# An Assessment of Bio-active Conformation of CAAX-based Tetrapeptide in Ras Farnesyltransferase Inhibition

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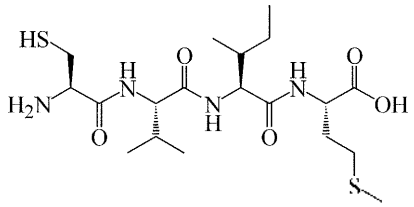
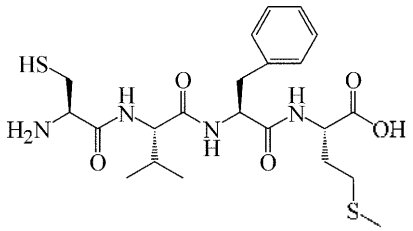
The protein product from the *ras* oncogene is a small G-protein, $p21^{ras}$ (Ras), which is known to play a key role in the signal transduction cascade and cell differentiation and proliferation. Mutated Ras is unable to regulate itself and remains constantly activated, leading to uncontrolled cell growth.[1] The function of Ras in signal transduction requires its location near the growth factor receptor at the cell membrane. However, Ras does not have a transmembrane domain. Ras requires farnesylation to increase its hydrophobicity and subsequent plasma membrane association for its transforming activity. The zinc-containing enzyme, Ras farnesyltransferase (FTase) catalyzes the key post-translational modification, transfering a farnesyl group from farnesylpyrophosphate to the *C*-terminal cysteine of the Ras protein. The requirement provides a focused attention on FTase as a target for therapeutic intervention.[2]

In 1990, Goldstein and Brown discovered that small peptides containing the $CA_1A_2X$ sequence as shown in Figure 1 (C is cysteine, A is any aliphatic amino acid, and X is preferentially methionine) could act as alternative substrates and therefore competitive inhibitors of FTase. The tetrapeptide CVIM (**1**, $IC_{50}$ = 150 nM) inhibits FTase, but acts as a substrate for farnesylation.[3] When the $A_2$ position is occupied by an aromatic acid as in CVFM (**2**, $IC_{50}$ – 20 nM), it is no longer farnesylated and becomes a very potent inhibitor of FTase.[4] This provides structural information for peptidomimetic inhibitor design of CAAX-based tetrapeptides.

Several groups suggested that the two interior residues of the CAAX peptides, typical hydrophobic residues, served to confer an active conformation similar to a $\beta$-turn. The replacement of the interior peptide bonds with benzodiazepine in BZA-2B (**3**, $IC_{50}$ – 0.8 nM) might serve to stabilize such a bent conformation.[5] However, other groups have postulated extended conformations for potent peptidomimetic inhibitors.[6] The peptidomimetic inhibitor, FTI-276 (**4b**, $IC_{50}$ – 0.5 nM)[6b] incorporates a lipophilic 2-phenyl-4-aminobenzoic acid as an isosteric replacement for the internal Val-Phe dipeptide. The rigidity of **4b** only allows for this molecule to adopt the extended conformation. Because molecules incorporating extended peptide mimics as well as turn-mimics have yielded equally strong inhibitors, there has



**1**



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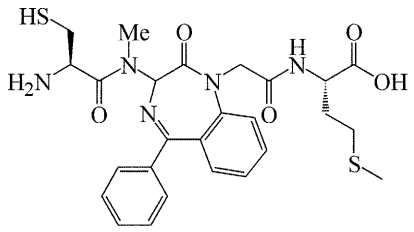

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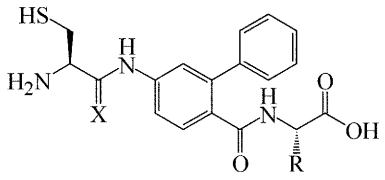


**4a:** X = O; R = MTE
**4b:** X = $H_2$, R = MTE
**4c:** X = O; R = *s*-Bu
**4d:** X = $H_2$; R = *s*-Bu

**Figure 1**. Representative FTase inhibitors based on CAAX-motif (MTE methylthioethyl).

been considerable debate concerning the active conformations of CAAX-peptide substrates and inhibitors.

One of advantage of aromatic spacer is that by attaching amino and carboxyl groups at different positions on the aromatic rings can control the distance between cysteine and methionine. In this study, we designed and synthesized 2-phenyl-5-aminobenzoate as a turn-scaffold. We envisioned that this study confer an assessment of bio-active conformation of CAAX-based tetrapeptide by the changes of aromatic substitution patterns. Here, we would like to report the synthesis and biological evaluation of tetrapeptide turn-mimetics.

## Results and Discussion

The required 2-phenyl-5-nitrobenzoic acid **7** was easily prepared from the commercially available 2-bromo-5-nitrotoluene **5**. The bromide **5** was coupled with phenylboronic acid to give 2-phenyl-5-nitrotoluene **6** under Suzuki coupling conditions.[7] The toluene derivative **6** was transformed to 2-phenyl-5-nitrobenzoic acid **7** by potassium permanganate oxidation. The benzoic acid **7** was coupled with Met-OMe and Leu-OMe using EDCI-HOBT coupling, followed by reduction with stannous chloride,[8] respectively, to furnish the amines **9a** and **9b**. The resulting 5-amino-2-phenylbenzoate **9a** was coupled with *N*-Boc-*S*-trityl-cysteine using isobutyl chloroformate (IBCF) and reductively alkylated with *N*-Boc-*S*-trityl-cysteinal[9] by the action of sodium cyanoborohydride to give the cysteine derivatives **10a** and **10b**, respectively. The leucine derivatives **10c** and **10d** were also similarly prepared from **9b**. The methyl esters **10a-d** were hydrolyzed by LiOH and then deprotected by trifluoroacetic acid in the presence of triethylsilane, as shown in Scheme 1. The final compounds **11a-d** were obtained through Prep HPLC separation and showed purity greater than 97%.

In **1**, the number of conformations with similar low energies is large due to the many possible bond rotations. Conformational calculations were carried out in the absence of solvent using the Amber force field within the Macro-

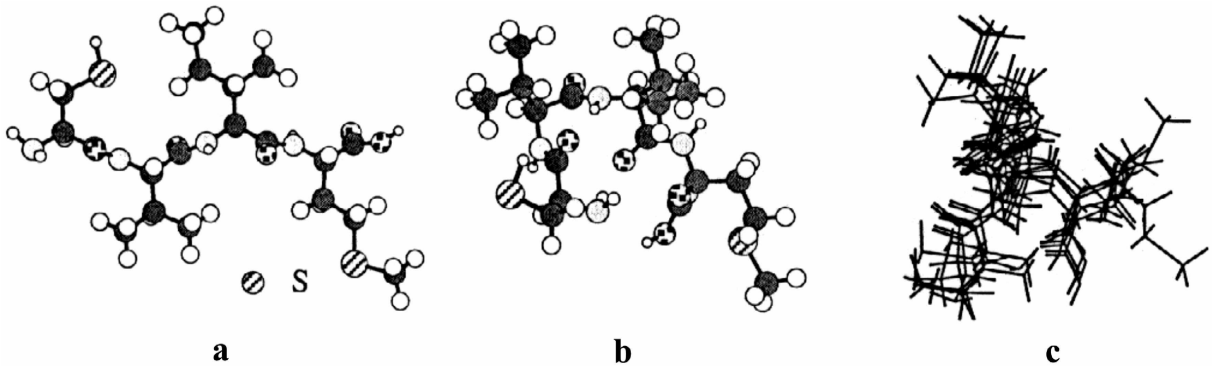
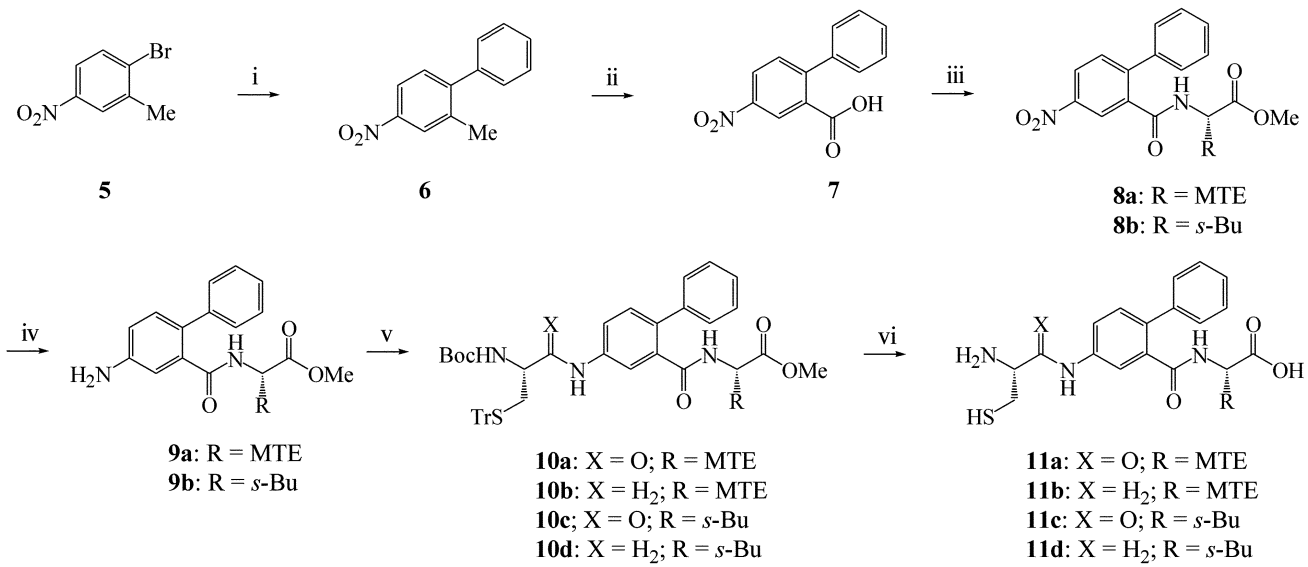



**Figure 2.** Energy-minimization of **1** in (a) extended, (b) turn conformation, (c) overplay of the five lowest energy conformations.




**Scheme 1.** *Reagents and conditions:* i. $Ph(OH)_2$, $Pd(PPh_3)_4$, $Ba(OH)_2$, DMF-$H_2O$, 120 °C, 5h, 84%; ii. $KMnO_4$, pyridine-$H_2O$, 90 °C, 20h, 86%; iii. EDCI, HOBT, $Et_3N$, Met-OMe, 70% (for **8a**), Leu-OMe, 83% (for **8b**); iv. $SnCl_2$, 88% (for **9a**), 92% (for **9b**); v. IBCF, NMM, −20 °C, *N*-Boc-*S*-Tr-Cys, 75% (for **10a**), 76% (for **10c**); $NaBH_3CN$, *N*-Boc-*S*-Tr-Cysteinal, 79% (for **10b**), 78% (for **10d**); vi. LiOH; TFA, $Et_3SiH$; Prep-HPLC, 40% (for **11a**), 38% (for **11b**), 78% (for **11c**), 72% (for **11d**); MTE = methylthioethyl.

Model program. Figure 2 shows two possible conformations in which **1** takes up either an extended or a turn-like conformation. Molecular modeling indicates the distance from $C_\alpha$ of Cys to $C_\alpha$ of Met in **1** is about 10.3 Å in an extended, whereas 5.6 Å in a turn conformation. Within a turn conformation, the thiol group of Cys points to the carboxylate moiety of Met. It adopts a non-ideal turn conformation characterized by the lack of a transannular hydrogen bond. As an application of global energy minimization, it converged to a turn conformation.

An energy-minimized structure for **4b** maintains at a distance of 9.8 Å analogous to the extended conformation of **1**, because *para*-relationship makes it impossible for this molecule to adopt $\beta$-turn conformations. Whereas, a *meta*-isomer of **11b** has a similar shape and the distance of 5.9 Å to the turn conformation of **1**, as shown in Table 1. This examination suggests that 3-aminobenzoic acid may serve as a turn-scaffold. Further examination of electrostatic potential maps for the two molecules, **4b** and **11b**, was carried out using the AM1 semi-empirical within Spartan program. Molecular mechanics indicates the surface area and volume of the *meta*-isomer **11b** are about 413.72 $Å^2$ and 251.13 $Å^3$, and about 376.91 $Å^2$ and 219.11 $Å^3$ in the *para*-isomer **4b**. In a given substrate, a turn structure of **11b** may take up a larger effective hydrophobic surface than an extended structure of **4b**. Thus, a simple transposition of the substitution pattern greatly changes the distance and shape of an entire molecule, and may allow a unique mimicking of a turn conformation.

Whereas **4a** retains good inhibitory activity, its *meta*-isomer **11a** is 2,000-fold less active against FTase, as shown in Table 2.[10] Then, the amide bond linking cysteine and 3-aminobenzoate was reduced to a secondary amine, which is expected to reduce susceptibility to protease degradation and to improve cellular uptake. Remarkably, the reduction of the cysteine amide (in **11a**) to an amine (in **11b**) provides a 2200-fold enhancement of potency. Presumably, the enhancement of potency is due to the relaxation of an unfavorable conformational restriction provided by the reduction of cysteine amide to the amine.[11] The reduced **4b** (**FTI-276**) still retains its high potency and is 8-fold more active than **11b** towards FTase. Inhibitory activity is dependent on the ring-substitution pattern. Indeed, the introduction of 3-aminobenzoic acid onto the *meta*-isomers (**11a-d**) does not improve inhibition activity comparing to 4-aminobenzoic acid of the *para*-isomers (**4a-d**). These results clearly suggest that *para*-isomers **4a**, **b** containing 4-aminobezoic acid provide exact positioning of the Cys and Met residues in the enzyme active site, while *meta*-isomers **11a**, **b** do not. In the given structures, the results emphasize that an extended conformation is pretty much important in FTase inhibition.

**Table 1**. Molecular shape

| CAAX | Distance[a] (Å) | Surface Area[b] ($Å^2$) | Volume[c] ($Å^3$) |
|---|---|---|---|
| **1a**[d] | 10.3 | nd[f] | nd |
| **1b**[e] | 5.6 | nd | nd |
| **4b** | 9.8 | 376.91 | 219.11 |
| **11b** | 5.9 | 413.72 | 251.13 |

[a]Distance from $C_\alpha$ of Cys to $C_\alpha$ of Met (Amber force field within Macromodel). [b]Electrostatic potential surface area (AM1 semi-empirical within Spartan). [c]Electrostatic potential volume (AM1 semi-empirical). [d]Extended conformation of **1** (Amber force field). [e]Turn conformation of **1** (Amber force field). [f]Not determined

In conclusion, the aromatic spacers are designed to control the distance between Cys and Met to probe a bioactive conformation of the CAAX tetrapeptide. Changing the substitution pattern probes the distance and shape of the entire molecule in order to explore bio-active conformations.[12] Our results demonstrate that a turn conformation is not required for inhibitory activity, presumably, it does not provide exact positioning of the Cys and Met residues in the enzyme active site. More recent evidence, particularly from the disclosure of the ternary complex of the FTase has provided insight into the extended conformation of the CAAX peptidomimetics is more favorable.[13]

## Experimental Section

**5-Nitro-2-phenylbenzoyl-methionine methyl ester (8a).** The acid **7** (1.43 g, 5.96 mmol) was suspended in methylene chloride (30 mL). To this solution were added methionine methyl ester hydrochloride (1.31 g, 6.55 mmol), triethylamine (0.92 mL, 6.55 mmol), EDCI (1.37 g, 7.15 mmol) and HOBT (0.97 g, 7.17 mmol) in an ice bath. The mixture was stirred for 10 hr at room temperature and then partitioned with methylene chloride and 1*N* HCl. The organic layer was washed with 5% sodium bicarbonate and water and dried over anhydrous magnesium sulfate. After evaporating solvents, the solid residue was recrystallized from ethyl acetate to give **8a** (1.64 g, 70.8 %): $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 8.54 (d, 1H, $J$ = 3.0 Hz), 8.32 (dd, 1H, $J$ = 9.0, 3.0 Hz), 7.56 (d, 1H, $J$ = 9.0 Hz), 7.46-7.25 (m, 5H), 6.07 (d, 1H, $J$ = 7.5 Hz), 4.68 (dd, 1H, $J$ = 12.2, 7.0 Hz ), 3.68 (s, 3H), 2.06 (t, 2H, $J$ = 9.0 Hz), 2.00 (s, 3H), 1.95 (m, 1H), 1.78 (m, 1H); $^{13}$C NMR ($CDCl_3$) $\delta$ 171.7, 167.0, 147.2, 146.1, 138.2, 136.4, 131.8, 129.3, 129.2, 128.7, 125.0, 124.4, 52.8, 52.1, 31.3, 29.5, 15.5; HRMS (EI) calculated for $C_{19}H_{20}N_2O_5S$: 388.1092, found: 388.1084.

***N*-Boc-*S*-Tr-cysteinyl-(5-amino-2-phenyl)benzoyl-methionine methyl ester (10a).** The commecially available *N*-Boc-*S*-Trity-cysteine (278 mg, 0.6 mmol) was dissolved in dichloromethane (7 mL). To this solution was added NMM

**Table 2**. FTase inhibition ($IC_{50}$, nM)

| CAAX[a] | FTase | CAAX[b] | FTase |
|---|---|---|---|
| **4a**[c] | 4.5 | **11a** | 9,100 |
| **4b**[d] | 0.5 | **11b** | 4 |
| **4c** | na[e] | **11c** | 5,300 |
| **4d**[d] | 25 | **11d** | 90 |

[a]Corresponding *p*-isomers to **11**. [b]Corresponding *m*-isomers to **4**. [c]See ref. 6(a). [d]See ref. 6(b). [e]Not available.

(132 mL, 1.2 mmol) at −20 °C under argon, followed by IBCF (78 mL, 0.6 mmol). The reaction mixture was allowed to stir for 15 min. At this time TLC showed the absence of the starting material. To this solution the amine **9a** (227 mg, 0.57 mmol) was introduced. The reaction mixture was stirred 2 hr at the same temperature. The reaction mixture was washed with 1 *N* HCl, 5% sodium bicarbonate and water, dried over magnesium sulfate, and solvent was removed. The residue was chromatographed on silica gel using 30% ethyl acetate in hexanes to yield **10a** (344 mg, 75 %): $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 8.57 (br s, 1H), 7.81 (dd, 1H, $J$ = 8.4, 2.2 Hz), 7.45-7.19 (m, 22H), 6.27 (br s, 1H), 5.07 (br s, 1H), 4.65 (dd, 1H, $J$ = 12.6, 7.2 Hz), 4.12 (br s, 1H), 3.66 (s, 3H), 2.75 (dd, 1H, $J$ = 12.8, 7.1 Hz), 2.63 (dd, 1H, $J$ = 12.8, 5.5 Hz), 2.12 (t, 2H, $J$ = 7.5 Hz), 2.00 (s, 3H), 1.92 (m, 1H), 1.77 (m, 1H), 1.43 (s, 9H); FAB-MS (MNBA, NaCl) $m/z$ 826 $[M + Na]^+$.

**5-[(2R)-*N*-Boc-amino-3-*S*-Tr-propyl]amino-2-phenyl-benzoyl-methionine methyl ester (10b).** To a solution of 2-phenyl-5-aminobenzoyl-methionine methyl ester **9a** (158 mg, 0.4 mmol) and *N*-Boc-*S*-tritylcysteinal (1.2 equiv) in methanol (10 mL) were added acetic acid (0.5 mL), followed by sodium cyanoborohydride (38 mg, 0.6 mmol). The reaction mixture was allowed to react overnight. After removal of the solvent, the residue was partitioned with ethyl acetate and 5% sodium bicarbonate. The organic layer was washed with water and brine, dried over magnesium sulfate, and solvent was removed. The residue was chromatographed on silica gel using 30% ethyl acetate in hexanes to yield **10b** (252 mg, 79.7 %): $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 7.56-7.23 (m, 21H), 6.89 (d, 1H, $J$ = 2.4 Hz), 6.76 (dd, 1H, $J$ = 8.3, 2.5 Hz), 5.93 (d, 1H, $J$ = 7.7 Hz), 4.76 (dd, 1H, $J$ = 12.5, 7.1 Hz), 4.66 (br s, 1H), 4.09 (br s, 1H), 3.88 (br s, 1H), 3.75 (s, 3H), 3.21 (t, 2H), 2.57 (d, 2H), 2.12 (m, 2H), 2.10 (s, 3H), 2.03-2.19 (m, 1H), 1.85-1.78 (m, 1H), 1.54 (s, 9H); FAB-MS (MNBA, NaCl) $m/z$ 812 $[M + Na]^+$.

**Cysteinyl-(5-amino-2-phenyl)benzoyl-methionine (11a).** To a solution of the methyl ester **10a** (285 mg, 0.35 mmol) in THF (5 mL) and methanol (3 mL) was added 1 *N* LiOH (1 mL) in an ice-salt bath. The reaction mixture was stirred for 4 hr. The reaction mixture was adjusted to pH 2-3 with 1 *N* HCl at the same temperature and the solvent was evaporated. The resulting residue was partitioned between chloroform and water. The organic phase was dried over anhydrous magnesium sulfate, and concentrated in vacuo to give the resulting free acid in a quantitative yield. Without purification, the acid was used to the next reaction. To a solution of the acid in dichloromethane (5 mL) were added TFA (2 mL) and a few drops of triethylsilane. After 3 h, the reaction mixture was thoroughtly evaporated under high vacuum to give an oily residue. The residue was triturate with anhydrous ether and the white solid was collected by filtration to give the crude compound, which was then purified by Prep-HPLC to afford **11a** (80 mg, 40.7%): HPLC 98% (purity): $^1$H NMR (300 MHz, CD3OD) $\delta$ 7.83 (d, 1H, $J$ = 1.9 Hz), 7.75 (dd, 1H, $J$ = 8.4, 2.1 Hz), 7.44-7.32 (m, 6H), 4.49 (dd, 1H, $J$ = 9.5, 3.9 Hz), 4.17 (t, 1H, $J$ = 6.6 Hz), 3.17 (dd, 1H, $J$ = 14.6, 5.1 Hz), 3.05 (dd, 1H, $J$ = 14.5, 7.0 Hz ), 2.20-2.14 (m, 2H), 2.12-2.04 (m, 1H), 2.00 (s, 3H), 1.85-1.77 (m, 1H); FAB-MS (MNBA, NaCl) $m/z$ 470 $[M + Na]^+$.

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