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PEPTIDE BASED P21^{RAS} FARNESYL TRANSFERASE INHIBITORS: SYSTEMATIC MODIFICATION OF THE TETRAPEPTIDE CA$_1$A$_2$X MOTIF

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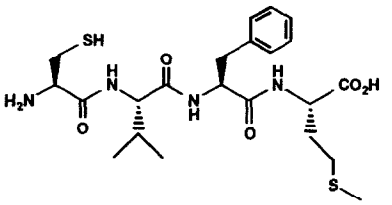
Abstract

A systematic study of CVFM, a CAAX-derived farnesyl transferase inhibitor, was undertaken to determine the structural elements important for intrinsic activity as well as substrate character. Results indicate a narrowly defined profile for nonsubstrate FT inhibition .

Introduction

A family of guanine-nucleotide binding proteins including the p21^{ras} proteins, nuclear lamins and transducin, carry a conserved carboxy-terminal CA$_1$A$_2$X sequence (where C is cysteine, A can be a wide variety of uncharged amino acids, and X is methionine or serine).[1] This motif cues a three-step sequence of post-translational modifications, initiated by cysteine S-farnesylation by the enzyme farnesyl transferase (FT), that enables the protein to anchor to cell membranes.[2,3] Such membrane localization is critical to the function of oncogenic p21^{ras}. Therefore, inhibition of farnesyl transferase may be therapeutic in the treatment of certain cancers mediated by oncogenic p21^{ras} including colon, lung and pancreatic carcinomas. It is known that the CA$_1$A$_2$X sequence in tetrapeptides is sufficient for FT recognition/inhibition, and specific features of this sequence can provide selectivity to FT inhibitors.[4] Two structural features, a free amino terminus and an aromatic residue at the A$_2$ position, are associated with the non-farnesylated class of peptide inhibitors.[5] We undertook a systematic study to probe the recognition elements and bioactive conformation of CVFM, a potent, nonsubstrate FT inhibitor (Figure 1). Recent work has demonstrated that the peptide backbone can be modified[6,7] or replaced entirely by a non-peptide scaffold[8,9] provided the correct disposition of the sidechain functional groups is maintained.



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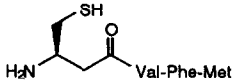
Sidechain alterations probed the contribution of each component of the CAAX box (compounds **3 -17**), including $C^{\alpha,\alpha}$-tetrasubstituted amino acids (**9** and **10**) associated with turns in short linear peptides[10]. Compounds **3** and **7** addressed the importance of the distances between the thiol and amine from the remainder of the tripeptide, while **11** and **12** addressed the importance of the phenyl ring-to-backbone distance. In analogs **18** - **22** the peptide backbone was modified to allow a greater range of ϕ,ψ angles. All four nitrogens were independently N-methylated as a probe of *cis/trans* amide bond geometry and hydrogen bonding requirements (compounds **23-26**).

Materials and Methods

Peptides **1-6** and **8-17** were assembled on a Wang resin using standard BOP/HOBt coupling, N^{α}Fmoc protection, and compatible sidechain protecting groups.[11] Cleavage from the resin and global deprotection were effected in trifluoroacetic acid in the presence of 5% v/v/v [3:1:1]thioanisole/dimethylsulfide/ethanedithiol. Both isomers of **3** were synthesized as a diastereomeric mixture starting from racemic Hcy and separated by preparative HPLC. Compound **7** was synthesized using the Arndt-Eistert rearrangement of N^{α}Boc(S-trityl)cysteine methyl ester.[12] Following methyl ester deprotection, conventional solution peptide synthesis afforded the product.[13] Compounds **18-21** were synthesized in solution by reductive amination[14] of the corresponding aldehyde, prepared by the method of Fehrenz, Heitz and Castro.[15] The thioamide derivative **22** was prepared by treating Boc-Phe-Met-OBzl with Lawesson's reagent[16] followed by standard solution amide bond couplings. Global deprotection using HF afforded the product. Compound **23** was prepared by Na/NH_3 reduction of thiazolidine-4-carboxylic acid followed by *in-situ* benzyl protection of the free thiol[17] and N^{α}Boc protection to afford the protected N-methyl cysteine. Coupling to Val-Phe-Met-OMe using BOP/HOBt followed by global deprotection generated the product. The N-methyl residues in compounds **24-26** were prepared according to literature procedures.[18] N-terminal couplings of N-methyl amino acid derivatives were carried out using BOP-Cl[19] and all standard amide bond couplings were performed using BOP/HOBt. The *in vitro* inhibition assay was carried out essentially as described[20] and was

adapted to TCA precipitation in microtiter plates. The tlc procedure used for the substrate assay was taken from a previously described method.[4]

Table 1 *Peptide-based Inhibitors of p21^{ras} Protein Farnesyl Transferase*

	Structure	IC_{50} (nM)	Substrate
1	Cys-Val-Phe-Met	37	no
2	Cys-Val-Val-Met	68	yes
3a	Hcy-Val-Phe-Met (Isomer A)	510	no
3b	Hcy-Val-Phe-Met (Isomer B)	2700	no
4	Asp-Val-Phe-Met	250000	NT
5	His-Val-Phe-Met	6800	NT
6	Glu-Val-Phe-Met	120000	NT
7		1800	no
8	Cys-Ala-Phe-Met	241	no
9	Cys-Aib-Phe-Met	400	no
10	Cys-Ac_6c-Phe-Met	1200	no
11	Cys-Val-Phg-Met	696	yes
12	Cys-Val-Hph-Met	120	yes
13	Cys-Val-dPhe-Met	5200	no
14	Cys-Val-Nap-Met	12	no
15	Cys-Val-Trp-Met	14	no
16	Cys-Val-Phe-Nle	950	no
17	Cys-Val-Phe-Phe	400	no
18	Cysψ[CH_2NH]Val-Val-Met	29	yes
19	Cysψ[CH_2NH]Val-Phe-Met	12	no
20	Cys-Valψ[CH_2NH]Phe-Met	126	no
21	Cys-Val-Pheψ[CH_2NH]Met	2220	no
22	Cys-Val-Pheψ[CSNH]Met	17	no
23	[N-MeCys]-Val-Phe-Met	420	yes
24	Cys-[N-MeVal]-Phe-Met	250	no
25	Cys-Val-[N-MePhe]-Met	360	no
26	Cys-Val-Phe-[N-MeMet]	1400	no

Ac_6c, 1-aminocyclohexylcarboxylic acid; Aib, aminoisobutyric acid; Hcy, homocysteine; Hph, homophenylalanine; Nap, 2-Naphthylalanine; Nle, norleucine; Phg, phenylglycine.

Results and Discussion

As shown in Table 1, CVFM has rigidly defined requirements at the cysteine residue (compounds **3**-**7**). Replacing the sidechain with other functionalities inclined to chelate metals (**4-6**) or altering the length of either the sidechain or the backbone (**3,7**) dramatically reduced intrinsic activity. Two turn-inducing replacements at the A_1 position were investigated to explore the possibility of a turn as the preferred conformation (**9,10**). These compounds were weak nonsubstrate inhibitors. In view of reports that inhibitors bind in a turn conformation in this region[21], the poor activity of these compounds is difficult to explain. Replacing Val with Ala at this position (**8**) also led to a weak inhibitor. The distance between the aromatic ring at the A_2 position and the backbone was found to be critical for nonsubstrate inhibition. Positioning of the aromatic ring either closer to (**11**) or farther from (**12**) the backbone resulted in a less potent inhibitor that *reverted to a substrate*. Aromatic ring replacements such as naphthylalanine (**14**) and Trp (**15**) retained the correct distance, maintained intrinsic potency, and were nonsubstrates. Replacing the methionine sidechain with either a carbon analog (**16**) or Phe (**17**) led to a substantial decrease in intrinsic potency.

As found by others[6,7] replacing the amide bond between Cys and Val with a methyleneamine (**19**) conferred improved potency over the progenitor peptide for both CVVM and CVFM. This same modification between Val and Phe (**20**) resulted in a four fold reduction in intrinsic potency. Between Phe and Met (**21**) the methyleneamine modification led to a very poor inhibitor, suggesting that either the Phe-Met amide carbonyl is involved in a critical hydrogen bond or a rigid geometry is required at that position. The *improved intrinsic potency* of the analog with a Phe-Met thioamide bond (**22**) suggests that a rigid geometry may be required since thioamides are known to be stronger acids and weaker bases than amides leading to enhanced hydrogen bond donating ability of the thioamide proton and weaker hydrogen bond acceptance of the C=S fragment.[22] N-methylation in any position (**23-26**) generally led to a weaker nonsubstrate inhibitor. Interestingly, methylating the N-terminus of CVFM (**23**) generated a substrate, despite the preservation of a positive charge and H-bonding capability. In the CVVM substrate series, methylation was well-tolerated at both the A_1 and A_2 positions.[23] These findings suggest there are interactions required for nonsubstrate (CVFM-like) inhibitor binding which may be less necessary for substrate (i.e.CVVM-like) inhibitor binding. In both the CVVM and the CVFM series, methylating the C-terminal methionine nitrogen led to very weak inhibition, suggesting that the A_2-X amide plays a similar important role in both substrate and inhibitor peptides.

Several conclusions may be drawn from these studies. Few ostensibly conservative replacements are tolerated for any of the residues of CVFM. The distances from the peptide backbone to the thiol, amine, and aromatic ring functions must be maintained, although the nature

of the aromatic ring can be altered. Increasing or decreasing the aromatic nucleus-to-backbone distance positions the peptide in a substrate binding mode, as does N-methylation of the primary amine. Releasing the conformational constraint of the Cys-Val peptide bond enables the reduced bond pseudotetrapeptide[24] to adopt a more tolerated conformation than the parent compound. In contrast, maintaining the rigidity imparted by the C-terminal peptide bond appears requisite for inhibitor binding. These observations will inform the design of the next generation of modified CAAX inhibitiors.

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