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INHIBITORS OF ACYL-COA:CHOLESTEROL *O*-ACYLTRANSFERASE (ACAT) AS HYPOCHOLESTEROLEMIC AGENTS: SYNTHESIS AND STRUCTURE–ACTIVITY RELATIONSHIPS OF NOVEL SERIES OF SULFONAMIDES, ACYLPHOSPHONAMIDES AND ACYLPHOSPHORAMIDATES

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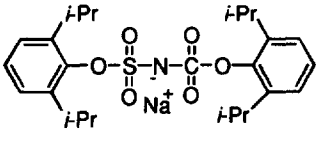
Abstract: Sulfoacetic acid, phosphoramidate, and phosphonamide analogs of the ACAT inhibitors, CI-999 and CI-1011 were synthesized. The structure–activity relationships of these compounds as ACAT inhibitors are described.

Introduction: Acyl-CoA:cholesterol acyltransferase (ACAT) is an ubiquitous intracellular enzyme that catalyzes the reaction of cholesterol with CoA-activated fatty acids to form cholesteryl esters.[1] Several ACAT inhibitors reduce plasma and hepatic cholesteryl ester levels in cholesterol-fed animals.[1] Furthermore, the systemically available ACAT inhibitor CI-976 causes the regression of atherosclerotic lesions in cholesterol-fed rabbits.[2] Some of these agents have been evaluated clinically, however, efficacy in humans has been disappointing.[3] Recently, our laboratories identified a series of oxysulfonyl carbamate ACAT inhibitors,[1,4] including the highly bioavailable and water soluble inhibitor CI-999 (**1**).[5] Despite excellent hypolipidemic effects, compound **1** degraded in solution, especially at acidic pH. We overcame the solution instability problem employing an isosteric replacement strategy yielding CI-1011 (**2**).[6] Although both **1** and **2** display outstanding in vivo efficacy, they are modestly potent ACAT inhibitors in our standard in vitro assay. To improve the potency of both compounds, we synthesized several series of closely related amides wherein N-acylphosphonamide, N-acylphosphoramidate, sulfonamide amide, sulfonamide ester, sulfonamide thioester, and amide sulfonate analogs of **1** and **2** were made. The effects of these substitutions on biological activity are herein reported.



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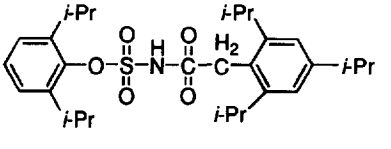

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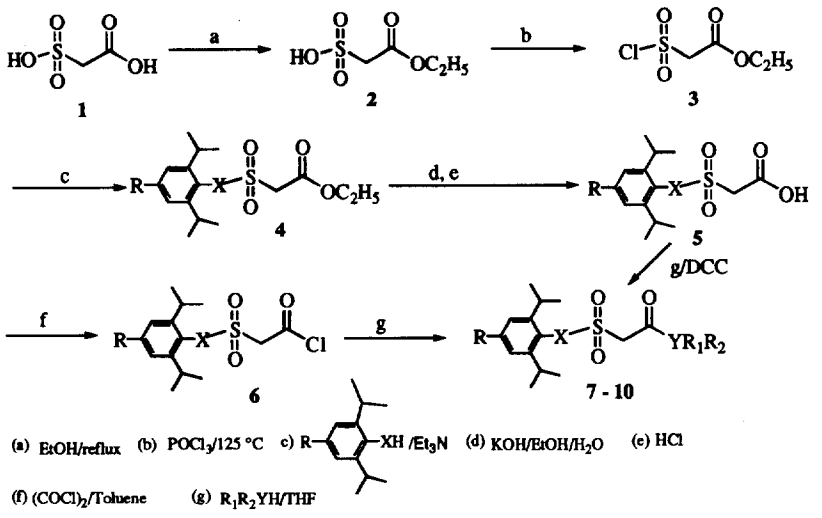
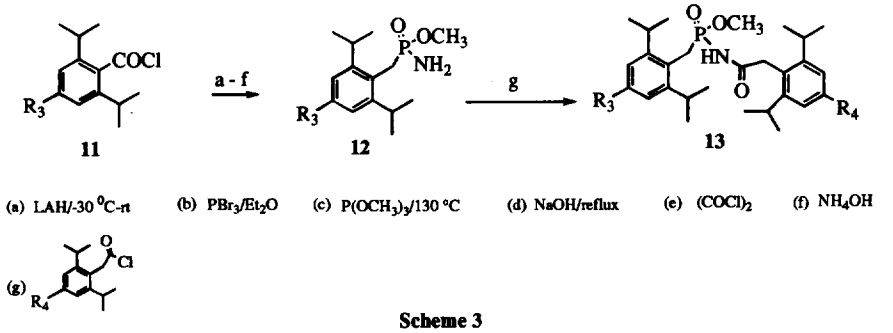
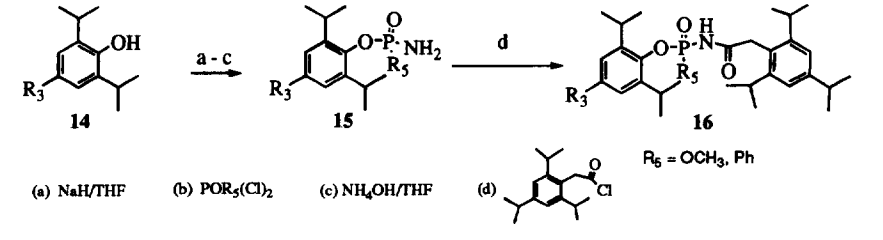
Scheme 1



Scheme 2



Scheme 3



Chemistry: The synthetic routes for compounds represented in Table 1 are shown in Scheme 1. The routes for compounds in Table 2 are shown in Schemes 2 and 3.

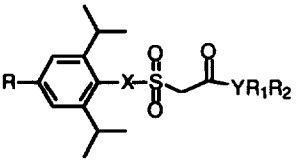
Esterification of sulfoacetic acid **1** in refluxing ethanol gave ethyl ester **2** (100%). Conversion to the sulfonyl chloride **3** with $POCl_3$ at 125 °C, then treatment with anilines or phenols (trisubstituted or disubstituted) in the presence of Et_3N in THF afforded the compound **4** (44%). Hydrolysis using KOH in aqueous ethanol, then reaction with $(COCl)_2$ in toluene gave **6** (>95%). Treatment with thiols, alcohols, or anilines (trisubstituted or disubstituted) gave compounds **7** (**a–h,** X = NH, Y = N), **8** (**a–m,** X = NH, Y = O), **9** (**a–g,** X = NH, Y = S) (40-80%). Coupling of compound **5** and anilines or amines (trisubstituted or disubstituted) in the presence of DCC in CH_2Cl_2 gave compounds **10** (**a–g,** X = O, Y = N) (40% to 80%).

The N-acylphosphonamides were prepared as shown in Scheme 2. 2,6-Diisopropylbenzoyl chloride or 2,4,6-triisopropylbenzoyl chloride **11** were reduced with lithium aluminum hydride and then converted to the bromides with phosphorus tribromide. Arbuzov reaction of the bromides with trimethylphosphite gave the phosphonic diesters. Monohydrolysis, conversion to the chlorophosphonic ester, and treatment with ammonia produced the phosphonamides **12**. Acylation with the appropriately substituted phenylacetyl chlorides gave the N-acylphosphonamides **13** (**a–c**). The acylphosphoramidates were prepared as shown in Scheme 3. Addition of the sodium phenolates derived from **14** to methylphosphorodichloridate followed by treatment with ammonia gave the phosphoramidates **15**. Acylation with the 2,4,6-triisopropylphenylacetyl chloride afforded the N-acylphosphoramidates **16** (**a–c**).

Biological Methods: ACAT inhibition in vitro was determined by incubation with [1-^{14}C]oleoyl-CoA and intestinal microsomes isolated from cholesterol-fed rabbits.[7] Results are reported as the micromolar concentration of the drug required to inhibit the enzymatic activity by 50% (IC_{50}). In vivo activity was assessed in rats by giving a single dose of compound in an aqueous suspension (1.5% carboxymethylcellulose, CMC/0.2% Tween 20) vehicle, at a dose of 30 mg/kg and the rats were then fed a high fat , cholesterol-rich diet (5.5% peanut oil, 1.5% cholesterol, and 0.5% cholic acid) overnight (APCC). Plasma cholesterol levels were measured and the results were expressed as the percent change from control animals given vehicle and diet only (ΔTC).

Result and Discussion: The biological activities of the sulfoacetic acid and phosphorus derived ACAT inhibitors are shown in Tables 1 and 2. Based on previous SAR.[7-10] we incorporated 2,6-diisopropylphenyl, and 2,4,6-triisopropylphenyl moieties to obtain optimal ACAT inhibitory activity. From Table 1, when X = Y = nitrogen, compounds with long chain substituents on nitrogen are more potent ACAT inhibitors in vitro than compounds with shorter chain lengths, and are also slightly more potent in vivo (**7a-7c**). Changing the nitrogen substituent to benzyl gave **7d** the most potent compound in vitro (IC_{50} = 0.15 μM), while changing the substituent to 2,6-diisopropylphenyl gave **7f** the most potent compound in vivo in the series (ΔTC = -51%). When X = nitrogen and Y = oxygen, increasing the chain length to at least 12 carbons on the carbonyl ester increased potency of the compounds **8e** and **8f**, and a branching chain substituent lead to a dramatic increase in in vitro potency (**8h**, IC_{50} = 0.021 μM). When X = nitrogen and Y = sulfur, very potent in vitro and in vivo activity resulted (**9d**, IC_{50} = 0.073 μM, ΔTC = -71%). However, changing the substituent on carboxy from dodecyl (**9d**) to hexyl (**9a**) or octodecyl (**9f**) resulted in diminished activities bothin vitro and in vivo. When X = oxygen and Y = nitrogen, two

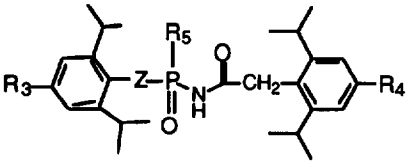
Table 1



compd	X	R	Y	R_1	R_2	IC_{50} (μM)	%Δ TC
1	--	--	--	--	--	5.2	-74**
2	--	--	--	--	--	12	-73**
7a	NH	H	N	hexyl	H	>5	-16*
7b	NH	H	N	octyl	octyl	4.2	-19(N/S)
7c	NH	H	N	dodecyl	H	0.32	-29**
7d	NH	H	N	benzyl	*i*-Pr	0.15	-31**
7e	NH	H	N	CH_2-1-Ph-cyclopentyl	H	>1	ND
7f	NH	H	N	2,6-(*i*-Pr)$_2$-Ph	H	0.35	-51***
8a	NH	H	O	C_2H_5	--	>1	+2(N/S)
8b	NH	H	O	hexyl	--	>1	-5(N/S)
8c	NH	H	O	octyl	--	>1	-4(N/S)
8d	NH	H	O	decyl	--	>1	-16*
8e	NH	H	O	dodecyl	--	0.11	-34**
8f	NH	H	O	tetradecyl	--	0.23	-45**
8g	NH	H	O	2,6-(*i*-Pr)$_2$-Ph	--	>1	ND
8h	NH	H	O	1-CH_3-tridecyl	--	0.021	-39***
8i	NH	H	O	2,4,6-(*i*-Pr)$_3$-Ph	--	2.8	-19(N/S)
8j	NH	H	O	2,4,6-(OMe)$_3$-Ph	--	>1	-6(N/S)
8k	NH	*i*-Pr	O	2,6-(*i*-Pr)$_2$-Ph	--	>1	-29**
9a	NH	H	S	hexyl	--	1.0	ND
9b	NH	H	S	octyl	--	0.15	-32*
9c	NH	H	S	decyl	--	0.13	ND
9d	NH	H	S	dodecyl	--	0.073	-71**
9e	NH	H	S	tetradecyl	--	0.036	-23**
9f	NH	H	S	octadecyl	--	>5	-35(N/S)
9g	NH	H	S	2,6-(*i*-Pr)$_2$-Ph	--	>50	-16(N/S)
10a	O	H	N	2,6-(*i*-Pr)$_2$-Ph	H	0.007	-42**
10b	O	*i*-Pr	N	2,6-(*i*-Pr)$_3$-Ph	H	0.007	-60**
10c	O	H	N	dodecyl	H	3.0	-3(N/S)
10d	O	H	N	$CH(Ph)_2$	H	1.8	-43**

Statistical difference from control values: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. ND (not determined). %Δ TC (APCC, dose were 30 mg/kg). N/S (not significant).

Table 2



compd	R_3	R_4	R_5	Z	IC_{50} (μM)	%Δ TC*
13a	*i*-Pr	H	OMe	CH_2	0.29	-44(3)**
13b	H	*i*-Pr	OMe	CH_2	1.2	-2(10)(N/S)
13c	*i*-Pr	*i*-Pr	OMe	CH_2	0.74	-17(10)(N/S)
16a	H	*i*-Pr	OMe	O	4.8	-18(1)(N/S)
16b	Cl	*i*-Pr	OMe	O	>5	-12(10)(N/S)
16c	H	*i*-Pr	Ph	O	2.5	-25(10)*

For footnote see Table 1. *Numbers in parentheses indicate dose (mg/kg)

of the most potent compounds in vitro result (**10a** and **10b**, IC_{50} = 0.007 μM). These compounds also maintain very good in vivo activities (ΔTC = -42% and -60%, respectively). Replacement of 2,6-diisopropylphenyl or 2,4,6-triisopropylphenyl with dodecyl, giving **10c**, reduced in vitro and eliminated in vivo activity (IC_{50} = 3.0 μM, ΔTC = -3%). Overall, the SAR in the sulfoacetic acid derivatives show that biological activity is sensitive to the variation of the substituents attached to the carbonyl group, especially with shorter (<10 carbons) alkyl chains which reduced both in vitro and in vivo activities. Comparison of sulfonamide esters , thioesters and amides of equal chain length show that thio analogs are more potent both in vivo and in vitro than corresponding oxygen and nitrogen analogs (**9d** > **8e** > **7c**). When R_1R_2 = 2,6-diisopropylphenyl and Y = nitrogen, **7f** is the most potent compound (**7f** > **8g** > **9g**). Transposition of the nitrogen and oxygen in compound **8k** gave compound **10b**, in which both in vitro and in vivo activities have changed from inactive to the most potent compound in the series (IC_{50} > 1.0 μM, ΔTC = -29%, IC_{50} = 0.007 μM, ΔTC = -60%). When X = oxygen and Y = nitrogen, 2,6-diisopropylphenyl and 2,4,6-triisopropylphenyl gave the best in vitro and in vivo activities (compounds **10a** and **10b**).

From Table 2, the acylphosphonamides (**13a–c**) were slightly more potent than the acylphosphoramidates (**16a–c**). Replacement of the *O*-methyl in **16a** with the phenyl (**16c**) caused significant losses in potency in vitro and in vivo. Thus, the phosphonamides **13(a–c)** were more potent in vitro but less efficacious in vivo than CI-1011 (**2**, IC_{50} = 12 μM, ΔTC = -73%, 30 mg/kg). Compound **13a** gave good in vitro potency (IC_{50} = 0.29 μM) and was the most efficacious phosphorus analog tested (ΔTC = -44% at 3 mg/kg).

In conclusion, sulfoacetic acid and phosphorus containing bioisosteric substitutions in CI-1011 have produced compounds that are much more potent than both CI-999 and CI-1011 in vitro while maintaining high efficacy in cholesterol-fed rats. Thus, the best compound from these series, **10b**, shows similar in vivo potency as compared with CI-999 and CI-1011 when dosed at 30 mg/kg PO to cholesterol-fed rats and is 1700-fold more potent in vitro. The reason for the greater in vitro potency of **10b** is unclear. However, the compound CI-1011 is known to avidly bind BSA,[6] which could result in its plasma sequestration. Additionally, since the compound was developed to inhibit liver ACAT, it is possible that it is not as potent against intestinal ACAT. These findings have prompted further pharmacological investigation of these compounds.

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