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RP 70676: A POTENT SYSTEMICALLY AVAILABLE INHIBITOR OF ACYL-CoA:CHOLESTEROL O-ACYL TRANSFERASE (ACAT)

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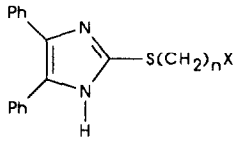
Abstract: RP 70676 (**3d**) is a potent inhibitor of ACAT. It is an effective hypocholesterolaemic agent in the cholesterol-fed rabbit, and reduces the accumulation of both cholesterol and cholesterol ester in rabbit aorta and thoracic artery. The compound is readily bioavailable in rabbits with significant levels of parent compound present in plasma up to 6 hours after an oral dose.

The intracellular accumulation of cholesterol esters in the artery is a marked feature of the atherosclerotic plaque. Cholesterol accumulation mediated by ACAT is thought to contribute both to the stasis of macrophages in the artery and to the formation of foam cell lesions which, following the development of more complex lesions, lead to the eventual occlusion of arteries such as the coronary artery. The inhibition of ACAT may be of value in preventing the accumulation of cholesterol ester in the artery and promoting reverse cholesterol transport, thus arresting the progression or initiating the regression of atherosclerosis.[1]

The esterification of dietary cholesterol in the intestine appears to play a pivotal role in chylomicron assembly and subsequent secretion of lipid into the lymphatic system.[2] ACAT is also present in the liver, where it is responsible for the formation of cholesterol esters prior to their inclusion into very low density lipoproteins (VLDL). It is not clear, however, whether or not this process is limiting on the formation of VLDL and thus if inhibition of hepatic ACAT would reduce the release of the lipoprotein into the circulation with a consequent hypolipidaemic effect. [2]

A systemically available ACAT inhibitor may, therefore, have a beneficial therapeutic effect on atherosclerosis by acting in the gut, liver, and artery, and a number of groups have targeted ACAT as a potential site of intervention in the atherosclerotic disease process. Potent ACAT inhibitors have been described which exert a hypocholesterolaemic effect in cholesterol fed animals by the inhibition of cholesterol absorption from the intestine.[3] As a result of their poor water solubility and highly lipophilic nature these compounds would be expected to have (very) low systemic bioavailability.

It has been demonstrated that 2-(alkylthio)-4,5-diphenyl-1*H*-imidazoles (**1**) are potent inhibitors of ACAT.[4,5] In this letter we describe our efforts to modify this class of compound to give potent, water soluble and moderately lipophilic ACAT inhibitors with good systemic bioavailability. In order to introduce a degree of water solubility into compounds of general structure **1** a series of analogues was synthesised in which the "X" group was a (substituted)pyrazolyl or -imidazolyl moiety. The syntheses of these compounds are shown in the scheme.[6]



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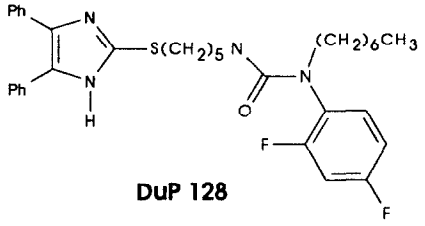
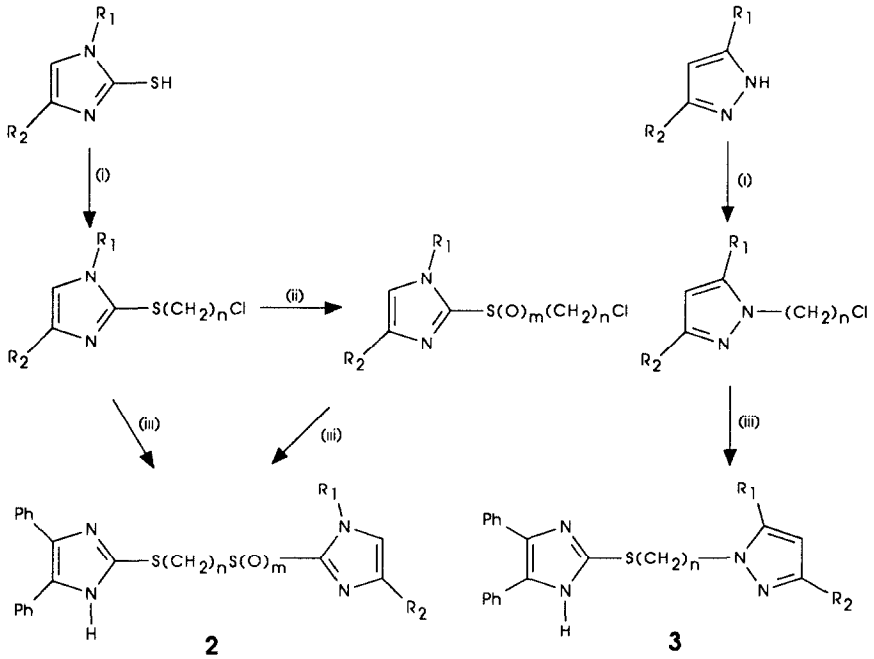


DuP 128

Scheme



Reagents: (i) KO-*t*-Bu / DMF; Br-$(CH_2)_n$-Cl. (ii) m-CPBA / CH_2Cl_2. (iii) 4,5-diphenyl-1*H*-imidazole-2-thiol / KO-*t*-Bu / DMF

Compounds **2a-d, 3a-d** were very potent inhibitors of ACAT derived from rat liver microsomes (IC_{50} < 50 nM), but significant enzyme selectivity was observed when the compounds were examined for their inhibitory effect on ACAT obtained from rabbit liver microsomal preparations (Table 1).[7] Subtle structural modifications led to marked differences in the inhibitory potency of these compounds for rat and rabbit hepatic ACAT. All of the compounds were significantly less lipophilic in nature than **DuP 128**, an ACAT inhibitor currently under development by Du Pont Merck,[9] as shown by their calculated partition coefficients[10] (ClogP - Table 1). The 3,5-dimethylpyrazole compound, RP 70676 (**3d**), was the only compound with an IC_{50} of less than 50 nM in both assays, and was selected for further investigation.

Table 1-Inhibition of hepatic microsomal ACAT and ClogP values

No.	m	n	R_1	R_2	ClogP	Inhibition of ACAT IC_{50} nM	
						Rat	Rabbit
2a	0	3	CH_3	H	6.7[11]	14	970
2b	2	3	CH_3	H	4.3[12]	11	8000
2c	0	4	CH_3	H	7.2	38	180
2d	0	5	H	H	7.7	44	880
3a	-	3	H	H	6.3	50	>1000
3b	-	5	H	H	7.4	24	280
3c	-	6	H	H	7.9	48	590
3d	-	5	CH_3	CH_3	7.9[13]	25	44
DuP 128					10.4	10[14]	

RP 70676 was a potent inhibitor of rabbit arterial ACAT (IC_{50} = 40 nM) and has been shown to be an effective inhibitor of ACAT derived from a number of tissues and species including man (Figure 1). The IC_{50} values ranged from 21 nM for hamster liver ACAT to 108 nM for enzyme from the intestine of cholesterol fed rabbits; in human hepatic tissues the mean IC_{50} was 44 nM. In whole cell $P388D_1$ murine macrophages the compound had an IC_{50} of 540 nM. RP 70676 was a mixed inhibitor of rat hepatic microsomal ACAT with respect to oleoyl Co-A with a marked competitive component (Figure 2).

Figure 1 - Inhibition of ACAT from various tissues by RP 70676

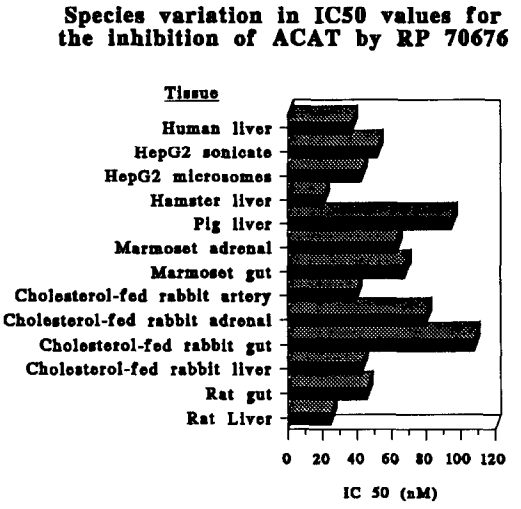
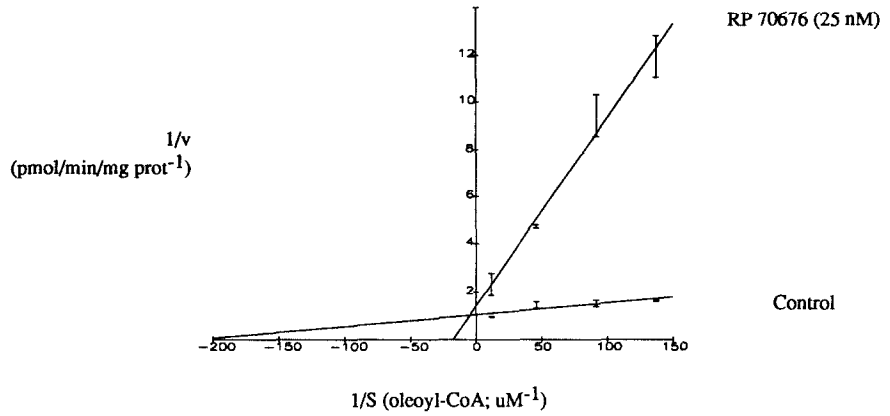


Figure 2 - Inhibition of ACAT by RP 70676 in rat hepatic microsomes
Lineweaver-Burk Plot



In cholesterol fed New Zealand White (NZW) rabbits the compound reduced plasma cholesterol levels and also reduced the concentrations of both arterial cholesterol and cholesterol ester (Table 2). A similar reduction in arterial and plasma cholesterol was obtained by reducing the dietary cholesterol intake, suggesting that the effect of RP 70676 on arterial cholesterol levels in this test was secondary to its hypolipidaemic effect, rather than a direct effect on arterial ACAT.

Table 2-Reduction of plasma and tissue cholesterol concentrations in NZW rabbits by RP 70676[15]

		Control (0.5% chol. diet)	RP 70676 1 mg/kg bid	% Change from control	0.25% Chol. diet	%Change from control
Plasma Cholesterol (AUC; mM.days)		1438 (88)	857 (108)	-40***	776 (43)	-46***
Thoracic Aorta	FC[a]	4.4 (0.5)	2.8 (0.4)	-36*	2.5 (0.2)	-44**
(mg/g)	CE[a]	1.9 (0.2)	1.1 (0.2)	-42*	1.1 (0.2)	-40**
Abdominal Aorta	FC[a]	4.6 (0.5)	3.4 (0.4)	-27*	3.0 (0.3)	-36**
(mg/g)	CE[a]	2.8 (0.4)	1.7 (0.4)	-40*	1.5 (0.2)	-46**

a: FC = free cholesterol, CE = cholesterol esters; * $p<0.05$, ** $p<0.01$, *** $p<0.001$ (student's T-test)

In NZW rabbits RP 70676, given orally at a dose of 10 mg/kg as either the free base or the hydrochloride salt, was well absorbed with plasma levels of parent compound being of the order of 500 nM over a period of 6 hours post dose. This is more than ten times greater than the IC_{50} required for the inhibition of microsomal arterial ACAT and is approximately equivalent to the IC_{50} for the inhibition of cholesterol synthesis in macrophages.

In summary RP 70676 (**3d**) is a potent, systemically available ACAT inhibitor which demonstrates a hypocholesterolaemic effect in the NZW rabbit, as well as reducing arterial cholesterol concentrations. This compound could be of value both as a hypocholesterolaemic agent and as a direct disease modifying agent by preventing the progression or inducing the regression of atherosclerotic plaque.

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References and notes:

1. Bell, F.P.; *Pharmacological Control of Hyperlipidaemia*; Fears, R., Ed.; J.R.Prous: Barcelona, 1986; p 409

2. Suckling, K.E.; Stange, E.F.; *J. Lipid. Res.* **1985**, *26*, 647

3. Sliskovic, D.R.; White, A.D.; *Trends Pharm. Sci.* **1991**, *12*, 194

4. Coffee, E.C.J.; Eur. Pat. 391796 (10 October 1990)

5. Billheimer, J.T.; Gillies, P.J.; Wexler, R.R.; Higley, C.A.; Maduskuie, T.P.; Eur. Pat. 372445 (13 June 1990)

6. Typical experimental details: A stirred solution of 3,5-dimethylpyrazole (9.6 g, 100 mmol) in anhydrous DMF (100 mL) was cooled in an ice bath and treated with potassium *t*-butoxide (11.2 g, 100 mmol). The ice bath was removed and the mixture stirred for 1 hour. 1-Bromo-5-chloropentane (18.6 g, 100 mmol) was added and the mixture allowed to stir at room temperature overnight. A mixture of 4,5-diphenyl-1*H*-imidazole-2-thiol (25.2 g, 100 mmol) and potassium *t*-butoxide (11.2 g, 100 mmol) in anhydrous DMF (200 mL) was stirred at room temperature for 30 minutes and then treated with the previously prepared solution of 1-(5-chloropentyl)-3,5-dimethylpyrazole. After stirring at room temperature overnight the mixture was filtered and the filtrate evaporated to low bulk. The residue was partitioned between dichloromethane (400 mL) and water (200 mL), the layers separated, and the organic layer washed with water (200 mL), dried (magnesium sulphate) and evaporated. The residue was triturated with ether (250 mL), filtered, and the filtrate evaporated. This residue was purified by flash chromatography, eluting with a mixture of dichloromethane and ethyl acetate (1:1 by volume) to give a colourless gum. Trituration with pentane gave compound **3d** as a fine white powder, 16.9 g (41%); m.p. 75°C. Analysis:- C, 71.9; H, 6.78; N, 13.5; S, 8.0%; $C_{25}H_{28}N_4S$ requires:- C, 72.1; H, 6.73; N, 13.5; S, 7.69%. ^{1}H-nmr (CD_3SOCD_3): 1.37 (2H, m, $S(CH_2)_2CH_2(CH_2)_2$), 1.69 (4H, m,

$SCH_2CH_2CH_2CH_2CH_2$), 2.05 & 2.13 (6H, 2s, $(CH_3)_2$), 3.09 (2H, t, $SCH_2(CH_2)_4$, J = 8 Hz), 3.88 (2H, t, $S(CH_2)_4CH_2$, J = 8 Hz), 5.73 (1H, s, pyrazole-*H*), 7.15 - 7.50 (10H, m, $(C_6H_5)_2$).

7. The enzyme screen was based on a method described in the literature[8] with some modifications: assays were performed using 60µg of microsomal protein in a reaction volume of 200µL with a concentration of 90µM of oleoyl-CoA. Conversion of radiolabelled oleoyl-CoA to cholesterol ester was determined by scintillation counting after separation of the reaction products by TLC.

8. Lichtenstein, A.H.; Brecher, T.; *J. Biol. Chem.* **1980**, *255*, 19098

9. Billheimer, J.T.; Cromley, D.A.; Higley, C.A.; Wexler, R.R; Robinson, C.S.; Gillies, P.J.; poster presented at the 9th International Symposium on Atherosclerosis; Chicago, USA; 6-11 October **1991**

10. ClogP values were calculated using the MedChem software (Pomona College Medicinal Chemistry Project, Release 3.63); for a discussion on the relevance of partition coefficients to the absorption of drugs from the gut see: Dennis, M.J.; *Comprehensive Medicinal Chemistry*, Taylor, J.B., Ed.; Pergammon Press: Oxford, 1990; p 7f

11. Experimental logP = 5.2 (octanol/phosphate buffer, pH 7.5) (shake-flask)

12. Experimental logP = 3.9 (octanol/phosphate buffer, pH 7.5) (shake-flask)

13. Experimental logP = 5.65 (octanol/phosphate buffer, pH 7.5) (HPLC using an octanol coated silica stationary phase, and octanol saturated buffer as mobile phase).

14. Literature value (see reference 9)

15. New Zealand White rabbits (2.5 kg body weight) received bovine serum albumin (200 mg/kg i.v.) on day -4 and 0.5% cholesterol supplemented chow diet on day -5 and then for 7 weeks. Treatment of the animals with RP 70676 or diet was begun on day 0. The drug was dosed twice daily by gavage and the dietary treatment was designed to reduce plasma cholesterol levels by a degree similar to that achieved by drug treatment (this required 0.25% cholesterol in the diet). Aortic lipid was extracted into chloroform/methanol and free cholesterol and esterified cholesterol were separated using disposable silica Sep-pack columns. Sterol concentrations were determined using the Liebermann-Burchard method. Data presented are means (+/−SEM), n = 12 to 14 per group.