz), 6.34 (1H, d, J=15.6 Hz), 6.69 (2H, s), 7.24—7.48 (10H, m), 7.56 (1H, d, J=15.6 Hz). IR (KBr) cm^{-1}: 3252, 1652, 1276. MS *m/z*: 403 (M^+), 312, 226, 92. *Anal.* Calcd for $C_{25}H_{25}NO_4$: C, 74.42; H, 6.25; N, 3.47. Found: C, 74.36; H, 6.46; N, 3.24.

N-(2-Phenyl)ethyl-(2*E*)-3-(4-benzyloxy-3,5-dimethoxyphenyl)-2-propenamide (**14**): Yield 46.5%. mp 116—119 °C. ^{1}H-NMR ($CDCl_3$) δ: 2.88 (2H, t, J=6.8 Hz), 3.66 (2H, q, J=6.6 Hz), 3.82 (6H, s), 5.02 (2H, s), 5.69 (1H, br t, J=5.6 Hz), 6.23 (1H, d, J=15.4 Hz), 6.69 (2H, s), 7.20—7.55 (11H, m). IR (KBr) cm^{-1}: 3291, 1648, 1275. MS *m/z*: 417 (M^+), 326, 226, 91. *Anal.* Calcd for $C_{26}H_{27}NO_4$: C, 74.80; H, 6.52; N, 3.35. Found: C, 75.04; H, 6.68; N, 3.26.

N-(4-Phenyl)butyl-(2*E*)-3-(4-benzyloxy-3,5-dimethoxyphenyl)-2-propenamide (**15**): Yield 4.2%. mp 133—135 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.52—1.72 (4H, m), 2.63 (2H, t, J=7.4 Hz), 3.38 (2H, q, J=6.5 Hz), 3.80 (6H, s), 5.02 (2H, s), 5.78 (1H, br t, J=5.2 Hz), 6.29 (1H, d, J=15.4 Hz), 6.69 (2H,

s), 7.13—7.54 (11H, m). IR (KBr) cm^{-1}: 3279, 1648, 1239. MS *m/z*: 445 (M^+), 354, 226, 91. *Anal.* Calcd for $C_{28}H_{31}NO_4$: C, 75.48; H, 7.01; N, 3.14. Found: C, 75.60; H, 7.23; N, 3.08.

N-(2,4-Dimethylphenyl)-(2*E*)-3-(4-benzyloxy-3,5-dimethoxyphenyl)-2-propenamide (**17**): Yield 30.5%. mp 215—216 °C. ^{1}H-NMR ($CDCl_3$) δ: 2.26 (3H, s), 2.30 (3H, s), 3.84 (6H, s), 5.05 (2H, s), 6.48 (1H, d, J=15.4 Hz), 6.74 (2H, s), 7.00—7.50 (8H, m), 7.65 (1H, d, J=15.4 Hz), 7.75 (1H, br s). IR (KBr) cm^{-1}: 3201, 1664, 1239. MS *m/z*: 417 (M^+), 326, 120, 92. *Anal.* Calcd for $C_{26}H_{27}NO_4$: C, 74.80; H, 6.52; N, 3.35. Found: C, 75.05; H, 6.45; N, 3.56.

N-(3,4-Dimethylphenyl)-(2*E*)-3-(4-benzyloxy-3,5-dimethoxyphenyl)-2-propenamide (**18**): Yield 55.3%. mp 148—149 °C. ^{1}H-NMR ($CDCl_3$) δ: 2.21 (3H, s), 2.22 (3H, s), 3.80 (6H, s), 5.04 (2H, s), 6.48 (1H, d, J=15.6 Hz), 6.70 (2H, s), 7.07 (1H, d, J=8.0 Hz), 7.26—7.48 (8H, m), 7.63 (1H, d, J=15.6 Hz). IR (KBr) cm^{-1}: 3310, 1658, 1271. MS *m/z*: 417 (M^+), 326, 120, 91. *Anal.* Calcd for $C_{26}H_{27}NO_4$: C, 74.80; H, 6.52; N, 3.35. Found: C, 74.67; H, 6.54; N, 3.57.

***N*-(4-Benzyloxy-3,5-dimethoxycinnamoyl)-*N′*-phenylpiperazine (16)** EDC (6.40 g, 33.4 mmol) and 1-phenylpiperazine (8.80 g, 54.2 mmol) were added to a solution of **9a** (10.00 g, 31.9 mmol) in $CHCl_3$ (500 ml) and stirred overnight at room temperature. The reaction mixture was directly purified by silica gel column chromatography ($CHCl_3$) and recrystallized from EtOH to give **16** as pale yellow crystals (9.64 g, yield 66.0%). mp 98—99 °C. ^{1}H-NMR ($CDCl_3$) δ: 3.16 (4H, br s), 3.81 (4H, br s), 3.82 (6H, s), 5.03 (2H, s), 6.74 (2H, s), 6.81 (1H, d, J=15.4 Hz), 6.85—6.92 (3H, m), 7.23—7.48 (7H, m), 7.62 (1H, d, J=15.4 Hz). IR (KBr) cm^{-1}: 1646, 1336, 1272. MS *m/z*: 458 (M^+), 367, 208, 132. *Anal.* Calcd for $C_{28}H_{30}N_2O_4$: C, 73.34; H, 6.59; N, 6.11. Found: C, 73.74; H, 6.83; N, 5.87.

***N*-(4-Benzyloxy-3,5-dimethoxycinnamoyl)-*N′*-(2,4-dimethylphenyl)-piperazine (19)** Compound **10a** (5.00 g, 12.6 mmol) was dissolved in DMF (400 ml) and then sodium hydride (0.66 g, 16.4 mmol) was very carefully added to this solution. Benzyl chloride (2.07 g, 16.4 mmol) was added to this mixture portionwise and the mixture was stirred at room temperature for 96 h. The reaction mixture was poured into water (4 l) and neutralized with 1 N aq. HCl. The resulting precipitate was collected by filtration. The precipitate was purified by silica gel column chromatography ($CHCl_3$) and recrystallized from EtOH to give **19** as pale yellow crystals (3.24 g, yield 52.9%). mp 102—104 °C. ^{1}H-NMR ($CDCl_3$) δ: 2.28 (3H, s), 2.31 (3H, s), 2.91 (4H, br s), 3.82 (4H, br s), 3.86 (6H, s), 5.04 (2H, s), 6.74 (2H, s), 6.80 (1H, d, J=15.4 Hz), 6.90 (1H, d, J=8.0 Hz), 6.98 (1H, dd, J=8.0, 2.1 Hz), 7.03 (1H, d, J=2.1 Hz), 7.27—7.36 (3H, m), 7.47—7.49 (2H, m), 7.61 (1H, d, J=15.4 Hz). IR (KBr) cm^{-1}: 1636, 1338, 1273. MS *m/z*: 486 (M^+), 395, 190, 161. *Anal.* Calcd for $C_{30}H_{34}N_2O_4$: C, 74.05; H, 7.04; N, 5.76. Found: C, 73.71; H, 7.25; N, 5.54.

The following compounds **21**—**29** were prepared in a manner similar to that described for **19** from **10a** and the appropriate halide.

N-[3,5-Dimethoxy-4-(3-phenyl)propyloxycinnamoyl]-*N′*-(2,4-dimethylphenyl)piperazine (**21**): Yield 96.8%. mp 103—104 °C. ^{1}H-NMR ($CDCl_3$) δ: 2.06 (2H, m), 2.28 (3H, s), 2.31 (3H, s), 2.84 (2H, t, J=7.7 Hz), 2.91 (4H, br s), 3.82 (4H, br s), 3.87 (6H, s), 4.04 (2H, t, J=6.3 Hz), 6.75 (2H, s), 6.81 (1H, d, J=15.4 Hz), 6.87—7.04 (3H, m), 7.15—7.31 (5H, m), 7.62 (1H, d, J=15.4 Hz); IR (KBr) cm^{-1}: 2908, 1636, 1241. MS *m/z*: 514 (M^+), 325, 190, 161. *Anal.* Calcd for $C_{32}H_{38}N_2O_4$: C, 74.68; H, 7.44; N, 5.44. Found: C, 74.50; H, 7.29; N, 5.63.

N-[3,5-Dimethoxy-4-(2-phenoxy)ethyloxycinnamoyl]-*N′*-(2,4-dimethylphenyl)piperazine (**22**): Yield 20.9%. mp 123—124 °C. ^{1}H-NMR ($CDCl_3$) δ: 2.27 (3H, s), 2.30 (3H, s), 2.91 (4H, br s), 3.82 (4H, br s), 3.83 (6H, s), 4.27 (2H, t, J=5.1 Hz), 4.39 (2H, t, J=5.0 Hz), 6.74 (2H, s), 6.80 (1H, d, J=15.4 Hz), 6.87—7.05 (6H, m), 7.23—7.30 (2H, m), 7.62 (1H, d, J=15.4 Hz). IR (KBr) cm^{-1}: 2934, 1643, 1241. MS *m/z*: 516 (M^+), 327, 208, 161. *Anal.* Calcd for $C_{31}H_{36}N_2O_5$: C, 72.07; H, 7.02; N, 5.42. Found: C, 71.88; H, 6.78; N, 5.63.

N-(3,5-Dimethoxy-4-geranyloxycinnamoyl)-*N′*-(2,4-dimethylphenyl)-piperazine (**23**): Yield 84.1%. mp 69—71 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.59 (3H, s), 1.66 (3H, s), 1.67 (3H, s), 1.93—2.10 (4H, m), 2.28 (3H, s), 2.31 (3H, s), 2.91(4H, br s), 3.81 (4H, br s), 3.89 (6H, s), 4.57 (2H, d, J=7.0 Hz), 5.08 (1H, t, J=6.0 Hz), 5.56 (1H, t, J=6.5 Hz), 6.75 (2H, s), 6.81 (1H, d, J=15.4 Hz), 6.89 (1H, d, J=8.0 Hz), 6.98 (1H, dd, J=8.0, 2.1 Hz), 7.03 (1H, d, J=2.1 Hz), 7.62 (1H, d, J=15.4 Hz). IR (KBr) cm^{-1}: 2930, 1636, 1241. MS *m/z*: 532 (M^+), 397, 208, 161. *Anal.* Calcd for $C_{33}H_{44}N_2O_4$: C, 74.40; H, 8.33; N, 5.26. Found: C, 74.42; H, 8.13; N, 5.22.

N-(3,5-Dimethoxy-4-farnecyloxycinnamoyl)-*N′*-(2,4-dimethylphenyl)-piperazine (**24**): Yield 68.8%. mp 58—59 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.59 (6H, s), 1.66 (3H, s), 1.68 (3H, s), 1.94—2.12 (8H, m), 2.27 (3H, s), 2.30 (3H, s), 2.90 (4H, br s), 3.81 (4H, br s), 3.88 (6H, s), 4.57 (2H, d, J=7.0 Hz), 5.05—5.13 (2H, m), 5.57 (1H, t, J=7.3 Hz), 6.75 (2H, s), 6.82 (1H, d, J=15.4 Hz), 6.88 (1H, d, J=8.0 Hz), 6.97 (1H, dd, J=8.0, 2.1 Hz), 7.02 (1H, d, J=2.1 Hz), 7.62 (1H, d, J=15.4 Hz). IR (KBr) cm^{-1}: 2956, 1636, 1241. MS *m/z*: 600 (M^+), 397, 208, 161. *Anal.* Calcd for $C_{38}H_{52}N_2O_4$: C, 75.96; H, 8.72; N, 4.66. Found: C, 76.02; H, 8.66; N, 4.63.

N-(3,5-Dimethoxy-4-*n*-hexyloxycinnamoyl)-*N′*-(2,4-dimethylphenyl)-piperazine (**25**): Yield 71.0%. mp 113—114 °C. ^{1}H-NMR ($CDCl_3$) δ: 0.90 (3H, t, J=6.7 Hz), 1.30—1.50 (6H, m), 1.70—1.78 (2H, m), 2.26 (3H, s), 2.29 (3H, s), 2.88 (4H, br s), 3.81 (4H, br s), 3.86 (6H, s), 3.99 (2H, t, J=6.7 Hz), 6.76 (2H, s), 6.85 (1H, d, J=15.4 Hz), 6.87 (1H, d, J=8.0 Hz), 6.96 (1H, dd, J=8.0, 2.1 Hz), 7.01 (1H, d, J=2.1 Hz), 7.62 (1H, d, J=15.4 Hz). IR (KBr) cm^{-1}: 2924, 1636, 1243. MS *m/z*: 480 (M^+), 255, 190, 161. *Anal.* Calcd for $C_{29}H_{40}N_2O_4$: C, 72.47; H, 8.39; N, 5.83. Found: C, 72.23; H, 8.56; N, 5.68.

N-(3,5-Dimethoxy-4-*n*-heptyloxycinnamoyl)-*N′*-(2,4-dimethylphenyl)-piperazine (**26**): Yield 51.9%. mp 88—89 °C. ^{1}H-NMR ($CDCl_3$) δ 0.89 (3H, t, J=6.8 Hz), 1.27—1.48 (8H, m), 1.72—1.80 (2H, m), 2.28 (3H, s), 2.31 (3H, s), 2.91 (4H, br s), 3.81 (4H, br s), 3.86 (6H, s), 3.99 (2H, t, J=6.7 Hz), 6.76 (2H, s), 6.82 (1H, d, J=15.4 Hz), 6.89 (1H, d, J=8.0 Hz), 6.98 (1H, dd, J=8.0, 2.1 Hz), 7.02 (1H, d, J=2.1 Hz), 7.62 (1H, d, J=15.4 Hz). IR (KBr) cm^{-1}: 3002, 1636, 1242. MS *m/z*: 494 (M^+), 305, 190, 161. *Anal.* Calcd for $C_{30}H_{42}N_2O_4$: C, 72.84; H, 8.56; N, 5.66. Found: C, 72.82; H, 8.75; N, 5.44.

N-(3,5-Dimethoxy-4-*n*-octyloxycinnamoyl)-*N′*-(2,4-dimethylphenyl)-piperazine (**27**): Yield 68.2%. mp 107—109 °C. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (3H, t, J=6.8 Hz), 1.26—1.48 (10H, m), 1.72—1.80 (2H, m), 2.27 (3H, s), 2.30 (3H, s), 2.89 (4H, br s), 3.81 (4H, br s), 3.87 (6H, s), 3.99 (2H, t, J=6.8 Hz), 6.76 (2H, s), 6.83 (1H, d, J=15.4 Hz), 6.88 (1H, d, J=8.0 Hz), 6.97 (1H, dd, J=8.0, 2.1 Hz), 7.01 (1H, d, J=2.1 Hz), 7.62 (1H, d, J=15.4 Hz). IR (KBr) cm^{-1}: 3000, 1636, 1241. MS *m/z*: 508 (M^+), 319, 208, 161. *Anal.* Calcd for $C_{31}H_{44}N_2O_4$: C, 73.19; H, 8.72; N, 5.51. Found: C, 73.26; H, 8.66; N, 5.72.

N-(3,5-Dimethoxy-4-*n*-nonyloxycinnamoyl)-*N′*-(2,4-dimethylphenyl)-piperazine (**28**): Yield 70.4%. mp 100—101 °C. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (3H, t, J=6.8 Hz), 1.23—1.47 (12H, m), 1.72—1.78 (2H, m), 2.28 (3H, s), 2.31 (3H, s), 2.91 (4H, br s), 3.82 (4H, br s), 3.88 (6H, s), 3.99 (2H, t, J=6.8 Hz), 6.75 (2H, s), 6.80 (1H, d, J=15.4 Hz), 6.90 (1H, d, J=8.0 Hz), 6.98 (1H, dd, J=8.0, 2.1 Hz), 7.03 (1H, d, J=2.1 Hz), 7.62 (1H, d, J=15.4 Hz). IR (KBr) cm^{-1}: 2916, 1636, 1242. MS *m/z*: 522 (M^+), 333, 190, 161. *Anal.* Calcd for $C_{32}H_{46}N_2O_4$: C, 73.53; H, 8.87; N, 5.36. Found: C, 73.45; H, 8.85; N, 5.51.

N-(4-*n*-Decyloxy-3,5-dimethoxycinnamoyl)-*N′*-(2,4-dimethylphenyl)-piperazine (**29**): Yield 53.1%. mp 97—99 °C. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (3H, t, J=6.8 Hz), 1.22—1.48 (14H, m), 1.71—1.77 (2H, m), 2.28 (3H, s), 2.31 (3H, s), 2.91 (4H, br s), 3.82 (4H, br s), 3.88 (6H, s), 3.99 (2H, t, J=6.7 Hz), 6.75 (2H, s), 6.80 (1H, d, J=15.4 Hz), 6.90 (1H, d, J=8.0 Hz), 6.98 (1H, dd, J=8.0, 2.1 Hz), 7.03 (1H, d, J=2.1 Hz), 7.62 (1H, d, J=15.4 Hz). IR (KBr) cm^{-1}: 2915, 1636, 1241. MS *m/z*: 536 (M^+), 347, 208, 161. *Anal.* Calcd for $C_{33}H_{48}N_2O_4$: C, 73.84; H, 9.01; N, 5.22. Found: C, 73.67; H, 9.12; N, 5.44.

***N*-(4-*n*-Decyloxy-3,5-dimethoxycinnamoyl)-*N′*-phenylpiperazine (30)** EDC (1.58 g, 8.2 mmol) and 1-phenylpiperazine (1.34 g, 8.2 mmol) were added to a solution of **9b** (3.00 g, 8.2 mmol) in $CHCl_3$ (200 ml) and stirred for 3 d at room temperature. The reaction mixture was directly purified by silica gel column chromatography ($CHCl_3$) and recrystallized from EtOH to give **30** as pale yellow crystals (1.29 g, yield 30.9%). mp 77—78 °C. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (3H, t, J=6.8 Hz), 1.22—1.49 (14H, m), 1.73—1.79 (2H, m), 1.86 (6H, s), 3.19 (4H, br s), 3.83 (4H, br s), 4.00 (2H, t, J=6.8 Hz), 6.76 (2H, s), 6.82 (1H, d, J=15.4 Hz), 6.86—6.94 (3H, m), 7.24—7.30 (2H, m), 7.63 (1H, d, J=15.4 Hz). IR (KBr) cm^{-1}: 2949, 1642, 1236. MS *m/z*: 508 (M^+), 347, 208, 132. *Anal.* Calcd for $C_{31}H_{44}N_2O_4$: C, 73.19; H, 8.72; N, 5.51. Found: C, 73.20; H, 8.65; N, 5.63.

The following compounds **31**—**41** were prepared in a manner similar to that described for **30** from **9b** and the appropriate phenylpiperazine.

N-(4-*n*-Decyloxy-3,5-dimethoxycinnamoyl)-*N′*-(2-methylphenyl)piperazine (**31**): Yield 33.3%. mp 85—87 °C. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (3H, t, J=6.8 Hz), 1.22—1.48 (14H, m), 1.72—1.79 (2H, m), 2.34 (3H, s), 2.94 (4H, br s), 3.82 (4H, br s), 3.88 (6H, s), 3.99 (2H, t, J=6.8 Hz), 6.76 (2H, s), 6.82 (1H, d, J=15.4 Hz), 6.97—7.22 (4H, m), 7.63 (1H, d, J=15.4 Hz). IR (KBr) cm^{-1}: 2915, 1647, 1238. MS *m/z*: 522 (M^+), 347, 208, 147. *Anal.* Calcd for $C_{32}H_{46}N_2O_4$: C, 73.53; H, 8.87; N, 5.36. Found: C, 73.48; H, 8.96; N, 5.40.

N-(4-*n*-Decyloxy-3,5-dimethoxycinnamoyl)-*N′*-(3-methylphenyl)piperazine (**32**): Yield 17.4%. mp 76—77 °C. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (3H, t,

J=6.7 Hz), 1.22—1.48 (14H, m), 1.71—1.79 (2H, m), 2.32 (3H, s), 3.19 (4H, br s), 3.85 (4H, br s), 3.87 (6H, s), 4.00 (2H, t, J=6.8 Hz), 6.70—6.77 (5H, m), 6.81 (1H, d, J=15.4 Hz), 7.16 (1H, t, J=7.7 Hz), 7.62 (1H, d, J=15.4 Hz). IR (KBr) cm^{-1}: 2989, 1642, 1240. MS m/z: 522 (M$^+$), 347, 208, 147. *Anal.* Calcd for $C_{32}H_{46}N_2O_4$: C, 73.53; H, 8.87; N, 5.36. Found: C, 73.29; H, 8.85; N, 5.53.

N-(4-*n*-Decyloxy-3,5-dimethoxycinnamoyl)-*N*′-(4-methylphenyl)piperazine (**33**):Yield 6.7%. mp 108—110 °C. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (3H, t, J=6.8 Hz), 1.22—1.48 (14H, m), 1.72—1.78 (2H, m), 2.27 (3H, s), 3.15 (4H, br s), 3.84 (4H, br s), 3.90 (6H, s), 3.99 (2H, t, J=6.8 Hz), 6.75 (2H, s), 6.80 (1H, d, J=15.4 Hz), 6.82 (2H, d, J=8.2 Hz), 7.09 (2H, d, J=8.2 Hz), 7.62 (1H, d, J=15.4 Hz). IR (KBr) cm^{-1}: 2917, 1642, 1238. MS m/z: 522 (M$^+$), 347, 208, 147. *Anal.* Calcd for $C_{32}H_{46}N_2O_4$: C, 73.53; H, 8.87; N, 5.36. Found: C, 73.56; H, 8.96; N, 5.24.

N-(4-*n*-Decyloxy-3,5-dimethoxycinnamoyl)-*N*′-(4-*tert*-butylphenyl)piperazine (**34**): Yield 2.0%. mp 122—123 °C. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (3H, t, J=6.8 Hz), 1.23—1.48 (23H, m), 1.72—1.78 (2H, m), 3.19 (4H, m), 3.84 (4H, br s), 3.88 (6H, s), 3.99 (2H, t, J=6.8 Hz), 6.75 (2H, s), 6.80 (1H, d, J=15.4 Hz), 6.86—6.91 (2H, m), 7.28—7.34 (2H, m,), 7.62 (1H, d, J=15.4 Hz). IR (KBr) cm^{-1}: 2950, 1642, 1229. MS m/z: 564 (M$^+$), 347, 208, 189. *Anal.* Calcd for $C_{35}H_{52}N_2O_4$: C, 74.43; H, 9.28; N, 4.96. Found: C, 74.29; H, 9.41; N, 4.93.

N-(4-*n*-Decyloxy-3,5-dimethoxycinnamoyl)-*N*′-(4-methoxyphenyl)piperazine (**35**): Yield 17.8%. mp 113—114 °C. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (3H, t, J=6.8 Hz), 1.22—1.48 (14H, m), 1.75 (2H, m), 3.08 (4H, br s), 3.76 (3H, s), 3.84 (4H, br s), 3.87 (6H, s), 3.99 (2H, t, J=6.8 Hz), 6.76 (2H, s), 6.80—6.93 (5H, m), 7.62 (1H, d, J=15.4 Hz). IR (KBr) cm^{-1}: 2949, 1643, 1223. MS m/z: 538 (M$^+$), 347, 208, 163. *Anal.* Calcd for $C_{32}H_{46}N_2O_5$: C, 71.34; H, 8.61; N, 5.20. Found: C, 71.29; H, 8.57; N, 5.11.

N-(4-*n*-Decyloxy-3,5-dimethoxycinnamoyl)-*N*′-(4-fluorophenyl)piperazine (**36**): Yield 40.2%. mp 65—66 °C. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (3H, t, J=6.8 Hz), 1.21—1.49 (14H, m), 1.72—1.79 (2H, m), 3.12 (4H, m), 3.84 (4H, br s), 3.87 (6H, s), 4.00 (2H, t, J=6.8 Hz), 6.76 (2H, s), 6.81 (1H, d, J=15.4 Hz), 6.85—7.01 (4H, m), 7.62 (1H, d, J=15.4 Hz). IR (KBr) cm^{-1}: 2914, 1641, 1236. MS m/z: 526 (M$^+$), 347, 208, 151. *Anal.* Calcd for $C_{31}H_{43}FN_2O_4$: C, 70.69; H, 8.23; N, 3.61. Found: C, 70.70; H, 8.36; N, 3.63.

N-(4-*n*-Decyloxy-3,5-dimethoxycinnamoyl)-*N*′-(4-nitrophenyl)piperazine (**37**): Yield 44.8%. mp 117—119 °C. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (3H, t, J=6.8 Hz), 1.22—1.48 (14H, m), 1.72—1.80 (2H, m), 3.52 (4H, br s), 3.88 (6H, s), 3.91 (4H, br s), 4.00 (2H, t, J=6.7 Hz), 6.76—6.85 (5H, m), 7.65 (1H, d, J=15.2 Hz), 8.08 (2H, d, J=9.2 Hz). IR (KBr) cm^{-1}: 2916, 1643, 1599, 1246. MS m/z: 553 (M$^+$), 347, 208, 178. *Anal.* Calcd for $C_{31}H_{43}N_3O_6$: C, 67.25; H, 7.83; N, 7.59. Found: C, 67.29; H, 7.95; N, 7.55.

N-(4-*n*-Decyloxy-3,5-dimethoxycinnamoyl)-*N*′-(2,3-dimethylphenyl)piperazine (**38**): Yield 30.4%. mp 98—100 °C. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (3H, t, J=6.7 Hz), 1.22—1.48 (14H, m), 1.72—1.78 (2H, m), 2.26 (3H, s), 2.28 (3H, s), 2.91 (4H, br s), 3.82 (4H, br s), 3.87 (6H, s), 3.99 (2H, t, J=6.84 Hz), 6.75 (2H, s), 6.82 (1H, d, J=15.4 Hz), 6.87 (1H, d, J=7.8 Hz), 6.93 (1H, d, J=7.8 Hz), 7.08 (1H, t, J=7.7 Hz), 7.62 (1H, d, J=15.4 Hz). IR (KBr) cm^{-1}: 2914, 1644, 1205. MS m/z: 536 (M$^+$), 347, 208, 161. *Anal.* Calcd for $C_{33}H_{48}N_2O_4$: C, 73.84; H, 9.01; N, 5.22. Found: C, 73.59; H, 9.30; N, 5.24.

N-(4-*n*-Decyloxy-3,5-dimethoxycinnamoyl)-*N*′-(2,5-dimethylphenyl)piperazine (**39**): Yield 29.9%. mp 66—67 °C. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (3H, t, J=6.8 Hz), 1.22 (14H, m), 1.72—1.78 (2H, m), 2.29 (6H, s), 2.92 (4H, br s), 3.83 (4H, br s), 3.87 (6H, s), 3.99 (2H, t, J=6.8 Hz), 6.76 (2H, s), 6.78—6.86 (3H, m), 7.07 (1H, d, J=15.4 Hz), 7.62 (1H, d, J=15.4 Hz). IR (KBr) cm^{-1}: 2988, 1646, 1237. MS m/z: 536 (M$^+$), 347, 208, 161. *Anal.* Calcd for $C_{33}H_{48}N_2O_4$: C, 73.84; H, 9.01; N, 5.22. Found: C, 73.65; H, 8.96; N, 5.49.

N-(4-*n*-Decyloxy-3,5-dimethoxycinnamoyl)-*N*′-(2,6-dimethylphenyl)piperazine (**40**): Yield 27.6%. mp 65—67 °C. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (3H, t, J=6.8 Hz), 1.23—1.48 (14H, m), 1.72—1.78 (2H, m), 2.32 (6H, s), 3.13 (4H, br s), 3.85 (4H, br s), 3.87 (6H, s), 3.99 (2H, t, J=6.8 Hz), 6.76 (2H, s), 6.83 (1H, d, J=15.4 Hz), 6.94—7.02 (3H, m), 7.63 (1H, d, J=15.4 Hz). IR (KBr) cm^{-1}: 2913, 1636, 1243. MS m/z: 536 (M$^+$), 347, 208, 161. *Anal.* Calcd for $C_{33}H_{48}N_2O_4$: C, 73.84; H, 9.01; N, 5.22. Found: C, 73.84; H, 9.23; N, 5.03.

N-(4-*n*-Decyloxy-3,5-dimethoxycinnamoyl)-*N*′-(3,5-dimethylphenyl)piperazine (**41**): Yield 36.8%. mp 79—80 °C. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (3H, t, J=7.0 Hz), 1.22—1.48 (14H, m), 1.72—1.78 (2H, m), 2.29 (6H, s), 3.20 (4H, m), 3.84 (4H, br s), 3.88 (6H, s), 4.00 (2H, t, J=6.8 Hz), 6.57 (3H, s), 6.75 (2H, s), 6.80 (1H, d, J=15.4 Hz), 7.62 (1H, d, J=15.4 Hz). IR (KBr) cm^{-1}: 2949, 1642, 1230. MS m/z: 536 (M$^+$), 347, 208, 161. *Anal.* Calcd for $C_{33}H_{48}N_2O_4$: C, 73.84; H, 9.01; N, 5.22. Found: C, 74.00; H, 8.93; N, 5.12.

***N*-(4-Benzyloxy-3,5-dimethoxycinnamoyl)-*N*′-(3,4-dimethylphenyl)-piperazine (20)** Compound **10b** (7.00 g, 17.7 mmol) was dissolved in DMF (700 ml) and then sodium hydride (0.92 g, 23.0 mmol) was very carefully added to this solution. Benzyl chloride (2.90 g, 23.0 mmol) was added to this mixture portionwise followed by stirring at room temperature for 96 h. The reaction mixture was poured into water (5 l) and the resulting precipitate was collected by filtration. The precipitate was purified by silica gel column chromatography ($CHCl_3$) and recrystallized from EtOH to give **20** as pale yellow crystals (7.50 g, yield 87.1%). mp 99—100 °C. ^{1}H-NMR ($CDCl_3$) δ: 2.19 (3H, s), 2.23 (3H, s), 3.14 (4H, m), 3.82 (4H, br s), 3.84 (6H, s), 5.04 (2H, s), 6.68 (1H, dd, J=8.0, 2.4 Hz), 6.74 (2H, s), 6.75 (1H, d, J=2.4 Hz), 6.80 (1H, d, J=15.4 Hz), 7.04 (1H, d, J=8.0 Hz), 7.22—7.50 (5H, m), 7.61 (1H, d, J=15.4 Hz). IR (KBr) cm^{-1}: 1644, 1334, 1272. MS m/z: 486 (M$^+$), 395, 190, 161. *Anal.* Calcd for $C_{30}H_{34}N_2O_4$: C, 74.05; H, 7.04; N, 5.76. Found: C, 73.71; H, 7.27; N, 5.56.

The following compounds **42**—**46** were prepared in a manner similar to that described for **20** from **10b** and the appropriate halide.

N-(4-*n*-Decyloxy-3,5-dimethoxycinnamoyl)-*N*′-(3,4-dimethylphenyl)-piperazine (**42**): Yield 78.8 %. mp 100—101 °C. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (3H, t, J=6.7 Hz), 1.22—1.48 (14H, m), 1.70—1.78 (2H, m), 2.19 (3H, s), 2.24 (3H, s), 3.15 (4H, m), 3.85 (4H, br s), 3.87 (6H, s), 3.99 (2H, t, J=6.8 Hz), 6.69 (1H, dd, J=8.0, 2.4 Hz), 6.75 (2H, s), 6.77 (1H, d, J=2.4 Hz), 6.81 (1H, d, J=15.4 Hz), 7.04 (1H, d, J=8.0 Hz), 7.62 (1H, d, J=15.4 Hz). IR (KBr) cm^{-1}: 2917, 1642, 1240. MS m/z: 536 (M$^+$), 347, 208, 161. *Anal.* Calcd for $C_{33}H_{48}N_2O_4$: C, 73.84; H, 9.01; N, 5.22. Found: C, 73.75; H, 8.98; N, 5.45.

N-(3,5-Dimethoxy-4-*n*-hexyloxycinnamoyl)-*N*′-(3,4-dimethylphenyl)-piperazine (**43**): Yield 48.5%. mp 92—93 °C. ^{1}H-NMR ($CDCl_3$) δ: 0.90 (3H, t, J=6.8 Hz), 1.30—1.50 (6H, m), 1.72—1.80 (2H, m), 2.19 (3H, s), 2.24 (3H, s), 3.16 (4H, m), 3.86 (4H, br s), 3.88 (6H, s), 4.00 (2H, t, J=6.8 Hz), 6.70 (1H, dd, J=8.0, 2.4 Hz), 6.75 (2H, s), 6.77 (1H, d, J=2.4 Hz), 6.80 (1H, d, J=15.4 Hz), 7.04 (1H, d, J=8.0 Hz), 7.62 (1H, d, J=15.4 Hz). IR (KBr) cm^{-1}: 2918, 1642, 1246. MS m/z: 480 (M$^+$), 291, 207, 160. *Anal.* Calcd for $C_{29}H_{40}N_2O_4$: C, 72.47; H, 8.39; N, 5.83. Found: C, 72.45; H, 8.46; N, 5.80.

N-(3,5-Dimethoxy-4-*n*-heptyloxycinnamoyl)-*N*′-(3,4-dimethylphenyl)-piperazine (**44**): Yield 46.0%. mp 102—104 °C. ^{1}H-NMR ($CDCl_3$) δ: 0.89 (3H, t, J=6.5 Hz), 1.26—1.48 (8H, m), 1.72—1.80 (2H, m), 2.19 (3H, s), 2.24 (3H, s), 3.16 (4H, m), 3.85 (4H, br s), 3.88 (6H, s), 3.99 (2H, t, J=6.7 Hz), 6.70 (1H, dd, J=8.0, 2.4 Hz), 6.75 (2H, s), 6.77 (1H, d, J=2.4 Hz), 6.80 (1H, d, J=15.4 Hz), 7.04 (1H, d, J=8.0 Hz), 7.62 (1H, d, J=15.4 Hz). IR (KBr) cm^{-1}: 2916, 1641, 1245. MS m/z: 494 (M$^+$), 305, 208, 161. *Anal.* Calcd for $C_{30}H_{42}N_2O_4$: C, 72.84; H, 8.56; N, 5.66. Found: C, 72.76; H, 8.63; N, 5.63.

N-(3,5-Dimethoxy-4-*n*-octyloxycinnamoyl)-*N*′-(3,4-dimethylphenyl)-piperazine (**45**): Yield 49.5%. mp 104—106 °C. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (3H, t, J=6.7 Hz), 1.28—1.50 (10H, m), 1.72—1.80 (2H, m), 2.19 (3H, s), 2.24 (3H, s), 3.16 (4H, t, J=5.0 Hz), 3.86 (4H, br s), 3.88 (6H, s), 3.99 (2H, t, J=6.7 Hz), 6.70 (1H, dd, J=8.0, 2.4 Hz), 6.75 (2H, s), 6.77 (1H, d, J=2.4 Hz), 6.79 (1H, d, J=15.4 Hz), 7.05 (1H, d, J=8.0 Hz), 7.62 (1H, d, J=15.4 Hz). IR (KBr) cm^{-1}: 2916, 1641, 1245. MS m/z: 508 (M$^+$), 319, 208, 161. *Anal.* Calcd for $C_{31}H_{44}N_2O_4$: C, 73.19; H, 8.72; N, 5.51. Found: C, 73.18; H, 8.81; N, 5.46.

N-(3,5-Dimethoxy-4-*n*-nonyloxycinnamoyl)-*N*′-(3,4-dimethylphenyl)-piperazine (**46**): Yield 41.4%. mp 98—100 °C. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (3H, t, J=6.8 Hz), 1.23—1.48 (12H, m), 1.70—1.78 (2H, m), 2.20 (3H, s), 2.24 (3H, s), 3.17 (4H, m), 3.86 (4H, br s), 3.88 (6H, s), 3.99 (2H, t, J=6.8 Hz), 6.70 (1H, dd, J=8.0, 2.4 Hz), 6.75 (2H, s), 6.77 (1H, d, J=2.4 Hz), 6.79 (1H, d, J=15.4 Hz), 7.05 (1H, d, J=8.0 Hz), 7.62 (1H, d, J=15.4 Hz). IR (KBr) cm^{-1}: 2917, 1641, 1245. MS m/z: 522 (M$^+$), 333, 208, 161. *Anal.* Calcd for $C_{32}H_{46}N_2O_4$: C, 73.53; H, 8.87; N, 5.36. Found: C, 73.50; H, 8.90; N, 5.32.

Assay of ACAT Activity Microsome fractions were prepared from livers of male Sprague–Dawley rats given a normal diet (F2: Funabashi Farmer Co., Ltd., Chiba, Japan). Microsome fractions were also prepared from cell lines including Caco2 (Riken Cell Bank No. RCB0988; human colon adenocarcinoma) that were cultured for 14 d after confluence and from HepG2 (Riken Cell Bank No. RCB0459; human hepatocarcinoma). Microsomes were prepared according to the method described by Field and Salome.[45] ACAT activity was measured using [1-^{14}C]oleoyl-CoA as a substrate according to the method described by Helgerud *et al.*[46]

Plasma Cholesterol Levels Male Sprague–Dawley rats (5-weeks-old) were given a diet, F2 (Funabashi Farmer Co., Ltd., Chiba, Japan), containing

0.5% cholic acid, 10% sucrose, 10% coconut oil, 0.005 % 6-propyl-2-thiouracil and 0.005 or 0.1% test compounds for four days. For the three following days, the rats were given the F2 diet, which contained 1.5% cholesterol, 0.5% cholic acid, 10% sucrose, 10% coconut oil, 0.005% 6-propyl-2-thiouracil and 0.005 or 0.1% test compounds. Normal group rats were fed the F2 diet containing 0.5% cholic acid, 10% sucrose, 10% coconut oil and 0.005% 6-propyl-2-thiouracil. The rats were bled *via* the abdominal aorta while under pentobarbital anesthesia. The total plasma cholesterol concentration was determined enzymatically using a commercially available kit (Determiner TC555, Kyowa Medex Co., Tokyo, Japan).

References

1) Kannel W. B., Castelli W. P., Gordon T., McNamara P. M., *Ann. Intern. Med*., **74**, 1—12 (1971).
2) Martin M. J., Hulley S. B., Browner W. S., Kuller L. H., Wentworth D., *Lancet*, **2**, 933—936 (1986).
3) Badimon J. J., Fuster V., Chesebro J. H., Badimon L., *Circulation*, **87** (Suppl. II), 3—16 (1993).
4) McCarthy P. A., *Med. Res. Rev*., **13**, 139—159 (1993).
5) Brown G., Albers J. J., Fischer L. D., Schaefer S. M., Lin J. T., Kaplan C., Zhao X. Q., Bisson B. D., Fitzpatrick V. F., Dodge H. T., *N. Engl. J. Med*., **323**, 1289—1298 (1990).
6) Spector A. A., Mathur S. N., Kaduce T. L., *Prog. Lipid Res*., **18**, 31—53 (1979).
7) Suckling K. E., Stange E. F., *J. Lipid Res*., **26**, 647—671 (1985).
8) Krause B. R., Anderson M., Bisgaier C. L., Bocan T., Bousley R., DeHart P., Essenburg A., Hamelehle K., Homan R., Kieft K., McNally W., Stanfield R., Newton R. S., *J. Lipid Res*., **34**, 279—294 (1993).
9) Largis E. E., Wang C. H., DeVries V. G., Schaffer S. A., *J. Lipid Res*., **30**, 681—690 (1989).
10) Carr T. P., Rudel L. L., *Arteriosclerosis*, **10**, 823a (1990).
11) Carr T. P., Parks J. S., Rudel L. L., *Arterioscler. Thromb*., **12**, 1274—1283 (1992).
12) Burrier R. E., Deren S., McGregor D. G., Hoos L. M., Smith A. A., Davis H. R., Jr., *Biochem. Pharmacol*., **47**, 1545—1551 (1994).
13) Brown M. S., Goldstein J. L., *Annu. Rev. Biochem*., **52**, 223—261 (1983).
14) Gillies P. J., Robinson C. S., Rathgeb K. A., *Atherosclerosis*, **83**, 177—185 (1990).
15) Sliskovic D. R., White A. D., *Trends Pharmacol. Sci*., **12**, 194—199 (1991).
16) Itokawa H., Aiyama R., Ikuta A., *Phytochemistry*, **21**, 241—243 (1982).
17) Itokawa H., Aiyama R., Ikuta A., *Chem. Pharm. Bull*., **31**, 2491—2496 (1983).
18) Roth B. D., Blankley C. J., Hoefle M. L., Holmes A., Roark W. H., Trivedi B. K., Essenburg A. D., Kieft K. A., Krause B. R., Stanfield R. L., *J. Med. Chem*., **35**, 1609—1617 (1992).
19) Field F. J., Albright E., Mathur S., *Lipids*, **26**, 1—8 (1991).
20) Sakuma Y., Hagihara H., Ohne K., Nagayoshi A., Mutoh S., Ito Y., Notsu Y., Okuhara M., *Jpn. J. Pharmacol*., **70**, 35—41 (1996)
21) Ito T., Matsuda K., Iwaoka K., *Japan patent provisional publication*, No. 170727/93, (1993).
22) Picard J. A., *Curr. Opin. Ther. Pat*., **3**, 151—160 (1993).
23) Matsuda K., *Med. Res. Rev*., **14**, 271—305 (1994).
24) Sliskovic D. R., Trivedi K. K., *Curr. Med. Chem*., **1**, 204—225 (1994).
25) Roark W. H., Roth B. D., *Exp. Opin. Invest. Drugs*, **3**, 1143—1152 (1994).
26) Lee H. T., Picard J. A., *Exp. Opin. Ther. Patents*, **5**, 397—416 (1995).
27) Trivedi B. K., Purchase T. S., Holmes A., Augelli-Szafran C. E., Essenburg A. D., Hamelehle K. L., Stanfield R. L., Bousley R. F., Krause B. R., *J. Med. Chem*., **37**, 1652—1659 (1994).
28) Dominick M. A., Bobrowski W. A., MacDonald J. R., Gough A. W., *Toxicol. Pathol*., **21**, 54—62 (1993).
29) Dominick M. A., McGuire E. J., Reindel J. F., Bobrowski W. F., Bocan T. M. A., Gough A. W., *Fundam. Appl. Toxicol*., **20**, 217—224 (1993).
30) Reindel J. F., Dominick M. A., Bocan T. M. A., Gough A. W., McGuire E. J., *Toxicol Pathol*., **22**, 510—518 (1994).
31) Smith C., Ashton M. J., Bush R. C., Facchini V., Harris N. V., Hart T. W., Jordan R., McKenzie R., Riddell D., *Bioorg. Med. Chem. Lett*., **6**, 47—50 (1996).
32) White A. D., Creswell M. W., Chucholowski A. W., Blankey C. J., Wilson M. W., Bousley R. F., Essenburg A. D., Hamelehle K. L., Krause B. R., Stanfield R. L., Dominick M. A., Neub M., *J. Med. Chem*., **39**, 4382—4395 (1996).
33) Sliskovic D. R., Picard J. A., O'Brien P. M., Liao P., Roark W. H., Roth B. D., Anderson M. A., Mueller S. B., Bocan T. M. A., Bousley R. F., Hamelehle K. L., Homan R., Reindel J. F., Stanfield R. L., Turluck D., Krause B. R., *J. Med. Chem*., **41**, 682—690 (1998).
34) Warner G. J., Stoudt G., Bamberger M., Johnson W. J., Rothblat G. H., *J. Biol. Chem*., **270**, 5772—5778 (1995).
35) Matsuo M., Hashimoto M., Suzuki J., Iwanami K., Tomoi M., Shimomura K., *Toxicol. Appl. Pharmacol*., **140**, 387—392.
36) Tanaka A., Terasawa T., Hagihara H., Sakuma Y., Ishibe N., Sawada M., Takasugi H., Tanaka H., *J. Med. Chem*., **41**, 2390—2410 (1998).
37) Tanaka A., Terasawa T., Hagihara H., Ishibe N., Sawada M., Sakuma, Y., Hashimoto M., Takasugi H., Tanaka H., *J. Med. Chem*., **41**, 4408—4420 (1998).
38) Reindel J. F., Dominick M. A., Krause B. R., *Toxicol. Pathol*., **20**, 642—643 (1992).
39) Joyce C., Skinner K., Anderson R. A., Rudel L. L., *Curr. Opin. Lipidol*., **10**, 89—95 (1999).
40) Miyazaki A., Sakashita N., Lee O., Takahashi K., Horiuchi S., Hakamata H., Morganelli P. M., Chang C. C., Chang T. Y., *Arterioscler. Thromb. Vasc. Biol*., **18**, 1568—1574 (1998).
41) Cases S., Novak S., Zheng Y. M., Myers H. M., Lear S. R., Sande E., Welch C. B., Lusis A. J., Spencer T. A., Krause B. R., Erickson S. K., Farese R. V., Jr., *J. Biol. Chem*., **273**, 26755—26764 (1998).
42) Chang C. C., Sakashita N., Ornvold K., Lee O., Chang E. T., Dong R., Lin S., Lee C. Y., Strom S., Kashyap R., Fung J., Farese J., Patoiseau J. F., Delhon A., Chang T. Y., *J. Biol. Chem*., **275**, 28083—28092 (2000).
43) Matsuda H., Hakamata H., Kawasaki T., Sakashita N., Miyazaki A., Takahashi K., Shichiri M., Horiuchi S., *Biochim. Biophys. Acta*, **1391**, 193—203 (1998).
44) Mishani E., Dence C. S., McCarthy T. J., Welch M. J., *Tetrahedron Lett*., **37**, 319—322 (1996).
45) Field F. J., Salome R. G., *Biochim. Biophys. Acta*, **712**, 557—570 (1982).
46) Helgerud P., Saarem K., Norum K. R., *J. Lipid Res*., **22**, 271—277 (1981).