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SULFOBETAINE ZWITTERIONIC INHIBITORS OF SQUALENE SYNTHASE

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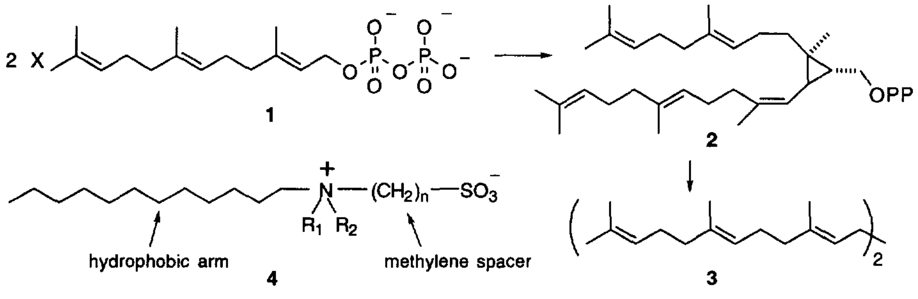
Abstract: Zwitterions such as farnesyl sulfobetaine **8** are a new type of inhibitor of squalene synthase that, while maintaining overall neutrality, mimic both the carbocationic and anionic moieties of presumed reaction intermediates. The most effective zwitterionic sulfobetaines discovered to date are aromatic derivatives **17** (IC_{50} = 2 μM) and **20** (IC_{50} = 5 μM). Copyright © 1996 Elsevier Science Ltd

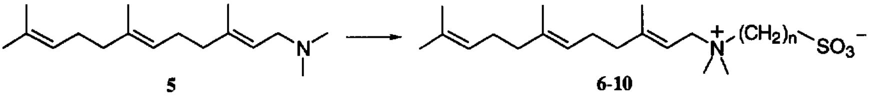
In recent years there has been intensive effort directed toward development of inhibitors of squalene synthase (SS),[1] the enzyme that catalyzes the remarkable reductive dimerization of two farnesyl diphosphates (FPP, **1**) via presqualene diphosphate (**2**) to form squalene (**3**). As the catalyst of the first step in cholesterol biosynthesis committed exclusively to sterol formation, SS presents an attractive target for inhibition that does not also have the potential for depletion of essential non-sterol isoprenoid metabolites. An impressive array of structurally diverse inhibitors of SS has been reported.[1] Extensive modifications of both the hydrophobic and polar portions have been explored in analogs of **1** and **2**, and natural product screening revealed the extremely potent zaragozic acids (squalestatins).[1] Compounds containing ammonium ions have been successfully used as mimics of putative carbocation intermediates in the conversions of both **1** to **2** and **2** to **3**.[1] Incorporation of an appropriate surrogate for the diphosphate group has been essential in the design of SS inhibitors because compounds containing that moiety are labile to esterases and have difficulty crossing cell membranes due to their strong charge.[1] On the other hand, anionic character appears to be necessary for binding to SS.[2] This Letter describes a new approach to incorporation of the required attributes in SS inhibitors by the use of simple sulfobetaines, a well known, readily prepared type of zwitterion,[3] shown schematically in **4**.



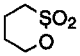
1 **2** **3** **4**

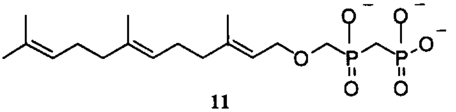
Initial efforts were to determine whether such sulfobetaines would, in fact, inhibit SS, and if so, what the optimum separation between the ionic centers would be. To this end, zwitterions **6-10**, containing bridges of 2-6 methylene groups between the ammonium and sulfonate ions, were prepared from farnesyldimethylamine (**5**)[4] as indicated in Scheme 1. The resulting sulfobetaines were evaluated in a standard microsomal assay for SS inhibition,[5] yielding the IC_{50} values indicated in Scheme 1. Compound **8**, with a four methylene group spacer is clearly the best inhibitor among these compounds. Interestingly, the total chain length of **8** is the same as that in Biller's potent SS inhibitor **11**,[6] rather than that in FPP (**1**). To determine if the methyl substituents on N in **8** were undesirably bulky, zwitterion **12** was prepared analogously from farnesylamine and found to be inferior to **8** as an SS inhibitor, having IC_{50} = 150 μM.

Scheme 1

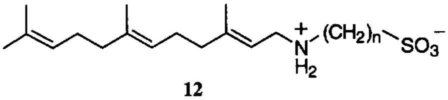


5 **6-10**

Reagents Used with **5**	Compound Produced	IC_{50} (μM)
(1) $BrCH_2CH_2Br$; (2) Na_2SO_3	**6**, n = 2	200
	**7**, n = 3	200
	**8**, n = 4	15
(1) $Br(CH_2)_5Br$; (2) Na_2SO_3	**9**, n = 5	200
(1) $Br(CH_2)_6Br$; (2) Na_2SO_3	**10**, n = 6	200



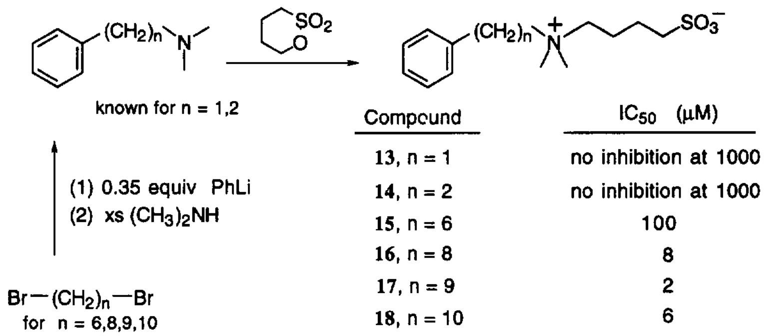
11



12

Aromatic rings are found at the ends of at least one of the side chains in the zaragozic acids, and their introduction into the hydrophobic portion of a variety of SS inhibitors has proved effective,[1] so it was decided to incorporate a phenyl ring into zwitterionic sulfobetaine inhibitors. Accordingly, compounds **13-18**, with methylene connectors of varied length between the ammonium ion moiety and a phenyl group, were synthesized as shown in Scheme 2. Evaluation of **13-18** in the microsomal SS assay gave the IC_{50} values indicated, and showed that **17**, with a nine methylene group connector, is the best inhibitor, having IC_{50} = 2 μM, almost an order of magnitude more potent than **8**.

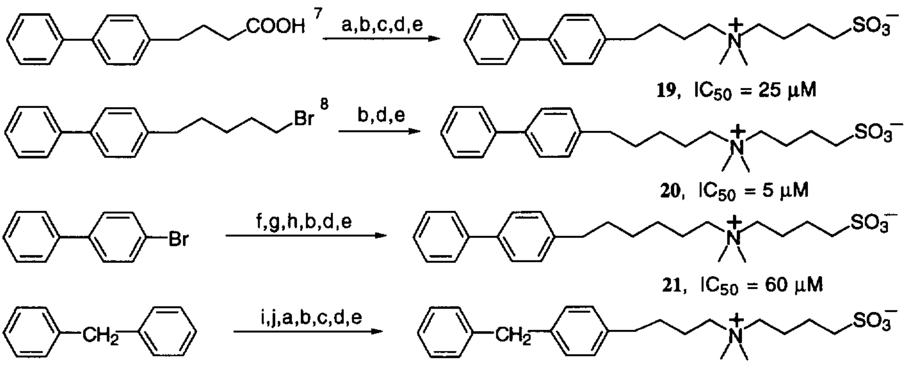
Scheme 2



Compound	IC_{50} (μM)
13, n = 1	no inhibition at 1000
14, n = 2	no inhibition at 1000
15, n = 6	100
16, n = 8	8
17, n = 9	2
18, n = 10	6

Incorporation of a second phenyl group was explored next with compounds **19**-**22**. The syntheses of these compounds are outlined in Scheme 3. Preparation of **19** proceeded from the known intermediate shown, prepared via Friedel-Crafts acylation,[7] and synthesis of **22** followed an analogous sequence. Preparation of **20** and **21** was based on a work by Lee et al.,[8] followed by standard transformation into the target zwitterions. The IC_{50} values for **19**-**22**, indicated in Scheme 3, show that **20**, with 5 methylene groups between the phenyl group and the nitrogen, is the best SS inhibitor, with an IC_{50} value of 5 μM, almost as low as that of **17**. The diphenylmethylene derivative **22** proved to be relatively ineffective, with IC_{50} = 200 μM.

Scheme 3



19, IC_{50} = 25 μM

20, IC_{50} = 5 μM

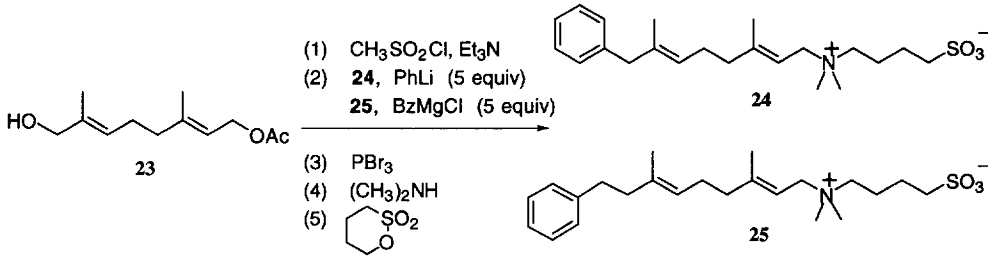
21, IC_{50} = 60 μM

22, IC_{50} = 200 μM

Reagents: (a) ClCOCOCl; (b) $(CH_3)_2NH$; (c) $LiAlH_4$; (d) $Br(CH_2)_4Br$; (e) Na_2SO_3; (f)[8] $(Ph_3)Pd$, CuI, Et_3N, $HCC(CH_2)_3CH_2OH$; (g) H_2, Pd/C; (h) PBr_3; (i)[7] O O O , $AlCl_3$; (j) H_2NNH_2, KOH, 180 °C.

It seemed obviously desirable to try to combine the effectiveness of a terminal phenyl group of **17** with that of the natural farnesyl group of **8**. To that end, compounds **24** and **25** were synthesized from the known geraniol-derived **23**,[9] as described in Scheme 4. Both of these compounds proved to be disappointing as SS inhibitors, exhibiting IC_{50} values of 500 and 600 μM, respectively. Nonetheless, this study has demonstrated that relatively simple sulfobetaine zwitterions can be effective SS inhibitors in vitro, and efforts continue to design such zwitterions with even greater potency.

Scheme 4



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