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From Pure FPP to Mixed FPP and CAAX Competitive Inhibitors of Farnesyl Protein Transferase

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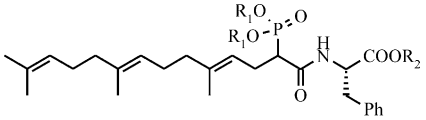
Abstract—Starting from a FPP analogue with nanomolar inhibitory activity against isolated FPTase, yet lacking activity in cellular assays, structural modifications were performed to enhance cellular activity by removing all acidic functionalities. Overall, these changes resulted in the transformation of a pure FPP to a mixed FPP and CAAX competitive inhibitor with nanomolar activity on isolated FPTase and micromolar inhibitory activity in the farnesylation of H-Ras in cultured DLD-1 cells.

Farnesyl protein transferase (FPTase) is a zinc metalloenzyme that has emerged as a potential target in cancer chemotherapy.[1] Using farnesylpyrophosphate (FPP), FPTase catalyzes the farnesylation of several proteins involved in tumorigenesis, particularly Ras proteins[2] whose oncogenic forms can be found in about 30% of all human cancers, Rho B[3] involved in receptor trafficking and centromeric proteins[4] implicated in mitosis. FPTase recognizes the last four C-terminal amino acids of these proteins that match the CAAX formula, where A's are essentially aliphatic amino acids and X is a wild card. Farnesylation occurs at the cysteine of these CAAX sequences and is essential for the function of these proteins.

Rationally-designed FPTase inhibitors[5] can be divided into three categories, namely FPP competitive inhibitors which occupy the FPP site of FPTase, CAAX competitive inhibitors which can displace the protein substrate and bisubstrate analogues which are designed to occupy both sites simultaneously. Following our early work on FPP analogues,[6] which identified farnesylphosphonic acid derivative **1a** (Fig. 1) as a potent inhibitor of FPTase in vitro (IC_{50} = 83 nM), structural modifications were necessary to provide cellular activity. We first turned our efforts towards the preparation of some of the most common prodrugs of phosphonic acids such as bis POM ester[7] **1b** and bis SATE ester[8] **1c**. However both compounds failed to provide any trace of cellular activity[9] even at high concentration (10 μM). Herein our second approach is described, consisting of structural modifications of **1a**.



1a : R_1 = H; R_2 = H
1b : R_1 = CH_2OCO^tBu; R_2 = Me
1c : R_1 = $(CH_2)_2SCO^tBu$; R_2 = Me

Figure 1.

The first X-ray analyses of rat FPTase complexed with FPP[10] showed that the head of the farnesyl group is only a few angstroms away from the zinc ion. We decided to take advantage of this close proximity and to replace the phosphonic acid moiety of **1a** by a functional group capable of chelating the zinc cation. It was also postulated that this strategy would probably lead to a bisubstrate instead of a pure FPP analogue.

The syntheses performed in standard solution chemistry are described in Scheme 1. Direct farnesylation of bis protected glycine was successful and gave easy access to

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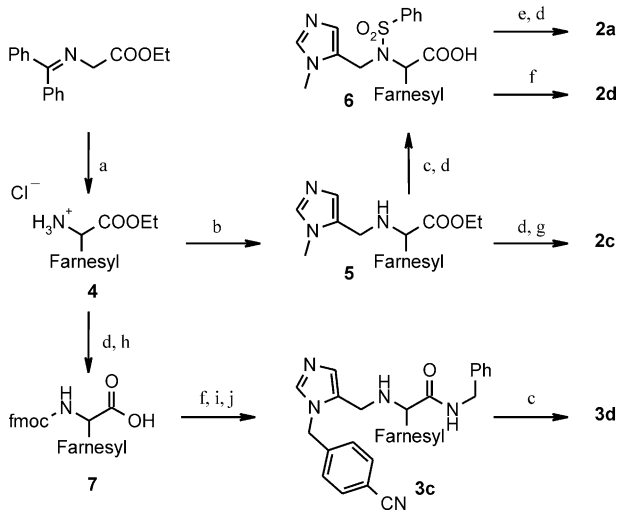
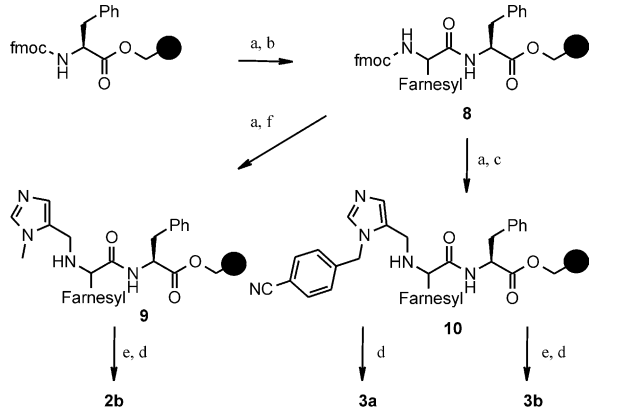
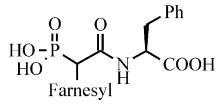
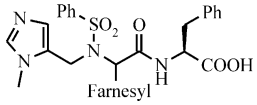
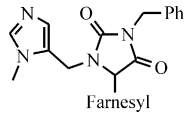
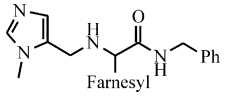
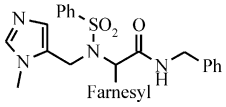
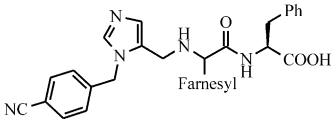
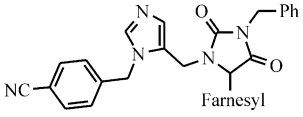
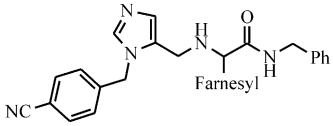
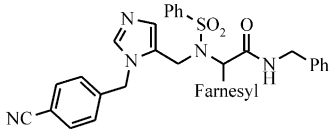


Scheme 1. Reagents and conditions: (a) (i) LDA, Farnesyl-Br, THF, −78 °C to rt; (ii) HCl 2N, 85%. (b) (i) 1-Methyl-*3H*-imidazole-4-carbaldehyde, DIEA, 1,2-DCE, rt; (ii) AcOH, $NaBH(OAc)_3$, rt, 57%. (c) $PhSO_2Cl$, pyridine, rt, 84% from **5**, 20% from **3c**. (d) LiOH, THF, H_2O, rt, 98% for **6**, 38% for **2a**, 84% from **4**, 48% from **5**. (e) H-Phe-OMe.HCl, EDC, HOOBT, DIEA, DCM, rt, 52%; (f) $PhCH_2NH_2$, EDC, HOOBT, DIEA, DCM, rt, 70% from **6**, 82% from **7**. (g) (i) $PhCH_2NH_2$, PS-carbodiimide, HOBT, DMF; (ii) MP-carbonate, 65%. (h) FmocOSu, Na_2CO_3, dioxane, H_2O, 0 °C to rt, 94%. (i) Piperidine, DMF, rt, 93%. (j) 4-(5-Formyl-imidazol-1-ylmethyl)-benzonitrile, AcOH, $NaBH(OAc)_3$, 1,2-DCE, rt, 46%.



Scheme 2. Reagents and conditions: (a) Piperidine, DMF. (b) **7**, BOP, DIEA, NMP, 97%. (c) 4-(5-Formyl-imidazol-1-ylmethyl)-benzonitrile, AcOH, $NaBH(OAc)_3$, 1,2-DCE, 97%. (d) LiOH, THF, MeOH, H_2O, 55 °C, 37% for **2b**, 93% for **3a**, 65% for **3b**. (e) $PhCH_2NCO$, DCM. (f) 1-Methyl-*3H*-imidazole-4-carbaldehyde, AcOH, $NaBH(OAc)_3$, 1,2-DCE, 82%.

Table 1. Farnesyltransferase inhibitory activities of compounds **1**, **2a–d** and **3a–d**

Compd	Structure	IC_{50}, μM	
		In vitro[a]	Cell[b]
1a		0.083	> 10
2a		0.47	10
2b		0.7	nd
2c		> 10	nd
2d		0.67	1
3a		0.091	5
3b		0.2	nd
3c		0.006	1
3d		0.04	> 10

[a]FPTase inhibitory assay was performed as described in ref 15, using purified bovine FPTase, 2 μM dansyl-GCVLS and 2μM FPP.
[b]Inhibition of farnesylation of H-Ras in DLD-1 cells as described in ref 9.

the central core of all molecules, as a racemic mixture. Reductive amination with 1-methyl-*3H*-imidazole-4-carbaldehyde[11] could then be performed in two steps to give **5**, followed by saponification and amidation to yield the benzylamide derivative **2c**. Sulfonylation of **5** was achieved in pyridine using 1.5 equiv of phenylsulfonyl chloride. It was followed by sequences of saponifications and amidations to give compounds **2a** and **2d**. The cyanobenzyl analogue **3d** was prepared using the same chemistry as that developed for **2d**. However, saponification of the ethyl ester had to be carried out before the reductive amination with 4-(5-formyl-imidazol-1-ylmethyl)-benzonitrile[12] so as to avoid hydrolysis of the nitrile group. Several attempts were made to keep the diphenylmethylene amino protecting group of the starting protected glycine, however it was hydrolyzed under saponification conditions. Therefore two steps of protection and deprotection had to be incorporated in order to prepare compound **3d**. Other modifications were performed on solid phase (Scheme 2), leading to the preparation of analogues **2b**, **3a** and **3b**. Yields of the intermediate reactions were determined after cleavage of a small fraction of the resin under LiOH conditions. Loading of **7** on the deprotected fmoc-Phe-Wang[13] resin was completed in one cycle using 1.5 equivalents of the farnesylated glycine.

Then, reductive amination could be performed in one step using 3 equiv of the corresponding aromatic aldehyde to give **9** and **10** with no trace of dialkylation products. Both resins were treated with phenyl isocyanate to form the supported urea intermediates. Unexpectedly, under cleavage conditions these intermediates underwent cyclization into hydantoins **2b** and **3b**,[14] an event resulting in loss of the phenylalanine residue. Compound **3a** was obtained by direct cleavage of resin **10**.

In the methyl imidazole series, removal of the phosphonic acid was possible, but at the cost of a 5-fold decrease in activity (Table 1, compare **1a** with **2a**). None of the modifications performed thereafter improved activity (**2b**, **2c** and **2d**). However, for the first time in this farnesyl series, some activity could be detected using the cellular assay (**2d**). In the cyanobenzyl imidazole series very potent inhibitors of FPTase in vitro were obtained. Replacement of the phosphonic acid present in **1a** by the cyanobenzyl imidazole group (**3a**) maintained the inhibitory activity, and further removal of the carboxylic acid (**3c**) gave a 15-fold decrease in IC_{50} value. In an attempt to further improve the cellular activity, substitution of the newly introduced nitrogen was performed, but this gave a less potent inhibitor (**3d**). However, the most striking difference between these two imidazole series is exemplified by the in vitro results obtained with compounds **2c** and **3c**. Hence the mode of inhibition of the cyanobenzyl imidazole derivatives was investigated, choosing **3c** as a representative example and comparing it with **1a**. The pharmacological behavior of both compounds was determined qualitatively by observation of FPP- and CAAX-dependent modulations of inhibitory potencies (Table 2). While FPTase inhibition by **1a** was only affected by FPP concentration, enzyme inhibition by **3c** was markedly affected by the peptide concentration and only partially by FPP concentration. From these results, we can conclude that **3c** behaves as a mixed FPP and CAAX competitive inhibitor.

Table 2. Mode of FPTase inhibition by **1** and **3c**

FPP (μM)	0.5	1	2	5	10	20
1a (100 nM)[a]	80	72	37	9	9	10
3c (10 nM)[a]	54	55	42	38	36	32
Dansyl-GCVLS (μM)	0.5	1	2	5	10	20
1a (100 nM)[b]	43	42	41	34	33	38
3c (10 nM)[b]	49	41	38	27	14	3

[a]Per cent inhibitions at fixed concentrations of peptide substrate (2 μM).
[b]Per cent inhibitions at fixed concentrations of FPP (2 μM).

Docking studies using the flexible docking program FlexX[16] suggested two docking hypotheses for **3c** that can explain the pharmacological behavior observed. Using the coordinates of two published X-ray crystal structures of human FPTase,[17] two models were built to allow the docking of **3c** in the FPP competitive inhibitor hypothesis (active site empty) and in the CAAX competitive inhibitor hypothesis (active site containing FPP). In the first model, the imidazole ring was used as the anchor fragment, then FlexX positioned the rest of the molecule, fragment by fragment, into the active site searching for favorable interactions between the ligand and the amino acid residues. The same protocol was used to test the second hypothesis using the cyanobenzyl group as the anchor fragment. 3D conformations generated from these calculations are represented in Figure 2. In both cases, the docking program calculated an interaction between the imidazole ring and the zinc cation. In the FPP competitive inhibitor model, no solution was found that could allow both imidazole chelation with the zinc cation and farnesyl full occupancy of the FPP hydrophobic pocket. Indeed, the best solution shows a partial occupancy of this lipophilic site where only the central isoprene unit can fit. This model hypothesis fits well with the partial competitive nature of **3c** with regard to FPP, since **3c** cannot interact with the ionic site of FPP due to the removal of all acidic residues, and can only partially occupy the hydrophobic site. In the second model, compound **3c** can adopt a conformation that will allow strong interactions between the three parts of the molecule and the enzyme. The cyanobenzyl group can adopt the same position as the cyanobenzyl in the crystallized reference complex,[17] making stacking with FPP isoprenoid and pointing towards Arg202β and Tyr166α. The flexible farnesyl unit can fold to find hydrophobic interactions in one of the CAAX substrate lipophilic sites and the benzyl residue comes across a new hydrophobic pocket (far aryl binding site[18]) made of Leu295β, Lys294β and Tyr300β.

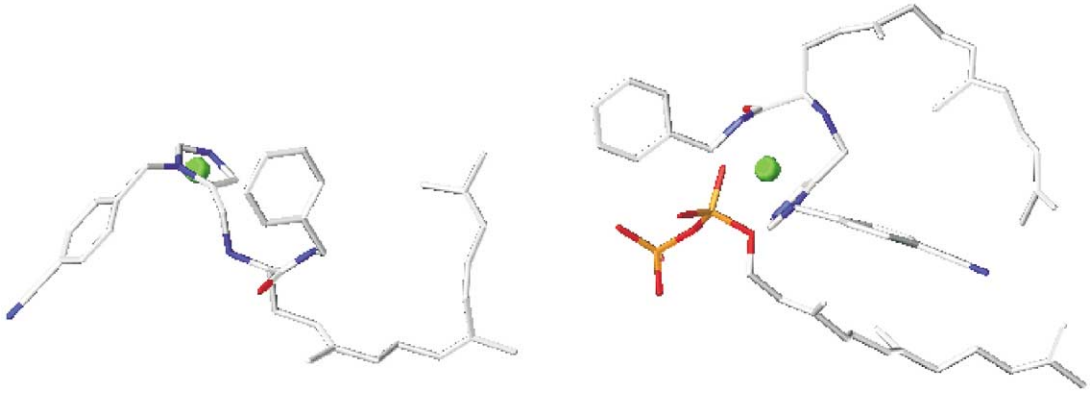


Figure 2. 3D conformations of **3c** generated from docking in either empty FPTase (left view) or FPTase-FPP complex (right view). The dark blue ball represents the zinc ion. The right view (CAAX competitive inhibitor hypothesis) shows **3c** interacting with FPP.

This FPP analogue approach has allowed the identification of very potent inhibitors of FPTase in vitro. However, results still need to be improved with regard to their cellular activities. Compound **3c** which has nanomolar inhibitory potency on the enzyme and does not bear any highly acidic or basic functionalities, still remains active only in the micromolar range in the cellular assay. It can be questioned whether such behavior could be attributed to the presence of the very lipophilic farnesyl moiety. Several groups have been working on the replacement of this isoprenoid chain with aromatic residues,[19] but it still has to be demonstrated whether such structural modifications could convey some improvement in cellular activity. Overall it has been shown that, removal of all acidic functionalities of **1a** and introduction of 4-cyanobenzylimidazolyl group as a strong zinc chelator and FPP interacting fragment, resulted in the transformation of a pure FPP competitive inhibitor into a mixed FPP and CAAX competitive inhibitor.

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References and Notes

1. Cox, A. D.; Der, C. J. *Curr. Opin. Pharmacol.* **2002**, *2*, 388.
2. Barbacid, M. *Annu. Rev. Biochem.* **1987**, *56*, 779.
3. Prendergast, G. C.; Rane, N. *Expert Opin. Investig. Drugs* **2001**, *10*, 2105.
4. Ashar, H. R.; James, L.; Gray, K.; Carr, D.; Black, S.; Armstrong, L.; Bishop, W. R.; Kirschmeier, P. *J. Biol. Chem.* **2000**, *275*, 30451.
5. Gibbs, R. A.; Zahn, T. J.; Sebolt-Leopold, J. S. *Curr. Med. Chem.* **2001**, *8*, 1437.
6. Lamothe, M.; Perrin, D.; Blotières, D.; Leborgne, M.; Gras, S.; Bonnet, D.; Hill, B. T.; Halazy, S. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 1291.
7. Starrett, E. J.; Tortolani, D. R.; Russell, J.; Hitchcock, M. J. M.; Whiterock, V.; Martin, J. C.; Mansuri, M. M. *J. Med. Chem.* **1994**, *37*, 1857.
8. Benzaria, S.; Pélicano, H.; Johnson, R.; Maury, G.; Imbach, J.-L.; Aubertin, A.-M.; Obert, G.; Gosselin, G. *J. Med. Chem.* **1996**, *39*, 4958.
9. Gibbs, J. B.; Pompliano, D. L.; Mosser, S. D.; Rands, E.; Lingham, R. B.; Singh, S. B.; Scolnick, E. M.; Kohl, N. E.; Oliff, A. *J. Biol. Chem.* **1993**, *268*, 7617.
10. (a) Dunten, P.; Kammlott, U.; Crowther, R.; Weber, D.; Palermo, R.; Birktoft, J. *Biochemistry* **1998**, *37*, 7907. (b) Long, S. B.; Casey, P. J.; Beese, L. S. *Biochemistry* **1998**, *37*, 9612.
11. Daninos-Zeghal, S.; Mourabit, A. A.; Ahond, A.; Poupat, C.; Potier, P. *Tetrahedron* **1997**, *53*, 7605.
12. 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. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 3301.
13. Fmoc-Phe-Wang was purchased from Senn Chemical (Dielsdorf Switzerland).
14. Lamothe, M.; Lannuzel, M.; Perez, M. *J. Comb. Chem.* **2002**, *4*, 73.
15. Pompliano, D. L.; Gomez, R. P.; Anthony, N. J. *J. Am. Chem. Soc.* **1992**, *114*, 7945.
16. FlexX version 1.11 part of SYBYL molecular modeling software from Tripos Inc.
17. (a) Long, S. B.; Hancock, P. J.; Kral, A. M.; Hellinga, H. W.; Beese, L. S. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 12948. (b) Bell, I. M.; Gallicchio, S. N.; Abrams, M.; Beese, L. S.; Beshore, D. C.; Bhimnathwala, H.; Bogusky, M. J.; Carolyn, A.; Culberson, J. C.; Davide, J.; Ellis, H.; Fernandes, C.; Gibbs, J. B.; Graham, S. L.; Hamilton, K. A.; Hartman, G. D.; Heimbrook, D. C.; Homnick, C.; Huber, H. E.; Huff, J. R.; Kassahun, K.; Koblan, K. S.; Nancy, E.; Lobell, R. B.; Lynch, J. J., Jr.; Robinson, R.; Rodrigues, A. D.; Taylor, J. S.; Walsh, E. S.; Williams, T. M.; Zartman, C. B. *J. Med. Chem.* **2002**, *45*, 2388.
18. Schlitzer, M. *Curr. Pharm. Des.* **2002**, *8*, 1713.
19. (a) Moreno Barber, A.; Hardcastle, I. R.; Rowlands, M. G.; Nutley, B. P.; Marriott, J. H.; Jarman, M. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 623. (b) Schlitzer, M.; Satter, I. *Bioorg. Med. Chem.* **1999**, *7*, 2391. (c) Chehade, K. A. H.; Andres, D. A.; Morimoto, H.; Spielmann, H. P. *J. Org. Chem.* **2000**, *65*, 3027. (d) Turek, T. C.; Gaon, I.; Distefano, M. D. *J. Org. Chem.* **2001**, *66*, 3253. (e) Zhou, C.; Shao, Y.; Gibbs, R. A. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 1417.