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Atropisomeric Trihalobenzocycloheptapyridine Analogues Provide Stereoselective FPT Inhibitors with Antitumor Activity†

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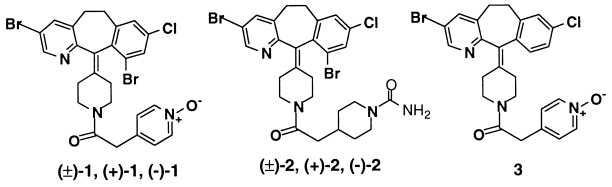
Abstract—Introduction of bromine at the 10-position of 3-bromo-8-chloro-benzocycloheptapyridine analogues of type **3** results in formation of atropisomeric compounds of type (±)-**1** and (±)-**2** that are easily separable at room temperature on a ChiralPak® AD column providing pure atropisomers, (+)-**1**, (−)-**1**, and (+)-**2** (−)-**2**, respectively. Evaluation of the FPT activity of these atropisomers revealed that compounds (+)-**1** and (+)-**2** were more potent in the FPT enzyme and cellular assay than their (−)-isomer counterparts. Compounds (+)-**1** and (+)-**2** were found to inhibit FPT processing in COS cells at low micro molar range. They were also found to have excellent cellular antitumor activity. Evaluation of compound (+)-**1** and (+)-**2** in DLD-tumor model in nude mice revealed that they were efficacious, inhibiting tumor growth by 55 and 63% at 50 mpk, respectively. © 1999 Published by Elsevier Science Ltd. All rights reserved.

Introduction

The notion of chiral recognition in drug–receptor interactions has been an important subject in medicinal chemistry for a long time.[1] Many of the biologically active compounds are chiral in nature and in most cases their respective enantiomers have different biochemical and pharmacological effects.[2] In general, the therapeutic action resides in one of the antipodes.[3] Most of the studies reported to date have been carried out on molecules whose chirality arise from one or more asymmetric centers. However, in recent years a number of cases have been reported where chirality has been observed on various molecules by virtue of molecular asymmetry rather than the presence of a chiral center.[4]

During our search for potent farnesyl protein transferase (FPT) inhibitors as antitumor agents,[5] we discovered that the 3,10-dibromo 8-chlorotricyclic compounds of types **1** and **2** existed as a pair of isomers that were stable at room temperature and could be separated on a chiral HPLC column. This was in contrast to the 3-bromo tricyclic compound **3** which was found to exist only as a single isomer at room temperature.



In a previous report, Piwinski and co-workers had observed that some bridgehead modified loratadine® analogues existed as a pair of atropisomers at low temperatures (∼−20 °C) but these isomers equilibrated very quickly at room temperature.[6]

A similar phenomenon has been demonstrated in telenzapine **4**, a selective muscarinic antagonist developed for the treatment of peptic ulcer diseases.[7] Telenzapine exists as a mixture of enantiomeric atropisomers that are resolvable at ambient temperature. The two isomers are interconvertable at higher temperatures (90–120 °C) but are stable at room temperature (37 °C) where racemization is only ∼0.1% with a calculated half-life of 1000 years![7]

More recently, a number of substituted cyproheptadine compounds exemplified by **5** and **6** have been synthesized and resolved into enantiomeric atropisomers.[8] Whereas compound **5** was shown to be a potent antipsychotic

Key words: Ras; farnesyl protein transferase; atropisomerism; trihalobenzocycloheptapyridine; antitumor.
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† Dedicated to the late Professor Sir Derek H. R. Barton.

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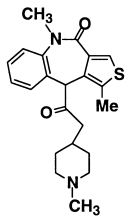
agent, its corresponding atropisomeric enantiomer **6** was virtually inactive.



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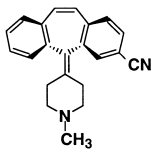

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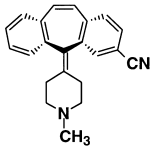

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In our present study, resolution of (±)-**1** and (±)-**2** afforded the atropisomers (+) and (−)-**1** as well as (+) and (−)-**2** whose biological and pharmacological properties are discussed in this paper.

Chemistry

Compounds prepared for this study are shown in Tables 1–3, and their synthetic routes are illustrated in Scheme 1.

Reaction of previously prepared amine (±)-**7**[5e] with 4-pyridinylacetyl *N*-oxide using standard DEC-HOBT-NMM coupling procedures provided tricyclic pyridine acetamide *N*-oxide (±)-**1** in 99% yield. Separation of the racemic acetamide on ChiralPak® AD column eluting with 40% isopropanol–hexanes with 0.2% DEA provided the (+)-**1** and the (−)-**1** atropisomers. Preparation of carbamoyl analogues was carried out by first coupling amine (±)-**7** to *N*-Boc acetylpiperidine to give the tricyclic amide (±)-**8**. Removal of Boc group with TFA afforded amine (±)-**9** which was subsequently reacted with TMSNCO followed by basic work up to give the target carbamoyl (±)-**2**. Separation of (±)-**2** chiral on ChiralPak® AD afforded the atropisomeric compounds (+)-**2** and (−)-**2**.

Table 1. FPT, COS, GGPT activities of compounds (±)-**1**, (±)-**2**, (+)-**1**, (−)-**1**, (+)-**2**, and (−)-**2**

Compound	FPT (IC_{50}) μM	GGPT (IC_{50}) μM	COS cell (IC_{50}) μM
(±)-**1**	0.012	38	< 0.25
(+)-**1**	< 0.003	> 38	0.025
(−)-**1**	> 0.1	> 33	> 1.0
(±)-**2**	0.021	38	< 0.25
(+)-**2**	0.013	> 38	0.28
(−)-**2**	> 1.57	> 33	> 1.0

Table 2. Pharmacokinetic profile for FPT compounds dosed at 25 mg/kg in cynomolgus monkey as solutions of HCl salts

Compound	AUC (po)[a] μg·h/mL)	Cmax (po) μg/mL
(±)-**1**	6.93	2
(+)-**1**	7.8	2
(±)-**2**	3.9	1
(+)-**2**	6.48	1

[a]AUC (0–48 h); po = oral, AUC = area under the concentration time curve.

Results and Discussion

Compounds prepared in this study were tested both for their ability to inhibit the transfer of [^{3}H] farnesyl from farnesyl pyrophosphate to H-Ras-CVLS, a process that is mediated by FPT and their inhibitory activity towards the closely related enzyme, GGPT-1 that catalyzes the transfer of [^{3}H]-geranylgeranyl moiety from geranylgeranyl pyrophosphate to H-Ras-CVLL using conditions previously described.[9] These compounds were also evaluated in a cellular ras processing assay (COS cell assay).[9] Biological and pharmacokinetic data for these compounds are summarized in Tables 1–3 and in Figure 2.

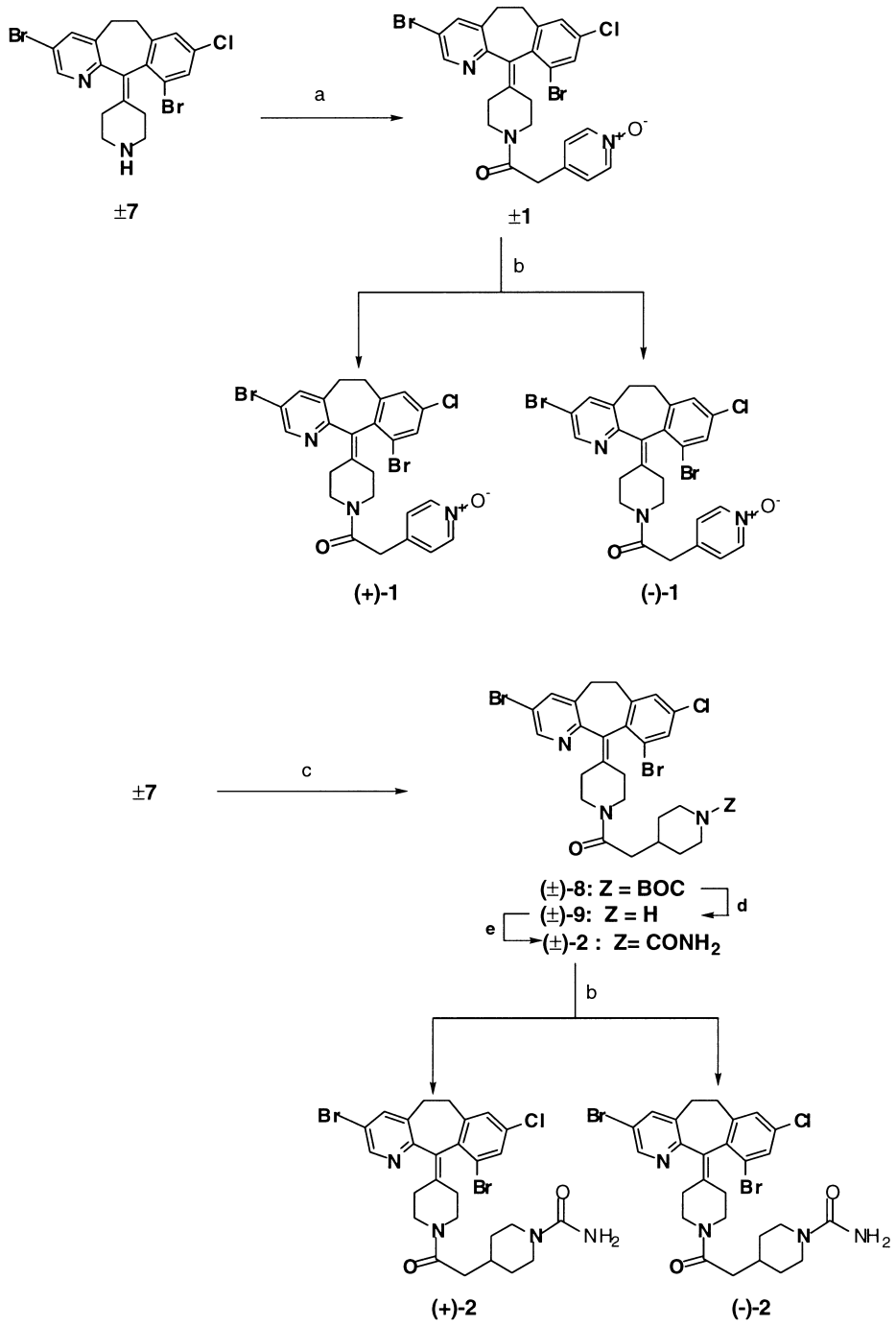
The discovery of 3-bromo tricyclic compound **3** as potent FPT inhibitor (IC_{50} = 95 nM),[5b] prompted us to investigate whether substitution on the tricyclic ring system would improve FPT activity. Elaboration of the tricyclic ring system by introduction of a bromine atom at the 10-position led to the discovery of acetamide (±)-**1** and carbamoyl (±)-**2** as very potent FPT inhibitors. It was realized that **1** and **2** existed as a pair of enantiomeric atropisomers that were separated using ChiralPak® AD column to give (+) and (−)-**1** and (+) and (−)-**2**. As shown in Figure 1, each pair of atropisomers was baseline resolved, thus, whereas (+)-**1** had a retention time of 18.5 min, the (−)-**1** eluted after 36.9 min. Similarly (+)-**2** and (−)-**2** had retention times of 9.2 and 12.9 min, respectively (Fig. 1). While the racemic mixture (±)-**1** inhibited 50% of FPT at 0.012 μM, the (+)-**1** atropisomer was found to be a highly potent FPT inhibitor (IC_{50}∼0.03 μM) and inhibited farnesyl processing in COS cells with an IC_{50} = 0.25 μM. On the other hand, the corresponding (−)-**1**-isomer did not have any appreciable FPT activity at 0.1 mM and neither did it show any cellular activity on the FPT processing of COS cells.

Similar results were obtained with the piperidine carbamoyl analogues whereby, racemic (±)-**2** had an FPT activity of 0.021 μM (Table 1). As in the case of pyridylacetamide (+)-**1** above, it was also found that

Table 3. Percent inhibition of tumour growth in mice DLD tumor model, compound given po qid for 14–21 days[a]

Compound	10 mpk	50 mpk
(+)-**1**	34	55
(+)-**2**	29	63

[a]$p < 0.005$.



Scheme 1. a = 4-Pyridine acetic acid *N*-Oxide-DEC-HOBT-NMM; b = ChiralPak® AD separation; c = *N*-Boc piperidinylacetic acid-DEC-HOBT-NMM; d = TFA; e = TMS-NCO.

the biological activity in this series resided in the (+) antipode. Thus (+)-**2** had FPT activity of 0.013 μM and inhibited the FPT processing of COS cells with an $IC_{50} = 0.28$ μM. The corresponding (−)-isomer compound (−)-**2** did not have appreciable FPT activity at 1.57 μM and neither did it show cellular activity at 1.0 μM in COS cell assay.

As observed with previously prepared tricyclic compounds,[5] compounds evaluated in this series also showed very good selectivity for FPT versus the closely related enzyme, GGPT-1. Thus, all the potent compounds discussed in this report, were found to be inactive in inhibition of GGPT-1 mediated prenyl transfer at concentrations as high as 30 μM (Table 1).

Results obtained from evaluation of both the (+) and the (−) antipodes of tricyclic analogues (±)-**1** and (±)-**2** indicated that the biological activity of 3,10-dibromo, 8-chloro tricyclic piperidine compounds of types **1** and **2** resided in the (+)-antipode, a factor that would reflect the mode of binding of these molecules to FPT. Thus, the

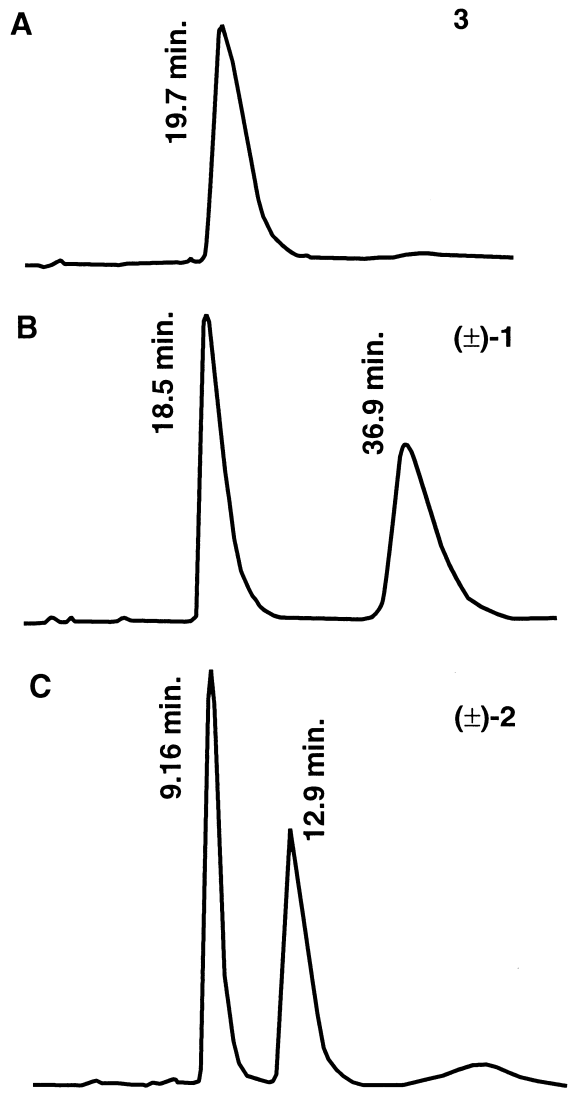
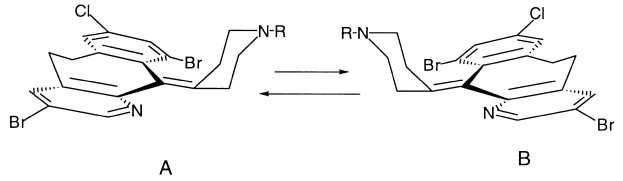


Figure 1. Analytical Chiral Pak® AD HPLC profile of (A) compound **3**, (B) (±)-**1**, (C) (±)-**2**.

10-bromo substituted tricyclic exists in two forms. In one case (A) the piperidine ring would be in be in pseudo-axial position and the bromine would be above the plane of the piperidine; whereas in case (B), the bromine would be below the plane of the piperidine ring. Conversion of A to B and vice versa can only be achieved at high temperatures in the case where R = H (detailed result of this phenomenon will be reported shortly).



Pharmacokinetics

Evaluation of pharmacokinetic (PK) profile of compounds (±)-**1**, (±)-**2**, (+)-**1**, and (+)-**2** in cynomolgus monkeys, dosed at 25 mpk showed that these compounds had serum AUC between 4–8 μg·h/mL and C_{max} between 1 and 2 μM (Table 2). The most pharmacokinetically stable compound is (+)-**1** with AUC = 7.8 μg·h/mL. The effective concentration of these tricyclic FPT inhibitors in tumors was sufficient for these compounds to be efficacious in nude mice.

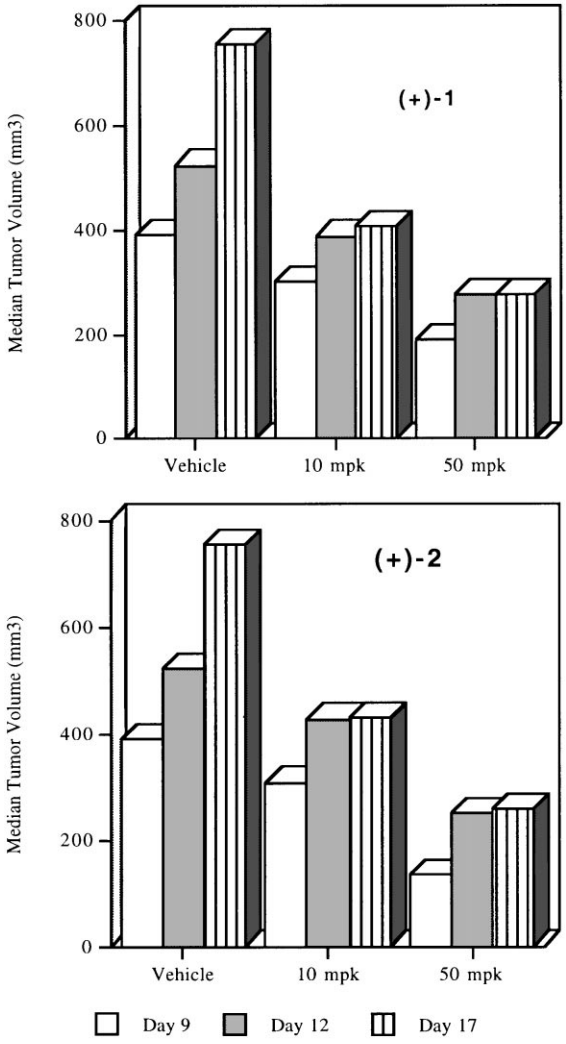


Figure 2. Antitumor activity of targets (+)-**1** and (+)-**2** in DLD-1 tumor model (See Experimental for details).

In vivo studies

In vivo evaluation of (+)-**1**, and (+)-**2** showed that these compounds were potent antitumor agents. As shown in Table 2 and in Figure 2, (+)-**1** inhibited the growth of tumor in DLD-1 model in a dose-dependent way. Thus, (+)-**1** inhibited growth of tumor cells in DLD-1 model by 34 and 55% at 10 and 50 mpk, respectively. Similar results were obtained in the carbamoyl series where (+)-**2** inhibited the growth of tumor in the DLD-1 model by 29 and 63% at 10 and 50 mpk, respectively.

Conclusion

Synthesis of 3,10 dibromo 8-chloro tricylic acetamides and ureas of type **1** and **2** have been prepared and found

to exist as a pair of atropisomers that are stable at room temperature. Separation of individual atropisomers was achieved by ChiralPak® AD and it was clearly established that the biological activity resided in the (+)-isomer of each of the components. Thus the (+)-**1** and (+)-**2** isomers were the most potent antipodes in both series. Evaluation of the PK profile in cynomolgus monkeys of the potent atropisomers showed that they had reasonable pharmacokinetic stability. This also translated into the in vivo activity whereby, compounds (+)-**1**, and (+)-**2** inhibited tumor growth in DLD-1 model at 10 and 50 mpk in a dose dependent way. On the basis of the present results, the (+)-antipodes of the 3,10 dibromo 8-chlorotricylic analogues disclosed in this report, represent a promising class of potential cancer chemotherapeutics.

Experimental

Melting points were determined with an electrothermal digital melting point apparatus and are uncorrected. Elemental analyses 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), spectrometers. In general, structures of the compounds were determined by coupling constants, coupling information from the COSY spectra and 1D NOE experiments. The ^{1}H and ^{13}C NMR spectra were obtained on either Varian VXR-200 (200 MHz, ^{1}H), Varian Gemini-300 (300 MHz, ^{1}H; 75.5 MHz, ^{13}C) or XL-400 (400 MHz, ^{1}H; 100 MHz, ^{13}C) and are reported as ppm down field from Me_4Si with number of protons, multiplicities, and coupling constants in hertz (Hz) indicated parenthetically. For ^{13}C NMR, a Nalorac Quad nuclei probe was used. Rotations were recorded on a Perkin–Elmer 243B polarimeter.

4-(8-Chloro-3,10-dibromo-6,11-dihydro-5*H*-benzo-[5,6]-cyclohepta[1,2-*b*]-pyridin-11-yl)-1-(4-pyridinylacetyl)-piperidine *N*1-oxide (±)-1. A mixture of (±)-**7** (0.2 g, 0.43 mmol), pyridylacetic acid *N*-oxide (0.085 g, 0.55 mmol), HOBT (0.075 g, 0.55 mmol), DEC (0.11 g, 0.55 mmol), *N*-methylmorpholine (0.1 mL, 0.55 mmol) and dry DMF (7 mL) was stirred at 25 °C for 16 h. The mixture was concentrated in vacuo, diluted with CH_2Cl_2 and washed with saturated $NaHCO_3$ and 10% NaH_2PO_4 (aqueous). The organic phase was dried over anhydrous $MgSO_4$ and concentrated in vacuo to provide a residue which was purified by flash column chromatography (silica gel, 5% MeOH/CH_2Cl_2 + saturated with NH_3) to give the product (±)-**1** as a solid (0.25 g, 99% yield): mp 167–168 °C; δ 1.95–2.10 (m, 1H), 2.20–2.40 (m, 2H), 2.60–2.95 (m, 3H), 3.20–3.55 (m, 4H), 3.60–3.80 (m, 3H), 3.90–3.96 (m, 1H), 7.17–7.22 (m, 3H), 7.48 (d, J = 2 Hz, 2H), 7.53 (d, J = 2 Hz, 1H), 8.16 (d, J = 6.4 Hz, 1H), 8.44 (m, 1H); ^{13}C NMR (300 MHz, $CDCl_3$) δ (mixture of rotamers) 29.67, 30.50, 31.25, 31.30, 31.42, 32.28, 38.87, 42.31, 42.75, 45.92, 46.40, 129.24, 127.06, 130.33, 130.42, 131,54, 133.90, 133.94, 134.24, 134.44, 134.61, 138.34, 139.08, 139.22, 139.33, 141.03, 141.07, 141.79, 141.95, 147.78, 147.93, 167.11, 167.17; IR (film) u_{max} 993, 1178, 1225, 1443, 1637, 2913, 3110, 3414; MS m/z 604 (MH^+). Anal. calcd for $C_{26}H_{22}N_3O_2Br_2Cl$ 1.5 H_2O: C, 49.51; H, 3.99; N, 6.66. Found: C, 49.43; H, 3.67; N, 6.60.

4-[2-[4-(8-Chloro-3,10-dibromo-6,11-dihydro-5*H*-benzo-[5,6]-cyclohepta[1,2-*b*]pyridin-11-yl)-1-piperidinyl]-2-oxo-ethyl]-1-piperidinecarboxamide (+)-1 and 4-[2-[4-(8-chloro-3,10-dibromo-6,11-dihydro-5*H*-benzo[5,6]-cyclohepta[1,2-*b*]pyridin-11-yl)-1-piperidinyl]-2-oxo-ethyl]-1-piperidinecarboxamide (−)-1. Resolution of **1** on prep ChiralPak® AD column eluting with 40% isopropanol, 60% hexane, 0.2% diethyl amine afforded the enantiomeric amines (+)-**1**: mp 112–114 °C (retention time on prep column = 26.9 min), $[\alpha]_D^{25}$ +159.7° (*c* 0.52, MeOH) and (−)-**1**: mp 114–115 °C (retention time on prep column = 55.3 min), $[\alpha]_D^{25}$ −154.6° (*c* 0.44, MeOH). All other data for compounds (+)-**1** and (−)-**1** were identical to those of (±)-**1** above.

1,1-Dimethylethyl[[[4-(8-chloro-3,10-dibromo-6,11-dihydro-5*H*-benzo[5,6]cyclohepta[1,2-*b*]pyridin-11-yl)-1-piperidinyl]-carbonyl]-methyl]-1-piperidinecarboxylate (±)-8. A mixture of (±)-**7** (6.0 g, 12.8 mmol), piperidylacetic acid *N*-Boc (3.78 g, 16.6 mmol), HOBT (2.25 g, 16.6 mmol), DEC (3.19 g, 16.6 mmol) and dry DMF (50 mL) was stirred at 25 °C for 24 h. The mixture was concentrated in vacuo, diluted with CH_2Cl_2 and washed with saturated $NaHCO_3$ and 10% NaH_2PO_4 (aqueous). The organic phase was dried over anhydrous $MgSO_4$ and concentrated in vacuo to provide (±)-**8** as an off-white solid (8.52 g), which was used in the next step without further purification. ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.95–2.53 (m, 14H), 2.50–2.99 (m, 4H), 3.25–3.50 (m, 4H), 3.90–4.20 (m, 3H), 7.20 (br s, 1H), 7.45 (s, 1H), 7.50 (br s, 1H), 8.50 (s, 1H); MS (FAB) (relative intensity) m/z 694 (100, MH^+).

4-(8-Chloro-3,10-dibromo-6,11-dihydro-5*H*-benzo-[5,6]-cyclohepta[1,2-*b*]-pyridin-11-yl)-1-[(4-piperidinyl)-acetyl]-piperidine (±)-9. A solution of (±)-**8** (0.26 g, crude residue from previous step), anhydrous dichloromethane (10 mL) and trifluoroacetic acid (10 mL) was stirred at 0 °C for 2 h. The solution was cooled in ice-water and treated slowly with 1 N NaOH until basic. The mixture was poured into dichloromethane and washed with water. The organic phase was dried over anhydrous $MgSO_4$ and concentrated in vacuo to give (±)-**9** as a white solid: 0.16 g, 68% yield; mp 153–154 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.4–1.65 (m, 2H), 1.95–2.50 (m, 5H), 2.60–3.60 (m, 14H), 3.79 (m, 1H), 3.65–3.80 (m, 1H), 3.90–4.15 (m, 1H), 7.21 (s, 1H), 7.48 (d, J = 2 Hz, 1H), 7.53 (s, 1H), 8.51 (d, J = 2 Hz, 1H); ^{13}C NMR (300 MHz, $CDCl_3$) δ (mixture of rotamers) 29.15, 29.87, 30.65, 30.71, 31.30, 31.40, 31.48, 32.31, 38.96, 41.94, 42.35, 44.44, 45.42, 45.91, 119.13, 123.32, 123.38, 127.06, 127.06, 130.36, 130.39, 131.18, 133.84, 134.43, 134.54, 139.03, 140.98, 141.04, 141.82, 141.91, 147.80, 147.90, 152.13, 169.15, 169.24; IR (film) u_{max} 993, 1130,

1202, 1436, 1631, 2512, 2859, 2928, 3444; MS *m/z* 594 (MH^+). Anal. calcd for $C_{26}H_{22}N_3O_2Br_2Cl$ 0.2 H_2O 1.0 TFA: C, 47.27; H, 4.17; N, 5.91. Found: C, 47.01; H, 4.32; N, 6.11.

4-[2-[4-(8-Chloro-3,10-dibromo-6,11-dihydro-5*H*-benzo-[5,6]-cyclohepta[1,2-*b*]pyridin-11-yl)-1-piperidinyl]-2-oxo-ethyl]-1-piperidinecarboxamide (±)-2. To a solution of (±)-**9** (0.12 g, 0.21 mmol) in anhydrous dichloromethane (7 mL) was added trimethylsilylisocyanate (0.19 g, 0.22 mL, 1.65 mmol). After stirring at 25 °C for 16 h the solution was poured into dichloromethane and washed with saturated aqueous $NaHCO_3$. The organic phase was dried over anhydrous $MgSO_4$ and concentrated in vacuo to give (±)-**2** as a white solid (0.05 g, 37% yield): mp 155–156 °C; MS (FAB) *m/z* 637 (MH^+); ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.10–1.30 (m, 2H), 1.70–1.90 (m, 2H), 2.20–2.45 (m, 4H), 2.55–2.95 (m, 5H), 3.20–3.45 (m, 4H), 3.70 (m, 1H), 3.20–3.50 (m, 4H), 4.50 (s, 2H), 7.20 (s, 1H), 7.45 (d, J = 2 Hz, 1H,), 7.55 (s, 1H), 8.50 (d, J = 2 Hz, 1H); ^{13}C NMR (300 MHz, $CDCl_3$) δ (mixture of rotamers) 29.87, 30.70, 31.25, 31.55, 31.98, 32.03, 3.29, 32.95, 33.02, 39.41, 41.85, 42.27, 44.29, 44.45, 44.49, 45.57, 46.03, 119.03, 119.07 123.27, 123.32, 126.96, 130.25, 130.33, 131.04, 133.74, 133.77, 134.37, 134.52, 139.14, 139.34, 139.44, 140.92, 140.97, 141.76, 141.89 147.69, 147.82, 151.99, 152.12, 157.90, 169.79, 169.87; IR (film) u_{max} 992, 1123, 1435, 1636, 2852, 2917, 3426; MS *m/z* 637 (MH^+). Anal. calcd for $C_{27}H_{29}N_4O_2Br_2Cl$ 0.8 H_2O 0.4 CH_2Cl_2: C, 48.39; H, 4.59; N, 8.12. Found: C, 48.15; H, 4.63; N, 8.06.

(+)-4-[2-[4-(8-Chloro-3,10-dibromo-6,11-dihydro-5*H*-benzo[5,6]-cyclohepta[1,2-*b*]pyridin-11-yl)-1-piperidinyl]-2-oxo-ethyl]-1-piperidinecarboxamide (+)-2 and (−)-4-[2-[4-(8-chloro-3,10-dibromo-6,11-dihydro-5*H*-benzo[5,6]-cyclohepta[1,2-*b*]pyridin-11-yl)-1-piperidinyl]-2-oxo-ethyl]-1-piperidinecarboxamide (−)-2. Resolution of (±)-**2** on prep ChiralPak® AD column eluting with 20% isopropanol-80% hexane-0.2% diethyl amine afforded the enantiomeric amines (+)-**2**: mp 126–127 °C (retention time on prep column = 20.44 min), $[\alpha]_D^{25}$ +145.6½ (c 0.44, MeOH) and (−)-2: mp = 143–144 °C (retention time on prep column = 29.12 min), $[\alpha]_D^{25}$ −151.7° (*c* 0.55, MeOH). All other data for compounds (+)-**2** and (−)-**2** were identical to those of (±)-**2** above.

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.[9] GGPT-1 activity was similarly determined using [^{3}H]geranylgeranyl diphosphate and Ha-Ras-CVLL as substrates.[9]

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.[9]

Cell lines for in vivo studies. The human colon carcinoma DLD-1 cell line was obtained from the 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 milligrams per kilogram 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 $V = 1/6\times\pi\times L\times W\times T$, where L, W, and T represent length, width, and thickness respectively.[10] T/C value in percent was calculated for each measurement where T and C were the median tumor volume 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. The pharmacokinetics of the potent FPT inhibitors discussed in this report were studied in male cynomologus monkeys. These inhibitors were suspended in 20% HPβCD and given by oral administration at doses of 25 mg/kg. Blood samples were collected up to 48 h post-dosing. 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. A detailed description of the analytical methodology has been described for an earlier analogue in this series.[11]

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