Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 5, No. 15, pp. 1581-1586, 1995

0960-894X/95 $9.50+0.00

0960-894X(95)00275-8

SYNTHESIS AND PHARMACOLOGICAL PROFILE OF FCE 28654: A WATER-SOLUBLE AND INJECTABLE ACAT INHIBITOR

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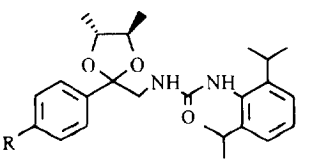
Abstract: The synthesis and the pharmacological evaluation of FCE 28654, a novel water-soluble ACAT inhibitor, are reported. The compound positively modifies blood and hepatic cholesterol levels when orally or intravenously administered to hypercholesterolemic animals.

The inhibition of the enzyme acylCoA: cholesterol acyltransferase (ACAT) is generally considered as one of the most appealing approaches to the treatment of dyslipidemias and to the prevention of the atherosclerotic process.[1-3] The ACAT inhibitors of the first generation showed generally marked hypolipidemic and, sometimes, antiatherosclerotic activities in the experimental models of exogenous hypercholesterolemia where the pharmacological treatment is associated to a simultaneous administration of a cholesterol enriched diet.[4-6] These compounds were characterized by high lipophilicity, low aqueous solubility and low bioavailability; by consequence their effects on blood and tissutal cholesterol levels were indirect and appeared almost exclusively related to a reduction of the intestinal cholesterol absorption. In these recent years this pharmacological feature has been partially revised, as further experimental data demonstrated that the therapeutical potential of an ACAT inhibitor can be markedly enhanced when the compound directly affects ACAT activity in target tissues such as the liver[7-9] or the arterial wall.[10] Therefore an aqueous solubility sufficient to achieve high systemic bioavailability is now considered a crucial requirement for an ACAT inhibitor to be developed as a hypolipidaemic as well as antiatherosclerotic agent.[11] During our program of synthesis and evaluation of new ACAT inhibitors we identified a series of disubstituted ureas structurally characterized by the presence of a cyclic ketal or thioketal ring[12] and endowed with good *in vitro* and *in vivo* ACAT inhibitory activity. Among them compound **1** showed to be one of the most active when tested either in *in vitro* or *in vivo* studies.[12] This ACAT inhibitor however resulted completely water-insoluble and poorly bioavailable. Thus, different chemical strategies were attempted with the aim of synthesizing more hydrosoluble pharmacologically active analogues. The hydroxy derivative **2** was designed as a possible metabolite of **1** endowed with slightly higher hydrophilicity and as a potential parent compound of water-soluble pro-drugs.



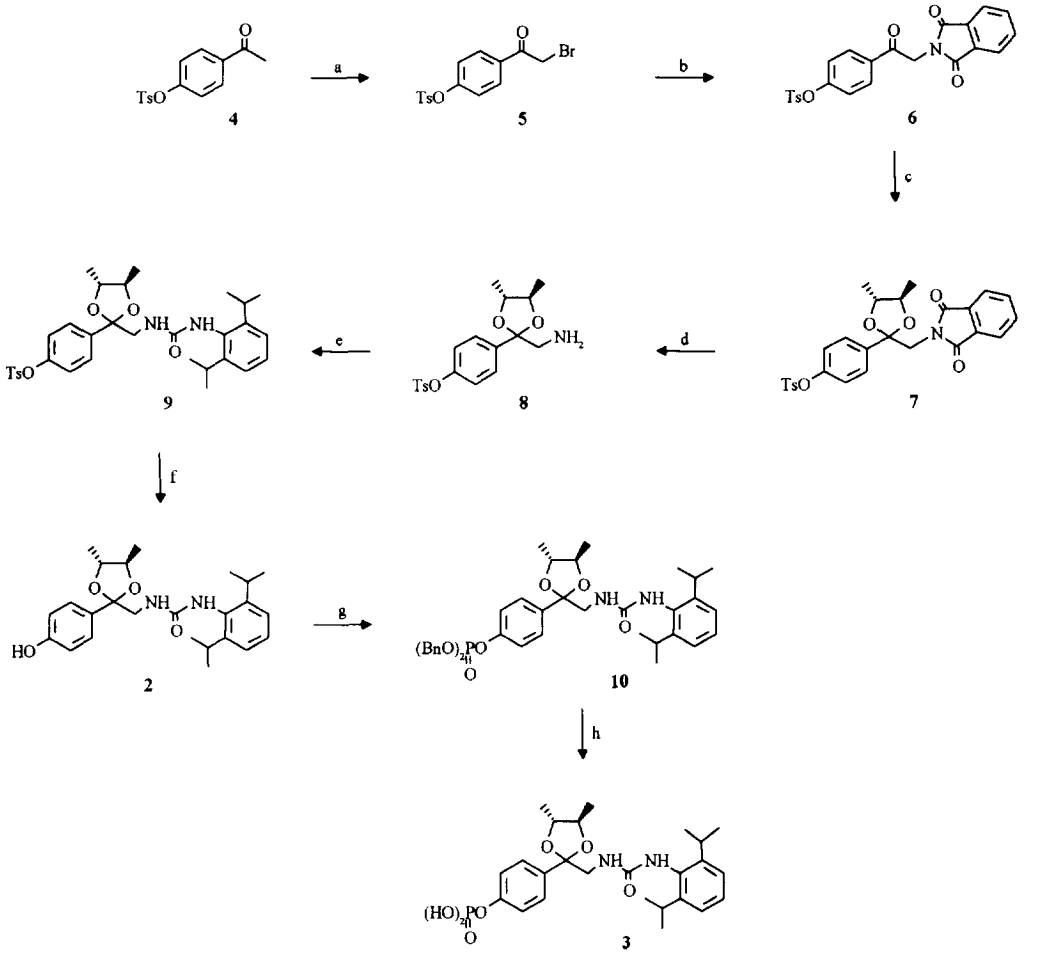
1 R = H

2 R = OH

3 R = $OPO(OH)_2$

In this paper we describe the synthesis and the pharmacological profile of the phosphate derivative **3**: 4-{(4R,5R)-2-[3-(2,6-diisopropylphenyl)ureidomethyl]-4,5-dimethyl-1,3-dioxolan-2-yl}phenylphosphate (internal code FCE 28654).

Scheme 1



FCE 28654

(a) Br_2, CH_2Cl_2, 22°C, 0.5h, 95%. (b) potassium phthalimide, DMF, 22°C, 16h, 90%. (c) (2R,3R)-(-)-2,3-butanediol, triflic acid, trimethyl orthoformate, CH_3NO_2, 100°C, 1.5h, 95%. (d) hydrazine hydrate, EtOH, 1.5h, 70°C, 90%. (e) 2,6-diisopropyl-phenylisocyanate, AcOEt, 22°C, 2h, 90%. (f) NaOH, H_2O, PEG-400, 100°C, 0.5h, 78%. (g) tBuOK, DMF, dibenzyl pyrophosphate, 0-22°C, 0.5h, 65%. (h) H_2, 10% Pd-C, EtOH, 2 atm, 15°C, 98%.
Satisfactory analytical and spectral data were obtained for new compounds[13].

Chemistry

Compounds **2** and **3** were prepared by the route described in scheme 1. The key intermediate α-aminoketal **8** was obtained in four steps from the known substituted acetophenone **4**[14] using a slight modification of the method reported by Adachi et al.[15]. Sequential coupling of **8** with 2,6-diisopropyl-phenylisocyanate using standard means to give the disubstituted urea **9**, followed by hydrolysis with NaOH in H_2O/PEG-400, gave the hydroxy compound **2**. Treatment of the anion of **2** with dibenzyl pyrophosphate in DMF afforded the diester **10**, which upon hydrogenolysis was finally converted to the target compound **3**. Subsequently this acid was converted to its corresponding monosodium salt by treatment with 1 eq. of NaOAc in ethyl alcohol. Compound **1** was obtained in 5 steps from acetophenone by an analogous procedure.

Biological results

Compound **3** showed a good water solubility (8500 µg/ml were dissolved into a pH 7.4 PBS buffer) and was readily cleaved to give the hydroxy derivative **2** (>90% in 45 min. at 37°C) in the presence of alkaline phosphatase preparations from bovine liver (79395 - Fluka) at pH 7.4, fulfilling one of the requirements as prodrug. When assayed *in vitro*[16] **3** weakly inhibited microsomal ACAT in each tissue tested and resulted significantly less potent than either **2** or **1** (Table 1).

Table 1 ACAT inhibitory activity of compounds **1**, **2**, and **3** in microsomes from different tissues.

compound	IC_{50}[a] (nM) (rabbit aorta)	IC_{50}[b] (nM) (rabbit intestine)	IC_{50}[c] (nM) (monkey liver)
1	24.2	27.0	63.0
2	119	26.6	332
3	2550	1080	5690

a) ACAT inhibitory activity in microsomes from cholesterol-fed rabbit thoracic aorta.
b) ACAT inhibitory activity in microsomes from cholesterol-fed rabbit intestinal mucosa.
c) ACAT inhibitory activity in microsomes from monkey liver.

In hypercholesterolemic rats[18] a single oral administration of **3** at the dose of 15mg/kg significantly reduced blood cholesterol and favourably modified the hepatic cholesterol pattern by enhancing the free/esterified cholesterol ratio (Table 2). In the same model **2** and **1** gave similar results.

Pharmacological effects comparable to those evidenced after an oral treatment were obtained intravenously administering **3** to rats or mice made hypercholesterolemic by diet[19]: a significant reduction of blood cholesterol and a modest enhancement of the hepatic free/esterified cholesterol ratio were obtained in the rat at the dose of 2mg/Kg (Table 3); similar results were produced in the mouse at the dose of 10mg/Kg (Table 3).

Table 2 Effects of a single oral administration (15mg/Kg) of compounds **1**,**2** and **3** on plasma and liver lipids of hypercholesterolemic rats.

Treatment	Plasma lipids (mg/dl)[a]				Hepatic lipids FC/CE ratio[a]
	FC	CE	TG	PL	
Control	51±15	200±58	114±25	155±27	0.19±0.03
1	18±8**	57±29**	76±36	77±19**	0.27±0.11
2	33±5**	75±13**	126±36	96±6**	0.35±0.06**
3	28±8**	73±14**	98±31	84±11**	0.38±0.08**

a) Values are mean ± SD * $p < 0.05$, ** $p < 0.01$ (Dunnett's test).
FC = free cholesterol, CE = cholesterol esters, TG = triglycerides, PL = phospholipids.

Table 3 Effects of a single intravenous administration of compound **3** on plasma and liver lipids of hypercholesterolemic rats and mice.

Treatment	Plasma lipids (mg/dl)[a]				Hepatic lipids FC/CE ratio[a]
	FC	CE	TG	PL	
Control rats	65±18	226±51	109±35	178±30	0.169±0.016
3 (2 mg/Kg)	39±10*	134±36**	126±34	138±19*	0.188±0.020
Control mice	34±13	105±17	80±25	183±42	0.270±0.031
3 (10 mg/Kg)	26±4	79±8**	71±25	142±11	0.316±0.082

a) Values are mean ±SD * $p < 0.05$, ** $p < 0.01$ (Dunnett's test).
FC = free cholesterol, CE = cholesterol esters, TG = triglycerides, PL = phospholipids.

A preliminary evaluation of the oral bioavailability of **3** was carried out by *in vitro* testing the ACAT activity[21] when plasma from rats orally treated at the dose of 100 mg/kg (about 7-fold higher than the dose required to induce the lipid lowering effect in the hypercholesterolemic diet model) was added into the assay system. The results reported in table 4 show that compound **3** treated rat plasma significantly reduced microsomal ACAT activity, while none inhibitory effect could be detected when plasma from compound **1** treated animals was used.

Table 4 Microsomal ACAT inhibition in the presence of plasma from rats orally treated with compounds **1** and **3**.

Treatment	Dose (mg/Kg)	Samples	ACAT activity[a]
Control	-	6	287 ± 38
1	100	3	286 ± 58
3	100	3	148 ± 46*

a) pmoles ^{14}C-Cholesteryl oleate formed / mg protein / min incubation.
* $p < 0.01$ (Dunnett's test)

In summary compound **3**, despite its weak *in vitro* inhibitory potency, markedly improves its *in vivo* profile of activity and in plasma from orally treated animals a significant residual ACAT inhibitory effect can be detected. These results are consistent with the idea that the increased water-solubility of the compound strongly enhances its oral bioavailability. The lack of significant differences among the activities of **3** and its water-insoluble analogues after a single oral administration to hypercholesterolemic rats may be related to the fact that in the adopted *in vivo* model intestinal ACAT still plays a significant role in modulating cholesterolemia; thus, more appropriate *in vivo* tests have probably to be chosen for further comparisons. Finally, the compound, when intravenously administered even at lower doses, maintains the hypolipidaemic activity shown after the oral treatment. As the intravenous route of administration doesn't involve the intestine in the determination of the pharmacological effect, FCE 28654 (**3**) may represent a useful tool to unambigously evaluate the therapeutic potential of an ACAT inhibitor circulating in sufficient amounts to directly affect hepatic and arterial ACAT activities.

Acknowledgement: The authors thank Mrs. M. Rei Rosa for her valuable contribution to the typing of this manuscript.

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13. Selected analytical data for compounds **1**, **2** and **3**:
compound **1** : mp 200-202°C; $[\alpha]^{23}_D$ -18.6 (c = 0.787, AcOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ : 1.0-1.4 (18H, m), 3.23 (2H, m), 3.40 (3H, m), 3.54 (1H, dd, J=6.2Hz, J=14.1Hz), 4.5 (1H, bs), 5.6 (1H, bs), 7.1-7.5 (8H, m),; FAB MS : 411 (100, $[M+H]^+$), 136 (67);
compound **2** : $[\alpha]^{23}_D$ -12.3 (c = 0.909, MeOH); ^{1}H NMR (200 MHz, $CDCl_3$) δ : 1.0-1.04 (18H, m), 3.09 (2H, m), 3.3-3.5 (3H, m), 3.81 (1H, m), 6.1 (1H, bs), 7.0-7.3 (7H, m), 7.5 (1H, bs); FAB MS : 427 (79.5, $[M+H]^+$), 333 (6.9), 193 (72.7), 152 (100);
compound **3** (FCE 28654A) : mp 142-144°C; $[\alpha]^{23}_D$ -13.2 (c = 0.980, MeOH); ^{1}H NMR (400 MHz, DMSO) δ : 1.0-1.04 (18H, m), 3.09 (2H, m), 3.3-3.5 (3H, m), 3.81 (1H, m), 6.1 (1H, bs), 7.0-7.3 (7H, m), 7.5 (1H, bs); FAB MS : 551 (100, $[M+Na]^+$), 529 (52.3, $[M+H]^+$), 449 (49.1).
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16. *In vitro* ACAT assay was carried out according to a method described in the literature[17] with slight modifications. Briefly, 120-200µg of microsomal proteins (suspended in 345µl of phosphate buffer at pH7.4) were incubated at 37°C in the presence of the test compound vehicled by 25µl of dimethyl sulfoxide (DMSO). Control samples contained the same volume of DMSO alone. The enzymatic reaction was started by the addiction of 10µl of ^{14}C-OleolylCoA and was stopped by methanol 10 minutes later. The formation of radiolabelled cholesteryl esters was evaluated by scintillation counting after separation of the reaction products by TLC. ACAT activity was expressed as pmoles ^{14}C-Cholesteryl oleate formed/mg microsomal protein/min incubation.
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18. Male rats (mean weight 235 g) received a 1.5% cholesterol - 0.5% cholic acid diet for 5 days. At the end of this period compounds **1**, **2** and **3** were orally administered suspended in Methocel-Tween. Six hours after dosing rats were sacrified in a fed condition. Blood and hepatic (after extraction into chloroform/methanol according to the method of Folch[17]) lipids were dosed by enzymatic methods. Data presented are means (± SD), n = 7 per group.
19. Male rats (mean weight 300 g) and male mice (mean weight 40 g) were treated with a 1.5% cholesterol - 0.5% cholic acid diet for 5 or 10 days respectively. Compound **3**, dissolved in sterile PBS at pH 7.4, was intravenously administered through the tail vein. Six hours after dosing fed animals were sacrified and blood and hepatic lipids were assayed following the same procedure of reference 15. Data presented are means (± SD), n = 7 per group.
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21. Male fasting rats (mean weight 200 g) were orally treated with **1** or **3** (both suspended in Methocell-Tween). Sixty minutes after dosing animals were sampled from the aorta and plasma samples recovered. ACAT activity was assayed as previously reported (see reference 13) adding in the incubation system aliquots of plasma from control or treated animals. Microsomes from rabbit intestinal mucosa were used as source of enzyme. Data presented are means (± SD).

(*Received in Belgium* 20 *March* 1995; *accepted* 2 *June* 1995)