

Development of Non-peptidic Farnesyltransferase Inhibitors based on the Ca₁a₂ of Ras-CaaX

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Protein prenylation that is joining farnesyl group or geranylgeranyl group to a specific cysteine residue of protein is catalyzed by protein farnesyltransferase (FTase) and protein geranylgeranyltransferase-I and II (GGTase-I and GGTase-II).¹ Since their discovery in early nineties, they were found to be associated with Ras and G-protein prenylations that play important roles in signal transduction pathway.² Though it is not clear at this point whether FTase inhibition is directly related to the anticancer activity or not, development of FTI is still attractive to find a cure for cancer as well as to find the real target of these compounds. Furthermore, FTIs were also found to have great potential for treatment of malaria, African sleeping disease, and hepatitis,³ and could be a treatment for progerias that are rare genetic disease characterized by premature aging.⁴

As a part of our research in designing nonpeptide inhibitors of FTase based on peptide ligands, we reported tripeptidyl inhibitors of FTase that are lacking the crucial carboxyl group for the binding activity designed based on Ca₁a₂ of Ras protein.⁵ The lost binding energy of the inhibitors was compensated by a hydrophobic interaction from the extended hydrophobic side chain at the terminal amino-acid. Based on this result, we started designing non-peptidic compounds as the FTI since the peptide bonds and carboxyl group of **1** do not appear to play an important role for the binding activity other than conformational bias for proper placement of hydrophobic side chains.⁶ We designed a non-peptidyl structure with amino-thiol group and hydrophobic group connected through a rigid spacer as shown in figure 1 based on the assumption that the peptide bonds adopt extended conformation for enzyme binding.

Preparation of these non-peptidic compounds with aromatic

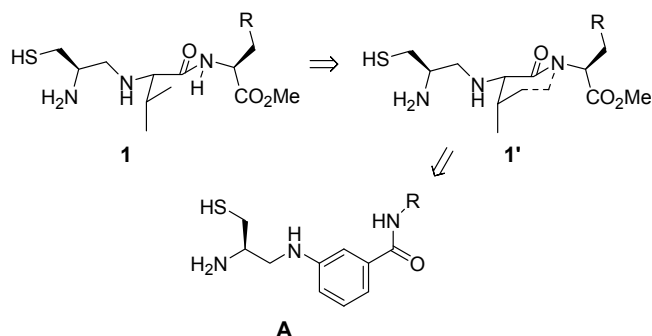
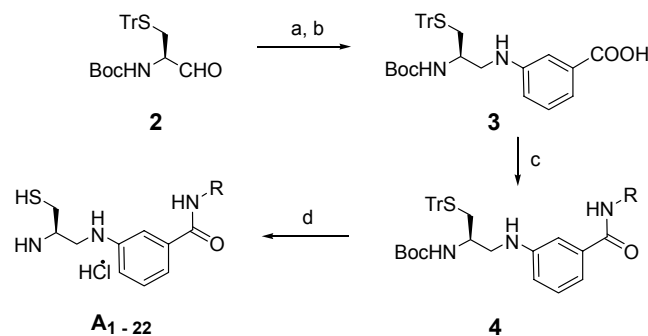


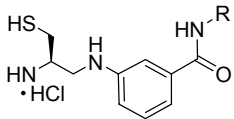
Figure 1. Designed Nonpeptidyl-Farnesyltransferase Inhibitors


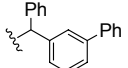






































spacer was carried out from the properly protected cysteinyl aldehyde, methyl *m*-aminobenzoate and various amines (scheme 1). Amines were selected based on the structure activity relationship of tripeptide inhibitors of FTase to provide enough hydrophobic interaction.

The inhibitory activity of the compounds for farnesyltransferase was evaluated as *in vitro* IC₅₀ values. *In vitro* IC₅₀ values were determined against partially purified bovine farnesyltransferase using SPA assay (scintillation proximity assay, Amersham, Arlington heights).^{5,7} Since extra-hydrophobic interaction was believed to be required to compensate the lack of carboxyl group for the binding activity, various phenyl substituted alkyl groups were introduced through the amide bonds (Table 1). When phenyl, benzyl or phenethyl group was introduced (compounds A_{1,2,9}), moderate binding activity was observed. Introduction of a naphthyl group that is known to increase hydrophobic binding activity either for enzymes or for receptors,⁸ significant improvement of binding activity was observed depending on the location of the second benzene ring (compounds A_{14,15,18}). The binding activity of A₁₆ that can be viewed as ring constrained benzyl or reduced naphthyl group indicated that restriction of conformational freedom of the phenyl group of the A₃ improved the binding activity by five fold while the reduction of an aromatic ring of A₁₄ to the cyclohexyl ring lowered the activity by ten fold. A polar group, especially the hydroxyl group attached to the naphthyl group interfered with the binding of the inhibitor (compounds A_{6,17,19}). All these results



Scheme 1. (a) methyl *m*-aminobenzoate, NaBH₃CN, AcOH, MeOH, r.t., 4 h, 80%; (b) aq. KOH, MeOH-H₂O-THF(1:1:2), r.t., 1 h., 99%; (c) RNH₂, EDC, DMAP, DIPEA, CH₂Cl₂, 70~90%; (d) Et₃SiH, HCl, Et₂O, CH₂Cl₂, 2-4 h., 70~90%

Table 1. *In vitro* binding activity against FTase


Compound	R	IC ₅₀ (nM)	Compound	R	IC ₅₀ (nM)
A ₁	 Ph	800	A ₁₂		7
A ₂	 Ph	570	A ₁₃		420
A ₃	 - 	140	A ₁₄	 - 	9
A ₄	 - 	720	A ₁₅	 - 	2100
A ₅	 - 	140	A ₁₆	 - 	100
A ₆	 - 	600	A ₁₇	 - 	62
A ₇	 - 	7	A ₁₈	 - 	420
A ₈	 - 	7	A ₁₉	 - 	3450
A ₉	 - 	750	A ₂₀	 - 	140
A ₁₀	 - 	570	A ₂₁	 - 	2400
A ₁₁	 - 	240	A ₂₂	 - 	120

were in good agreement with the idea that these amide groups bind to the hydrophobic binding site created by the hole in the FTase and FPP bound to the enzyme.⁹ When another alkyl substitution was introduced to the benzyl group of A₂, improvement of binding activity was observed depending on the stereochemistry of the substituent. The (S)-isomer (A₅) improved the binding activity and the (R)-isomer (A₄) did not affect the binding activity. When a phenyl group was attached instead of methyl group of A₅, great enhancement of the binding activity was observed (A₇). Even when a phenyl group was attached to the phenethyl compound (A₉) similar enhancement of the activity was observed (A₈). The structure activity relationship of these compounds strongly indicated that there should be an extra-hydrophobic binding pocket off from the existing hydrophobic pocket and the existing hydrophobic binding site is large enough to accommodate biphenyl ring as indicated by the binding activity of A₁₁ and A₁₂ that have another phenyl group attached as a biphenyl group at the *meta*- and the *para*-position respectively. The difference in binding activity of these compounds also provided an idea for the shape of the hydrophobic binding site. Binding activity of the

dibenzyl compound (A₁₀) that was similar to the binding activity of benzyl compound (A₂), indicated that the amide-NH group did not have important role in the binding of inhibitors.

Preliminary molecular modeling study with these data provided an insight into the SAR of the synthesized compounds.¹¹ The substitution from the benzyl group of A₂ appeared to bind to the hydrophobic binding site created along the bound FPP. The molecular modeling study also explained the activity difference between two isomeric phenethyl groups (A_{4,5}). The modeling showed that the methyl groups of A₄ and A₅ occupied the shallow hydrophobic binding pocket and the phenyl groups were located in the main hydrophobic binding site. This newly located hydrophobic binding site explained the activity of symmetric diphenyl compound (A₇) as one of the phenyl groups had to occupy that site.

In summary, a series of non-peptidic inhibitors of FTase based on the *m*-aminobenzoic acid spacer were designed and an optimized low *nano* molar inhibitor was identified that was presumed to utilize the newly identified hydrophobic binding interaction near the isoprene part of bound FPP in the enzyme.

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11. The molecular modeling was carried out using Discover in Insight II[®]. Full details of the molecular modeling study including FPP surrogate model will be reported in due course.