Potent Inhibitors of Acyl-CoA:Cholesterol Acyltransferase. 2. Structure−Activity Relationships of Novel *N*-(2,2-Dimethyl-2,3-dihydrobenzofuran-7-yl)amides

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Novel *N*-(2,2-dimethyl-2,3-dihydrobenzofuran-7-yl)amide derivatives **1** were synthesized and tested for their ability to inhibit rabbit small intestinal ACAT (acyl-CoA:cholesterol acyltransferase) and lower serum total cholesterol in cholesterol-fed rats. Among the synthesized compounds, *N*-(2,2,4,6-tetramethyl-2,3-dihydrobenzofuran-7-yl)amide derivatives showed potent ACAT inhibitory activity. The synthesis and structure−activity relationships of these compounds are described. A methyl group at position 6 of the 2,3-dihydrobenzofuran moiety was important for potent ACAT inhibitory activity. In the series of *N*-(2,2,4,6-tetramethyl-2,3-dihydrobenzofuran-7-yl)amides, lipophilicity of the acyl moiety was necessary for the potent ACAT inhibitory activity. The highly lipophilic acid amides *N*-(2,2,4,6-tetramethyl-2,3-dihydrobenzofuran-7-yl)-2,2-dimethyldodecanamide (**10**) and 6-(4-chlorophenoxy)-*N*-(2,2,4,6-tetramethyl-2,3-dihydrobenzofuran-7-yl)-2,2-dimethyloctanamide (**50**) showed potent activity. Introduction of a dimethylamino group at position 5 of the 2,3-dihydrobenzofuran moiety resulted in highly potent activity. The most potent compound, *N*-[5-(dimethylamino)-2,2,4,6-tetramethyl-2,3-dihydrobenzofuran-7-yl]-2,2-dimethyldodecanamide (**13**, TEI-6620), showed highly potent ACAT inhibitory activity (rabbit small intestine IC_{50} = 0.020 μM, rabbit liver IC_{50} = 0.009 μM), foam cell formation inhibitory activity (rat peritoneal macrophage IC_{50} = 0.030 μM), extremely potent serum cholesterol-lowering activity in cholesterol-fed rats (71% at a dose of 0.3 mg/kg/day po), and good bioavailability in fed dogs (C_{max} = 2.68 μg/mL at 1 h, 10 mg/kg po).

Introduction

Acyl-CoA:cholesterol acyltransferase (ACAT)[1] is the intracellular enzyme responsible for catalyzing the esterification of free cholesterol in various tissues. In a developing atherosclerotic lesion, ACAT-mediated cholesterol esterification is believed to play an important role in the accumulation of cholesteryl esters by macrophages. Therefore, the inhibition of ACAT in the arterial wall is expected to prevent the formation of cholesteryl ester-loaded macrophages (foam cell).[2,3] This enzyme also plays a key role in intestinal dietary cholesterol absorption[4,5] and in the secretion of very low density lipoproteins (VLDL) from the liver.[6,7] For these reasons, a systematically available ACAT inhibitor is an attractive target for new treatments of atherosclerosis and hyperlipidemia.

In recent years, a number of ACAT inhibitors have been reported.[8] As amide class ACAT inhibitors, amide derivatives consist of an aromatic ring moiety and a lipophilic long-chain moiety: CI-976 (**4**)[9] and CP-113818 (**5**)[10] were reported (Figure 1). We have reported that a series of *N*-(7-alkoxy-4-oxochroman-8-yl)amides [*e.g.*, **2** (TEI-6522), **3** (TEI-6657)] had highly potent ACAT inhibitory activities.[11] Structure−activity relationship studies have shown that this *N*-(7-alkoxy-4-oxochroman-8-yl)amide moiety is one of the optimum arylamide moieties for potent ACAT inhibitory activities. The most potent compound, TEI-6522, showed *ca.* 10-fold more potent ACAT inhibitory activities than CI-976 both in vitro and in vivo. The finding that modification of the aryl moiety furnished potent ACAT inhibitors encouraged us to evaluate other novel aryl moieties to find more potent systematically available inhibitors

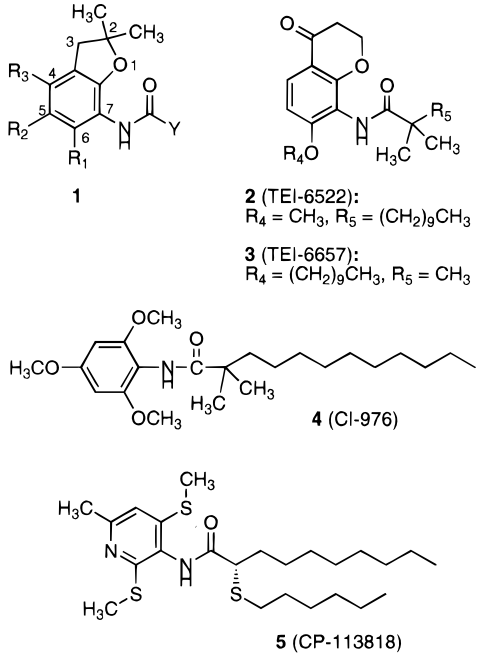

Figure 1. General structures for *N*-(2,2-dimethyl-2,3-dihydrobenzofuran-7-yl)amide derivatives **1** and *N*-(4-oxochroman-8-yl)amide derivatives TEI-6522 (**2**) and TEI-6657 (**3**). ACAT inhibitors: CI-976 (**4**) and CP-113818 (**5**).

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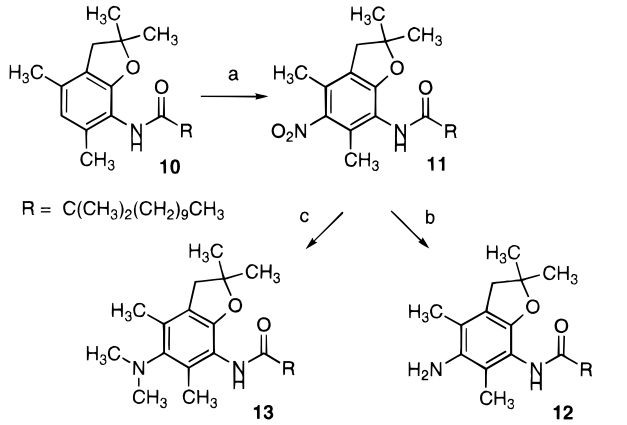
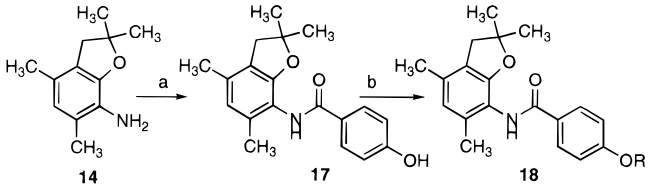
Scheme 1. Preparation of Target Molecules by Method B[a]



[a] Reagents: (a) HNO_3, Ac_2O, 10 °C; (b) H_2 (1 atm), Pd/C (10%), EtOH, 50 °C; (c) H_2 (18 atm), Pd/C (10%), HCHO, H_2O/EtOH, 90 °C.

Scheme 2. Preparation of Target Molecules by Method C[a]



[a] Reagents: (a) 4-hydroxybenzoic acid, DCC, CH_2Cl_2, room temperature; (b) RBr, K_2CO_3, CH_3CN, reflux.

than TEI-6522. We now report on the synthesis and ACAT inhibitory activity of a series of *N*-(2,2-dimethyl-2,3-dihydrobenzofuran-7-yl)amides **1**. The structure−activity relationships of these amides are discussed on the basis of in vitro ACAT inhibitory activity and percent change of serum total cholesterol in cholesterol-fed rats.

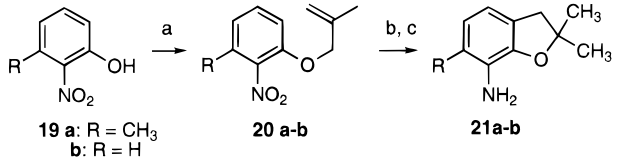
Chemistry

A coupling of the corresponding aromatic amine with the appropriate acid chloride in the presence of triethylamine (method A) was used to synthesize the majority of compounds (general structure **1**) described in this paper. Preparation of compounds **11**−**13**, which have a nitro or an amino group at position 5 of the 2,3-dihydrobenzofuran, was achieved by method B as outlined in Scheme 1. Nitration of *N*-(2,2,4,6-tetramethyl-2,3-dihydrobenzofuran-7-yl)amide **10** afforded *N*-(2,2,4,6-tetramethyl-5-nitro-2,3-dihydrobenzofuran-7-yl)amide **11**, which was reduced by catalytic hydrogenation to the corresponding *N*-(5-amino-2,2,4,6-tetramethyl-2,3-dihydrobenzofuran-7-yl)amide **12**. Reductive methylation of the 5-nitro derivative **11** using formaldehyde to 5-dimethylamino derivative **13** was achieved by catalytic hydrogenation in the presence of Pd/C.

Some benzamide analogs were prepared as shown in Scheme 2 (method C). 7-Amino-2,2,4,6-tetramethyl-2,3-dihydrobenzofuran (**14**) was acylated with 4-hydroxybenzoic acid using DCC as a coupling agent. Alkylation of the resulting 4-hydroxybenzamide with alkyl bromide/K_2CO_3 gave **18**.

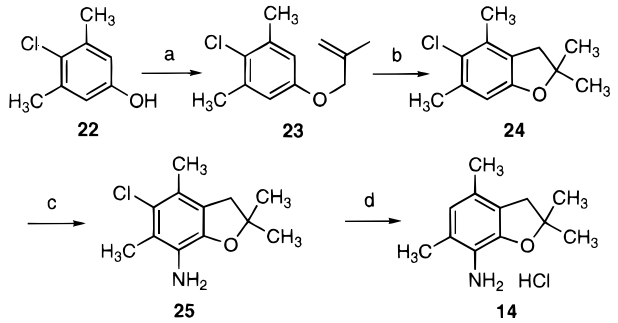
Preparations of 2,3-dihydrobenzofuranylamine intermediates which were used in methods A and C are outlined in Schemes 3 and 4. 7-Amino-2,2-dimethyl-2,3-dihydrobenzofuran (**21b**) and 7-amino-2,2,6-trimethyl-2,3-dihydrobenzofuran (**21a**) were prepared as shown in Scheme 3[12] from the corresponding 2-nitrophenols **19a,b** by (a) *O*-methallylation using NaOH, (b) cyclization at 180 °C in the presence of $MgCl_2$, and (c) catalytic hydrogenation in the presence of Pd/C. 7-Amino-2,2,4,6-tetramethyl-2,3-dihydrobenzofuran (**14**) was synthesized as shown in Scheme 4, that is, methallylation of 4-chloro-3,5-xylenol (**22**) with 3-chloro-2-methylpropene gave *O*-methallylphenol **23**. Cyclization of **23** at 210 °C using $MgCl_2$ afforded 5-chloro-2,2,4,6-tetramethyl-2,3-dihydrobenzofuran (**24**). Nitration of the 2,3-dihydrobenzofuran followed by reduction of the nitro group by catalytic hydrogenation in the presence of Pd/C under a mild condition gave 7-amino-5-chloro-2,2,4,6-tetramethyl-2,3-dihydrobenzofuran (**25**). It was necessary to carry out the reduction under stronger conditions (H_2, 18 atm, 120 °C) in order to complete hydrogenolysis of the C−Cl bond at position 5 to give the 7-amino-2,2,4,6-tetramethyl-2,3-dihydrobenzofuran (**14**). To develop a useful process for the reduction under mild conditions, electrochemical reduction was examined. Electroreduction of the 5-chloro-7-nitro-2,2,4,6-tetramethyl-2,3-dihydrobenzofuran in DMF−^{t}BuOH/Bu_4NBF_4/Zn−Zn system effected conversion of the nitro group into amino group and ensuing removal of the chlorine atom in one pot to give **14** (Scheme 5).[13]

Scheme 3. Preparation of 7-Amino-2,2-dimethyl-2,3-dihydrobenzofurans[a]



[a] Reagents: (a) 3-chloro-2-methylpropene, NaOH/H_2O; (b) $MgCl_2$, 180 °C; (c) H_2 (1 atm), Pd/C (5%), EtOH.

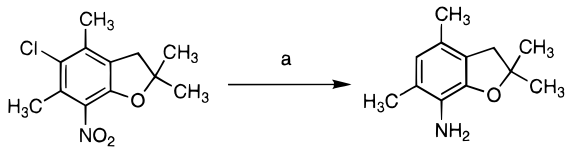
Scheme 4. Preparation of 7-Amino-2,2,4,6-tetramethyl-2,3-dihydrobenzofuran[a]



[a] Reagents: (a) 3-chloro-2-methylpropene, $NaOCH_3$/CH_3OH; (b) $MgCl_2$, 210 °C; (c) (1) HNO_3, Ac_2O, room temperature, (2) H_2 (1 atm), Pd/C (5%), room temperature; (d) H_2 (18 atm), Pd/C (10%), EtOH, 115 °C.

The acid chlorides used in the general method A were prepared as follows. α,α-Dimethyl carboxylic acids were prepared by alkylation of the dianion[14] of isobutyric acid by treatment with 2 equiv of LDA. Alternatively, alkylation of the anion of methyl isobutyrate by treatment with 1 equiv of LDA followed by hydrolysis gave the carboxylic acids. The acid chlorides were prepared from the corresponding carboxylic acids by treatment with thionyl chloride. 4-Alkoxybenzoyl chlorides were prepared from methyl 4-hydroxybenzoate.

Scheme 5. Preparation of 7-Amino-2,2,4,6-tetramethyl-2,3-dihydrobenzofuran[a]



14

[a] Reagents: (a) electroreduction, DMF−^{t}BuOH/Bu_4NBF_4/Zn−Zn.

Results and Discussion

The structures and biological activities of the synthesized compounds are shown in Tables 1−4. The ACAT inhibitory activities were determined using [1-^{14}C]-oleoyl-CoA as an enzyme substrate and small intestinal microsomes from cholesterol-fed rabbits as an enzyme source. The IC_{50}, which is the concentration that inhibited 50% of the enzyme activity, was determined for all compounds. Serum total cholesterol-lowering activity was assessed in the cholesterol-fed animal model in which male Wistar rats were administered an oral dose of a tested compound once a day and then allowed to consume overnight a diet supplemented with cholesterol (2%), cholic acid (1%), casein (20%), sucrose (45%), coconut oil (12%), KC flock (4%), vitamin mixture (1%), mineral mixture (7%), and dried fish powder (8%). After 3 days of oral administration and cholesterol feeding, the serum total cholesterol was measured, and the percent change vs the difference between the control and normal levels was determined. These assays were carried out by the method reported.[11]

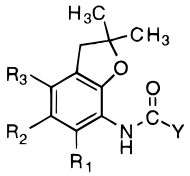
The compounds listed in Table 1 were synthesized to study the effects of substituents on the 2,3-dihydrobenzofuran ring of the *N*-(2,2-dimethyl-2,3-dihydrobenzofuran-7-yl)amides. We used the acyl moieties A−C listed in this table. Roth *et al.* reported[9b] that the anilides of saturated fatty acids possessing group A, B, or C were found to have ACAT inhibitory activities in the series of *N*-(2,4,6-trimethoxyphenyl)amides. Group B was reported to have reduced activity in vivo in spite of having potent activity in vitro. On the other hand, we reported in our previous paper[11] that 2,2-dimethyldodecanamides have potent in vitro and in vivo activity in the series of *N*-(7-alkoxy-4-oxochroman-8-yl)amides. For these reasons, these acyl structures A−C were selected to examine the potential of novel aryl moieties. Using these acyl structures A−C, *N*-(2,2-dimethyl-2,3-dihydrobenzofuran-7-yl)amides were synthesized.

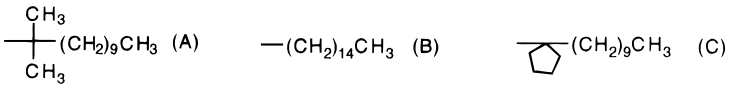
A methyl group at the 6-position of the 2,3-dihydrobenzofuran moiety was important for potent ACAT inhibitory activity in the series of *N*-(2,2-dimethyl-2,3-dihydrobenzofuran-7-yl)amide derivatives. Introduction of a methyl group at the 6-position of the *N*-(2,2-dimethyl-2,3-dihydrobenzofuran-7-yl)amide derivatives **30** and **31** increased in vitro activity (**32** and **33**). Introduction of an additional methyl group at the 4-position of the 2,3-dihydrobenzofuran moiety (**34** and **35**) retained potent activity in vitro. *N*-(2,2,4,6-Tetramethyl-2,3-dihydrobenzofuran-7-yl)-2,2-dimethyldodecanamide (**10**) showed potent in vitro activity and lowered serum cholesterol 61% at a dose of 1.0 mg/kg/day. Thus, it became obvious that the *N*-(2,2,4,6-tetramethyl-2,3-dihydrobenzofuran-7-yl)amide is an optimal structure for potent ACAT inhibitory activity.

Next, effects of substituents at the 5-position of the 2,3-dihydrobenzofuran (R_2) were examined. A chloro (**36**), nitro (**11**), and amino (**12**) group introduced at this position retained potent activity, while a dimethylamino group introduced at this position (**13**) resulted in highly potent activity. **13** also showed more potent in vivo cholesterol-lowering activity (81% at a dose of 1.0 mg/kg/day) than the nonsubstituted (R_2 = H) analog **10**.

On the basis of our study of the *N*-(7-alkoxy-4-oxochroman-8-yl)amides,[11] we anticipated that molecular lipophilicity of the *N*-(2,2,4,6-tetramethyl-2,3-dihydrobenzofuran-7-yl)amide derivatives might be a important factor for potent ACAT inhibitory activity. To examine the effect of the acyl moiety (-CO-Y group in general structure **1**), the analogs having a lipophilic group in the acyl moiety were synthesized; the results are shown in Tables 2−4. The 2,2,4,6-tetramethyl-2,3-

Table 1. Chemical Properties and Biological Data for *N*-(2,2-Dimethyl-2,3-dihydrobenzofuran-7-yl)amides

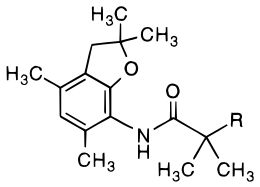


Y = 

compd	R_1	R_2	R_3	Y	yield, %[a] (method)	mp, °C	formula[b]	ACAT inhibition IC_{50}, μM[c]	change TC,[d,e] % (mg/kg)[f]
30	H	H	H	B	90 (A)	90−92	$C_{26}H_{43}NO_2$	0.89	
31	H	H	H	C	91 (A)	oil	$C_{26}H_{41}NO_2$	0.50	
32	CH_3	H	H	B	82 (A)	76−77	$C_{27}H_{45}NO_2$	0.024	
33	CH_3	H	H	C	96 (A)	97−107	$C_{27}H_{43}NO_2$	0.24	−52 (10)
34	CH_3	H	CH_3	B	89 (A)	84−86	$C_{28}H_{47}NO_2$	0.026	
35	CH_3	H	CH_3	C	73 (A)	65−66	$C_{28}H_{45}NO_2$	0.17	
10	CH_3	H	CH_3	A	92 (A)	57−59	$C_{26}H_{43}NO_2$	0.042	−61 (1)
36	CH_3	Cl	CH_3	C	98 (A)	69−70	$C_{28}H_{44}NO_2Cl$	0.29	
11	CH_3	NO_2	CH_3	A	58 (B-a)	87−90	$C_{26}H_{42}N_2O_4$	0.036	
12	CH_3	NH_2	CH_3	A	72 (B-b)	125	$C_{26}H_{44}N_2O_2$	0.059	
13	CH_3	$N(CH_3)_2$	CH_3	A	70 (B-c)	87−88	$C_{28}H_{48}N_2O_2$	0.020	−88 (1)

[a] Yield (%) of final step. [b] Satisfactory elemental analyses were obtained for C, H, and N unless otherwise indicated. [c] IC_{50} (μM) for the enzyme obtained from rabbit intestine microsomes. [d] Serum cholesterol-lowering activity in the cholesterol-fed rat expressed as the ratio of the observed reduction to the difference between the control and base-line levels × 100. [e] Compounds with no change indicated were not tested. [f] Dose: compounds were administered orally to rats at the indicated dose once a day for 3 days.

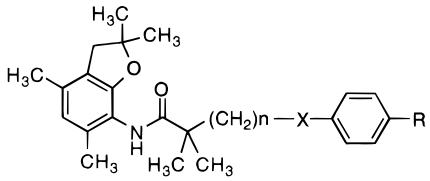
Table 2. Chemical Properties and Biological Data for *N*-(2,2,4,6-Tetramethyl-2,3-dihydrobenzofuran-7-yl)amides



compd	R	yield, %[a] (method)	mp, °C	formula[b]	ACAT inhibition IC_{50}, μM[c]	change TC,[d,e] % (mg/kg)[f]
37	$(CH_2)_7CH_3$	93 (A)	81−82	$C_{24}H_{39}NO_2$	0.14	
10	$(CH_2)_9CH_3$	92 (A)	57−59	$C_{26}H_{43}NO_2$	0.042	−61 (1)
38	$(CH_2)_{11}CH_3$	68 (A)	36−38	$C_{28}H_{47}NO_2$	0.033	
39	$(CH_2)_6OCH_2CH(CH_3)_2$	66 (A)	45−47	$C_{26}H_{43}NO_3$	0.059	−62 (10)
40	$(CH_2)_6$-piperidin-1-yl	26 (A)	177−180	$C_{27}H_{44}N_2O_2$·HCl	>0.5	

[a−f] See corresponding footnotes of Table 1.

Table 3. Chemical Properties and Biological Data for *N*-(2,2,4,6-Tetramethyl-2,3-dihydrobenzofuran-7-yl)amides



compd	*n*	X	R	yield, %[a] (method)	mp, °C	formula[b]	ACAT inhibition IC_{50}, μM[c]	change TC,[d,e] % (mg/kg)[f]
41	4	bond	H	29 (A)	73−74	$C_{26}H_{35}NO_2$	0.21	
42	5	bond	H	78 (A)	69−71	$C_{27}H_{37}NO_2$	0.12	−66 (10)
43	6	bond	H	85 (A)	51−52	$C_{28}H_{39}NO_2$	0.081	
44	3	bond	$CH_2CH(CH_3)_2$	82 (A)	98−100	$C_{29}H_{41}NO_2$	0.040	−57 (3)
45	3	O	Cl	75 (A)	117	$C_{25}H_{32}NO_3Cl$	0.26	
46	4	O	Cl	87 (A)	91−92	$C_{26}H_{34}NO_3Cl$	0.33	−61 (10)
47	5	O	Cl	83 (A)	90−91	$C_{27}H_{36}NO_3Cl$	0.15	−64 (1)
48	5	O	H	71 (A)	108	$C_{27}H_{37}NO_3$	0.086	
49	4	OCH_2	H	54 (A)	78−79	$C_{27}H_{37}NO_3$	0.40	−50 (10)
50	6	O	Cl	75 (A)	72−73	$C_{28}H_{38}NO_3Cl$	0.076	−75 (1)
51	6	O	CH_3	38 (A)	79−80	$C_{29}H_{41}NO_3$	0.050	

[a−f] See corresponding footnotes of Table 1.

dihydrobenzofuran-7-yl group was selected as the common aryl moiety.

The results of α,α-dimethylalkanoic acid amides are shown in Table 2. As the R group shown in this table, alkyl groups in the C_{10}−C_{12} range functioned well for ACAT inhibitory activity. A branched alkoxyl group introduced into the R group to increase metabolic stability (**39**) retained activity in vitro but resulted in less in vivo activity. Introduction of the 1-piperidinyl group into the R group to increase water solubility (**40**) resulted in a dramatic loss of activity.

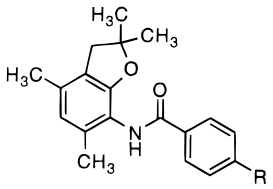
The compounds listed in Table 3 were synthesized to examine the effect of introduction of a benzene ring into the acyl moiety. In the series of phenylalkyl analogs (X = bond), when the acyl moiety had a lipophilic group with a range of more than C_6 in addition to the benzene ring (**43**), potent in vitro activities were obtained. A benzene ring introduced in the middle of the alkyl moiety (**44**) retained potent in vitro activity as compared with the corresponding 2,2-dimethyldodecanamide **10**; however, it showed reduced in vivo activity.

In the series of phenoxyalkyl analogs (X = O), the in vitro activity was increased depending on the length of the alkyl chain, in the order of caron number of 3 (**45**) = 4 (**46**) < 5 (**47**) < 6 (**50**). Substituents on the benzene ring of the phenoxyalkyl group, such as *p*-chloro and *p*-methyl, did not affect the activity. (Benzyloxy)alkyl analog **49** (X = OCH_2) showed reduced activity compared with the phenoxyalkyl analog **48**. In the series of phenoxyalkyl analogs, **47** and **50** showed similar potent in vivo cholesterol-lowering activity to the corresponding 2,2-dimethyldodecanamides **10**. Thus, it became obvious that the 8-(4-chlorophenoxy)-2,2-dimethyloctanamide (*n* = 6) is an optimal structure for potent in vivo activity.

The effects of substituents on the benzene ring of *N*-(2,2,4,6-tetramethyl-2,3-dihydrobenzofuran-7-yl)benzamides were examined; the results are shown in Table 4. The benzamide derivatives were found to have potent ACAT inhibitory activity in vitro, when they had a highly lipophilic substituent like decyloxy (**53**) at the para position. However, **53** was inactive in vivo in spite of potent activity in vitro. Interestingly, the 4-[[6-(4-chlorophenoxy)hexyl]oxy] group, which showed potent activity in the case of *N*-(7-methoxy-4-oxochroman-8-yl)benzamide,[11] showed rather reduced activity. These results suggest that the benzamide structure was not beneficial for *N*-(2,2,4,6-tetramethyl-2,3-dihydrobenzofuran-7-yl) derivatives.

Selected compounds, with **2** and CI-976, were further evaluated for the ability to inhibit rabbit liver ACAT and acetyl-LDL-induced cholesteryl ester accumulation in rat peritoneal macrophages (foam cell formation). Assays of these activities were carried out by using the

Table 4. Chemical Properties and Biological Data for *N*-(2,2,4,6-Tetramethyl-2,3-dihydrobenzofuran-7-yl)benzamides



compd	R	yield, %[a] (method)	mp, °C	formula[b]	ACAT inhibition IC_{50}, μM[c]	change TC,[d,e] % (mg/kg)[f]
52	$O(CH_2)_7CH_3$	79 (C)	85−88	$C_{27}H_{37}NO_3$	>0.2	
53	$O(CH_2)_9CH_3$	99 (A)	97−98	$C_{29}H_{41}NO_3$	0.030	0 (3)
54	$O(CH_2)_{11}CH_3$	99 (C)	57−60	C_{31}[g]$H_{45}NO_3$	0.090	
55	$O(CH_2)_6O(CH_2)_2CH_3$	98 (C)	69−71	$C_{28}H_{39}NO_4$	>0.2	
56	$O(CH_2)_6OC_6H_4$(4-Cl)	86 (A)	135−136	$C_{31}H_{36}NO_4Cl$	>0.2	

[a−f] See corresponding footnotes of Table 1. [g] Calcd, 77.62; found, 78.14.

Table 5. Biological Activities of Selected Compounds

compd	ACAT inhibition IC_{50}, μM: rabbit intestine[a]	ACAT inhibition IC_{50}, μM: rabbit liver[b,c]	hypocholesterolemic activity (rat) change TC,[d] % (mg/kg)[e]	foam cell formation inhibition (rat) IC_{50}, μM[f]
50	0.076		−64 (0.3)	1.5
10	0.042		−44 (0.3)	0.65
13 (TEI-6620)	0.020	0.009	−71 (0.3)	0.030
2 (TEI-6522)	0.013	0.016	−66 (0.3)	0.16
CI-976	0.14	0.149	−50 (3.0)	3.8

[a] IC_{50} (μM) for the enzyme obtained from rabbit intestine microsomes. [b] IC_{50} (μM) for the enzyme obtained from rabbit liver microsomes. [c] Compounds with no IC_{50} indicated were not tested. [d] Serum total cholesterol-lowering activity in the cholesterol-fed rat expressed as the ratio of the observed reduction to the difference between the control and normal levels × 100. [e] Dose: compounds were administered orally to rats at the indicated dose once a day for 3 days. [f] IC_{50} (μM) for acetyl-LDL-induced cholesteryl ester accumulation in rat peritoneal macrophages.

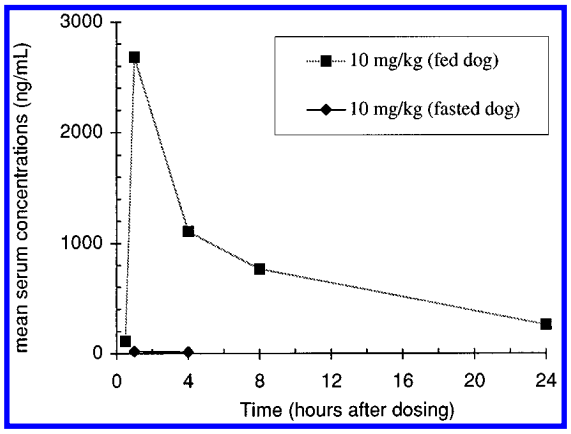


Figure 2. Serum concentrations of **13** in dogs.

method described.[11] The results of these in vitro assays and the in vivo effect on total plasma cholesterol concentrations are summarized in Table 5. In vitro, **13** displayed high ACAT inhibitory potency (rabbit intestine $IC_{50} = 0.020$ μM, rabbit liver $IC_{50} = 0.009$ μM) and foam cell formation inhibitory activity (rat peritoneal macrophage $IC_{50} = 0.030$ μM). Under the same conditions, the IC_{50} value of CI-976 was 0.14 μM (rabbit intestine), 0.149 μM (rabbit liver), and 3.8 μM (rat peritoneal macrophage), respectively. Furthermore, **13** was *ca.* 10-fold more potent with respect to hypocholesterolemic activity in vivo than CI-976, that is, **13** lowered serum cholesterol 71% at a dose of 0.3 mg/kg/day in cholesterol-fed rats. Under the same conditions, CI-976 lowered serum cholesterol 25% at a dose of 1.0 mg/kg/day and 50% at a dose of 3.0 mg/kg/day.

Serum concentrations of **13** were measured in dogs ($n = 2$) following a single oral administration of the drug suspended in 0.5% methyl cellulose solution at a dose of 10 mg/kg. As shown in Figure 2, the bioavailability of **13** was found to increase dramatically in the fed state as compared with the fasted state. The peak serum concentration in fed dogs was 2.68 μg/mL at 1 h after oral administration. Even at the 24 h point, the serum concentration of **13** (260 ng/mL) exceeded its in vitro rat foam cell formation IC_{50} concentration. These results suggest that **13** has potential to inhibit foam cell formation in the developing atherosclerotic lesion.

Conclusion

This study has identified a novel series of *N*-(2,2,4,6-tetramethyl-2,3-dihydrobenzofuran-7-yl)amides with potent ACAT inhibitory activities. Examination of structure−activity relationships in this series revealed the following features. (1) The methyl group at position 6 of the 2,3-dihydrobenzofuran was essential for potent activity. (2) Introduction of a dimethylamino group at position 5 of the 2,3-dihydrobenzofuran moiety resulted in highly potent activity. (3) The necessary factor to elicit the potent ACAT inhibitory activity was high lipophilicity of the molecules.

Introduction of a lipophilic group at the acyl moiety, except for the benzamide analogs, resulted in potent activity. Among the synthesized derivatives, *N*-[5-(dimethylamino)-2,2,4,6-tetramethyl-2,3-dihydrobenzofuran-7-yl]-2,2-dimethyldodecanamide (**13**) showed highly potent ACAT inhibitory activity (rabbit small intestine $IC_{50} = 0.020$ μM, rabbit liver $IC_{50} = 0.009$ μM) and extremely potent serum cholsterol-lowering activity in cholesterol-fed rats (71% at a dose of 0.3 mg/kg/day po, *ca.* 10-fold more potent than CI-976). This compound was the most potent inhibitor of foam cell formation (rat

peritoneal macrophage $IC_{50} = 0.030\ \mu M$, 127-fold more potent than CI-976) among the synthesized compounds including our earlier studies [*N*-(4-oxochroman-8-yl)-amides].

The preliminary pharmacokinetics data showed that **13** had improved bioavailability (fed dogs, $C_{max} = 2.68\ \mu g/mL$ at 1 h, 10 mg/kg po) in comparison with our earlier oxochroman compound **2**. On the basis of these findings, we conclude that **13** has an excellent profile for development as therapeutic agent for the treatment of hyperlipidemia and atherosclerosis. **13** was selected for further pharmacological and toxicological studies. A detailed study of **13** in WHHL rabbits, as well as studies of **13** in several cholesterol-fed animals, will be the subjects of future papers from this laboratory.

Experimental Section

Melting points were determined on a Yanagimoto micromelting point apparatus and are uncorrected. Infrared spectra were recorded on a JASCO FT/IR-5300 spectrometer. NMR spectra were acquired in the indicated solvent on a JEOL-EX270, JEOL-GX400 FTNMR, or Hitachi R-90H spectrometer with Me_4Si as an internal standard. Elemental analyses were performed by Toray Research Center Inc. or the Analytical Research Department of Teijin Ltd. Where analyses are indicated only by symbols of the elements, analytical results are within ±0.4% of the theoretical values. Thin layer chromatography (TLC) was performed on E. Merck Kieselgel 60 F-254 plates. Column chromatography was performed with E. Merck silica gel 60 (40−63 μm, 230−400 mesh) under low pressure. Unless otherwise noted, materials were obtained from a commercial source and used without further purification. CI-976 was synthesized in our laboratory following the published procedure[9b] and characterized by NMR and elemental analysis (C, H, N).

Assay of in Vitro ACAT Inhibitory Activity of Rabbit Intestinal Microsomes. ACAT inhibitory activity of rabbit intestine in vitro was evaluated with rabbit intestinal mucosa microsome as reported.[11]

Assay of in Vitro ACAT Inhibitory Activity of Rabbit Liver. ACAT inhibitory activity of rabbit liver in vitro was evaluated with rabbit liver microsome as reported.[11]

Assay of Percent Change of Serum Total Cholesterol Level. Serum total cholesterol-lowering activity was determined in cholesterol-fed rats. The experimental details of the in vivo assay have been described.[11]

Assay of Inhibitory Activity of Macrophage Foam Cell Formation. Peritoneal macrophages were prepared from rats and used for IC_{50} determinations of acetyl-human-LDL-induced cellular cholesteryl ester accumulation as described.[11]

5-Chloro-2,2,4,6-tetramethyl-7-nitro-2,3-dihydrobenzofuran. Sodium methoxide (296 g, 28% solution in methanol, 1.2 equiv) and 3-chloro-2-methyl-1-propene (200 g, 1.73 equiv) were added to a solution of 4-chloro-3,5-xylenol (200 g, 1.28 mol) in methanol (200 mL). The mixture was heated to 70 °C with stirring for 24 h. After the reaction mixture was cooled, the solvent was removed under reduced pressure. H_2O (500 mL) was added to the residue, and the mixture was extracted with ethyl acetate (500 mL). The organic layer was washed with 5 N NaOH (500 mL), H_2O (500 mL), and brine (100 mL) and dried over $MgSO_4$. The solvent was removed under reduced pressure to give crude *O*-methallyl-4-chloro-3,5-xylenol (**23**): 263.66 g, pale yellow oil; ^1H NMR ($CDCl_3$, 90 MHz) δ 1.81 (s, 3 H), 2.33 (s, 6 H), 4.37 (s, 2 H), 4.98 (br, 1 H), 5.06 (br, 1 H), 6.65 (s, 2 H).

To the crude **23** (262 g) was added 100 g of anhydrous $MgCl_2$, and the mixture was heated at 210 °C with stirring for 18 h. Ethyl acetate (500 mL) was added to the cooled reaction mixture, and $MgCl_2$ was removed by filtration through Celite and washed with ethyl acetate (200 mL). The combined filtrate was washed successively with 2 N HCl (300 mL), 2 N NaOH (500 mL), H_2O (100 mL), and brine (100 mL) and dried over $MgSO_4$. The solvent was removed under reduced pressure to give crude 5-chloro-2,2,4,6-tetramethyl-2,3-dihydrobenzofuran (**24**): 225.2 g, pale red oil; ^1H NMR ($CDCl_3$, 270 MHz) δ 1.46 (s, 6 H), 2.23 (s, 3 H), 2.31 (s, 3 H), 2.93 (s, 2 H), 6.48 (s, 1 H).

The crude **24** (63.21 g) was dissolved in 160 mL of acetic anhydride, and 28.35 g of nitric acid (70%, 1.05 equiv) was slowly added with stirring at 0−15 °C (ice−water bath). The reaction mixture was stirred for 0.5 h at room temperature and then poured into ice−water (600 mL). The precipitated product was filtered, washed with water (3 × 300 mL), and dried. Recrystallization from EtOH (500 mL) afforded 5-chloro-2,2,4,6-tetramethyl-7-nitro-2,3-dihydrobenzofuran: 48.57 g, 63% yield from crude **24**, pale yellow solid; mp 114.7−115.3 °C; ^1H NMR ($CDCl_3$, 270 MHz) δ 1.52 (s, 6 H), 2.28 (s, 3 H), 2.38 (s, 3 H), 3.01 (s, 2 H).

7-Amino-5-chloro-2,2,4,6-tetramethyl-2,3-dihydrobenzofuran (25). A solution of 5-chloro-2,2,4,6-tetramethyl-7-nitro-2,3-dihydrobenzofuran (800 mg, 3.13 mmol) in EtOH (10 mL) was hydrogenated at 1 atm for 3 h in the presence of 5% palladium on charcoal (300 mg) at room temperature. The catalyst was removed by filtration through Celite and washed with EtOH (30 mL). The combined filtrate was evaporated to give a crude solid which was purified by silica gel column chromatography (hexane−AcOEt) to give 7-amino-5-chloro-2,2,4,6-tetramethyl-2,3-dihydrobenzofuran (**25**): 494 mg, 70.0% yield, colorless needles; mp 90−91 °C; ^1H NMR ($CDCl_3$, 270 MHz) δ 1.47 (s, 6 H), 2.17 (s, 3 H), 2.22 (s, 3 H), 2.95 (s, 2 H), 3.45 (br, 2 H).

7-Amino-2,2,4,6-tetramethyl-2,3-dihydrobenzofuran Hydrochloride (14). In a 500 mL autoclave were placed 5-chloro-2,2,4,6-tetramethyl-7-nitro-2,3-dihydrobenzofuran (20.00 g, 78.2 mmol), 10% palladium on charcoal (6.0 g), and EtOH (160 mL). The mixture was hydrogenated at 18 atm for 12 h at 115 °C. The catalyst was removed by filtration through Celite and washed with EtOH (100 mL). To the combined filtrate was added 6 N HCl solution in EtOH (20 mL), and the solvent was removed under reduced pressure to give crude hydrchloride. The crude product was recrystallized (from CH_2Cl_2−AcOEt, 1:2) to give 7-amino-2,2,4,6-tetramethyl-2,3-dihydrobenzofuran hydrochloride (**14**): 14.02 g, 78.8% yield, pale yellow needles; mp 202.2−204.8 °C; ^1H NMR ($CDCl_3$ 90 MHz) δ 1.49 (s, 6 H), 2.13 (s, 3 H), 2.57 (s, 3 H), 2.91 (s, 2 H), 3.47 (s, 3 H), 6.45 (s, 1 H). Anal. ($C_{12}H_{18}NOCl$) C, H, N.

7-Amino-2,2,4,6-tetramethyl-2,3-dihydrobenzofuran (free base) was prepared from hydrochloride **14**. Saturated $NaHCO_3$ aqueous solution (50 mL) was added to a solution of **14** (1.0 g, 4.39 mmol) in CH_2Cl_2 (50 mL). Extraction with CH_2Cl_2, drying over $MgSO_4$, evaporation of the solvent, and column chromatography (hexane−AcOEt, 10:1) gave 7-amino-2,2,4,6-tetramethyl-2,3-dihydrobenzofuran: 798 mg, 95% yield, colorless needles; mp 37.5 °C; ^1H NMR ($CDCl_3$, 270 MHz) δ 1.47 (s, 6 H), 2.10 (s, 3 H), 2.13 (s, 3 H), 2.91 (s, 2 H), 3.33 (br s, 2 H), 6.40 (s, 1 H).

7-Amino-2,2,6-trimethyl-2,3-dihydrobenzofuran (21a). 3-Chloro-2-methyl-1-propene (7.69 g, 1.3 equiv) was added to a solution of 3-methyl-2-nitrophenol (10 g, 65.3 mmol) and NaOH (2.16 g, 1.0 equiv) in H_2O (20 mL). The mixture was heated to 90 °C with stirring for 6 h. After the reaction mixture was cooled, H_2O (50 mL) was added and the mixture was extracted with ethyl acetate (100 mL). The organic layer was washed with H_2O (10 mL) and brine (10 mL) and dried over $MgSO_4$. The solvent was removed under reduced pressure to give an oil which was purified by distillation under reduced pressure to give *O*-methallyl-3-methyl-2-nitrophenol (**20a**): 8.05 g, 60% yield, yellow oil; bp 78−82 °C/0.1 mmHg; ^1H NMR ($CDCl_3$, 90 MHz) δ 1.78 (s, 3 H), 2.30 (s, 3 H), 4.50 (s, 2 H), 4.9−5.1 (m, 2 H), 6.83 (d-like, 2 H, $J = 7.9$ Hz), 7.15−7.4 (m, 1 H).

To **20a** (7.0 g, 33.78 mmol) was added 322 mg of anhydrous $MgCl_2$, and the mixture was heated at 180 °C with stirring for 5 h. Ethyl acetate (50 mL) was added to the cooled reaction mixture, and $MgCl_2$ was removed by filtration through Celite and washed with ethyl acetate (20 mL). The combined filtrate was washed with H_2O (20 mL) and brine (20 mL) and dried over $MgSO_4$. The solvent was removed under reduced pressure to give an oil which was purified by column chromatography

(hexane−AcOEt, 12:1) to give 2,2,6-trimethyl-7-nitro-2,3-dihydrobenzofuran: 2.26 g, 32% yield, pale yellow oil; ^{1}H NMR ($CDCl_3$, 90 MHz) δ 1.52 (s, 6 H), 2.39 (s, 3 H), 3.03 (s, 2 H), 6.68 (d, 1 H, J = 7.5 Hz), 7.12 (d, 1 H, J = 7.5 Hz).

A solution of 2,2,6-trimethyl-7-nitro-2,3-dihydrobenzofuran (2.10 g, 10.1 mmol) in EtOH (30 mL) was hydrogenated at 1 atm for 15 h in the presence of 5% palladium on charcoal (600 mg) at room temperature. The catalyst was removed by filtration through Celite and washed with EtOH (30 mL). The combined filtrate was evaporated to give an oil which was purified by silica gel column chromatography (hexane−AcOEt, 10:1) to give 7-amino-2,2,6-trimethyl-2,3-dihydrobenzofuran (**21a**): 1.40 g, 78% yield, pale red oil; ^{1}H NMR ($CDCl_3$ 270 MHz) δ 1.47 (s, 6 H), 2.15 (s, 3 H), 2.98 (s, 2 H), 3.46 (br, 2 H), 6.51 (d, 1 H, J = 7.6 Hz), 6.57 (d, 1 H, J = 7.6 Hz).

***N*-(2,2,4,6-Tetramethyl-2,3-dihydrobenzofuran-7-yl)-2,2-dimethyldodecanamide (10). Method A.** 2,2-Dimethyldodecanoyl chloride (247 mg, 1.00 mmol) was slowly added to a solution of 7-amino-2,2,4,6-tetramethyl-2,3-dihydrobenzofuran hydrochloride (**14**; 233 mg, 1.02 mmol) and triethylamine (0.28 mL, 2.0 mmol) in CH_2Cl_2 (3.0 mL) with stirring. The reaction mixture was stirred for 3 h at room temperature, and then 2 N HCl (15 mL) was added. The organic layer was separated, and the water layer was further extracted with AcOEt (2 × 25 mL). The combined extracts were washed with brine (25 mL) and dried over $MgSO_4$. The solvent was removed under reduced pressure to give a solid which was purified by column chromatography (hexane−AcOEt, 9:1) to give *N*-(2,2,4,6-tetramethyl-2,3-dihydrobenzofuran-7-yl)-2,2-dimethyldodecanamide (**10**): 370 mg, 92% yield, white solid; mp 57−58.5 °C; ^{1}H NMR ($CDCl_3$, 270 MHz) δ 0.88 (t, 3 H, J = 6.6 Hz), 1.2−1.5 (m, 16 H), 1.26 (s, 6 H), 1.43 (s, 6 H), 1.57 (m, 2 H), 2.13 (s, 3 H), 2.14 (s, 3 H), 2.89 (s, 2 H), 6.50 (s, 1 H), 6.79 (br, 1 H). Anal. ($C_{26}H_{43}NO_2$) C, H, N.

***N*-(2,2,4,6-Tetramethyl-5-nitro-2,3-dihydrobenzofuran-7-yl)-2,2-dimethyldodecanamide (11). Method B.** 2,2-Dimethyldodecanoyl chloride (65.03 g, 264 mmol) was slowly added to a solution of 7-amino-2,2,4,6-tetramethyl-2,3-dihydrobenzofuran hydrochloride (**14**; 60.00 g, 263 mmol) and triethylamine (58.66 g, 2.2 equiv) in CH_2Cl_2 (600 mL) with stirring at 10 °C (ice bath). The reaction mixture was stirred for 3 h at room temperature and then cooled with an ice bath; 2 N HCl (200 mL) was added to the reaction mixture, the organic layer was separated, and the water layer was further extracted with CH_2Cl_2 (50 mL). The combined extracts were washed with H_2O (150 mL) and brine (50 mL) and dried over $MgSO_4$. The solvent was removed under reduced pressure to give *N*-(2,2,4,6-tetramethyl-2,3-dihydrobenzofuran-7-yl)-2,2-dimethyldodecanamide (**10**): 104.80 g.

The crude **10** (104.80 g, 261 m mol) was dissolved in 500 mL of acetic anhydride, and 24.7 g of nitric acid (70%, 1.05 equiv) was slowly added with stirring at 0−15 °C (ice−water bath). The reaction mixture was stirred for 3 h at 10 °C and then poured into ice−water (1 L). The mixture was extracted with AcOEt (1 L). The organic layer was washed with H_2O (2 × 1 L), and 500 mL of H_2O was added. The mixture was basified (pH > 8) by adding K_2CO_3 with vigorous stirring, and the organic layer was separated. The organic layer was washed successively with saturated K_2CO_3 aqueous solution (2 × 500 mL), H_2O (300 mL), and brine (100 mL) and dried over $MgSO_4$. The solvent was removed under reduced pressure to afford a crude solid (120.2 g) which was recrystallized from hexane (200 mL) at 0 °C to give *N*-(2,2,4,6-tetramethyl-5-nitro-2,3-dihydrobenzofuran-7-yl)-2,2-dimethyldodecanamide (**11**): 67.89 g, 57.7% yield, pale yellow solid; mp 87.2−90.0 °C; ^{1}H NMR ($CDCl_3$, 270 MHz) δ 0.88 (t, 3 H, J = 6.6 Hz), 1.2−1.7 (m, 18 H), 1.26 (s, 6 H), 1.47 (s, 6 H), 2.11 (s, 3 H), 2.15 (s, 3 H), 2.97 (s, 2 H), 6.80 (br, 1 H). Anal. ($C_{26}H_{42}N_2O_4$) C, H, N.

***N*-(5-Amino-2,2,4,6-tetramethyl-2,3-dihydrobenzofuran-7-yl)-2,2-dimethyldodecanamide (12). Method B.** A solution of *N*-(2,2,4,6-tetramethyl-5-nitro-2,3-dihydrobenzofuran-7-yl)-2,2-dimethyldodecanamide (**11**; 56.2 g, 126 mmol) in EtOH (600 mL) was hydrogenated at 1 atm for 8 h in the presence of 10% palladium on charcoal (20 g) at 50 °C. The catalyst was removed by filtration through Celite and washed with EtOH (150 mL). The combined filtrate was evaporated. The crude product was recrystallized (from EtOH) to give *N*-(5-amino-2,2,4,6-tetramethyl-2,3-dihydrobenzofuran-7-yl)-2,2-dimethyldodecanamide (**12**): 37.73 g, 72.0% yield, colorless needles; mp 125 °C; ^{1}H NMR ($CDCl_3$, 270 MHz) δ 0.88 (t, 3 H, J = 6.6 Hz), 1.2−1.5 (m, 22 H), 1.40 (s, 6 H), 1.55−1.65 (m, 2 H), 1.99 (s, 3 H), 2.04 (s, 3 H), 2.92 (s, 2 H), 2.7−3.7 (br, 2 H), 6.87 (s, 1 H). Anal. ($C_{26}H_{44}N_2O_2$) C, H, N.

***N*-[5-(Dimethylamino)-2,2,4,6-tetramethyl-2,3-dihydrobenzofuran-7-yl]-2,2-dimethyldodecanamide (13). Method B.** In a 500 mL autoclave were placed *N*-(2,2,4,6-tetramethyl-5-nitro-2,3-dihydrobenzofuran-7-yl)-2,2-dimethyldodecanamide (**11**; 26.30 g, 58.9 mmol), formaldehyde (37% solution in H_2O, 20 g), 10% palladium on charcoal (7.9 g), and EtOH (160 mL). The mixture was hydrogenated at 18 atm for 5 h at 90 °C. The catalyst was removed by filtration through Celite and washed with EtOH (100 mL). The combined filtrate was evaporated to afford a crude solid, which was dissolved in AcOEt (200 mL). The solution was washed successively with saturated $NaHCO_3$ aqueous solution (2 × 60 mL), H_2O (2 × 60 mL), and brine (50 mL) and dried over $MgSO_4$. The solvent was removed under reduced pressure to afford a crude solid (26.25 g) which was purified by short silica gel column chromatography (hexane−AcOEt, 19:1) and recrystallization (CH_3CN-H_2O, 25:2) to give *N*-[5-(dimethylamino)-2,2,4,6-tetramethyl-2,3-dihydrobenzofuran-7-yl]-2,2-dimethyldodecanamide (**13**, TEI-6620): 18.38 g, 70.2% yield, colorless needles; mp 86.7−87.5 °C; ^{1}H NMR ($CDCl_3$, 270 MHz) δ 0.88 (t, 3 H, J = 6.8 Hz), 1.2−1.5 (m, 22 H), 1.42 (s, 6 H), 1.50−1.65 (m, 2 H), 2.09 (s, 3 H), 2.10 (s, 3 H), 2.79 (s, 6 H), 2.89 (s, 2 H), 6.82 (s, 1 H). Anal. ($C_{28}H_{48}N_2O_2$) C, H, N.

***N*-(2,2,4,6-Tetramethyl-2,3-dihydrobenzofuran-7-yl)-4-(octyloxy)benzamide (52). Method C.** 4-Hydroxybenzoic acid (361 mg, 2.61 mmol) was added to a solution of 7-amino-2,2,4,6-tetramethyl-2,3-dihydrobenzofuran (500 mg, 2.61 mmol) and dicyclohexylcarbodiimide (539 mg, 2.61 mmol) in CH_2Cl_2 (20 mL). The reaction mixture was stirred at room temperature for 3 days. Filtration through Celite, evaporation of the solvent, and column chromatography (hexane−AcOEt, 2:1) gave 4-hydroxy-*N*-(2,2,4,6-tetramethyl-2,3-dihydrobenzofuran-7-yl)benzamide (**17**): 600 mg, 73.8% yield, white solid; ^{1}H NMR (DMSO-d_6, 270 MHz) δ 1.41 (s, 6 H), 2.08 (s, 3 H), 2.15 (s, 3 H), 2.91 (s, 3 H), 6.50 (s, 1 H), 6.81 (br d, 2 H, J = 7.4 Hz), 7.85 (br d, 2 H, J = 7.4 Hz), 9.18 (br, 1 H), 9.85 (br, 1 H).

K_2CO_3 (66 mg, 1.5 equiv) and octyl bromide (62 mg, 0.32 mmol) were added to a solution of 4-hydroxy-*N*-(2,2,4,6-tetramethyl-2,3-dihydrobenzofuran-7-yl)benzamide (100 mg, 0.32 mmol) in CH_3CN (2 mL). The mixture was refluxed for 6 h with stirring. H_2O (30 mL) was added to the mixture, and the mixture was extracted with AcOEt (50 mL). The organic layer was washed with brine (10 mL) and dried over $MgSO_4$. The solvent was removed under reduced pressure to give a solid which was purified by column chromatography (hexane−AcOEt, 10:1) to give *N*-(2,2,4,6-tetramethyl-2,3-dihydrobenzofuran-7-yl)-4-(octyloxy)benzamide (**52**): 107 mg, 79% yield, white solid; mp 85−88 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.89 (t, 3 H, J = 6.7 Hz), 1.2−1.6 (m, 16 H), 1.72−1.85 (m, 2 H), 2.16 (s, 3 H), 2.22 (s, 3 H), 2.91 (s, 2 H), 4.01 (t, 2 H, J = 6.6 Hz), 6.56 (s, 1 H), 6.94 (br d, 2 H, J = 8.3 Hz), 7.24 (br, 1 H), 7.86 (br d, 2 H, J = 8.3 Hz). Anal. ($C_{27}H_{37}NO_3$) C, H, N.

1-Decyl-*N*-(2,2-dimethyl-2,3-dihydrobenzofuran-7-yl)-1-cyclopentanecarboxamide (31). This product was prepared in a similar manner to that described in method A using 7-amino-2,2-dimethyl-2,3-dihydrobenzofuran (**21b**; 200 mg, 1.23 mmol) and 1-decyl-1-cyclopentanecarbonyl chloride (367 mg, 1.1 equiv) to give a solid which was purified by column chromatography (hexane−AcOEt) to give **31**: 322 mg, 91% yield, pale yellow oil; ^{1}H NMR ($CDCl_3$, 90 MHz) δ 0.87 (br t, 3 H J = 7.0 Hz), 1.0−1.9 (m, 30 H), 2.0−2.3 (m, 2 H), 3.04 (s, 2 H), 6.7−6.9 (m, 2 H), 7.4 (br 1 H), 8.0−8.15 (m, 1 H). Anal. ($C_{26}H_{41}NO_2$) C, H, N.

1-Decyl-*N*-(2,2,6-trimethyl-2,3-dihydrobenzofuran-7-yl)-1-cyclopentanecarboxamide (33). This product was prepared in a similar manner to that described in method A using 7-amino-2,2,6-trimethyl-2,3-dihydrobenzofuran (**21a**; 100 mg, 0.56 mmol) and 1-decyl-1-cyclopentanecarbonyl chloride (169 mg, 1.1 equiv) to give a solid which was purified by

column chromatography (hexane−AcOEt) to give **33**: 224 mg, 96% yield, white solid; mp 97−107 °C; ^{1}H NMR ($CDCl_3$, 90 MHz) δ 0.87 (br t, 3 H, J = 7.0 Hz), 1.05−1.80 (m, 30 H), 2.0−2.35 (m, 2 H), 2.18 (s, 3 H), 2.97 (s, 2 H), 6.65 (d, 1 H, J = 8.0 Hz), 6.80 (br s, 1 H), 6.89 (d, 1 H, J = 8.0 Hz). Anal. ($C_{27}H_{43}NO_2$) C, H, N.

***N*-(2,2,4,6-Tetramethyl-2,3-dihydrobenzofuran-7-yl)-hexadecanamide (34).** This product was prepared in a similar manner to that described in method A using 7-amino-2,2,4,6-tetramethyl-2,3-dihydrobenzofuran (**14**; 19.4 mg, 0.10 mmol) and palmitoyl chloride (30 mg, 1.1 equiv) to give a solid which was purified by column chromatography (hexane−AcOEt, 10:1) to give **34**: 38.7 mg, 89% yield, white solid; mp 84−85.5 °C; ^{1}H NMR ($CDCl_3$, 90 MHz) δ 0.88 (br t, 3 H, J = 5.0 Hz), 1.05−1.55 (m, 24 H), 1.45 (s, 6 H), 2.15 (s, 6 H), 2.2−2.5 (m, 2 H), 2.90 (s, 2 H), 6.51 (s, 1 H), 6.6 (br, 1 H). Anal. ($C_{28}H_{47}NO_2$) C, H, N.

***N*-(5-Chloro-2,2,4,6-tetramethyl-2,3-dihydrobenzofuran-7-yl)-1-decyl-1-cyclopentanecarboxamide (36).** This product was prepared in a similar manner to that described in method A using 7-amino-5-chloro-2,2,4,6-tetramethyl-2,3-dihydrobenzofuran (**25**; 100 mg, 0.44 mmol) and 1-decyl-1-cyclopentanecarbonyl chloride (133 mg, 1.1 equiv) to give a solid which was purified by column chromatography (hexane−AcOEt) to give **36**: 201 mg, 98% yield, white solid; mp 69−70 °C; ^{1}H NMR ($CDCl_3$, 90 MHz) δ 0.88 (br t, 3 H, J = 7.0 Hz), 1.1−1.8 (m, 30 H), 2.20 (s, 6 H), 2.0−2.35 (m, 2 H), 2.95 (s, 2 H), 6.78 (br, 1 H). Anal. ($C_{28}H_{44}NO_2Cl$) C, H, N.

***N*-(2,2,4,6-Tetramethyl-2,3-dihydrobenzofuran-7-yl)-2,2-dimethyl-8-[(2-methylpropyl)oxy]octanamide (39).** This product was prepared in a similar manner to that described in method A using 7-amino-2,2,4,6-tetramethyl-2,3-dihydrobenzofuran hydrochloride (**14**; 160 mg, 0.70 mmol) and 2,2-dimethyl-8-[(2-methylpropyl)oxy]octanoyl chloride (193 mg, 0.73 mmol) to give a solid that was purified by column chromatography (hexane−AcOEt, 19:1) to give **39**: 192 mg, 66% yield, white solid; mp 45−47 °C; ^{1}H NMR ($CDCl_3$, 90 MHz) δ 0.89 (br t, 6 H, J = 6.5 Hz), 1.1−1.9 (m, 23 H), 2.13 (s, 6 H), 2.88 (s, 2 H), 3.15 (d, 2 H, J = 6.5 Hz), 3.38 (t, 2 H, J = 6.5 Hz), 6.49 (s, 1 H), 6.8 (br s, 1 H). Anal. ($C_{26}H_{43}NO_3$) C, H, N.

***N*-(2,2,4,6-Tetramethyl-2,3-dihydrobenzofuran-7-yl)-2,2-dimethyl-8-piperidin-1-yloctanamide Hydrochloride (40).** This product was prepared in a similar manner to that described in method A using 7-amino-2,2,4,6-tetramethyl-2,3-dihydrobenzofuran hydrochloride (**14**; 128 mg, 0.56 mmol) and 2,2-dimethyl-8-piperidin-1-yloctanoyl chloride (211 mg, 0.86 mmol) to give a solid that was purified by column chromatography ($CHCl_3$−CH_3OH, 8:1) to give the free base of **40**. To an ether solution of this free base (5 mL) was added 3 N HCl solution in ether (1 mL), and the solvent was removed under reduced pressure to give hydrochloride **40**: 68 mg, 26% yield, white solid; mp 177−180 °C; ^{1}H NMR (free base, $CDCl_3$, 90 MHz) δ 1.27−1.65 (m, 16 H), 1.27 (s, 6 H), 1.43 (s, 6 H), 2.12 (s, 6 H), 2.32−2.56 (m, 6 H), 2.88 (s, 2 H), 6.48 (s, 1 H), 6.86 (br, 1 H). Anal. ($C_{27}H_{44}N_2O_2HCl$) C, H, N.

2,2-Dimethyl-*N*-(2,2,4,6-tetramethyl-2,3-dihydrobenzofuran-7-yl)-8-phenyloctanamide (43). This product was prepared in a similar manner to that described in method A using 7-amino-2,2,4,6-tetramethyl-2,3-dihydrobenzofuran hydrochloride (**14**; 200 mg, 0.88 mmol) and 2,2-dimethyl-8-phenyloctanoyl chloride (234 mg, 1.0 equiv) to give a solid which was purified by column chromatography (hexane−AcOEt) to give **43**: 315 mg, 85% yield, white solid; mp 51−52 °C; ^{1}H NMR ($CDCl_3$, 90 MHz) δ 1.27 (s, 6 H), 1.40 (s, 6 H), 1.0−1.8 (m, 10 H), 2.13 (s, 6 H), 2.59 (t, 2 H, J = 6.5 Hz), 2.87 (s, 2 H), 6.48 (s, 1 H), 6.75 (br s, 1 H), 7.1−7.4 (m, 5 H). Anal. ($C_{28}H_{39}NO_2$) C, H, N.

2,2-Dimethyl-*N*-(2,2,4,6-tetramethyl-2,3-dihydrobenzofuran-7-yl)-5-[4-(2-methylpropyl)phenyl]pentanamide (44). This product was prepared in a similar manner to that described in method A using 7-amino-2,2,4,6-tetramethyl-2,3-dihydrobenzofuran hydrochloride (**14**; 246 mg, 1.08 mmol) and 2,2-dimethyl-5-[4-(2-methylpropyl)phenyl]pentanoyl chloride (296 mg, 1.06 mmol) to give a solid which was purified by column chromatography (hexane−AcOEt, 19:1) to give **44**: 379 mg, 82% yield, white solid; mp 98−100 °C; ^{1}H NMR ($CDCl_3$, 90 MHz) δ 0.89 (d, 6 H, J = 6.5 Hz), 1.27 (s, 6 H), 1.38 (s, 6 H), 1.4−1.95 (m, 5 H), 2.13 (s, 6 H), 2.2−2.7 (m, 4 H), 2.87 (s, 2 H), 6.49 (s, 1 H), 6.75 (br s, 1 H), 6.9−7.2 (m, 4 H). Anal. ($C_{29}H_{41}NO_2$) C, H, N.

6-[(4-Chlorophenyl)oxy]-*N*-(2,2,4,6-tetramethyl-2,3-dihydrobenzofuran-7-yl)-2,2-dimethylhexanamide (46). This product was prepared in a similar manner to that described in method A using 7-amino-2,2,4,6-tetramethyl-2,3-dihydrobenzofuran hydrochloride (**14**; 150 mg, 0.66 mmol) and 6-(4-chlorophenoxy)-2,2-dimethylhexanoyl chloride (210 mg, 1.1 equiv) to give a solid which was purified by column chromatography (hexane−AcOEt, 4:1) to give **46**: 255 mg, 87% yield, white solid; mp 91−91 °C; ^{1}H NMR ($CDCl_3$, 90 MHz) δ 1.31 (s, 6 H), 1.41 (s, 6 H), 1.1−1.9 (m, 6 H), 2.13 (s, 3 H), 2.16 (s, 3 H), 2.88 (s, 2 H), 3.93 (t, 2 H, J = 6 Hz), 6.50 (s, 1 H), 6.79 (d, 2 H, J = 9 Hz), 6.85 (br s, 1 H), 7.20 (d, 2 H, J = 9 Hz). Anal. ($C_{26}H_{34}NO_3Cl$) C, H, N.

7-[(4-Chlorophenyl)oxy]-*N*-(2,2,4,6-tetramethyl-2,3-dihydrobenzofuran-7-yl)-2,2-dimethylheptanamide (47). This product was prepared in a similar manner to that described in method A using 7-amino-2,2,4,6-tetramethyl-2,3-dihydrobenzofuran hydrochloride (**14**; 150 mg, 0.66 mmol) and 7-(4-chlorophenoxy)-2,2-dimethylheptanoyl chloride (220 mg, 1.1 equiv) to give a solid which was purified by column chromatography (hexane−AcOEt, 4:1) to give **47**: 184 mg, 82.8% yield, white solid; mp 90−91 °C; ^{1}H NMR ($CDCl_3$, 90 MHz) δ 1.29 (s, 6 H), 1.42 (s, 6 H), 1.2−1.95 (m, 8 H), 2.13 (s, 6 H), 2.88 (s, 2 H), 3.91 (t, 2 H, J = 6 Hz), 6.49 (s, 1 H), 6.7 (br s, 1 H), 6.7 (br s, 1 H), 6.79 (d, 2 H, J = 9 Hz), 7.20 (d, 2 H, J = 9 Hz). Anal. ($C_{27}H_{36}NO_3Cl$) C, H, N.

***N*-(2,2,4,6-Tetramethyl-2,3-dihydrobenzofuran-7-yl)-2,2-dimethyl-6-[(phenylmethyl)oxy]hexanamide (49).** This product was prepared in a similar manner to that described in method A using 7-amino-2,2,4,6-tetramethyl-2,3-dihydrobenzofuran hydrochloride (**14**; 150 mg, 0.66 mmol) and 2,2-dimethyl-6-[(phenylmethyl)oxy]hexanoyl chloride (300 mg) to give an oil which was purified by column chromatography (hexane−AcOEt, 4:1) to give **49**: 149 mg, 53.5% yield, white solid; mp 78−79 °C (recrystallized from hexane); ^{1}H NMR ($CDCl_3$, 90 MHz) δ 1.28 (s, 6 H), 1.41 (s, 6 H), 1.2−1.8 (m, 6 H), 2.13 (s, 6 H), 2.88 (s, 2 H), 3.47 (t, 2 H, J = 6.5 Hz), 4.48 (s, 2 H), 6.49 (s, 1 H), 6.80 (br s, 1 H), 7.2−7.4 (m, 5 H). Anal. ($C_{27}H_{37}NO_3$) C, H, N.

8-[(4-Chlorophenyl)oxy]-*N*-(2,2,4,6-tetramethyl-2,3-dihydrobenzofuran-7-yl)-2,2-dimethyloctanamide (50). This product was prepared in a similar manner to that described in method A using 7-amino-2,2,4,6-tetramethyl-2,3-dihydrobenzofuran hydrochloride (**14**; 150 mg, 0.66 mmol) and 8-(4-chlorophenoxy)-2,2-dimethyloctanoyl chloride (230 mg, 1.1 equiv) to give an oil which was purified by column chromatography (hexane−AcOEt) to give **50**: 234 mg, 75% yield, white solid; mp 72−73 °C; ^{1}H NMR ($CDCl_3$, 90 MHz) δ 1.29 (s, 6 H), 1.42 (s, 6 H), 1.3−1.9 (m, 10 H), 2.13 (s, 6 H), 2.88 (s, 2 H), 3.90 (t, 2 H, J = 6.5 Hz), 6.49 (s, 1 H), 6.79 (d, 2 H, J = 6.9 Hz), 6.8 (br s, 1 H), 7.19 (d, 2 H, J = 6.9 Hz). Anal. ($C_{28}H_{38}NO_3Cl$) C, H, N.

***N*-(2,2,4,6-Tetramethyl-2,3-dihydrobenzofuran-7-yl)-4-[6-(propyloxy)hexyl]benzamide (55).** This product was prepared in a similar manner to that described in method C using 4-hydroxy-*N*-(2,2,4,6-tetramethyl-2,3-dihydrobenzofuran-7-yl)benzamide (**17**; 94 mg, 0.30 mmol) and 6-(propyloxy)-hexyl chloride (69.6 mg, 0.31 mmol) to give an oil which was purified by column chromatography (hexane−AcOEt, 4:1) to give **55**: 133 mg, 98% yield, white solid; mp 69−71 °C; ^{1}H NMR ($CDCl_3$, 90 MHz) δ 1.4−1.7 (m, 16 H), 1.82 (m, 2 H), 2.16 (s, 3 H), 2.22 (s, 3 H), 2.91 (s, 2 H), 3.37 (t, 2 H, J = 6.8 Hz), 3.42 (t, 2 H, J = 6.5 Hz), 4.01 (t, 2 H, J = 7.4 Hz) 6.56 (s, 1 H), 6.93 (br d, 2 H, J = 8.3 Hz), 7.26 (s, 1 H), 7.85 (br d, 2 H, J = 8.3 Hz). Anal. ($C_{28}H_{39}NO_4$) C, H, N.

4-[[6-[(4-Chlorophenyl)oxy]hexyl]oxy]-*N*-(2,2,4,6-tetramethyl-2,3-dihydrobenzofuran-7-yl)benzamide (56). This product was prepared in a similar manner to that described in method A using 7-amino-2,2,4,6-tetramethyl-2,3-dihydrobenzofuran hydrochloride (**14**; 150 mg, 0.66 mmol) and 4-[[6-(4-chlorophenoxy)hexyl]oxy]benzoyl chloride (166 mg, 2.5 equiv) to give a solid which was purified by column chromatography

(hexane−AcOEt; 3:1) to give **56**: 295 mg, 85.7% yield, white solid; mp 135−136 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.4−1.7 (m, 14 H), 1.83 (m, 4 H), 2.16 (s, 3 H), 2.21 (s, 3 H), 2.92 (s, 2 H), 3.94 (t, 2 H, J = 6.5 Hz), 4.03 (t, 2 H, J = 6.5 Hz), 6.56 (s, 1 H), 6.81 (d, 2 H, J = 9 Hz), 6.93 (br d, 2 H, J = 8 Hz), 7.2 (br s, 1 H), 7.22 (d, 2 H, J = 9 Hz), 7.86 (br d, 2 H, J = 8 Hz). Anal. ($C_{31}H_{36}NO_4Cl$) C, H, N.

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References

(1) (a) Chang, C. C. Y.; Huh, H. Y.; Cadigan, K. M.; Chang, T. Y. Molecular Cloning and Functional Expression of Human Acyl-Coenzyme A:Cholesterol Acyltransferase cDNA in Mutant Chinese Hamster Ovary Cells. *J. Biol. Chem.* **1993**, *268*, 20747−20755. (b) Chang, T. Y.; Chang, C. C. Y.; Cadigan, K. M. The Structure of Acyl Coenzyme A-Cholesterol Acyltransferase and Its Potential Relevance to Atherosclerosis. *Trends Cardiovasc. Med.* **1994**, *4*, 223−230. (c) Chang, D.; Chang, C. C. Y.; Qu, X.; Chang, T. Y. Activation of Acyl-Coenzyme A:Cholesterol Acyltransferase by Cholesterol or by Oxysterol in a Cell-free System. *J. Biol. Chem.* **1995**, *270*, 685−695.

(2) Suckling, K. E.; Stange, E. F. Role of Acyl-CoA Cholesterol Acyltransferase in Cellular Cholesterol Metabolism. *J. Lipid Res.* **1985**, *26*, 647−671.

(3) Gillies, P. J.; Robinson, C. S.; Rathgeb, K. A. Regulation of ACAT Activity by a Cholesterol Substrate Pool during the Progression and Regression Phases of Atherosclerosis: Implications for Drug Discovery. *Atherosclerosis* **1990**, *83*, 177−185.

(4) Sliskovic, D. R.; White, A. D. Therapeutic Potential of ACAT Inhibitors as Lipid Lowering and Anti-atherosclerotic Agents. *Trends Pharmacol. Sci.* **1991**, *12*, 194−199.

(5) Marzetta, C. A.; Savoy, Y. E.; Freeman, A. M.; Long, C. A.; Pettini, J. L.; Hagar, R. E.; Inskeep, P. B.; Davis, K.; Stucchi, A. F.; Nicolosi, R. J.; Hamanaka, E. S. Pharmacological Properties of a Novel ACAT Inhibitor (CP-113,818) in Cholesterol-fed Rats, Hamsters, Rabbits, and Monkeys. *J. Lipid Res.* **1994**, *35*, 1829−1838.

(6) Cianflone, K. M.; Yasruel, Z.; Rodriguez, M. A.; Vas, D.; Sinderman, A. D. Regulation of ApoB Secretion from HepG2 Cells: Evidence for a Critical Role for Cholesteryl Ester Synthesis in the Response to a Fatty Acid Challenge. *J. Lipid Res.* **1990**, *31*, 2045−2055.

(7) Burrier, R. E.; Deren, S.; McGregor, D. G.; Hoos, L. M.; Smith, A. A.; Davis, H. R., Jr. Demonstration of a Direct Effect on Hepatic Acyl CoA:Cholesterol Acyl Transferase (ACAT) Activity by Orally Administered Enzyme Inhibitor in the Hamster. *Biochem. Pharmacol.* **1994**, *47*, 1545−1551.

(8) (a) Sliskovic, D. R.; Trivedi, B. K. ACAT Inhibitors: Potential Anti-atherosclerotic Agents. *Curr. Med. Chem.* **1994**, *1*, 204−225. (b) Higley, C. A.; Wilde, R. G.; Maduskuie, T. P.; Johnson, A. L.; Pennev, P.; Billheimer, J. T.; Robinson, C. S.; Gillies, P. J.; Wexler, R. R. Acyl CoA:Cholesterol Acyltransferase (ACAT) Inhibitors: Synthesis and Structure−Activity Relationship Studies of a New Series of Trisubstituted Imidazoles. *J. Med. Chem.* **1994**, *37*, 3511−3522. (c) Maduskuie, T. P., Jr.; Wilde, R. G.; Billheimer, J. T.; Cromley, D. A.; Germain, S.; Gillies, P. J.; Higley, C. A.; Johnson, A. L.; Pennev, P.; Shimshick, E. J.; Wexler, R. R. Design, Synthesis, and Structure−Activity Relationship Studies for a New Imidazole Series of J774 Macrophage Specific Acyl−CoA:Cholesterol Acyltransferase (ACAT) Inhibitors. *J. Med. Chem.* **1995**, *38*, 1067−1083. (d) Clader, J. W.; Berger, J. G.; Burrier, R. E.; Davis, H. R.; Domalski, M.; Dugar, S.; Kogan, T. P.; Salisbury, B.; Vaccaro, W. Substituted (1,2-Diarylethyl)amide Acyl−CoA:Cholesterol Acyltransferase Inhibitors: Effect of Polar Groups on *in Vitro* and *in Vivo* Activity. *J. Med. Chem.* **1995**, *38*, 1600−1607.

(9) (a) Woolf, T. F.; Bjorge, S. M.; Black, A. E.; Halmes, A.; Chang, T. Metabolism of the Acyl CoA:Cholesterol Acyltransferase Inhibitor 2,2-Dimethyl-*N*-(2,4,6-trimethoxyphenyl)dodecanamide in Rat and Monkey. *Drug Metab. Dispos.* **1991**, *19*, 696−702. (b) 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. Inhibitors of Acyl-CoA:Cholesterol Acyltransferase. 1. Identification and Structure-Activity Relationships of a Novel Series of Fatty Acid Anilide Hypocholesterolemic Agents. *J. Med. Chem.* **1992**, *35*, 1609−1617.

(10) McCarthy, P. A.; Hamanaka, E. S.; Marzetta, C. A.; Bamberger, M. J.; Gaynor, B. J.; Chang, G.; Kelly, S. E.; Inskeep, P. B.; Mayne, J. T.; Beyer, T. A.; Walker, F. J.; Goldberg, D. I.; Savoy, Y. E.; Davis, K. M.; Diaz, C. L.; Freeman, A. M.; Johnson, D. A.; LaCour, T. G.; Long, C. A.; Maloney, M. E.; Martingano, R. J.; Pettini, J. L.; Sand, T. M.; Wint, L. T. Potent, Selective, and Systematically-Available Inhibitors of Acyl-Coenzyme A:Cholesterol Acyl Transferase (ACAT). *J. Med. Chem.* **1994**, *37*, 1252−1255.

(11) Kataoka, K.-i.; Shiota, T.; Takeyasu, T.; Mochizuki, T.; Taneda, K.; Ota, M.; Tanabe, H.; Yamaguchi, H. Potent Inhibitors of Acyl-CoA:Cholesterol Acyltransferase. Structure−Activity Relationships of Novel *N*-(4-Oxochroman-8-yl)amides. *J. Med. Chem.* **1995**, *38*, 3174−3186.

(12) FMC Corp. Neth. Appl. 6 602 601, 1966; *Chem. Abstr.* **1996**, 46319.

(13) Torii, S.; Okumoto, H.; Minoshima, T.; Kurozumi, S. Electroreduction of 5-Chloro-7-nitrobenzofuran Derivative: A Facile Access to A Key Intermediate for ACAT Inhibitors. *Synlett* **1994**, 68.

(14) Creger, P. L. Metalated Carboxylic Acid. III. Monoalkylation of Alkylacetic Acids. A Possible Alternative to the Malonic Ester Synthesis for the Preparation of Dialkylacetic Acids. *J. Am. Chem. Soc.* **1970**, *92*, 1397−1398.

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