Expedited Articles

Potent, Selective, and Orally Bioavailable Tricyclic Pyridyl Acetamide *N*-Oxide Inhibitors of Farnesyl Protein Transferase with Enhanced in Vivo Antitumor Activity

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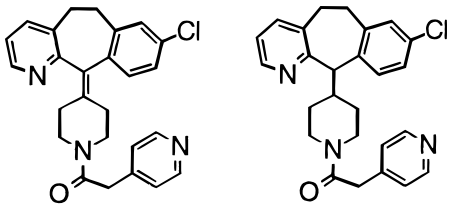
We previously reported compound **1** as a potent farnesyl protein transferase (FPT) inhibitor that exhibited reasonable pharmacokinetic stability and showed moderate in vivo activity against a variety of tumor cell lines. The analogous C-11 single compound, pyridylacetamide **2**, was found to be more potent than **1** in FPT inhibition. Further studies showed that modification of the ethano bridge of the tricyclic ring system by conversion into a double bond with concomitant introduction of a single bond at C-11 piperidine resulted in compound **3** that had superior FPT activity and pharmacokinetic stability. Compound **4**, a 5-bromo-substituted analogue of **3**, showed improved FPT activity, had good cellular activity, and demonstrated a remarkably improved pharmacokinetic profile with AUC of 84.9 and $t_{1/2}$ of 82 min. Compound **4** inhibited the growth of solid tumor in DLD-1 model by 70% at 50 mpk and 52% at 10 mpk.

The ras oncogenes are found activated in a variety of tumors, including colorectal, pancreatic, and lung carcinomas.[1] Ras functions as a molecular switch that regulate the transduction of biological information from the cell surface to the nucleus, being "on" when GTP bound and "off" when bound to GDP. In mutated ras p21, the GTPase activity is much reduced and the protein remains in a locked GTP bound form, leading to uncontrolled cell proliferation.[2]

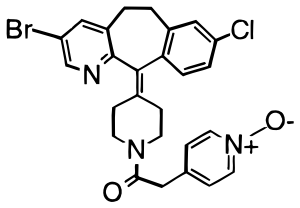
The crucial role played by Ras p21 in signal transduction is mainly dependent on its plasma membrane association[3], thus, Ras is synthesized as a cytosolic precursor and then posttranslationally modified by a C-15 isoprenoid moiety which eventually assists it to anchor to the cell membrane.[4] The first and obligatory step in this modification is the attachment of a farnesyl group to the cysteine residue of the Ras carboxyl terminal sequence CAAX.[5] Farnesyl protein transferase (FPT) catalyzes the transfer of the farnesyl group from farnesyl diphosphate (an intermediate in cholesterol biosynthesis) to p21 ras proteins.[6] It is therefore conceivable that inhibition of FPT would provide an attractive strategy for developing potential anticancer agents.[7]

A number of FPT inhibitors based on the CAAX motif of ras p21 have been reported.[8] Although some of these inhibitors showed very good in vitro activity, they were found to be inactive in the whole cells and in vivo studies, possibly due to poor cellular uptake and metabolic instability.[9] To circumvent this problem, a number of groups have prepared peptidomimetics and pseudopeptides as FPT inhibitors and some of these compounds have shown promising results in cellular and in vivo activity.[10]



SCH 44342

SCH 55495



1

We have previously reported SCH 44342, a nonpeptidic tricyclic compound with FPT and COS activities of 0.25 and 1 μM, respectively.[11] Although this compound did not show any in vivo efficacy, its C-11 saturated analogue, SCH 55495, which was equipotent in FPT activity, demonstrated moderate in vivo activity in mice model studies (inhibiting 30% of tumor growth in SW-620 at 50 mpk bid). More recently, this laboratory has reported a 3-bromo tricyclic pyridineacetic acid *N*-oxide compound **1**,[13] a potent, selective, nonpeptidic,

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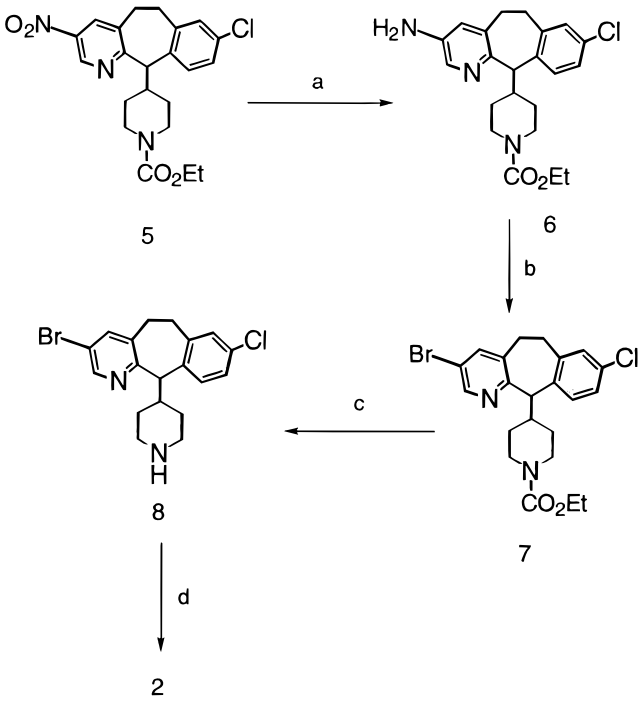
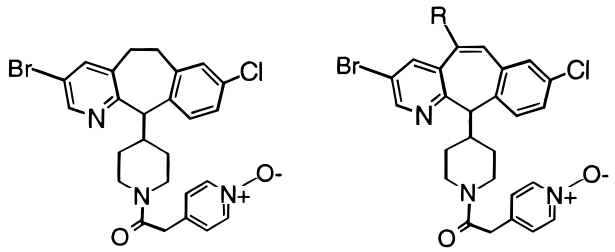
Scheme 1. Preparation of Tricyclic Pyridyl Acetamide *N*-Oxide **2**[a]



[a] (a) iron fillings−$CaCl_2$; (b) concentrated HBr−$NaNO_2$, Br_2; (c) concentrated HCl reflux; (d) DEC−HOBT−NMM, pyridineacetic acid *N*-oxide.

nonsulfhydryl FPT inhibitor with considerably enhanced pharmacological stability and with better in vivo efficacy than the tricyclic benzocycloheptapyridine compounds previously reported. On the basis of these studies, we have extended this work by designing and synthesizing pyridylacetamides **2**, **3**, and **4** and evaluated their FPT, pharmacokinetics profile, and in vivo activities. Modification of the ethano bridge has provided compound **4** which showed superior pharmacokinetics and demonstrated good in vivo activity in DLD-1 tumor model (a human colon carcinoma). These compounds are potential anticancer drugs and will be the subject matter of this report.



2

3, R = H
4, R = Br

Chemistry

Synthesis of compound **2** is outlined in Scheme 1; thus, reduction of nitro carbamate **5**[14] gave the desired amine **6**. Diazotization of **6** followed by treatment with bromine−HBr afforded the 3-bromo carbamate **7.** Hydrolysis of **7** in refluxing HCl followed by coupling the resulting amine with pyridineacetic acid *N*-oxide gave the target pyridylacetamide **2** in 50% yield.

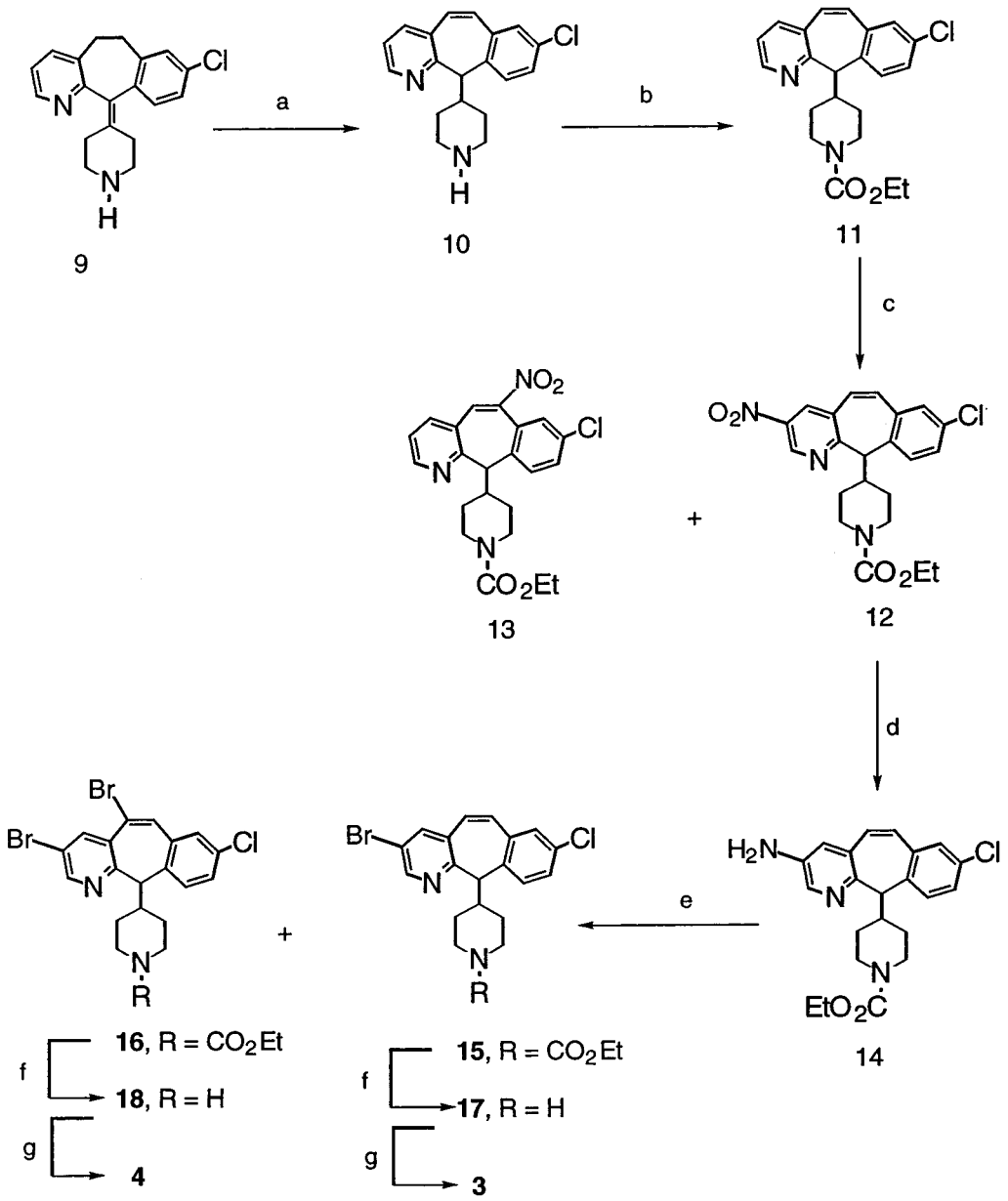
Preparation of target compounds **3** and **4** is outlined in Scheme 2. Synthesis of these two compounds start from the readily available amine **9**.[15] Thus, using similar procedure to that described by Schumacher and co-workers,[16] amine **9** was heated in triflic acid at temperatures around 180 °C for 16 h to give the 5,6-ene amine **10** in 52% yield. Amine **10** was then treated with ethyl chloroformate to give the 5,6-ene carbamate **11** in 57% yield. Reaction of the 5,6-ene carbamate **11** with tetrabutylammonium nitrate−trifluroacetic anhydride nitrating conditions[14] gave compounds **12** and **13** in 26 and 11% yield, respectively. Whereas ^{1}H NMR of **12** exhibited two vinyl protons at δ 6.9 and 7.1, that of **13** showed only one vinyl proton at δ 8.08 as a singlet. The position of the nitro group on compound **13** was established by NOE experiments; thus upon irridation of the singlet at δ 8.08, an NOE enhancement on H-4 was observed, clearly indicating that the vinyl proton was at 5-position of the tricyclic ring system and that the nitro group was at the 6-position. Reduction of **12** with iron filings in the presence of $CaCl_2$ in refluxing 85% aqueous ethanol gave the amine **14** in quantitative yield. Diazotization with HBr−$NaNO_2$ in the presence of molecular bromine provided the 3-bromo carbamate **15** and the 3, 5-dibromo carbamate **16** in 57% and 21% yield, respectively**.** The latter is presumably formed through bromination−dehydrobromination of **15.** 3-Bromo carbamate **15** was hydrolyzed in refluxing HCl to afford the 3-bromo amine **17**. Coupling of **17** with pyridineacetic acid *N*-oxide using DEC−HOBT gave amide **3** in 54% yield. In a similar manner, 3,5-dibromo carbamate **16** was hydrolyzed to amine **18**. The latter was subsequently coupled with pyridineacetic acid *N*-oxide to afford pyridyl acetamide *N*-oxide **4** in 68% yield.

Results and Discussions

Compounds prepared in this study were tested for their ability to inhibit the FPT-catalyzed transfer of [^{3}H]farnesyl moiety from farnesyl pyrophosphate to H-Ras-CVLS and also to the closely related GGPT-1 that catalyzes the transfer of [^{3}H]geranylgeranyl moiety from geranyl-geranyl pyrophosphate to H-Ras-CVLL using conditions previously described.[17] These compounds were also evaluated in a COS cell assay for their cellular activity using previously described procedure.[17] Biological and pharmacokinetic data for these compounds are summarized in Tables 1 and 2. In vivo antitumor efficacy and dose response results are presented in Table 3 and Figure 1A−C, respectively.

The discovery that introduction of a bromine at the 3-position of the tricyclic ring system pyridine resulted in greatly enhanced FPT activity,[13] coupled to the fact that pyridylacetyl *N*-oxide derivatives demonstrated superior pharmacokinetic profile, prompted us to prepare tricyclic analogues that incorporated some of these features. Our earlier observations that SCH 55495[11] showed some antitumor activity encouraged us to make the C-11 saturated analogue **2**. Compound **2** inhibited 50% of FPT at 40 nM and was also found to be active in the inhibition of Ras p21 modification in whole cells as evidenced by its COS cell inhibitory activity of IC_{50} = 0.4 μM (Table 1). However, compound **2** showed poorer po pharmacokinetic stability than the analogous double bond compound **1** with AUC of 4.1 and $t_{1/2}$ of 46 min

Scheme 2. Preparation of Tricyclic Pyridyl Acetamide *N*-Oxides **3** and **4**[a]



[a] (a) triflic acid, Δ; (b) EtOCOCl−Et_3N; (c) *n*-Bu_4NNO_3−TFAA; (d) iron filings−$CaCl_2$; (e) HBr−$NaNO_2$, Br_2; (f) concentrated HCl reflux; (g) DEC−HOBT−NMM, pyridine acetic acid *N*-oxide.

Table 1. FPT, COS, and GGPT Activities of Compounds **1**, **2**, **3**, and **4**

	IC_{50} (μM)		
compd	FPT	GGPT	COS cell
1	0.09	38	0.6
2	0.04	>38	0.4
3	0.03	>38	0.7
4	0.04	>33	0.6

Table 2. Pharmacokinetic Profile for FPT Compounds (Dosed at 25 mg/kg in Mice as Solutions of HCl Salts)

compound	AUC (po) (μg·h/mL)	C_{max} (po) μg/mL	AUC (iv) (μg·h/mL)	$t_{1/2}$ (iv) (min)	bioavailability % (po)
1	12.9[a]	10	17.3[a]	48	75
2	4.1[b]	10	10.6[b]	46	38
3	19[b]	11	18.6[b]	26.6	100
4	84.9[b]	12	43.7[b]	82.2	(100)[c]

[a] AUC (0−7 h). [b] AUC (0−24 h). [c] See pharmacokinetic studies in the Experimental Section for discussion: po = oral, iv = intravenous, AUC = area under the concentration time curve.

(cf. AUC of 12.9 for **1** (Table 2)). Compound **2** had a similar half-life to **1** but demonstrated lower bioavailablity (38%) than **1** (75%). Compound **2** did not show any GGPTase activity at 38 μM and was found to inhibit growth of tumors in mice by 38% when given orally at 10 mpk qid and 60% at 50 mpk.

In an earlier publication, Piwinski and co-workers[18] had shown that Loratadine, a tricyclic benzacycloheptapyridine, was metabolized to give, among other products, hydroxyl metabolites at the 5- and 6-positions of the bridgehead. We therefore sought to protect the bridgehead by introducing a double bond with and without substitution. Compound **3,** the unsubstituted analogue, had similar FPT activity to that of compound **2** (Table 1) but had substantially better pharmacokinetics (po and iv AUC 19 and 18.6 μg·h/mL respectively (Table 2)). Although compound **3** was 100% bioavailable, it exhibited a shorter half-life (27 min)

Table 3. Percent Inhibition of Tumor Growth in DLD-1 Tumor Model, Compound Given po qid for 14−28 days

compd	10 mpk	50 mpk
1	28[b]	40[a]
2	38[d]	60[b]
3	21[c]	31[a]
4	52[b]	70[a]

[a] $p < 0.01$. [b] $0.01 < p < 0.025$. [c] $0.05 < p < 0.1$. [d] $0.1 < p < 0.25$.

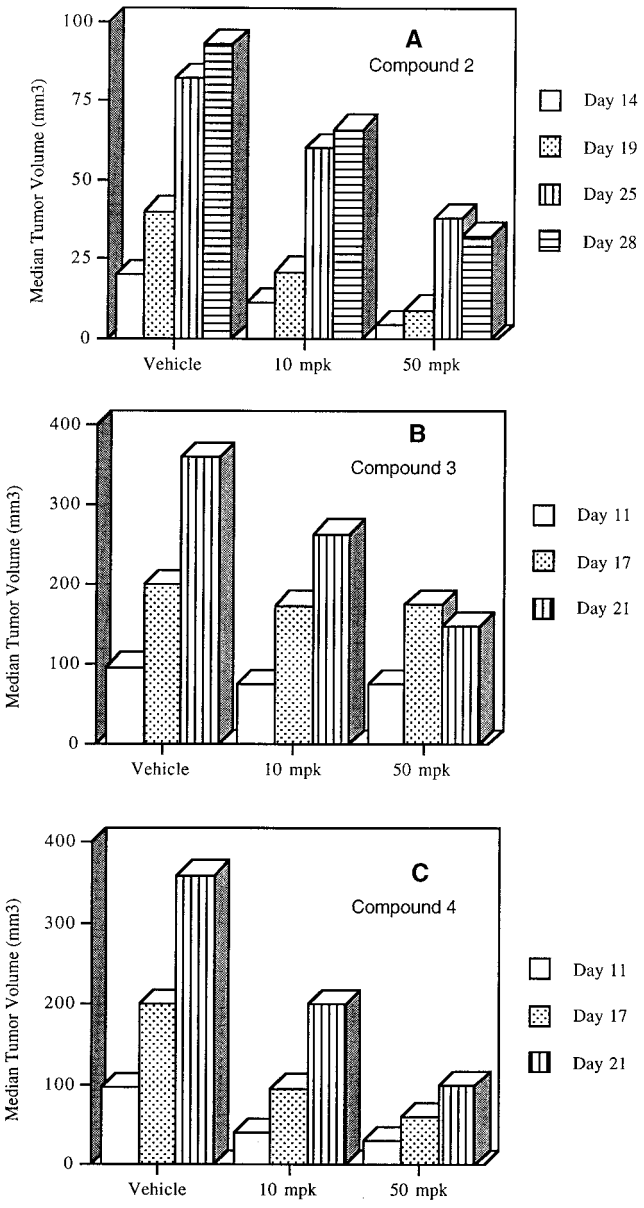


Figure 1. Antitumor activity of targets 2−4 in DLD tumor model. (See Experimental Section for assay conditions.)

than either compound **1** or **2** (Table 2). Compound **3** was found to be less potent in the antitumor efficacy studies than **2,** probably due to its shorter half-life. Compound **3** inhibited growth of tumor cells in nude mice by 21% at 10 mpk and 37% at 50 mpk when given po.

Further elaboration of the bridgehead was achieved by preparation of the 5-bromo-ene compound **4**. Although **4** had FPT activity similar to that of **3** (30 nM vs 40 nM), it had greatly improved pharmacokinetic stability (AUC of 84.9, C_{max} of 12 and $t_{1/2}$ of 82 min (Table 2)), with 100% bioavailability. The improved pharmacokinetic stability of **4** was clearly manifested in the efficacy studies; thus, compound **4** reduced the growth of solid tumors in DLD-1 model by 52% at 10 mpk and 70% at 10 mpk PO. Theses results showed that **4** was remarkably better than compounds **1**−**3** as antitumor agents (Table 3 and Figure 1A−C).

Conclusion

Modification of the bridgehead of the tricyclic ring system has provided compounds that have better pharmacokinetic properties than the unmodified ones. The 5,6-ene compound **3** is pharmacokinetically more stable than its saturated counterpart compound **2**. However, compound **3** suffered from a major set-back in that it had a shorter half-life than its closely related analogues. Introduction of a bromine at the 5-position of the bridgehead resulted in compound **4** which was found to be the most pharmacokinetically stable compound in this series. The enhanced pharmacokinetic properties of compound **4** was clearly reflected in its enhanced efficacy in the DLD tumor model compared to its closely related counterparts, compounds **1**−**3**. The present study indicates that appropriate modification of the bridgehead of the tricyclic ring system would give rise to potent FPT inhibitors with greatly enhanced pharmacokinetic properties. The study reveals that improved pharmacokinetics could be translated into improved antitumor activity.

Experimental Section

Melting points were determined with an Electrothermal digital melting point apparatus and are uncorrected. Elemental analysis were performed by the Physical-Analytical Chemistry Department, Schering-Plough Research Institute, on either a Leeman CE 440 or a FISONS EA 1108 elemental analyzer. FT-IR spectra were recorded using a BOMEN Michelson 120 spectrometer. Mass spectra were recorded using either EXTREL 401 (Chemical Ionization) JEOL, or MAT-90 (FAB), VG ZAB-SE (SIMS), or Finnigan MAT-CH-5 (EI) spectrometer. In general, structures of the compounds were determined by coupling constants, coupling information from the COSY spectra, and 1D NOE experiments. The ^1H and ^{13}C NMR spectra were obtained on either Varian VXR-200 (200 MHz, ^1H), Varian Gemini-300 (300 MHz, ^1H; 75.5 MHz, ^{13}C) or XL-400 (400 MHz, ^1H; 100 MHz, ^{13}C) and are reported as ppm downfield from Me_4Si with number of protons, multiplicities, and coupling constants in hertz indicated parenthetically. For ^{13}C NMR, a Nalorac Quad nuclei probe was used.

4-[3-Bromo-8-chloro-6,11-dihydro-5H-benzo[5,6]-cyclohepta[1,2-*b*]pyridin-11-yl]-1-[(4-pyridinyl)acetyl]-piperidine *N*1-Oxide (2). Nitrocarbamate **5** (2.03 g, 4.7 mmol) was dissolved in 150 mL of 85% EtOH−H_2O. To this solution were added iron powder (2.4 g, 42.3 mmol) and $CaCl_2$ (0.24 g, 2.1 mmol), and the reaction mixture was refluxed for 16 h. The reaction mixture was filtered through Celite and extensively washed with hot EtOH. It was then treated with decolorized charcoal and filtered, and the organic solvents were removed to give amine **6** in 100% crude yield: ^1H NMR (200 MHz, DMSO) δ 0.90−1.20 (m, 4H), 1.10 (t, J = 7.5 Hz, 3H), 2.10−2.20 (m, 1H), 2.50−3.00 (m, 2H), 3.10−3.50 (m, 3H), 3.60−4.10 (m, 2H), 4.06 (q, J = 7.5 Hz, 2H), 4.40 (t, J = 5.0 Hz, 2H) 5.20 (brs, 2H), 6.70 (d, J = 2.5 Hz, 1H), 7.15−7.30 (m, 3H),7.70 (d, J = 2.5 Hz, 1H); MS m/z 400.3 (MH^+).

Amino carbamate **6** (2.06 g, 5.20 mmol) was dissolved in 20 mL of 48% HBr. The reaction mixture was then cooled to −5 °C, and 1.4 mL of molecular bromine was then added. The reaction mixture was stirred at that temperature for 15 min

after which $NaNO_2$ (1.1 g, 15.5 mmol) dissolved in 10 mL of H_2O was slowly added. The reaction mixture was stirred for 45 min and then neutralized with 40% NaOH to pH = 10. The aqueous phase was extracted with EtOAc. Combined EtOAc fractions were dried over $MgSO_4$ and concentrated to give ~2.4 g of 3-bromo carbamate **7**: ^{1}H NMR (200 MHz, $CDCL_3$) δ 1.10−1.50 (m, 4H), 1.25 (t, J = 7.5 Hz, 3H), 1.80−1.90 (m, 1H), 2.40−2.70 (m, 2H), 2.90−3.10 (m, 1H), 3.50−3.80 (m, 2H), 4.06 (q, J = 7.5 Hz, 2H), 4.40 (t, J = 5.0 Hz, 2H), 7.15−7.40 (m, 3H), 8.35 (brs, 1H), 8.75 (brs, 1H); MS m/z 465 (MH^+).

To 15 mL of concentrated HCl was added 3-bromo carbamate **7** (2.3 g, 5.00 mmol). The reaction mixture was refluxed for 16 h. It was then cooled, poured into ice, and neutralized with 50% aqueous NaOH. The aqueous phase was extracted with CH_2Cl_2. Concentration of the organic phase afforded 1.39 g of amine **8** (69% yield) which was used for subsequent reaction without further purification: δ ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.10−1.60 (m, 4H), 2.20−2.70 (m, 3H), 2.80−3.60 (m, 6H), 3.90 (d, J = 10.0 Hz, 1H), 7.10−7.20 (m, 3H), 7.55 (d, J = 2.5 Hz, 1H), 8.40 (d, J = 2.5 Hz, 1H).

To a solution of amine **8** (3.00 g, 7.66 mmol) in 40 mL of DMF were added 4-pyridylacetic acid *N*-oxide (2.78 g, 15.32 mmol), HOBT (2.1 g, 15.32 mmol), DEC (2.9 g, 15.32 mmol), and *N*-methylmorpholine (7.7 g, 8.4 mL, 76.6 mmol), and the mixture was stirred at room temperature for 16 h. All of the volatiles were removed by rotary evaporation. The crude mixture was dissolved in CH_2Cl_2. The organic phase was washed with saturated $NaHCO_3$ and brine and then dried over $MgSO_4$. It was then concentrated and purified on silica gel, eluting with 5% MeOH−CH_2Cl_2 to afford the 2.01 g of **2** (50% yield) as a white solid: mp 134−135 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.10−1.70 (m, 4H), 2.30−2.60 (m, 2H), 2.80−3.10 (m, 3H), 3.20−3.50 (m, 2H), 3.60 (s, 2H), 3.70−3.80 (m, 1H), 3.89−3.90 (d, J = 10 Hz, 1H), 4.55 (d, J = 15 Hz, 1H), 7.00−7.20 (m, 5H), 7.50 (d, J = 2.5 Hz, 1H), 8.15 (d, J = 7.5 Hz, 2H), 8.35 (d, J = 2.5 Hz, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 30.7, 31.6, 38.5, 38.7, 42.1, 46.0, 46.7, 61.6, 118.7, 126.2, 126.3, 126.7, 126.8 129.7, 130.0, 132.8, 132.9, 133.2, 134.2, 138.9, 140.8, 140.9, 146.9, 147.1, 147.2, 166.8; IR (film) ν_{max} 805, 1241, 1439, 1637; MS m/z 528 (MH^+). Anal. Calcd for $C_{26}H_{25}N_3O_2BrCl\cdot1.5H_2O$: C, 56.38; H, 5.10; N, 7.59. Found: C, 56.64; H, 4.72; N, 7.63.

4-[3-Bromo-8-chloro-11*H*-benzo[5,6]cyclohepta[1,2-*b*]-pyridin-11-yl]-1-[(4-pyridinyl)acetyl]piperidinecarboxylic Acid Ethyl Ester (15) and 4-[8-Chloro-3,5-dibromobromo-11*H*-benzo[5,6]cyclohepta[1,2-*b*]pyridin-11-yl]-1-piperidinecarboxylic Acid Ethyl Ester (16). Amine **9**[2c] (20.55 g, 66 mmol) was dissolved in 120 mL of triflurometh ane sulfonic acid and refluxed for 72 h. The reaction mixture was then cooled, poured into ice water, and basified with 50% aqueous NaOH. The resulting crude product was extracted with EtOAc and purified on silica gel chromatography, eluting with 3% MeOH (saturated with NH_3)−CH_2Cl_2 to afford 10.4 g of amine **10** (52% yield): ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.98−1.55 (m, 3H), 2.20 (d, J = 15 Hz, 1H), 2.10−2.30 (m, 1H) 2.60−2.80 (m, 2H), 3.20−3.40 (m, 2H), 4.15 (d, J = 15 Hz, 1H), 6.85 (d, J = 12.5 Hz, 1H), 6.95 (d, J = 12.5 Hz, 1H), 7.13−7.38 (m, 4H), 7.66 (dd, J = 7.5, 2.5 Hz, 1H), 8.52 (dd, J = 5.0 Hz, 2.5 Hz, 1H); MS m/z (rel intens) 311 (100, MH^+).

Amine **10** (1.14 g, 3.7 mmol) was dissolved in 50 mL of anhydrous toluene, and Et_3N (2.9 g 29.4 mmol, 4.10 mL, 8 equiv) and ethyl chloroformate (3.89 g, 35.7 mmol, 3.4 mL, 9.7 equiv) were added. The reaction mixture was refluxed for 12 h. It was then cooled to room temperature, and all of the volatiles were removed. The crude material was partitioned between EtOAc and water, and the aqueous phase was extracted with EtOAc. Combined EtOAc fractions were dried over $MgSO_4$, and the resulting extract was purified on normal phase HPLC eluting with 2% MeOH (saturated with NH_3)−CH_2Cl_2 to afford 0.78 g (57% yield) of carbamate **11** as a white solid: ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.98−1.12 (m, 3H), 1.21 (t, J = 7.5 Hz, 3H), 1.95−2.20 (m, 1H), 2.31−2.57 (m, 2H), 3.86−4.18 (m, 4H), 4.08 (q, J = 7.5 Hz, 2H), 6.85 (d, J = 12.5 Hz, 1H), 6.95 (d, J = 12.5 Hz, 1H), 7.13−7.38 (m, 4H), 7.66 (dd, J = 7.5 Hz, 2.5 Hz, 1H), 8.52 (dd, J = 5.0 Hz, 2.5 Hz, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) d 15.1, 31.1, 31.3, 33.7, 43.9, 44.0, 61.5, 62.8, 122.3, 129.2, 129.3, 129.7, 131.3, 132.8, 132.9, 135.0, 136.9, 137.7, 149.4, 155.8, 156.9, 159.9; IR (film) ν_{max} 1108, 1694, 2934, 2980; MS m/z (rel intens) 226 (6), 337 (9), 383 (100, MH^+).

Tricyclic carbamate **11** (14.95 g, 39.1 mmol) was dissolved in 150 mL of CH_2Cl_2 under N_2 atmosphere and stirred at ~0 °C. To this solution was added a mixture of Bu_4NNO_3 (13.07 g, 43 mmol) and trifluoroacetic anhydride (9.02 g, 6.1 mL, 43 mmol) dissolved in 20 mL of CH_2Cl_2 and cooled to 0 °C. The reaction mixture was stirred at 0 °C for 2 h and then allowed to come to room temperature overnight. It was then basified with saturated $NaHCO_3$ and extracted with CH_2Cl_2. The combined organic phase was dried over $MgSO_4$ and concentrated. Purification on silica gel eluting with 20−70% EtOAc−hexanes gradient gave the 3-nitro tricyclic carbamate **12** as the more polar product in 26% yield: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.98−1.28 (m, 3H), 1.22 (t, J = 7.11 Hz, 3H), 2.06−2.19 (m, 1H), 2.37−2.57 (m, 2H), 3.89−4.14 (m, 3H), 4.08 (q, J = 7.2 Hz, 2H), 4.23 (d, J = 11.0 Hz, 1H), 6.90 (d, J = 11.9 Hz, 1H), 7.10 (d, J = 12.0 Hz, 1H), 7.23−7.29 (m, 1H), 7.34−7.44 (m, 2H), 8.43 (d, J = 2.0 Hz, 1H), 9.31 (d, J = 2.0 Hz, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) d14.7, 30.7, 33.3, 43.4, 43.5, 61.3, 62.4, 126.6, 128.5, 129.8, 131.6,132.7, 133.2, 133.6, 134.2, 135.6, 143.1, 143.8, 155.4, 162.0; IR (film) ν_{max} 1114, 1432, 1523, 1699, 2939, 2981, 3069, 3448; MS m/z (rel intens) 428.1 (100, MH^+).

The 6-nitro carbamate **13** was obtained as the less polar compound in 11% yield: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.09−1.35 (m, 3H), 1.22 (t, J = 7.5 Hz, 3H), 1.87−2.02 (m, 1H), 2.40−2.60 (m, 2H), 3.94−4.21 (m, 4H), 4.09 (q, J = 7.5 Hz, 2H), 7.29−7.37 (m, 2H), 7.40 (d, J = 3.8 Hz, 1H), 7.46 (dd, J = 7.5, 3.8 Hz, 1H), 7.88 (dd, J = 7.5, 3.8 Hz, 1H), 8.08 (s, 1H), 8.68 (dd, J = 5.6, 2.0 Hz, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 14.7, 31.0, 34.7, 43.3, 43.6, 61.2, 62.6, 121.1, 122.4, 127.0, 128.3, 128.8, 131.4, 132.5, 133.0, 139.4, 150.6, 151.8, 155.4, 158.5; IR (film) ν_{max} 755, 1111, 1436, 1694, 2851, 2934, 3057, 3438; MS m/z (rel intens) 428.3 (100, MH^+).

Nitrocarbamate **12** (22 g, 51.42 mmol) was dissolved in 150 mL of 85% EtOH−H_2O. To this solution were added iron powder (25.85 g, 462.7 mmol) and $CaCl_2$ (2.42 g, 21.8 mmol), and the reaction mixture was refluxed for 16 h. The reaction mixture was filtered through Celite and extensively washed with hot EtOH. It was then treated with decolorized charcoal and filtered, the organic solvents were removed, and resulting crude product was chromatographed on silica gel, eluting with 10% MeOH−CH_2Cl_2 to give amine **14** in 100% crude yield: ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.81−1.28 (m, 4H), 1.23 (t, J = 7.5 Hz, 3H), 1.67−1.82 (m, 1H), 1.90−2.13 (m, 1H), 2.37−2.56 (m, 2H), 3.68 (s, 2H), 3.82−4.16 (m, 1H), 3.92 (d, J = 10.0 Hz, 1H), 4.06 (q, J = 7.5 Hz, 2H), 6.72 (d, J = 10.0 Hz, 1H), 6.88 (d, J = 10.0 Hz, 1H), 6.95 (d, J = 2.5 Hz, 1H), 7.17−7.36 (m, 3H), 8.01 (d, J = 2.5 Hz, 1H); MS m/z 398.2 (MH^+).

Amino carbamate **14** (16.47 g, 41.4 mmol) was dissolved in 150 mL of 48% HBr. The reaction mixture was cooled to −5 °C, and 18 mL of molecular bromine was then added. The reaction mixture was stirred at that temperature for 20 min after which $NaNO_2$ (8.55 g, 123.9 mmol) dissolved in 85 mL of H_2O was slowly added. The reaction mixture was stirred for 45 min and then neutralized with 50% NaOH to pH = 10. The aqueous phase was extracted with EtOAc. Combined EtOAc fractions were dried over Na_2SO_4 and concentrated. Purification on silica gel eluting with 30% EtOAc−hexane gave the 3-bromo carbamate **15** (10.61 g, 23 mmol) as the more polar compound in 57% yield: ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.76−1.32 (m, 2H), 1.23 (t, J = 7.2 Hz, 3H), 1.60−1.87 (m, 2H), 1.94−2.18 (m, 2H), 2.34−2.58 (m, 3H), 3.85−4.23 (m, 2H), 4.08 (q, J = 7.0 Hz, 2H), 6.76 (d, J = 15.0 Hz, 1H), 6.98 (d, J = 15.0 Hz, 1H), 7.18−7.40 (m, 3H), 7.78 (d, J = 2.0 Hz, 1H), 8.55 (d, J = 2.2 Hz, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 14.66, 30.73, 30.791, 33.33, 43.49, 43.58, 61.16, 61.85, 118.62, 127.24, 129.27, 129.57, 130.26, 132.30, 132.52, 132.65, 134.35, 136.38,

139.14, 149.77, 154.96, 155.39; IR (film) ν_{max} 1112, 1247, 1433, 1693, 2849, 2934, 2979, 3450; MS *m/z* 463 (MH^+).

The less polar product, 3,5-dibromo carbamate **16**, was obtained in 21% yield: ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.94−1.33 (m, 4H), 1.22 (t, J = 7.5 Hz, 3H), 1.98−2.21 (m, 2H), 2.46−2.67 (m, 2H), 3.93−4.20 (m, 2H), 4.06 (q, J = 7.5 Hz, 2H), 7.17−7.39 (m, 3H), 7.62 (s, 1H), 8.32 (d, J = 2.5 Hz, 1H), 8.57 (d, J = 2.5 Hz, 1H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 15.0, 31.3, 33.9, 34.0, 43.9, 44.0, 61.6, 62.3, 119.6, 120.4, 129.4, 130.0, 131.2, 132.7, 133.3, 133.8, 135.0, 137.2, 141.3, 151.6, 155.4, 155.7; IR (film) ν_{max} 1113, 1247, 1427, 1695, 2849, 3449; MS *m/z* (rel intens) 541.0 (100).

4-[3-Bromo-8-chloro-11*H*-benzo[5,6]cyclohepta[1,2-*b*]-pyridin-11-yl]-1-[(4-pyridinyl)acetyl]piperidine *N*1-Oxide (3). To 150 mL of concentrated HCl was added 3-bromo carbamate **15** (10.6 g, 23 mmol). The reaction mixture was refluxed for 16 h. It was then cooled, poured into ice, and neutralized with concentrated NH_4OH. The aqueous phase was extracted with EtOAc. Concentration of the organic phase afforded 9 g of amine **17** which was used in the subsequent reaction without further purification: ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.76−1.32 (m, 4H), 1.90−2.14 (m, 1H), 2.23−2.42 (m, 1H), 2.85−3.03 (m, 1H), 4.05 (br, d, J = 10.0 Hz, 1H), 6.73 (d, J = 15.0 Hz, 1H), 6.98 (d, J = 15.0 Hz, 1H), 7.19−7.41 (m, 3H), 7.79 (d, J = 2.5 Hz, 1H), 8.55 (d, J = 2.5 Hz, 1H); IR (film) ν_{max} 475, 562, 715, 790, 815, 899, 1108, 1376, 1433, 1481, 2845, 2924, 3026, 3270, 3434; MS *m/z* (rel intens) 306.9 (12.04), 389.0 (82.39), 390.0 (21.32), 391.0 (100 MH^+), 392.0 (24.57), 393.0 (28.62).

To a solution of the amine **17** (0.2 g, 0.51 mmol) in 6 mL of DMF was added 4-pyridylacetic acid *N*-oxide (0.16 g, 1.02 mmol), HOBT (0.152 g, 1.1 mmol), DEC (0.25, 1.3 mmol), and *N*-methylmorpholine (0.114 g, 124 mL, 1.1 mmol), and the mixture was stirred at room temperature for 16 h. The organic phase was washed with saturated $NaHCO_3$ and brine and then dried over Na_2SO_4. It was then concentrated and purified on silica gel, eluting with 1−10% MeOH (saturated with ammonia)−CH_2Cl_2 to afford the **3** in 54% yield as a white solid: ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.80−1.20 (m, 4H), 2.05−2.20 (m, 1H), 2.25−2.50 (m, 1H), 2.70−2.95 (m, 1H), 3,65 (s, 2H), 3.60−3.80 (m, 1H), 4.00 (d, J = 10 Hz, 1H), 4.45 (d, J = 15 Hz, 1H), 6.75 (dd, J = 5 Hz, J = 20 Hz, 1H), 6.95 (dd, J = 5 Hz, J = 20 Hz, 1H), 7.10 (d, J = 5 Hz, 1H), 7.20−7.45 (m, 4H), 7.80 (d, J = 2.5 Hz,1H), 8.10 (d, J = 5 Hz, 2H), 8.55 (d, J = 2.5 Hz, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 30.6, 31.5, 33.4, 38.6, 38.7, 41.8, 45.7, 61.6, 119.3, 119.9, 120.2, 127.2, 129.0, 129.7, 130.6, 132.2, 133.3, 134.6, 134.4, 136.3, 138.9, 140.9, 154.6, 166.6; IR (film) ν_{max} 806, 1096, 1342, 1426, 1640; MS *m/z* (rel intens) 526 (100, MH^+). Anal. Calcd for $C_{26}H_{23}N_3O_2BrCl\cdot1.7H_2O$: C, 56.22; H, 4.79; N, 7.56. Found: C, 56.16; H, 4.38; N, 7.56.

4-[8-Chloro-3,5-dibromobromo-11*H*-benzo[5,6]cyclohepta[1,2-*b*]pyridin-11-yl]-1-[(4-pyridinyl)acetyl]piperidine *N*1-Oxide (4). To 15 mL of concentrated HCl was added 3,5-dibroromo carbamate **16** (1.0 g, 1.9 mmol). H_2O (15 mL) was also added. The reaction mixture was refluxed for 16 h. It was then cooled, poured into ice, and neutralized with 50% aqueous NaOH. The aqueous phase was extracted with EtOAc. Concentration of the organic phase afforded 0.5 g of amine **18** which was used in the subsequent reaction without further purification: ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.80−1.30 (m, 4H), 1.90−2.20 (m, 1H), 2.30−2.50 (m, 2H), 2.90−3.00 (m, 3H), 4.10 (d, J = 10 Hz, 1H), 7.10−7.40 (m, 3H), 8.30 (d, J = 2.5 Hz, 1H), 8.50 (d, J = 2.5 Hz, 1H).

To a solution of the amine **18** (0.15 g, 0.32 mmol) in 3 mL of CH_2Cl_2 was added 4-pyridylacetic acid *N*-oxide (0.098 g, 0.64 mmol), HOBT (0.87 g, 0.64 mmol), DEC (0.122, 0.64 mmol), and 15 drops of DMF, and the mixture was stirred at room temperature for 16 h. The organic phase was washed with saturated $NaHCO_3$ and brine and then dried over Na_2SO_4. It was then concentrated and purified on silica gel Prep plate eluting with 10% MeOH (saturated with ammonia)−CH_2Cl_2 to afford **4** in 68% yield as a white solid: ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.80−1.30 (m, 4H), 2.05−2.30 (m, 1H), 2.35−2.55 (m, 1H), 2.70−2.95 (m, 1H), 3.65 (s, 2H), 3.60−3.80 (m, 1H), 4.00 (d, J = 10 Hz, 1H), 4.45 (d, J = 15 Hz, 1H), 7.15 (d, J = 5 Hz, 1H), 7.20−7.45 (m, 4H), 7.60 (d, J = 2.5 Hz, 1H), 8.15 (d, J = 5 Hz, 2H), 8.30 (d, J = 2.5 Hz, 1H), 8.55 (d, J = 2.5 Hz, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 30.3, 31.4, 33.63, 33.3, 38.5, 38.7, 41.8, 45.6, 61.6, 118.7, 126.7, 127.0, 127.1, 129.0, 129.3, 129.4, 129.6, 132.1, 132.4, 135.9, 136.0, 138.9, 139.1, 138.9, 149.9, 154.6, 166.7; IR (film) ν_{max} 820, 1104, 1175, 1482, 1637; MS *m/z* (rel intens) 604 (100, MH^+). Anal. Calcd for $C_{26}H_{22}N_3O_2Br_2Cl\cdot1.7H_2O\cdot0.2CH_2Cl_2$: C, 48.31; H, 3.99; N, 6.42. Found: C, 48.09; H, 3.62; N, 6.42.

In Vitro Enzyme Assays. FPT activity was determined by measuring transfer of [^{3}H]farnesyl from [^{3}H]farnesyl pyrophosphate to trichloroacetic acid−precipitable Ha-Ras-CVLS as previously described.[17] GGPT-1 activity was similarly determined using [^{3}H]geranylgeranyl diphosphate and Ha-Ras-CVLL as substrates.[17]

Cellular Assays for Inhibition of Ha-Ras Processing and Transforming Function. Inhibition of intracellular processing of H-Ras by inhibitors was measured in transfected Cos cells as described previously.[17]

Cell Lines for in Vivo Studies. The human colon carcinoma DLD-1 cell line was obtained from American Type Culture Collection (Rockville, MD).

In Vivo Efficacy Studies. All animal studies were carried out in the animal facility of Schering-Plough Research Institute in accordance with institutional guidelines. After a week of acclimation, 5−7-week-old female nude mice (Crl:Nu/Nu-nu Br, Charles River Laboratories, Wilmington, MA) were subcutaneously inoculated with various cell lines on day 0. The number of cells inoculated were 5×10^6 for DLD-1. Animals were randomly assigned to control and treatment groups (10 animals per group) before the first treatment. Drug treatment at either 10 mg/kg body weight (mg/kg) or 50 mg/kg was initiated on day 1. Appropriate tricyclic inhibitor was dissolved in 20% (w/v) hydroxypropyl-β-cyclodextrin (HPβCD). Vehicle controls received 20% HPβCD. Vehicle or drug solution (0.1 mL) was administrated by oral gavage every 6 h (qid) for 14−28 days. A no treatment control was always included along with the vehicle control to evaluate the influence of vehicle and of the qid gavage treatment. Once palpable, tumor volume was measured in three dimensions twice weekly and calculated with the formula of $V = {}^1/_6\pi LWT$, where L, W, and T represent length, width, and thickness, respectively.[17] T/C value in percent was calculated for each measurement where T and C were the median tumor volumes of the treated and control groups, respectively. Growth inhibition was used to compare efficacy of various treatments and was derived by subtracting the final T/C values of each treatment from 100. Single-tailed Student's t test was used for statistical analysis.

Pharmacokinetic Studies. Nude mice were also used to study the pharmacokinetic properties of the tricyclic inhibitors. Blood samples were collected at nine time points (2 min, 5 min, 15 min, 30 min, 1 h, 2 h, 4 h, 7 h, and 24 h) after a single oral or intravenous (tail vein) dose of 25 mg/kg inhibitor in 20% HPβCD. Two mice were used for each time point, and samples were collected by cardiac puncture after euthanasia with carbon dioxide. After clotting on ice, serum was isolated by centrifugation. Quantitation of inhibitor serum levels was achieved using acetonitrile precipitation, followed by high-performance liquid chromatography−atmospheric pressure chemical ionization (APCI) tandem mass spectrometry. The mean serum concentration at each point was used to construct a plot of the serum concentration versus time. The area under this curve (AUC) was calculated using the linear trapezoidal rule for both the oral and the intravenous experiments. The bioavailability was calculated by determination of the ratio of the AUC after oral and intravenous administration. Since the animals utilized for each oral and intravenous portions of the experiment were different, bioavailability numbers exceeding 100% were occasionally determined. The numbers were attributed to somewhat high interanimal variability observed in this species. The highest observed mean concentration after oral administration was reported as C_{max} value. The serum

half-life after intravenous administration was determined by graphical estimation. A detailed description of the analytical methodology has been described for an earlier analogue in this series.[19]

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