Pergamon

*Bioorganic & Medicinal Chemistry Letters*, Vol. 4, No. 23, pp. 2775-2780, 1994

0960-894X/94 $7.00+0.00

0960-894X(94)00419-6

# CYSTEINE AND METHIONINE LINKED BY CARBON PSEUDOPEPTIDES INHIBIT FARNESYL TRANSFERASE

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**Abstract.** Compounds in which cysteine and methionine have been linked by amino acids replacing the $A_1A_2$ portion of the $CA_1A_2X$ box template inhibit farnesyl and geranylgeranyl transferases. The expected specificity for FTase over GGTase I is observed. A variety of linkers are accepted with the most potent compounds possessing a hydrophobic substituent at C-2 of the $A_1A_2$ replacement.

The ras oncogene has been shown to be present in several clinically important human tumors, including tumors of the pancreas and colon. Current thought postulates that ras protein is involved in cellular growth regulation effected through its molecular role in signal transduction. Oncogenic ras mutations found in cancer cells differ from the protooncogene found in normal cells by loss of regulatory and catalytic function.[1]

The proteins encoded by the ras gene family are members of a superfamily of small GTP-hydrolyzing proteins (small G-proteins) which must be post-translationally modified in order to retain their full function.[2] In the case of ras, post-translational processing is necessary to ensure that the finished protein is properly localized to the plasma membrane.[3] For ras proteins the first step of this post-translational processing consists of the attachment of a farnesyl group to a cysteine residue mediated by the enzyme farnesyl transferase (FTase). This is followed by the cleavage of three C-terminal amino acids and subsequent methylation of the carboxyl terminus. These processes are signaled by a short consensus sequence at the C-terminus of the protein known as the $CA_1A_2X$ box, in which C = cysteine, $A_1$, $A_2$ = aliphatic amino acid, X = methionine, serine, or glutamine. Other proteins that posses the $CA_1A_2X$ box and have been indicated to undergo attachment of farnesyl are lamin A[4] and the $\gamma$ subunit of transducin.[5]

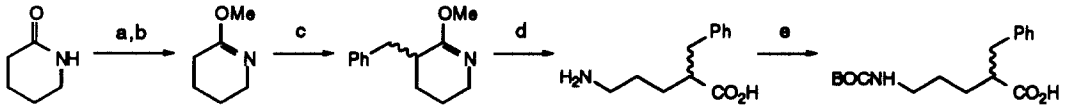
On the other hand, a far greater number of small G-proteins are modified by the attachment of a geranylgeranyl residue mediated by geranylgeranyl transferases (GGTases). At least two distinct GGTases have been identified and characterized so far. One of these GGTase I, recognizes the $CA_1A_2X$ box in which X = leucine or phenylalanine.[6] Proteins processed by GGTase I are also further processed by proteolysis and methylation. The role and function of different proteins modified by geranylgeranyl is quite diverse. Unresolved issues with the selection of FTase as a therapeutic target are possible side effects arising through inhibition of GGTase(s).

The characterization and inhibition of FTase by tetrapeptides such as CVFM has been previously reported.[7] For several reasons peptides do not readily make useful therapeutics, and we and others have reported on strategies toward the preparation of improved peptidomimetic inhibitors.[8-10] Results thus far have indicated that inhibition of ras post-translational processing both *in vitro* and in whole cells is possible through the use of compounds in which either one or two of the internal peptide bonds have been replaced by either an amine[8], aromatic[9], or diazepam[10] linkage. Herein we report novel extensions of this strategy employing compounds in which the two internal amino acids have been replaced by carbon peptidomimetic linkers.

Because the $CA_1A_2X$ box is characterized both by terminal specificity and internal variability, an obvious simplifying approach toward examining the spatial requirements for inhibition of FTase might be the connection of cysteine and methionine residues by ω-amino acids of varying chain length. Such compounds are compared by their intrinsic *in vitro* inhibition of FTase and GGTase I in Figure 1. The maximum inhibitory activity is seen for the longer analog n = 7 (**2**) rather than the analog n = 5 (**1**) which maintains the same number of backbone atoms between the cysteine and methionine residues as in the tetrapeptide CVFM. One possible interpretation of this data is that the two extra carbon atoms in **2** place a bend or kink in the linker which is contributing to a greater hydrophobic enzyme interaction in the center portion of the molecule while maintaining the active distance geometry of the cysteine and methionine. It is interesting to note the potency of **2** ($IC_{50}$ for inhibition of FTase = 1.0 μM), which although an extremely simple analog, is comparable to the tetrapeptide CVLS ($IC_{50}$ = 0.9 μM) which corresponds to the C-terminal sequence of H-ras.

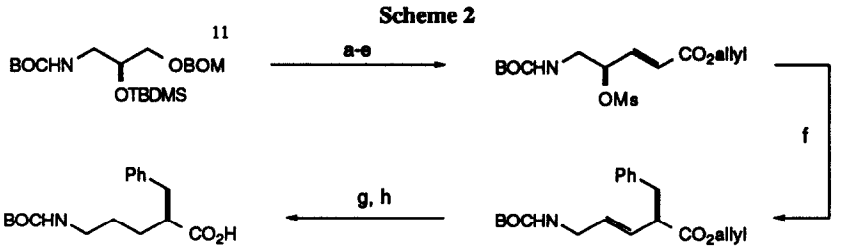
The above results suggest that additional hydrophobic character might be expected to enhance binding of these simple structures; consequently attachment of C-2 arylmethyl groups to the $A_2$ position was pursued. These compounds might be considered more analogous to CVFM. The required 2-arylmethyl-δ-N-BOC amino acid intermediates were prepared in racemic form in expeditious fashion (Scheme 1). The C-2 enantiomers produced from this route were resolved chromatographically as diastereomers subsequent to coupling with the terminal methionine and cysteine amino acids. Assignment of the absolute configuration of C-2 of analogs **3** and **4** was performed by independent enantiospecifc synthesis (Scheme 2). If separable, assignment of the configuration of the other analogs **6** - **12** was based on comparison of relative HPLC retention times .

**Scheme 1**




a. $Me_2SO_4$ b. NaOH, 38%, 2 steps c. LDA, HMPA, BnBr d. NaOH, 22%, 2 steps e. $BOC_2O$, NaOH, 74%

**Scheme 2**




a. $H_2$, 20% $Pd(OH)_2$, 87% b. DMSO, $(COCl)_2$, TEA c. $Ph_3PCHCO_2$allyl, 76% d. TBAF, 62% e. MsCl, TEA, 97% f. CuCN, BnMgCl, $BF_3$•$OEt_2$, -78 °C, 29% g. $Pd(PPh_3)_4$, $PPh_3$, sodium 2-ethylhexanoate, 81% h. $H_2$, 20% $Pd(OH)_2$, quant.

Several C-2-arylmethyl $A_2$ analogs (**3** - **14**) were evaluated as inhibitors of both FTase and GGTase (Table 1). It is readily apparent that the addition of a variety of C-2 aryl groups results in much improved inhibition of both

**Figure 1. Straight Chain $A_1A_2$ Replacements are Inhibitors of FTase and GGTase.**[12]

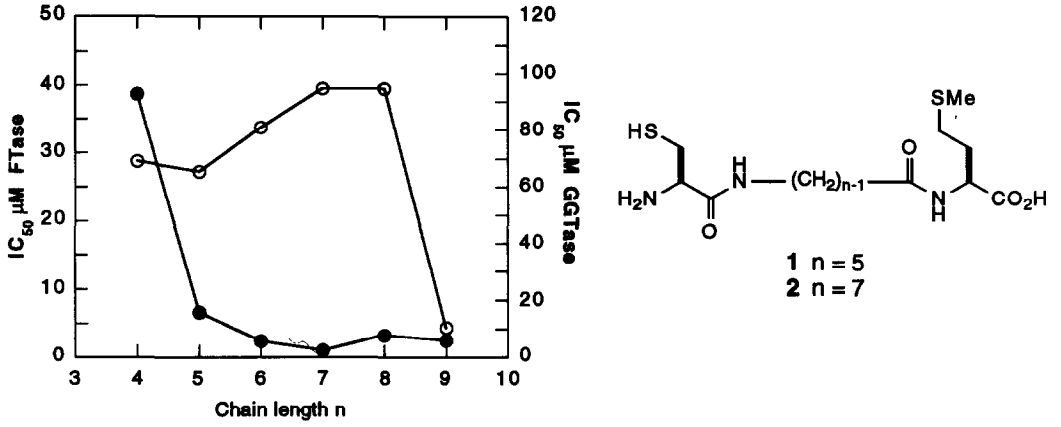
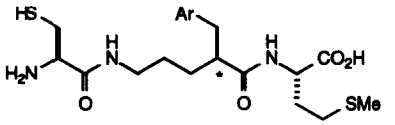



**Table 1. Addition of a C-2-Arylmethyl Substituent to $A_1A_2$ Replacements Result in Improved Inhibition of Both FTase and GGTase.**[12]




| Analog | Ar | Configuration | FTase $IC_{50}$ (μM) | GGTase $IC_{50}$ (μM) | Ratio GGTase/FTase |
|---|---|---|---|---|---|
| 3 | Ph | R | 0.018 | 0.33 | 18.5 |
| 4 | Ph | S | 0.020 | 4.1 | 205 |
| 5 | Ph | R/S mixture | 0.016 | 1.01 | 63.1 |
| 6 | *p*-$C_6H_4OMe$ | R/S mixture | 0.170 | 1.12 | 6.59 |
| 7 | *p*-$C_6H_4F$ | R/S mixture | 0.009 | 0.090 | 10.0 |
| 8 | *p*-$C_6H_4Me$ | R | 0.315 | 0.51 | 1.62 |
| 9 | *p*-$C_6H_4Me$ | S | 0.105 | 26.5 | 252 |
| 10 | 2-$C_4H_3S$ | R | 0.007 | 0.7 | 100 |
| 11 | 2-$C_4H_3S$ | S | 0.008 | 5.1 | 638 |
| 12 | 3,5-Di-F-$C_6H_3$ | R | 0.016 | 0.99 | 61.9 |
| 13 | 3,5-Di-F-$C_6H_3$ | S | 0.032 | 2.2 | 68.8 |
| 14 | Ph, Ph (*bis* benzyl) | R and S | 1.86 | 19.0 | 10.2 |

prenyl transferases. For example, both analogs **3** and **4** are at least 50 times more inhibitory toward either FTase or GGTase than the unsubstituted parent analog **1**. Interestingly, for **3** and **4**, inhibition of FTase is insensitive to the configuration of the C-2 center (which corresponds to the α position of the $A_2$ amino acid), while GGTase is less sensitive to inhibition by material of the natural amino acid configuration (**S**). This trend is seen throughout the table suggesting that GGTase may be more specific in its hydrophobic requirements for binding to this region of the inhibitor. No evidence from this data suggests any significant π-stacking interactions between C-2 $A_2$ arylmethyl substituents and the complementary region of the transferases. On the other hand, for FTase, there exists steric restrictions on the nature of the C-2 $A_2$ arylmethyl substituents. Compounds that have substituents isosteric with benzyl (**7**, **10**, **11**, **12**, **13**) are equipotent relative to **3** while those that have substituents with greater steric requirements (**6**, **8**, **9**) exhibit decreased potency. Disubstitution by two C-2 arylmethyl substituents as in analog **14** results in a large loss of potency indicating that the corresponding arylmethyl substituents on **3** and **4** probably occupy the same hydrophobic pocket of the enzyme.

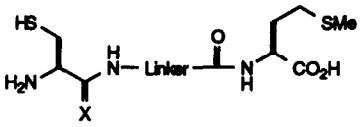
Because the FTase seems rather promiscuous with respect to the precise composition of the $A_1A_2$ region of the inhibitors, several other linker structures were examined (see Table 2). Addition of an olefin amide bond isostere in **17** was nonproductive when compared to **4** suggesting that these analogs may not be binding to FTase strictly analogously to CVFM. Interestingly, analog **24** is slightly less potent than **4**, even though it differs only by the addition of an isopropyl group in the same orientation as in the valine residue of CVFM. Replacement of the C-2 benzyl substituent in **24** by a cyclohexylmethyl substituent as in compound **26** dramatically reduces inhibitory potency. This further supports steric limitations in this portion of the inhibitor for the complementary regions of FTase.

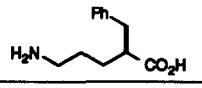
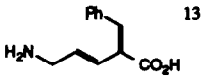
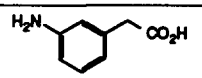
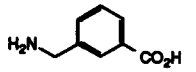
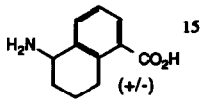
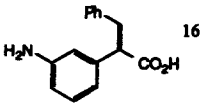
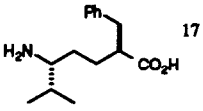
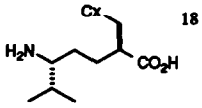
Tolerance of FTase to aliphatic ω-amino acid linkers longer than the CVFM template suggested that backbone aromatic residues might also be tolerated as linkers. Analogs **18** - **23** were prepared to examine this possibility. The precise position of the phenyl ring appears crucial; aminomethyl benzoic acid derivatives **20** and **22** both have $IC_{50}$'s below that of straight chain analog **1**, while aminophenylacetic acid derivatives **18** and **23**, which have the phenyl ring displaced just one carbon, are almost an order of magnitude less potent than compounds **20** and **22**.

Several groups have examined the replacement of the C-$A_1$ peptide bond by a reduced amine linkage[8] which appears to increase both intrinsic potency and stability to exogenous peptidases. Five analogs in this series were evaluated with this modification, **15**, **16**, **19**, **21**, and **25**, which were prepared by conventional reductive amination with S-trityl-N-BOC-cysteinal and the corresponding amine. Unlike the lead tetrapeptides CVFM and CVIM in which this modification increases potency[8], only one compound, **19**, was more potent than its parent amide (**18**). This increase in potency seen with **19** is probably related to the recovery of flexibility suppressed by the non optimal planar relationship of the amide and phenyl group in **18**. It appears that there may be substantial differences in the binding modes of the isoprenyl transferases across different classes of similar substrates.

The compounds described herein have been useful in the exploration of structural requirements for FTase inhibition. This information is being used for the further development of more potent inhibitors that are intended to meet the more rigorous requirements for ultimate use against human cancers.

**Table 2. A Variety of Amino Acids Linking Cysteine and Methionine Inhibit Isoprenyl Transferases[12]**




| Compound | X | Linking Amino Acid | FTase $IC_{50}$ (μM) | GGTase $IC_{50}$ (μM) | Ratio: GGTase/FTase |
|---|---|---|---|---|---|
| 1<br>15 | O<br>$H_2$ |  | 6.6<br>20.4 | 65.5<br>>100 | 9.92<br>>4.90 |
| 4<br>16 | O<br>$H_2$ |  | 0.020<br>0.080 | 4.1<br>5.95 | 205<br>74.4 |
| 17 | O |  13 | 0.23 | 2.0 | 8.70 |
| 18<br>19 | O<br>$H_2$ |  | 3.3<br>0.24 | 38.6<br>9.74 | 11.7<br>40.6 |
| 20<br>21 | O<br>$H_2$ |  | 0.35<br>1.20 | 18.4<br>21.2 | 52.6<br>17.7 |
| 22[14] | O |  15 | 0.94 | 18.6 | 19.8 |
| 23 | O |  16 | 9.7 | 11.7 | 1.21 |
| 24<br>25 | O<br>$H_2$ |  17 | 0.026<br>0.033 | 8.2<br>3.4 | 315<br>103 |
| 26 | O |  18 | 1.9 | 18.4 | 9.68 |

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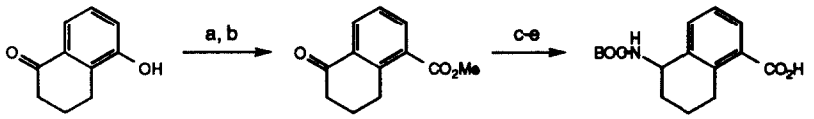
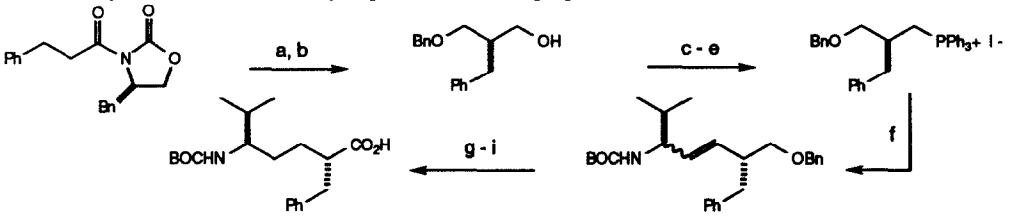
11. Prepared from D-glyceraldehyde acetonide by consecutive treatment with 1) benzyl amine, $NaH_3BCN$ 2) $H_2/Pd(OH)_2$ 3) HCl 4) $BOC_2O$, NaOH 5) BOM-Cl, DIEA 6) TBDMSCl, imidazole.
12. FTase and GGTase inhibition assays were performed as previously described in Garcia, A. M. *et. al.*, reference 8.
13. *trans* 2-(**R**)-benzyl-5-*t*-butoxycarbonylamino-3-pentenoic acid was prepared as depicted in Scheme 2.
14. Compound **21** is a diastereomeric mixture obtained from coupling with *racemic* 6-amino- 7, 8, 9, 10-tetrahydronaphthoic acid[15].
15. *Racemic* 6-*t*-butoxycarbonylamino- 7, 8, 9, 10-tetrahydronaphthoic acid was prepared as follows:




a. $Tf_2O$, lutidine b. $Pd(OAc)_2$, CO, MeOH, 75 °C c. $NH_4OAc$, $NaH_3BCN$ d. $BOC_2O$ e. NaOH

16. 2-(**R**)-benzyl-aminophenyl acetic acid was prepared by alkylation of the lithium enolate of the (**R**)-4-benzyl-3-oxizolidinone derivative with benzyl bromide.
17. 2-(**R**)-benzyl-5-(**S**) amino-6-methylheptanoic acid was prepared as follows:




a. $TiCl_4$, DIPEA, 0 °C; BOM-Cl b. $LiBH_4$ c. MsCl d. NaI e. $PPh_3$ f. $KN(SiMe_3)_2$, THF; N-BOC-valinal g. $H_2$, 20% $Pd(OH)_2$ h. DMSO, $(COCl)_2$, TEA i. $NaClO_2$.

18. 2-(**R**) cyclohexylmethyl-5-(**S**)-amino-6-methylheptanoic acid was prepared in a manner similar to that used for the prepartion of the 2-(**R**)-benzyl acid in reference 15.


(*Received in USA* 14 *September* 1994; *accepted* 21 *October* 1994)