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Probing the Hydrophobic Pocket of Farnesyltransferase: Aromatic Substitution of CAAX Peptidomimetics Leads to Highly Potent Inhibitors

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Abstract—Cysteine farnesylation at the carboxylate terminal tetrapeptide CAAX of Ras protein is catalyzed by farnesyltransferase. This lipid modification is necessary for regulatory function of both normal and oncogenic Ras. The high frequency of Ras mutation in human cancers has prompted an intensive study on finding ways of controlling oncogenic Ras function. Inhibition of farnesyltransferase is among the most sought after targets for cancer chemotherapy. We report here the design, synthesis and biological characterization of a series of peptidomimetics as farnesyltransferase inhibitors. These compounds are extremely potent towards farnesyltransferase with IC_{50} values ranging from subnanomolar to low nanomolar concentrations. They have a high selectivity for farnesyltransferase over the closely related geranylgeranyltransferase-I. Structure–activity relationship studies demonstrated that a properly positioned hydrophobic group significantly enhanced inhibition potency, reflecting an improved complementarity to the large hydrophobic pocket in the CAAX binding site.

Introduction

Protein isoprenylation plays a key role in protein subcellular localization, protein–protein interaction, and signal transduction. In this process C_{15} farnesyl and C_{20} geranylgeranyl groups are covalently linked to the cysteine residue near the carboxyl terminus through a thiol alkylation reaction that is catalyzed by the isoprenyltransferase enzymes.[1] Three different isoprenyltransferases have been identified, farnesyltransferase (FTase), type-I geranylgeranyltransferase (GGTase-I), and type-II geranylgeranyltransferase (GGTase-II).[2–4] FTase catalyzes farnesylation of proteins with a carboxyl terminal sequence CAAX, where C is cysteine, A is an aliphatic amino acid, X is methionine, serine, alanine, glutamine, or cysteine.[5] GGTase-I catalyzes geranylgeranylation of proteins with the same carboxyl terminal sequence, except that the X residue is restricted to leucine, isoleucine, or, to a less extent, phenylalanine.[6] Unlike FTase and GGTase-I, GGTase-II only modifies proteins with carboxyl terminal sequences CXC, CCXX, or XXCC and requires a Rab escort protein to function.[7,8]

Among the three isoprenyltransferases, FTase has been most intensively studied, owing to its involvement in the post-translational modification of the low molecular weight GTPase, Ras.[9] Ras cycles between GDP (inactive) and GTP (active) states to transduce growth factor signals from cell surface receptor tyrosine kinases to the nucleus.[10] Single point mutations of Ras abolish its GTPase activity and lead to uncontrolled signaling.[11] These mutations to Ras are found in over 30% of all human tumors, particularly pancreatic and colon cancers.[12,13]

The regulatory function of Ras is dependent on its association with the plasma membrane.[14] It is now recognized that Ras undergoes a three step post-translational modification to convert its cytosolic form to a membrane bound form. The first, obligatory and

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sufficient step in these modifications is the attachment of a farnesyl group to the cysteine residue of the CAAX sequence at the carboxyl terminal.[15] After farnesylation, the tripeptide AAX is cleaved by a peptidase and the resulting carboxylic acid is methylated by a methyltransferase.[16] Site directed mutagenesis has shown that farnesylation alone is necessary and sufficient for Ras transforming activity.[17] Thus, the inhibition of FTase provides a new target for potential anticancer drug design.[18–20]

FTase is a heterodimeric metalloenzyme composed of α and β subunits with molecular weights of 49 and 46 kDa, respectively.[21,22] Both farnesylpyrophosphate (FPP) and Ras bind to the β subunit.[23,24] However, FTase does not require the full length of Ras for the farnesylation reaction. Small peptides, as short as four amino acids with the consensus CAAX sequence can be farnesylated with kinetic parameters similar to that of Ras protein.[5,25] A recent X-ray structure of FTase with tetrapeptide Ac-CVIM(Se) and an FPP analogue bound into the active site shows that the active site is at the interface of the two subunits.[26,27] The tetrapeptide is in an extended conformation with the Cys-SH bound to the catalytically important zinc ion (Fig. 1). Importantly for the present paper, a single hydrogen bond is formed to the C-terminal amide and a large pocket formed by two Trp and one Tyr residues provides a binding cavity for the aliphatic side chain of the penultimate residue.

The discovery that CAAX tetrapeptides can competitively inhibit FTase has played a key role in the design of peptidomimetics as FTase inhibitors. So far, several different approaches have been taken in the search for in vitro active inhibitors of FTase. These include the screening of natural products and synthetic libraries, the design of FPP analogues, bisubstrate transition state analogues, and most importantly, CAAX peptidomimetics.[28,29] We and others have previously reported peptidomimetics and pseudopeptides as FTase inhibitors.[30–41] In this paper, we report that systematic hydrophobic functionalization of our peptidomimetics to fit the hydrophobic pocket in the FTase active site has led to the discovery of FTase inhibitors with subnanomolar inhibition potency.

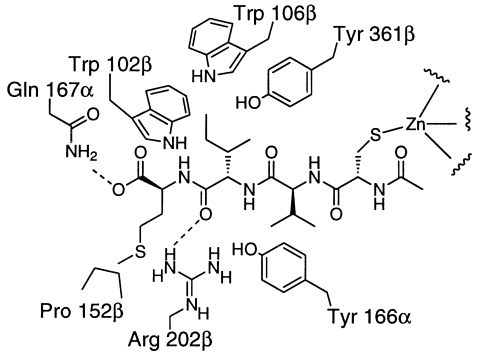


Figure 1. Active site contacts to the CAAX substrate for FTase.[26,27]

Design and Synthesis

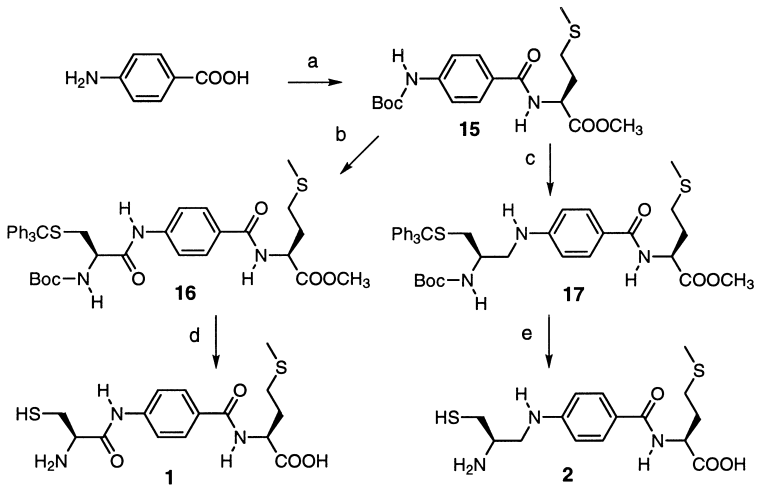
We initiated this project with the goal of identifying peptidomimetics and nonpeptides as FTase inhibitors. We first replaced the central dipeptide in CAAX with a variety of spacers including 3-aminomethylbenzoic acid which could replace the Val-Ile dipeptide in CVIM without significant loss of inhibition activity.[38,39] Further rigidification was achieved by using a 4-aminobenzoic acid spacer between the methionine and cysteine derivatives. The synthesis of this parent peptidomimetic included coupling (L)-methionine methyl ester with *N*-Boc-aminobenzoic acid, deprotection and amino group coupling with *N*-Boc-*S*-trityl-(L)-cysteine by using EDCI or isobutylchloroformate. A further modification was the replacement of the cysteine amide bond with a methyleneamino group using a reductive amination of the corresponding amine with *N*-Boc-*S*-trityl-(L)-cysteinal.[31] Scheme 1 describes the synthesis of compounds **1** and **2**.

While the rigidity of the 4-aminobenzoic acid spacer significantly reduces the possible conformations of the corresponding Val-Ile dipeptide, the simple aromatic ring does not reach the volume of the side chains of the Val-Ile dipeptide. In order to probe the size and complementarity of the hydrophobic pocket in FTase, we have attached different groups to the aromatic ring. Figure 2 shows the structures of inhibitors with differently designed hydrophobic substituents.

The synthesis of compounds **3** and **4** was similar to that of **1** except 4-amino-3-methylbenzoic acid and 4-amino-3-methoxybenzoic acid, respectively, were used. Compound **7** was prepared from 4-amino-1-naphthoic acid, itself prepared from the hydrolysis of 4-amino-1-naphthalenecarbonitrile. The spacers in compounds **5** and **6** were prepared from the coupling of 4-nitro-2-bromotoluene with phenyl or xylylboronic acid under Suzuki conditions.[42] Oxidation of 4-nitro-2-phenyltoluene with potassium permanganate provided 4-nitro-2-phenylbenzoic acid. The carboxylic acid was coupled with (L)-methionine methyl ester and the nitro group was reduced with stannous chloride.[43] The resulting amine was coupled with *N*-Boc-*S*-trityl-(L)-cysteine to give a fully protected precursor which was deprotected and purified to give the final product.

The structure–activity relationship of compounds **3**–**7** revealed that a phenyl substitution at the 2-position significantly enhanced potency for FTase inhibition (Table 1). We next investigated different aromatic substitutions at the 2-position of the 4-aminobenzoic acid spacer. This substitution is expected not only to increase the hydrophobicity of the molecule, but also to restrict rotation about the phenyl–CO bond. Figure 3 shows the structures of compounds **8**–**13**, which also include pseudopeptide modifications of the cysteine residue.

The synthesis of compound **8** (FTI-276) and its methyl ester (FTI-277) is described in Scheme 2. Compounds **8**–**13** were purified by reverse phase preparative HPLC (C18 column, linear gradient from 5% acetonitrile to



Scheme 1. Replacement of dipeptide in CVIM with 4-aminobenzoic acid. Reagents: (a) (1) $(Boc)_2O$, NaOH, (2) (L)-methionine methyl ester; (b) (1) gaseous HCl in methanol, (2) *N*-Boc-*S*-trityl-(L)-cysteine, isobutyl chloroformate, Et_3N; (c) (1) gaseous HCl in methanol, (2) *N*-Boc-*S*-trityl-(L)-cysteinal, $NaB(CN)H_3$; (d) (1) aqueous LiOH, THF, (2) gaseous HCl in ether; (e) (1) aqueous LiOH, THF, (2) TFA, Et_3SiH.

60% acetonitrile in 0.1% TFA aqueous solution over 30 min). The methyl ester of compound **8** (compound **14**, FTI-277) was prepared from deprotection of the fully protected precursor using mercuric chloride and hydrogen sulfide. FTI-277 was isolated as a hydrochloride salt without the requirement of HPLC purification.

The spacer in **9** and **10** was prepared by oxidation of 4-nitro-2-bromotoluene followed by methylation to form methyl 4-nitro-2-bromobenzoate. The required 2-thienyl- and 1-naphthylboronic acids were prepared from the corresponding aryl bromides. Scheme 3 shows the synthesis of peptidomimetics **9** and **10**. The ^1H NMR spectra of **10** showed two isomers due to slow aryl–aryl bond rotation on the NMR time scale.

Incorporation of a pyridine ring into the aromatic spacer was accomplished through the coupling of methyl 4-nitro-2-bromobenzoate with trimethylstannylpyridine (Scheme 4).[44] The methyl ester was hydrolyzed with aqueous lithium hydroxide in methanol and the corresponding carboxylic acid was coupled with (L)-methionine methyl ester. The nitro group was reduced using stannous chloride with the 4-pyridyl derivative, but the same conditions caused amide bond cleavage in the 2-pyridyl and 3-pyridyl analogues. For these compounds reduction was best achieved with Raney nickel and hydrazine in methanol.[45] The resulting amine was coupled with *N*-Boc-*S*-trityl-(L)-cysteinal to give the fully protected precursor, which was deprotected and purified to give the pyridine substituted inhibitors.

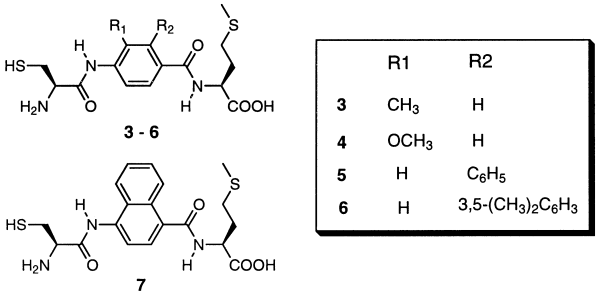


Figure 2. Structures of peptidomimetics with improved hydrophobic spacer.

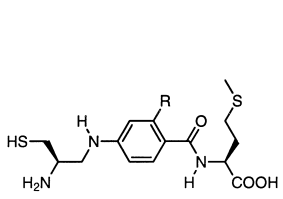
Results and Discussion

The ability of the peptidomimetics to inhibit FTase and GGTase-I in vitro was investigated by using partially purified FTase and GGTase-I from human Burkitt lymphoma (Daudi) cells.[40] Enzyme preparations were incubated with [^{3}H]FPP and recombinant H-Ras-CVLS (FTase) or [^{3}H]GGPP and H-Ras-CVLL (GGTase-I) in the presence of different concentrations of inhibitors. After incubation for 30 min at 37°C, the reaction was stopped and filtered on glass fiber filters to separate free from incorporated label, as described earlier.[40] The

Table 1. Inhibition of FTase and GGTase-I by CAAX peptidomimetics[a]

Peptidomimetics	IC_{50} (nM) ± SEM FTase	IC_{50} (nM) ± SEM GGTase-I	Selectivity FTase/GGTase-I
1	213 ± 71 (4)	1200 ± 216 (3)	6
2	267 ± 104 (6)	3675 ± 653 (4)	14
3	825 ± 225 (2)	9000 (1)	11
4	2550 ± 1250 (2)	50000 (1)	20
5	4.5 ± 2.9 (5)	267 ± 94 (3)	59
6	405 ± 105 (2)	400 (1)	1
7	143 ± 26 (3)	3150 ± 150 (2)	22
8 (FTI-276)	0.61 ± 0.16 (17)	50 ± 20 (13)	82
9	1.3 ± 0.2 (2)	40 (1)	31
10	1.9 ± 0.3 (2)	7.0 (1)	4
11	3.0 (1)	400 (1)	133
12	5.5 (1)	680 (1)	124
13	5.3 ± 2.1 (2)	310 (1)	59
14 (FTI-277)	63 ± 21 (5)	1800 ± 200 (2)	28

[a] Numbers in parenthesis indicate the times of independent enzyme inhibition assay.



	R
8 (FTI-276)	C_6H_5
9	2-C_4H_3S
10	1-$C_{10}H_7$
11	2-C_5H_4N
12	3-C_5H_4N
13	4-C_5H_4N

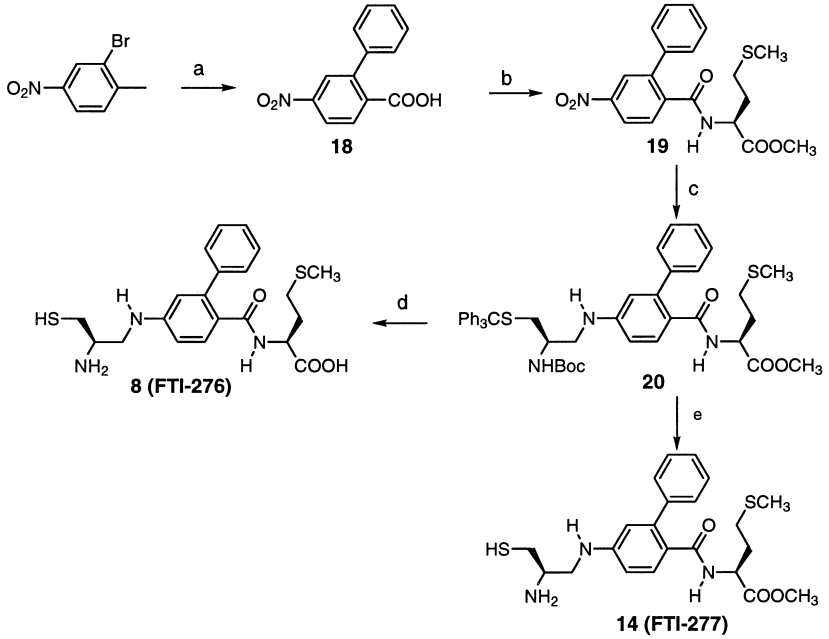
Figure 3. Peptidomimetics with aromatic substitution at the 2-position of 4-aminobenzoic acid.

activity of the inhibitors is reported in Table 1 as IC_{50} values, the concentration at which FTase or GGTase-I activity was inhibited by 50%.

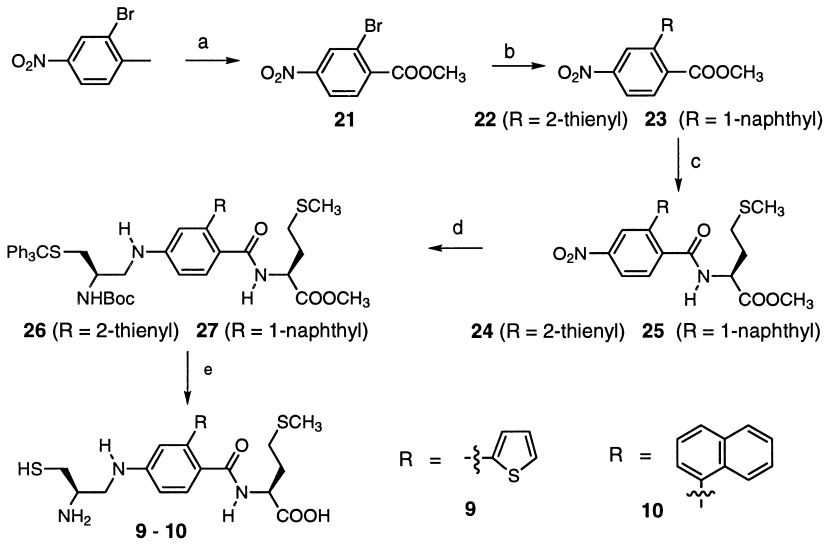
Tetrapeptide CVIM from the K_B-Ras terminal sequence can inhibit FTase in vitro with an IC_{50} value around 165 nM.[31] In our FTase inhibition studies, CVIM was used as a control with a measured IC_{50} value of 150–340 nM.[39] Our initial proposal was that the central dipeptide of CAAX might simply play a hydrophobic role in binding to the FTase active site. This postulation was consistent with the observation that in the CAAX sequence the cysteine residue is absolutely required, the methionine residue is favored and the central two residues can tolerate a variety of aliphatic or aromatic amino acids. The recent X-ray structure of FTase bound to a tetrapeptide[26,27] shows no significant interaction to the amide bond between the central Val and Ile residues (Fig. 1). To test this hypothesis, we replaced the central two amino acids in CVIM with a hydrophobic 4-aminobenzoic acid spacer. This replacement maintains the FTase inhibition activity (IC_{50} = 213 nM, Table 1) and gives a sixfold selectivity for FTase over GGTase-I.[46] The methyl ester of **1** inhibited H-Ras processing in NIH 3T3 cells at 200 μM without affecting the geranylgeranylation of Rap1A at concentrations as high as 400 μM. Although the potency of **1** is in the submicromolar range, it became a key starting point for the design of subnanomolar FTase inhibitors.

Reduction of the cysteine amide to its methyleneamino isostere has been shown to be tolerated in CAAX pseudopeptides.[31] In our series of compounds reduction of cysteine amide did not affect inhibition (**2**, IC_{50} = 267 nM) but gave improved selectivity for FTase over GGTase-I (14-fold for **2**). Furthermore, the methyl ester of **2** inhibited H-Ras farnesylation in NIH 3T3 cells at 25 μM, a 10-fold improvement over the methyl ester of **1**. This result clearly confirmed that one advantage afforded by the pseudopeptide modification is to reduce peptidase cleavage.

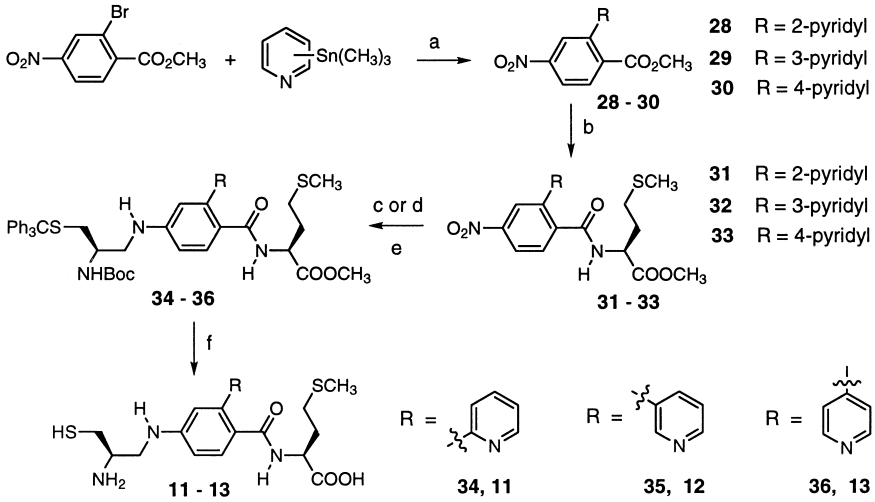
Although **1** and **2** are as potent inhibitors as CVIM, it is clear that the 4-aminobenzoic acid spacer is a poor mimic of the large hydrophobic side chains in valine and isoleucine. Therefore, increasing the size and hydrophobicity of the 4-aminobenzoic acid spacer should allow better occupation of the FTase substrate binding pocket. Attachment of methyl and methoxy substituents at the 3-position of the 4-aminobenzoic acid spacer led to a decrease in inhibition potency (IC_{50} = 825 and 2250 nM for **3** and **4**, respectively). In contrast, substitution at the 2-position gave a startling improvement in activities with phenyl-substituted **5** giving an IC_{50} of 4.5 nM. This corresponds to an increase in FTase inhibition potency of 50-fold when compared to **1**. Compound **5** is sevenfold more potent than CIFM, the most potent FTase inhibitor in CAAX series. The increased



Scheme 2. Synthesis of peptidomimetic **FTI-276** and **FTI-277**. Reagents: (a) (1) phenylboric acid, $Pd(OAC)_2$, aqueous acetone, (2) $KMnO_4$, aqueous pyridine; (b) (L)-methionine methyl ester, EDCI, HOBT; (c) (1) $SnCl_2$ hydrate, (2) *N*-Boc-*S*-trityl-(L)-cysteinal $NaB(CN)H_3$; (d) (1) LiOH, THF, (2) TFA, Et_3SiH; (e) (1) $HgCl_2$, methanol, (2) H_2S, methanol.



Scheme 3. Preparation of peptidomimetic **9** and **10**. Reagents: (a) (1) $KMnO_4$, aqueous pyridine, (2) methanol, $SOCl_2$; (b) (1) 2-thienylboric acid or 1-naphthylboric acid, $Pd(PPh_3)_4$, DMF; (c) (1) aqueous LiOH, MeOH, THF, (2) (L)-methionine methyl ester, EDCI, HOBT; (d) (1) $SnCl_2$ hydrate, (2) *N*-Boc-*S*-trityl-(L)-cysteinal, $NaB(CN)H_3$, methanol; (e) (1) aqueous LiOH, THF; (2) TFA, Et_3SiH.



Scheme 4. Preparation of pyridine containing peptidomimetics **11**–**13**. Reagents; (a) $Pd(PPh_3)_2Cl_2$, CuI, LiCl, toluene; (b) (1) aqueous LiOH, methanol, (2) (L)-methionine methyl ester, EDCI, HOBT; (c) $SnCl_2$ hydrate; (d) Raney Ni, hydrazine, methanol; (e) *N*-Boc-*S*-trityl-(L)-cysteinal, $NaB(CN)H_3$, methanol; (f) (1) aqueous LiOH, THF, (2) TFA, Et_3SiH.

potency of **5** clearly suggested a favorable hydrophobic interaction of FTase active site with the phenyl ring. When the phenyl group was replaced by a xylyl ring, inhibition activity decreased 100-fold. The potency of **6** is reduced compared to **1**, possibly due to unfavorable steric effects from the methyl groups. It is interesting to note that when 4-amino-1-naphthoic acid was used as the spacer, inhibition potency was not improved (compare **7** and **1** in Table 1).

As described for compound **2**, the pseudopeptide strategy was also applied to modify the cysteine amide bond in **5**. Compound **8** (FTI-276) showed subnanomolar inhibition activity with an average IC_{50} of 0.61 nM (IC_{50} ranged from 0.33 to 0.90 nM in 17 independent assays) and a selectivity for FTase over GGTase-I of 82-fold. Lineweaver–Burk analysis confirmed that **8** (FTI-276) is competitive to H-Ras for binding to FTase with a K_i of 0.74 nM. Methylation of the carboxylic acid in **8** decreased inhibition potency by 100-fold ($IC_{50} = 63$ nM, **14** in Table 1). However, the methyl ester showed significant potency in inhibiting Ras farnesylation in whole cells. Compound **14** (FTI-277) inhibited H-Ras processing with an IC_{50} of 100 nM and retained selectivity in whole cells, inhibiting the processing of Rap1A (a geranylgeranylated protein) with an IC_{50} of 50 μM.[41]

Results from Table 1 also indicate that substitution at the 2-position of the aminobenzoic acid spacer is not restricted to phenyl groups since both 2-thienyl and 1-naphthyl attachments also lead to potent inhibitors with IC_{50} values in the 1–2 nM range. Interestingly, the selectivity for FTase over GGTase-I is decreased from 82-fold in **5** to only fourfold in **10**. This indicates that large hydrophobic substituents such as naphthalene are more optimal for strong binding to GGTase-I.

Comparison of the active site residues in the two enzymes[26,27] suggests that the hydrophobic pocket is larger in GGTase-I, formed by smaller Thr, Phe and Leu residues in place of Trp102, Trp106 and Tyr361 seen in the crystal structure of FTase (Fig. 1). This difference has recently been exploited in the development of a family of inhibitors for GGTase-I.[46]

Finally, we linked a pyridine ring to the 2-position of the 4-aminobenzoic acid spacer in an attempt to probe the presence of hydrogen bond donors in the enzyme binding region. The recent crystal structure of FTase (Fig. 1) shows three potential acidic centers in the hydrophobic pocket for the penultimate Leu side chain, a Tyr-OH and two Trp-NH groups.[26,27] We used 2-, 3-, and 4-pyridyl groups (**11**, **12** and **13**, respectively) in an attempt to position the hydrogen bond accepting pyridine-*N* at different locations in the pocket. Table 1 shows that the three pyridine derived inhibitors share similar inhibition activity for FTase (IC_{50} values 3–6 nM) with good selectivity for FTase over GGTase-I. These three compounds are five- to 10-fold less active as FTase inhibitors than FTI-276 (compare **8** with **11**, **12** and **13** in Table 1), suggesting that no additional interactions have been exploited. In fact, the drop in activity presumably reflects the less hydrophobic and more strongly solvated character of pyridyl compared to phenyl. A computational study of the binding and solvation consequences of a phenyl to pyridyl modification in FK506 analogues has recently been published by Jorgensen.[47]

The structure–activity relationships of our peptidomimetics in Table 1 clearly indicates that the strategy of hydrophobic substitution can lead to highly potent inhibitors of FTase. These inhibitors share some structural similarity with the CVIM tetrapeptide including the methionine residue, the methionine amide bond, the cysteine amino group and the cysteine side chain. Recent work by us[48] and by Augeri et al.[49] has shown that the N-terminal cysteine group in these inhibitors can be replaced by nitrogenous base derivatives and that an extended hydrophobic spacer can be used in place of the C-terminal methionine.[50] In the present paper we have investigated the third region of the molecules and identified an aryl-substituted hydrophobic spacer that gives both potent inhibition of FTase and high selectivity over GGTase-I.

Experimental

Nuclear magnetic resonance spectra were acquired using Bruker AM-300 series spectrometers (300 MHz for ^1H, 75 MHz for ^{13}C). Chemical shifts were expressed as parts per million and referenced to internal tetramethylsilane (TMS), $CDCl_3$ or CD_3OD. Electron impact (EI) and fast atom bombardment (FAB) mass spectra were obtained on a Varian MAT CH-5 (high resolution) or VG 7070 (low resolution) mass spectrometer. Elemental analysis were performed by Atlantic Microlabs, Inc., Norcross, GA. Optical rotations $[\alpha]_D^{25}$ were measured using a Perkin–Elmer 241 polarimeter. Concentrations are expressed in g/100 mL. Melting points (mp) were determined using an Electrothermal capillary melting point apparatus and are uncorrected. All synthesized final compounds (amine hydrochloride or amine trifluoroacetate salts) were checked for purity by analytical high pressure liquid chromatography which was performed using a Rainin HP controller and a Rainin UV-C detector with a Rainin 250×4.6 mm, 5 μm Microsorb C18 column. Binary solvents consisting of 10–90% acetonitrile and 0.1% TFA in water were used. Flow rate was set as 1 mL/min and separation was programmed for 30-min running. Preparative HPLC was performed on a Waters 600E controller and a Waters 490E multi-wavelength UV detector with a 25×10 cm Delta-Pak C-18 300 Å cartridge column inside a Waters 25×10 cm Radial Compression Module. Solvents consisting of 20–80% acetonitrile and 0.1% TFA in water were used with a flow rate of 15 mL/min in 40 min.

Farnesyltransferase inhibition assay was determined by measuring the amount of [^3H]-farnesyl group transferred from [^3H]-FPP to recombinant H-Ras-CVLS protein according to literature procedure.[40] Each assay solution contained the following concentrations of components in a final volume of 50 μL: 50 mM Tris–chloride (pH 7.5), 50 μM $ZnCl_2$, 20 mM KCl, 3 mM $mgCl_2$, 500 nM [^3H]-FPP (specific activity 20 Ci/mmol), and 15 μM H-Ras. For kinetic assay, the different concentrations of inhibitors and certain amount of partially purified farnesyltransferase from human Burkitt lymphoma (Daudi) cells were added to the above solution at 0°C in 12×75 mm borosilicate tubes. After incubation at 37°C for 15 min, the reaction was stopped by addition of 0.5 mL of 4% SDS and 0.5 mL of 30% trichloroacetic acid (TCA) solution. The mixture was left on ice for 45 min and then 2 mL of 2% SDS and 6% TCA was added. The solution was filtered on glass fiber filters and each filter was washed five times with 2 mL of 6% TCA solution. The filters were dried and counted in a scintillation counter. For GGTase-I assays, the reaction mixture was similar except that the substrates used were H-Ras-CVLL and [^3H]-GGPP (specific activity 20 Ci/mmol). GGTase-I was also partially purified from Daudi cells.[40]

***N*-(4-*N*-Boc-Aminobenzoyl)-methionine methyl ester (15).** To a mixture of 4-aminobenzoic acid (13.7 g, 100 mmol) in 100 mL of 1 N NaOH and 100 mL of dioxane was added di-*tert*-butyl dicarbonate (24.0 g, 110 mmol) at 0°C. The mixture was stirred at rt overnight and then acidified with 3 N HCl. After extraction with ethyl acetate and evaporation of solvents, a crude solid product was obtained. This solid was recrystallized from methanol and water to give *N*-Boc-4-aminobenzoic acid as white crystals (15.2 g, 65%). Mp 190–191°C. ^1H NMR (CD_3OD) δ 7.92 (d, J = 8.5 Hz, 2H), 7.50 (d, J = 8.5 Hz, 2H), 1.51 (s, 9H); ^{13}C NMR (CD_3OD) δ 169.8, 155.0, 145.8, 131.8, 125.3, 118.5, 81.2, 28.6; HRMS (EI) calcd for $C_{12}H_{15}O_4N$ 237.0961, obsd 237.0956. This acid (3.55 g, 15 mmol) was dissolved in 150 mL of methylene chloride. To this solution was added L-methionine methyl ester hydrochloride (3.0 g, 15 mmol) and triethylamine (2.48 mL). This mixture was cooled in an ice bath and

then EDCI (2.87 g, 15 mmol) was added. The clear solution was stirred at rt overnight and then extracted with methylene chloride and 1 N HCl. The organic phase was washed with concentrated sodium bicarbonate and dried over sodium sulfate. After evaporating the solvents, a crude solid was obtained which was recrystallized from ethyl acetate and hexane to give a pure product (4.92 g, 86%). Mp 184–185°C. ^{1}H NMR ($CDCl_3$) δ 7.78 (d, J = 8.6 Hz, 2H, aromatic), 7.47 (d, J = 8.6 Hz, 2H, aromatic), 7.01 (d, J = 7.0 Hz, 1H, amide), 6.91 (s, 1H, amide), 4.92 (q, J = 7.0 Hz, 1H, Met α H), 3.79 (s, 3H, OCH_3), 2.59 (t, J = 5.9 Hz, 2H, CH_2S), 2.18 (m, 1H, Met CH_2), 2.11 (m, 4H, Met CH_2, SCH_3), 1.53 (s, 9H, Bu^t); ^{13}C NMR ($CDCl_3$) δ 172.8, 166.6, 152.3, 141.9, 128.3, 127.8, 117.9, 81.2, 52.7, 52.1, 31.6, 30.2, 28.3, 15.6. Anal. calcd for $C_{18}H_{26}N_2O_5S$: C 56.53, H 6.85, N 7.29; found C 56.46, H 6.83, N 7.30.

***N*-Boc-*S*-trityl-cysteinyl-4-aminobenzoyl-methionine methyl ester (16).** Compound **15** (298 mg, 0.78 mmol) was dissolved in a mixture of 2 mL of methylene chloride and 2 mL of 3 N HCl in methanol. After 1.5 h, TLC showed disappearance of starting material. This clear solution was evaporated and the residue was dried under vacuum. Amide coupling was carried out through a mixed anhydride which was prepared by stirring a mixture of *N*-Boc-*S*-trityl-L-cysteine (370 mg, 0.80 mmol), isobutyl chloroformate (0.105 mL, 0.80 mmol) and triethylamine (0.10 mL, 0.80 mmol) in 5 mL of THF at −15°C for 30 min. The hydrochloride salt from Boc deprotection was then suspended in 3 mL of methylene chloride and neutralized with 0.15 mL of triethylamine. This solution was added to the mixed anhydride solution prepared above and the mixture was stirred at rt for 12 h. After work up and column chromatography purification, a pure product was obtained (450 mg, 79.5%). Mp 98–99°C. $[\alpha]_D^{25}$ −3.1 (*c* 1.4, EtOAc). ^{1}H NMR ($CDCl_3$) δ 8.52 (br s, 1H, amide), 7.72 (d, J = 8.5 Hz, 2H, aromatic), 7.50 (m, 8H, aromatic), 7.29 (m, 9H, aromatic), 7.03 (d, J = 6.8 Hz, 1H, amide), 4.93 (q, J = 7.4 Hz, Met α H), 4.85 (d, J = 7.2 Hz, 1H, amide), 3.98 (m, 1H, Cys α H), 3.79 (s, 3H, OCH_3), 2.75 (dd, J = 13.0, 7.3 Hz, 1H, CH_2SCPh_3), 2.63 (dd, J = 13.0, 5.3 Hz, 1H, CH_2SCPh_3), 2.59 (t, J = 7.3 Hz, 2H, CH_2SCH_3), 2.29 (m, 1H, Met CH_2), 2.12 (m, 4H, Met CH_2, SCH_3), 1.44 (s, 9H, Bu^t); ^{13}C NMR ($CDCl_3$) δ 173.9, 169.7, 166.7, 158.0, 144.2, 140.8, 129.3, 128.3, 128.0, 127.8, 126.7, 110.6, 80.6, 67.1, 54.9, 52.8, 51.9, 33.3, 30.9, 30.4, 28.1, 15.3. Anal. calcd for $C_{40}H_{45}O_6N_3S_2$: C 65.99, H 6.24, N 5.77; found C 65.94, H 6.28, N 5.71.

***N*-[4-[*N*-[2(*R*)-(*tert*-Butoxycarbonyl)amino-3-(triphenylmethyl)thio]propyl]-aminobenzoyl]-methionine methyl ester (17).** To a solution of *N*-(4-aminobenzoyl)-methionine methyl ester hydrochloride (1.78 g, 5.6 mmol, prepared from the deprotection of compound **15**) in 60 mL of methanol and 4 mL of glacial acetic acid was added one equivalent of *N*-Boc-*S*-tritylcysteinal (according to ^{1}H NMR determination of aldehyde percentage) in 10 mL of methanol. Sodium cyanoborohydride (528 mg, 8.40 mmol) was added to this deep colored solution at 0°C. The mixture was stirred at rt for 15 h. After evaporating solvents, the residue was extracted with ethyl acetate and concentrated sodium bicarbonate. The organic layer was dried and solvents were evaporated. The residue was purified by flash column chromatography (ethyl acetate:hexane, 1:1) to give the desired product (2.52 g, 65%). Mp 85°C (decomp). ^{1}H NMR ($CDCl_3$) δ 7.63 (d, J = 8.6 Hz, 2H, aromatic), 7.43 (m, 6H, trityl), 7.21–7.32 (m, 9H, trityl), 6.73 (d, J = 7.6 Hz, 1H, Met amide), 6.50 (d, J = 8.6 Hz, 2H, aromatic), 4.91 (ddd, J = 5.5, 7.6 Hz, 1H, Met α H), 4.59 (d, J = 7.3 Hz, 1H, Boc amide), 4.25–4.40 (br, 1H, NHPh), 3.80 (m, 1H, Cys α H), 3.78 (s, 3H, OCH_3), 3.09 (d, J = 6.3 Hz, 2H, CH_2N), 2.55–2.60 (m, 2H, CH_2 $SCPh_3$), 2.46 (d, J = 5.0 Hz, 2H, CH_2SCH_3), 2.23–2.28 (m, 1H, Met CH_2), 2.07–2.12 (m, 1H, Met CH_2), 2.09 (s, 3H, SCH_3), 1.43 (s, 9H, Boc); ^{13}C NMR ($CDCl_3$) δ 172.8, 166.8, 155.7, 150.8, 144.3, 129.4, 128.8, 127.9, 126.8, 121.5, 115.2, 79.7, 67.0, 52.4, 51.8, 49.2, 46.8, 34.2, 31.6, 30.0, 28.2, 15.4; Anal. calcd for $C_{40}H_{47}O_5$ $N_3S_2 \cdot 0.4CH_2Cl_2$: C 64.89, H 6.39, N 5.62, S 8.56; found C 64.66, H 6.43, N 5.69, S 8.50.

Cysteinyl-4-aminobenzoyl-methionine hydrochloride (1). The fully protected compound **16** (400 mg, 0.55 mmol) was hydrolyzed with 1.0 equiv of 0.5 N LiOH in 3 mL of THF at 0°C. The solvents were evaporated and the residue was first acidified with 1 N HCl and then extracted with methylene chloride. After evaporation of solvents, the carboxylic acid was dissolved in 3 mL of methylene chloride, 1 mL of THF and 3 mL of 3 N HCl in dry ether. The solution was maintained at rt for 25 min. The white precipitate was collected and recrystallized twice from ethanol and ether to give **1** shown to be pure by HPLC (108 mg, 48%). Mp 157°C (decomp). $[\alpha]_D^{25}$ −22.5 (*c* 0.5, MeOH). ^{1}H NMR (CD_3OD) δ 7.87 (d, J = 8.7 Hz, 2H, aromatic), 7.73 (d, J = 8.7 Hz, 2H, aromatic), 4.75 (dd, J = 4.6, 6.5 Hz, 1H, Met α H), 4.16 (dd, J = 5.1, 7.0 Hz, 1H, Cys α H), 3.18 (dd, J = 14.7, 5.0 Hz, 1H, CH_2SH), 3.04 (dd, J = 14.7, 7.3 Hz, 1H, CH_2SH), 2.62 (m, 2H, CH_2SCH_3), 2.20 (m, 1H, Met CH_2), 2.11 (m, 4H, SCH_3, Met CH_2); ^{13}C NMR (CD_3OD) δ 175.5, 169.8, 166.9, 142.5, 131.0, 129.6, 120.5, 56.7, 53.2, 31.8, 31.6, 26.3, 15.2. Anal. calcd for $C_{15}H_{21}O_4N_3S_2 \cdot HCl$: C 44.16, H 5.44, N 10.30; found C 44.40, H 5.87, N 10.01.

***N*-[4-*N*-[2(*R*)-Amino-3-mercaptopropyl]aminobenzoyl]-methionine hydrochloride (2).** The fully protected compound **17** (567 mg, 0.79 mmol) was dissolved in 3.0 mL of 0.5 N lithium hydroxide and 3.0 mL of tetrahydrofuran. The mixture was stirred at 0°C for 1 h. After evaporating solvents, the residue was dissolved in water and extracted with methylene chloride and 1 N hydrochloric acid. The organic layer was dried and the solvents were evaporated. The residue was dissolved in a mixture of 1 mL of methylene chloride and 2 mL of trifluoroacetic acid. Triethylsilane was added dropwise until the deep brown color disappeared. The mixture was maintained at rt for 1 h. The solvents were evaporated and the residue was dried. This residue was dissolved in 2 mL of 1.7 N HCl in acetic acid and then 5 mL of 3 N HCl in ether was added followed by further addition of 20 mL of ether. The white precipitate was filtered and dried to give a hydrochloride salt (159 mg,

46%). Analytical HPLC showed purity over 98%. Mp 164–165°C. $[\alpha]_D^{25}$ −11.9 (*c* 0.15, MeOH). ^{1}H NMR (CD_3OD) δ 7.74 (d, *J* = 8.7 Hz, 2H, aromatic), 6.75 (d, *J* = 8.7 Hz, 2H, aromatic), 4.73 (dd, *J* = 4.6, 9.3 Hz, 1H, Met α H), 3.45–3.58 (m, 3H, CH_2NH and Cys α H), 2.93 (dd, *J* = 4.5, 14.6 Hz, 1 H, CH_2SH), 2.80 (dd, *J* = 5.3, 14.6 Hz, 1H, CH_2SH), 2.53–2.64 (m, 2H, CH_2SCH_3), 2.15–2.23 (m, 1H, Met CH_2), 2.07–2.13 (m, 1H, Met CH_2), 2.10 (s, 3H, SCH_3); ^{13}C NMR (CD_3OD) δ 175.6, 170.3, 152.5, 130.4, 123.4, 113.0, 53.7, 53.1, 44.7, 32.0, 31.6, 25.3, 15.2; LRMS for $C_{15}H_{23}O_3N_3S_2$ 357 (M^+, 10), 282 (60), 133 (100).

Cysteinyl-4-amino-3-methylbenzoyl methionine trifluoroacetate (3). 4-Amino-3-methylbenzoic acid was protected to give *N*-Boc-4-amino-3-methylbenzoic acid (60%). This acid was coupled with (L)-methionine methyl ester as described in compound **15** to give *N*-Boc-4-amino-3-methylbenzoyl methionine methyl ester (86%). The Boc group was deprotected and the resulting amine was coupled with *N*-Boc-*S*-trityl-(L)-cysteine using a mixed anhydride method. The fully protected precursor was first treated with aqueous LiOH in THF and then deprotected with TFA and 1.0 equiv of triethylsilane. Compound **3** was purified by reverse phase preparative HPLC. Mp 158–163°C. $[\alpha]_D^{25}$ +21.0 (*c* 1.0, H_2O). ^{1}H NMR (CD_3OD) δ 7.80 (1H, s), 7.69–7.73 (1H, m), 7.64 (1H, d, *J* = 8.4 Hz), 4.70–4.75 (1H, m), 4.25 (1H, dd, *J* = 6.9 and 5.2 Hz), 3.19 (1H, dd, *J* = 14.6 and 5.2 Hz), 3.08 (1H, dd, *J* = 14.6 and 6.9 Hz), 2.57–2.65 (2H, m), 2.36 (3H, s), 2.19–2.30 (1H, m), 2.09–2.17 (4H, m); ^{13}C NMR (CD_3OD) δ 175.6, 169.7, 167.5, 139.3, 133.2, 131.0, 126.7, 125.8, 125.5, 56.7, 53.5, 32.1, 31.6, 26.5, 18.3, 15.2.

Cysteinyl-4-amino-3-methoxybenzoyl methionine trifluoroacetate (4). This compound was prepared with the same method as described in compound **3**. 4-Amino-3-methoxybenzoic acid was protected to give *N*-Boc-4-amino-3-methoxybenzoic acid (93%). This acid was coupled with (L)-methionine methyl ester and the Boc group was then deprotected. The free amine was coupled with *N*-Boc-*S*-trityl-(L)-cysteine to provide a fully protected precursor. After deprotection, the crude product was purified by reverse phase preparative HPLC. Mp 191–193°C (decom). $[\alpha]_D^{25}$ +19.0 (*c* 1.0, H_2O); ^{1}H NMR (CD_3OD) δ 8.23 (1H, d, *J* = 8.4 Hz), 7.56 (1H, s), 7.50 (1H, d, *J* = 8.4 Hz), 4.77 (1H, dd, *J* = 7.5 and 4.8 Hz), 3.92 (3H, s), 3.15 (1H, dd, *J* = 14.6 and 4.8 Hz), 3.00 (1H, dd, *J* = 14.6 and 7.5 Hz), 2.53–2.71 (2H, m), 2.20–2.32 (1H, m), 2.12–2.18 (1H, m), 2.10 (3H, s); ^{13}C NMR (CD_3OD) δ 175.4, 169.6, 167.2, 150.9, 131.9, 130.9, 122.1, 121.1, 111.0, 56.8, 56.6, 53.3, 31.8, 31.6, 26.7, 15.2; *m*/*z* (FAB) for the free amine 402 (M + 1).

Cysteinyl-4-amino-2-phenylbenzoyl methionine trifluoroacetate (5). 4-Nitro-2-phenylbenzoic acid (0.30 g, 1.23 mmol) was coupled with (L)-methionine methyl ester (0.27 g, 1.35 mmol) in the presence of 1.0 equiv of EDCI and HOBT. The crude product was recrystallized from ethyl acetate and hexane (86%). The nitro group was reduced with $SnCl_2$ dihydrate (5.0 equiv) in ethyl acetate to give the free amine as a yellow solid. This amine was coupled with *N*-Boc-*S*-trityl-(L)-cysteine using a mixed anhydride method. Mp 100–103°C. ^{1}H NMR ($CDCl_3$) δ 8.69 (1H, s), 7.58 (1H, d, *J* = 8.2 Hz), 7.50 (1H, s), 7.37–7.44 (11H, m), 7.18–7.30 (10H, m), 6.10 (1H, d, *J* = 6.6 Hz), 4.90 (1H, d, *J* = 7.4 Hz), 4.58–4.63 (1H, m), 4.02 (1H, br s), 3.64 (3H, s), 2.72 (1H, dd, *J* = 12.7 and 5.5 Hz), 2.63 (1H, dd, *J* = 12.7 and 6.9 Hz), 2.05 (2H, t, *J* = 7.6 Hz), 2.00 (3H, s), 1.84 1.95 (1H, m), 1.61 1.78 (1H, m), 1.41 (9H, s); ^{13}C NMR ($CDCl_3$) δ 171.8, 169.1, 168.5, 156.0, 130.1, 129.8, 129.4, 128.7, 128.6, 128.0, 127.9, 126.9, 120.9, 118.2, 80.8, 67.3, 54.2, 52.3, 51.8, 33.0, 31.2, 29.4, 28.2, 15.2. This fully protected compound was deprotected using the same method described for compound **2**. The final product was purified by reverse phase preparative HPLC. Mp 150–154°C (decomp). $[\alpha]_D^{25}$ +21.5 (*c* 0.7, H_2O/CH_3OH). ^{1}H NMR (CD_3OD) δ 7.65–7.67 (2H, m), 7.52–7.55 (1H, m), 7.37–7.41 (5H, m), 4.46 (1H, br s), 3.15 (1H, dd, *J* = 14.7 and 4.8 Hz), 3.03 (1H, dd, *J* = 14.7 and 7.3 Hz), 2.16–2.18 (1H, m), 2.00–2.10 (5H, m), 1.62–1.79 (1H, m); ^{13}C NMR (CD_3OD) δ 172.5, 167.8, 167.0, 142.5, 141.4, 140.5, 133.5, 130.1, 129.7, 129.6, 129.0, 122.3, 119.4, 57.0, 32.8, 31.8, 26.3, 15.0. Anal. calcd for $C_{23}H_{26}F_3N_3O_6S_2$: C 49.20, H 4.67, N 7.48; found C 49.14, H 4.71, N 7.42.

Cysteinyl-4-amino-2-(3,5-dimethylphenyl)benzoyl methionine trifluoroacetate (6). This compound was prepared in exactly the same way described for the preparation of compound **5** except 4-nitro-2-(3,5-dimethylphenyl)-benzoic acid was used. Final product was purified by reverse phase preparative HPLC. Mp 135–138°C. ^{1}H NMR (CD_3OD) δ 7.66 (2H, m), 7.53 (1H, d, *J* = 8.0 Hz), 7.02 (3H, s), 4.45–4.48 (1H, m), 4.15 (1H, dd, *J* = 7.3 and 4.9 Hz), 3.17 (1H, dd, *J* = 14.6 and 4.9 Hz), 3.05 (1H, dd, *J* = 14.6 and 7.3 Hz), 2.33 (6H, s), 2.00–2.17 (6H, m), 1.76–1.84 (1H, m); ^{13}C NMR (CD_3OD) δ 174.8, 172.6, 166.9, 142.9, 141.3, 140.4, 139.3, 133.4, 130.6, 130.1, 127.5, 122.3, 119.3, 57.0, 53.0, 31.7, 31.0, 26.3, 21.5, 15.0.

Cysteinyl-4-amino-1-naphthoyl methionine trifluoroacetate (7). 4-Amino-1-naphthalenecarbonitrile (1.50 g, 8.91 mmol) was dissolved into a 50% KOH solution (18 mL). The heterogeneous solution was heated at reflux for 2–3 days. Once the solution became homogeneous and TLC showed no more starting material, the deep red solution was cooled and acidified with concentrated HCl. The solid was filtered and treated with activated carbon to obtain 1.51 g of 4-amino-1-naphthoic acid (91%). Mp 169–171°C. ^{1}H NMR (CD_3OD) δ 9.09 (1H, d, *J* = 8.5 Hz), 8.13 (1H, d, *J* = 8.2 Hz), 8.03 (1H, d, *J* = 8.5 Hz), 7.48–7.54 (1H, m), 7.38–7.43 (1H, m), 6.69 (1H, d, *J* = 8.2 Hz); ^{13}C NMR (CD_3OD) δ 171.4, 151.3, 135.0, 128.5, 127.4, 125.2, 123.9, 123.0, 114.6, 107.4; HRMS calcd for $C_{11}H_7NO_2$ 187.0633; found 187.0642. The amino group of this product was protected with Boc anhydride and the carboxylic acid was coupled with (L)-methionine methyl ester as described before. The Boc group was deprotected and the free amine was coupled with *N*-Boc-*S*-trityl-(L)-cysteine to give a fully protected compound. This compound was deprotected as described before to give the final product. Mp 121–125°C. $[\alpha]_D^{25}$ +2.4 (*c* 0.8, H_2O). ^{1}H NMR

(CD_3OD) δ 8.98 (1H, d, J = 7.7 Hz), 8.29–8.32 (1H, m), 8.08–8.11 (1H, m), 7.70 (1H, d, J = 7.7 Hz), 7.64 (1H, d, J = 7.7 Hz), 7.57–7.61 (1H, m), 4.84–4.89 (1H, m), 4.42 (1H, m), 3.16–3.33 (2H, m), 2.59–2.74 (2H, m), 2.22–2.36 (1H, m), 2.03–2.13 (4H, m); ^{13}C NMR (CD_3OD) δ 175.2, 172.4, 168.1, 135.1, 134.1, 132.3, 129.5, 128.3, 128.0, 127.0, 126.1, 126.4, 123.4, 122.5, 56.7, 53.2, 31.6, 31.5, 26.4, 15.2. Anal. calcd for $C_{21}H_{23}F_3N_3O_6S_2$: C 47.19, H 4.34, N 7.86; found C 47.53, H 4.56, N 7.59.

4-Nitro-2-phenylbenzoic acid (18). 4-Nitro-2-bromotoluene (6.48 g, 30 mmol) and phenyl boronic acid (3.84 g, 31.5 mmol) was dissolved in 70 mL of acetone. To this mixture was added 85 mL of water, potassium carbonate hydrate (12.4 g, 75 mmol, 2.5 equiv) and $Pd(OAc)_2$ (0.33 g, 5% equiv). The mixture was refluxed for 10 h and then cooled. The deep black solution was extracted with ether and 3 N HCl. The ether fraction was passed through a layer of Celite. After evaporating solvents, the residue was dried and then recrystallized from methanol to give flake crystals (4.60 g, 88%). Mp 77–78°C. ^{1}H NMR ($CDCl_3$) δ 8.09–8.11 (m, 2H, aryl), 7.40–7.49 (m, 4H, aryl), 7.30–7.33 (m, 2H, aryl), 2.37 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 146.1, 143.5, 143.0, 139.4, 131.1, 128.8, 128.4, 127.8, 124.5, 122.0, 20.7. Anal. calcd for $C_{13}H_{11}NO_2$: C 73.24, H 5.16, N 6.57; found C 73.11, H 5.15, N 6.53; LRMS (EI) 213. This compound (3.20 g, 15 mmol) was dissolved in 15 mL of pyridine and 30 mL of water. The mixture was heated to 90°C and then $KMnO_4$ (14.2 g, 90 mmol) was added in portions. The mixture was refluxed for 5 h. The black solid was filtered off and the filtrate was acidified with 6 N HCl. The mixture was cooled in an ice bath and the white precipitate was collected (3.1 g, 85%). Mp 175–176°C. ^{1}H NMR ($CDCl_3$) δ 8.25–8.33 (m, 2H, aryl), 8.08 (d, J = 8.9 Hz, 1H, aryl), 7.41–7.51 (m, 3H, aryl), 7.31–7.39 (m, 2H, aryl); ^{13}C NMR (CD_3OD) δ 170.6, 150.2, 144.4, 140.4, 139.3, 131.6, 129.5, 129.4, 129.3, 126.1, 123.0; LRMS (EI) 243. Anal. calcd for $C_{13}H_9O_4N$: C 64.19, H 3.70, N 5.76; found C 64.23, H 3.74, N 5.70.

***N*-[4-Nitro-2-phenylbenzoyl]-methionine methyl ester (19).** Compound **18** (2.43 g, 10 mmol) was suspended in 50 mL of methylene chloride. To this solution in an ice bath was added (L)-methionine methyl ester hydrochloride (2.0 g, 10 mmol), triethylamine (1.38 mL, 10 mmol), EDCI (2.01 g, 10.5 mmol) and HOBT (1.35 g, 10 mmol). The mixture was stirred at rt for 10 h and then extracted with methylene chloride and 1 N HCl. The organic layer was washed with concentrated sodium bicarbonate and dried. After evaporating solvents, the residue was recrystallized from ethyl acetate and hexane to give **19** (3.22 g, 83%). Mp 104–105°C. $[\alpha]_D^{25}$ –5.2 (c 0.9, EtOAc). ^{1}H NMR ($CDCl_3$) δ 8.24–8.28 (m, 2H, aryl), 7.85 (d, J = 8.9 Hz, 1H), 7.43–7.52 (m, 5H), 6.01 (d, J = 7.5 Hz, 1H, amide), 4.69 (ddd, J = 4.5, 7.4 Hz, 1H, Met α H), 3.68 (s, 3H, OCH_3), 2.05 (t, J = 7.2 Hz, 2H, CH_2S), 1.98 (s, 3H, SCH_3), 1.88–1.96 (m, 1H, Met CH_2), 1.72–1.81 (m, 1H, Met CH_2); ^{13}C NMR ($CDCl_3$) δ 171.2, 167.3, 148.1, 141.0, 140.6, 137.5, 129.5, 128.7, 128.6, 128.3, 124.7, 121.9, 52.3, 51.5, 30.7, 29.2, 15.0. Anal. calcd for $C_{19}H_{20}O_5N_2S$: C 58.76, H 5.15, N 7.21, S 8.24; found C 58.79, H 5.22, N 7.26, S 8.42.

***N*-[4-[*N*-[2(*R*)-(*tert*-Butoxycarbonyl)amino-3-(triphenylmethyl)thio]propyl]amino-2-phenylbenzoyl]-methionine methyl ester (20).** Compound **19** (3.04 g, 7.83 mmol) was dissolved in 100 mL of ethyl acetate. Stannous chloride hydrate (8.84 g, 39 mmol) was added and the mixture was refluxed for 2 h. This solution was poured into 200 mL of concentrated sodium bicarbonate and extracted with ethyl acetate. After evaporating solvents, the residue was dissolved in 10 mL of methylene chloride and 15 mL of 3 N HCl in ether was added. Solvents were evaporated and a hydrochloride salt was obtained (2.97 g, 96%). Mp 229–230°C. ^{1}H NMR (CD_3OD) δ 7.65 (d, J = 8.1 Hz, 1H, aryl), 7.39–7.46 (m, 7H, aryl), 4.53 (dd, J = 4.3, 9.5 Hz, 1H, Met α H), 3.69 (s, 3H, OCH_3), 2.15–2.23 (m, 1H, Met CH_2), 2.00 (s, 3H, SCH_3), 1.93–2.11 (m, 2H, CH_2S), 1.74–1.83 (m, 1H, Met CH_2); ^{13}C NMR (CD_3OD) δ 173.4, 171.7, 143.4, 140.1, 137.4, 134.0, 130.9, 129.7, 129.4, 125.5, 122.7, 53.0, 52.9, 31.3, 30.9, 15.1 (expect 10 aromatic C, observed 9).

The above hydrochloride salt (1.27 g, 3.22 mmol) was dissolved in 10 mL of methanol and then *N*-Boc-*S*-tritylcysteinal (1.0 equiv, according to ^{1}H NMR determination of aldehyde percentage) was added. After stirring for 10 min, sodium cyanoborohydride (305 mg, 1.5 equiv) was added and the mixture was stirred for 12 h at rt. After evaporating solvents, the residue was extracted with ethyl acetate and concentrated sodium bicarbonate. Solvents were evaporated and the residue was purified by flash column chromatography (1:1 = hexane:ethyl acetate, silica) to give **20** as a fluffy foam (1.84 g, 74%). Mp 82–83°C (decomp). $[\alpha]_D^{25}$ 0.3 (c 1.6, EtOAc). ^{1}H NMR ($CDCl_3$) δ 7.65 (d, J = 8.6 Hz, 1H, aryl), 7.34–7.42 (m, 11H, aryl), 7.18–7.29 (m, 9H, aryl), 6.52 (dd, J = 2.3, 8.1 Hz, 1H, aryl), 6.34 (d, J = 2.3 Hz, 1H, aryl), 5.65 (d, J = 7.7 Hz, 1H, amide), 4.64 (ddd, J = 4.5, 9.3 Hz, 1H, Met α H), 4.55 (d, J = 8.1 Hz, 1H, Boc amide), 4.19 (br t, 1H, NH), 3.78 (br m, 1H, Cys α H), 3.64 (s, 3H, OCH_3), 3.09 (t, J = 6.1 Hz, 2H, CH_2N), 2.44 (m, 2H, CH_2SCPh_3) 2.04–2.10 (m, 2H, CH_2SCH_3), 2.00 (s, 3H, SCH_3), 1.81–1.90 (m, 1H, Met CH_2), 1.60–1.70 (m, 1H, Met CH_2), 1.41 (s, 9H, Boc); ^{13}C NMR ($CDCl_3$) δ 172.0, 168.3, 155.7, 149.4, 144.3, 141.6, 141.1, 131.3, 129.5, 128.7, 128.5, 127.9, 127.7, 126.8, 122.6, 113.6, 111.3, 79.8, 67.1, 52.2, 51.7, 49.5, 47.2, 34.3, 31.6, 29.4, 28.2, 15.2. Anal. calcd for $C_{46}H_{51}O_5N_3S_2 \cdot 0.4CH_2Cl_2$: C 67.65, H 6.29, N 5.10, S 7.77; found C 67.68, H 6.40, N 5.18, S 7.69.

***N*-[4-[2(*R*)-Amino-3-mercaptopropyl]amino-2-phenylbenzoyl]-methionine trifluoroacetate (8, FTI-276).** The *N*-Boc-*S*-trityl protected peptide methyl ester **20** (500 mg, 0.63 mmol) was hydrolyzed with 2.0 equiv of 0.5 N lithium hydroxide at 0°C for 1 h. The obtained free carboxylic acid was treated with trifluoroacetic acid (2 mL) in methylene chloride (1 mL). Triethylsilane was added dropwise until the deep yellow color disappeared. The mixture was maintained at rt for 1 h. After evaporating solvents, the residue was dried and washed with dry ether. The solid was purified by reverse phase preparative HPLC (Waters 25×10 cm, C-18 column, 220 nm UV detector, flow rate 15 mL/min, linear gradient from 5% acetonitrile and 95% water with 0.1% TFA to 60% acetonitrile in 40 min) to give **FTI-276** as a

TFA salt (270 mg, 78%). Mp 90°C (decomp). $[\alpha]_D^{25}$ −13.6 (*c* 0.5, MeOH). ^{1}H NMR (CD_3OD) δ 7.44 (d, *J* = 8.4 Hz, 1H, aryl), 7.30–7.39 (m, 5H, aryl), 6.75 (d, *J* = 8.4 Hz, 1H, aryl), 6.67 (s, 1H, aryl), 4.45 (dd, *J* = 4.2, 5.1 Hz, 1H, Met α H), 3.42–3.58 (m, 3H, CH_2N, Cys α H), 2.90 (dd, *J* = 4.3, 14.5 Hz, 1H, CH_2SH), 2.81 (dd, *J* = 5.5, 14.5 Hz, 1H, CH_2SH), 2.17–2.23 (m, 1H, CH_2SCH_3), 2.09–2.15 (m, 1H, CH_2SCH_3), 2.00 (s, 3H, SCH_3), 1.90–1.99 (m, 1H, Met CH_2), 1.71–1.81 (m, 1H, Met CH_2); ^{13}C NMR (CD_3OD) δ 176.4, 173.5, 150.4, 143.0, 141.5, 131.0, 129.7, 129.4, 128.9, 124.6, 115.0, 112.3, 53.3, 49.6, 44.4, 30.8, 30.1, 24.9, 14.8. Anal. calcd for $C_{21}H_{27}O_3N_3S_2 \cdot CF_3COOH \cdot H_2O$: C 48.85, H 5.31, N 7.43, S 11.32; found C 48.90, H 5.35, N 7.41, S 11.38.

***N*-[4-[2(*R*)-Amino-3-mercaptopropyl]amino-2-phenylbenzoyl]-methionine methyl ester hydrochloride (14, FTI-277).** The above *N*-Boc-*S*-trityl protected peptide methyl ester **20** (900 mg, 1.14 mmol) was dissolved in 5 mL of methanol. To this mixture was added a solution of mercuric chloride (774 mg, 2.85 mmol) in 5 mL of methanol. The mixture was refluxed for 20 min. The clear solution was decanted and the precipitate was collected and dried. This solid was suspended in 10 mL of methanol and treated with gaseous hydrogen sulfide. After the removal of black solid, the clear solution was evaporated to dryness. The residue was then dissolved in 2 mL of methylene chloride and 5 mL of 3 N hydrogen chloride in ether was added. The white solid was collected and dried to give the pure product **FTI-277** (476 mg, 81%). Mp 125°C (foaming). $[\alpha]_D^{25}$ −12.0 (*c* 0.6, MeOH). ^{1}H NMR (CD_3OD) δ 7.42 (d, *J* = 8.4 Hz, 1H, aryl), 7.30–7.38 (m, 5H, aryl), 6.77 (d, *J* = 8.4 Hz, 1H, aryl), 6.71 (s, 1H, aryl), 4.48 (dd, 4.2 and 5.1 Hz, 1H, Met α H)), 3.68 (s, 3H, OCH_3), 3.44–3.58 (m, 3H, CH_2N, Cys α H), 2.90–2.95 (dd, *J* = 4.1, 14.5 Hz, 1H, CH_2SH), 2.79–2.85 (dd, *J* = 4.7, 14.5 Hz, 1H, CH_2SH), 2.18–2.22 (m, 1H, CH_2SCH_3), 2.03–2.16 (m, 1H, CH_2SCH_3), 2.00 (s, 3H, SCH_3), 1.91–1.97 (m, 1H, Met CH_2), 1.73–1.82 (m, 1H, Met CH_2); ^{13}C NMR (CD_3OD) δ 173.7, 173.4, 150.7, 143.5, 142.3, 131.2, 129.8, 129.5, 128.6, 125.6, 115.6, 112.2, 53.7, 53.2, 52.8, 45.0, 31.4, 30.9, 25.3, 15.0; LRMS (EI) for $C_{22}H_{29}O_3N_3S_2$ 447 (M^+, 15), 372 (25), 285 (35), 209 (100). Anal. calcd for $C_{22}H_{29}O_3N_3S_2 \cdot 1.6HCl$: C 52.32, H 6.05, N 8.31, S 12.63; found C 52.08, H 6.25, N 8.11, S 12.30.

Methyl 4-nitro-2-bromobenzoate (21). 4-Nitro-2-bromotoluene was oxidized by $KMnO_4$ using the same method as for the preparation of compound **18** to give 4-nitro-2-bromobenzoic acid (75%). Mp 175–176°C. This carboxylic acid (4.92 g, 20 mmol) was mixed with 50 mL of methanol and 4 mL of thionyl chloride (54 mmol) and the mixture was refluxed for 3 h. After cooling in an ice bath, white crystals were filtered and washed with 5 mL of cold methanol to give 4-nitro-2-bromobenzoic acid methyl ester (4.67 g, 90%). Mp 81–82°C. ^{1}H NMR ($CDCl_3$) δ 8.52 (s, 1H), 8.23 (d, *J* = 8.6 Hz, 1H), 7.92 (d, *J* = 8.6 Hz, 1H), 3.99 (s, 3H).

4-Nitro-2-(2-thienyl)benzoic acid methyl ester (22). 2-Thienylboronic acid was prepared by reacting Grignard reagent of 2-thienyl bromide with trimethyl borate. The crude product was recrystallized from water to give a pure 2-thienylboronic acid (65%). Mp 146–147°C. ^{1}H NMR (acetone-d_6) δ 7.67 (m, 2H), 7.31 (br s, 2H), 7.15 (t, *J* = 3.7 Hz, 1H).

Compound **21** (1.30 g, 5.0 mmol) and 2-thienylboronic acid (1.27 g, 10 mmol) were dissolved in 30 mL of dry DMF. Trisodium phosphate (3.0 equiv) and $Pd(PPh_3)_4$ (300 mg, 5% equiv) were added and the mixture was heated at 100°C for 12 h. After work up with ether and 2 N HCl, the ether layer was dried and evaporated. The residue was recrystallized from methanol to give **22** as pale-brown crystals (0.75 g, 57%). Mp 97–98°C. ^{1}H NMR ($CDCl_3$) δ 8.35 (s, 1H), 8.22 (d, *J* = 8.4 Hz, 1H), 7.83 (d, *J* = 8.6 Hz, 1H), 7.45 (d, *J* = 5.0 Hz, 1H), 7.09–7.14 (m, 2H), 3.80 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 169.6, 150.8, 141.2, 139.1, 137.6, 132.3, 129.7, 129.4, 127.5, 124.1, 54.8 (expect 10 aromatic C, obsd 9).

4-Nitro-2-(1-naphthyl)benzoic acid methyl ester (23). 1-Naphthylboronic acid was prepared with the same method described in **22**. The crude boronic acid was used directly without purification. This boronic acid (2.40 g, 13.95 mmol) was coupled with 4-nitro-2-bromobenzoic acid methyl ester (1.95 g, 7.5 mmol) in 50 mL of DMF at 100°C in the presence of Na_3PO_4 (22.4 mmol, 3.0 equiv) and $Pd(PPh_3)_4$ (430 mg, 5% equiv). The crude product was purified by flash column chromatography (hexane:ethyl acetate, 5:1) to give **23** as an oil (1.69 g, 73%). ^{1}H NMR ($CDCl_3$) δ 8.34 (d, *J* = 8.5 Hz, 1H), 8.28 (s, 1H), 8.14 (d, *J* = 8.5 Hz, 1H), 7.92 (m, 2H), 7.47–7.56 (m, 2H), 7.41 (d, *J* = 3.8 Hz, 2H), 7.34 (d, *J* = 6.8 Hz, 1H), 3.40 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 166.4, 149.2, 142.7, 137.2, 136.8, 133.2, 131.3, 131.0, 128.6, 128.4, 126.6, 126.2, 126.0, 125.0, 124.7, 122.3, 52.3 (expect 16 aromatic C, observed 15). Anal. calcd for $C_{18}H_{13}O_4N$: C 70.35, H 4.23, N 4.56; found, C 70.21, H 4.14, N 4.37.

***N*-[4-Nitro-2-(2-thienyl)benzoyl]-methionine methyl ester (24).** Compound **22** was suspended in a mixture of 1 N NaOH (2.0 equiv) in methanol and the solution was refluxed for 2 h. After acidification with 2 N HCl, a carboxylic acid derivative was obtained (100%). This carboxylic acid was coupled with L-methionine methyl ester using EDCI and HOBT as coupling reagents as described before. After flash column chromatography purification, **24** was obtained (90%). Mp 94–95°C. ^{1}H NMR ($CDCl_3$) δ 8.29 (s, 1H, aryl), 8.16 (d, *J* = 8.4 Hz, 1H, aryl), 7.70 (d, *J* = 8.4 Hz, 1H, aryl), 7.45 (d, *J* = 5.3 Hz, 1H, thienyl), 7.24 (d, *J* = 3.7 Hz, 1H, thienyl), 7.11 (t, *J* = 3.7 Hz, 1H, thienyl), 6.59 (d, *J* = 7.8 Hz, 1H, amide), 4.76 (ddd, *J* = 5.2, 7.8 Hz, 1H, Met α H), 3.72 (s, 3H, OCH_3), 2.25 (t, *J* = 7.5 Hz, 2H, CH_2S), 2.00–2.11 (m, 1H, Met CH_2), 2.04 (s, 3H, SCH_3), 1.82–1.94 (m, 1H, Met CH_2); ^{13}C NMR ($CDCl_3$) δ 171.4, 167.2, 148.1, 140.8, 138.2, 133.6, 129.5, 128.1, 128.0, 127.7, 125.1, 122.3, 52.5, 51.8, 30.8, 29.4, 15.2. Anal. calcd for $C_{17}H_{18}O_5N_2S_2$: C 51.77, H 4.56, N 7.10; found C 51.84, H 4.61, N 7.08.

***N*-[4-Nitro-2-(1-naphthyl)benzoyl]-methionine methyl ester (25).** Compound **23** was first hydrolyzed as described

for compound **24** to give a free carboxylic acid (98%). Mp 197–198°C. ^{1}H NMR ($CDCl_3$) δ 8.33 (d, J = 8.6 Hz, 1H), 8.23 (s, 1H), 8.17 (d, J = 8.6 Hz, 1H), 7.88 (d, J = 8.2 Hz, 2H), 7.46–7.52 (m, 2H), 7.38–7.42 (m, 2H), 7.33 (d, J = 7.0 Hz, 1H); ^{13}C NMR (CD_3COCD_3) δ 167.2, 150.1, 143.1, 139.0, 138.2, 134.4, 132.5, 132.1, 129.2, 127.3, 126.7, 125.8, 125.9, 123.3 (expect 16 aromatic C, obsd 13); HRMS calcd for $C_{17}H_{11}O_4N$ 293.0688, obsd 293.0686.

The coupling of 4-nitro-2-(1-naphthyl)benzoic acid with L-methionine methyl ester in the presence of EDCI and HOBT provided the desired product (yield 95%). TLC of the product showed a single spot, but ^{1}H NMR showed the presence of diastereomers caused by the restricted C–C bond rotation. Variable temperature ^{1}H NMR spectrum indicated that peaks at 8.14, 8.00, 3.56, 3.51, 1.83 and 1.74 became broad as temperature was increased and coalescence temperatures were ~373 K for methionine side chain methyl group, 383 K for methyl ester group and 393 K for aromatic hydrogen. Mp 114–115°C. $[\alpha]_D^{25}$ +37.4 (*c* 0.7, EtOAc). ^{1}H NMR ($CDCl_3$) δ 8.33–8.38 (m, 1H), 8.26 (m, 1H), 8.14 (d, J = 8.5 Hz, 0.5H), 8.00 (d, J = 8.5 Hz, 0.5H), 7.94–7.98 (m, 2H), 7.42–7.65 (m, 5H), 5.98 (t, 1H, amide), 4.42 (m, 1H, Met α H), 3.56 (s, 1.5H, OCH_3), 3.51 (s, 1.5H, OCH_3), 1.83 (s, 1.5H, SCH_3), 1.74 (s, 1.5H, SCH_3), 1.56–1.64 (m, 1H, Met CH_2), 1.33–1.45 (m, 2H, CH_2S), 1.09–1.14 (m, 1H, Met CH_2); ^{13}C NMR ($CDCl_3$) δ 171.4, 171.1, 166.2, 165.5, 148.8, 148.3, 141.5, 140.6, 139.4, 135.8, 135.1, 133.5, 131.3, 130.9, 130.4, 129.6, 129,4, 128.8, 128.6, 127.4, 127.3, 126.7, 126.6, 126.3, 125.5, 124.7, 124.4, 122.9, 52.4, 51.5, 31.0, 28.8, 28.6, 15.0. Anal. calcd for $C_{23}H_{22}O_5N_2S$: C 63.01, H 5.02, N 6.39, S 7.31; found C 62.91, H 5.12, N 6.40, S 7.24.

***N*[4-[*N*-[2(*R*)-(*tert*-Butoxycarbonyl)amino-3-(triphenylmethyl)thio]propyl]amino-2-(2-thienyl)benzoyl]-methionine methyl ester (26).** Compound **24** was first reduced to its corresponding amine and this amine was coupled with *N*-Boc-*S*-trityl-L-cysteinal using the same method as for the preparation of compound **20**. After purification by flash column chromatography (ethyl acetate:hexane, 1:1), pure **26** was obtained (46%, two steps). Mp 79–80°C (decomp). $[\alpha]_D^{25}$ +0.9 (*c* 0.85, EtOAc). ^{1}H NMR ($CDCl_3$) δ 7.60 (d, J = 8.5 Hz, 1H, aryl), 7.39–7.42 (m, 6H, trityl), 7.30 (d, J = 4.8 Hz, 1H, thienyl), 7.17–7.29 (m, 9H, trityl), 7.07 (m, 2H, thienyl), 6.50 (d, J = 8.5 Hz, 1H, aryl), 6.43 (s, 1H, aryl), 6.04 (d, J = 5.8 Hz, 1H, amide), 4.70 (ddd, J = 5.4, 5.8 Hz, 1H, Met α h), 4.63 (br d, J = 7.6 Hz, Boc amide), 4.22 (br t, 1H, NH), 3.75 (br, 1H, Cys α h), 3.66 (s, 3H, OCH_3), 3.06 (t, J = 5.7 Hz, 2H, CH_2N), 2.45 (br d, 2H, CH_2SCPh_3), 2.26 (t, J = 7.6 Hz, 2H, CH_2SCH_3), 2.00 (s, 3H, SCH_3), 1.96 2.02 (m, 1H, Met CH_2), 1.73–1.83 (m, 1H, Met CH2), 1.41 (s, 9H, Boc); ^{13}C NMR ($CDCl_3$) δ 172.0, 168.2, 155.6, 149.2, 144.3, 141.9, 133.5, 131.1, 129.4, 127.9, 127.4, 126.9, 128.8, 126.1, 123.4, 114.3, 111.7, 79.7, 67.0, 52.3, 51.7, 49.4, 47.0, 34.2, 31.6, 29.5, 28.2, 15.2; Anal. calcd for $C_{44}H_{49}O_5N_3S_3 \cdot 0 \cdot 4C_6H_{14}$: C 67.13, H 6.58, N 5.06, S 11.57; Found C 67.00, H 6.76, N 5.06, S 11.47.

***N*-[4-[*N*-[2(*R*)-(*tert*-Butoxycarbonyl)amino-3-(triphenylmethyl)thio]propyl]amino-2-(1-naphthyl)benzoyl]-methionine methyl ester (27).** Compound **25** was reduced to the corresponding amine by stannous chloride as described for compound **20**. This amine was coupled with *N*-Boc-*S*-trityl-L-cysteinal under reductive amination conditions. After column chromatography (ethyl acetate:hexane, 1:1), compound **27** was obtained (40%, two steps). TLC showed a single spot, but ^{1}H NMR showed diastereomers caused by the restricted rotation of C–C bond. Mp 91–92°C. ^{1}H NMR ($CDCl_3$) δ 7.84–7.96 (m, 3H, aryl), 7.49–7.66 (m, 4H, aryl), 7.37–7.43 (m, 7H, aryl), 7.14–7.27 (m, 9H, aryl), 6.60–6.63 (d, J = 8.6 Hz, 1H, aryl), 6.33 (m, 1H, aryl), 5.67 (d, J = 7.8 Hz, 0.6H, amide), 5.60 (d, J = 7.8 Hz, 0.4H, amide), 4.56 (br d, J = 6.2 Hz, 1H, Boc amide), 4.35–4.44 (m, 1H, Met α H), 4.30 (br, 1H, NH), 3.78 (br m, 1H, Cys α H), 3.55 (s, 1.9H, OCH_3), 3.38 (s, 1.1H, OCH_3), 3.06 (t, J = 5.8 Hz, 2H, CH_2N), 2.44 (m, 2H, CH_2SCPh_3), 1.90 (s, 1H, SCH_3), 1.79 (s, 2H, SCH_3), 1.57–1.68 (m, 0.5H, Met CH_2), 1.36–1.45 (m, 10H, Boc, Met CH_2), 1.23–1.32 (m, 2H, CH_2SCH_3), 0.94–0.98 (m, 0.5H, Met CH_2). Anal. calcd for $C_{50}H_{53}O_5N_3S_2 \cdot 0.5CH_3COOC_2H_5$: C 70.67, H 6.45, N 4.75; found C 70.63, H 6.47, N 4.86.

***N*-[4-[2(*R*)-Amino-3-mercaptopropyl]amino-2-(2-thienyl)-benzoyl]-methionine hydrochloride (9).** The fully protected compound **26** was first hydrolyzed by 1 equiv of 0.5 N LiOH and then deprotected by TFA in the presence of triethylsilane. The TFA salt was dried and washed with ether. The crude product (80% purity according HPLC analysis) was purified by preparative HPLC as described for the preparation of **FTI-276** (60%). The highly hydroscopic TFA salt was dissolved in 1 N HCl and the aqueous solution was lyophilized to give compound **9** as a hydrochloride salt. Mp 110°C (foaming). $[\alpha]_D^{25}$ −15.5 (*c* 0.2, MeOH). ^{1}H NMR (CD_3OD) δ 7.40 (m, 2H, phenyl and thienyl), 7.15 (d, J = 3.3 Hz, 1H, thienyl), 7.03 (t, J = 4.2 Hz, 1H, thienyl), 6.77 (s, 1H, phenyl), 6.71 (d, J = 8.4 Hz, 1H, phenyl), 4.55 (dd, J = 4.3, 4.9 Hz, 1H, Met α H) 3.39–3.57 (m, 3H, CH_2N, Cys α H), 2.92 (dd, J = 4.5, 14.6 Hz, 1H, CH_2SH), 2.80 (dd, J = 5.7, 14.6 Hz, 1H, CH_2SH), 2.20–2.38 (m, 2H, CH_2SCH_3), 2.03–2.10 (m, 1H, Met CH_2), 2.00 (s, 3H, SCH_3), 1.81–1.92 (m, 1H, Met CH_2); ^{13}C NMR (CD_3OD) δ 175.0, 173.0, 150.7, 143.2, 135.2, 131.1, 128.6, 127.7, 127.0, 126.3, 115.4, 112.5, 53.8, 53.1, 44.8, 31.7, 31.1, 25.3, 15.1; LRMS (EI) for $C_{19}H_{25}O_3N_3S_3$ 439 (M^+, 95), 202 (100); HRMS (EI) calcd 439.1058, obsd 439.1063.

***N*-[4-[2(*R*)-Amino-3-mercaptopropyl]amino-2-(1-naphthyl)-benzoyl]-methionine trifluoroacetate (10).** The fully protected compound **27** was deprotected using the same method as for the preparation of compound **9**. Final product was isolated from preparative HPLC as described before (51%). The purity of the final product was over 99% as indicated from HPLC analysis. Mp 89–90°C (decomp). $[\alpha]_D^{25}$ −41.2 (*c* 0.4, MeOH). ^{1}H NMR showed complicated diastereomers caused by the restricted rotation of aryl–aryl bond. ^{1}H NMR (CD_3OD) δ 7.86–7.94 (m, 2H, naphthyl), 7.73 (d, J = 8.6 Hz, 0.6H, phenyl), 7.35–7.67 (m, 5.4H, naphthyl,

phenyl), 6.83–6.88 (m, 1H, phenyl), 6.63–6.67 (m, 1H, phenyl), 4.17–4.23 (m, 1H, Met α H), 3.41–3.58 (m, 3H, CH_2N, Cys α H), 2.91 (dd, $J=4.2$, 14.5 Hz, 1H, CH_2SH), 2.80 (dd, $J=5.3$, 14.5 Hz, 1H, CH_2SH), 1.82 (s, 1.4H, SCH_3), 1.80 (s, 1.6H, SCH_3), 1.65–1.77 (m, 1H, Met CH_2), 1.41–1.52 (m, 2H, CH_2SCH_3), 1.09–1.32 (m, 1H, Met CH_2) Anal. calcd for $C_{25}H_{31}O_3N_3S_2{\cdot}CF_3\ COOH{\cdot}H_2O$: C 52.68, H 5.20, N 6.83; found C 52.73, H 5.19, N 6.78.

Methyl 4-nitro-2-(2-pyridyl)benzoate (28). Methyl-2-bromo-4-nitro benzoate (3 g, 12.41 mmol), $(Ph_3P)_2PdCl_2$ (435 mg, 0.62 mmol) and CuI (236 mg, 1.24 mmol) were dissolved in anhydrous toluene (10 mL) and LiCl (2.63 g, 62.04 mmol) and 2-trimethylstannylpyridine in anhydrous toluene (5 mL) were added. The mixture was refluxed for 13 h and then cooled and filtered. Evaporation in vacuum and flash chromatography (from hexane:ether, 7:3 to ether) afforded 2 g (62% yield) of pure compound **28**. ^{1}H NMR (Cl_3CD) δ 3.69 (s, 3H), 7.29 (dd, $J=4.6$, 7.2 Hz, 1H), 7.56 (d, $J=7.8$ Hz, 1H), 7.79 (m, 1H), 7.85 (d, $J=8.4$ Hz, 1H), 8.24 (dd, $J=2.2$, 8.4 Hz, 1H), 8.40 (d, $J=2.2$ Hz, 1H), 8.62 (d, $J=4.6$ Hz, 1H); ^{13}C NMR (Cl_3CD) δ 52.49, 122.42, 122.99, 123.06, 124.27, 130.56, 136.84, 137.42, 141.73, 148.82, 149.30, 155.60, 167.90.

Methyl 4-nitro-2-(3-pyridyl)benzoate (29). This compound was prepared with the same method described for compound **28** (53%). ^{1}H NMR (Cl_3CD) δ 3.69 (s, 3H), 7.38 (dd, $J=4.9$, 7.8 Hz, 1H), 7.67 (d, $J=7.8$ Hz, 1H), 8.05 (d, $J=8.4$ Hz, 1H), 8.19 (d, $J=2.0$ Hz, 1H), 8.26 (dd, $J=2.0$, 8.4 Hz, 1H), 8.54 (m, 1H), 8.64 (m, 1H); ^{13}C NMR (Cl_3CD) δ 52.71, 122.75, 123.12, 125.60, 131.56, 134.87, 135.62 135.98, 140.46, 148.36, 149.16, 149.73, 166.42.

Methyl 4-nitro-2-(4-pyridyl)benzoate (30). This compound was prepared with the same method described for compound **28** (45%). ^{1}H NMR (Cl_3CD) δ 3.58 (s, 3H), 7.15 (d, $J=5.5$ Hz, 2H), 7.92 (d, $J=8.5$ Hz, 1H), 8.07 (d, $J=2.2$ Hz, 1H), 8.16 (dd, $J=2.2$, 8.5 Hz, 1H), 8.54 (d, $J=5.5$ Hz, 2H); ^{13}C NMR (Cl_3CD) δ 52.41, 122.69, 122.82, 124.80, 131.20, 135.58, 140.91 146.52, 148.87, 149.50, 166.14.

***N*-[4-Nitro-2-(2-pyridyl)benzoyl]-methionine methyl ester (31).** The methyl benzoate of compound **28** was hydrolyzed with 2.0 equiv of lithium hydroxide (0.5 N) in methanol at rt for 2 h. The mixture was acidified with 1 N HCl and the aqueous solution was lyophilized. The solid was coupled with (L)-methionine methyl ester using coupling reagent EDCI (1.0 equiv) and HOBT (1.0 equiv) and triethylamine. Solvents were evaporated and the residue was purified, without extraction, through flash column chromatography (hexane:ethyl acetate, 1:1) to give pure **31** (83%). ^{1}H NMR (Cl_3CD) δ 1.88–2.09 (m, AB, 2H), 2.01 (s, 3H), 2.35 (t, 4.18 Hz, 2H), 3.69 (s, 3H), 4.74 (m, 1H), 7.15 (d, $J=7.8$ Hz, 1H), 7.33 (dd, $J=4.6$, 7.5 Hz, 1H), 7.57 (d, $J=7.8$ Hz, 1H), 7.79 (m, 2H), 8.23 (dd, $J=2.2$, 8.5 Hz, 1H), 8.37 (d, $J=2.2$ Hz, 1H), 8.62 (d, $J=4.6$ Hz, 1H).

***N*-[4-Nitro-2-(3-pyridyl)benzoyl]-methionine methyl ester (32).** This compound was prepared with the same method described for compound **31** (92%). ^{1}H NMR (Cl_3CD) δ 1.84–1.97 (m, AB, 2H), 1.96 (s, 3H), 2.18 (t, 7.3 Hz, 2H), 3.64 (s, 3H), 4.62 (m, 1H), 7.19 (d, $J=7.7$ Hz, 1H), 7.30 (m, 1H), 7.73 (m, 2H), 8.14 (d, $J=2.2$ Hz, 1H), 8.19 (dd, $J=2.2$, 8.5 Hz, 1H), 8.44 (d, $J=2.0$ Hz, 1H), 8.48 (dd, $J=1.4$, 8.5 Hz, 1H); ^{13}C NMR (Cl_3CD) δ 15.21, 29.60, 30.68, 51.80, 52.59, 122.89, 123.41, 124.97, 129.60, 133.61, 136.09, 137.71, 141.27, 148.37, 148.70, 149.44, 167.18, 171.48.

***N*-[4-Nitro-2-(4-pyridyl)benzoyl]-methionine methyl ester (33).** This compound was prepared with the same method described for compound **31** (86%). ^{1}H NMR (Cl_3CD) δ 1.89–2.04 (m, AB, 2H), 2.02 (s, 3H), 2.25 (t, 6.9 Hz, 2H), 3.69 (s, 3H), 4.70 (m, 1H), 6.76 (d, $J=7.4$ Hz, 1H), 7.36 (d, $J=4.6$ Hz, 2H), 7.78 (d, $J=8.4$ Hz, 1H), 8.22 (d, $J=2.2$ Hz, 1H), 8.27 (dd, $J=2.2$, 8.4 Hz, 1H), 8.64 (d, $J=4.6$ Hz, 2H); ^{13}C NMR (Cl_3CD) δ 15.43, 29.73, 30.83, 52.01, 52.76, 123.25, 123.48, 124.84, 129.76, 138.79, 140.97, 145.54, 148.60, 150.17, 166.92, 171.55.

***N*-[4-[*N*-[2(*R*)-(*tert*-Butoxycarbonyl)amino-3-(triphenylmethyl)thio]propyl]amino-2-(2-pyridyl)benzoyl]-methionine methyl ester (34).** Compound **31** (370 mg, 0.95 mmol) and Raney Ni (1 mg, catalyst) were dissolved in MeOH (15 mL). The mixture was refluxed and then hydrazine (92 mg, 2.87 mmol) was added. After 10 min of refluxing, Raney Ni was removed. After evaporation of the solvent, the residue was purified by flash column chromatography to provide an amino derivative (97% yield). Longer time of refluxing caused ester cleavage by the hydrazine. ^{1}H NMR (Cl_3CD) δ 1.94 (m, 2H), 2.13 (s, 3H), 2.34 (m, 2H), 3.72 (s, 3H), 4.59 (dd, $J=4.4$, 8.9 Hz, 1H), 6.75 (dd, $J=1.6$, 8.2 Hz, 1H), 6.81 (br s, 1H), 7.35 (m, 1H), 7.44 (d, $J=8.2$ Hz, 1H), 7.54 (m, 1H), 7.81 (m, 1H), 8.55 (d, $J=4.6$ Hz, 1H); ^{13}C NMR (CD_3OD) δ 15.16, 31.03, 31.67, 52.81, 53.01, 114.71, 116.82, 123.59, 124.92, 125.32, 131.04, 138.16, 141.91, 149.51, 151.62, 160.09, 172.60, 173.86. This amino derivative was coupled with *N*-Boc-*S*-trityl-(L)-cysteinal under the reductive amination conditions described before. After the evaporation of methanol, the residue was purified by flash column chromatography (hexane:ethyl acetate 7:3) to provide compound **34** (53%). ^{1}H NMR (Cl_3CD) δ 1.31 (s, 9H), 1.73–1.95 (m, AB, 2H), 1.93 (s, 3H), 2.17 (t, $J=7.6$ Hz, 2H), 2.35 (br s, 2H), 2.98 (br s, 2H), 3.55 (s, 3H), 3.57 (br s, 1H), 4.22 (br s, 1H), 4.61 (m, 2H), 6.41 (s, 1H), 6.44 (m, 1H), 6.90 (d, $J=7.7$ Hz, 1H), 7.19 (m, 10H), 7.31 (m, 6H), 7.47 (d, $J=8.1$ Hz, 1H), 7.61 (m, 1H), 8.53 (d, $J=4.6$ Hz, 1H); ^{13}C NMR (Cl_3CD) δ 15.26, 28.26, 29.64, 31.65, 34.30, 46.95, 49.46, 51.86, 52.22, 67.00, 79.64, 111.87, 113.65, 122.26, 123.33, 124.12, 126.78, 127.93, 129.44, 130.85, 136.51, 140.40, 144.34, 148.68, 149.39, 155.68, 159.26, 168.88, 172.13.

***N*-[4-[*N*-[2(*R*)-(*tert*-Butoxycarbonyl)amino-3-(triphenylmethyl)thio]propyl]amino-2-(3-pyridyl)benzoyl]-methionine methyl ester (35).** Compound **32** was reduced with Raney Ni as described for compound **34** to give the corresponding amine (45%). ^{1}H NMR (Cl_3CD) δ 1.97 (m, 2H), 1.99 (s, 3H), 2.23 (t, 7.4 Hz, 2H), 3.64 (s, 3H), 4.17 (br s, 1H), 4.61 (m, 1H), 6.29 (d, $J=7.0$ Hz, 1H), 6.47 (d, $J=2.2$ Hz, 1H), 6.61 (dd, $J=2.2$, 8.2 Hz, 1H), 7.26 (m,

1H), 7.46 (d, $J = 8.2$ Hz, 1H), 7.64 (d, $J = 7.7$ Hz, 1H), 8.50 (m, 2H); ^{13}C NMR (Cl_3CD) δ 15.32, 29.70, 31.32, 51.83, 52.45, 113.82, 116.20, 123.08, 124.44, 130.57, 136.07, 136.46, 138.20, 148.53, 148.83, 148.96, 168.74, 172.13. This amine was coupled with *N*-Boc-*S*-trityl-(L)-cysteinal as described before to give **35** (46%). ^{1}H NMR (Cl_3CD) δ 1.39 (s, 9H), 1.83–1.97 (m, AB, 2H), 2.00 (s, 3H), 2.27 (t, $J = 7.4$ Hz, 2H), 2.44 (br s, 2H), 3.07 (m, 2H), 3.66 (s, 3H), 3.78 (br s, 1H), 4.63 (m, 1H), 4.78 (br s, 1H), 6.16 (m, 1H), 6.34 (d, $J = 2.0$ Hz, 1H), 6.50 (dd, $J = 2.0$, 8.2 Hz, 1H), 7.28 (m, 10H), 7.43 (m, 6H), 7.51 (d, $J = 8.2$ Hz, 1H), 7.71 (d, $J = 7.8$ Hz, 1H), 8.57 (br s, 2H); ^{13}C NMR (Cl_3CD) δ 15.33, 28.27, 29.70, 31.34, 34.25, 46.90 48.97, 51.86, 52.44, 67.11, 79.50, 111.46, 113.99, 123.22, 126.85, 127.97, 129.47, 130.64, 132.07, 136.58, 136.98, 138.14, 144.32, 148.00, 148.56, 149.69, 159.73, 168.58, 172.15.

***N*-[4-[*N*-[2(*R*)-(*tert*-Butoxycarbonyl)amino-3-(triphenylmethyl)thio]propyl]amino-2-(4-pyridyl)benzoyl]-methionine methyl ester (36).** Compound **33** was reduced with $SnCl_2$ dihydrate as described before to give the corresponding amine (60%). ^{1}H NMR (Cl_3CD) δ 2.00–2.20 (m, AB, 2H), 2.04 (s, 3H), 2.37 (m, 2H), 3.69 (s, 3H), 4.50 (m, 1H), 6.67 (d, $J = 2.2$ Hz, 1H), 6.73 (dd, $J = 2.2$, 8.3 Hz, 2H), 7.36 (d, $J = 8.3$ Hz, 1H), 7.43 (d, $J = 5.5$ Hz, 2H), 8.48 (d, $J = 5.5$ Hz, 2H); ^{13}C NMR (Cl_3CD) δ 15.13, 31.15, 31.43, 52.81, 53.13, 114.81, 116.53, 124.92, 125.45, 131.25, 140.45, 149.69, 153.07, 159.21, 172.90, 173.87. This amine was coupled with *N*-Boc-*S*-trityl-(L)-cysteinal under reductive amination conditions to give **36** (74%). ^{1}H NMR (Cl_3CD) δ 1.20 (br s, 9H), 1.96–1.99 (m, AB, 2H), 1.98 (s, 3H), 2.26 (t, $J = 7.0$ Hz, 2H), 2.42 (br s, 2H), 3.04 (br s, 2H), 3.62 (s, 3H), 3.76 (br s, 1H), 4.60 (m, 2H), 4.93 (d, $J = 7.4$ Hz, 1H), 6.35 (br s, 2H), 6.48 (d, $J = 8.4$ Hz, 1H), 7.18 (m, 11H), 7.23 (m, 6H), 7.36 (d, $J = 7.4$ Hz, 1H), 8.50 (br s, 2H); ^{13}C NMR (Cl_3CD) δ 15.20, 28.14, 29.65, 31.09, 34.10, 46.80 49.28, 51.79, 52.29, 66.91, 79.51, 111.68, 113.26, 122.55, 123.62, 126.71, 127.84, 129.34, 130.42, 139.04, 144.24, 148.98, 149.39, 149.60, 155.61, 168.55, 172.00.

***N*-[4-[2(*R*)-Amino-3-mercaptopropyl]amino-2-(2-pyridyl)-benzoyl]-methionine trifluoroacetate (11).** Compound **34** was hydrolyzed with 2.0 equiv of aqueous lithium hydroxide (0.5 N) in methanol at rt for 2 h. The reaction mixture was acidified with 1 N HCl and the solvents were evaporated. The residue was lyophilized to give a crude solid. This solid was treated with trifluoroacetic acid in methylene chloride in the presence of triethylsilane. After evaporation of solvents, the residue was washed with ether and saturated HCl in ether. The crude solid was purified by preparative reverse phase HPLC to give the product. ^{1}H NMR (D_2O) δ 2.02 (s, 3H), 2.14 (m, 2H), 2.50 (m, 2H), 2.90 (m, 2H), 3.61–3.45 (m, 4H), 4.44 (m, 1H), 6.85 (br s, 1H), 6.92 (d, $J = 8.1$ Hz, 1H), 7.65 (m, 1H), 7.98 (m, 2H), 8.50 (m, 1H), 8.66 (d, $J = 5.58$ Hz, 1H); ^{13}C NMR (D_2O/CD_3OD) δ 15.69, 30.02, 31.30, 37.92, 42.71, 50.93, 53.90, 115.46, 116.33, 123.77, 127.32, 129.29, 132.46, 134.58, 142.43, 147.93, 151.76, 154.79, 171.23, 177.28.

***N*-[4-[2(*R*)-Amino-3-mercaptopropyl]amino-2-(3-pyridyl)-benzoyl]-methionine trifluoroacetate (12).** This compound was prepared from the deprotection of **35** as described in the preparation of **11**. ^{1}H NMR (D_2O) δ 2.09 (s, 3H), 2.10 (m, 2H), 2.53 (m, 2H), 2.78 (m, 2H), 3.53 (m, 4H), 4.44 (m, 1H), 6.72 (d, $J = 2.0$ Hz, 1H), 6.82 (dd, $J = 2.1$, 8.5 Hz, 1H), 7.52 (d, $J = 8.5$ Hz, 1H), 8.02 (m, 1H), 8.50 (d, $J = 8.1$ Hz, 1H), 8.70 (br s, 2H); ^{13}C NMR (CD_3OD) δ 15.23, 25.05, 30.67, 30.74, 44.38, 53.14 (2C), 113.94, 115.77, 123.68, 128.11, 131.81, 136.24, 140.85, 141.15, 141.63, 147.69, 151.24, 172.39, 176.24.

***N*-[4-[2(*R*)-Amino-3-mercaptopropyl]amino-2-(4-pyridyl)-benzoyl]-methionine trifluoroacetate (13).** This compound was prepared from the deprotection of **36** as described in the preparation of **11**. ^{1}H NMR (CD_3CN/D_2O) δ 2.00–2.17 (m, AB, 2H) 2.13 (s, 3H), 2.45–2.60 (m, 2H), 2.99–2.93 (m, 2H), 3.63–3.48 (m, 4H), 4.49 (m, 1H), 6.80 (d, $J = 2.0$ Hz, 1H), 6.88 (dd, $J = 2.0$, 8.5 Hz, 1H), 7.56 (d, J 8.5 Hz, 1H), 7.99 (d, $J = 6.6$ Hz, 2H), 8.73 (d, $J = 6.6$ Hz, 1H); ^{13}C NMR (CD_3CN/D_2O) δ 15.08, 24.81, 30.52, 30.60, 44.05, 53.02 (2C), 114.20, 115.23, 123.52, 127.81, 131.70, 138.26, 141.53, 150.97, 160.64, 171.49, 175.93; ESMS 435.0 (calcd molecular weight 434.57). Anal. calcd for $C_{20}H_{26}O_3N_4S_2 \cdot 2CF_3COOH \cdot 4H_2O$:C 42.42, H 4.30, N 8.24, S 9.43; found C 42.20, H 4.28, N 8.16, S 9.26.

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