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Synthesis and Biological Activity of J-104,118, A Novel, Potent Inhibitor of Squalene Synthase

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Abstract: A new structural class of squalene synthase (SQS) inhibitor, J-104,118, was developed by chemical modification of L-592,901. The absolute configuration of J-104,118 was determined by X-ray crystal analysis. An oral dose of J-104,118 potently inhibited cholesterol synthesis in mice.

Elevated serum cholesterol levels are an established risk factor for atherosclerosis. Inhibitors of 3-hydroxy-3-methylglutaryl(HMG)-CoA reductase, a major regulatory enzyme in the cholesterol biosynthetic pathway, effectively lower serum cholesterol levels in humans and are widely used clinically; these drugs include 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

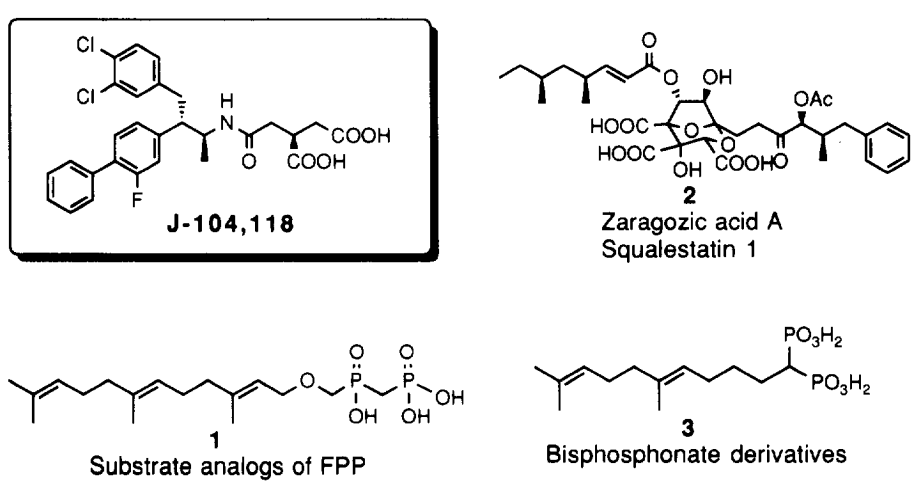


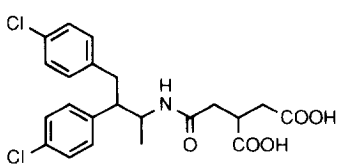
Fig. I. Structures of known SQS inhibitors and J-104,118

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 expected to be ideal cholesterol-lowering agents because they do not prevent the biosynthesis of ubiquinone, dolichol and isopentenyl t-RNA. Some potent inhibitors of SQS have been reported, such as substrate analogs of FPP (**1**)[2], zaragozic acids/squalestatins (**2**)[3], bisphosphonate derivatives (**3**)[4], transition state analogs[5] and others[6] (Fig. 1). We have developed a novel, potent inhibitor of squalene synthase, J-104,118, which has a different structure from the known SQS inhibitors. Herein, we describe the synthesis and the biological profile of J-104,118.

J-104,118 was developed by the chemical modification of L-592,901, a SQS inhibitor discovered at Merck Research Labs (MRL)[7]. The structure of L-592,901 is shown in Scheme II. This compound has a hydrophobic arylalkyl group on the left side and a hydrophilic dicarboxylic acid function on the right side that are connected by an amide linkage. This compound inhibited SQS obtained from Hep G2 cells (HB8065, a human hepatoma cell line) with an IC_{50} of 660 nM.

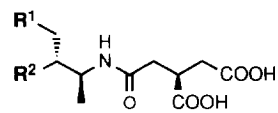
Table I briefly summarizes the inhibitory activitiy of J-104,118 and its related compounds against HepG2 SQS [8]. Through modifications of the hydrophobic part of L-592,901, we found great enhancement of potency against SQS by changing the 4-chlorophenyl group (R^2) of L-592,901 to a 4-biphenyl structure. The IC_{50} of compound **4** is 3.2nM, which is approximately 200-fold more potent than L-592,901. Among the compounds

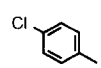
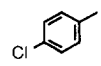
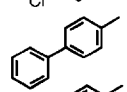
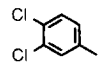
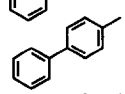
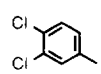
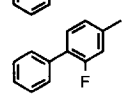
Table I. Structure and enzyme activities of L-592,901 and its related compounds.



L-592,901

HepG2 SQS IC_{50} 660nM

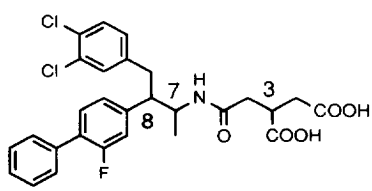


No.	R^1	R^2	HepG2 SQS IC_{50}(nM)
L-592,901			660
4			3.2
J-104,117			1.2
J-104,118			0.52

synthesized to date, the most favorable R^1 group is a 3,4-dichlorophenyl structure. The IC_{50} value of J-104,117 having 3,4-dichlorophenyl and 4-biphenyl groups is 1.2 nM. J-104,118 was discovered by introducing a fluorine atom into the biphenyl moiety, and its IC_{50} value was determined to be less than 1nM.

We have examined the stereochemistry of J-104,118. This compound has three asymmetric carbons: one is in the hydrophilic di-carboxylic part (position 3) and the other two are in the hydrophobic group (positions 7 and 8); therefore, eight stereoisomers exist. We have prepared most of the stereoisomers and compared their inhibitory activities against SQS. The results are summarized in Table II. The stereochemistries of the 7 and 8 positions are critical for the inhibitory activity. The isomer with the 7S,8S configuration (J-104,118 and **5**) was over 20-fold more potent than the other isomers (**6**,**7** and **8**). The stereochemistry of the 3 position is not as important; the 3S isomer is a little bit more potent than the 3R isomer. The absolute configuration of the most potent isomer (J-104,118) was determined by X-ray crystal analysis (Fig. II).

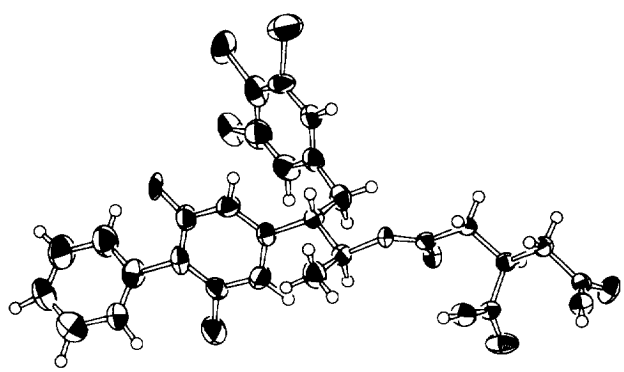
Table II. IC50 of stereoisomers of J-104,118



No.	Configuration	HepG2 SQS IC_{50}(nM)
J-104,118	3S 7S 8S	0.52
5	3R 7S 8S	0.73
6	3S 7R 8R	16
7	3R 7R 8R	19
8 *	3RS 7R 8S 3RS 7S 8R	>300

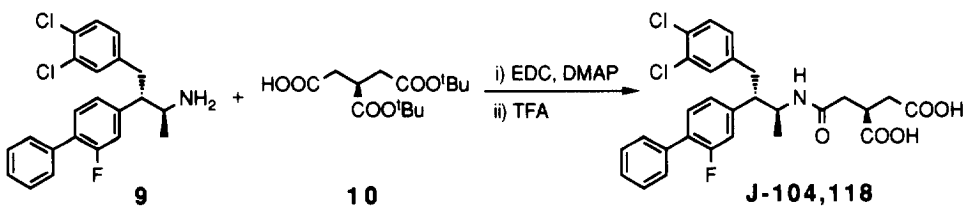
* mixture of four isomers

Fig. II. Crystal structure of J-104,118[9]



The synthesis of J-104,118 is outlined in Scheme I. The racemic amine **9** was synthesized according to the method reported by Schultz *et al.*[10]. Optical resolution of the racemic amine **9** was performed by recrystallization of its L-tartaric acid salt from ethanol. Racemic 2,3-di-tert-butyl 1,2,3-propanetri-carboxylate **10** was prepared by Michael reaction of lithium enolate of benzyl acetate with di-tert-butyl maleate[11], followed by debenzylation by hydrogenation. The optical resolution was successively carried out via recrystallization of its cinchonidine salt from carbon tetrachloride. The coupling reaction of the amine **9** with the carboxylic acid **10** in the presence of EDC and the subsequent deesterification with trifluoroacetic acid afforded J-104,118 as white crystals[12].

Scheme I. Synthesis of J-104,118



9 **10** **J-104,118**

J-104,118 potently inhibited cholesterol synthesis *in vivo*. The acute inhibitory effect of J-104,118 on cholesterol synthesis from [^{14}C]acetate was examined in mice. A single oral 3 mg/kg dose of this compound decreased the radioactivity in serum cholesterol by approximately 84%. In liver, J-104,118 also inhibited cholesterol synthesis. Zaragozic acid A is one of the most potent inhibitor of SQS (IC_{50} 0.24 nM). In spite of its potent inhibitory activity against SQS, oral administration of zaragozic acid A weakly inhibited cholesterol synthesis (38% inhibition at 10 mg/kg) in mice. Squalestatin 1, the same compound as zaragozic acid A, has been reported to lower serum cholesterol by up to 75% at an oral dose of 10-100 mg/kg/day in marmosets[3b]. We are now evaluating the cholesterol lowering effect of J-104,118 and analogous compounds in marmosets and dogs.

We have developed J-104,118, a potent and orally active inhibitor of mammalian SQS. We are hopeful that J-104,118 or its analogous compounds will proved to be useful cholesterol-lowering agents in man.

Acknowledgements:

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References and Notes:

1. Scandinavian Simvastatin Survival Study Group, *Lancet* **1994**, *344*, 1383.
2. (a) Oritz de Montellano, P. R.; Wei, J. S.; Castillo, R.; Hsu, C. K.; Boparai, A. *J. Med. Chem.* **1977**, *20*, 243. (b) Biller, S.A.; Sofia, M. J.; DeLange, B.; Forster, C.; Gordon, E. M.; Harrity, T.; Rich, L. C.; Ciosek, C. P., Jr. *J. Am. Chem. Soc.* **1991**, *113*, 8522.
3. (a) Bergstorm, J. D.; Kurtz, M. M.; Rew, D. J.; Amend, A. M.; Karkas, J. D.; Bostedor, R. G. Bansal, V. S.; Dufresne, C.; VanMiddlesworth, F. L.; Hensens, O. D.; Liesch, J. M.; Zink, D. L.; Wilson, K. E.; Onishi, J.; Milligan, J. A.; Bills, G.; Kaplan, L; Nallin Omstead, M.; Jenkins, R. G.; Huang, L; Meinz, M. S.; Quinn, L.; Burg, R. W.; Kong, Y. L.; Mochales, S.; Mojena, M.; Martin, I.; Pelaez, F.; Diez, M. T.; Alberts, A. W. *Proc. Natl. Acad. Sci. USA* **1993**, *90*, 80. (b) Baxter, A.; Fitzgerald, B. J.; Hutson, J. L.; McCarthy, A. D.; Motteram, J. M.; Ross, B. C.; Sapra, M.; Snowden, M. A.; Watson, N. S.; Williams, R. J.; Wright, C. *J. Biol. Chem.* **1992**, *267*, 11705.
4. (a) Ciosek, C. P., Jr.; Magnin, D. R.; Harrity, T. W.; Logan, J. V. H.; Dickson, J. K., Jr.; Gordon, E. M.; Hamilton, K. A.; Jolibois, K. G.; Kunselman, L. K.; Lawrence, R. M.; Mookhtiar, K. A.; Rich, L. C.; Slusarchk, D. A.; Sulsky, R. B.; Biller, S. A. *J. Biol. Chem.* **1993**, *268*, 248832. (b) Amin, D.; Cornell, S. A.; Gustafson, S. K.; Needle, S. J.; Ullrich, J. W.; Bilder, G. E.; Perrone, M. H. *J. Lipid Res.* **1992**, *33*, 1657.
5. (a) Corey, E.J.; Volante R. P. *J. Am. Chem. Soc.* **1976**, *98*, 1291. (b) Poulter, C. D.; Capson, T. L.; Thompson, M. D.; Bard, R. S. *J. Am. Chem. Soc.* **1989**, *111*, 3734. (c) Oehlschlager, A. C.; Singh, S. M.; Sharma, S. *J. Org. Chem.* **1991**, *56*, 3856. (d) Ortitz de Montellano, P. R.; Castillo, R. *Tetrahedron Lett.* **1976**, 4115.
6. (a) Bertolino, A.; Altman, L. J.; Vasak, J.; Rilling, H. C. *Biochim. Biophys. Acta* **1978**, *530*, 17. (b) Prashad, M.; Kathawala, F. G.; Scallen, T. *J. Med. Chem.* **1993**, *36*, 1501.
7. Mosley, R.; Bergstrom, J. personal communication.
8. The structure-activity relationships of J-104,118 will be described elsewhere in detail.
9. Crystal data; F.W.=546.42; Space group: $P2_1$ (#4); a =5.839(2)A, b =9.041(2)A, c =24.799(1)A, β =95.43(1); V =1303.3(5)A3; Z= 2; Radiation =Cu Kα (λ =1.54184A); μ =26.42cm^{-1}; Temperature =25.0±1.0°; Final R= 0.067; Final ωR =0.064; Number of Obserbed Reflections =4656; Number of Unique Reflections =2097; Rmerge =0.06. An absolute configulation was determined using program teXsan.

10. Schultz, E. M.; Bolhoffer, W. A.; Augenblick, A.; Bicking, J. B.; Habecker, C. N.; Horner, J. K.; Kwong, S. F.; Pietruszkiewicz, A. M. *J. Med. Chem.* **1967**, *10*, 717.
11. A Similar reaction was reported. Yamaguchi M.; Tsukamoto, M.; Hirao, I. *Chem. Lett.* **1984**, 375.
12. J-104,118: ^{1}H NMR (CD_3OD, 300MHz) δ 0.99 (d, J = 6.9 Hz, 3H), 2.53 (dd, J = 6.9, 15.9 Hz, 1H), 2.61 (dd, J = 6.0, 17.1 Hz, 1H), 2.69 (dd, J = 6.9, 15.6 Hz, 1H), 2.74 (dd, J = 7.5, 17.1 Hz, 1H), 2.82 (dd, J = 11.7, 12.6 Hz, 1H), 2.89~2.96 (m, 1H), 3.21~3.28 (m, 1H), 4.23 (dq, J = 8.7, 6.9 Hz, 1H), 6.91 (dd, J = 1.8, 8.0 Hz, 1H), 6.95 (s, 1H), 6.99 (s, 1H), 7.16 (d, J = 1.8 Hz, 1H), 7.26 (d, J = 8.0 Hz, 1H), 7.29~7.43 (m, 4H), 7.46~7.51 (m, 2H); Anal. Calcd for $C_{28}H_{26}NO_5FCl_2$: C, 61.55; H, 4.80; N, 2.56. Found: C, 61.46; H, 4.77; N, 2.53.; mp 170~171°C; $[\alpha]^{20}_D$ +124° (c = 1.0, MeOH).

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