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SYNTHESIS AND EVALUATION OF HYDROXYPROLINE-DERIVED ISOPRENYLTRANSFERASE INHIBITORS

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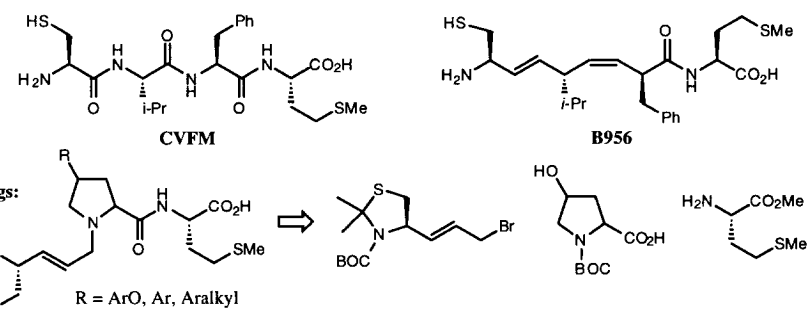
Abstract: A series of peptidomimetics based on a hydroxyproline scaffold was prepared and evaluated for inhibition of farnesyltransferase and geranylgeranyltransferase I in both enzymatic and cell-based assays. A number of analogs were potent and selective inhibitors of FTase, while one compound (**22**) was nonselective in the enzymatic assays but eight-fold selective for inhibition of GGTase in the cellular assay (IC_{50} = 0.39 μM).

Introduction: The *ras* oncogene plays a role in mammalian growth regulatory signaling pathways and exists in mutated forms in a high percentage of human tumors, most notably colon and pancreatic carcinomas.[1] The mature protein product of *ras* is produced via a series of four post-translational modifications, and the first of these is farnesylation of the thiol group of a C-terminal cysteine residue. *S*-Farnesylation is catalyzed by the enzyme farnesyltransferase (FTase) and is crucial to the ability of the protein to associate with the inner surface of the plasma membrane where protein mediation of signaling takes place.[2] Accordingly, inhibition of FTase represents a rational approach to novel anticancer agents and is the goal of numerous medicinal chemistry programs.[3] However, it has been demonstrated in whole cell experiments that inhibition of FTase can lead to prenylation of *K-ras* protein by the enzyme geranylgeranyltransferase I (GGTase I),[4] suggesting that GGTase I might be an additional target for new anticancer agents. As part of an ongoing program to develop inhibitors of ras prenylation, we now report the preparation and biological evaluation of a series of tetrapeptide mimetics with a core scaffold derived from hydroxyproline.

Design and synthesis: Our development of small molecule FTase inhibitors began, as in many other groups, with the tetrapeptide CVFM (Scheme 1). This peptide is a potent, competitive FTase inhibitor and is not a substrate for the enzyme.[5] The diene **B956**[4a,6a] was a potent CVFM peptidomimetic that showed in vivo activity,[6b] and led us to consider, among various issues, the bioactive conformation of our FTase inhibitors.

Scheme 1



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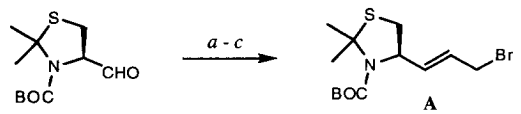
There has been some debate over the question of whether peptide and peptidomimetic FTase inhibitors exert their activity from an extended[7a,b] or a turn-like conformation.[7c,d] Analogs of **B956** where the *cis*-alkene/phenylalanine moiety was replaced by a hydroxyproline core[8] (Scheme 1) were viewed as compounds which might demonstrate a more pronounced turn-like conformation. In addition, the modular synthesis of these analogs should make them readily accessible and allow for a rapid accumulation of SAR data. For these reasons, the hydroxyproline-based compounds were attractive targets and their syntheses are outlined below.

The cysteine-derived allylic bromide **A** was prepared as shown in Scheme 2. The aldehyde[9] was homologated to the unsaturated ester, and this was easily converted in two steps to the desired electrophile.

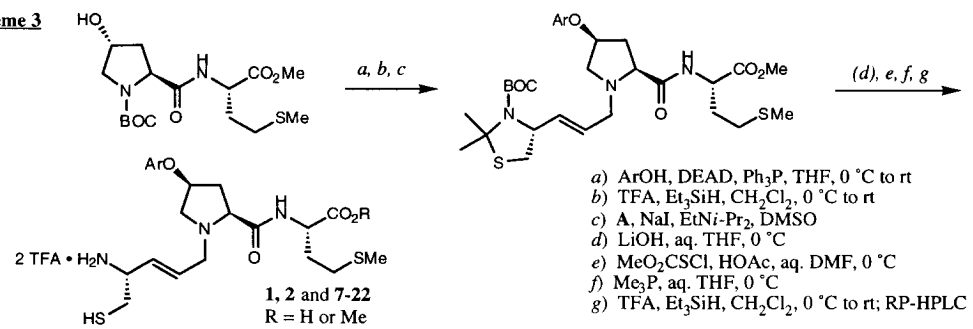
Scheme 2



a) $Ph_3P{=}CHCO_2Me$, toluene, 85 °C
b) DIBAL, THF, 0 °C
c) CBr_4, Ph_3P, THF, 0 °C

The BOC-protected Hyp-Met dipeptide[10] (Scheme 3) was converted to a variety of aryl ethers in high yield using Mitsunobu conditions.[11] *N*-BOC-removal and alkylation of the 2°-amine with **A** gave the protected tetrapeptide mimetics. Cleavage of the dimethylketal with methoxycarbonylsulfenyl chloride was followed by reduction of the resulting mixed disulfide with trimethylphosphine.[6a] Deprotection of the 1°-amine under acidic conditions gave the methyl ester of each target analog which was purified by RP-HPLC. Methyl ester hydrolysis prior to the N-terminal deblock sequence afforded each target analog as the C-terminal carboxylic acid.

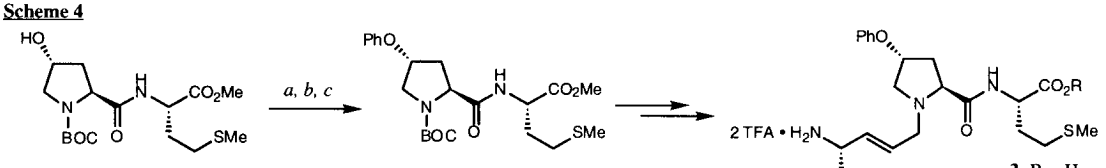
Scheme 3



a) ArOH, DEAD, Ph_3P, THF, 0 °C to rt
b) TFA, Et_3SiH, CH_2Cl_2, 0 °C to rt
c) **A**, NaI, $EtNi\text{-}Pr_2$, DMSO
d) LiOH, aq. THF, 0 °C
e) MeO_2CSCl, HOAc, aq. DMF, 0 °C
f) Me_3P, aq. THF, 0 °C
g) TFA, Et_3SiH, CH_2Cl_2, 0 °C to rt; RP-HPLC

Stereochemical variation about the central ring of the phenyl ether analog was also examined. The hydroxy group of the Hyp-Met dipeptide was inverted using a Mitsunobu reaction with acetic acid (Scheme 4), and following cleavage of the acetate group, the β-alcohol was converted to the α-phenyl ether via another Mitsunobu reaction. This intermediate was processed as in Scheme 3 to provide the desired α-ether analogs **3/4**.

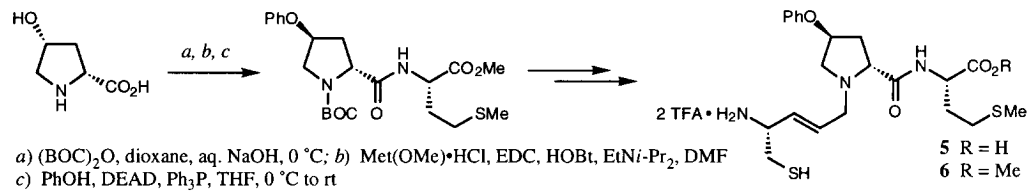
Scheme 4



a) AcOH, DEAD, Ph_3P, THF, 0 °C to rt; *b*) K_2CO_3, MeOH, 0 °C
c) PhOH, DEAD, Ph_3P, THF, 0 °C to rt;

Another pair of stereoisomers was readily available from *cis*-4-hydroxy-D-proline (Scheme 5). *N*-BOC protection of the free amino acid, dipeptide formation, and installation of the β-phenyl ether was followed by the *N*-alkylation / deprotection sequence to provide the desired α-amide isomers **5/6**.

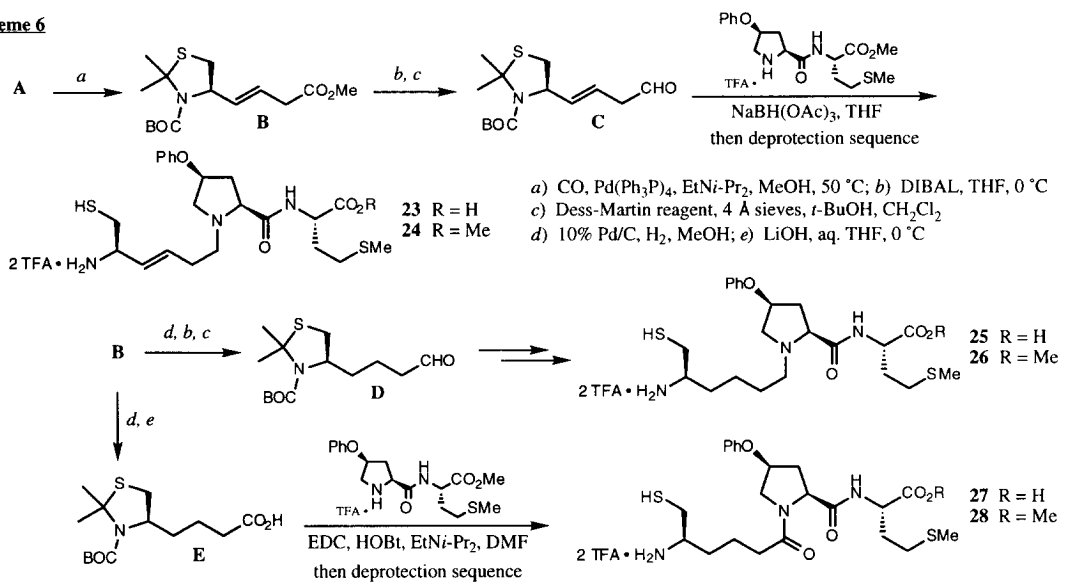
Scheme 5



a) $(BOC)_2O$, dioxane, aq. NaOH, 0 °C; *b*) Met(OMe)•HCl, EDC, HOBt, Et*N*i-Pr_2, DMF
c) PhOH, DEAD, Ph_3P, THF, 0 °C to rt

Three homologated cysteine-derived sidechains were also prepared (Scheme 6). Palladium-mediated alkoxycarbonylation[12] of **A** provided ester **B**, and this could be converted to aldehyde **C** which was used directly in a reductive amination[13] with the Hyp-Met dipeptide. Deprotection of the aminothiol functionality followed the usual course to provide the homologated analogs **23/24**. Ester **B** was also converted to the saturated aldehyde **D**, which was carried on in a similar fashion to provide analogs **25/26**. Hydrogenation and basic hydrolysis of **B** gave the acid **E** which was coupled with the dipeptide and deprotected to afford the amide derivatives **27/28**.

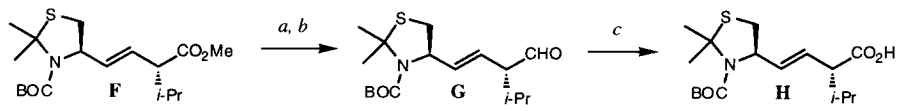
Scheme 6



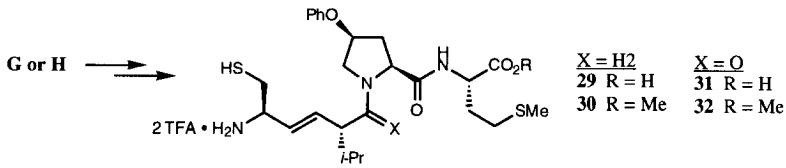
a) CO, $Pd(Ph_3P)_4$, Et*N*i-Pr_2, MeOH, 50 °C; *b*) DIBAL, THF, 0 °C
c) Dess-Martin reagent, 4 Å sieves, *t*-BuOH, CH_2Cl_2
d) 10% Pd/C, H_2, MeOH; *e*) LiOH, aq. THF, 0 °C

The isopropyl-substituted ester **F** was prepared as previously described[6a] (Scheme 7), and a reduction/oxidation sequence provided the aldehyde **G**. Direct ester hydrolysis of **F** resulted in epimerization of the isopropyl group, but oxidation[14] of **G** provided the carboxylic acid **H** as a single diastereomer. Reductive amination (**G**) or amide coupling (**H**) with the Hyp-Met dipeptide provided the desired targets after deprotection.

Scheme 7



a) DIBAL, THF, 0 °C; *b*) $(ClCO)_2$, DMSO, Et_3N, CH_2Cl_2, -78 °C to 0 °C
c) $NaClO_2$, Na_2HPO_4, t-BuOH, aq. THF, isobutylene, 0 °C



Discussion: Table 1 shows the screening results for the peptidomimetics prepared from the hydroxyproline scaffold. The in vitro assays[15] used recombinant FTase or GGTase, and measured the intrinsic activity and selectivity of the analogs for inhibition of protein prenylation. The cell-based assays[15] are more meaningful in that any drug designed to act through prenyltransferase inhibition will do so only after permeating the target cells.

An early analog, phenyl ether **1**, showed good in vitro inhibition of both FTase and GGTase, although **1** was not as potent as **B956**. As observed for other series of prenylation inhibitors, the C-terminal carboxylic acid derivatives such as **1** were generally not active in the cell-based assay and we relied on methyl ester functionality to provide cell permeability. In this case, ester **2** showed promising, albeit not very selective FTase activity in cells and prompted us to continue with the hydroxyproline series. Altering the stereochemistry about the pyrrolidine core (**3/4** and **5/6**) resulted in dramatic losses of activity in vitro and in whole cells, and the stereochemistry of **1/2** was retained in subsequent analogs.

We speculate that the β-aryl ether substituent binds in fairly large pockets or along large hydrophobic surfaces of the enzymes as changing the phenyl ether to a tetrahydronaphthyl, naphthyl, or 4-biphenyl ether did not significantly change the in vitro activity. In two of these examples, however, the cellular selectivity was altered relative to that of **2**; ester **12** was nonselective and ester **8** was somewhat GGTase-selective. The electronic nature of the aryl ether was also probed (**13/14** and **15/16**). The electron-withdrawing CF_3-group did not significantly alter the in vitro activity/selectivity but did make ester **14** much less active in cells relative to **2**. The electron-rich dimethoxyphenyl group made a very FTase-selective inhibitor in vitro (**15**), but also resulted in a large loss of cellular activity (**16**). This may simply reflect the loss of inherent inhibitory activity of acid **15** relative to **1**. For ester **14**, the loss of cellular activity may result from a decreased ability of the ester to penetrate the cell, but could also arise from a reduced level of prodrug hydrolysis in the cell. Ortho *n*-alkyl substitution on the phenyl ether (**17/18**) appeared to be less well tolerated than para *n*-alkyl substitution (**19/20**) for cellular activity, and a branched alkyl group in the para-position (**21/22**) provided a compound that was a potent, slightly FTase-selective inhibitor in vitro but about eight-fold selective for GGTase in cells. Ester **22** is the most potent and selective CVFM peptidomimetic inhibitor of GGTase I in cells that we have tested.[16]

Adding one carbon to the cysteine-derived portion of the analogs (**23-28**) provided more potent and more selective FTase-inhibitors. Replacing the positively charged 3°-amine with a neutral amide led to a very potent FTase inhibitor in vitro (**27**), but somewhat surprisingly did not provide a similar increase to the inhibition observed in whole cells (**28**). This suggests that cellular penetration relies on more than just membrane

permeability. Incorporating the isopropyl group of **B956** into this homologated sidechain (**29-32**) provided an active compound (**30**) which showed no significant loss of activity in the cellular assay relative to the in vitro assay. Again, the amide analog (**32**) was less active in cells than the positively charged amine analog (**30**).

While we have no direct evidence that any of the above analogs adopt a turn-like conformation when binding to FTase or GGTase I, the introduction of the hydroxyproline core has provided a number of potent and selective FTase inhibitors, and has also provided us with an analog selective for GGTase I in cells. The ester **22** should be a useful tool to help elucidate the contributions of protein farnesylation or geranylgeranylation to growth regulation. In addition, our initial exploration of substitution on the aryl ether as well as the *N*-terminal portion of these peptidomimetics suggests that a number of subtle and not readily separable factors contribute to the enzymatic selectivity as well as to cellular activity.

Table 1. Biological Evaluation of Hydroxyproline-derived Peptidomimetics

Ar	Cmpd R = H	FTase in vitro[a] IC_{50} (μM)	GGTase in vitro[b] IC_{50} (μM)	Cmpd R = Me	FTase whole cells[c] IC_{50} (μM)	GGTase whole cells[d] IC_{50} (μM)
---	**B956**	0.026	0.001	**B956**	0.37	>100
phenyl	**1**	0.057	0.053	**2**	5.3	21.9
"	**3**	4.0	8.0	**4**	>50	>50
"	**5**	3.3	11.0	**6**	>50	nd[e]
5,6,7,8-tetrahydronaphthyl	**7**	0.047	0.089	**8**	>10	3.1
1-naphthyl	**9**	0.10	0.20	**10**	22.5	>50
4-biphenyl	**11**	0.035	0.25	**12**	3.3	3.2
4-(trifluoromethyl)phenyl	**13**	0.11	0.11	**14**	>50	>50
3,4-dimethoxyphenyl	**15**	0.45	67.0	**16**	>50	>50
2-*n*-propylphenyl	**17**	0.21	0.17	**18**	>10	>10
4-*n*-butylphenyl	**19**	0.087	0.18	**20**	3.2	3.1
4-isopropylphenyl	**21**	0.082	0.21	**22**	3.0	0.39
phenyl	**23**	0.020	0.57	**24**	0.49	>50
"	**25**	0.020	3.3	**26**	0.35	>50
"	**27**	0.002	0.1	**28**	2.0	>50
"	**29**	0.017	0.15	**30**	0.04	>25
"	**31**	0.018	0.011	**32**	0.84	>50

[a]Farnesylation of H-ras protein; [b]Geranylgeranylation of H-ras-CAIL protein; [c]Farnesylation of H-ras protein in NIH 3T3 cells transformed with activated H-ras; [d]Geranylgeranylation of Rap-1A protein in NIH 3T3 cells transformed with activated H-ras; [e]nd = not determined

References and Notes

1. (a) Barbacid, M. *Ann. Rev. Biochem.* **1987**, *56*, 779. (b) Khosravi-Far, R.; Der, C. J. *Cancer Metastasis Rev.* **1994**, *13*, 67.

2. (a) Reiss, Y.; Goldstein, J. L.; Seabra, M. C.; Casey, P. J.; Brown, M. S. *Cell* **1990**, *62*, 81. (b) Scaber, M. D.; O'Hara, M. B.; Garsky, V. M.; Mosser, S. C.; Bergstrom, J. D.; Moores, S. L.; Marshall, M. S.; Freidman, P. A.; Dixon, R. A.; Gibbs, J. B. *J. Biol. Chem.* **1990**, *265*, 14701. (c) Kato, K.; Der, C.; Buss, J. E. *Semin. Cancer Biol.* **1992**, *3*, 179.
3. (a) Ayral-Kaloustian, S.; Skotnicki, J. S. *Ann. Rep. Med. Chem.* **1996**, *31*, 171. (b) Williams, T. M. *Exp. Opin. Ther. Patents* **1998**, *8*, 553.
4. (a) Rowell, C. A.; Kowalczyk, J. J.; Lewis, M. D.; Garcia, A. M. *J. Biol. Chem.* **1997**, *272*, 14093. (b) Whyte, D. B.; Kirschmeier, P.; Hockenberry, T. N.; Nunez-Oliva, I.; James, L.; Catino, J. J.; Bishop, W. R.; Pai, J.-K. *ibid.* **1997**, *272*, 14459.
5. Brown, M. S.; Goldstein, J. L.; Paris, K. J.; Burnier, J. P.; Marsters, J. C., Jr. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 8313.
6. (a) Yang, H.; Sheng, X. C.; Harrington, E. M.; Ackermann, K.; Garcia, A. M.; Lewis, M. D. *J. Org. Chem.* **1999**, *64*, 242. (b) Nagasu, T.; Yoshimatsu, K.; Rowell, C.; Lewis, M. D.; Garcia, A. M. *Cancer Res.* **1995**, *55*, 5310.
7. (a) Qian, Y.; Blaskovich, M. A.; Saleem, M.; Seong, C. M.; Wathen, S. P.; Hamilton, A. W.; Sebti, S. M. *J. Biol. Chem.* **1994**, *269*, 12410. (b) Dunten, P.; Kammlott, U.; Crowther, R.; Weber, D.; Palermo, R.; Birktoft, J. *Biochemistry* **1998**, *37*, 7907. (c) James, G. L.; Goldstein, J. L.; Brown, M. S.; Rawson, T. E.; Somers, T.; McDowell, R. S.; Crowley, C. W.; Lucas, B. K.; Levinson, A. D.; Marsters, J. C., Jr. *Science* **1993**, *260*, 1937. (d) Stradley, S. J.; Rizo, J.; Gierasch, L. M. *Biochemistry* **1993**, *32*, 12586.
8. For related peptidomimetic FTase inhibitors incorporating a substituted proline residue, see: (a) Clerc, F.-F. **1999**, US Patent #5,856,439. (b) de Solms, S. J.; Graham, S. L. **1995**, US Patent #5,439,918.
9. Duthaler, R. O. *Angew. Chem.; Int. Ed. Engl.* **1991**, *30*, 705.
10. Prepared via EDC-mediated coupling of *N*-BOC-*trans*-4-hydroxy-L-proline and L-methionine methyl ester hydrochloride (HOBt, EtN*i*-Pr_2, DMF).
11. (a) Mitsunobu, O. *Synthesis* **1981**, 1. (b) Krapcho, J.; Turk, C.; Cushman, D. W.; Powell, J. R.; DeForrest, J. M.; Spitzmiller, E. R.; Karanewsky, D. S.; Duggan, M.; Rovnyak, G.; Schwartz, J.; Natarajan, S.; Godfrey, J. D.; Ryono, D. E.; Nuebeck, R.; Atwa, K. S.; Petrillo, E. W., Jr. *J. Med. Chem.* **1988**, *31*, 1148.
12. (a) Tsuji, J.; Sato, K.; Okumoto, H. *Tetrahedron Lett.* **1982**, *23*, 5189. (b) Murahashi, S.-I.; Imada, Y.; Taniguchi, Y.; Higashiura, S. *J. Org. Chem.* **1993**, *58*, 1538.
13. Abdel-Magid, A. F.; Maryanoff, C. A.; Carson, K. G. *Tetrahedron Lett.* **1990**, *31*, 5595.
14. (a) Kraus, G. A.; Taschner, M. J. *J. Org. Chem.* **1980**, *45*, 1175. (b) Bal, B. S.; Childers, W. E.; Pinnick, H. W. *Tetrahedron* **1981**, *37*, 2091.
15. Garcia, A. M.; Rowell, C.; Ackermann, K.; Kowalczyk, J. J.; Lewis, M. D. *J. Biol. Chem.* **1993**, *268*, 18415.
16. For other GGTase-selective peptidomimetics, see: (a) Vogt, A.; Qian, Y.; McGuire, T. F.; Hamilton, A. D.; Sebti, S. M. *Oncogene* **1996**, *13*, 1991. (b) Vogt, A.; Qian, Y.; McGuire, T. F.; Hamilton, A. D.; Sebti, S. M. *J. Biol. Chem.* **1997**, *272*, 27224. (c) Vasudevan, A.; Qian, Y.; Vogt, A.; Blaskovich, M. A.; Ohkanda, J.; Sebti, S. M.; Hamilton, A. D. *J. Med. Chem.* **1999**, *42*, 1333.