Structure−Activity Relationships of Cysteine-Lacking Pentapeptide Derivatives That Inhibit ras Farnesyltransferase

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Mutational activation of ras has been found in many types of human cancers, including a greater than 50% incidence in colon and about 90% in pancreatic carcinomas. The activity of both native and oncogenic ras proteins requires a series of post-translational processing steps. The first event in this process is the farnesylation of a cysteine residue located in the fourth position from the carboxyl terminus of the ras protein, catalyzed by the enzyme farnesyltransferase (FTase). Inhibitors of FTase are potential candidates for development as antitumor agents. Through a high-volume screening program, the pentapeptide derivative PD083176 (**1**), Cbz-His-Tyr(OBn)-Ser(OBn)-Trp-DAla-NH_2, was identified as an inhibitor of rat brain FTase, with an IC_{50} of 20 nM. Structure−activity relationships were carried out to determine the importance of the side chain and chirality of each residue. This investigation led to a series of potent FTase inhibitors which lack a cysteine residue as found in the ras peptide substrate. The parent compound (**1**) inhibited the insulin-induced maturation of *Xenopus* oocytes (concentration: 5 pmol/oocyte), a process which is dependent on the activation of the ras pathway.

Introduction

Post-translational modifications of proteins include glycosylation, phosphorylation, methylation, acetylation, fatty acid acylation, and prenylation. Prenylation is referred to as the covalent modification of a molecule by the attachment of a lipophilic isoprenoid group. There are two types of prenylation, namely, farnesylation and geranylgeranylation. The enzymes for each of the respective prenylations have been isolated and characterized. Farnesyltransferase (FTase) has been identified and characterized from pig[1] and bovine extracts[2] and purified to homogeneity and cloned from rat brain cytosol.[3,4] Geranylgeranyl transferase (GGTase-I) was characterized[5] and purified from bovine brain cytosol.[6] Both enzymes contain two subunits: the α-subunit which is common to both and distinct β-subunits.[7−10] Their substrates are proteins with the C-terminal motif: CAAX, where C is the amino acid cysteine, A is any aliphatic amino acid, and X is either Ser or Met for proteins which undergo farnesylation[1−3] or Leu or Phe for proteins which undergo geranylgeranylation.[5,6] However, since these two enzymes share a common α-subunit, possible overlapping substrate specificity can occur.[6]

The ras proteins undergo farnesylation at the cysteine residue in the presence of the heterodimeric enzyme that uses farnesyl pyrophosphate as a donor, forming a thioether linkage.[11−13] The three terminal amino acids are then proteolytically cleaved[14−16] followed by methyl esterification at the new C-terminal cysteine residue by a protein methyltransferase.[17,18] The ras protein then associates with the membrane, which enables signaling events leading to cell replication and transformation. There are four closely related forms of ras proteins: H-Ras, N-Ras, K-Ras-A, and K-Ras-B.[19] Oncogenic versions of these proteins are also farnesylated and subsequently localized to the cell membrane, leading to uncontrolled growth.[20] The *ras* oncogenes have been implicated in a wide variety of human cancers, including colon, pancreatic, breast, lung, liver, kidney, ovary and stomach carcinomas.[19,21] Inhibition of the ras FTase enzyme offers potential utility in the treatment of proliferative diseases including cancer.

Initial reports of FTase inhibitors were based on the CAAX motif. The tetrapeptide Cys-Val-Ile-Met was found to be a competitive inhibitor with ras p21.[4] Modification of Ile to Phe in the tetrapeptide led to a potent inhibitor of bovine FTase (IC_{50} = 25 nM), and this peptide was not a substrate.[22] Further modifications of the aliphatic amino acids and introduction of reduced bond isosteres in the CAAX motif gave rise to potent ras FTase inhibitors with potency in the range of 20−300 nM.[23−31] In vivo evaluation of some of these inhibitors[25,29,30] has shown efficacy in different animal models. Replacement of the two aliphatic amino acids with either a benzodiazepine group[32] or 3- and 4-(aminomethyl)benzoic acids[33−36] led to potent FTase inhibitors in vitro, although the 4-aminobenzoic acid mimetic of the tetrapeptide was active in vivo.[36]

The cysteine residue has also been substituted by a 4-imidazolyl group[37] and a phenolic benzyl group.[38] These inhibitors have IC_{50} values in the range of 0.003−1 μM and are active in cells. A novel series of tricyclic inhibitors were reported[39] to be inhibitors of FTase with IC_{50} values in the range of 250 nM, and at low micromolar concentrations they were effective at blocking farnesylation in Cos cells.

We used high-volume screening of our compound library with rat brain FTase to identify the potent selective ras FTase inhibitor PD083176: Cbz-His-Tyr-(OBn)-Ser(OBn)-Trp-DAla-NH_2 (Figure 1).[40,41] This pentapeptide was found to have an IC_{50} of 20 nM when tested against FTase purified from rat brain, similar

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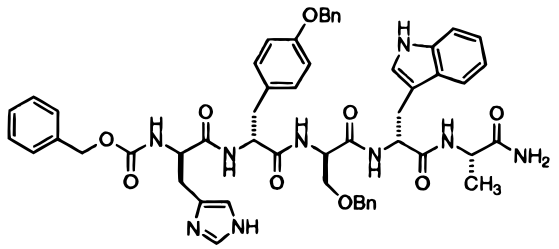

Cbz - His - Tyr(OBn) - Ser(OBn) - Trp - DAla - NH_2

PD083176: IC_{50} = 0.020 μM (rat FTase)

IC_{50} = 0.010 μM (human FTase)

IC_{50} = 1.25 μM (rat GGTase-I)

Figure 1. Structure and data of PD083176.

activity against human FTase (IC_{50} = 10 nM), and an IC_{50} of 1.25 μM against rat GGTase-I, in 30 mM phosphate buffer. A novel feature of this inhibitor is the absence of a cysteine residue common to most of the previously reported farnesyltransferase inhibitors. In a microinjection experiment, PD083176 was found to inhibit insulin-induced ras-dependent maturation of *Xenopus* oocytes.

Herein we report the structure−activity relationships of this novel FTase inhibitor to identify critical residues for activity. Key cysteine substitutions were incorporated in order to attempt to identify a correlation of our series of ras peptide inhibitors with the CAAX motif.

Chemistry

Peptide Synthesis, Purification, and Characterization. The peptide analogues were synthesized by solid phase peptide synthetic (SPPS) methodologies.[42,43] The peptide analogues were prepared using an *N*-α-Fmoc protecting group strategy on a Rink-amide resin (4-2′,4′-dimethoxyphenyl)-Fmoc-(aminomethyl)phenoxy resin)[44] or on a SASRIN resin (super acid sensitive resin, 2-methoxy-4-alkoxybenzyl alcohol resin).[45]

The *N*-α-Fmoc group was removed with 20% piperidine in *N*-methylpyrrolidone (NMP) prior to coupling with the next protected amino acid. All amino acids were doubled coupled as their *N*-hydroxybenzotriazole (HOBt)-activated esters or as their PyBOP-activated esters, and the completion of the couplings was verified by the Kaiser test.[46] Acetylation of the peptides was carried out on the resin using an excess of 1-acetylimidazole in methylene chloride. The peptides were simultaneously deprotected and cleaved from the resin by treatments with 10−70% trifluoroacetic acid (TFA) in methylene chloride, depending on the side chain protecting groups, at room temperature for 2−3 h.

Crude peptides were then purified to homogeneity by preparative reversed-phase high-performance liquid chromatography (RP/HPLC) eluting with a linear gradient of 0.1% aqueous TFA with increasing concentrations of 0.1% TFA in acetonitrile (CH_3CN). Peptide fractions found to be homogeneous by analytical RP/HPLC were combined, concentrated, and lyophilized. The peptides were analyzed for homogeneity by analytical RP/HPLC (eluent A, 0.1% aqueous TFA and 0.1% TFA in acetonitrile; eluent B, 0.1 M phosphoric acid (pH 2.25) and isopropyl alcohol) and characterized by amino acid analysis, elemental analysis, fast atom bombardment or electrospray mass spectrometry (FABMS or ESMS), and proton nuclear magnetic resonance (^{1}H-NMR) spectroscopy (Table 1).

Phosphorylation of the tyrosine residue was carried out while the peptide was still linked to the resin using an excess of di-*tert*-butyl *N*,*N*-diethylphosphoramidite and tetrazole followed by oxidation with 70% *tert*-butyl hydroperoxide in methylene chloride. The *tert*-butyl groups were removed simultaneously under the cleavage conditions (60% TFA in methylene chloride).

Results and Discussion

Structure−activity relationships were explored with the pentapeptide PD083176 (**1**) to identify critical features for activity and in an attempt to optimize affinity for FTase. During the course of the study, kinetic analysis was carried out with some of the inhibitors in a Hepes buffer system. We observed that the activity of this series of compounds was dependent on the concentration of phosphate ion in the buffer system. This interesting observation was followed by the synthesis of selected phosphate analogues to explore phosphate-dependent activity in more detail.[47]

N-Terminal Modifications (Table 2). The N-terminal group benzyloxycarbonyl (Cbz) was found to be preferred for activity against ras FTase (**1**). A free amino terminus (**2**) or acetylation (Ac) of the N-terminus gave rise to less potent inhibitors of ras FTase. Substitutions with cyclobutyloxycarbonyl (CB, **4**), cyclohexylcarboxy (CH, **5**), pyroglutamic acid (Glp, **6**), and (fluorenylmethyl)oxycarbonyl (Fmoc, **7**) at the N-terminus also led to decreases in activity over the parent lead, compound **1**.

Histidine Position Modifications (Table 3). The histidine residue in PD083176 (**1**) was found to be critical for the activity against ras FTase. D-Stereochemistry (**8**) led to a 4-fold decrease in activity. Alanine substitution (**9**) for histidine led to an inactive analogue. Since the imidazole side chain of the histidine has aromatic and basic features, substitutions with phenylalanine (Phe, **10**) and ornithine (Orn, **11**) were carried out. The phenylalanine-containing pentapeptide **10** was found to be inactive at concentrations up to 100 μM. The ornithine substitution (**11**) also showed a marked decrease in activity. Tryptophan substitution (**12**) caused a loss in activity. These results suggest that the imidazole group plays an important role in the activity of PD083176.

The present series of FTase inhibitors lack a cysteine residue. To investigate if a correlation exists between the present analogues and the CAAX-based inhibitors, cysteine was introduced at some of the residues in PD083176. Cysteine substitution for histidine (**13**) led to a 10-fold decrease in activity.

Tyrosine Position Modifications (Table 4). The tyrosine residue was found to be more tolerant to a variety of modifications. D-Stereochemistry (**14**) caused only a 2-fold decrease in activity. The pentapeptides containing a free tyrosine residue, in both the L- and D-configurations, (**16, 17**), were found to be 2- and 4-fold, respectively, less active against ras FTase than the corresponding *O*-benzyl protected analogues. Substitution of tyrosine (O-benzylated) by the aromatic amino

Table 1. Analytical Data for the Synthetic Pentapeptides

compd	HPLC t_R (min)[a–d] (purity, %)	FABMS[1] or ES[2] (*m/z*) calcd	obsd	amino acid anal.[e] (calcd) obtained
1	19.8[a] (94) 18.9[d] (99)	976.1	976.6[2]	His (1) 1.03, Tyr (1) 1.00, Ser (1) 1.08, Ala(1) 1.16
2	14.8[a] (93) 16.1[d] (96)	842.0	842.4[2]	His (1) 0.85, Tyr (1) 0.66, Ser (1) 0.75, Ala (1) 1.00
7[f]	17.22[a] (99)	1064.2	1064.4[2]	His (1) 0.96, Tyr (1) 1.00, Ser (1) 1.21, Ala (1) 1.21
8	16.4[b] (99)	976.1	977.0[1]	His (1) 0.96, Tyr (1) 0.98, Ser (1) 1.02, Ala (1) 1.04
9	22.7[a] (98) 20.8[d] (99)	910.0	910.1[1]	Tyr (1) 0.92, Ser (1) 0.88, Ala (2) 1.88
10	20.3[a] (95)	986.1	1009.5[1] (M + Na)	Phe (1) 1.00, Tyr (1) 0.71, Ser (1) 1.00, Ala (1) 1.06
11	16.9[b] (93)	953.1	953.5[1]	Tyr (1) 1.00, Ser (1) 1.12, Ala (1) 1.27
12	21.3[b] (99)	1025.2	1025.3[1]	Tyr (1) 1.02, Ser (1) 0.93, Ala (1) 1.06
13	19.7[b] (95)	942.1	942.2[1]	Tyr (1) 0.94, Ser (1) 1.03, Ala (1) 1.03
14	15.7[b] (99)	976.1	976.9[1]	His (1) 1.02, Tyr (1) 1.00, Ser (1) 1.03, Ala (1) 1.14
15	16.0[a] (98)	793.9	794.3[2]	His (1) 1.00, Ser (1) 0.97, Ala (2) 2.00
16	15.9[a] (98) 15.3[d] (99)	886.0	886.2[1]	His (1) 1.04, Tyr (1) 0.97, Ser (1) 0.98, Ala (1) 1.02
17	15.6[b] (93)	886.0	886.2[2]	His (1) 1.03, Tyr (1) 1.00, Ser (1) 0.97, Ala (1) 1.00
18	14.6[d] (98)	870.0	870.4[2]	His (1) 0.82, Phe (1) 0.93, Ser (1) 0.94, Ala (1) 1.00
19	16.8[b] (94) 17.2[d] (99)	870.0	870.2[2]	His (1) 0.88, Phe (1) 0.88, Ser (1) 0.83, Ala (1) 0.96
20	15.7[b] (95)	884.0	884.3[2]	His (1) 0.90, Ser (1) 0.88, Ala (1) 1.02
21	11.9[c] (99)	837.9	838.2[2]	His (1) 1.00, Asp (1) 1.01, Ser (1) 0.84, Ala (1) 1.06
22	12.4[b] (99)	851.9	852.3[2]	His (1) 1.05, Glu (1) 0.98, Ser (1) 0.95, Ala (1) 1.01
23	11.7[b] (99)	851.0	851.6[2]	His (1) 1.00, Lys (1) 1.01, Ser (1) 0.95, Ala (1) 1.00
24	12.7[b] (93)	850.9	851.6[2]	His (1) 0.99, Gln (1) 1.02, Ser (1) 0.94, Ala (1) 0.99
25	14.8[b] (99)	876.0	876.3[2]	His (1) 1.55, Ser (1) 0.97, Ala (1) 1.03
26	14.3[a] (93) 15.7[d] (96)	826.0	826.4[2]	His (1) 1.03, Ser (1) 1.00, Ala (1) 0.98
27	13.4[b] (99)	869.9	870.5[2]	His (1) 1.06, Tyr (1) 0.90, Ala (2) 2.00
28	17.4[b] (99)	976.1	976.4[2]	His (1) 0.98, Tyr (1) 0.96, Ser (1) 1.01, Ala (1) 1.05
29	13.0[c] (97)	886.0	886.2[1]	His (1) 1.03, Tyr (1) 0.95, Ser (1) 1.00, Ala (1) 1.02
30	19.6[b] (93)	946.1	946.6[1]	His (1) 1.12, Tyr (1) 0.82, Phe (1) 1.00, Ala (1) 1.16
31	14.8[b] (99)	928.0	928.5[2]	His (1) 1.03, Tyr (1) 0.96, Glu (1) 0.99, Ala (1) 1.02
32	13.6[c] (95)	990.1	990.4[2]	His (1) 0.99, Tyr (1) 1.00, Thr (1) 1.00, Ala (1) 1.16
33	13.9[c] (99)	902.0	902.2[2]	His (1) 1.01, Tyr (1) 1.07, Ala (1) 0.92
34	17.5[b] (98)	992.2	992.7[2]	His (1) 1.09, Tyr (1) 0.97, Ala (1) 1.25
35	13.6[b] (97)	861.0	861.4[2]	His (1) 0.92, Tyr (1) 0.81, Ser (1) 0.90, Ala (2) 2.00
36	17.0[b] (97)	937.0	937.2[2]	
37	16.8[b] (97)	976.1	976.5[2]	His (1) 1.39, Tyr (1) 1.00, Ser (1) 1.17, Ala (1) 1.38
38	18.3[a] (99) 18.8[d] (99)	976.1	976.4[2]	His (1) 1.01, Tyr (1) 0.96, Ser (1) 1.01, Ala (1) 1.01
39	19.6[a] (98) 19.3[d] (99)	977.1	977.5[1]	His (1) 1.03, Tyr (1) 0.96, Ser (1) 1.01, Ala (1) 1.01
43[g]	15.5[b] (92)	966.0	966.0[2]	His (1) 1.01, Ser (1) 0.95, Ala (1) 1.04
44	12.8[b] (93)	964.0	964.1[1]	His (1) 0.96, Ser (1) 0.94, Ala (1) 1.10
45	12.8[c] (99)	1020.1	1020.4[2]	His (1) 1.03, Ser (1) 0.97, Ala (1) 1.00
46	14.6[b] (99)	966.0	966.2[2]	not available

[a] 90:10 to 24:76 (0.1% aqueous TFA:0.1% TFA in acetonitrile) over 22 min, 1.5 mL/min. [b] 80:20 to 14:86 (0.1% aqueous TFA:0.1% TFA in acetonitrile) over 22 min, 1.5 mL/min. [c] 70:30 to 4:96 (0.1% aqueous TFA:0.1% TFA in acetonitrile) over 22 min, 1.5 mL/min. [d] 80:20 to 14:86 (0.1 M phosphoric acid:isopropyl alcohol) over 22 min, 1.5 mL/min. [e] Trp recovery is low under the hydrolysis conditions, and therefore it is not determined. Unnatural amino acids were not determined. [f] Compounds **3**–**6** were compounds from the sample collection. [g] Compounds **40**–**42** were compounds from the sample collection.

Table 2. N-Terminal Modifications of **1**

compound	IC_{50} (μM)[e]
1, Cbz-His-Tyr(OBn)-Ser(OBn)-Trp-DAla-NH_2[a]	0.020[f] (±0.0042)
2, H-His-Tyr(OBn)-Ser(OBn)-Trp-DAla-NH_2	4.5
3, Ac-His-Tyr(OBn)-Ser(OBn)-Trp-DAla-NH_2	10
4, CB[b]-His-Tyr(OBn)-Ser(OBn)-Trp-DAla-NH_2	3.2
5, CH[c]-His-Tyr(OBn)-Ser(OBn)-Trp-DAla-NH_2	3.8
6, Glp[d]-His-Tyr(OBn)-Ser(OBn)-Trp-DAla-NH_2	>20
7, Fmoc-His-Tyr(OBn)-Ser(OBn)-Trp-DAla-NH_2	1.6[g]

[a] PD083176. [b] CB: cyclobutyloxycarbonyl. [c] CH: cyclohexylcarboxy. [d] Glp: pyroglutamic acid. [e] Activity against rat brain FTase. n = 1 determination, unless otherwise noted; values are estimated to be reliable within 2-fold. [f] n = 6 determinations. [g] n = 2 determinations; values are estimated to be reliable within 2-fold.

acids L- and D-phenylalanine (**18**, **19**) and homophenylalanine (**20**) was found to be tolerated, indicating that the *O*-benzyl group is not required for activity. In the case of the Phe-containing pentapeptide **18**, a 2-fold increase in activity was observed. Acidic residues, aspartic (**21**) and glutamic (**22**) acids, at the tyrosine position led to a 2-fold loss in activity for the aspartic acid peptide **21**, while **22** (Glu) showed similar activity to the parent pentapeptide. Basic residues, such as lysine (**23**), led to a loss in activity, while substitution with a neutral amino acid, such as glutamine (**24**), decreased the activity by 3-fold. Cyclohexylalanine

Table 3. Histidine Position Modifications of **1**

compound	IC_{50} (μM)[c]
1, Cbz-His-Tyr(OBn)-Ser(OBn)-Trp-DAla-NH_2[a]	0.020[d] (±0.0042)
8, Cbz-DHis-Tyr(OBn)-Ser(OBn)-Trp-DAla-NH_2	0.082[e] (±0.019)
9, Cbz -Ala-Tyr(OBn)-Ser(OBn)-Trp-DAla-NH_2	>20
10, Cbz-Phe-Tyr(OBn)-Ser(OBn)-Trp-DAla-NH_2	>20
11, Cbz-Orn[b]-Tyr(OBn)-Ser(OBn)-Trp-DAla-NH_2	4.70
12, Cbz -Trp-Tyr(OBn)-Ser(OBn)-Trp-DAla-NH_2	>20
13, Cbz -Cys-Tyr(OBn)-Ser(OBn)-Trp-DAla-NH_2	0.17

[a] PD083176. [b] Orn: ornithine. [c] Activity against rat brain FTase. $n = 1$ determination, unless otherwise noted; values are estimated to be reliable within 2-fold. [d] $n = 6$ determinations. [e] $n = 3$ determinations.

Table 4. Tyrosine Position Modifications of **1**

compound	IC_{50} (μM)[d]
1, Cbz-His-Tyr(OBn)-Ser(OBn)-Trp-DAla-NH_2[a]	0.020[e] (±0.0042)
14, Cbz-His-DTyr(OBn)-Ser(OBn)-Trp-DAla-NH_2	0.035
15, Cbz-His -Ala-Ser(OBn)-Trp-DAla-NH_2	1.00
16, Cbz-His-Tyr-Ser(OBn)-Trp-DAla-NH_2	0.035
17, Cbz-His-DTyr-Ser(OBn)-Trp-DAla-NH_2	0.170
18, Cbz-His-Phe-Ser(OBn)-Trp-DAla-NH_2	0.009
19, Cbz-His-DPhe-Ser(OBn)-Trp-DAla-NH_2	0.079
20, Cbz-His-HomoPhe[b]-Ser(OBn)-Trp-DAla-NH_2	0.018
21, Cbz-His-Asp-Ser(OBn)-Trp-DAla-NH_2	0.038
22, Cbz-His-Glu-Ser(OBn)-Trp-DAla-NH_2	0.018
23, Cbz-His-Lys-Ser(OBn)-Trp-DAla-NH_2	1.90
24, Cbz-His-Gln-Ser(OBn)-Trp-DAla-NH_2	0.054
25, Cbz-His-Cha[c]-Ser(OBn)-Trp-DAla-NH_2	0.066
26, Cbz-His-Cys-Ser(OBn)-Trp-DAla-NH_2	4.90

[a] PD083176. [b] HomoPhe: homophenylalanine. [c] Cha: cyclohexylalanine. [d] Activity against rat brain FTase. $n = 2$ determinations, unless otherwise noted; values are estimated to be reliable within 2-fold. [e] $n = 6$ determinations.

Table 5. Serine Position Modifications of **1**

compound	IC_{50} (μM)[b]
1, Cbz-His-Tyr(OBn)-Ser(OBn)-Trp-DAla-NH_2[a]	0.020[c] (±0.0042)
27, Cbz-His-Tyr(OBn)-Ala-Trp-DAla-NH_2	1.1[d]
28, Cbz-His-Tyr(OBn)-DSer(OBn)-Trp-DAla-NH_2	13[d]
29, Cbz-His-Tyr(OBn)-Ser-Trp-DAla-NH_2	1.8[d]
30, Cbz-His-Tyr(OBn)-Phe-Trp-DAla-NH_2	0.13
31, Cbz-His-Tyr(OBn)-Glu-Trp-DAla-NH_2	0.20
32, Cbz-His-Tyr(OBn)-Thr(OBn)-Trp-DAla-NH_2	0.015
33, Cbz-His-Tyr(OBn)-Cys-Trp-DAla-NH_2	0.004
34, Cbz-His-Tyr(OBn)-Cys(SBn)-Trp-DAla-NH_2	0.015

[a] PD083176. [b] Activity against rat brain FTase. $n = 2$ determinations, unless otherwise noted; values are estimated to be reliable within 2-fold. [c] $n = 6$ determinations. [d] $n = 1$ determination; values are estimated to be reliable within 2-fold.

Table 6. Tryptophan and D-Alanine Position Modifications of **1**

compound	IC_{50} (μM)[b]
1, Cbz-His-Tyr(OBn)-Ser(OBn)-Trp-DAla-NH_2[a]	0.020[c] (±0.0042)
35, Cbz-His-Tyr(OBn)-Ser(OBn)-Ala-DAla-NH_2	0.25[d] (±0.080)
36, Cbz-His-Tyr(OBn)-Ser(OBn)-Phe-DAla-NH_2	2.50
37, Cbz-His-Tyr(OBn)-Ser(OBn)-DTrp-DAla-NH_2	8.70
38, Cbz-His-Tyr(OBn)-Ser(OBn)-Trp-Ala-NH_2	0.0085

[a] PD083176. [b] Activity against rat brain FTase. $n = 2$ determinations, unless otherwise noted; values are estimated to be reliable within 2-fold. [c] $n = 6$ determinations. [d] $n = 3$ determinations.

(Cha, **25**) substitution decreased the activity against FTase by about 3-fold. Cysteine substitution (**26**) was not tolerated at the tyrosine position. Alanine substitution (**15**) led to a 100-fold loss in activity.

Serine Position Modifications (Table 5). Alanine substitution (**27**), inversion of stereochemistry (**28**), or side chain deprotection (**29**) were all found to be detrimental to activity. The substitution with the aromatic amino acid phenylalanine (**30**) and the acidic amino acid glutamic acid (**31**) caused 7- and 10-fold losses in activity, respectively. The threonine-O-benzylated-containing pentapeptide **32** had similar activity to the parent compound **1**.

Cysteine substitution at the serine position (**33**) led to the most potent ras FTase inhibitor in this series (4 nM) in 30 mM phosphate buffer. The cysteine-S-benzylated-containing inhibitor **34** was found to show good potency as a ras FTase inhibitor.

Tryptophan and D-Alanine Position Modifications (Table 6). Alanine (**35**) or phenylalanine (**36**) substitution or inversion of stereochemistry (**37**) for tryptophan all led to decreased activity against ras FTase. However, the L-stereochemistry for alanine (**38**) was tolerated.

C-Terminal Modifications (Table 7). The C-terminal of **1** could be modified without causing a significant loss in the activity. The amide **1** was found to be 2−3-fold more potent than the corresponding acid **39**, methyl ester **40**, and hydrazide **41**. However, the ethyl amide pentapeptide **42** was less active by about 10-fold.

Phosphate Effect. The present FTase inhibitors were assayed against rat FTase in a 30 mM potassium

Table 7. C-Terminal Modifications of **1**

compound	IC_{50} (μM)[b]
1, Cbz-His-Tyr(OBn)-Ser(OBn)-Trp-DAla-NH_2[a]	0.020[c] (±0.0042)
39, Cbz-His-Tyr(OBn)-Ser(OBn)-Trp-DAla-OH	0.048
40, Cbz-His-Tyr(OBn)-Ser(OBn)-Trp-DAla-OCH_3	0.048
41, Cbz-His-Tyr(OBn)-Ser(OBn)-Trp-DAla-$NHNH_2$	0.064[d] (±0.007)
42, Cbz-His-Tyr(OBn)-Ser(OBn)-Trp-DAla-NHEt	0.170[e] (±0.025)

[a] PD083176. [b] Activity against rat brain FTase. $n = 2$ determinations, unless otherwise noted; values are estimated to be reliable within 2-fold. [c] $n = 6$ determinations. [d] $n = 4$ determinations. [e] $n = 3$ determinations.

Table 8. Modifications of **1** and the Phosphate Effect

	IC_{50} (μM)[b]		
compound	A	B	C
1, Cbz-His-Tyr(OBn)-Ser(OBn)-Trp-DAla-NH_2[a]	0.020[c]	0.076	1.60
16, Cbz-His-Tyr-Ser(OBn)-Trp-DAla-NH_2	0.035	0.102	0.51
43, Cbz-His-Tyr(OPO_3H_2)-Ser(OBn)-Trp-DAla-NH_2	0.013	0.009	0.006
44, Cbz-His-p($CH_2PO_3H_2$)Phe-Ser(OBn)-Trp-DAla-NH_2	0.20	0.28	0.19
45, Cbz-His-p($CH_2PO_3Et_2$)Phe-Ser(OBn)-Trp-DAla-NH_2	0.025	0.049	0.075
46, Cbz-His-Tyr(OBn)-Ser(OPO_3H_2)-Trp-DAla-NH_2	0.24	0.36	0.66

[a] PD083176. [b] Activity against rat brain FTase. $n = 2$ determinations, unless otherwise noted; values are estimated to be reliable within 2-fold. [c] $n = 6$ determinations. Assay buffer: (A) 30 mM potassium phosphate, (B) 5 mM potassium phosphate and 50 mM Hepes, and (C) 50 mM Hepes.

phosphate buffer. Kinetic experiments carried out in Hepes buffer showed that **1** was a competitive inhibitor with respect to FPP.[42] Interestingly, the activity of **1** was found to be 60-fold less when compared to the activity found in 30 mM potassium phosphate buffer (IC_{50}'s were 1.6 and 0.020 μM, respectively). Further studies have shown that the inhibitory activity of **1** is clearly dependent on the concentration of phosphate anion in the buffer.[47]

The effect of the concentration of potassium phosphate in the assay buffer led us to incorporate a phosphate group at the tyrosine residue to check if this modification would cause equipotency in the three buffer systems: 30 mM potassium phosphate (A), 50 mM Hepes (C), and a combination of the two buffers leading to a 5 mM concentration of phosphate anion (B). Phosphorylation of the tyrosine residue (**43**) led to a potent ras inhibitor in all three buffer systems (Table 8); no effect of the potassium phosphate concentration was observed on the activity. Due to the instabiltity of the phosphate group to phosphatases, a phosphonate group was introduced, in both the unprotected (**44**) and ethyl-protected (**45**) forms. A decrease in activity of 30-fold was seen for **44** when compared to **43**, while the ethyl-protected phosphonate analogue **45** was more potent than the free phosphonate by 2−4-fold but less potent than the phosphate-containing pentapeptide **43** by 7-fold. These results indicate that the effect seen in the presence of potassium phosphate in the medium may be overcome by introducing phosphate groups in the pentapeptide inhibitor.

Phosphorylation of the serine residue (**46**) was also carried out, and it showed a 4-fold loss in activity in 30 mM phosphate buffer. However, the phosphate effect was decreased.

This synergistic effect of the phosphate anion with FTase inhibitors has not been reported by other groups, as most of the FTase inhibitors disclosed so far are competitive against the ras protein, and such an effect has not been observed.[47] It would be of interest to examine inhibitors such as the non-thiol tetrapeptide reported by Hunt et al.[37] or some of the bisubstrate inhibitors[48,49] which could also show a unique binding mode toward FTase. The present series of peptidic FTase inhibitors are competitive against FPP.[42] Investigations are underway to ascertain whether the pentapeptide binds to the enzyme in the farnesyl pocket and the phosphate anion at the pyrophosphate site thereby leading to improved inhibition of FTase. Since tumor cells generally contain between 3 and 12 mM phosphate anions, the anionic synergism exhibited by PD083176 may make this unique FTase inhibitor a promising prototype for analogue development.

Inhibition of Insulin-Mediated Maturation of *Xenopus* Oocytes. The pentapeptide PD083176 was shown to be a potent ras FTase inhibitor. However, the compound proved to be impermeable to cells. In order to verify that PD083176 is capable of blocking ras function, a microinjection experiment was carried out. In *Xenopus* oocytes, maturation can be induced by progesterone via a ras-independent pathway or by insulin via a ras-dependent pathway.[50] Stage VI *Xenopus* oocytes were injected with 5 pmol/oocyte **1**. Insulin or progesterone was present in the medium at a final concentration of 1 μM. Oocytes were examined 20 h after the addition of insulin/progesterone for evidence of maturation. The progesterone-induced *Xenopus* oocyte maturation was not affected by the addition of **1** (Figure 2). However, the insulin-induced *Xenopus* oocyte maturation was about one-half the control value, when **1** was microinjected (Figure 2). These results demonstrate that **1** inhibits ras activity in these cells after microinjection.

Conclusions

We have discovered a novel series of potent pentapeptide ras farnesylation inhibitors through compound library screening. Exploration of the structure−activity relationships of PD083176 (**1**) has identified critical residues for potent inhibition of FTase. The Cbz-His moiety was determined as being key to the activity of the pentapeptide. The tyrosine-O-benzylated residue tolerated modifications; tyrosine, phenylalanine, and homophenylanine substitutions led to FTase inhibitors of similar activity, as well as acidic amino acids such as aspartic and glutamic acid at this position. The serine-O-benzylated residue was sensitive to changes, but threonine-O-benzylated residues led to an inhibitor

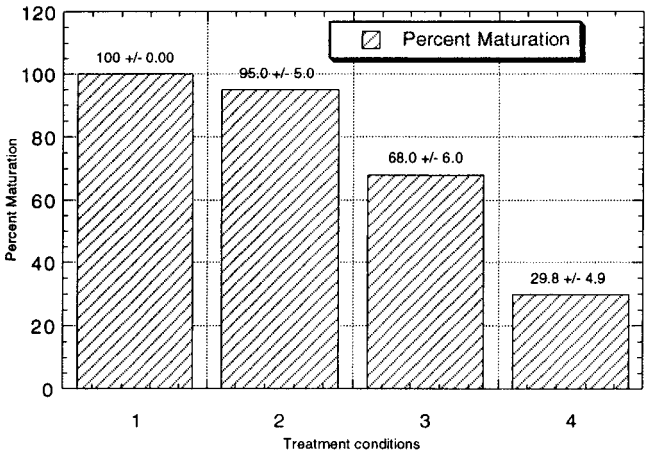

Figure 2. Results for the microinjection experiment of PD083176. Treatment conditions: (1) DMSO and progesterone, (2) compound **1** and progesterone, (3) DMSO and insulin, and (4) compound **1** and insulin.

with comparable activity. Cysteine incorporation as both the free sulfhydryl or S-benzylated also gave potent inhibitors of ras FTase. All tryptophan residue modifications carried out were detrimental to activity, while at the alanine position either D- or L-stereochemistry was tolerated.

The parent compound **1** was tested against human FTase and found to have similar activity (IC_{50} of 10 nM) to that of the rat enzyme (IC_{50} of 20 nM). It was also found to be selective for FTase since it had an IC_{50} of 1.25 μM against rat GGTase-I. Kinetic analysis of **1** showed that it was competitive for the farnesyl pyrophosphate substrate.[47]

The effect of phosphate concentration in the assay buffer upon activity was an interesting finding. The introduction of a phosphate group into the pentapeptide inhibitor decreased the difference in activity when measured in buffers containing 0, 5, and 30 mM phosphate anion. The proposed mechanism for this phosphate dependency is discussed in more detail elsewhere, but the relevance of the effect will depend on the concentration of free phosphate available in tumor cells.[47]

From this series of pentapeptides, it was not possible to obtain cellular activity, due to either a lack of cell permeability and/or proteolytic degradation. To evaluate the stability of compound **1**, it was incubated with NIH 3T3 cell lysates at concentrations of 0.01, 0.1, and 1 μM and compared to the tetrapeptide FTase inhibitor Cys-Val-Phe-Met. After a 30 min incubation at 37 °C, the inhibition of rat FTase activity was measured. As seen in Figure 3, the tetrapeptide Cys-Val-Phe-Met was less stable to proteolysis than **1** which retained activity throughout the 30 min time period. This could indicate that the cellular inactivity of compound **1** is mainly due to its inability to cross the cell membrane. Indeed, in a microinjection experiment, PD083176 was able to inhibit 50% of the insulin-induced maturation of *Xenopus* oocytes, demonstrating the ability of this pentapeptide to block ras activity in cells.

The structure−activity relationship studies around **1** led to a series of potent ras FTase inhibitors. The cysteine residue, common to most FTase inhibitors, was not present in this series. The pentapeptides were determined to be competitive against farnesyl pyrophosphate and also shown to be synergistic with the concentration of phosphate anions in the buffer. Compound **1** showed a high degree of selectivity for FTase versus GGTase-I. Continuing research in our laboratories has led to a series of truncated peptides which have shown the importance of the N-terminal Cbz-His for activity, as was found for the present series of FTase inhibitors.[51−54]

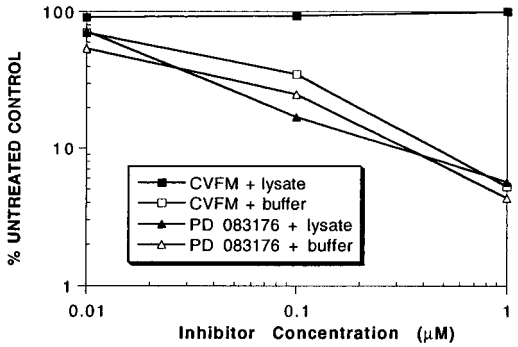

Figure 3. Proteolytic stability of PD083176 and Cys-Val-Phe-Met after treatment with NIH 3T3 lysates.

Experimental Section

Materials and Methods. Orthogonally protected *N*-α-Fmoc amino acids, *N*-α-Cbz amino acids, Rink amide resin, and SASRIN resin were purchased from either Bachem Bioscience, Bachem California, Advanced Chemtech, Applied Biosystems Inc., or Novabiochem. The unnatural *N*-α-Fmoc amino acids homoPhe (homophenylalanine) and Cha (cyclohexylalanine) were purchased from Bachem Bioscience; p($CH_2PO_3Et_2$)Phe was purchased from Neosystem Laboratoire.

Trifluoroacetic acid (TFA) was purchased from Halocarbon, and N,N′-dicyclohexylcarbodiimide (DCC), diisopropylethylamine (DIEA), and *N*-hydroxybenzotriazole (HOBt) were purchased from Applied Biosystem Inc. (Benzotriazolyl)-*N*-oxypyrrolidinium phosphonium hexafluorophosphate (PyBOP) was purchased from Advanced ChemTech. 1-Acetylimidazole was purchased from Aldrich. *N*-Methylpyrrolidone (NMP), methylene chloride, and dimethylformamide (DMF) were purchased from Burdick & Jackson and were of reagent grade quality or better. HPLC grade acetonitrile, isopropyl alcohol, and water were from EM Science or Mallinckrondt. For the biological assay the solvents and reagents were obtained from Sigma, except for DMSO and H_3PO_4, which were purchased from EM Science. Labeled farnesyl pyrophosphate was obtained from American Radiochemicals. The Mono-Q-purified FTase was from rat brain tissue, obtained from Pel-Freez; the Mono-Q-purified FTase from human brain was purchased from ABS.

The peptides were synthesized on an ABI-431A peptide synthesizer and on a manual shaker (MilliGen Model 504). Reversed phase high-pressure liquid chromatography (RP/HPLC) was carried out on a Waters HPLC system, consisting of a Model 600E system controller, a Model 600 solvent delivery system, and a Model 490 variable wavelength detector operating at 214 and 280 nm. The autosampler was from Bio-Rad Laboratories, Model AS-100. Preparative RP/HPLC was carried out using a C18 preparative scale Vydac column (218TP1022) (22 × 250 mm, 10 μm particle size). Analytical RP/HPLC was carried out using a C18 analytical scale Vydac column (218TP54) (4.6 × 250 mm, 5 μm particle size). The Vydac columns were purchased from the Nest Group. Elution was carried out with a linear gradient of 0.1% aqueous TFA with increasing concentrations of 0.1% TFA in acetontitrile, at 13 mL/min for the preparative work and at 1.5 mL/min for the analytical work. A second gradient was also used for the analytical work which was a linear gradient of 0.1 M phosphoric acid (pH 2.25) with increasing concentrations of isopropyl alcohol, at 1.0 mL/min. The gradients for the analytical RP/HPLC were as follows: (a) 90:10 to 24:76 (0.1% aqueous

TFA:0.1% TFA in acetonitrile) over 22 min, (b) 80:20 to 14:86 (0.1% aqueous TFA:0.1% TFA in acetonitrile) over 22 min, (c) 70:30 to 4:96 (0.1% aqueous TFA:0.1% TFA in acetonitrile) over 22 min, or (d) 80:20 to 14:86 (0.1 M phosphoric acid:isopropyl alcohol) over 22 min.

Proton and phosphorous NMR spectra were measured with a Varian Unity 400 NMR spectrophotometer. Fast atom bombardment spectra were measured with either a Finnigan TSQ70 mass spectrometer, a VG Trio 2 mass spectrometer, or a Finnigan MAT 900 mass spectrometer. Electrospray mass spectra were obtained on a VG Trio 2000 mass spectrometer.

Amino acid analyses were carried out using an ABI automated derivatizer/analyzer (Model 420A) with a two-component aqueous TFA/acetonitrile buffer system. Automated hydrolysis was carried out using 6 N hydrochloric acid at 150−160 °C for 75 min. Phenyl isothiocyanate derivatization in the presence of DIEA was carried out, and separation of the derivatized amino acid was carried out using an ABI Model 130A separation system. The data were analyzed on an ABI Model 610A instrument.

Peptide Synthesis. The peptides were synthesized by solid phase peptide chemistry.[42,43] The syntheses were carried out on an ABI Model 431A peptide synthesizer, but the coupling of Cbz-His was performed on a manual shaker. The peptides were prepared via *N*-α-Fmoc strategies using the Rink resin or the SASRIN resin. The amino acid side chains were protected as follows: O-*tert*-butyl (Asp, Glu, Ser, Tyr), *S*-trityl (Cys), *S*-benzyl (Cys), *N*-Boc (Orn, Lys), *O*-benzyl (Ser, Tyr, Thr). Cbz-His was used in the deprotected form. The couplings were carried out with DCC/HOBt in DMF. The peptides were Fmoc-deprotected with 20% piperidine in NMP. The coupling of Cbz-His was done manually, using a shaker, in the presence of DCC/HOBt, for 20−24 h. The peptides were then cleaved with 10−70% TFA in methylene chloride for 2 h at room temperature. The crude peptides were obtained by removal of the TFA/methylene chloride solution followed by precipitation with diethyl ether and filtration.

Phosphorylation of the hydroxyl group of tyrosine and serine was carried out while the peptide was still linked to the resin. The peptide-resin was suspended in THF, and 150 equiv of tetrazole (Aldrich) was added followed by 50 equiv of di-*tert*-butyl *N*,*N*-diethylphosphoramidite (Aldrich). The reaction was shaken at room temperature for 1 h, and the peptide-resin was washed with THF and methylene chloride. The oxidation was carried out by suspending the peptide-resin in methylene chloride and adding 20 equiv of *tert*-butyl hydroperoxide. The reaction mixture was shaken at room temperature for 1 h. The resin was then washed with methylene chloride, and the *tert*-butyl groups were removed by treatment with 60% TFA in methylene chloride.

In the preparation of **44**, the ethyl groups were removed by treating **45** with an excess of trimethylsilyl bromide (Aldrich) in methylene chloride for 20 h at room temperature.

Peptide Purification. The crude peptides were dissolved in a mixture of aqueous TFA and acetonitrile and purified by preparative RP/HPLC, under conditions as described above. The peptide fractions which were pure by analytical RP-HPLC were combined, concentrated in vacuo, resuspended in water, and lyophilized.

Peptide Homogeneity and Characterization. All peptides were analyzed for their purity by analytical RP/HPLC. The peptides were characterized by amino acid analysis, mass spectrometry (ES or FAB), proton NMR spectroscopy, and elemental analysis (Table 1).

Synthesis of Compound 1, D-Alanimade, *N*-[(Phenylmethoxy)carbonyl]-L-histidyl-*O*-(phenylmethyl)-L-tyrosyl-*O*-(phenylmethyl)-l-seryl-L-tryptophyl. The Fmoc-Rink amide resin (0.70 mmol/g, 0.36 g, 0.25 mmol) was used to prepare the peptide: H-Tyr(OBn)-Ser(OBn)-Trp-DAla-Rink, on an ABI 431A peptide synthesizer, by stepwise coupling of the amino acids N-α-Fmoc-DAla-OH, N-α-Fmoc-Trp-OH, N-α-Fmoc-Ser(OBn)-OH, and N-α-Fmoc-Tyr(OBn)-OH. Each coupling cycle consisted of the following: (1) wash three times with DMF, (2) treat twice with 20% piperidine in DMF for 20 min each time, (3) wash five times with DMF, (4) couple the amino acid, (5) wash three times with DMF, (6) couple the amino acid, and (7) wash three times with DMF.

The first cycle included a preliminary wash with methylene chloride. The coupling of the last amino acid was as described above, but additional steps were added as follows: (8) treat twice with 20% piperidine in DMF for 20 min each time, (9) wash five times with DMF, and (10) wash five times with methylene chloride. Each amino acid was double-coupled with 4 equivalents for each coupling reaction. The amino acid was activated as the HOBt ester.

The peptide-resin was then transferred to a reaction vessel equipped with a fritted disk and a stopcock and suspended in DMF. Cbz-His-OH (4 equiv) was then added followed by HOBt and DCC. The coupling reaction mixture was shaken on a manual shaker for 18−22 h at room temperature. The peptide-resin was then filtered and rinsed thoroughly with DMF followed by methylene chloride. Cleavage of the peptide from the resin was carried out with 20% TFA in methylene chloride for 2 h at room temperature. The solution was filtered and the resin rinsed with 20% TFA in methylene chloride followed by methylene chloride. The filtrate was concentrated in vacuo. The residue was treated with diethyl ether to precipitate the peptide. The peptide was filtered, washed with diethyl ether, and dried. The crude peptide was purified by preparative HPLC, as described above, with a linear gradient of 20−70% 0.1% TFA in acetonitrile against 0.1% aqueous TFA, over 100 min, at 13 mL/min. The lyophilized product was a white amorphous solid. Overall yield: 7%. A similar protocol was carried out for the synthesis of the pentapeptides in this manuscript.

ras Farnesyltransferase Assay. The farnesyltransferase inhibitory activity of the compounds was carried out in 30 mM potassium phosphate buffer (or 5 mM phosphate buffer and 50 mM Hepes, or 50 mM Hepes), at pH 7.4. The buffer solution also contained 7 mM DTT, 1.2 mM $MgCl_2$, 0.1 mM leupeptin, 0.1 mM pepstatin, and 0.2 mM phenylmethanesulfonyl fluoride. The assays were performed in 96-well plates (Wallec) with varying concentrations of the particular compound in 100% DMSO. Upon addition of both substrates, radiolabeled farnesyl pyrophosphate (1-^{3}H, specific activity 15−30 Ci/mmol, final concentration 0.12 μM) and (biotinyl)-7-aminoheptanoic acid-Thr-Lys-Cys-Val-Ile-Met peptide (final concentration 0.1 μM), the enzyme reaction was started by addition of 40-fold purified rat brain farnesyltransferase. After a 30 min incubation at 37 °C, the reaction was quenched by the addition of 2.5-fold of a stop buffer containing 1.5 M magnesium acetate, 0.2 M H_3PO_4, 0.5% bovine serum albumin, and streptavidin beads (Amersham), at a concentration of 1.3 mg/mL. After allowing the plate to settle for 30 min at room temperature, radioactivity was quantified on a microBeta counter (Model 1450, Wallec).

Geranylgeranyltransferase Assay. The geranylgeranyltransferase inhibitory activity of compound **1** was determined in a similar manner as the farnesyltransferase activity. The enzyme was rat geranylgeranyltransferase from Sf9 cells (gift from M. Gelb, University of Washington). (Biotinyl)-7-aminoheptanoic acid-Thr-Lys-Cys-Val-Ile-Leu was used at the peptide and geranylgeranyl pyrophosphate as the substrate.

***Xenopus* Ooocyte Maturation Studies.** Oocyte isolation was carried out as previously described.[50] Adult female South African clawed frogs, *X. Laevis* (Xenopus 1, Ann Arbor, MI), were anesthetized with a 0.3% solution of ethyl *m*-aminobenzoate for 15 min and then placed on an ice bed. Ovarian fragments were surgically removed and placed in modified Barth's saline (MBS) [88 mM NaCl, 1.0 mM KCl, 2.4 mM $NaHCO_3$, 0.3 mM $Ca(NO_3)_2$, 0.41 mM $CaCl_2$, 0.82 $MgSO_4$, 15 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (Hepes)-NaOH, pH 7.6, 10 U of penicillin/mL, 10 μg of streptomycin sulfate/mL, 0.5 μg of gentamicin/mL, and 2.5 mM sodium pyruvate]. Using forceps, the ovary lobe was separated into clumps and manually teared apart prior to staging. Healthy stage VI oocytes, previously washed with several changes of MBS, were selected for maturation studies. Subsequent to microinjection of the farnesyltransferase inhibitor, oocytes were incubated in the presence or absence of either insulin or progesterone at the indicated concentrations. Oocytes were

incubated at 19 °C for 20 h prior to determination of maturation response.

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References

(1) Manne, V.; Roberts, D.; Tobin, A.; O'Rourke, E.; DeVirgilio, M.; Meyers, C.; Ahmed, N.; Kurz, B.; Resh, M.; Kung, H.-F.; Barbacid, M. Identification and preliminary characterization of protein-cysteine farnesyl transferase. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 7541−7545.

(2) Schaber, M. D.; O'Hara, M. B.; Garsky, V. M.; Mosser, S. D.; Bergstrom, J. D.; Moores, S. L.; Marshall, M. S.; Friedman, P. A.; Dixon, R. A. F.; Gibbs, J. B. Polyisoprenylation of Ras in vitro by a farnesyl-protein transferase. *J. Biol. Chem.* **1990**, *265*, 14701−14704.

(3) Reiss, Y.; Seabra, M. C.; Goldstein, J. L.; Brown, M. S. Purification of ras farnesyl protein transferase. *Methods: Companion Methods Enzymol.* **1990**, *1*, 241−245.

(4) Reiss, Y.; Goldstein, J. L.; Seabra, M. C.; Casey, P. J.; Brown, M. S. Inhibition of purified p21 ras farnesyl:protein transferase by CysAAX tetrapeptides. *Cell* **1990**, *62*, 81−88.

(5) Casey, P. J.; Thissen, J. A.; Moomaw, J. F. Enzymatic modification of protein with a geranylgeranyl isoprenoid. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 8631−8635.

(6) Yokoyama, K.; Goodwin, G.; Ghomsashchi, F.; Glomset, J. A.; Gelb, M. H. A protein geranylgeranyl transferase from bovine brain: implications for protein prenylation specificity. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 5302−5306.

(7) Chen, W.-J.; Andres, D. A.; Goldstein, J. L.; Russell, D. W.; Brown, M. S. cDNA cloning and expression of the peptide-binding β subunit of rat p21ras farnesyltransferase, the counterpart of yeast DPR1/RAM1. *Cell* **1991**, *66*, 327−334.

(8) Chen, W.-J.; Andres, D. A.; Goldstein, J. L.; Brown, M. S. Cloning and expression of a cDNA encoding the α-subunit of rat p21ras protein farnesyltransferase. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 11368−11372.

(9) Kohl, N. E.; Diehl, R. E.; Schaber, M. D.; Rands, E.; Soderman, D. D.; He, B.; Moores, S. L.; Pompliano, D. L.; Ferro-Novick, S.; Powers, S.; Thomad, K. A.; Gibbs, J. B. Structural homology among mammalian and *saccharomyces cerevisiae* isoprenyl-protein transferase. *J. Biol. Chem.* **1991**, *266*, 18884−18888.

(10) Seabra, M. C.; Reiss, Y.; Casey, P. J.; Brown, M. S.; Goldstein, J. L. Protein farnesyltransferase and geranylgeranyltransferase share a common α subunit. *Cell* **1991**, *65*, 429−434.

(11) Casey, P. J.; Solski, P. A.; Der, C. J.; Buss, J. E. p21ras is modified by a farnesyl isoprenoid. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 8323−8327.

(12) Glomset, J.; Gelb, M.; Farnsworth, C. The prenylation of proteins. *Curr. Opin. Lipidol.* **1991**, *2*, 118−124.

(13) Hancock, J. F.; Magee, A. I.; Chids, J. E.; Marshall, C. J. All ras proteins are polyisoprenylated but only some are palmitoylated. *Cell* **1989**, *57*, 1167−1177.

(14) Ashby, M. N.; King, D. S.; Rine, J. Endoproteolytic processing of a farnesylated peptide in vitro. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 4613−4617.

(15) Ma, Y.-T.; Rando, R. R. A microsomal endoprotease that specifically cleaves isoprenylated peptides. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 6275−6279.

(16) Akopyan, T. N.; Couedel, Y.; Beaumont, A.; Fournie-Salurki, M.-C.; Roques, B. P. Cleavage of farnesylated COOH-teminal heptapeptide of mouse N-ras by brain microsomal membranes: Evidence for a carboxypeptidase which specifically removes the COOH-terminal methionine. *Biochem. Biophys. Res. Commun.* **1992**, *187*, 1336−1342.

(17) Clarke, S.; Voge, J. P.; Deschenens, R. J.; Stock, J. Posttranslational modification of the Ha-ras oncogene protein: evidence for a third class of protein carboxyl methyl transferases. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 4643−4647.

(18) Perez-Sala, D.; Tan, E. W.; Canada, F. J.; Rando, R. R. Methylation and demethylation reactions of guanine nucleotide-binding proteins of retinal rod outer segments. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 3043−3046.

(19) Barbacid, M. The ras oncogene. *Annu. Rev. Biochem.* **1987**, *56*, 779−828.

(20) Cox, A. D.; Der, C. J. The ras/cholesterol connection: Implications for ras oncogenicity. *Crit. Rev. Oncogen.* **1992**, *3*, 365−400.

(21) Bos, J. L. *ras* Oncogenes in human cancer: A review. *Cancer Res.* **1989**, *49*, 4682−4689.

(22) Goldstein, J. L.; Brown, M. S.; Stradley, S. J.; Reiss, Y.; Gierasch, L. M. Non-farnesylated tetrapeptide inhibitors of protein farnesyl transferase. *J. Biol. Chem.* **1991**, *266*, 15575−15578.

(23) Kohl, N. E.; Mosser, S. D.; deSolms, S. J.; Giuliani, E. A.; Pompliano, D. L.; Graham, S. L.; Smith, R. L.; Scolnick, E. M.; Oliff, A.; Gibbs, J. B. Selective inhibition of ras dependent transformation by a farnesyl transferase inhibitor. *Science* **1993**, *260*, 1934−1937.

(24) Graham, S. L.; deSolms, S. J.; Giuliani, E. A.; Kohl, N. E.; Mosser, S. D.; Oliff, A. I.; Pompliano, D. L.; Rands, E.; Breslin, M. J.; Deana, A. A.; Garsky, V. M.; Scholx, T. H.; Gibbs, J. B.; Smith, R. L. Pseudopeptide inhibitors of ras farnesyl protein transferase. *J. Med. Chem.* **1994**, *37*, 725−732.

(25) Kohl, N. E.; Wilson, F. R.; Mosser, S. D.; Giuliani, E.; deSolms, S. J.; Conner, M. W.; Anthony, N. J.; Holtz, W. J.; Gomez, R. P.; Lee, T.-J.; Smith, R. L.; Graham, S. L.; Hartman, G. D.; Gibbs, J. B.; Oliff, A. Protein farnesyltransferase inhibitors block the growth of ras-dependent tumors in nude mice. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 9141−9145.

(26) Prendergast, G. C.; Davide, J. P.; deSolms, S. J.; Giuliani, E. A.; Graham, S. L.; Gibbs, J. B.; Oliff, A.; Kohl, N. E. Farnesyl transferase inhibition causes morphological reversion of ras-transformed cells by a complex mechanism that involves regulation of the actin skeleton. *Mol. Cell. Biol.* **1994**, *14*, 4193−4202.

(27) Garcia, A. M.; Rowell, C.; Ackerman, K.; Kowalczyck, J. J.; Lewis, M. D. Peptidomimetic inhibitors of ras farnesylation and function in whole cells. *J. Biol. Chem.* **1993**, *268*, 18415−18418.

(28) Cox, A. D.; Garcia, A. M.; Westwick, J. K.; Kowalczyck, J. J.; Lewis, M. D.; Brenner, D. A.; Der, C. J. The CAAX peptidomimetic compound B581 specifically blocks farnesylation but not geranylgeranylated or myristylated, oncogenic ras signalling and transformation. *J. Biol. Chem.* **1994**, *269*, 19203−19206.

(29) Nagasu, T.; Yoshimatsu, K.; Rowell, C.; Lewis, M. D.; Garcia, A. M. Inhibition of human tumor xenograft growth by treatment with the farnesyl transferase inhibitor B956. *Cancer Res.* **1995**, *55*, 5310−5314.

(30) Qian, Y.; Vogt, A.; Sebti, S. M.; Hamilton, A. D. Design ans synthesis of non-peptide ras CAAX mimetics as potent farnesyl transferase inhibitors. *J. Med. Chem.* **1996**, *39*, 217−223.

(31) Leftheris, K.; Kline, T.; Vite, G. D.; Cho, V. H.; Bhide, R. S.; Patel, D. V.; Patel, M. M.; Schmidt, R. J.; Weller, H. N.; Andahazy, M. L.; Carboni, J. M.; Gullo-Brown, J. L.; Lee, F. Y. F.; Ricca, C.; Rose, W. C.; Yan, N.; Barbacid, M.; Hunt, J. T.; Meyers, C. A.; Seizinger, B. R.; Zahler, R.; Manne, V. Development of highly potent inhibitors of ras farnesyl transferase possessing cellular and in vivo activity. *J. Med. Chem.* **1996**, *39*, 224−236.

(32) James, G. L.; Goldstein, J. L.; Brown, M. S.; Rawson, T. E.; Somers, T. C.; McDowell, R. S.; Crowley, C. W.; Lucas, B. K.; Levinson, A. D.; Marsters, J. C., Jr. Benzodiazepine peptidomimetics: potent inhibitors of ras farnesylation in animal cells. *Science* **1993**, *260*, 1937−1942.

(33) Nigam, M.; Seong, C.-M.; Qian, Y.; Hamilton, A. D.; Sebti, S. M. Potent inhibition of human tumor p21 ras farnesyl transferase by A_1A_2-lacking p21ras CA_1A_2X peptidomimetics. *J. Biol. Chem.* **1993**, *268*, 20695−20698.

(34) Qian, Y.; Blaskovich, M. A.; Saleem, M.; Seong, C. M.; Wathen, S. P.; Hamilton, A. D.; Sebti, S. M. Design and structural requirements of potent peptidomimetic inhibitors of p21ras farnesyl transferase. *J. Biol. Chem.* **1994**, *269*, 12410−12413.

(35) Qian, Y.; Blaskovich, M. A.; Seong, C.-M.; Vogt, A.; Hamilton, A. D.; Sebti, S. M. Peptidomimetic inhibitors of p21ras farnesyl transferase: hydrophobic functionalization leads to disruption of p21ras membrane association in whole cells. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 2579−2584.

(36) Lerner, E. C.; Qian, Y.; Blaskovich, M. A.; Fossum, R. D.; Vogt, A.; Sun, J.; Cox, A. D.; Der, C. J.; Hamilton, A. D.; Sebti, S. M. Ras CAAX peptidomimetic FTI-277 selectively blocks oncogenic ras signaling by inducing cytoplasmic accumulation of inactive ras-raf complex. *J. Biol. Chem.* **1995**, *270*, 26802−26806.

(37) Hunt, J. T.; Lee, V. G.; Leftheris, K.; Seizinger, B.; Carboni, J.; Mabus, J.; Ricca, C.; Yan, N.; Manne, V. Potent, cell active, non-thiol tetrapeptide inhibitors of farnesyltransferase. *J. Med. Chem.* **1996**, *39*, 353−358.

(38) Kowalczyk, J. J.; Ackermann, K.; Garcia, A. M.; Lewis, M. D. Phenolic replacements for cysteine in farnesyltransferase inhibitors based on CVFM. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 3073−3078.

(39) Bishop, W. R.; Bond, R.; Petrin, J.; Wang, L.; Patton, R.; Doll, R.; Njoroge, G.; Catino, J.; Schwartz, J.; Windsor, W.; Syto, R.; Schwartz, J.; Carr, D.; James, L.; Kirschmeier, P. Novel tricyclic inhibitors of farnesyl protein transferase: biochemical characterization and inhibition of ras modification in transfected Cos cells. *J. Biol. Chem.* **1995**, *270*, 30611−30618.

(40) (a)Leonard, D. M.; Eaton, S. R.; Sawyer, T. K.; Bolton, G.; Hodges, J.; Gowan, R.; Sebolt-Leopold, J. S. Structure-activity relationships of cysteine-lacking pentapeptide derivatives that inhibit ras:farnesyltransferase. Presented at the 207th National Meeting of the American Chemical Society, San Diego, CA, March 1994; MEDI 281. (b) Leonard, D. M.; Eaton, S. R.; Sawyer, T. K.; Doherty, A. M.; Bolton, G.; Hodges, J.; Scholten, J.; Gowan, R.; Sebolt-Leopold, J. S. Structure-activity relationships of

cysteine-lacking pentapeptide derivatives that inhibit ras: farnesyltransferase. In *Peptides 1994: Proceedings of the Twenty-Third European Peptide Symposium*; Maia, H. L. S., Ed.; ESCOM Science: Leiden, The Netherlands, 1995; pp 658−659.

(41) Sebolt-Leopold, J. S.; Gowan, R.; Su, T.-Z.; Leonard, D.; Sawyer, T.; Bolton, G.; Hodges, J.; Hupe, D. Inhibition of ras farnesyltransferase by a novel class of peptides containing no cysteine or thiol moieties. *Proc. Am. Assoc. Cancer Res.* **1994**, *35*, Abstract 3535.

(42) Stewart, J. M.; Young, J. D. *Solid Phase Peptide Synthesis*; Pierce Chemical Co.: Rockford, IL, 1984.

(43) *The Practice of Peptide Synthesis*; Bodansky, M., Bodansky, A., Eds.; Springer-Verlag: Berlin, Heidelberg, 1984.

(44) Rink, H. Solid-phase synthesis of protected peptide fragments using trialkoxy-diphenyl-methyl ester. *Tetrahedron Lett.* **1987**, *28*, 3787−3790.

(45) Mergler, M.; Tanner, R.; Gosteli, J.; Grogg, P. Peptide synthesis by a combination of solid phase and solution methods I: a new very acid-labile anchor group for the solid phase synthesis of fully protected fragments. *Tetrahedron Lett.* **1988**, *29*, 4005−4008.

(46) Kaiser, E.; Colescott, R. L.; Bossinger, C. D.; Cook, P. I. Color test for detection of free terminal amino groups in the solid-phase synthesis of peptides. *Anal. Biochem.* **1970**, 34, 595−598.

(47) Scholten, J. D.; Zimmerman, K.; Oxender, M.; Sebolt-Leopold, J.; Gowan, R.; Leonard, D.; Hupe, D. J. Inhibitors of farnesyl: protein transferase: A possible cancer chemotherapeutic. *Bioorg. Med. Chem. Lett.* **1996**, *4*, 1537−1543.

(48) Patel, D. V.; Gordon, E. M.; Schmidt, R. J.; Weller, H. N.; Young, M. G.; Zahler, R.; Barbacid, M.; Carboni, J. M.; Gullo-Brown, J. L.; Hunihan, L.; Ricca, C.; Robinson, S.; Seizinger, B. R.; Tuomari, A. V.; Manne, V. Phosphinyl acid-based bisubstrate analog inhibitors of ras farnesyl protein transferase. *J. Med. Chem.* **1995**, *38*, 435−442.

(49) Manne, V.; Yan, N.; Carboni, J. M.; Tuomari, A. V.; Ricca, C. S.; Gullo-Brown, J.; Andahazy, M. L.; Schmidt, R. J.; Patel, D.; Zahler, R.; Weinmann, R.; Der, C. J.; Cox, A. D.; Hunt, J. T.; Barbacid, M.; Seizinger, B. R. Bisubstrate inhibitors of farnesyl transferase: a novel class of specific inhibitors of ras transformed cells. *Oncogene* **1995**, *10*, 1763−1779.

(50) Su, T.-Z.; Logsdon, C. D.; Oxender, D. L. Chinese hamster ovary mRNA-dependent, Na+-independent L-leucine transport in *Xenopus laevis oocytes*. *Mol. Cell. Biol.* **1992**, *12*, 5281−5287.

(51) Bolton, G. L.; Creswell, M. W.; Hodges, J. C.; Gowan, R. C.; Scholten, J.; Sebolt-Leopold, J. S. The SAR of tripeptide inhibitors of ras farnesylprotein transferase. Presented at the 209th National Meeting of the American Chemical Society, Anaheim, CA, April 1995; MEDI 032.

(52) Bolton, G. L.; Creswell, M. W.; Hodges, J. C.; Gowan, R. C.; Scholten, J.; Sebolt-Leopold, J. S. Modified peptide inhibitors of ras farnesylprotein transferase. Presented at the 209th National Meeting of the American Chemical Society, Anaheim, CA, April 1995; MEDI 033.

(53) Shuler, K. R.; Leonard, D. M.; Bolton, G. L.; Scholten, J.; Sebolt-Leopold, J.; Doherty, A. M. The inhibition of ras farnesyl transferase by a modified dipeptide: Structure-activity relationships. Presented at the 211th National Meeting of the American Chemical Society, New Orleans, LA, April 1996; MEDI 086.

(54) Bolton, G. L.; Creswell, M. W.; Hodges, J. C.; Gowan, R. C.; Scholten, J.; Sebolt-Leopold, J. S. Modified dipeptide inhibitors of ras farnesyl transferase. Presented at the 25th National Medicinal Chemistry Symposium, Ann Arbor, MI, June 1996.

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