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J-104,123, A Novel and Orally-active Inhibitor of Squalene Synthase: Stereoselective Synthesis and Cholesterol Lowering Effects in Dogs

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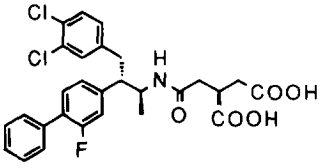
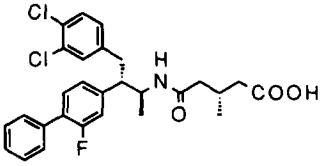
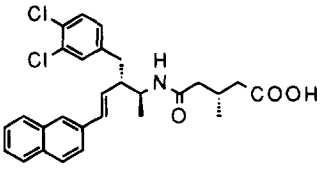
Abstract: J-104,123, a potent inhibitor of squalene synthase having monocarboxylic acid structure, was discovered by chemical modification of J-104,118. An oral dose of J-104,123 lowered serum cholesterol levels in dogs. J-104,123 was synthesized stereoselectively from methyl (*R*)-3-hydroxybutyrate.

Elevated serum cholesterol levels are an established risk factor for atherosclerosis. 3-Hydroxy-3-methylglutaryl(HMG)-CoA reductase is a major regulatory enzyme in the cholesterol biosynthetic pathway. Inhibitors of this enzyme effectively lower serum cholesterol levels in humans and are widely used clinically. (i.e., lovastatin, simvastatin and pravastatin) A recent clinical study (the Scandinavian Simvastatin Survival Study) showed that long-term treatment with simvastatin improved survival rates in patients with coronary artery disease[1].

The enzyme squalene synthase (SQS) catalyzes the reductive dimerization of two molecules of farnesyl pyrophosphate (FPP) to form squalene in the middle stage of the cholesterol biosynthetic pathway. Inhibitors of SQS would be ideal cholesterol-lowering agents because they do not prevent the biosynthesis of ubiquinone, dolichol and isopentenyl t-RNA. We recently described a novel class of SQS inhibitor (J-104,118) which potently inhibited SQS and cholesterol synthesis in mice[2]. A subsequent structure-activity study led to the discovery of another structural class of potent inhibitor, J-104,123. In this paper, we describe the discovery and stereoselecctive synthesis as well as the cholesterol-lowering effect of J-104,123 in dogs.

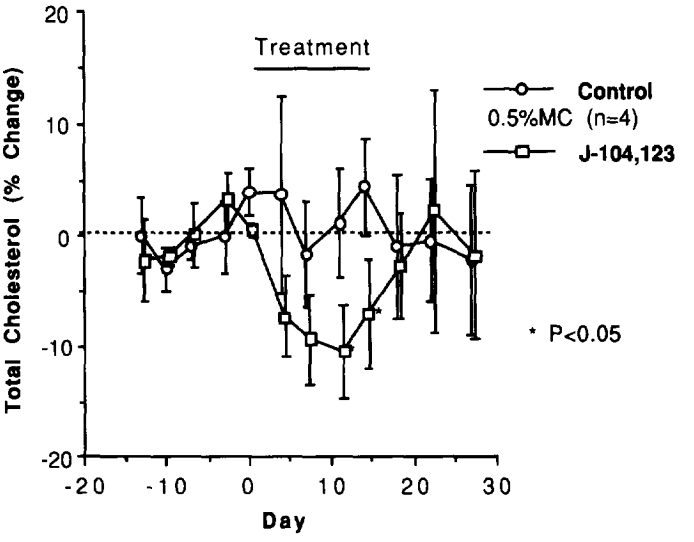
Table I shows the structures of J-104,118 and newly developed inhibitors, their inhibitory activities against SQS obtained from HepG2 cells and AUC values in dogs after oral administration. In a previous paper[2], we reported that the 3-fluoro-4-biphenyl group located in the hydrophobic part of J-104,118 greatly enhanced the inhibitory activity against SQS. We further conducted a strucure-activity study of this class of inhibitors. We found that a monocarboxylic compound **1** showed potent inhibitory activity against SQS with an IC_{50} value of 3.9nM. A naphtylvinyl derivative J-104,123 was slightly more potent than compound **1** with regard to inhibitory activity.

Table I. Structures, IC50 and AUC of J-104,118 and newly-developed inhibitors.

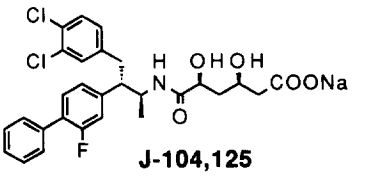
Compound No.	Structure	SQS IC_{50} (nM)	AUC (Dog) (μg hr/ml)
J-104,118		**0.52**	0.9 (20mg/kg)
1		**3.9**	9.9 (10mg/kg)
J-104,123		**2.5**	54 (20mg/kg)

In a previous paper[2], we reported that oral J-104,118 potently inhibited acute cholesterol synthesis in mice. We investigated the cholesterol-lowering effects of J-104,118 and its analogues in dogs. However, oral administration of the inhibitors having the dicarboxylic acid structure did not lower cholesterol in dogs. These results were probably due to the poor oral absorption of the dicarboxylic acid inhibitors in dogs (the plasma AUC value of J-104,118 was less than

Fig. I. Cholesterol lowering effect of J-104,123 in dogs.



1 μg•hr/ml). On the other hand, the monocarboxylic acid inhibitors provided better oral absorption as compared with the dicarboxylic inhibitors. The plasma AUC value for J-104,123 was approximately fifty-fold higher than that for J-104,118. By oral dosing in dogs, J-104,123 significantly lowered the plasma cholesterol level by 11% (30 mg/kg, 7 days then 30 mg/kg b.i.d.,7 days, Fig. I). Squalestatin 1, the same compound as zaragozic acid A, has been reported to lower serum cholesterol levels by up to 75% at an oral dose of 10-100 mg/kg/day in marmosets[3]. However, none of the SQS inhibitors have been reported to lower plasma cholesterol levels in dogs, the most commonly used animal model for the evaluation of cholesterol biosynthesis inhibitors. Although the cholesterol-lowering effect of J-104,123 was insufficient as compared with HMG-CoA reductase inhibitors[4] and squalene epoxidase inhibitors[5], this is the first report of a SQS inhibitor reducing plasma cholesterol levels in dogs.

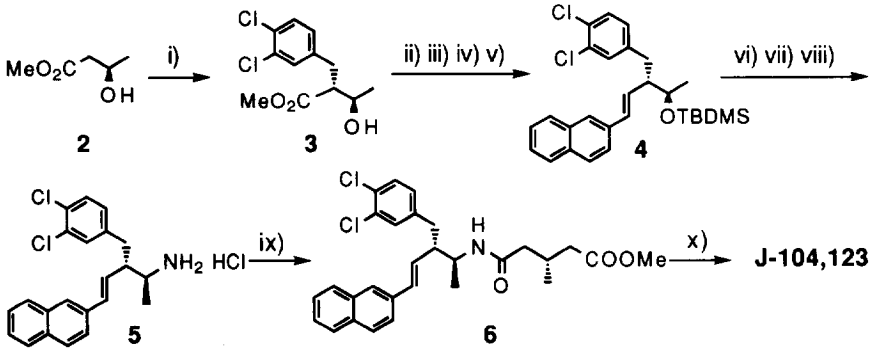


J-104,125

Recently, we found a unique monocarboxylic analog (J-104,125) with a dihydroxycarboxylic acid structure. This compound inhibited SQS with an IC_{50} value of 0.13 nM, which is comparable in potency with the most potent inhibitor, zaragozic acid A/squalestatin 1[6]. The in vivo study of J-104,125 and its related compounds are now in progress.

J-104,123 has three asymmetric carbons, and the most potent stereoisomer was known to have 3*R*,7*S* 8*S* configurations[2]. We developed an efficient method to synthesize optically active J-104,123 outlined in Scheme I. Methyl (2*R*,3*R*)-2-(3,4-dichlorobenzyl)-3-hydroxybutyrate **3** was prepared from commercially available methyl (*R*)-3-hydroxybutyrate **2** according to the method reported by Frater[7]. Thus, the stereoselective alkylation of the dianion of **2** with 3,4-dichlorobenzylbromide afforded **3** having a desired stereochemistry in 78% yield. After protecting the hydroxy functionality with a TBDMS group, the ester moiety was converted to aldehyde in a

Scheme I. Synthesis of J-104,123



i) 2LDA, THF then 3,4-dichlorobenzylbromide, 83% ii) TBDMSCl, imidazole, DMF, 84% iii) DIBAL, toluene iv) PCC, CH_2Cl_2 v) 2-naphtylmethylphosphonium bromide, NaH, THF 45% from **3** vi) TBAF, THF vii) DEAD, PPh_3, DPPA, THF viii) PPh_3, H_2O, THF then HCl, 67% ix) methyl (*R*)-3-methylglutarate (**7**), EDCI, DMAP, CH_2Cl_2, 97% x) NaOH, 94%.

stepwise manner. A subsequent Wittig reaction yielded a trans olefin derivative **4**. The amine **5** was obtained in the usual manner, and the optical purity was determined to be more than 95% by HPLC analysis of its MTPA amide derivative. The optically active methyl (*R*)-3-methylglutarate **7** was synthesized according to a procedure using pig liver esterase[8]. J-104,123 was easily obtained by the coupling reaction of the amine **5** and carboxylic acid **7** in the presence of EDCI and subsequent alkaline hydrolysis[9].

It is concluded that J-104,123 (a mono-carboxylic acid analog of J-104,118) reduced serum cholesterol levels in dogs. J-104,125 inhibited SQS with a potency almost comparable to zaragozic acid A/squalestatin 1. We are hopeful that J-104,125 and its analogues will lower cholesterol levels in dogs and prove to be useful cholesterol-lowering agents in man.

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9. J-104,123: ^{1}H NMR (CD_3OD, 300MHz) δ 1.04 (d, J = 6.3 Hz, 3H), 1.19 (d, J = 6.9 Hz, 3H), 2.11-2.30 (m, 3H), 2.35-2.47 (m, 2H), 2.57-2.68 (m, 2H), 2.94 (d, J = 10.2 Hz, 1H), 4.03 (quint., J = 6.6 Hz), 6.15 (dd, J = 15.3, 8.4 Hz, 1H), 6.30 (d, J = 15.3 Hz, 1H), 7.10 (d, J=8.1 Hz, 1H), 7.31-7.44 (m, 4H), 7.55 (d, J = 8.7 Hz, 1H), 7.62 (s, 1H), 7.73-7.78 (m, 3H); mp 146~147°C; $[\alpha]^{20}_D$ +103° (c = 1.02, MeOH).

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