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INHIBITORS OF ACYL-COA:CHOLESTEROL ACYLTRANSFERASE (ACAT). 15. SULFONYLUREA INHIBITORS WITH EXCELLENT HYPOCHOLESTEROLEMIC ACTIVITY IN VIVO.

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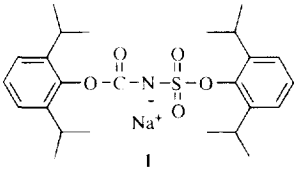
Abstract: A series of sulfonylureas were prepared and tested for the ability to inhibit the enzyme acyl-CoA: cholesterol acyltransferase (ACAT) in vitro and lower plasma cholesterol in cholesterol-fed rats in vivo. Although compounds from this series were generally weak inhibitors of ACAT in vitro, several displayed excellent hypocholesterolemic activity in vivo.

Introduction: Over the past two decades, considerable effort has been directed toward the discovery and development of novel therapies for the treatment of hypercholesterolemia and atherosclerotic disease.[1] Recently, inhibitors of the enzyme acyl-CoA:cholesterol acyltransferase (ACAT) have received significant attention due to evidence that this enzyme may be important, and possibly rate-limiting, in absorption of dietary cholesterol from the intestine, secretion of very-low density lipoproteins by liver and esterification and storage of cholesteryl esters in the arterial wall.[2] This latter function of ACAT has made it a particularly attractive target for pharmacological intervention due to the potential that inhibitors of this enzyme that penetrate the artery wall may not only lower plasma cholesterol, but also possess a direct effect on arterial lesions.[3] This possibility has become more attractive since recent clinical studies suggest that cholesteryl ester/macrophage-rich lesions, the putative target of arterial wall directed ACAT inhibitors, may be most prone to rupture, resulting in myocardial infarction.[4]

Although most strategies for identifying efficacious ACAT inhibitors have focused on identifying potent, but often highly lipophilic inhibitors of this enzyme,[2] we recently reported remarkable efficacy in vivo with a structurally novel compound **1** which is a relatively water soluble, but weakly potent ACAT inhibitor in vitro.[5] To build on this observation and previous studies of bioisosterism in ACAT inhibitors,[6] we have been preparing closely related analogs to determine whether they would possess properties similar to **1**. This report describes our investigations of a series of sulfonylureas from which several compounds with potency and efficacy profiles similar to **1** were identified.



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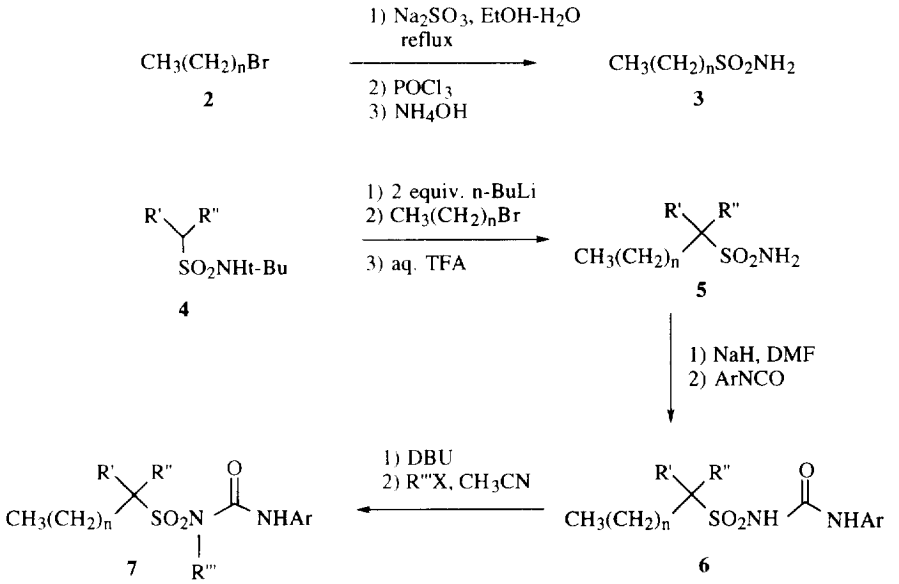
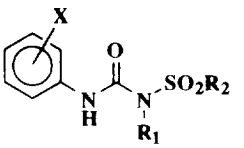
Chemistry: The compounds examined in this study were prepared as shown in Scheme 1. The requisite sulfonamides (**3**, **5**) were prepared most conveniently by the alkylation of the dilithioanion of an *N*-*t*-Bu-alkylsulfonamide (**4**) followed by TFA hydrolysis employing the procedure of Thompson.[7] Several analogs derived from primary alkylhalides (**2**) were prepared by displacement of the halide by sodium sulfinate followed by treatment first with refluxing $POCl_3$ and then with conc. NH_4OH. This method was limited, however, to primary alkylhalides. The sulfonamides (**3**, **5**) were then deprotonated with NaH and reacted with the requisite isocyanate in dry DMF at room temperature. Compounds substituted on the central nitrogen (**7**) could be prepared by treatment of **6** with a mixture of DBU and an alkylhalide in CH_3CN.

Scheme 1



Biological Methods: ACAT inhibition in vitro was measured employing two separate assays; one measuring ACAT inhibition by incubating test compounds with [1-^{14}C]-oleoyl-CoA and microsomes isolated from livers of cholesterol-fed rats (LAI) and a second using microsomes isolated from intestines of cholesterol-fed rabbits (IAI).[5] Hypocholesterolemic activity was assessed in rats both acutely (APCC) and chronically (CPCC). In the acute assay, rats were given a single dose of test compounds by gavage suspended in carboxymethyl-cellulose (CMC) and Tween-20 in water, followed by a single high-fat, high-cholesterol meal.[5] In the chronic assay, rats were fed a high-fat, high-cholesterol diet for 14 consecutive days and the test compound was administered by gavage in CMC-Tween 20 on the last 7 days.[5] Serum cholesterol levels were determined on the day after the final dose of test compound and the data expressed as percent decrease relative to controls.

Table of ACAT Inhibition In Vitro and Hypocholesterolemic Activity In Vivo



Cpd. No.	X	R_1	R_2	IC_{50} (μM) LAI[a]	IC_{50} (μM) IAI[b]	%Δ APCC[c] (mg/kg) 3	%Δ APCC[c] (mg/kg) 30	%Δ CPCC[d] (mg/kg) 3	%Δ CPCC[d] (mg/kg) 30	Formula[e]
1				5.3		-59**	-74**	-64**	-64**	
8	2,6-(iPr)$_2$	H	$(CH_2)_7CH_3$	22.6	24(5)[f]	-31**	-74**		-59**	$C_{21}H_{36}N_2O_3S$
9	2,6-(iPr)$_2$	H	$CH(CH_3)(CH_2)_7CH_3$	1.3		-32**	-69***	-46**	-58**	$C_{23}H_{40}N_2O_3S$
10	2,6-(iPr)$_2$	H	$CH(Ph)(CH_2)_7CH_3$	9.2	1.1	-62***	-75***	-48**	-74***	$C_{28}H_{42}N_2O_3S$
11	2,6-(iPr)$_2$	H	$(CH_2)_9CH_3$	13.6	11.1	-40**	-52***		-47**	$C_{23}H_{40}N_2O_3S$[g]
12	2,6-(iPr)$_2$	H	$(CH_2)_{11}CH_3$	3.30	2.33	-59***	-63***	-35*	-53***	$C_{25}H_{44}N_2O_3S$
13	2,6-(iPr)$_2$	CH_3	$(CH_2)_{11}CH_3$	0.675	0.086	-18*	-49**			$C_{26}H_{46}N_2O_3S$
14	2,6-(iPr)$_2$	H	$(CH_2)_{12}CH_3$	2.0	1.2	-42***	-62***		-69**	$C_{26}H_{46}N_2O_3S$
15	2,6-(iPr)$_2$	H	$(CH_2)_{13}CH_3$	0.94	0.83	-60***	-56***	-46*	-58*	$C_{27}H_{48}N_2O_3S$
16	2,4-F_2	H	$(CH_2)_{13}CH_3$	0 (10)[f]	49(5)[f]	-14				$C_{21}H_{34}F_2N_2O_3S$
17	2,4,6-(OMe)$_3$	H	$(CH_2)_{13}CH_3$	40 (5)[f]		-31*	-47**			$C_{24}H_{42}N_2O_3S$
18	2,6-(iPr)$_2$	CH_3	$(CH_2)_{13}CH_3$	8.83	34(5)[f]	-20*	-48**			$C_{28}H_{50}N_2O_3S$
19	2,6-(iPr)$_2$	CH_2Ph	$(CH_2)_{13}CH_3$	38(5)[f]	0.52		NC			$C_{34}H_{54}N_2O_3S$
20	2,6-(iPr)$_2$	H	$CH(CH_3)(CH_2)_{12}CH_3$	0.83		-61***	-76***	-26	-61***	$C_{28}H_{50}N_2O_3S$
21	2,6-(iPr)$_2$	H	$C(CH_3)_2(CH_2)_{12}CH_3$	0.682		-55***	-52***	-26*	-59**	$C_{29}H_{52}N_2O_3S$
22	2,4,6-(OMe)$_3$	H	$C(CH_3)_2(CH_2)_{12}CH_3$	8.1		-18	-76***	-13		$C_{26}H_{46}N_2O_3S$
23	2,6-(iPr)$_2$	H	$CH(Ph)(CH_2)_{12}CH_3$	2.3	2.0	-69***	-72***	NC	-62**	$C_{33}H_{52}N_2O_3S$
24	2,6-(iPr)$_2$	H	$(CH_2)_{15}CH_3$	1.27	1.35	-58***	-64***		-10	$C_{29}H_{52}N_2O_3S$
25	2,6-(iPr)$_2$	H	$CH(CH_3)(CH_2)_{15}CH_3$	1.39		-58***	-76***		-36**	$C_{31}H_{56}N_2O_3S$
26	2,4,6-(OMe)$_3$	H	$CH(CH_3)(CH_2)_{15}CH_3$	0.085		-12*	-53***	-18		$C_{28}H_{50}N_2O_3S$

*Significantly different from control using the unpaired, two-tailed t-test. *$p<0.05$, **$p<0.01$, ***$p<0.001$. [a] ACAT inhibition in vitro measured in rat liver microsomes. See ref. 5 for details. [b] ACAT inhibition in vitro measured in rabbit intestinal microsomes. See ref. 5 for details. [c] Denotes percent change in total cholesterol in the acute cholesterol-fed rat model of hypercholesterolemia. See ref. 5 for details. [d] Denotes percent change in total cholesterol in the chronic cholesterol-fed rat model of hypercholesterolemia. See ref. 5 for details. [e] Analytical results are within 0.4% of the theoretical value unless otherwise noted. [f] Percent inhibition (conc. μM). [g] Found N: 5.07. NC = no change. Data for **1** is from ref. 5.

Results and Discussion: Based on results in previous ACAT inhibitor studies,[2] substitution in the aryl ring was limited to 2,6-diisopropyl, 2,4,6-trimethoxy and 2,4-difluoro. For compounds evaluated in both assays, when R_1=H, there was good correlation between inhibiton of ACAT derived from rat liver and rabbit intestinal microsomes. With the exception of compound **26**, the 2,6-diisopropyl-substituted analogs possessed best activity in vitro and in vivo; therefore, this moiety was retained in most subsequent analogs. In vitro potency in the straight-chain containing analogs (**8**, **11**-**15**, **24**) generally increased with increasing chain length; however, this was not necessarily correlated with improved hypocholesterolemic activity in vivo. Thus, **8** and **15**, differing twenty-fold in their in vitro activity, were essentially equipotent in vivo. Alkyl-substitution on the central nitrogen also produced inconsistent results. Thus, introduction of an *N*-methyl led to increases in potency in some cases (**13** vs. **12**), decreases in potency in others (**18** vs. **15**) and reductions in in vivo activity in all cases. The introduction of α-substitution in the alkyl chain (R_2, Table) produced better results and, in general, these analogs displayed improved potency in vitro and in vivo. This strategy produced a compound (**10**) which possessed potency and efficacy for lowering total cholesterol approaching that of **1**, even at the low dose of 3 mg/kg in the chronic rat assay. **10** also increased HDL-cholesterol as effectively as **1** (+476%). In fact, both compounds normalized lipids as effectively as switching the rats to a cholesterol free-diet in the second week. Even in the α-substituted series, however, anamolies occurred. Thus, trimethoxy-substituted analog **26** was twenty times more potent than **25** in vitro, but was significantly less efficacious in vivo. The poor relationship between ACAT inhibition in vitro and hypocholesterolemic activity in vivo in this series may be due to a variety of factors. In the case of **1**, since no activities other than ACAT inhibiton could be identified, it was postulated that its weak ACAT inhibition in vitro was offset by high aqueous solubility and enhanced bioavailability.[5] In the series described in this communication, where aqueous solubility is low, it is not clear whether pharmacokinetic factors can explain the discrepancies between in vitro and in vivo data. Nonetheless, by modification of **1**, we identified a series of moderately potent, highly efficacious ACAT inhibitors, which included a compound (**10**) which lowered TC and elevated HDL-C as effectively as **1** in a chronic rat model of hypercholesterolemia.

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