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CYCLOPENTANEDI- AND TRICARBOXYLIC ACIDS AS SQUALENE SYNTHASE INHIBITORS: SYNTHESES AND EVALUATION

Wang Shen,* David S. Garvey, Jerry Cohen, Herman Stein, and Saul H. Rosenberg
Cancer Research, Pharmaceutical Products Division, Abbott Laboratories, Abbott park, IL 60064, U.S.A.

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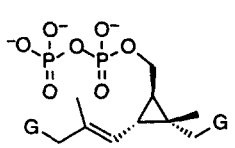
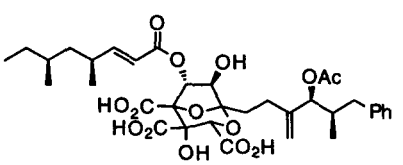
Abstract: Based on earlier lead squalene synthase inhibitor A-87049 (**3**) and zaragozic acids, a series of cyclopentanedi- and tricarboxylic acids were synthesized and evaluated against the enzyme. Some exhibited good potency and SAR revealed the importance of conformation and substitution pattern of these synthetic inhibitors.

It has been well established that elevated serum cholesterol level is associated with the increased risk of atherosclerosis and coronary heart disease, and numerous clinical trials have demonstrated the benefits of lowering serum cholesterol by diet or drug intervention.[1,2] In particular, statins[3] suppress cholesterol biosynthesis by inhibiting 3-hydroxy-3-methylglutrayl-coenzyme A (HMG-CoA) reductase, an early stage enzyme in the cholesterol biosynthesis pathway.[4] Squalene synthase (SS) catalyzes the dimerization of farnesyl pyrophosphate (FPP) to squalene in the first committed step of *de novo* cholesterol biosynthesis. Inhibition at this step of the sterol synthesis pathway would effectively inhibit cholesterol biosynthesis,[5,6] but unlike HMG-CoA reductase inhibitors it would not prevent the formation of other biologically important products of the mevalonic acid pathway, such as dolichol and ubiquinone.[7] This strategy could potentially have advantages over other existing drugs, such as lovastatin for fewer side effects, and higher efficiency.

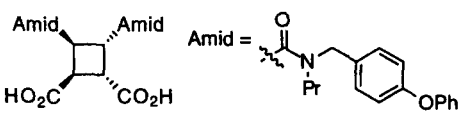
It has been demonstrated that SS catalyzed dimerization involves a key cyclopropane intermediate (**1**).[8] Recently, a class of natural product SS inhibitors, zaragozic acids (zaragozic acid A, **2**), were discovered.[9] Based on these discoveries, a rationally designed, and quickly optimized potent SS inhibitor, cyclobutanedicarboxylic acid A-87049 (**3**) was synthesized.[10] In this communication, we described the syntheses of cyclopentanedi- and tricarboxylic acids, and the evaluation of them against rat liver SS assay.



1 (G = Geranyl)



2 (Zaragozic acid A), IC_{50} = 12 nM



3 (A-87049, racemic), IC_{50} = 37 nM

Chemistry

Tetrasubstituted cyclopentanes have far more isomers and than the corresponding cyclobutanes. Earlier work on the cyclobutane derivatives[10] demonstrated that vicinal diacids (or vicinal diamides) had higher inhibitory activity against SS than the corresponding 1,3-diacids (or diamides). With that in mind, we first prepared some easily accessible derivatives. Compound **6** and **7** were prepared by opening the commercially available bisanhydride **4** with amine **5**. Compounds **8** and **9** were synthesized using methods shown in Scheme 1.

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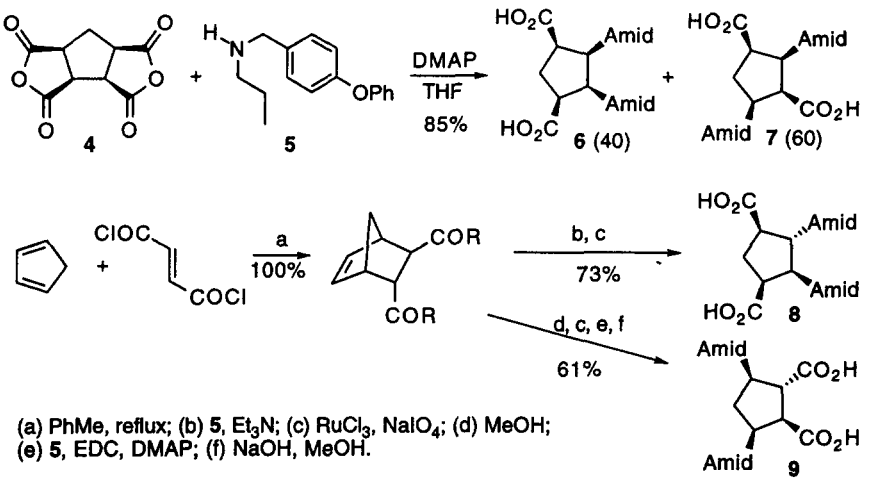
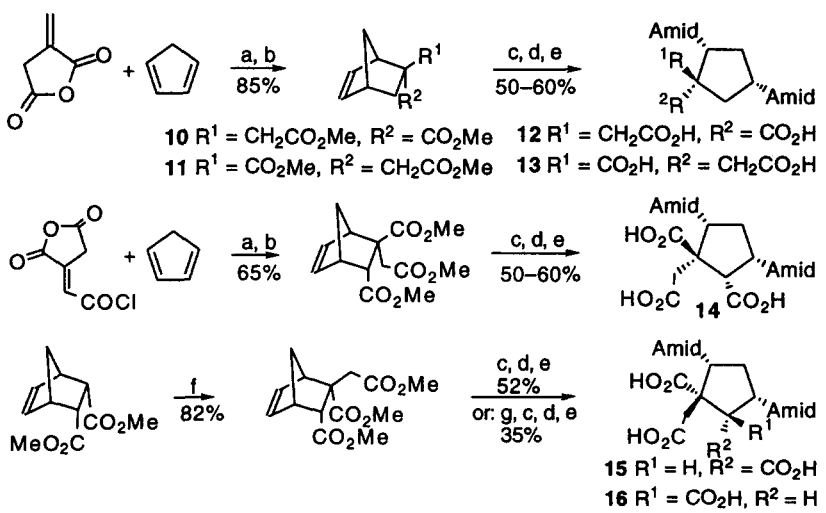
Scheme 1



(a) PhMe, reflux; (b) **5**, Et_3N; (c) $RuCl_3$, $NaIO_4$; (d) MeOH; (e) **5**, EDC, DMAP; (f) NaOH, MeOH.

Both the rigid cyclopropane intermediate (**1**) and zaragozic acids (A, **2**) have their two lipophilic side chains on the same face of their corresponding rings. Based on these two structures, a series of cyclopentanedi- and tricarboxylic acids were designed. Diels–Alder reaction of cyclopentadiene with itaconic anhydride and subsequent esterification gave two separable diesters **10** (66%) and **11** (19%). Further manipulation gave rise to **12** and **13** (Scheme 2). Similarly, the three triacids **14** to **16** were synthesized. Of the six compounds, **12** and **16** have similar steric arrangement to that of zaragozic acids.

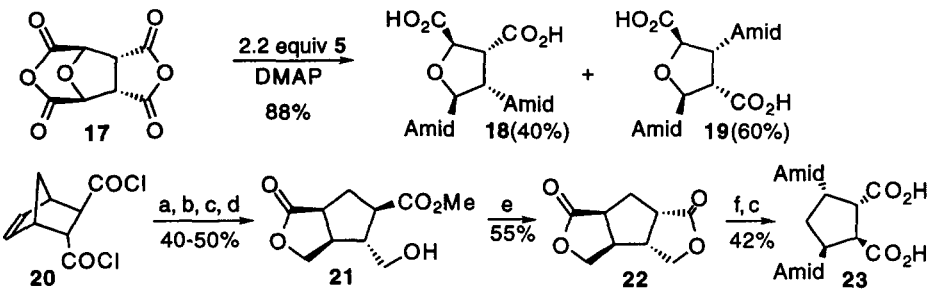
Scheme 2



(a) BF_3; (b) MeOH, $TMSCHN_2$; (c) $RuCl_3$, $NaIO_4$; (d) $(COCl)_2$, **5**; e) LiOH; (f) NaHMDS, $BrCH_2CO_2Me$; (g) 3 equiv LDA, AcOH.

While this work was going on, Rockway et al. synthesized all possible stereoisomers of A-87049, which established that A-87049 possessed the optimal orientation for cyclobutane-derived inhibitors.[12] With this information, the effort was redirected towards the syntheses of all 3 five-membered ring analogs with similar steric arrangements. Because the bisanhydride **17** was commercially available, the first compound (**18**) was synthesized with a tetrahydrofuran ring. The commercially available diacid chloride (**20**) was converted to alcohol **21**, which was refluxed in toluene with DBU to generate bislactone **22**. Opening of lactone with amine **5** and a mild Lewis acid,[13] and subsequent oxidation of the alcohol gave the desired diacid **23** in good yield.

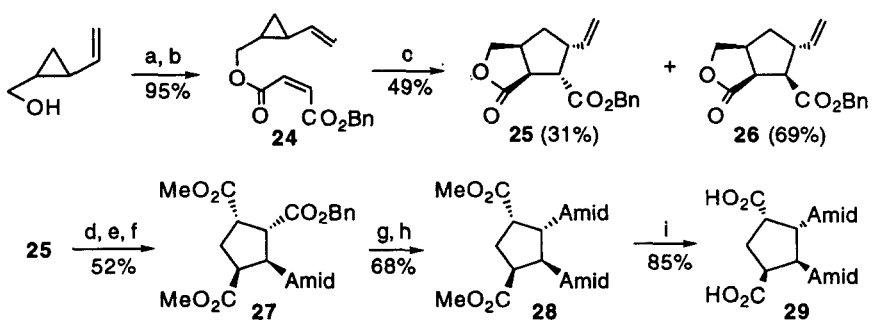
Scheme 3



(a) LAH; (b) AcCl; (c) $RuCl_3$, $NaIO_4$; (d) MeOH, H^+; (e) DBU, PhMe, 110 °C; (f) **5**, $Sn[N(TMS)_2]_2$.

To synthesize the last analog (**27**) with similar steric arrangement to A-87049, a new novel tandem radical cyclization was developed based on the research done by Feldman et al.[14] Irradiation of the cyclopropane intermediate (**24**) in presence of 1.1 equiv of phenyl disulfide gave two products in moderate yield. Further manipulation of the minor product **25** yielded the desired diacid **29**.

Scheme 4



(a) maleic anhydride; (b) BnOH, DCC, DMAP; (c)PhSSPh, *hν*; (d) [on **25**], **5**, $Sn[N(TMS)_2]_2$; (e) $RuCl_3$, $NaIO_4$; (f) $TMSCHN_2$; (g) H_2/Pd–C; (h) EDC, DMAP, **5**; (i) LiOH, MeOH, 70 °C.

Biological Results and Discussion

Rat liver micrsomes were used as the source of squalene synthase, with ^{3}H-Farnesyl pyrophosphate (FPP) as the

substrate. The assay was buffered with KH_2PO_4 0.055 M, pH 7.4, dithiothreitol 2 mM, 1 mM $MgCl_2$ and 1 mM NADPH. Oxidation of squalene to squalene oxide was prevented by excluding oxygen from the assay. Squalene was extracted with hexane and radioactivity was evaluated by scintillation counting.[6] The IC_{50} was estimated from the log concentration – % inhibition curve. The results were summarized in Table 1.

Table 1

Compound	IC_{50} (μM)	Compound	IC_{50} (μM)
3 (A-87049)	0.037	14	0.24
6	9.1	15	25% inh @ 1 μM
7	2.9	16	38% inh @ 1 μM
8	45% inh @ 1 μM	18	3.0
9	0.58	19	n.a.
12	44% inh @ 1 μM	23	0.17
13	n.a.[1]	29	24% inh @ 1 μM

[1] n.a. indicates less than 20% inhibition when checked at 1 μM.

Some information could be obtained from the limited SAR in Table 1. The orientations of the two lipophilic side chains are not critical for the activity, because they may adopt different conformation to reach the active sites in squalene synthase. However, the *trans* arrangement gives slightly more potent activity (**9** compared to **23**). The more active compounds (**9**, **14**, **23**) have two carboxylic acid groups *trans* on vicinal carbons. This was also observed in the cyclobutane series.[10,12] This steric arrangement of carboxylic groups may be required for potent inhibitors of squalene synthase. Compounds (**6**, **7**, **8**, **19**, **29**) with 1,3-dicarboxylic acids are weaker inhibitors than those with *trans*-1,2-dicarboxylic acids, even if it (compound **9**) has similar structure to A-87049 (**3**). Compounds **12** and **16** do not exhibit good potency against SS, even though they have the similar steric arrangement to that of zaragozic acids. Unlike the very rigid skeleton of zaragozic acids, these two compounds are more flexible and may adopt totally different conformations. Both **14** and **16** possess *trans*–1,2-dicarboxylic acid groups similar to **9**, but unlike **14**, **16** is a weak inhibitor. This again may reflect the steric requirements for potent SS inhibitors.

In summary, several potent SS inhibitors were identified, though they are weaker than the cyclobutane analog A-87049 (**3**). The SAR of this class of compounds may lead to better understanding of squalene synthase inhibition, and help the designing of better inhibitors.

Acknowledgment: The authors wish to thank Dr. Roger Henry for structure determination of both bisanhydrides **4** and **17** by X-ray crystallography. We are also grateful to Dr. Xiaolin Zhang for assigning the structures of regioisomers **10/11**, and **25/26** based on two dimensional NMR analysis.

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