Inhibitors of Acyl-CoA:Cholesterol O-Acyltransferase. 11. Structure–Activity Relationships of Several Series of Compounds Derived from N-(Chlorocarbonyl) Isocyanate[1]

Joseph A. Picard,* Richard F. Bousley,† Helen T. Lee, Katherine L. Hamelehle,† Brian R. Krause,† Laura L. Minton,† Drago R. Sliskovic, and Richard L. Stanfield†

Departments of Medicinal Chemistry and Atherosclerosis Therapeutics, Parke-Davis Pharmaceutical Research, Division of Warner-Lambert Company, 2800 Plymouth Road, Ann Arbor, Michigan 48105

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Five series of compounds (**4**–**9**) derived from *N*-(chlorocarbonyl) isocyanate have been synthesized and evaluated for their ability to inhibit the enzyme acyl-CoA:cholesterol *O*-acyltransferase and lower plasma cholesterol levels in cholesterol-fed rats. Structure–activity relationships indicate that the imino dicarboxylates (**6** and **7**) and the oxycarbonyl thiocarbamates (**8**) are the most potent and efficacious series. In these series, the combination of a 2,6-diisopropylphenyl group and an aliphatic alkyl group with a chain length between 6 and 14 carbon atoms gives good activity in vitro and in vivo. In addition, a hydrogen donor is required to maintain good in vitro activity, and the acidic proton on the central nitrogen in these series appears to be important for in vivo activity.

The enzyme acyl-CoA:cholesterol *O*-acyltransferase (ACAT) catalyzes the esterification of cholesterol intracellularly and has been implicated in several aspects of lipoprotein metabolism and atherosclerosis. Specifically, inhibition of ACAT in intestinal mucosal cells has been shown to decrease cholesterol absorption in a variety of animal models, with a consequent decrease in plasma total cholesterol (TC) levels.[2] In the liver, ACAT inhibition has been linked to a decreased secretion of very low-density lipoproteins (VLDL), the precursor of the atherogenic low-density lipoprotein (LDL) particle.[3] On the basis of the accepted role of ACAT in the formation of cholesteryl ester droplets in macrophages, inhibition of ACAT in the macrophages of the artery wall would be expected to prevent or reverse the formation of the fatty streak, an early lesion in the atherosclerotic process.[4] These three drug targets have made inhibition of ACAT a very attractive approach in the development of novel hypolipidemic and antiatherosclerotic agents.[2] Initial studies in our laboratories identified a series of potent anilide ACAT inhibitors (**1**, R = alkyl).[5] A subsequent study of bioisosteric replacements of the amide moiety showed that a hydrogen donor (anilide NH) and acceptor (amide carbonyl) were both needed to maintain potent inhibitory activity. In the analysis of the various bioisosteres, a series of phenylureas (**1**, R = NH-alkyl) were identified as the most effective replacement for the anilide functionality.[6] Since then, we have developed several series of ureas[7] and amides[8] which maintain potent ACAT inhibitory activity and hypocholesterolemic activity in cholesterol-fed animal models. This paper describes our continued effort in this area and examines several phenylureas, isosteric carbamates, and thiocarbamates, additionally functionalized at the nitrogen with a carboxyl moiety. The structural variations of these compounds allow us to further investigate the positional requirements of the hydrogen donor/acceptor functionalities in the maintenance of good ACAT inhibitory activity. Two series of compounds in this work (**4a**–**n** and **5a**–**l**) are also recognized as isosteres of the previously published malondiamides (**2**)[8b] and malonester amides (**3**),[8c] in which the acidic methylene group of these amides is replaced by a nitrogen atom. This results in a very acidic proton on the nitrogen between the two carboxyl groups, which may serve to improve the absorption of these compounds in vivo, as exemplified recently in a novel series of oxysulfonyl carbamates.[9] These compounds possess modest in vitro activity but excellent hypocholesterolemic effects in vivo, which may be due, in part, to the ability of the compounds to form potentially soluble base salts at the acidic site between the carbonyl and sulfonyl moieties. Several examples in this report possess similar structural properties and also exhibit excellent ACAT inhibitory activity and hypocholesterolemic effects in cholesterol-fed rats.

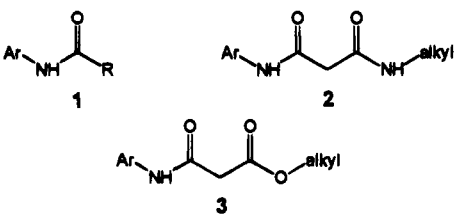

Figure 1.

Chemistry

The reaction of *N*-(chlorocarbonyl) isocyanate (CCI) with various nucleophiles has been extensively studied and reviewed.[10] The stepwise addition of nitrogen, oxygen, and sulfur nucleophiles to CCI yields a variety of *N*-carboxyureas (**4** and **5**), carbamates (**6** and **7**), and thiocarbamates (**8** and **9**) (Scheme 1). The extreme reactivity of CCI allows the formation of symmetrical byproducts (**4′**, **6′**, and **9′**) by reaction of two identical nucleophiles at both reactive centers. We attempted to minimize the formation of the symmetrical byproducts by keeping the reaction temperature cold and adding the less reactive nucleophile first.

Five series of compounds were examined, biurets (**4a**–**n**, employing two nitrogen nucleophiles), aminocarbonyl

† Department of Atherosclerosis Therapeutics.
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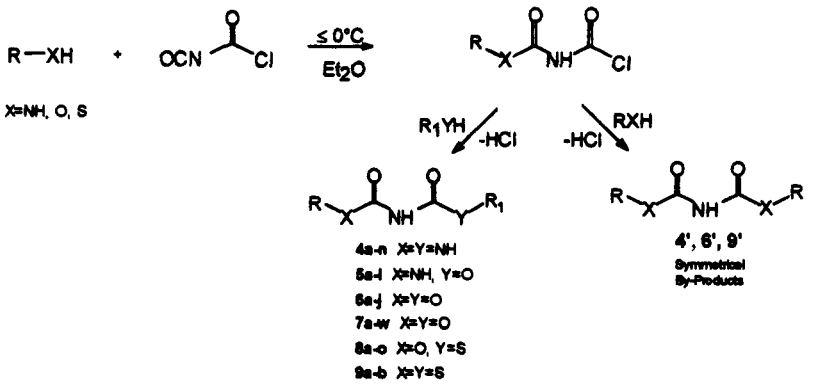
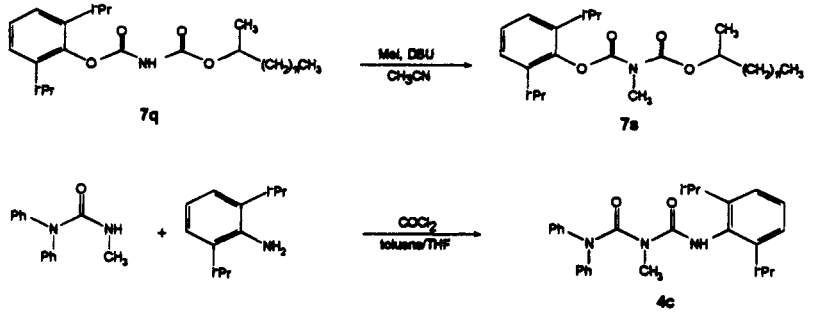
Scheme 1



Scheme 2



carbamates (**5a**–l, from one nitrogen and one oxygen nucleophile), imino dicarboxylates (**6a**–**j** and **7a**–**w**, from two oxygen nucleophiles), oxycarbonyl thiocarbamates (**8a**–**o**, from one oxygen and one sulfur nucleophile), and imino dicarbonothioates (**9a**,**b**, from two sulfur nucleophiles). A typical procedure (Scheme 1) involved the addition of a solution of the first nucleophile in diethyl ether to an ether solution of the CCI at low temperature (≤0 °C) and stirring for 30 min. An ether solution of the second nucleophile and triethylamine was then added, and the resulting reaction mixture was warmed to room temperature. The desired products were isolated by chromatography on silica gel; the unoptimized yields ranged from 45% to 75%. N-methylated compounds (e.g., **4c** and **7s**) could be prepared by N-methylation using methyl iodide and DBU. Alternatively, the N-methylated compounds could be prepared by reaction of the corresponding *N*-methyl-*N'*-substituted urea with phosgene and the appropriate nucleophile (Scheme 2).

Results and Discussion

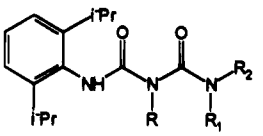
The in vitro ACAT inhibitory activity (IAI) was determined by measuring the incorporation of [1-^{14}C]-oleoyl-CoA into cholesterol esters by intestinal microsomes isolated from cholesterol-fed rabbits.[5] Results are reported as the micromolar concentration of drug required to inhibit the enzymatic activity by 50% (IC_{50}). The in vivo hypocholesterolemic activity was measured in an acute, cholesterol-fed rat model (APCC).[11] This acute screen measures the ability of a single dose of drug to prevent the rise in plasma cholesterol induced by a single cholesterol-rich meal (5.5% peanut oil, 1.5% cholesterol, and 0.5% cholic acid). The drugs were administered by gavage at a dose of 30 mg/kg, and the animals (n = 5/group/cage) were then fed the test diet overnight. Plasma cholesterol levels were measured by standard enzymatic methods, and the results were expressed as the percent change from the levels of control animals given vehicle (CMC/Tween in water) and diet only. For comparison, Lederle's ACAT inhibitor, CL 277,082,[12] was used as a reference agent and gave an IC_{50} of 0.47 μM in vitro and a 58% lowering of plasma TC in vivo.

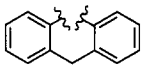
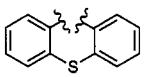
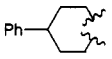
The 2,6-diisopropylphenyl substitution pattern has previously been shown to be optimal for ACAT inhibitory activity in other series[6-8] and was initially incorporated into each of the series of compounds examined here.

As shown in Table 1, neither the aryl (**4a**–**e**) nor alkyl (**4f**–**n**) biurets showed remarkable ACAT inhibitory activities in vitro or significant hypolipidemic effects in vivo. The *N*,*N*-diphenyl compound (**4b**) showed modest potency in vitro (IC_{50} = 0.34 μM) and good efficacy in vivo (−49% lowering of plasma TC). Addition of a methyl group to the central nitrogen (**4c**) maintained most of the in vitro activity (IC_{50} = 0.44 μM) but drastically decreased in vivo efficacy (−9% TC). The *N*,*N*-dibutyl compound (**4l**) was the most potent of the biurets in vitro (IC_{50} = 0.25 μM). However, it possessed only modest in vivo activity (−30% TC). Longer and shorter chain lengths were considerably less potent both in vitro and in vivo.

In the aminocarbonyl carbamate series (**5a**–l; Table 2), compounds containing a long chain alkyl group (7–15 carbons) on the carbamate portion of the molecule, while maintaining the (2,6-diisopropylphenyl)urea group on the other side, were the most potent (e.g., **5c**–**j**). The straight chain dodecyl compound (**5c**) was a potent inhibitor in vitro (IC_{50} = 0.12 μM) and modestly active in vivo (−38% TC). Interestingly, adding an α-methyl branch on the alkyl chain and increasing the overall length led to **5h**, which showed good in vitro potency (IC_{50} = 0.046 μM) and maintained the in vivo activity (−33% TC). Other compounds possessing the α-methyl branch but having chain lengths longer or shorter than **5h** were much less potent in vitro. Addition of another

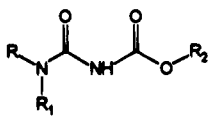
Table 1. SAR of Biurets



cmpd	R	R_1	R_2	IAI[a] IC_{50} (μM)	APCC[b] (%Δ TC)	formula[c]	mp (°C)
4a	H	2,6-(*i*-Pr)$_2$Ph	H	>5.0	0	$C_{26}H_{37}N_3O_2$	222–224
4b	H	Ph	Ph	0.34	−49***	$C_{26}H_{29}N_3O_2$	135–139
4c	CH_3	Ph	Ph	0.44	−9	$C_{27}H_{31}N_3O_2 \cdot 0.33H_2O$	115–120
4d	H			0.89	−18	$C_{28}H_{31}N_3O_2$	178–179
4e	H			1.9	−6	$C_{26}H_{27}N_3O_2S$[d]	176–177
4f	H	$CHPh_2$	H	>1.0	4	$C_{27}H_{31}N_3O_2$	139–141
4g	H	CH_2Ph	CH_2Ph	1.04	−14	$C_{28}H_{33}N_3O_2$	163–166
4h	H			0.89	3	$C_{28}H_{33}N_3O_2$	205–207
4i	H	*i*-Pr	CH_2Ph	0.75	−22*	$C_{24}H_{33}N_3O_2$	92–95
4j	H	CH_2CH_3	CH_2CH_3	>5.0	−16	$C_{18}H_{29}N_3O_2$	160–163
4k	H			>5.0	−7	$C_{18}H_{27}N_3O_2$	175–177
4l	H	$(CH_2)_3CH_3$	$(CH_2)_3CH_3$	0.25	−30*	$C_{22}H_{37}N_3O_2$	112–114
4m	H	$(CH_2)_7CH_3$	$(CH_2)_7CH_3$	0.35	3	$C_{30}H_{53}N_3O_2$	44–48
4n	H	CH_3	$(CH_2)_{13}CH_3$	1.7	−8	$C_{29}H_{51}N_3O_2$	47–49

[a] In vitro ACAT inhibition, determined in rabbit intestinal microsomes from cholesterol-fed animals. [b] Reported as percent change of total cholesterol as compared to controls. Animals were administered a single dose of compound (30 mg/kg) and then fed a single meal containing cholic acid (0.5%), cholesterol (1.5%), and peanut oil (5.5%). *Significantly different from controls, $P < 0.05$. ***Significantly different from controls, $P < 0.0001$. [c] Analyses are within ±0.4%, unless otherwise noted. [d] H: calcd, 6.11; found, 6.53.

Table 2. SAR of Aminocarbonyl Carbamates



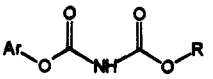
cmpd	R	R_1	R_2	IAI[a] IC_{50} (μM)	APCC[b] (%Δ TC)	formula[c]	mp (°C)
5a	2,6-(*i*-Pr)$_2$Ph	H	2,6-(*i*-Pr)$_2$Ph	0.98	−25**	$C_{26}H_{36}N_2O_3$	184–186
5b	2,6-(*i*-Pr)$_2$Ph	H	$CHPh_2$	7.8	−3	$C_{27}H_{30}N_2O_3$	169–172
5c	2,6-(*i*-Pr)$_2$Ph	H	$(CH_2)_{11}CH_3$	0.12	−38**	$C_{26}H_{44}N_2O_3$	100–102
5d	2,6-(*i*-Pr)$_2$Ph	H	$CH(CH_3)(CH_2)_4CH_3$	1.06	−22*	$C_{21}H_{34}N_2O_3$	113–115
5e	2,6-(*i*-Pr)$_2$Ph	H	$CH(CH_3)(CH_2)_6CH_3$	0.39	−24**	$C_{23}H_{38}N_2O_3$	100–102
5f	2,6-(*i*-Pr)$_2$Ph	H	$CH(CH_3)(CH_2)_7CH_3$	0.75	−22*	$C_{24}H_{40}N_2O_3$	84–86
5g	2,6-(*i*-Pr)$_2$Ph	H	$CH(CH_3)(CH_2)_9CH_3$	0.35	−26*	$C_{26}H_{44}N_2O_3$	59–61
5h	2,6-(*i*-Pr)$_2$Ph	H	$CH(CH_3)(CH_2)_{11}CH_3$	0.046	−33**	$C_{28}H_{48}N_2O_3$	65–66
5i	2,6-(*i*-Pr)$_2$Ph	H	$CH(CH_3)(CH_2)_{13}CH_3$	6.8	−26***	$C_{30}H_{52}N_2O_3$	68–70
5j	2,6-(*i*-Pr)$_2$Ph	H	$C(CH_3)_2(CH_2)_{11}CH_3$	0.065	−14	$C_{29}H_{50}N_2O_3$	65–67
5k	$CHPh_2$	H	2,6-(*i*-Pr)$_2$Ph	>1.0	−4	$C_{27}H_{30}N_2O_3$	174–176
5l	Ph	Ph	2,6-(*i*-Pr)$_2$Ph	0.17	−3	$C_{26}H_{28}N_2O_3$[d]	142–146

[a] In vitro ACAT inhibition, determined in rabbit intestinal microsomes from cholesterol-fed animals. [b] Reported as percent change of total cholesterol as compared to controls. Animals were administered a single dose of compound (30 mg/kg) and then fed a single meal containing cholic acid (0.5%), cholesterol (1.5%), and peanut oil (5.5%). *Statistically significant from controls, $P < 0.05$. **Statistically significant from controls, $P < 0.001$. ***Statistically significant from controls, $P < 0.0001$. [c] Analyses are within ±0.4%, unless otherwise noted. [d] C: calcd, 74.97; found, 74.22.

α-methyl gave the *gem*-dimethyl compound **5j** which retained in vitro potency ($IC_{50} = 0.065$ μM), as compared to **5h**. However, in vivo activity diminished considerably (−14% TC). Interestingly, the aminocarbonyl carbamate **5l** possesses good potency in vitro ($IC_{50} = 0.17$ μM). This demonstrates that the hydrogen donor (NH) need not be adjacent to the aryl ring, as in the arylureas and amides previously disclosed,[6] but may be three atoms away and still maintain ACAT inhibitory activity. This finding prompted us to examine other aryl carbamates.

In the imino dicarboxylate series (**6a–j** and **7a–w**), there is a distinct difference in activity between the compounds containing two aryl groups (**6a–f**; Table 3) and those containing one aryl group and one alkyl group (**7a–w**; Table 4). With the diaryl compounds (**6a–d**), variation of the 2,6-substituents from methyl (**6a**, $IC_{50} = >5.0$ μM) to isopropyl (**6b**, $IC_{50} = 0.11$ μM) and then to *tert*-butyl (**6c**, $IC_{50} = 1.2$ μM) confirmed the earlier observations that the 2,6-diisopropylphenyl is the best for ACAT inhibition in a given series.[6–8] Interestingly, the corresponding 2,6-diphenyl substitution (**6d**) also gave good in vitro activity ($IC_{50} = 0.23$ μM). Nevertheless, none of these diaryl iminodicarboxylates gave good in vivo activity. As the alkyl character of one side of the imino dicarboxylates is increased (**6g–j**), more

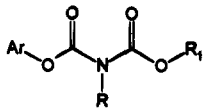
Table 3. SAR of Aryl Imino Dicarboxylates



cmpd	Ar	R	IAI[a] IC_{50} (μM)	APCC[b] (%Δ TC)	formula[c]	mp (°C)
6a	2,6-$(CH_3)_2$Ph	2,6-$(CH_3)_2$Ph	>5.0	4	$C_{18}H_{19}NO_4$[d]	270–273
6b	2,6-(*i*-Pr)$_2$Ph	2,6-(*i*-Pr)$_2$Ph	0.11	−24	$C_{30}H_{43}NO_4$	152–153.5
6c	2,6-(*t*-Bu)$_2$Ph	2,6-(*t*-Bu)$_2$Ph	1.2	−23*	$C_{38}H_{27}NO_4$[e]	195–197
6d	2,6-$(Ph)_2$Ph	2,6-$(Ph)_2$Ph	0.23	0	$C_{26}H_{35}NO_4$	>275
6e	2,6-(*i*-Pr)$_2$Ph	Ph	1.8	−29***	$C_{20}H_{23}NO_4$	144–147
6f	2,6-(*i*-Pr)$_2$Ph	1-naphthyl	3.7	NT	$C_{24}H_{28}NO_4$	152–154
6g	2,6-(*i*-Pr)$_2$Ph	$CH(Ph)_2$	0.35	−44**	$C_{27}H_{29}NO_4$	146–148
6h	2,6-(*i*-Pr)$_2$Ph	$CH(Ph)CO_2CH_3$	0.94	−3	$C_{23}H_{27}NO_6$	130–134
6i	2,6-(*i*-Pr)$_2$Ph	CH_2Ph	0.077	−23	$C_{21}H_{25}NO_4$	108–110
6j	2,6-(*i*-Pr)$_2$Ph	$(CH_2)_3Ph$	0.66	−24	$C_{23}H_{29}NO_4$	85–87

[a] In vitro ACAT inhibition, determined in rabbit intestinal microsomes from cholesterol-fed animals. [b] Reported as percent change of total cholesterol as compared to controls. Animals were administered a single dose of compound (30 mg/kg) and then fed a single meal containing cholic acid (0.5%), cholesterol (1.5%), and peanut oil (5.5%). *Statistically significant from controls, $P < 0.05$. **Statistically significant from controls, $P < 0.001$. ***Statistically significant from controls, $P < 0.0001$. [c] Analyses are within ±0.4%, unless otherwise noted. [d] C: calcd, 68.99; found, 68.58. [e] C: calcd, 81.26; found, 80.57.

Table 4. SAR of Aryl Alkyl Imino Dicarboxylates

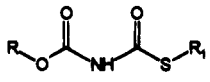


cmpd	Ar	R	R_1	IAI[a] IC_{50} (μM)	APCC[b] (%Δ TC)	formula[c]	mp (°C)
7a	2,6-(*i*-Pr)$_2$Ph	H	$(CH_2)_5CH_3$	0.053	−38***	$C_{20}H_{33}NO_4$·0.33H_2O[d]	53–55
7b	2,6-(*i*-Pr)$_2$Ph	H	$(CH_2)_7CH_3$	0.25	−47***	$C_{22}H_{35}NO_4$·0.33H_2O	oil
7c	2,6-(*i*-Pr)$_2$Ph	H	$(CH_2)_9CH_3$	0.074	−44***	$C_{24}H_{39}NO_4$	45–47
7d	2,6-(*i*-Pr)$_2$Ph	H	$(CH_2)_{10}CH_3$	0.14	−65***	$C_{25}H_{41}NO_4$	oil
7e	2,6-(*i*-Pr)$_2$Ph	H	$(CH_2)_{11}CH_3$	0.021	−52***	$C_{28}H_{43}NO_4$	60–62
7f	2,4,6-$(OCH_3)_3$Ph	H	$(CH_2)_{13}CH_3$	>5.0	−15	$C_{23}H_{37}NO_7$	87–89
7g	2,6-(*i*-Pr)$_2$Ph	H	$(CH_2)_{12}CH_3$	0.009	−69***	$C_{27}H_{45}NO_4$	65–68
7h	2,6-(*i*-Pr)$_2$Ph	H	$(CH_2)_{13}CH_3$	0.01	−59***	$C_{28}H_{47}NO_4$	58–60
7i	2,6-(*i*-Pr)$_2$Ph	H	$(CH_2)_{15}CH_3$	0.05	−70***	$C_{30}H_{51}NO_4$·0.35C_6H_{14}	67–69
7j	2,6-(*i*-Pr)$_2$Ph	H	$(CH_2)_{17}CH_3$	1.0	−35**	$C_{32}H_{55}NO_4$·0.23H_2O	57–59
7k	2,6-(*i*-Pr)$_2$Ph	H	(±)-$CH(CH_3)(CH_2)_4CH_3$	0.102	−60***	$C_{21}H_{33}NO_4$·0.5H_2O	60–62
7l	2,6-(*i*-Pr)$_2$Ph	H	(*R*)-$CH(CH_3)(CH_2)_4CH_3$	0.130	−49***	$C_{21}H_{33}NO_4$·0.21C_6H_{14}[e]	128–130
7m	2,6-(*i*-Pr)$_2$Ph	H	(*S*)-$CH(CH_3)(CH_2)_4CH_3$	0.098	−25	$C_{21}H_{33}NO_4$·0.16H_2O	73–75
7n	2,6-(*i*-Pr)$_2$Ph	H	(±)-$CH(CH_3)(CH_2)_8CH_3$	0.097	−56***	$C_{23}H_{37}NO_4$	oil
7o	2,6-(*i*-Pr)$_2$Ph	H	(±)-$CH(CH_3)(CH_2)_7CH_3$	0.27	−54***	$C_{24}H_{39}NO_4$	50–52
7p	2,6-(*i*-Pr)$_2$Ph	H	(±)-$CH(CH_3)(CH_2)_9CH_3$	0.049	−66***	$C_{28}H_{43}NO_4$	50–52
7q	2,6-(*i*-Pr)$_2$Ph	H	(±)-$CH(CH_3)(CH_2)_{11}CH_3$	0.017	−50***	$C_{28}H_{47}NO_4$·0.3$C_4H_8O_2$·0.45C_8H_{14}	55–57
7r	2,4-$(F)_2$Ph	H	(±)-$CH(CH_3)(CH_2)_{11}CH_3$	>1.0	−35***	$C_{22}H_{33}NO_4F_2$	65–67
7s	2,6-(*i*-Pr)$_2$Ph	CH_3	(±)-$CH(CH_3)(CH_2)_{11}CH_3$	>1.0	−8	$C_{29}H_{49}NO_4$	oil
7t	2,6-(*i*-Pr)$_2$Ph	H	(±)-$CH(CH_3)(CH_2)_{13}CH_3$	0.058	−43**	$C_{30}H_{51}NO_4$·0.09C_6H_{14}	50–52
7u	2,6-(*i*-Pr)$_2$Ph	H	$C(CH_3)_2(CH_2)_{11}CH_3$	0.021	−51***	$C_{29}H_{49}NO_4$	oil
7v	2,6-(*i*-Pr)$_2$Ph	H	$C(CH_3)_2(CH_2)_3CH_3$	0.064	−29	$C_{21}H_{38}NO_4$	95–97
7w	2,6-(*i*-Pr)$_2$Ph	H	$CH_2C(CH_3)_2(CH_2)_9CH_3$	0.020	−72***	$C_{28}H_{47}NO_4$	74–76

[a] In vitro ACAT inhibition, determined in rabbit intestinal microsomes from cholesterol-fed animals. [b] Reported as percent change of total cholesterol as compared to controls. Animals were administered a single dose of compound (30 mg/kg) and then fed a single meal containing cholic acid (0.5%), cholesterol (1.5%), and peanut oil (5.5%). **Statistically significant from controls, $P < 0.001$. ***Statistically significant from controls, $P < 0.0001$. [c] Analyses are within ±0.4%, unless otherwise noted. [d] C: calcd, 67.67; found, 68.11. [e] N: calcd, 3.67; found, 3.21.

potent compounds are observed. For example, the compound with phenol on one side (**6e**) is not a potent ACAT inhibitor (IC_{50} = 1.8 μM), but the compound obtained from benzyl alcohol (**6i**) is very potent (IC_{50} = 0.077 μM). This observation led us to examine several compounds with an alkyl chain on one side of the imino dicarboxylate (**7a–w**). In this series, the 2,6-diisopropylphenyl group is again identified as the preferred substitution pattern for the aryl portion of the molecule and any variation in aryl substitution resulted in a loss of activity in vitro and in vivo (cf. **7e** vs **7f** and **7q** vs **7r**). Table 4 shows that a combination of the 2,6-diisopropylphenyl group on one side and a simple straight chain alkyl group on the other gives several very potent ACAT inhibitors. In general, an overall trend of increasing in vitro activity and in vivo efficacy is seen with increased chain length of the alkyl group. For example, **7a**, with a *n*-hexyl chain, is fairly potent and efficacious (IC_{50} = 0.053 μM, in vivo = −38% TC), while **7g**, with a *n*-tridecyl chain, is extremely potent, with an IC_{50} of 0.009 μM, and produces a 69% decrease in plasma TC in vivo. The range for the optimal chain length is from *n*-dodecyl to *n*-hexadecyl (**7e,g–i**), with IC_{50} values of 0.009–0.050 μM and decreases in plasma TC from 52% to 70% in vivo. The upper limit to the chain length is shown by the *n*-octadecyl compound **7j** (IC_{50} = 1.0 μM, in vivo = −35% TC). Branching the long chain alkyl group by adding an α-methyl produced additional potent compounds (**7k–q, t–v**). The trend of increasing activity with increased chain length is

Table 5. SAR of Oxycarbonyl Thiocarbamates



cmpd	R	R_1	IAI[a] IC_{50} (μM)	APCC[b] (%Δ TC)	formula[c]	mp (°C)
8a	2,6-(*i*-Pr)$_2$Ph	2,6(*i*-Pr)$_2$Ph	3.2	−20*	$C_{26}H_{35}NO_3S$	160−164
8b	2,6-(*i*-Pr)$_2$Ph	Ph	>5	−37**	$C_{20}H_{23}NO_3S$	150−152
8c	2,6-(*i*-Pr)$_2$Ph	CH_2Ph	0.44	−43**	$C_{21}H_{25}NO_3S$	122−124
8d	2,6-(*i*-Pr)$_2$Ph	CH_2(4-ClPh)	0.16	−50**	$C_{21}H_{24}ClNO_3S{\cdot}0.24C_6H_{14}$	115−117
8e	2,6-(*i*-Pr)$_2$Ph	CH_2-2-furanyl	0.47	−51**	$C_{19}H_{23}NO_4S$	121−123
8f	2,6-(*i*-Pr)$_2$Ph	$(CH_2)_2$Ph	0.88	−30	$C_{22}H_{27}NO_3S{\cdot}0.18H_2O$	117−120
8g	2,6-(*i*-Pr)$_2$Ph	$(CH_2)_2$-*N*-morpholine	0.9	−33*	$C_{20}H_{30}N_2O_4S$	156−157
8h	2,6-(*i*-Pr)$_2$Ph	$(CH_2)_3$Ph	0.069	−63***	$C_{23}H_{29}NO_3S$	102−105
8i	2,6-(*i*-Pr)$_2$Ph	cyclohexyl	0.097	−63***	$C_{20}H_{29}NO_3S{\cdot}0.44H_2O$	100−104
8j	2,6-(*i*-Pr)$_2$Ph	$(CH_2)_7CH_3$	0.16	−63***	$C_{22}H_{35}NO_2S{\cdot}1.2C_6H_{14}$	44−47
8k	2,6-(*i*-Pr)$_2$Ph	$(CH_2)_9CH_3$	0.13	−52***	$C_{24}H_{39}NO_3S{\cdot}1.0H_2O$	50−52
8l	2,6-(*i*-Pr)$_2$Ph	$(CH_2)_{10}CH_3$	0.21	−54***	$C_{25}H_{41}NO_3S$	oil
8m	2,6-(*i*-Pr)$_2$Ph	$(CH_2)_{11}CH_3$	0.016	−63***	$C_{26}H_{43}NO_3S$	55−57
8n	2,4,6-$(OCH_3)_3$Ph	$(CH_2)_{11}CH_3$	0.25	−59***	$C_{23}H_{37}NO_6S$	100−102
8o	$(CH_2)_{11}CH_3$	2,6-(*i*-Pr)$_2$Ph	>5.0	−4	$C_{26}H_{43}NO_3S{\cdot}0.64C_4H_8O_2$[d]	oil
8p	2,6-(*i*-Pr)$_2$Ph	(±)-CH(CH_3)$(CH_2)_{11}CH_3$	0.008	−60***	$C_{28}H_{47}NO_3S$	oil

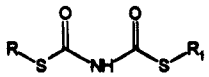
[a] In vitro ACAT inhibition, determined in rabbit intestinal microsomes from cholesterol-fed animals. [b] Reported as percent change of total cholesterol as compared to controls. Animals were administered a single dose of compound (30 mg/kg) and then fed a single meal containing cholic acid (0.5%), cholesterol (1.5%), and peanut oil (5.5%). *Statistically significant from controls, $P < 0.05$. **Statistically significant from controls, $P < 0.001$. ***Statistically significant from controls, $P < 0.0001$. [c] Analyses are within ±0.4%, unless otherwise noted. [d] H: calcd, 9.58; found, 9.08.

somewhat evident in the biological activity of these compounds. The optimal chain length in this series of compounds occurs around the 2-dodecyl or 2-tetradecyl chains (**7p**, IC_{50} = 0.049 μM, in vivo = −66% TC; and **7q**, IC_{50} = 0.017 μM, in vivo = −50% TC, respectively), and the activity decreases with longer chains like the 2-hexadecyl (**7t**, IC_{50} = 0.058 μM, in vivo = −43% TC). Adding the α-methyl introduces a chiral center which, as demonstrated by the racemic compound **7k** and its two enantiomers **7l** (*R*) and **7m** (*S*), appears to have little effect on in vitro activity (IC_{50} = 0.102, 0.130, and 0.098 μM, respectively). However, **7m** is significantly less active in vivo as compared to **7k** and **7l** (−25% vs −60% and −49% TC, respectively). Adding a second α-methyl to the very potent 2-tetradecyl compound (**7q**) removes the chirality while retaining the activity (cf. **7u**, IC_{50} = 0.021 μM, in vivo = −51% TC; and **7q**, IC_{50} = 0.017 μM, in vivo = −50% TC). Moving the *gem*-dimethyl substituents to the β-carbon (**7w**) also has no adverse effect on activity in vitro (IC_{50} = 0.020 μM) but did improve the in vivo activity (-72% TC). In this series, like the biurets, substituents on the central nitrogen are not tolerated in vivo. However, contrary to the result observed in the biurets, the in vitro potency is also drastically decreased, as seen by comparing the 2-tetradecyl compound **7q** and the N-methylated analogue **7s** (IC_{50} = >1.0 μM, in vivo = −8% TC). This is not surprising, since adding the substituent on the imino removes the hydrogen donor previously identified as essential for ACAT inhibitory activity.[6]

The excellent activity displayed by this series of imino dicarboxylates prompted us to examine the thiocarbonyl carbamates (**8a**−**o**; Table 5) in which one of the oxygens of the imino dicarboxylates has been replaced by sulfur. Similar trends were observed in this series. Compounds incorporating two aryl rings (**8a**−**h**) were typically less active than those containing one aryl group and one alkyl group (**8i**−**p**). In **8a**−**h**, it is apparent that replacing one of the aryl groups with an arylalkyl group (i.e., benzyl and phenethyl, etc.) results in more potent compounds (i.e., **8h**, IC_{50} = 0.069 μM, in vivo = −63% TC), while those containing solely aromatic groups (i.e., **8b**, IC_{50} = >5.0 μM, in vivo = −37% TC) gave poor activity. Compounds **8j**−**l** with medium length, straight alkyl chains (8−11 carbons) showed very little variation in in vitro and in vivo activity. However, compound **8m**, with an *n*-dodecyl chain, is very potent in vitro (IC_{50} = 0.016 μM). This agrees with the optimal activity associated with a chain length of 12−16 carbon atoms observed in the imino dicarboxylates. Similarly, branching in the alkyl chain in this series also gave a very potent compound, **8p** (IC_{50} = 0.008 μM, in vivo = −60% TC). Interestingly, replacement of the 2,6-diisopropylphenyl group with 2,4,6-trimethoxyphenyl in this series (cf. **8m** and **8n**) moderately decreased in vitro activity (IC_{50} = 0.016 vs 0.25 μM) but had no effect on the in vivo activity (−63% vs −59% TC). This is different from the effect seen in the imino dicarboxylate series where the 2,4,6-trimethoxyphenyl compound (**7f**) was completely inactive both in vitro and in vivo when compared to the corresponding 2,6-diisopropylphenyl compound (**7e**). It is necessary to have the alkyl group attached to the sulfur and the aryl group attached to the oxygen as in **8m** in order to maintain ACAT inhibitory activity in this series. Compound **8o**, having the reversed *O*-alkyl, *S*-aryl attachment, is completely inactive in vitro and in vivo. With this information, one would predict that the imino dicarbonothiates (**9a**,**b**) in which an *S*-aryl attachment is inevitable would also be inactive. In fact, as shown in Table 6, these compounds were inactive. Even **9b**, with an optimal substitution pattern (2,6-diisopropylphenyl and *n*-dodecyl), had an IC_{50} > 5.0 μM.

In summary, we have evaluated several series of compounds, obtained by the stepwise addition of nucleophiles to CCI, as potential ACAT inhibitors. The biurets (**4a**−**n**) were found to possess only modest activity in vitro and no significant activity in vivo. Replacing one nitrogen with oxygen gave a series of compounds (**5a**−**l**) with some improvement of ACAT inhibitory activity in vitro and a modest hypocholesterolemic effect in vivo. Significant improvements of both

Table 6. SAR of Imino Dicarbonothioates



cmpd	R	R_1	IAI[a] IC_{50} (μM)	APCC[b] (%Δ TC)	formula[c]	mp (°C)
9a	2,6-$(CH_3)_2$Ph	2,6-$(CH_3)_2$Ph	>5.0	−13	$C_{18}H_{19}NO_2S_2$	218−222
9b	2,6-(*i*-Pr)$_2$Ph	$(CH_2)_{11}CH_3$	>5.0	−36**	$C_{26}H_{43}NO_2S_2$	50−52

[a] In vitro ACAT inhibition, determined in rabbit intestinal microsomes from cholesterol-fed animals. [b] Reported as percent change of total cholesterol as compared to controls. Animals were administered a single dose of compound (30 mg/kg) and then fed a single meal containing cholic acid (0.5%), cholesterol (1.5%), and peanut oil (5.5%). **Statistically significant from controls, $P < 0.001$. [c] Analyses are within ±0.4%, unless otherwise noted.

in vitro and in vivo activity were observed by replacement of a second nitrogen by oxygen, giving a series of imino dicarboxylates (**6a−j** and **7a−w**). While the diaryl compounds in this series (**6a−j**) showed only modest ACAT inhibitory activity, the aryl alkyl compounds (**7a−w**) were extremely potent ACAT inhibitors in vitro and also possessed a marked hypolipidemic effect in vivo. Compounds **8a−p** demonstrated that the replacement of the alkyl alcohol by a thiol gave similarly potent and efficacious compounds. However, the data obtained for compounds **8m,o** and **9b** demonstrate that replacement of the 2,6-diisopropylphenol with 2,6-diisopropylthiophenol totally diminishes all activity. This indicates that the 2,6-diisopropylphenyl carbamate is essential for potent ACAT inhibition and excellent hypolipidemic effect in these series. Substitution at the central nitrogen diminishes the activity of these compounds. In the biurets (**4c**), methylation removes the central acidic proton but a hydrogen-donor source is still present adjacent to the aryl ring. This resulted in a loss of in vivo activity but a retention of in vitro activity. In the imino dicarboxylates (**7s**), substitution at the nitrogen not only removes the acidic central proton but also removes the last hydrogen-donor source, thereby resulting in a total loss of activity in vitro and in vivo. This may indicate that the central acidic proton is important for in vivo activity, and it confirms the previously stated hypothesis that a hydrogen donor is required for ACAT inhibitory activity in vitro. The excellent in vitro activity observed in the imino dicarboxylates also demonstrates that the hydrogen donor does not need to be adjacent to the aryl ring but is tolerated in a position three atoms away. Additional studies on the ACAT activity of other series incorporating the 2,6-diisopropylphenyl carbamate group will be the subject of future communications from this laboratory. Also, the hypolipidemic effect of other compounds possessing an acidic hydrogen will be reported in order to expand on the possible importance of this acidic site with respect to in vivo activity.

Experimental Section

Unless otherwise noted, reagents and solvents obtained from commercial sources were used without further purification. The starting thiols, if not commercially available, were obtained through reaction of the corresponding alcohol with Lawesson reagent.[13] Column chromatography was performed with Merck silica gel 60 (230−400 mesh). Proton NMR spectra were recorded with a Varian XL-200 spectrometer; chemical shifts are expressed in parts per million (ppm) relative to internal tetramethylsilane. Melting points were measured with a Thomas-Hoover capillary melting point apparatus and are uncorrected. Elemental analyses were performed on a Perkin-Elmer Model 240C elemental analyzer and were ±0.4% of the theoretical values, unless otherwise noted. Tetrahydrofuran (THF) was distilled from sodium−benzophenone ketyl prior to use. The general scheme for the synthesis of the various compounds is below.

General Method for Biuret Synthesis. Preparation of *N*-[2,6-Bis(1-methylethyl)phenyl]-*N'*-(diphenylmethyl)-iminodicarbonic Diamide (4f). A solution of 2,6-diisopropylaniline (1.5 g, 8.5 mmol) in 50 mL of Et_2O was added dropwise to a solution of *N*-(chlorocarbonyl) isocyanate (0.68 mL, 8.5 mmol) in 40 mL of Et_2O at −50 °C under an atmosphere of N_2. The resulting solution was stirred for 3 h, allowing the temperature to rise to −30 °C. A solution of benzhydrylamine (1.46 mL, 8.5 mmol) and excess triethylamine (1.0 mL) in 50 mL of Et_2O was added dropwise. The resulting suspension was warmed to room temperature and stirred for 16 h. The reaction mixture was partitioned between EtOAc and 1 N HCl. The organic layer was dried over $MgSO_4$, filtered, and evaporated to give a white foam. Chromatography (SiO_2, 10% EtOAc/hexanes) gave 0.86 g (23%) of the title compound as a white solid, mp 139−141 °C. ^{1}H-NMR ($CDCl_3$): δ 10.34 (s, 1H), 7.26−7.11 (m, 15H), 6.07−6.04 (d, 1H), 3.15−2.99 (m, 2H), 1.27−1.13 (d, 12H). Anal. ($C_{27}H_{31}N_3O_2$) C, H, N.

General Method for Aminocarbonyl Carbamate Synthesis. Preparation of [[[2,6-Bis(1-methylethyl)phenyl]-amino]carbonyl]carbamic Acid, 2,6-Bis(1-methylethyl)-phenyl Ester (5a). A solution of 2,6-diisopropylphenol (1.69 g, 9.5 mmol) in 50 mL of Et_2O was added dropwise to a solution of *N*-(chlorocarbonyl) isocyanate (0.76 mL, 9.5 mmol) in 50 mL of Et_2O at −50 °C under an atmosphere of N_2. The temperature was raised to 0 °C over 2 h. A solution of 2,6-diisopropylaniline (1.68 g, 9.5 mmol) and excess triethylamine (1 mL) in 50 mL of Et_2O was added dropwise to the reaction. The resulting mixture was stirred at room temperature for 16 h and then partitioned between 1 N HCl and EtOAc. The organic layer was dried ($MgSO_4$), filtered, and concentrated to give a white solid. Chromatography (10% EtOAc/hexanes) gave the title compound (1.5 g, 37%), mp 184−186 °C. ^{1}H-NMR ($CDCl_3$): δ 9.11 (s, 1H), 7.99 (s, 1H), 7.31−7.17 (m, 6H), 3.16-3.02 (m, 4H), 1.26−1.2 (m, 24H). Anal. ($C_{26}H_{36}N_2O_3$) C, H, N.

General Method for the Synthesis of Imino Dicarboxylates. Preparation of (±)-Iminodicarbonic, 2,6-Bis-(1-methylethyl)phenyl 1-Methyltridecyl Ester (7q). A solution of 2,6-diisopropylphenol (2.67g, 15 mmol) in 45 mL of Et_2O was added dropwise to a solution of *N*-(chlorocarbonyl) isocyanate (1.46 mL, 18 mmol) in 45 mL of Et_2O at −15 °C. The reaction mixture was stirred for 45 min before a solution of 1-methyltridecanol (3.21 g, 15 mmol) and excess triethylamine (2.5 mL) in 75 mL of Et_2O was added dropwise. The resulting mixture was warmed to room temperature for 1 h and then partitioned between 1 N HCl and EtOAc. The organic layer was dried over $MgSO_4$, filtered, and evaporated to give a yellow oil. Chromatography gave the title compound (5.28 g, 76%) as a white solid, mp 55−57 °C. ^{1}H-NMR ($CDCl_3$): δ 7.35−7.10 (m, 4H), 5.05−4.85 (m, 1H), 3.15−2.90 (m, 2H), 1.40−1.10 (m, 38H), 0.88 (t, 3H). Anal. ($C_{28}H_{47}NO_4 \cdot 0.3C_4H_8O_2 \cdot 0.45C_8H_{14}$). C, H, N.

General Method for N-Alkylation. Preparation of (±)-(Methylimino)dicarbonic, 2,6-Bis(1-methylethyl)phenyl 1-Methyltridecyl Ester (7s). 1,8-Diazabicyclo[5.4.0]undec-7-ene (DBU, 1.6 mL, 10.7 mmol) was added dropwise to a

mixture of **7q** (4.47 g, 9.7 mmol) and CH_3I (1.52 g, 10.7 mmol) in 100 mL of CH_3CN at −15 °C. The resulting mixture was stirred at room temperature overnight. The solvent was evaporated under vacuum, and the residue was partitioned between 20 mL of dilute HCl and 20 mL of EtOAc. The organic layer was separated, dried over $MgSO_4$, and evaporated. The title compound (3.48 g, 75%) was isolated by chromatography (eluant = hexane:CH_2Cl_2, 4:1). ^{1}H-NMR ($CDCl_3$): δ 7.1−7.23 (m, 3H), 4.95−5.1 (m, 1H), 3.39 (s, 3H), 2.95−3.1 (m, 2H), 0.87−1.7 (m, 40H). Anal. ($C_{29}H_{49}NO_4$) C, H, N.

Alternate Synthesis of N-Alkylated Compounds. Preparation of *N'*-[2,6-Bis(1-methylethyl)phenyl]-2-methyl-*N,N*-diphenyliminodicarbonic Diamide (4c). Phosgene (in toluene, 7.92 mL, 10 mmol) was added to a solution of *N*-methyl-*N',N'*-diphenylurea (2.26 g, 10 mmol) in 20 mL at THF at room temperature. The mixture was stirred at room temperature for 2 days and then at 60 °C for 2 weeks. The solvent and excess phosgene were removed under vacuum. The residue was redissolved in 20 mL of THF, and 2,6-diisopropylaniline (3.55 g, 20 mmol) was added. A white precipitate appeared, and the mixture was stirred at room temperature overnight. The solvent was removed, and 50 mL of EtOAc was added to the residue. The mixture was filtered, and the filtrate was concentrated under vacuum. The title compound was isolated by chromatography (eluant = hexane:EtOAc, 8:1) to give the title compound (2.6 g, 65%) as an oil. ^{1}H-NMR ($CDCl_3$): δ 9.42 (s, 1H), 6.8−7.5 (m, 13H), 3.1−3.3 (m, 2H), 2.9 (s, 3H), 1.25 (d, 12H). Anal. ($C_{27}H_{31}N_3O_2 \cdot 0.33H_2O$) C, H, N.

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