Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 4, No. 13, pp. 1591-1594, 1994

0960-894X/94 $7.00+0.00

0960-894X(94)00210-X

Design and Synthesis of Squalene Synthase Inhibitors - Acyclic Mimics of the Zaragozic Acids

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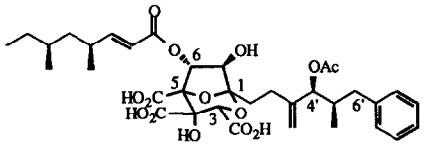
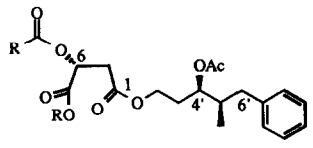
Abstract: A simple and versatile synthetic scheme employing D-malic acid as the principal building block permits the ready preparation of acyclic mimics of the squalene synthase inhibitor zaragozic acid A (ZA-A). Several compounds which were synthesized according to this approach have rat liver squalene synthase (RLSS) enzyme activities ranging from 200 to 470 nM, as compared to 0.3 nM for ZA-A. These analogs represent the first examples in this series which possess biological activity.

Zaragozic acid A (ZA-A), a naturally-occurring fungal metabolite, was discovered as a potent competitive inhibitor of squalene synthase with a K_i of 78 pM.[1] Structurally, ZA-A represents a class of natural products that is comprised of a bicyclic polar core and two lipophilic side-chains; the arms differentiate members of the class.[2] We initially anticipated that the bicyclic core structure with its spatially defined arrangement of polar functionalities would be important for the high level potency of this class. Modifications of this core and the discovery of several natural acyclic inhibitors led us to question the essential nature of the rigid ring system.[3] Thus, in addition to studying semi-synthetic analogs, we have investigated simplified structures based upon the zaragozic acid SAR profile.



Zaragozic Acid A
L-694,599

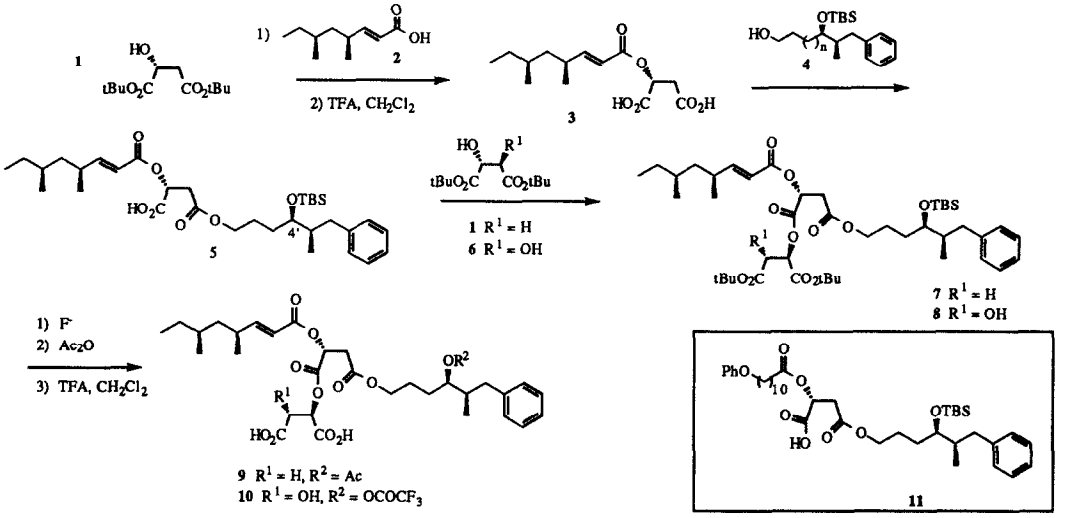


Acyclic Series:
C-6 Side-chain
C-1 Side-chain
Polar Core

In extensive SAR studies with ZA-A, the C-6 side-chain was substituted with a number of lipophilic fatty esters without loss of activity.[4] Though semi-synthetic modifications could be made at C-1, this arm was much less tolerant of change; the arrangement presented by the natural products was optimal in all biological assays.[4] A synthetic scheme was devised that allowed the use of either commercially available or naturally degraded C-6 fatty esters and synthetic C-1 side-chains with the natural configuration. The ready access to C-1 and C-6 arms from the most potent zaragozic acid series was deemed to be essential for this strategy. In this way, potential lead structures would not be missed owing to sub-optimally active side-chains. The initial strategy focused on developing a route amenable to variations of the polar core as well as the distance between the essential features of the side-chains.

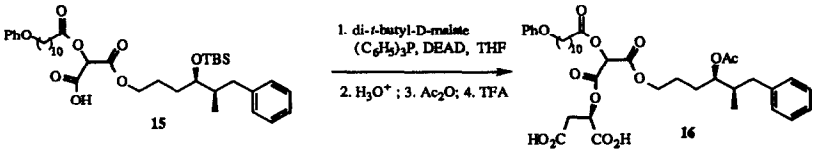
Diester **1** (prepared from D-malic acid)[5] was esterified using DCC and (2E,4S,6S)-4,6-dimethyl-2-octenoic acid **2**, which was readily available *via* base cleavage of ZA-A.[6] Subsequent *t*-butyl ester removal with

TFA in CH_2Cl_2 furnished the dicarboxylic acid **3**. Selective esterification of the less hindered carboxyl of **3** with 6-phenyl-5β-methyl-4β-*t*-butyldimethylsilyloxyhexanol **4**[7] (n=1), afforded the mixed diester **5** in excellent yield. Attachment of the "polar core" units was accomplished by treatment of **5** with the corresponding di-*t*-butyl malate **1** and di-*t*-butyl tartrate **6** to afford the triesters **7** and **8**, respectively, in >64% overall yield. Removal of the silyl protecting group of **7** followed by acetylation and hydrolysis of the *t*-butyl esters gave the initial analog **9** in excellent yield. Alternatively, removal of the silyl group of **8** and subsequent TFA treatment afforded the trifluoroacetate diester **10** directly.[8]



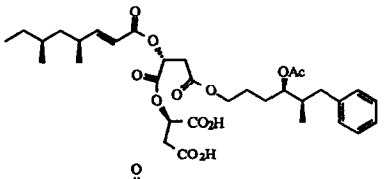
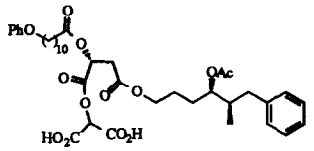
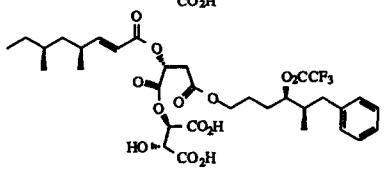
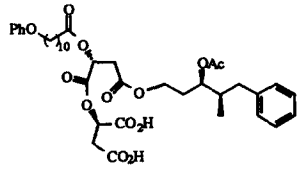
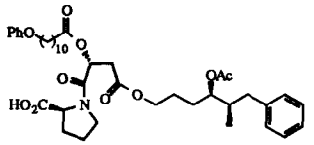
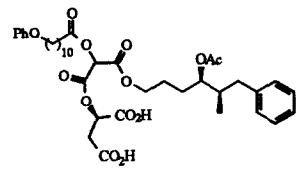
As previously mentioned, substitution of long-chain lipophilic arms at C-6 for the enoate of the natural product afforded analogs of comparable activity; terminal phenoxy substitution was often desirable. Additional derivatives were prepared incorporating this feature following the general procedures outlined above. Treatment of di-*t*-butyl malate **1** with 11-phenoxyundecanoic acid, hydrolysis of the *t*-butyl esters and selective esterification with alcohol **4** (n=1) as before, gave the mixed ester **11** in 76% yield. Amidation of the carboxyl residue of **11** with proline benzyl ester followed by removal of the silyl protecting group, acetylation of the resultant alcohol and debenzylation afforded monocarboxylic acid **12** (see Table). Similarly, condensation of intermediate **11** with di-*t*-butyl tartronate followed by the usual desilylation, acetylation and *t*-butyl ester hydrolysis yielded the dicarboxylic acid **13** in good yield.

The presumed critical spacing of the C-6 and C-4' hydroxyl moieties on the biological activity was examined by the preparation of additional derivatives. The assembly of the lower homolog **14** was accomplished *via* the procedure employed for the synthesis of analog **9** utilizing the one carbon shorter C-1 side-chain[9] **4** (n=0) and phenoxyundecanoic acid as the C-6 ester counterpart. A related chain-shortened derivative **16** was prepared employing tartronic acid as the backbone as opposed to the malate skeletal framework. Preparation of di-*t*-butyl tartronate followed by selective monoesterification with 11-phenoxyundecanoic acid and subsequent *t*-butyl ester hydrolysis with TFA in CH_2Cl_2 furnished the tartronyl phenoxyundecanoate.



Elaboration to the monocarboxylic acid was performed as usual affording derivative **15** in good yield. However, the final esterification with di-*t*-butyl malate under normal conditions failed to yield any desired ester. This was overcome by employing Mitsunobu conditions involving treatment of **15** with one mole-equiv. of diethylazodicarboxylate, triphenylphosphine, and di-*t*-butylmalate **1**. Sequential desilylation, acetylation and deprotection yielded the dicarboxylic acid **16**.

TABLE

Compound and #	RLSS (ZA-A = 0.35 nM)	Compound and #	RLSS (ZA-A = 0.35 nM)
 **9**	470 nM	 **13**	200 nM
 **10**	300nM	 **14**	200 nM
 **12**	>3000 nM	 **16**	>3000 nM

Based on the rat liver squalene synthase (RLSS) enzyme assay, a few general conclusions can be drawn from the bioactivities of compounds shown in the Table. As seen throughout the SAR studies of the zaragozic acids, compounds with lipophilic C-6 side-chains show comparable potencies in the RLSS enzyme assay (compare **9** and **13**). The structure of the polar core seems to have little effect on the bioactivity as long as it is a dicarboxylic acid (compare **9** and **12**).[10] Optimal activity in the acyclic series was anticipated for derivatives that best approximate the spatial relationship between the C-6 and C-4' esters of the natural products. Neglecting the constraints imposed by the bicyclic ring system of the natural products, the simplest approach involved examination of the 7-atom tether length between the C-6 and C-4' positions. Comparison of the analogs **13** and **14**, which are equipotent in the RLSS assay, indicates some flexibility in the chain length requirement. However, the contrast of activities for compounds **14** and **16** reveal that the C6/C4' spacing is not the only factor of importance. The absence of spacing in the original malate backbone, while maintaining the

seven atom tether and dicarboxylic acid moiety, afforded a derivative (**16**) with no comparable activity. This may be due more to the increased acidity of this tartronate diester, as opposed to that of the malate, than to the actual tether length between the C-6 and C-4' hydroxyl appendages.

References and notes

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8. The yields of all intermediates in like reaction in each preparation are comparable and typically as represented in this scheme and all compounds gave spectra that are consistent with their assigned structures and the final products **9, 10, 12, 13, 14, 16** are recorded as follows: Compound (**9**): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34-7.08 (m, 5H), 6.89 (dd, J=15.75, 8.37Hz, 1H), 5.81 (d, J=15.75 Hz, 1H), 5.50 (t, J=5.61Hz, 2H), 4.89-4.86 (m, 1H), 4.17-4.09 (m, 2H), 3.08-2.86 (m, 4H), 2.71 (dd, J=13.15, 4.89 Hz, 1H), 2.46-2.23 (m, 2H), 2.09 (s, 3H), 2.94 (br s, 1H), 1.72-1.50 (m, 4H), 1.40-1.18 (m, 4H), 1.14-1.03 (m, 2H), 1.00 (d, J=6.59 Hz, 3H), 0.98-0.78 (m, 8H); MS FAB-POS m/z 657.0 [M+Na]; FAB-POS-MB/Li 647.2 [M+2Li], 653.1 [M+3Li].; (**10**) ^{1}H NMR (400 MHz, CD_3OD) δ 7.27-7.11 (m, 5H), 6.87 (dd, J=15.63, 8.42 Hz, 1H), 5.84 (d, J=15.63 Hz, 1H), 5.53-5.40 (m, 2H), 5.08-5.03 (m, 1H), 4.72 (br s, 1H), 4.27-4.09 (m, 2H), 3.08-2.98 (m, 2H), 2.72 (dd, J=13.33, 5.44 Hz, 1H), 2.5-2.32 (m, 2H), 2.14 (br s, 1H), 1.87-1.73 (m, 2H), 1.70-1.57 (m, 2H), 1.56-1.23 (m, 4H), 1.18-1.07 (m, 2H), 1.01 (d, J=6.64 Hz, 3H), 0.90 (d, J=6.83 Hz, 3H), 0.89-0.78 (m, 8H).; MS FAB-NEG m/z 703.; (**12**) ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.27-6.85 (m, 10H), 5.52-5.49 (m, 1H), 4.88-4.86 (m, 1H), 4.58-4.56 (m, 1H), 4.23-4.04 (m, 2H), 3.92 (t ,J=6.58Hz, 2H), 3.62-3.58 (m, 1H), 3.47-3.44 (m, 1H), 2.97-2.85 (m, 2H), 2.74 & 2.71 (2d, J=5.13 & 4.76 resp. total 1H), 2.42-2.20 (m, 4H), 2.06 (s, 3H), 2.06-1.87 (m, 3H), 1.78-1.71 (m, 2H), 1.66-1.56 (m, 6H), 1.45-1.38 (m, 2H), 1.30 (br s, 10H), 0.83 (d, J=6.87 Hz, 3H).; MS FAB-NEG m/z 722.; (**13**) ^{1}H NMR (400 MHz, CD_3OD) δ 7.26-7.12 (m, 8H), 6.90-6.86 (m, 2H), 5.48-5.43 (m, 2H), 4.86-4.82 (m, 1H), 4.24-4.10 (m, 2H), 3.96 (t , J=6.46 Hz, 2H), 3.31-3.29 (m, 2H), 3.08-3.04 (m, 2H), 2.73 (dd, J=13.33, 5.54 Hz, 1H), 2.41-2.33 (m, 2H), 2.05 (s, 3H), 2.03-1.94 (m, 1H), 1.81-1.53 (m, 6H), 1.51-1.41 (m, 2H), 1.32 (br s, 12H), 1.86 (d, J=6.87 Hz, 3H).; MS FAB-POS m/z 751.4 [M+Na].; (**14**) ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.27-7.08 (m, 8H), 6.92-6.8 (m, 2H), 5.50 (br s, 1H), 5.44 (t , J=5.74 Hz,1H), 4.94-4.90 (m, 1H), 4.26-4.13 (m, 1H), 4.14-4.10 (m, 1H), 3.92 (t , J=6.59 Hz, 2H), 3.06-2.85 (m, 3H), 2.72 (dd, J=13.47, 5.13 Hz, 1H), 2.4-2.21 (m, 3H), 2.06 (s, 3H), 2.01-1.86 (m, 2H), 1.79-1.67 (m, 2H), 1.64-1.52 (m, 2H), 1.47-1.37 (m, 2H), 1.27 (br s, 12H), 0.85 (d, J=6.67 Hz, 3H).; MS FAB-POS m/z 751.6 [M+Na]; (**16**) ^{1}H NMR (400 MHz CD_3OD) δ 7.26-7.13 (m, 8H), 6.90-6.86 (m, 2H), 5.64 & 5.61 (2s, 1H), 5.50-5.48 (m, 1H), 4.29-4.18 (m, 2H), 3.94 (t, J=6.46 Hz, 2H), 3.0-2.7 (m, 3H), 2.45 (t , J=7.38 Hz, 2H), 2.38-2.31 (m, 1H), 2.06 (s, 3H), 2.01-1.98 (m, 1H), 1.77-1.61 (m, 7H), 1.52-1.42 (m, 2H), 1.32 (br s, 12H), 0.86 (d, J=6.82 Hz, 3H).; MS FAB-POS m/z 751.5 [M+Na].
9. This compound, a one carbon shorter chain than **4** was prepared from **4** by sequential Swern oxidation, enolsilylation, ozonolysis followed by reductive work-up ($NaBH_4$). This sequence was first carried out by Dr. H. Koyama of this laboratory.
10. In addition, monocarboxylic acid derivatives of **5** (*i.e.* C-4' = OH, OAc) showed activity not less than 3000 nM in the RLSS enzyme assay.

(*Received in USA* 22 *March* 1994; *accepted* 17 *May* 1994)