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INHIBITION OF FARNESYL PROTEIN TRANSFERASE BY NEW FARNESYL PHOSPHONATE DERIVATIVES OF PHENYLALANINE

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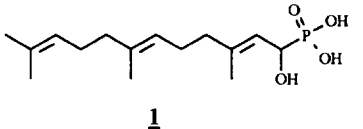
Abstract: New farnesyl phosphonate derivatives of phenylalanine have been prepared as inhibitors of farnesyl-protein transferase (FPT). Enzyme inhibition studies with bovine brain FPT and rat liver squalene synthase show that compound **3a** is a new, potent and selective FPT inhibitor. Moreover, structural modifications from **3a** have provided some useful information concerning the key structural subunits necessary for optimum and selective FPT inhibition.

The ras genes products (Ras) are monomeric proteins that bind reversibly, and hydrolyze guanosine triphosphate[1]. Mutated ras genes are frequently found in a variety of human malignancies and are presumed to play an important role in human tumor growth. Indeed, mutated ras oncogenes are present in 50% of colon and more than 90% of pancreatic cancers[2]. The function of Ras proteins in signal transduction and cell growth requires several post-translational modifications to increase its hydrophobicity[3]. These events appear to be critically required for efficient cell transformation activity of Ras and it has been well established that farnesylation of the C-terminal cysteine side-chain of p21Ras is the essential step to achieve that goal[4]. Farnesyl-protein transferase (FPT) is the enzyme that catalyzes the S-prenylation process[5] and inhibition of FPT has recently attracted attention[6] as a potential approach toward the discovery of novel anti-tumor agents. Recent *in vivo* data[7] describing the efficacy of some FPT inhibitors in controlling tumor growth have strongly reinforced the potential for these type of compounds in cancer chemotherapy.

Farnesyl-protein transferase catalyzes the attachment of a 15-carbon isoprenoid moiety (farnesyl) to the cysteine residue of the so-called "CAAX" carboxy terminus of the Ras protein, thus involving participation of two substrates, namely the Ras protein and the prenyl group donor farnesyl pyrophosphate (FPP).



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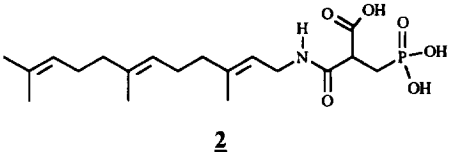

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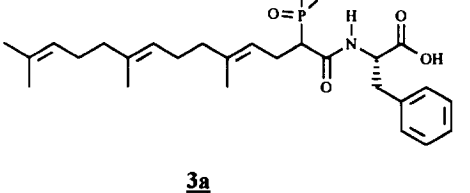


3a

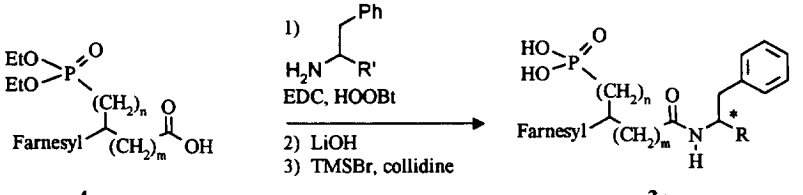
Rationally designed FPT inhibitors can be divided into three categories: first, the "multisubstrate-analog" type of inhibitors[8] which combine together structural features from both FPP and the CAAX C-terminus of Ras; secondly, CAAX analogs (now including non-peptidic type of inhibitors[6-9]), and thirdly, FPP analogs in which the labile polyanionic pyrophosphate group is replaced by stable synthetic surrogates like phosphonates[10]. The first reported example[11] of such a FPP analog was compound **1**, a potent FPT inhibitor which has demonstrated cellular activity against ras-transformed cells.

More recently, Patel and co-workers have reported[12] a new series of β-carboxylphosphonic acid derivatives of farnesyl as potent FPT inhibitors. For example, compound **2** was found to inhibit FPT with an IC_{50} of 75nM.

These results have prompted this report of our preliminary work regarding the synthesis and biological properties of compound **3a**, a new carboxylphosphonic acid derivative in which a (L)-phenylalanine residue has been combined with a farnesyl phosphonate moiety. The role of the carboxylate function, the importance of the phenylalanine configuration and the distances between the farnesyl lipophilic part and the phosphonic or carboxylic acid moieties in compound **3a** have also been investigated in order to determine the key sub-structural features necessary for the binding of **3a** to FPT. Finally, we also report on the inhibitory properties of the above-mentioned compounds at the squalene synthase level, since this enzyme (involved in the biosynthesis of cholesterol) also uses FPP as a substrate and is known to be inhibited by many FPP analogs.[13,14]

The phosphonic acid derivatives **3a-g** suitable for biochemical evaluation have all been obtained from the carboxylic acid derivatives of phosphonate diethyl esters **4** according to scheme 1.

Scheme 1



4 **3a-g**

Chemical yields:

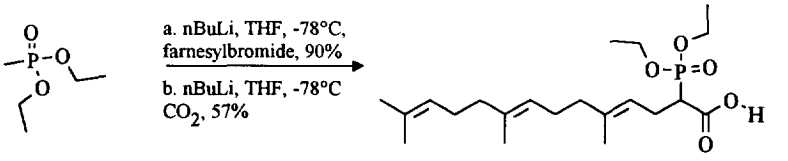
Cpd	R	C*[a]	n	m	R'	Step 1: amide formation	Step 2: ester saponification	Step 3: phosphonate hydrolysis
3a	CO_2H	S	0	0	CO_2Me	93%	42%	89%
3b	CO_2Me	S	0	0	CO_2Me	93%	NA	88%
3c	CO_2H	R	0	0	CO_2Me	93%	79%	62%
3d	H	NA	0	0	H	77%	NA	47%
3e	CO_2H	S	1	0	CO_2Me	91%	61%	50%
3f	CO_2H	S	2	0	CO_2Me	96%	74%	45%
3g	CO_2H	S	0	1	CO_2Me	96%	58%	16%

NA: not applicable; a. stereochemistry at the amino acid chiral center.

The first step involves an amide bond formation which was achieved in high yield by condensation of either phenethylamine, (L) or (D)-phenylalanine with a carboxylic acid derivative **4** in the presence of EDC and HOOBt. The intermediate carboxylic acid methyl esters (except **3b**) are then saponified using lithium hydroxide in THF/water at 20°C. Finally, compounds **3a-g** were obtained by hydrolysis of the phosphonate ester upon treatment with trimethylsilyl bromide in dichloromethane in the presence of 2,4,6-collidine. All molecules suitable for biochemical testing were isolated as lysine salts[15].

The phosphonocarboxylic acid **4a** (which is the precursor of the final compounds **3a**, **3b**, **3c** and **3d**) was prepared from diethyl methylphosphonate by two deprotonation-alkylation sequences using farnesyl bromide and carbon-dioxide successively as electrophiles (scheme 2).

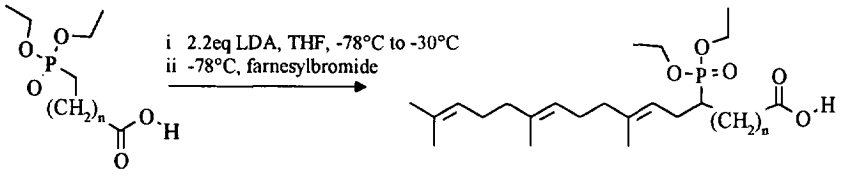
Scheme 2



4a

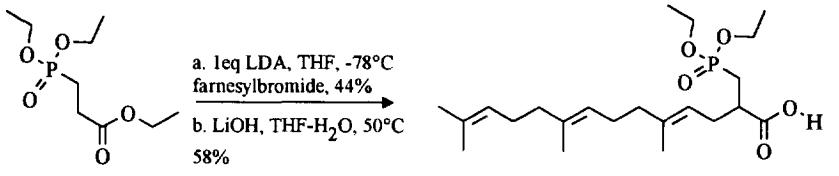
The phosphono carboxylic acid intermediates **4e**, **4f** and **4g** were obtained (scheme 3) by farnesylation of the *in situ* generated carbanions prepared by regioselective deprotonation of the phosphono carboxylate **5e** and **5f** or the phosphono carboxylic acid ester **5g** following the Janecki and Bodalski procedure[16].

Scheme 3



5e n = 1
5f n = 2

4e n = 1, 38%
4f n = 2, 40%

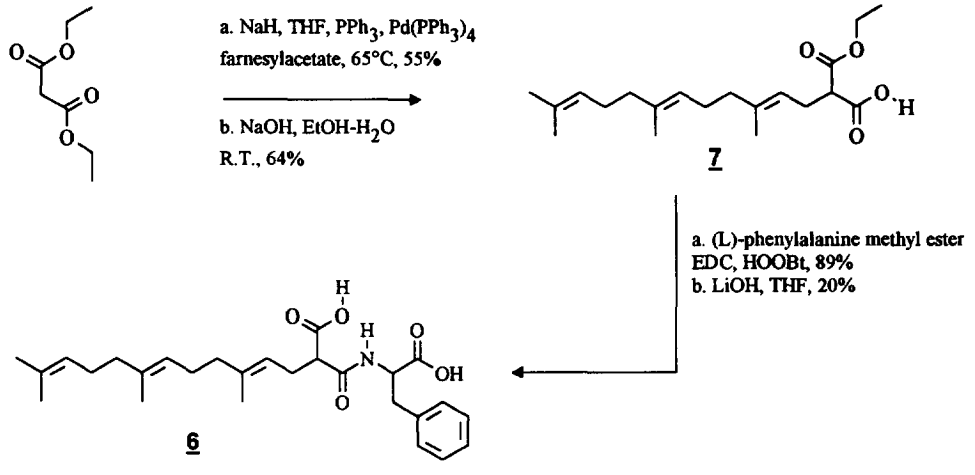


5g **4g**

In order to assess the importance of the phosphonic acid residue in compound **3a** for FPT inhibition, the bis-carboxylic acid derivative **6** was prepared (scheme 4). The palladium(0) catalyzed coupling of farnesyl acetate[17] with diethyl malonate gave 55% of mono-farnesyl malonate along with 24% of the bi-farnesyl coupling

product which could be separated by flash chromatography. Intermediate 7 was obtained in 64% yield by chemoselective mono-saponification of diethyl farnesylmalonate using 1 equivalent of NaOH in ethanol/water at 20°C. Finally, the dicarboxylic acid 6 was prepared by sequential amide formation with (L)-phenylalanine methyl ester and mild saponification of the remaining ethyl and methyl esters.

Scheme 4



It is noteworthy that the α-phosphono carboxylic acid ester derivative 4a could also be prepared by palladium(0) catalyzed allylic coupling of farnesyl acetate with the anion of diethyl methylphosphonoacetate (45% yield) in the presence of 10% tri *o*-tolylphosphine; however this method also leads to the formation of the bis-farnesylation product (15%) which was difficult to separate by chromatography.

The inhibitory properties of compounds 4a-g, 6 as well as compound 1 and SQ 34919 (a well known[14] squalene synthase (SS) inhibitor included for comparative purposes) have been determined for FPT from bovine brain and SS from rat liver microsomes. The results obtained are summarized in table 1.

Table 1

IC_{50} (μM) values of compounds 3a-g, 6, 1 and SQ34919 as FPT and SS inhibitors

Cpd	3a	3b	3c	3d	3e	3f	3g	6	1	SQ34919
FPT[18]	0.08	>100	0.98	13	0.17	10	0.47	37	0.41	80
SS[19]	1.3	1.4	0.5	0.48	0.09	4.2	0.22	2.4	2.3	0.017

Results from table 1 show that compound 3a, the (L)-phenylalanine derivative of farnesyl phosphonate, emerges as the most potent inhibitor of FPT in the series and compares favourably with the previously reported α -hydroxyphosphonate 1, since 3a appears as a better and more selective FPT inhibitor. As expected, both phosphonate derivatives 1 and 3a have been found to be competitive inhibitors with respect to farnesyl pyrophosphate and non-competitive inhibitors with respect to the peptide co-substrate (data not shown). These results show that compound 3a, despite the presence of an amino-acid residue, behaves as a FPP analog and does not interfere with important residues within the peptide binding site of FPT. The inhibitory potency of

compound **3a** is very dependent on the presence of the carboxylic acid function since the carboxylic acid methyl ester derivative **3b** and the decarboxylated analog **3d** are much weaker FPT inhibitors. The (L) absolute configuration of the phenylalanine residue is also very important since the (D) epimer **3c** is 10 times less potent as a FPT inhibitor. Interestingly enough, the structure-activity analysis does not reach the same conclusions when considering squalene synthase inhibition: in that particular case, the methyl ester **3b** is equipotent to the carboxylic acid derivative **3a** while both the decarboxylated analog **3d** and the D-Phe epimer **3c** are better SS inhibitors than the parent compound **3a**. These observations may find some interesting applications in future design of selective SS inhibitors. The same discrepancy is observed when the distance between (L)-Phe-carboxylic acid or the phosphonate residue with farnesyl is increased by one methylene group (compare **3e** and **3g** with **3a**). In both cases, this modification leads to a lower inhibitory potency at the FPT level but to a better inhibitory profile for SS. Increasing the distance between the carboxylic acid function and the farnesyl residue by 2 methylene groups (compare **3f** with **3a**) is however deleterious for both enzymes. Finally, the high IC_{50} value obtained with compound **6** (in which the phosphonate residue of compound **3a** has been replaced by a carboxylate moiety) clearly demonstrates the importance of the phosphonate group in such types of inhibitors. A similar observation has been previously reported[12] in the case of compound **2** in which replacement of the phosphonate residue by a carboxylate anion led also to a dramatic loss of FPT inhibitory potency.

In conclusion, compound **3a** which can be easily prepared in 5 steps from diethyl methylphosphonate, farnesyl bromide and (L)-phenylalanine methyl ester is a novel, potent, non peptidic inhibitor of FPT that may find utility as a potential antitumor agent. The next step toward that goal will be to assess the ability of **3a** to control ras processing in intact cells. It must be pointed out here that cell-penetration of phosphonate derivatives is no longer a major problem since very efficient prodrugs of the phosphonic diacid moiety have recently been proposed[20] and some of them selected for clinical evaluation[21].

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