Inhibitors of Acyl-CoA:cholesterol O-Acyl Transferase (ACAT) as Hypocholesterolemic Agents. 6. The First Water-Soluble ACAT Inhibitor with Lipid-Regulating Activity

Drago R. Sliskovic,*,† Brian R. Krause,*
Joseph A. Picard,† Maureen Anderson,
Richard F. Bousley, Katherine L. Hamelehle,
Reynold Homan, Thomas N. Julian,‡
Zara A. Rashidbaigi,‡ and Richard L. Stanfield

Departments of Chemistry and Atherosclerosis Therapeutics, Parke-Davis Pharmaceutical Research, Division of Warner-Lambert Company, 2800 Plymouth Road, Ann Arbor, Michigan 48105, and Product Development, Morris Plains, New Jersey 07950

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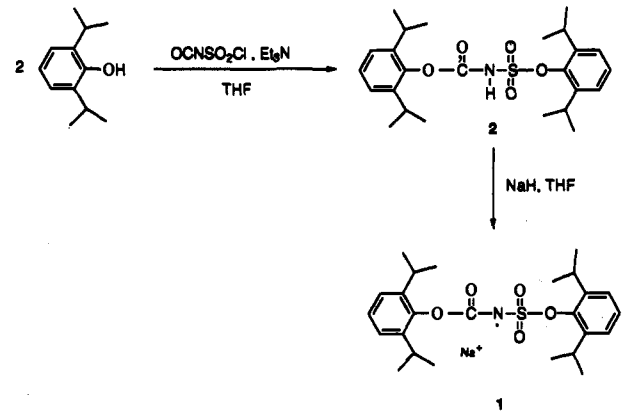
From our recent reviews of the literature,[1-3] it has become clear that inhibitors of acyl-CoA:cholesterol acyltransferase (ACAT) are uniformly lipophilic in nature, as exemplified by melinamide,[4] SaH 57-118,[5] CL 277,082,[6] PD 129337,[7] DuP 128,[8] RP 64477,[9a] and RP 70676.[9b] Although all of these compounds have been shown to inhibit cholesterol absorption in rodent species, most are poorly or erratically absorbed, especially in higher species, and thus may not reach the liver and arterial wall target enzymes.[9b,10,11] This feature may limit their therapeutic potential since liver ACAT is thought to determine the size, cholesteryl ester content, and atherogenicity of plasma LDL, at least in nonhuman primates[12] and to regulate the amount of apo B secreted from cultured human hepatoma cells.[13,14] Moreover, it is generally accepted that macrophage ACAT in the arterial wall is responsible for foam cell and fatty streak formation.[15] Thus, compounds with low systemic bioavailability would not possess direct antiatherosclerotic activity. This paper describes the synthesis of a novel, water-soluble inhibitor of ACAT, 2,6-bis(1-methylethyl)phenyl [[2,6-bis(1-methylethyl)phenoxy]-sulfonyl]carbamate, monosodium salt (1), which shows unexpected biological activity.

Our strategy to design ACAT inhibitors with improved bioavailability involved combining the features found in some of our most potent inhibitors with structural features of other classes of compounds which are known to be highly absorbed and bioavailable.

The sulfonylureas are one of the two main categories of oral hypoglycemic agents available. One of the striking features of this class of drug is the fact that they are completely absorbed after oral administration.[16] These compounds contain the sulfonylurea nucleus (SO_2-NHCONH) which results in all of these compounds having both measured and calculated LOGP values between 1.79 and 2.44.[17] This CLOGP range is considerably less than the one commonly encountered in the ACAT inhibitor field, i.e. 6-12.[18] It is known that many drugs with log P values between -2 and +4 are well absorbed and that drugs with log P values <0 or >3 are generally poorly or variably absorbed and have low aqueous solubility; however, drugs which can form water-soluble salts are often well absorbed, even with log P values outside the range previously discussed.[19] In addition, it is well-known that the hypoglycemic agents exhibit greater water solubility under basic conditions rather than at neutral pH. For example, the second-generation sulfonylurea, glyburide, is 40-fold more soluble at pH 9.53 (768 μg/mL) than at 7.39 (18.8 μg/mL).[20] This is an interesting observation, since the absorption of drugs occurs mainly in the small intestine, which tends to be more basic in nature.[21] Thus, the ability to form salts at basic pH's may lead to more water-soluble compounds. In order to procure some ACAT inhibition with these compounds, we planned to incorporate the 2,6-diisopropylphenyl moiety into our target compound, since it has previously been shown that introduction of this moiety into a wide variety of series has yielded potent ACAT inhibitors in vitro which were also effective hypocholesterolemic agents.[2-3]

Scheme 1



By combining modifications of these two structural features into one molecule, we hoped to design an ACAT inhibitor with a relatively low lipophilicity which, like the sulfonylureas, would be completely absorbed and thus exhibit improved bioavailability.

The synthesis of 1 is a two-step process utilizing commercially available starting materials (Scheme 1). Nucleophilic addition of 2 equiv of 2,6-diisopropylphenol to chlorosulfonyl isocyanate (CSI) and triethylamine at 0 °C in THF resulted in attack at both the carbonyl carbon and the sulfur of the sulfonyl chloride group of CSI to yield 2 in 62% yield. Treatment of 2 with NaH in THF at 0 °C gave the sodium salt, 1, in 91% yield.

The partition coefficient (log P) of 1, as measured by the shake flask method, was 2.98 at pH 7.4. The aqueous solubility of 1 at several pH values was determined. At pH values of 4 and 5 the solubility of 1 ranged from 20 to 150 μg/mL; however, at pH values between 6.87 and 9.85, the aqueous solubility was constant at 21 000 μg/mL. This combination of low lipophilicity and aqueous solubility is unique in the ACAT inhibitor field.

ACAT inhibition, by 1, was demonstrated using microsomes isolated from the livers of cholesterol-fed rats. In this system the IC_{50} values ranged from 3.5 to 7.6 μM (mean = 5.3 μM) in four separate assays using four separate drug concentrations in each assay. For comparison, the IC_{50} values for DuP-128 and CL 277,082 were 0.018 and 0.47 μM, respectively.[22] Similar ACAT inhibitory activities by 1 and DuP 128 were also found using microsomes isolated from the intestinal mucosa of cholesterol-fed rabbits,[23] but CL 277,082 was more potent in this system (0.2 μM).[22] To demonstrate activity in intact cells, 1 was added to CaCo-2 cells incubated with radiolabeled oleic

† Department of Chemistry.
‡ Product Development.

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Table 1. Prevention of Acute Hypercholesterolemia by 1 in Rats

dose (mg/kg)	1	DuP-128	CL 277,082
0.3	142 ± 10[a] (-26%)		
1	98 ± 8[a,b] (-49%)	158 ± 23 (-17%)	
3	78 ± 7[a,b] (-59%)	122 ± 3[a] (-36%)	164 ± 14 (-14%)
10	56 ± 4[a-c] (-70%)	77 ± 8[a,b] (-60%)	142 ± 25[a] (-26%)
30	50 ± 5[a-c] (-74%)	74 ± 10[a] (-61%)	101 ± 13[a] (-47%)
100		79 ± 11[a] (-59%)	78 ± 9[a] (-59%)
ED_{50} (mg/kg)[d]	1.8	15.2	48.0

Values are the mean ± SEM (n = 5/group) with percent changes from PCC (191 ± 18) controls in parentheses. [a] Significantly less than PCC controls (191 ± 18), $p < 0.05$. [b] Less than CL 277,082 at same dose. [c] Less than chow controls (70 ± 4). [d] Dose required to lower plasma cholesterol by 50%.

acid.[24] In this established in vitro model of intestinal cell function,[25] the IC_{50} value averaged 0.93 μM in three separate experiments (IC_{50} for CL 277,082 was 1.3 μM). Compound 1 did not affect the incorporation of oleic acid into triglycerides or phospholipid in this experiment at concentrations up to 100 μM, making it unlikely that it inhibited the acyltransferases involved in the synthesis of these lipids (e.g. acyl-CoA:monoglyceride acyltransferase, MGAT). In separate experiments 1 also had no inhibitory effect on lecithin:cholesterol acyltransferase (LCAT), acyl-CoA:retinol acyltransferase (ARAT), carnitine acyltransferase (CAT), pancreatic cholesterol ester hydrolase (p-CEH), hepatic lipase, or HMG-CoA reductase.

To determine the biological activity of 1, we used three separate approaches. First, we demonstrated that 1 inhibits the absorption of cholesterol by using a rat lymph-fistula model.[22] The lymphatic transport (mg/h) of ACAT-derived cholesteryl esters was inhibited 50–60% after a single dose of 10 mg/kg in rats during active lipid absorption. The effect lasted up to 10 h. As expected from the in vitro data, there was no effect on the lymphatic transport of triglyceride. At this dose, CL 277,082 had no effect on cholesteryl ester transport.[22] Secondly, we tested for acute efficacy by administering a single dose to rats, followed by overnight ad libitum feeding of a normal, chow diet supplemented with peanut oil (5.5%), cholesterol (1.5%), and cholic acid (0.5%) (PCC diet).[26] Table 1 demonstrates that 1 was more potent than the lipophilic reference agents at preventing acute, diet-induced hypercholesterolemia in rats. The ED_{50} values obtained for DuP-128 and CL 277,082 were 8-fold and 27-fold higher than that for 1 using this acute model. In addition, only 1 lowered plasma cholesterol to levels below those found in chow-fed controls. In the third type of experiment the PCC diet was fed chronically for 2 weeks with oral drug dosing during the second week as previously described (Table 2).[22] Under these conditions, hypercholesterolemia is associated with not only marked increases in nonHDL cholesterol (nonHDL-C) but also decreases in HDL-C, as determined by dextran sulfate precipitation of whole plasma.[22,23] Compound 1 potently lowered total and nonHDL-C (ED_{50} = 1.1 mg/kg) and also dose-dependently elevated HDL-C (by approximately 126% at 10 mg/kg). In this animal model DuP-128 and CL 277,082 both had ED_{50}'s (16.5 and 16.4 mg/kg, respectively) for lowering nonHDL-C that were almost 15-fold higher than 1. In this experiment, and in previous experiments,[22] CL 277,-082 failed to elevate HDL-C at any dose below 100 mg/kg.

In addition, when 1 was dosed to chow-fed hamsters (at doses between 10 and 60 mg/kg) or chow-fed dogs (at up to 300 mg/kg), no change in plasma glucose level was observed.

In summary, a novel, water-soluble inhibitor of ACAT has been identified which thus far possesses selectivity toward ACAT as opposed to other acylating enzymes or enzymes involved in lipoprotein metabolism. Despite relatively weak in vitro activity, this compound shows excellent lipid-regulating activity in vivo compared to lipophilic reference agents. Cholesterol absorption inhibition has been identified as one mechanism for this efficacy. Our current working hypothesis is that this water-soluble compound is well absorbed and therefore also inhibits liver ACAT in the intact animal. In fact, preliminary data indicate that after a single dose (50 mg/kg) in chow-fed rats, a plasma concentration of 17 μg/mL (34 μM, 7 times the IC_{50}) is attained 3 h postdose. Thus, the observed efficacy could be due in part to the decreased secretion of cholesteryl ester–rich lipoproteins by the livers of our cholesterol-fed animals.[12] Although further work is required to support this hypothesis, supportive data is accumulating such as (1) reduction of plasma cholesterol

Table 2. Chronic Effect of 1 on Preestablished Dyslipidemia in Rats

		mg/dL ± SEM (% change)		
treatment group	dose (mg/kg)	total cholesterol	nonHDL-C	HDL-C
PCC controls		255 ± 40	242 ± 41	13 ± 2
chow controls		72 ± 6 (-72)[a]	31 ± 3 (-87)[a]	41 ± 3 (+213)[a]
1	0.3	204 ± 14 (-20)	192 ± 15 (-20)	11 ± 1 (-14)
1	1	127 ± 15 (-50)[a,b]	102 ± 16 (-58)[a,b]	25 ± 3 (+88)[a,b]
1	3	92 ± 2 (-64)[a,c]	53 ± 4 (-78)[a,c]	39 ± 3 (+192)[a,c,d]
1	10	111 ± 8 (-57)[a,c]	59 ± 6 (-76)[a,c]	52 ± 3 (+290)[a,c,d]
1	30	91 ± 7 (-64)[a,d]	47 ± 5 (-81)[a,c]	44 ± 3 (+233)[a,c,d]
DuP-128	1	244 ± 24 (-4)	234 ± 25 (-3)	10 ± 1 (-25)
DuP-128	3	217 ± 15 (-15)	203 ± 15 (-16)	14 ± 1 (+4)
DuP-128	10	154 ± 14 (-40)[a]	135 ± 15 (-44)[a]	18 ± 1 (+38)[a]
DuP-128	30	120 ± 14 (-53)[a]	98 ± 16 (-59)[a]	21 ± 2 (+61)[a]
DuP-128	100	119 ± 10 (-54)[a]	87 ± 11 (-64)[a]	32 ± 3 (+140)[a]
CL277,082	3	172 ± 25 (-33)	157 ± 27 (-35)	14 ± 2 (+8)
CL277,082	10	178 ± 17 (-30)	167 ± 18 (-31)	11 ± 1 (-14)[b]
CL277,082	30	106 ± 13 (-58)[a]	92 ± 13 (-62)[a]	15 ± 1 (+11)[b]
CL277,082	100	82 ± 4 (-68)[a,b]	63 ± 4 (-74)[a]	19 ± 1 (+42)[a,b]

(n = 5/group) with percent changes from PCC controls in parentheses. [a] Significantly different from PCC controls, $p < 0.05$. [b] Significantly different from DuP-128 at same dose, $p < 0.05$. [c] Significantly different from DuP-128 and CL277,082 at same dose, $p < 0.05$. [d] Not different from chow controls.

and liver cholesteryl esters in rats or rabbits fed cholesterol-free diets, (2) prevention of cholesteryl ester deposition in the livers of chow-fed rats treated with ethinylestradiol, a model previously described,[22] and (3) activity with parenteral dosing. These and other data will be presented separately to further address questions relating to the site of action of this water-soluble ACAT inhibitor.

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Supplementary Material Available: Full experimental details (2 pages). Ordering information is given on any current masthead page.

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