N-(Arylalkyl)farnesylamines: New Potent Squalene Synthetase Inhibitors

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Because of its strategic location in the cholesterol biosynthesis pathway, squalene synthetase enzyme is an attractive target for inhibition. Squalene synthetase catalyzes the transformation of farnesyl pyrophosphate (FPP) to squalene in two steps.[1] The first step is the condensation of two molecules of FPP to presqualene pyrophosphate (PSPP), and the second step involves the reductive rearrangement of PSPP to squalene. Both steps have been hypothesized to involve several putative carbocation intermediates.[2] Ammonium and sulfonium ion analogs that mimic the electrostatic properties of the putative carbocation intermediates have been reported[2-4] to inhibit the first and second steps of the FPP to squalene conversion. Potency of these inhibitors was rather low when compared to newly described potent inhibitors derived from a pyrophosphate mimic approach[5] and from natural sources[6] (squalestatins and zaragozic acids). In a program to synthesize squalene synthetase inhibitors as agents for the treatment of hypercholesterolemia, we sought to design analogs of the carbocation **I** (Figure 1), a carbocation that may undergo reduction with NADPH to produce squalene in the second step of squalene biosynthesis from FPP. Based on mimicking the electrostatic properties of the carbocation **I** by an ammonium ion, we report herein *N*-(arylalkyl)farnesylamine derivatives, which would get protonated at physiological pH to generate an ammonium ion, as potent squalene synthetase inhibitors.

Replacement of the carbon possessing the positive charge in carbocation **I** by an ammonium ion led to the structure **II** (Figure 1), which, being an enamine, would present a stability problem. We envisioned two possible ways to overcome the instability problem associated with **II**. The first possibility was to insert a methylene unit between the nitrogen and the double bond, leading to the structure **III**. The second possibility was to hydrogenate the double bond adjacent to the nitrogen, and this led to the structure **IV**. Structures **III** and **IV** would be generated at the physiological pH by the protonation of amines **1** and **3**, respectively (Figure 2).

N,N-Difarnesylamine (**1**) was prepared by N-alkylation of *N*-farnesyltrifluoroacetamide[7] with farnesyl bromide in THF in the presence of sodium hydride and tetrabutylammonium bromide (TBAB) followed by hydrolysis with potassium hydroxide in refluxing aqueous methanol (Figure 2). To study the effect of steric bulk around nitrogen on the biological activity, *N*-ethyl-*N,N*-difarnesylamine (**2**) was synthesized by N-alkylation of *N*-farnesylacetamide[7] with farnesyl bromide in THF in the presence of sodium hydride and TBAB followed by

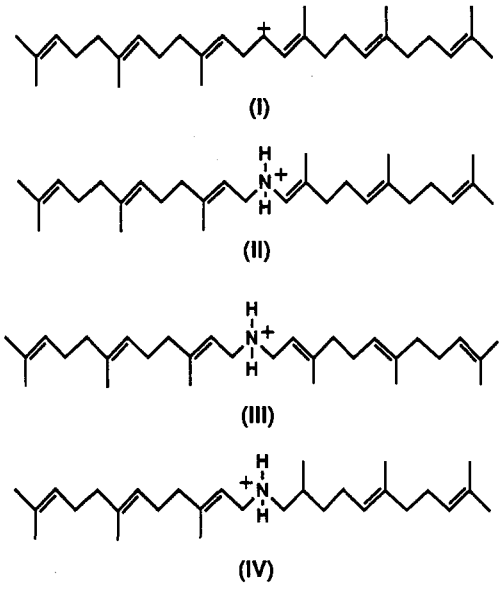

Figure 1.

reduction of the resulting *N,N*-difarnesylacetamide with LAH in refluxing ether. Synthesis of amine **3** involved several steps as depicted in Figure 2. Treatment of propionic acid with LDA in THF and HMPA followed by the alkylation of the resulting dianion[8] with homogeranyl iodide[9] yielded the acid **14**. Reaction of **14** with oxalyl chloride in benzene in the presence of catalytic amounts of DMF gave the corresponding acid chloride **15**. Condensation of **15** with farnesylamine[7] in methylene chloride in the presence of triethylamine followed by the reduction of the resulting amide with LAH in refluxing ether furnished the desired amine **3** in racemic form. In order to carry out a structure–activity relationship study in **3** to further optimize the biological activity, several analogs were synthesized (Figure 3). Analogs **4–6** were prepared from an appropriate N-substituted trifluoroacetamide (**16–18**) in two steps. N-Alkylation of **16–18** with farnesyl bromide in THF in the presence of sodium hydride and TBAB gave **19–21** in 60, 62, and 50% yields, respectively. Hydrolysis of **19–21** with potassium hydroxide in refluxing aqueous methanol furnished **4–6** in 80, 91, and 93% yields, respectively. The tertiary amine **7** was synthesized by N-alkylation of **22** with farnesyl bromide in THF in the presence of sodium hydride and TBAB followed by the reduction of the resulting amide with LAH in refluxing ether. *N*-Ethylfarnesylamine (**8**) was obtained by the reduction of *N*-farnesylacetamide with LAH in refluxing ether. Compound **10** was prepared by the condensation of acid chloride **15** with (3-pyridyl)methylamine in methylene chloride using triethylamine as a base followed by the reduction of the resulting amide with LAH in refluxing ether. *N*-Geranyl-*N*-(3-pyridyl)methylamine (**11**) was synthesized by N-alkylation of **17** with geranyl bromide in the presence of sodium hydride and TBAB followed by the hydrolysis with potassium hydroxide in refluxing aqueous methanol. Compound **9** was obtained as an inseparable mixture of isomers by the catalytic hydrogenation of **5** with 10% Pd–C in methanol. To probe the importance of the secondary amine nitrogen, which is necessary to mimic the positive charge of the cation, corresponding ether analogs **12** and **13** of amines **1** and **5** were prepared in 55 and 65% yields, respectively, by etherification of farnesol and (3-pyridyl)methanol with

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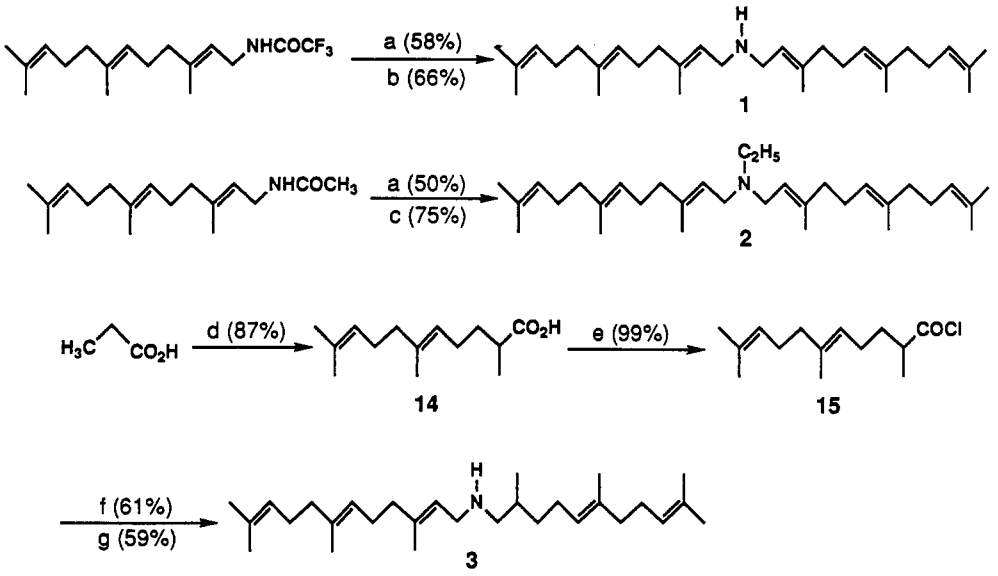

Figure 2. (a) NaH, THF, Bu_4NBr, farnesyl bromide, room temperature, 16 h; (b) KOH, MeOH, H_2, reflux, 16 h; (c) $LiAlH_4$, ether, reflux, 16 h; (d) LDA, THF, HMPA, homogeranyl iodide, 0 °C to room temperature, 2 h; (e) oxalyl chloride, DMF, benzene, 0 °C to room temperature, 1.5 h; (f) farnesylamine, triethylamine, CH_2Cl_2, 0 °C to room temperature, 2 h; (g) $LiAlH_4$, ether, reflux, 16 h.

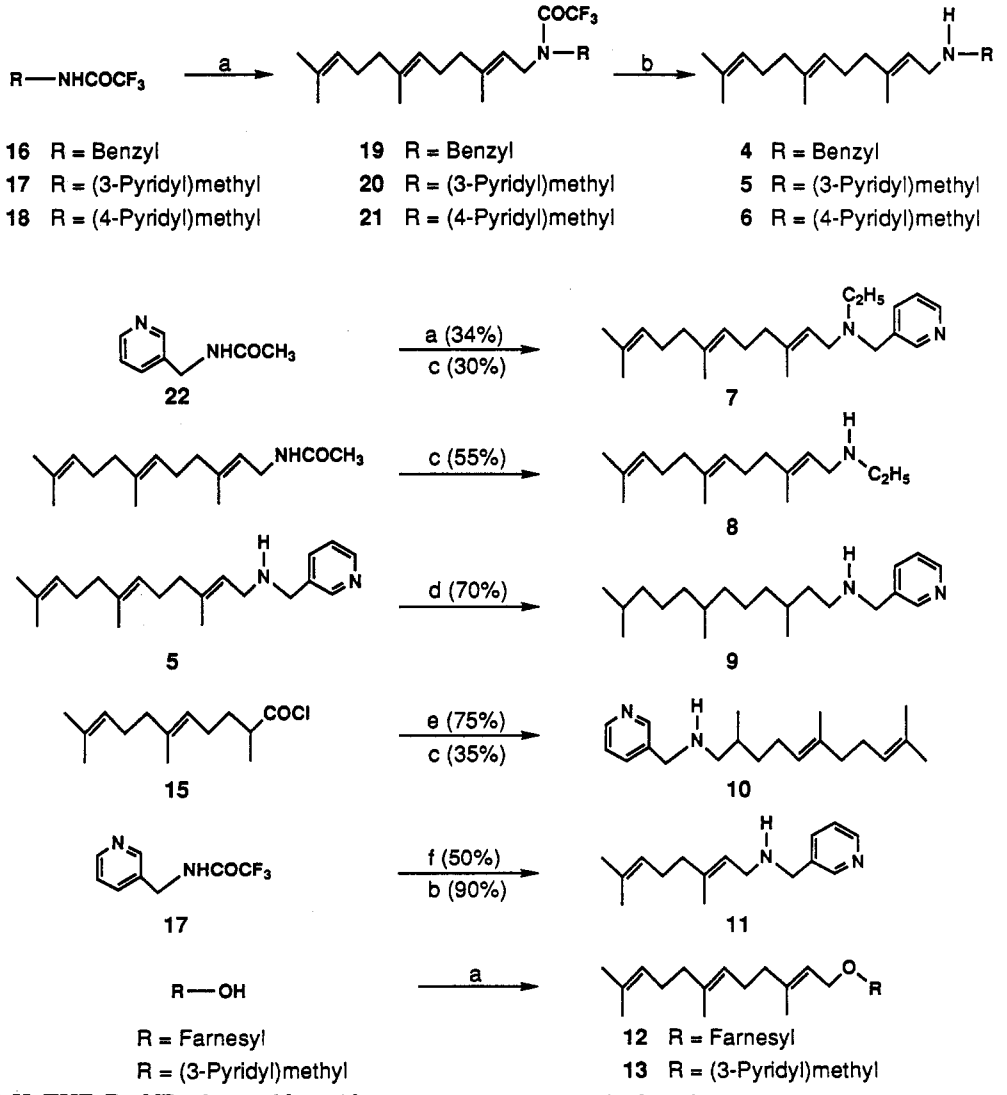

Figure 3. (a) NaH, THF, Bu_4NBr, farnesyl bromide, room temperature, 16 h; (b) KOH, MeOH, H_2O, reflux, 16 h; (c) $LiAlH_4$, ether, reflux, 16 h; (d) H_2, 10% Pd–C, MeOH, 6 h; (e) (3-pyridyl)methylamine, triethylamine, CH_2Cl_2, 0 °C to room temperature, 12 h; (f) NaH, THF, Bu_4NBr, geranyl bromide, room temperature, 16 h.

farnesyl bromide in THF in the presence of sodium hydride and TBAB.

Compounds 1–13 were tested in a rat liver microsomal assay[10] for squalene synthetase inhibitory activity, and

Table I

compound[a]	squalene synthetase inhibitory activity IC_{50} (μM)	compound[a]	squalene synthetase inhibitory activity IC_{50} (μM)
1	48	8	0.6
2	182	9	7
3	1	10	23
4	0.1 (0.01)[b]	11	23
5	0.05 (0.004)[b]	12	828
6	0.1	13	33
7	4.9		

[a] All the compounds gave satisfactory spectral and analytical data. [b] IC_{50} with added PPi.

their IC_{50} values are listed in Table I. Amines 1 and 2 possessed poor activity. A comparison of the IC_{50} values of 1 and 2 suggested that an increase in the steric bulk on the nitrogen led to a decrease in the activity. Amine 3 exhibited good activity and represented the most active squalene synthetase inhibitor designed based on mimicking carbocation intermediates involved in squalene biosynthesis when compared to the biological activities of the known inhibitors designed based on this concept.[3,4] These results suggested that carbocation I is a possible intermediate in the conversion of PSPP to squalene. The biological activity associated with 3 prompted us to undertake a structure–activity relationship study in 3. We sought to design analogs by keeping the farnesyl chain as such on the nitrogen in 3 and modifying the other chain. Of the several possibilities, we selected to design conformationally restricted analogs of this chain coupled with shortening its length to further simplify the structure. This analysis produced the amine 4 which exhibited an IC_{50} value of 0.1 μM, leading to a 10 times more active analog than 3. When 4 was tested in the presence of added inorganic pyrophosphate (PPi) it showed a significant increase in the biological activity exhibiting an IC_{50} value of 10 nM. Such an enhancement of the squalene synthetase inhibitory activity in the presence of added PPi was first reported by the Poulter group[3] and serves as good evidence for both the involvement of the carbocation in the rearrangement as well as its existence as a tight ion pair with PPi in the active site. Replacement of the phenyl ring in 4 by a methyl group led to a slight loss in the activity as is evident by the IC_{50} value of compound 8. A comparison of the IC_{50} values of 3 and 8 suggested that the replacement of one of the chains in 3 by an ethyl group led to slight increase in the activity, implying that only one farnesyl chain is important for the biological activity. To further enhance the biological activity by an additional hydrogen bonding site, the phenyl ring in 4 was replaced by 3-pyridyl and 4-pyridyl rings leading to analogs 5 and 6, respectively. While amine 6 was as potent as 4, analog 5 exhibited a slight increase in the activity and was found to be the most potent squalene synthetase inhibitor in this series. Compound 5 also showed significant increase in activity, exhibiting an IC_{50} value of 4 nM when tested in the presence of added PPi. An increase in the steric bulk on the nitrogen in 5 also led to a significant decrease in the biological activity as was evident from the IC_{50} value of compound 7. These results are consistent with those observed previously for analogs 1 and 2. Any modifications of the farnesyl chain in 5, such as hydrogenation of all the double bonds as in 9, significantly shortening its length as in 11, or shortening its length by only one methylene unit coupled with hydrogenating only one of the double bonds as represented in analog 10, led to a significant loss in the activity in each case. These results suggested that the farnesyl chain is important for the biological activity in 5. Replacement of the secondary amine nitrogen in 1 and 5 by an oxygen atom led to loss in the activity as was evident by the IC_{50} values of ethers 12 and 13 and their comparison with those of their corresponding amines 1 and 5. These results clearly demonstrated that the secondary amine nitrogen, necessary to mimic the positive charge of the carbocation I, is critical for squalene synthetase inhibitory activity.

In summary, mimicking of the putative high-energy carbocation I involved in squalene biosynthesis from farnesyl pyrophosphate, by an ammonium ion, led to amine 3 as a good inhibitor of squalene synthetase. Structure–activity relationship in 3 produced structurally simple secondary amines 5 and 6 as potent squalene synthetase inhibitors.

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(10) Squalene synthetase assay: Rat liver microsomes (ca. 1 mg/mL of protein) were made in buffer P (20 mM phosphate buffer containing 0.1 mM EDTA, pH 7.4) and frozen at −80 °C until used in the assay. The solution of test compound (5 mL) in buffer (20 mM, pH 7.4, no EDTA) and 15 mL of microsomes (1 mg/mL of protein stock microsomal suspension) were added to 190 mL of buffer and incubated in a shaking water bath at 37 °C under nitrogen for 5 min; 20 mL of an NADPH/$MgCl_2$ solution was added to the sample

to yield a final assay concentration of 1.2 mM NADPH and 5 mM $MgCl_2$. Immediately, the enzymatic reaction is initiated by adding 20 mL of tritium-labeled farnesyl pyrophosphate (500 000 dpm, 125 mM) to yield a final concentration of 10 mM farnesyl pyrophosphate in the sample. The samples were then incubated for 10 min at 37 °C in an atmosphere of nitrogen. The reaction was quenched by adding 20 mL of 190 mM EDTA to each sample. The samples were then removed from the incubator and placed on ice. Ten milliliters of 5% squalene in *N,N*-dimethylacetamide was then added to each sample. Toluene in hexane (25%, 2.5 mL) was then added to each sample followed by sonication (Bronson sonicator, micro tip) for 6 s at setting of 6. The sonicated samples were each poured onto a column (6 cm × 0.7-cm diameter) containing 600 mg of silicic acid in 3 mL of 25% toluene in hexane. Another 2.5 mL of 25% toluene in hexane was added to each sample tube. The samples were sonicated again and poured onto the column. A final 2.5-mL rinse of 25% toluene in hexane was poured directly onto the column after the first two applications had drained. Merit liquid scintillation counter fluid (2.5 mL) was then added to each sample and the samples were counted in a scintillation counter. IC_{50} values were determined by linear regression analysis of the combined data. All reactions were run in duplicate. All the inhibitors were tested at several different concentrations in at least four independent assays.