sodium chloride solution (~0.8 L), dried over sodium sulfate, and evaporated, finally at 80 °C under high vacuum to give 536.5 g of oily residue. Distillation gave 483.3 g (62.3%) of pure product as a liquid (solidifies on standing at room temperature), bp 160 °C (0.75 mm). Anal. ($C_{12}H_{13}NO_3$) C, H, N.

2-Amino-α-cyano-1,6-dihydro-α-[(2-methoxyphenyl)-methyl]-5-nitro-6-oxo-4-pyrimidineacetic Acid, Methyl Ester (10d). To a stirred solution of compound **9d** (285 g, 1.3 mol) in dimethyl sulfoxide (650 mL) at 0–30 °C (ice–salt bath cooling) was added potassium *tert*-butoxide (146 g, 1.3 mol) portionwise over 15 min. The reaction mixture was stirred at room temperature for 40 min (internal temperature ~30 °C) and then was cooled to 6 °C, and a solution of 2-amino-6-chloro-5-nitro-4-(3*H*)-pyrimidinone monohydrate (108.4, 0.527 mol) in dimethyl sulfoxide (500 mL) was added over a 15-min period (internal temperature 6–12 °C reprecipitated with ice–water bath cooling). After the addition the mixture was stirred at room temperature for 80 min and then was heated in a water bath at 65 °C (internal temperature) for 19 h. After cooling to 12 °C, the mixture was poured into ice water (~8.0 L), acidified to pH ~5.0 with acetic acid (~100 mL), and stirred at room temperature for 40 min. The precipitate was separated by filtration and washed with cold water. Waxy solid was suspended in ether (~2.0 L) and the mixture stirred at room temperature for 30 min, and the solid was collected, washed with cold water (2 × 2 L), ether (1 L), and dried at 78 °C under vacuum for 18 h to give 154.8 g (78.6%) of a light yellow solid, mp 244 °C dec. The solid was suspended in methanol (1 gal) refluxed with stirring on a stream bath for 2 h and cooled, and the precipitate was separated, washed with cold methanol (500 mL) and ether (~800 mL) and dried at 78 °C under vacuum for 4 h to give 116.2 g (59%) of a solid, mp 249 °C dec. Recrystallization (3.3 g) from DMF (40 mL) and ether (~950 mL) gave analytically pure product, mp 247 °C dec. Anal. ($C_{16}H_{15}N_5O_6$) C, N; H: calcd, 4.05; found 4.62.

2-Amino-α-[(2-methoxyphenyl)methyl]-1,6-dihydro-5-nitro-6-oxo-4-pyrimidineacetonitrile (12d). A solution of **10d** (112 g, 0.3 mol) in 1 N sodium hydroxide (1.5 L) was stirred at room temperature under nitrogen atmosphere for 3.75 h. The solution was carefully acidified to pH ~1 with 6 N hydrochloric acid (~425 mL) by dropwise addition over a 15-min period and then stirred for 45 min, 6 N hydrochloric acid (100 mL) was added, and the mixture was stirred for 20 min (internal temperature 25–32 °C). After cooling, the precipitate was collected by filtration, washed with cold water, and dried (house vacuum) overnight to give 74.1 g (78.3%) of a solid, mp ~185 °C dec. This was used in the next step without any further characterization.

2,6-Diamino-3,5-dihydro-7-[(2-methoxyphenyl)methyl]-4*H*-pyrrolo[3,2-*d*]pyrimidin-4-one (11d). To a solution of **12d** (74.1 g, 0.235 mol) in 1 N sodium hydroxide (3.0 L) was added sodium dithionite (263.3 g, 1.51 mol). The reaction mixture was heated on a steam bath with stirring under nitrogen for 50 min, then one additional portion of sodium dithionite (25 g, 0.14 mol) was added, and the mixture heated for 20 min. The hot mixture was filtered (folded paper). After cooling to 30 °C, the solution was acidified to pH ~1 with 6 N hydrochloric acid (~200 mL) (internal temperature was kept at ~30 °C by ice–water cooling) and then stirred at room temperature for 30 min. The mixture was cooled to ~20 °C and the precipitate was separated by filtration, washed with cold water and then with ether (~1.2 L), and dried at 78 °C under vacuum for 17 h to give 56.75 g of **14d**, mp 235–7 °C.

The solid **14d** (56.75 g) was added to concentrated hydrochloric acid (1.2 L), and the mixture was stirred at room temperature for 2 h, then heated to 55 °C, and stirred at 47–55 °C for 45 min. After cooling to 20 °C, the precipitate was separated by filtration, washed with ether (2 × 500 mL), cold water (2 × 250 mL), and then with ether (~500 mL), and dried at 78 °C for 16 h to give 28.7 g of a light-orange colored solid, mp 215–235 °C. A solution of the solid in warm methanol (~700 mL) was filtered, concentrated under reduced pressure to a 250-mL volume, diluted with ether (~1.6 L), and cooled. The precipitate was separated, washed with ether, and dried at 78 °C for 19 h to give 26.95 g (35%) of analytically pure product (**11d**), mp 215–240 °C. Anal. ($C_{14}H_{15}N_5O_2$·HCl·0.33H_2O) C, H, Cl, H_2O; N: calcd 21.37; found, 20.45.

Inhibitors of Acyl-CoA:Cholesterol Acyltransferase. 1. Identification and Structure–Activity Relationships of a Novel Series of Fatty Acid Anilide Hypocholesterolemic Agents

Bruce D. Roth,*,† C. John Blankley,† Milton L. Hoefle,† Ann Holmes,† W. Howard Roark,† Bharat K. Trivedi,† Arnold D. Essenburg,‡ Karen A. Kieft,‡ Brian R. Krause,‡ and Richard L. Stanfield‡

Departments of Medicinal Chemistry and Pharmacology, Parke-Davis Pharmaceutical Research Division, Warner-Lambert Company, 2800 Plymouth Road, Ann Arbor, Michigan 48105. *Received September 20, 1991*

A series of fatty acid anilides was prepared, and compounds were tested for their ability to inhibit the enzyme acyl-CoA:cholesterol acyltransferase (ACAT) in vitro and to lower plasma total cholesterol and elevate high-density lipoprotein cholesterol in cholesterol-fed rats in vivo. The compounds reported were found to fall into two subclasses with different anilide SAR. For nonbranched acyl analogues, inhibitory potency was found to be optimal with bulky 2,6-dialkyl substitution. For α-substituted acyl analogues, there was little dependence of in vitro potency on anilide substitution and 2,4,6-trimethoxy was uniquely preferred. Most of the potent inhibitors (IC_{50} < 50 nM) were found to produce significant reductions in plasma total cholesterol in cholesterol-fed rats. Additionally, in vivo activity could be improved significantly by the introduction of α,α-disubstitution into the fatty acid portion of the molecule. A narrow group of α,α-disubstituted trimethoxyanilides, exemplified by 2,2-dimethyl-*N*-(2,4,6-trimethoxyphenyl)dodecanamide (**39**), was found to not only lower plasma total cholesterol (–60%) in cholesterol-fed rats but also elevate levels of high-density lipoprotein cholesterol (+94%) in this model at the screening dose of 0.05% in the diet (ca. 50 mg/kg).

Acyl-CoA:cholesterol acyltransferase (ACAT, EC 2.3.1.26) is the intracellular enzyme responsible for catalyzing the formation of cholesteryl esters, utilizing cholesterol and fatty acyl-CoA as substrates. Although ACAT activity has been demonstrated in most mammalian tissues, the enzyme itself is poorly characterized.[1] It has been hypothesized that this enzyme may play an important

† Department of Medicinal Chemistry.
‡ Department of Pharmacology.

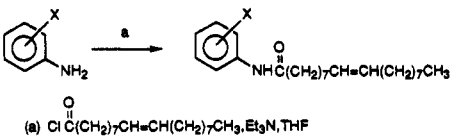
(1) Myant, N. B. Acyl-CoA: Cholesterol Acyltransferase. In *Cholesterol Metabolism, LDL, and the LDL Receptor*; Academic Press, Inc.: San Diego, CA, 1990; pp 85–98.

0022-2623/92/1835-1609$03.00/0

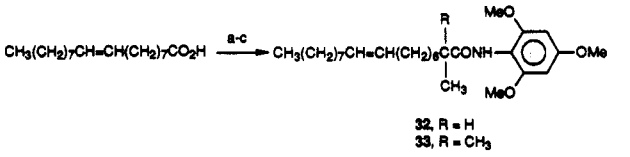
role in the absorption of dietary cholesterol, the secretion of hepatic very-low-density lipoproteins (VLDL) and the accumulation of cholesteryl esters in arterial lesions.[2] It is, in fact, known that the distribution of intestinal ACAT activity reflects the predominant sites of cholesterol absorption[3] and that ACAT activity is increased with cholesterol feeding in several animal species.[4] Additionally, it has been demonstrated that potent inhibitors of this enzyme both decrease the absorption of dietary cholesterol and reduce plasma total cholesterol (TC) concentrations in several cholesterol-fed animal models of hypercholesterolemia.[5] Recently, it has been reported that a systemically bioavailable inhibitor will prevent the progression of atherosclerotic lesions in cholesterol-fed rabbits.[6] Despite the evidence supporting the intermediacy of ACAT in cholesterol absorption in animal models and reports confirming the presence of ACAT in the human intestine and liver,[7] the role of ACAT in human cholesterol metabolism is still controversial.[8]

Several publications have appeared describing series of fatty acid amide hypocholesterolemic agents.[5a-d,f,g] Both the linoleic acid amide of *dl*-α-methylbenzylamine[5f] and the oleic acid amide of *dl*-tryptophan ethyl ester[5a] had been reported to be moderately potent inhibitors of ACAT in vitro (both inhibit rabbit intestinal ACAT 50% at approximately 0.5 μM) and to reduce plasma TC in cholesterol-fed animal models.[5g] A series of (aralkylamino)- and (alkylamino)benzoic acid analogues of the antiatherosclerotic agent cetaben (sodium 4-(hexadecylamino)-benzoate) had also been studied extensively.[5b-d] In ad-

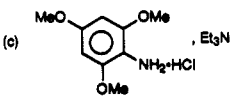
Scheme I. Method A



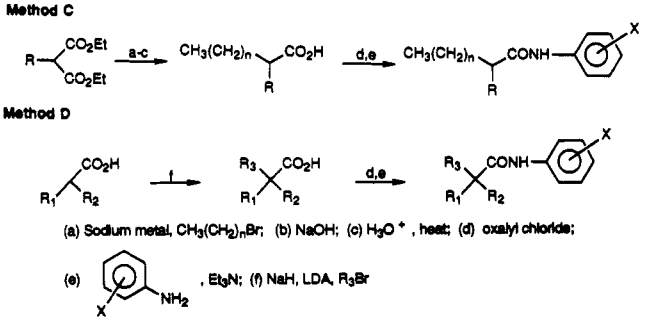
Scheme II. Method B



(a) I) NaH, THF; ii) LDA, CH_3I; iii) LDA, CH_3I; (b) oxalyl chloride;



Scheme III



(2) (a) Suckling, K. E.; Stange, E. F. Role of Acyl-CoA: Cholesterol Acyltransferase in Cellular Cholesterol Metabolism. *J. Lipid Res.* **1985**, *26*, 647–671. (b) Krause, B. R.; Newton, R. S. ACAT Inhibitors: Preclinical Pharmacologic Profiles and Implications for Plasma Lipid Regulation and Prevention of Atherosclerosis. In *Atherosclerosis VIII*; Crepaldi, G., Gotto, A. M., Manzato, E., Baggio, G., Eds.; Elsevier Science Pub.: Amsterdam, 1989; pp 707–710. (c) Sliskovic, D. R.; White, A. D. Therapeutic Potential of ACAT Inhibitors as Lipid Lowering and Anti-atherosclerotic Agents. *Trends Pharmacol. Sci.* **1991**, *11*, 194–199.

(3) Sylven, C.; Nordstrom, C. The Site of Absorption of Cholesterol and Sitosterol in the Rat Small Intestine. *Scand. J. Gastroenterol.* **1970**, *5*, 57–63.

(4) (a) Field, F. J.; Cooper, A. D.; Erickson, S. K. Regulation of Rabbit Intestinal Acyl Coenzyme A: Cholesterol Acyltransferase In Vivo and In Vitro. *Gastroenterology* **1982**, *83*, 873–880. (b) Heller, F. R. Cholesterol Esterifying Capacity of Various Organs in Cholesterol-Fed Guinea Pigs. *Lipids* **1983**, *18*, 18–24. (c) Norum, K. R.; Helgerud, P.; Petersen, L. B.; Groot, P. H. E.; de Jonge, H. R. Influence of Diets on Acyl-CoA: Cholesterol Acyltransferase and on Acyl-CoA: Retinol Acyltransferase in Villous and Crypt Cells from Rat Small Intestinal Mucosa and in the Liver. *Biochim. Biophys. Acta* **1983**, *751*, 153–161.

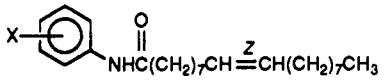
(5) (a) Heider, J. G.; Pickens, C. E.; Kelly, L. A. Role of Acyl CoA: Cholesterol Acyltransferase in Cholesterol Absorption and its Inhibition by 57-118 in the Rabbit. *J. Lipid Res.* **1983**, *24*, 1127–1134. (b) Albright, J. D.; DeVries, V. G.; Largis, E. E.; et al. Potential Antiatherosclerotic Agents. 2. (Aralkylamino)- and (Alkylamino)benzoic Acid Analogues of Cetaben. *J. Med. Chem.* **1983**, *26*, 1378–1393. (c) Albright, J. D.; DeVries, V. G.; Du, M. T.; et al. Potential Antiatherosclerotic Agents. 3. Substituted Benzoic and Nonbenzoic Acid Analogues of Cetaben. *J. Med. Chem.* **1983**, *26*, 1393–1411. (d) DeVries, V. G.; Largis, E. E.; Miner, T. G.; Shepherd, R. G.; Upeslacis, J. Potential Antiatherosclerotic Agents. 4. [(Functionalized-alkyl)amino]benzoic Acid Analogues of Cetaben. *J. Med. Chem.* **1983**, *26*, 1411–1421. (e) DeVries, V. G.; Schaffer, S. A.; Largis, E. E.; et al. Potential Antiatherosclerotic Agents. 5. An Acyl-CoA: Cholesterol O-Acyltransferase Inhibitor with Hypocholesterolemic Activity. *J. Med. Chem.* **1986**, *29*, 1131–1133. (f) Natori, K.; Okazaki, Y.; Nakajima, T.; Hirohashi, T.; Aono, S. Mechanism of the Inhibition of Cholesterol Absorption by DL-Melinamide: Inhibition of Cholesterol Esterification. *Jpn. J. Pharmacol.* **1986**, *42*, 517–523. (g) Matsubara, K.; Matsuzawa, Y.; Jiao, S.; et al. Cholesterol-Lowering Effect of N-(α-Methylbenzyl)linoleamide (Melinamide) in Cholesterol-Fed Diabetic Rats. *Atherosclerosis* **1988**, *72*, 199–204. (h) Largis, E. E.; Wang, C. H.; DeVries, V. G.; Schaffer, S. A. CL277,082: A Novel Inhibitor of ACAT-Catalyzed Cholesterol Esterification and Cholesterol Absorption. *J. Lipid Res.* **1989**, *30*, 681–690. (i) DeVries, V. G.; Bloom, J. D.; Dutia, M. D.; Katocs, A. S., Jr.; Largis, E. E. Potential Antiatherosclerotic Agents. 6. Hypocholesterolemic Trisubstituted Urea Analogues. *J. Med. Chem.* **1989**, *32*, 2318–2325. (j) Balasubramanian, S.; Simons, L. A.; Chang, S.; Roach, P. D.; Nestel, P. J. On the Mechanism by Which an ACAT Inhibitor (CL 277,082) Influences Plasma Lipoproteins in the Rat. *Atherosclerosis* **1990**, *82*, 1–5. (k) Larsen, S. D.; Spilman, C. H.; Bell, F. P.; et al. Synthesis and Hypocholesterolemic Activity of 6,7-Dihydro-4H-pyrazolo[1,5-a]pyrrolo[3,4-d]pyrimidine-5,8-diones, Novel Inhibitors of Acyl CoA: Cholesterol O-Acyltransferase. *J. Med. Chem.* **1991**, *34*, 1721–1727.

(6) Gammill, R. B.; Bell, F. P.; Bell, L. T.; Bisaha, S. N.; Wilson, G. J. Antiatherosclerotic Agents. A Structurally Novel Bivalent Inhibitor of Acyl CoA: Cholesterol O-Acyltransferase with Systemic Activity. *J. Med. Chem.* **1990**, *33*, 2685–2687.

(7) (a) Norum, K. R.; Lilljequist, A.-C.; Helgerud, P.; Normann, E. R.; Mo, A.; Selbekk, B. Esterification of Cholesterol in Human Small Intestine: The Importance of Acyl-CoA: Cholesterol Acyltransferase. *Eur. J. Clin. Invest.* **1979**, *9*, 55–62. (b) Smith, J. L.; de Jersey, J.; Pillay, S. P.; Hardie, I. R. Hepatic Acyl-CoA:Cholesterol Acyltransferase. Development of a Standard Assay and Determination in Patients with Cholesterol Gallstones. *Clin. Chim. Acta* **1986**, *158*, 271–282. (c) Reihner, E.; Angelin, B.; Bjorkhem, I.; Einarsson, K. Hepatic Cholesterol Metabolism in Cholesterol Gallstone Disease. *J. Lipid Res.* **1991**, *32*, 469–475.

(8) Harris, W. S.; Dujovne, C. A.; von Bergmann, K.; et al. Effects of the ACAT inhibitor CL 277,082 on Cholesterol Metabolism in Humans. *Clin. Pharm. Ther.* **1990**, *48*, 189–194.

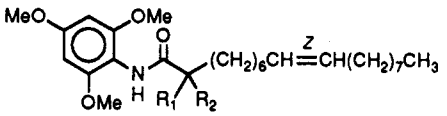
Table I. Oleic Acid Anilides



no.	X	mp (°C)	formula[a]	IC_{50} (μM)[b]	% change[c] TC
1	H	wax[d]	$C_{24}H_{39}NO$	26.0	*i*
2	2-OMe	wax[d]	$C_{25}H_{41}NO_2$	8.0	*i*
3	2-Cl	wax[d]	$C_{24}H_{38}ClNO$	75	*i*
4	2-Et	e	$C_{26}H_{43}NO$	1.2	*i*
5	2-iPr	*f*	$C_{27}H_{45}NO$	0.60	*i*
6	2-Ph	wax[d]	$C_{30}H_{43}NO$	0.91	*i*
7	2-COPh	oil[d]	$C_{31}H_{43}NO_2$[h]	>10	*i*
8	2,4-$(OMe)_2$	46–8	$C_{26}H_{43}NO_3$	3.0	*i*
9	2,4-F_2	39–40	$C_{24}H_{37}F_2NO$	32.0	−19
10	3,4-$(OMe)_2$	62–3	$C_{26}H_{43}NO_3$	4.5	*i*
11	2-Me, 3-CO_2Me	wax[d]	$C_{27}H_{43}NO_3$	>5	*i*
12	2-OMe, 5-CO_2Me	wax[d]	$C_{27}H_{43}NO_4$	0.43	*i*
13	2-CO_2Me, 4-Me	oil[d]	$C_{27}H_{43}NO_3$	9.2	*i*
14	2,3-$(CH_2)_4$	oil[d]	$C_{28}H_{45}NO$	0.35	−7
15	2-NO_2, 3-Me	oil[d]	$C_{25}H_{40}N_2O_3$	12.0	*i*
16	3-Me, 4-CH_2CO_2Et	*g*	$C_{29}H_{47}NO_3$	3.0	−10
17	2,4-$(Me)_2$, 5-CO_2Me	75–8	$C_{28}H_{45}NO_3$	0.06	−41[j]
18	2,4-$(OMe)_2$, 5-CO_2Me	oil[d]	$C_{28}H_{45}NO_5$	1.10	*i*
19	2,4-$(OMe)_2$, 5-Cl	47–9	$C_{26}H_{42}ClNO_3$	0.52	−7
20	2,4,5-$(OMe)_3$	44–6	$C_{27}H_{45}NO_4$	1.10	*i*
21	2,4,6-$(OMe)_3$	93–4	$C_{27}H_{45}NO_4$	0.05	−38[j]
22	2,4,6-Me_3	wax[d]	$C_{27}H_{45}NO$	0.70	−27
23	2,4,6-Cl_3	wax[d]	$C_{24}H_{36}Cl_3NO$	0.03	−44[j]
24	2,4,6-F_3	41–2°	$C_{24}H_{36}F_3NO$	6.3	+2
25	2,6-Me_2	51–3°	$C_{26}H_{43}NO$	0.043	−38[j]
26	2-Me, 6-OMe	wax[d]	$C_{26}H_{43}NO_2$	0.020	−30[j]
27	2-Me, 6-OEt	wax[d]	$C_{27}H_{45}NO_2$	0.015	−45[j]
28	2,6-Et_2	oil[d]	$C_{28}H_{47}NO$	0.017	−51[j]
29	2,6-iPr_2	oil[d]	$C_{30}H_{51}NO$	0.007	−21
30	2-iPr, 6-Me	35–7	$C_{28}H_{47}NO$	0.023	−45[j]
31	2,4-$(OMe)_2$, 6-OH	48–9	$C_{26}H_{43}NO_4$	0.15	*i*

[a] Analytical results are within ±0.4% of theoretical unless otherwise noted. [b] ACAT inhibition in vitro, intestinal microsomes isolated from cholesterol-fed rabbits. Each determination performed in triplicate. See Experimental Section for complete protocol. [c] Denotes percent change in total cholesterol in cholic acid (0.3%) – cholesterol (1.5%) – peanut oil (5.5%) – fed rats. All compounds were dosed at 0.05% of the diet for 1 week. In a typical experiment, TC and HDL-C in cholesterol-fed controls would range from 150 to 250 mg/dL and 15 to 20 mg/dL, respectively. All animals gained weight normally. See ref 14. [d] Purified by flash chromatography on silica gel. [e] Bp 210–15 °C/0.15 mmHg. [f] Bp 200–4 °C/0.1 mmHg. [g] Bp 255–60 °C/0.07 mmHg. [h] H: calcd; 9.38; found, 9.91. High mass: calcd, 461.3294; found, 461.3299. [i] Not tested. [j] Significantly different from control, $p < 0.05$, using analysis of variance followed by Fisher's multiple range test.

Table II. α-Substituted Oleic Acid Anilides



no.	R_1	R_2	mp (°C)	formula[a]	IC_{50} (μM)[b]	% change TC[c]
21	H	H	93–4	$C_{27}H_{45}NO_4$	0.051	−38[f]
32	H	CH_3	wax[d]	$C_{28}H_{47}NO_4$	0.036	−40[f]
33	CH_3	CH_3	*e*	$C_{29}H_{49}NO_4$	0.044	−60[f]

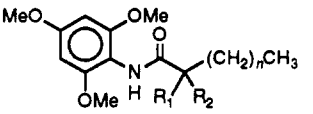
[a] Analytical results are within ±0.4% of theoretical unless otherwise noted. [b] ACAT inhibition in vitro. See footnote in Table I. [c] Denotes percent change in total cholesterol in cholic acid–cholesterol–peanut oil fed rats. See footnote in Table I. [d] Purified by flash chromatography on silica gel. [e] Bp 225–30 °C/0.01 mmHg. [f] Significantly different from control, $p < 0.05$, using analysis of variance followed by Fisher's multiple range test.

dition to the fatty acid amides, a series of potent aryl urea inhibitors had also been reported from the same laboratories.[5e,h-j] Other unrelated series have been reported subsequently.[5k,6] Despite these reports, we felt that the potential of the fatty acid amide class of inhibitors had not been adequately defined. Thus, in order to better understand the structural requirements for potent ACAT inhibition in vitro, a systematic examination of fatty acid amide ACAT inhibitors was initiated. The present study identifies a number of compounds which potently inhibit ACAT in vitro, reduce plasma TC, and, in some cases, elevate levels of plasma high-density lipoprotein cholesterol (HDL-C) in cholesterol-fed rats.

Chemistry

The general synthetic strategies used to prepare the compounds employed in this study are illustrated in Schemes I–III. The oleic acid anilides (compounds 1–31, Table I) were all prepared by reacting the appropiate anilines with oleoyl chloride (Scheme I, method A). Three methods were used to prepare the α-mono- and α,α-disubstituted anilides listed in Tables II–IV. Compounds 32 and 33 were prepared by alkylation of oleic acid via treatment with NaH followed by 2 equiv each of LDA and

Table III. Modifications in Chain Length and α-Substitution in Saturated 2,4,6-Trimethoxyanilides



no.	n	R_1	R_2	mp (°C)	formula[a]	IC_{50} (μM)[b]	% change[c] TC	% change[c] HDL-C
34	6	CH_3	CH_3	70–2	$C_{20}H_{33}NO_4$	0.18	−13	+2
35	7	CH_3	CH_3	53–5	$C_{21}H_{35}NO_4$	0.059	−43[d]	+53[d]
36	8	CH_3	CH_3	56–7	$C_{22}H_{37}NO_4$	0.059	−53[d]	+86[d]
37	9	H	CH_3	109–110	$C_{22}H_{37}NO_4$	0.090	[e]	[e]
38	9	H	CH_2CH_3	92–4	$C_{23}H_{39}NO_4$	0.073	[e]	[e]
39	9	CH_3	CH_3	59–60	$C_{23}H_{39}NO_4$	0.073	−60[d]	+94[d]
40	9	CH_2CH_3	CH_2CH_3	oil	$C_{25}H_{43}NO_4$	0.097	[e]	[e]
41	9	$=CH_2$		59–61	$C_{22}H_{35}NO_4$	0.20	[e]	[e]
42	9	$-CH_2CH_2CH_2-$		89–90	$C_{24}H_{39}NO_4$	0.039	−57[d]	+73[d]
43	9	$-(CH_2)_4-$		73–4	$C_{25}H_{41}NO_4$	0.032	−67[d]	+45
44	9	$-(CH_2)_5-$		71–3	$C_{26}H_{43}NO_4$	0.070	−43[d]	NC[g]
45	9	$-(CH_2)_6-$		54–5	$C_{27}H_{45}NO_4$	0.093	−42[d]	+9
46	11	H	H	105–6	$C_{23}H_{39}NO_4$	0.19	−44[d]	[f]
47	11	H	CH_3	109–11	$C_{24}H_{41}NO_4$	0.13	−39[d]	[f]
48	11	H	CH_2CH_3	98–9	$C_{25}H_{43}NO_4$	0.052	−11	[f]
49	11	CH_3	CH_3	61–3	$C_{25}H_{43}NO_4$	0.063	−45[d]	+19
50	11	$-CH_2CH_2-$		61–2	$C_{25}H_{41}NO_4$	0.058	−40[d]	+27
51	12	H	H	105–6	$C_{24}H_{41}NO_4$	0.065	[e]	[e]
52	13	H	H	100–2	$C_{25}H_{43}NO_4$	0.054	−38[d]	[f]
53	13	H	CH_3	109–11	$C_{26}H_{45}NO_4$	0.031	[e]	[e]
54	13	CH_3	CH_3	63–5	$C_{27}H_{47}NO_4$	0.044	−55[d]	+26
55	13	$-CH_2CH_2-$		61–3	$C_{27}H_{45}NO_4$	0.049	−43[d]	+16
56	15	H	H	105–6	$C_{27}H_{47}NO_4$	0.052	−11	+31
57	15	CH_3	CH_3	68–70	$C_{29}H_{51}NO_4$	0.087	−34[d]	+13

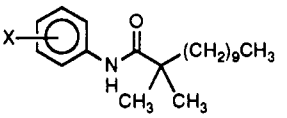
[a] Analytical results are within ±0.4% of theoretical unless otherwise noted. [b] ACAT inhibition in vitro. See footnote in Table I. [c] Denotes percent change in total (TC) or high density lipoprotein (HDL-C) cholesterol in cholesterol-fed rats. See footnote in Table I. [d] Significantly different from control, $p < 0.05$. See footnote in Table I. [e] Not tested. [f] Not determined. [g] NC = no change.

methyl iodide. This afforded a 1:1 mixture of mono- and dimethylated compounds which, without separation, were converted to a mixture of acid chlorides employing oxalyl chloride. Reaction of this mixture with 2,4,6-trimethoxyaniline[9] afforded a 1:1 mixture of **32** and **33**, which was separated by silica gel chromatography (Scheme II, method B). The α-monosubstituted fatty acids needed to prepare anilides **37**, **38**, **47**, **48**, and **53** were prepared by alkylation of an alkyl malonate with a long-chain alkyl halide followed by hydrolysis and decarboxylation (Scheme III, method C).[10] The resultant acids were converted to the anilides as described in method A. The α,α-disubstituted carboxylic acids needed to prepare the anilides listed in Tables III and IV were all prepared by alkylation of the dianion of the requisite carboxylic acid, generated by treatment with 1 equiv each of NaH and LDA (Scheme III, method D).[11] The α-methylene analogue **41** was prepared from known 2-methylenedodecanoic acid.[12]

Results

The compounds prepared for this study were evaluated in two primary biological assays. Each compound was evaluated for its ability to inhibit intestinal ACAT in vitro by incubation with [1-^{14}C]oleoyl-CoA and microsomes isolated from the intestines of cholesterol-fed rabbits.[5a] In vivo activity was assessed in rats by admixing the compounds into a diet (at a level of 0.05%[13]) supplemented

Table IV. 2,2-Dimethyldodecanoic Acid Anilides



no.	X	mp (°C)	formula[a]	IAI[b] IC_{50} (μM)
58	2,4-Me_2	47–9	$C_{22}H_{37}NO$	0.68
59	3,4-Me_2	58–9	$C_{22}H_{37}NO$	1.66
60	2,6-Me_2	53–4	$C_{22}H_{37}NO$	1.30
61	2-OMe, 5-CO_2Me	oil	$C_{23}H_{37}NO_4$	0.13
62	2-Me, 6-Cl	52–4	$C_{21}H_{34}ClNO$	0.75
63	2-Me, 6-OMe	39–40	$C_{22}H_{37}NO_2$	0.21
64	2-Me, 6-OEt	60–2	$C_{23}H_{39}NO_2$	0.18
65	2-Me, 6-iPr	66–8	$C_{24}H_{41}NO$	0.24
66	2,6-Et_2	70–2	$C_{24}H_{41}NO$	0.39
67	2,6-iPr_2	119–120	$C_{26}H_{45}NO$	0.21
39	2,4,6-$(OMe)_3$	59–60	$C_{23}H_{39}NO_4$	0.073
68	2,4,6-Me_3	50–2	$C_{23}H_{39}NO$	0.194
69	2,4,6-F_3	75–6	$C_{20}H_{30}F_3NO$	0.26
70	2,6-Me_2, 4-Br	59–60	$C_{22}H_{36}BrNO$	0.41
71	2,4-$(OMe)_2$, 6-OH	oil	$C_{22}H_{37}NO_4$	0.59
72	2,4-$(OMe)_2$, 6-OCH_2CO_2Me	54–6	$C_{25}H_{41}NO_6$	2.3
73	2,4-$(OMe)_2$, 6-OCH_2CO_2H	95–7	$C_{24}H_{39}NO_6$	>1.0
74	2,4-$(OMe)_2$, 6-$O(CH_2)_6CH_3$	oil	$C_{29}H_{51}NO_4$	0.14

[a] Analytical results are within ±0.4% of theoretical unless otherwise noted. [b] Intestinal ACAT inhibition in vitro. See footnote in Table I.

(9) Fukui, Y.; Kuwahara, Y.; Saheki, K.; Mori, M. Synthesis of 2,4,6-Trimethoxyaniline. *Yakugaku Zasshi* **1960**, *80*, 1472.
(10) Cason, J.; Allinger, N. L.; Williams, D. E. Branched-Chain Fatty Acids. XXIV. Synthesis of 2-Methyldodecanoic Acid and of 2-Methylenedodecanoic Acid. *J. Org. Chem.* **1953**, *18*, 842–849.
(11) Creger, P. L. Metalated Carboxylic Acids. III. Monoalkylation of Alkylacetic Acids. A Possible Alternative to the Malonic Ester Synthesis for the Preparation of Dialkyl Acids. *J. Am. Chem. Soc.* **1970**, *92*, 1397–1398.
(12) Allen, C. F.; Kalm, M. J. 2-Methylenedodecanoic Acid. In *Organic Syntheses*; Wiley: New York, 1963; Collect. Vol. IV, pp 616–620.
(13) This corresponds to a dose of approximately 50 mg/kg on a body weight basis.

Table V. Correlation Matrix (r) of Selected Variables (n = 74)

	σ	CLOGP	CMR	υ_26	υ_6	L_triOMe
σ	1					
CLOGP	0.54	1				
CMR	−0.00	0.74	1			
υ_26	−0.06	0.05	0.11	1		
υ_6	−0.40	−0.33	−0.09	0.72	1	
L_triOMe	−0.72	−0.46	−0.01	−0.02	0.26	1
log ($1/IC_{50}$)	−0.55	−0.20	0.20	0.46	0.65	0.51

with peanut oil (5.5%), cholesterol (1.5%), and cholic acid (0.3%).[14] After 1 week of ad libitum feeding, the animals were sacrificed in the fed state. Plasma total (TC) and/or HDL cholesterol (HDL-C) were measured and the percent change vs control was determined.

Since it was known that the preferred substrate for the enzyme was oleoyl-CoA[15] and that aryl ureas afforded potent inhibitors, the initial set of compounds prepared were oleic acid anilides (Table I). From the analogues prepared, it can be concluded that, in general, highest potency for ACAT inhibition is obtained with anilides containing 2,6-substitution in the aryl ring (compounds **21–31**). This is in contrast to the aryl ureas, where 2,4-difluoro and 2,4,6-trifluoro substitution were the two patterns found to confer optimum potency.[5h] In this study, similar fluoro substitution resulted in weak inhibitors (compounds **9** and **24**). In the homologous series of 2,6-dialkyl-substituted analogues (compounds **25**, **28**, and **29**), potency increased with increasing size of the substituents, with maximum potency obtained in the 2,6-diisopropyl analogue **29** (IC_{50} = 7 nM). In general, there was a good relationship between in vitro and in vivo activity and all of the compounds possessing IC_{50} values less than 60 nM produced reductions in plasma total cholesterol (>30% reduction) which were significantly different from control values ($p < 0.05$) at the screening dose (0.05%). The sole exception was the 2,6-diisopropyl analogue **29**, the most potent compound in the series, which was relatively ineffective in vivo. The reason for the weak activity in vivo for this compound is not clear, but may be related to poor absorption. Since the 2,4,6-trimethoxy substitution pattern had been identified early in the study as providing a good profile of in vitro and in vivo activity, and since no obvious advantage was offered by other substitution patterns, this moiety was selected for incorporation into analogues in which the fatty acid moiety had been modified.

In order to determine whether the efficacy of the oleic acid amides was being limited by hydrolysis of the amide bond in vivo, the α-mono and α,α-dimethyl analogues of **21** (compounds **32** and **33**, Table II) were prepared. It was found that, whereas α-monomethyl substitution produced no enhancement of either in vitro or in vivo activity, α,α-dimethyl substitution led to a significant improvement in activity in vivo, although in vitro potency was unchanged. Having identified α,α-disubsitution on the fatty acid as an important factor for optimizing in vivo activity, we systematically evaluated the effects of varying both chain length and α substitution on ACAT inhibition in vitro and plasma TC lowering in vivo. As shown in Table III, in vitro potency was relatively insensitive to changes in either α substitution or chain length over the range of compounds prepared. In fact, all of the trimethoxyanilides prepared (**34–57**, Table III) possessed in vitro potencies below 200 nM. The fact that the enzyme is insensitive to changes in this portion of the molecule is not surprising, perhaps, in light of the range of fatty acyl-CoA substrates accepted by ACAT.[15] Most of the test compounds in this group also produced marked and statistically significant reductions in plasma TC in cholesterol-fed rats, with only a few exceptions (**34**, **48**, and **56**). In addition to reducing non-HDL-C (i.e., apo-B containing particles), several of these compounds (**35**, **36**, **39**, and **42**) prevented the diet-induced reduction in HDL-C.[14] The most effective compound at elevating HDL-C in this model was **39**, which produced a near doubling in HDL-C, compared to cholesterol-fed controls, at the dose level of 0.05%. In the series of the α-cycloalkyldodecanoic acid trimethoxyanilides (**42–45**), the magnitude of the HDL-C elevation decreased with increasing ring size. It is also interesting to note that only the shorter chain length α,α-disubstituted fatty acid trimethoxyanilides (dodecanoic acid and shorter) produce an HDL effect and it appears to be related neither to the potency of ACAT inhibition nor to the lowering of plasma TC. Another series of ACAT inhibitors which produce elevations in HDL-C (albeit to a lesser degree) in a related cholesterol-fed rat model have been reported recently.[5k] Although it is not clear whether the elevation of HDL-C is related to the inhibition of cholesterol absorption in this model, other cholesterol absorption inhibitors (diosgenin, β-sitosterol) have been reported to elevate HDL-C when administered to cholesterol-fed rats at high doses.[16]

Since it was not clear whether 2,4,6-trimethoxy substitution in the anilide was in fact preferred when the fatty acid moiety contained α,α-disubstitution we repeated the structure–activity study used to optimize activity in the oleic acid anilides with 2,2-dimethyldodecanoic acid (Table IV). Surprisingly, in contrast to the results in the oleic acid anilides where 2,6-dialkyl substitution, especially 2,6-diisopropyl substitution, was found to be optimal for ACAT inhibition in vitro (compare compounds **22** and **25**, Table I), in the case of the 2,2-dimethyldodecanoic acid anilides, 2,4,6-trisubsitution was found to produce significantly more potent inhibition (compare compounds **67** and **39**, Table IV). In fact, unlike the oleamides, 2,4,6-trimethoxyanilide **39** was found to be the most potent inhibitor in this series in vitro.

QSAR

In order to gain further insight into the SAR of this series of compounds, we applied a QSAR analysis to the in vitro activities. A number of potential variables were considered on the basis of the qualitative observations made above. The parameters are defined as follows: σ is the sum of Hammett σ for the anilide substituents,[17]

(14) Maxwell, R. E.; Nawrocki, J. W.; Uhlendorf, P. D. Effects of Gemfibrozil (Cl-719), Gemcadiol (Cl-720), Clofibrate and U-41792 on Cholesterol Distribution in Rat Plasma Fraction by Polyanion Precipitation. *Artery* **1978**, *4*, 303–313.

(15) Heider, J. G. Agents Which Inhibit Cholesterol Esterification in the Intestine and Their Potential Value in the Treatment of Hypercholesterolemia. In *Pharmacological Control of Hyperlipidemia*; Fears, R., Ed.; J.R. Prous Science Pub.: Barcelona, Spain, 1986; pp 423–438.

(16) Cayen, M. N.; Dvornik, D. Effect of Diosgenin on Lipid Metabolism in Rats. *J. Lipid Res.* **1979**, *20*, 162–164.

(17) Hansch, C.; Leo, A. *Substituent Constants for Correlation Analysis in Chemistry and Biology*: Wiley & Sons, New York, 1979.

CLOGP and CMR are the calculated total molecular log P and MR,[18] v_26 and v_6 are Charton's steric parameters[19] for either the combined 2,6-substituents or the 6-substituent of the anilide moiety, position 6 being defined as the smaller of the two ortho substituents, and L_triOMe is an indicator variable denoting the presence or absence of 2,4,6-trimethoxy substitution in the aryl moiety. An examination of Table V suggests that for the total compound set, CLOGP and CMR are moderately correlated, as are σ and the indicator variable L_triOME.

Stepwise regression generated a significant equation with only moderate fitting ability which contained terms in v_6, CMR, and L_triOMe (eq 1). Coefficients are shown with standard errors. All compounds were included except the three without established IC_{50}s (7, 11, 73).

$$\log (1/IC_{50}) = 3.26 + 0.20\ (\pm 0.06)\text{CMR} + 2.41\ (\pm 0.33)v\_6 + 0.63\ (\pm 0.14)\text{L\_triOMe} \quad (1)$$

$$n = 71,\ R^2 = 0.61,\ s = 0.55,\ F = 35.0$$

All terms are highly significant, and the included parameters are essentially uncorrelated. In particular, CMR performed better than CLOGP, v_6 performed better than v_26, and L_triOMe performed better than σ, although an equation of somewhat poorer quality could be obtained using σ in place of L_triOMe. Other variables or indicators characterizing side-chain type, steric effects of α-side chain substitution, or other electronic effects could not improve this equation.

When the total compound set was partitioned into straight-chain acyl analogues and those containing α-substitution, an interesting result was obtained. The subset containing only the straight-chain analogues (Table I plus 46, 51, 52, and 56 from Table III) gave eq 2.

$$\log (1/IC_{50}) = 4.29 + 1.64\ (\pm 0.39)\text{MR\_ar} - 0.37\ (\pm 0.14)(\text{MR\_ar})^2 + 3.19\ (\pm 0.37)v\_6 \quad (2)$$

$$n = 33,\ R^2 = 0.82,\ s = 0.49,\ F = 44.9,\ (\text{MR\_ar})^0 = 2.93$$

The statistics are much improved over eq 1. MR_ar, calculated for the aryl portion only as CMR(X) - CMR(H) (39), gave a better result than total molecule CMR, and a second-order term became significant, implying a size optimum very close to the 2,6-diisopropyl value ($\text{MR\_ar}^{26\text{diipr}} = 2.78$). The steric term v_6 is even more influential and no term in σ or the indicator L_triOMe was significant. For this reduced set, v_6 and MR_ar are essentially uncorrelated ($r = 0.15$) and hence convey different structural information. Furthermore, MR_ar and CLOGP for this subset are much less correlated than in the full set ($r = 0.36$), and thus the preference for MR_ar implies that the region of the enzyme contacted by the aryl moiety of these inhibitors is polar, as opposed to hydrophobic in nature.[20] This improved correlation suggests that the straight-chain analogues may interact with the enzyme in a different manner from the α-branched analogues. Indeed when the branched analogues were examined separately, it was found that simply the presence of the 2,4,6-trimethoxyanilide function could account for 65% of the activity variance (eq 3) and that no other property parameter examined could significantly improve this.

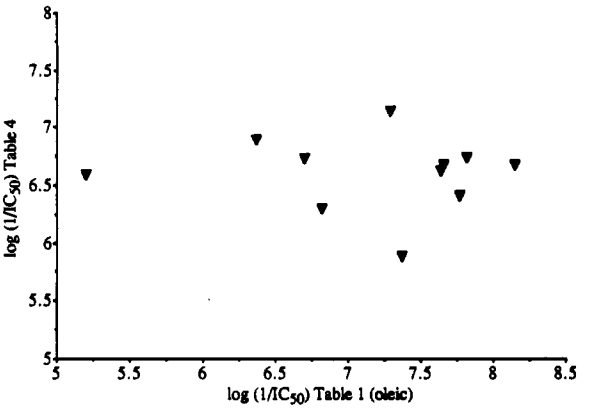


Figure 1. Comparison of in vitro activities of straight chain and α,α-dimethyl series with equivalent aryl substitution.

$$\log (1/IC_{50}) = 6.40 + 0.77\ (\pm 0.10)\text{L\_triOMe} \quad (3)$$

$$n = 38,\ R^2 = 0.63,\ s = 0.30,\ F = 60.4$$

In spite of the fact that the α-branched subset (from Tables III and IV) contains primarily ortho substituted analogues, no significant relation including ortho substituent parameters could be derived. The dichotomy of behavior can be further illustrated by plotting the activities of equivalent aryl substituted analogues of the two series against each other (Figure 1). It is clear from the plot that the factors which increase activity in the straight-chain series do not cause the same increase in the α-branched series. Clearly it is the combination of these two very different relations that gives eq 1.

Discussion

Several fatty acid amides have been reported to inhibit ACAT in vitro and to produce modest reductions of plasma TC in cholesterol-fed animal models. However, no structure–activity studies examining the effects of structural changes in either the fatty acid or amine moieties were reported. In the present study, by systematically examining both substituent effects in the aniline and structural modifications of the fatty acid moiety, it was found that optimum in vitro potency was obtained with the 2,6-di- and 2,4,6-trialkyl- or -alkoxy-substituted anilides. QSAR analysis demonstrated that the set of compounds reported in this study is not homogeneous in its behavior toward ACAT and can be divided into two subsets based on the presence of α-substitution in the acyl side chain portion of the molecule. Those analogues with straight-chain acyl groups show a characteristic strong dependence on the size of ortho substituents, which probably is best interpreted as a requirement to assure a perpendicular orientation of the required amide function with the aromatic ring. In contrast, the presence of α-substitution greatly reduces the sensitivity of in vitro activity to aryl substituent effects with the rather unique enhancement, 6-fold on average, by the 2,4,6-trimethoxy function. Whether this is a special electronic, H-bonding or steric effect cannot be determined from the series reported here.

With respect to in vivo activity, feeding a high-fat, high-cholesterol diet to rats results in both an increase in TC and a redistribution of cholesterol from apo A_1/apo E containing lipoproteins (HDL) to apo B containing particles (non-HDL).[14] It has been reported that blocking cholesterol absorption in this model prevents both the

(18) CLOGP and CMR calculated using MedChem software, v 3.54; Daylight Systems.

(19) Charton, M. The Prediction of Chemical Lability Through Substituent Effects. In *Design of Biopharmaceutical Properties through Prodrugs and Analogs*; Roche, E. B., Ed. American Pharmaceutical Association, Washington, D.C., 1977; pp 228–280.

(20) Yoshitomo, M.; Hansch, C. Quantitative Structure–Activity Relationships of D- and L-N-Acyl-α-aminoamide Ligands binding to Chymotrypsin. On the Problem of Combined Treatment of Stereoisomers. *J. Org. Chem.* **1976**, *41*, 2269–2273.

increase in TC and the decrease in HDL-C induced by the dietary regimen.[16] We (and others[5,14,16]) have defined these changes as a reduction in plasma TC and an elevation in HDL-C. However, one could also interpret these changes as a normalization of plasma lipoprotein distribution to those found in chow-fed animals.

Consistent with previous results,[5] we have found that a variety of potent ACAT inhibitors, when coadministered with the high-fat, high-cholesterol diet, will prevent the changes in plasma lipoprotein concentrations normally induced by this dietary regimen. In this series of fatty acid anilides, it was found that α,α-disubstitution in the fatty acid was important for optimal cholesterol lowering, possibly by preventing the hydrolysis of the amide bond in vivo.[21] Furthermore, several compounds were found to elevate HDL-C, a somewhat unexpected feature in an ACAT inhibitor. The best profile of in vivo activity was found with **39**, the 2,4,6-trimethoxyanilide of 2,2-dimethyldodecanoic acid, which produced both significant reductions in non-HDL-C and elevations in HDL-C as compared to cholesterol-fed controls. Since it has been demonstrated clinically that morbidity and mortality from coronary heart disease can be reduced by either lowering low-density lipoprotein cholesterol (LDL-C)[22] or elevating HDL-C,[23] the profile displayed by **39** may prove desirable in a lipid-regulating agent. Recently, **39** was reported to inhibit ACAT in a manner competitive with respect to oleoyl-CoA.[24] This may, in part, explain the differences in structure–activity relationships between the compounds described in this study and the trisubstituted ureas, which have been found to be noncompetitive inhibitors.[5h] Compound **39** has also been found to produce marked reductions in atherosclerotic lesions in cholesterol-fed rabbits.[25] In fact, regression of lesions was observed not only at doses where plasma cholesterol was significantly reduced but at doses where plasma cholesterol was unchanged, possibly suggesting a direct effect on arterial wall ACAT.[25] Based in part on this data, **39** (CI-976) has been selected for further detailed preclinical and clinical evaluation.

Experimental Section

Unless otherwise noted, materials were obtained from commerical suppliers and were used without purification. Oleic acid was purchased from NuChek Prep. and was >99.5% pure. All organic extracts were dried over $MgSO_4$ except where otherwise noted. Melting points were determined on a Thomas-Hoover melting point apparatus and are uncorrected. Infrared spectra were determined on a Nicolet MX-1 FT-IR spectrophotometer. NMR spectra were determined on either a Varian EM-390 spectrophotometer, a Varian XL-200 spectrophotometer, or a Bruker 250 MHz instrument. Chemical shifts are expressed as parts per million downfield from internal tetramethylsilane. Elemental analyses for carbon, hydrogen, and nitrogen were determined on a Perkin-Elmer Model 240C elemental analyzer and are within 0.4% of theory unless noted otherwise. Routine HPLC analyses were performed using a Varian 5500 unit equipped with a Reodyne 7126 loop injector, a Dupont variable-wavelength detector, and an octadecylsilane (Alltech C18 600RP, CH_3CN–H_2O eluant, 60:40, v/v) or silica gel column (Beckman Altex Ultrasphere 5 μ) interfaced to Varian 402 data system for computation of peak areas.

Method A. (*Z*)-*N*-(2,4,6-Trimethoxyphenyl)-9-octadecenamide (21). 2,4,6-Trimethoxyaniline hydrochloride (18.23 g, 0.083 M) was suspended in a solution of 22.9 mL (0.166 M) of Et_3N in 300 mL of distilled THF. This mixture was stirred for approximately 10 min at room temperature. Oleoyl chloride (25 g, 0.083 M) (99% from Sigma) in 200 mL of distilled THF was added with stirring. The reaction mixture was stirred at room temperature overnight and then filtered. The white solid (Et_3N·HCl) was washed with THF. The combined THF filtrates were concentrated in vacuo. The resulting waxy solid was taken up in ethyl acetate, washed with 2 N HCl, saturated aqueous $NaHCO_3$, and brine, and dried. The filtered solution was concentrated in vacuo. The waxy solid was taken up in a mixture of 50:50 v/v ethyl acetate–hexane and purified by flash chromatography on silica gel. The yield was 31.4 g (84.5%) of **21** (mp 93–94 °C): IR (KBr) ν 2926, 2855, 1649, 1609, 1529, 1152, 1140 cm^{-1}; 200-MHz NMR ($CDCl_3$) δ 0.88 (br t, 3 H, J = 7 Hz), 1.1–1.9 (m, 22 H), 2.02 (m, 4 H), 2.38 (br t, 2 H, J = 7 Hz), 3.80 (s, 9 H), 5.35 (m, 2 H), 6.15 (s, 2 H), 6.43 (br s, 1 H). Anal. ($C_{27}H_{45}NO_4$) C, H, N.

Method B. (*Z*)-2-Methyl-*N*-(2,4,6-trimethoxyphenyl)-9-octadecenamide (32) and (*Z*)-2,2-Dimethyl-*N*-(2,4,6-trimethoxyphenyl)-9-octadecanamide (33). Diisopropylamine (30.3 g, 300 mmol) was dissolved in 300 mL of dry THF. NaH (14.4 g of 50% in oil, 300 mmol) was added, followed by oleic acid (84.75 g, 300 mmol) dropwise (gas evolution). When gas evolution was complete, a further 200 mL of THF was added. The mixture was heated to reflux for 15 min and then cooled to 10 °C, and *n*-BuLi (115.4 mL of 2.6 M hexanes solution) was added dropwise. When addition was complete, the resultant brown solution was stirred 30 min at room temperature and cooled to 10 °C, and CH_3I (18.6 mL, 300 mmol) was added dropwise. The mixture was allowed to warm to room temperature and stirred for 2 d. A further 30.3 mL (300 mmol) of diisopropylamine was added and the mixture cooled to 10 °C, where 115.4 mL (300 mmol) of 2.6 M *n*-BuLi was added dropwise. After stirring a further 1 h at room temperature, the mixture was cooled to 10 °C and a second equivalent of CH_3I (18.6 mL, 300 mmol) was added dropwise. The mixture was stirred for 3 h at room temperature and filtered and the filtrate concentrated in vacuo. Water was added, followed by concentrated HCl until pH 1 was achieved. A heavy oil separated which was redissolved in ether. The organic layer was washed with H_2O (2×), dried, filtered, and concentrated in vacuo. Distillation (bp 169–78 °C/0.2 mmHg) afforded 63.4 g (68%) of a 50:50 mixture of 2-methyl- and 2,2-dimethyl-9-octadecenoic acid.

Thirty-one grams (31 g, 100 mmol) of this mixture was dissolved in benzene (100 mL). Oxalyl chloride (21.1 mL, 100 mmol) was added dropwise at room temperature. The solution was stirred overnight and then concentrated in vacuo. The residue was dissolved in THF (150 mL) and added dropwise to a room temperature solution of 2,4,6-trimethoxyaniline hydrochloride (22 g, 100 mmol) and Et_3N (20 g, 200 mmol) in 250 mL of THF. This mixture was stirred overnight, filtered, and concentrated in vacuo. The residue was dissolved in ethyl acetate, washed with 1 N HCl, saturated aqueous $NaHCO_3$, and brine, dried, filtered, and concentrated in vacuo. This mixture was chromatographed twice on silica gel eluting with 50:50 v/v ethyl acetate–hexane. Fraction A (R_f = 0.53, 1:1 ethyl acetate–hexane) was Kugelrohr distilled

(21) It has been demonstrated with **39** that hydrolysis of the amide bond does not occur in vivo. (a) Woolf, T. F.; Bjorge, S. M.; Black, A. E.; Holmes, A.; Chang, T. Metabolism of the Acyl-CoA: Cholesterol Acyltransferase (ACAT) Inhibitor 2,2-Dimethyl-N-(2,4,6-trimethoxyphenyl)-dodecanamide in Rat and Monkey. *Drug Metab. Dispos.* **1991**, *19*, 696–702.

(22) Lipid Research Clinics Program. The Lipid Research Clinics Coronary Primary Prevention Trial Results. II. The Relationship of Reduction in Incidence of Coronary Heart Disease to Cholesterol Lowering. *J. Am. Med. Assoc.* **1984**, *251*, 365–374.

(23) Helsinki Heart Study: Primary-Prevention Trial with Gemfibrozil in Middle-Aged Men with Dyslipidemia. Safety of Treatment, Changes in Risk Factors, and Incidence of Coronary Heart Disease. *N. Engl. J. Med.* **1987**, *317*, 1237–1245.

(24) Field, F. J.; Albright, E.; Mathur, S. Inhibition of Acylcoenzyme A: Cholesterol Acyltransferase Activity by PD 128042: Effect on Cholesterol Metabolism and Secretion in CaCo-2 Cells. *Lipids* **1991**, *26*, 1–8.

(25) (a) Bocan, T. M. A.; Bak Mueller, S.; Uhlendorf, P. D.; et al. Effect of ACAT Inhibitors and Selected Lipid Lowering Agents on Atherosclerotic Lesions in a Cholesterol-Fed Rabbit Model. *Abstracts of the X International Symposium on Drugs Affecting Lipid Metabolism.* Houston, TX, Nov 8–11, 1989; p 55. (b) Bocan, T. M. A.; Bak Mueller, S.; Uhlendorf, P. D.; Newton, R. S.; Krause, B. R. Comparison of Cl-976, an ACAT Inhibitor, and Selected Lipid-Lowering Agents for Antiatherosclerotic Activity in Iliac-Femoral and Thoracic Aortic Lesions. *Arteriosclerosis Thrombosis* **1991**, *11*, 1830–1843.

(bp 225–230 °C/0.1 mmHg) to afford 22.5 g of **33**: 200-MHz NMR ($CDCl_3$) δ 0.88 (m, 3 H), 1.2–1.7 (m, 28 H), 2.00 (m, 4 H), 3.77 (s, 6 H), 3.79 (s, 3 H), 5.35 (m, 2 H), 6.14 (s, 2 H), 6.60 (s, 1 H). Anal. ($C_{29}H_{49}NO_4$) C, H, N. Fraction B (R_f = 0.4) contained 14.4 g of **32** as a low-melting wax: 200-MHz NMR ($CDCl_3$) δ 0.82 (m, 3 H), 1.0–1.5 (m, 23 H), 1.5–1.8 (m, 2 H), 1.96 (m, 4 H), 2.32 (m, 1 H), 3.72 (s, 9 H), 5.28 (m, 2 H), 6.08 (s, 2 H), 6.33 (br s, 1 H). Anal. ($C_{28}H_{47}NO_4$) C, H, N.

Method C. 2-Methyl-*N*-(2,4,6-trimethoxyphenyl)hexadecanamide (53). Sodium metal (12.06 g, 0.52 mol) was dissolved in 400 mL of absolute ethanol. Diethyl 2-methyl-1,3-propanedioate (95.8 g, 0.55 mol) was added dropwise with stirring. When addition was complete, the mixture was heated under reflux for 15 min. 1-Bromotetradecane (138.65 g, 0.5 mol) was added dropwise with stirring and the resulting mixture was stirred and heated at reflux overnight. The mixture was cooled, neutralized with acetic acid, and concentrated under vacuum to half its original volume. This residue was diluted with water and the aqueous phase was separated and extracted twice with ether. The organic layers were combined, washed with water, dried, and evaporated to yield an oil.

This oil was mixed with 112 g (1.7 mol) of 85% potassium hydroxide in 900 mL of 95% ethanol. The resulting mixture was heated at reflux overnight, cooled to room temperature, and made strongly acidic with concentrated HCl. The mixture was cooled and filtered. The solid was taken up in ether, dried, and evaporated under vacuum to yield 2-methyl-2-tetradecyl-1,3-propanedioic acid, mp 83–85 °C. The solid was heated with stirring to 165 °C, whereupon evolution of CO_2 began. The temperature rose rapidly to 190 °C with rapid evolution of CO_2. The solid was heated for an additional 0.5 h at 185–190 °C to yield 81.3 g of 2-methylhexadecanoic acid, mp 44–46 °C after cooling.

To 100 mL of $SOCl_2$ was added 27.0 g (0.1 mol) of 2-methylhexadecanoic acid and the resulting mixture was stirred and heated under reflux for 8 h and then cooled to room temperature overnight. The mixture was concentrated under vacuum, ether was added, and the mixture was again concentrated under vacuum. The residue was distilled to yield 25.8 g of 2-methylhexadecanoyl chloride (bp 120–125 °C/0.25 mmHg).

2,4,6-Trimethoxyaniline hydrochloride (6.58 g, 0.03 mol) and 8.3 mL (0.06 mol) of Et_3N were dissolved in 100 mL of THF. To this mixture was slowly added, with stirring, 8.65 g of 2-methylhexadecanoyl chloride. The resulting mixture was stirred at room temperature overnight and filtered and the filtrate concentrated under vacuum. Water was added to the residue. The resulting solid was collected by filtration and recrystallized from diisopropyl ether to yield 12.0 g of **53**: mp 109–111 °C; 200-MHz NMR ($CDCl_3$) δ 0.88 (m, 3 H), 1.0–1.9 (m, 29 H), 2.30 (m, 1 H), 3.80 (s, 6 H), 3.81, (s, 3 H), 6.15 (s, 2 H), 6.41 (br s, 1 H). Anal. ($C_{26}H_{45}NO_4$) C, H, N.

Method D. 2,2-Dimethyl-*N*-(2,4,6-trimethoxyphenyl)dodecanamide (39). To a suspension of NaH (14.4 g of 50% in oil, 300 mmol) in 250 mL of anhydrous THF and 42 mL (300 mmol) of diisopropylamine was added dropwise isobutyric acid (27.8 mL, 300 mmol). When gas evolution was complete, the mixture was heated at reflux for 20 min. After cooling to 0 °C, *n*-BuLi (120 mL of a 2.5 M hexane solution) was added dropwise. The resulting solution was stirred a further 15 min at 0 °C and then at ambient temperature for 2 h. It was then cooled to 0 °C and 1-bromodecane (66.3 g, 300 mmol) was added dropwise. The mixture was stirred 60 min at 0 °C and then allowed to warm to ambient temperature overnight. It was then cooled to 0 °C and H_2O (400 mL) was added. The solution was poured into a separatory funnel and ether was added. Three layers formed. The top and bottom layers were discarded, and the middle layer was made acidic with concentrated HCl. The two layers that formed were separated. The bottom layer was extracted with ether (2×). The combined organic layers were washed with brine and dried. Filtration and concentration afforded an oil which was distilled in vacuo (bp 120–123 °C/0.1 mmHg) to afford 53 g (61%) of 2,2-dimethyldodecanoic acid as a colorless oil.

To 100 mL of $SOCl_2$ was added 22.8 g (0.1 mol) of 2,2-dimethyldodecanoic acid. This mixture was stirred overnight at ambient temperature, concentrated in vacuo, and distilled (bp 90–92 °C/0.2 mmHg) to afford 22 g (89%) of the acid chloride as an oil. A 7.4-g amount of the acid chloride (30 mmol) in THF (100 mL) was added dropwise to a stirred solution of Et_3N (8.3 mL, 60 mmol) and 2,4,6-trimethoxyaniline hydrochloride (6.6 g, 30 mmol) in 150 mL of THF at ambient temperature. The resulting mixture was stirred overnight at ambient temperature and filtered, and the filtrate was concentrated in vacuo. The residue was taken up in ethyl acetate, washed with 2 N HCl, saturated aqueous $NaHCO_3$, and brine, and dried. Filtration and concentration afforded a waxy solid which was flash chromatographed (silica gel, 50:50 v/v hexanes–ethyl acetate eluant) to afford 10.5 g of **39** as a low-melting solid (mp 59–61 °C): 200-MHz ($CDCl_3$) δ 0.88 (m, 3 H), 1.1–1.6 (m, 24 H), 3.78 (s, 6 H), 3.80 (s, 3 H), 6.15 (s, 2 H), 6.61 (br s, 1 H); IR ($CHCl_3$) ν 2920, 1670, 1610, 1520, 1140 cm^{-1}. Anal. ($C_{23}H_{39}NO_4$) C, H, N.

Biological Methods. In Vitro ACAT Assay. Male New Zealand rabbits weighing 2–3 kg were fed a cholesterol diet (1% cholesterol in 10% corn oil) for 1 week and sacrificed in a fasted state. The entire small intestine was removed and the middle third (ca. 60–70 cm long) was placed in ice-cold saline. This section was divided into four equal parts and the lumen of each rinsed with ice-cold saline until all debris was removed. Each part was opened longitudinally along the mesenteric border and placed with the mucosal side up on a stainless steel tray over ice. The mucosa was scraped off with a glass microscope slide and placed in a conical polyethylene tube with 20 mL of buffer (0.04 M KH_2PO_4, 0.05 M KCl, 0.03 mM EDTA, 0.3 M sucrose), capped, vortexed, and centrifuged at 2000 rpm (900*g*) for 5 min at 10 °C. The supernatant was poured off, and the cells were washed two more times with buffer and then homogenized in 20 mL of buffer in a glass homogenizer with a motor-driven Teflon pestle for 10 strokes. The homogenate was centrifuged in a Servall RC-2 centrifuge at 9500 rpm (10000*g*) for 15 min at 10 °C. The supernatant was centrifuged in polycarbonate bottles in a 50 Ti rotor at 40 000 rpm (100000*g*) 60 min at 10 °C. The pellet was gently washed with 1 mL of buffer before being resuspended in 2 mL of buffer. This was accomplished by adding a small magnetic stirring bar and mixing until the microsomes were in suspension. An aliquot was removed to determine the protein content by a method modified from Lowry et al.[26] The remainder of the microsomal preparation was divided into 2-mL plastic tubes and kept frozen at −70 °C until needed.

Compounds were weighed to make a 1000 μM solution, placed in vials, and solubilized in 400 μL of dimethyl sulfoxide. Buffer was added (1.6 mL of 0.05% Tween-20), and the samples were sonicated by using a Kontes Microultrasonic cell disrupter for 20–30 s. The samples were brought to volume of 10 mL with buffer and vortexed before making dilutions. All dilutions were made with a stock solution containing 4% dimethyl sulfoxide and 0.008% Tween-20 in buffer.

ACAT activity was measured using endogenous cholesterol of the microsomal fraction and exogenous [1-^{14}C]oleoyl-CoA as the substrate. Specifically, the incubation mixture of 50 μL of microsomal preparation (0.3 mg of protein), 50 μL of BSA (0.5 mg), and 50 μL of potential ACAT inhibitor compound at the appropriate concentration was placed in a 12-mL conical polyethylene tube and preincubated in a shaking water bath at 37 °C for 5 min. Fifty microliters of [1-^{14}C]oleoyl-CoA (10 nmol, 0.05 μCi) was added and incubated an additional 5 min.

The reaction was stopped by the addition of 5 mL of chloroform/methanol (2:1 plus 1 mL of dilute H_2SO_4 (1 mL of concentrated H_2SO_4/L of H_2O). The tubes were capped and placed in a mechanical shaker for 5 min and centrifuged at 2000 rpm (900*g*) for 3 min in an IEC PR-6000 centrifuge.

The aqueous layer was aspirated and the chloroform/methanol layer dried under N_2. The residue was redissolved in 100 μL of chloroform/methanol (2:1), spotted on silica gel G TLC plates, and developed in methanol/ether/glacial acetic acid (80:20:1) until the solvent front reached the top of the plate. After the plate was dried, the cholesterol oleate in the standard lanes was visualized by iodine vapors, and the unknown lanes were scraped into scintillation vials containing 8 mL of RPI 3a20 counting for liquid scintillation counting. Each compound was tested at four dilutions

(26) Lowry, O. H.; Rosebrough, N. J.; Farr, A. L.; Randell, R. J. Protein Measurement with the Folin Phenol Reagent. *J. Biol. Chem.* **1951**, *193*, 265–275.

and each concentration was run in triplicate. IC_{50} values were calculated from the sigmoidal inhibition curves.

In Vivo Cholesterol-Fed Rat Assay. Male Sprague–Dawley rats (approximately 200 g) were randomly divided into groups of 10 and provided ad libitum a regular rat chow diet (Purina No. 5002) supplemented with 5.5% peanut oil, 1.5% cholesterol, and 0.3% cholic acid. Compounds were admixed by blender in the diet at a level of 0.05% (w/w). After 1 week the animals (nonfasted) were anesthetized with ether, and blood was taken from the heart into EDTA (0.14% final concentration) to measure total and HDL cholesterol using the Abbott VP Analyzer and Abbott reagents.

Acknowledgment. We thank Dr. Gary McClusky and staff for spectral and analytical determinations and Ms. Patty Elka and Beth McMillan for manuscript preparation.

Registry No. **1**, 5429-85-6; **2**, 140112-39-6; **3**, 140112-40-9; **4**, 140112-41-0; **5**, 140112-42-1; **6**, 140112-43-2; **7**, 140112-44-3; **8**, 140112-45-4; **9**, 140112-46-5; **10**, 140112-47-6; **11**, 140112-48-7; **12**, 140112-49-8; **13**, 140112-50-1; **14**, 140112-51-2; **15**, 140112-52-3; **16**, 140112-53-4; **17**, 140112-54-5; **18**, 140112-55-6; **19**, 140112-56-7; **20**, 140112-57-8; **21**, 122705-93-5; **22**, 140112-58-9; **23**, 140112-59-0; **24**, 140112-60-3; **25**, 140112-61-4; **26**, 140112-62-5; **27**, 140112-63-6; **28**, 140112-64-7; **29**, 140112-65-8; **30**, 140112-66-9; **31**, 140112-67-0; **32**, 114289-55-3; **33**, 114289-56-4; **34**, 140112-68-1; **35**, 140112-69-2; **36**, 140112-70-5; **37**, 140112-71-6; **38**, 140112-72-7; **39**, 114289-47-3; **40**, 140112-73-8; **41**, 140112-74-9; **42**, 114289-54-2; **43**, 114289-53-1; **44**, 140112-75-0; **45**, 140112-76-1; **46**, 140112-77-2; **47**, 114289-48-4; **48**, 114289-49-5; **49**, 114289-50-8; **50**, 140112-78-3; **51**, 140112-79-4; **52**, 140112-80-7; **53**, 114289-51-9; **54**, 140112-81-8; **55**, 140112-82-9; **56**, 140112-83-0; **57**, 114289-52-0; **58**, 140112-84-1; **59**, 140112-85-2; **60**, 114289-39-3; **61**, 140112-86-3; **62**, 140112-87-4; **63**, 140112-88-5; **64**, 114289-42-8; **65**, 140112-89-6; **66**, 114289-40-6; **67**, 114289-41-7; **68**, 140112-90-9; **69**, 140112-91-0; **70**, 140112-92-1; **71**, 140112-93-2; **72**, 140112-94-3; **73**, 140112-95-4; **74**, 140112-96-5; ACAT, 9027-63-8; aniline, 62-53-3; 2-methoxyaniline, 90-04-0; 2-chloroaniline, 95-51-2; 2-ethylaniline, 578-54-1; 2-isopropylaniline, 643-28-7; 2-benzoylaniline, 2835-77-0; 2-phenylaniline, 90-41-5; 2,4-dimethoxyaniline, 2735-04-8; 2,4-difluoroaniline, 367-25-9; 3,4-dimethoxyaniline, 6315-89-5; methyl 3-amino-2-methylbenzoate, 18583-89-6; methyl 3-amino-4-methoxybenzoate, 24812-90-6; methyl 2-amino-5-methylbenzoate, 18595-16-9; 1,2,3,4-tetrahydro-5-methylnaphthalene, 2809-64-5; 2-nitro-3-methylaniline, 601-87-6; ethyl 4-amino-2-methylphenylacetate, 50712-55-5; methyl 5-amino-2,4-dimethylbenzoate, 140112-97-6; methyl 5-amino-2,4-dimethoxybenzoate, 70752-22-6; 5-chloro-2,4-dimethoxyaniline, 97-50-7; 2,4,5-trimethoxyaniline, 26510-91-8; 2,4,6-trimethoxyaniline hydrochloride, 102438-99-3; 2,4,6-trimethylaniline, 88-05-1; 2,4,6-trichloroaniline, 634-93-5; 2,4,6-trifluoroaniline, 363-81-5; 2,6-dimethylaniline, 87-62-7; 2-methoxy-6-methylaniline, 50868-73-0; 2-ethoxy-6-methylaniline, 53982-02-8; 2,6-diethylaniline, 579-66-8; 2,6-diisopropylaniline, 24544-04-5; 2-isopropyl-6-methylaniline, 5266-85-3; 2-hydroxy-4,6-dimethoxyaniline, 140112-98-7; oleoyl chloride, 112-77-6; oleic acid, 112-80-1; diethyl 2-methyl-1,3-propanedioate, 609-08-5; diethyl 2-ethyl-1,3-propanedioate, 133-13-1; 1-bromodecane, 112-29-8; 1-bromododecane, 143-15-7; 1-bromotetradecane, 112-71-0; 2-methyldodecanoic acid, 2874-74-0; 2-ethyldodecanoic acid, 2874-75-1; 2-methyltetradecanoic acid, 6683-71-2; 2-ethyltetradecanoic acid, 25354-93-2; 2-methylhexadecanoic acid, 27147-71-3; 2-methylhexadecanoyl chloride, 114289-74-6; isobutyric acid, 79-31-2; 2-ethylbutyric acid, 88-09-5; acrylic acid, 79-10-7; cyclobutanecarboxylic acid, 3721-95-7; cyclopentanecarboxylic acid, 3400-45-1; cyclohexanecarboxylic acid, 98-89-5; cycloheptanecarboxylic acid, 1460-16-8; acetic acid, 64-19-7; cyclopropanecarboxylic acid, 1759-53-1; 1-bromoheptane, 629-04-9; 1-bromooctane, 111-83-1; 1-bromononane, 693-58-3; 1-bromotridecane, 765-09-3; 1-bromohexadecane, 112-82-3; 2,2-dimethylnonanoic acid, 14250-75-0; 2,2-dimethyldecanoic acid, 5343-54-4; 2,2-dimethylundecanoic acid, 13005-29-3; 2,2-dimethyldodecanoic acid, 2874-73-9; 2,2-diethyldodecanoic acid, 14276-81-4; 2-methylenedodecanoic acid, 52756-21-5; 1-decyl-1-cyclobutanecarboxylic acid, 140112-99-8; 1-decyl-1-cyclopentanecarboxylic acid, 122098-86-6; 1-decyl-1-cyclohexanecarboxylic acid, 140113-00-4; 1-decyl-1-cycloheptanecarboxylic acid, 140113-01-5; tetradecanoic acid, 544-63-8; 2,2-dimethyltetradecanoic acid, 85216-66-6; 1-dodecyl-1-cyclopropanecarboxylic acid, 140113-02-6; pentadecanoic acid, 1002-84-2; hexadecanoic acid, 57-10-3; 2,2-dimethylhexadecanoic acid, 22890-23-9; 1-tetradecyl-1-cyclopropanecarboxylic acid, 70858-10-5; octadecanoic acid, 57-11-4; 2,2-dimethyloctadecanoic acid, 4691-93-4; 2,4-dimethylaniline, 95-68-1; 3,4-dimethylaniline, 95-64-7; 2-chloro-6-methylaniline, 87-63-8; 4-bromo-2,6-dimethylaniline, 24596-19-8; methyl 2-(2-amino-3,5-dimethoxyphenoxy)-acetate, 140113-03-7; 2-(2-amino-3,5-dimethoxyphenoxy)acetic acid, 140113-04-8; 2-(heptyloxy)-4,6-dimethoxyaniline, 140113-05-9; 2-methyl-2-tetradecyl-1,3-propanedioic acid, 114289-76-8; 2,2-dimethyldodecanoyl chloride, 60631-34-7.

Supplementary Material Available: Table of parameters employed in the QSAR study (4 pages). Ordering information is given on any current masthead page.