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Communications to the Editor

C-Terminal Modifications of Histidyl-*N*-benzylglycinamides To Give Improved Inhibition of Ras Farnesyltransferase, Cellular Activity, and Anticancer Activity in Mice

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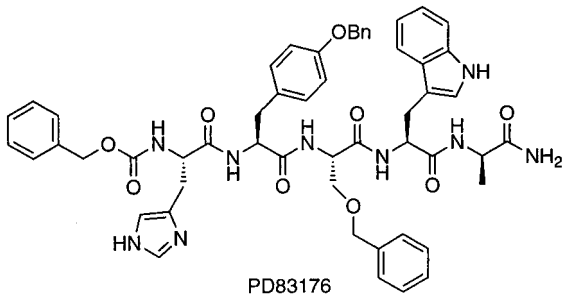
To accomplish its critical role in transmitting the signal for cell growth, the Ras protein (H, N, K-4A, and K-4B) must be attached to the inside of the cell membrane.[1] This is achieved in large part by lipophilic modifications to the four amino acids at its C-terminus, termed the CAAX box, where C is cysteine, A is any amino acid, and X is serine or methionine.[2] The first and most important of these modifications is the attachment of a farnesyl group, a 15-carbon isoprenoid unit, to the sulfur of cysteine. This reaction is catalyzed by the enzyme farnesyltransferase (FT) and utilizes farnesylpyrophosphate (FPP) as the farnesyl donor.

Mutations in Ras are frequently encountered in cancer: 30% of all human cancers contain a Ras mutation, including 90% of pancreatic, 50% of colon, and 30% of lung cancers.[3] Since mutated Ras must be farnesylated in order to transform cells to a malignant state,[4] inhibition of FT is an attractive target for anticancer activity. In fact, several groups have reported anticancer activity in mice utilizing this strategy.[5−9]

Chart 1. Structures of PD83176 and PD152440 (**5a**)



PD152440 (**5a**)

We have previously reported that PD83176 (Chart 1), a protected pentapeptide discovered by screening our compound library, inhibited the isolated rat FT enzyme with an $IC_{50} = 0.076\ \mu M$.[10] However, it did not inhibit Ras farnesylation in cells, probably due to an inability to enter cells. By truncating the C-terminus of PD83176 at the serine α-carbon and transposing, in peptoid fashion, the benzylated side chain of the tyrosine to its α-nitrogen, PD152440 **(5a)** (Chart 1) was designed.[11] While less active than PD83176 against the isolated FT enzyme with an $IC_{50} = 0.32\ \mu M$, **5a** did inhibit Ras farnesylation in cells at 1 μM. We here report on modifications to the C-terminal amide of **5a**, which result in dramatically improved enzymatic and cellular activities, culminating in anticancer activity in mice.

Chemistry. The synthesis of **5a** is illustrated in Scheme 1. Glycine methyl ester hydrochloride was reductively aminated with 4-(benzyloxy)benzaldehyde using sodium triacetoxyborohydride to give the *N*-benzylglycine **1** in 40% yield. Acylation with CbzHis-

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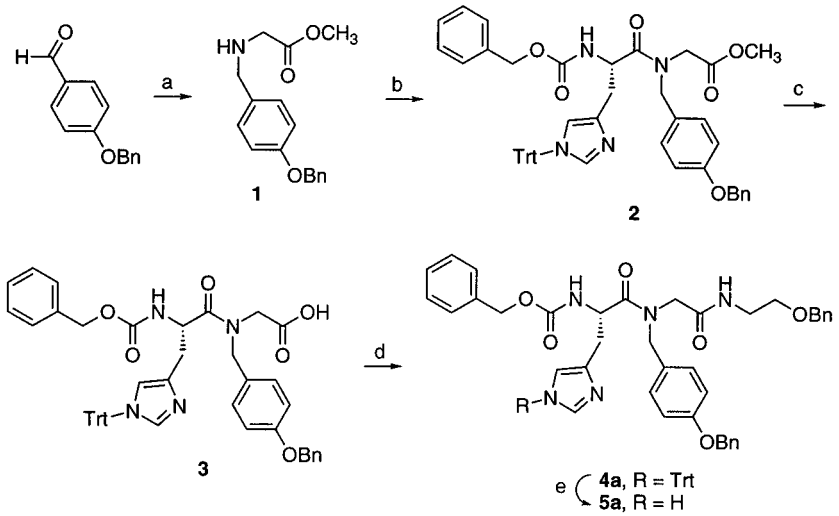
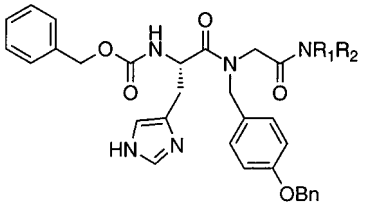
Scheme 1. Synthesis of **5a**[a]



[a] Reagents: (a) $HCl{\cdot}H_2NCH_2CO_2CH_3$, $Na(OAc)_3BH$, CH_2Cl_2 (40%); (b) CbzHis(Trt), PyBOP, DIEA, CH_2Cl_2 (55%); (c) LiOH, THF, H_2O (89%); (d) $HCl{\cdot}H_2NCH_2CH_2OBn$, HBTU, DIEA, CH_2Cl_2; (e) TFA, CH_2Cl_2 (54%, two steps).

Table 1. Inhibitory Activities against the Isolated FT Enzyme and in Transformed Cells



compd	R_1	R_2	FT IC_{50} (μM)[a]	Ras farnesylation MED (μM)[b]	colony formation IC_{50} (μM)[c]
PD83176		see Chart 1	0.076	>100	NT[d]
5a	H	CH_2CH_2OBn	0.32 ± 0.07 (6)	1	14.3 ± 3.6 (4)
5b	H	CH_2CH_2Ph	0.062 ± 0.007 (9)	0.1	4.3 ± 0.8 (7)
5c	Me	CH_2CH_2Ph	4	NT	NT
5d	H	$CH(Me)CH_2Ph$ (*R*)	0.003 ± 0.001 (4)	0.05	0.71 ± 0.23 (3)
5e	H	$CH(Me)CH_2Ph$ (*S*)	0.041 ± 0.012 (5)	0.2	6.3
5f	H	$C(Me)_2CH_2Ph$	0.27	1	NT
5g	H	$CH_2CH(Me)Ph$	0.008 ± 0.001 (5)	0.05	0.31 ± 0.06 (4)
5h	H	$CH_2CH(Me)Ph$ (*R*)	0.005	0.05	0.30
5i	H	$CH_2CH(Me)Ph$ (*S*)	0.016	0.2	0.57
5j	H	$CH_2CH(CN)Ph$	0.002	0.05	0.19 ± 0.06 (3)
5k	H	$CH_2CH(Et)Ph$	0.005 ± 0.001 (3)	0.05	0.40 ± 0.08 (3)
5l	H	$CH_2CH(n\text{-}Pr)Ph$	0.025	0.2	>1
5m	H	$CH_2C(Me)_2Ph$	0.004 ± 0.001 (3)	0.05	0.18 ± 0.04 (8)
5n	H	$CH_2C(Et)_2Ph$	0.032	0.2	>1
5o	H	$CH_2C(CH_2)_2Ph$	0.007	0.02	0.18
5p	H	$CH_2C(CH_2)_3Ph$	0.006	0.02	0.088 ± 0.022 (6)
5q	H	$CH_2C(CH_2)_4Ph$	0.017	0.2	0.33

[a] Concentration of compound required to inhibit by 50% the incorporation of [^{3}H]FPP into a biotinylated hexapeptide from the C-terminus of K_B-Ras by Sf9-affinity-purified rat FT. Values are averages of the number of determinations in parentheses ± SEM or, where unnoted, of two determinations. [b] Minimum effective dose (MED) of compound required to detect unfarnesylated Ras in H-Ras-F cells as determined by Western transfer techniques. Values are confirmed data from at least two determinations. [c] Concentration of compound required to inhibit by 50% the number of colonies of H-Ras-F cells when grown on soft agar. Values are averages of the number of determinations in parentheses ± SEM or, where unnoted, of two determinations. [d] Not tested.

(Trt)[12] gave **2** in 55% yield**.** The methyl ester was hydrolyzed to the acid **3** (88% yield), which was coupled to 2-(benzyloxy)ethylamine hydrochloride[13] to give the protected intermediate **4a**. Detritylation of the imidazole furnished the target **5a** in 54% yield over two steps. Compounds **5b**−**q** (Table 1) were synthesized analogously by coupling the required phenethylamines to **3**.

Results and Discussion. The compounds in this series were first tested for inhibition of the isolated rat FT enzyme (Table 1).[14] The cellular activity of these compounds was then investigated, first by measuring the inhibition of Ras farnesylation in H-Ras-transformed NIH3T3 (H-Ras-F) cells[15] and then by measuring the inhibition of colony formation of these same cells when grown in soft agar. This latter assay is especially relevant to anticancer activity since colony formation in soft agar is well correlated with tumorogenicity in the nude mouse.[16] In general there was very good agreement between these three assays for this series of compounds.

The greater activity of PD83176 relative to **5a** against the FT enzyme suggested that the activity of **5a** could be enhanced by substituting the C-terminal amide. Initial efforts to simplify the scaffold on which to carry out structure−activity studies led to the unsubstituted phenethylamine **5b**, which displayed a 5-fold improvement in activity against FT, with a corresponding improvement in cellular activity (Table 1). Methylation

of the C-terminal amide nitrogen (**5c**) markedly decreased activity against the enzyme. Substitution on the α-carbon of the phenethylamine with a (*R*)-methyl group (**5d**) resulted in a 20-fold improvement in activity against FT relative to **5b**, with improved cellular activity. (*S*)-Methyl substitution (**5e**) did not result in a significant improvement in activity, and dimethyl substitution (**5f**) resulted in decreased activity. Methyl substitution on the *β*-carbon (**5g**), especially in the *R*-configuration (**5h** vs **5i)**, resulted in a 10-fold improvement in activity against FT relative to **5b**, with improved cellular activity. Other small substituents, as with the cyano (**5j**) and ethyl (**5k**) groups, maintained this enzymatic and cellular activity, while the larger *n*-propyl substituent (**5l**) did not. Dimethyl substitution (**5m**) resulted in increased activity, whereas diethyl substitution (**5n**) resulted in decreased activity. Cyclopropyl (**5o**) and cyclobutyl (**5p**) substitution also resulted in increased activity, whereas cyclopentyl (**5q**) resulted in decreased activity. In general, small substituents on the α and *β* positions of the phenethylamine scaffold resulted in compounds with dramatically improved activity against the isolated FT enzyme and in cells.

Compound **5m**, which inhibited the isolated FT enzyme with an $IC_{50} = 0.004$ *μ*M, inhibited Ras farnesylation in transformed cells at 0.05 *μ*M, and inhibited the colony formation of transformed cells in soft agar with an $IC_{50} = 0.18$ *μ*M, was one of the most active compounds in this series. Investigation of its inhibition of FT using steady state kinetic analysis revealed it to be competitive with the FPP substrate (data not shown), with a $K_i = 0.74 \pm 0.04$ nM.

A further requirement for these FT inhibitors as potential anticancer drugs is selectivity for the FT enzyme compared to the closely related geranylgeranyltransferase-1 (GGT1) enzyme. GGT1 catalyzes the transfer of a geranylgeranyl group, a 20-carbon isoprenoid unit, to proteins containing a CAAX box, where X is leucine.[2] Since many more proteins in the body are geranylgeranylated than farnesylated, we believed that selectivity for FT was important. Our lead compound in this series, **5a**, displayed only 2-fold selectivity for FT ($IC_{50} = 0.32$ *μ*M) over GGT1 ($IC_{50} = 0.6$ *μ*M). However **5m** was a very selective inhibitor (4500-fold), with an IC_{50} of 0.004 *μ*M against FT and an IC_{50} of 18 *μ*M against GGT1.

Finally, we evaluated **5m** for anticancer activity in mice (Figure 1). Athymic mice were implanted subcutaneously with H-Ras-F cells and treated intraperitoneally with **5m**, once a day for 14 consecutive days after tumor implantation. When administered at 150 mg/kg/day, **5m** inhibited tumor growth by 88% relative to untreated controls when assessed on day 15. At 100 mg/kg/day, **5m** inhibited tumor growth by 53%.

Thus, simple chemical modification of the C-terminal amide of our lead compound, **5a**, has resulted in potent, selective inhibition of the FT enzyme, potent cellular activity, and significant anticancer activity in mice, as exemplified by **5m**. Further in vivo evaluation of this compound in other types of tumors is ongoing.

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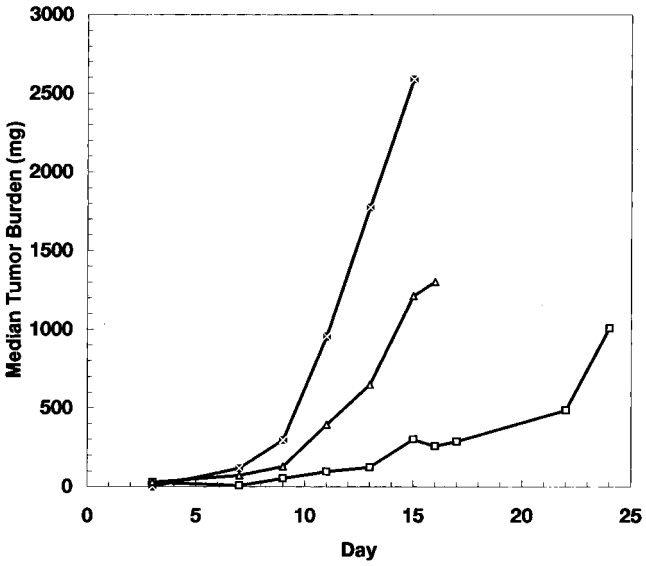

Figure 1. Mean tumor burden of mice inoculated subcutaneously with H-Ras-F cells on day 0 and treated intraperitoneally with **5m** at 150 mg/kg/day (□) or 100 mg/kg/day (△) or vehicle control (×) once a day for 14 consecutive days after tumor implantation.

Supporting Information Available: Details for the synthesis of **5a**−**q**; assays for inhibition of the FT enzyme, the GGT1 enzyme, cellular Ras farnesylation, and colony formation; and testing of **5m** in mice (10 pages). Ordering information is given on any current masthead page.

References

(1) Barbacid, M. Ras Genes. *Annu. Rev. Biochem.* **1987**, *56*, 779−827.
(2) Zhang, F. L.; Casey, P. J. Protein Prenylation: Molecular Mechanisms and Functional Consequences. *Annu. Rev. Biochem.* **1996**, *65*, 241−269.
(3) Bos, J. L. Ras Oncogenes in Human Cancer: A Review. *Cancer Res.* **1989**, *49*, 4682−4689.
(4) Jackson, J. H.; Cochrane, C. G.; Bourne, J. R.; Solski, P. A.; Buss, J. E.; Der, C. J. Farnesol Modification of Kirsten-Ras Exon 4B is Essential for Transformation. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 3042−3046.
(5) Kohl, N. E.; Wilson, F. R.; Mossor, S. D.; Giuliani, E.; de Solms, S. J.; Conner, M. W.; Anthony, N. J.; Holtz, W. J.; Gomez, R. P.; Lee, T.-J.; Smith, R. L.; Graham, S. L.; Hartmann, 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.
(6) Leftheris, K.; Kline, T.; Vite, G. D.; Cho, Y. 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 Farnesyltransferase Possessing Cellular and In Vivo Activity. *J. Med. Chem.* **1996**, *39*, 224−236.
(7) Nagasu, T.; Yoshimatsu, K.; Rowell, C.; Lewis, M. D.; Garcia, A. M. Inhibition of Human Tumor Xenograft Growth by Treatment with Farnesyl Inhibitor B956. *Cancer Res.* **1995**, *55*, 5310−5314.
(8) Mallams, A. K.; Njoroge, F. G.; Doll, R. J.; Snow, M. E.; Kaminski, J. J.; Rossman, R. R.; Vibulbhan, B.; Bishop, W. R.; Kirschmeier, P.; Liu, M.; Bryant, M. S.; Alvarez, C.; Carr, D.; James, L.; King, I.; Li, Z.; Lin, C.-C.; Nardo, C.; Petrin, J.; Remiszewski, S. W.; Taveras, A. G.; Wang, S.; Wong, J.; Catino, J.; Girijavallabhan, V.; Ganguly, A. K. Antitumor 8-Chlorobenzocycloheptapyridines: A New Class of Selective, Nonpeptidic, Nonsulfydryl Inhibitors of Ras Farnesylation. *Bioorg. Med. Chem.* **1997**, *5*, 93−99.
(9) Sun, J.; Quian, Y.; Hamilton, A. D.; Sebti, S. Ras CAAX Peptidomimetic FTI 276 Selectively Blocks Tumor Growth in Nude Mice of a Human Lung Carcinoma with K-Ras Mutation and p53 Deletion. *Cancer Res.* **1995**, *55*, 4243−4247.

(10) Leonard, D. M.; Shuler, K. R.; Poulter, C. J.; Eaton, S. R.; Sawyer, T. K.; Hodges, J. C.; Su, T.-Z.; Scholten, J. D.; Gowan, R. C.; Sebolt-Leopold, J. S.; Doherty, A. M. Structure-Activity Relationships of Cysteine-Lacking Pentapeptide Derivatives that Inhibit Ras Farnesyltranferase. *J. Med. Chem.* **1997**, *40*, 192−200.

(11) Bolton, G. L.; Creswell, M. W.; Hodges, J. C.; Gowan, R. C.; Scholten, J.; Sebolt-Leopold, J. S.; Zimmerman, K. Modified Dipeptide Inhibitors of Ras Farnesyl Transferase. Presented at the 25th Medicinal Chemistry Symposium, Ann Arbor, MI, June 1996; Poster 6.

(12) Hudspeth, J. P.; Kaltenbronn, J. S.; Repine, J. T.; Roark, W. H.; Stier, M. A. Renin Inhibitors III. United States Patent No. 4,735,933, 1988.

(13) Miller, T. L.; Rowley, G. L.; Stewart, C. J. Coenzyme A Analogs. Synthesis of D-Oxypantetheine-4'-Phosphate and Oxy-Coenzyme A. *J. Am. Chem. Soc.* **1966**, *88*, 2299−2304.

(14) The potency of these compounds in this enzyme assay was dependent upon the concentration of phosphate ion in the assay buffer as described in Scholten, J. D.; Zimmerman, K.; Oxender, M. G.; Leonard, D.; Sebolt-Leopold, J.; Gowan, R.; Hupe, D. J. Synergy Between Anions and Farnesyldiphosphate Competitive Inhibitors of Farnesyl Protein Transferase. *J. Biol. Chem.* **1997**, *272*, 18077−18081 and references therein. The concentration of phosphate used in this assay was 5 mM (see Supporting Information for details).

(15) Cox, A. D.; Hisaka, M. M.; Buss, J. E.; Der, C. J. Specific Isoprenoid Modification Is Required for Function of Normal, but Not Oncogenic, Ras Protein. *Mol. Cell. Biol.* **1992**, *12*, 2606−2615.

(16) Shin, S.; Freeman, V. H.; Risser, R.; Pollack, R. Tumorigenicity of Virus-Transformed Cells in Nude Mice is Correlated Specifically with Anchorage Independent Growth in Vitro. *Proc. Natl. Acad. Sci. U.S.A.* **1975**, *72*, 4435−4439.

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