Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 7, No. 23, pp. 3039-3044, 1997

0960-894X/97 $17.00 + 0.00

PII: S0960-894X(97)10133-0

N-HYDROXYGLYCINE DERIVATIVES AS NOVEL INHIBITORS OF SQUALENE SYNTHASE[1]

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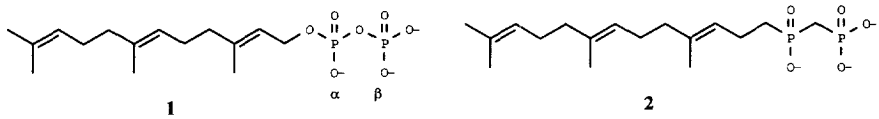
Abstract: The squalene synthase inhibiting properties of farnesyl diphosphate (FPP) mimics, **3** and related analogues, are described. The results indicate that the nonphosphorus-containing N-hydroxyglycine is a novel replacement for the diphosphate group. Further optimization of **3** has led to **12**, a new and potent squalene synthase inhibitor. © 1997 Elsevier Science Ltd.

The inhibition of cholesterol biosynthesis constitutes an important approach to the reduction of low density lipoprotein cholesterol (LDL-C).[2] Squalene synthase occupies a branchpoint in the isoprenoid pathway, catalyzing the reductive dimerization of farnesyl diphosphate (FPP, **1**) to squalene via the intermediate cyclopropane, presqualene diphosphate.[3a] Because its inhibition maintains the biosynthesis of nonsterol mevalonate derived products, squalene synthase is an attractive target for pharmacological intervention in order to lower serum cholesterol.[3b]

Early efforts at inhibiting squalene synthase used diphosphate-containing compounds.[3] Both negatively ionizable phosphate groups of FPP are crucial for substrate binding, presumably through electrostatic interactions. However, problems associated with the labile and highly charged diphosphate moiety made it unsuitable as a component of an orally administered drug substance, and thus considerable effort has been invested in an attempt to find a replacement.[4]

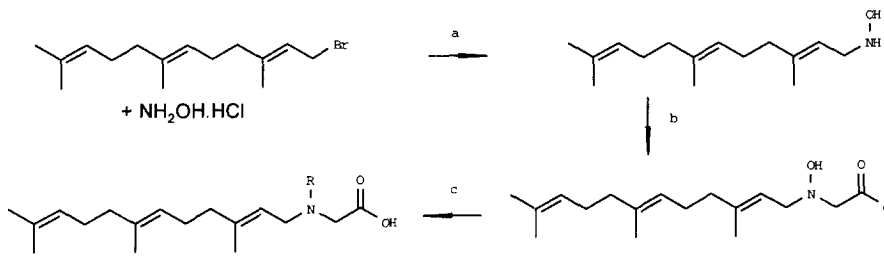
In search for new and therapeutically useful FPP analogues as squalene synthase inhibitors,[5] we were interested in identifying a nonphosphorus-containing mimic of the diphosphate group and its optimization. We chose to replace the β-phosphate group of FPP with the carboxyl group based on prior studies with inorganic diphosphate analogues[6] and sought a better replacement of the α-phosphate group. We expected that a replacement of this weakly basic moiety[7a] with a more strongly basic group should bind better with the enzyme via electrostatic interactions[7b] and this might compensate for the loss of other interactions due to the structural changes.[7c] The N-hydroxyl group, with an estimated pK_a of 5, seemed to be an ideal moiety[8] and led to the design of N-hydroxyglycine **3** and related analogues.



1 2

Results and Discussion

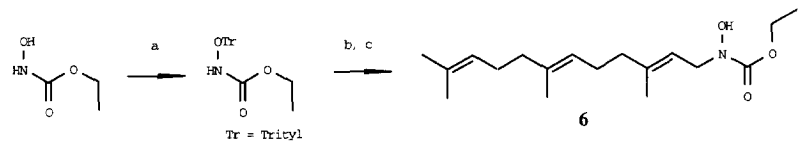
The syntheses of **3–5** are summarized in Scheme 1. Treatment of *trans,trans*-farnesyl bromide with hydroxylamine hydrochloride in dimethylformamide followed by the alkylation with methyl bromoacetate in dichloromethane gave **4**. Hydrolysis of **4** with bis(tributyltin) oxide[9] in toluene afforded the N-hydroxyamino acid **3**.[10] Similar treatment of methyl bromoacetate with *trans,trans*-farnesylamine[5] in dichloromethane (Et_3N, –5 °C, 4.5 h) followed by basic hydrolysis in ethanol (1 N NaOH, rt, 2 days) and subsequent neutralization (1 N HCl) gave **5**.



3: R = OH; 5: R = H **4**

Scheme 1: (a) Et_3N, DMF, 65 °C, 6 h; (b) methyl bromoacetate, CH_2Cl_2, Et_3N, rt, 17 h; (c) bis(tributyltin)oxide, toluene, 18 h.

The synthesis of **6** is outlined in Scheme 2. Oxygen protection of N-hydroxyurethane with the trityl group followed by the Mitsunobu reaction with *trans,trans*-farnesol and removal of the trityl group gave the N-hydroxycarbamate **6**.



Tr = Trityl **6**

Scheme 2: (a) Trityl chloride, Et_3N, DMF, rt, 24 h; (b) *trans,trans*-farnesol, DEAD, Ph_3P, THF, rt, 24 h; (c) 70% TFA/H_2O, CH_2Cl_2, 0 °C, 10 min.

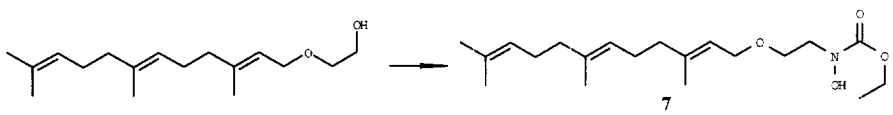
Compounds were evaluated for squalene synthase inhibitory activity in a rat liver microsomal assay[5b] and their IC_{50} values are listed in Table 1. The nonphosphorus-containing compound **3** inhibited squalene synthase with an IC_{50} of 5.0 μM. Its inhibitory activity was essentially the same as that of the known FPP

mimic, phosphinylmethyl phosphonate **2**, when tested side by side in the same assay.[4a,11] This result suggested that the N-hydroxyglycinate moiety is a suitable surrogate for the diphosphate group. The pK_a's of the carboxy and N-hydroxy groups of **3** have been determined to be 4.07 and 5.05, respectively, suggesting the ionizability of both groups at physiological pH.[12] Both ionizable anionic groups are important for its enzyme binding. Blocking the carboxyl group as an ester **4** or removing the N-hydroxy group as in **5** resulted in a loss of more than 100-fold in potency. The N-hydroxycarbamate **6** is about sixfold less active than **3**. Its ability to tautomerize to an iminol form may partly explain its 17-fold better binding than the monoanionic N-hydroxy ester **4**.[13]

Table 1. In vitro Squalene Synthase Inhibitory Activity[4b]

Compound	IC_{50} (μM)	Compound	IC_{50} (μM)
1	-	**7**	21
2	7	**8**	27
3	5	**9**	14
4	523	**10**	29
5	640	**11**	87
6	30	**12**	0.23

Once the nonphosphorus mimic of the diphosphate was identified, our effort was devoted to potency optimization of **3**. An earlier report[14] indicated that an ether oxygen insertion between the farnesyl chain and the diphosphate mimic group of **2**, created a profound increase in inhibitory activity. Hydrogen bonding of the ether oxygen linker to an active-site acid catalyst was postulated as a key contributor to this activity enhancement.[14] In order to reveal the H-bond linker that is important for the binding improvement of **3**, compounds **7**–**12** were synthesized.



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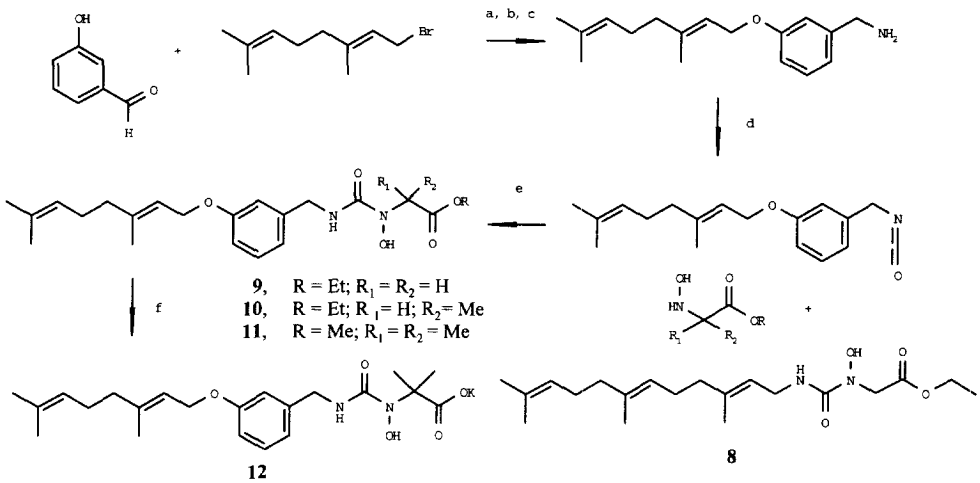
Compound **7** was prepared via Misunobu reaction in an analogous manner to the synthesis of **6** (Scheme 2). Compounds **9**–**12** were synthesized as shown in Scheme 3. Their isocyanate precursor was readily prepared from the commercially available 3-hydroxybenzaldehyde and geranyl bromide. Condensation of the isocyanate with known N-hydroxyaminoacid esters[15] gave compounds **9**–**11**. Basic hydrolysis of esters **9** and **10** failed to give cleanly the corresponding free acids. Saponification of the gem-dimethyl ester **11**, however, gave the desired carboxylate **12**. Compound **8** was prepared similarly from farnesylamine.[16]



Scheme 3: (a) K_2CO_3, DMF; (b) $H_2NOH.HCl$, 1 N NaOH; (c) Zn, NH_4OH, NH_4OAc, EtOH, 78 °C; (d) $COCl_2$, toluene, 50 °C; (e) THF, 0 °C, rt, 17 h; (f) KOTMS, THF, rt then MeOH.

The ether analogue of compound **6** (**7**) showed no improvement in binding (Table 1). The amide linker **8**, however, showed significant activity increase (~19-fold) in comparison with the ester **4**. The corresponding phenoxy chain analogue **9** showed an additional twofold improvement in activity. Its methyl substituted analog **10** showed a twofold reduction in activity. Failures in saponifying esters **9** and **10** led to the synthesis of the gem-dimethyl analogs **11** and **12**. The ester **11** showed further loss of activity in comparison with **9** and **10**. However, despite the bulky gem-dimethyl substituent, the carboxylate **12** demonstrated a 21-fold enhancement in inhibitory activity than its parent **3**. These results suggest that the amide linker contributed remarkably to the binding affinity of this class of squalene synthase inhibitors potentially through its hydrogen bonding interactions. Additional studies were carried out with **12** using purified rat hepatic squalene synthase and the results are summarized in Table 2. The truncated form of rat squalene synthase was purified and assayed foractivity as previously described.[17] The K_m value for NADPH with the truncated rat enzyme is 0.04 mM. The compound was thus tested below and above the K_m value for NADPH. These results indicate that **12** does not bind in the NADPH binding site since the increased occupancy of the NADPH binding site enhances inhibitor effectiveness 60-fold. In addition, inorganic pyrophosphate further enhanced inhibitor effectiveness by decreasing the apparent IC_{50} value to 0.17 μM. Such an effect with inorganic pyrophosphate has previously been observed with other squalene synthase inhibitors.[3b]

Table 2. Effects of NADPH and Inorganic Pyrophosphate on Squalene Synthase Inhibition

Compound	IC_{50} (μM) @ 0.025 mM NADPH	IC_{50} (μM) @ 1.2 mM NADPH	IC_{50} (μM) @ 1.2 mM NADPH + 1.0 mM PP_i
12	57 ± 6	0.95 ± 0.07	0.17 ± 0.005

In conclusion, we have shown that the N-hydroxyglycinate moiety is a novel replacement of the diphosphate group of FPP. This is the first report on the use of N-hydroxyglycine as a surrogate for an organic diphosphate. Using **3** as a starting point, we have identified **12** as a potent and new structural class of non-phosphorus-containing squalene synthase inhibitors. The results reported herein provide a novel direction for further development of useful squalene synthase inhibitors.

Acknowledgments. We would like to thank Jeff Chin and Dr. Mike Shapiro for NMR characterization of key compounds. We also thank Mr. G. Diamantidis for technical assistance and Professor R. Abeles (Brandeis) for helpful discussions.

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8 The pK_a value was estimated by Computer Assisted Mechanistic Evaluation of Organic reactions (CAMEO) program, Version 89, developed by W. L. Jorgensen, Mechanistic Evaluation of Organic reaction (CAMEO) program, Version 89, developed by W. L. Jorgensen, Purdue University, IN, USA.

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10. The poor yield (20–30%) obtained is most likely due to the β-elimination of the N-hydroxy group of **3** under basic conditions.

11. Though **2** was inactive as a cholesterol biosynthesis inhibitor in rat hepatocytes, a failure that was attributed to the inability of polyanions to penetrate the cell membrane, it has been reported to bind to squalene synthase with binding affinities comparable to those of the natural substrate **1**. Our results in Hep G2 cells showed that **2** inhibited cholesterol synthesis with an IC_{50} of greater than 500 μM, under these assay conditions, **3** showed an IC_{50} of 30 μM.

12. The pK_a's in water of **3** were determined by pH titration with the Mettler DL21 at Robertson Microlit Lab, NJ.

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16. All compounds were characterized by NMR, Mass Spectroscopy, TLC or HPLC, and elemental analysis for selected compounds.

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(*Received in USA* 18 *September* 1997; *accepted* 28 *October* 1997)