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Development of Tripeptidyl Farnesyltransferase Inhibitors

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Abstract—The first example of tripeptide inhibitors of farnesyltransferase with sub-micromolar inhibition activity was developed based on the fact that CVFM is not a substrate for farnesyltransferase.

Significant progress has been made in the development of non-cytotoxic anti-tumor agents during the last three decades.[1] Among them, inhibitors of farnesyltransferase have been developed extensively for cancer chemotherapy since blockage or attenuation of the Ras farnesylation pathway was shown to suppress Ras related cancer cell growth.[2] During the farnesylation step, CaaX motif present at the C-terminal of Ras where C is cysteine, a is an aliphatic amino acid and X is preferably serine or methionine, is recognized along with farnesyl pyrophosphate (FPP) by farnesyltransferase and then the cysteine of CaaX is farnesylated by the enzyme. It was also found out that the CaaX tetrapeptide sequence renders enough specificity for recognition by farnesyltransferase and even is farnesylated at the cysteine site. While various substrate-mimetic inhibitors of farnesyltransferase were developed based either on tetrapeptide, FPP, or even on the farnesylated peptides,[3] only the peptide-based inhibitors were successfully developed as potent inhibitors of farnesylation.

During the search for farnesyltransferase inhibitors from nonpeptidyl or natural sources,[4] we became interested in the observation that some of the tetrapeptidyl inhibitors of farnesyltransferase such as CVFM, CVWM and CVYM were not substrates for farnesyltransferase. The overall structures[5] of ternary complexes using a FPP analogue and peptides containing CVIM from X-ray crystallographic study strongly suggested that the phenyl rings of the peptidyl inhibitors blocked entry of FPP to the binding site and prevented farnesylation of these peptide-inhibitors. Thus it appears that there might be more space for hydrophobic interaction than a space for the binding of phenyl group of a_2 and room for extending the hydrophobic side chain of a_2 into the FPP binding site.

We became interested in exploring a possibility of further shortening the tetrapeptide CaaX to the tripeptide Caa in an anticipation that tripeptide analogues that consist of Ca_1a_2 with a_2-side chain having a large hydrophobic group without both terminal X residue and the carboxylate group would find enough hydrophobic binding energy from both the a_2 binding site and FPP binding site (Fig. 1). We hoped that the large hydrophobic group would occupy the binding site where the phenyl group blocked the entry of FPP and also picked up the extra binding energy by extending itself toward the FPP binding site.

To establish the structure–activity relationship (SAR) of tripeptides for the binding pocket, we prepared Ca_1a_2 with varying size of the side chain of a_2. To simplify the SAR, a_1 residue was fixed as valine and all the variation was placed on the a_2 residue. Substituted tyrosine, and *meta*-tyrosine with various hydrophobic groups as esters attached to their hydroxyl groups were prepared to explore the extent of the hydrophobic interaction with

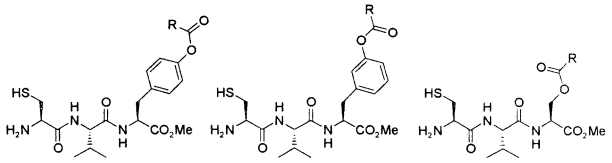

Figure 1. Designed tripeptide-farnesyltransferase inhibitors.

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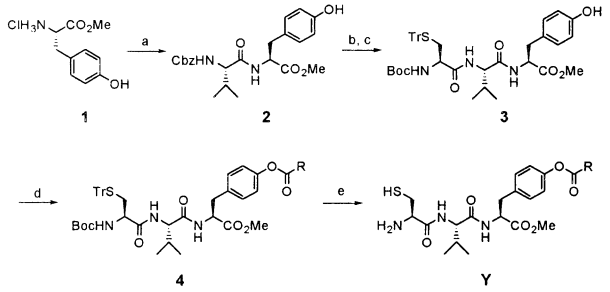
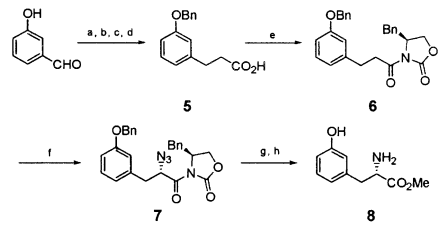
the binding pocket and to explore a possibility of extending the interaction into the FPP binding site.[6] Serine analogues were also prepared to introduce flexibility to the side chain that could allow the side chain to find the FPP binding site without much conformational restriction.

Derivatives of Cys-Val-Tyr-OMe were prepared by EDC coupling of (L)-tyrosine methyl ester hydrochloride with *N*-Cbz-valine followed by deprotection of Cbz group and subsequent EDC coupling with *N*-Boc-S-Tr-(L)-cysteine without protecting the phenolic OH group of tyrosine (Scheme 1). The hydroxyl group of tyrosine was esterified with acid chloride or with carboxylic acid using EDC and DMAP to produce **4**. Cysteine of **4** was deprotected using trifluoroacetic acid and triethylsilane to yield **Y**.

Cys-Val-*meta*-Tyr-OMe analogues were synthesized from *meta*-(L)-tyrosine methyl ester in the same manner as the syntheses of **Y** series starting from *meta*-tyrosine methyl ester. *meta*-Tyrosine methyl ester was synthesized from *m*-hydroxybenzaldehyde according to the literature preparation (Scheme 2).[7] After protection of the phenolic hydroxyl group of *m*-hydroxybenzaldehyde as the benzyl ether, Wittig reaction followed by selective hydrogenation of the double bond with platinum on charcoal and subsequent ester hydrolysis produced **5**. After attaching the benzyl oxazolidinone as the chiral auxiliary via the pivaloyl mixed anhydride method,[8] stereoselective introduction of α-amino group was accomplished via α-azidation followed by catalytic reduction of the azide that was accompanied by the



Scheme 1. (a) *N*-Cbz-valine, EDC, HOBT, DIPEA, DMF, 16 h, 83%; (b) H_2, 10% Pd/C, MeOH, 30 min, 94%; (c) *N*-Boc-*S*-Tr-cysteine, EDC, HOBT, THF, 80%; (d) RCO_2H, EDC, DMAP, CH_2Cl_2 or RCOCl, DIPEA, CH_2Cl_2, 75–88%; (e) Et_3SiH, TFA, CH_2Cl_2, 58–86%.



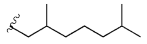
Scheme 2. (a) NaH, BnBr, TBAI, THF, 85%; (b) Ph_3PCHCO_2Me PhH, reflux, 89%; (c) H_2, 10% Pt/C, AcOEt, 99%; (d) LiOH, THF/H_2O, 90%; (e) PivCl, DIPEA, THF, −78 °C, *N*-(Li)-(*R*)-phenyloxazolidinone, 87%; (f) KHMDS, THF, trisyl azide, −78 °C, AcOH, rt, 51%; (g) MeMgBr, MeOH, 0 °C, 81%; (h) H_2, Pd/C, AcOEt, 86%.

removal of benzyl group[7,9] after removing the chiral auxiliary. The chiral auxiliary was replaced with methyl ester using MeMgBr in methanol solvent[10] to produce *m*-tyrosine methyl ester. Derivatives of Cys-Val-Ser-OMe, were also prepared in the same manner as in the preparation of the Cys-Val-Tyr-OMe series.

The inhibitory activity of the compounds for farnesyltransferase was evaluated as in vitro IC_{50} values. In vitro IC_{50} values were determined against partially purified bovine farnesyltransferase using SPA assay (scintillation proximity assay, Amersham, Arlinton heights).[11] ^{3}H-FPP and a biotin-linked C-terminal undecapeptide of K-Ras4B (KKKSKTKCVIM) were used as the substrates. The radioactivity captured by the SPA beads were counted by a Packard Topcount, and data were stored and analyzed in an Oracle-based database. CVFM, one of the most potent tetrapeptide inhibitors and its methyl ester was used as the references with IC_{50} values of 0.06 and 5.8 μM respectively in our farnesyltransferase inhibition studies.

Since all the prepared compounds did not possess the crucial terminal carboxyl group for the binding activity, all the synthesized compounds were screened first at the concentration of 1 μg/mL for the inhibitory activity (Table 1). While all the compounds showed appreciable inhibitory activity, CVS-OMe that lacks the extended hydrophobic group at a_2 did not show any inhibitory activity. This result demonstrated the importance of the hydrophobic binding group at a_2 and it also showed that as the hydrophobic group became larger, inhibitory activity became higher. This result indicates that the hydrophobic group at the a_2 plays an important role in binding to the Ca_1a_2X box of farnesyltransferase but the hydrophobic binding pocket of farnesyltransferase has the limited space in it as the hydrophobic group attached to a_2 became large the binding activity did not improve after a certain point. To evaluate the SAR more accurately, IC_{50} values of the compounds were

Table 1.

Compd[a]	R	% Inhibition[b]	Compd[a]	R	% Inhibition[b]
Y_1	Me	6.3	Ym_1	*i*-Pr	30
Y_2	*i*-Pr	26.7	Ym_2		61
Y_3		79.6	Ym_3	Ph	69.3
Y_4	*i*-Bu	74	Ym_4		17
Y_5	Pentyl	42	Ym_5	Me	12
Y_6	Bn	32	S_1	Ph	69.3
Y_7	Heptyl	17	S_2	p-Ph-Ome	57
Y_8		10	S_3	p-Tolyl	67
Y_9	Ph	23.4	S_4	p-Ph-Cl	75
Y_{10}		12.6	S_5		38.6
	CVS-OMe	0	S_6		16.3

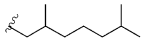
[a]Concentration of sample; 1 μg/mL.
[b]% Of inhibition = 100[1−{sample-blank2(sample, no enzyme)}/{control-blank1(no enzyme, no sample)}].

measured and the result is summarized in the Table 2. The fact that CVFM methyl ester (CVFM-OMe) is 100-fold less potent than CVFM ($IC_{50} = 60$ nM) clearly indicated that the presence of a free carboxylate at the C-terminus is important for a strong inhibition of the enzyme. The IC_{50} value of $\mathbf{Y_1}$ indicated that deletion of the 4th amino acid decreased the activity by another 10-fold from CVFM-OMe.

Substituting the methyl group of $\mathbf{Y_1}$ with a larger group improved the binding activity. However, when the substituent became longer than the *iso*-butyl group ($\mathbf{Y_5}$–$\mathbf{Y_{10}}$), no more binding energy was gained. This result indicates that the space in the hydrophobic binding pocket for a_2 might not be as large as we hoped for to pick up enough binding energy to compensate for the lost binding energy from the lack of the carboxyl group. Interestingly, compounds with branched side chains showed markedly improved activity, especially when the branching is at the β-position of the ester group ($\mathbf{Y_2}$–$\mathbf{Y_4}$). This result is quite interesting since coincidentally the *iso*-butyl side chain of arteminolide[4a] played an important role in inhibiting farnesyltransferase. When the substitution was moved from *para*-position to *meta*-position of the phenyl ring, the extended hydrophobic group did not seem to fit into the binding pocket well as only Ym_3 showed a significantly improved binding activity. When serine analogues that were prepared with an anticipation that would be free from the conformational restriction imposed by the phenyl rings of tyrosines were tested, improved binding activity was observed with substituted benzoate esters attached to the serine ($\mathbf{S_1}$–$\mathbf{S_4}$). When R group of **S** was smaller or bigger than the phenyl group ($\mathbf{S_5}$, $\mathbf{S_6}$), only a small binding energy gain was observed. All these results strongly suggest that there is a binding site for an extended hydrophobic group of a_2 and supplementing this binding energy to the tripeptide inhibitors is large enough to compensate the loss of the 4th amino acid unit from CVFM-OMe and even offset a part of the binding energy lost from the lack of the terminal carboxylate group.

In summary, we were able to identify a hydrophobic binding pocket associated with the binding site of a_2 residue and FPP binding site, and by utilizing this binding site we were able to reduce the size of the peptide inhibitors to tripeptide from tetrapeptide. Though there have been reports of tripeptide analogues of farnesyltransferase inhibitors that either mimic tetrapeptides or tripeptidyl-FPP, this is the first example of the tripeptide based analogues of farnesyltransferase inhibitors with sub-micromolar inhibitory activity.[12] While our investigation has not been able to fully extend the hydrophobic binding interaction to the FPP binding site, the current result strongly suggests that one could pick up more hydrophobic binding energy from CaaX based nonpeptidyl inhibitors[13] since the hydrophobic groups of those inhibitors appear to reach only to the part of the hydrophobic group binding pocket of a_2. Currently, we are pursuing to find effective bisubstrate inhibitors of farnesyltransferase, since combination of Y_3, Ym_2 and Ym_3 into another hydrophobic group could also lead us to design better inhibitors as our SAR result in connection to the X-ray ternary structures of farnesyltransferase with inhibitors suggests that the binding site of side chain of a_2 residue should exist in close proximity to the FPP binding site. We should be able to extend this hydrophobic binding interaction into the FPP binding site with the properly located extension of the side chain.

Table 2.

Compd	R	IC_{50} (μM)	Compd	R	IC_{50} (μM)
$\mathbf{Y_1}$	Me	48	$\mathbf{Ym_1}$	*i*-Pr	10
$\mathbf{Y_2}$	*i*-Pr	12	$\mathbf{Ym_2}$		8.4
$\mathbf{Y_3}$		0.5	$\mathbf{Ym_3}$	Ph	1.1
$\mathbf{Y_4}$	*i*-Bu	0.82	$\mathbf{Ym_4}$		17
$\mathbf{Y_5}$	Pentyl	7.5	$\mathbf{S_1}$	Ph	0.9
$\mathbf{Y_7}$	Heptyl	22	$\mathbf{S_2}$	*p*-Ph-OMe	1.8
$\mathbf{Y_8}$		8.1	$\mathbf{S_3}$	*p*-Tolyl	0.92
$\mathbf{Y_9}$	Ph	20	$\mathbf{S_4}$	*p*-Ph-Cl	0.82
$\mathbf{Y_{10}}$		43	**CVFM-OMe**		5.8
			CVFM		0.06

References and Notes

1. (a) Goldenberg, G. J.; Moore, M. J.; Wright, J. E.; Ludlum, D. B.; Kelland, L. R.; Sweatman, T. W.; Israel, M.; Giovanella, B. C.; Pommier, Y.; Bissery, M.-C.; Lavelle, F.; Zunino, F.; Capranico, G.; Romerdahl, C. A.; Braña, M. F.; Schor, N. F.; Stetler-Stevenson, W. G.; Hendrzak, J. A.; Brunda, M. J.; Ingber, D. E.; Crooke, S. T.; Sausville, E. A.; Longo, D. L.; Blättler, W. A.; Chari, R. V. J.; Lambert, J. M.; Sebolt-Leopold, J. S.; Schrier, P. I.; Osanto, S. In *Cancer Therapeutics: Experimental and Clinical Agents*; Teicher, B. A., Ed.; Humana: Totowa, 1997; Vol. 1, p 3. (b) Traxler, P.; Bold, G.; Buchdunger, E.; Caravatti, G.; Furet, P.; Manley, P.; O'Reilly, T.; Wood, J.; Zimmermann, J. *Med. Res. Rev.* **2001**, *21*, 499.

2. Gibbs, R. A.; Zahn, T. J.; Sebolt-Leopold, J. S. *Curr. Med. Chem.* **2001**, *8*, 1437.

3. (a) Patel, D. V.; Young, M. G.; Robinson, S. P.; Hunihan, L.; Dean, B. J.; Gordon, E. M. *J. Med. Chem.* **1996**, *39*, 4197. (b) Schlitzer, M.; Bohm, M.; Sattler, I.; Dahse, H.-M. *Bioorg. Med. Chem.* **2000**, *8*, 1991 and references therein.

4. (a) Lee, S.-H.; Kim, M.-J.; Bok, S. H.; Lee, H.; Kwon, B.-M.; Shin, J.; Seo, Y. *J. Org. Chem.* **1998**, *63*, 7111. (b) Lee, H.-Y.; Sohn, J.-H.; Kim, H. Y. *Tetrahedron Lett.* **2001**, *42*, 1695.

5. (a) Long, S. B.; Casey, P. J.; Beese, L. S. *Structure* **2000**, *8*, 209. (b) Strickland, C. L.; Windsor, W. T.; Syto, R.; Wang, L.; Bond, R.; Wu, Z.; Schwartz, J.; Le, H. V.; Beese, L. S.; Weber, P. C. *Biochemistry* **1998**, *37*, 16601.

6. Attachment of hydrophobic groups as allylic or benzylic ether linkage that could provide more flexibility than ester linkage turned out to be unstable under the screening condition and produced unreliable inhibitory activities.

7. Bänteli, R.; Brun, I.; Hall, P.; Metternich, R. *Tetrahedron Lett.* **1999**, *40*, 2109.

8. Evans, D. A.; Gage, J. R.; Leighton, J. L. *J. Am. Chem. Soc.* **1992**, *114*, 9434.

9. Evans, D. A.; Britton, T. C.; Ellman, J. A.; Dorow, R. L. *J. Am. Chem. Soc.* **1990**, *112*, 4011.

10. Evans, D. A.; Britton, T. C.; Dorow, R. L.; Dellaria, J. F., Jr. *Tetrahedron* **1988**, *44*, 5525.

11. Reiss, Y.; Goldstein, J. L.; Seabra, M. C.; Casey, P. J.; Brown, M. S. *Cell* **1990**, *62*, 81.
12. (a) Patel, D. V.; Patel, M. M.; Robinson, S. S.; Gordon, E. M. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 1883. (b) Kothare, M. A.; Ohkanda, J.; Lockman, J. W.; Qian, Y.; Blaskovich, M. A.; Sebti, S. M.; Hamilton, A. D. *Tetrahedron* **2000**, *56*, 9833.
13. (a) Qian, Y.; Marugan, J. J.; Fossum, R. D.; Vogt, A.; Sebti, S. M.; Hamilton, A. D. *Bioorg. Med. Chem.* **1999**, *7*, 3011. (b) Williams, T. M.; Bergman, J. M.; Brashear, K.; Breslin, M. J.; Dinsmore, C. J.; Hutchinson, J. H.; MacTough, S. C.; Stump, C. A.; Wei, D. D.; Zartman, C. B.; Bogusky, M. J.; Culberson, C.; Buser-Doepner, C.; Hamilton, K. A.; Koblan, K. S.; Kohl, N.; Liu, D.; Lobell, R. B.; Mosser, S. D.; O'Neill, T. J.; Rands, E.; Schaber, M. D.; Wilson, F.; Senderak, M. D.; Motzel, S. L.; Gibbs, J. B.; Graham, S. L.; Heimbrook, D. C.; Hartman, G. D.; Oliff, A. I.; Huff, J. R. *J. Med. Chem.* **1999**, *42*, 3779.