Design and in Vivo Analysis of Potent Non-Thiol Inhibitors of Farnesyl Protein Transferase

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Inhibitors of farnesyl protein transferase (FPTase) based upon a pseudotripeptide template are described that comprise an imidazole group substituted with a hydrophobic substituent. (1,5)-Disubstitution of the imidazole group is shown to be the optimal array that leads to potent and selective inhibitors of FPTase. A variety of aryl and isoprenyl substituents are shown to afford effective inhibitors, and the mechanism by which these compounds inhibit FPTase has been investigated. The biochemical behavior of these compounds suggests that they bind to FPTase at the site usually occupied by the protein substrate. In experiments in cell culture, the methyl ester prodrugs of these inhibitors are cell permeant and potently inhibit the posttranslational modification of H-Ras protein. Additionally, these molecules revert the phenotype of *ras* transformed cells as evidenced by their ability to slow the growth of *ras* transformed cell lines in soft agar. One of the inhibitors, as its methyl prodrug, was evaluated in two in vivo models of tumor growth. The compound selectively inhibited the growth of tumors derived from H-*ras* transformed cells, in nude mice, and caused the regression of preexisting tumors in an H-*ras* transgenic animal model.

Introduction

Approximately 20−30% of a wide variety of human cancers including 50% of colon and over 90% of pancreatic cancers have been found to contain a mutated or activated *ras* gene.[1,2] This suggests that therapeutic agents directed against the proteins encoded by these genes might be effective anticancer agents.[3,4] Ras proteins are synthesized in the cell as cytosolic precursor molecules that require a series of posttranslational modifications to acquire full biological activity.[5] The first and obligatory of these posttranslational modifications is isoprenylation by the enzyme farnesyl protein transferase (FPTase).

FPTase is a heterodimeric protein that is composed of 48-kDa (α) and 46-kDa (*β*) subunits. The enzyme catalyzes the transfer of the 15-carbon atom isoprenoid chain, farnesyl, from the prenyl donor farnesyl diphosphate (FPP) to the thiol of a cysteine residue in a substrate protein. The protein substrates for FPTase contain the consensus sequence motif CAAX at their carboxy terminus, where C is cysteine, A is generally an aliphatic amino acid, and X is commonly methionine or serine. The related enzyme geranylgeranyl protein transferase type 1 (GGPTase 1) is comprised of the same 48-kDa α subunit and a unique *β* subunit. This enzyme catalyzes the transfer of the 20-carbon isoprenoid geranylgeranyl from geranylgeranyl diphosphate (GGPP) to proteins that contain a CAAX motif in which the carboxy terminal X residue is most commonly the branched amino acid leucine.[6]

The mechanism of the FPTase enzyme-catalyzed farnesylation reaction has been studied in detail by steady-state[7] and pre-steady-state kinetic analysis.[8,9] The reaction occurs via a functionally ordered mechanism in which FPTase initially binds FPP to form a binary complex. The enzyme subsequently recognizes and binds its substrate protein through coordination of the cysteinyl thiol group to an active site zinc atom.[10] This interaction in conjunction with an attractive Coulombic interaction between the substrates C-terminal carboxylate and the guanidinium group from arginine 202 in the proteins *β* subunit[11] are key determinants of substrate recognition. Nucleophilic activation of the thiol group occurs upon its coordination to the zinc atom, and the transfer of the farnesyl residue proceeds from the ternary complex. The reaction occurs with inversion of configuration at the allylic carbon through a transition state that is thought to involve partial ionization prior to carbon−sulfur bond formation.[12,13] The rate-limiting step in the overall reaction is the slow release of the product from the enzyme, a process that is catalyzed by an additional molecule of the FPP cosubstrate.[14] Recently the three-dimensional structure of the FPTase·FPP binary complex has been reported.[15,16] This information may lead to a more complete understanding of the details of substrate recognition and catalysis.

The current interest[17−19] in obtaining inhibitors of FPTase (FTIs) stems from the discovery that the protein products of the *ras* oncogenes are substrates for FPTase. Importantly, attachment of the farnesyl group to oncogenic variants of Ras is essential for their ability to transform cells. Treatment of *ras* transformed cells with specific inhibitors of FPTase has been shown to inhibit the farnesylation of Ras protein and revert the phenotype and morphology of *ras* transformed cell lines in tissue culture.[20,21] Additionally, FTIs have been shown

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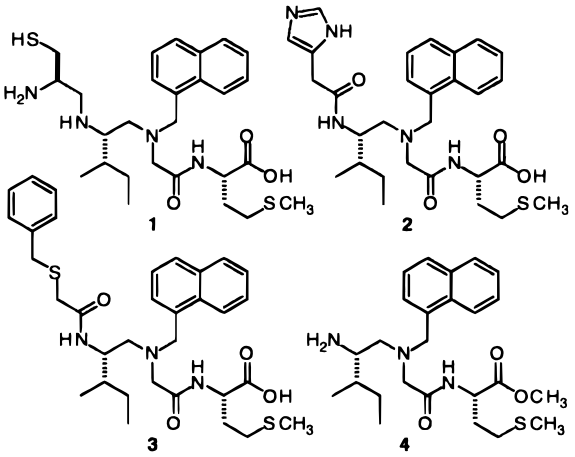
Chart 1. Pseudotetrapeptide Inhibitors of Farnesyl Protein Transferase



to slow the growth of tumors in xenograft experiments in nude mice.[22−24] Similarly, in studies involving transgenic animal models, FTIs have been demonstrated to shrink preexisting tumors.[25] While these studies have suggested the potential utility of FTIs as anticancer agents, evidence is accumulating that the mechanism of FTI action is complex and may involve the combined effect of inhibiting the farnesylation of a variety of cellular proteins.[26−30]

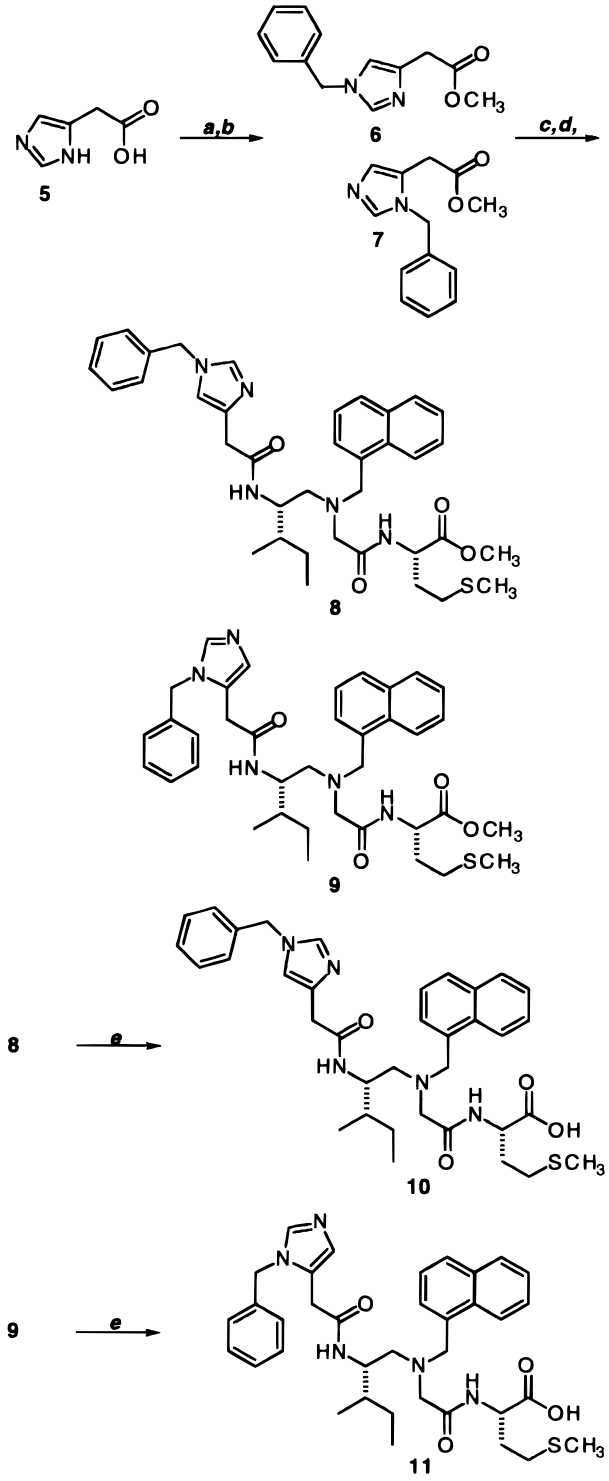
The *N*-(naphthylmethyl)glycine pseudotripeptide template **4** has been shown to function as an effective platform for the discovery of potent cysteinyl,[31] **1** (Chart 1), and non-cysteinyl,[32,33] **2** and **3**, inhibitors of FPTase. Comparison of the structures of the cysteinyl inhibitor **1** with those of the imidazole and thioether derivatives **2** and **3** suggested that the thiol and imidazole[19,34−39] functionalities share the common property of being capable of acting as effective ligands to zinc. In contrast, the thioether **3** lacks a putative zinc ligand and presumably derives its affinity for FPTase from productive hydrophobic interactions that the benzyl substituent makes with suitable active site residues. This analysis suggested that zinc ligation and occupancy of a nearby hydrophobic binding site might be accomplished simultaneously by a single molecule bearing suitable appendages. In this paper we describe the synthesis and biological properties of potent inhibitors of FPTase that feature a substituted imidazole moiety that appears to satisfy these requirements.

Chemistry

To elucidate which regioisomeric substitution of the imidazole was most consistent with FPTase inhibitory potency, substituted imidazoleacetic acid derivatives were prepared, coupled to the previously described amine **4**,[31] and screened for FPTase inhibitory activity. Thus, imidazoleacetic acid methyl ester was treated with sodium hydride and alkylated with benzyl bromide to afford an inseparable (3:1) mixture of the regioisomeric benzyl derivatives **6** and **7** (Scheme 1). The major isomer, the 1,4-substituted imidazole **6**, resulted from preferential alkylation of the least sterically hindered nitrogen atom.

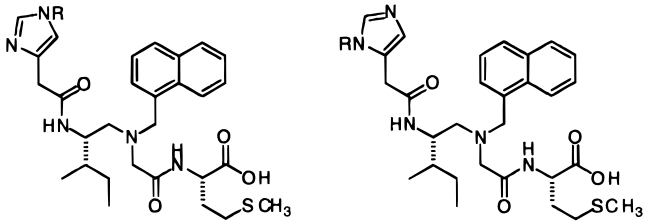
Acidic hydrolysis of the methyl esters **6** and **7** occurred cleanly to afford the corresponding acids. These acids underwent efficient EDC coupling reactions with the amine **4** to afford the corresponding amides **8** and **9** which were separated by HPLC. The major isomer was assigned structure **8** based upon appropriate nuclear Overhauser effect experiments. Hydrolysis of the carboxy terminal methyl esters **8** and **9** afforded the corresponding acids **10** and **11** in good yield. The ability of the regioisomeric compounds **10** and **11** to inhibit FPTase was assessed in an in vitro assay, and the results are presented in Table 1.

Scheme 1. Synthesis of Regioisomeric Benzylimidazole Derivatives[a]



[a] Reagents: (a) HCl, methanol; (b) NaH, $PhCH_2Br$, DMF; (c) HCl, H_2O; (d) EDC, NEt_3, HOOBT, **4**; (e) LiOH, methanol, H_2O.

Table 1. Dependence of FPTase Inhibitory Potency upon the Imidazole Substitution Pattern



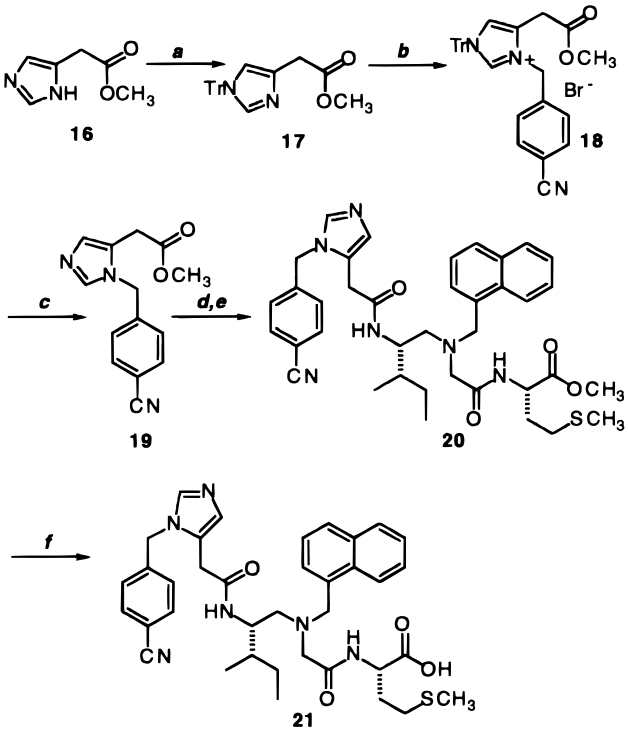
R	compd	FPTase IC_{50} (nM)[a]	compd	FPTase IC_{50} (nM)[b]
$C_6H_5CH_2$	**10**	82 ± 2 (2)	**11**	0.15 (1)
4-$C_5H_4NCH_2$	**12**	74 ± 26 (3)	**13**	2.8 ± 0.8 (3)
4-$NO_2C_5H_4CH_2$	**14**	10 ± 5 (3)	**15**	0.16 ± 0.05 (3)

[a] The concentration of compound required to inhibit 50% of FPTase-catalyzed incorporation of [^{3}H]FPP into recombinant [Leu^{68}]RAS1(term.)CVIM protein[5] by 50%. Determined using FPTase at a concentration of approximately 1 nM. [b] Determined using FPTase at a concentration of 10 pM. The assay results are reported as a concentration ± SEM for the number of determinations shown in parentheses. With one determination, the values are estimated to be reliable to within 2-fold.

The 1,5-disubstituted imidazole isomer **11** was significantly more potent than its regioisomer **10**. Importantly, the benzylimidazole **11** was also more potent than both the unsubstituted imidazole derivative **2** which has an IC_{50} of 6.2 nM[32] and the benzyl thioether **3** which has an IC_{50} of 36 nM.[33] To assess the generality of the regiochemical dependence of FPTase inhibitory potency, the corresponding 4-pyridylmethyl and 4-nitrobenzyl derivatives were subsequently prepared. Again the 1,5-disubstituted imidazole derivatives **13** and **15** were found to have superior FPTase inhibitory activity. These results are presented in Table 1.

Since the most potent isomer in each of these examples was derived from the minor product of the imidazole alkylation reaction, our attention turned toward securing a regioselective synthesis of the required 1,5-disubstituted imidazoles. This particular array formally requires the alkylation of the more sterically encumbered imidazole nitrogen atom. We, therefore, adopted a blocking/alkylation strategy similar to that used previously to prepare regioselectively alkylated histidine derivatives.[40,41] The synthetic route is outlined in Scheme 2. This methodology has proven suitable for the preparation of the benzylic and allylic imidazole derivatives illustrated in Table 2. For example, 4-imidazoleacetic acid methyl ester **16** was protected in a highly regioselective manner with the sterically demanding triphenylmethyl (trityl) group. Subsequent alkylation of the imidazole, by heating the tritylimidazole derivative **17** with 4-cyanobenzyl bromide in acetonitrile, resulted in the formation of the imidazolium salt **18**. In this case, the intermediate imidazolium salt **18** precipitated from the reaction mixture and could be isolated in high purity by filtration. We have found that tritylimidazolium salts of this general type are stable but chemically reactive species. Upon warming the imidazolium salt **18** in methanol, the molecule undergoes regiospecific solvolysis to liberate the desired 1,5-disubstituted imidazole derivative **19**, as its hydrobromide salt. In cases where the requisite imidazolium salt did not precipitate from the reaction mixture, the crude imidazolium salt was subjected to methanolysis. The desired imidazole derivatives could then be isolated by column chromatography.[42] We have found this synthetic methodology best suited to the use of activated halides. The chief limitation of the method is that the intermediate imidazolium salts can undergo premature loss of the trityl group during the time course of the alkylation reaction. This can lead to the formation of the corresponding bis-alkylated imidazolium salts. The methyl ester **19** was saponified to the corresponding imidazoleacetic acid derivative, and subsequent EDC coupling with the amine **4** provided the prodrug methyl ester **20** in good yield. Base-catalyzed hydrolysis of the ester **20** to the corresponding carboxylic acid occurred straightforwardly to afford the FTI **21**.

Scheme 2. Synthesis of (1,5)-Disubstituted Imidazole Derivatives[a]

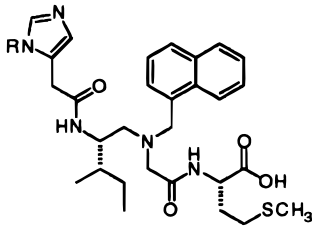


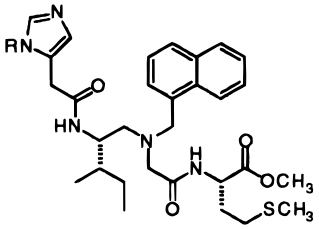
[a] Reagents: (a) TrBr, NEt_3, DMF; (b) 4-$CNC_6H_4CH_2Br$, acetonitrile, 55 °C; (c) methanol, reflux; (d) LiOH, THF, H_2O; (e) EDC, NEt_3, HOOBT, **4**; (f) NaOH, methanol, H_2O.

Structure−Activity Relationships

The compounds prepared in this manner were characterized as in vitro inhibitors of FPTase using purified recombinant human FPTase at a concentration of 1 nM. To more accurately determine the potency of the more active compounds, the inhibitors were further tested in a similar protocol that utilized the enzyme at a concentration of 10 pM.[31] The in vitro substrates used in the enzyme inhibition assay were [1-^{3}H]FPP and [Leu^{68}]-RAS1(term.)CVIM protein[5]. The inhibitory activities of the compounds are presented in Table 2, as an IC_{50} value. This value corresponds to the concentration of inhibitor required to reduce the incorporation of radiolabeled isoprenoid into the Ras protein to one-half of the level observed in the absence of inhibitor. The compounds were similarly assayed against the closely related enzyme GGPTase 1 using either bovine or, in the case of more potent inhibitors, the recombinant human enzyme.

Table 2. In Vitro Activity of FPTase Inhibitors





							soft agar MIC[h]	
R	compd	FPTase IC_{50} (nM)[a]	GGPTase IC_{50} (nM)[b]	compd	CTE (μM)[f]	Raspro IC_{50} (μM)[g]	H-Ras (μM)	Raf (μM)
$C_6H_5CH_2$	**11**	0.15 (1)	8200 (1)	**9**	10−25 (2)	2.5−10 (1)	1 (2)	10 (2)
4-$C_5H_4NCH_2$	**13**	2.8 ± 0.8 (3)	nd[c]	**22**	50 (1)	1−10 (2)	10 (1)	>25 (1)
4-$NO_2C_6H_4CH_2$	**15**	0.16 ± 0.05 (3)	92[d] (1)	**23**	10 (1)	0.1−1 (4)	1 (1)	>10 (1)
4-$CNC_6H_4CH_2$	**21**	0.15 (1)	67 ± 39[d,e] (4)	**20**	10−25 (2)	≤1 (9)	1 (3)	>2.5 (3)
4-$FC_6H_4CH_2$	**24**	0.45 ± 0.02 (2)	5000 (1)	**31**	10 (1)	1−10 (1)	1 (1)	10 (1)
4-$CH_3OC_6H_4CH_2$	**25**	0.55 ± 0.05 (2)	2200 ± 1000 (2)	**32**	10 (1)	1 (1)	1 (1)	>10 (1)
1-$C_{10}H_7CH_2$	**26**	0.12 (1)	10000 (1)	**33**	5−10 (2)	1−10 (2)	1 (2)	10 (2)
2-$C_{10}H_7CH_2$	**27**	0.12 (1)	10000 (1)	**34**	10 (1)	1 (3)	1 (2)	10 (2)
2-$C_9H_6NCH_2$	**28**	4.0 ± 0.5 (2)	17500 ± 2000 (2)	**35**	25 (1)	1−10 (1)	2.5 (1)	>10 (1)
geranyl	**29**	0.41 (1)	10000 (1)	**36**	10 (1)	1−10 (1)	1 (2)	10 (2)
farnesyl	**30**	2.8 ± 0.8 (3)	10000 (1)	**37**	25 (1)	1−10 (1)	2.5 (2)	2.5−10 (2)
	L-749,750[22]	1.8 ± 0.2 (7)	3000 (1)	L-744,832[25]	>50 (2)	0.1−1 (1)	1 (1)	>10 (2)

[a] The concentration of compound required to inhibit 50% of FPTase-catalyzed incorporation of [^{3}H]FPP into recombinant [Leu^{68}]RAS1(term.)CVIM protein[5] by 50%. Determined using FPTase at a concentration of 10 pM. The assay results are reported as a concentration ± SEM for the number of determinations shown in parentheses. [b] The concentration of compound required to inhibit 50% of GGPTase-catalyzed incorporation of [^{3}H]GGPP into the recombinant human Ha-RasCVLL protein by 50%. Determined using GGPTase from bovine brain. [c] nd, not determined. [d] Determined using human recombinant GGPTase. [e] This compound did not inhibit the posttranslational modification of the GGPTase substrate H-RasCVLL at a concentration of 10 μM in NIH 3T3 cells. [f] Highest nontoxic concentration for cultured NIH 3T3 cells as assessed by MTT staining. [g] Inhibition of posttranslational processing of v-Ras protein in cultured NIH 3T3 cells. [h] Minimum inhibitory concentration (MIC) required to achieve a reduction in size and number of colonies of Rat-1 v-*ras* or Rat-1 v-*raf* transformed cells in soft agar relative to vehicle-treated control.

As previously stated, the 1,5-disubstituted imidazole derivatives bearing the benzyl (**11**), 4-nitrobenzyl (**15**), and 4-pyridylmethyl (**13**) imidazole substituents are significantly more potent than their corresponding 1,4-disubstituted isomers (compounds **10**, **14**, and **12**; Table 1). It is interesting that the pyridylmethyl derivative **13** is significantly less active than the benzyl compound **11**. This may indicate that these residues occupy a hydrophobic region within the FPTase active site.

To evaluate if the electronic nature of the benzylic substituent was an important determinant of the potency of this class of inhibitors, a series of benzylimidazole derivatives was prepared in which the electronic character of the para substituent was varied. Inspection of Table 2 shows that FPTase inhibitory potency is relatively insensitive to the electronic influence of this substituent. In contrast, inhibition of GGPTase 1 was most effectively achieved by compounds bearing the strongly electron-deficient 4-nitrobenzyl (**10**) and 4-cyanobenzyl (**2l**) substituents. Despite this GGPTase inhibitory activity, these compounds are still selective inhibitors of FPTase (400-fold). Enlargement of the imidazole substituent from benzyl to 1- or 2-naphthylmethyl was also tolerated by FPTase. The 1- and 2-naphthyl derivatives **27** and **28** are potent and selective inhibitors of the enzyme. In contrast, the 2-quinoline derivative **28**, which may be regarded as a hybrid of the 2-naphthylmethyl **27** and the 4-pyridylmethyl **13**, was significantly less potent than the 2-naphthyl derivative **27**. This behavior parallels that seen in the previous comparison of the benzyl and pyridylmethyl derivatives **11** and **13** and suggests that the pyridyl and quinoline groups occupy a common site within the FPTase enzyme.

To address the possibility that the benzylic imidazole substituent may bind to FPTase by occupying the part of the enzyme active site that binds FPP, the corresponding farnesyl (**30**) and geranyl (**29**) derivatives were prepared. These compounds were also found to be selective inhibitors of FPTase. The geranyl derivative was particularly potent.

To gain more detailed information about the mechanism by which these compounds inhibit FPTase, the 4-cyanobenzyl derivative **21** was characterized by steady-state enzyme kinetic analysis. A Lineweaver−Burk analysis for compound **21** against the protein substrate K-Ras is shown in Figure 1. The 4-cyanobenzylimidazole derivative was found to be a competitive inhibitor with respect to the protein substrate. The K_i for the inhibitor **21** was 1.8 ± 0.1 nM in this assay. Additional experiments suggest that the compound is not competitive with respect to the isoprenoid substrate FPP. These results indicate that the cyanobenzyl group takes advantage of a binding site that is distinct from that usually occupied by the isoprene donor FPP. A similar kinetic analysis of the geranyl derivative **29** showed that this compound was similarly competitive with protein substrate, was not competitive with FPP, and therefore does not function as a product-like inhibitor.[43,44]

Since the identity of the C-terminal residue is an important determinant for the faithful discrimination of substrate proteins by FPTase and GGPTase 1, we evaluated the effects of replacing the C-terminal

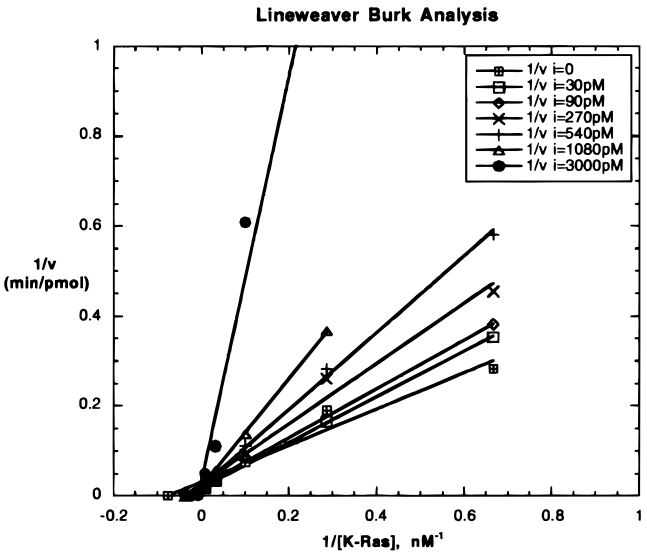


Figure 1. FPTase inhibitor **21** is competitive with K-Ras.

methionine residue of the 4-cyanobenzylimidazole inhibitor **21** with leucine (Table 3). As anticipated, this substitution causes a turnover in enzyme selectivity, with the leucinyl derivative **38** now being 4−5-fold more potent against GGPTase 1 than FPTase. This behavior is also consistent with the mechanistic classification of these inhibitors as being CAAX competitive.

The presence of a free carboxylate at the C-terminus of substrate proteins is also an important determinant in the recognition of substrate peptides by FPTase.[11] This structural feature in a substrate protein appears to be identified by FPTase through attractive Coulombic interactions between the C-terminal carboxylate of the substrate and the guanidine moiety of arginine 202 β from the enzyme. To test the sensitivity of these inhibitors to the absense of a free terminal carboxylate, the carboxylate in **21** was replaced with an isosteric C-terminal amide **40**. In accord with expectation, this substitution results in a 100-fold decrease in FPTase inhibitory potency. However, the potency of this amide derivative (IC_{50} 15 nM) is quite comparable to that of the carboxylate-containing inhibitors **2** and **3**. This finding is important as it indicates that the 4-cyanobenzylimidazole moiety may be incorporated into FPTase inhibitors that may not require a prodrug strategy to gain efficient entry into cells.[45]

To test the facility with which these imidazole derivatives inhibit FPTase in whole cells, the compounds were examined in a series of cell-based assays. The methyl ester prodrugs of the FTIs were used in these experiments. While the ester prodrugs are intrinsically weak inhibitors of FPTase, they appear to be significantly more cell penetrant than their acid counterparts (see Table 3, entries **20** and **21**). Upon entry into the cell, the prodrug esters can be hydrolyzed to their corresponding active acid by the action of intracellular esterases.

The cytotoxicity of the prodrug methyl esters of the FPTase inhibitors was assessed using a viable staining assay with MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide.[21] Cells were incubated with the test compound over a range of concentrations. The cytotoxic endpoint (CTE) defines the highest concentration of compound that is tolerated by NIH 3T3 cells in a 48-h assay. The practical significance of the CTE value is that it defines a concentration of inhibitor above which the effect the compound has on cells may be ascribed to nonspecifc toxicity. For the compounds presented in Table 2 the CTE value ranged from 5 to 50 μM.

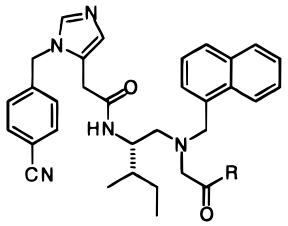
To assess the ability of these compounds to inhibit the posttranslational processing of Ha-Ras in NIH 3T3 cells, an assay based upon the difference in electrophoretic mobility of posttranslationally modified and unmodified Ha-Ras protein on an SDS page gel was utilized.[21] Briefly, cells were incubated in the presense of test compound or solvent control for 24 h, and the newly synthesized proteins were labeled using [^{35}S]-methionine during the following 20 h. The cells were lysed, and the total Ha-Ras protein was immunoprecipitated and subjected to SDS gel electrophoretic separation. The intensity of the unprocessed and processed Ha-Ras bands were then quantitated. An IC_{50} value in this assay is the concentration of inhibitor that results in equal amounts of unfarnesylated and posttranslationally modified Ha-Ras. The results from this assay are shown in Table 2. The HMG CoA reductase inhibitor lovastatin was used as a positive control in this assay, and at a concentration of 15 μM, lovastatin causes >90% inhibition of Ha-Ras processing.

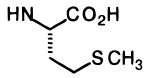
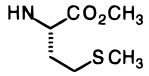
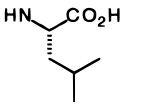
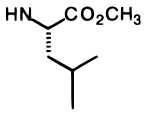
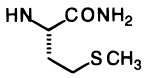
Inspection of the data obtained from this study shows that the 4-nitrobenzyl (**23**), 4-cyanobenzyl (**20**), 4-methoxybenzyl (**32**), and the 2-naphthyl (**34**) derivatives were the most efficient inhibitors of Ha-Ras farnesylation in whole cells. The compounds **23** and **20**, bearing the strongly electron-withdrawing nitro and cyano substituents, were particularly effective. The remaining compounds, while possessing significant activity in this assay, were less potent with IC_{50} values in the 1−10 μM range. The efficacy in inhibiting Ha-Ras farnesylation in whole cells does not straightforwardly correspond to the potency of the compounds in the in vitro enzyme inhibition assay. Whether these differences are due to the differential cell permeability of the compounds or reflect differences in the cleavage efficacy of the prodrug esters has not been determined.

Since the 4-cyanobenzyl derivative **21** has significant GGPTase 1 activity in vitro (IC_{50} 67 nM), the corresponding methyl ester prodrug **20** of this compound was further investigated to examine its ability to inhibit GGPTase 1 in whole cells. For this assay, NIH 3T3 cells expressing a chimeric H-Ras protein that has a CVLL motif at its carboxy terminus was used. However compound **20** at a concentration of 10 μM was unable to significantly inhibit the geranylgeranylation of this substrate. This result suggests that the effect prodrug **20** has on whole cells is due to its FPTase inhibitory activity.

One of the phenotypical properties that cells acquire upon transformation with an oncogenic *ras* gene is an ability to grow in an anchorage-independent manner in soft agar. The ester prodrugs were therefore evaluated for their ability to inhibit the growth of Rat-1 cells transformed by H-*ras* in this medium.[21] A minimum inhibitory concentration (MIC) in this assay is the lowest concentration of test compound that causes a

Table 3. In Vitro Effects of Carboxy Terminal Modifications



R	compd	FPTase IC_{50} (nM)[a]	GGPTase IC_{50} (nM)[b]	CTE (μM)[e]	Raspro IC_{50} (μM)[f]	soft agar MIC[g] H-Ras (μM)	soft agar MIC[g] Raf (μM)
	**21**	0.15 (1)	67 ± 39[d] (4)	≥50 (1)	10	2.5−10	nd
	**20**	nd[c]	nd	10−25 (2)	≤1 (9)	1 (3)	>2.5 (3)
	**38**	430	90[d] (1)	nd	nd	nd	nd
	**39**	nd	nd	10 (1)	nd	10 (1)	>10 (1)
	**40**	13 (1)	nd	25 (1)	10 (1)	10 (1)	25 (1)

[a] The concentration of compound required to inhibit 50% of FPTase-catalyzed incorporation of [^{3}H]FPP into recombinant[Leu^{68}]-RAS1(term.)CVIM protein by 50%. Determined using FPTase at a concentration of 10 pM. The assay results are reported as a concentration ± SEM for the number of determinations shown in parentheses. [b] The concentration of compound required to inhibit 50% of GGPTase-catalyzed incorporation of [^{3}H]GGPP into the recombinant [Leu^{68}]RAS1(term.)CVLL protein by 50%. Determined using GGPTase from bovine brain. [c] nd, not determined. [d] Determined using human recombinant GGPTase. [e] Highest nontoxic concentration for cultured NIH 3T3 cells as assessed by MTT staining. [f] Inhibition of posttranslational processing of v-Ras protein in cultured NIH 3T3 cells. [g] Minimum inhibitory concentration (MIC) required to achieve a reduction in size and number of colonies of Rat-1 v-*ras* or Rat-1 v-*raf* transformed cells in soft agar relative to vehicle-treated control.

reduction in the number and size of colonies of transformed cells in soft agar, relative to control cells that were treated with vehicle. To evaluate the compound's ability to specifically inhibit H-Ras-induced cell growth, the ester prodrugs were also tested for their affect on the growth of *v-raf* transformed Rat-1 cells. The underlying mechanistic rationale for this counterscreen stems from the fact that Raf is a downstream effector of Ras in the signal transduction pathway and can transform cells independently of Ras.[46] The results of this study are shown in Table 2.

All of the compounds tested in these assays were able to selectively inhibit the growth of H-Ras transformed Rat-1 cells. Somewhat surprisingly, there is not a strong correspondence between the ability of the compounds to inhibit H-Ras processing in NIH 3T3 cells and their growth inhibitory effect on H-Ras transformed Rat-1 cells in soft agar. For example, the 4-nitrobenzyl (**23**) and 4-cyanobenzyl (**20**) derivatives inhibit H-Ras farnesylation at 0.1−1 μM, whereas the unsubstituted benzyl (**11**) derivative requires concentrations of 2.5−10 μM to be as effective; yet these compounds have similar potency in the soft agar assay (MIC 1 μM). This discrepancy may be due to differences between the cell lines used in the two experiments, or it may indicate that other farnesylated proteins play a significant role in determining the phenotype of transformed cells.[47]

It should be noted that these compounds are somewhat more potent than the prototypical thiol inhibitor **1** which has an MIC in the soft agar assay of 2.5−5 μM.[31] Thus, it appears that FPTase inhibitors that contain a suitably substituted imidazole moiety can have improved cell-based potency over their thiol counterparts.

Previous reports from these laboratories have demonstrated that the thiol FTI (Table 2) L-744,832 is efficacious in slowing the growth of tumors derived from H-*ras* transformed cells in nude mice.[22] Due to its in vitro potency, favorable tissue distribution,[48] and suitable physical properties, the non-thiol prodrug **20** was selected for examination in a similar nude mouse xenograft study. Briefly, 36 athymic mice were divided into three treatment groups and innoculated subcutaneously with 10^6 Rat-1 cells transformed with either Zip H1a-*ras* or *mos* 3a2 oncogenes. The following day one group of 12 mice received 40 mpk subcutaneously[49] b.i.d. of the imidazole prodrug **20**, and one group received vehicle alone (phosphate-buffered saline) once daily. As a positive control in this assay, the thiol prodrug L-744,-832 was administered 40 mpk subcutaneously once daily to the remaining 12 animals. After 12 and 14 days the animals that received the *mos* and *ras* transformed cells, respectively, were sacrificed and the tumors were excised and weighed. The efficacy of the two compounds was measured by comparing the average tumor weight

Table 4. Effect of the FPTase Inhibitor **20** on the Growth of Tumors in Nude Mice[a]

	average tumor weight (g)		% difference (relative to vehicle)	
compd[b,c,d]	Rat-1 ZipH1a[e]	Rat-1 mos3a2[f]	ZipH1a[g]	Rat-1 mos3a2
vehicle	0.302 ± 0.032	0.563 ± 0.050		
20	0.171 ± 0.032	0.534 ± 0.069	−43	−5
L-744,832	0.020 ± 0.007	0.475 ± 0.058	−93	−16

[a] 12 mice were randomly assigned to each vehicle or treatment group. [b] The vehicle phosphate-buffered saline was administered by sc injection of a 0.1-mL solution once daily. [c] Mice received 40 mkd of compound **20** by sc injection twice daily. [d] Mice received 40 mkd of L-744,832 by sc injection once daily. [e] Rat-1 ZipH1a tumors were harvested after 14 days of dosing. [f] Rat-1 mos3a2 tumors were harvested after 12 days of dosing. [g] Statistical significance of the difference between the average tumor weight in the vehicle- and drug-treated groups was evaluated using Students one-sided *t*-test ($P < 0.01$).

from the drug-treated groups of animals with that of the average tumor weight from the animals that received the vehicle. The results from this experiment are tabulated in Table 4.

As reported previously[22] the thiol prodrug L-744,832 was highly effective in inhibiting the growth of H-*ras* tumors, showing a 93% reduction in tumor mass compared to vehicle and having little effect (−16%) on the control tumors that derived from implantation of *mos* transformed cells. The non-thiol prodrug **20** was also effective at slowing the growth of the H-*ras* tumors, causing a 43% reduction in the average tumor size. Again, growth of the *mos* tumors was not significantly affected (−5%). No overt toxic effects due the compounds were noted except for some local scarring at the site of drug administration.

The tumors that develop in a nude mouse xenograft experiment are localized and well-encapsulated and are not wholly reminiscent of those found in naturally arising cancers. In contrast tumors that arise spontaneously in transgenic animals exhibit a pathology that is more similar to that encountered clinically. Previous reports from these laboratories with the thiol inhibitor L-744,832 have demonstrated that this compound caused regression of spontaneously arising tumors in an Ha-*ras* transgenic mouse model. It was therefore of interest to evaluate the non-thiol agent **20** in this system.[25]

Mice harboring the viral Ha-*ras* oncogene under the control of the mouse mammary tumor virus long terminal repeat (MMTV) were calipered periodically and entered into study when a primary tumor of approximately 100 mm^3 was detected. The animals were divided into two groups: one received daily subcutaneous injections of the vehicle (10% EtOH, 30% propylene glycol), and the other received 120 mpk of compound **20** in the same vehicle, subcutaneously, once a day. The tumors were measured and a mean daily growth rate (MGR) calculated. The results of this study are presented in Table 5. After 14 days of treatment the growth of the primary tumors in the vehicle group had an average MGR of +19.8 mm^3/day. In contrast, the tumors in the animals that were receiving the imidzole derivative **20** shrank in size with an average MGR of −4.1 mm^3/day. This result is similar to that seen with the prototypical thiol inhibitor L-744,832, which had an MGR of −5.4 ± 1.9 mm^3/day following its daily administration at a dose of 40 mpk.[25]

Table 5. Efficacy of the FPTase Inhibitor **20** in MMTV v-Ha-*ras* Transgenic Mice[c]

animal no.	14-day MGR (mm^3/day)	average 14-day MGR (mm^3/day)
	Vehicle Group[a]	
1	17.1	
2	26.0	19.8 ± 3.1
3	16.2	
	Compound **20**[b] Group	
4	−10.2	
5	−5.5	−4.8 ± 2.4
6	−4.7	
7	1.3	

[a] 10% Ethanol−30% polypropylene glycol. [b] Mice received 120 mkd of compound **20** by sc injection once daily. [c] Tumor volume was calculated twice weekly from caliper measurements according to the approximate formula for a prolate ellipsoid: $(W^2 \times L)/2$, where W and L are in millimeters and $L \geq W$. The area under the curve (AUC) for a particular tumor was calculated according to the formula: $(vol_1 - vol_2)/2 \times (day_2 - day_1)$. The mean growth rate was calculated according to the formula: $[(sumAUC) - (vol_1 \times (day_n - day_1))]/(day_n - day_1)^2$.

Conclusion

It has been demonstrated that an imidazole, in combination with a suitable hydrophobic substituent, may be successfully combined to obtain effective replacements for the cysteinyl moiety in the FTI **1**. This finding may be of value since there is some concern that non-mechanism-based side effects may occur upon chronic administration of a FPTase inhibitor that contains a thiol moiety. The successful union of the imidazole and its hydrophobic substituent is highly dependent upon the substitution pattern of the imidazole moiety. The 1,5-disubstituted imidazole has been shown to be the optimal array that leads to potent and selective inhibitors of FPTase. A variety of aryl and isoprenyl substituents have been shown to afford effective inhibitors that bind to FPTase at the site usually occupied by the protein substrate. In experiments in cell culture, the methyl ester prodrugs of these inhibitors have been shown to be cell permeant and to potently inhibit the posttranslational modification of H-Ras protein. Additionally, these molecules are able to revert the phenotype of *ras* transformed cells as evidenced by their ability to slow the growth of *ras* transformed cell lines in soft agar. On the basis of these findings, the methyl prodrug **20** was evaluated in two in vivo models of tumor growth. This compound is able to selectively inhibit the growth of tumors derived from H-*ras* transformed cells in nude mice at doses that did not affect the growth of *mos* transformed xenografts. Similarly, the prodrug **20** was shown to cause the regression of preexisting tumors in an H-*ras* transgenic animal model. The efficacy of this compound in these models approaches that of the prototypical thiol inhibitor L-744,832.

These results demonstrate that an imidazole moiety in conjunction with a suitably positioned 4-cyanobenzyl group can function as a highly effective cysteine replacement within the *N*-arylalkyl pseudopeptide class of FTIs. This new pharmacophore may be of value in the development of farnesyltransferase inhibitors as chemotherapeutic agents.

Experimental Section

General Methods. Proton NMR spectra were run at 400 MHz on a varian Unity 400 or VRX-400 spectrometer, and chemical shifts are reported in parts per million (δ) downfield from tetramethylsilane as internal standard. Fast atom bombardment mass spectra were recorded on a VG-ZAB-HF spectrometer using glycerol as matrix. Elemental analyses were performed using a Perkin-Elmer 2400 model II elemental analyzer. Silica gel 60 (230−400) mesh from EM Science was used for column chromatography, and analytical thin-layer chromatography was conducted using EM Science Kieselgel 60 F254 plates. Preparative HPLC was performed on a Septech Novaprep 5000 with either a C-18Vydac or PrepPak reverse-phase column. For reactions performed under anhydrous conditions, glassware was either oven- or flame-dried and the reaction was run under a positive pressure of argon. Solvents and reagents were obtained from commercial sources and used without furthur purification. The reported yields are the actual isolated yields of purified material and are not optimized. The following examples are illustrative of the procedures employed.

Preparation of *N*-[2(*S*)-(1-(Phenylmethyl)-1*H*-imidazol-4-ylacetyl)amino-3(*S*)-methylpentyl]-*N*-(1-naphthylmethyl)glycylmethionine Bis-trifluoroacetate Salt (10) and *N*-[2(*S*)-(1-(Phenylmethyl)-1*H*-imidazol-5-ylacetyl)amino-3(*S*)-methylpentyl]-*N*-(1-naphthylmethyl)glycylmethionine Bis-trifluoroacetate Salt (11). Preparation of 1*H*-Imidazole-4-acetic Acid Methyl Ester Hydrochloride. A solution of 1*H*-imidazole-4-acetic acid hydrochloride (3.89 g, 23.9 mmol) in methanol (100 mL) was saturated with hydrogen chloride gas. The solution was allowed to stand for 18 h at room temperature, and then the solvent evaporated in vacuo to give the title compound (4.20 g,100%) as a white solid: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.85 (1H, s), 7.45 (1H, s), 3.89 (2H, s) and 3.75 (3H, s) ppm.

Preparation of 1-(Phenylmethyl)-1*H*-imidazol-4-ylacetic Acid Methyl Ester (6) and 1-(Phenylmethyl)-1*H*-imidazol-5-ylacetic Acid Methyl Ester (7) (3:1 mixture). To a solution of sodium hydride (37.3 mg, 1.56 mmol) in dimethylformamide (DMF) (2 mL) at 0 °C was added, via cannula, a solution of 1*H*-imidazole-4-acetic acid methyl ester hydrochloride (115 mg, 0.71 mmol) in DMF (3 mL). The reaction was stirred at 0 °C for 15 min. To the resulting suspension was added benzyl bromide (0.084 mL, 0.71 mmol), and the mixture was stirred at room temperature for 2 h. The reaction was quenched with saturated aqueous sodium bicarbonate (15 mL) and water (20 mL) and extracted into CH_2Cl_2 (2 × 50 mL). The combined organic extracts were washed with brine (20 mL) and dried ($MgSO_4$), and the solvent was evaporated in vacuo. The residue was purified by flash chromatography (eluting with acetonitrile) to afford an inseparable 3:1 mixture of **6** and **7** (106 mg, 65%) as an oil: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.53 (0.25H, s), 7.48 (0.75H, s), 7.35 (3H,m), 7.18 (1.5H, d, J = 7.4 Hz), 7.06 (0.5H, d, J = 7.2 Hz), 7.00 (0.25H, s), 6.87 (0.75H, s), 5.16 (0.5H, s), 5.08 (1.5H, s), 3.72 (1.5H, s), 3.65 (2.25H, s), 3.63 (0.75H, s) and 3.48 (0.5H, s) ppm.

Preparation of 1-(Phenylmethyl)-1*H*-imidazol-4-ylacetic Acid Hydrochloride and 1-(Phenylmethyl)-1*H*-imidazol-5-ylacetic Acid Hydrochloride (3:1 mixture). A solution of **6** and **7** (3:1 mixture, 106 mg, 0.046 mmol) in HCl (3 mL of a 1 M aq solution) was heated at 45 °C for 4 h. The solution was evaporated in vacuo to afford an inseparable 3:1 mixture of the title compounds (120 mg, 100%) as a glass: ^{1}H NMR (DMSO-d_6, 400 MHz) δ 9.26 (0.75H, s), 9.23 (0.25H, s), 7.60 (0.25H, m), 7.58 (0.75H, s), 7.45−7.26 (5H, m), 5.43 (0.5H, s), 5.41 (0.5H, s), 3.77 (1.5H, s), 3.75 (0.5H, s) ppm.

Preparation of 2(*S*)-[2-({2(*S*)-[2-(1-Benzyl-1*H*-imidazol-4-yl)acetylamino]-3(*S*)-methylpentyl}naphthalen-1-ylmethylamino)acetylamino]-4-(methylsulfanyl)-butyric Acid Methyl Ester Bis-trifluoroacetate (8) and 2(*S*)-[2(*S*)-({2-[2-(3-Benzyl-3*H*-imidazol-4-yl)acetylamino]-3-methylpentyl}naphthalen-1-ylmethylamino)-acetylamino]-4-(methylsulfanyl)butyric Acid Methyl Ester Bis-trifluoroacetate 9. To a solution of 1-(phenylmethyl)-1*H*-imidazol-4-ylacetic acid hydrochloride and 1-(phenylmethyl)-1*H*-imidazol-5-ylacetic acid hydrochloride (3:1 mixture, 115 mg, 0.455 mmol), 2(*S*)-[2-({2(*S*)-amino-3(*S*)-methylpentyl}naphthalen-1-ylmethylamino)acetylamino]-4-(methylsulfanyl)butyric acid methyl ester bis-hydrochloride (**4**) (244 mg, 0.455 mmol), 3-hydroxy-1,2,3-benzotriazin-4(3*H*)-one (HOOBT) (74 mg, 0.46 mmol), and triethylamine (0.19 mL, 1.36 mmol) in DMF (5 mL) was added 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) (87 mg, 0.455 mmol), and the solution stirred at room temperature overnight. The reaction was quenched with saturated aq sodium bicarbonate (20 mL) and water (25 mL) and extracted into EtOAc (2 × 50 mL). The combined organic extracts were washed with brine (5 mL), and the solvent was evaporated in vacuo. The residue was purified by flash chromatography (2−5% MeOH/CH_2Cl_2 gradient elution) to provide the amides **8** and **9** as a glass (3:1 mixture (237 mg, 79%)). For use in biological assays these isomers were separated by preparative HPLC (Nova Prep 5000 Semipreparative HPLC system and a Waters PrepPak cartridge, 47 × 300 mm, C18, 15 μm, 100A) eluting with 5−95% acetonitrile/water (0.1% TFA) at 100 mL/min) to give after lyophilization pure **8** (121 mg) and **9** (21 mg) as the bis-trifluoroacetate salts. **8**: ^{1}H NMR (CD_3OD, 400 MHz) δ 8.95 (1H, s), 8.27 (1H, m), 7.96 (2H, m), 7.68 (1H, d), 7.60−7.37 (9H, m), 5.38 (2H, s), 5.0−4.8 (1H, m), 4.52 (1H, t, J = 10.6 Hz), 4.42 (1H, dd, J = 4.0 and 6.6 Hz), 4.14 (1H, m), 3.92 (1H, d, J = 13.3 Hz), 3.83 (1H, d, J = 13.3 Hz), 3.70 (1H, s), 3.64 (1H, m), 3.54 (2H, m), 3.22 (1H, dd, J = 7.0 and 8.0 Hz), 2.37 (1H, m), 2.10 (1H, m), 2.00 (3H, s), 1.98 (1H, m), 1.79 (1H, m), 1.58 (1H, m), 1.42 (1H, m), 1.17 (1H, m) and 0.90 (6H, m) ppm; FAB HRMS exact mass calcd for $C_{37}H_{48}N_5O_4S$ 658.342702 (MH^+), found 658.341278. Anal. ($C_{37}H_{47}N_5O_4S\cdot 3.0TFA\cdot 0.15H_2O$) C,H,N. **9**: ^{1}H NMR (CD_3OD, 400 MHz) δ 8.80 (1H, s), 8.26 (1H, m), 7.89 (2H, m), 7.66−7.24 (8H, m), 7.21 (2H, s), 5.36 (2H, m), 4.37 (3H, m), 4.09 (1H,br s), 3.66 (3H, s), 3.56 (3H, m), 3.50−2.90 (3H, m), 2.27 (1H, br s), 2.20 (1H, br s), 1.96 (3H, s), 1.90 (1H, br s), 1.68 (1H, br s), 1.58 (1H, br s), 1.40 (1H, m), 1.18 (1H, m) and 0.89 (6H, m) ppm; FAB HRMS exact mass calcd for $C_{37}H_{48}N_5O_4S$ 658.342702 (MH^+), found 658.343754. Anal. ($C_{37}H_{47}N_5O_4S\cdot 1.85TFA\cdot 0.10H_2O$) C,H,N.

Preparation of 2(*S*)-[2-({2(*S*)-[2-(1-Benzyl-1*H*-imidazol-4-yl)acetylamino]-3(*S*)-methylpentyl}naphthalen-1-ylmethylamino)acetylamino]-4-(methylsulfanyl)butyric Acid Bis-trifluoroacetate (10) and 2(*S*)-[2-({2(*S*)-[2-(3-Benzyl-3*H*-imidazol-4-yl)acetylamino]-3(*S*)-methylpentyl}naphthalen-1-ylmethylamino)acetylamino]-4-(methylsulfanyl)butyric Acid Bis-trifluoroacetate (11). To a solution of **8** and **9** (2:1 mixture, 50 mg, 0.057 mmol) in methanol (5 mL) at room temperature was added lithium hydroxide (0.57 mL of a 1 M aq solution, 0.547 mmol), and the reaction was stirred for 4 h. After this time, the reaction was quenched by the addition of trifluoroacetic acid (to pH <3), and the solvent was evaporated in vacuo. The residue was purified by preparative HPLC (Nova Prep 5000 Semipreparative HPLC system and a Waters PrepPak cartridge, 47 × 300 mm, C18, 15 μm, 100A) eluting with 5−95% acetonitrile/water (0.1% TFA) at 100 mL/min) to give after lyophilization pure **10** (29 mg, 87%) and **11** (12 mg, 72%) as the bis-trifluoroacetate salts. **10**: ^{1}H NMR (CD_3OD, 400 MHz) δ 8.83 (1H, s), 8.21 (1H, d, J = 9.5 Hz), 7.88 (2H, m), 7.54 (1H, d, J = 6.9 Hz), 7.50−7.30 (9H, m), 5.32 (2H, s), 4.56 (1H, br d, J = 10.0 Hz), 4.36 (2H, m), 4.09 (1H, m), 3.55 (4H, m), 3.17 (1H, br d, J = 10 Hz), 2.98 (1H, t, J = 10.0 Hz), 2.29 (1H, m), 2.18 (1H, m), 1.96 (1H, m), 1.95 (3H, s), 1.67 (1H, m), 1.56 (1H, m), 1.37 (1H, m), 1.11 (1H, m) and 0.88 (6H, m) ppm; FAB HRMS exact mass calcd for $C_{36}H_{46}N_5O_4S$ 644.327052 (MH^+), found 644.326691. Anal. ($C_{36}H_{45}N_5O_4S\cdot 2.15TFA$) C,H,N. **11**: ^{1}H NMR (CD_3OD, 400 MHz) δ 8.80 (1H, s), 8.29 (1H, m), 7.92 (2H, m), 7.61 (1H, br), 7.53−7.32 (7H, m), 7.21 (2H, br s), 5.37 (2H, s), 4.37 (2H, m), 4.08 (1H, m), 3.57 (4H, br m), 3.05 (2H, m), 2.29 (2H, m), 2.20 (1H, m), 1.96 (3H, s), 1.70 (1H, m), 1.62 (1H, m), 1.57 (1H, m), 1.39 (1H, m), 1.13 (1H, m) and 0.88 (6H, m) ppm;

FAB HRMS exact mass calcd for $C_{36}H_{46}N_5O_4S$ 644.327052 (MH^+), found 644.327917.

2(*S*)-[2-({3(*S*)-Methyl-2(*S*)-[2-(1-pyridin-4-ylmethyl-1*H*-imidazol-4-yl)acetylamino]pentyl}naphthalen-1-ylmethylamino)acetylamino]-4-(methylsulfanyl)butyric acid methyl ester tris-trifluoroacetate: ^{1}H NMR (CD_3OD, 400 MHz) δ 8.99 (1H, s), 8.65 (2H, d, J = 4.9 Hz), 8.28 (1H, d, J = 9.4 Hz), 7.91 (2H, m), 7.69 (1H, d, J = 6.5 Hz), 7.61−7.44 (6H, m), 5.59 (2H, s), 4.90 (1H, m), 4.68 (1H, d, J = 13.4 Hz), 4.42 (1H, m), 4.16 (1H, m), 3.90 (1H, d, J = 15.6 Hz), 3.82 (1H, d, J = 15.6 Hz), 3.75−3.55 (2H, m), 3.69 (3H, s), 3.50 (1H, d, J = 13.1 Hz), 3.20 (1H, m), 2.37 (1H, m), 2.29 (1H, m), 1.99 (3H, s), 1.96 (1H, m), 1.77 (1H, m), 1.58 (1H, m), 1.23 (1H, m), 1.19 (1H, m), and 0.91 (6H, m) ppm; FAB HRMS exact mass calcd for $C_{36}H_{47}N_6O_4S$ 659.337951 (MH^+), found 659.336943. Anal. ($C_{36}H_{46}N_6O_4S$·4.95TFA·2.2H_2O) C,H,N.

2(*S*)-[2-({3(*S*)-Methyl-2(*S*)-[2-(1-pyridin-4-ylmethyl-1*H*-imidazol-4-yl)acetylamino]pentyl}naphthalen-1-ylmethylamino)acetylamino]-4-(methylsulfanyl)butyric acid tris-trifluoroacetate (12): ^{1}H NMR (CD_3OD, 400 MHz) δ 8.96 (1H, s), 8.55 (2H, d, J = 5.2 Hz), 8.21 (1H, d, J = 7.2 Hz), 7.97 (2H, m), 7.69 (1H, d, J = 7.2 Hz), 7.60−7.40 (6H, m), 5.58 (2H, s), 4.91 (1H, d, J = 13.2 Hz), 4.69 (1H, d, J = 13.2 Hz), 4.38 (1H, dd, J = 4.6 and 8.8 Hz), 4.15 (1H, m), 3.89 (1H, d, J = 16.1 Hz), 3.81 (1H, d, J = 16.1 Hz), 3.71 (1H, d, J = 17 Hz), 3.62 (1H, d, J = 17 Hz), 3.50 (1H, dd, J = 3.4 and 12 Hz), 3.21 (1H, m), 2.38 (1H, m), 2.27 (1H, m), 1.99 (1H, m), 1.99 (3H, s), 1.77 (1H, m), 1.58 (1H, m), 1.43 (1H, m), 1.16 (1H, m), and 0.88 (6H, m) ppm; FAB HRMS exact mass calcd for $C_{35}H_{45}N_6O_4S$ 645.322301 (MH^+), found 645.323649.

2(*S*)-[2-({3(*S*)-Methyl-2(*S*)-[2-(3-pyridin-4-ylmethyl-3*H*-imidazol-4-yl)acetylamino]pentyl}naphthalen-1-ylmethylamino)acetylamino]-4-(methylsulfanyl)butyric acid tris-trifluoroacetate (13): ^{1}H NMR (CD_3OD, 400 MHz) δ 8.97 (1H, s), 8.58 (2H, s), 8.27 (1H, m), 7.95 (2H, m), 7.64 (1H, m), 7.50 (4H, m), 7.31 (2H, d, J = 4.4 Hz), 5.57 (2H, s), 4.63 (2H, m), 4.38 (1H, m), 4.09 (1H, m), 3.78 (2H, m), 3.60 (2H, m), 3.42 (1H, m), 3.15 (1H, m), 2.36 (1H, m), 2.15 (1H, m), 2.01 (1H, m), 1.98 (3H, s), 1.76 (1H, m), 1.55 (1H, m), 1.41 (1H, m), 1.15 (1H, m), and 0.88 (6H, m) ppm; FAB HRMS exact mass calcd for $C_{35}H_{45}N_6O_4$ 645.322301 (MH^+), found 645.321321.

2(*S*)-{2-[(3(*S*)-Methyl-2(*S*)-{2-[1-(4-nitrobenzyl)-1*H*-imidazol-4-yl]acetylamino}pentyl)naphthalen-1-ylmethylamino]acetylamino}-4-(methylsulfanyl)butyric acid methyl ester bis-trifluoroacetate: ^{1}H NMR (CD_3OD, 400 MHz) δ 8.96 (1H, s), 8.17 (1H, m), 8.23 (2H, d, J = 8.7 Hz), 7.92 (2H, d, J = 8.9 Hz), 7.61 (1H, d, J = 6.9 Hz), 7.56 (2H, d, J = 8.9 Hz), 7.50 (2H, m), 7.44 (2H, m), 5.52 (2H, s), 4.70 (1H, d, J = 9.4 Hz), 4.49 (1H, d, J = 11.9 Hz), 4.38 (1H, dd, J = 4.7 and 8.9 Hz), 4.13 (1H, m), 3.67 (3H, s), 3.65 (4H, m), 3.30 (1H, m), 3.06 (1H, m), 2.31 (1H, m), 2.23 (1H, m), 1.97 (3H, s), 1.94 (1H, m), 1.71 (1H, m), 1.57 (1H, m), 1.42 (1H, m), 1.17 (1H, m), 0.90 (3H, d, J = 6.9 Hz) and 0.87 (3H, t, J = 7.4 Hz) ppm; FAB MS calcd for $C_{37}H_{47}N_6O_6S$ 703 (MH^+), found 703. Anal. ($C_{37}H_{46}N_6O_6S$·2.40TFA·0.25H_2O) C,H,N.

2(*S*)-{2-[(3(*S*)-Methyl-2(*S*)-{2-[1-(4-nitrobenzyl)-1*H*-imidazol-4-yl]acetylamino}pentyl)naphthalen-1-ylmethylamino]acetylamino}-4-(methylsulfanyl)butyric acid bis-trifluoroacetate (14): ^{1}H NMR (CD_3OD, 400 MHz) δ 8.86 (1H, s), 8.23 (2H, d, J = 8.8 Hz), 8.22 (1H, m), 7.90 (2H, d, J = 7.3 Hz), 7.55 (2H, d, J = 8.4 Hz), 7.44−7.28 (5H, m), 5.50 (2H, s), 4.53 (1H, m), 4.35 (2H, m), 4.12 (1H, m), 3.79−3.25 (4H, m), 3.26−2.86 (2H, m), 2.27 (1H, m), 2.18 (1H, m), 1.96 (3H, s), 1.9 (1H, m), 1.67 (1H, m), 1.57 (1H, m), 1.42 (1H, m), 1.15 (1H, m), 0.90 (3H, d, J = 6.9 Hz) and 0.86 (3H, t, J = 7.3 Hz) ppm; FAB HRMS exact mass calcd for $C_{36}H_{45}N_6O_6S$ 689.31213 (MH^+), found 689.31262.

2(*S*)-{2-[(3(*S*)-Methyl-2(*S*)-{2-[3-(4-nitrobenzyl)-3*H*-imidazol-4-yl]acetylamino}pentyl)naphthalen-1-ylmethylamino]acetylamino}-4-(methylsulfanyl)butyric acid bis-trifluoroacetate (15): ^{1}H NMR (CD_3OD, 400 MHz) δ 8.89 (1H, s), 8.25 (1H, m), 8.21 (2H, d, J = 9.0 Hz), 7.89 (2H, m), 7.64−7.34 (7H, m), 5.52 (2H, s), 4.59−3.88 (4H, m), 3.77−3.38 (4H, m), 3.18−2.75 (2H, m), 2.27 (1H, m), 2.18 (1H, m), 1.96 (3H, s), 1.9 (1H, m), 1.67 (1H, m), 1.57 (1H, m), 1.42 (1H, m), 1.15 (1H, m), 0.89 (6H, m) ppm; FAB HRMS exact mass calcd for $C_{36}H_{45}N_6O_6S$ 689.31213 (MH^+), found 689.31135.

2(*S*)-[2-({3(*S*)-Methyl-2(*S*)-[2-(3-pyridin-4-ylmethyl-3*H*-imidazol-4-yl)acetylamino]pentyl}naphthalen-1-ylmethylamino)acetylamino]-4-(methylsulfanyl)butyric acid methyl ester (22): ^{1}H NMR (CD_3OD, 400 MHz) δ 9.01 (1H, s), 8.63 (2H, m), 8.28 (1H, m), 7.98 (2H, m), 7.70 (1H, d, J = 6.0 Hz), 7.52 (4H, m), 7.41 (2H, d, J = 6.2 Hz), 5.62 (2H, s), 4.94 (1H, m), 4.72 (1H, m), 4.42 (1H, m), 4.07 (1H, m), 3.89 (2H, m), 3.68 (1H,m), 3.69 (3H, s), 3.55 (2H, m), 3.24 (1H, m), 2.39 (1H, m), 2.31 (1H, m), 2.00 (3H, s), 1.98 (1H, m), 1.79 (1H, m), 1.58 (1H, m), 1.42 (1H, m), 1.18 (1H, m), and 0.91 (6H, m) ppm; FAB HRMS exact mass calcd for $C_{36}H_{47}N_6O_4S$ 659.337951 (MH^+), found 659.336826.

2(*S*)-{2-[(3(*S*)-Methyl-2(*S*)-{2-[3-(4-nitrobenzyl)-3*H*-imidazol-4-yl]acetylamino}pentyl)naphthalen-1-ylmethylamino]acetylamino}-4-(methylsulfanyl)butyric acid methyl ester bis-trifluoroacetate (23): ^{1}H NMR (CD_3OD, 400 MHz) δ 8.91 (1H, s), 8.26 (1H, d, J = 12.8 Hz), 8.21 (2H, d, J = 10.7 Hz), 7.91 (2H, m), 7.65−7.36 (7H, m), 5.51 (2H, s), 4.72−3.99 (4H, m), 3.66 (3H, s), 3.66−3.24 (4H, m), 3.20−2.85 (2H, m), 2.29 (1H, m), 2.20 (1H, m), 1.96 (3H, s), 1.91 (1H, br s), 1.70 (1H, d, J = 16 Hz), 1.56 (1H, m), 1.38 (1H, m), 1.13 (1H, m) and 0.88 (6H, m) ppm; FAB HRMS exact mass calcd for $C_{37}H_{47}N_6O_6S$ 703.32778 (MH^+), found 703.32852.

Regioselective Preparation of 2(*S*)-{2-[(2(*S*)-{2-[3-(4-Cyanobenzyl)-3*H*-imidazol-4-yl]acetylamino}-3(*S*)-methylpentyl)naphthalen-1-ylmethylamino]acetylamino}-4-(methylsulfanyl)butyric Acid Methyl Ester Bis-trifluoroacetate Salt (20). Preparation of 1-(Triphenylmethyl)-1*H*-imidazol-4-ylacetic Acid Methyl Ester (17). To a suspension of 1*H*-imidazole-4-acetic acid methyl ester hydrochloride (**16**) (5.11 g, 28.9 mmol) in DMF (60 mL) were added triethylamine (10.1 mL, 72.3 mmol) and triphenylmethyl bromide (10.3 g, 31.8 mmol), and the reaction was stirred for 72 h. After this time, the reaction mixture was diluted with EtOAc (600 mL) and washed with saturated aqueous sodium bicarbonate (200 mL), water (100 mL), and brine (150 mL). The organic extract was dried ($MgSO_4$) and evaporated in vacuo. The residue was purified by flash chromatography (25−100% ethyl acetate/hexanes gradient elution) to provide **17** (8.30 g, 75%) as a white solid: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.35 (1H, s), 7.31 (9H, m), 7.22 (6H, m), 6.76 (1H, s), 3.68 (3H, s) and 3.60 (2H, s) ppm.

Preparation of 1-(4-Cyanophenylmethyl)-1*H*-imidazol-5-ylacetic Acid Methyl Ester (19). To a solution of 1-(triphenylmethyl)-1*H*-imidazol-4-ylacetic acid methyl ester (**17**) (8.0 g, 20.9 mmol) in acetonitrile (10 mL) was added 4-cyanobenzyl bromide (4.10 g, 20.9 mmol) and heated at 55 °C for 3 h. After this time, the reaction was cooled to room temperature, and the resulting precipitate **18** was collected by filtration. The filtrate was collected, and this solution was heated at 55 °C for 15 h. After this time, the reaction mixture was evaporated to dryness and treated with EtOAc (70 mL), and the solids were collected by filtration. The two solids were combined, suspended in methanol (140 mL), and heated at reflux for 30 min. The resulting solution was evaporated in vacuo. The solid residue was triturated with EtOAc (75 mL) and then partitioned between CH_2Cl_2 (100 mL) and saturated aq sodium bicarbonate (100 mL). The organic extract was washed with brine and dried ($MgSO_4$), and the solvent evaporated in vacuo to provide **19** (3.88 g, 70%) as a white solid: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.65 (2H, d, J = 8.1 Hz), 7.53 (1H, s), 7.15 (2H, d, J = 8.1 Hz), 7.04 (1H, s), 5.24 (2H, s), 3.63 (3H, s) and 3.46 (2H, s) ppm.

Preparation of 1-(4-Cyanophenylmethyl)-1*H*-imidazol-5-ylacetic Acid. 1-(4-Cyanophenylmethyl)-1*H*-imidazol-5-ylacetic acid methyl ester (**19**) (4.44 g, 17.4 mmol) was dissolved in THF (100 mL), lithium hydroxide (18.8 mL of a 1 M aq solution, 18.8 mmol) was added, and the resulting mixture stirred at room temperature for 16 h. The reaction was neutralized with hydrochloric acid (18.8 mL of a 1 M aq solution, 18.8 mmol) and the solvent removed by lyophilization.

The acid obtained by this protocol was of sufficient quality to be used in the following reaction without further purification: ^{1}H NMR (CD_3OD, 400 MHz) δ 8.22 (1H, s), 7.74 (2H, d, J = 8.3 Hz), 7.36 (2H, d, J = 8.3 Hz), 7.15 (1H, s), 5.43 (2H, s) and 3.49 (2H, s) ppm.

Preparation of 2(*S*)-{2-[(2(*S*)-{2-[3-(4-Cyanobenzyl)-3*H*-imidazol-4-yl]acetylamino}-3(*S*)-methylpentyl)naphthalen-1-ylmethylamino]acetylamino}-4-(methylsulfanyl)-butyric Acid Methyl Ester Bis-trifluoroacetate Salt (20). To a solution of 1-(4-cyanophenylmethyl)-1*H*-imidazol-5-ylacetic acid (1.30 g, 3.91 mmol), amine hydrochloride **4** (2.08 g, 3.91 mmol), triethylamine (1.71 mL, 12.3 mmol), and HOOBT (0.67 g, 4.10 mmol) in DMF (30 mL) was added EDC (0.79 g, 4.10 mmol), and the reaction was stirred at room temperature 16 h. The reaction was quenched with saturated aq sodium bicarbonate (100 mL) and water (100 mL) and extracted into EtOAc (150 mL × 3). The combined organic extracts were washed with brine (100 mL), and the solvent was evaporated in vacuo. The residue was purified by flash chromatography (4−7% MeOH/CH_2Cl_2 gradient elution) to provide the amide **20** (1.98 g, 74%) as a white solid. For use in biological assays this material was furthur purified by preparative HPLC (Nova Prep 5000 Semipreparative HPLC system and a Waters PrepPak cartridge, 47 × 300 mm, C18, 15 μm, 100A) eluting with 5−95% acetonitrile/water (0.1% TFA) at 100 mL/min to give **20** as its bis-trifluoroacetate salt after lyophilization): ^{1}H NMR (CD_3OD, 400 MHz) δ 8.92 (1H, s), 8.31 (1H, m), 8.01 (1H, d, J = 8.3 Hz), 7.96 (1H, m), 7.75 (2H, d, J = 8.2 Hz), 7.72 (1H, m), 7.58−7.48 (3H, m), 7.45 (1H, m), 7.41 (2H, d, J = 8.3 Hz), 5.51 (2H, s), 4.97 (1H, m), 4.76 (1H, m), 4.41 (1H, m), 4.10 (1H, m), 3.92 (2H, m), 3.75−3.47 (3H, m), 3.69 (3H, s), 3.25 (1H, m), 2.37 (1H, m), 2.30 (1H, m), 2.00 (3H, s), 1.97 (1H,m), 1.79 (1H, m), 1.58 (1H, m), 1.43 (1H, m), 1.19 (1H, m) and 0.93−0.88 (6H, m) ppm; FAB HRMS exact mass calcd for $C_{38}H_{47}N_6O_4S$ 683.337951 (MH^+), found 683.338437. Anal. ($C_{38}H_{46}N_6O_4S$·2.40TFA·1.90H_2O) C,H,N.

Preparation of 2(*S*)-{2-[(2(*S*)-{2-[3-(4-Cyanobenzyl)-3*H*-imidazol-4-yl]acetylamino}-3(*S*)-methylpentyl)naphthalen-1-ylmethylamino]acetylamino}-4-(methylsulfanyl)-butyric Acid Bis-trifluoroacetate (21). To a solution of the methyl ester **20** (25.6 mg, 0.028 mmol) in methanol (1 mL) was added sodium hydroxide (0.28 mL of a 1.0 M solution, 0.28 mmol), and the solution was stirred at room temperature for 2 h. After this time, the reaction was acidified to pH 3 by the addition of trifluoroacetic acid, and the solvent was evaporated in vacuo. The residue was purified by preparative HPLC (Nova Prep 5000 Semipreparative HPLC system and a Waters PrepPak cartridge, 47 × 300 mm, C18, 15 μm, 100A) eluting with 5−95% acetonitrile/water (0.1% TFA) at 100 mL/min to give **21** (21 mg, 83%) as its bis-trifluoroacetate salt after lyophilization: ^{1}H NMR (CD_3OD, 400 MHz) δ 8.87 (1H, s), 8.27 (1H, d, J = 9.2 Hz), 7.90 (2H, m), 7.73 (2H, d, J = 8.2 Hz), 7.60 (1H, s), 7.46 (4H, m), 7.36 (2H, d, J = 8.2 Hz), 5.48 (2H, s), 4.95−4.28 (2H, m), 4.36 (1H, m), 4.09 (1H, m), 3.59 (4H, m), 3.51−2.73 (2H, m), 2.29 (1H, m), 2.19 (1H, m), 2.03−1.85 (1H, m), 1.97 (3H, s), 1.70 (1H, m), 1.56 (1H, m), 1.39 (1H, m), 1.14 (1H, m) and 0.90−0.79 (6H, m) ppm; FAB HRMS exact mass calcd for $C_{37}H_{44}N_6O_4S$ 669.322301 (MH^+), found 669.323148. Anal. ($C_{37}H_{44}N_6O_4S$·2.45TFA·1.3H_2O) C,H,N.

2(*S*)-{2-[(2(*S*)-{2-[3-(4-Fluorobenzyl)-3*H*-imidazol-4-yl]-acetylamino}-3(*S*)-methylpentyl)naphthalen-1-ylmethylamino]acetylamino}-4-(methylsulfanyl)butyric acid bis-trifluoroacetate (24): ^{1}H NMR (CD_3OD, 400 MHz) δ 8.79 (1H, s), 8.30 (1H, m), 8.00−7.80 (2H, m), 7.65−7.40 (5H, m), 7.30−7.20 (2H, m), 7.13 (2H, t, J = 8.7 Hz), 5.35 (2H, m), 4.38 (2H, m), 4.13 (1H, m), 3.80−3.40 (4H, m), 3.10 (1H, m), 2.40−2.15 (2H, m), 1.97 (3H, s), 1.95 (1H, m), 1.70 (1H, m), 1.60 (1H, m), 1.43 (1H, m), 1.07 (1H, m), and 1.00−0.80 (6H, m) ppm; FAB MS m/z = 662 (M + 1). Anal. ($C_{36}H_{44}N_5O_4S$·0.60H_2O·2.30TFA) C,H,N.

2(*S*)-{2-[(2(*S*)-{2-[3-(4-Methoxybenzyl)-3*H*-imidazol-4-yl]acetylamino}-3(*S*)-methylpentyl)naphthalen-1-ylmethylamino]acetylamino}-4-(methylsulfanyl)butyric acid bis-trifluoroacetate (25): ^{1}H NMR (CD_3OD, 400 MHz) δ 8.70 (1H, s), 8.27 (1H, m), 7.92 (2H, m), 7.63 (1H, s), 7.56−7.35 (4H, m), 7.18 (2H, d, J = 8.6 Hz), 6.93 (2H, d, J = 8.6 Hz), 5.27 (2H, s), 4.93−4.29 (2H, m), 4.36 (1H, m), 4.12 (1H, m), 3.79 (3H, s), 3.63 (4H, m), 3.07 (2H, m), 2.28 (1H, m), 2.19 (1H, m), 2.02−1.88 (1H, m), 1.95 (3H, s), 1.70 (1H, m), 1.60 (1H, m), 1.43 (1H, m), 1.18 (1H, m), and 0.91 (6H, m) ppm; FAB HRMS exact mass calcd for $C_{37}H_{48}N_5O_5S$ 674.337617 (MH^+), found 674.338053.

2(*S*)-[2-({3(*S*)-Methyl-2(*S*)-[2-(3-naphthalen-1-ylmethyl-3*H*-imidazol-4-yl)acetylamino]pentyl}naphthalen-1-ylmethylamino)acetylamino]-4-(methylsulfanyl)butyric acid bis-trifluoroacetate (26): ^{1}H NMR (CD_3OD, 400 MHz) δ 8.41 (1H, s), 8.19 (1H, d, J = 7.7 Hz), 7.99 (2H, m), 7.87 (3H, m), 7.64 (1H, m), 7.56 (1H, t, J = 7 Hz), 7.46 (6H, m), 7.16 (1H, d, J = 8 Hz), 5.79 (2H, s), 5.04−4.71 (1H, m), 4.61−4.38 (1H, m), 4.38−4.21 (1H, m), 4.14 (1H, m), 3.97−3.51 (4H, m), 3.51−3.21 (1H, m), 3.21−2.85 (1H, m), 2.21 (1H, m), 2.13 (1H, m), 1.98 (1H, m), 1.91 (3H, s), 1.66 (1H, m), 1.56 (1H, m), 1.40 (1H, m), 1.15 (1H, m), and 0.87 (6H, m) ppm; FAB HRMS exact mass calcd for $C_{40}H_{48}N_5O_4S$ 694.342702 (MH^+), found 694.342837. Anal. ($C_{40}H_{47}N_5O_4S$·2.70TFA·0.5H_2O) C,H,N.

2(*S*)-[2-({3(*S*)-Methyl-2(*S*)-[2-(3-naphthalen-2-ylmethyl-3*H*-imidazol-4-yl)acetylamino]pentyl}naphthalen-1-ylmethylamino)acetylamino]-4-(methylsulfanyl)butyric acid bis-trifluoroacetate (27): ^{1}H NMR (CD_3OD, 400 MHz) δ 8.88 (1H, s), 8.28 (1H, d, J = 9 Hz), 7.96−7.78 (5H, m), 7.67 (1H, s), 7.57−7.41 (7H, m), 7.32 (1H, d, J = 9 Hz), 5.55 (2H, s), 4.81(1H, m), 4.56 (1H, m), 4.37 (1H, m), 4.06 (1H, m), 3.89−3.50 (4H, m), 3.42 (1H, m), 3.10 (1H, m), 2.28 (1H, m), 2.19 (1H, m), 2.03−1.86 (1H, m), 1.93 (3H, s), 1.90 (1H, m), 1.71 (1H, m), 1.52 (1H, m), 1.37 (1H, m) and 0.87 (6H, m) ppm; FAB HRMS exact mass calcd for $C_{40}H_{48}N_5O_4S$ 694.342702 (MH^+), found 694.342837. Anal. ($C_{40}H_{47}N_5O_4S$·2.95TFA·0.5H_2O) C,H,N.

2(*S*)-[2-({3(*S*)-Methyl-2(*S*)-[2-(3-quinolin-4-ylmethyl-3*H*-imidazol-4-yl)acetylamino]pentyl}naphthalen-1-ylmethylamino)acetylamino]-4-(methylsulfanyl)butyric acid bis-trifluoroacetate (28): ^{1}H NMR (CD_3OD, 400 MHz) δ 8.87 (1H, s), 8.82 (1H, d, J = 5 Hz), 8.28 (1H, m), 8.15 (1H, d, J = 8.6 Hz),8.06−7.82 (4H, m), 7.67 (2H, m), 7.58 (1H, s), 7.48 (3H, s), 6.96 (1H, m), 6.03 (2H, s), 4.93−4.57 (2H, m), 4.22 (1H, m), 4.08 (1H, m), 3.72 (4H, m), 3.47 (1H, m), 3.13 (1H, m), 2.28 (1H, m), 2.21 (1H, m), 1,95 (3H, s), 1.87 (1H, m), 1.70 (1H, m), 1.48 (1H, m), 1.35 (1H, m), 1.09 (1H, m), and 0.84 (6H, m) ppm; FAB HRMS exact mass calcd for $C_{39}H_{47}N_6O_4S$ 695.33795 (MH^+), found 695.33893.

2(*S*)-{2-[(2(*S*)-{2-[3-(3,7-Dimethylocta-2,6-dienyl)-3*H*-imidazol-4-yl]acetylamino}-3(*S*)-methylpentyl)naphthalen-1-ylmethylamino]acetylamino}-4-(methylsulfanyl)-butyric acid bis-trifloroacetate (29): ^{1}H NMR (CD_3OD, 400 MHz) δ 8.67 (1H, s), 8.27 (1H, m), 7.92 (2H, m), 7.59 (1H, m), 7.52 (2H, m), 7.46 (1H, t, J = 7.8 Hz), 7.38 (1H, s), 5.28 (1H, t, J = 11.2 Hz), 5.04 (1H, m), 4.96−4.54 (1H, m), 4.72 (2H, s), 4.54−4.31 (1H, m), 4.39 (1H, m), 4.13 (1H, m), 3.82−3.31 (4H, m), 3.68 (2H, m), 3.31−2.79 (2H, m), 2.30 (1H, m), 2.12 (5H, m), 1.97 (3H, s), 1.97 (1H, m), 1.73 (1H, m), 1.71 (3H, s), 1.70 (3H, s), 1.60 (3H, s), 1.44 (1H, m), 1.18 (1H, m) and 0.92 (3H, d, J = 6.8 Hz), and 0.90 (3H, t, J = 7.5 Hz) ppm; FAB HRMS exact mass calcd for $C_{39}H_{56}N_5O_4S$ 690.405303 (MH^+), found 690.405157.

4-(Methylsulfanyl)-2(*S*)-{2-[(3(*S*)-methyl-2(*S*)-{2-[3-(3,7,-11-trimethyldodeca-2,6,10-trienyl)-3*H*-imidazol-4-yl]-acetylamino}pentyl)naphthalen-1-ylmethylamino]-acetylamino}butyric acid bis-trifluoroacetate (30): ^{1}H NMR (CD_3OD, 400 MHz) δ 8.68 (1H, s), 8.18 (1H, m), 7.90 (2H, m), 7.52 (3H, m), 7.44 (1H, t, J = 7.5 Hz), 7.37 (1H, s), 5.29 (1H, br t, J = 7.0 Hz), 5.08 (2H, m), 4.95−4.64 (1H, m), 4.73 (2H, m), 4.37 (2H, m), 4.12 (1H, m), 3.71 (2H, m), 3.47 (2H, m), 3.11 (1H, m), 2.95 (1H, m), 2.27 (1H, m), 2.23−2.01 (9H, m), 2.01−1.89 (1H, m), 1.97 (3H, s), 1.77−1.54 (2H, m), 1.71 (3H, s), 1.65 (3H, s), 1.60 (3H, s), 1.58 (3H, s), 1.42 (1H, m), 1.16 (1H, m), 0.91 (3H, t, J = 7.0 Hz) and 0.87 (3H, d, J = 7.5 Hz) ppm; FAB HRMS exact mass calcd for $C_{44}H_{64}N_5O_4S$ 758.467903 (MH^+), found 758.467591.

2(*S*)-{2-[(2(*S*)-{2-[3-(4-Fluorobenzyl)-3*H*-imidazol-4-yl]-acetylamino}-3(*S*)-methylpentyl)naphthalen-1-ylmethylamino]acetylamino}-4-(methylsulfanyl)butyric acid methyl ester bis-trifluoroacetate (31): ^{1}H NMR (CD_3-OD, 400 MHz) δ 8.77 (1H, s), 8.28 (1H, m), 8.00−7.80 (2H, m), 7.65−7.40 (5H, m), 7.30−7.20 (2H, m), 7.14 (2H, t, J = 8.6 Hz), 5.34 (2H, m), 4.39 (2H, m), 4.13 (1H, m), 3.68 (3H, s), 3.65−3.40 (4H, m), 2.95 (1H, m), 2.40−2.15 (2H, m), 1.97 (3H, s), 1.95 (1H, m), 1.70 (1H, m), 1.60 (1H, m), 1.43 (1H, m), 1.07 (1H, m), and 1.00−0.80 (6H, m) ppm; FAB MS m/z = 676 (M + 1). Anal. ($C_{37}H_{46}N_5O_4S$·0.45H_2O·1.65TFA) C,H,N.

2(*S*)-{2-[(2(*S*)-{2-[3-(4-Methoxybenzyl)-3*H*-imidazol-4-yl]acetylamino}-3(*S*)-methylpentyl)naphthalen-1-ylmethylamino]acetylamino}-4-(methylsulfanyl)butyric acid methyl ester bis-trifluoroacetate (32): ^{1}H NMR (CD_3-OD, 400 MHz) δ 8.70 (1H, s), 8.27 (1H, m), 7.92 (2H, m), 7.70−7.35 (5H, m), 7.18 (2H, d, J = 8.5 Hz), 6.92 (2H, d, J = 8.5 Hz), 5.27 (2H, s), 4.60−4.00 (4H, m), 3.79 (3H, s), 3.67 (3H, s), 3.61 (4H, m), 3.40−2.75 (2H, m), 2.28 (1H, m), 2.19 (1H, m), 1.96 (3H, s), 1.91 (1H, m), 1.70 (1H, m), 1.60 (1H, m), 1.43 (1H, m), 1.18 (1H, m), and 0.91 (6H, m) ppm. FAB HRMS exact mass calcd for $C_{38}H_{50}N_5O_5S$ 688.353267 (MH^+), found 688.352186. Anal. Calcd for $C_{38}H_{49}N_5O_5S$·1.75TFA·1.75H_2O: C, 54.45; H, 5.98; N, 7.67. Found: C, 54.44; H, 5.95; N, 7.85.

2(*S*)-[2-({3(*S*)-Methyl-2(*S*)-[2-(3-naphthalen-1-ylmethyl-3*H*-imidazol-4-yl)acetylamino]pentyl}naphthalen-1-ylmethylamino)acetylamino]-4-(methylsulfanyl)butyric acid methyl ester bis-trifluoroacetate (33): ^{1}H NMR (CD_3-OD, 400 MHz) δ 8.42 (1H, s), 8.31 (1H, d, J = 8.9 Hz), 8.04−7.80 (5H, m), 7.69 (1H, m), 7.59−7.39 (7H, m), 7.20 (1H, d, J = 8.2 Hz), 5.80 (2H, s), 5.0−4.5 (2H, m), 4.26 (1H, m), 4.13 (1H, m), 4.0−3.6 (4H, m), 3.64 (3H, s), 3.49 (1H, m), 3.18 (1H, m), 2.17 (2H, m), 1.91 (3H, s), 1.86 (1H, m), 1.67 (1H, m), 1.55 (1H, m), 1.41 (1H, m), 1.16 (1H, brs), and 0.88 (6H, m) ppm; FAB HRMS exact mass calcd for $C_{41}H_{50}N_5O_4S$ 708.358352 (MH^+), found 708.357618. Anal. ($C_{41}H_{49}N_5O_4S$·3.10TFA·0.55H_2O) C,H,N.

2(*S*)-[2-({3(*S*)-Methyl-2(*S*)-[2-(3-naphthalen-2-ylmethyl-3*H*-imidazol-4-yl)acetylamino]pentyl}naphthalen-1-ylmethylamino)acetylamino]-4-(methylsulfanyl)butyric acid methyl ester bis-trifluoroacetate (34): ^{1}H NMR (CD_3-OD, 400 MHz) δ 8.89 (1H, s), 8.29 (1H, d, J = 9 Hz), 7.92 (4H, m), 7.83 (1H, d, J = 9 Hz), 7.68 (1H, s), 7.58−7.42 (7H, m), 7.33 (1H, d, J = 9 Hz), 5.54 (2H, s), 4.90−4.50 (2H, m), 4.38 (1H, m), 4.05 (1H, m), 3.93−3.32 (5H, m), 3.65 (3H, s), 3.12 (1H, m), 2.24 (2H, m), 1.93 (3H, s), 1.87 (1H, brs), 1.72 (1H, br s), 1.52 (1H, brs), 1.38 (1H, brs), 1.13 (1H, brs) and 0.87 (6H, m) ppm; FAB HRMS exact mass calcd for $C_{41}H_{50}N_5O_4S$ 708.358352 (MH^+), found 708.357618. Anal. ($C_{41}H_{49}N_5O_4S$·3.20TFA·0.75H_2O) C,H,N.

2(*S*)-[2-({3(*S*)-Methyl-2(*S*)-[2-(3-quinolin-4-ylmethyl-3*H*-imidazol-4-yl)acetylamino]pentyl}naphthalen-1-ylmethylamino)acetylamino]-4-(methylsulfanyl)butyric acid methyl ester bis-trifluoroacetate (35): ^{1}H NMR (CD_3-OD, 400 MHz) δ 8.88 (1H, s), 8.83 (1H, d, J = 4.8 Hz), 8.28 (1H, m), 8.15 (1H, d, J = 8.6 Hz), 7.99−7.85 (4H, m), 7.67 (2H, m), 7.57 (1H, s), 7.48 (3H, m), 6.96 (1H, m), 6.02 (2H, s), 4.90 (1H, m), 4.62 (1H, m), 4.18 (1H, m), 4.07 (1H, m), 3.94−3.50 (4H, m), 3.64 (3H, s), 3.45 (1H, m), 3.13 (1H, m), 2.28 (1H, m), 2.21 (1H, m), 1.95 (3H, s), 1.87 (1H, m), 1.69 (1H, m), 1.48 (1H, m), 1.35 (1H, m), 1.11 (1H, m), and 0.84 (6H, m) ppm; FAB HRMS exact mass calcd for $C_{40}H_{49}N_6O_4S$ 709.353601 (MH^+), found 709.353711.

2(*S*)-{2-[(2(*S*)-{2-[3-(3,7-Dimethylocta-2,6-dienyl)-3*H*-imidazol-4-yl]acetylamino}-3(*S*)-methylpentyl)naphthalen-1-ylmethylamino]acetylamino}-4-(methylsulfanyl)-butyric acid methyl ester bis-trifluoroacetate (36): ^{1}H NMR (CD_3OD, 400 MHz) δ 8.67 (1H, s), 8.27 (1H, m), 7.92 (2H, m), 7.57 (1H, m), 7.53 (2H, m), 7.46 (1H, dd, J = 9.0 Hz), 7.36 (1H, s), 5.29 (1H, t, J = 6.0 Hz), 5.08 (1H, t, J = 6.0 Hz), 4.71 (1H, m), 4.71−4.12 (1H, m), 4.38 (1H, m), 4.12 (1H, m), 3.80−3.33 (4H, m), 3.68 (3H, s), 3.14 (1H, m), 2.96 (1H, m), 2.29 (1H, m), 2.21 (1H, m), 2.12 (4H, m), 2.11 (1H, m), 1.97 (3H, s), 1.97 (1H, m), 1.70 (3H, s), 1.68 (3H, s), 1.65 (1H, m), 1.60 (3H, s), 1.41 (1H, m), 1.15 (1H, m), 0.91 (3H, d, J = 7.0 Hz) and 0.88 (3H, t, J = 7.5 Hz) ppm; FAB HRMS exact mass calcd for $C_{40}H_{58}N_5O_4S$ 704.420953 (MH^+), found 704.420223. Anal. ($C_{40}H_{57}N_5O_4S$·1.80TFA·0.25H_2O) C,H,N.

4-(Methylsulfanyl)-2(*S*)-{2-[(3(*S*)-methyl-2(*S*)-{2-[3-(3,7,-11-trimethyldodeca-2,6,10-trienyl)-3*H*-imidazol-4-yl]-acetylamino}pentyl)naphthalen-1-ylmethylamino]-acetylamino}butyric acid methyl ester bis-trifluoroacetate (37): ^{1}H NMR (CD_3OD, 400 MHz) δ 8.70 (1H, s), 8.26 (1H, m), 7.91 (2H, m), 7.52 (3H, m), 7.48 (1H, m), 7.37 (1H, s), 5.40 (1H, m), 5.08 (2H, m), 4.94−4.72 (3H, m), 4.71 (1H, m), 4.40 (1H, m), 4.13 (1H, m), 3.95−2.80 (6H, m), 3.68 (3H, s), 2.27 (1H, m), 2.21 (1H, m), 2.09 (8H, m), 1.97 (3H, s), 1.92 (2H, m), 1.72 (3H, s), 1.65 (1H, m), 1.65 (3H, s), 1.60 (3H, s), 1.58 (3H, s), 1.42 (1H, m), 1.18 (1H, m) and 0.90 (6H, m) ppm; FAB HRMS exact mass Calcd for $C_{45}H_{66}N_5O_4S$ 772.483553 (MH^+), found 772.481709.

2(*S*)-{2-[(2(*S*)-{2-[3-(4-Cyanobenzyl)-3*H*-imidazol-4-yl]-acetylamino}-3(*S*)-methylpentyl)naphthalen-1-ylmethylamino]acetylamino}-4-methylpentanoic acid bis-trifluoroacetate salt (38): ^{1}H NMR (CD_3OD, 400 MHz) δ 8.89 (1H, s), 8.28 (1H, d, J = 8.9 Hz), 8.00−7.90 (2H, m), 7.73 (2H, d, J = 8.1 Hz), 7.70−7.60 (1H, m), 7.58−7.42 (4H, m), 7.37 (2H, d, J = 8.1 Hz), 5.49 (2H, m), 4.28−4.04 (2H, m), 3.90−3.00 (9H, m), 1.70−1.30 (5H, m), 1.16 (1H, m), and 1.00−0.70 (12H, m) ppm; FAB HRMS exact mass calcd for $C_{38}H_{47}N_6O_4$ 651.365879 (MH^+), found 651.365115. Anal. ($C_{38}H_{46}N_6O_4$·2.65TFA·0.25H_2O) C,H,N.

2(*S*)-{2-[(2(*S*)-{2-[3-(4-Cyanobenzyl)-3*H*-imidazol-4-yl]-acetylamino}-3(*S*)-methylpentyl)naphthalen-1-ylmethylamino]acetylamino}-4-methylpentanoic acid methyl ester (39): ^{1}H NMR (CD_3OD, 400 MHz) δ 8.25 (1H, d, J = 8.8 Hz), 7.87 (1H, d, J = 7.3 Hz), 7.80 (1H, d, J = 7.9 Hz), 7.70−7.64 (3H, m), 7.50−7.35 (4H, m), 7.18 (2H, d, J = 8.2 Hz), 6.90 (1H, s), 5.36 (1H, d, J = 16.8 Hz), 5.30 (1H, d, J = 16.8 Hz), 4.22 (1H, dd, J = 9.2 and 5.5 Hz), 4.15 (1H, d, J = 13.2 Hz), 4.10 (1H, d, J = 13.2 Hz), 4.02 (1H, m), 3.65 (3H, s), 3.30−3.10 (4H, m), 2.83 (1H, dd, J = 13.3 and 3.5 Hz), 2.69 (1H, dd, J = 13.3 and 9.6 Hz), 1.55 (1H, m), 1.41−1.11 (4H, m), 1.10−0.97 (1H, m), and 0.90−0.70 (12H, m) ppm; FAB HRMS exact mass calcd for $C_{39}H_{49}N_6O_4$ 665.381530 (MH^+), found 665.380331. Anal. ($C_{39}H_{48}N_6O_4$·0.80H_2O) C,H,N.

2(*S*)-{2-[(2(*S*)-{2-[3-(4-Cyanobenzyl)-3*H*-imidazol-4-yl]-acetylamino}-3(*S*)-methylpentyl)naphthalen-1-ylmethylamino]acetylamino}-4-(methylsulfanyl)butyramide bis-trifluoroacetate salt (40): ^{1}H NMR (CD_3OD, 400 MHz) δ 8.82 (1H, s), 8.19 (1H, m), 7.83 (2H, m), 7.66 (2H, d, J = 8.2 Hz), 7.54 (1H, m), 7.50−7.35 (4H, m), 7.30 (2H, d, J = 8.2 Hz), 5.41 (2H, s), 4.13 (1H, m), 3.95 (1H, m), 3.75−3.25 (4H, m), 3.20−2.90 (2H, m), 2.30−2.00 (3H, m), 1.89 (3H, s), 1.90 (1H, m), 1.70−1.40 (3H, m), 1.31 (1H, m), 1.07 (1H, m), and 0.95−0.75 (6H, m) ppm; FAB HRMS exact mass calcd for $C_{37}H_{46}N_7O_3S$ 683.338286 (MH^+), found 683.339559. Anal. ($C_{37}H_{45}N_7O_3S$·2.60TFA·0.20H_2O) C,H,N.

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(49) This dose was selected based upon pharmacodynamic data: sc dosing of the ester **20** (40 mpk) to nude mice resulted in relatively constant drug levels in both the plasma (1−2 μM) and tissues (spleen 0.4−1 μM) for a period of 6 h. These drug concentrations were sufficient to significantly (>90%) inhibit the farnesyltransferase activity present in cytosolic fractions of homogenized spleen tissue derived from drug-treated animals verses non-drug-treated control animals.

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