JOURNAL OF
MEDICINAL
CHEMISTRY

Volume 40, Number 12 June 6, 1997

Communications to the Editor

Novel Conformationally Extended Naphthalene-Based Inhibitors of Farnesyltransferase

Christopher J. Burns,*,† Jean-Dominique Guitton,* Bernard Baudoin, Yves Lelièvre, Marc Duchesne, Fabienne Parker, Nadine Fromage, and Alain Commerçon

Rhône-Poulenc Rorer S. A., Centre de Recherches de Vitry-Alfortville, 13, Quai Jules Guesde, B. P. 14, 94403 Vitry-sur-Seine, France

Received February 27, 1997

Oncogenic mutations of the mammalian *ras* proto-oncogene have been implicated in 20−30% of all human cancers including more than 50% of colon and 90% of pancreatic carcinomas.[1,2] A key step in a series of posttranslational modifications of the oncogene product Ras is the S-farnesylation of a cysteine residue near the C-terminus in a reaction catalyzed by the enzyme farnesyltransferase (FTase). This step is important for the association of the GTP-binding Ras protein to the inner surface of the plasma membrane, where it mediates cellular transformation.[3] Inhibition of this farnesylation event may lead to novel anti-cancer agents, and thus has been the subject of much recent research.[4]

It has been appreciated for several years now that conformationally flexible tetrapeptides corresponding to the C-terminal CA_1A_2X sequence (where *A* is an aliphatic amino acid and *X* is Met or Ser) of Ras can serve as effective inhibitors of Ras farnesylation.[5] From efforts to increase not only enzyme-inhibitory activity but also cellular activity, a number of effective CA_1A_2X-based peptidomimetics have emerged.[6] While initial reports focused on flexible pseudopeptidic (ψ[CH_2X]) variants, other inhibitors have been designed to incorporate conformationally constrained A_1A_2 replacements, particularly for CA_1A_2M peptides and pseudopeptides. These have ranged from classical amino acid replacements such as CVTicM (where Tic = L-1,2,3,4-tetrahydroisoquinolin-3-ylcarbonyl),[6l,7] to inhibitors in which the two internal amino acids of the C*AAX* sequence are replaced by surrogates such as benzodiazepines,[6b] aminobenzoic acids, and aminomethylbenzoic acids.[6e,i] More recently, several studies have appeared in which the entire A_1A_2X portion of the CA_1A_2X box has been replaced by non-peptidic units.[8]

Largely as a consequence of these efforts, discussion surrounding the likely active conformation of the C*AAX*-based inhibitors has been increasingly fruitful. Early opinion favored the possibility of a "turn-like" backbone conformation, based upon analogy to Cys-X-X-Cys tetrapeptide motifs found in aspartate transcarbamylase and "zinc finger" domains,[9] as well as on solution NMR studies using moderately potent C*AAX*-based derivatives.[10] More recent evidence, particularly that from the aminomethylbenzoic acid based C*AAX* mimetics, has led to the suggestion that an "extended" (or nonturned) conformation is more accurate.[4]

Our initial molecular modeling simulation studies on the conformationally constrained Tic-containing peptides and pseudopeptides demonstrated a direct correlation between FTase inhibitory activity and the proclivity of these inhibitors to adopt an extended conformation.[7] These studies precipitated a search for hydrophobic scaffolds capable of orienting the cysteine (or modified cysteine) and methionine units according to a pharmacophore model derived from the potent prototype Cys-(*N*-Me)Val-Tic-Met (Figure 1). We intentionally restricted this search to scaffolds that were incapable of accessing low-energy turned conformations.

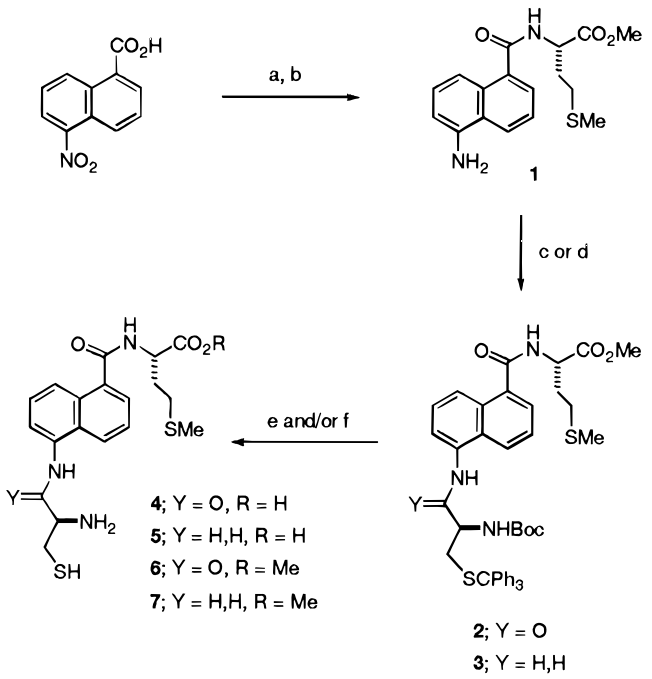
The 1,5-naphthyl scaffold proved to be worthy of further investigation.[11] It satisfies the CO(Met)−NH(Cys) distance relationship outlined in Figure 1 and also provides a rigid hydrophobic template that directs the Cys and Met amino acids unambiguously into an extended conformation. Indeed, a modeling simulation calculates a difference of at least 15−20 kcal between the low-energy extended conformations and the lowest energy "pseudocurved" conformation. In no case was a true turned arrangement manifested.[12]

Synthesis of the dipeptide mimetic scaffold and several analogues with the cysteine and methionine residues affixed is described in Scheme 1. Thus 5-nitro-1-naphthoic acid[13] was coupled to L-methionine-OMe

† Author to whom correspondence should be addressed. Present address: Rhône-Poulenc Rorer, 500 Arcola Rd, SW 8, Collegeville, PA 19426. E-Mail: burnscj@rpr.rpna.com.

S0022-2623(97)00117-9 CCC: $14.00

Scheme 1[a]



[a] (a) H-Met-OMe, DCC, HOBT, Et_3N, CH_2Cl_2, DMF, 20 h (72%); (b) $SnCl_2$, EtOH, EtOAc, 0.5 h, 70 °C (90%); (c) Boc-Cys(Trt)-OH, NMM, *i*-BuOC(O)Cl, THF, 2 d (71%); (d) L-*N*-Boc-*S*-tritylcysteine aldehyde, $NaCNBH_3$, AcOH, MeOH, 3 Å sieves, 24 h (24%); (e) LiOH, THF, H_2O; (f) TFA, EDT, H_2O.

and then reduced with $SnCl_2$ to afford **1**. Naphthylamine **1** was then either coupled to Boc-Cys(Trt)-OH to afford amide **2** or reductively alkylated with L-*N*-Boc-*S*-tritylcysteine aldehyde[6f] to give the aminomethyl (Cys-ψ[CH_2NH]) derivative **3**. Saponification of esters **2** and **3** followed by removal of the Boc and Trt protective groups with TFA then afforded carboxylic acids **4** and **5**. Alternatively, independent treatment of **2** and **3** directly with TFA provided esters **6** and **7**, respectively.

The parent structure **4** demonstrated potent (IC_{50} = 48 nM) inhibitory activity against FTase. The aminomethyl variant **5** showed an increase in potency by almost 10-fold (IC_{50} = 5.6 nM, see Tables 1 and 2). Neither compound served as a substrate for FTase, and both were competitive with respect to the Ras substrate used in the assay.[7] The phenomenon of augmented FTase activity upon reduction of the cysteine amide bond, initially reported by Kohl[6a] and by Garcia[6c] for nonconstrained C*AAX* peptides, has been observed for a number of inhibitor series despite considerable diversity in the scaffolding for each class.[6,8]

It is believed that the cysteinyl sulfur atom of the C*AAX*-based inhibitors is involved in an important binding interaction with a Zn^{2+} atom of FTase, based on binding studies of the C*AAX*-containing proteins.[14] Our observation that the 1,5-naphthalene-substituted C*AAX*-based inhibitor demonstrated an SAR correlation with the nonconstrained C*AAX* peptides implies that there are similarities in their binding interactions, at least in the region of the cysteine residue. If, in fact, these two classes of inhibitors were bound to FTase in two distinct conformations (extended for one, and turned for the other), then it would appear unlikely that the SAR correlation observed at the cysteine terminus would be maintained at the methionine terminus as well. We hypothesized therefore that both the naphthalene-based inhibitors and the C*AAX*-based peptides adopt similar conformations when bound in the active site of the enzyme. This hypothesis precipitated a retrospective analysis of both in-house and published SAR data surrounding the methionine terminus to ascertain whether the naphthalene-based inhibitors exhibited the same global binding profile as that reported for the conformationally-flexible peptide-based inhibitor, CVFM.[15] This comparison is described in Table 1 and displayed graphically in Figure 2.

Using the IC_{50} values obtained for inhibitors **5** and **8** as a reference, conversion of the Met C(O) to the thioamide (**9** and **10**) maintained the same level of inhibitory activity in both series as the parent compounds **5** and **8**. Replacement of Met with the isostere norleucine (**11** and **12**) provided a modest decrease in activity within both series, albeit somewhat greater in the peptide-based series than in the naphthyl series. Methylation of the methionine nitrogen (**13** and **14**) gave a 40-fold loss of activity in both series, perhaps suggesting the loss of an important hydrogen bond. Modification of the Met amide to the aminomethyl (ψ[CH_2-NH]Met) analogues **15** and **16** resulted in a significant loss (>50-fold) of activity in both series as did esterification of methionine to the methyl esters (**7** and **17**; >50-fold loss for both). While not exhaustive, the data clearly suggest a direct correlation between the two series of inhibitors, implying that similar inhibitor−enzyme interactions are involved for both this new rigid naphthyl-based scaffold and the flexible peptide-based scaffolds. Additional comparative analysis will be required to fully substantiate this postulate.

Further examination of the naphthalene scaffold by molecular modeling suggested that the isomeric 1,6-naphthalene-substituted analogue would also be capable of maintaining the approximate distances and conformational constraints between the Cys and Met termini projected from the earlier analysis. The parent diamide **18** and the aminomethyl derivatives **19** (RPR 113829) and **20** (RPR 114334) were prepared according to the methodology outlined in Scheme 1, starting from 6-nitro-1-naphthoic acid.[13] Both carboxylic acids **18** and **19** exhibited potent FTase inhibitory activity (Table 2), the latter demonstrating significant inhibition of both Ha-Ras and Ki-Ras *in vitro*. This result is particularly pertinent since activated Ki-Ras tumors are much more prevalent than activated Ha-Ras tumors[16] and because Ki-Ras exhibits a 50-fold higher affinity for FTase than does Ha-Ras.[17]

Historically, one of the major challenges in the research on C*AAX* peptides and peptidomimetics has been the translation of potent isolated-enzyme inhibitory activity to activity in cells. Considerable progress has been made in this area due largely to the structural backbone modifications alluded to earlier and the employment of ester "prodrugs" on the methionine carboxylate.[6] Application of the ester prodrug approach to the 1,5- and 1,6-naphthyl regioisomers (Table 2) revealed a modest improvement in inhibition of Ha-Ras processing in THAC cells for the 1,5-substituted analogue **7** compared to the free carboxylic acid **5**. A more dramatic improvement was observed for the 1,6-substituted ester RPR 114334 (**20**). The cellular activity of **20** is many orders of magnitude greater than that

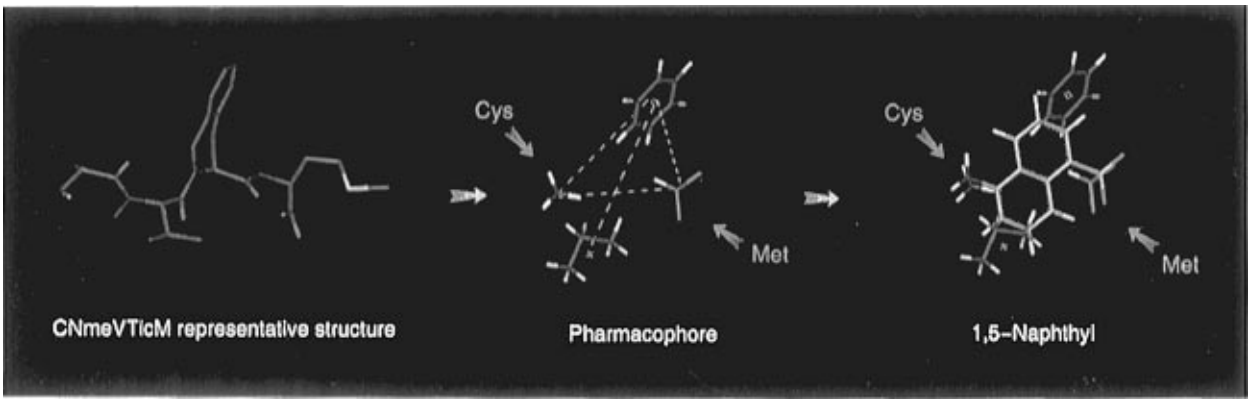
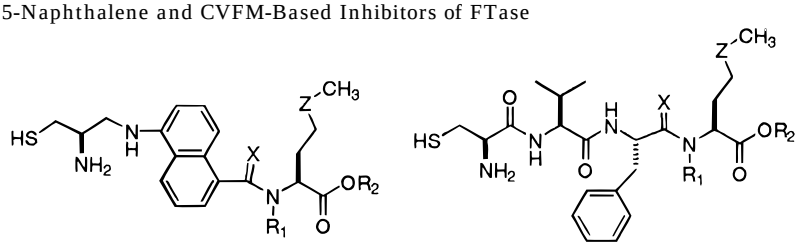


Figure 1. Development of extended pharmacophore model from Cys-(*N*-Me)Val-Tic-Met.

Table 1. Comparison of 1,5-Naphthalene and CVFM-Based Inhibitors of FTase



Series A **Series B**

compound[a]						FTase			
						series A[b]		series B[c]	
series A	series B	X	R_1	Z	R_2	IC_{50} (nM)	(no./**5**)[d]	IC_{50} (nM)	(no./**8**)[e]
5	**8**	O	H	S	H	5.6	1.0	37	1.0
9	**10**	S	H	S	H	4.8	0.9	17	0.5
11	**12**	O	H	CH_2	H	44	8	950	26
13	**14**	O	Me	S	H	210	37.5	1400	38
15	**16**	H,H	H	S	H	2570[f]	54[g]	2220	60
7	**17**	O	H	S	Me	325	58	3400	92

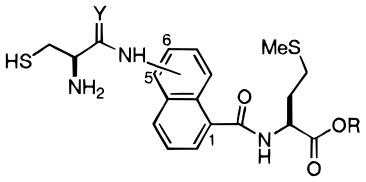
[a] Preparations of **9**, **11**, **13**, and **15** are provided in the Supporting Information. [b] IC_{50} values for FTase-catalyzed transfer of [^{3}H]FPP to human Ha-Ras protein; values are means of two or more IC_{50} determinations. Assay conditions are described in ref 7. [c] IC_{50} values are reproduced from ref 15, except for the value for **17** (BMS-187912, Dr. K. Leftheris, Bristol-Myers Squibb, personal communication). [d] Ratio of FTase IC_{50} values for series A compounds relative to **5**. [e] Ratio of FTase IC_{50} values for series B compounds relative to **8**. [f] IC_{50} value for ψ[CH_2NH]Met derivative of **4**. [g] Ratio of FTase IC_{50} values is obtained using value for **15** divided by that for **4** (IC_{50} 48 nM).

exhibited by the peptide derivative Cys-(*N*-Me)Val-Tic-Met (**21**),[7] which displayed similar *in vitro* activity in the FTase enzyme assay. The improvement in cellular activity of **20** when compared to that of **21** may be due to better cell membrane penetration and/or enhanced resistance to proteolytic degradation due to the replacement of two amino acids and two amide bonds.

RPR 114334 (**20**) potently inhibits the anchorage-independent growth of several cell lines (Table 3). Activated Ha-*ras* and Ki-*ras* transformed cell lines were both inhibited in their ability to form colonies in soft agar, an important finding in light of the prevalence of cells harboring mutations in Ki-*ras* in human carcinomas. Several recent studies have pointed out the lack of a direct correlation between inhibition of activated Ras processing and inhibition of cell growth,[18] a phenomenon which appears to be dependent on cell type and class of FTase inhibitor. A number of explanations (parallel processing by other prenyltransferases, multiple gene mutations, inhibition of non-Ras targets, etc.) were proposed, some or all of which might be operative for a given cell line. In addition, it has also been shown that cells harboring activated upstream tyrosine kinases and wild-type Ras can also be inhibited by some FTase inhibitors,[18] potentially expanding the therapeutic application of these inhibitors. These phenomena are also observed within this new series of FTase inhibitors (Table 3). Indeed, the sensitivity toward growth inhibition in soft agar by RPR 114334 (**20**) appears to be cell-dependent (cf., NIH 3T3, H 460, and HCT 116 cells all bearing mutant forms of Ki-Ras); however, in all of these cases, the inhibitor is able to significantly block the anchorage-independent cell growth. Interestingly, we have found that **19** (the acid form of **20**) demonstrates good inhibitory activity against GGTase *in vitro* (Ki-Ras, IC_{50} 73 nM). This additional enzyme activity may be contributing to the cellular activity of **20**, particularly for Ki-*ras* transformed cells.[19] Finally, RPR 114334 (**20**) also potently inhibited the anchorage-independent growth of c-*src*-transformed NIH 3T3 cells, which possess the activated tyrosine kinase Src and wild-type Ras.

In summary, we report here on two new series of potent, conformationally fixed, naphthalene-based inhibitors of FTase. These series were designed based on the premise that the active conformation of the inhibitory C*AAX*-based peptides and pseudopeptides is "ex-

Table 2. In Vitro and Intracellular Activity of Regioisomeric Naphthalene Inhibitors



				IC$_{50}$ (nM)		
compd	position	Y	R	Ha-Ras FTase[a]	Ki-Ras FTase[b]	% inhibition of Ras processing THAC (10 μM)[c]
21 (Cys-(*N*-Me)Val-Tic-Met)				5		0 at 1 mM
4	5	O	H	48		0
5	5	H,H	H	5.6		0
7	5	H,H	Me	325		20
18	6	O	H	60	1250	0
19 (RPR 113829)	6	H,H	H	1.8	17.5	10
20 (RPR 114334)	6	H,H	Me	80	500	>50

[a] See Table 1, footnote *b*. [b] IC$_{50}$ using Ki-Ras as substrate; value is the average of two or more IC$_{50}$ measurements by SPA of FTase using as first substrate a biotinylated fragment of the *C*-terminus of Ki-Ras: [Biotin]-(A)$_3$-SKDG-(K)$_6$-SKTKCVIM (at a concentration equal to its K_m, 55 nM) and as second substrate [^{3}H]FPP (at a concentration equal to its K_m, 120 nM). [c] Percentage inhibition of Ras protein processing in THAC cells (CCL39 cells transformed with activated Ha-Ras)—assay conditions are described in ref 20.

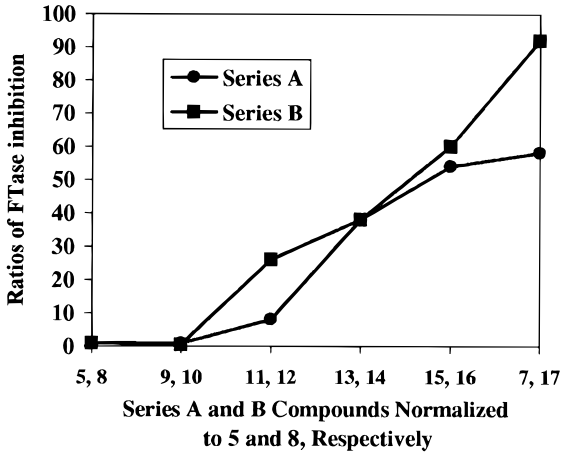


Figure 2. Graphical illustration of ratios derived from Table 1.

Table 3. Inhibition of Anchorage-Independent Cell Growth by RPR 114334 (**20**)

	inhibition of cell growth in soft agar (IC$_{50}$ μM)[a]				
compound	NIH 3T3 Ha-Ras[b]	NIH 3T3 Ki-Ras[c]	NIH 3T3 Src[d]	H 460 Ki-Ras[e]	HCT 116 Ki-Ras[f]
RPR 114334	5	10	5	5	50

[a] Assay conditions are described in ref 20. [b] NIH 3T3 cells transformed by Ha-*ras* (Val-12). [c] NIH 3T3 cells transformed by Ki-*ras* (Val-12). [d] NIH 3T3 cells transformed by c-*src* (Y527F). [e] Human lung carcinoma cells containing a point mutation in Ki-Ras (Gln^{61} to His^{61}). [f] Human colon carcinoma cells containing a point mutation in Ki-Ras (Gly^{13} to Asp^{13}).

tended" rather than "turned". Correlation of the SAR trends for the 1,5-naphthalene-based series compared with a representative nonconstrained peptide, CVFM, provides further evidence that the biologically active conformations for *both* the new series and CVFM are closely related and are therefore extended. The new prototypes, particularly members of the 1,6-naphthalene-based series, demonstrate potent cellular activity against Ras processing and in clonogenic assays of tumor cell growth in soft agar (activated Ha-*ras* and Ki-*ras*, as well as activated tyrosine kinase with wild-type Ras) and has formed the basis for further research which will be reported in due course.

Acknowledgment. The authors thank N. Baudouy, R. Boulay, F. Clerc, C. Colonna, H. Dubois, T. X. Q. K. Faitg, M. Leroux, M.-F. Marzin, P. Mouton, and B. Sans for their expert technical assistance. The financial support of the Bioavenir program is gratefully acknowledged.

Supporting Information Available: Experimental details and characterization data for compounds **1**−**7**, **9**, **11**, **13**, **15**, and **18**−**20** (7 pages). Ordering information is given on any current masthead page.

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(12) Molecular modeling simulation studies were performed using INSIGHT II/Discover software (Biosym-MSI), employing systematic analysis of conformational space by molecular dynamics (2 ps 900 K, 2 ps 300 K, minimization 100 cycles). For Cys-(*N*-Me)Val-Tic-Met, over 100 structures were generated; the average of the 30 lowest energy conformations was used to derive the conformation shown in Figure 1. A measurement of the mean distance value between Cα (Cys) and Cα (Met) for a "turned" peptide (such as Cys-D-Tic-Phe-Met) is approximately 5.5 Å. In the 1,5-naphthyl series, the mean value is approximately 11 Å. The smallest calculated distance in the 1,5-naphthyl series, and representing 3% of the accessible conformations, is 8.5 Å. These "pseudocurved" structures are the highest energy conformations. A plot of energy versus Cα (Cys to Met) distance reveals a direct correlation between lowest energy and greatest distance.

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JM9701177