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RATIONAL DESIGN OF POTENT CARBOXYLIC ACID BASED BISUBSTRATE INHIBITORS OF RAS FARNESYL PROTEIN TRANSFERASE

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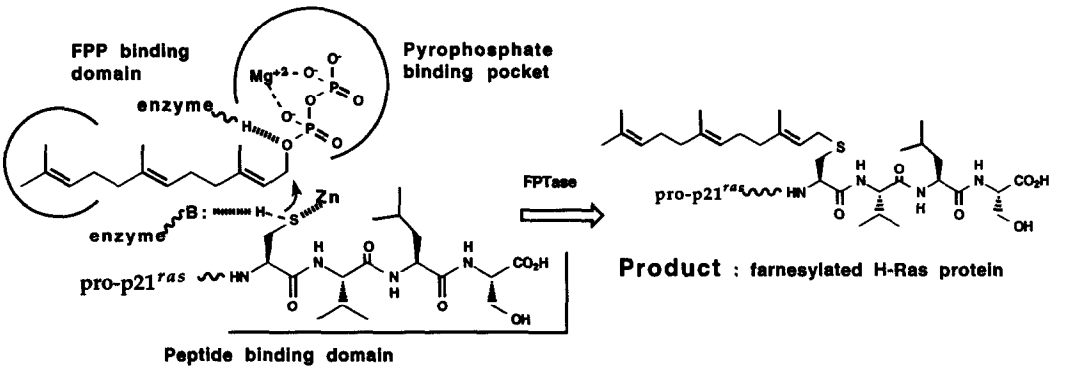
Abstract: Bisubstrate analog inhibitors in which a substrate mimetic tripeptide is attached to a homologated farnesyl carboxylic acid were synthesized and evaluated for *in vitro* inhibition versus ras farnesyl protein transferase (FPT). Our results demonstrate that such bisubstrate analogs are potent inhibitors of FPT.

Mutated *ras* genes are frequently found in a variety of human malignancies and are presumed to play an important role in human tumor growth.[3] Ras proteins, which are part of growth regulatory signal transduction pathway, are post-translationally modified in the cytosol and then migrate to their site of action, the cell membrane. Because post-translational modifications are necessary for attachment of the normal as well as mutated ras proteins to the membrane, obstruction of one of the steps in the process could potentially control oncogenic activity of the ras proteins. The first and mandatory modification in this sequence is farnesylation of the thiol group of cysteine located at the fourth amino acid position from the ras C-terminus. Farnesyl protein transferase (FPT) is the enzyme that catalyzes this reaction and is an attractive target for inhibition to control oncogenic activity caused by mutated ras protein. This hypothesis has led to vigorous research activity in the area of FPT inhibitors.[4]

Figure 1 : Hypothetical "Active Site" Model for Farnesylation of p21^{ras}

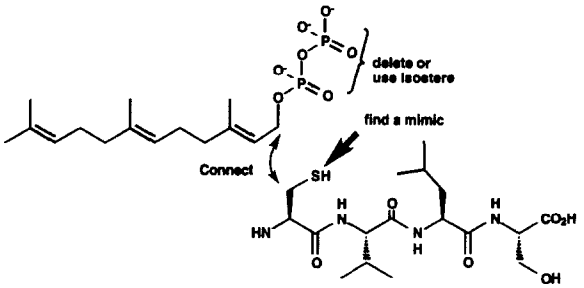


A hypothetical 'active site' model of FPT based on general information[5b] is depicted in **Figure 1**. A highly conserved C-terminal CAAX sequence, where C is Cys, A is an aliphatic amino acid and X is any amino acid, is prevalent in ras proteins. Simple tetrapeptides of general formula Cys-A_1-A_2-X inhibit FPT[4a] and most inhibitor research has focused on these types of sulfhydryl containing tetrapeptides and peptidomimetics.[4]

Since we were concerned about the specificity and chemical lability of such compounds, we undertook the design and synthesis of novel inhibitors that bear a functionally equivalent replacement for the sulfhydryl group.

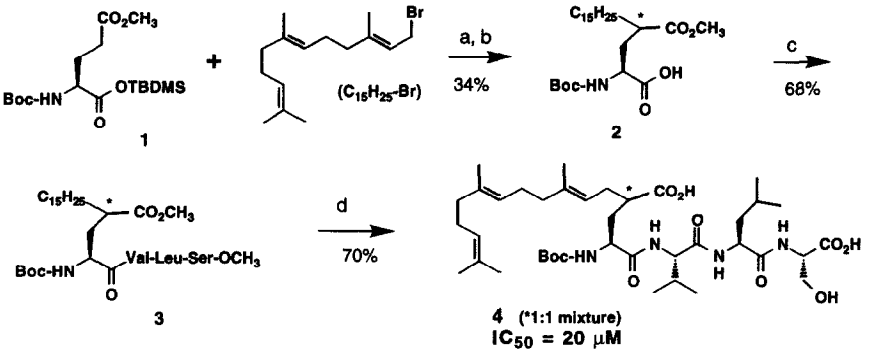
A carboxylic acid group has been successfully employed as a replacement for the sulfhydryl group in ACE inhibitors. Substituting aspartic or glutamic acid for cysteine in CAAX based tetrapeptides, DVLS and EVLS, led to inactive compounds.[5a] We reasoned that a collected substrate analog might have better affinity and specificity for the enzyme. Our approach to the design of these types of inhibitors is depicted in **Figure 2.**

Figure 2: Model for Collected Substrate Inhibitors



A peptide group attached to a farnesyl group would constitute a simple bisubstrate analog. We reasoned that in the early transition state the thiol group would be coordinated with an enzymic base and/or with the putative zinc atom. It was, therefore, considered necessary to incorporate a free sulfhydryl or its mimic in these collected substrate analogs. Based on this model, compounds in which a small peptide is attached to a sulfhydryl mimic and a farnesyl group by a tether were prepared. Thus, alkylation of fully protected glutamic acid **1** with farnesyl bromide,[6] followed by mild and selective deprotection (**Scheme I**) of the α-carboxyl group afforded **2**. Coupling of **2** to Val-Leu-Ser-OMe followed by hydrolysis of the esters afforded inhibitor **4** as a 1:1 diastereomeric mixture.

Scheme I[a]

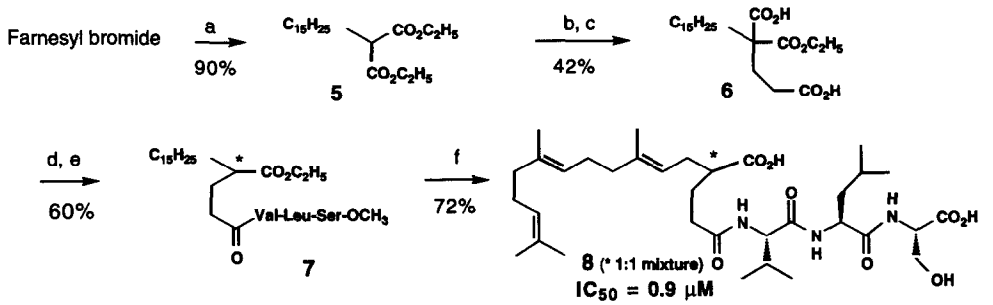


[a]Reagents: a) LiHMDS, THF, -78°C ; b) AcOH:H_2O:THF, 1:1:8; c) Val-Leu-Ser-OCH_3, EDCI, HOBT, ; d) NaOH, MeOH

The activity of **4** (IC_{50} = 20 μM),[7] though moderate, indicated that the rational design of collected substrate based non-sulfhydryl inhibitors was a viable approach to novel FPT inhibitors. An extensive SAR study was conducted to optimize the activity of this lead compound.

First, desamino analog **8** was prepared as shown in **Scheme II**. Alkylation of farnesyl bromide with diethyl malonate afforded the key intermediate **5** that was used to make several other analogs shown in **Table I**. Michael addition to ethyl acrylate with catalytic potassium-t-butoxide followed by hydrolysis afforded diacid monoester **6**. Upon refluxing in xylene, **6** underwent smooth decarboxylation to give a monoacid monoester that was coupled with tripeptide Val-Leu-Ser-OMe. Hydrolysis of **7** afforded the desired diacid **8** that has an **IC_{50} = 0.9 μM** versus FPT.[7] Since this modification improved activity and simplified the synthesis, we performed further SAR studies with **8** as the new starting point.

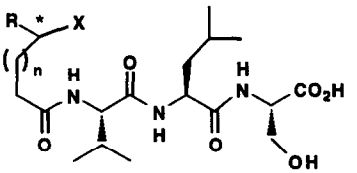
Scheme II[a]



[a]Reagents: a) NaH, diethyl malonate; b) K-O-t-Bu, t-BuOH, ethyl acrylate; c) 5 eq. NaOH, EtOH; d) Xylene, reflux; e) EDCI, HOBT, Val-Leu-Ser-OCH_3; f) NaOH, MeOH

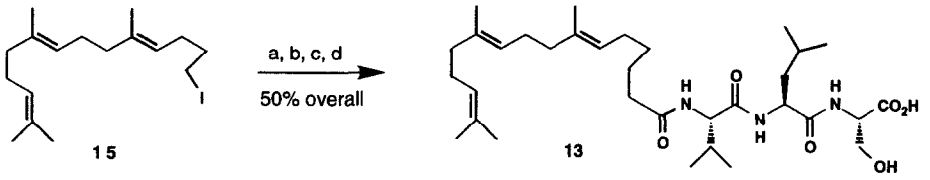
The importance of the α-carboxylic group, the optimal length of the tether that connected the farnesyl and peptide fragments, and chemically more stable farnesyl replacements were studied. **Table I** summarizes the structural changes and the biological results.

Table I



Compound #	X	n	R	IC_{50} μM
8	CO_2H	1	Farnesyl	0.9
9	CO_2Et	1	Farnesyl	>360
10	CO_2H	0	Farnesyl	4.5
11	CO_2H	2	Farnesyl	1.2
12	CO_2H	3	Farnesyl	1.8
13	H	1	Farnesyl	>360
14	CO_2H	1	Dodecyl	10.0

All these compounds were prepared according to the general procedure outlined in **Scheme II**. Compound **5** was alkylated with an appropriate alkyl bromide followed by coupling and hydrolysis. For the preparation of **9,** the serine methyl ester was selectively cleaved in the presence of the carboxyethyl group adjacent to farnesyl (α-carboxyl) by employing 1.2 equivalents of sodium hydroxide at 0°C for 1 hour. Compound **13** was prepared by alkylation of t-butyl acetate with **15**[8] in the presence of HMPA. Deprotection with TMSI, coupling with tripeptide and hydrolysis afforded the desired monoacid **13**.

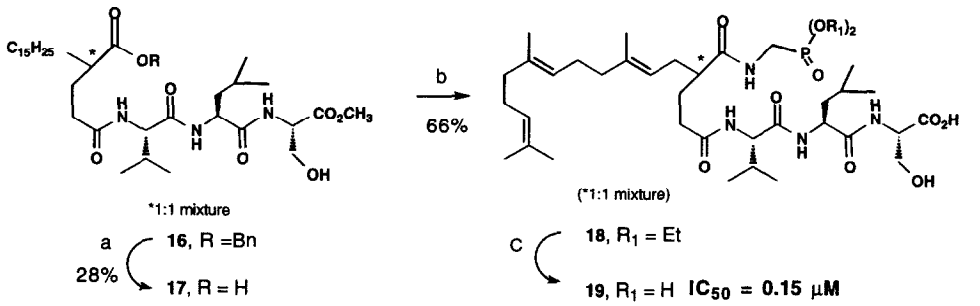


Reagents: a) $LiN(TMS)_2$, HMPA, t-Butyl acetate; b) TMSI, lutidine;
c) EDCI, HOBT, $VLS\text{-}OCH_3$; d) NaOH, MeOH

The moderate 10-fold loss in activity of the dodecyl analog **14** suggests that the farnesyl group could be replaced with other more biochemically robust groups. The carboxylic acid is important as both the descarboxy and ethyl ester analogs (see compounds **9** and **13, Table I**) are inactive. Because of the positioning of the α-carboxyl group relative to the farnesyl chain, we believe that it may be acting as sulfhydryl mimic rather than a pyrophosphate surrogate. Since the α-carboxyl group and the farnesyl group (*vide supra*) are important for reasonable activity, these inhibitors appear to be collected substrate type inhibitors.

In order to increase the potency of inhibitor **8**, we sought to incorporate a group that would extend into the pyrophosphate binding region of the enzyme. **Scheme III** describes the synthesis of **19** in which the α-carboxyl group of inhibitor **8** is replaced by an amidomethylphosphonic acid that may extend into such a binding pocket.

Scheme III[a]



[a]Reagents: a) 1.3 eq. Et_3SiH, $Pd(OAc)_2$; b) BOP, DIPEA, $NH_2CH_2P(O)OC_2H_5$;
c) BSTFA, TMSBr, then NaOH, MeOH

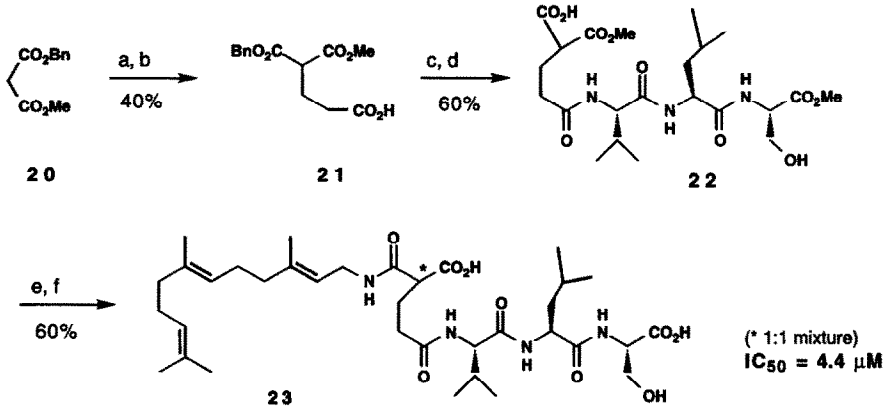
Intermediate **16** was prepared using the same reaction sequence as for the synthesis of intermediate **7** (**Scheme II**) except dibenzyl malonate was used in place of diethyl malonate. This change allowed us to selectively deprotect the α-carboxyl group in the presence of the methyl ester as well as the chemically sensitive farnesyl chain to afford **17**. Thus, on treatment of **16** with 1.3 equivalents of triethylsilane in the presence of catalytic

palladium(0), the benzyl ester was cleaved without any reduction of the trisubstituted double bonds of the farnesyl group.[9] Coupling of **17** with known diethyl aminomethylphosphonate[10] gave **18** which when treated sequentially with trimethylsilyl bromide and sodium hydroxide afforded the inhibitor **19**. A six-fold increase in potency (**19 vs 8**, IC_{50} = 0.15 vs 0.9 μM) was obtained by this change where a putative sulfhydryl mimic was replaced by a putative pyrophosphate surrogate.

Another approach we took to increase the potency of inhibitor **8** was to incorporate a group that may mimic a hypothetical hydrogen bonding interaction of the FPP oxygen with the enzyme (see **Figure 1**). An amide or an amine group was thus introduced between the farnesyl and the tether (see **Schemes IV** and **V**).

We prepared the 1, 3-dicarbonyl analog **23** as shown in **Scheme IV**. Mixed malonate **20** was added to t-butyl acrylate in Michael fashion and the t-butyl group was cleaved to afford **21.** The choice of these three ester protecting groups was critical for their stepwise deprotection. Coupling of **21** with the tripeptide followed by hydrogenation afforded **22**. Coupling with farnesyl amine and hydrolysis afforded **23** as a mixture of diastereomers. Compound **23,** which contains two additional atoms in the linker between the farnesyl and the the α-carboxyl, was 4-fold less active than compound **8.**

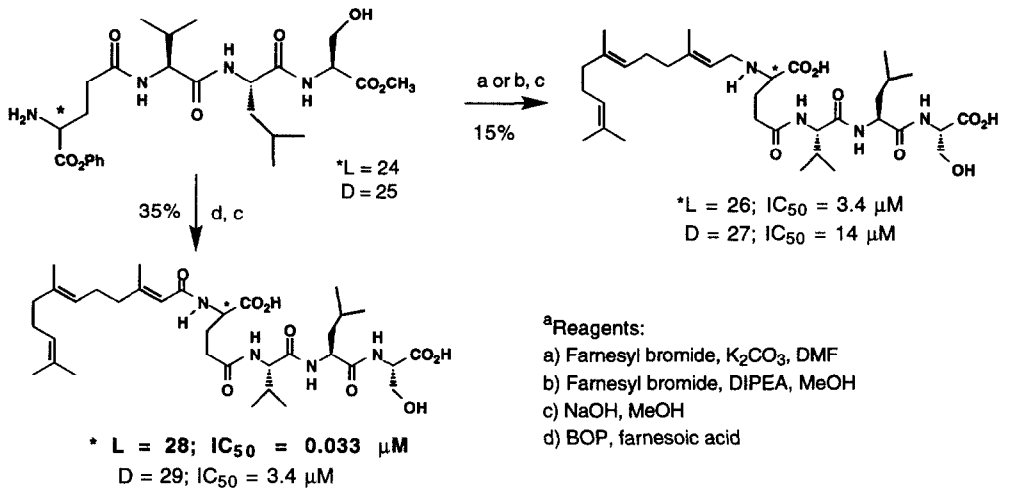
Scheme IV[a]



[a]Reagents: a) t-Butyl acrylate, K-O-t-Bu; b) CF_3COOH; c) EDCI, HOBT, VLS-OCH_3; d) H_2,$Pd(OH)_2$; e) EDCI, HOBT, farnesyl amine; f) 3 eq. NaOH

Synthesis of amines **26, 27** and amides **28** and **29** in enantiomerically pure forms is described in **Scheme V**. Protected L and D-glutamic acids were coupled at the α-carboxyl with tripeptide VLS-OCH_3 followed by deprotection of the amino group to obtain the common intermediates **24** and **25**. Alkylation with farnesyl bromide followed by hydrolysis afforded amine analogs **26** and **27** both of which showed weak inhibition. Coupling with farnesoic acid followed by hydrolysis afforded the amides **28** and **29.** The diastereomer **28** was 30-fold more potent than **8** and 100 fold more potent than its isosteric amine **26** and structurally related amide **23**. This increased potency of **28** may be due to the appropriately placed amide carbonyl that may participate in a hydrogen bonding interaction analogous to the oxygen of the pyrophosphate in the substrate FPP binding.

Scheme V[a]



In summary, we have demonstrated the rational design of carboxylic acid based collected substrate inhibitors of FPT. *Bisubstrate analog 28 was found to be 100 fold more potent than the parent sulfhydryl-based inhibitor CVLS* (4 μM).[5a] Unlike sulfhydryl-based tetrapeptides, these inhibitors may exhibit better specificity for FPT *vis a vis* other competing intracellular prenylation events.

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