2-Azetidinone Cholesterol Absorption Inhibitors: Structure−Activity Relationships on the Heterocyclic Nucleus

John W. Clader,* Duane A. Burnett, Mary Ann Caplen, Martin S. Domalski, Sundeep Dugar, Wayne Vaccaro, Rosy Sher, Margaret E. Browne, Hongrong Zhao, Robert E. Burrier, Brian Salisbury, and Harry R. Davis, Jr.

Schering-Plough Research Institute, 2015 Galloping Hill Road, Kenilworth, New Jersey 07033-0539

Received June 7, 1996[⊗]

A series of azetidinone cholesterol absorption inhibitors related to SCH 48461 ((−)-**6**) has been prepared, and compounds were evaluated for their ability to inhibit hepatic cholesteryl ester formation in a cholesterol-fed hamster model. Although originally designed as acyl CoA: cholesterol acyltransferase (ACAT) inhibitors, comparison of *in vivo* potency with *in vitro* activity in a microsomal ACAT assay indicates no correlation between activity in these two models. The molecular mechanism by which these compounds inhibit cholesterol absorption is unknown. Despite this limitation, examination of the *in vivo* activity of a range of compounds has revealed clear structure−activity relationships consistent with a well-defined molecular target. The details of these structure−activity relationships and their implications on the nature of the putative pharmacophore are discussed.

Introduction

Atherosclerotic coronary artery disease (CAD) is a major cause of death and morbidity as well as a significant and preventable drain on healthcare resources in the western world.[1] There is now a clear association between reduction in serum lipids and decreased incidence of CAD. Although reducing dietary fat and cholesterol is still considered the appropriate first-line therapy, the advent of more effective pharmacological agents has resulted in increased use of drug therapy to control serum cholesterol.[2] Serum cholesterol can be reduced by inhibiting endogenous cholesterol biosynthesis, promoting hepatic cholesterol clearance from the plasma, and inhibiting the absorption of dietary and biliary cholesterol from the intestines.[3] Several agents are available which inhibit biosynthesis and/or promote clearance. However, of the variety of agents shown to inhibit cholesterol absorption in animals, only the bile acid sequestrants have shown sufficient efficacy and safety to warrant extensive clinical use.[4] Even here, the modest efficacy and unpleasant side effects associated with resins have limited their use.

Several classes of compounds have been investigated as cholesterol absorption inhibitors (Figure 1). Acyl CoA:cholesterol acyltransferase (ACAT) inhibitors such as CI-976 (**1**),[5] CL 277082 (**2**),[6] and SCH 46442 (**3**),[7] which inhibit absorption by blocking the formation of intestinal cholesteryl esters, have received considerable preclinical attention, but none has shown clinical efficacy. Recently, it has been suggested that cholesterol absorption can be decreased by inhibiting pancreatic cholesteryl ester hydrolase.[8] Compounds such as WAY 121,898 (**4**)[9] have been effective in rodent models, but their effects in clinical trials remain to be determined. Saponins such as CP 88,818 (**5a**) have been effective clinically, but the gram-quantity doses required in compounds disclosed to date make these less attractive. It remains to be seen if more potent analogs such as CP 148,623 (**5b**) will be as effective at a more reasonable dose.[10]

In a preliminary report, we disclosed that 2-azetidinones such as SCH 48461 ((−)-**6**) are effective inhibitors of cholesterol absorption in a cholesterol-fed hamster model.[11] Subsequently, SCH 48461 has been shown to reduce serum cholesterol in human clinical trials.[12] Although this class of compounds was initially designed as ACAT inhibitors, early structure−activity studies demonstrated a striking divergence of *in vitro* ACAT inhibition and *in vivo* activity in the cholesterol-fed hamster. A detailed examination of the hypocholesterolemic activity of (−)-**6** indicates that it acts at the intestintal wall to inhibit cholesterol absorption through a novel and as yet undetermined mechanism.[13] Initial follow-up studies on SCH 48461 have shown that the azetidinone nucleus is a critical element for *in vivo* activity (Chart 1). For instance, neither the thioazetidinone **7** nor amino acid **8** derived from SCH 48461 possess any significant activity in the hamster, and other lactams show, at best, severely attenuated activity.[14] On the basis of these findings, we initiated an investigation of structure−activity relationships around the 2-azetidinone nucleus. These studies, the first of which is the subject of this report, have revealed clear structure−activity relationships (SAR) for cholesterol absorption inhibition which are distinct from the modest ACAT inhibitory activity shown by these compounds. Thus, these compounds appear to be acting *via* a novel mechanism which may be fundamentally important in the intestinal absorption of cholesterol.

Chemistry

3-Substituted 2-azetidinones such as **6** were prepared as described in Scheme 1. Ester enolate−imine reaction as previously described[11,15] gave primarily the 3,4-cis isomer (method A). Subsequent treatment with potassium *tert*-butoxide (method B) converted this to a mixture of isomers from which the major trans isomer was separated either chromatographically or by crystallization. Subsequently, we have found that *N*-arylazetidinones can be prepared with high trans diastereoselectivity *via* the ketene−imine reaction (method C).[16]

4,4-Disubstituted 2-azetidinone **9** was prepared from the corresponding imine *via* a modified ketene−imine

[⊗] Abstract published in *Advance ACS Abstracts,* August 1, 1996.

S0022-2623(96)00405-0 CCC: $12.00

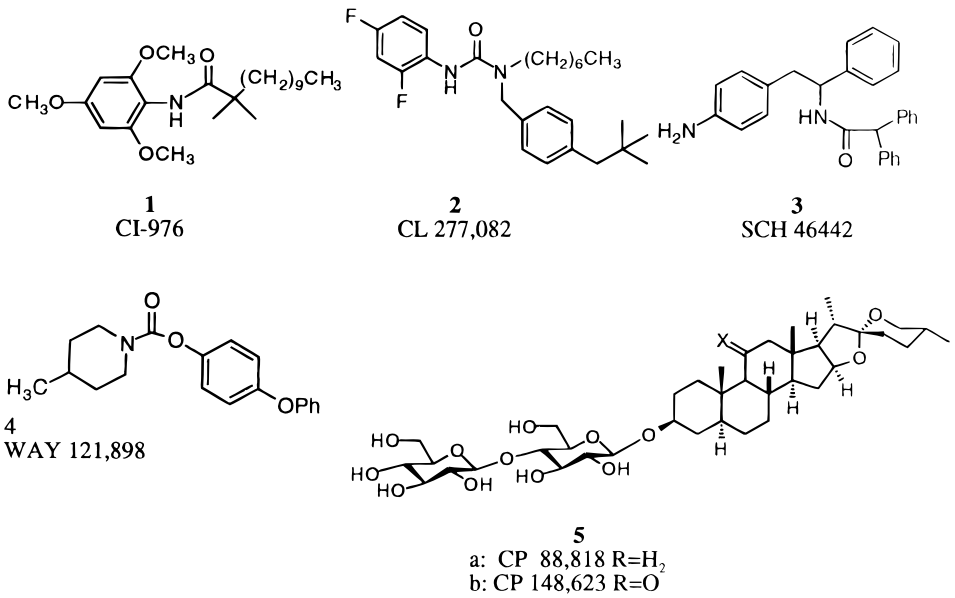
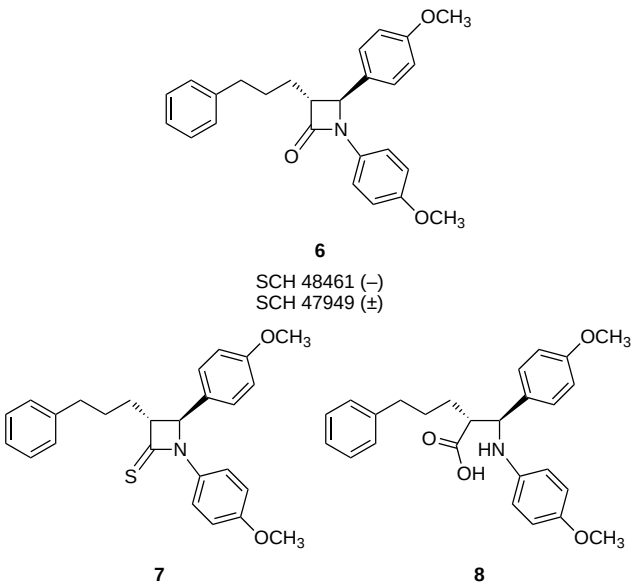


Figure 1. Cholesterol absorption inhibitors.

Chart 1

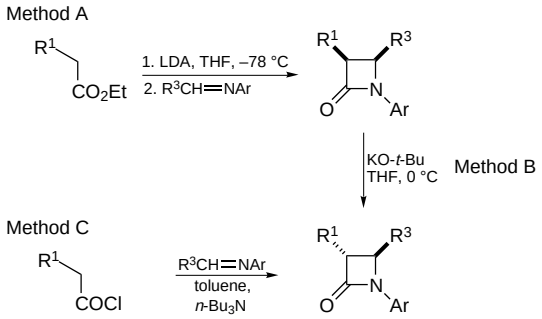


	cholesterol-fed hamster, % change[a]	
no.	serum cholesterol	cholesteryl esters
6	-29	-77.5
7	0	-23
8	-8	0

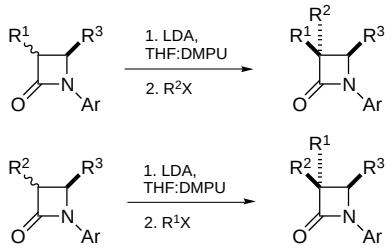
reaction with 5-phenylvaleric acid in the presence of phenyl dichlorophosphate as activating agent.[17] Additional 4,4-disubstituted 2-azetidinones **10** and **11** were prepared from the corresponding imine *via* methods C and A. In all three cases, the hindered imine starting material was prepared from 4-methoxyaniline and the corresponding ketone in the presence of $TiCl_4$.

3,3-Disubstituted 2-azetidinones were prepared as shown in Scheme 2. Alkylation of 2-azetidinone enolates derived from C-3-monosubstituted compounds (method D) proceeded with high diastereofacial selectivity to give compounds which result from alkylation on the face opposite from the C-4 aryl moiety. By exchanging the acid chloride or ester in the azetidinone synthesis and the subsequent alkylating agent, either diastereomer of the product could be obtained.

Scheme 1. Preparation of C-3-Monosusbtituted 2-Azetidinones



Scheme 2. Preparation of C-3-Disubstituted 2-Azetidinones



Compounds with an acyl, alkyl, or phenylalkyl group on nitrogen were prepared by acylation or alkylation of 1(*H*)-2-azetidinone (**74**) (methods E and F) as shown in Scheme 3. The azetidinone **74** was prepared by ester enolate-imine condensation using an *N*-(trimethylsilyl)-imine followed by *in situ* hydrolysis of the *N*-TMS-azetidinone.[15] Additional 2-azetidinones were prepared by standard functional group manipulations as described in the Experimental Section.

Biology

Compounds were evaluated for cholesterol absorption inhibitory activity using a 7-day cholesterol-fed hamster model. Some compounds were also evaluated for *in vitro* activity in a rat liver microsomal ACAT assay. Details of both models have been described previously.[18] The change in hepatic cholesterol ester content is the most sensitive and reproducible end point in the cholesterol-fed hamster model. This end point has also

Scheme 3. Preparation of *N*-Alkyl- and *N*-Acyl-2-azetidinones

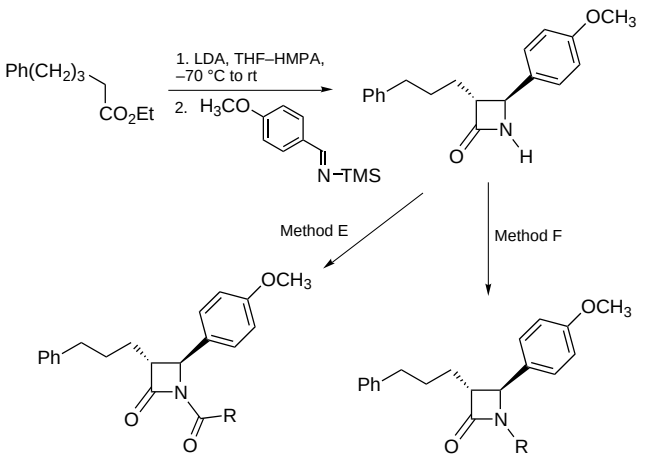
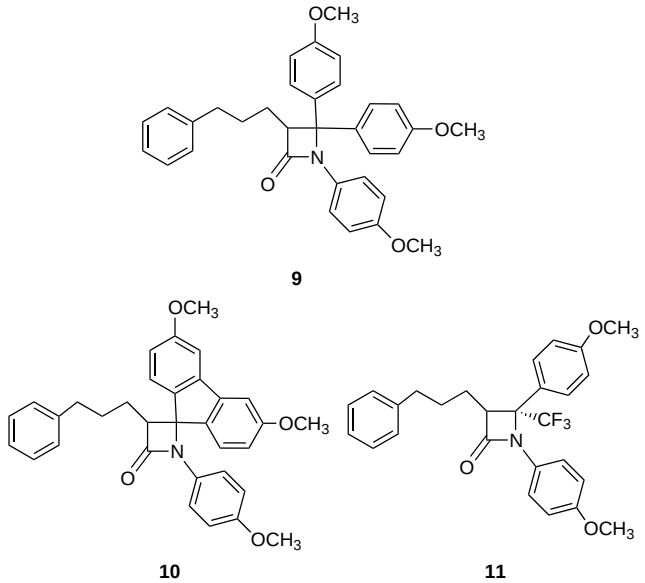


Chart 2



9 **10** **11**

cholesterol-fed hamster, % change[a]

no.	serum cholesterol	cholesteryl esters
9	-9	0
10	-22	-22
11	0	-11

been shown to correlate well with the degree of inhibition of cholesterol absorption. Unlike most ACAT inhibitors, potent azetidinone cholesterol absorption inhibitors also show a substantial reduction in serum cholesterol in this model. This difference provided one of the first indications that the mechanism of action of these compounds did not involve ACAT inhibitions.

Results

Structure−Activity Relationships at C-4. Early work in this series[11] established that the *p*-methoxyphenyl group at C-4 of the azetidinone was critical for *in vivo* activity in the cholesterol-fed hamster model. The same study also demonstated that *in vivo* activity resided primarily in a single configuration at C-4. In a limited follow-up study (Chart 2), several C-4-disubstituted compounds were prepared, including two symmetrically disubstitued compounds, **9** and **10**, which combine the 4(*S*)- and 4(*R*)-*p*-methoxyphenyl moiety in a single molecule. Neither compound showed significant activity in the cholesterol-fed hamster. Compound **11**, which substitutes a trifluoromethyl group for the C-4 hydrogen was also nearly devoid of activity, suggesting that disubstitution at C-4 is not tolerated.

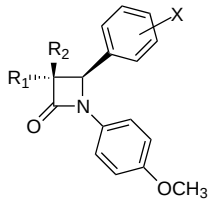
With the importance of the C-4 substituent established, we began a detailed study of the effect of the substituent pattern on the C-4 phenyl moiety on *in vivo* and *in vitro* activity. The results of this study are given in Table 1. These data show that activity in the cholesterol-fed hamster is extremely sensitive to both the nature and pattern of substituents at C-4. For example, the 3-methoxyphenyl (**15** and **16**), 3,4-dimethoxyphenyl (**18** and **19**), and 3,4-(methylenedioxy)phenyl (**20** and **21**) analogs are all significantly less active than 4-methoxyphenyl compound **6**, while the 2,4-dimethoxy derivative **22** is equipotent with **6**. Except for benzyl ether **33**, a variety of ethers (**23**−**32**) are well tolerated, although in compounds such as **23** and **24** there is also an increase in the ACAT inhibitory activity which may contribute to the overall reduction in cholesteryl ester content. Phenolic derivatives **34** and **35** are slightly less active than the corresponding methyl ethers. This may be partly due to reduced absorption of these poorly soluble compounds. Trifluoromethyl ether **37** is significantly less active than the corresponding methyl ether. Insertion of an extra methylene unit to give a hydroxymethyl (**39**) or methoxymethyl (**40**) group also reduces activity. These data suggest that a properly placed hydrogen-bonding moiety in this position is critical for activity. Consistent with this idea, the 4-methyl derivative **38** shows markedly reduced activity. However, even slightly perturbing the location of the alkoxy moiety as in **39** and **40** or substitution with other potential hydrogen bond acceptor or donor groups at the para position (compounds **41**−**50**) reduces activity compared to **6**.

Studies on **(−)-6** and related optically pure compounds have indicated that activity resides predominantly in a one absolute stereochemistry at C-4.[19] As a part of the investigation of substituents on the C-4 phenyl group, the importance of the relative stereochemistry between C-3 and C-4 was also investigated. The data in Table 1 indicate no consistent preference for the relative stereochemistry between C-3 and C-4. Since several early leads showed better dose−response curves in the 3*R*,4*S* (trans) series, most of the remaining structure−activity work focused on trans isomers with a 4-methoxyphenyl group at C-4.

In Figure 2, the ACAT inhibitory activity of the compounds in Table 1 is compared with their activity in the cholesterol-fed hamster model. Not only does the *in vivo* activity not correlate with the *in vitro* activity but the level of ACAT activity in many instances is inconsistent with any level of *in vivo* activity due to ACAT inhibition.[20] As a result, ACAT activity was no longer routinely determined, and subsequent SAR studies focused entirely on activity in the cholesterol-fed hamster.

Structure−Activity Relationships at N-1. Unlike the phenyl group at C-4, the phenyl group on nitrogen was found to tolerate a variety of substituents or could be unsubstituted with no loss in activity (Table 2). For example, 3- and 2-methoxyphenyl derivatives **51** and **52** as well as the unsubstituted phenyl derivative **65** are each at least as active as **6** in the cholesterol-fed

Table 1. Structure−Activity Relationships at the C-4 Phenyl Group



								cholesterol-fed hamster, % change[b]	
no.	R_1	R_2	X	method	formula[a]	mp (°C)	ACAT % inhib at 50 μM	serum cholesterol	cholesteryl esters
6	$Ph(CH_2)_3$	H	4-CH_3O	B	$C_{26}H_{27}NO_3$	96.0−97.5	33	−29	−77.5
12	H	$Ph(CH_2)_3$	4-CH_3O	A	$C_{26}H_{27}NO_3$	90−93	38	−45	−95
13	$Ph(CH_2)_3$	H	H	A	$C_{25}H_{25}NO_2$	92.0−92.5	22	−6	−15
14	H	$Ph(CH_2)_3$	H	A	$C_{25}H_{25}NO_2$	120.5−121.5	28	0	0
15	$Ph(CH_2)_3$	H	3-CH_3O	B	$C_{26}H_{27}NO_3$	109.5−110.0	21	−17	−24
16	H	$Ph(CH_2)_3$	3-CH_3O	A	$C_{26}H_{27}NO_3$	90.5−91.0	39	−19	−54
17	$Ph(CH_2)_3$	H	2-CH_3O	A	$C_{26}H_{27}NO_3$	115−117		0	0
18	$Ph(CH_2)_3$	H	3,4-CH_3O	B	$C_{27}H_{29}NO_4$	70.5−71.5	53	−11	−33
19	H	$Ph(CH_2)_3$	3,4-CH_3O	A	$C_{27}N_{29}NO_4$	86.0−87.0	−16	0	0
20	$Ph(CH_2)_3$	H	3,4-(OCH_2O)	B	$C_{26}H_{25}NO_4$	79.5−81.0	−36	0	−19
21	H	$Ph(CH_2)_3$	3,4-(OCH_2O)	A	$C_{26}H_{25}NO_4$	144.0−144.5	−28	0	0
22	$Ph(CH_2)_3$	H	2,4-CH_3O	C	$C_{27}H_{29}NO_4$[d]	oil		−26	−78
23	$Ph(CH_2)_3$	H	4-CH_3CH_2O	B	$C_{27}H_{29}NO_3$	85−87	68	−52	−98
24	H	$Ph(CH_2)_3$	4-CH_3CH_2O	A	$C_{27}H_{29}NO_3$	84−85	79	−48	−93
25	$Ph(CH_2)_3$	H	4-$^nC_2H_7O$	C	$C_{28}H_{31}NO_3$	92.5−93.5		−40	−95
26	H	$Ph(CH_2)_3$	4-$^nC_3H_7$	A	$C_{28}H_{31}NO_3$	96.0−97.0		−37	−91
27	$Ph(CH_2)_3$	H	4-$^iC_3H_7O$	C	$C_{28}H_{31}NO_3$	96.5−97.5	59	−38	−94
28	H	$Ph(CH_2)_3$	4-$^iC_3H_7O$	A	$C_{28}H_{31}NO_3$[d]	96−97		−28	−78
29	$Ph(CH_2)_3$	H	4-$^nC_4H_9O$	C	$C_{29}H_{33}NO_3$	79−82		−34	−64
30	H	$Ph(CH_2)_3$	4-$^nC_4H_9O$	A	$C_{29}H_{33}NO_3$	71.0−72.5		−15	−70
31	$Ph(CH_2)_3$	H	4-$^tC_4H_9O$	C	$C_{29}H_{33}NO_3$	101.5−102.5		−26	−80
32	$Ph(CH_2)_3$	H	4-PhO	B	$C_{31}H_{29}NO_3$	103.5−105.5		−37	−77
33	$Ph(CH_2)_3$	H	4-$PhCH_2O$	B	$C_{32}H_{31}NO_3$	111−112		−15	−48
34	$Ph(CH_2)_3$	H	4-OH	*c*	$C_{25}H_{25}NO_3$	170.0−170.5	8	−16	−48
35	H	$Ph(CH_2)_3$	4-OH	*c*	$C_{25}H_{25}NO_3$	152.2−155.0	20	−31	−75
36	$Ph(CH_2)_3$	H	3,4-OH	*c*	$C_{24}H_{23}NO_3$[d]	54−55		0	−39[e]
37	$Ph(CH_2)_3$	H	4-CF_3O	C	$C_{26}H_{24}F_3NO_3$	66−67		0	−29
38	$Ph(CH_2)_3$	H	4-CH3	B	$C_{26}H_{27}NO_2$	74.5−77.0	18	−20	−21
39	$Ph(CH_2)_3$	H	4-$HOCH_2$	*c*	$C_{26}H_{27}NO_3$	112−113	−6	−21	−46
40	$Ph(CH_2)_3$	H	4-CH_3OCH_2	*c*	$C_{27}H_{29}NO_3$	oil		−27	−56
41	$Ph(CH_2)_3$	H	4-NH_2	*c*	$C_{25}H_{26}N_2O_2$[d]	155−157	63	0	0
42	$Ph(CH_2)_3$	H	4-$N(CH_3)_2$	C	$C_{27}H_{30}N_2O_2$	96.5−97.5		−29	−60
43	$Ph(CH_2)_3$	H	4-$NHSO_2CH_3$	*c*	$C_{26}H_{28}N_2O_4S$[d]	oil		−8	−46
44	$Ph(CH_2)_3$	H	4-$NHCOCH_3$	E	$C_{27}H_{28}N_2O_3$[d]	153−154		−4	−26
45	H	$Ph(CH_2)_3$	4-SH	*c*	$C_{25}H_{25}NO_2S$	134−135		−19	0
46	$Ph(CH_2)_3$	H	4-CH_3S	A	$C_{26}H_{27}NO_2S$	104.5−105.5		0	0
47	$Ph(CH_2)_3$	H	4-CH_3SO	*c*	$C_{26}H_{27}NO_3S$	111−115		−17	−32
48	$Ph(CH_2)_3$	H	4-CH_3SO_2	*c*	$C_{26}H_{27}NO_4S$	57−58		−26	−75
49	$Ph(CH_2)_3$	H	4-NO_2	A	$C_{25}H_{24}N_2O_4$	116−117	21	−11	0
50	$Ph(CH_2)_3$	H	4-F	A	$C_{25}H_{24}FNO_2$	83.0−84.0	−27	0	12

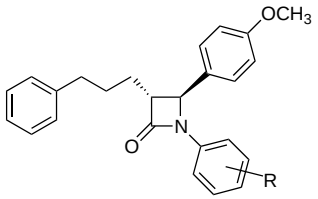
[a] Except where noted, elemental analysis within 0.4% of theoretical values was obtained. [b] All compounds were tested at 50 mg/kg/day unless otherwise stated. [c] Prepared by functional group changes as described in the Experimental Section. [d] High-resolution mass spectrum and/or NMR data availabile from the author. [e] Tested at 10 mg/kg/day.

hamster model. Hydrogen-bonding potential does not appear to be important, as evidenced by the 4-(trifluoromethoxy)phenyl, phenyl, 4-fluorophenyl, and 4-methylphenyl derivatives **57**, **65**, **66**, and **72**. On the other hand, the reduced activity of the 4-phenoxyphenyl derivative **58** suggests some steric limitations on the *N*-aryl group.

While a variety of substituted phenyl groups are tolerated on nitrogen, the aryl moiety itself is critical for activity (Table 3). Desphenyl analog **74** is completely inactive. Compounds with either small alkyl groups (**75**−**78**) or even larger cycloalkyl analogs (**79**, **80**) on nitrogen are either weak or inactive, as are benzyl derivatives **81** and **82**. These data show that the aromatic moiety on nitrogen is an essential part of the pharmacophore. The only exception is the *N*-cyclopropyl derivative **83**. The π character of the cyclopropyl moiety may be contributing to the modest activity of this compound. *N*-Acyl substitution (**84**, **85**) dramatically reduces activity, suggesting that activation of the azetidinone toward nucleophiles not only is not required but is detrimental to activity.

Structure−Activity Relationships at C-3. Lastly, the effect of substitution at C-3 was examined (Table 4). This study examined both the nature of the substituent at C-3 and the degree of substitution at this position. Decreasing the length of the phenylalkyl chain at C-3 (compounds **86** and **87**) reduces activity. Increasing the chain length to four or five carbons (**88**−**90**) causes a modest decrease in activity, while a six-carbon chain (**91**, **92**) results in a marked reduction in activity. Replacing the phenyl alkyl group with a simple alkyl group (**93**−**96**) or saturation of the phenyl group (**97**, **98**) both result in a reduction in activity. Thus, a properly positioned phenylalkyl moiety at C-3 is also an important part of the pharmacophore.

Table 2. Structure−Activity Relationships at the N-1 Phenyl Group



					cholesterol-fed hamster, % change[b]	
no.	R	method	formula[a]	mp (°C)	serum cholesterol	cholesteryl esters
51	3-CH_3O	B	$C_{26}H_{27}NO_3$	86−87	−38	−91
52	2-CH_3O	C	$C_{26}H_{27}NO_3$	oil	−49	−87
53	2,4,6-$(CH_3O)_3$	C	$C_{28}H_{31}NO_5$	98−99	0	0
54	3,4-(OCH_2O)	C	$C_{26}H_{25}NO_4$	118	−19	−52
55	4-OH	*c*	$C_{24}H_{23}NO_3$	160−161	−35	−90
56	4-CH_3CH_2O	B	$C_{27}H_{29}NO_3$	96−97	−57	−96
57	4-CF_3O	A	$C_{26}H_{24}F_3NO_3$	oil	−34	−85
58	4-PhO	B	$C_{31}H_{29}NO_3$	99−100	−28	−49
59	4-CH_2=$CHCH_2O$	F	$C_{28}H_{29}NO_3$	oil	−44	−90
60	4-CH_3S	B	$C_{26}H_{27}NO_2S$	78−80	−26	−70
61	4-CH_3SO	*c*	$C_{26}H_{27}NO_3S$	52−54	−11	0
62	4-CH_3SO_2	*c*	$C_{26}H_{27}NO_4S$	56−58	0	−39
63	4-$CH_3CH_2CO_2$	C	$C_{28}H_{29}NO_4$	75−77	−22	−54
64	4-CH_3CO	C	$C_{27}H_{27}NO_3$	oil	−40	−90
65	H	C	$C_{25}H_{25}NO_2$	75−76	−49	−95[e]
66	4-F	B	$C_{25}H_{24}FNO_2$[d]	oil	−38	−95
67	4-Cl	B	$C_{25}H_{24}ClNO_2$[d]	oil	−44	−93
68	4-CN	C	$C_{26}H_{24}N_2O_2$	79−81	−35	−74
69	3,5-difluoro	C	$C_{25}H_{23}F_2NO_2$	oil	−52	−93
70	4-$(CH_3CH_2)_2N$	C	$C_{29}H_{34}N_2O_2$	91−93	−51	−95
71	3-NH_2	B	$C_{25}H_{26}N_2O_2$[f]	46−48	−25	−54
72	4-CH_3	B	$C_{26}H_{27}NO_2$	98−99	−38	−90
73	3,4-$(CH_3)_2$	C	$C_{27}H_{29}NO_2$	129−130	−21	−39

[a−c] Refer to the corresponding footnotes in Table 1. [d] High-resolution mass spectrum and/or NMR data available from the author. [e] Tested at 40 mg/kg/day. [f] Anal. C: calcd, 77.69; found, 76.91.

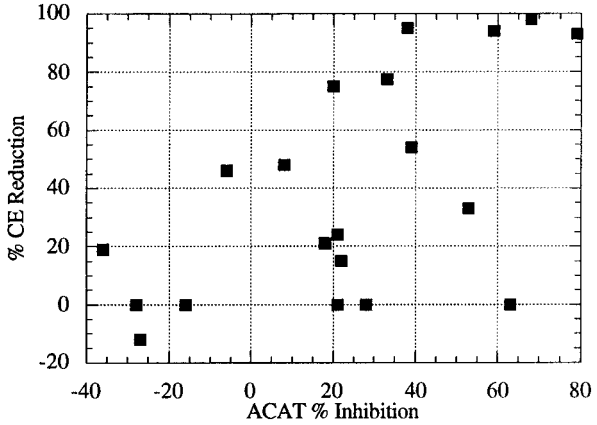


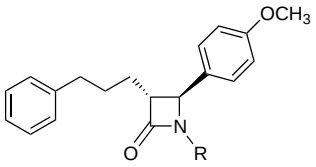
Figure 2. Comparison of *in vitro* activity and *in vivo* activity of compounds in Table 1, shown in order of increasing *in vitro* potency. *In vitro* activity is expressed as percent ACAT inhibition at 10 μM. *In vivo* activity is expressed as percent reduction in hepatic cholesteryl esters (CE) at 50 mg/kg.

Several disubstituted analogs were prepared (**99**−**110**) with a variety of substitution patterns. In nearly every case, disubstitution caused a marked reduction in activity, the only exception being fluoro derivative **105**. This intolerance to disubstitution probably reflects an effect on the conformation of the phenylalkyl side chain and again is indicative of the importance of this group in the pharmacophore. Given this importance, the lack of sensitivity to the absolute stereochemistry at C-3 (see also Table 1) seemed counterintuitive.[20] However, subsequent SAR studies on conformationally constrained analogs have suggested that the bioactive conformation of 2-azetidinone cholesterol absorption inhibitors has the C-3 side chain in a "cisoid" conformation. This conformation is equally accessible to either stereochemistry when C-3 is monosubstituted but inaccessible in C-3-disubstituted compounds.[21]

Discussion

The mechanism of action of 2-azetidinone cholesterol absorption inhibitors has not been determined, and the absence of an appropriate *in vitro* model complicates the interpretation of SAR data. In particular, it is impossible to distinguish effects on intrinsic activity from effects on pharmacokinetics and metabolism. Nonetheless, the studies described in this report show that, even with only an *in vivo* assay, the compounds in this class show some consistent structure−activity trends which can be used in the design of new targets. The basic stucture−activity relationships in the 2-azetidinone series identified in these studies are summarized in Figure 3. The presence of a 4-methoxyphenyl or similar hydrogen bonding moiety and proper absolute stereochemistry at C-4 are both critical determinants of activity. The phenylalkyl group at C-3 and monosusbtitution at C-3 are also important, but the relative geometry of the groups at C-3 and C-4 is less critical. An *N*-aryl group is required, but there is considerable tolerance for substitution on the phenyl ring. The azetidinone ring is required, but there is no evidence that it acts as anything more than a scaffold to correctly position the pharmacophore groups.[14]

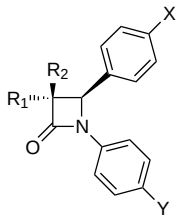
Table 3. Effect of Other Groups at N-1



no.	R	method	formula[a]	mp (°C)	cholesterol-fed hamster, % change[b] serum cholesterol	cholesterol-fed hamster, % change[b] cholesteryl esters
74	H	*c*	$C_{19}H_{21}NO_2$	oil	0	0
75	CH_3	F	$C_{20}H_{23}NO_2$	oil	0	0
76	CH_3CH_2	F	$C_{21}H_{25}NO_2$	oil	−13	−21
77	$^iC_3H_7$	F	$C_{22}H_{27}NO_2$	oil	−16	−30
78	$CH_2{=}CHCH_2$	F	$C_{22}H_{25}NO_2$	oil	0	0
79	adamantyl	C	$C_{29}H_{35}NO_2$	100−104.5	0	0
80	cyclo-C_6H_{11}	C	$C_{25}H_{31}NO_2$	oil	0	0
81	$C_6H_5CH_2$	F	$C_{26}H_{27}NO_2$	oil	0	16
82	4-CH_3O-$C_6H_5CH_2$	F	$C_{27}H_{29}NO3$	oil	0	−26
83	cyclo-C_3H_5	C	$C_{22}H_{25}NO_2$	oil	−20	−61
84	PhCO	E	$C_{26}H_{25}NO_3$	73.5−75	0	16
85	4-CH_3O-C_6H_5CO	E	$C_{27}H_{27}NO_4$	67−69	−25	0

[a−c] Refer to the corresponding footnotes in Table 1.

Table 4. Structure−Activity Relationships at C-3



no.	R_1	R_2	X	Y	method	formula[a]	mp (°C)	cholesterol-fed hamster, % change[b] serum cholesterol	cholesterol-fed hamster, % change[b] cholesteryl esters
86	$Ph(CH_2)_2$	H	CH_3O	CH_3O	A	$C_{25}H_{25}NO_3$	oil	0	0
87	H	$Ph(CH_2)_2$	CH_3O	CH_3O	C	$C_{25}H_{25}NO_3$[d]	oil	0	−19
88	$Ph(CH_2)_4$	H	CH_3O	CH_3O	A	$C_{27}H_{29}NO_3$	oil	−8	−23
89	H	$Ph(CH_2)_4$	CH_3O	CH_3O	B	$C_{27}H_{29}NO_3$	oil	−28	−72
90	H	$Ph(CH_2)_5$	CH_3O	CH_3O	C	$C_{28}H_{31}NO_3$	oil	−24	−70
91	$Ph(CH_2)_6$	H	CH_3O	CH_3O	A	$C_{29}H_{33}NO_3$	oil	−19	−17
92	H	$Ph(CH_2)_6$	CH_3O	CH_3O	B	$C_{29}H_{33}NO_3$[d]	oil	−29	0
93	H	$C_{10}H_{21}$	CH_3O	CH_3O	C	$C_{27}H_{37}NO_3$	oil	0	−15
94	$C_{10}H_{21}$	H	CH_3O	CH_3O	A	$C_{27}H_{37}NO_3$	oil	0	−18
95	H	CH_3	CH_3O	CH_3O	C	$C_{18}H_{19}NO_3$	63−67	0	0
96	CH_3CH_2	H	CH_3O	CH_3O	A	$C_{19}H_{21}NO_3$	96−103	0	0
97	$C_6H_{11}(CH_2)_3$	H	CH_3O	CH_3O	A	$C_{26}H_{33}NO_3$	oil	−23	−47
98	H	$C_6H_{11}(CH_2)_3$	CH_3O	CH_3O	C	$C_{26}H_{33}NO_3$	oil	12	−28
99	$Ph(CH_2)_3$	CH_3CH_2	H	CH_3O	D	$C_{27}H_{29}NO_2$	100.0−101.0	−12	−29
100	$Ph(CH_2)_3$	CH_3CH_2	CH_3O	CH_3O	D	$C_{28}H_{31}NO_3$	oil	0	0
101	CH_3CH_2	$Ph(CH_2)_3$	H	CH_3O	D	$C_{27}H_{29}NO_2$	8.25−83.5	0	0
102	CH_3CH_2	$Ph(CH_2)_3$	CH_3O	CH_3O	D	$C_{28}H_{31}NO_3$[h]	oil	0	0
103	CH_3	$Ph(CH_2)_3$	CH_3O	CH_3O	A	$C_{27}H_{29}NO_3$	75.0−76.5	0	−56
104	$Ph(CH_2)_3$	CH_3	CH_3O	CH_3O	A	$C_{27}H_{29}NO_3$	oil	0	21
105	F	$Ph(CH_2)_3$	CH_3O	H	A	$C_{25}H_{24}FNO_2$	96.0−97.5	−30	−94[e]
106	$Ph(CH_2)_3$	F	CH_3O	H	A	$C_{25}H_{24}FNO_2$	128.0	−10	−17[f]
107	CH_3CH_2	C_6H_5	CH_3O	CH_3O	A	$C_{25}H_{25}NO_3$	oil	0	−28
108	C_6H_5	C_6H_5	CH_3O	CH_3O	C	$C_{29}H_{25}NO_3$	oil	0	0
109	C_6H_5	$C_6H_5CH_2$	CH_3O	CH_3O	C	$C_{30}H_{27}NO_3$	oil	23	−15
110	$C_6H_5CH_2$	C_6H_5	CH_3O	CH_3O	C	$C_{30}H_{27}NO_3$[g]	oil	16	16

[a−c] Refer to the corresponding footnotes in Table 1. [d] High-resolution mass spectral data available from the author. [e] Tested at 20 mg/kg/day. [f] Tested at 25 mg/kg/day. [g] Anal. C: calcd, 80.15; found, 79.59. [h] Anal. C: calcd, 78.29; found, 77.73.

Taken together, the data in Tables 1−4 indicate that activity in this series follows clearly defined structure−activity relationships consistent with a precise molecular target. As indicated in the preliminary account of this work, the identity of this target has yet to be determined. However, data so far indicate that this target is not ACAT.

Both ACAT inhibitors and 2-azetidinones reduce the formation of intestinal and heptatic cholesteryl esters. Recent data have shown that **6** inhibits the accumulation of both esterified and unesterified [^{14}C]cholesterol in the intestinal wall of cholesterol-fed hamsters, while CI-976, a potent ACAT inhibitor, prevents only the accumulation of esterified cholesterol.[13] Given the weak

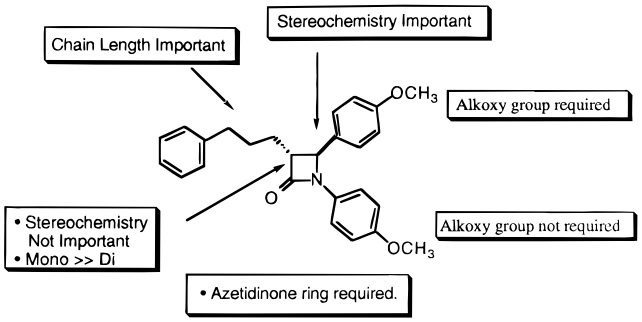


Figure 3. Summary of azetidinone structure−activity relationships.

ACAT activity of **6**, the observed reduction in cholesteryl esters probably reflects a reduction in the supply of free cholesterol substrate. These same studies also indicate that **6** has no direct effect on luminal cholesterol physicochemistry or micelle integrity. Thus, the compounds in this series appear to inhibit cholesterol absorption by a novel and as yet undefined mechanism which mediates intestinal cholesterol absorption. Studies toward further insight into this mechanism are currently in progress and will no doubt help in the design of new compounds. Even in the absence of a clear mechanism, the studies presented here have delineated the important pharmacophore features and provided the foundation for future generations of azetidinone cholesterol absorption inhibitors.

Experimental Section

General. All melting points were taken on a Thomas-Hoover melting point apparatus and are uncorrected. Chromatography was performed over Universal Scientific or Selecto Scientific flash silica gel 32−63 mesh. HPLC was performed on a Rainin HPLX system using Rainin Dynamax 60A analytical or preparative SiO_2 columns at flow rates of 1.5 and 33 mL/min, respectively, for solvent mixtures as described in the Experimental Section. ^{1}H NMR spectra were determined with a Varian VXR 200 or Gemini 300 MHz instrument using Me_4Si as an internal standard. *J* values are given in hertz. IR were obtained on a Perkin Elmer 727B series IR spectrophotometer or on a Nicolet MX-1 FTIR instrument. Unless otherwise noted, elemental analyses were within 0.4% of the theoretical value.

General Method A: Rel-(3*S*,4*S*)-1-(4-methoxyphenyl)-3-(3-phenylpropyl)-4-(4-methoxyphenyl)-2-azetidinone (12). LDA was freshly prepared by dissolving 23.96 mL (17.39 g, 172 mmol) of diisopropylamine in 230 mL of dry THF at −78 °C under nitrogen and adding 103.9 mL (166 mmol of 1.6 M solution in hexanes) of *n*-butyllithium. This cold solution was stirred at −78 °C for 1 h followed by the addition of 32.58 g (158 mmol) of the 5-phenylvaleric acid ethyl ester in 195 mL of dry THF over ∼1 h, keeping the reaction temperature below −65 °C. The reaction mixture was stirred for 1 h at −78 °C; then 38.13 g (158 mmol) of *N*-(4-methoxybenzylidene)aniline in 350 mL of dry dichloromethane was added. The reaction mixture was allowed to slowly come to room temperature and the precipitate that forms dissolved. The reaction mixture was stirred for 16 h at room temperature. The mixture was partitioned between 1.2 L of 1 N aqueous HCl and 1 L of ether. The ether layer was washed with 300 mL of 1 N HCl. The acid layers were combined and extracted with 1 L of ether. The ether extracts were combined, dried ($MgSO_4$), and concentrated in vacuo. The residue (35.08 g, 55%) was crystallized from ∼200 mL of ethyl acetate−hexane (1:1) to give the desired *cis*-2-azetidinone as off-white crystals (32.05 g, 51%): mp 90−93 °C; IR (NaCl, cm^{-1}) 2900, 1726, 1593, 1491, 1432, 1370, 1278, 1227, 1155, 910, 800; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.28−7.11 (m, 7H), 6.99 (d, 2H, J = 7) 6.87 (d, 2H, J = 9), 6.79 (d, 2H, J = 9), 5.11 (d, 1H, J = 6), 3.83 (s, 3H), 3.75 (s, 3H), 3.52 (dt, 1H, J = 9, 6), 2.49−2.36 (m, 2H), 1.62−1.24 (m, 4H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 167.27, 159.25, 155.53, 141.51, 130.96, 128.05, 127.92, 127.86, 126.40, 125.30, 117.99, 113.83, 113.65, 57.35, 54.81, 54.64, 54.03, 34.99, 28.18, 24.26; FABMS *m*/*z* 402 (M + 1), 252. Anal. ($C_{26}H_{27}NO_3$) C,H,N.

General Method B: Rel-(3*R*,4*S*)-1-(4-methoxyphenyl)-3-(3-phenylpropyl)-4-(4-methoxyphenyl)-2-azetidinone ((±)6). The racemic *cis*-2-azetidinone **12** (32.05 g, 79.8 mmol) was dissolved in 500 mL of THF. Potassium *tert*-butoxide (1.79 g, 16.0 mmol) was added, and the mixture was stirred at 0 °C for 1.5 h. The reaction mixture was partitioned between 600 mL of 1 N aqueous HCl and 1.2 L of ether. The aqueous layer was extracted with 400 mL of ether. The ether layers were combined, dried ($MgSO_4$), and concentrated in vacuo to give 32.0 g of a mixture of *cis*- and *trans*-2-azetidinone (∼3:1). The pure *trans*-2-azetidinone **6** was isolated *via* silica gel HPLC eluting with 10% ethyl acetate−hexane. Crystallization from ethyl acetate−hexane gave white crystals: mp 96.0−97.5 °C; IR (NaCl, cm^{-1}) 2933, 1742, 1463, 1454, 1388, 1297, 1175, 1032, 830; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.31−7.16 (m, 9H), 6.90 (d, 2H, J = 9), 6.78 (d, 2H, J = 9), 4.56 (d, 1H, J = 2), 3.81 (s, 3H), 3.74 (s, 3H), 3.08−3.06 (m, 1H), 2.65 (t, J = 7, 2H), 1.98−1.81 (m, 4H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 167.07, 159.40, 155.58, 141.44, 131.07, 129.68, 128.08, 128.05, 126.83, 125.56, 117.84, 114.13, 113.85, 60.29, 59.91, 54.82, 54.70, 35.06, 28.27, 27.73; FABMS *m*/*z* 402 (M + 1), 252. Anal. ($C_{26}H_{27}NO_3$) C,H,N.

General Method C: Rel-(3*R*,4*S*)-1-phenyl-4-(4-methoxyphenyl)-3-(3-phenylpropyl)-2-azetidinone (65). To a refluxing solution of 4.33 g (0.0205 mol) of *N*-(4-methoxybenzylidene)aniline and 7.6 g (0.0410 mol) of tributylamine in 40 mL of heptane was added a solution of 4.03 g (0.0205 mol) of 5-phenylpentanoyl chloride in 15 mL of heptane over the course of 2 h. When the addition was complete, the mixture was heated at reflux for an additional 4 h and then evaporated to dryness. The residue was dissolved in 150 mL of ethyl acetate and washed with 1 N HCl (2 × 30 mL) followed by saturated sodium bicarbonate (1 × 30 mL) and brine (1 × 30 mL). The organic layer was dried over magnesium sulfate and evaporated to dryness to give 7.69 g of a semisolid. This was recrystallized from 15% ethyl acetate in hexane to give 3.08 g (41%) of compound **65**. Chromatography of the mother liquors on silica gel eluting with 5% ethyl acetate yielded and additional 2.31 g (30%): total yield = 5.39 g (71%); mp 75−76 °C. Anal. ($C_{25}H_{25}NO_2$) C,H,N.

General Method D: Rel-(3*S*,4*S*)-1-(4-methoxyphenyl)-4-phenyl-3-ethyl-3-(3-phenylpropyl)-2-azetidinone (100). To a −78 °C solution of lithium diisopropylamide (1.88 mL of 1.5 M solution) in 8 mL dry of THF and 4 mL of DMPU was added 0.40 g (1.42 mmol) of 1-(4-methoxyphenyl)-4-phenyl-3-ethyl-2-azetidinone (**96**) at −78 °C, then 0.25 mL (1.64 mmol) of 3-phenyl-1-bromopropane was added, and the mixture was stirred for 3 h while slowing coming to room temperature. The reaction was quenched with 150 mL of 1 N HCl and the mixture extracted with two 100 mL portions of ether. The combined ether layers were washed with saturated sodium bicarbonate, dried over magnesium sulfate, and concentrated under vacuum. The residue was chromatographed over 100 g of silica gel, eluting with 10% ethyl acetate−hexane to give 0.453 g (80%) of the title compound as an oil. Anal. ($C_{19}H_{21}NO_3$) C,H,N.

General Method E: Rel-(3*R*,4*S*)-3-(3-phenylpropyl)-4-(4-methoxyphenyl)-1-(4-methoxybenzoyl)-2-azetidinone (85). To a 0.5 M solution of 0.51 g (1.7 mmol) of *N*-1-unsubstituted azetidinone **74** in 3.5 mL of dry methylene chloride was added 0.36 mL (2.6 mmol) of triethylamine followed by 21 mg (0.17 mmol) of 4-(dimethylamino)pyridine. The resultant solution was cooled to 0 °C, and 0.28 mL (2.08 mmol) of *p*-anisoyl chloride was added dropwise using a syringe. The cold bath was removed, and the solution was allowed to warm to room temperature. Within 4 h, all starting material was consumed, as determined by TLC (40% ethyl acetate−hexane). The reaction mixture was partitioned between ether and water and the ether layer washed with three portions of saturated sodium bicarbonate and one portion of brine. The aqueous phase was extracted with three portions of ether. The combined organic phases were dried over sodium sulfate and concentrated under vacuum to give 0.78 g of a

yellow-orange oil. Flash chromatography of the product adsorbed onto 3.6 g of silica gel using 15% ethyl acetate−hexane as eluent afforded 0.70 g (94% yield) of **85** as a clear, nearly colorless oil. An analytical sample was prepared by recrystallizing twice from ether−hexane, first at −78 °C and then at 0 °C, to give a pale yellow crystalline solid: mp 67−69 °C. Anal. ($C_{27}H_{27}NO_4$) C,H,N.

General Method F: Rel-(3*R*,4*S*)-3-(3-phenylpropyl)-4-(4-methoxyphenyl)-1-(phenylmethyl)-2-azetidinone (81). To a 0.2 M solution of 0.51 g (1.7 mmol) of *N*-1-unsubstituted azetidinone **74** in 8.5 mL of dry THF was added 0.25 mL (2.08 mmol) of benzyl bromide followed by 59 mg (0.17 mmol) of tetrabutylammonium hydrogen sulfate. The resultant solution was cooled to 0 °C, and 106 mg (1.9 mmol) of freshly powdered potassium hydroxide was added. The cold bath was removed, and the solution was allowed to warm to room temperature. After 24 h, an additional 0.2 equiv of benzyl bromide and 0.15 equiv of potassium hydroxide were added to the solution. After another 3 h, all starting material was consumed, as determined by TLC (20% ethyl acetate−hexane). The solution was diluted with saturated aqueous ammonium chloride and ether. The ether was washed with saturated aqueous ammonium chloride, water, and brine, in succession, and the combined aqueous phases were extracted with three portions of ether. The combined organic phases were dried over sodium sulfate and concentrated under vacuum to afford 0.70 g of a yellow oil. This was purified by flash chromatography over silica gel eluting with 15% ethyl acetate−hexane, affording 0.58 g (87%) of **81** as a clear, nearly colorless oil. Anal. ($C_{26}H_{27}NO_2$) C,H,N.

Rel-(3*R*,4*S*)-1,4-bis(4-methoxyphenyl)-3-(3-phenylpropan-1-yl)-2-thioazetidinone (7). A solution of 300 mg (0.75 mmol) of compound **6** and 302 mg (0.75 mmol) of Lawesson's reagent in 10 mL of toluene was heated at 78 °C for 16 h. The mixture was concentrated onto silica gel and chromatographed over 40 g of silica gel, eluting with 20% ethyl acetate−hexane to give 275 mg of a white solid. This was recrystallized from ether−hexanes to give 250 mg (80% yield) of white crystals: mp 93.0−94.0 °C. Anal. ($C_{26}H_{27}NO_2S$) C,H,N.

***trans*-2-(α-((4-Methoxyphenyl)amino)-4-methoxybenzyl)-5-phenylpentanoic Acid (8).** A suspension of 300 mg (0.75 mmol) of compound **6** in 8 mL of absolute ethanol was added to a solution of 500 mg (11.9 mmol) of lithium hydroxide in 4 mL of water. The resulting suspension was stirred at 70−80 °C for 2 h, at which point TLC (20% ethyl acetate−hexane) indicated complete consumption of starting material. After cooling to room temperature, the solution was made slightly acidic by addition of concentrated HCl and then was brought to pH 7 by addition of 10% aqueous sodium bicarbonate. The mixture was extracted three times with 75 mL of ethyl acetate. The combined extracts were washed with 50 mL of brine, dried over magnesium sulfate, and evaporated to give 276 mg (88% yield) of **7** as a white solid: mp 153.5−154.5 °C. An analytical sample was prepared by recrystallization from ethyl acetate hexane: mp 154.0−154.5 °C. Anal. ($C_{26}H_{29}NO_4$) C,H,N.

1-(4-Methoxyphenyl)-3-(3-phenylpropyl)-4,4-(bis-(4-methoxyphenyl)-2-azetidinone (9). $TiCl_4$ (2.0 mL, 2.0 mmol, 1 M in CHI_2CL_2) was added to a solution of 4,4′-dimethoxybenzophenone (0.98 g, 4.0 mmol) and 4-methoxyaniline (0.50 g, 4.0 mmol) in anhydrous toluene (40 mL). Tri-*n*-butylamine (2.9 mL, 12.2 mmol) was added. The resulting dark purple mixture was stirred overnight. Tri-*n*-butylamine (2.0 mL, 8.4 mmol), 5-phenylvaleric acid (0.80 g, 4.4 mmol), and phenyl dichlorophosphate (0.67 mL, 4.4 mmol) were added sequentially. The resulting mixture was heated to reflux and refluxed overnight. The mixture was cooled to room temperature, the reaction quenched with water, and the mixture transferred to a separatory funnel, diluted with ethyl acetate, washed with 1 M HCl, saturated $NaHCO_3$, water, and brine, dried over anhydrous sodium sulfate, and concentrated onto enough silica gel such that a free flowing powder was obtained. The powder was loaded onto a chromatography column packed with 30% ethyl acetate−hexanes. Elution with the same solvent provided 1.45 g of partially pure title compound. The title compound was further purified by HPLC (silica gel, 10% ethyl acetate−hexanes) to give 0.85 g (41%) of the title compound as a clear oil: ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.39 (2H, d, J = 8.8), 7.21 (7H, m), 7.03 (2H, dd, J = 8, 1.8), 6.86 (4H, dd, J = 8.8, 1.8), 6.72 (2H, d, J = 9.2), 3.85 (3H, s), 3.80 (3H, s), 3.73 (3H, s), 3.72 (1H, t, J = 7.8), 2.44 (2H, m), 1.77 (1H, m), 1.57 (1H, m), 1.37 (2H); MS (EI) 507 (M^+, 2), 358 (100), 253 (37). Anal. Calcd: C, 78.08; H, 6.55; N, 2.76. Found: C, 77.50; H, 6.54; N, 2.90.

3′,6′-Dimethoxy-1-(4-methoxyphenyl)-3-(3-phenylpropyl)spiro(azetidine-2,9′-(9*H*)-fluoren)-4-one (10). To a flask containing 0.215 g (0.90 mmol) of 3,6-dimethoxy-9-fluorenone[22] were added 55 mL of dry toluene and 0.496 g (0.90 mmol) of *p*-anisidine. The light yellow-brown solution was cooled to 0 °C, and 0.67 mL (0.67 mmol) of titanium tetrachloride in toluene was added dropwise over 1 h. The brown mixture was stirred for 1 h at room temperature followed by heating to 127 °C overnight. The orange mixture was cooled to 0 °C and stirred for approximately 4 h. The mixture was filtered through Celite and the precipitate washed with ether. The filtrate was concentrated to an orange oil and recrystallized from hexane−ethyl acetate to give 0.230 g (74%) of the desired imine as bright yellow crystals: mp 189−190 °C.

To a flask containing 0.273 g (0.79 mmol) of the fluorenimine were added 0.235 g (0.79 mmol) of 5-phenylpentanoyl chloride, 20 mL of toluene, and 0.40 mL (0.79 mmol) of tributylamine. The dark orange solution was heated to reflux overnight. The solution was cooled to room temperature and then extracted with 2 × 20 mL of 1 N HCl, washed with brine, dried over Na_2SO_4, and concentrated to a yellow oil. Purification *via* SiO_2 HPLC eluting with 20% ethyl acetate in hexane gave 0.320 g (79%) of the desired product as a white flaky solid: mp 67−72 °C. Anal. ($C_{33}H_{41}NO_4$) C,H,N.

Rel-(3*R*,4*S*)-4-(trifluoromethyl)-4-(4-methoxyphenyl)-1-phenyl-3-(3-phenylpropyl)-2-azetidinone (11). 4-Bromoanisole (3.07 mL, 24.5 mmol) was added to a rapidly stirring suspension of magnesium turnings (1.19 g, 49.0 mmol) in THF (40 mL) at such a rate that a gentle reflux was maintained. Upon completion of addition, the mixture was refluxed for an additional 30 min, cooled to room temperature, and added *via* cannula to a 0 °C solution of triflic anhydride (6.92 mL, 49.0 mmol). The mixture was stirred for 30 min, the reaction quenched with HCl (1 M), and the mixture transferred to a separatory funnel, diluted with ether, washed with HCl (1 M), NaOH (1 M), water, and brine, dried over anhydrous sodium sulfate, filtered, and concentrated onto enough silica gel such that a free flowing powder was obtained. The powder was loaded onto a chromatography column prepacked with silica gel and 20% ethyl acetate−hexane. Elution with the same solvent provided 2.33 g of the 2,2,2-trifluoro-4′-methoxyacetophenone and 1.34 g of the corresponding hydrate. The products were combined for a total yield of 17.4 mmol (71%) and used as a mixture as follows.

Titanium tetrachloride (4.12 mL, 4.12 mmol, 1 M in CHI_2-Cl_2) was added to a room temperature solution of a mixture of 2,2,2-trifluoro-4′-methoxyacetophenone and the corresponding hydrate (1.71 g, 8.25 mmol) and triethylamine (3.5 mL, 24.72 mmol) in CHI_2Cl_2 (20 mL). The resulting mixture was stirred overnight at room temperature, filtered through Celite, concentrated, redissolved in ethyl acetate, filtered, and concentrated onto enough silica gel such that a free flowing powder was obtained. The powder was loaded onto a chromatography column prepacked with silica gel and 20% ethyl acetate−hexane. Elution with the same solvent provided 0.995 g of the imine: ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.78 (3H, s), 6.78 (4H, m), 7.06 (1H, m), 7.22 (4H, m); MS (CI) 295 (M^{16+}, 94), 280 (M^{1+}, 16), 205 (100).

n-Butyllithium (3.8 mL, 6 mmol, 1.6 M in hexane) was added to a −78 °C solution of diisopropylamine (0.9 mL, 6.4 mmol) in THF (15 mL). After 2 h, ethyl 5-phenylvalerate (0.82 g, 4 mmol) in THF (2 mL) was dropwise added. After an additional 2 h, the imine (0.995 g, 4 mmol) in THF (15 mL) was slowly added. The resulting mixture was stirred at −78 °C for 1 h and then allowed to warm to room temperature overnight. The reaction was quenched with NH_4Cl (saturated), diluted with ethyl acetate, washed with water and brine, dried over anhydrous sodium sulfate, filtered, and concentrated onto enough silica gel that a free flowing powder was obtained. The powder was loaded onto a chromatography column prepacked with silica gel and 20% ethyl acetate−hexanes. Elution with

the same solvent provided 0.66 g (37%) of a yellow oil. ^{1}H NMR indicated that the oil was impure. Further purification by preparative HPLC (silica gel column, 7% ethyl acetate−hexanes, 50 mL/min) provided 0.366 g of the title compound as a single peak by analytical silica gel HPLC (7% ethyl acetate−hexanes, 1.5 mL/min, t_R = 5.02 min). NOE studies indicated that the C-4 phenyl group and C-3 phenylpropyl substituents are cis to each other: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42 (2H, d, J = 7.7), 7.34 (2H, d, J = 8.5), 7.24 (4H, m), 7.13 (2H, m), 6.98 (2H, d, J = 7.0), 6.91 (2H, d, J = 9.1), 3.84 (3H, s), 3.65 (1H, dd, J = 6.6, 8.8), 2.47 (1H, m), 2.35 (1H, m), 1.63 (1H, m), 1.48 (2H, m), 1.18 (1H, m); MS (EI) 439 (M^+, 23), 320 (51), 279 (23), 229 (74), 210 (83), 202 (46), 189 (77), 91 (100); HRMS (FAB) calcd for M + 1, $C_{26}H_{25}NO_2F_3$ 440.1837, found 440.1841.

Rel-(3*S*,4*S*)-1-(4-methoxyphenyl)-3-(3-phenylpropyl)-4-(4-(hydroxymethyl)phenyl)-2-azetidinone (39). To a solution of 5.1 g (9.8 mmol) of the *tert*-butyldimethylsilyl ether of **40** (prepared by method C) in 25 mL of THF was added 49 mL (49 mmol) of 1 M tetra-*n*-butylammonium fluoride. The mixture was stirred overnight at room temperature and then partitioned between saturated ammonium chloride and ether. The aqueous layer was extracted with ether, and the combined ether layers were dried over sodium sulfate and evaporated. The residue was chromatographed on silica gel, eluting with 30% ethyl acetate−hexane to give 3.31 g (84%) of the desired compound as an oil. Anal. ($C_{27}H_{29}NO_3$) C,H,N. Compounds **34** and **35** were prepared *via* an analogous procedure starting from the corresponding *tert*−butyldimethylsilyl ether.

Rel-(3*R*,4*S*)-4-(4-hydroxyphenyl)-1-(4-methoxyphenyl)-3-(3-phenylpropyl)-2-azetidinone (34): mp 170.0−170.5 °C. Anal. ($C_{25}H_{25}NO_3$) C,H,N.

Rel-(3*S*,4*S*)-1-(4-methoxyphenyl)-3-(3-phenylpropyl)-4-(4-hydroxyphenyl)-2-azetidinone (35): mp 152.5−155.0 °C. Anal. ($C_{25}H_{25}NO_3$) C,H,N.

Rel-(3*R*,4*S*)-1-(4-methoxyphenyl)-3-(3-phenylpropyl)-4-(4-(methoxymethyl)phenyl)-2-azetidinone (40). Freshly prepared silver oxide (0.35 g, 1.21 mmol) was added to a solution of 0.37 g (0.92 mmol) of 4-*p*-(hydroxymethyl)phenyl derivative **39** and 0.18 mL (2.8 mmol) of methyl iodide in 5 mL of dry DMF. The mixture was heated to 40−45 °C until TLC (50% ethyl acetate−hexane) indicated the reaction was complete. After cooling to room temperature, the mixture was partitioned between water and ethyl acetate and extracted with ethyl acetate. The combined ethyl acetate layers were washed with water, dried over sodium sulfate, and evaporated. The residue was chromatographed over silica gel, eluting with 30% ethyl acetate−hexanes to give 0.31 g (81%) of **40** as an oil. Anal. ($C_{27}H_{29}NO_3$) C,H,N.

Rel-(3*R*,4*S*)-4-(4-aminophenyl)-3-(3-phenylpropan-1-yl)-1-(4-methoxyphenyl)-2-azetidinone (41). To 217 mg (0.52 mmol) of the 4-p-nitrophenyl derivative **49** in 10 mL of ethyl acetate was added 30 mg of 10% palladium on carbon. The compound was placed under a balloon of hydrogen for 18 h. The mixture was filtered through Celite and concentrated in vacuo. The residue was chromatographed over 40 g of SiO_2 eluting with 30% ethyl acetate in hexanes to give 155 mg of the desired amine as an off-white solid: mp 155−157 °C. Anal. ($C_{25}H_{26}N_2O_2 \cdot 0.75$) C,H,N.

Rel-(3*R*,4*S*)-1-(4-methoxyphenyl)-3-(3-phenylpropyl)-4-(4-((methylsulfonyl)amino)phenyl)-2-azetidinone (43). Methanesulfonyl chloride (0.05 mL, 0.67 mmol) was added to a solution of 0.26 g (0.67 mmol) of 4-*p*-aminophenyl derivative **41** and 3 drops of pyridine in 5 mL of dichloromethane, and the mixture was stirred overnight at room temperature. The mixture was diluted with dichloromethane and washed with 0.5 M HCl, 5% sodium bicarbonate, water, and brine. The organic layer was dried over sodium sulfate and evaporated under vacuum. The residue was chromatographed over silica gel, eluting with 50% ethyl acetate−hexanes to give 0.18 g (58%) of **43** as an oil: HRMS calcd for $C_{26}H_{28}N_2O_4S$ 465.1848, found 465.1834.

Rel-(3*R*,4*S*)-1-(4-methoxyphenyl)-3-(3-phenylpropyl)-4-(4-(acetylamino)phenyl)-2-azetidinone (44). Acetyl chloride (0.4 mL, 0.57 mmol) was added to a solution of 0.20 g (0.52 mmol) of 4-*p*-aminophenyl derivative **41** and 0.11 mL (0.79 mmol) of triethylamine in 3 mL of dichloromethane. After about 3 h, TLC monitoring (50% ethyl acetate−hexane) indicated consumption of the starting aniline. The mixture was diluted with dichloromethane, washed with 5% sodium bicarbonate, water, and brine, dried over sodium sulfate, and evaporated to give 0.19 g (85%) of **37**: mp 153−154 °C; HRMS calcd for $C_{27}H_{28}N_2O_3$ 428.2100, found 428.2083.

Rel-(3*S*,4*S*)-1-(4-methoxyphenyl)-3-(3-phenylpropyl)-4-(4-mercaptophenyl)-2-azetidinone (45). A solution of 3.2 g (7.38 mmol) of 4-*p*-(methylthio)phenyl derivative **46** in 15 mL of dichloromethane and 15 mL of trifluoroacetic anhydride was heated at reflux for 15 min, at which point TLC (ethyl acetate) indicated complete consumption of the sulfoxide. After cooling to room temperature, the solvent was removed under vacuum. The residue was dissolved in 30 mL of 50% triethylamine in methanol and stirred at room temperature for 15 min. The solvent was again removed under vacuum, and the residue was taken up in dichloromethane and washed with saturated ammonium chloride. The organic layer was dried over sodium sulfate and evaporated. The residue was chromatographed over silica gel, eluting with 30−60% ethyl acetate−hexanes to afford 2.65 g (89%) of the desired compound. An analytical sample was prepared by recrystallization from ether−dichloromethane: mp 134−135 °C. Anal. ($C_{25}H_{25}NO_2S$) C,H,N.

Rel-(3*R*,4*S*)-1-(4-methoxyphenyl)-3-(3-phenylpropyl)-4-(4-(methylsulfinyl)phenyl)-2-azetidinone (47) and Rel-(3*R*,4*S*)-1-(4-methoxyphenyl)-3-(3-phenylpropyl)-4-(4-(methylsulfonyl)phenyl)-2-azetidinone (48). To a solution of 0.31 g (0.74 mmol) of 4-*p*-(methylthio)phenyl derivative **46** in 4 mL of dichloromethane was added 0.16 g (0.79 mmol) of 85% *m*-chloroperoxybenzoic acid. The mixture was stirred for 60 min at room temperature, at which time TLC (silica gel, 50% ethyl acetate−hexane) indicated compete consumption of **46**. The reaction was quenched by addition of 0.1 g of $Ca(OH)_2$ followed by stirring for an additional 15 min. The mixture was filtered through Celite, washing with dichloromethane, and the filtrate was concentrated onto silica gel and chromatographed over silica gel, eluting with 5% methanol−dichloromethane to give 0.26 g (81%) of **47** (mp 111−115 °C) and 0.50 g of **48** (mp 57−58 °C). Anal. ($C_{26}H_{27}NO_4S$) C,H,N. Compounds **61** and **62** were prepared *via* an analogous procedure starting from 1-*p*-(methylthio)phenyl derivative **60**.

Rel-(3*R*,4*S*)-1-(4-(methylsulfinyl)phenyl)-3-(phenylpropyl)-4-(4-methoxyphenyl)-2-azetidinone (61): mp 52−54 °C. Anal. ($C_{26}H_{27}NO_3S$) C,H,N.

Rel-(3*R*,4*S*)-1-(4-(methylsulfonyl)phenyl)-3-(phenylpropyl)-4-(4-methoxyphenyl)-2-azetidinone (62): mp 56−58 °C. Anal. ($C_{26}H_{27}NO_4S$) C,H,N.

Rel-(3*R*,4*S*)-1-(4-hydroxyphenyl)-3-(phenylpropyl)-4-(4-hydroxyphenyl)-2-azetidinone (55). The bis-benzyl ether of compound **55** (0.686 g, 1.2 mmol), prepared according to method C, was dissolved in 25 mL of 1:1 ethanol−ethyl acetate and hydrogenated over 0.7 g of 10% Pd on carbon at 50 psi for 16 h. After filtering the catalyst, the solvent was removed under vacuum and the residue was chromatographed over silica gel, eluting with 80:20 hexane−ethyl acetate to give 0.432 g (93%) of **55**: mp 54−55 °C. Anal. ($C_{24}H_{23}NO_3$) C,H,N. Compound **36** was prepared by an analogous procedure starting from the corresponding bis-benzyl ether.

Rel-(3*R*,4*S*) 4-(3,4-dihydroxyphenyl)-1-phenyl-3-(3-phenylpropyl)-2-azetidinone (36): mp 54−55 °C; HRMS calcd 373.1678, found 373.1696.

Rel-(3*R*,4*S*)-4-(4-methoxyphenyl)-3-(3-phenylpropyl)-2-azetidinone (74). A 250 mL 3-neck round bottom flask equipped with stir bar, thermometer adapter, and low-temperature thermometer was charged with 8.5 mL (40.4 mmol, 1.1 equiv) of 1,1,1,3,3,3-hexamethyldisilazane and anhydrous tetrahydrofuran. To the solution, cooled to −78 °C, was added 29.9 mL (38.9 mmol, 1.06 equiv) of freshly titrated *n*-butyllithium in hexanes (1.3 M). The resultant solution was stirred at −78 °C for 15 min. After this time, a 3.75 M solution of 5.0 g (36.7 mmol, 1 equiv) of *p*-anisaldehyde in 9.8 mL of dry tetrahydrofuran was added dropwise to the lithium hexamethyldisilylamide at such a rate such that the internal temperature of the solution never exceeded −60 °C. The resulting solution of *N*-trimethylsilylimine was stirred at −78 °C for 60 min and then used immediately in the following reaction.

A flame-dried 500 mL 3-neck round bottom flask equipped with stir bar, thermometer adapter, and low-temperature thermometer was charged with 5.9 mL (42.2 mmol, 1.15 equiv) of diisopropylamine and 29.5 mL of dry tetrahydrofuran. To the solution, cooled to −78 °C, was added 32.8 mL (42.6 mmol, 1.01 equiv, 1.3 M in hexanes) of freshly titrated *n*-butyllithium. The resultant solution was stirred at −78 °C for 15 min, and then 19.2 mL (110.2 mmol, 3 equiv) of hexamethylphosphoramide (HMPA) was added to the cooled solution. A 1.9 M solution of 7.56 g (36.7 mmol, 1 equiv) of ethyl 5-phenylvalerate in 20 mL of dry tetrahydrofuran was added dropwise using a double-ended needle to the cooled LDA/HMPA solution at such a rate that the internal temperature did not exceed −60 °C. The resultant solution was stirred at −78 °C for 60 min. A solution of 36.7 mmol of the *N*-(trimethylsilyl)-*p*-anisaldimine in tetrahydrofuran cooled to −78 °C was added to the enolate solution at −78 °C, dropwise, using a double-ended needle at a rate such that the internal temperature never exceeded −60 °C. The addition takes approximately 35 min. After completion of addition, the resultant solution was stirred at −78 °C for 1 h. After 1 h, the cold bath was removed, and the solution was allowed to warm to room temperature. After 4 h, the reaction solution was diluted with ether and washed with two portions of 1 N aqueous hydrogen chloride and one portion of brine. The combined aqueous phases were extracted with three portions of ether. The combined organic phases were dried over sodium sulfate and concentrated under vacuum to afford 17.32 g of an orange oil. The oil (a 1:2 mixture of *cis*:*trans*-azetidinones by NMR) was purified by flash chromatography, using 35% ethyl acetate−hexanes as eluent to afford 6.34 g (58.5%) of the trans diastereomer **74** and an additional 2.81 g of a cis/trans mixture. Anal. ($C_{19}H_{21}NO_2$) C,H,N.

Cholesterol-Fed Hamster Assay. *In vivo* activity was assessed using the 7-day cholesterol-fed hamster model as previously described.[18] Briefly, male golden Syrian hamsters weighing from 100−125 g were fed chow supplemented with 0.5% cholesterol for 7 days. During this period, the animals were gavaged once daily with test compound dissolved in 0.2 mL of corn oil. On the last day, animals were sacrificed and hepatic and serum lipids determined as previously described. Data are reported as percent change in hepatic cholesteryl ester concentration or serum cholesterol concentration versus control animals receiving the high-cholesterol diet (oral gavaged in 0.2 mL of corn oil/day) without drug. All changes were statistically different from non-drug treated controls.

Microsomal ACAT Assay. Assays for acyl CoA:cholesterol acyltransferase (ACAT; EC 2.3.1.26) activity were performed by measuring the formation of cholesteryl [^{3}H]oleate from cholesterol and [^{3}H]oleaoyl CoA in rat liver microsomes using conditions previously described.[17] Data are reported as the percent inhibition at 10 uM.

Acknowledgment. We gratefully acknowledge Drs. A. K. Ganguly, Michael J. Green, and Joel G. Berger for their continued support and encouragement throughout this project.

References

(1) McGill, H. C., Jr. *Geographical Pathology of Atherosclerosis*; Williams and Wilken Co.: Baltimore, 1985.

(2) (a) The Nutrition Committee of the American Heart Association. Dietary guidelines for healthy American Adults: a statement for physicians and health professionals. *Circulation* **1988**, *77*, 721A−724A. (b) The Expert Panel. Summary of the Second Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel II). *J. Am. Med. Assoc.* **1993**, *269*, 3015−3023.

(3) For a recent review of pharmacological approaches to the treatment of atherosclerosis, see: Krause, B. R.; Sliskovic, D. R.; Bocan, T. M. A. Emerging therapies in atherosclerosis. *Exp. Opin. Invest. Drugs* **1995**, *4*, 353−387.

(4) Although bile acid sequestrants have been shown to inhibit cholesterol absorption in animals (see ref 13), their effect on serum cholesterol in humans is due mostly to inhibition of bile acid reabsorption and a resultant increase in hepatic 7α-hydroxylase activity.

(5) Roth, B. D.; Blankley, C. J.; Hoefle, M. L.; Holmes, A.; Roark, W. H.; Trevedi, 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.

(6) 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.

(7) Clader, J. W.; Berger, J. G.; Burrier, R. E.; Davis, H. R.; Domalski, M.; Dugar, S.; Kogan, T. P.; Salisbury, B.; Vaccaro, W. Substituted (1,2-Diarylethyl)amide Acyl-CoA:Cholesterol Acyltransferase Inhibitors: Effects of Polar Groups on *in Vitro* and *in Vivo* Activity. *J. Med. Chem.* **1995**, *38*, 1600−1607.

(8) Lopenzcandales, A.; Bosner, M. S.; Spilburg, C. A.; Lange, L. G. Cholesterol transport function of pancreatic cholesterol esterase: directed sterol uptake and esterification in enterocytes. *Biochemistry* **1993**, *32*, 12085−12089.

(9) Mckean, M. L.; Commons, T. J.; Berens, M. S.; Hsu, P. L.; Ackerman, D. M.; Steriner, K. E.; Adelman, S. J. Effects of inhibitors of pancreatic cholesterol ester hydrolase (PCEH) on [^{14}C]-cholesterol absorption in animal models. *FASEB J.* **1992**, *6*, A1388.

(10) (a) Davignon, J. Prospects for drug therapy for hyperlipoproteinaemia. *Diabete Metab.* **1995** *21* (2), 139−146 and references cited therein. (b) McCarthy, P. A.; DeNinno, M. P.; Morehouse, L. A.; Chandler, C. E.; Bangerter, F. W.; Wilson, T. C.; Urban, F. J.; Walinsky, S. W.; Cosgrove, P. G.; Duplantier, K.; Etienne, J. B.; Fowler, M. A.; Lambert, J. F.; O'Donnel, J. P.; Pezzullo, S. L.; Watson, H. A., Jr.; Wilkins, R. W.; Zaccaro, L. M.; Zawistoski, M. P. 11-Ketotigogenin Cellobioside (Pamaqueside): A Potent Cholesterol Absorption Inhibitor in the Hamster. *J. Med. Chem.* **1996**, *39* (10), 1935−1937.

(11) Burnett, D. A.; Caplen, M. A.; Davis, H. R., Jr.; Burrrier, R. E.; Clader, J. W. 2-Azetidinones as Inhibitors of Cholesterol Absorption. *J. Med. Chem.* **1994**, *37* (12), 1733−1736.

(12) Bergman, M.; Morales, H.; Mellars, L.; Kosoglou, T.; Burrier, R.; Davis, H. R.; Sybertz, E. J.; Pollare, T. 12th International Symposium on Drugs Affecting Lipid Metabolism, Houston, TX, Nov. 7−10, 1995.

(13) Salisbury, B. G.; Davis, H. R.; Burrier, R. E.; Burnett, D. A.; Boykow, G.; Caplen, M. A.; Clemmons, A. L.; Compton, D. S; Hoos, L. M.; McGregor, D. G.; Schnitzer-Polokoff, R.; Smith, A. A.; Weig, B. C.; Zilli, D. L.; Clader, J. W.; Sybertz, E. J. Hypocholesterolemic activity of a novel inhibitor of cholesterol absorption, SCH 48461. *Atherosclerosis* **1995**, *115*, 45−63.

(14) For a discussion of the activity of other lactams, see: Dugar, S.; Kirkup, M. P.; Clader, J. W.; Lin, S.-I.; Rizvi, R.; Snow, M. E.; Davis, H. R.; McCombie, S. W. *Gamma*-Lactams and related compounds as Cholesterol Absorption Inhibitors: Homologs of the *beta*-Lactam Cholesterol Absorption Inhibitor SCH 48461. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 2947−2952.

(15) Hart, D. J; Ha, D.-C. The Ester Enolate-Imine Condensation Route to β-Lactams. *Chem. Rev.* **1989**, *89*, 1447−1465.

(16) Browne, M.; Burnett, D. A.; Caplen, M. A.; Chen, L.-Y.; Clader, J. W.; Domalski, M.; Dugar, S.; Pushpavanem, P.; Sher, R.; Vaccaro, W.; Viziano, M.; Zhao, H. *trans* Diastereoselective synthesis of 3-alkyl substituted beta lactams via the acid chloride-imine reaction of non-activated acid chlorides. *Tetrahedron Lett.* **1995**, *36* (15), 2555−2558

(17) Arrieta, A.; Cossio, F. P.; Palomo, P. Synthesis of β-lactams from acetic acids and imines induced by phenyl dichlorophosphate reagent. *Tetrahedron* **1985**, 1703−1712.

(18) For details on both models as well as leading references, see refs 7, 11, and 13.

(19) The absolute stereochemistry of SCH 48461 has been shown unambiguously to be 3*R*,4*S*; see: Burnett, D. A. Asymmetric Synthesis and Absolute Stereochemistry of Cholesterol Absorption Inhibitor, SCH 48461. *Tetrahedron Lett.* **1994**, *35* (40), 7339−7342 and references cited therein.

(20) An investigation of the metabolism of SCH 48461 shows no evidence for ACAT inhibitory metabolites and no evidence for loss of stereochemistry at either of the chiral centers. Details of these studies will be presented elsewhere.

(21) Dugar, S.; Clader, J. W.; Chan, T.-M.; Davis, H., Jr. Substituted 2-Azaspiro[5.3]nonan1-ones as Potent Cholesterol Absorption Inhibitors: Defining a Binding Conformation for SCH 48461. *J. Med Chem.* **1995**, *38*, 4875−4877.

(22) Chuang, C.; Lapin, S. C.; Schrock, A. K.; Schuster, G. B. *J. Am. Chem Soc.* **1985**, *14*, 4238.

JM960405N