# *Expedited Articles*

## Potent, Cell Active, Non-Thiol Tetrapeptide Inhibitors of Farnesyltransferase

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All previously reported CAAX-based farnesyltransferase inhibitors contain a thiol functionality. We report that attachment of the 4-imidazolyl group, via 1-, 2-, or 3-carbon alkyl or alkanoyl spacers, to Val-Tic-Met or tLeu-Tic-Gln provides potent FT inhibitors. (*R**)-*N*-[[1,2,3,4-Tetrahydro-2-[*N*-[2-(1*H*-imidazol-4-yl)ethyl]-L-valyl]-3-isoquinolinyl]carbonyl]-L-methionine ([imidazol-4-yl-ethyl]-Val-Tic-Met), with FT $IC_{50}$ = 0.79 nM, displayed potent cell activity in the absence of prodrug formation (SAG $EC_{50}$ = 3.8 $\mu$M).

Mutated *ras* genes are found in a wide variety of human tumors, with the highest rates observed in cancers of the pancreas (90%), colon (50%), and lung (30%).[1,2] This incidence has prompted considerable efforts at elucidating the pathways of Ras transformation and developing therapeutic agents which might interfere with this pathway. These latter efforts have focused primarily on the development of inhibitors of the enzyme farnesyltransferase (FT).

In order to perform both its normal and oncogenic functions, the Ras protein must be membrane associated. The carboxy terminus of proteins of the Ras family consists of a tetrad referred to as a CAAX box, where C is cysteine, A is an aliphatic amino acid, and X is a member of a limited set of amino acids. Membrane localization of Ras is the result of a three-step posttranslational modification of its CAAX box, involving farnesylation of the cysteine by farnesyl pyrophosphate (FPP), hydrolysis of the three C-terminal amino acid residues, and methyl esterification of the resulting C-terminal farnesylcysteine. Of these steps, farnesylation appears to be a necessary and sufficient step for membrane localization, implicating FT as a preferred target for interrupting oncogenic Ras signaling.[3]

A number of groups have reported thiol FT inhibitors based on the CAAX sequence. These include tetrapeptides,[4] reduced tetrapeptides,[5−8] constrained tetrapeptides,[9,10] and tetrapeptide-like molecules in which spacers have been used to replace one or more of the central amides or amino acid residues.[11−15] While many of these thiol-containing compounds are effective inhibitors of both the isolated enzyme and cellular Ras processing, the presence of the oxidizable thiol functionality confers disadvantages on the development of such compounds as therapeutic agents. Although non-thiol inhibitors based upon FPP analogs (see ref 16) or bisubstrate analogs[17] have been reported, there is only one report of a non-thiol CAAX-based inhibitor, and these analogs were weak inhibitors.[18]

Farnesyltransferase requires for its function the presence of two metal ions, zinc and magnesium.[19] In one plausible catalytic mechanism, the magnesium coordinates to the oxygens of FPP, and the CAAX thiol of the Ras protein coordinates to the zinc, perhaps with the formation of a zinc thiolate assisted by a nearby general base. In considering alternate zinc ligands, we were drawn to consider imidazole because of the prevalence of histidine as a key ligand in metalloenzymes.[20] Despite its ubiquitous use by enzymes as a metal ligand, the use of the imidazole ring as a metal ligand in metalloenzyme inhibitors has received scant attention.[21] In this report, we describe the discovery of extremely potent imidazole-based tetrapeptide inhibitors of FT. These compounds display cellular effects in the absence of prodrug formation.

### Chemistry, Biological Results, and Discussion

We have discovered that cysteine-based tetrapeptides which contain (*S*)-1,2,3,4-tetrahydro-3-isoquinolinecarboxylic acid (Tic) in place of the $A_2$ residue of the CAAX box provide potent FT inhibitors.[22] We have also previously demonstrated that the imidazole-containing tetrapeptide His-Val-Phe-Met was a poor inhibitor (FT $IC_{50}$ = 6.8 $\mu$M).[8] Nevertheless, we were interested in determining whether this poor activity was due to inappropriate spacing of the imidazole ring with respect to the remainder of the tripeptide. Direct coupling of commercially available 4-imidazoleacetic acid to the HCl salt of Val-Tic-Met-OMe using 1-[3-(dimethylamino)-propyl]-3-ethylcarbodiimide hydrochloride, HOBt, and *N*-methylmorpholine in DMF, followed by ester hydrolysis, afforded **2**, which inhibited FT with an $IC_{50}$ value of 6 nM (Table 1). Compound **2** displayed 300-fold selectivity for inhibition of FT versus GGTI; in fact, all of the imidazole tetrapeptides were more potent inhibitors of FT versus GGTI, with selectivities ranging from 300− to 40 000-fold. Compound **2** was also active in cell-based assays, as demonstrated by its ability to inhibit the anchorage independent growth of H-Ras

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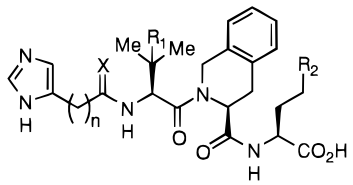
**Table 1.** Imidazole-Containing Inhibitors of Farnesyltransferase



| compd | *n* | $R_1$ | $R_2$ | X | FT $IC_{50}$ (nM) | GGTI $IC_{50}$ (nM) | SAG $IC_{50}$ ($\mu$M) | MTD ($\mu$M) |
|---|---|---|---|---|---|---|---|---|
| 1[a] | 0 | H | SMe | O | 84 ± 10 | 25 800 ± 5600 | 100 | >150 |
| **2**[a] | 1 | H | SMe | O | 6.6 ± 1 | 2020 ± 700 | 50 | >150 |
| **3**[a] | 0 | H | SMe | H,H | 114 ± 25 | 17 400 ± 330 | 100 | NT |
| **4**[a] | 1 | H | SMe | H,H | 0.79 ± 0.07 | 234 ± 41 | 3.8 | >150 |
| **5**[a] | 1 | Me | $CONH_2$ | O | 28 ± 0.5 | 28% (360 $\mu$M) | 3.9 | >150 |
| **6**[a] | 2 | Me | $CONH_2$ | O | 4.4 ± 0.25 | 168 000 ± 54 000 | 27 | >150 |
| **7**[b] | 1 | Me | $CONH_2$ | H,H | 3.4 ± 0.05 | 30 500 ± 5300 | 3.2 | NT |
| **8**[b] | 2 | Me | $CONH_2$ | H,H | 20 ± 3.5 | 106 000 ± 42 000 | 32 | >150 |

[a] Elemental analysis for C, H, N as hydrated TFA salts. [b] Elemental analysis for C, H, N as hydrated Li salts. NT = not tested.

transformed rat-1 cells in soft agar (SAG $EC_{50}$ = 50 $\mu$M). Compound **2** and the other imidazole tetrapeptide inhibitors did not display cytotoxicity to untransformed NIH 3T3 cells. The high MTD values (no toxicity observed at 150 $\mu$M, the highest concentration tested) indicate that SAG activity was not due to general cytotoxicity. Encouraged by these findings, we synthesized other imidazole-containing tetrapeptides. Compound **1** was prepared by coupling the tripeptide with 1-(triphenylmethyl)imidazole-4-carboxylic acid and subsequent deprotection. Compound **3** was prepared by reductive amination ($NaCNBH_3$) of the tripeptide with [1-(triphenylmethyl)imidazole-4-carboxaldehyde and subsequent deprotection. Compound **4** was prepared by reductive amination of the tripeptide with [1-(ethoxycarbonyl)imidazol-4-yl]acetaldehyde and subsequent deprotection. Both the amide and methyleneamine 1-carbon linkers afforded moderate, equipotent inhibitors. However, the 2 carbon methyleneamine analog **4** afforded a subnanomolar inhibitor of FT. More importantly, **4** was a potent inhibitor in the SAG assay. Almost complete (95%) inhibition of the growth of H-Ras-transformed cells occurred with 25 $\mu$M **4** (Figure 1B), and in three independent assays, the $EC_{50}$ value for **4** was 3.8 ± 0.1 $\mu$M. This potent cell activity was observed despite the fact that **4** contains an unesterified and therefore charged carboxylic acid group.

We have previously demonstrated that Tic-containing tetrapeptides in which *t*-Leu and Gln occupied the $A_1$ and X sites, respectively, show increased cell activity relative to their FT inhibitory potency (decrease in the SAG $EC_{50}$/FT $IC_{50}$ ratio).[22] These changes were incorporated into imidazole tetrapeptides with the more potent 2-carbon linker. In addition, the effect of lengthening the linker to three atoms was also investigated in this series. These compounds were prepared by procedures analogous to those described above. As shown in Table 1, the acetamide analog **5** was a moderately potent FT inhibitor. The 4-imidazolylethyl analog **7**, while 4-fold less potent than **4** as an FT inhibitor, was equipotent in the SAG assay. The 3-carbon linkers also afforded good FT inhibitors, although in this case the 4-imidazolylpropionyl analog **6** was 5-fold more potent than the 4-imidazolylpropyl analog **8**.

The high inhibitory potency of the 3-carbon amide analog **6**, which maintains the same imidazole to tripeptide spacing as histidine, suggested that optimal spacing was not the reason for the effectiveness of these compounds as FT inhibitors compared to His-Val-Phe-Met. To further confirm this, the L-histidine analog **9** (Figure 2) was prepared and found to be a relatively poor inhibitor (FT $IC_{50}$ = 520 nM; SAG $EC_{50}$ = 100 $\mu$M; 30% inhibition of GGTI at 360 $\mu$M; MTD >150 $\mu$M). Similarly, the D,L-imidazol-4-ylglycine analog **10** (FT $IC_{50}$ = 180 nM; SAG $EC_{50}$ = 100 $\mu$M; 6% inhibition of GGTI at 180 $\mu$M) was also a moderate inhibitor compared to its desamino analog **4**. These results indicate that the presence of the α-amine is detrimental to the inhibitory potency of imidazole-based tetrapeptide FT inhibitors. One possible explanation for this effect is

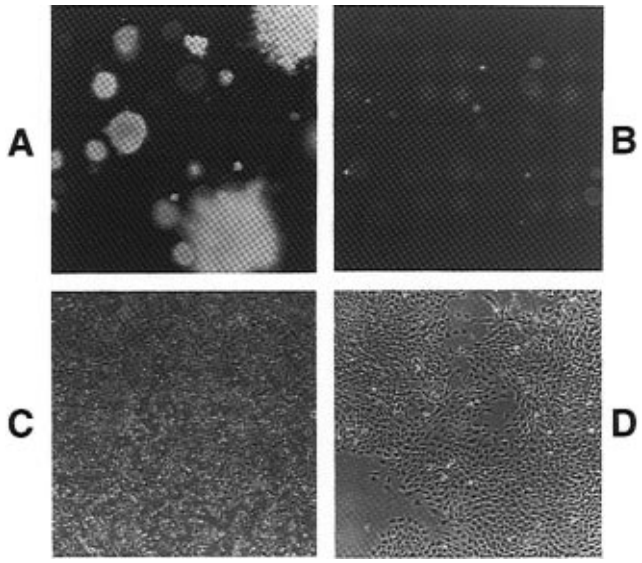



**Figure 1.** Whole cell activity of **4.** A and B: Effect on growth of H-Ras-transformed NIH 3T3 cells (44−911 cells) in soft agar. Experimental conditions used to determine anchorage independent growth in soft agar were as described.[26] After 14 days of growth at 37 °C, colonies were photographed at 40× magnification using phase contrast ring 2. Colonies larger than 0.1 mm were counted in the untreated (A) and treated (B) samples to determine the percent inhibition of colony growth. Colony growth was inhibited by 95% with **4** at 25 $\mu$M (B). The total number of colonies per 35 mm well in untreated samples was 1856. C and D: Effect on morphology of H-Ras-transformed rat-1 cells. The initial plating conditions and the treatment protocol with inhibitor were essentially as described.[26] Representative areas of the wells were photographed on day 4 at 40× magnification: C, no treatment; D, 25 $\mu$M **4**.

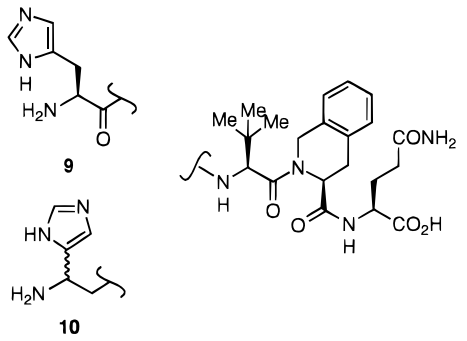


**Figure 2.** Structures of α-amine-containing imidazole tetrapeptides.

that the α-ammonium ion may provide an internal hydrogen bond to the imidazole, tying up the lone pair of electrons which would otherwise be available for the putative zinc coordination.

The most potent imidazole tetrapeptide inhibitor, **4** (BMS-193269), was further characterized to ensure that its effects in the SAG assay were due to mechanism-based inhibition of FT. The posttranslational processing status of the Ras protein in cells can be monitored by SDS-PAGE of lysed cell extracts. In the presence of an FT inhibitor, Ras protein farnesylation is blocked, and the unprocessed protein migrates as a slower band. Treatment of H-Ras-transformed rat-1 cells with 100 $\mu$M **4** led to complete inhibition of Ras processing, and the $IC_{50}$ for this inhibition was found to be about 5 $\mu$M. Compared to their untransformed counterparts, H-Ras-transformed rat-1 cells round up, appear refractile, and pile up due to a loss of contact inhibition (Figure 1C). Treatment of these cells with 25 $\mu$M **4** for 4 days led to a complete restoration of the untransformed phenotype, with the cells flattening and growing in a monolayer (Figure 1D). The $IC_{50}$ for reversion of the transformed phenotype by **4** was about 5 $\mu$M.

In conclusion, we have discovered potent tetrapeptide inhibitors of FT in which the key thiol functionality is replaced by the chemically stable imidazole group. In the absence of prodrug formation, these compounds are potent inhibitors in cell-based assays, leading to inhibition of Ras processing, restoration of the untransformed phenotype, and inhibition of anchorage independent growth in soft agar.

## Experimental Section

**General Chemical Procedures.** Melting points were recorded on a Thomas-Hoover capillary apparatus and are reported uncorrected. IR spectra were recorded on a Mattson Sirius 100 spectrometer. Proton NMR ($^1$H-NMR) and carbon NMR ($^{13}$C-NMR) spectra were obtained on JOEL FX-270 and GX-400 spectrometers and are reported relative to tetramethylsilane (TMS) reference. Analytical and preparative HPLC were performed on YMC columns (A-302, S-5, 120A ODS, 4.6 × 150 mm; SH-345-15, S-15, 120A ODS, 20 × 500 mm) with acetonitrile:water gradients containing 0.1% trifluoroacetic acid. Chromatography was performed under flash conditions using EM Science silica gel, 0.040−0.063 mm particle size. THF was distilled from Na/benzophenone. Solutions were dried with magnesium sulfate unless otherwise noted.

**(*R**)-*N*-[[2-[(1,1-Dimethylethoxy)carbonyl]-1,2,3,4-tetrahydro-3-isoquinolinyl]carbonyl]-L-methionine, Methyl Ester (11).** A solution of (*S*)-3,4-dihydro-2,3(1*H*)-isoquinolinedicarboxylic acid, 2-(1,1-dimethylethyl) ester (2.0 g, 7.21 mmol) and L-methionine, methyl ester, hydrochloride (1.44 g, 7.21 mmol) in 5:15 *N*-methylpyrrolidinone-methylene chloride was stirred at 4 °C. *N*,*N*-Diisopropylethylamine (DIEA; 1.23 mL, 7.21 mmol) was added followed by *N*-hydroxybenzotriazole (HOBt; 974 mg, 7.21 mmol). The mixture was stirred for 5 min, and 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride (EDC; 1.38 g, 7.21 mmol) was added. The mixture was allowed to come to room temperature, stirred overnight, and partitioned between methylene chloride and brine. The organic phase was washed successively with citric acid, $NaHCO_3$, and brine, dried, and concentrated under vacuum to give 2.64 g (86%) of **11**: MS $(M + H)^+$ 423; $^1$H-NMR ($CDCl_3$, 270 MHz) $\delta$ 7.25−7.13 (4H, m), 4.86−4.40 (4H, m), 3.69 (3H, s), 3.32 (2H, m), 3.05 (2H, m), 1.93 (3H, m), 1.90−1.78 (2H, m), 1.50 (9H, s).

**(*R**)-*N*-[(1,2,3,4-Tetrahydro-3-isoquinolinyl)carbonyl]-L-methionine, Methyl Ester (12).** A solution of **11** (2 g, 6.22 mmol) in methylene chloride (10 mL) was treated at room temperature with trifluoroacetic acid (TFA; 10 mL) and dimethyl sulfide (0.5 mL) and stirred for 0.5 h. The mixture was concentrated under vacuum, dissolved in methylene chloride, and concentrated. This procedure was repeated five times to yield **12** (99%) as a clear glass: $^1$H-NMR ($CDCl_3$, 270 MHz) $\delta$ 7.24−7.10 (4H, m), 4.53−4.39 (4H, m), 3.64 (3H, s), 3.26 (2H, m), 2.52 (1H, m), 2.44 (1H, m), 1.98 (5H, m).

**(*R**)-*N*-[[2-[*N*-[(1,1-Dimethylethoxy)carbonyl]-L-valyl]-1,2,3,4-tetrahydro-3-isoquinolinyl]carbonyl]-L-methionine, Methyl Ester (13).** To a solution of **12** (4.73 mmol) in methylene chloride (20 mL) at 0 °C was added *N*-(*tert*-butyloxycarbonyl)-L-valine (2.06 g, 9.47 mmol), bis(2-oxo-3-oxazolidinyl)phosphinic chloride (BOP-Cl, 1.2 g, 4.7 mmol) and DIEA (1.2 g, 1.6 mL, 9.5 mmol). The mixture was stirred for 24 h at 0 °C. Additional BOP-Cl; (1.2 g, 4.7 mmol), and DIEA (590 mg, 0.8 mL, 4.73 mmol) were added, and the mixture was stirred for an additional 12 h at 0 °C. The mixture was concentrated, and the residue was chromatographed (silica gel, 50% ethyl acetate/hexane). Appropriate fractions were collected and concentrated to yield **13** as a clear oil (2.4 g, 97%): MS $(M + H)^+$ 522; $^1$H-NMR ($CD_3OD$, 400 MHz) $\delta$ 7.25−7.18 (4H, m), 5.29 (1H, m), 5.01 (1H, m), 4.91 (1H, m), 4.70−4.54 (2H, m), 3.71 (3H, s), 3.32 (1H, m), 3.09 (1H, m), 2.25−2.02 (4H, m), 2.00 (3H, s), 1.82 (1H, m), 1.42 (9H, s), 1.14−0.90 (6H, m).

**(*R**)-*N*-[(2-L-Valyl-1,2,3,4-tetrahydro-3-isoquinolinyl)-carbonyl]-L-methionine, Methyl Ester, Hydrochloride (14).** A solution of **13** (100 mg, 0.192 mmole) in 4 N HCl in dioxane was stirred at 0 °C for 1 h. The solution was concentrated under vacuum to afford a pink oil which was chased with diethyl ether (3 × 20 mL) and triturated with diethyl ether to afford 81 mg (91%) of **14** as a pink solid: MS $(M + H)^+$ 422; $^1$H-NMR ($CDCl_3$, 270 MHz) $\delta$ 7.32−7.23 (4H, m), 4.83 (1H, d, $J$ = 15.0 Hz), 4.72 (1H, m), 4.61 (1H, d, $J$ = 15.0 Hz), 4.58 (1H, m), 4.52 (1H, d, $J$ = 4.7 Hz), 3.70 (3H, s), 3.25 (1H, m), 3.11 (1H, m), 2.58−2.46 (2H, m), 2.37 (1H, m), 2.06 (3H, s), 1.94 (1H, m), 1.20 (3H, m), 1.08 (3H, m); $^{13}$C-NMR ($CD_3OD$, 100 MHz) 173.64, 173.44, 169.60, 135.11, 129.10, 128.47, 128.16, 126.88, 56.94, 52.97, 52.59, 47.54, 32.61, 32.24, 31.19, 30.96, 19.07, 16.20, 15.23 ppm.

**4-(Hydroxymethyl)-1-(triphenylmethyl)imidazole (15).** A solution of triphenylmethyl chloride (2.2 g, 7.9 mmol) in DMF (5 mL) was added to a mixture of 4-(hydroxymethyl)-imidazole (1 g, 7.4 mmol) and triethylamine (2.5 mL, 18 mmol) in DMF (7.5 mL). The mixture was stirred for 18 h and filtered, and the solid was washed with water and dried under vacuum to afford 2.33 g (93%) of **15** as a white solid: MS $(M + H)^+$ 341.

**1-(Triphenylmethyl)imidazole-4-carboxaldehyde (16).** A mixture of **15** (2.33 g, 6.85 mmol) in dioxane (100 mL) was heated to 85 °C to obtain a homogenous solution. The heat source was removed, solid $MnO_2$ (5 g, 57.5 mg) was added all at once, and the mixture was stirred until the temperature began to drop. The mixture was heated for 6 h at 85 °C and filtered through a pad of Celite, and the filtrate was concentrated. The resulting white solid was dissolved in methylene chloride (20 mL), hexane was added, and the mixture was allowed to stand overnight. The solid was filtered and dried to afford 1.86 g (80%) of **16** as a white solid. $^1$H-NMR ($CDCl_3$) $\delta$ 9.9 (s, 1H), 7.63 (s, 1H), 7.55 (s, 1H), 7.38 (m, 9H), 7.13 (m, 6H).

**1-(Triphenylmethyl)imidazole-4-carboxylic Acid (17).** To a suspension of $Ag_2O$ (0.68 g, 3.0 mmol) in water (4 mL) at 55 °C was added NaOH (570 mg, 14.4 mmol) followed by **16** (1 g, 3.0 mmol). The mixture was stirred at 55 °C for 15 min, filtered hot through a pad of Celite, and the filtrate was acidified to pH 3.5 with 6 N HCl. The resulting tan solid was collected, rinsed with water, dried under vacuum to afford 110 mg (10%) of **17**: MS $(M + H)^+$ 355.

**(*S*)-*N*-[[1,2,3,4-Tetrahydro-2-[*N*-[[1-(triphenylmethyl)-imidazol-4-yl]carbonyl]-L-valyl]-3-isoquinolinyl]carbonyl]-L-methionine, Methyl Ester (18).** To a solution of **17** (89 mg, 0.192 mmol), HOBt (25.9 mg, 0.192 mmol), and **14** (74.6 mg, 0.21 mmol), in 5 mL of dichloromethane at 0 °C was added *N*-methylmorpholine (NMM; 21 mL, 0.192 mmol) followed by EDC (40 mg, 0.21 mmol). The mixture was stirred at room temperature for 18 h and partitioned between water and dichloromethane. The organic phase was extracted with saturated sodium bicarbonate (2×), 3 N aqueous HCl, water, and brine and dried. The solvent was removed under vacuum to afford 110 mg (76%) of **18**: MS $(M + H)^+$ 780.

**(*S*)-*N*-[[1,2,3,4-Tetrahydro-2-[*N*-[[1-(triphenylmethyl)-imidazol-4-yl]carbonyl]L-valyl]-3-isoquinolinyl]carbonyl]-L-methionine (19).** To a cooled solution of **18** (110 mg, 0.145 mmol) in methanol (5 mL) was added a solution of 1 N aqueous NaOH (5 mL). The mixture was stirred for 2 h and concentrated to remove methanol, and the residue was partitioned between water and dichloromethane. The aqueous layer was acidified to pH 4 with 6 N HCl and extracted with methylene chloride (4×). The combined organic phases were washed with water and brine and dried. The solvent was removed under vacuum to afford 106 mg (98%) of crude **19**.

**(*S*)-*N*-[[1,2,3,4-Tetrahydro-2-[*N*-(1*H*-imidazol-4-ylcarbonyl)-L-valyl]-3-isoquinolinyl]carbonyl]-L-methionine, Trifluoroacetate (1:1) Salt (1).** To a degassed solution of triethylsilane (0.23 mL, 1.4 mmol) and TFA (5 mL) in methylene chloride (5 mL) was added **19**. The mixture was stirred for 4 h and concentrated, and the residue was dissolved in 25 mL of (80:20) water/acetonitrile/0.1% TFA. The mixture was centrifuged and decanted to remove insoluble materials. The solution was subjected to preparative HPLC on an octadecylsilane column (S-10, 30 × 500) using a gradient system from 80% water:20% acetonitrile:0.1% TFA to 55% water:45% acetonitrile:0.1% TFA over 50 min at 40 mL/min. Fractions were analyzed by analytical reversed phase HPLC, and those containing product with a minimum purity of 99% were pooled and lyophilized to afford 14 mg (19.6%) of **1** as a white solid: MS $(M + H)^+$ 502; $^1$H-NMR ($CD_3OD$) $\delta$ 8.74 (d, 1H, $J$ = 9.3 Hz), 8.05 (d, 1H, $J$ = 9.3 Hz), 7.21 (m, 5H), 4.88 (m, 2H), 4.69 (m, 1H), 3.3 (m, 1H), 3.2 (m, 4H), 2.55 (m, 2H), 2.2 (m, 2H), 2.06−1.94 (d, 3H), 1.9 (m, 1H), 1.1−1.04 (m, 6H); $^{13}$C-NMR ($CD_3OD$) 173.27, 172.22, 171.58, 172.0, 170.3, 135.52, 134.02, 133.80, 132.12, 127.78, 127.38, 126.90, 126.78, 126.49, 125.78, 125.42, 120.27, 120.00, 55.71, 55.44, 55.00, 50.99, 46.14, 31.24, 31.02, 30.91, 30.59, 29.44, 18.39, 17.25, 13.70 ppm; IR (KBr) 3435, 3032, 2971, 2926, 1645, 1549, 1415, 1206, 1140, 801, 750, 723 $cm^{-1}$.

***S*-Ethyl[1-(ethoxycarbonyl)imidazol-4-yl)thioacetic Acid (20).** To a mixture of 4-imidazoleacetic acid (2 g, 12 mmol) and triethylamine (5.1 mL, 37 mmol) in methylene chloride (40 mL) at 0 °C was added a solution of ethyl chloroformate (2.5 mL, 26 mmol) in methylene chloride (10 mL) over 20 min to maintain the reaction temperature between 0 and 5 °C. The mixture was stirred for 15 min, and ethanethiol (2.0 mL, 28 mmol) and (*N*,*N*-dimethylamino)-pyridine (DMAP; 100 mg) were added consecutively. The mixture was stirred for 30 min at 0 °C, warmed to room temperature, washed with water and brine, and dried. The solvent was evaporated, the residue was adsorbed on a plug of silica gel, and the plug was eluted with dichloromethane to remove the unreacted ethanethiol followed by 10% methanol: dichloromethane. Fractions containing product were combined and evaporated to yield 0.55 g of **20** as an oil: MS $(M + H)^+$ 243.

**[1-(Ethoxycarbonyl)imidazol-4-yl]acetaldehyde (21).** To a degassed solution of **20** (0.5 g, 2.1 mmol) and triethylsilane (1.64 mL, 10.3 mmol) in acetone (15 mL) was added 10% palladium on carbon (100 mg). The mixture was stirred for 1 h and filtered through a pad of Celite and the filtrate concentrated to afford 0.37 g (98%) of **21** as an oil: MS $(M + H)^+$ 183.

**(*R**)-*N*-[[1,2,3,4-Tetrahydro-2-[*N*-[2-(1*H*-imidazol-4-yl)-ethyl]-L-valyl]-3-isoquinolinyl]carbonyl]-L-methionine, Trifluoroacetate (2:5) Salt (4).** To a solution of **14** (83 mg, 0.18 mmol) and **21** (100 mg) in methanol (5 mL) was added solid $NaBH_3CN$ (23 mg, 0.36 mmol). After 10 min, additional amounts of $NaBH_3CN$ (23 mg, 0.36 mmol) and acetic acid (21 $\mu$L, 0.36 mmol) were added, and the mixture was stirred for 30 min. Two additional portions of **21** (150 mg), $NaBH_3CN$ (46 mg, 0.72 mmol), and acetic acid (41 $\mu$L, 0.72 mmol) were added over 1 h. The mixture was stirred for 18 h and evaporated, and the residue was dissolved in methanol (5 mL). Sodium hydroxide (1 N, 4 mL) was added, the solution was stirred for 1 h, and the methanol was evaporated. The aqueous phase was diluted to 10 mL with water, and 1 mL of TFA was added. The mixture was stirred for 10 min and concentrated, and the residue was dissolved in 25 mL of (80:20) water/acetonitrile containing 0.1% TFA. The mixture was centrifuged and decanted to remove insoluble materials. The solution was subjected to preparative HPLC on an octadecylsilane column (S-10, 30 × 500) using a gradient system from 80% water:20% acetonitrile:0.1% TFA to 55% water:45% acetonitrile:0.1% TFA over 50 min at 40 mL/min. Fractions containing product with a minimum purity of 99% were pooled and lyophilized to afford 22 mg of **4** (24%) as a white solid: MS $(M + H)^+$ 502. $^1$H-NMR ($CD_3OD$) $\delta$ 8.79 (d, 1H, $J$ = 9.3 Hz), 7.26 (m, 5H), 4.85 (m, 2H), 4.65 (m, 1H), 4.28 (m, 1H), 3.4 (m, 1H), 3.2 (m, 4H), 2.55 (m, 2H), 2.2 (m, 2H), 2.06−1.94 (d, 3H), 1.9 (m, 1H), 1.2 (m, 6H); $^{13}$C-NMR ($CD_3OD$) 173.25, 171.74, 170.57, 167.65, 133.96, 133.89, 133.68, 132.05, 131.33, 128.74, 127.78, 127.68, 126.97, 126.71, 125.91, 125.26, 116.71, 62.92, 56.02, 55.81, 50.89, 45.25, 31.08, 30.99, 30.17, 29.53, 20.86, 17.80, 16.52, 16.51, 13.64 ppm; IR (KBr) 2976, 1674, 1433, 1204, 1136, 835, 721 $cm^{-1}$.

***N*-Methyl-*N*-methoxy[2-amino-*N*-[(1,1-dimethylethoxy)carbonyl]-2-*N*-[(1,1-dimethylethoxy)carbonyl]-imidazol-4-yl]acetamide (22).** To a solution of 2-amino-*N*-[(1,1-dimethylethoxy)carbonyl]-2-*N*-[(1,1-dimethylethoxy)-carbonyl]imidazole-4-acetic acid[23,24] (330 mg, 0.97 mmol), *N*,*O*-dimethyl hydroxylamine hydrochloride (97 mg, 1 mmol), and bromotris(pyrrolidinophosphonium) hexafluorophosphate (466 mg, 1 mmol) in methylene chloride (10 mL) at 0 °C were added DIEA (0.523 mL, 3 mmol) and DMAP (12.2 mg, 0.1 mmol). The solution was stirred at room temperature for 2 h and concentrated, the residue was dissolved in ethyl acetate (40 mL), and the solution was washed with 1 N $KHSO_4$ (3 × 30 mL) and water (1 × 30 mL). The organic layer was dried, filtered, and concentrated. The residue was chromatographed (silica gel, 1:1 ethyl acetate:hexanes). Fractions containing the product were pooled and concentrated to afford 260 mg (70%) of **22** as a white solid: MS $(M + H)^+$ $385^+$; $^1$H-NMR ($CDCl_3$) $\delta$ 8.08 (1H, s), 7.41 (1H, s), 5.81 (1H, s), 3.72 (1H, s), 3.21 (1H, s), 1.61 (9H, s), 1.42 (9H, s).

**(*S*)-1,2,3,4-Tetrahydro-2-[3-methyl-*N*-[(phenylmethoxy)-carbonyl]-L-valyl]-3-isoquinolinecarboxylic Acid, Methyl Ester (23).** To a solution of 3-methyl-*N*-[(phenylmethoxy)-carbonyl]-L-valine (5.31 g, 20 mmol) in methylene chloride (80 mL) at 0 °C under argon were sequentially added DIEA (10.6 mL, 60 mmol), BOP (5.08 g, 20 mmol), and (*S*)-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid, methyl ester, hydrochloride (5.68 g, 25 mmol). The mixture was allowed to warm to 5 °C over 2 h, stirred for 16 h, and washed with 1 N hydrochloric acid, saturated sodium bicarbonate and brine. The organic layer was dried, filtered, and concentrated to afford an oil. Chromatography (silica gel, 20% ethyl acetate/hexanes) afforded **23** (4.0 g, 45%): MS $(M + H)^+$ 439.

**(*S*)-1,2,3,4-Tetrahydro-2-[3-methyl-*N*-[(phenylmethoxy)-carbonyl]-L-valyl]-3-isoquinolinecarboxylic Acid (24).** To a solution of **23** (830 mg, 1.9 mmol) in THF/methanol (4 mL/5 mL) was added lithium hydroxide (1 N, 1.9 mL). After 1 h, additional lithium hydroxide (1 N, 1.5 mL) was added. After 2 h, sodium hydroxide (1 N, 1.9 mL) was added. After another 1 h, solvent was removed and the residue was partitioned

between 1 N hydrochloric acid and ethyl acetate. The organic layer was dried and concentrated to afford **24** (820 mg).

**(*R**)-*N*$^2$-[[1,2,3,4-Tetrahydro-2-[3-methyl-*N*-[(phenylmethoxy)carbonyl]-L-valyl]-3-isoquinolinyl]carbonyl]-L-glutamine, 1,1-Dimethylethyl Ester (25).** To a solution of **24** (0.9 g, 2.1 mmol), L-glutamine, *tert*-butyl ester, hydrochloride (0.50 g, 2.1 mmol), and BOP (0.93 g, 2.1 mmol) in 3:1 $CH_3$-CN:DMF (26 mL) was added DIEA (1.1 mL, 6.3 mmol). The solution was stirred for 16 h, the reaction quenched with 1 N HCl (100 mL), and the mixture extracted four times with ethyl acetate, and the combined organic extracts were washed three times with 10% LiCl, dried, filtered, and concentrated. The residue was chromatographed (silica gel, 1:1 hexanes:acetone) to afford **25** (1.1 g, 87%) as a white solid: mp 60−68 °C; MS $(M + H)^+$ 609.

**(*R**)-*N*$^2$-[1,2,3,4-Tetrahydro-2-(3-methyl-L-valyl)-3-isoquinolinyl]carbonyl]-L-glutamine, 1,1-Dimethylethyl Ester, Hydrochloride (26).** Palladium hydroxide on carbon (10%, 91 mg) was added to a solution of **25** (0.91 g, 1.5 mmol) in THF (9.1 mL) with 1 N HCl (1.5 mL). A balloon containing hydrogen was attached to the flask, and the mixture was stirred for 3 h. The mixture was filtered through Celite and the filtrate concentrated under vacuum to afford **26** (0.77 g, 100%) which was used without further purification: MS $(M + H)^+$ 475 (free base).

***N*$^2$-[[(*S*)-2-[*N*-[2-Amino-*N*-[(1,1-dimethylethoxy)carbonyl]-2-[*N*-[(1,1-dimethylethoxy)carbonyl]imidazol-4-yl]-ethyl]-3-methyl-L-valyl]-1,2,3,4-tetrahydro-3-isoquinolinyl]-carbonyl]-L-glutamine, 1,1-Dimethylethyl Ester (27).** To a solution of **22** (0.18 g, 0.47 mmol) in THF (20 mL) at 0 °C under argon was added 1 M lithium aluminum hydride in THF (0.47 mL, 0.47 mmol) dropwise. The solution was stirred for 30 min followed by dropwise addition of 1 M $KHSO_4$ to pH 4. The mixture was stirred for 1 h at 0 °C, ether (40 mL) and water (40 mL) were added, and the layers were separated. The aqueous layer was washed with ether, and the organic layers were pooled, dried, and concentrated. The highly unstable residue was immediately dissolved in dry methanol (20 mL), along with 3 Å molecular sieves (0.5 g), acetic acid (0.2 mL), and **26** (300 mg, 0.58 mmol). The solution was stirred for 10 min followed by the portionwise addition of $NaBH_3CN$ (29 mg, 0.47 mmol) over 1 h. The solution was stirred for 4 h and concentrated, and the residue was chromatographed (silica gel, 19:1 chloroform:methanol). Fractions containing the product were pooled and concentrated to afford 90 mg (25%) of **27** as a slightly yellow oil. Due to instability of the Boc groups, this compound was minimally characterized and carried on to the next step: MS $(M + H)^+$ 784$^+$.

***N*$^2$-[[(*S*)-2-[*N*-[2-Amino-2-(1*H*-imidazol-4-yl)ethyl]-3-methyl-L-valyl]-1,2,3,4-tetrahydro-3-isoquinolinyl]carbonyl]-L-glutamine, Trifluoroactate (1:3) Salt (10).** A solution of **27** (40 mg, 0.05 mmol) in TFA (5 mL) and methylene chloride (5 mL) was stirred for 2.5 h and concentrated. The residue was dissolved in 3 mL of a 50/50 mixture of 0.1% TFA in methanol and 0.1% TFA in water and subjected to HPLC purification (YMC C18 column (S-10, ODS 30 × 500 mm); solvent A, 0.1% TFA in 90% water, 10% methanol; solvent B, 0.1%TFA in 90% methanol, 10% water; 10−35% B in A over 60 min). Fractions containing the major peak were pooled and lyophilized to yield 20 mg (45%) of **10** as a fluffy white solid: MS $(M + H)^+$ 528$^+$; HRMS $(M + H)^+$ 528.2939; MS/MS 110$^+$, 178$^+$, 195$^+$, 256$^+$, 273$^+$, 306$^+$, 365$^+$, 511$^+$, 528$^+$; $^1$H-NMR ($CDCl_3$) $\delta$ 8.40 (1H, m), 7.46 (1H, m), 7.22 (4H, m), 5.10 (1H, m), 4.8−4.3 (5H, m), 4.19 (1H, m), 3.3−3.0 (2H, m), 2.28 (1H, m), 1.92 (2H, m), 1.80 (1H, m), 1.10 (9H, 2s).

**Biological Testing.** Assays for FT and GGTI inhibition,[25] Ras processing inhibition,[26] inhibition of soft agar growth,[26] and phenotypic reversion[26] were performed as previously described. The gross *in vitro* cytotoxicity of FT inhibitors was assessed using untransformed NIH 3T3 ("normal") cells. NIH 3T3 cells were plated at 2500 cells/well in a 96-well microtiter plate, and 24 h later serial dilutions of drugs were added. After incubation at 37 °C for 48 h, the tetrazolium dye XTT (2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-5-[(phenylamino)carbonyl]-2*H*-tetrazolium hydroxide) containing phenazine methosulfate was added. The reduction of XTT as a measure of live cells was determined spectrophotometrically at 450 nm. The results are expressed as MTD (maximum tolerated dose), the highest tested nontoxic concentration of the inhibitor.

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