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POTENT, NON-THIOL INHIBITORS OF FARNESYLTRANSFERASE

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Abstract: The structure–activity relationship of a series of non-thiol CaaX analogs, which are inhibitors of farnesyltransferase, is described. These inhibitors contain a substituted phenyl group at the N terminus, which may occupy a novel binding domain on the Ras protein.

The Ras protein, when localized in the cell membrane, plays a key role in cell replication by mediating signal transduction between external growth factors and the cell nucleus.[1] This localization requires a series of post-translational modifications, the first of which is farnesylation of the cysteine residue present in the terminal CaaX motif found in all Ras proteins.[2] The CaaX motif serves as the recognition sequence for the enzyme farnesyltransferase (FTase), which catalyzes the farnesylation reaction.[3] Oncogenic Ras proteins cause cell division to remain unregulated and are found in over 30% of human cancers.[4] Thus, the pharmacological inhibition of FTase by CaaX peptidomimetics presents an attractive anticancer drug target since inhibition would prevent oncogenic Ras from associating with the cell membrane and interrupt its role in signal transduction.[5]

Many CaaX peptides and peptidomimetics that inhibit FTase have been described,[6] although a preponderance of these compounds contain a terminal cysteine residue as a requirement for good inhibitory activity. Since concerns over thiol-dependent toxicity render these undesirable, we and others have investigated the replacement of the cysteine residue by imidazole,[7] pyroglutamine,[8] and phenolic rings[9] with modest success.

This paper describes the structure–activity relationships of a series of pseudotetrapeptides leading to the discovery of novel, potent non-thiol containing FTase inhibitors that utilize a novel binding domain on the Ras protein.

Chemistry

The route used to produce the pseudotetrapeptides is shown in Scheme 1. The synthesis of the key intermediates **3** and **4** have already been described.[10] Briefly, successive reductive couplings of **1** to glycine methyl ester then 1-naphthaldehyde, followed by ester hydrolysis gave the acid **2**. Standard peptide coupling to methionine methyl ester followed by HCl deprotection of the Boc group yielded the key intermediate **3**. Similar chemistry yielded the phenyl analog **4**. The amine **3** or **4** was coupled to prospective cysteine replacements by

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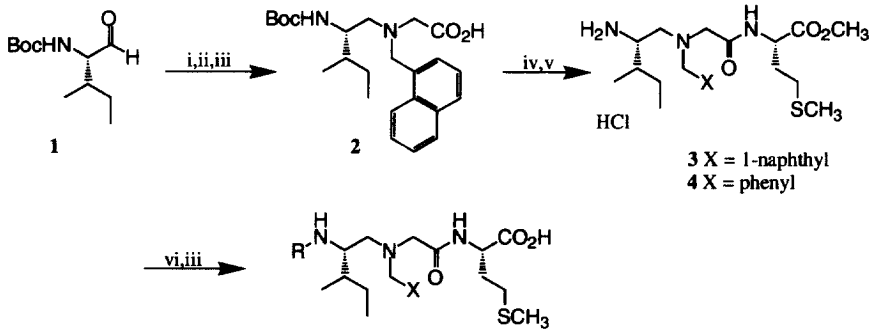
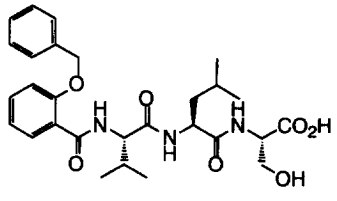
one of four general methods. Method A involved the pretreatment of **3** (or **4**) with bromoacetyl bromide in DMF with Et_3N followed by the addition of an appropriate thiol with Et_3N. Method B involved coupling to an acid chloride and Method C utilized an EDC coupling with the appropriate carboxylic acid. The acids were either commercially available or synthesized by coupling benzyl chlorides to mercaptoacetic acid in aqueous sodium hydroxide and THF. Method D utilized a reductive amination with an appropriate aldehyde. The resulting methyl esters were then saponified to give the final compounds **5** or **6** which were assayed for their ability to inhibit the incorporation of [^{3}H] FPP into recombinant Ha-Ras by FTase[11] (see Tables 1 and 2).

Scheme 1[a]



[a]Reagents:(i) Glycine methyl ester HCl, $NaCNBH_3$, MeOH; (ii) 1-naphthaldehyde, $NaB(OAc)_3H$, DCE, 4 Å sieves LiOH, H_2O, MeOH, THF; (iv)Methionine methyl ester HCl, HOBT, EDC, NMM, DMF; (v) HCl(g), EtOAc; (vi) Method A: (1) bromoacetyl bromide, Et_3N, DMF; (2) R'SH, Et_3N; Method B: $R'CH_2COCl$; Method C: $R'CH_2CO_2H$ HOBT, EDC, Et_3N, DMF; Method D: R'CHO, $NaCNBH_3$, KOAc, MeOH, 4 Å sieves.



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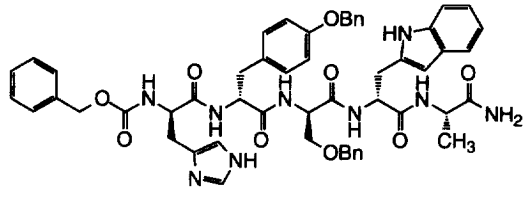
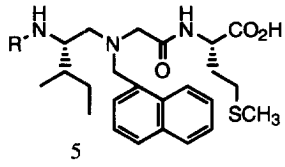


8 PD083176

Table 1



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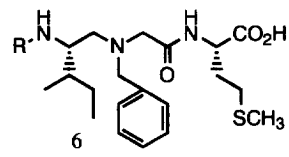

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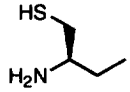
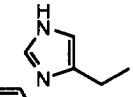
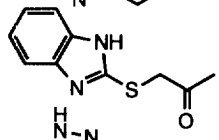
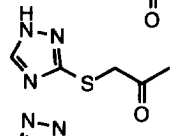
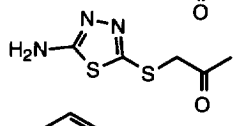
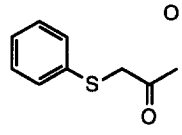
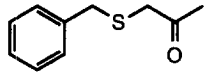
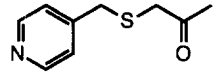
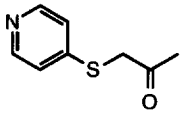
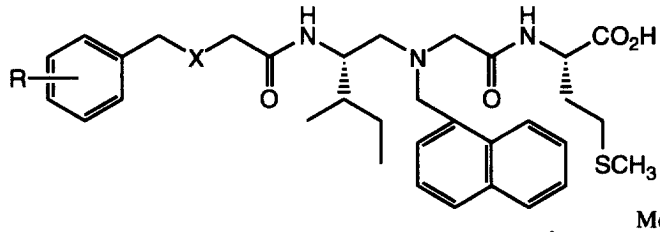
Compound	R	IC_{50} (nM) [a]	Method of Preparation[c]
5a		0.123 [b]	D
6a		1.2[b]	
5b		31	D
6b		>10,000	A
6c		>10,000	A
6d		>10,000	A
5c		51	A
5d		36	C
5e		230	C
5f		17	C

[a]Concentration of compound required to inhibit the FTase-catalyzed incorporation of [^{3}H] FPP into recombinant Ha-Ras by 50% at an enzyme concentration of 1 nM.[11] [b]Determined at an enzyme concentration of 10 pM. [c]See Scheme 1.

Table 2



Compound	X	R	IC_{50} (nM)[a]	Method of Preparation[b]
5d	S	H	36	C
5g	SCH_2	H	120	C
5h	SO	H	160	C
5i	SO_2	H	310	C
5j	CH_2CH_2	H	230	C
5k	O	H	230	B
5l	NBoc	H	260	C
5m	NH	H	190	deprotection of 5l
5n	$NCOCH_3$	H	70	acetylation of 5m
5o	S	2-NO_2	86	C
5p	S	3-NO_2	23	C
5q	S	4-NO_2	6.2	C
5r	CH_2	4-NO_2	19	C
5s	S	4-CF_3	37	C
5t	S	4-Ph	29	C
5u	S	4-CH_3O	17	C
5v	S	4-SO_2CH_3	16	C
5w	S	4-CH_3	14	C
5x	S	4-CN	5.4	C

[a]See Table 1, footnote a. [b]See Scheme 1.

Results and Discussion

The parent CaaX peptidomimetic (**5a**), which contains a reduced cysteine residue, has been shown to be a very potent inhibitor of Ras FTase with an IC_{50} of 0.123 nM.[10] Since the CaaX thiol is postulated to be involved in coordination to zinc during the catalytic mechanism of FTase,[12] replacement of the cysteine moiety (Table 1) with alternate ligands capable of binding to zinc, such as imidazole, led to the moderately potent inhibitor **5b** with an IC_{50} of 31 nM. Heterocycles such as mercaptobenzimidazole, thiadiazole, and triazole were incorporated as ligands in the benzyl series (**6b–d**) and led to significant losses in potency (all >10 μM). It quickly became apparent that the simple phenyl substituted compounds, **5c** and **5d**, were of similar potency (51 nM and 36 nM, respectively) to that of the imidazole cysteine replacements. Substituting a 4-pyridyl for the phenyl group in **5c** led to a threefold increase in potency (**5f**; IC_{50} = 17 nM), but a four- to fivefold decrease in potency (**5e**; IC_{50} = 230 nM) when substituted for the benzyl in **5d**.

We next investigated the spacing of the phenyl group (Table 2) by altering the chain length and the nature of the heteroatom in the linker. Extending or shortening the phenyl position by one carbon as seen in compounds **5c** and **5g** (51 and 120 nM, respectively) led to losses in potency over **5d** (36 nM). Oxidation of the sulfur to give the sulfoxide (**5h**; IC_{50} = 160 nM) and the sulfone (**5i**; IC_{50} = 310 nM) yielded successive losses in potency for each oxidation. Replacement of the sulfur by an ethyl spacer (**5j**), oxygen (**5k**), and nitrogen (**5m**) all led to a five- to sixfold loss in potency. In contrast, acetylation of **5m** to give **5n** gained back some potency (70 nM), but substitution with larger groups such as Boc proved detrimental (**5l**; IC_{50} = 260 nM). In the 4-nitro substituted series, replacement of the sulfur of **5q** by a methylene (**5r**) led to a three fold loss in potency.

The effect of the introduction of a substituent on the phenyl ring was then investigated. A nitro group was placed at each position on the phenyl ring and it was found that substitution at the ortho position (**5o**) decreased potency by two- to threefold over **5d** while *meta* (**5p**) and *para* (**5q**) substitution increased potency (23 and 6.2 nM, respectively). Based on the observation that substitution in the *para* position is preferred, a series of 4-substituted phenyl analogs were prepared and evaluated (**5s-x**). The 4-trifluoromethyl (**5s**; IC_{50} = 37 nM) and 4-phenyl (**5t**; IC_{50} = 29 nM) were approximately equal in potency to the unsubstituted compound **5d**. A twofold boost in potency was noted for the 4-methoxy (**5u**), the 4-methylsulfone (**5v**), and the 4-methyl (**5w**) derivatives. Interestingly, a 4-cyano substituent (**5x**) proved to be equipotent to **5q** (5.4 nM and 6.2 nM, respectively).

Patel and coworkers recently described a series of Ras FTase inhibitors in which the cysteine group had been replaced with a phenol.[9] Interestingly, they also reported that, for the *meta* derivative, the benzylated phenol precursor **7** was more potent than the corresponding phenol. However, the potency of **7** was modest (IC_{50} = 63 μM).

In a similar vein, Leonard et al. have recently reported on pentapeptide analogs lacking a cysteine group.[13] From this series (typified by PD083176, **8**, IC_{50} = 20 nM) it was found that a critical residue for activity is the Cbz-His moiety. Removal of either the Cbz group or the imidazole group resulted in over a 200-fold drop in potency.

Our data taken together with the above results indicate that there is an aromatic binding region at the active site of the Ras FTase protein. Perhaps this aromatic binding region is involved in binding the farnesyl pyrophosphate cosubstrate. In addition, it is apparent from our data that the presence of a *para*-substituent on the

phenyl ring can further improve the binding affinities of these pseudotetrapeptide analogs to afford potent FTase inhibitors.

In conclusion, through a combination of rapid analog and directed synthesis we have been successful in obtaining a replacement for cysteine in a pseudotetrapeptide series of Ras FTase inhibitors. The key finding is the identification of the 4-nitro and 4-cyanophenyl group (**5q** and **5x**) which appears to occupy an aromatic binding domain at the active site of the enzyme. The results of the combination of an imidazole with a *para*-substituted phenyl group (cf. Leonard, et al.[13]) will be described in a future publication.

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