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SQUALENE SYNTHASE INHIBITORS: ISOSTERIC REPLACEMENTS OF THE FARNESYL CHAIN OF BENZYL FARNESYL AMINE

John A. Brinkman, Robert E. Damon, Jay B. Fell,*Lawerence B. Perez,
Terence J. Scallen,† and T.R. Vedananda,

Department of Atherosclerosis and Vascular Biology, Preclinical Research, Sandoz Research Institute, Sandoz Pharmaceuticals Corporation, East Hanover, N.J. 07936 and †Department of Biochemistry, University of New Mexico School of Medicine, Albuquerque, N.M. 87131

Abstract. Squalene synthase catalyzes the committed step of cholesterol biosynthesis. We report here the synthesis and in vivo activity of a series of squalene synthase inhibitors that contain isosteric replacements for the farnesyl chain of the known inhibitor benzyl farnesyl amine. Copyright © 1996 Elsevier Science Ltd

Introduction. Elevated plasma low-density lipoprotein (LDL) cholesterol levels are an established risk factor for atherosclerosis. One strategy for lowering serum LDL-cholesterol is to decrease cholesterol biosynthesis.[1] Recently HMG-CoA reductase inhibitors, the statins for example, have proven to be an effective therapy for serum LDL-cholesterol lowering.[2] They inhibit cholesterol biosynthesis by reducing levels of mevalonic acid, an early cholesterol precursor, in the liver. As an alternate strategy we chose squalene synthase as a target for therapeutic intervention. Squalene synthase(SS) is a microsomal enzyme that catalyses the reductive dimerization of farnesyl pyrophosphate (FPP) via presqualene pyrophosphate to produce one molecule of squalene in the committed step of cholesterol biosynthesis.[3] Inhibition at this stage is attractive because the use of mevalonate in nonsteroidal pathways will be minimally affected. For a recent review on SS inhibitors see Biller et al.[4] Previous studies have suggested that several putative carbocationic intermediates are involved in the mechanism by which SS catalyzes the linking of two FPP molecules to generate squalene.[3,5] Compounds containing ammonium or sulfonium cations designed to be mimics of these carbocationic intermediates are known inhibitors of the microsomal enzyme.[5] Benzyl farnesyl amine, a putative carbocationic mimic, inhibits SS in vitro with an IC_{50} of 100 nM.[6] We report here the synthesis and activity of a series of secondary amine squalene synthase inhibitors with isosteric replacement of the farnesyl chain of benzyl farnesyl amine (Figure 1).

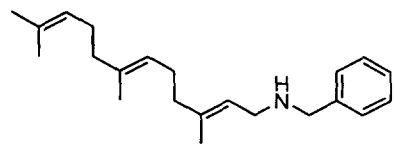

Figure 1. Benzyl Farnesyl amine

Chemistry. Figure 2 highlights the synthesis of the target compounds **1-8** from commercially available materials. Analog **1** was prepared from benzyloxybenzyl alcohol **9.** The alcohol is converted to the bromide by treatment with PBr_3 in THF. This halide was used to alkylate benzylamine to give compound **1**. Analog **2** was prepared from benzyloxyphenol **10**. Alkylation with 1,2-dibromoethane in the presence of K_2CO_3 followed by N-alkylation of benzylamine in DMF gave the desired product. Analogs **3** and **4** were prepared similarly by N-alkylation of phenethylamine and 2-phenylpropylamine respectively. Compound **5** was obtained by initial alkylation of benzyloxyphenol **10** with 2-bromoethylether followed by N-alkylation of benzylamine. Alkylation of 4-methoxyphenol **11** using 1,2-dibromoethane followed by N-alkylation of benzylamine as previously described gave the product **6**. Products **7** and **8** were synthesized from 4-hydroxystilbene **12**. Alkylation with 1,2-dibromoethane in the presence of K_2CO_3 followed by alkylation of benzylamine or 2-phenylpropyl amine gave **7** and **8**, respectively.

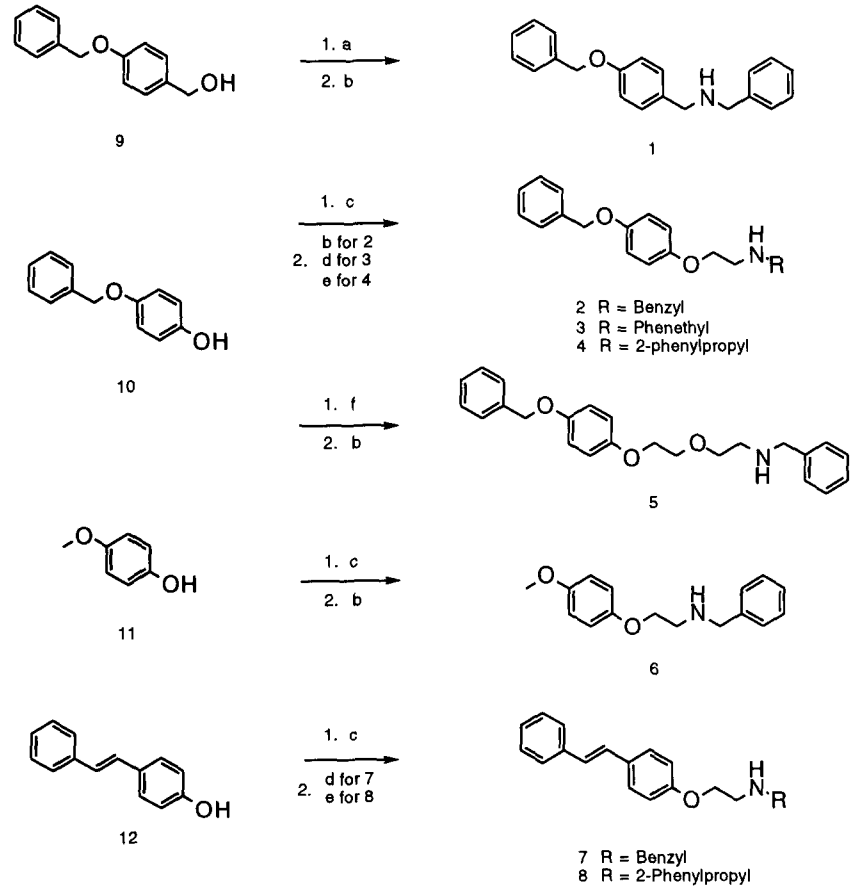

Figure 2. (a) PBr_3, THF, 0 °C, 1 h; (b) benzylamine, K_2CO_3, DMF, reflux, 18 h; (c) 1,2-dibromoethane, K_2CO_3, reflux, 18 h; (d) phenethylamine, K_2CO_3, DMF, reflux, 18 h; (e) 2-phenylpropylamine, K_2CO_3, DMF, reflux, 18 h; (f) dibromoethylether, K_2CO_3, 130 °C, 18 h.

Discussion and Conclusion. Compounds **1-8** were tested for squalene synthase inhibitory activity in a rat liver microsomal assay without added PPi[7], their IC_{50}s are listed in Table 1 along with the IC_{50} of benzyl farnesyl amine. Compound **1** was designed with an isosteric replacement of the farnesyl chain of benzyl farnesyl amine (Figure 1). The IC_{50} of **1** is comparable to the IC_{50} that was previously reported for benzyl farnesyl amine[6]. In an effort to maximize the activity of compound **1**, the length requirements of the side chain were investigated. Lengthening the side-chain by addition of "O-CH_2" gave analog **2** that maintained activity in the squalene synthase microsomal assay. However, compounds **5** obtained by further lengthening of the side chain resulted in loss of activity. Compound **6** in which the terminal phenyl group was removed was also inactive.

Table I

compound[a]	squalene synthetase inhibitory activity IC_{50} (nM)	compound[a]	squalene synthetase inhibitory activity IC_{50} (nM)
Benzylfarnesyl amine[b]	100	**5**	29000
1	55	**6**	89000
2	70	**7**	9
3	15	**8**	9
4	10		

[a] All of the compounds gave satisfactory spectral and analytical data.
[b]Previously synthesized (see reference 6).

It has been hypothesized that benzyl farnesyl amine is a mimic of one of the carbocationic intermediates in the biosynthesis of squalene from FPP[7]. The secondary amine of benzyl farnesyl amine is expected to be protonated at physiological pH to give a carbocation mimic. If this is true, replacement of the farnesyl chain may cause a positional shift of the proposed carbocation mimic in the binding pocket of the enzyme. Compounds **3** and **4** were synthesized to modify the ammonium ion placement in the binding pocket. Both compounds showed enhanced potency relative to **1**, compound **4** had an in vitro IC_{50} of 10 nM.

Further rigidification of the side chain by replacement of the "CH_2-O" in **2** with a trans-olefin led to compound **7**. This compound showed an IC_{50} of 9 nM in the microsomal assay. Compound **8** was synthesized to combine our most interesting farnesyl chain replacement with the modified ammonium ion placement. This compound was equipotent with compounds **4** and **7**.

In summary, benzyl farnesyl amine is a hypothesized mimic of a putative carbocationic intermediate in the biosynthetic conversion of farnesyl pyrophosphate to squalene by squalene synthase. Isosteric replacements of the farnesyl chain led to the series of secondary amines **1-8**. Several analogs **4**,**7**, and **8** possessed activity that was an order of magnitude better in vitro than the parent benzyl farnesyl amine.

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7. Assay details previously reported reference 6. Note. The FPP concentration in the assay was incorrectly reported as 10 mM. The correct value should be 10 μM.
8. Note. During the preparation of this manuscript a communication reporting phenoxypropylamines as squalene synthase inhibitors appeared. Brown, G. R.; Butlin, R. J.; Chapman, S.; Eakin, M. A.; Foubister, A. J.; Freeman, S.; Griffiths, D.; Harrison, P. J.; Johnson, M. C.; Mallion, K. B.; McTaggart, F.; Reid, A. C.; Smith, G. J.; Taylor, M. J.; Walker, R. P.; Whittamore, P. R. O. *J. Med. Chem.* **1995**, *38*, 4157.

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