(1α,2β,3β,4α)-1,2-Bis[[*N*-propyl-*N*-(4-phenoxybenzyl)amino]carbonyl]cyclobutane-3,4-dicarboxylic Acid (A-87049): A Novel Potent Squalene Synthase Inhibitor

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Squalene synthase is a microsomal enzyme which catalyzes the reductive dimerization of two molecules of farnesyl pyrophosphate (FPP) to form squalene (**1**, Figure 1). This enzyme is involved in the first committed step of the *de novo* cholesterol biosynthetic pathway.[1] As suggested by *in vivo* experiments, inhibition of squalene synthase will block cholesterol biosynthesis and thus should act to suppress elevated serum cholesterol (*vide infra*). Elevated serum cholesterol is well established as a risk factor for coronary heart disease.[2]

Developments in the last few years have shown that one of the more effective ways of reducing serum cholesterol is inhibition of sterol biosynthesis, and a number of therapeutic agents are available which work by inhibiting hydroxymethylglutaryl-Co A reductase. However, they are not very selective as they inhibit the biosynthetic pathway early on, and thus they may prevent the formation of important compounds that are derived from the mevalonic acid pathway[3] such as dolicols and ubiquinones. A more selective inhibitor may be one that inhibits the enzymatic step after the biosynthetic pathway branches to other isoprene-derived compounds.[4] Several years ago, groups of Biller[5] and Poulter[6] described studies using inhibitors of this enzyme based on substrate and transition-state analogs (**2a,b**). Recently, the Glaxo[7] and Merck[8] groups discovered that Squalestatin 1 (Zaragozic Acid A) (**3**) is a potent, selective inhibitor of squalene synthase both *in vitro* and *in vivo*.[9] Interestingly, the oral activity of zaragozic acid A (including other members of the family) was species dependent. Its squalene synthase inhibitory potency was only marginally active when administered po (ED_{50} = 100 mg/kg) in mice.[10] On the other hand, it was demonstrated that the natural product lowered serum cholesterol by up to 75% at an oral dose of 10−100 mg/kg/day in primates.[7] Further studies are required to establish whether the species dependence is unique to **3** or is a characteristic of all squalene synthase inhibitors. More recently, Biller,[11] Iwasawa,[12] and Brown[13] have also reported orally active squalene synthase inhibitors. There are no reports thus far of human clinical trials, so it remains to be determined whether inhibition of squalene synthase will prove advantageous compared to existing marketed agents.

Squalestatin 1 (**3**) is a member of a novel family of fermentation products isolated from a previously unknown Phoma species (Coelomycetes). It is a bicyclic molecules substituted by several carboxy and hydroxy groups and two long lipophilic side chains. It is also one of the most potent reported inhibitors of squalene synthase. In our laboratories, we chose to use the squalestatins/zaragozic acids as a general template from which new, wholly synthetic squalene synthase inhibitors could be designed. We hypothesized that the central 2,8-dioxabicyclo[3.2.1]octane core served simply to position two lipophilic groups and two carboxylates in the proper orientation for efficient enzyme inhibition. Models suggested that a 4-, 5-, or 6-membered aliphatic ring might position these groups in a similar fashion. We chose to focus on cyclobutane-derived compounds because commercially available *trans*-1,2,3,4-cyclobutanetetracarboxylic acid dianhydride would provide easy access to a variety of 1,2-*trans*-dicarboxylic acid structures in which lipophilic groups could be appended through amide linkages to the remaining two carboxylates. Thus, a series of cyclobutanedicarboxylic acid diamide analogs (**5**) were prepared (Table 1).

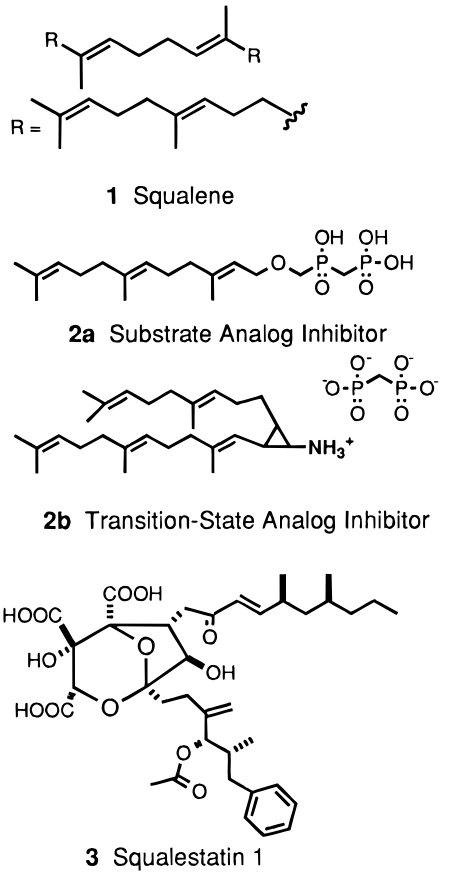

Figure 1.

The syntheses of the intermediate amines (**4**) are shown in Scheme 1, and the syntheses of the target compounds (**5**) are shown in Scheme 2. The intermediate *N*-methylhomogeranylamine (**4a**) was prepared in 47% distilled yield by alkylation of geranyl bromide with lithium salt of *N′-tert*-butyl-*N*,*N*-dimethylformamidine followed by KOH hydrolysis of the formamidine intermediate. The *N*-propylgeranylamine (**4b**) was prepared as follows: geranyl bromide was reacted with sodium succinimide, and the resulting geranylsuccinimide was hydrolyzed by KOH to provide geranylamine in 48% overall yield. Geranylamine was then acylated with propionyl chloride followed by LAH reduction to give 84% yield of the target intermediate. The intermediate

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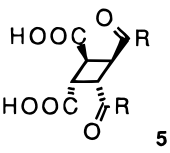
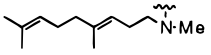
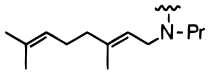
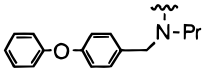
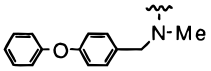
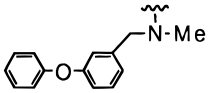
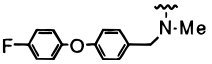
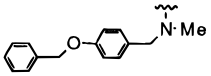
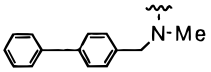
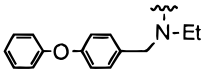
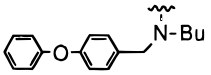
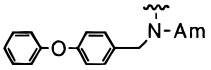
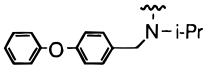
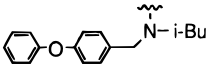
Table 1. *In Vitro* Inhibitory Activity



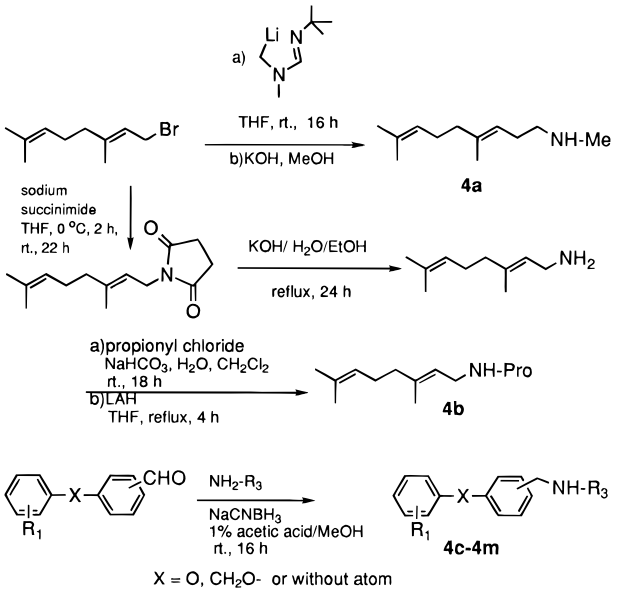
5

Compound	R =	IC_{50} (nM)
5a		8000
5b		720
5c (A-87049)		37
5d		1400
5e		8000
5f		1400
5g		10000
5h		10000
5i		180
5j		13
5k		130
5l		140
5m		14
5n	**Zaragozic Acid A**	33

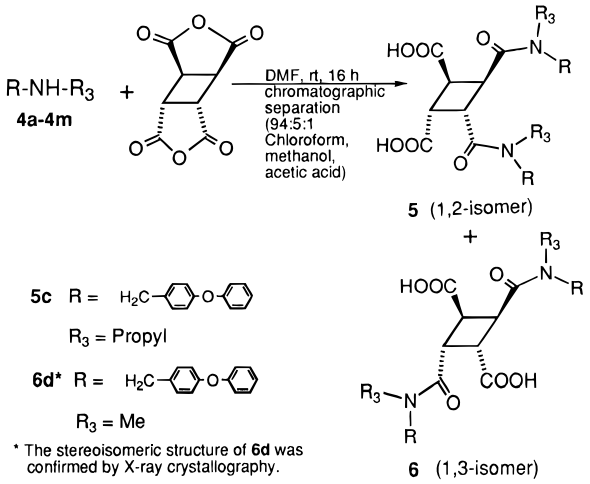
secondary amines (**4c**−**m**)[14] were synthesized by reductive amination of the commercially available amines and the corresponding aldehydes (either commercially available or known in the literature[15]) with sodium cyanoborohydride. The yields were approximately 60−75%. Finally, *trans*-1,2,3,4-cyclobutanetetracarboxylic dianhydride[16] was treated with the appropriate secondary amine in DMF for 16 h. After flash silica gel chromatography, there was obtained, in general, a 30−45% yield of the desired 1,2-isomer (**5**)[17] and similar yield of the 1,3-isomer (**6**).[17] Squalene synthase activity was measured using rat liver microsomes as the enzyme source.[18]

Initially, we prepared cyclobutane analogs (see Table 1) containing the geranyl and homogeranyl side chains. The inhibitory activities of compounds **5a** and **5b** were very weak. However, by replacing the geranyl unit with a diphenyl ether moiety, the inhibitory potency (IC_{50}) improved dramatically from 720 nM (**5b**) to 37 nM (**5c**).[19] Compound **5c** compared favorably to zaragozic

Scheme 1



Scheme 2



acid A (33 nM) under the same assay condition. Surprisingly, the inhibitory potency decreased 38 times by simply replacing the propyl group of **5c** with methyl as in compound **5d**. All other methyl analogs (**5e**−**h**) were either equivalent to or less potent than that of the compound **5d**. In order to further explore the inhibitory activity of this series of compounds, various *N*-(lower alkyl)-substituted analogs were synthesized. A desirable and improved inhibitory potency of 13 and 14 nM (**5j** and **5m**) was achieved as the alkyl chain length increased from propyl to butyl and isobutyl.

The *in vivo* lipid lowering efficacy of compound **5c** (A-87049) was studied according to the following protocol. Cynomolgus monkeys (two males and two females per group) were fasted overnight and bled in the morning. Plasma samples were taken from the femoral vein and analyzed for total cholesterol, HDL cholesterol, and triglycerides. The treated animals were dosed with **5c** or lovastatin, 20 mg/kg po, and control monkeys were dosed with vehicle containing 0.2% methocel. Dosing was continued daily for 5 days. The animals were fasted overnight prior to the last dose. They were bled 2 h after dosing on day 5 but before feeding. Plasma samples were prepared and analyzed as before. One-sample *t*-tests were calculated to test for significant

Table 2. *In Vivo* Activity of A-87049 in Monkeys (20 mg/kg/day for 5 days)

	% change (from pretreatment control)[a]	
	5c (A-87049)	lovastatin
total cholesterol	−12 ± 1*	−19 ± 9*
HDL cholesterol	−4 ± 2	−12 ± 5
LDL cholesterol	−36 ± 10*	−21 ± 16

[a] An asterisk (*) denotes values significantly different from zero in one-sample *t*-test ($p < 0.02$).

change within a group across the experiment. Compound **5c** caused a statistically significant decrease in total plasma cholesterol of 12% and LDL cholesterol of 36%, compared to pretreatment values. There was no significant change in HDL cholesterol. Similar results were obtained with lovastatin (Table 2). Plasma drug level analysis revealed no circulating drug (limit of detection = 100 ng/mL), suggesting that **5c** is cleared rapidly from the plasma.

In summary, several potent squalene synthase inhibitors have been identified. Their inhibitory potencies were very significant (13−37 nM) in the rat liver microsomal enzyme assay. Daily po administration of 20 mg/kg of A-87049 (**5c**) for 5 days in cynomolgus monkeys ($n = 4$) resulted in approximately 12% decrease in total cholesterol and 36% in LDL cholesterol, while HDL cholesterol and triglycerides[20] remained unchanged. Further work on A-87049 (inhibition of cholesterol biosynthesis and oral efficacy in other species) and its more potent analogs is underway to confirm the clinical utility of these inhibitors.

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Supporting Information Available: Full experimental procedures, NMR data, and analytical data for the final products (11 pages). Ordering information is given on any current masthead page.

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(14) Compound **4m** was alternately prepared by replacing sodium cyanoborohydride with Pd/C and 1 atm of H_2. The yield was 95%.
(15) (a) Aldehyde intermediate for **4f**: Harling, John; Orlek, Barry Sidney. (SmithKline Beecham PLC, UK). PCT Int. Appl. WO 9504027, p 57. (b) Aldehyde intermediate for **4g**: Guziec, Frank S.; Luzzio, Frederick A. *J. Org. Chem.* **1982**, *47*, 1787−1789. All the other aldehyde intermediates are commercial products obtained from Aldrich Chemical Co.
(16) The dianhydride was obtained from Aldrich.
(17) The isomeric structures of **5** and **6** are determined by ^{1}H NMR analyses. Furthermore, the 1,3-isomer **6d** (where R = Me and R_3 = 4-phenoxybenzyl; see Scheme 2) is confirmed by X-ray crystallography. The total yield of both isomers of all inhibitors are approximately 70−80% except **5a** and **6a** isomers whose total yield is only 10%.
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(19) The IC_{50} of the 1,3-isomer (**6c**) is 1200 nM.
(20) Data not shown.

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