Synthesis and Evaluation of Some Hydroxyproline-Derived Peptidomimetics as Isoprenyltransferase Inhibitors

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Received September 20, 1999; accepted December 17, 1999

CA_1A_2X peptidomimetics containing a modified proline at position A_2 were prepared and evaluated for their ability to inhibit farnesyltransferase (FTase) and geranylgeranyltransferase I (GGTase I) in enzymatic and cell-based assays. These compounds inhibited farnesylation of H-ras *in vitro* in the high nanomolar to low micromolar IC_{50} range.

Key words farnesyltransferase inhibitor; CAAX; peptidomimetic; hydroxyproline; H-ras

During the course of an ongoing project to prepare farnesyltransferase (FTase) inhibitors as potential drug candidates in an anti-cancer program targeting tumors with ras mutations,[1] we found CAAX peptidomimetics, such as **1**—**6**, to have very good *in vitro* potency against FTase (Fig. 1).[2] *Trans*-isomers, such as **1**, were less active against the enzyme than *cis*-isomers (*e.g.*, **3**). Furthermore, one such compound (**6**) showed interesting specificity for inhibition of geranylgeranyltransferase I (GGTase I) *vs*. FTase in a whole cell assay.

In order to expand the structure–activity relationship of this class of inhibitor, we examined the effects of changes at the 4-position of the hydroxyproline in compounds of type **3** (and the corresponding esters, **4**) by preparing a number of analogs in which the ether oxygen was replaced by a sulfur atom or an alkyl chain or removed completely, while keeping the remainder of the peptidomimetic scaffold intact. In particular, we wished to examine how replacement of oxygen with carbon or sulfur might affect the lipophilicity or stability of such compounds.

Chemistry Thioether compounds[3] were prepared by S_N2 displacement of mesylate **7** with 4-isopropylthiophenol to give **8**. The yield of this reaction was low (15%), but **7** could be recovered (81%) and recycled. Attempts to prepare **8** using standard Mitsunobu conditions (as used in the preparation of compounds **1**—**6**) failed to give any of the desired product. Arylalkyl derivatives with a one-carbon linker connecting the aromatic group to the proline were prepared as shown in Chart 2. *N*-Boc-*trans*-4-hydroxy-L-proline methyl ester (**9**) was oxidized to ketone **10** under Swern conditions. The ketone was converted under Wittig conditions[3a] to a mixture of olefins (**11**). Hydrogenation of **11** gave **12** as a 2.5 : 1 mixture of diastereomers. These isomers were not readily separable neither at this stage nor at subsequent stages in the synthetic sequence. The major isomer was assumed to be the *cis*-isomer (hydrogenation from the less hindered face) based on a literature precedent[4] and ^{1}H-NMR analysis. This mixture was carried forward, and, as the final compounds did not display any improved qualities over analogs **3**—**6** (see Discussion), the individual isomers were never separated. Arylalkyl derivatives with a two-carbon linker were prepared as shown in Chart 3. *N*-Boc-*trans*-4-hy-

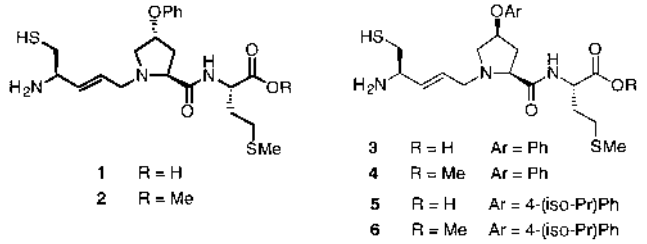
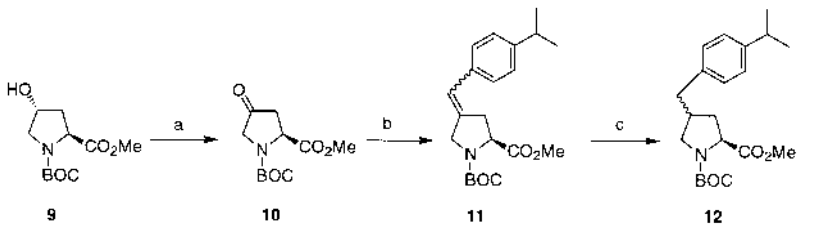


Fig. 1

Chart 1



(a) $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2, −78°C to 0°C (100%); (b) 4-(iso-Pr)$PhCH_2PPh_3Br$, KO *tert*-Bu, Et_2O, 0°C to 35°C (37%); (c) H_2, 5% Pd/C, MeOH (75%)

Chart 2

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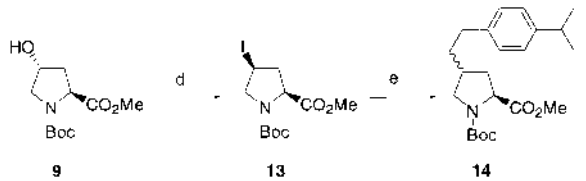
droxy-L-proline methyl ester (**9**) was converted to the *cis*-iodide[5] (**13**) under Mitsunobu-type conditions. The iodide was treated with AIBN/*n*-Bu_3SnH in the presence of *p*-isopropylstyrene to give **14** as a mixture of *cis*- and *trans*-isomers (as determined by ^{1}H-NMR).[6] Once again, this pair of compounds was evaluated as a mixture since the isomers were not readily separable by silica gel chromatography or reverse phase HPLC.

Aryl-substituted derivatives with no linker were prepared as shown in Chart 4. Ketone **10** was converted into enol triflate **15** in moderate yield.[7] This triflate was cross-coupled with various arylboronic acids under palladium catalysis (Suzuki coupling[8]) to give **16** in good yield.[7,8] We found it more convenient to use Stille's catalyst[9] in place of the more air-sensitive $Pd(PPh_3)_4$ in these cross-coupling reactions. Hydrogenation of each alkene gave a single isomer (^{1}H-NMR), assumed to be the *cis*-isomer by a literature precedent for related hydrogenations.[3a]

For each target series, the remainder of the synthesis[2] was completed by carrying out the necessary steps as shown in Charts 5 and 6.



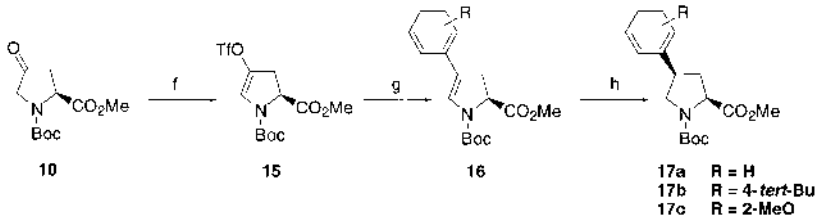
(d) CH_3I, diethyl azodicarboxylate (DEAD), PPh_3, THF, 0°C to room temp. (76%); (e) *n*-Bu_3SnH, AIBN, 4-(iso-Pr)PhCH=CH_2, benzene, 80°C (16-31%)

Chart 3

Discussion

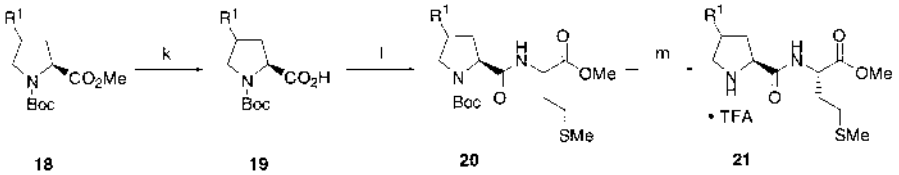
Screening results for the compounds described above are summarized in Table 1. The values for compounds **1**—**6** are presented for comparison. The *in vitro* assays[10] used recombinant FTase or GGTase, and measured the intrinsic activity and selectivity of the carboxylic acid forms of the analogs for inhibition of protein prenylation. The whole-cell assays used activated H-*ras* transfected NIH3T3 fibroblasts[10] and measured protein processing (H-ras or Rap 1A) in the presence of the methyl ester form of the inhibitors.

As can be seen in Table 1, every modification described herein led to compounds less active than compounds **3**—**6**.



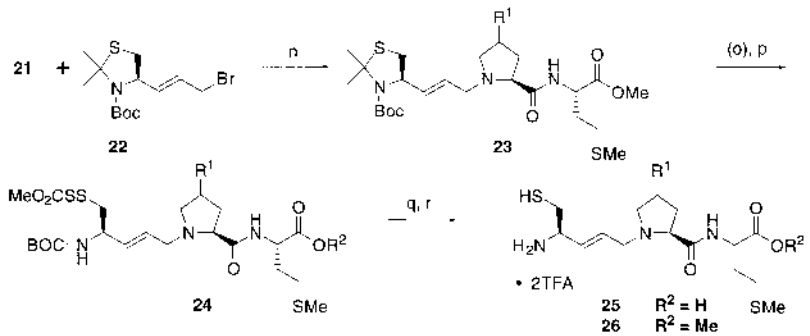
(f) i. LiN[Si(CH$_3$)$_3$]$_2$, THF, –78°C; ii. PhN(Tf)$_2$, –78°C to room temp. (30%); (g) ArB(OH)$_2$, aq. Na_2CO_3, LiCl, cat. (PhCH$_2$)(Cl)Pd(PPh$_3$)$_2$, 1,2-dimethoxyethane, 86°C (40-80%); (h) H_2, 5% Pd/C, EtOAc (quant.)

Chart 4



(k) LiOH, aq. dioxane, 0°C to room temp. (94-100%); (l) HCl•H_2N-Met-OMe, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide•HCl, HOBt•H_2O, *N*-Methylmorpholine, DMF (*ca.*94%); (m) Trifluoroacetic acid (TFA), Et_3SiH, CH_2Cl_2, 0°C (quant.)

Chart 5



(n) NaI, EtN(iso-Pr)$_2$, DMSO (*ca.* 70%); (o) LiOH, aq. THF, 0°C (quant.); (p) $ClSCO_2Me$, HOAc, H_2O, DMF, 0°C ; (q) PMe_3, H_2O, THF, 0°C; (r) TFA, Et_3SiH, CH_2Cl_2, 0°C (40-60%).

Chart 6

Table 1. Biological Evaluation of 4-Substituted L-Proline CAAX Mimetics (**25**, **26**)

R^1 isomer	R^1	Compd. R^2=H	FTase *in vitro*[a] IC_{50} (μM)	GGTase I *in vitro*[b] IC_{50} (μM)	Compd. R^2=Me	FTase whole cells[c] IC_{50} (μM)	GGTase I whole cells[d] IC_{50} (μM)
Trans	PhO–	**1**	4.0	8.0	**2**	>50	>50
Cis	PhO–	**3**	0.057	0.053	**4**	5.3	21.9
Cis	4-(iso-Pr)PhO–	**5**	0.082	0.21	**6**	3.0	0.39
Cis	4-(iso-Pr)PhS–	**25a**	0.16	0.34	**26a**	>25	3.1
Cis	Ph–	**25b**	0.79	1.8	**26b**	4.1	>30
Cis	4-(*tert*-Bu)Ph–	**25c**	1.0	0.27	**26c**	>10	~10
Cis	2-(MeO)Ph–	**25d**	0.19	0.19	**26d**	3.8	>30
Cis/trans 2.5 : 1	4-(iso-Pr)-–$PhCH_2$	**25e**	8.3	16.3	**26e**	>25	13.3
Cis/trans 1 : 1.7	4-(iso-Pr)–$PhCH_2CH_2$–	**25f**	1.8	2.8	**26f**	>10	>10

a) Farnesylation of H-ras protein. *b*) Geranylgeranylation of H-ras-CAIL protein. *c*) Farnesylation of H-ras protein in NIH3T3 cells transformed with activated H-*ras*. *d*) Geranylgeranylation of Rap-1A protein in NIH3T3 cells transformed with activated H-*ras*.

Thus, replacement of the ether oxygen with sulfur or carbon neither improved *in vitro* activity, nor improved activity in whole cells. In general, the carboxylic acids (**25**) were more active against FTase than GGTase I *in vitro*, with the exception of compound **25c** which was somewhat more potent against GGTase I than FTase *in vitro* (Table 1), and the corresponding methyl ester (**26c**) showed the same trend in whole cells. Interestingly, compounds **26a** and **26e** were slightly more potent in their inhibition of Rap 1A processing (GGTase I) than in their inhibition of H-ras processing in whole cells, although their corresponding carboxylic acids (**25a**, **25e**) were slightly more potent in their inhibition of FTase *in vitro*. We currently have no explanation for this curious phenomenon.

In summary, we have prepared a series of CA_1A_2X peptidomimetic isoprenyltransferase inhibitors based on hydroxyproline at the A_2 position, and although none of the compounds prepared in this study offered significant improvement over the most potent that we reported previously (**3**—**6**),[2] nonetheless several were relatively potent inhibitors of FTase or GGTase I *in vitro*. We found no evidence that replacing or removing the ether oxygen in compounds such as **3**—**6** improved the stability or lipophilicity. However, this class of compounds expands the field of isoprenyltransferase inhibitors and may be useful in future development of anticancer drugs.

Acknowledgements We thank Dr. C. Eric Schwartz for helpful discussions.

References and Notes

1) *a*) Barbacid M., *Ann. Rev. Biochem*., **56**, 779—827 (1987); *b*) Khosravi-Far R., Der C. J., *Cancer Metastasis Rev*., **13**, 67—89 (1994); *c*) Sebti S. M., Hamilton A. D., *Drug Discovery Today*, **3**, 26—33 (1998).
2) O'Connell C. E., Ackermann K., Rowell C. A., Garcia A. M., Lewis M. D., Schwartz C. E., *Bioorg. Med. Chem. Lett*., **9**, 2095—2100 (1999).
3) 4-(Arylthio)hydroxyproline derivatives have been prepared by the reaction of 4-hydroxyproline derivatives with *N*-(arylthio)succinimides and by displacement of a *trans*-tosylate with the sodium salt of thiophenol or an alkyl mercaptan: *a*) 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., Sesha N., Godfrey J. D., Ryono D. E., Neubeck R., Atwal K. S., Petrillo, Jr., E. W., *J. Med. Chem*., **31**, 1148—1160 (1998); *b*) Smith E. M., Swiss G. F., Neustadt B. R., Gold E. H., Sommer J. A., Brown A. D., Chiu P. J. S., Moran R., Sybertz E. J., Baum T., *ibid*., **31**, 875—885 (1988).
4) *a*) *N*-CBZ-4-Benzylidene-(2*S*)-proline has been hydrogenated using H_2 and Pd/C to give exclusively *cis*-4-benzyl-(2*S*)-proline: ref. 3*a*; *b*) Likewise, *N*-Boc-4-benzylidene-(2*S*)-proline ethyl ester has been hydrogenated using H_2 and PtO_2 to give the *cis*-isomer: Ezquerra J., Pedregal C., Yruretagoyena B., Almudena R., *J. Org. Chem.*, **60**, 2925—2930 (1995); *c*) Ezquerra J., Pedregal C., Rubio A., Escribano A., Sánchez-Ferrando F., *Tetrahedron*, **49**, 8665—8678 (1993); *d*) *cis*-*N*-Boc-4-alkyl-(2*S*)-proline *tert*-butyl esters have been prepared by hydrogenation and reduction of alkylidene pyroglutamates: Moody C. M., Young D. W., *J. Chem. Soc., Perkin Trans. 1*, **1997**, 3519—3530.
5) Dormoy J.-R., *Synthesis*, **1982**, 753.
6) *a*) The reaction of the *trans*-iodide with styrene under *n*-Bu_3SnH/AIBN conditions has been reported to yield the *trans* product (no yield was given): Klein S. I., Dener J. M., Molino B. F., Gardner C. J., D'Alisa R., Dunwiddie C. T., Kasiewski C., Leadley R. J., *Bioorg. Med. Chem. Lett.*, **6**, 2225—2230 (1996); *b*) The chemical shift and splitting pattern of the C2 methine proton was used for identification of *cis*- and *trans*-isomers, but the ^{1}H-NMR spectrum of **14** is further complicated by the presence of Boc rotamers at room temperature. For the synthesis and characterization of *trans*-*N*-Boc-(4*R*)-(3-phenylpropyl)proline, see example 2, p. 51, in: Llinas-Brunet M., Bailey M. D., Halmos T., Poupart M.-A., Tsantrizos Y., Wernic D., PCT Int. Appl. WO 9907734 A2 19990218, 1999.
7) Similar enol triflates have been prepared and used in Suzuki couplings: *a*) Baldwin J. E., Bamford S. J., Fryer A. M., Wood M. E., *Tetrahedron Lett.*, **36**, 4869—4872 (1995); *b*) Baldwin J. E., Fryer A. M., Pritchard G. J., Spyvee M. R., Whitehead R. C., Wood M. E., *ibid*., **39**, 707—710 (1998); *c*) Baldwin J. E., Fryer A. M., Pritchard G. J., Spyvee M. R., Whitehead R. C., Wood M. E., *Tetrahedron*, **54**, 7465—7484 (1998).
8) *a*) Shieh W.-C., Carlson J. A., *J. Org. Chem.*, **57**, 379—381 (1992); *b*) Oh-e T., Miyaura N., Suzuki A., *ibid*., **58**, 2201—2208 (1993); *c*) Suzuki A., Miyaura N., *Chem. Rev*., **95**, 2457—2483 (1995).
9) *a*) Labadie J. W., Stille J. K., *J. Am. Chem. Soc*., **105**, 6129 (1983); *b*) Stille J. K., *Angew. Chem. Int. Ed. Engl*., **25**, 508—524 (1986).
10) *a*) Garcia A. M., Rowell C., Ackermann K., Kowalczyk J. J., Lewis M. D., *J. Biol. Chem*., **268**, 18415 (1993). *b*) At concentrations>10—30 μM most of the methyl esters exhibited some toxicity towards whole cells.