Inhibitors of Acyl-CoA:Cholesterol Acyltransferase. 5. Identification and Structure-Activity Relationships of Novel β-Ketoamides as Hypocholesterolemic Agents[1]

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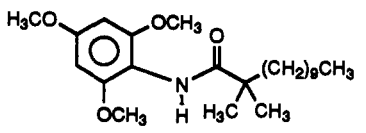
Received April 28, 1993•

A study of structure-activity relationships of substituted β-ketoamide ACAT inhibitors I and II was performed. The results of this study suggest that whereas the β-keto group was tolerated with no loss in activity, β-hydroxy and oxime moieties led to significantly reduced activity in vitro and in vivo. The most potent inhibitor from the acyclic series (I) (11, IC_{50} = 0.006 μM) contained a C-13 alkyl chain. This compound reduced plasma total cholesterol by 38% and 66% at 3 and 30 mg/kg, respectively, in cholesterol-fed rats. Dimethylation α to the anilide core (5) and subsequent N-methylation of the amide NH (6) decreased in vitro potency significantly. It was also found that high potency was retained with inhibitors which incorporated the carbonyl into a lactam ring (II).

Introduction

Acyl-coenzyme A:cholesterol acyltransferase (ACAT, EC 2.3.1.26) is the intracellular enzyme responsible for the esterification of cholesterol in tissues from all mammalian species.[2] There is considerable evidence in the literature supporting a role for ACAT in intestinal cholesterol esterification and, hence, in the absorption of most dietary and biliary cholesterol.[3] It has also been demonstrated that potent inhibitors of this enzyme decrease the absorption of dietary cholesterol and reduce plasma total cholesterol concentrations in several cholesterol-fed animal models.[4] Therefore, it has been postulated that potent ACAT inhibitors could be useful in the treatment of hypercholesterolemia. It has also been postulated that inhibition of ACAT in the arterial wall may have a beneficial effect on atherosclerotic lesions.[3] Recently, the diverse chemical structures and pharmacologic profiles of many ACAT inhibitors have been described.[5] Despite the high interest of ACAT inhibitors to date, their clinical efficacy is unproven.[6]

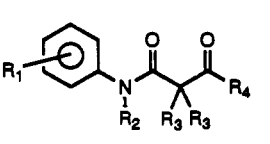
Previous work in our laboratories led to the development of a series of fatty acid anilides which were potent both at inhibiting ACAT in vitro and at lowering plasma total cholesterol in vivo.[7,8] One member of this series, CI-976



CI-976

[2,2-dimethyl-*N*-(2,4,6-trimethoxyphenyl)dodecanamide], not only was found to lower plasma total cholesterol (−60%) in cholesterol-fed rats but also elevated levels of high-density lipoprotein cholesterol (HDL-C) (+94%) in this model at the screening dose of 0.05% in the diet (ca. 50 mg/kg).[7a] CI-976 has also been shown to decrease the involvement of monocyte-macrophages within atherosclerotic lesions, blunt lesion progression, and potentiate lesion regression in cholesterol-fed rabbits.[9] It has been hypothesized that this is due to a direct inhibition of arterial wall ACAT, since it occurs even at doses where plasma total cholesterol is unaffected. Recently, another systemically bioavailable inhibitor has been reported to prevent the progression of atherosclerotic lesions in cholesterol-fed rabbits.[10]

Due to the encouraging biological results found with CI-976, we felt that exploration of other modifications of these fatty acid anilides might expand our understanding of the structural requirements for potent ACAT inhibition both in vitro and in vivo. Our continued interest in this field has now led to the development of substituted β-ketoamide ACAT inhibitors (I and II) in which a second



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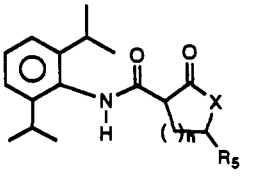

II

carbonyl moiety and conformational constraints have been added to the anilide core of CI-976. These modifications led to the identification of a number of substituted β-ketoamide ACAT inhibitors which potently inhibit ACAT in vitro and effectively lower plasma total cholesterol in vivo.

Chemistry

Most of the compounds listed in Table I were prepared by the synthetic route shown in Scheme I (method A). Many of the methyl ketones (VI) were commercially available. However, some were prepared from readily available carboxylic acids (IV). Treatment of IV with 1,1'-carbonyldiimidazole in THF at 0 °C followed by addition of the magnesium salt of ethyl malonate in acetonitrile at 25 °C yielded β-ketoester V. Decarboxylation of V using 1,4-diazo-bicyclo[2.2.2.]octane in xylenes afforded ketone VI.[11] The kinetic enolate of ketone VI was formed with

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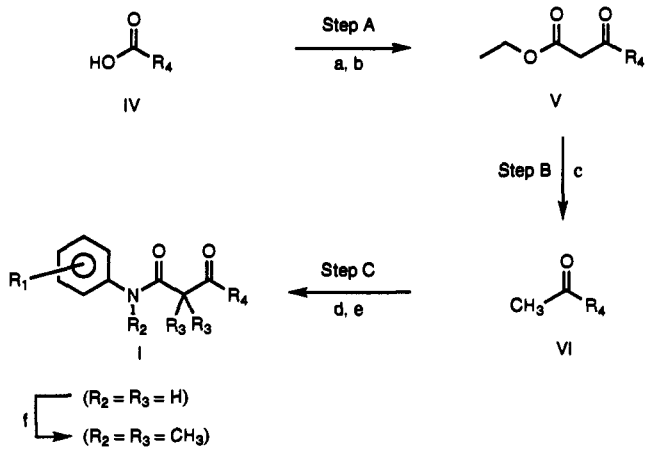
• Abstract published in *Advance ACS Abstracts*, September 1, 1993.

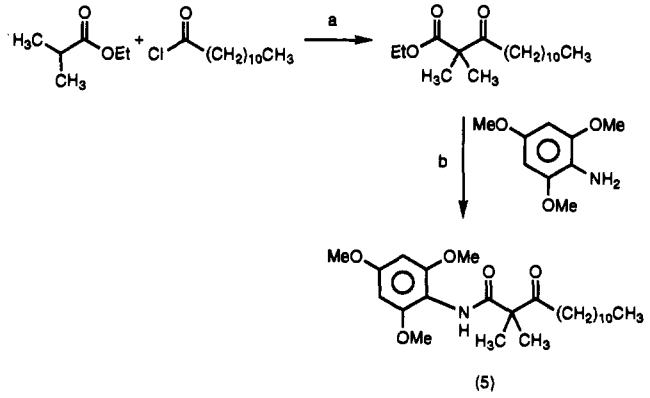
0022-2623/93/1836-2943$04.00/0

Scheme I. Method A[a]



[a](a) CDI, THF, 0 °C; (b) (Mg^{2+}), CH_3CN, 25 °C; (c) DABCO, xylenes, Δ; (d) LDA, Et_2O, 0 → 25 °C; (e) (f) NaH, CH_3I, THF, 0 → 25 °C

Scheme II. Method B[a]



[a] (a) LDA, Et_2O, 0 °C → 25 °C; (b) Me_3Al, benzene, 5 °C → reflux.

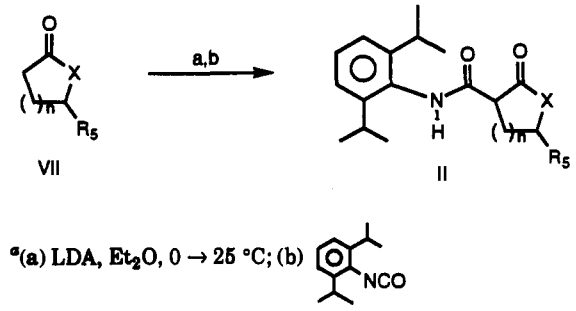
lithium diisopropylamide at 0 °C. Addition of the appropriate aryl isocyanate yielded β-ketoamide I.[12] The 2,4,6-trimethoxyphenyl isocyanate used for the synthesis of I (R_1 = 2,4,6-trimethoxy) was prepared from 2,4,6-trimethoxyaniline and phosgene in toluene.[13] Alkylation of **4** and **3** with methyl iodide in the presence of sodium hydride yielded **6** and **7**, respectively. Removal of the THP ether from **8** using *p*-toluenesulfonic acid in 95% methanol yielded unprotected alcohol **9**.

A second method utilized for the synthesis of these β-ketoamides (method B) is shown is Scheme II. Treatment of ethyl isobutyrate with LDA followed by the addition of lauroyl chloride yielded 2,2-dimethyl-3-oxotetradecanoic acid ethyl ester.[14] Addition of 2,4,6-trimethoxyaniline in the presence of trimethylaluminum[15] yielded the α,α-disubstituted β-ketoamide **5**.

Compounds **17–21** were prepared using the procedure shown in Scheme III (method C) involving the same reagents and conditions as that in method A, step C, but with lactone or lactam (VII) as the substrate in place of the acyclic methyl ketone. The appropriate lithioenolate is obtained by treating VII with lithium diisopropylamide, followed by the addition of 2,6-diisopropylphenyl isocyanate to give the corresponding β-dicarbonyl compounds.

Compounds of the generic structure III were synthesized from **15** using standard conditions. Treatment of **15** with

Scheme III. Method C[a]



[a](a) LDA, Et_2O, 0 → 25 °C; (b)

sodium borohydride reduced the ketone to β-hydroxyamide **22**. Oxime **23** was obtained by reacting **15** with hydroxylamine hydrochloride and sodium acetate.

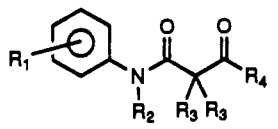
Results

Two primary biological assays were used to evaluate the compounds prepared in this study. The ability of each compound to inhibit intestinal ACAT in vitro (IAI) was evaluated by incubating the compound with [1-^{14}C]oleoyl-CoA and intestinal microsomes isolated from cholesterol-fed rabbits.[4a, 7a] Activity is expressed as the micromolar concentration of compound required to inhibit enzyme activity by 50% (IC_{50}). Cholesterol-lowering activity was assessed in an acute in vivo screen (APCC)[16] in which male rats (Sprague–Dawley, 200 g) were given a single oral dose (3 or 30 mg/kg) of compound or vehicle by gavage (4 p.m.) and then allowed to consume overnight a diet supplemented with peanut oil (5.5%), cholic acid (0.5%), and cholesterol (1.5%) (PCC diet). Those animals given the drug vehicle (CMC/Tween in water) (1.5%/0.2%, w/w)[7c] generally experienced a rise in plasma total cholesterol from an average of 73 mg/dL to an average of 214 mg/dL. Total serum cholesterol was then determined and expressed as a percent decrease relative to PCC controls. However, this is most accurately interpreted as preventing the rise in total cholesterol seen in control animals.

It had been previously determined[17] that 2,6-disubstitution on the phenyl ring was necessary for potent ACAT inhibition. It was also found that by increasing the size of the 2,6-alkyl substituents[18] (i.e., from methyl to isopropyl) on the phenyl ring, activity was improved significantly. This strong dependence on the size of the ortho substituents is probably best interpreted as a requirement to assure a perpendicular orientation of the required amide ketone function with the aromatic ring. Because of this requirement, the aryl substituents utilized in this study were mainly 2,6-diisopropyl.

The primary modifications to CI-976 illustrated in this study were the addition of a second carbonyl group and various ring systems positioned β to the amide group. Introduction of the β-keto group (**2**), while maintaining the same chain length as CI-976, resulted in similar in vitro potency (IC_{50} = 0.06 μM vs IC_{50} = 0.074 μM, respectively). Examination of various alkyl chain lengths (**1-3**, **10-12**, Table I) led to an exceptionally potent ACAT inhibitor (**11**), having a C-13 alkyl chain and an IC_{50} = 0.006 μM. Plasma total cholesterol was lowered by 66% at 30 mg/kg and 38% at 3 mg/kg, with this compound. Thus, potency appears to be greater than that observed for CI-976, which typically lowers plasma total cholesterol by 19% and 56% at 3 and 30 mg/kg, respectively, in this acute efficacy screen.[19] Also demonstrating high in vitro

Table I. In Vitro and in Vivo Activity of Substituted Acyclic β-Ketoamides (I)



I

example	R_1	R_2	R_3	R_4	method of prepn	IAI[a] IC_{50} (μM)	APCC[b] (% Δ in TC) 3 mg	APCC[b] (% Δ in TC) 30 mg	CLOGP[c] (measd values)	mp (°C)	formula[d]
1	2,6-$(CHMe_2)_2$	H	H	$(CH_2)_8CH_3$	A	0.097	−5	−54[e]	6.23	*f*	$C_{24}H_{39}NO_2$
2	2,6-$(CHMe_2)_2$	H	H	$(CH_2)_9CH_3$	A	0.060	g	−30[e]	6.76	*f*	$C_{25}H_{41}NO_2$
3	2,6-$(CHMe_2)_2$	H	H	$(CH_2)_{10}CH_3$	A	0.069	−25[e]	−43[e]	7.29	*h*	$C_{26}H_{43}NO_2$[i]
4	2,4,6-$(OMe)_3$	H	H	$(CH_2)_{10}CH_3$	A	0.15	g	−31[e]	5.16 (5.02)	95–98	$C_{23}H_{37}NO_5$
5	2,4,6-$(OMe)_3$	H	CH_3	$(CH_2)_{10}CH_3$	B	0.37	0	−44[e]	5.78 (4.88)	92–94	$C_{25}H_{41}NO_5$[j]
6	2,4,6-$(OMe)_3$	CH_3	CH_3	$(CH_2)_{10}CH_3$	A	>1	*g*	−40[e]	6.93 (6.67)	*h*	$C_{26}H_{43}NO_5$[k]
7	2,6-$(CHMe_2)_2$	CH_3	CH_3	$(CH_2)_{10}CH_3$	A	1.0	0	−60[e]	9.73 (>8.6)	*h*	$C_{29}H_{49}NO_2$[l]
8	2,6-$(CHMe_2)_2$	H	H	$(CH_2)_{10}OTHP$	A	0.057	−17	−67[e]	6.60	*h*	$C_{30}H_{49}NO_3$[m]
9	2,6-$(CHMe_2)_2$	H	H	$(CH_2)_{10}OH$	A	0.23	−2	−29	5.30 (4.20)	*f*	$C_{25}H_{41}NO_3$[n]
10	2,6-$(CHMe_2)_2$	H	H	$(CH_2)_{11}CH_3$	A	0.011	−31[e]	−56[e]	7.81	*h*	$C_{27}H_{45}NO_2$
11	2,6-$(CHMe_2)_2$	H	H	$(CH_2)_{12}CH_3$	A	0.006	−38[e]	−66[e]	8.34	*h*	$C_{28}H_{47}NO_2$
12	2,6-$(CHMe_2)_2$	H	H	$(CH_2)_{13}CH_3$	A	0.024	−34[e]	−57[e]	8.87	*f*	$C_{29}H_{49}NO_2$[o]
13	2,6-$(CHMe_2)_2$	H	H	Ph	A	0.81	0	−4	3.59 (2.87)	124–126	$C_{21}H_{25}NO_2$
14	2,6-$(CHMe_2)_2$	H	H	$CHPh_2$	A	0.035	−21[e]	−65[e]	5.00 (3.96)	153–155	$C_{23}H_{31}NO_2$[p]
15	2,6-$(CHMe_2)_2$	H	H	CH_2CHPh_2	A	0.044	−23[e]	−43[e]	5.23	146–148	$C_{29}H_{33}NO_2$
16	2,6-$(CHMe_2)_2$	H	H		A	0.011	*g*	−37[e]	5.11 (4.55)	145–147	$C_{26}H_{33}NO_2$

[a] ACAT inhibition in vitro, intestinal microsomes isolated from cholesterol-fed rabbits. Each determination performed in triplicate. See ref 7a for complete protocol. [b] Denotes percent change in total cholesterol in cholic acid (0.5%)–cholesterol (1.5%)–peanut oil (5.5%)-fed rats. All animals were dosed at 30 mg/kg, and the more active compounds at 3 and 30 mg/kg. [c] Experimental values denoted in parentheses were determined by HPLC correlation method.[22] [Alltech C18, 250 × 4.6 mm, 10 μm column; 80:20 MeOH/buffer (0.05 M $NH_4H_2PO_4$), pH 7.4]. [d] Analytical results are within ±0.4% of theoretical values unless otherwise noted. Some difficulty was found in obtaining combustion analysis on the indicated compounds due to the propensity of these compounds to retain solvents. [e] Significantly different from control, $p < 0.05$, using unpaired two-tailed t-test. The average vehicle control value for total cholesterol was 214 mg/dL. [f] Isolated material was analytical. [g] Not tested. [h] Purified by flash chromatography on silica gel. [i] High mass calcd 401.3293, found 401.3296. [j] High mass calcd 435.2974, found 435.2990. [k] High mass calcd 449.3130, found 449.3146. [l] High mass calcd 443.3751, found 443.3788. [m] High mass calcd 501.3805, found 501.3816. [n] High mass calcd 417.3232, found 417.3242. [o] High mass calcd 443.3763, found 443.3745. [p] High mass calcd 413.2354, found 413.2337.

potency was 10 (IC_{50} = 0.011 μM), which has a C-12 side chain. Plasma total cholesterol was also lowered substantially by this compound (−31/−56%).

To mimic the α,α-dimethyl substitution pattern of CI-976, the α,α-dimethyl analog (**5**) was prepared. It was found that the α,α-dimethyl substitution produced no enhancement of in vitro or in vivo activity. This is in contrast to the simple fatty acid anilides reported previously, where α,α-disubstitution led to a significant improvement in in vitro activity.[7] However, N-methylation of the anilide nitrogen (**6** and **7**) caused a marked reduction in in vitro activity (e.g., **6** vs **5**, Table I). This may indicate that the amide NH is essential for ACAT inhibition, something not addressed in the previously reported series of amides[4f,g, 7] and ureas.

A survey of in vivo activity indicates that most of the inhibitors prepared in this study are effective at preventing the rise in plasma total cholesterol caused by the high fat/high cholesterol meal when dosed at 30 mg/kg, regardless of in vitro potency, suggesting that it is not difficult to block cholesterol absorption at this dose. However, at low dose (3 mg/kg), only the more potent ACAT inhibitors are effective. For example, at 30 mg/kg, compound **7** (IC_{50} = 1.0 μM) reduced plasma total cholesterol 60%, but at 3 mg/kg, no effect was observed. Compounds **8** and **11** (IC_{50} = 0.057 and 0.006 μM, respectively) reduced plasma total cholesterol 17/67% and 38/66%, respectively, at 3 and 30 mg/kg. Overall, the reduction of plasma total cholesterol observed at 3 mg/kg seems to correlate with the observed in vitro potency.

Addition of a free hydroxyl group at the end of the alkyl chain decreased lipophilicity by two log units and decreased in vitro activity by ca. 3.5-fold (**9** vs **3**). In vivo activity observed was −29% vs −43% at 30 mg/kg and −2% vs −25% at 3 mg/kg, respectively. Masking this hydroxyl unit with a tetrahydropyran ring (**8**) restored both in vitro and in vivo activity (IC_{50} = 0.057 μM, 17/67% reduction in plasma total cholesterol). Apparently, polarity on the terminus of the alkyl chain is unfavorable. Also, the hydroxyl group may readily be metabolized, rendering less active metabolites.

Since potency was maintained by the addition of a β-keto group to the fatty acid moiety structure of CI-976, other modifications (illustrated in **13-23**) were examined. Based on our previous observation for a series of disubstituted ureas, where a phenyl group three atoms away from the urea carbonyl seemed to be the optimal spatial arrangement for potent ACAT inhibition,[18] we synthesized **13-15** (Table I). Highest in vitro and in vivo potency for these compounds was observed for **14** (IC_{50} = 0.035 μM, 21/65% reduction in plasma total cholesterol), which has a phenyl group three atoms away from the amide carbonyl, confirming the original observation. Also, a second phenyl group is attached α to the keto group which resembles the preferred β,β-disubstituted phenethyl side chains reported.[18]

Compound 16 incorporates the phenyl group located three atoms from the amide carbonyl and a cyclopentyl moiety substituted α to the keto group rather than a phenyl group as in 14. This modification originated from a series of *N*-phenyl-*N'*-(1-phenylcycloalkyl)ureas studied previously[17] where cyclopentyl was determined to be optimal. The potency of 16 (IC_{50} = 0.011 μM, -28/-37%) is similar to our most potent alkyl chain analog 11 (IC_{50} = 0.006 μM, -38/66%).

Additional analogs (17-21, Table II) were synthesized in which the second carbonyl was directly incorporated into a ring. Addition of this rigidity did not affect activity significantly. The seven-membered lactam, with a C-12 alkyl chain substituted on the ring (21), yielded slightly better in vitro and in vivo activity (IC_{50} = 0.022 μM, 47% reduction in plasma total cholesterol at 30 mg/kg) than the five-membered lactam with the same chain length (20, IC_{50} = 0.053 μM, -20% at 30 mg/kg).

Conversion of the keto group of 15 to both the alcohol and the oxime (22 and 23, respectively, Table III) showed much reduced activity both in vitro and in vivo.

Discussion

A comprehensive structure–activity study examining the effects of structural changes in both the fatty acid or amine moieties was recently reported.[7a] Extension of this structure–activity study by examining the effects of the addition of a β-keto moiety and various side chains and ring systems to the anilide core of CI-976 on ACAT inhibition is reported in this present study.

All analogs containing straight alkyl chains ($>C_8$) were potent ACAT inhibitors possessing IC_{50} values less than 0.09 μM. The most potent ACAT inhibitor of this type had a C-13 alkyl chain (11, IC_{50} = 0.006 μM). This in vitro potency was significantly reduced when hydrophilic groups were added to the alkyl chain (9, 22, and 23). This reduction in in vitro potency may reflect the lipophilic nature of the fatty acid binding region of the enzyme.

Contrary to the potent in vitro activity of the α,α-dimethyl substituted analogs reported previously for the fatty acid anilide series,[7a] the dimethyl substitution α to the amide bond of β-ketoamide (5) produced a significant decrease in potency in vitro and in vivo.

The N-methylated, α,α-dimethyl substituted β-ketoamides (6 and 7) were even less potent than 5 in vitro but had similar in vivo activity. Compound 7 illustrates the correlation of in vitro to in vivo potency for compounds dosed at 3 mg/kg. Even though compound 7 showed a statistically significant reduction in plasma total cholesterol at the screening dose of 30 mg/kg, no reduction was observed at 3 mg/kg, which is consistent with its observed in vitro potency (IC_{50} = 1.0 μM).

In general, replacement of the alkyl chain with a diphenyl methylene group maintained potency both in vitro and in vivo. Substituting the alkyl chain with substituted lactones and lactams (17-21) caused a reduction in both in vitro and in vivo potency. However, the larger ring system (21) containing a C-12 alkyl chain exhibited the least reduction in in vitro potency (ca. 4-fold, 21 vs 11). This observation may suggest that more flexible ring systems in this series of β-ketoamides (II) are preferred for ACAT inhibition.

The relationship of in vitro activity to lipophilicity observed in other ACAT series[20] was also examined in this series. All compounds, excluding 6 and 7 (*N*-methyl) and 22 and 23 (non-keto), showed a significant trend towards increased in vitro potency with increased lipophilicity as calculated by CLOGP[21] or measured by the HPLC correlation method.[22] Three compounds, the aralkyl acyclic analogs (14-16), are significant outliers, being much more potent than expected on the basis of this relationship. From studies on potent aralkyl ureas of related structures, we know that properly oriented phenyl rings can impart a special contribution to potency.[17,18] It may be that the flexible analogs 14-16 can allow their aryl moieties to interact with the same accessory binding region available to the ureas, but the rigidified analogs of Table II are prevented from doing so. Equations 1 and 2 and Figure 1 show the relationship of CLOGP to in vitro potency. Compounds 14-16 are excluded in the derivation of eq 2.

$$-\log(\mathrm{IAI}) = 5.53 + 0.25\mathrm{CLOGP} \quad (1)$$

$$n = 19,\ R^2 = 0.36,\ S = 0.49,\ F = 20.8\ (p = 0.0065)$$

$$-\log(\mathrm{IAI}) = 4.61 + 0.37\mathrm{CLOGP} \quad (2)$$

$$n = 16,\ R^2 = 0.76,\ s = 0.31,\ F = 43.2\ (p = 0.0001)$$

Thus, in summary, addition of a second carbonyl, β to the amide of the fatty acid anilides reported previously, results in compounds which are equipotent and, in some cases, more potent than the parent anilides. The placement of this carbonyl in a ring is also tolerated without significant loss in potency in vitro. All of the compounds reported prevented the rise in plasma cholesterol induced by the high fat meal at the screening dose of 30 mg/kg, except the β-hydroxy and oxime analogs, which were much less potent both in vitro and in vivo. The more potent ACAT inhibitors maintained in vivo activity when the dose was lowered to 3 mg/kg.

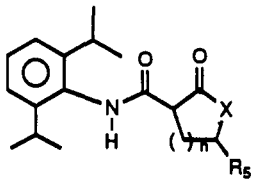
The application of the observation that a β-carbonyl leads to a retention of potent ACAT inhibition in the anilide series will be the topic of future communications from these laboratories.

Experimental Section

High-field nuclear magnetic resonance (NMR) spectra were recorded in deuterochloroform ($CDCl_3$) as a solvent on a Varian XL-200 or a Bruker 250 MHz Spectrometer. All chemical shifts are reported in ppm downfield from internal tetramethylsilane. Infrared spectra were determined on a Nicolet MX-1 FT-IR spectrophotometer. 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. Mass spectra were obtained by using a VG Masslab Trio-2A, Finnigan TSQ-70, or VG Analytical 7070E/HF mass spectrometer. Melting points were determined on a Thomas-Hoover melting point apparatus and are uncorrected. All chemicals and reagents used were of commercial purity unless otherwise specified.

Method A. *N*-[2,6-Bis(1-methylethyl)phenyl]-3-oxo-13-[(tetrahydro-2*H*-pyran-2-yl)oxy]tridecanamide (8). Step A: Preparation of Ethyl 3-Oxo-14-[(tetrahydro-2*H*-pyran-2-yl)oxy]tetradecanoate. To a solution of 12-hydroxydodecanoic acid (10.0 g, 0.046 mol) in 150 mL of THF was added *p*-toluenesulfonic acid (0.087 g, 0.01 equiv) followed by 3,4-dihydro-2*H*-pyran (5.95 g, 0.070 mol). After stirring this homogeneous reaction mixture for 16 h at room temperature under a nitrogen atmosphere, $NaHCO_3$ saturated solution was added to the mixture. The mixture was extracted with methylene chloride, dried ($MgSO_4$), and concentrated in vacuo (30 °C) to yield 13.0 g (94%) of 12-[(tetrahydro-2*H*-pyran-2-yl)oxy]dodecanoic acid. Anal. ($C_{17}H_{32}O_4$) C,H,N. To a cooled solution (0 °C) of 12-[(tetrahydro-2*H*-pyran-2-yl)oxy]dodecanoic acid (10.0 g, 0.033 mol) in 50 mL of THF was added 1,1'-carbonyldiimidazole (6.47 g, 0.039 mol) portionwise (exotherm to 10 °C),

Table II. In Vitro and in Vivo Activity of Substituted Cyclic β-Ketoamides (II)



II

example	X	n	R_5	method of prepn	IAI[a] IC_{50} (μM)	APCC[b] (% Δ in TC) 3 mg	APCC[b] (% Δ in TC) 30 mg	CLOGP[c] (measd values)	mp (°C)	formula[d]
17	O	1	$(CH_2)_6CH_3$	C	0.26	*g*	−26	5.82 (4.72)	95–98	$C_{24}H_{37}NO_3$
18	O	2	$(CH_2)_6CH_3$	C	0.34	*g*	−11	6.38	101–103	$C_{23}H_{39}NO_3$[f]
19	O	2	$(CH_2)_5CH_3$	C	0.40	*g*	−43[e]	5.85	113–115	$C_{24}H_{37}NO_3$
20	NH	1	$(CH_2)_{11}CH_3$	C	0.053	*g*	−20[e]	7.67 (7.58)	*h*	$C_{28}H_{48}N_2O_2$
21	NH	3	$(CH_2)_{11}CH_3$	C	0.022	*g*	−47[e]	8.79 (8.55)	99–104	$C_{30}H_{52}N_2O_2$[i]

[a] ACAT inhibition in vitro. See footnote a, Table I. [b] Denotes percent change in total cholesterol in cholic acid–cholesterol–peanut oil-fed rats. See footnote *b*, Table I. [c] Experimental values denoted in parentheses were determined by HPLC method.[22] See footnote *c*, Table I. [d] Analytical results are within ±0.4% of theoretical values unless otherwise noted. See footnote *d*, Table I. [e] Significantly different from control, $p < 0.05$. See footnote *e*, Table I. [f] High mass calcd 401.2920, found 401.2926. [g] Not tested. [h] Isolated material was analytical. [i] High mass calcd 484.4016, found 484.4034.

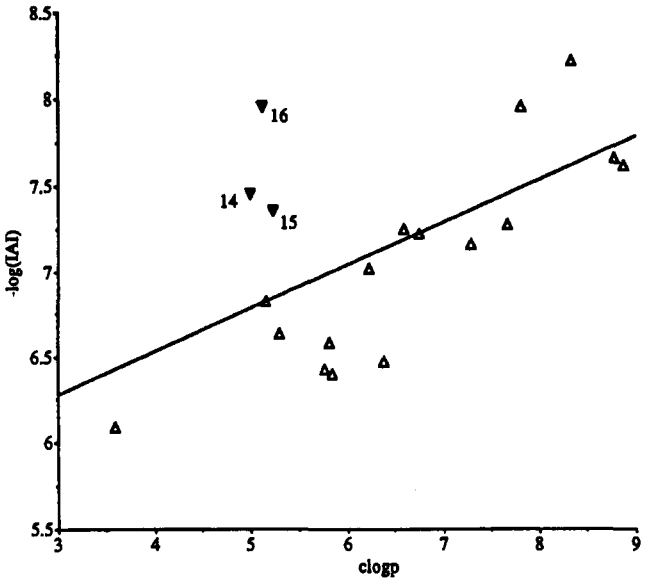


Figure 1. Activity vs lipophilicity.

and the solution was stirred at 0 °C for 3 h. After 3 h, in a separate flask (equipped with an overhead stirrer), magnesium chloride (6.02 g, 0.063 mol) was added to a cooled solution (0 °C) of ethyl potassium malonate (9.06 g, 0.053 mol) in 50 mL of acetonitrile (exotherm to 30 °C). Triethylamine (7.40 g, 0.073 mol) was then added, and this mixture was allowed to gradually warm to room temperature and stir for 4 h. The dodecanoic acid mixture was now also allowed to gradually warm to room temperature and stir for 4 h. This dodecanoic acid mixture was then added dropwise to the malonate mixture (exotherm to 30 °C), and this reaction mixture was stirred at room temperature for 16 h. This heterogeneous reaction mixture was then carefully quenched with a solution of 28.5 g of sodium bisulfate in 90 mL of water. Ethyl acetate (200 mL) was then added to the homogeneous solution, the layers were separated, and the organic layer was washed with 50 mL of 5% NaOH solution and 50 mL of saturated NaCl solution. The organic layer was then diluted with 30 mL of hexane, dried ($MgSO_4$), and concentrated in vacuo (30 °C) to give 9.97 g (82%) of the desired product: 250 MHz NMR ($CDCl_3$) 1.26–1.82 (m, 20H), 2.50–2.55 (t, 3H, CH_2CH_3, J = 8.79 Hz), 3.33–3.87 (m, 8H), 3.43 (s, $COCH_2CO$), 4.15–4.24 (q, 2H, CH_2CH_3, J = 8.57 Hz), 4.57 (m, 1H).

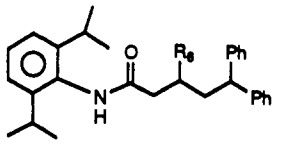
Step B: Preparation of 13-(Tetrahydro-2*H*-pyran-2-yl)oxy-2-tridecanone. To a solution of ethyl 3-oxo-14-[(tetrahydro-2*H*-pyran-2-yl)oxy]tetradecanoate (7.97 g, 0.021 mol) in xylenes (39.3 mL, 15 equiv) was added 1,4-diazabicyclo[2.2.2]octane (24.13 g, 0.215 mol), and the reaction mixture was first heated to reflux for 5 h and then stirred at room temperature for 16 h.[11] The reaction mixture was then acidified (pH 1) with approximately 10 mL of 5% HCl. This heterogeneous mixture was then filtered through Celite, thoroughly washing the bed of Celite with ether. The organic layer was washed with water, dried ($MgSO_4$), and concentrated in vacuo to give a yellow oil. Purification by flash chromatography (silica gel, 10% EtOAc/hexane) yielded 2.53 g (40%) of the desired product: MS 299.3 (MH^+), 298.3 (M^+).

Step C: Preparation of *N*-[2,6-Bis(1-methylethyl)phenyl]-3-oxo-13-[(tetrahydro-2*H*-pyran-2-yl)oxy]tridecanamide (8). To a cooled (−78 °C) solution of diisopropylamine (0.612 g, 0.006 mol) in 40 mL of ether was added *n*-BuLi (0.006 mol) dropwise followed by 20 mL of ether. After stirring for 5 min at −78 °C under a nitrogen atmosphere, a solution of 13-(tetrahydro-2*H*-pyran-2-yl)oxy-2-tridecanone (2.0 g, 0.006 mol) in 10 mL of ether was added dropwise, and the resulting solution was stirred for 20 min at −78 °C. A solution of 2,6-diisopropylphenyl isocyanate (1.36 g, 0.006 mol) in 10 mL of ether was then added dropwise, and the reaction mixture was allowed to gradually warm to room temperature and stir for 16 h under a nitrogen atmosphere.[12] The reaction mixture was then quenched with a saturated solution of ammonium chloride and extracted with methylene chloride. The layers were separated, and the organic layer was washed two times with water, dried (Na_2SO_4), and concentrated in vacuo (30 °C) to afford an oil. Purification by flash chromatography (silica gel, 10% EtOAc/hexane) yielded 3.11 g (93%) of the desired product: 250 MHz NMR ($CDCl_3$) 1.14–1.81 (m, 34H), 2.55–2.60 (t, 2H, J = 6.12 Hz), 2.92–3.01 (m, 2H), 3.30–3.85 (m, 4H), 3.58 (s, 2H), 4.52–4.54 (m, 1H), 7.10–7.27 (m, 3H), 8.38 (br.s, 1H); high mass calcd 501.3805, found 501.3816.

***N*,2,2-Trimethyl-3-oxo-*N*-(2,4,6-trimethoxyphenyl)tetradecanamide (6).** To a cooled solution (0 °C) of NaH (0.290 g, 0.006 mol) in 10 mL of THF was added 4 (1.23 g, 0.003 mol) in 8 mL of THF dropwise (exotherm from 0–5 °C). The resulting reaction mixture was then stirred for 30 min at 0 °C under a nitrogen atmosphere. Iodomethane (0.850 g, 0.006 mol) was then added dropwise, and the reaction mixture was then allowed to gradually warm to room temperature and stirred for 16 h. The mixture was then quenched with H_2O and extracted with ether. The layers were separated, and the organic layer was washed with brine, dried ($MgSO_4$), and concentrated in vacuo (30 °C) to yield 0.460 g (34%) of 4: 250 MHz NMR ($CDCl_3$) 0.81–0.88 (m, 3H), 1.18–1.28 (br.s, 18H), 1.40 (s, 6H), 2.35–2.41 (m, 2H), 3.05 (s, 3H), 3.74 (s, 9H), 6.05–6.12 (m, 2H); high mass calcd 449.3130, found 449.3146.

***N*-[2,6-Bis(1-methylethyl)phenyl]-13-hydroxy-3-oxotridecanamide (9).** To a solution of 8 (0.97 g, 0.001 mol) in 190 mL of 95% MeOH was added *p*-toluenesulfonic acid (0.055 g, 0.15 equiv), and the reaction mixture was stirred for 16 h at room

Table III. In Vitro and in Vivo Activity of Compounds with Modifications on the Keto Functionality (III)



III

example	R_6	method of prepn	IAI[a] IC_{50} (μM)	APCC[b] (% Δ in TC) 3 mg	APCC[b] (% Δ in TC) 30 mg	CLOGP[c] (measd values)	mp (°C)	formula[d]
15	=O	A	0.044	-23[e]	-43[e]	5.23	146–148	$C_{29}H_{33}NO_2$
22	OH	A	3.5	g	-10	5.30 (4.01)	138–140	$C_{29}H_{35}NO_2$[f]
23	=NOH	A	0.21	0	-1	5.80 (3.93)	69–72[h]	$C_{29}H_{34}N_2O_2$[i]

[a] ACAT inhibition in vitro. See footnote *a*, Table I. [b] Denotes percent change in total cholesterol in cholic acid–cholesterol–peanut oil-fed rats. See footnote *b*, Table I. [c] Experimental values denoted in parentheses were determined by HPLC correlations method.[22] See footnote *c*, Table I. [d] Analytical results are within ±0.4% of theoretical values unless otherwise noted. See footnote *d*, Table I. [e] Significantly different from control, $p < 0.05$. See footnote *e*, Table I. [f] High mass calcd 429.2659, found 429.2666. [g] Not tested. [h] Triturated with diethyl ether. [i] High mass calcd 442.2612, found 442.2631.

temperature under a nitrogen atmosphere. The reaction mixture was then concentrated in vacuo (30 °C) to give a tacky residue. This residue was diluted with water and chloroform, the layers were separated, and the aqueous layer was then extracted three times with chloroform. The combined organic layers were then washed two times with a saturated NaCl solution, dried ($MgSO_4$), and concentrated in vacuo to yield 0.63 g (79.4%) of the desired product: MS 418.4 (MH^+), 417.4 (M^+); 250 MHz ($CDCl_3$) 1.07–1.57 (m, 31H), 2.51–2.57 (t, 2H, J = 7.36 Hz), 2.85–3.02 (m, 2H), 3.52–3.57 (m, 4H), 7.09–7.25 (m, 3H), 8.38 (br.s, 1H); high mass calcd 417.3232, found 417.3242.

***N*-[2,6-Bis(1-methylethyl)phenyl]-β-hydroxy-δ-phenylbenzenepentanamide (22).** To a cooled (0 °C) solution of sodium borohydride (0.090 g, 0.002 mol) in 20 mL of ether was added dropwise *N*-[2,6-bis(1-methylethyl)phenyl]-β-oxo-δ-phenylbenzenepentanamide (15, 1.0 g, 0.002 mol) in 20 mL of THF. The mixture was allowed to gradually warm to room temperature and stir for 16 h under a nitrogen atmosphere. The reaction mixture was then quenched with water and extracted with ethyl acetate. The organic layer was then washed with brine, dried (Na_2SO_4), and concentrated in vacuo to yield a crude solid. Purification by flash chromatography (silica gel, 20% EtOAc/hexane) yielded 0.34 g (40%), mp 138–140 °C, of the desired product: 250 MHz NMR ($CDCl_3$) 1.06–1.25 (m, 12H), 1.90–2.09 (m, 2H), 2.29–2.36 (m, 2H), 2.46–2.64 (m, 2H), 2.93–3.01 (m, 2H), 3.41–3.43 (d, 1H, J = 4.22 Hz), 4.20–4.32 (m, 1H), 7.11–7.35 (m, 13H); high mass calcd 429.2659, found 429.2666.

***N*-[2,6-Bis(1-methylethyl)phenyl]-β-(hydroxyimino)-γ-phenylbenzenepentanamide (23).** To a solution of *N*-[2,6-bis(1-methylethyl)phenyl]-β-oxo-δ-phenylbenzenepentanamide (15) (1.0 g, 0.002 mol) in 20 mL of ethanol were added hydroxylamine hydrochloride (0.162 g, 0.002 mol), and sodium acetate (0.131 g, 0.0016 mol) in 2 mL of H_2O.[23] After stirring for 1 h at room temperature, the mixture was then concentrated in vacuo to evaporate excess ethanol. This residue was dissolved in ether and then concentrated in vacuo to afford 0.715 g (80.8%) mp 69–72 °C, of the desired product: MS 443 (MH^+), 442 (M^+); 250 MHz NMR ($CDCl_3$) 1.04–1.34 (m, 12H), 2.89–3.53 (m, 6H), 4.44–4.64 (m, 1H), 7.12–7.26 (m, 13H); high mass calcd 442.2612, found 442.2631.

Method B. 2,2-Dimethyl-3-oxo-*N*-(2,4,6-trimethoxyphenyl)tetradecanamide (5). To a cooled solution (−78 °C) of diisopropylamine (2.22 g, 0.022 mol) in 25 mL of ether was added *n*-BuLi (0.022 mol) dropwise, and the solution was stirred for 20 min under a nitrogen atmosphere. Ethyl isobutyrate (2.65 g, 0.022 mol) was then added dropwise, and the mixture was stirred for 20 min. In a separate flask, lauroyl chloride (5.0 g, 0.022 mol) was stirred in 10 mL of ether and cooled to 0 °C. The enolate solution was added dropwise to the lauryl chloride solution over a 5-min period. The reaction mixture was allowed to gradually warm to room temperature and stir for 16 h.[14] The mixture was quenched with saturated ammonium chloride solution and extracted with ether two times. The combined organic layers were washed with brine, dried ($MgSO_4$), and concentrated in vacuo (30 °C) to yield 6.5 g (98.9%) of 2,2-dimethyl-3-oxotetradecanoic acid ethyl ester: MS 299.2 (MH^+), 298.2 (M^+); 250 MHz NMR ($CDCl_3$) 0.85–0.90 (t, 3H), 1.21–1.28 (m, 21H), 1.35 (s, 6H), 2.40–2.46 (m, 2H), 4.16–4.22 (m, 2H).

To a flask containing 10 mL of benzene which had been flushed with nitrogen was added trimethylaluminum (2.0 M hexane solution, 0.003 mol, 1.14 equiv) dropwise and the solution was cooled to 5 °C. A solution of 2,4,6-trimethoxyaniline (0.653 g, 0.003 mol) in 1.5 mL of benzene was added dropwise (exotherm to 10 °C) and stirred for 20 min at 5 °C. The ice bath was removed, and the contents were allowed to gradually warm to room temperature over a 45-min period. A solution of 2,2-dimethyl-3-oxotetradecanoic acid ethyl ester (1.0 g, 0.003 mol) in 1.5 mL of benzene was added dropwise, and the resulting reaction mixture was heated for 16 h.[15] After cooling to room temperature, the mixture was hydrolyzed slowly with 2% HCl solution (5 mL) and stirred for 20 min. The layers were then separated and the aqueous layer was extracted three times with ethyl acetate. The combined organic layers were washed with brine, dried ($MgSO_4$), and concentrated in vacuo (30 °C) to yield a residue which was washed with hot hexanes, cooled, filtered, and oven-dried to yield 0.340 g (26%) of 2,2-dimethyl-3-oxo-*N*-(2,4,6-trimethoxyphenyl)tetradecanamide, mp 92–94 °C: 250 MHz NMR ($CDCl_3$) 0.85–0.90 (m, 3H), 1.08–1.34 (br.s, 18H), 1.46 (s, 6H), 2.36–2.57 (m, 2H), 3.78 (s, 9H), 6.13 (s, 2H), 6.46 (br.s, 1H); high mass calcd 435.2974, found 435.2990.

Biological Methods. In Vitro ACAT Assay. The ability of each compound to inhibit intestinal ACAT in vitro (IAI) was evaluated by the method described by Roth et al.[7a]

In Vivo Cholesterol-Fed Rat Assay. Cholesterol-lowering activity was assessed in an acute in vivo screen (APCC) as follows: Male Sprague–Dawley rats (200–225 g) were dosed po at 4 p.m. with test compound suspended in carboxymethylcellulose (1.5%) and Tween-20 (0.2%) in water. The control group received vehicle alone. Immediately after dosing, all animals received ad libitum, a chow diet supplemented with peanut oil (5.5%), cholesterol (1.5%), and a cholic acid (0.5%) (PCC diet). At 8 a.m. the following day, the rats were anesthetized with ether and exsanguinated via cardiac puncture. This type of acute in vivo test has been described previously.[16] Total serum cholesterol concentration was determined using an Abbott VP Analyzer with Boehringer–Mannheim reagents. The animals were dosed at 3 and/or 30 mg/kg, as indicated. The data are expressed as percent decrease in total cholesterol relative to PCC controls. The average vehicle control value for total cholesterol was 214 mg/dL.

Acknowledgment. We thank Dr. G. McClusky and staff for spectral and analytical determinations, Dr. F. MacKellar and staff for the experimental log *P* values, Mr. D. DeJohn (Parke-Davis), Mr. T. Stevenson (Parke-Davis) and Mr. J. Windak (U of M, Chem. Dept.) for the high resolution mass spectral data, and Ms. B. Barth, Ms.

P. Elka, Ms. Diane Honeycutt, Ms. J. Lipcaman, Ms. S. Koschay, and Ms. B. McMillan for manuscript preparation.

References

(1) Presented in part at the 202nd National Meeting, American Chemical Society, New York, New York, 1991; MEDI 108. Augelli-Szafran, C. E.; Blankley, C. J.; Roth, B. D.; Trivedi, B. K.; Bousley, R. F.; Essenburg, A. D.; Hamelehle, K. L.; Minton, L.; Sekerke, C. S.; Stanfield, R. L.; Krause, B. R. Novel Substituted β-Ketoamide ACAT Inhibitors: Chemistry and Biological Activity.

(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) (a) 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 Publishers B. V. (Biomedical Division): Amsterdam, 1989; pp 707–710. (b) 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) 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-4*H*-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.

(5) Sliskovic, D. R.; White, A. D. Therapeutic Potential of ACAT Inhibitors as Lipid Lowering and Anti-Atherosclerotic Agents. *TIPS* **1991**, *12*, 194–199.

(6) Harris, S. W.; Dujovne, C. A.; von Bergmann, K.; Neal, J.; Akester, J.; Windsor, S. L.; Green, D. Effects of the ACAT Inhibitor CL 277,082 on Cholesterol Metabolism in Humans. *Clin. Pharmacol. Ther.* **1990**, *48*, 189–194.

(7) (a) 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. (b) Krause, B. R.; Bousley, R. F.; Kieft, K. A.; Stanfield, R. L. Effect of the ACAT Inhibitor CI-976 on Plasma Cholesterol Concentrations and Distribution in Hamsters Fed Zero- and Low-Cholesterol Diets. *Clin. Biochem.* **1992**, *25*, 371–377. (c) Krause, B. R.; Anderson, M.; Bisgaier, C. L.; Bocan, T.; Bousley, R.; DeHart, P.; Essenburg, A.; Hamelehle, K.; Homan, R.; Kieft, K.; McNalley, W.; Stanfield, R.; Newton, R. S. In Vivo Evidence that the Lipid-Regulating Activity of the ACAT Inhibitor CI-976 in Rats is Due to Inhibition of Both Intestinal and Liver ACAT. *J. Lipid Res.* **1993**, *34*, 279–294.

(8) 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.

(9) Bocan, T. M. A.; BakMueller, S.; Uhlendorf, P. D.; Newton, R. S.; Krause, B. R. Comparision of CI-976, an ACAT Inhibitor, and Selected Lipid-Lowering Agents for Antiatherosclerotic Activity in Iliac-Femoral and Thoracic Aortic Lesions. *Arteriosclerosis and Thrombosis* **1991**, *11*, 1830–1843.

(10) 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.

(11) Miles, D. H.; Huang, B.-S.; Parish, E. J. Selective Cleavage of β-Keto Esters by 1,4-Diazobicyclo[2.2.2]octane (Dabco). *J. Org. Chem.* **1974**, *39*, 2647–2648.

(12) Wolfe, J. F.; Hendi, S. B.; Hendi, M. S. A New Synthesis of β-Keto Amides via Reaction of Ketone Lithium Enolates with Isocyanates. *Synth. Commun.* **1987**, *17*, 13–18.

(13) Roark, W. H.; Roth, B. D.; Holmes, A.; Trivedi, B. K.; Kieft, K. A.; Essenburg, A. D.; Krause, B. R.; Stanfield, R. L. Inhibitors of Acyl-CoA:Cholesterol Acyltransferase (ACAT). 2. Modification of Fatty Acid Anilide ACAT Inhibitors: Bioisosteric Replacement of the Amide Bond. *J. Med. Chem.* **1993**, *36*, 1662–1668.

(14) Rathke, M. W.; Deitch, J. The Reaction of Lithium Ester Enolates with Acid Chlorides. A Convenient Procedure for the Preparation of β-Keto Esters. *Tetrahedron Lett.* **1971**, 2953–2956.

(15) Lipton, M. F.; Basha, A.; Weinreb, S. M. Conversion of Esters to Amides with Dimethylaluminum Amides: N,N-Dimethylcyclohexanecarboxamide. *Organic Syntheses*; Wiley: New York, Collect. Vol. VI, pp 492–495.

(16) Williams, R. J.; McCarthy, A. D.; Sutherland, C. D. Esterification and Absorption of Cholesterol: In Vitro and In Vivo Observations in the Rat. *Biochem. Biophys. Acta* **1989**, 213–216.

(17) Trivedi, B. K.; Holmes, A. H.; Stoeber, T. L.; Shaw, M. K.; Essenburg, A. D.; Stanfield, R. L.; Krause, B. R. A Series of Substituted *N*-Phenyl-*N'*-(1-Phenylcycloalkyl)Ureas as Inhibitors of ACAT: Chemistry and SAR Studies. In Abstracts of Papers, American Chemical Society, 200th National ACS Meeting, Washington, DC, 1990; MEDI 122.

(18) Trivedi, B. K.; Holmes, A. H.; Stoeber, T. L.; Shaw, M. K.; Essenburg, A. D.; Stanfield, R. L.; Krause, B. R. Novel Disubstituted Ureas as Inhibitors of ACAT: Chemistry and SAR Studies. In Abstracts of Papers, American Chemical Society, 200th National ACS Meeting, Washington, DC, 1990; MEDI 121.

(19) Krause, B. R.; Stanfield, R. L. Unpublished observations.

(20) (a) O'Brien, P. M.; Sliskovic, D. R.; Blankley, C. J.; Essenburg, A. D.; Stanfield, R. L.; Krause, B. R. ACAT Inhibitors: Urea Derivatives Containing Alkyl Substituted Amide or Amine Functionalities. In Abstracts of Papers, American Chemical Society, 201st National Meeting, Atlanta, GA, 1991; MEDI 120. (b) Padia, J. K.; Blankley, C. J.; Bolton, G. L.; Roark, W. H.; Roth, B. D.; Essenburg, A. D.; Hamelehle, K. L.; Krause, B. R.; Stanfield, R. L. Substituted Glycine Anilides: Inhibitors of Acyl-CoA:Cholesterol Acyltransferase. In Abstracts of Papers, American Chemical Society, 201st National Meeting, Atlanta, GA, 1991; MEDI 116. (c) Roark, W. H.; Roth, B. D.; Blankley, C. J.; Essenburg, A. D.; Hamelehle, K. L.; Krause, B. R.; Stanfield, R. L. *N,N*-Disubstituted Glycine Anilides as Inhibitors of Acyl-CoA:Cholesterol Acyltransferase (ACAT). In Abstracts of Papers, American Chemical Society, 201st National Meeting, Atlanta, GA, 1991; MEDI 115.

(21) CLOGP calculated using MedChem software, V. 3.54; Daylight Systems.

(22) Haky, J. E.; Young, A. M. Evaluation of a Simple HPLC Correlation Method for the Estimation of the Octanol-Water Partition Coefficients of Organic Compounds. *J. Liq. Chromatog.* **1984**, *7*, 675–689.

(23) Khalil, A. M.; Abd El-Gawad, I. I.; Ali, M. I.; Girges, M. M. Some Reactions with 3-oxyobutyriodoanilides. *Indian J. Chem.* **1979**, *17B*, 627–629.