Structure-based design of imidazole-containing peptidomimetic inhibitors of protein farnesyltransferase

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A series of imidazole-containing peptidomimetic PFTase inhibitors and their co-crystal structures bound to PFTase and FPP are reported. The structures reveal that the peptidomimetics adopt a similar conformation to that of the extended CVIM tetrapeptide, with the imidazole group coordinating to the catalytic zinc ion. Both mono- and bis-imidazole-containing derivatives, **13** and **16**, showed remarkably high enzyme inhibition activity against PFTase *in vitro* with IC_{50} values of 0.86 and 1.7 nM, respectively. The peptidomimetics were also highly selective for PFTase over PGGTase-I both *in vitro* and in intact cells. In addition, peptidomimetics **13** and **16** were found to suppress tumor growth in nude mouse xenograft models with no gross toxicity at a daily dose of 25 mg kg^{-1}.

Introduction

During the last decade protein prenylation has become a major focus in anti-cancer research due to its critical role in the Ras- and Rho-mediated signaling pathways that are aberrantly activated in many human cancers.[1–3] Protein prenylation is a post-translational modification that involves the attachment of a C-15 farnesyl or a C-20 geranylgeranyl group through a thioether linkage to a cysteine residue near the carboxyl terminus of a target substrate protein.[4] Three enzymes comprise the protein prenyltransferase family that is responsible for catalyzing the prenylation of substrate proteins in mammalian cells: protein farnesyltransferase (PFTase), type I protein geranylgeranyltransferase (PGGTase-I), and type II protein geranylgeranyltransferase (PGGTase-II). PFTase transfers a C_{15} farnesyl residue to a cysteine within the CaaX tetrapeptide sequence found at the carboxyl terminus of prenylated proteins. The central "aa" residues are usually aliphatic amino acids while X is commonly methionine or serine. The related protein PGGTase-I attaches a C_{20} geranylgeranyl lipid to the cysteine of the CaaX sequence of target substrates, where the X residue is predominantly leucine, isoleucine or phenylalanine. Thus, the prenylation state of a protein, farnesylated or geranylgeranylated, is dictated by the nature of the terminal X specificity residue. A third prenyltransferase, PGGTase-II, transfers two geranylgeranyl residues to protein trafficking Rab proteins containing Cys–Cys or Cys–Ala–Cys sequences at the C-terminus.

The crystal structure of PFTase bound to a CVIM tetrapeptide (the CaaX tetrapeptide terminal sequence of K-Ras) and a farnesylpyrophosphate (FPP) analog has been solved.[5–7] The structure reveals that the CVIM binds to a large hydrophobic pocket in the enzyme in an extended conformation forming two hydrogen bonds with Gln 167α and Arg 202β, while the Cys thiol group coordinates to the zinc ion. In earlier generations of our peptidomimetic PFTase inhibitors, the central aliphatic dipeptide in CaaX was replaced by a 4-amino-2-phenylbenzamide spacer to afford the lead inhibitor, FTI-276[8,9] (Fig. 1). FTI-276 was found to be a remarkably potent inhibitor *in vitro* against PFTase with an IC_{50} value of 0.5 nM, however, the metabolically unstable thiol group hampered further development. We[10] and others[11] have demonstrated that replacement of the thiol group by an imidazole maintains enzyme inhibition activity and that non-thiol-containing peptidomimetics inhibit human tumor growth in animal models in a more selective and potent manner than the corresponding thiol contaning inhibitors.[10b] In this study, we have replaced the free thiol group with differently lengthed imidazole

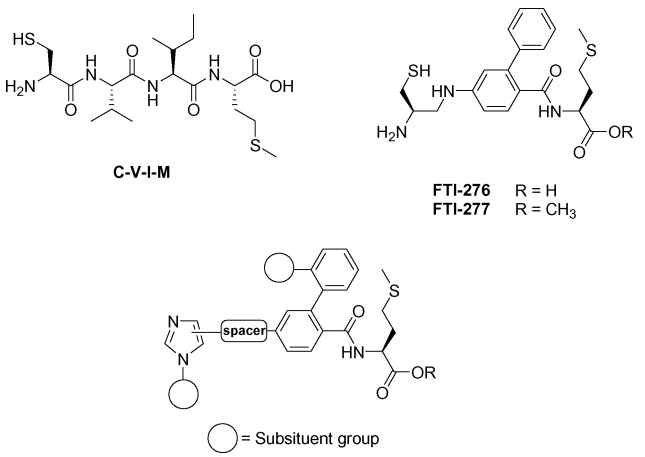

Fig. 1 Design of imidazole-containing peptidomimetics.

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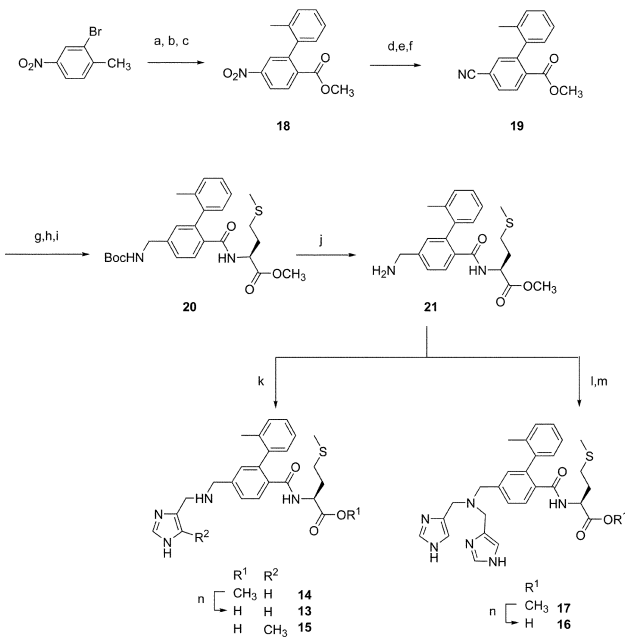
groups and investigated the scope of this change by introducing various substituents on the *N*-1 of the imidazole and the *ortho* position of the 2-phenyl group in the scaffold (Fig. 1). In addition, we have solved the crystal structures of several ternary complexes of PFTase bound to imidazole-containing peptidomimetics and have assessed their antitumor potency in nude mouse xenograft models of cancer.

Results and discussion

Chemistry

The synthetic approach to compounds **1**–**17** is shown in Scheme 1. Compounds **1**–**14** were synthesized by the previously reported procedures.[9,12] Briefly, Suzuki coupling of 2-bromo-4-nitrobenzoic acid methyl ester, which was derived from commercially available 2-bromo-4-nitrotoluene, afforded *ortho*-methyl substituted biphenyl scaffold **18**. The conversion of the nitro group by reduction and Sandmeyer reaction to the corresponding iodide, followed by palladium catalyzed reaction with zinc cyanide and $Pd(PPh_3)_4$ gave **19**. Hydrogenation in the presence of Boc_2O afforded the Boc-protected benzylamine derivative, which was followed by hydrolysis of the methyl ester, and subsequent coupling with L-methionine methyl ester to give **20**. Reductive amination of free benzylamine **21** with the respective imidazolecarboxyaldehydes proceeded efficiently to give the methyl ester derivatives, and subsequent hydrolysis of the methyl esters afforded **13** and **15**. The bis-imidazole-containing compounds were prepared by alkylation of **21** with *N*-Boc-4-chloromethylimidazole[13] followed by deprotection, and hydrolysis to give **16** and **17**, respectively.



Scheme 1 Reagents and conditions: (a) $KMnO_4$, 75%; (b) $SOCl_2$, MeOH, 78%; (c) *o*-tolylB$(OH)_2$, $Pd(PPh_3)_4$, K_2CO_3, 83%; (d) $SnCl_2$, 100%; (e) $NaNO_2$, KI, 81%; (f) $Zn(CN)_2$, $Pd(PPh_3)_4$, 91%; (g) H_2, 60 psi, 10% Pd–C, Boc_2O, 99%; (h) LiOH, 100%; (i) H–Met–OCH_3, EDCI, HOBt, 92%; (j) 50% TFA in CH_2Cl_2, 100%; (k) imidazolecarboxaldehyde, $NaBH_4$, 86%; (l) 1-*t*-butoxycarbonyl-4-chloromethylimidazole, $NaHCO_3$, DMF, 88%; (m) 50% TFA in CH_2Cl_2, 74%; (n) LiOH in THF, 77–100%.

Crystal structures

The crystal structure of the ternary complex of PFTase: FPP: FTI-2148 (**13**) was solved and a view of the inhibitor bound into the active site with the corresponding position of the CVIM tetrapeptide superimposed, is shown in Fig. 2. The structure confirmed our peptidomimetic design strategy, since FTI-2148 adopts a similar conformation to that of the extended conformation of CVIM in the active site of PFTase. The two hydrogen bonds between Gln167α/Met carboxylate, and Arg202β/amido carbonyl group of the benzoate scaffold are well conserved. The *N*-1 position of the imidazole group coordinates to the catalytic zinc ion, and the hydrophobic biphenyl spacer binds to a hydrophobic pocket created by aromatic amino acid residues Trp102β, Trp106β, and Tyr361β.[5] The crystal structure of PFTase bound to bis-imidazole FTI-2287 (**16**) was also solved (Fig. 3). In this structure the *N*-1 of the first imidazole group coordinates to the zinc ion, and the biphenyl scaffold and Met residue of the inhibitor adopt a similar orientation to that observed in the mono-imidazole derivative FTI-2148 (**13**), with the two key hydrogen bonds conserved. The second imidazole group in **16** does not coordinate the zinc but projects towards the α-subunit as shown in Fig. 3. The 2-H of this imidazole is located within van der Waals contact distance (*ca.* 4.5 Å) with the phenol ring system in Tyr166α suggesting that a CH–π interaction may contribute to stabilizing the complex.

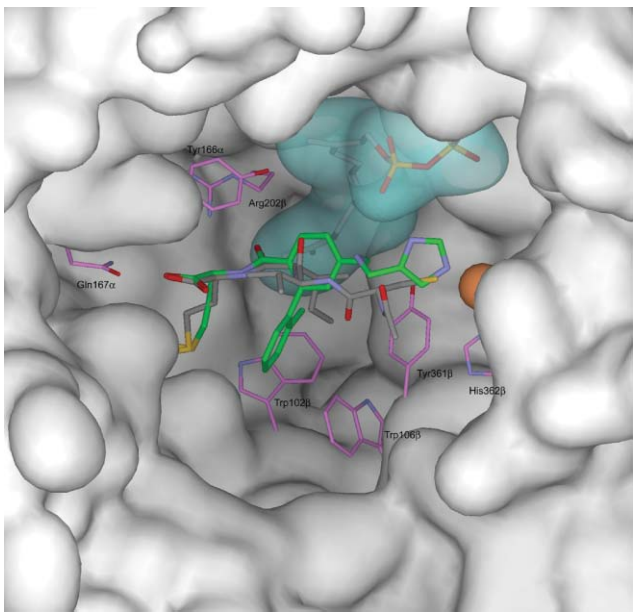


Fig. 2 View of the active site of PFTase ternary complex with FTI-2148 (**13**) (green) and FPP (gray with blue surface). The zinc ion is shown in orange. The structure of CVIM tetrapeptide (gray) bound to the active site of PFTase is superimposed.

In vitro and *in vivo* inhibition activity

The ability of these compounds to inhibit PFTase and PGGTase-I *in vitro* was investigated by using partially purified PFTase and PGGTase-I from human Burkitt lymphoma (Daudi) cells.[14] Enzyme preparations were incubated with [^{3}H]FPP and recombinant H-Ras-CVLS (PFTase) or [^{3}H]GGPP and recombinant H-Ras-CVLL (PGGTase-I) in the presence of different concentrations

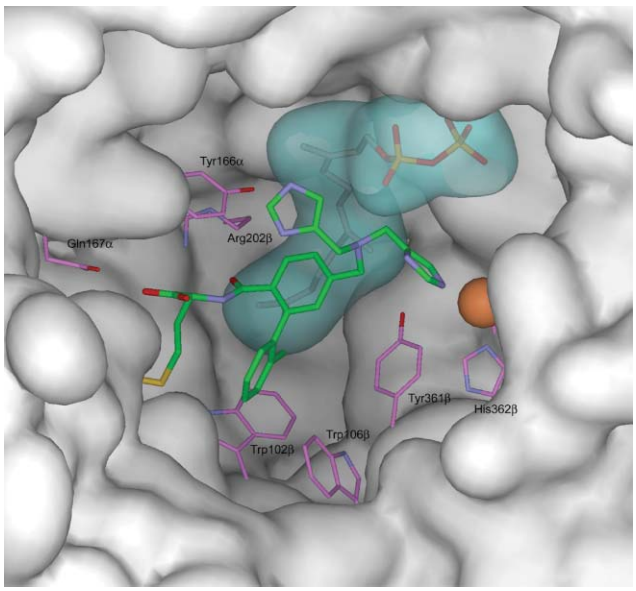


Fig. 3 View of the active site of PFTase ternary complex with FTI-2287 (**16**) (green) and FPP (gray with blue surface).

of inhibitors. After incubation for 30 min at 37 °C, the reaction was stopped and filtered onto glass fiber filters to separate free [^{3}H]FPP from [^{3}H]H-Ras. The IC_{50} values for the inhibition of PFTase of a series of compounds **1**–**9** based on the aniline type scaffold are reported in Table 1. The unsubstituted imidazole derivative **1** showed good inhibition activity against PFTase (IC_{50} = 28 nM). Introducing a benzyl group onto the *N*-1 position of the imidazole group as in **2b** retained the activity against PFTase but gave higher selectivity over PGGTase-I compared to **1** (IC_{50} PGGTase-I/PFTase: 41 for **1**; 105 for **2b**), whereas the corresponding regioisomer, **2a**, in which the imidazole ring is linked to the aniline scaffold at its 4-position (series **a**), showed lower activity. All compounds containing a bulky aromatic group at *N*-1 (such as **3**–**5**) showed lower activity against PFTase compared to **2**. The alkyl group containing compounds **6**–**9** were also effective inhibitors. Again, the compounds in series **b** show higher inhibition potency than in series **a**, and cyclohexylmethyl substituted **9b** showed higher selectivity, consistent with the fact that the binding environment around the zinc metal in the active site of PFTase is considerably different to that of PGGTase-I.[15]

Modification of the second phenyl group in the scaffold led to compounds **10**–**12** (Table 2). Interestingly, introducing a methyl group at the *ortho* position of **1** led to compound **10**, which showed 10-fold higher potency against PFTase and improved the selectivity over PGGTase-I (PGGTase-I/PFTase = 40 for **1**; 100 for **10**). Compound **11**, in which the imidazole ring system was attached to the scaffold at C-2, was less active, indicating that the position of the imidazole nitrogen is critical for binding to the active site. Replacement of the *ortho*-methyl group by a methoxy group to give **12** did not lead to an improvement in inhibition activity.

Replacement of the aniline-type scaffold in **10** by a benzylamine-based system led to a highly potent PFTase inhibitor, **13** (Table 3, FTI-2148) which showed an IC_{50} value of 0.86 nM. The corresponding methyl ester prodrug **14** (FTI-2153) potently inhibited H-Ras processing in whole cell-based assays (IC_{50} = 0.02 μM) (Table 3). A related compound **15**, which possesses a methyl group at the C-5 position of the imidazole group, showed reduced activity compared to **13**. Furthermore, installing a second imidazole group onto the benzyl amine nitrogen as in **16**, gave remarkably high inhibition potency in whole cells with single digit nanomolar IC_{50} value of 0.005 μM (Fig. 4 and Table 3). Somewhat surprisingly, the methyl ester prodrug **17** (FTI-2295), which might have been expected to have improved membrane penetration was found to inhibit farnesylation in whole cells with a correspondingly higher IC_{50} value of 0.02 μM. As shown in Fig. 4, **16** inhibits H-Ras processing at 5 nM (lanes 5 and 6) without any disruption of Rap1A processing, whereas **17** inhibits 50% processing only at 20 nM (lanes 13 and 14), suggesting the methyl ester prodrug did not improve inhibition activity in whole cells. A comparison of the whole cell activity of **13** (IC_{50} = 0.2 μM) and that displayed by **16**, reveals that installing a second imidazole group leads to an improvement in inhibition by 40-fold. This result suggests that the second imidazole group may contribute to an increase in membrane permeability of the molecule, less protein binding, better stability, or higher intracellular concentration.[16] We further investigated the *in vivo* activity of **13** and **16** using xenograft nude mice models of human lung carcinomas. Fig. 5 shows the tumor growth suppression after treatment with each compound at a 25 mg kg^{-1} d^{-1} dose administered subcutaneously. After four weeks, tumor growth was suppressed by 59 and 47% for **13** and **16**, respectively. The tumor growth inhibition activity of **16** was less marked than **13** in contrast to the high inhibition potency in whole cells. Preliminary pharmacokinetic results (data not shown)

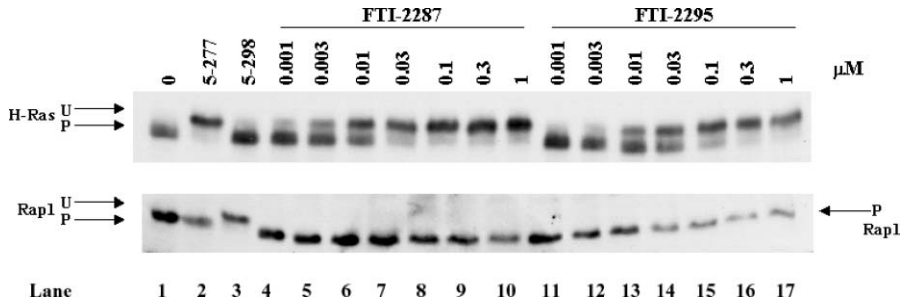
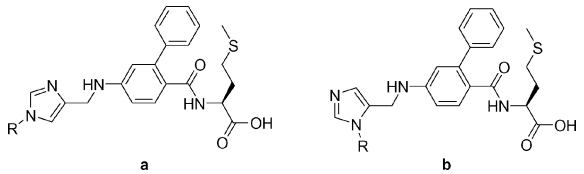
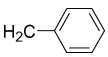
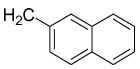
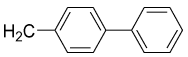
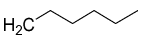
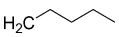
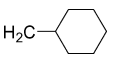


Fig. 4 Effect of bis imidazole-containing compounds on processing of H-Ras and Rap1A in NIH 3T3 cells. Western blot analysis demonstrates the inhibition of farnesylated H-Ras or geranylgeranylated Rap1A as seen by the band shift from processed (P) to unprocessed (U) protein. Lane 1: vehicle control; lane 2: 5 μM FTI-277; lane 3: 5 μM GGTI-298; lanes 4 to 10: 0.001, 0.003, 0.01, 0.03, 0.1, 0.3 and 1 μM **16** (FTI-2287); lanes 11 to 17: 0.001, 0.003, 0.01, 0.03, 0.1, 0.3, and 1 μM **17** (FTI-2295).

Table 1 Inhibition activity of **1**–**9** against mammalian PFTase and PGGTase-I



		a		b	
		IC_{50}/nM		IC_{50}/nM	
Compound	R	PFTase	PGGTase-I	PFTase	PGGTase-I
1 (FTI-2118)	H	28 ($n = 2$)	1150 ($n = 2$)	—	—
2a (FTI-2208) **2b** (FTI-2209)		603 ± 201	3900 ± 700	46 ± 28	4833 ± 1305
3a (FTI-2218)		4200 ($n = 2$)	2600 ($n = 2$)	—	—
4a (FTI-2219) **4b** (FTI-2221)		1400 ($n = 2$)	6950 ($n = 2$)	130 ($n = 2$)	4550 ($n = 2$)
5a (FTI-2217) **5b** (FTI-2220)		5600 ($n = 2$)	3500 ($n = 2$)	435 ($n = 2$)	4550 ($n = 2$)
6b (FTI-2239)		—	—	36 ± 8	>10 000 ($n = 3$)
7a (FTI-2236)		1230 ($n = 2$)	>10 000 ($n = 2$)	—	—
8a (FTI-2240)		1980 ($n = 2$)	5950 ($n = 2$)	22	6900
8b (FTI-2241) **9a** (FTI-2237) **9b** (FTI-2238)		1060 ($n = 2$)	6250 ($n = 2$)	41 ± 18	>10 000 ($n = 2$)

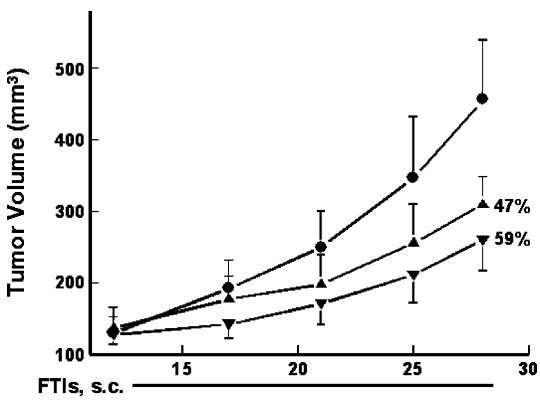


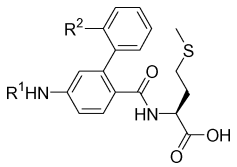
Fig. 5 *In vivo* activity of **13** and **16** using xenograft nude mice models of human lung carcinomas: **13**, 25 mg^{-1} kg^{-1} d^{-1} (▼); **16**, 25 mg^{-1} kg^{-1} d^{-1} (▲); control, (●).

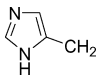
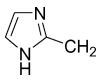
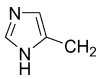
suggest that **16** is less stable in serum than **13**, presumably as a result of its rapid metabolic degradation due to the second imidazole group. It is worth noting that there was no gross toxicity observed during the course of these experiments, as documented by no change in the weight, appetite and activity of the mice.

Conclusions

A series of imidazole-containing peptidomimetics was prepared, and the crystal structures of their complexes with PFTase bound to FPP showed that the peptidomimetics adopt a similar conformation to that of the extended CVIM tetrapeptide. In addition, two distinct hydrogen bonds seen between the CVIM tetrapeptide and PFTase active site were well conserved, and the *N*-1 position of the imidazole group coordinates to the zinc ion. However, in the case of bis-imidazole derivatives, the second imidazole group projects away from the active site and is located near Tyr166α within van der Waals contact distance, suggesting a possible CH–π interaction. Both mono- and bis-imidazole-containing derivatives, **13** and **16** showed remarkably high enzyme inhibition activity (IC_{50} = 0.86 and 1.7 nM, respectively), and suppressed tumor growth in nude mouse xenograft models with no gross toxicity. Furthermore, compound **16** showed higher inhibition activity in whole cells than **13**, suggesting that the role of the second imidazole group

Table 2 Inhibition activity of **10**–**12** against mammalian PFTase and PGGTase-I



Compound	R^1	R^2	IC_{50}/nM PFTase	IC_{50}/nM PGGTase-I
10 (FTI-2128)		CH_3	2.8 ± 2.0	260 ± 130
11 (FTI-2130)		CH_3	155 ($n = 2$)	388 ($n = 2$)
12 (FTI-2134)		OCH_3	9.5 ($n = 2$)	3710 ($n = 2$)

may not only be to stabilize the complex but also to increase its cell permeability. Additional SAR studies on the second imidazole group of **16** are currently underway.

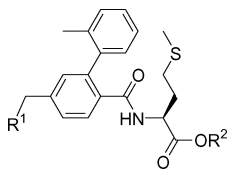
Experimental

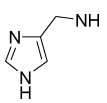
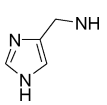
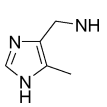
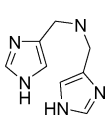
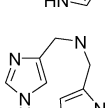
General procedures

Melting points were determined with an electrothermal capillary melting point apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AM-500, 400, and 300 spectrometer. Chemical shifts were reported in δ (ppm) relative to tetramethylsilane. All coupling constants were described in Hz. Elemental analyses were performed by Atlantic Microlab, Inc., GA. Flash column chromatography was performed on silica gel (40–63 μm) under a pressure of about 4 psi. Solvents were obtained from commercial suppliers and purified as follows: tetrahydrofuran and ether were distilled from sodium benzophenone ketyl; dichloromethane was distilled over calcium hydride. Synthesized final compounds were checked for purity by analytical HPLC, which was performed using a Rainin HP controller and a Rainin UV-C detector with a Rainin 250 × 4.6 mm, 5 μm Microsorb C-18 column, eluted with gradient 10 to 90% of CH_3CN in 0.1% TFA in H_2O in 30 min. High-resolution mass spectra (HRMS) and low-resolution mass spectra (LRMS) were performed on a Varian MAT-CH-5 (HRMS) or VG 707 (LRMS) mass spectrometer. Compounds **5**, **8**–**9**, **11**, **13**–**14**[9,12,17] were prepared by the previously reported methods.

4-[*N*-(1*H*-Imidazol-4-yl)methylamino]-2-phenylbenzoylmethionine (1, FTI-2118). 4-Hydroxymethylimidazole hydrochloride salt

Table 3 Inhibition activity of **13**–**17** against mammalian PFTase and PGGTase-I



Compound	R^1	R^2	IC_{50}/nM PFTase	IC_{50}/nM PGGTase-I	Processing IC_{50}/μM H-Ras	Processing IC_{50}/μM Rap1A
13 (FTI-2148)		H	0.86 ± 0.43	1700 ± 840	0.2	>100
14 (FTI-2153)		CH_3	60 (n = 2)	>10 000 ($n = 2$)	0.02	>30
15 (FTI-2288)		H	4.0 ± 2.3	660 ± 330	>10	>10
16 (FTI-2287)		H	1.7 ± 0.5	400 ± 130	0.005	>30
17 (FTI-2295)		CH_3	46 ($n = 2$)	2700 ($n = 2$)	0.02	>10

(1.0 g, 7.4 mmol) and *p*-toluenesulfonyl chloride were suspended in 1 cm^3 of distilled water and 3 cm^3 of THF. Sodium hydroxide (1 N) was added and the pH maintained at approximately 9 over a period of 3 h. The reaction mixture was extracted with diethyl ether (3 × 50 cm^3). The extracts were combined, dried over magnesium sulfate and concentrated. The residue was purified by flash column chromatography (100% ethyl acetate) to give 4-hydroxymethyl-1-*p*-toluenesulfonylimidazole as a white solid (1.1 g, 58%). Mp 109–112 °C. ^{1}H NMR ($CDCl_3$) δ 7.98 (s, 1H, imidazole), 7.82 (d, $J = 8.4$ Hz, 2H), 7.34 (d, $J = 8.3$ Hz, 2H), 7.22 (s, 1H, imid), 4.51 (s, 2H, CH_2OH), 2.42 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$) δ 146.68, 144.68, 136.82, 134.72, 130.58, 127.54, 114.28, 57.66, 21.82. HRMS: *m/e* calcd for $C_{11}H_{12}N_2O_3S$, 252.0569, found 252.0576.

This compound (0.7 g, 2.8 mmol) was dissolved into 7 cm^3 of methylene chloride and manganese(IV) oxide (2.0 g, 23 mmol) was added. The reaction mixture was stirred at room temperature under a nitrogen atmosphere for 20 h. The solvent, methylene chloride was filtered through a celite pad, and the combined filtrates were evaporated. The residue was purified by flash column chromatography (2 : 3 ethyl acetate–hexanes) to give (1-*p*-toluenesulfonylimidazol-4-yl)carboxaldehyde as a white solid (0.45 g, 64%). Mp 94–97 °C. ^{1}H NMR ($CDCl_3$) δ 9.79 (s, 1H), 8.00 (s, 1H), 7.87 (s, 1H), 7.81 (d, $J = 8.2$ Hz, 2H), 7.33 (d, $J = 8.0$ Hz, 2H), 2.38 (s, 3H). HRMS(FAB) *m/e* calcd for $C_{11}H_{10}N_2O_3S$: 250.0412, found 250.0419.

The above carboxaldehyde (0.10 g, 0.4 mmol) and *N*-(4-amino-2-phenylbenzoyl)-methionine methyl ester hydrochloride salt (0.16 g, 0.4 mmol) were dissolved in 10 cm^3 of 95% methanol and 5% acetic acid and stirred for 15 min before adding sodium cyanoborohydride (0.05 g, 0.8 mmol). The reaction was stirred for 1/2 h and twice an additional amount of the carboxaldehyde (0.165 g, 0.66 mmol) and sodium cyanoborohydride (0.83 g, 1.3 mmol) were added. The reaction was stirred at room temperature under a nitrogen atmosphere for 16 h. The reaction mixture was concentrated, the residue taken up in ethyl acetate and washed with a saturated solution of sodium bicarbonate. The organic phase was dried over magnesium sulfate and concentrated. The residue was purified by flash column chromatography (3 : 2 ethyl acetate–hexanes) to give 4-[*N*-(1-*p*-toluenesulfonylimidazol-4-yl)methylamino]-2-phenylbenzoylmethionine methyl ester as a white solid (0.075 g, 7.5%). ^{1}H NMR ($CDCl_3$) δ 7.97 (s, 1H, imid), 7.79 (d, $J = 8.2$ Hz, 2H), 7.65 (d, $J = 8.5$ Hz, 1H), 7.41–7.34 (m, 8H), 7.18 (s, 1H, imid), 6.60 (d, $J = 6.7$ Hz, 1H), 6.46 (s, 1H,), 6.46 (s, 1H), 5.71 (d, 1H), 4.62 (dd, $J = 6.10$, 6.21 Hz, 1H), 4.27 (s, 2H), 3.64 (s, 3H), 2.44 (s, 3H), 2.10 (t, $J = 7.56$ Hz, 2H), 2.00 (s, 3H), 1.93–1.82 (m, 1H), 1.69–1.60 (m, 1H).

The above protected sulfonamide (0.075 g, 0.13 mmol) was dissolved into 2 cm^3 of THF and cooled to 0 °C. Lithium hydroxide (2 cm^3, 0.5 M) was slowly added to the reaction mixture and stirred for 4 h. The pH was adjusted to 4 with 1 N HCl and excess THF was removed under vacuum. The aqueous layer was lyophilized and the resulting solid was purified by reverse phase preparative HPLC. All fractions containing not less than 98.8% were pooled and lyophilized to give **1** as a TFA salt (0.03 g, 52%). ^{1}H NMR (DMSO-d_6) δ 14.44 (s, 2H), 12.60 (s, 1H), 9.01 (s, 1H), 8.07 (d, $J =$ 7.8 Hz, 1H), 7.56 (s, 1H), 7.30–7.24 (m, 8H), 6.65 (d, $J = 1.5$ Hz, 1H), 6.59 (s, 1H), 4.41 (s, 2H), 4.21 (dd, $J = 4.5$, 4.2 Hz, 1H), 2.27–2.14 (m, 1H), 1.98 (s, 3H), 1.86–1.75 (m, 1H). FAB [M + H]$^+ = 425^+$.

4-[*N*-(1-Benzyl-1*H*-imidazol-4-yl)methylamino]-2-phenylbenzoylmethionine (2a, FTI-2208). 4(5)-Hydroxy-methylimidazole (5.0 g, 37 mmol) was dissolved in 20 cm^3 of dry DMF containing triethylamine (15.0 g, 148 mmol). Benzyl chloride (9.5 g, 55.5 mmol) was added to the reaction mixture *via* an addition funnel over a 5 min period and refluxed for 3 h. Additional benzyl chloride (3.2 g, 18.7 mmol) and triethylamine (2.6 g, 25.7 mmol) were added and the reaction mixture refluxed an additional 1 h. The solvents were concentrated and the residue taken up into chloroform. The solvent was dried over magnesium sulfate and concentrated. The oily material was crystallized from acetone to give 1-benzyl-5-hydroxymethylimidazole (0.4 g, 5.7%). Mp 137–139 °C (Lit.[18] 139–140 °C). ^{1}H NMR ($CDCl_3$) δ 7.50 (s, 1H, imid), 7.31–7.35 (m, 3H, aromatic), 7.12–7.15 (m, 2H, aromatic), 6.99 (s, 1H, imid), 5.24 (s, 1H, $CH_2C_6H_5$), 4.51 (s, 2H, CH_2OH), 2.16 (br s, 1H, OH). An additional 0.3 g of 1-benzyl-5-hydroxymethylimidazole was recovered from the column and combined to give a total of 0.7 g (10%).

The acetone soluble material was further purified by a silica gel column using 100% acetone as the eluent to give 1-benzyl-4-hydroxymethylimidazole as a white solid (1.4 g, 20%). Mp 75–77 °C, (Lit.[18] 79.5–80 °C). ^{1}H NMR ($CDCl_3$) δ 7.52 (s, 1H, imid), 7.34–7.36 (m, 3H, aromatic), 7.12–7.18 (m, 2H, aromatic), 5.07 (s, 2H, $CH_2C_6H_5$), 4.58 (s, 2H, CH_2OH), 3.51 (br s, 1H, OH).

1-Benzyl-4-hydroxymethylimidazole (0.6 g, 3.2 mmol) was dissolved into 15 cm^3 of dioxane and heated to 80 °C. Manganese dioxide (2.23 g, 25.6 mmol) was added at once and heating continued for 6 h. The reaction was monitored by TLC using 100% acetone as the eluent. After cooling slightly, the reaction mixture was filtered through a celite plug followed by several washings of methylene chloride. The filtrate was dried over magnesium sulfate, concentrated, and the residue was purified by flash column chromatography using 1 : 1 ethyl acetate–hexanes as the eluent to give 1-benzyl-4-imidazolecarboxaldehyde as an oil (0.55 g, 93%).

The above carboxaldehyde (0.21 g, 1.1 mmol) and *N*-(4-amino-2-phenylbenzoyl)-methionine methyl ester hydrochloride[9] (0.4 g, 1.0 mmol) were dissolved in 10 cm^3 of methanol and stirred for 15 min before adding dropwise 2 cm^3 of a methanol solution containing sodium cyanoborohydride (0.034 g, 1.1 mmol). The reaction was stirred at room temperature for 4 h. The reaction mixture was concentrated and the residue taken up in ethyl acetate and washed with a saturated solution of sodium bicarbonate followed by a saturated solution of sodium chloride. The organic phase was dried over magnesium sulfate and concentrated. The residue was purified by flash column chromatography using a gradient of 3 : 2 ethyl acetate–hexanes to 100% ethyl acetate as the eluent. The product was crystallized from ethyl acetate and hexanes to give 4-[*N*-(1-benzyl-1*H*-imidazol-4-yl)methylamino]-2-phenylbenzoylmethionine methyl ester as a white solid (0.38 g, 72%). Mp 105–107 °C. ^{1}H NMR ($CDCl_3$) δ 7.67 (s, 1H), 7.66 (d, $J = 8.6$ Hz, 1H, aromatic), 7.35–7.45 (m, 9H, aromatic), 7.18 (m, 1H, aromatic), 6.86 (s, 1H), 6.66 (dd, $J = 8.6$, 2.4 Hz, 1H, aromatic), 6.51 (d, $J = 2.4$ Hz, 1H, aromatic), 5.67 (d, $J = 7.7$ Hz, 1H), 5.10 (s, 2H, $CH_2C_6H_5$), 4.61 (m, 1H, α met-H), 4.33 (s, 2H, CH_2NH), 3.64 (s, 3H, OCH_3), 2.09 (t, $J = 7.7$ Hz, 2H, CH_2SCH_3), 2.00 (s, 3H, SCH_3), 1.87 (m, 1H, $CH_2CH_2SCH_3$), 1.65 (m, 1H,

$CH_2CH_2SCH_3$). ^{13}C NMR ($CDCl_3$) δ 172.3, 168.7, 149.8, 141.7, 141.4, 140.0, 137.4, 136.1, 131.4, 129.2, 128.9, 128.7, 128.5, 127.9, 127.5, 123.0, 116.9, 114.3, 111.8, 52.5, 51.9, 51.1, 41.8, 31.8, 29.6, 15.4.

This compound (0.10 g, 0.19 mmol) was dissolved into 5 cm^3 of THF and cooled to 0 °C. Lithium hydroxide (0.017 g, 0.4 mmol) was dissolved into 5 cm^3 of distilled water and was slowly added to the reaction mixture and stirred for 2 h. The pH was adjusted using 0.5 M HCl and extracted with ethyl acetate. The extracts were combined, washed first with a saturated solution of sodium chloride, dried over magnesium sulfate and concentrated to give **2a** as a white solid (0.058 g, 60%). Mp 143–146 °C. ^1H NMR (CD_3OD) δ 8.99 (s, 1H, imid), 7.51 (s, 1H, imid), 7.29–7.45 (m, 9H, aromatic), 6.65 (dd, $J = 8.3$, 2.2 Hz, 1H, aromatic), 6.58 (d, $J = 2.3$ Hz, 1H, aromatic), 5.38 (s, 2H, $CH_2C_6H_5$), 4.41–4.47 (m, 3H, CH_2NH; α met-H), 2.01–2.12 (m, 2H, CH_2SCH_3), 2.00 (s, 3H, SCH_3), 1.90–1.96 (m, 1H, $CH_2CH_2SCH_3$), 1.73–1.78 (m, 1H, $CH_2CH_2SCH_3$). ^{13}C NMR (CD_3OD) δ 175.1, 173.1, 150.5, 143.5, 142.5, 136.5, 135.6, 135.0, 131.2, 130.6, 130.5, 129.8, 129.6, 129.6, 128.7, 126.2, 120.8, 115.5, 112.4, 54.1, 53.2, 39.0, 31.9, 31.1, 15.2.

4-[*N*-(1-Benzyl-1*H*-imidazol-5-yl)methylamino]-2-phenylbenzoylmethionine (2b, FTI-2209). 1-Benzyl-5-hydroxymethylimidazole (0.6 g, 3.2 mmol) was dissolved into 15 cm^3 of dioxane and heated to 85 °C. Manganese dioxide (2.22 g, 26.0 mmol) was added at once and heated for 6 h. After cooling, the reaction mixture was filtered through a celite plug. The celite was washed several times with methylene chloride. The filtrate was concentrated and the oily residue was crystallized from hexanes to give 1-benzyl-5-imidazolecarboxaldehyde[19] as a white solid (0.46 g, 78%). Mp 52–54 °C; ^1H NMR ($CDCl_3$) δ 9.77 (s, 1H, C(O)H), 7.85 (s, 1H, imid), 7.75 (s, 1H, imid), 7.34–7.36 (m, 3H, aromatic), 7.20–7.24 (m, 2H, aromatic), 5.53 (s, 2H, $CH_2C_6H_5$).

This carboxaldehyde (0.21 g, 1.1 mmol) and *N*-(4-amino-2-phenylbenzoyl)-methionine methyl ester hydrochloride (0.4 g, 1.0 mmol) were dissolved in 10 cm^3 of methanol and stirred for 15 min before adding dropwise a methanol (2 cm^3) solution containing sodium cyanoborohydride (0.034 g, 1.1 mmol). The reaction was stirred at room temperature for 6 h. The reaction mixture was concentrated and the residue was taken up in ethyl acetate and washed with a saturated solution of sodium bicarbonate followed by a saturated solution of sodium chloride. The organic phase was dried over magnesium sulfate and concentrated. The residue was purified by flash column chromatography using 100% ethyl acetate as the eluent. The product was crystallized from ethyl acetate and hexanes to give 4-[*N*-(1-benzyl-1*H*-imidazol-5-yl)methylamino]-2-phenylbenzoylmethionine methyl ester as a white solid (0.38 g, 72%). Mp 182–183 °C; ^1H NMR ($CDCl_3$) δ 7.68 (s, 1H, imid), 7.30–7.46 (m, 9H, aromatic), 7.10 (s, 1H, imid), 7.04–7.07 (m, 2H, aromatic), 6.49 (dd, $J = 8.5$, 2.2 Hz, 1H, aromatic), 6.35 (d, $J = 2.3$ Hz, 1H, aromatic), 5.70 (d, $J = 7.7$ Hz, 1H, C(O)NH), 5.19 (s, 2H, $CH_2C_6H_5$), 4.62 (dd, $J = 7.3$ Hz, 2H, α met-H), 4.17 (d, $J = 5.0$ Hz, 2H, CH_2NH), 3.94 (br t, 1H), 3.64 (s, 3H, OCH_3), 2.09 (t, $J = 7.7$ Hz, 2H, CH_2SCH_3), 1.88 (m, 1H, $CH_2CH_2SCH_3$), 1.66 (m, 1H, $CH_2CH_2SCH_3$). ^{13}C NMR (CD_3OD) δ 172.2, 168.6, 149.0, 141.8, 141.1, 136.0, 131.3, 129.3, 128.8, 128.5, 128.0, 126.9, 123.8, 114.2, 111.9, 52.5, 51.9, 49.2, 37.9, 31.7, 29.6, 15.4.

This compound (0.10 g, 0.19 mmol) was dissolved into 5 cm^3 of THF and cooled to 0 °C. Lithium hydroxide (0.017 g, 0.4 mmol) dissolved into 5 cm^3 of distilled water was slowly added to the reaction mixture and stirred for 2 h. The pH was adjusted using 0.5 M HCl and the reaction mixture was lyophilized. The lyophilized mixture was purified by reverse phase preparative HPLC and fractions having assays greater than 99% were pooled and lyophilized to give **2b** as the TFA salt (0.058 g, 60%). Mp 120–122 °C. ^1H NMR (CD_3OD) δ 8.99 (d, $J = 1.2$ Hz, 1H, imid), 7.49 (s, 1H, imid), 7.28–7.40 (m, 11H, aromatic), 6.57 (dd, $J = 8.7$, 2.4 Hz, 1H, aromatic), 6.45 (d, $J = 2.4$ Hz, 1H), 5.53 (s, 2H, $CH_2C_6H_5$), 4.44 (dd, $J = 9.3$, 4.2 Hz, 1H, α met-H), 4.38 (s, 2H, CH_2NH), 2.12–2.22 (m, 1H, $CH_2CH_2SCH_3$), 2.01 (s, 3H, SCH_3), 1.93–2.09 (m, 2H, CH_2SCH_3, 1.74–1.79 (m, 1H, $CH_2CH_2SCH_3$). ^{13}C NMR (CD_3OD) δ 175.1, 173.2, 150.3, 143.4, 142.5, 137.7, 135.0, 134.6, 131.2, 130.7, 130.4, 129.9, 129.6, 129.0, 128.8, 126.2, 120.0, 115.4, 112.2, 53.2, 52.0, 38.2, 31.9, 31.1, 15.2.

4-[*N*-(1-(1-Naphthyl)methyl-1*H*-imidazol-4-yl)methylamino]-2-phenylbenzoylmethionine (3a, FTI-2218). 1-(1-Naphthylmethyl)-4-(hydroxymethyl)imidazole was prepared from 4-(hydroxymethyl)-imidazole hydrochloride (2.5 g, 18.6 mmol), 1-(chloromethyl)-naphthalene (4.0 g, 20.4 mmol), and Na_2CO_3 (3.94 g, 37.2 mmol) in DMF (30 cm^3) by stirring at 100 °C for 10 h. After removal of DMF by distillation under reduced pressure, the residue was dissolved in acetone (30 cm^3). The resulting white precipitates were collected by filtration, and recrystallized from acetone to give 1-naphthylmethyl-4-(hydroxymethyl)imidazole as a sole product (1.05 g, 24%). Mp 175–176 °C; ^1H NMR (CD_3OD) δ 8.04 (d, $J = 8$ Hz, 1H, aryl H), 7.92 (d, $J = 8$ Hz, 1H, aryl H), 7.89 (d, $J = 8$ Hz, 1H, aryl H), 7.71 (s, 1H, imid-2H), 7.55 (m, 2H, aryl H), 7.47 (t, $J = 7.0$ Hz, 1H, aryl H), 7.35 (d, $J = 7.0$ Hz, 1H, aryl H), 7.02 (s, 1H, imid-5H), 5.68 (s, 2H, CH_2O), 4.46 (s, 2H, CH_2). Anal. calcd for $C_{15}H_{14}N_2O_1$: C, 75.61; H, 5.92; N, 11.76. Found: C, 75.52; H, 5.96; N, 11.61. The characterization of the isomer was based on the result of the NOE measurement in DMSO-d_6 at room temperature. NOE between both imidazole-ring protons and naphthylmethyl protons were 6%, respectively.

To a solution of 1-naphthylmethyl-4-hydroxymethylimidazole (239 mg, 1 mmol) in dioxane (5 cm^3) was added MnO_2 (869 mg, 10 mmol) at 80 °C. After stirring for 1 h at 80 °C, the reaction mixture was filtered through celite washing the solid with CH_2Cl_2 (100 cm^3), and then the filtrate was concentrated to give the corresponding aldehyde as colorless oil (253 mg, 100%). ^1H NMR ($CDCl_3$) δ 9.82 (s, 1H, CHO), 7.89–7.92 (m, 2H, aryl H), 7.79 (m, 1H), 7.64 (s, 1H, imid-2H), 7.57 (s, 1H, imid-5H), 7.52–7.55 (2H, m, aryl H), 7.46 (t, $J = 8.4$ Hz, 1H, aryl H), 7.28 (d, $J = 7.4$ Hz, 1H, aryl H), 5.59 (s, 2H, NCH_2).

A solution of 1-(1-naphthylmethyl)-4-imidazolecarboaldehyde (236 mg, 1 mmol) and *N*-(4-amino-2-phenylbenzoyl)methionine methyl ester hydrochloride (395 mg, 1 mmol) in MeOH (10 cm^3) was stirred for 15 min at room temperature. To the solution was added $NaBCNH_3$ (69 mg, 1.1 mmol) in MeOH (2 cm^3). After stirring for 4 h, the solvent was evaporated and the residue was dissolved in CH_2Cl_2 (100 cm^3), and then the organic layer was washed with sat. $NaHCO_3$ (30 $cm^3 \times 2$), brine, and dried (Na_2SO_4). The product was purified by flash column chromatography (100 : 10 : 2 $CHCl_3$–acetone–EtOH) to afford the product as a foam; 311 mg (54%) ^1H NMR ($CDCl_3$) δ 7.84–7.96 (m, 3H), 7.67 (d,

J = 8.5 Hz, 1H), 7.59 (s, 1H), 7.53–7.55 (m, 2H), 7.36–7.49 (m, 6H), 7.22 (d, J = 7.0 Hz, 1H), 6.86 (s, 1H), 6.65 (dd, J = 7.7 and 1.8 Hz, 1H), 6.50 (d, J = 1.8 Hz, 1H), 5.67 (d, J = 7.7 Hz, 1H), 5.55 (s, 2H, NCH), 4.68 (br s, 1H, NH), 4.61 (q, J = 7.4 Hz, 1H, αCH), 4.30 (s, 2H, NCH), 3.65 (s, 3H, OCH), 2.12 (t, J = 7.7 Hz, 2H, CH S), 2.01 (s, 3H, SCH_3), 1.84–1.98 (m, 1H, C*H*HCH$_2$), 1.84–1.98 (m, 1H, C*H*HCH$_2$). HRMS (ESI) calcd for $C_{34}H_{34}N_4O_3SH^+$ 579.2430. Found 579.2422.

To a solution of 4-[*N*-((1-naphthylmethyl-1*H*-imidazol-4-yl)methyl)amino]-2-phenylbenzoylmethionine methyl ester (150 mg, 0.26 mmol) in THF (5 cm^3) was added 0.1 N LiOH (5.2 cm^3) at 0 °C, and then the mixture was stirred at room temperature for 1 h. The mixture was concentrated and acidified with 10% citiric acid. The product was extracted with CH_2Cl_2 (200 cm^3), brine, and dried (Na_2SO_4). Evaporation of the solvent gave the product **3a** as an amorphous (115 mg, 78%). ^{1}H NMR (CD_3OD) δ 7.90–8.03 (m, 3H), 7.89 (s, 1H), 7.27–7.56 (m, 11H), 7.01 (s, 1H), 6.61 (d, J = 8.8 Hz, 1H), 6.52 (s, 1H), 5.65 (s, 2H), 4.36 (m, 1H), 4.25 (s, 2H), 2.15 (m, 1H), 2.05 (m, 1H), 2.01 (s, 3H), 1.90 (m, 1H), 1.73 (m, 1H). HRMS (ESI) calcd for $C_{33}H_{32}N_4O_3SH^+$ 565.2273. Found 565.2277.

4-[*N*-(1-(2-Naphthyl)methyl-1*H*-imidazol-4-yl)methylamino]-2-phenylbenzoylmethionine (4a, FTI-2219). 1-(2-Naphthyl-methyl)-4-(hydroxymethyl)imidazole was prepared using 4-(hydroxymethyl)-imidazole hydrochloride and 2-(bromomethyl)naphthalene by a similar procedure to that described for 1-benzyl-4 or 5-hydroxymethylimidazole, white powder (16%). Mp 109–110 °C. ^{1}H NMR ($CDCl_3$) δ 7.81–7.86 (m, 3H, aryl H), 7.64 (s, 1H, imid-2H), 7.60 (s, 1H, aryl H), 7.50–7.54 (m, 2H, aryl H), 7.29 (dd, J = 8.4 and 1.7 Hz, 1H, aryl H), 6.90 (s, 1H, imid-5H), 5.25 (s, 2H, NCH_2), 4.61 (s, 2H, CH_2OH), 2.18 (br s, 1H, OH).

This compound was converted to 1-(2-naphthylmethyl)-4-imidazolecarboxyaldehyde by a similar procedure to that described for 1-benzyl-4 or 5-imidazolecarboxyaldehyde. Without purification this aldehyde was reacted with *N*-(4-amino-2-phenylbenzoyl)methionine methyl ester hydrochloride by a similar procedure to that described for **3a** to give 4-[*N*-(1-(2-naphthyl)methyl-1*H*-imidazol-4-yl)methylamino]-2-phenylbenzoylmethionine methyl ester as an oil, 241 mg (93%). ^{1}H NMR ($CDCl_3$) δ 1.63–1.68 (m, 1H, C*H*HCH$_2$), 1.85–1.90 (m, 1H, C*H*HCH$_2$), 2.00 (s, 3H, SCH_3), 2.10 (t, J = 7.5 Hz, 2H, CH_2S), 3.64 (s, 3H, CO_2CH_3), 4.31 (br s, 2H, CH_2NH), 4.60–4.64 (m, 2H, αCH and NH), 5.23 (s, 2H, NCH_2), 5.68 (d, J = 7.5 Hz, 1H, CONH), 6.50 (d, J = 2.5 Hz, 1H, aryl H), 6.66 (dd, J = 8.0 and 2.5 Hz, 1H, aryl H), 6.85 (s, 1H, imid-5H), 7.25–7.86 (m, 14H, aryl H and imid-2H). HRMS (ESI) calcd for $C_{34}H_{34}N_4O_3SH^+$ 579.2430. Found 579.2422.

The methyl ester was hydrolyzed in a similar manner to that described for **3a** to give **4a** as an amorphous solid (82%). ^{1}H NMR (CD_3OD) δ 7.96 (s, 1H), 7.79–7.83 (m, 3H), 7.71 (s, 1H, imid-2H), 7.47–7.50 (m, 2H, aryl H), 7.38 (d, J = 8 Hz, 1H), 7.29 (m, 6H, aryl H), 7.11 (s, 1H, imid-5H), 6.63 (d, J = 8 Hz, 1H, aryl H), 6.53 (s, 1H, aryl H), 5.33 (s, 2H, CH_2N), 4.39–4.41 (m, 1H, αCH), 4.29 (br s, 2H, CH_2N), 2.14–2.17 (m, 1H, C*H*HS), 2.05–2.07 (m, 1H, C*H*HS), 2.00 (s, 3H, SCH_3), 1.73–1.74 (m, 2H, CH_2). HRMS (FAB) m/z calcd for $C_{33}H_{32}N_4O_3S$ H^+ 565.2273. Found 565.2271.

4-[*N*-(1-(2-Naphthyl)methyl-1*H*-imidazol-5-yl)methylamino]-2-phenylbenzoylmethionine (4b, FTI-2221). 1-(2-Naphthylmethyl)-5-(hydroxymethyl)imidazole was prepared using 4-(hydroxymethyl)-imidazole hydrochloride and 2-(bromomethyl)naphthalene by a similar procedure to that described for 1-benzyl-4 or 5-hydroxymethylimidazole (8%). Mp 150–151 °C. ^{1}H NMR ($CDCl_3$) δ 7.83–7.84 (m, 2H, aryl H), 7.76–7.80 (m, 1H, aryl H), 7.58 (s, 1H, aryl H), 7.55 (s, 1H, imid-2H), 7.49–7.52 (m, 2H, aryl H), 7.26 (dd, J = 8.5 and 1.8 Hz, aryl H), 7.04 (s, 1H, imid-4H), 5.41 (s, 2H, NCH_2), 4.54 (s, 2H, CH_2OH), 2.05 (br s, 1H, OH).

This compound was converted to 1-(2-naphthylmethyl)-4-imidazolecarboxyaldehyde by a similar procedure to that described for 1-benzyl-4- or 5-imidazolecarboxyaldehyde (100%). Without purification this aldehyde was reacted with *N*-(4-amino-2-phenylbenzoyl)methionine methyl ester hydrochloride by a similar procedure to that described for **3a** to give 4-[*N*-(1-(2-naphthyl)methyl-1*H*-imidazol-5-yl)methylamino]-2-phenylbenzoylmethionine methyl ester as an oil (100%). ^{1}H NMR ($CDCl_3$) δ 7.15–7.80 (m, 14H, aryl H and imid-2H), 7.07 (s, 1H, imid-4H), 6.43 (d, J = 8.0 Hz, 1H, aryl H), 6.24 (s, 1H, aryl H), 5.72 (d, J = 7.5 Hz, 1H, CONH), 5.31 (s, 2H, NCH_2), 4.60 (q, J = 7 Hz, 1H, αCH), 4.15 (d, J = 4 Hz, 2H, CH_2NH), 3.99 (br s, 1H, NH), 3.64 (s, 3H, CO_2CH_3), 2.08 (t, J = 7.5 Hz, 2H, CH_2S), 2.00 (s, 3H, SCH_3), 1.86 (m, 1H, C*H*HCH$_2$), 1.66 (m, 1H, C*H*HCH$_2$). HRMS (ESI) calcd for $C_{34}H_{34}N_4O_3SH^+$ 579.2430. Found 579.2432.

The methyl ester was hydrolyzed in a similar manner to that described for **3a** to give **4b** as white solid (74%). Mp 180–181 °C. ^{1}H NMR (CD_3OD) δ 7.98 (s, 1H), 7.79 (m, 2H, aryl H), 7.51 (s, 1H, imid-2H), 7.43 (m, 2H, aryl H), 7.29 (m, 4H, aryl H), 7.22 (m, 3H, aryl H), 7.06 (s, 1H, imid-4H), 6.48 (d, J = 7 Hz, 1H, aryl H), 6.31 (d, J = 1.8 Hz, 1H, aryl H), 5.47 (s, 2H, CH_2N), 4.43 (m, 1H, αCH), 4.22 (s, 2H, CH_2N), 2.15 (m, 1H, C*H*HS), 2.04 (m, 1H, C*H*HS), 1.99 (s, 3H, SCH_3), 1.91 (m, 1H, C*H*HCH$_2$), 1.72 (m, 1H, C*H*HCH$_2$). HRMS (FAB) m/z calcd for $C_{33}H_{32}N_4O_3S$ H^+ 565.2273. Found 565.2271.

4-[*N*-(1-(Isobutyl-1*H*-imidazol-5-yl)methylamino]-2-phenylbenzoylmethionine (6b, FTI-2239). 1-Isobutyl-5-(hydroxymethyl)-imidazole was prepared using 4-(hydroxymethyl)imidazole hydrochloride and 1-bromo-2-methylpropane by a similar procedure to that described for 1-benzyl-4 or 5-hydroxymethyl-imidazole (12%). ^{1}H NMR ($CDCl_3$) δ 7.42 (s, 1H, imid-2H), 6.91 (s, 1H, imid-4H), 4.62 (s, 2H, CH_2O), 3.81 (d, J = 7.5 Hz, 2H, NCH_2), 2.13 (m, 1H, CH), 0.92 (d, J = 6.5 Hz, 6H, CH_3 × 2).

The corresponding aldehyde was derived from the alcohol by a similar procedure to that described for **3a**, and used for the next reaction without further purification to give 4-[*N*-(1-(isobutyl-1*H*-imidazol-5-yl)methylamino]-2-phenylbenzoylmethionine methyl ester as an oil (100%). ^{1}H NMR ($CDCl_3$) δ 7.70 (d, J = 8.5 Hz, 1H), 7.37–7.45 (m, 6H, aryl H and imid-2H), 7.00 (s, 1H, imid-4H), 6.67 (dd, J = 2.2 and 8.5 Hz, 1H, aryl H), 5.75 (d, J = 7.7 Hz, 1H, NH), 4.60–4.64 (m, 1H, αCH), 4.28 (d, J = 4.8 Hz, 2H, CH_2), 4.15 (br s, 1H, NH), 3.71 (d, J = 7.7 Hz, 2H, CH_2), 3.64 (s, 3H, CO_2CH_3), 2.07 (t, J = 7.4 Hz, 2H, SCH_2), 2.01–2.04 (m, 1H, CH), 2.00 (s, 3H, SCH_3), 1.85–1.90 (m, 1H, C*H*H), 1.85–1.90 (m, 1H, C*H*H), 0.90 (d, J = 6.5 Hz, 6H, 2 × CH_3). HRMS (ESI) calcd for $C_{27}H_{34}N_4O_3SH^+$ 495.2430. Found 495.2414.

The methyl ester was hydrolyzed in a similar manner to that described for **3a** to give **6b** as an amorphous solid (51%). ^{1}H NMR (CD_3OD) δ 8.16 (s, 1H, imid-2H), 7.44 (d, J = 8.5 Hz, 1H, aryl H), 7.32–7.31 (m, 5H, aryl H), 7.16 (s, 1H, imid-4H), 6.71 (dd, J = 3.0 and 8.5 Hz, 1H, aryl H), 6.63 (d, J = 3.0 Hz, 1H, aryl H), 4.39 (dd, J = 4.5 and 8.3 Hz, 1H, αCH), 4.44 (s, 2H, CH_2), 3.95 (d, J = 7.5 Hz, 2H, CH_2), 2.14–2.23 (m, 2H, SCH and CH), 2.07–2.09 (M, 1H, SCH), 2.01 (s, 3H, SCH_3), 1.93–1.95 (m, 1H, C*H*H), 1.75–1.77 (m, 1H, C*H*H), 0.94 (t, J = 6.5 Hz, 6H, CH_3 × 2). HRMS (FAB) m/z calcd for $C_{26}H_{32}N_4O_3S$ H^+ 481.2273. Found 481.2272.

4-[*N*-(1-(*n*-Hexyl-1*H*-imidazol-4-yl)methylamino]-2-phenylbenzoylmethionine (7a, FTI-2236). 1-*n*-Hexyl-4-(hydroxymethyl)imidazole was prepared using 4-(hydroxymethyl)imidazole hydrochloride and 1-bromo-hexane by a similar procedure to that described for 1-benzyl-4 or 5-hydroxymethylimidazole, colorless oil (25%). ^{1}H NMR ($CDCl_3$) δ 7.34 (s, 1H, imid-2H), 6.84 (s, 1H, imid-5H), 4.55 (s, 2H, CH_2), 3.86 (t, J = 7.4 Hz, 2H, CH_2), 1.73 (m, 2H, CH_2), 1.27 (s, 1H), 1.24 (m, 6H, $(CH_2)_3$), 0.86 (t, J = 7.0 Hz, 3H).

The corresponding aldehyde was derived from the alcohol by a similar procedure to that described for **3a**, and used for the next reaction without further purification to give 4-[*N*-(1-(*n*-hexyl-1*H*-imidazol-4-yl)methylamino]-2-phenylbenzoyl-methionine methyl ester as an oil (85%). ^{1}H NMR ($CDCl_3$) δ 7.68 (d, J = 8.6 Hz, 1H, aryl H), 7.36–7.43 (m, 6H, aryl H and imid-2H), 6.81 (s, 1H, imid-5H), 6.66 (dd, J = 2.6 Hz, 1H, aryl H), 6.50 (d, J = 2.6 Hz, 1H, aryl H), 5.70 (d, J = 7.7 Hz, amido NH), 4.62 (m, 2H, αCH and NH), 4.28 (s, 2H, CH_2), 3.87 (t, J = 7.4 Hz, 2H, CH_2), 3.64 (s, 3H, CO_2CH_3), 2.10 (t, J = 7.7 Hz, CH_2), 2.00 (s, 3H, SCH_3), 1.88 (m, 1H, C*H*H), 1.75 (m, 2H, C*H*H), 1.66 (m, 1H, C*H*H), 1.29 (m, 6H, $(CH_2)_3$), 0.88 (t, J = 6.6 Hz, 3H, CH_3). HRMS (ESI) calcd for $C_{29}H_{38}N_4O_3SH^+$ 523.2743. Found 523.2725.

The methyl ester was hydrolyzed in a similar manner to that described for **3a** to give **7a** as an amorphous solid (77%). ^{1}H NMR (CD_3OD) δ 7.89 (s, 1H, imid-2H), 7.40 (d, J = 8.6 Hz, 1H, aryl H), 7.35 (m, 4H, aryl H), 7.31 (m, 1H, aryl H), 7.13 (s, 1H, imid-2H), 6.65 (dd, J = 1.8 and 8.6 Hz, 1H, aryl H), 6.58 (d, J = 1.8 Hz, 1H, aryl H), 4.37 (m, 1H, αCH), 4.31 (s, 2H, CH_2), 3.99 (t, J = 7.0 Hz, 2H, CH_2), 2.06 (m, 1H, C*H*H), 2.05 (m, 1H, C*H*H), 2.00 (s, 3H, SCH_3), 1.91 (m, C*H*H), 1.76 (m, 3H, CH_2 and C*H*H), 1.29 (m, 6H, $(CH_2)_3$), 0.88 (t, J = 4.4 Hz, 3H, CH_3). HRMS (FAB) m/z calcd for $C_{28}H_{36}N_4O_3S$ H^+ 509.2586. Found 509.2584.

4-[*N*-(1-*H*-Imidazol-4-yl)methylamino]-2-(2-methylphenyl)-benzoylmethionine (10, FTI-2128). 1-Triphenylmethyl-imidazole-4-carboxaldehyde (0.25 g, 0.7 mmol) and *N*-(4-amino-2-(2-methylphenylbenzoyl)methionine methyl ester hydrochloride (0.3 g, 0.7 mmol) were dissolved in 10 cm^3 of a 95% methanol–5% acetic acid solution and stirred for 30 min before adding 1 equivalent of sodium cyanoborohydride (0.044 g, 0.7 mmol). The reaction was stirred over a period of 3 h while additional aldehyde was added until all of the amine hydrochloride had disappeared. The reaction mixture was concentrated, taken up in ethyl acetate and washed with a saturated solution of sodium bicarbonate. The organic phase was dried over magnesium sulfate and concentrated. The residue was purified by flash chromatography (1 : 1 ethyl acetate–hexanes) to give 4-[*N*-(1-*H*-imidazol-4-yl)methylamino]-2-(2-methylphenyl)benzoylmethionine methyl ester as a white foam (0.36 g, 74%). ^{1}H NMR ($CDCl_3$) δ 7.45 (s, 1H), 7.25–7.35 (m, 13H), 7.09–7.13 (m, 6H), 6.74 (s, 1H), 6.65 (dd, J = 9.3, 1.5 Hz, 1H), 6.34 (d, J = 2.5 Hz, 1H), 5.69 (t, J = 7.35 Hz, 1H), 4.56–4.61 (m, 1H), 4.27 (d, J = 4.8 Hz, 2H), 3.64 (s, 3H), 2.00–2.15 (m, 8H), 1.79–1.86 (m, 1H), 1.48–1.56 (m, 8H); ^{13}C NMR ($CDCl_3$) δ 172.4, 167.6, 167.2, 150.3, 142.4, 141.4, 141.2, 139.1, 138.2, 136.6, 132.3, 132.0, 130.8, 130.6, 129.9, 129.2, 128.9, 128.3, 126.5, 126.4, 121.9, 121.4, 119.3, 114.2, 112.0, 75.6, 52.4, 51.7, 41.8, 32.0, 29.6, 20.1, 15.4; FAB $[M + H]^+ = 695^+$.

The above compound (0.15 g, 0.22 mmol) was dissolved into 3.5 cm^3 of THF and cooled to 0 °C. Lithium hydroxide (18.1 mg dissolved in 3.5 cm^3 of water) was slowly added to the reaction mixture and stirred for 1 h. The pH was adjusted using 1 N HCl and placed under vacuum to remove excess THF. The residue was taken up in ethyl acetate and dried over magnesium sulfate and concentrated under vacuum. The residue was taken up in 4 cm^3 of methylene chloride and 8 cm^3 of trifluoroacetic acid and the reaction mixture was immediately quenched with triethylsilane until colorless. The reaction was stirred for an addition 2 h and concentrated to an oil. The residue was taken up in methylene chloride and 3 N HCl added. The solid was vacuum filtered, collected and dried under vacuum to give **10** as a HCl salt (0.050 g, 50%). Mp 112–115 °C. ^{1}H NMR (300 MHz, CD_3OD) δ 8.65 (s, 1H), 7.58–7.66 (m, 1H), 7.49 (s, 1H), 7.11–7.25 (m, 4H), 6.80 (dd, J = 8.6, 2.4 Hz, 1H), 6.50 (d, J = 1.9 Hz, 1H), 4.53 (s, 2H), 4.39–4.40 (m, 1H), 2.04–2.12 (m, 5H), 1.98 (s, 3H), 1.81–1.90 (m, 1H), 1.46–1.64 (m, 1H); FAB $[M + H]^+ = 440^+$.

4-Nitro-2-(2-methoxyphenyl)benzoic acid. 2-Bromo-4-nitro-toluene (2.6 g, 11.9 mmol) and 2-methoxyboronic acid (2.4 g, 12 mmol) were dissolved into 35 cm^3 of acetone and 40 cm^3 of water. Potassium carbonate (4.2 g, 30 mmol) and palladium acetate (0.15 g, 0.67 mmol) were added and refluxed for 20 h. The reaction mixture was cooled, extracted with diethyl ether, filtered over a celite plug and evaporated to give 4-nitro-2-(2-methoxyphenyl)toluene (1.3 g, 45%) as yellow solid. Mp 76–77° C. ^{1}H NMR ($CDCl_3$) δ 8.11 (dd, J = 8.3, 2.4 Hz, 1H, aromatic), 8.07 (d, J = 2.2 Hz, 1H, aromatic), 7.40 (d, J = 8.1 Hz, 2H), 7.15 (dd, J = 7.3, 1.5 Hz, 1H), 7.06 (d, J = 7.3 Hz, 1H, aromatic), 7.00 (d, J = 8.3 Hz, 1H, aromatic), 3.78 (s, 3H, OCH_3), 2.24 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$) δ 156.5, 146.2, 145.4, 140.2, 130.8, 130.5, 129.9, 128.6, 125.3, 122.4, 120.9, 111.0, 55.6, 20.4; HRMS: calcd for $C_{14}H_{13}NO_3$: m/e 243.0895, found 243.0900.

The above compound (1.3 g, 5.7 mmol) was suspended in 10 cm^3 of pyridine and 20 cm^3 of water and heated to reflux. Potassium permanganate was added in portions and the reaction was heated for 16 h. The reaction mixture was filtered through a celite plug and the filtrate carefully acidified with concentrated HCl. The filtrate was extracted with diethyl ether, dried over magnesium sulfate and concentrated to give an oil which was recrystallized from ethyl acetate and hexanes to give 4-nitro-2-(2-methoxyphenyl)benzoic acid (0.65 g, 45%). Mp 171–175 °C. ^{1}H NMR ($CDCl_3$) δ 8.25 (d, J = 2.2 Hz, 1H, aromatic), 8.22 (s, 1H, aromatic), 8.06 (d, J = 8.6 Hz, 1H, aromatic), 7.40 (t, J = 7.6 Hz, 1H, aromatic), 7.31 (dd, J = 7.5, 1.5 Hz, 1H, aromatic), 7.09 (t, J = 7.3 Hz, 1H), 6.91 (d, J = 8.2 Hz, 1H), 3.71 (s, 3H, OCH_3). ^{13}C NMR ($CDCl_3$) δ 172.3,

155.9, 150.0, 141.0, 136.2, 131.0, 130.5, 129.9, 127.9, 126.6, 122.0, 121.5, 110.8, 55.1.

4-[*N*-(1-*H*-Imidazol-4-yl)methylamino]-2-(2-methoxyphenyl)-benzoylmethionine (12, FTI-2134). 4-Nitro-2-(2-methoxyphenyl)benzoic acid (0.55 g, 2 mmol) was coupled with L-methionine methyl ester hydrochloride in the manner described above to give 4-nitro-2-(2-methoxyphenyl)benzoyl-methionine methyl ester (0.8 g, 93%) as an oil after purification by flash column chromatography (3 : 2 ethyl acetate–hexanes, silica). ^{1}H NMR ($CDCl_3$) δ 8.25 (dd, J = 8.5, 2.3 Hz, 1H, aromatic), 8.14 (d, J = 2.2 Hz, 1H, aromatic), 7.90 (d, J = 8.5 Hz, 1H, aromatic), 7.45 (app t, 1H, aromatic), 7.25 (d, J = 6.5 Hz, 1H), 7.08 (app t, 1H, aromatic), 7.01 (d, J = 8.3 Hz, 1H), 6.5 (br s, 1H), 4.66 (m, 1H, α Met-H), 3.81 (s, 3H, OCH_3), 3.69 (s, 3H, OCH_3), 1.87–2.00 (m, 5H), 1.66–1.74 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 171.9, 166.9, 156.2, 148.7, 141.5, 137.9, 130.9, 130.6, 130.1, 127.2, 126.3, 122.7, 121.4, 111.1, 55.7, 52.7, 52.0, 31.8, 29.3, 15.4.

The above compound (0.7 g, 1.7 mmol) was reduced in a similar manner to that previously reported[9] to give 4-amino-2-(2-methoxyphenyl)benzoylmethionine methyl ester hydrochloride (0.65 g, 92%). ^{13}C NMR (CD_3OD) δ 173.4, 171.2, 157.5, 140.3, 138.4, 133.2, 131.8, 131.3, 130.7, 128.7, 127.0, 123.0, 121.7, 112.1, 55.9, 53.0, 31.4, 30.7, 15.0; FAB $[M + H]^+ = 389^+$.

This compound (0.3 g, 0.7 mmol) was coupled with triphenylimidazole carboxyaldehyde (0.29 g, 0.85 mmol) in a similar manner described above to give the fully protected compound as a white foam (0.26 g, 52%), which was deprotected by the same method described above to give **12** as the hydrochloride salt (0.09 g, 93%). The HPLC showed 91% purity.

2-[(2′-Methyl-5-{[(5-methyl-1*H*-imidazol-4-ylmethyl)amino]-methyl}biphenyl-2-carbonyl)amino]-4-methylsulfanylbutyric acid (15, FTI-2288). To a solution of the 2-[(5-aminomethyl-2′-methylbiphenyl-2-carbonyl)amino]-4-methylsulfanylbutyric acid methyl ester (**21**)[12] (33 mg, 0.06 mmol) in MeOH (0.5 cm^3) was added 5-methyl-4-imidazole carboxyaldehyde (6 mg, 0.06 mmol). After stirring at room temperature for 1 h, $NaBH_4$ (2.1 mg, 0.05 mmol) was added and stirred overnight at room temperature. Evaporation of the solvent, followed by extraction with 10% MeOH in CH_2Cl_2 (40 cm^3) from saturated $NaHCO_3$ (20 cm^3) afforded the crude product. The compound was purified by gel chromatography on Sephadex LH-20 with MeOH–$CHCl_3$ = 1 : 1 to give an amorphous solid (15 mg, 58%). ^{1}H NMR (CD_3OD) δ 1.70 (m, 1H, C*H*HCH$_2$S), 1.95–2.17 (m, 12H, 2 × CH_3, SCH_3, CH_2S, C*H*HCH$_2$), 3.67 (s, 3H, CO_2CH_3), 3.71 (s, 2H, NCH_2), 3.84 (s, 2H, NCH_2), 7.14–7.53 (m, 8H, aryl H).

Compound **15** was prepared by a similar procedure to that described for **16** (100%). ^{1}H NMR (CD_3OD) δ 7.42–7.13 (m, 8H), 4.20 (br s, 1H, αCH), 4H from CH_2NHCH_2 were overlapped under the D_2O peak at 3.90, 2.23–1.86 (m, 12 H), 1.60 (br s, 1H).

4-[*N*, *N*-Bis((1*H*-imidazol-4-yl)methyl)aminomethyl]-2-(1-(*o*-tolyl))benzoylmethionine methyl ester (17, FTI-2295). A solution of **21**[12] (490 mg, 1.27 mmol), 1-*t*-butoxycarbonyl-4-chloromethylimidazole (578 mg, 2.67 mmol), and $NaHCO_3$ (225 mg, 2.67 mmol) in DMF (10 cm^3) was stirred at 65 °C overnight. After distillation of DMF, the residue was dissolved in AcOEt (350 cm^3) and H_2O (200 cm^3), and the organic layer was washed with H_2O (200 cm^3 × 3) and brine, and dried over Na_2SO_4. The crude product was purified by SiO_2 column chromatography with $CHCl_3$–acetone–EtOH = 100 : 40 : 8 to afford the product as colorless oil (834 mg, 88%). ^{1}H NMR ($CDCl_3$) δ 8.02 (s, 2H, imid-2H × 2), 7.93 and 7.90 (d each, J = 8.4 Hz each, 1H, aryl H), 7.53 (d, J = 8.4 Hz, 1H, aryl H), 7.32–7.18 (m, 7H, aryl H and imid-5H × 2), 5.90 (t, J = 7.6 Hz, 1H, amido NH), 4.60 (m, 1H, αCH), 3.78 (s, 2H, NCH_2), 3.68 (s, 4H, CH_2N × 2), 3.65 (s, 3H, CO_2CH_3), 2.17–1.83 (m, 9H, CHH, CH_2S, SCH_3, tolyl-CH_3), 1.62 (s, 18H, Boc × 2).

To a solution of the Boc-protected bis-imidazole compound (834 mg, 1.12 mmol) in CH_2Cl_2 (5 cm^3) was added TFA (5 cm^3) dropwise at 0 °C, and the mixture was stirred at room temperature for 15 min. Evaporation of the solvent, followed by purification of the crude oily product by SiO_2 column chromatography with $CHCl_3$–MeOH–28% NH_4OH = 5 : 1 : 0.1 afforded the product as an amorphous solid (450 mg, 74%). ^{1}H NMR (DMSO-d_6) δ 1.86–1.94 (m, 2H, CH_2), 2.07–2.33 (m, 8H, CH_3, SCH_3, and CH_2), 3.67 (s, 3H, CO_2CH_3), 3.79 (s, 2H, NCH_2), 3.84 (s, 4H, $N(CH_2)_2$), 4.40 (br s, 1H, αCH),7.26–7.37 (m, 6H, aryl H), 7.58 (s, 2H, imid-5H), 7.76 (s, 2H, imid-2H), 1.92 (s, 1H, aryl H). ^{13}C NMR (DMSO-d_6) δ 14.4, 19.8, 29.3, 30.1, 46.8, 51.0, 51.8, 56.3, 72.2, 117.6, 118.0, 125.0, 127.2, 127.6, 129.2, 129.5, 129.8, 130.2, 134.4, 135.3, 139.0, 139.3, 158.0, 168.3, 171.8. LRMS (FAB) m/z calcd for $C_{29}H_{34}N_6O_3S$ H^+ 547. Found 547. Anal. calcd for $C_{29}H_{34}N_6O_3S{\cdot}3CF_3CO_2H{\cdot}0.5H_2O$: C,46.82; H, 4.23; N, 9.36. Found: C, 46.84; H, 4.22; N, 9.19.

4-[*N*, *N*-Bis((1*H*-imidazol-4-yl)methyl)aminomethyl]-2-(1-(*o*-tolyl))benzoylmethionine trifluoroacetate (16, FTI-2287). To a solution of **17** (450 mg, 0.82 mmol) in THF (20 cm^3) was added 0.1 N LiOH (16 cm^3) at 0 °C and the mixture was stirred at room temperature for 30 min. After neutralization with 0.5 M HCl, the solution was concentrated to almost dryness. The residue was purified by gel permiation on Sephadex LH-20 with MeOH–$CHCl_3$ = 1 : 1, and the product was dissolved in TFA at 0 °C. The solution was concentrated and the resulting residue was dried *in vacuo* overnight. To the yellow residue was added dry Et_2O to precipitate the compound as the TFA salt. The white powdery product was collected by centrifuge and dried *in vacuo* (549 mg, 77%). ^{1}H NMR (DMSO-d_6) δ 9.06 (s, 2H), 8.32–8.05 (m, 2H), 7.61 (s, 2H), 7.45 (s, 2H), 7.23–7.11 (m, 6H), 4.22 (br s, 1H, αCH), 3.87 (s, 4H, CH_2N × 2), 3.70 (s, 2H, NCH_2), 2.20–1.71 (m, 10H, SCH_3, CH_3, CH_2CH_2). HRMS (FAB) m/z calcd for $C_{28}H_{32}N_6O_3S$ H^+ 533.2335. Found 533.2334.

Crystal structures

Crystals of the FPT–**13** and FPT–**16** complexes were prepared by soaking the respective inhibitors into preformed crystals using methods previously described.[20] X-Ray diffraction data were collected at the IMCA-CAT 17-BM beamline equipped with a Mar 165 CCD detector. With the detector set at 130 mm, data were collected in 240 contiguous 0.30° oscillation images at 1 Å wavelength. The data for compound **13** extends to 2 Å resolution, has an R_{merge} of 5.3% and 4.5-fold multiplicity. The data for compound **16** extends to 1.9 Å resolution, has an R_{merge} of 3.6% and 4.6-fold multiplicity. The structures were refined using CNX (Accelrys Inc.) to an R_{factor} of approximately 19%.

In vitro assay and inhibition of protein prenylation in whole cells

In vitro inhibition assays for PFTase and PGGTase-I were carried out by measuring the incorporation of [^{3}H]FPP and [^{3}H]GGPP into recombinant H-Ras-CVLS and H-Ras-CVLL, respectively, as previously described.[14] The *in vivo* inhibition of farnesylation and geranylgeranylation was determined based on the level of inhibition by peptidomimetic compounds of H-Ras and Rap1 processing, respectively.[21] Briefly, oncogenic H-Ras-transformed NIH 3T3 cells were treated with various concentrations of inhibitors, and the cell lysates were isolated and proteins separated on a 12.5% SDS-PAGE gel. The separated proteins were transferred to nitrocellulose and immunoblotted using an anti-Ras antibody (Y13-258) or an anti-Rap1A antibody (Santa Cruz Biotechnology, Santa Cruz, CA). Antibody reactions were visualized using either peroxidase-conjugated goat anti-rat IgG or goat anti-rabbit IgG (Jackson ImmunoResearch, West Grove, PA) and an enhanced chemiluminescence detection system.

Antitumor activity in the nude mouse tumor xenograft model

Nude mice (Charles River, Wilmington, MA) were maintained in accordance with the Institutional Animal Care and Use Committee (IACUC) procedures and guidelines. A549 cells were harvested, resuspended in PBS and 1×10^7 cells injected s.c. onto both the right and left flanks of eight week old female nude mice as reported previously.[22] When tumors reached about 150 mm^3, animals were randomized and dosed s.c. with a mini-pump as described previously[22] with 25 mg^{-1} kg^{-1} d^{-1} of either FTI-2148, FTI-2287, or with an equal volume of vehicle control. The tumor volumes were determined by measuring the length (l) and the width (w) and calculating the volume ($V = lw^2/2$) as described previously.[23] Statistical significance between control and treated animals were evaluated by using Student's t-test.

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