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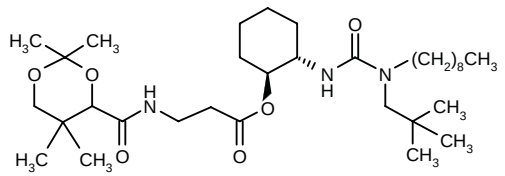
F-1394

Hypolipidemic
ACAT Inhibitor

N-(2,2,5,5-Tetramethyl-1,3-dioxan-4-ylcarbonyl)-β-alanine 2(*S*)-[*N*'-(2,2-dimethylpropyl)-*N*'-nonylureido]-1(*S*)-cyclohexyl ester



$C_{33}H_{61}N_3O_6$ Mol wt: 595.86

CAS: 135392-43-7

EN: 177583

Synthesis

By esterification of the acetonide of pantothenic acid (I) with (1*S*,2*S*)-2-[3-(2,2-dimethylpropyl)-3-nonylureido]-cyclohexanol (II) by means of dimethylaminopyridine (DMAP) and tosyl chloride in ethyl acetate (1) or DMAP and dicyclohexylcarbodiimide (DCC) in refluxing toluene (2). Scheme 1.

The starting compounds (I) and (II) have been prepared as follows:

1) The cyclization of pantothenic acid calcium salt (III) with 2,2-dimethoxypropane (IV) by means of oxalic acid and *p*-toluenesulfonic acid in refluxing acetones gives the acetonide (I) (1).

2) The condensation of the carbamate (V) with the secondary amine (VI) by heating at 120 °C yields the urea derivative (II) (1).

Description

Crystals, m.p. 77.1-79.4 °C (1).

Introduction

Several drug strategies have been investigated to facilitate reduction in plasma lipoproteins affecting intestinal processes. These include bile acid sequestrants, derivatized carbohydrates, saponins and compounds that target ACAT and cholesterol esterase enzymes.

Acyl-CoA:cholesterol *O*-acyltransferase (ACAT, EC 2.3.1.26) is the enzyme responsible for catalyzing the intracellular esterification of free cholesterol with fatty acyl-CoA to produce cholesteryl esters. This enzyme is known to play an important role in the absorption of dietary cholesterol, the secretion of hepatic VLDL and the accumulation of cholesteryl esters in arterial lesions. ACAT has been selected as a drug target to reduce the absorption of cholesterol, lower lipid levels and arrest progression and promote regression of atherosclerotic plaques.

In recent years intensive research efforts of many companies have focused on the design and synthesis of ACAT inhibitors. However, the hypocholesterolemic effects observed in human trials and adrenal toxicity evident from experimental studies have proved disappointing. Thus, the search for new ACAT inhibitors actively continues. At present, two compounds are in clinical trials, while others are presented regularly in current literature, congresses and patents. Table I presents information on last year's patents involving ACAT inhibitors. The chemical structures of ACAT inhibitors under development in clinical trials and preclinical testing, according to Prous Science databases, are shown in Table II.

As part of a research program directed towards the development of ACAT inhibitors, scientists at Fujirebio synthesized a series of pantothenic acid derivatives, and through structure-activity relationship studies selected compound F-1394 for further evaluation (3, 4).

Pharmacological Actions

F-1394 is a potent selective inhibitor of ACAT found to reduce ACAT activity in rat liver microsomes, homogenates of rabbit small intestinal mucosa and J774 macrophage lysates (IC_{50} = 6.4 nM, 10.7 nM, and 32 nM, respectively). Kinetic studies demonstrated that F-1394 inhibition was competitive and more potent than other ACAT inhibitors or hypolipidemic agents with K_i values of 4.0 nM and 9.9 nM for ACAT from liver and small intestine, respectively. F-1394 had no effect on 3-hydroxy-3-methylglutaryl CoA reductase acyl-CoA synthetase or cholesterol esterase. When compared to lecithine:cholesterol acyltransferase (LCAT) originating from rat plasma,

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Scheme 1: Synthesis of F-1394

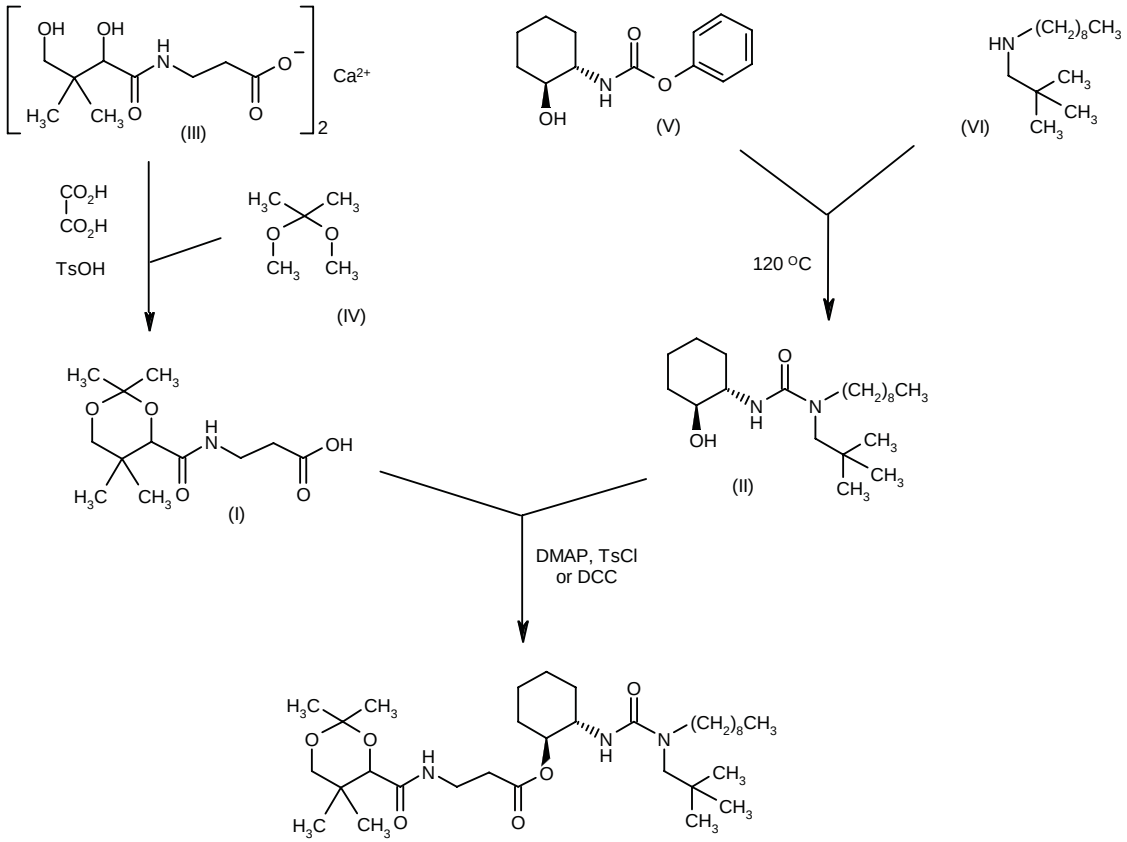


Table I: Recent patent literature on ACAT inhibitors.

Boehringer Mannheim	Pfizer
JP 96134053	US 5596001
DuPont Merck	Pierre Fabre
US 5491152	WO 9622279
US 5583147	WO 9719918
Fujisawa	Sankyo
WO 9610559	EP 763524
Grelan/Fournier	JP 96092222
WO 9634856	JP 96325218
Kitasato Institute	JP 97202775
JP 96056688	Sanwa
JP 96239385	JP 97301953
JP 96269062	JP 98095766
JP 96269063	Sumitomo
JP 96269064	WO 9638445
JP 96269066	Takeda
Kyoto	JP 96295667
WO 9712860	Warner-Lambert
Mitsubishi Chemical	US 5491170
JP 97194457	US 5510379
Nihon Nohyaku	WO 9744314
EP 761658	Yakult Honsha
Nippon Shoji	JP 96059461
EP 726247	JP 96059469
Nisshin Flour Milling	JP 96310949
JP 96041006	Yamanouchi
	WO 9802412

Source: Prous Science Ensemble database.

the ACAT inhibitory action of F-1394 was 4690-fold more potent and, in addition, F-1394 was found to weakly inhibit LCAT (5).

The hypocholesterolemic action of F-1394 was found to be rapid and due to inhibition of cholesterol absorption via the gut. Administration of 3-30 mg/kg F-1394 resulted in a 16-54% reduction in serum cholesterol levels 3 h postadministration in rats fed a 1% cholesterol diet. Moreover, 30 mg/kg F-1394 significantly reduced dietary cholesterol absorption via the gut as determined by the dual isotope ratio method; ACAT activity in the small intestinal mucosa was also significantly inhibited. The onset of F-1394 action was faster than that of DL-melinamide or CL-277082. When F-1394 was administered 1 or 2 h prior to or immediately following oral administration of a [^{14}C]-cholesterol tracer, approximately 90% of radioactivity did not appear in the circulation, indicating an immediate effect of the drug (6).

The inhibitory and hyperlipidemic actions of F-1394 on ACAT activity were also examined in HepG2 cells. Whole-cell ACAT activity was inhibited with an IC_{50} of 42 nM, a potency 5 times greater than that of other ACAT inhibitors such as YM-17E, CI-976, 57-118, CL-277082 and DL-melinamide. The hepatic secretion rate of cholesterol was also reduced in rats in which hyperlipidemia was induced by Triton WR-1339 (7).

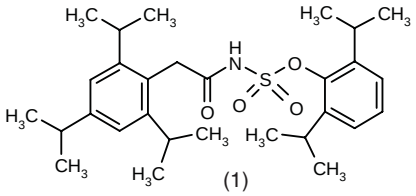
Table II: Chemical structures of ACAT inhibitors in clinical trials and preclinical testing.

Clinical

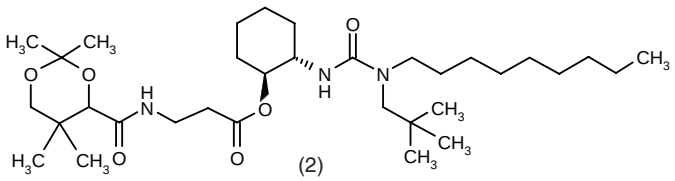
1. CI-1011
   Warner-Lambert
2. F-1394
   Fujirebio

Preclinical

3. F-12511[1]
   Pierre Fabre
4. FR-186054
   Fujisawa
5. KV-2920[1]
   Kyoto Pharmaceutical
6. KY-455[1,2]
   Kyoto Yakuhin
7. NTE-122
   Nisshin Food
8. R-755[1]
   Nihon Nokyaku
9. Epicochlioquinone A
   Sankyo
10. SKK-58099[1]
    Sanwa Kagaku
11. TS-962
    Taisho
12. YM-17E
    Yamanouchi



(1)



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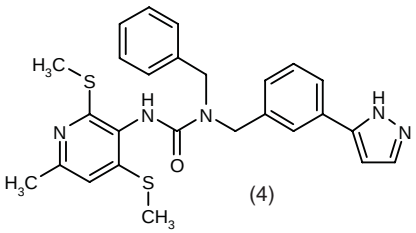
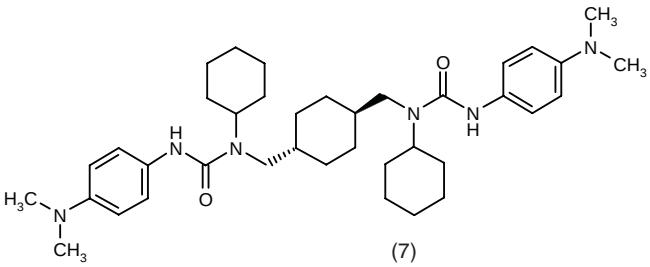
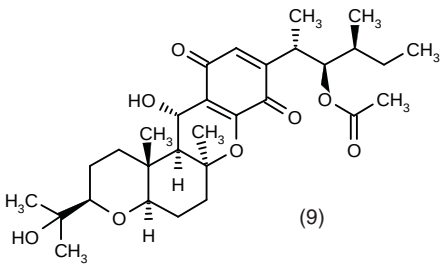


(4)



(7)



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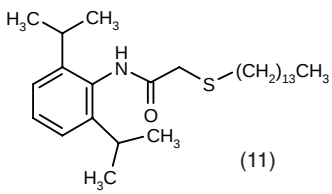

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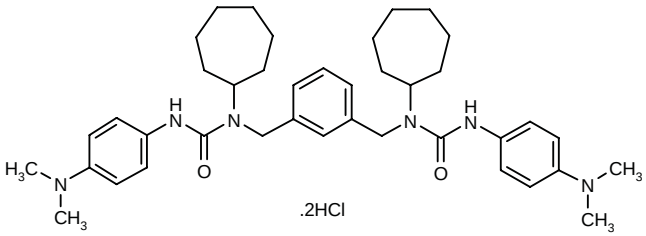

.2HCl

(12)

[1]Structure not yet detected; [2]Also lipid peroxidation inhibitor. Source: Prous Science Ensemble database.

Further studies have shown that increased ACAT activity in the gut can induce postprandial hyperlipidemia in rats. Thus, inhibition of ACAT activity by F-1394 administration may be a possible treatment for individuals with this condition. In the streptozotocin (STZ)-induced diabetic rat, small intestinal ACAT activity was 2-3 times higher than in normal rats in the absence of fat and hyperplasia in the gut; changes in total serum cholesterol (TC) and triglyceride (TG) levels were also greater in the postprandial state. When 3-30 mg/kg F-1394 was administered to STZ-diabetic rats, small intestinal ACAT activity was reduced and serum TG levels were inhibited. Moreover,

Table III: Biological activity of selected ACAT inhibitors according to the Prous Science MFline database.

Compound	ACAT inhibition (IC_{50} nM)	Material	Reference
EN: 207281	40	Rat liver microsomes	12
ACA-147 (Eldacimibe)	30	Rat liver microsomes	13
ACB-796	90	Human liver microsomes	14
	15	Monkey liver microsomes	14
	119	Hamster liver microsomes	14
	28	Rabbit liver microsomes	14
	20	Rat liver microsomes	14
CI-976	5800	Rabbit liver	15
PD-128042	930	Human Hep-G2 cells	7
	800	Human Hep-G2 cells	16
	300	Rat liver microsomes	16
	73	Rabbit intestinal microsomes	17
	16	Rabbit liver microsomes	16
CI-1011#	12000	Rabbit liver microsomes	18
	1000	Human Hep-G2 cells	19
CL-277082	8200	Human Hep-G2 cells	7
	3200	Human Hep-G2 cells	16
	2900	Rabbit liver microsomes	16
	33	Rabbit intestinal microsomes	20
	23	Rabbit liver microsomes	5
	56*	Rabbit liver microsomes	5
DuP-128 (Lecimibide)	10	Rat liver microsomes	21
E-5324	160	Rabbit liver microsomes	16
	110	Rat liver microsomes	16
	68	Human Hep-G2 cells	16
F-1394#	42	Human Hep-G2 cells	7
	6.4	Rat liver microsomes	5
	4.0*	Rat liver microsomes	5
F-12511	3.0-179	Human Hep-G2 cells	22
FR-186054	99	Rabbit intestinal microsomes	20
KY-455#,+	10-1000**	Rabbit tissues	23
NTE-122+	6.5	Human Hep-G2 cells	24
	1.1	Human Hep-G2 cells	25
	0.9*	Human Hep-G2 cells	25
RP-64477	503	Human Hep-G2 cells	26
	283	Pig liver microsomes	26
	194	Rat liver microsomes	26
	136	Marmoset liver microsomes	26
	42	Hamster liver microsomes	26
	20	Rabbit liver microsomes	26
RP-70676	108	Hamster liver microsomes	26
	44	Rabbit liver microsomes	27
	44	Human liver	27
	25	Rat liver microsomes	27
TS-962	4.0	Rabbit intestinal microsomes	28
	2.2	Rabbit liver microsomes	29
	1.7	Rabbit intestinal microsomes	29
YM-750	73	Rabbit liver	30
YM- 17E	80	Human Hep-G2 cells	7
	44	Rabbit liver	30
	45	Rabbit liver microsomes	31
	86*	Rabbit liver microsomes	31

*K_i nM; **Inhibitory concentration (nM); #Compound currently under clinical development; +Also peroxidation inhibitor (inhibitory concentrations: 0-1-10 µM, ref. 23; and 0.01-1µM, ref. 25, for KY-455 and NTE-122, respectively).

serum glucose was not affected and an 80% decrease in TG levels was observed with administration of 30 mg/kg of F-1394 (8).

Increased ACAT activity also contributes to the development of atherosclerosis and in this respect, F-1394 may possibly be an effective antiatherosclerotic drug. The effects of F-1394 were examined *in vivo* using rabbits fed a 0.5% cholesterol diet and administered F-1394 at a dose of 100 g/day for 3 months. F-1394 significantly decreased TC and aortal atherosclerotic involvement as compared to untreated rabbits. In addition, if F-1394 was administered in combination with probucol, a decrease in aortal cholesterol content was observed. Furthermore, F-1394 decreased cholesterol content in aortas with atherosclerotic lesions in rabbits fed a high-cholesterol diet (9).

F-1394 may also be used as a therapeutic agent of postprandial hypertriglyceridemia. Administration of graded doses of F-1394 (1-30 mg/kg/day) inhibited the elevation of TC in dogs fed a high-fat diet. Additionally, F-1394 at oral doses of 1, 3 or 10 mg/kg/day for 21 days reduced TC in a dose-dependent manner (ID_{50} = 7.2 ± 0.3 mg/kg/day). F-1394 at a dose of 10 mg/kg/day or greater also inhibited elevations in TG levels 3 h after ingestion of a high-fat diet, suggesting an inhibitory effect of the drug on TG absorption in the gut (10).

Finally, the inhibitory action of F-1394 as compared to other hypolipidemic agents on ACAT was demonstrated using a cultured human intestinal cell line, Caco-2, by following [^{14}C]-oleic acid incorporation into cholesteryl ester. Cholesterol esterification was significantly and dose-dependently inhibited by F-1394 treatment (IC_{50} = 22.5 μM). The IC_{50}s for YM-17E, CI-976, CL-277082, DL-melinamide and simvastatin were 121 nM, 702 nM, 21.5 μM, 20.9 μM and 22.5 μM, respectively; pravastatin sodium, probucol and clofibrate were ineffective. F-1394 also inhibited basal lateral cholesteryl ester secretion by 90% when Caco-2 cells were cultured on membrane filter (11).

The biological activities of F-1394 and other selected ACAT inhibitors are given in Table III.

Manufacturer

Fujirebio, Inc. (JP).

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