Design, Synthesis, and Pharmacological Evaluation of New Farnesyl Protein Transferase Inhibitors

Raymond Houssin, Jean Pommery, Marie-Catherine Salaün, Sophie Deweer, Jean-François Goossens, Philippe Chavatte, and Jean-Pierre Hénichart*

Institut de Chimie Pharmaceutique Albert Lespagnol, EA 2692, Université de Lille 2, 3 rue du Professeur Laguesse, BP 83, 59006 Lille, France

Received June 27, 2001

New CA_1A_2X peptidomimetics are described as Ras farnesyl transferase inhibitors (FTIs). They include cysteine and methionine as mimetics of the C-terminus sequence of farnesylated proteins. Furthermore, cysteine was replaced by heterocycles, taking into account the role of zinc and the metabolic instability of amino acids. The molecular docking of **8** in the active site of the enzyme and the pharmacological evaluation of the compounds are illustrative of a new class of FTIs.

Introduction

Ras oncogenes are found activated in a variety of tumors, with the highest incidence observed in cancers of the pancreas (ca. 90%), colon (ca. 50%), and lung (ca. 30%).[1−3] The important role played by Ras protein in the signal transduction process involved in cell division has been documented.[4,5] Molecular events that lead to Ras activation are mainly dependent on its membrane association.[6,7] A post-translational modification that includes the prenylation of Ras by farnesyl protein transferase (FTase) promotes its anchoring to the cell membrane.[8] It is therefore conceivable that inhibition of FTase would be a target for developing potential anticancer agents.[9]

Many classes of farnesyl transferase inhibitors (FTIs) have been reviewed.[10−13] In addition, the crystal structure of FTase was determined at 2.25 Å resolution and revealed essential information about the active site.[14] The existence of a high-affinity hydrophobic aromatic pocket[15,16] in the peptidic strategy based on the C-terminal CAAX motif of the Ras protein is the subject of increasing interest. Recent results are illustrated by (i) replacement of Cys by heterocycles such as pyridine[17,18] and imidazole[19] or by cyclohexylamine[20] and (ii) modulation of the A_1A_2 tensor with benzodiazepine,[21] diaryl ether, or diaryl sulfone.[22] Recent advances concerning competitive inhibitors are illustrated by non-peptide surrogates for the central moiety such as benzylamine substituted either by 1-(4-cyanobenzyl)-imidazole (IC_{50} = 0.1 nM)[23] or by pyridine (IC_{50} = 0.4 nM).[18]

On the other hand, the screening of chemical databases made it possible to identify randomly selected compounds interacting with the enzymatic active site.[24] Clinical trials of non-peptidic, non-thiol FTIs, 1-(4-*N*-carboxamidopiperidinylacetyl)-3,10-dibromo-8-chloro-5,6-dihydro-11*H*-benzo[5,6]cyclohepta[1,2-*b*] pyridin-11-yl)piperidine (SCH-66336) and 6-[amino-(4-chlorophenyl)-(1-methyl-1*H*-imidazol-5-yl)methyl]-4-(3-chlorophenyl)-1-methyl-2-1*H*-quinolinone (R-115777), suggested that their pharmacological profiles are largely comparable.[19,25−29] Taking into account these molecular and pharmacological results, we decided to design peptidomimetic compounds based on the cysteinyl−valinyl−phenylalanyl−methionyl (CVFM) motif and that contain the cysteine and methionine residues, which have been proven to be necessary for recognition, and hence farnesylation, of the K-Ras protein (oncogenic K-Ras 4B is known to be predominant in human cancers).[30]

The two aliphatic residues were replaced by a non-peptidic spacer that could suitably orient the essential elements for specific interaction with the enzyme. On the basis of molecular modeling studies (data not shown), it appeared that a piperidine bearing an exocyclic amino group could be an analogue but a better connector than the classically used heterocycles 1,4-benzodiazepine[31] or piperazine.[32,33] Furthermore, the FTase active site studies emphasized the crucial role of Zn^{2+}, which is required for the binding of the protein substrate and for coordination to the cysteine to facilitate deprotonation of the thiol group during catalysis.[12,14] These observations led us to design molecules with a cysteinyl substituent possibly replaced by heterocyclic groups to circumvent the metabolic instability of the amino acid (i.e., free amino and sulfhydryl moieties). Moreover, the tested molecules possess an ester function for enhancing cell penetration, which may be detrimental for FTase inhibition.[20,25,34]

Chemistry

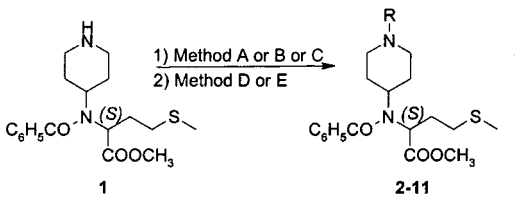
The key intermediate **1** (Scheme 1) was obtained by reductive amination of *N*-Boc-4-piperidone using H−Met−OMe followed by an acylation and a cleavage of the protecting Boc group.

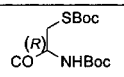
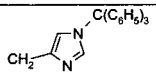
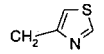
The synthesis of target compounds required the preparation of cysteinyl or adequate heterocyclic surrogates, i.e., thiazolidine, thiazole, and imidazole. (*R*)-Cysteine was N,S-protected by Boc groups by a conventional method. Meanwhile, (*R*)-thiazolidine-4-carboxylic acid was *N*-Boc-protected by a simpler method. Thiazole-4-carboxylic acid and 4-hydroxymethylthiazole were obtained by classical methods from ethyl 2-aminothiazole-

* To whom correspondence should be addressed. Phone: +33-3-2096-4374. Fax: +33-3-2096-4361. E-mail: henicha@phare.univ-lille2.fr.

10.1021/jm010297r CCC: $22.00
Published on Web 12/08/2001

Scheme 1[a,b]



compd	R	method	compd	R	method
2		A	**7**		C
3		A	**8**[b]		D
4		A	**9**[b]		D
5[b]		B	**10**		D
6[b]		C	**11**[b]		E

[a] Reagents: (method A) *N*,*S*-Bis-Boc-*(R)*-cysteine or *N*-Boc-*(R)*-thiazolidine-4-carboxylic acid or *N*-Boc-*(S)*-thiazolidine-4-carboxylic acid, PyBOP, DIPEA, CH_2Cl_2; (method B) thiazole-4-carboxylic acid hydrochloride, $SOCl_2$, $N(Et)_3$, CH_2Cl_2; (method C) 4-methanesulfonylmethylthiazole or 4-chloromethyl-1-tritylimidazole, $N(Et)_3$, acetone; (method D) THF, MeOH/HCl; (method E) TFA, CH_2Cl_2. [b] Hydrochloride.

4-carboxylate.[35,36] 4-Chloromethyl-1-tritylimidazole[37] was obtained in two steps from commercially available 4-hydroxymethylimidazole by N-tritylation and chlorination of the primary alcohol.

The target compounds **2**−**11** were obtained by acylation or alkylation of the key intermediate **1**. As depicted in Scheme 1, the coupling reaction (PyBOP) with *N*,*S*-bis-Boc-(*R*)-cysteine, *N*-Boc-(*R*)-thiazolidine-4-carboxylic acid, or *N*-Boc-(*S*)-thiazolidine-4-carboxylic acid gave the N-acylated compounds **2**, **3**, or **4**, respectively, while **5** resulted from the reaction involving thiazole-4-carbonyl chloride.[38] Deprotection of the amine and/or thiol function of **2**−**4** under acidic conditions (MeOH/HCl) led to **8**−**10**. The substitution of **1** with mesylate of 4-hydroxymethylthiazole or chloromethyl-1-tritylimidazole gave the corresponding *N*-alkyl compounds **6** or **7**, whereas the *N*-trityl derivative **7** gave **11** after deprotection with TFA.

Biological Data

The target compounds **1**, **5**, **6**, and **8**−**11** were tested for their ability to inhibit the FTase catalyzed transfer of the FPP moiety to dansyl−GCVLS and GGPT I, which catalyses the transfer of GGPP moiety to dansyl−GCVLL.[30,39] Commercially available *N*-[2(*S*)-[2(*R*)-amino-3-mercaptopropylamino]-3-methylbutyl]−Phe−Met−OH[40] (B.581) and H−Cys−4-Abz−Met−OH[41] were used respectively as FTI_1 and FTI_2 references for the tests. Biological data are summarized in Table 1.

Table 1. FTase and GGPT Activities of Reference Compounds and of **1**, **5**, **6**, **8**−**11**

	IC_{50} (μM)	
	FTase	GGPT
FTI_1	0.034 (±0.001)	5.1 (±0.7)
FTI_2	0.072 (±0.015)	>100
1	>100	
5	52.5 (±1.1)	>100
6	41.7 (±0.7)	>100
8	0.020 (± 0.002)	>100
9	2.14 (±0.84)	>100
10	>100	
11	16.2 (±0.6)	>100

Results and Discussion

Compound **1** (IC_{50} > 100 μM) demonstrates first that the structure that replaced AAX is not adequate for inhibition. Cysteine is certainly a key element for enzyme recognition. Thus, the presence of this residue on the piperidinic scaffold (compound **8**) found expression in high inhibitory potency (IC_{50} = 20 nM). Moreover, the importance of the thiol group was investigated by considering thiazolidine as a substitute for cysteine, and this change (compound **9**) was expressed by a moderate IC_{50} (2.1 μM). In an effort to understand the effect of stereochemistry on FTase inhibition, we compared the activity of the diastereomeric compounds **9** (*R*,*S*) and **10** (*S*,*S*). These compounds exhibit very little potency, indicating that the configuration, at least that of cysteinyl analogue, was essential for biological activity. The presence of aromatic thiazole (compound **5**) instead of thiazolidine was not beneficial.

We then focused on the restriction of conformational flexibility of cysteine analogues; the amide function of thiazole **5** was reduced, leading to a slight increase in inhibitory potency (IC_{50} = 41.7 μM). These results are relevant to other studies that described improved inhibitory potency[42] after reduction of a peptidic bond.

The prevalence of histidine as a key ligand in metalloenzymes[43] led us to incorporate an imidazole ring (compound **11**) whose IC_{50} was only 16.2 μM. This result was promising, and to prove the interaction of the thiol group with Zn^{2+} in the active site, it seemed interesting to determine whether a more appropriate spacer of the imidazole ring (relative to the remainder of the tripeptide mimetic scaffold) would not be favorable. A new spacer able to maintain the appropriate distances and conformational constraints between cysteine (or analogues) and methionine was therefore defined. Compound **8** is as potent as the reference compounds we used for the in vitro tests.[40,41] FTI_1 and FTI_2 were also designed along the CVFM model. Despite the presence of methionine in both compounds, a more striking FTase selective result was observed for compound **8** than for FTI_1.

Cellular activity was also investigated for final deprotected compounds. The whole cell activity profile of FTIs was evaluated using the growth assay on NIH/3T3 cells. The better results came from a comparison of the inhibitory potency of compounds in the antiproliferative test compared with the enzymatic test. Thiazolidine **9** presented nearly 70% proliferation inhibition at a concentration of 1 μM; such a property was not observed for **8** (cysteine) and **11** (imidazole). Its stereoisomer **10** is clearly inactive and confirms the results of the enzymatic study. Despite our objective to enhance

cellular uptake and to improve stability toward proteases using methyl methioninate, these results are likely to reflect poor cell penetration.

Molecular Modeling Data

It is known that the peptidic CA_1A_2X sequence is sufficient for FTase recognition/inhibition, whereas specific features of this sequence are selective of FTIs. Cysteine residue is present in all FTase protein substrates. Systematic amino acid replacements within the CA_1A_2X sequence showed that the A_1 position was the most flexible, the A_2 position could either be an aliphatic amino acid or an aromatic residue, and X was optimally a methionine. However, peptides with an aromatic residue in the A_2 position, as exemplified by CVFM (the most potent tetrapeptide FTI), were shown to be non-substrate FTI kinetically competitive with protein substrate.

By use of CVFM as a starting point, our design led to the synthesis of a dipeptide mimetic scaffold with the affixed cysteine and methionine residues and subsequently to the replacement of cysteine.

To justify our choice of hydrophobic scaffold able to correctly position the cysteine (or its surrogates) and methionine units and to establish a pharmacophoric model derived from the potent CVFM, molecular modeling was used. Comparison of the CVFM structure with that of **8**, which presented the best inhibition potency (IC_{50} = 20 nM), was carried out. It was noted that the lowest energy conformation of CVFM was globular and was stabilized by six intramolecular hydrogen bonds. Nevertheless, no true turn was identified. It was noteworthy that the conformational space created presented 50% of the extended conformation.

The superimposition of **8** and CVFM using genetic algorithm similarity program (GASP) made it possible to establish 50 molecular alignments. Among those that were visualized, four were chosen because of their ability to position key elements such as the thiol group in the same way, which is essential for farnesylation and the aromatic moiety. Afterward, we noted that they had the best-fitting scores. The pharmacophoric elements to be considered were the terminus NH_2, the carbonyl of cysteine, the aromatic ring, and the carbonyl group of phenylalanine of the CVFM peptide.

After discarding three out of four of the remaining alignments (on the basis of energetic criteria), the selected ones offered extended structures differing by about 20 kcal/mol from the global minimum and by 5 kcal/mol from the lowest energy extended conformation. This analysis favors extended conformation for inhibitory activity on the enzyme and agrees with previous results.[11]

On the other hand, enzyme crystallographic data at 2.25 Å resolution from the Brookhaven Protein Data Bank (PDB) enabled us to study the active site described as two clefts that intersect at a bound Zn^{2+}.[14] Lys164, Arg291, Tyr200, His248, and Tyr300 were selected because of their proven interaction with the terminal COOH of a nonapeptide inhibitor. Zn^{2+}, essential for the farnesylation of the thiol group, was also selected as a key element. After **8** was fit into the active site and when flexible docking was used, the appropriate geometry was identified that facilitated the potentiality of

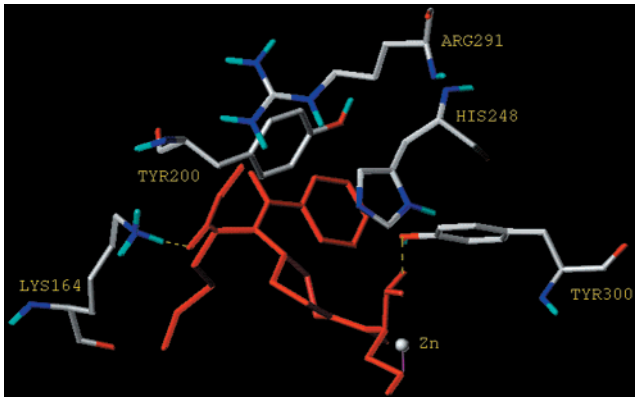


Figure 1. Interactions between **8** and key elements of the active site.

two hydrogen bonds with the Lys164 and Tyr300 of the active site and correctly positioned the essential thiol group in front of Zn^{2+} (Figure 1). In addition, a hydrophobic interaction between both aromatic moieties of compound **8** and His248 may reinforce docking. These results may be compared with those resulting from the crystal structure of the FTase−CVIM complex; the tetrapeptide substrate also adopts an extended conformation where binding and recognition are dominated by side chain interaction.[44]

Conclusion

The synthesized non-peptide Ras CAAX mimetics are potent inhibitors of FTase and are selective against GGTase. The potency in whole cells of **9**, as well as the discovery of a potent FTI, **8** (IC_{50} = 20 nM on isolated enzyme assay), confirms that these Ras CAAX mimetics have several desirable features for further drug design. Moreover, on the basis of modeling studies, we confirmed the design of the structure. Possible interactions in the active site were found, and they confirmed in vitro activity, leading to the hypothesis of a nonoccupied pocket. It would therefore be interesting to focus on the use of this free site to increase interaction with the enzyme. We are currently investigating this point and developing a new series of non-sulfhydryl molecules.

Acknowledgment. The authors are grateful to the "Ligue contre le Cancer" and the "Association pour la Recherche contre le Cancer" for their financial support.

Supporting Information Available: Detailed information on the synthesis and characterization of **1**−**11**, molecular modeling, and biological assays. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Barbacid, M. Ras genes. *Annual Review of Biochemistry*; Richardson, C., Ed.; Annual Reviews, Inc.: Palo Alto, CA, 1987; Vol. 56, pp 779−827.
(2) Bos, J. L. Ras oncogene in human cancer: a review. *Cancer Res.* **1989**, *26*, 85−88.
(3) Casey, P. J.; Solski, P. A.; Der, C. J.; Buss, J. E. p21 Ras is modified by a farnesyl isoprenoid. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 8323−8327.
(4) Hall, A. A biochemical function of Ras−At last. *Science* **1994**, *264*, 1413−1414.
(5) Spaargaren, M.; Bischoff, J. R.; McCormick, F. Signal transduction by Ras like GTPase: a potential target for anticancer drugs. *Gene Expression* **1995**, *4*, 345−356.
(6) Zhang, F. L.; Casey, P. J. Protein prenylation: Molecular mechanisms and functional consequences. *Annu. Rev. Biochem.* **1996**, *65*, 241−269.

(7) Kato, K. Isoprenoid addition to ras protein is the critical modification for its membrane association and transforming activity. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 6403−6407.
(8) Hancock, J. F.; Magee, J. E.; Marshall, C. J. All ras proteins are poly isoprenylated but only some are palmitoylated. *Cell* **1989**, *57*, 1167−1177.
(9) Gibbs, J. B. Ras C-terminal processing enzymes. New drug targets. *Cell* **1991**, *65*, 1−4.
(10) Leonard, D. M. Ras farnesyltransferase: A new therapeutic target. *J. Med. Chem.* **1997**, *40*, 2971−2990.
(11) Burns, C. J.; Guitton, J. D.; Baudoin, B.; Lelievre, Y.; Duchesne, M.; Parker, F.; Fromage, N.; Commerçon, A. Novel conformationally extended naphthalene-based inhibitors of farnesyltransferase. *J. Med. Chem.* **1997**, *40*, 1763−1767.
(12) Sebti, S. M.; Hamilton A. D. New approches to anticancer drug design based on the inhibition of farnesyltransferase. *Drug Discovery Today* **1998**, *3*, 26−33.
(13) Williams, T. M. Inhibitors of protein prenylation 1999. *Expert Opin. Ther. Pat.* **1999**, *9*, 1263−1280.
(14) Park, H. W.; Boduluri, S. R.; Moomaw, J. F.; Casey, P. J.; Beese, L. S. Crystal structure of protein farnesyltransferase at 2.25 angstrom resolution. *Science* **1997**, *275*, 1800−1804.
(15) Breslin, M. J.; Jane deSolms, S.; Giuliani, E. A.; Stokker, G. E.; Graham, S. L.; Pompliano, D. L.; Mosser, S. D.; Hamilton, K. A.; Hutchinson, J. H. Potent, non-thiol inhibitors of farnesyltransferase. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 3311−3316.
(16) Quian, Y.; Marugan, J. J.; Fossum, R. D.; Vogt, A.; Sebti, S. M.; Hamilton, A. D. Probing the hydrophobic pocket of farnesyltransferase: aromatic substitution of CAAX peptidomimetics leads to highly potent inhibitors. *Bioorg. Med. Chem.* **1999**, *7*, 3011−3024.
(17) Augeri, D. J.; O'Connor, S. J.; Janowick, D.; Szczepankiewicz, B.; Sullivan, G.; Larsen, J.; Kalvin, D.; Cohen, J.; Devine, E.; Zhang, H.; Cherian, S.; Saeed, B.; Ng, S.-C.; Rosenberg, S. Potent and selective non-cysteine-containing inhibitors of protein farnesyltransferase. *J. Med. Chem.* **1998**, *41*, 4288−4300.
(18) O'Connor, S. J.; Barr, K. J.; Wang, L.; Sorensen, B. K.; Tasker, A. S.; Sham, H.; Ng, S.-C.; Cohen, J.; Devine, E.; Cherian, S.; Saeed, B.; Zhang, H.; Lee, J. Y.; Warner, R.; Tahir, S.; Kovar, P.; Ewing, P.; Alder, J.; Mitten, M.; Leal, J.; Marsh, K.; Bauch, J.; Hoffman, D. J.; Sebti, S. M.; Rosenberg, S. H. Second-generation peptidomimetic inhibitors of protein farnesyltransferase demonstrating improved cellular potency and significant in vivo efficacy. *J. Med. Chem.* **1999**, *42*, 3701−3710.
(19) Henry, K. J.; Wasicak, J.; Tasker, A. S.; Cohen, J.; Ewing, P.; Mitten, M.; Larsen, J. J.; Kalvin, D. M.; Swenson, R.; Ng, S.-C.; Saeed, B.; Cherian, S.; Sham, H.; Rosenberg, S. H. Discovery of a series of cyclohexylethylamine-containing protein farnesyltransferase inhibitors exhibiting potent cellular activity. *J. Med. Chem.* **1999**, *42*, 4844−4852.
(20) Anthony, N. J.; Gomez, R. P.; Schaber, M. D.; Mosser, S. D.; Hamilton, K. A.; O'Neil, T. J.; Koblan, K. S.; Graham, S. L.; Hartman, G. D.; Shah, D.; Rands, E.; Kohl, N. E.; Gibbs, J. B.; Oliff, A. I. Design and in vivo analysis of potent non-thiol inhibitors of farnesyl protein transferase. *J. Med. Chem.* **1999**, *42*, 3356−3368.
(21) Ding, C. Z.; Batorsky, R.; Bhide, R.; Chao, H. J.; Cho, Y.; Chong, S.; Gullo-Brown, J.; Guo, P.; Kim, S. H.; Lee, F.; Leftheris, K.; Miller, A.; Mitt, T.; Patel, M.; Penhallow, B. A.; Ricca, C.; Rose, W. C.; Schmidt, R.; Slusarchyk, W. A.; Vite, G.; Yan, N.; Manne, V.; Hunt, J. T. Discovery and structure−activity relationships of imidazole-containing tetrahydrobenzodiazepine inhibitors of farnesyltransferase. *J. Med. Chem.* **1999**, *42*, 5241−5253.
(22) Dinsmore, C. J.; Williams, T. M.; O'Neill, T. J.; Liu, D.; Rands, E.; Culberson, J. C.; Lobell, R. B.; Koblan, K. S.; Kohl, N. E.; Gibbs, J. B.; Oliff, A. I.; Graham, S. L.; Hartman, G. D. Imidazole-containing diarylether and diarylsulfone inhibitors of farnesyl-protein transferase. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 3301−3306.
(23) Ciccarone, T. M.; MacTough, S. C.; Williams, T. M.; Dinsmore, C. J.; O'Neill, T. J.; Shah, D.; Culberson, J. C.; Koblan, K. S.; Kohl, N. E.; Gibbs, J. B.; Oliff, A. I.; Graham, S. L.; Hartman, G. D. Non-thiol 3-aminomethylbenzamide inhibitors of farnesyl-protein transferase. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 1991−1996.
(24) Perola, E.; Xu, K.; Kollmeyer, T. M.; Kaufman, S. H.; Prendergast, F. G.; Pang, Y. P. Successful virtual screening of a chemical database for farnesyltransferase inhibitor leads. *J. Med. Chem.* **2000**, *43*, 401−408.
(25) Taveras, A. G.; Deskus, J.; Chao, J.; Vaccaro, C. J.; Njoroge, F. G.; Vibulbhan, B.; Pinto, P.; Remiszewski, S.; del Rosario, J.; Doll, R. J.; Alvarez, C.; Lalwani, T.; Mallams, A. K.; Rossman, R. R.; Afonso, A.; Girijavallabhan, V. M.; Ganguly, A. K.; Pramanik, B.; Heimark, L.; Bishop, W. R.; Wang, L.; Kirshmeier, P.; James, L.; Carr, D.; Patton, R.; Bryant, M. S.; Nomeir, A. A.; Liu, M. Identification of pharmacokinetically stable 3,10-dibromo-8-chlorobenzocycloheptapyridine farnesyl protein transferase inhibitors with potent enzyme and cellular activities. *J. Med. Chem.* **1999**, *42*, 2651−2661.
(26) Eskens, F. A. L. M.; Stoter, G.; Verweij, J. Farnesyl transferase inhibitors: current developments and future perspectives. *Cancer Treat. Rev.* **2000**, *26*, 319−332.
(27) Zujewski, J.; Horak, I. D.; Bol, C. J.; Woestenborghs, R.; Bowden, C.; End, D. W.; Piotrovsky, V. K.; Chiao, J.; Belly, R. T.; Todd, A.; Kopp, W. C.; Kohler, D. R.; Chow, C.; Noone, M.; Hakim, F. T.; Larkin, G.; Gress, R. E.; Nussenblatt, R. B.; Kremer, A. B.; Cowan, K. H. Phase I and pharmacokinetic study of farnesyl protein transferase inhibitor R-115777 in advanced cancer. *J. Clin. Oncol.* **2000**, *18*, 927−941.
(28) Eskens, F. A. L. M.; Awada, A.; Cutler, D. L.; de Jonge, M. J.; Luyten, G. P.; Faber, M. N.; Statkevich, P.; Sparreboom, A.; Verweij, J.; Hanauske, A. R.; Piccart, M. J. Phase I and pharmacokinetic studies of the oral farnesyltransferase inhibitor SCH-66636 given twice daily to patients with advanced solid tumors. *Clin. Oncol.* **2001**, *19*, 1167−1175.
(29) Ashar, H. R.; James, L.; Gray, K.; Carr, D.; McGuirk, M.; Maxwell, E.; Black, S.; Armstrong, L.; Doll, R. J.; Taveras, A. G.; Bishop, W. R.; Kirschmeier, P. The farnesyl transferase inhibitor SCH-66336 induces a G2 → M or G1 pause in sensitive human tumor cell lines. *Exp. Cell Res.* **2001**, *262*, 17−27.
(30) Khan, S. G.; Mukthar, H.; Agarwal, R. A rapid and convenient filter-binding assay for Ras p21 processing enzyme farnesyl transferase. *J. Biochem. Biophys. Methods* **1995**, *30*, 133−144.
(31) 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.
(32) MacTough, S. C.; deSolms, S. J.; Shaw, A. W.; Abrams, M. T.; Ciccarone, T. M.; Davide, J. P.; Hamilton, K. A.; Hutchinson, J. H.; Koblan, K. S.; Kohl, N. E.; Lobell, R. B.; Robinson, R. G.; Graham, S. L. Diaryl ether inhibitors of farnesyl-protein transferase. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1257−1260.
(33) Bergman, J. M.; Abrams, M. T.; Davide, J. P.; Greenberg, I. B.; Robinson, R. G.; Buser, C. A.; Huber, H. E.; Koblan, K. S.; Kohl, N. E.; Lobell, R. B.; Graham, S. L.; Hartman, G. D.; Williams, T. M.; Dinsmore, C. J. Aryloxy substituted *N*-arylpiperazinones as dual inhibitors of farnesyltransferase and geranylgeranyltransferase-I. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1411−1415.
(34) 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 farnesyltransferase inhibitor. *Science* **1993**, *260*, 1934−1937.
(35) Campion, C.; Davidson, A. H.; Dickens, J. P.; Grimmin, M. J. Preparation of *N*-[4-(*N*-hydroxyamino)succinyl]amino acid amides as collagenase inhibitors. PCT Int. Appl. WO 9005719; Chem. Abstr. **1990**, *113*, 212677c.
(36) Erlenmeyer, H.; Morel, C. J. Structural and chemical study. VII About thiazole-4,5-dicarboxylic acid and thiazole-4-carboxylic acid. *Helv. Chim. Acta* **1945**, *28*, 362−364.
(37) Cordi, A. A.; Snyers, M. P.; Giraud-Mangin, D.; Van der Maesen, C.; Van Hoeck, J. P.; Beuze, S.; Ellens, E.; Napora, F.; Gillet, C. L.; Gorissen, H.; Calderon, P.; Remacle, M. D.; Janssens de Varebeke, P.; Van Dorsser, W.; Roba, J. Synthesis and structure−activity of 4(5)-(2,2-diphenylethyl)imidazoles as new α_2-adrenoceptor antagonists. *Eur. J. Med. Chem.* **1990**, *25*, 557−568.
(38) Martinez, J. Synthesis and biological activities of some pseudo peptide analogues of tetragastrin: the importance of the peptide backbone. *J. Med. Chem.* **1985**, *28*, 1874−1879.
(39) Cassidy, P. B.; Dolence, J. M.; Poulter, C. D. Continuous fluorescence assay for prenyl transferases. *Methods Enzymol.* **1995**, *250*, 30−43.
(40) Garcia, A. M.; Rowell, C.; Ackermann, K.; Kowalczyk, J. J.; Lewis, M. D. Peptidomimetic inhibitors of Ras farnesylation and function in whole cells. *J. Biol. Chem.* **1993**, *268*, 18415−18418.
(41) Hamilton, A. D.; Sebti, S. M. Inhibitors of Ras farnesyltransferase as novel antitumor agents. *Drug News Perspect.* **1995**, *8*, 138−145.
(42) Qian, Y.; Vogt, A.; Sebti, S.; Hamilton, A. Design and synthesis of non-peptide Ras CAAX mimetics as potent farnesyltransferase inhibitors. *J. Med. Chem.* **1996**, *39*, 217−223.
(43) Christiansen, D. W. Structural Biology of Zinc. In *Metalloproteins: Structural Aspects*; Edsall, J. T., Anfinsen C. B., Richards, F. M., Eisenberg, D. S., Eds.; Academic Press: San Diego, CA, 1991; Vol. 42, pp 281−355.
(44) Strickland, C. L.; Windsor, W. T.; Syto, R.; Wang, L.; Bond, R.; Wu, Z.; Schwartz, J.; Le, H. V.; Beese, L. S.; Weber, P. C. Crystal structure of farnesyl protein transferase complexed with a CaaX peptide and farnesyl diphosphate analogue. *Biochemistry* **1998**, *37*, 16601−16611.

JM010297R