Potent and Selective Farnesyl Transferase Inhibitors

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We recently described a novel series of CA_1A_2X peptidomimetics as farnesyl transferase inhibitors (FTIs). These compounds possess an *N*-(4-piperidinyl)benzamide scaffold mimicking A_1A_2 residue. Extensive exploration of structure−activity relationships revealed that replacement of cysteine by substituted benzylimidazoles provided nanomolar FTIs with in vitro activities (**18e**, $IC_{50} = 4.60$ nM on isolated enzyme, $EC_{50} = 20.0$ nM for growth inhibition on a tumor cell line). The molecular docking of **18e** and **19e** in the active site of the enzyme provided details of key interactions with the protein and showed that the methionine or phenylalanine residue fits into the aryl binding site.

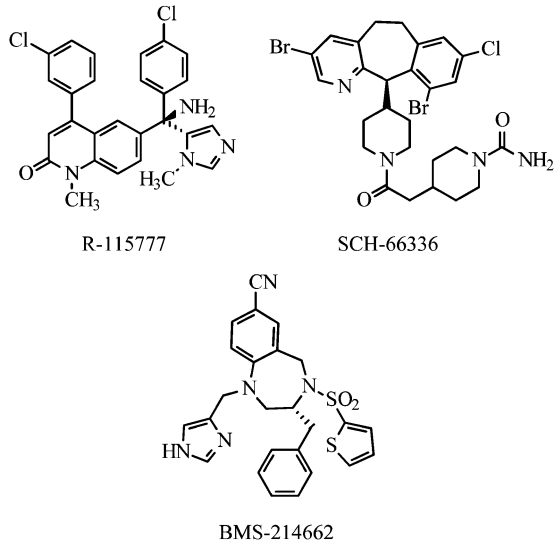
Introduction

The key role of Ras proteins in cell growth and cell proliferation inducing the MAP kinase signal transduction pathway and consequently the prevalence of Ras oncogene activation in a variety of human tumors (30% on average, reaching up to 50% in colon cancer and up to 90% in pancreas cancer) is well documented.[1] Biological activation of Ras proteins is directly related to their membrane association, which is mainly dependent on the prenylation of cysteine in the C-terminal tetrapeptide CA_1A_2X (C, Cys; A_1, A_2, any amino acid; X, Ser, Met, Phe, Leu, or Ile) by Zn−metalloenzyme farnesyltransferase (FTase).[2] As expected, inhibition of this enzyme prevents membrane localization of the Ras oncogene and constitutes, therefore, a valid target for the conception of new cytostatic anticancer drugs.[3]

However, if farnesyltransferase inhibitors (FTIs) are effective on the farnesylation and function of the H-Ras isoform, then they also prevent the farnesylation of K-Ras and N-Ras, which may undergo the alternative geranylgeranyltransferase reaction provoking an oncogenic effect. Unfortunately, H-Ras mutations are not prevalent in some common cancers, and other targets have to be considered. Experimental evidence supports the possibility that RhoB proteins are important targets.[4] Other candidate targets for proteins that mediate the antitumor effects of FTIs include other Ras-family GTPases such as Rheb[5] and centromer-associated proteins CENP-E and CENP−F, because functional association of CENP-E with microtubules seems to require farnesylation[6] or an unidentified protein that functions as activator of PI3K/AKT2 when farnesylated.[7]

Early screening of tetrapeptide libraries established that selective prenylation of the cysteine either by FTase or by closely related geranylgeranyltransferase-I (GGTI) is directed by the nature of X. Farnesylation is induced by Ser or Met whereas Leu or Ile provokes geranylgeranylation, and both prenylations are possible with Phe or Trp.[8] Stepwise modifications of the natural tetrapeptide resulted in a variety of FTIs when (a) Met was retained or suppressed,[9] (b) the A_1A_2 tensor was replaced by (i) a mono-[10] or a biaryl[11] moiety, (ii) piperazine,[12] (iii) benzodiazepine,[13] or (iv) a diaryl ether or a diaryl sulfone spacer,[14] and (c) cysteine was replaced by a heterocycle such as pyridine,[15] imidazole,[16] or cyclohexylamine.[17]

Chart 1



Random screening of chemical libraries also led to the identification of several nonpeptides, nonthiol compounds, which were optimized into R-115777, SCH-66336, and BMS-214662 presently engaged in clinical trials (Chart 1).[18]

We reported recently the synthesis and the pharmacological evaluation of new FTIs derived from the CVFM tetrapeptide and characterized by a piperidinyl spacer.[19] The most interesting molecule **1** (Chart 2) possessed a IC_{50} value (isolated enzyme FTase) as low as 22.8 nM but did not inhibit the proliferation of tumor cells in culture. Replacement of cysteine by thiazoline, thiazolidine, or imidazole was found to decrease inhibitory activity without disclosing any effect on cellular growth.

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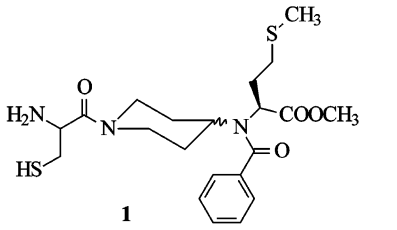
Chart 2



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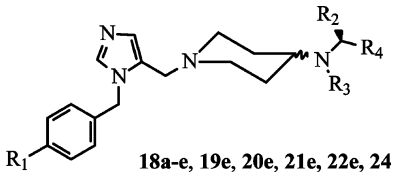


18a-e, 19e, 20e, 21e, 22e, 24

This paper reports the synthesis and study of the new FTIs **18a**−**e**, **19e**, **20e**, **21e**, **22e**, and **24** related to **1**, with potent isolated enzymatic and cellular activities, where cysteine is replaced by substituted 1-(4-benzyl)-4-imidazolylmethyl groups.

To obtain more insight into how this pharmacophore interacts with the active site of the enzyme, substituents on the benzyl ring ranged from withdrawing cyano and trifluoromethyl groups to electron-donating methyl or methoxy groups, with electronically inactive hydrogen atom as reference. Conformational restraint imposed by the carbonyl group of *N*-benzoylmethioninate was released with *N*-benzyl. Finally, to determine a specificity profile (FTase versus GGTase I) of the molecules, C-terminal methioninate was replaced by Phe and Ile esters.

Chemistry

The 5-formylimidazoles **7a**−**e** (Scheme 1) were prepared in five steps according to the standard procedure[9b,20] adopted for the synthesis of regiochemically substituted imidazoleacetic esters.

Thus, 4-hydroxymethylimidazole **2** was protected by a trityl group on the most accessible nitrogen to give exclusively the trityl-protected 1,4-disubstituted isomer following the methodology used to prepare regioselectively alkylated histidines (i.e., classical imidazole protection/deprotection strategy). Compound **3** was next acetylated with acetic anhydride in pyridine to give ester **4**. Alkylation of **4** was carried out with different commercially available 4-substituted benzyl bromides in EtOAc, with formation of the corresponding imidazolium salts as intermediates. Removal of the trityl protective group was effective (i) by solvolysis in refluxing methanol[9b] (18 h) for the preparation of **5a**,**c**,**d** or (ii) by the more rapid (1 h) classical method using trifluoroacetic acid giving **5b**,**e**; the yields for **5a**−**e** depend exclusively on the formation of imidazolium salt but not on the deprotection step. Further saponification of the ester function of **5a**−**e** with 2 N NaOH giving primary alcohols **6a**−**e** was followed by their oxidation (MnO_2, 30−40 °C, dioxane) providing 1-benzyl-5-formylimidazoles **7a**−**e**.

The key intermediates **15**−**17** were obtained (Scheme 2) from 4-piperidone according to our previously published protocol.[19]

Once N-protected by Boc, 4-piperidone **8** was condensed with an amino acid methyl ester (H-Met-OCH_3, H-Phe-OCH_3, or H-Ile-OCH_3), and reduction in situ by sodium cyanoborohydride gave the corresponding N-substituted amino esters **9**−**11**. After benzoylation and deprotection of piperidine with methanolic HCl, compounds **15**−**17** were subjected to reductive amination with imidazolecarbaldehydes **7a**−**e** to give final compounds **18a**−**e**, **19e**, and **20e**. Saponification of **19e** and **20e** with 2 N NaOH in methanol gave carboxylic acids **21e** and **22e**, respectively. The *N*-benzyl derivative **24** was prepared from **15** by reduction of the amide function with BH_3 (benzylaminopiperidine **23**) followed as above by reductive amination with 5-imidazolecarbaldehyde **7e**.

Biological Data

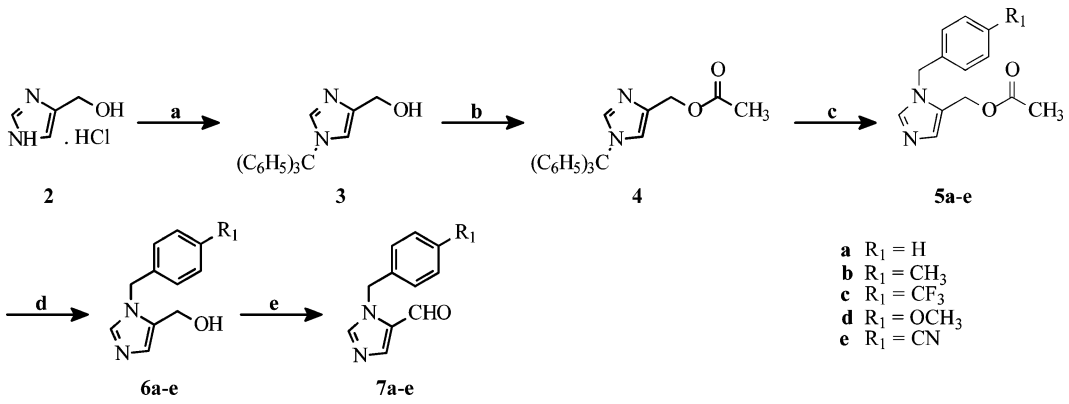
The compounds were tested for their ability to inhibit the FTase catalyzed transfer of the FPP moiety to dansyl-CVIM and GGT-I (which catalyses the transfer of the GGPP moiety to dansyl-GCVLL).[21] Biological data is summarized in Table 1.

Results and Discussion

Among FTIs based on the CA_1A_2X C-terminus sequence of farnesylated proteins, the most active compound **1**[19] (IC_{50} = 22.8 nM) includes Cys and Met, respectively, on its N- and C-termini and *N*-(4-piperidinyl)benzamide as a nonpeptidic template instead of the two central amino acid residues A_1A_2. The reported structure−activity relationship study[19] focused primarily on cysteine derivatives in which substituents were varied by different heterocycles such as thiazolidine, thiazole, or imidazole. The results revealed[19] decreased FTase inhibition (IC_{50} > 2.14 μM), showing the importance of Cys in recognizing the enzyme. Despite the modest activity of these analogues, we continued to investigate the 4-aminopiperidinyl core with a view to improving enzymatic and cellular potencies. We therefore decided to replace Cys by substituted 1-benzyl-4-imidazoylmethyl fragments because imidazole is known to be a putative binding element to the active site zinc atom[9b,20] and the 4-cyanobenzyl group a hydrophobic substituent to boost FTase activity.

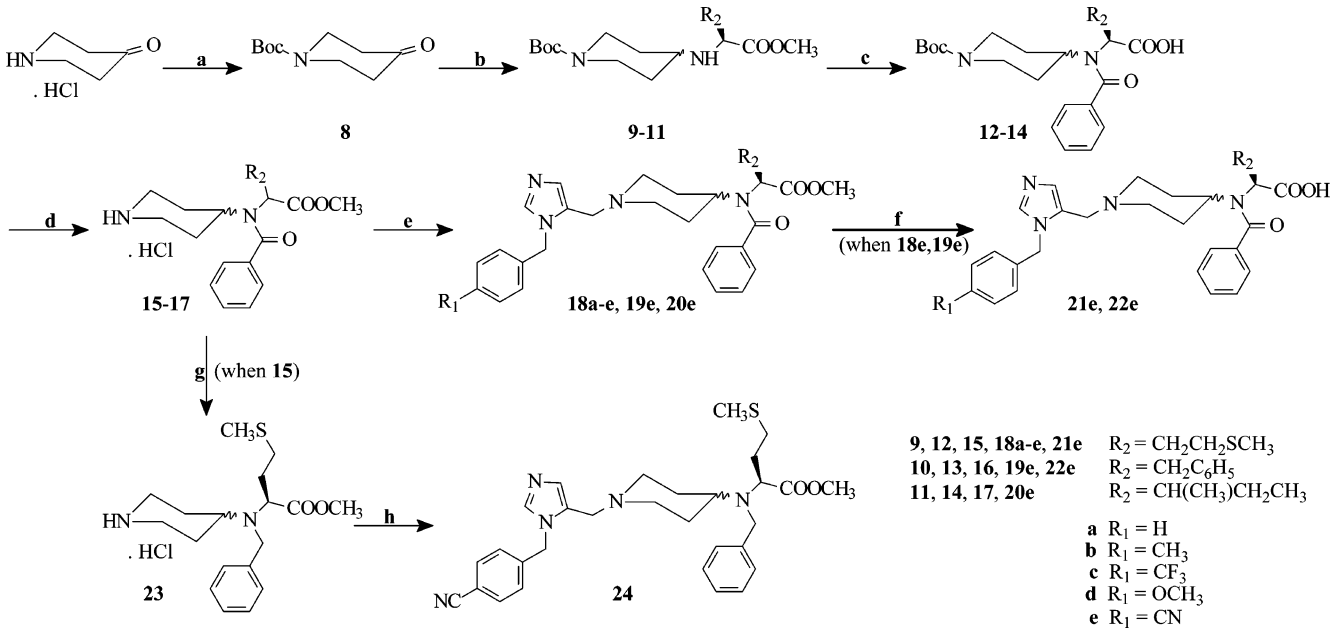
Compound **18e** displayed a more potent inhibition (4.5-fold) than that of **1** with an IC_{50} = 4.60 nM on an isolated enzyme assay. This finding concords with a previous report[9b] where imidazole may be successfully combined with a suitable hydrophobic substituent to obtain an effective cysteine substitute for FTIs. Moreover, no GGTase activity was detected in **18e**. GGT-I is responsible for most protein prenylation, and it has been shown that concomitant inhibition of FTase and GGT-I leads to substantial in vivo toxicity.[1c] Therefore, the highly significant FTase/GGTase selectivity led us to assess the role of Met-OCH_3 in recognizing the FTase enzyme versus GGTase. A previous study[8] revealed that selective prenylation of the cysteine residue either by FTase or by closely related GGT-I is triggered by the nature of the amino acid X in the CA_1A_2X sequence. Farnesylation is therefore induced by Ser or Met whereas Leu or Ile is responsible for geranylgeranylation, and both prenylations are possible with Phe or Trp. Changing Met for Phe (**19e**) or Ile (**20e**) varies the inhibition of FTase. Compared with that of the parent structure **18e**, the IC_{50} value of **19e** is 4-fold lower (IC_{50}

Scheme 1[a]



[a] Reagents and conditions: (a) $(C_6H_5)_3CCl$, NEt_3, DMF, rt, 72 h, 71%; (b) Ac_2O, pyridine, rt, 18 h, 84%; (c) (i) (*p*)$R_1-C_6H_4-CH_2Br$, EtOAc, 55 °C, 24 h; (ii) MeOH, reflux, 18 h, 30−77% for **5a**,**c**,**d** or TFA, rt, 1 h, 38−57% for **5b**,**e**; (d) 2 N NaOH, MeOH, rt, 0.5−2 h, 36−99%; (e) MnO_2, dioxane, 30−40 °C, 3 h, 70−97%.

Scheme 2[a]



[a] Reagents and conditions: (a) Boc_2O, DIEA, dioxane/H_2O (4:1), rt, 24 h, 75%; (b) H-(Met, Phe or Ile)-OCH_3, NEt_3, $NaBH_3CN$, MeOH, 3-Å molecular sieves, 50 °C, 48 h, 40−68%; (c) Benzoyl chloride, NEt_3, CH_2Cl_2, 0 °C, 1 h, then rt, 24 h, 30−55%; (d) HCl/MeOH, rt, 18 h, 95%; (e) (i) **7a**−**e**, NEt_3, MeOH, 3-Å molecular sieves, N_2, 50 °C, 4 h; (ii) $NaBH_3CN$, MeOH, 50 °C, 18 h, 42−52%; (f) (i) 2 N NaOH, MeOH, rt, 6 h; (ii) 2 N HCl, 80−90%; (g) (i) BH_3.THF, THF, 0 °C, 1 h, then rt, 18 h; (ii) 6 N HCl, H_2O, reflux, 10 min, 30%; (h) (i) **7e**, NEt_3, MeOH, 3-Å molecular sieves, N_2, 50 °C, 4 h; (ii) $NaBH_3CN$, MeOH, 50 °C, 18 h, 47%.

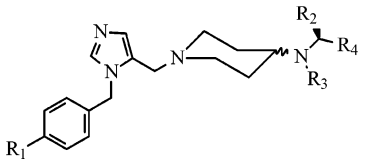
= 22.0 nM) whereas the Ile analogue **20e** is a comparable FTase inhibitor (IC_{50} = 32.7 nM), both of them being devoid of GGTase activity. The *N*-(4-piperidinyl)-benzamide scaffold therefore constitutes an interesting platform for FTase affinity and selectivity.

To assess the importance of the electronic nature of the benzylic substituent in this series of inhibitors, the electronic character of para-substituted benzylimidazole was varied. Inspection of Table 1 shows that FTase inhibitory potency is relatively sensitive to the electronic nature of this substituent rather than to its size.[25] Thus, an electron-donating group like methyl (**18b**, IC_{50} = 600 nM), methoxy (**18d**, IC_{50} = 575 nM), or a neutral hydrogen atom (**18a**, IC_{50} = 397 nM) led to a decrease in FTase inhibition. In contrast, the last was most effective with compounds containing strongly withdrawing groups such as 4-trifluoromethyl (**18c**, IC_{50} = 43.9 nM) or 4-cyano (**18e**, IC_{50} = 4.60 nM). Conformational restraints imposed by the carbonyl group of *N*-benzoyl-methioninate (**18e**) were finally released with *N*-benzyl (**24**) and gave a 2-fold increase in FTase activity (IC_{50} = 2.35 nM). Corresponding carboxylic acids of **18e** and **19e** revealed a good ability to inhibit the FTase enzyme (**21e**, IC_{50} = 10.0 nM; **22e**, IC_{50} = 54.1 nM).

Cellular proliferation (Table 2) was first investigated on L-1210 cells for the most potent 4-aminopiperidine derivatives (**1**, **18e**, **24**). The results showed a good response for **18e** (EC_{50} = 20.0 nM) and encouraged us to evaluate the affinity of these inhibitors in another cell type.

The DLD-1 (mutant K-Ras) human cell line was chosen because it is known that the K-Ras and N-Ras isoforms are most frequently mutated in human cancers. The results showed antiproliferative effects at low micromolar concentrations. The most active compound was **18e**. These results are in the same range as those

Table 1. FTase and GGTase Activities of Reference Compounds (Chart 3) and of **1**, **18a**−**e**, **19e**, **20e**, **21e**, **22e**, and **24** (Chart 2)



	R_1	R_2	R_3	R_4	IC_{50} (nM) FTase	IC_{50} (nM) GGTase
FTI_1[a]					34.0 ± 1.0	> 5000
FTI_2[b]					72.0 ± 15	> 100
$GGTI_1$[c]					56.2 ± 12.0	12.4 ± 7.3
1					22.8 ± 2.1	> 100
18a	H	$(CH_2)_2SCH_3$	COC_6H_5	$COOCH_3$	397 ± 84	nd[d]
18b	CH_3	$(CH_2)_2SCH_3$	COC_6H_5	$COOCH_3$	600 ± 87	nd[d]
18c	CF_3	$(CH_2)_2SCH_3$	COC_6H_5	$COOCH_3$	43.9 ± 4.2	nd[d]
18d	OCH_3	$(CH_2)_2SCH_3$	COC_6H_5	$COOCH_3$	575 ± 101	nd[d]
18e	CN	$(CH_2)_2SCH_3$	COC_6H_5	$COOCH_3$	4.60 ± 2.36	> 2000
19e	CN	$CH_2C_6H_5$	COC_6H_5	$COOCH_3$	22.0 ± 6.6	> 2000
20e	CN	$CH(CH_3)CH_2CH_3$	COC_6H_5	$COOCH_3$	32.7 ± 3.8	> 2000
21e	CN	$(CH_2)_2SCH_3$	COC_6H_5	COOH	10.0 ± 3.1	> 2000
22e	CN	$CH_2C_6H_5$	COC_6H_5	COOH	54.1 ± 12.0	> 2000
24	CN	$(CH_2)_2SCH_3$	$CH_2C_6H_5$	$COOCH_3$	2.35 ± 0.48	> 2000

[a] *N*-[2(*S*)-[2(*R*)-Amino-3-mercaptopropylamino]-3-methylbutyl]-Phe-Met-OH[22] (B.581). [b] H-Cys-4-Abz-Met-OH.[23] [c] H-Cys-4-(2-phenyl)-Abz-Leu-OH[24] (GGTI-287). FTI_1 and FTI_2 were used as positive controls for FTase activity and $GGTI_1$ for GGTase activity. [d] Not determined.

Table 2. Effect of 4-Aminopiperidine FTIs **1**, **18e**, and **24** on Cell Growth

	EC_{50} L-1210 (nM)	EC_{50} DLD-1 (μM)
1	> 10 000	18.1 ± 2.3
18e	20.0 ± 5.6	9.20 ± 2.47
24	> 5000	12.9 ± 3.8

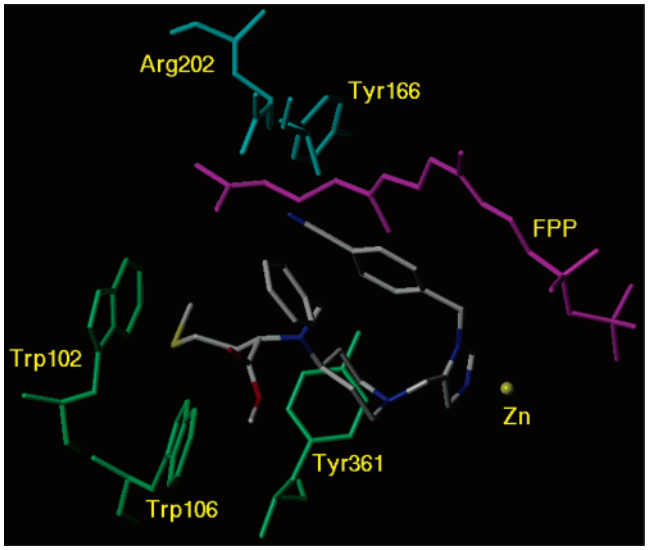


Figure 1. Docking of compound **18e** in the FTase binding site. The farnesyl group (FPP) is colored magenta. Arg202β and Tyr166α are shown in cyan, and the aromatic residue side chains that define a hydrophobic pocket (Trp102β, Trp106β, Tyr361β) are shown in green.

observed by Bell[16] showing high resistance of DLD-1 cells. In addition, **18e** blocks the growth of H-Ras transformed NIH3T3 fibroblasts (EC_{50} = 1.33 μM).

The docking experiment (Figure 1) shows how well **18e** fits with the active site of FTase. Figure 2 shows the superposition of compounds **18e** and **19e** after the docking of each molecule with the enzyme.

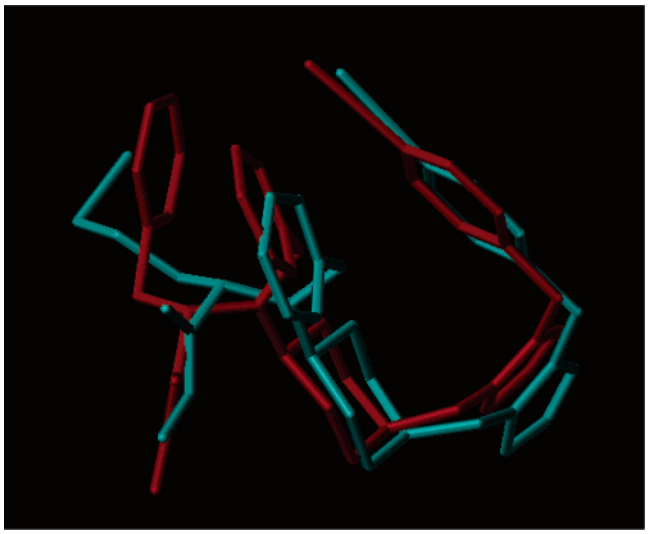


Figure 2. Overlap of compounds **18e** (cyan) and **19e** (red) docked in the FTase binding site.

The pharmacological results are in agreement with the predictions made by the modeling program GOLD and provide a reasonable explanation for the inhibitory activity of these compounds. In addition to the coordination observed between the distal nitrogen of imidazole and the zinc ion used as a starting fragment for the modeling study, the docking program revealed an additional interaction between Met-OCH_3 for **18e** (or Phe-OCH_3 for **19e**) and a hydrophobic pocket defined by Trp102β, Trp106β, and Tyr361β in the β-submit. Thus, the *N*-(4-piperidinyl)benzamide scaffold constitutes an interesting pattern to position Met-OCH_3 of **18e** or Phe-OCH_3 of **19e** in the aryl binding site of the FTase pocket and the imidazole to coordinate Zn^{2+}. This hydrophobic pocket has often been referred to as an important element for enzyme−inhibitor recognition.[26,27] In the case of CA_1A_2X peptide substrates, the hydrophobic pocket hosts a second aliphatic residue such as Ile in CVIM[27c] and could explain the FTase versus GGTase

selectivity of **18e** and **19e**. With regards to our modeling studies, compounds **18e** and **19e** constitute two CA_1A_2X competitive inhibitors. However, their interactions are quite different from those of classical CA_1A_2X inhibitors. The C-terminal side chain for X was positioned in the large hydrophobic pocket where the A_2 residue fits. This could explain why modification of the C-terminal residue X in our series failed to show dependence on FTase/GGTase selectivity. The CA_1A_2X competitive nature was also measured (**18e**, $K_{app} = 6.66 \pm 0.49$; **19e**, $K_{app} = 28.22 \pm 3.97$) and confirmed the modeling studies.

Furthermore, we observed that the cyanobenzyl group induces stacking with the FPP isoprenoid and fits Tyr166α and Arg202β with possible direct interactions. It is difficult to evaluate the importance of the electronic nature of the benzylic substituent with regard to the modeling study, but enzymatic studies show the important role of the electronic nature of para substituent, which could also enhance stacking with the isoprenoid FPP moiety.[26,27c] No interaction of the terminal $-COOCH_3$ residue in **18e** and **19e** as well as hydrogen bonds with the active site was observed, which confirms the good results of enzymatic inhibition by the corresponding carboxylic acids **21e** and **22e**.

Conclusion

This study has revealed that the *N*-(4-piperidinyl)-benzamide residue constitutes an effective platform for the discovery of noncysteinyl inhibitors of FTase. Compound **18e** shows a nanomolar affinity on an isolated FTase enzyme, significant FTase/GGTase selectivity, and cellular activity on L-1210 cell lines. Molecular modeling indicates possible interactions between the amino acids in the active site, and the two inhibitors **18e** and **19e** demonstrate their interaction with a hydrophobic pocket consisting of amino acids Trp102β, Trp106β, and Tyr361β.

The broad anticancer action of the *N*-(4-piperidinyl)-benzamide series merits further investigation as a potential lead structure in the search for new treatments against cancer. Molecular docking studies help to understand the interaction between these inhibitors and FTase. Other modulations are scheduled; the first step will consist of introducing diverse R_1 and R_4 substituents to increase bioavailability, antiproliferative activity, and enzymatic interaction. In addition, other proteins[28,29] such as Rheb or p70S6K have been shown to be functional when farnesylated and small nonpeptidic molecule inhibitors designed according to the CA_1A_2X box can block the Akt/mTOR/p70S6K signaling pathway.

Experimental Section

Chemistry. Melting points were determined with a Büchi 535 capillary melting point apparatus and remain uncorrected. Analytical thin-layer chromatography was performed on precoated Kieselgel $60F_{254}$ plates (Merck); the spots were located by UV (254 and 366 nm); R_f values are given for guidance. Silica gel 60 230−400 mesh purchased from Merck was used for column chromatography. The structures of all compounds were supported by IR (KBr pellets, FT-Bruker Vector 22 instrument) and by ^{1}H NMR at 300 MHz on a Bruker DRX-300 spectrometer. Chemical shifts were reported in ppm using tetramethylsilane as a standard, *J* values are in hertz, and the splitting patterns were designated as follows: s, singlet; d, doublet; t, triplet; m, multiplet. Mass spectra were recorded on a quadripolar Finnigan Mat SSQ 710 instrument. Elemental analyses were performed by the "Service Central d'Analyses" at the CNRS, Vernaison (France). Commercially available reagents and solvents were used throughout without further purification.

(1-Trityl-1*H*-4-imidazolyl)methanol (3). Trityl chloride (11.9 g, 40.7 mmol, 1.1 equiv) and NEt_3 (15.4 mL, 111.0 mmol, 3.0 equiv) were added to a suspension of 1*H*-4-imidazolylmethanol hydrochloride **2** (4.98 g, 37.0 mmol, 1.0 equiv) in dimethylformamide (100 mL). After being stirred at room temperature for 72 h, the solution was poured into 1.5 L of water. The precipitate was washed first with water and then with ether and recrystallized from dioxane to give **3** as a white solid (8.94 g, 71% yield). $R_f = 0.48$ (CH_2Cl_2/MeOH 9:1). Mp: 237−238 °C. IR: 3412 (OH), 1430 (C=C) cm^{-1}. ^{1}H NMR (DMSO-d_6): 4.62 (s, 2H), 6.58 (s, 1H), 7.05−7.17 (m, 7H), 7.28−7.40 (m, 9H), 7.54 (bs, 1H). Anal. ($C_{23}H_{20}N_2O$) C, H, N.

(1-Trityl-1*H*-4-imidazolyl)methyl Acetate (4). Acetic anhydride (4.2 mL, 43.5 mmol, 1.5 equiv) was added to a suspension of alcohol **3** (9.87 g, 29 mmol, 1.0 equiv) in pyridine (100 mL). The solution was stirred at room temperature for 18 h and then concentrated under reduced pressure. The solid obtained was recrystallized from cyclohexane to give **4** as a white powder (9.32 g, 84% yield). $R_f = 0.81$ (CH_2Cl_2/MeOH 9:1). Mp: 137−139 °C. IR: 1727 (CO) cm^{-1}. ^{1}H NMR (DMSO-d_6): 1.63 (s, 3H), 4.52 (s, 2H), 6.69 (s, 1H), 7.10−7.17 (m, 6H), 7.35−7.48 (m, 9H), 7.54 (s, 1H). Anal. ($C_{25}H_{22}N_2O_2$) C, H, N.

General Procedure for the Preparation of 1-Benzyl-1*H*-5-acetoxymethylimidazole Derivatives (5a−e). Compound **4** (7.65 g, 20.0 mmol, 1.0 equiv) in EtOAc (50 mL) was added to a solution of commercial (except for 4-methoxy) benzyl bromide derivatives (22.0 mmol, 1.1 equiv) in EtOAc (100 mL). After being stirred at 55 °C for 24 h and being cooled to room temperature, the resulting imidazolium salt was isolated by filtration and washed with EtOAc. The trityl protecting group was removed according to two procedures.

Procedure A. Imidazolium salt was added to MeOH (150 mL) and then stirred at reflux for 18 h. The solution was evaporated under reduced pressure, and the residue was triturated with a 5% citric acid solution. The solution was filtered and adjusted to pH 6 with a 5% Na_2CO_3 solution. The obtained precipitate was filtered, washed with ether, and used as such in the following reaction.

Procedure B. Imidazolium salt was added to TFA (30 mL), and the mixture was stirred for 1 h at room temperature. The solution was concentrated under reduced pressure, and TFA was evaporated with successive addition−evaporation cycles of CH_2Cl_2. The obtained solid was washed with ether and used as such in the following reaction.

(1-Benzyl-1*H*-5-imidazolyl)methyl Acetate (5a). Procedure A. White solid (4.79 g, 77% yield). $R_f = 0.59$ (CH_2Cl_2/MeOH 9:1). Mp: 99−100 °C. IR: 3122−2600 (CH), 1737 (CO) cm^{-1}. ^{1}H NMR (DMSO-d_6): 1.80 (s, 3H), 5.10 (s, 2H), 5.55 (s, 2H), 7.19−7.45 (m, 5H), 7.89 (s, 1H), 9.41 (s, 1H).

[1-(4-Methylbenzyl)-1*H*-5-imidazolyl]methyl Acetate Trifluoroacetate (5b). Procedure B. White solid (2.72 g, 38% yield). $R_f = 0.50$ (CH_2Cl_2/MeOH 9:1). Mp: 147−149 °C. IR: 3126−2601 (CH), 1736 (CO) cm^{-1}. ^{1}H NMR (DMSO-d_6): 1.85 (s, 3H), 2.30 (s, 3H), 5.08 (s, 2H), 5.47 (s, 2H), 7.18−7.29 (m, 4H), 7.85 (s, 1H), 9.33 (s, 1H).

[1-(4-Trifluoromethylbenzyl)-1*H*-5-imidazolyl]methyl Acetate (5c). Procedure A. White solid (5.23 g, 69% yield). $R_f = 0.53$ (CH_2Cl_2/MeOH 9:1). Mp: 76−78 °C. IR: 3054−2925 (CH), 1738 (CO) cm^{-1}. ^{1}H NMR (DMSO-d_6): 1.84 (s, 3H), 4.97 (s, 2H), 5.25 (s, 2H), 7.15 (s, 1H), 7.25−7.36 (m, 4H), 7.55 (s, 1H).

[1-(4-Methoxybenzyl)-1*H*-5-imidazolyl]methyl Acetate (5d). Procedure A. White solid (2.05 g, 30% yield). $R_f = 0.51$ (CH_2Cl_2/MeOH 9:1). Mp: 80−81 °C. IR: 3004−2837 (CH), 1739 (CO) cm^{-1}. ^{1}H NMR (DMSO-d_6): 1.93 (s, 3H), 3.78 (s, 3H), 4.97 (s, 2H), 5.08 (s, 2H), 6.85 (d, 2H, $J = 7.5$), 7.01 (d, 2H, $J = 7.5$), 7.13 (s, 1H), 7.54 (s, 1H).

[1-(4-Cyanobenzyl)-1*H*-5-imidazolyl]methyl Acetate Trifluoroacetate (5e). Procedure B. White solid (4.21 g, 57%

yield). R_f = 0.42 (CH_2Cl_2/MeOH 9:1). Mp: 198−200 °C. IR: 3093−2601 (CH), 2238 (CN), 1742 (CO) cm^{-1}. ^1H NMR (DMSO-d_6): 1.84 (s, 3H), 5.09 (s, 2H), 5.69 (s, 2H), 7.48−7.92 (m, 4H), 7.88 (s, 1H), 9.41 (s, 1H).

General Procedure for the Preparation of 1-Benzyl-1*H*-5-hydroxymethylimidazole Derivatives (6a−e). Compounds **5a−e** (10.0 mmol, 1.0 equiv) were dissolved in MeOH (40 mL), 2 N NaOH (40 mmol, 4.0 equiv) was added, and the resulting mixture was stirred at room temperature for 0.5−2 h. The solution was neutralized with 2 N HCl (41 mmol, 4.1 equiv), and the solvent was removed under reduced pressure. The solid was filtered, washed with water and ether, and finally purified by column chromatography on silica gel using CH_2Cl_2/MeOH as eluent.

1-Benzyl-1*H*-5-hydroxymethylimidazole (6a). White solid (1.05 g, 56% yield). R_f = 0.32 (CH_2Cl_2/MeOH 9:1). Mp: 135−136 °C. IR: 3203 (OH), 3118−2876 (CH) cm^{-1}. ^1H NMR (DMSO-d_6): 4.32 (s, 2H), 5.17 (s, 1H), 5.24 (s, 2H), 6.84 (s, 1H), 7.18 (m, 2H), 7.29−7.36 (m, 3H), 7.70 (s, 1H). Anal. ($C_{11}H_{12}N_2O$) C, H, N.

1-(4-Methylbenzyl)-1*H*-5-hydroxymethylimidazole (6b). White solid (2.00 g, 99% yield). R_f = 0.28 (CH_2Cl_2/MeOH 9:1). Mp: 136−138 °C. IR: 3217 (OH), 2945−2880 (CH) cm^{-1}. ^1H NMR (DMSO-d_6): 2.27 (s, 3H), 3.37 (s, 1H), 4.30 (s, 2H), 5.17 (s, 2H), 6.80 (s, 1H), 7.05−7.16 (m, 4H), 7.68 (s, 1H). Anal. ($C_{12}H_{14}N_2O$) C, H, N.

1-(4-Trifluoromethylbenzyl)-1*H*-5-hydroxymethylimidazole (6c). White solid (930 mg, 36% yield). R_f = 0.26 (CH_2Cl_2/MeOH 9:1). Mp: 134−136 °C. IR: 3229 (OH), 3118−2880 (CH) cm^{-1}. ^1H NMR (DMSO-d_6): 4.32 (s, 2H), 5.16 (s, 1H), 5.24 (s, 2H), 6.83 (s, 1H), 7.15−7.18 (m, 2H), 7.28−7.37 (m, 2H), 7.69 (s, 1H). Anal. ($C_{12}H_{11}F_3N_2O$) C, H, N.

1-(4-Methoxybenzyl)-1*H*-5-hydroxymethylimidazole (6d). White solid (1.83 g, 84% yield). R_f = 0.23 (CH_2Cl_2/MeOH 9:1). Mp: 129−130 °C. IR: 3196 (OH), 3118−2837 (CH) cm^{-1}. ^1H NMR (DMSO-d_6): 3.73 (s, 3H), 4.33 (s, 2H), 5.14 (s, 2H), 5.18 (s, 1H), 6.80 (s, 1H), 6.91 (d, 2H, J = 8.5), 7.15 (d, 2H, J = 8.5), 7.65 (s, 1H). Anal. ($C_{12}H_{14}N_2O_2$) C, H, N.

1-(4-Cyanobenzyl)-1*H*-5-hydroxymethylimidazole (6e). White solid (2.04 g, 96% yield). R_f = 0.19 (CH_2Cl_2/MeOH 9:1). Mp: 168−169 °C. IR: 3121 (OH), 2883−2753 (CH), 2232 (CN) cm^{-1}. ^1H NMR (DMSO-d_6): 4.31 (s, 2H), 5.37 (m, 3H), 6.87 (s, 1H), 7.73 (m, 2H), 7.74 (s, 1H), 7.83 (d, 2H, J = 8.5). Anal. ($C_{12}H_{11}N_3O$) C, H, N.

General Procedure for the Preparation of 1-Benzyl-5-formylimidazoles (7a−e). Imidazoles **6a−e** (6.0 mmol, 1.0 equiv) were dissolved in dioxane (50 mL) before adding MnO_2 (30.0 mmol, 5.0 equiv). The suspension was stirred at 30−40 °C for 3 h, then MnO_2 was filtered and washed with dioxane. The solution was evaporated under reduced pressure, and the residue was purified by column chromatography on silica gel using CH_2Cl_2/MeOH (97:3 → 99:1) as eluent.

1-Benzyl-1*H*-5-imidazolecarbaldehyde (7a). White solid (782 mg, 70% yield). R_f = 0.35 (CH_2Cl_2/MeOH 9:1). Mp: 135−136 °C. IR: 1670 (CO) cm^{-1}. ^1H NMR ($CDCl_3$): 5.52 (s, 2H), 7.21 (d, 2H, J = 8.5), 7.33 (m, 3H), 7.70 (s, 1H), 7.83 (s, 1H), 9.76 (s, 1H). Anal. ($C_{11}H_{10}N_2O$) C, H, N.

1-(4-Methylbenzyl)-1*H*-5-imidazolecarbaldehyde (7b). White solid (781 mg, 65% yield). R_f = 0.49 (CH_2Cl_2/MeOH 9:1). Mp: 92−94 °C. IR: 3087−2918 (CH), 1672 (CO) cm^{-1}. ^1H NMR ($CDCl_3$): 5.30 (s, 3H), 5.47 (s, 2H), 7.10−7.17 (m, 4H), 7.68 (s, 1H), 7.82 (s, 1H), 9.76 (s, 1H). Anal. ($C_{12}H_{12}N_2O$) C, H, N.

1-(4-Trifluoromethylbenzyl)-1*H*-5-imidazolecarbaldehyde (7c). White solid (991 mg, 97% yield). R_f = 0.58 (CH_2Cl_2/MeOH 9:1). Mp: 34−35 °C. IR: 3111−2831 (CH), 1673 (CO) cm^{-1}. ^1H NMR ($CDCl_3$): 5.58 (s, 2H), 7.28 (m, 2H), 7.59 (m, 2H), 7.76 (s, 1H), 7.86 (s, 1H), 9.74 (s, 1H). Anal. ($C_{12}H_9F_3N_2O$) C, H, N.

1-(4-Methoxybenzyl)-1*H*-5-imidazolecarbaldehyde (7d). White solid (986 mg, 76% yield). R_f = 0.59 (CH_2Cl_2/MeOH 9:1). Mp: 40−41 °C. IR: 3088−2837 (CH), 1673 (CO) cm^{-1}. ^1H NMR ($CDCl_3$): 3.78 (s, 3H), 5.43 (s, 2H), 6.86 (d, 2H, J = 6.7), 7.18 (d, 2H, J = 6.7), 7.66 (s, 1H), 7.80 (s, 1H), 9.75 (s, 1H). Anal. ($C_{12}H_{12}N_2O_2$) C, H, N.

4-[(5-Formyl-1*H*-1-imidazolyl)methyl]benzonitrile (7e). White solid (887 mg, 70% yield). R_f = 0.35 (CH_2Cl_2/MeOH 9:1). Mp: 135−136 °C. IR: 2227 (CN), 1670 (CO) cm^{-1}. ^1H NMR ($CDCl_3$): 5.58 (s, 2H), 7.26 (d, 2H, J = 8.5), 7.65 (d, 2H, J = 8.5), 7.78 (s, 1H), 7.88 (s, 1H), 9.73 (s, 1H). Anal. ($C_{12}H_9N_3O$) C, H, N.

***N*-Boc-4-piperidone (8).** DIEA (28.3 mL, 163 mmol, 2.5 equiv) was added to a solution of 4-piperidone hydrochloride (8.80 g, 65.0 mmol, 1.0 equiv) in 200 mL of a dioxane/H_2O (4:1) mixture. Di-*tert*-butyl dicarbonate (21.1 g, 97.5 mmol, 1.5 equiv) was added dropwise (over 1 h), and the resulting mixture was stirred at room temperature for 24 h. The solvent was evaporated under reduced pressure, and the residue was poured into a 5% citric acid solution and extracted with dichloromethane. The organic phase was dried ($MgSO_4$) and concentrated to give a solid which was recrystallized from cyclohexane: white solid (9.71 g, 75% yield). R_f = 0.46 (cyclohexane/EtOAc 1:1). Mp: 73−75 °C. IR: 1715 (CO), 1680 (CO) cm^{-1}. ^1H NMR ($CDCl_3$): 1.55 (s, 9H), 2.45 (t, 4H, J = 6.2), 3.75 (t, 4H, J = 6.2). Anal. ($C_{10}H_{17}NO_3$) C, H, N.

General Procedure for the Preparation of Piperidines 9−11. *N*-Boc-piperidone **8** (6.97 g, 35.0 mmol, 1.0 equiv) and NEt_3 (7.30 mL, 52.5 mmol, 1.5 equiv) in 100 mL of dry methanol were added to a mixture of methyl ester of Met, Phe, or Ile (52.5 mmol, 1.5 equiv) and 3-Å molecular sieves in 50 mL of dry methanol. The reaction was stirred at 50 °C for 4 h (nitrogen), and then $NaBH_3CN$ (3.30 g, 52.5 mmol, 1.5 equiv) was added in one batch. The mixture was stirred for 48 h. The sieves were filtered off, and the solvent was removed under reduced pressure. The residue was dissolved in EtOAc, NEt_3·HCl was filtered off, and the filtrate was washed with a 5% Na_2CO_3 solution, and then H_2O and brine. The organic phase was dried ($MgSO_4$) and concentrated under reduced pressure. The residue obtained was triturated in petroleum ether and recrystallized from pentane to give **9−11** as solids.

Methyl *N*-[4-(1-Boc-piperidinyl)]methioninate (9). White solid (7.91 g, 68% yield). R_f = 0.60 (hexane/EtOAc 2:8). Mp: 74−76 °C. IR: 1733 (CO), 1695 (CO) cm^{-1}. ^1H NMR ($CDCl_3$): 1.20−1.40 (m, 4H), 1.50 (s, 9H), 1.65−1.80 (m, 4H), 1.90 (m, 1H), 2.10 (s, 3H), 2.50−2.70 (m, 3H), 2.85 (m, 1H), 3.45−3.55 (m, 1H), 3.70 (s, 3H), 4.00 (m, 1H). Anal. ($C_{16}H_{30}N_2O_4S$) C, H, N.

Methyl *N*-[4-(1-Boc-piperidinyl)]phenylalaninate (10). White solid (7.99 g, 63% yield). R_f = 0.49 (cyclohexane/EtOAc 3:7). Mp: 53−54 °C. IR: 1733 (CO), 1694 (CO) cm^{-1}. ^1H NMR ($CDCl_3$): 1.30−1.70 (m, 4H), 1.44 (s, 9H), 2.45−4.11 (m, 11H), 4.20−4.25 (m, 1H), 7.13−7.33 (m, 5H). Anal. ($C_{20}H_{30}N_2O_4$) C, H, N.

Methyl *N*-[4-(1-Boc-piperidinyl)]isoleucinate (11). White solid (4.60 g, 40% yield). R_f = 0.63 (cyclohexane/EtOAc 3:7). Mp: 26−28 °C. IR: 1735 (CO), 1696 (CO) cm^{-1}. ^1H NMR ($CDCl_3$): 0.83−0.92 (m, 6H), 1.18−2.54 (m, 9H), 1.44 (s, 9H), 3.38−4.12 (m, 5H), 3.75 (s, 3H). Anal. ($C_{17}H_{32}N_2O_4$) C, H, N.

General Procedure for the Preparation of Benzoylpiperidines 12−14. Compounds **9−11** (14.5 mmol, 1.0 equiv) were added to a solution of NEt_3 (21.8 mmol, 1.5 equiv) in 100 mL of dry CH_2Cl_2. The mixture was cooled to 0 °C, then benzoyl chloride (17.4 mmol, 1.2 equiv) was added dropwise over 1 h, and the solution was stirred at room temperature for 24 h (reflux for **11**). The solvent was evaporated under reduced pressure, and the residue was partitioned between a 5% Na_2CO_3 solution and ether. The organic phase was washed successively with 1 N HCl and brine, dried ($MgSO_4$), and evaporated under reduced pressure. The resulting oil was flash chromatographied on a 5 cm × 40 cm column using cyclohexane/EtOAc (2:1; 7:3; 3:1, respectively) as eluent to give **12−14**.

Methyl *N*-Benzoyl,*N*-(1-Boc-4-piperidin-4-yl)methioninate (12). White solid (3.48 g, 55% yield). R_f = 0.32 (cyclohexane/EtOAc 1:1). Mp: 55 °C. IR: 1741 (CO), 1691 (CO), 1637 (CO) cm^{-1}. ^1H NMR (DMSO-d_6): 1.40 (s, 9H), 1.70−1.90 (m, 4H), 2.10 (s, 3H), 2.40−2.60 (m, 8H), 3.60 (s, 3H), 3.90 (bs, 1H), 4.10 (bs, 1H), 7.30−7.50 (m, 5H). Anal. ($C_{23}H_{34}N_2O_5S$) C, H, N.

Methyl *N*-Benzoyl,*N*-(1-Boc-4-piperidin-4-yl)phenylalaninate (13). Oil (3.04 g, 45% yield). $R_f = 0.37$ (cyclohexane/EtOAc 1:1). IR: 1742 (CO), 1694 (CO), 1640 (CO) cm^{-1}. ^1H NMR ($CDCl_3$): 1.40 (s, 9H), 1.72−2.04 (m, 4H), 2.95−3.15 (m, 2H), 3.42−3.71 (m, 4H), 3.83 (s, 3H), 4.00−4.31 (m, 2H), 7.20−7.47 (m, 10H). Anal. ($C_{27}H_{34}N_2O_5$) C, H, N.

Methyl *N*-Benzoyl,*N*-(1-Boc-4-piperidin-4-yl)isoleucinate (14). Oil (1.88 g, 30% yield). $R_f = 0.50$ (cyclohexane/EtOAc 1:1). IR: 1742 (CO), 1694 (CO), 1643 (CO) cm^{-1}. ^1H NMR ($CDCl_3$): 0.85−0.95 (m, 6H), 1.20−1.40 (m, 2H), 1.44 (s, 9H), 1.65−2.02 (m, 4H), 2.35−3.40 (m, 5H), 3.60−4.11 (m, 2H), 3.75 (s, 3H), 7.20−7.40 (m, 5H). Anal. ($C_{24}H_{36}N_2O_5$) C, H, N.

General Procedure for the Preparation of Piperidines 15−17. Compounds **12**−**14** (2.2 mmol, 1.0 equiv) were dissolved in MeOH (10 mL), and saturated methanolic hydrochloric acid (30 mL) was added. The solution was left standing for 18 h. The solvent was evaporated under reduced pressure, and the excess of HCl was eliminated by successive addition−evaporation cycles of MeOH. The residue obtained was triturated with dry ether to give white hygroscopic compounds **15**−**17**, which were directly used in the following reaction.

Methyl *N*-Benzoyl,*N*-(piperidin-4-yl)methioninate Hydrochloride (15). White hygroscopic solid (807 mg, 95% yield). $R_f = 0.06$ (CH_2Cl_2/MeOH 9:1, saturated NH_3). Mp: 109−110 °C. IR: 1738 (CO), 1637 (CO) cm^{-1}. ^1H NMR (DMSO-d_6): 1.62−1.80 (m, 4H), 2.11 (s, 3H), 2.50−2.70 (m, 4H), 3.10−3.30 (m, 4H), 3.62 (s, 3H), 3.80 (m, 1H), 4.01 (m, 1H), 7.30−7.50 (m, 5H), 8.82 (s, 2H).

Methyl *N*-Benzoyl,*N*-(piperidin-4-yl)phenylalaninate Hydrochloride (16). White hygroscopic solid (842 mg, 95% yield). $R_f = 0.12$ (CH_2Cl_2/MeOH 9:1, saturated NH_3). IR: 1739 (CO), 1633 (CO) cm^{-1}. ^1H NMR ($CDCl_3$): 1.88−2.26 (m, 4H), 2.54−3.02 (m, 6H), 3.83 (s, 3H), 4.01 (m, 1H), 5.20 (m, 1H), 7.17−7.56 (m, 10H), 9.23 (bs, 2H).

Methyl *N*-Benzoyl,*N*-(piperidin-4-yl)isoleucinate Hydrochloride (17). White hygroscopic solid (771 mg, 95% yield). $R_f = 0.23$ (CH_2Cl_2/MeOH 9:1, saturated NH_3). IR: 1741 (CO), 1637 (CO) cm^{-1}. ^1H NMR ($CDCl_3$): 0.82−0.93 (m, 6H), 1.20−2.12 (m, 5H), 2.33−3.20 (m, 6H), 3.53 (s, 3H), 4.08 (m, 1H) 5.14 (m, 1H), 7.02−7.26 (m, 5H), 8.80 (bs, 2H).

General Procedure for the Preparation of Imidazoles 18a−e, 19e, 20e, and 24. Amines **15**−**17** or **23** (7.5 mmol, 1.5 equiv), NEt_3 (7.5 mmol, 1.5 equiv), and the corresponding 1-benzyl-5-imidazolecarbaldehydes **7a**−**e** were dissolved in 50 mL of dry MeOH. Molecular sieves (3-Å) were added, and the solution was stirred at 50 °C for 4 h in a nitrogen atmosphere. Then $NaBH_3CN$ (0.32 g, 5.0 mmol, 1.0 equiv) was added, and the mixture was stirred for 18 h. The sieves were filtered off, and the solvent was removed under reduced pressure. The residue was dissolved in EtOAc, NEt_3·HCl was filtered off, and the filtrate was washed with a 5% Na_2CO_3 solution, then H_2O and brine. The organic phase was dried ($MgSO_4$) and concentrated under reduced pressure. The residue obtained was flash chromatographied on a 5 cm × 40 cm column using CH_2Cl_2/MeOH 96:4 as eluent to give **18a**−**e**, **19e**, **20e**, or **24**.

Methyl *N*-Benzoyl,*N*-[1-(1-benzylimidazol-5-ylmethyl)-piperidin-4-yl]methioninate (18a). White solid (1.14 g, 44% yield). $R_f = 0.47$ (CH_2Cl_2/MeOH 9:1). Mp: 64−66 °C. ^1H NMR ($CDCl_3$): 1.80−2.17 (m, 5H), 2.15 (s, 3H), 2.32−2.90 (m, 7H), 3.68 (s, 3H), 3.88−4.13 (m, 3H), 5.24 (s, 2H), 5.60 (m, 1H) 6.87 (s, 1H), 7.07−7.67 (m, 11H). MS (EI): 520. Anal. ($C_{29}H_{36}N_4O_3S$) C, H, N.

Methyl *N*-Benzoyl,*N*-{1-[1-(4-methylbenzyl)imidazol-5-ylmethyl]piperidin-4-yl}methioninate (18b). White solid (1.39 g, 52% yield). $R_f = 0.47$ (CH_2Cl_2/MeOH 9:1). Mp: 60−62 °C. ^1H NMR ($CDCl_3$): 1.81−2.18 (m, 5H), 2.14 (s, 3H), 2.30−2.94 (m, 7H), 2.32 (s, 3H), 3.77 (s, 3H), 3.88−4.13 (m, 3H), 5.18 (s, 2H), 5.58 (m, 1H) 6.84 (s, 1H), 6.97−7.50 (m, 10H). MS (EI): 534. Anal. ($C_{30}H_{38}N_4O_3S$) C, H, N.

Methyl *N*-Benzoyl,*N*-{1-[1-(4-trifluoromethylbenzyl)-imidazol-5-ylmethyl]piperidin-4-yl}methioninate (18c). White solid (1.23 g, 42% yield). $R_f = 0.52$ (CH_2Cl_2/MeOH 9:1). Mp: 84−86 °C. ^1H NMR ($CDCl_3$): 1.79−2.17 (m, 5H), 2.14 (s, 3H), 2.29−2.93 (m, 7H), 3.56 (s, 3H), 3.88−4.13 (m, 3H), 5.31 (s, 2H), 5.58 (m, 1H), 6.89 (s, 1H), 7.32−7.66 (m, 10H). MS (EI): 588. Anal. ($C_{30}H_{35}F_3N_4O_3S$) C, H, N.

Methyl *N*-Benzoyl,*N*-{1-[1-(4-methoxybenzyl)imidazol-5-ylmethyl]piperidin-4-yl}methioninate (18d). White solid (1.40 g, 51% yield). $R_f = 0.54$ (CH_2Cl_2/MeOH 9:1). Mp: 56−58 °C. ^1H NMR ($CDCl_3$): 1.80−2.18 (m, 5H), 2.14 (s, 3H), 2.32−2.91 (m, 7H), 3.76 (s, 3H), 3.80 (s, 3H), 3.89−4.14 (m, 3H), 5.15 (s, 2H), 5.57 (m, 1H) 6.80−6.91 (m, 3H), 7.03−7.52 (m, 8H). MS (EI): 550. Anal. ($C_{30}H_{38}N_4O_4S$) C, H, N.

Methyl *N*-Benzoyl,*N*-{1-[1-(4-cyanobenzyl)imidazol-5-ylmethyl]piperidin-4-yl}methioninate (18e). White solid (1.36 g, 50% yield). $R_f = 0.47$ (CH_2Cl_2/MeOH 9:1). Mp: 182−184 °C. ^1H NMR (DMSO-d_6): 1.78−2.17 (m, 5H), 2.14 (s, 3H), 2.30−2.54 (m, 4H), 2.80−3.10 (m, 2H), 3.66 (s, 3H), 3.73−4.05 (m, 3H), 5.58 (m, 1H), 5.79 (s, 2H), 6.86 (s, 1H), 6.92 (s, 1H), 7.11−7.82 (m, 10H). MS (EI): 545. Anal. ($C_{30}H_{35}N_5O_3S$) C, H, N.

Methyl *N*-Benzoyl,*N*-{1-[1-(4-cyanobenzyl)imidazol-5-ylmethyl]piperidin-4-yl}phenylalaninate (19e). White solid (1.40 g, 50% yield). $R_f = 0.61$ (CH_2Cl_2/MeOH 9:1). Mp: 103−105 °C. ^1H NMR ($CDCl_3$): 1.78−1.92 (m, 4H), 2.41−2.47 (m, 2H), 2.79−3.16 (m, 4H), 3.70 (s, 3H), 3.83 (s, 2H), 4.20 (m, 1H), 4.98 (m, 1H), 5.24 (s, 2H), 6.92 (s, 1H), 7.09−7.80 (m, 15H). MS (EI): 561. Anal. ($C_{34}H_{35}N_5O_3$) C, H, N.

Methyl *N*-Benzoyl,*N*-{1-[1-(4-cyanobenzyl)imidazol-5-ylmethyl]piperidin-4-yl}isoleucinate (20e). White solid (1.34 g, 51% yield). $R_f = 0.59$ (CH_2Cl_2/MeOH 9:1). Mp: 81−83 °C. ^1H NMR ($CDCl_3$): 0.82−0.93 (m, 6H), 1.18−1.38 (m, 2H), 1.79−2.01 (m, 5H), 2.44−2.83 (m, 4H), 3.45 (s, 3H), 3.77 (s, 2H), 3.88−4.12 (m, 3H), 5.19 (m, 1H), 6.92 (s, 1H), 7.20−7.80 (m, 10H). MS (EI): 527. Anal. ($C_{31}H_{37}N_5O_3$) C, H, N.

Methyl *N*-Benzyl,*N*-{1-[1-(4-cyanobenzyl)imidazol-5-ylmethyl]piperidin-4-yl}methioninate (24). White solid (1.25 g, 47% yield). $R_f = 0.50$ (CH_2Cl_2/MeOH 9:1). Mp: 189−191 °C. ^1H NMR ($CDCl_3$): 1.74−2.18 (m, 6H), 2.09 (s, 3H), 2.31−2.78 (m, 7H), 3.53 (m, 2H), 3.65 (s, 3H), 3.75−4.10 (m, 3H), 5.23 (s, 2H), 6.84 (s, 1H), 7.16−7.61 (m, 10H). MS (EI): 531. Anal. ($C_{30}H_{37}N_5O_2S$) C, H, N.

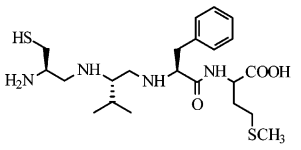
General Procedure for the Preparation of Carboxylic Acids 21e and 22e. Esters **18e** or **19e** (0.1 mmol, 1.0 equiv) were dissolved in methanol (10 mL), and 2 N NaOH (10 mL) was added. The reaction was monitored by analytical TLC and stirred for about 6 h. The solution was neutralized (2 N HCl, pH 4) and evaporated under reduced pressure, and the residue was purified by column chromatography on silica gel using CH_2Cl_2/MeOH (97:3 → 99:1) as eluent.

***N*-Benzoyl,*N*-{1-[1-(4-cyanobenzyl)imidazol-5-ylmethyl]piperidin-4-yl}methionine (21e).** White solid (42 mg, 80% yield). $R_f = 0.15$ (CH_2Cl_2/MeOH 8:2). Mp: > 250 °C. ^1H NMR (DMSO-d_6): 1.82−2.19 (m, 5H), 2.20 (s, 3H), 2.30−2.55 (m, 4H), 2.80−4.32 (m, 7H), 5.87 (s, 2H), 7.31−8.10 (m, 10H), 9.36 (s, 1H), 11.86 (bs, 1H). MS (EI): 531. Anal. ($C_{29}H_{33}N_5O_3S$) C, H, N.

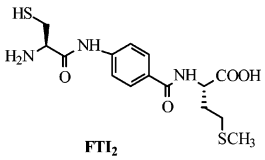
***N*-Benzoyl,*N*-{1-[1-(4-cyanobenzyl)imidazol-5-ylmethyl]piperidin-4-yl}phenylalanine (22e).** White solid (49 mg, 90% yield). $R_f = 0.15$ (CH_2Cl_2/MeOH 8:2). Mp: > 250 °C. ^1H NMR (DMSO-d_6): 1.80−1.95 (m, 4H), 2.41−2.47 (m, 2H), 2.78−3.15 (m, 4H), 3.83 (s, 2H), 4.00−4.30 (m, 2H), 5.85 (s, 2H), 7.08−7.90 (m, 15H), 9.10 (s, 1H), 11.80 (bs, 1H). MS (EI): 547. Anal. ($C_{33}H_{33}N_5O_3$) C, H, N.

Methyl *N*-Benzyl,*N*-(piperidin-4-yl)methioninate Hydrochloride (23). Compound **15** (1.0 g, 2.6 mmol, 1.0 equiv) was dissolved in 20 mL of dry THF. The solution was cooled to 0 °C, then BH_3 in THF (10.3 mL, 10.3 mmol, 4.0 equiv) was added dropwise over 1 h. The reaction was stirred at room temperature for 18 h and stopped by adding 5 mL of 6 N HCl. THF was evaporated under reduced pressure, and the aqueous residue was refluxed for 10 min. The solution was alkalinized (K_2CO_3) to pH > 10 and extracted with ether. The organic phase was dried ($MgSO_4$) and flash chromatographied on a 5 cm × 40 cm column using CH_2Cl_2/MeOH 9:1 as eluent. Piperidine **23** was finally obtained as a hydrochloride salt by adding a solution of HCl saturated ether and recrystallized from EtOAc. White hygroscopic solid (0.29 g, 30% yield). $R_f =$

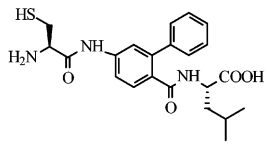
Chart 3



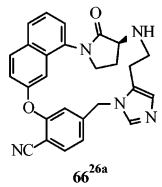
FTI$_1$



FTI$_2$



GGTI$_1$



66[26a]

0.18 (CH_2Cl_2/MeOH 9:1, saturated NH_3). Mp: 125 °C. IR: 1741 (CO) cm^{-1}. ^1H NMR ($CDCl_3$): 1.61−1.82 (m, 4H), 2.09 (s, 3H), 2.10−3.04 (m, 9H), 3.42 (m, 2H), 3.70 (s, 3H), 4.15 (m, 1H), 4.82 (m, 1H), 7.11−7.45 (m, 5H). MS (EI): 336.

Preparation of FTase from the Cytosolic Fraction of Rat Brain.[21a] Sprague−Dawley male rats (9−10 weeks old) were sacrified, and brains were placed in an ice-cold 0.1 M HEPES buffer, pH 7.4, 25 mM $MgCl_2$, and 10 mM DTT (dithiothreitol). The brains were cleaned and homogenized in a 0.1 M HEPES buffer, pH 7.4, 1 mM $MgCl_2$, and 1 mM DTT. The homogenates were centrifuged at 10 000*g* for 20 min at 4 °C. The supernatants were centrifuged at 100 000g for 1 h at 4 °C. The cytosolic fraction (the 100 000*g* supernatants) was stored at −80 °C. Protein concentration was determined by the bicinchonic acid protein assay method (Pierce) using bovine serum albumin as protein standard.

Protein Prenyl Transferase Assay. FTase activity was determined by a continuous fluorescence assay as previously described.[21b] The data were collected on a Spex Fluoro Max spectrofluorimeter. The optimal parameters are an excitation wavelength at 340 nm and an emission at 505 nm (slit 10,10) for dansyl-CVIM and dansyl-GCVLL. The standard reaction mixture containing 50 mM TrisHCl, pH 7.5, 5 mM $MgCl_2$, 10 μM $ZnCl_2$, 5 mM DTT, 0.01% *n*-dodecyl-β-D-maltoside, 5.4 mg/mL of cytosolic protein, 1.3 μM dansyl-CVIM, and 25 μM FPP for the FTase assay and 4 μM dansyl-GCVLL and 10 μM GGPP for the GGT-I assay was incubated at 25 °C, and fluorescence intensity was recorded for 10 min. Study of cross prenylation of dansyl-CVIM by GGTase with FPP or GGPP was performed. No variation of fluorescence was observed in any cases. Graphical representations of fluorescence variation $\Delta F/\Delta t = f$[ligand] gave sigmoid plots. Analysis of cell curves made it possible to calculate the IC_{50} values of each compound by using a theoretical equation (Graphpad Prism 3.03, Graphpad software, San Diego, CA).

Competitive inhibition patterns of **18e** and **19e** were performed as previously described[21b] by varying CVIM concentration in the presence of competitor concentration. The mathematical method of Lineweaver and Burk[21b] showed unvaried V_m and varied K_m.

Cell Culture and Growth Assays. DLD-1 (human colon adenocarcinoma transformed by K-Ras oncogene), L-1210 (mouse lymphocytic leukaemia), and H-Ras transformed NIH3T3 fibroblasts were cultured in RPMI medium. All of the media were supplemented with 10% fetal calf serum. Cells were allowed to grow for 24 h in 96-microwell plates and then treated with a range of inhibitor concentrations for 72 h (final 1‰ DMSO concentration). Cell growth was measured by means of the colorimetric MTT assay.

Molecular Modeling. Molecular modeling studies were performed using SYBYL software version 8[30] running on a Silicon Graphics workstation. Three-dimensional models of compounds **18e** and **19e** were built from a standard fragments library, and their geometry was subsequently optimized using the Tripos force field[31] including the electrostatic term calculated from Gasteiger and Hückel atomic charges. Powell's method available in the Maximin2 procedure was used for energy minimization until the gradient value was smaller than 0.001 kcal/mol·Å. The structure of the human FTase was obtained from its complexed X-ray crystal structure with FPP and inhibitor **66** (Chart 3) of the RCSB Protein Data Bank (1LD7).[26a] Flexible docking of **18e** and **19e** into the enzyme active site was performed using GOLD[32] software. The distance observed in the crystal structure between the distal nitrogen of compound **66** imidazole and the zinc cation was applied as a constraint. For each compound, the most stable docking model was selected according to the best scored conformation predicted by the GoldScore[32] and X-Score[33] scoring functions. The complexes were energy-minimized using the Powell method available in the Maximin2 procedure with the Tripos force field and a dielectric constant of 4.0 until the gradient value reached 0.01 kcal/mol·Å.

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