Inhibitors of Farnesyl Protein Transferase. 4-Amido, 4-Carbamoyl, and 4-Carboxamido Derivatives of 1-(8-Chloro-6,11-dihydro-5*H*-benzo[5,6]-cyclohepta[1,2-*b*]pyridin-11-yl)piperazine and 1-(3-Bromo-8-chloro-6,11-dihydro-5*H*-benzo[5,6]cyclohepta[1,2-*b*]pyridin-11-yl)piperazine[1]

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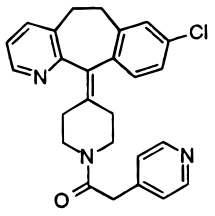
The synthesis of a variety of novel 4-amido, 4-carbamoyl and 4-carboxamido derivatives of 1-(8-chloro-6,11-dihydro-5*H*-benzo[5,6]cyclohepta[1,2-*b*]pyridin-11-yl)piperazine to explore the SAR of of this series of FPT inhibitors is described. This resulted in the synthesis of the 4- and 3-pyridylacetyl analogues **45a** and **50a**, respectively, both of which were orally active but were found to be rapidly metabolized in vivo. Identification of the principal metabolites led to the synthesis of a variety of new compounds that would be less readily metabolized, the most interesting of which were the 3- and 4-pyridylacetyl *N*-oxides **80a** and **83a**. Novel replacements for the pyridylacetyl moiety were also sought, and this resulted in the discovery of the 4-*N*-methyl and 4-*N*-carboxamidopiperidinylacetyl derivatives **135a** and **160a**, respectively. All of these derivatives exhibited greatly improved pharmacokinetics. The synthesis of the corresponding 3-bromo analogues resulted in the discovery of the 4-pyridylacetyl *N*-oxides **83b** (±) and **85b** [11*S*(−)] and the 4-carboxamidopiperidinylacetamido derivative **160b** (±), all of which exhibited potent FPT inhibition in vitro. All three showed excellent oral bioavailability in vivo in nude mice and cynomolgus monkeys and exhibited excellent antitumor efficacy against a series of tumor cell lines when dosed orally in nude mice.

Introduction

Mutations in the *ras* oncogene have been detected in a wide variety of human tumors, with the highest incidence being observed in cancers of the pancreas (ca. 90%), colon (ca. 50%), and lung (ca. 30%).[2] The important role played by Ras protein in the signal transduction process involved in cell division is well recognized.[2,3] Inhibition of farnesylation of the Ras protein, a key step in the posttranslational modification of this protein,[4,5] continues to be the subject of intense interest as a source of potential antitumor agents. This has led to the synthesis of several novel groups of farnesyl protein transferase (FPT) inhibitors which have recently been reviewed,[6] most of which are peptidic or peptidomimetic in nature. Most of these are CaaX mimetics, while others are farnesyl diphosphate analogues, bisubstrate analogues, or natural products derived from screening programs. Many of these contain free amino and sulfhydryl groups in the molecule, which are potentially metabolically labile.

Recently, this laboratory reported the discovery of a novel tricyclic FPT inhibitor **1**,[7] containing the 8-chloro-5,6-dihydro-11*H*-benzo[5,6]cyclohepta[1,2-*b*]pyridine moiety, which is of considerable interest as it is nonpeptidic, contains no sulfhydryl group, is selective for FPT over geranylgeranyl protein transferase (GGPT), and competes kinetically with the Ras protein in binding to FPT. A structure−activity relationship (SAR) study of these 11-piperidinylidene derivatives in which a series of aryl and pyridyl amides were prepared has been carried out in these laboratories.[8] Additional novel 11-piperidinyl[9] and 11-piperazinyl analogues[1,9] have been also been synthesized and found to be potent inhibitors of FPT, and we wish here to describe the latter series.



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The synthesis of a variety of 4-amido, 4-carbamoyl, and 4-carboxamido derivatives of both 1-(8-chloro-6,11-dihydro-5*H*-benzo[5,6]cyclohepta[1,2-*b*]pyridin-11-yl)-piperazine (**5a**) and of 1-(3-bromo-8-chloro-6,11-dihydro-5*H*-benzo[5,6]cyclohepta[1,2-*b*]pyridin-11-yl)piperazine (**5b**) has been carried out. This led initially to the preparation of the 4- and 3-pyridylacetamido derivatives **45a** and **50a** (Table 1). Both were found to be orally active, but unfortunately they were extensively metabolized. This information, coupled with the knowledge of the structures of two of the principal metabolites, led us to synthesize a variety of analogues in this series having reduced potential for metabolism, the most

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Table 1. Structures, Methods of Synthesis, and Antitumor Activity for the 4-Amido, 4-Carbamoyl, 4-Carboxamido, and 4-Alkyl Derivatives Derived from the Intermediates **5a** (Structure A), **46a** (Structure B), **48a** (Structure C), **90** (Structure D), **98a** (Structure E), **106** (Structure J), **5b** (Structure F), **46b** (Structure G), **48b** (Structure H), **98b** (Structure I), **112** (Structure K), and **114** (Structure L)

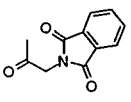
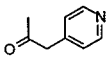
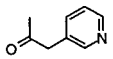
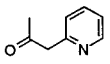
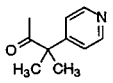
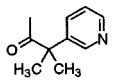
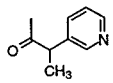
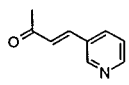
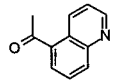
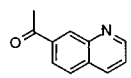
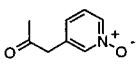
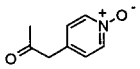
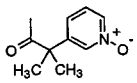
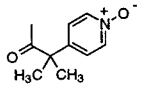
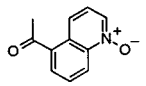
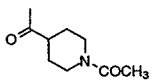
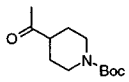
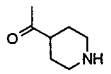
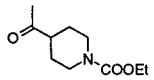
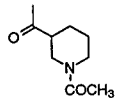
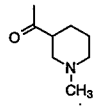
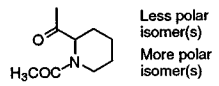
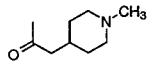
No	Str	R_3	Method	FPT IC_{50} (μM) H-*ras* [K-*ras*]	GGPT IC_{50} (μM)	COS IC_{50} (μM)
5a	A	-H		30.3		
5b	F	-H				
6	A		A	0.82	>40	39.9
13	A	$-COCH_2NHBoc$	A	4.8		
28	A	$-COCH_2NHCOCH(SH)CH_3$	A	2.78		
39	A	$-COCH(C_6H_5)_2$	A	2.5	>50	5.1
40	A	$-COCH_2C_6H_5$	A	2.73		12
41	A	$-COCH_2C_6H_4$-4-Br	A	2.04	>50	5.1
43	A	$-COCH_2C_6H_4$-4-NMe_2	A	2.44	42	7.0
44	A	$-COC_6H_4$-4-NMe_2	A	20.2		
45a	A		A	0.16[0.80]	>46	3.7
45b	F		A	0.012	>39	0.38
47	B		A	0.49		
49	C		A	0.14		
50a	A		A	0.53[3.17]	>46	6.22
50b	F		A	0.17	>10	0.85
51	B		A	2.1		10.5
52	C		A	0.19		1.3
91	D		A	0.91		11.5
99	E		A	2.1		3.7
107	J		A	0.88		0.89
54	A		A	0.36	>46	5.0
55	A	$-COC(CH_3)_2C_6H_5$	A	3.3		
73	A		A	1.74		
74a	A		A	0.85		6.1
74b	F		A	0.3		0.9
75	A		A	4.9		
76	A		A	2.6	>50	
77a	A		A	15% @ 1.3		
77b	F		A	38% @ 3.6		
78	F		A	1.8		
80a	A		A	1.17		11.1
80b	F		A	0.4		0.45
81	B		A	7.0		
82	C		A	2.3		
92	D		A	3.8		>20
100	E		A	4.5		
83a	A		A	0.74		
83b	F		A	0.047[0.13]	>38	0.45
84a	B		A	1.2		
84b	G		A	0.026	>38	0.5
85a	C		A	0.49		5.0
85b	H		A	0.023[0.12]	>76	0.125
101	I		A	0.028		0.35
108	J		A	0.33		
115	K		A	0.06	>34.2	0.63
116	L		A	0.034[0.24]		<0.25
93a	A		A	1.26		
93b	F		A	0.38		2.2
94a	A		A	2.2		
94b	F		A	0.39		5.0
86	F		A	38% @ 3.5		
118	A		A	1.24	>43	4.9
119	B		A	4.1		
120	C		A	1.8		6.5
128	A		A	20% @ 11		
129	A		B	11.3		
130	A		C	13.3		
122	A		A	4.3		
127	A		A	6.8		
124	A	 Less polar isomer(s)	A	10.8		
125	A	More polar isomer(s)	A	10.2		
135a	A		A	1.4		0.93
135b	F		A	0.12	>38	0.1
136a	B		A	1.8		
136b	G		A	0.054	>38	0.7
137a	C		A	0.64		

Table 1. (Continued)

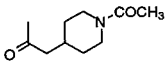
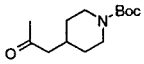
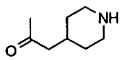
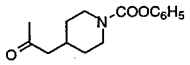
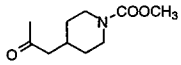
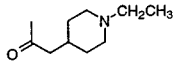
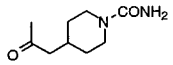
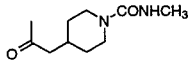
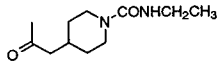
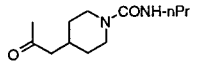
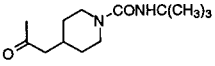
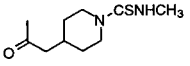
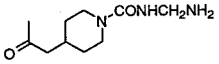
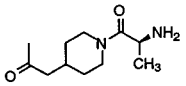
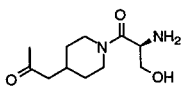
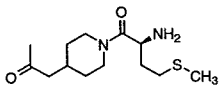
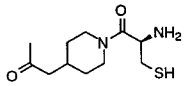
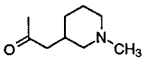
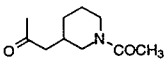
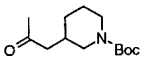
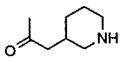
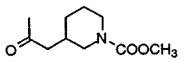
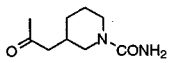
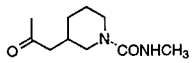
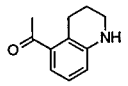
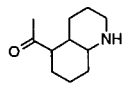
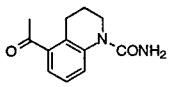
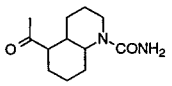
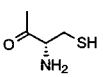
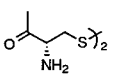
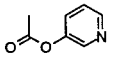
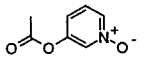
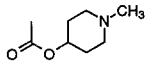
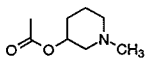
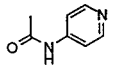
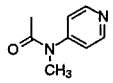
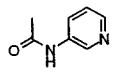
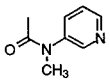
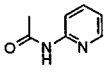
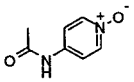
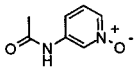
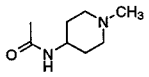
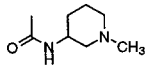
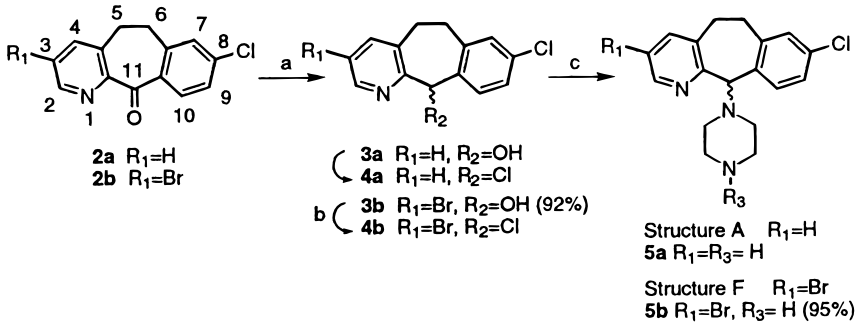
No	Str	R_3	Method	FPT IC_{50} (μM) H-*ras* [K-*ras*]	GGPT IC_{50} (μM)	COS IC_{50} (μM)
137b	H		A	0.05	>38	0.9
141	E		A	1.37		
143	J		A	0.45		5.7
144a	A		A	0.87		2.7
144b	F		D	0.102	>34	0.59
145	B		A	3.5		
146	C		A	1.6		6.2
148a	A		A	7.4		
148b	F		A	1.29		
150a	A		B	1.25		
150b	F		B	0.072	>38	0.17
151	G		A/B	0.087		0.56
152	H		A/B	0.078		
153	I		A/B	0.083		
154	L		A/B	0.069		
156	F		E	0.34		
157	A		C	1.3		
159	A		F	1.27		
160a	A		G	0.82		
160b	F		G	0.049	>35	0.59
161	G		G	0.043		0.35
162	H		G	0.048		0.5
164	I		G	0.045		
165	K		G	0.027		0.6
166	L		G	0.092		1.7
167a	A		H	1.05		
167b	F		H	0.05	>35	0.75
169	A		H	0.92		
170a	A		H	0.88		
170b	F		H	0.1	>33	1.5
171a	A		H	1.0		
171b	F		H	0.108		
172	A		I	0.88		
173	A		J	2.5		
174	A		J	2.5		
175a	A		J	1.1		
175b	F		J	0.104	>33	1.05
176	A		J	1.89		
177	F		K/L	0.23		
138a	A		A	1.19		2.4
138b	F		A	0.1	>38	0.45
139	B		A	0.7		
140	C		A	0.6		
142	E		A	1.66		
147	A		A	3.1		
149	A		A	12.2		
155	A		B	1.1		
158	A		C	7.0		
163	A		G	1.75		
168	A		H	7.0		
178	F		A/B	0% @ 0.1		
179	F		A/B	23% @1.1		
180	F		A/B	45% @1.0		
181	F		G	1.4		
184	F		L	2.1		
186	F		B	2.0[5.33]		1.8
209	A	-$COOC_6H_5$	N	0.8		
211	A		N	0.5		3.0
212	B		N	1.28		6.9
213	C		N	0.82		3.9
214a	A		M	3.1		
214b	F		P	0.28		6.0
216a	A		N	35% @ 1.3		
216b	F		N	0.31		>5.0

Table 1. (Continued)

No	Str	R_3	Method	FPT IC_{50} (μM) H-*ras* [K-*ras*]	GGPT IC_{50} (μM)	COS IC_{50} (μM)
217a	A		P / Q	2.1		
217b	F		P	0.3		5.7
218	E	$-COOC_6H_5$	N	0.88		
219a	A		O	9.8		
219b	F		O	1.77		
220	A	$-CONHCH_2C_6H_5$	R	4.1		
221	A	$-CONHC_6H_5$	R	1.09	>40	12.0
227	A		S	0.9	>40	10.5
232	A		T	0% @ 0.19		
228a	A		S	1.34	>46	4.8
228b	F		S	0.21	>10	3.7
229	B		S	3.2		
230	C		S	0.46		7.3
233	A		T	4.6		
231	A		S	0.95	>46	1.3
249a	A		U / V	1.8		
249b	F		U	0.21		5.8
250a	A		S / U	2.2		
250b	F		S / U	0.24		3.6
251a	A		U	0.47		
251b	F		W / U	0.041	>38	0.53
252a	A		W / U	0.66		
252b	F		W / U	0.054		
53	A			23% @ 14		
189	F			19% @ 10		
192				25.8		
196				38.3		
201				48% @ 15		
205				32% @ 58		

Scheme 1[a]



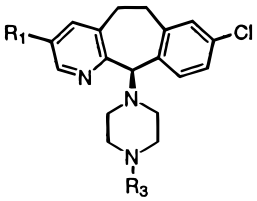
[a] (a) $NaBH_4$, MeOH, 0 °C, 2.5 h, 0−25 °C, 1 h; (b) $SOCl_2$, toluene, 0 °C, 2.5 h, 0−25 °C, 0.5 h; (c) piperazine, THF, 25 °C, 19 h.

interesting of which were the *N*-oxides **80a** and **83a**. Novel replacements for the pyridylacetyl moiety were also sought, and this resulted in the discovery of the 4-*N*-methyl- and 4-*N*-carboxamidopiperidinylacetyl derivatives **135a** and **160a**, respectively. All of the above derivatives exhibited greatly improved pharmacokinetics. The discovery in these laboratories that introduction of a 3-bromo substituent resulted in more potent FPT inhibitors in the 11-ylidene series[10] prompted us to prepare the more potent 4-amido, 4-carbamoyl, and 4-carboxamido derivatives described above, in the 3-bromo series starting from **5b**. This resulted in the synthesis of the 4-pyridylacetyl *N*-oxides **83b** (±) and **85b** [11*S*(−)] and the 4-carboxamidopiperidinylacetamido derivative **160b** (±), all of which exhibited more potent FPT inhibition in vitro. All three showed excellent oral bioavailability in vivo in nude mice and cynomolgus monkeys and exhibited excellent antitumor efficacy against a series of tumor cell lines when dosed orally in nude mice.

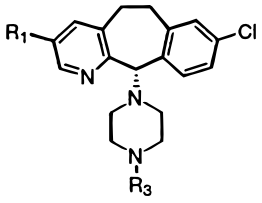
Chemistry

4-Amido Derivatives. The tricyclic piperazine derivative **5a** was prepared (Scheme 1) essentially as described previously.[11−13] The 3-bromotricyclic ketone[14] was synthesized by an alternative route from loratadine, by a sequence involving nitration, reduction, and bromination developed in these laborartories,[10] and was converted into the corresponding piperazine derivative **5b** (Scheme 1). In general the amides described in Table 1 were prepared by reacting either **5a** or **5b** with the appropriate acid in the presence of 1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide hydrochloride, 1-hydroxybenzotriazole, and 4-methylmorpholine in DMF (method A). In this manner, a variety of glycine and N-substituted analogues **6**−**28**, phenylalanine and N-

substituted analogues **29**−**31**, and tyrosine and N-substituted analogues **32**−**38** were prepared. A series of aryl **42**, **44**, arylacetyl **39**−**41** and **43**, and pyridylacetyl analogues **45a**,**b**, **50a**,**b**, and **54** were also prepared (method A). The resolved 11*R*(+)- and 11*S*(−)-piperazine **46a** and **48a**, respectively, were prepared



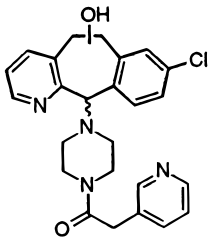
R(+)
Structure B R_1=H
46a R_1=R_3=H
Structure G R_1=Br
46b R_1=Br, R_3=H



S(-)
Structure C R_1=H
48a R_1=R_3=H
Structure H R_1=Br
48b R_1=Br, R_3=H

as described earlier[11] and used to synthesize the 11*R*(+) analogues **47** and **51**, as well as the 11*S*(−)-analogues **49** and **52**. Reduction of the amide **50a** with $LiAlH_4$ afforded the pyridylethyl derivative **53**. A series of α,α-dimethylphenylacetyl **55**, α,α-dimethylpyridylacetyl **73**, **74a**,**b**, and α-methylpyridylacetyl **75** analogues were also prepared using method A. The α-methyl-[15] and α,α-dimethylpyridylacetic[16] acids were prepared as described in Scheme 6 (Supporting Information). The 3-pyridylacrylicamide **76** was also prepared. The 5- and 7-carboxyquinolines[17] were also used to prepare the quinoline derivatives **77a**,**b** and **78**. The quinolines **77a**,**b** and **78** constitute rigid analogues of **50a**,**b**, respectively, having no possibility of metabolism at the carbon adjacent to the amide carbonyl.

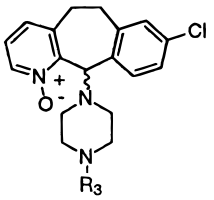
The identification of the principal metabolites **79** and **80a** formed when **50a** was dosed in mice prompted us to synthesize the 3- and 4-pyridylacetic acid N1-oxides **69**−**72** (Scheme 6). Using method A, the latter were



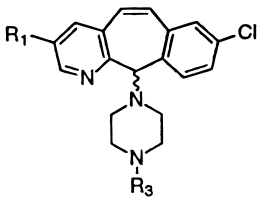
79

reacted with **5a**,**b** to give the *N*-oxides **80a**,**b**, **83a**,**b**, **93a**,**b**, and **94a**,**b**, with **46a**,**b** to give **81** and **84a**,**b** and with **48a**,**b** to give **82** and **85a**,**b**, respectively. The rigid quinoline *N*-oxide derivative **86** corresponding to **80b** was also prepared using similar techniques.[17] The tricyclicpiperazine N1-oxide **90** was also prepared by the method outlined in Scheme 7 (Supporting Information) and reacted with the appropriate pyridylacetic acids using method A to give the mono- and bis-*N*-oxides **91** and **92**, respectively.

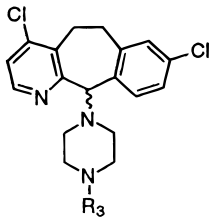
To prepare the 5,6-ene analogues, the ketones **2a** and **2b** were reacted with SeO_2[18,19] to give the 5,6-enes, which were converted into the corresponding piperazines **98a**,**b** as described in Scheme 8 (Supporting Information). The latter were used to prepare the



Sructure D
90 R_3=H

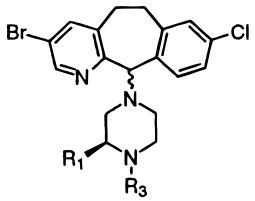


Structure E R_1=H
98a R_1=R_3=H
Structure I R_1=Br
98b R_1=Br, R_3=H

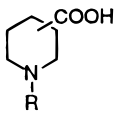


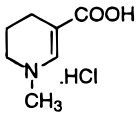
Structure J
106 R_3=H

amides **99**−**101**. The 4,8-dichloropiperazine **106** was synthesized from the tricyclic N1-oxide of **2a** by reacting it with $POCl_3$[20] as described in Scheme 9 (Supporting Information). Reaction of **106** with the appropriate pyridylacetic acid gave **107** and **108**. The 2*S*-*n*-butyl- and 2*S*-methoxyethyl-substituted piperazines **112** and **114**, respectively, were synthesized[21−23] as shown in Scheme 6. The latter were reacted with 4-pyridylacetic acid *N*-oxide to give **115** and **116**, respectively.



Structure K
112 R_1=n-Bu, R_3=H
Structure L
114 R_1=$MeO(CH_2)_2$, R_3=H





126

117a R=$COCH_3$ (4-)
117b R=$COOC(CH_3)_3$ (4-)
121a R=$COCH_3$ (3-)
121b R=CH_3 (3-)
123 R=$COCH_3$ (2-)

The search for a viable alternative to the pyridylacetyl moiety that would not be readily metabolized led us to synthesize a series of piperidine derivatives described below. Several compounds without the methylene spacer were synthesized from isonipecotic acid, which was converted into the derivatives **117a**[24] and **117b**,[25,26] which were in turn reacted with **5a** to give **118** and **128**, respectively. Deprotection of **128** afforded **129**. The 11*R*(+)- and 11*S*(−)-enantiomers **119** and **120** were also prepared from **46a** and **48a**, respectively. Nipecotic acid was converted into the derivative **121a**,[27] which was reacted with **5a** to give **122**. *N*-Methylnipecotic acid

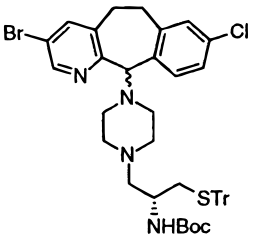
121b[28] was prepared either by reduction of **126** or by reductive formylation of nipecotic acid, and it was reacted with **5a** to give **127**. D,L-Pipecolinic acid was converted into the *N*-acetate **123**,[29] and the latter was reacted with **5a** to give the diastereoisomers **124** and **125**. Reaction of **129** with ethyl chloroformate afforded **130**.

To mimic the pyridylacetic acid derivatives, a series of *N*-methyl, *N*-acetyl,[30] and N-Boc[31] 4- and 3-piperidinylacetic acids were synthesized as described in Scheme 11 (Supporting Information). Using method A the following amides were prepared.The *N*-methyl acids were reacted with **5a**,**b**, **46a**,**b**, **48a**,**b**, **98a**, and **106** to give the amides **135a**,**b**−**143**. The *N*-acetyl acids were reacted with **5a**,**b**, **46a**, and **48a** to give **144a**−**147**, while **144b** was prepared by direct acetylation of **150b** as described in method D. The *N*-Boc acids were reacted with **5a**,**b**, **46b**, **48b**, **98b**, and **114** to give the corresponding *N*-Boc amides including **148a**,**b** and **149**. These were deprotected using method B to give the amines **150a**,**b**−**155**. Reaction of the amine **150b** with diphenyl carbonate (method E) afforded **156**, while reaction of **150a** and **155** with MeCOOCl (method C) gave **157** and **158**. Reductive amination of **150a** (method F) afforded the *N*-ethyl derivative **159**.

We next prepared a series of *N*-carboxamido derivatives **160a**,**b**−**166** from the amines **150a**,**b**−**155** and the piperidine derived from **112**, using method G. The amines **150a**,**b** and **155** were also reacted with a variety of isocyanates using method H to give the *N*-alkylcarboxamido derivatives **167a**,**b**−**171a**,**b**. The amine **150a** was also reacted with MeNCS using method I to give thiocarboxamido derivative **172**. Utilizing method J, the amines **150a**,**b** were converted using the appropriate *N*-Boc amino acids into a series of amide derivatives **173**−**176** after deprotection. The amine **150b** was also converted into the cysteinyl amide **177** using methods K and L.

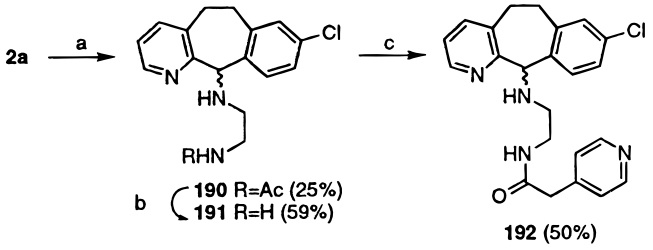
To prepare rigid analogues of **163**, 5-carboxyquinoline[17] was reduced to the 1,2,3,4-tetrahydro derivative[32] and to the perhydro derivative,[32] and each was converted into the *N*-Boc derivative and reacted with **5b** to give the *N*-Boc amides. The latter were deprotected (method B) to give **178** and **179**, which were reacted with TMSNCO (method G) to give **180** and **181**, respectively.

Using method A the amine **5b** was converted into the *N*-Boc-S-Tr-cysteinyl derivative, which was in turn deprotected to give **184**. The *N*-Boc-cystinyl derivative was prepared in a similar manner and deprotected to give **186**. The reduced cysteinyl analogues **187**−**189** were also prepared using simliar procedures to those described by others.[22]



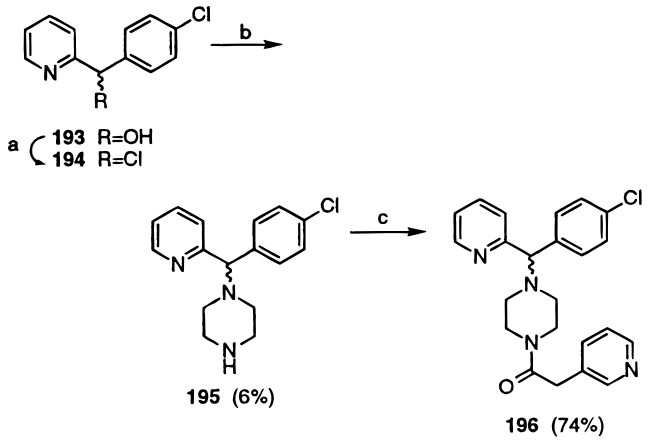
187 (less polar isomer)
188 (more polar isomer)

Scheme 2[a]



[a] (a) $NH_2CH_2CH_2NHAc$, MeOH, 28.8% HCl in EtOH, 3 Å sieves, $NaBH_3CN$, 60 °C, 236 h; (b) 85% hydrazine hydrate, MeOH, 90 °C, 13 days; (c) method A.

Scheme 3[a]

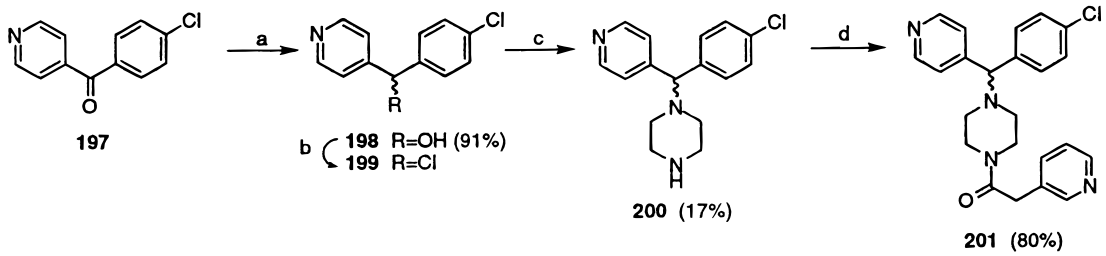


[a] (a) $SOCl_2$, toluene, 0 °C, 2 h, 0−25 °C, 1 h; (b) piperazine, THF, 25 °C, 18 h; (c) method A.

An analogue of **45a** in which the central piperazine ring has been replaced by an ethylenediamine moiety was prepared (Scheme 2) by reductive amination of **2a** with *N*-acetylethylenediamine to give **190**. Hydrazinolysis of the latter gave **191**, which was reacted with 4-pyridylacetic acid (method A) to give **192**. An analogue of **50a** lacking the 5,6-ethano bridge was also prepared (Scheme 3) by converting the alcohol **193** into the chloro derivative **194**. The latter was reacted with piperazine to give **195**, which was in turn reacted with 3-pyridylacetic acid (method A) to give **196**. The regio isomer **201** was prepared in a similar manner from the ketone **197** as shown in Scheme 4. An analogue of **196**, lacking the pyridyl ring, was also prepared from mono-*N*-Boc-piperazine **202** by reacting it with 3-pyridylacetic acid to give **203**. Deprotection of the latter gave **204** which was acylated to give **205** as described in Scheme 5.

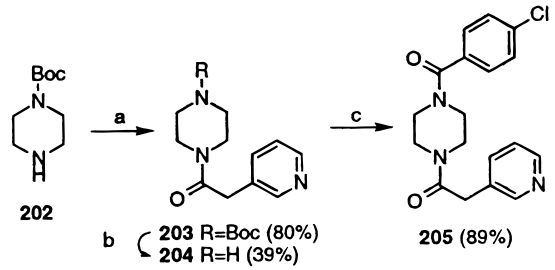
4-Carbamoyl Derivatives. We prepared a selected group of carbamoyl derivatives (Table 1) in order to determine what effect replacement of the methylene spacer with an oxygen atom would have on the FPT activity of these inhibitors. Reaction of **5a** with an excess of phosgene afforded **206**, which was reacted with the appropriate phenol to give **207** and **214a** (method M). Using method N, the appropriate chloroformates were reacted with **5a**, or **98a**, to give **208**, **209**, and **218**. The 3-pyridylchloroformate **210** was prepared from the phenol (method N) and reacted with **5a**, **46a**, and **48a** to give the carbamoyl derivatives **211**−**213**. The 4-*N*-methylpiperidinyl chloroformate **215**[33] was reacted with **5a**,**b** to give **216a**,**b** (method N). An alternative method for preparing these carbamoyl derivatives involved

Scheme 4[a]



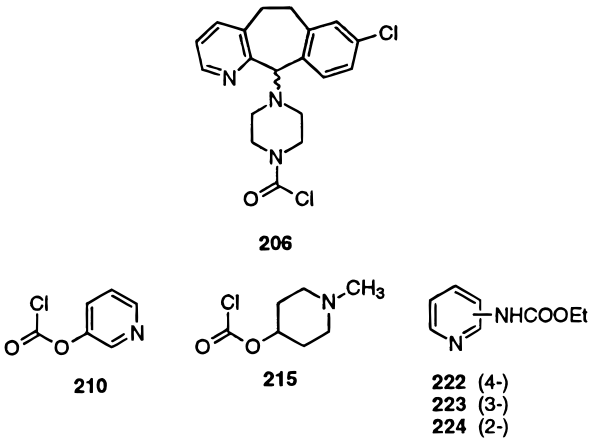
[a] (a) $NaBH_4$, MeOH, 0 °C, 1 h; (b) $SOCl_2$, toluene, 0 °C, 3.5 h; (c) piperazine, THF, CH_2Cl_2, 25 °C, 73 h; (d) method A.

Scheme 5[a]



[a] (a) Method 1; (b) 10% concentrated H_2SO_4−dioxane (v/v), 0 °C, 0.5 h; (c) 4-chlorobenzoyl chloride, THF, 25 °C, 1 h.

converting **5a**,**b** into the carbonylimidazole derivatives **219a**,**b** (Table 1) by reaction with carbonyldiimidazole (method O). Reaction of **219a**,**b** with 3-hydroxypyridine *N*-oxide or with 3-hydroxy-*N*-methylpiperidine, in the presence of $ZnBr_2$[34] (method P), gave **214b** and **217a**,**b**, respectively. The carbamoyl derivative **217a** was also prepared by reacting **219a** with 3-hydroxy-*N*-methylpiperidine and NaH (method Q).



4-Carboxamido Derivatives. A selected group of carboxamido derivatives was also prepared in which the methylene spacer had been replaced by an NH or N-Me moiety (Table 1). Reaction of **5a** with the appropriate isocyanate using method R afforded the carboxamides **220** and **221**. Alternatively **5a**,**b**, **46a**, and **48a** were fused with the appropriate ethyl or methyl cabamoylpyridine derivatives **222**−**224** and **226** (Scheme 12, Supporting Information), as described in method S, to give the carboxamido derivatives **227**−**231** and **250a**,**b**. N-Methylation of the carboxamides **227** and **228a** using KOH and MeI (method T) afforded the *N*-methyl analogues **232** and **233**, respectively. When **5a**,**b** were reacted with either 4-ethoxycarbonylamino-, or 3-ethoxycarbonylamino-*N*-methylpiperidine in the presence of β-chlorocatecholborane as described in method W, modest yields of the carboxamides **251b** and **252a**,**b** were obtained. Improved yields were obtained when the acyl azides **235**, **238**, **241**, and **242**, prepared as described in Schemes 13 and 14 respectively[35,36] (Supporting Information) were converted in situ into the isocyanates **236**, **239**, **243**, and **244**, which were then reacted with **5a**,**b** (method U) to give the carboxamides **249a**,**b**−**252a**,**b**. The carboxamide **249a** was also prepared by reacting **248** (Scheme 15, Supporting Information) with the imidazole derivative **219a** in the presence of NaH. When **219a** was reacted with 3-amino-*N*-methylpiperidine and DMAP in DMF (method X), only the carboxamide **253** was isolated, due to the Me_2NH present in the reaction medium. When the reaction was carried out in the absence of DMF under fusion conditions (method Y), only the dimer **254** was obtained. The *N*-methylpiperazinylcarboxamide **255** was also prepared by reaction of **206** with mono-*N*-methylpiperazine as described in method Z.

Biology

FPT and GGPT Activity in Vitro. The in vitro enzyme assays for measuring FPT and GGPT activity have been described earlier.[7] The results of these assays for the 4-amido, 4-carbamoyl, 4-carboxamido, and miscellaneous derivatives are listed in Table 1. The tricyclicpiperazine **5a** lacking any substituents at the 4-position of the piperazine was inactive in the FPT assay. Of the amides prepared from glycine, phenylalanine, and tyrosine and their N-substituted analogues, only **6** showed any activity. The remaining amides in this group were essentially inactive. The amide **6** showed good selectivity for FPT versus GGPT, the IC_{50} for the latter being >40 μM. The amides **39**−**41** and **43** derived from phenylacetic acid and substituted derivatives thereof were moderately active, while the amides **42** and **44** derived from substituted benzoic acids were inactive. This confirmed the importance of the methylene spacer adjacent to the amide carbonyl group in the piperazine series. Similar observations had been made in the 11-piperidinylidene series.[8,10]

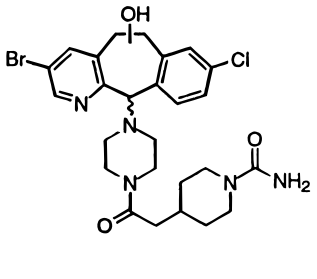
Following on the lead structure Sch 44342,[8,9] which contains a 4-pyridylacetamido moiety, we prepared a series of 4-, 3-, and 2-pyridylacetamides in the piperazine series from racemic **5a**,**b** as well as from the *R*(+) **46a** and *S*(−) **48a** enantiomers. All of them were found to be potent FPT inhibitors having good selectivity for FPT versus GGPT. The 11*S*(−)-enantiomers **49** and **52** were considerably more potent than the corresponding 11*R*(+)-enantiomers **47** and **51**. The presence of a 3-bromo substituent once again conferred much greater potency[10] on the piperazine **45b** relative to the unsub-

stituted analogue **45a**. The activity of **45a** against K-*ras*, was less than that observed against H-*ras* which was found to be true in general throughout the series. The 4-pyridylethyl analogue **53**, which lacks the amide carbonyl group of **50a**, was devoid of FPT activity (Table 1), indicating the important role that the 4-amido moiety plays in conferring activity in this series of FPT inhibitors, as well as the 11-piperidinylidene series.[10]

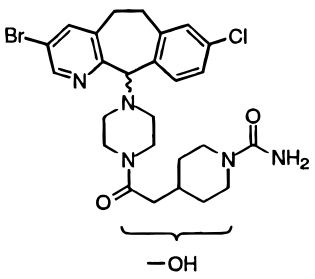
A study of the metabolites formed from **50a** when it was administered to mice, using tandem mass spectrometry, led to the identification of the two principal metabolites **79** and **80a** in the serum. Other potential sites of metabolism were the 3-position in the tricyclic pyridine ring and the methylene group adjacent to the amide carbonyl, although these possible metabolites were not observed from **50a**. A series of derivatives having reduced potential for metabolism was prepared, and these included the α,α-dimethylphenylacetamido derivative **55**, which was found to be less active than than either of its 4- or 3-pyridyl counterparts **73** and **74a**. The monomethyl analogue **75** was less potent than the dimethyl derivative **74a**, and both the mono- and dimethyl analogues were less potent than the corresponding unsubstituted analogues **45a** and **50a**. The 3-bromo derivative **74b** was again more potent than the unsubstituted analogue **74a**. The 3-pyridylacrylicamide **76** was also found to possess moderate FPT activity. The 5-carboxamidoquinoline derivatives **77a**,**b** were prepared as rigid mimics of **50a**,**b**, but they were found to be inactive as FPT inhibitors. The 7-carboxamidoquinoline derivative **78** showed moderate FPT activity. A series of 3- and 4-pyridylacetamido N4-oxides **80a**,**b**−**85a**,**b** and a 5-carboxamidoquinoline N4-oxide **86** were found to be slightly less potent FPT inhibitors than their parent pyridine and quinoline counterparts. It is interesting to note that, in the 3-bromo series, the 11*R*(+)-enantiomer **84b** was equipotent to the 11*S*(−)-enantiomer **85b**, in contrast to what was observed in the 3-unsubstituted series where the 11*S*(−)-enantiomers were more potent. The great advantage of the *N*-oxides **80a**,**b** and **83a**,**b**−**85a**,**b**, lay in their metabolic stability and enhanced bioavailability, which will be discussed later. The N1-oxide **91** was slightly less active than the pyridyl analogue **50a**, while the N1,N4-oxide **92** was less active than either the corresponding N1-oxide **91** or the N4-oxide **80a**. The α,α-dimethyl derivatives **93a**,**b** and **94a**,**b** were as active as their unsubstituted methylene counterparts **80a**,**b** and **83a**,**b**, respectively. The 3-pyridylacetamides **99** and **100**, having a 5,6-ene moiety[18,19] in place of the 5,6-ethano bridge, were less potent than **50a** and **80a**, while the corresponding 4-pyridylacetamide **101** was equipotent to **84b**. The 4-chloro derivative[20] in the 3-pyridyl series **107** was slightly less potent than the parent compound **50a**, while in the 4-pyridyl *N*-oxide series **108**, it was found to be slightly more potent than the parent **83a**. The effect of 2-substitution in the piperazine ring[21−23] was also investigated, and the 2*S*-*n*-butyl and 2*S*-methoxyethyl analogues **115** and **116**, respectively, showed potency similar to that of the unsubstituted piperazine **83b**.

The search for an alternative, metabolically resistant group to replace the pyridylacetyl moiety led first to the synthesis of a series of *N*-acetyl derivatives **118**−**120**, **122**, **124**, and **125**, of which only **118** and **120** showed modest FPT activity. The corresponding *N*-methyl, *N*-Boc, NH, and NCOOEt derivatives **127**−**130** were inactive. All of these derivatives lacked the methylene spacer adjacent to the amide carbonyl. The *N*-methyl-4- and -3-piperidinylacetamides **135a**,**b**−**140** on the other hand, which possess the methylene spacer, were potent FPT inhibitors. They again showed good selectivity for FPT over GGPT. The corresponding 5,6-ene derivatives **141** and **142** retained essentially the same potency as the ethano analogues **135a** and **138a**, respectively. The 4-chloro analogue **143** exhibited a potency intermediate between that of **135a** and **135b**. The *N*-acetyl-4- and -3-piperidinylacetamides **144a**,**b**−**147** were less potent than the *N*-methyl derivatives. The corresponding *N*-Boc derivatives in the 3-unsubstituted series **148a** and **149** were inactive, while in the 3-bromo series the *N*-Boc derivative **148b** showed modest FPT potency. Removal of the *N*-Boc group gave the free piperidinylacetamides **150a**,**b**−**155**, which were potent FPT inhibitors.

The availability of these free piperidines enabled us to explore the SAR at this site in the molecule to see what types of functional groups would be tolerated on the piperidine. The phenyl carbamate **156** in the 3-bromo series was quite active. The methyl carbamates **157** and **158** in the unsubstituted series were less potent than the corresponding free piperidines. The *N*-ethyl derivative **159** showed potency similar to that of the *N*-methyl analogue **135a**. An alternative neutral substituent was needed that would be superior to the *N*-acetyl and *N*-carbamoyl substituents, and this was found to be the *N*-carboxamido moiety. The *N*-carboxamido derivatives in both the unsubstituted series, as well as the 3-bromo series, namely **160a**,**b**−**166**, were found to be potent FPT inhibitors. When **160b** was administered to mice, the principal metabolites were found to be **256** and **257**. A series of *N*-alkyl-substituted carboxamides **167a**,**b**−**171a**,**b** were prepared, and these analogues were only slightly less potent than the unsubstituted carboxamides. The methylthiocarboxamide **172** was also a potent FPT inhibitor. A series of amido derivatives **173**−**177**, derived from various amino acids, was also prepared, and they were found in general to be somewhat less potent than the carboxamides. It was apparent from these studies that a wide variety of N-substituents could be tolerated in this series of piperidinylacetyl analogues.



256



257

Four tetra- and perhydroquinoline amides **178**−**181**, which constitute rigid analogues of **155** and **163**, were prepared, and only **180** and **181** were active as FPT inhibitors. The cysteinyl and cystinyl amido derivatives **182**−**186** were also prepared, and the deprotected amino

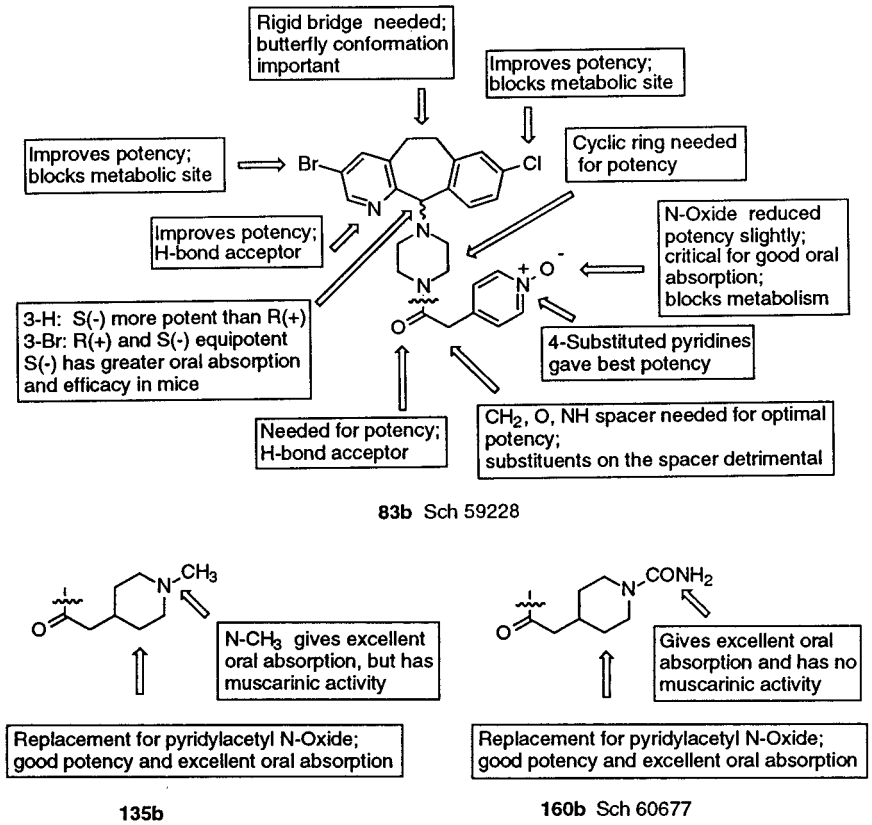


Figure 1. Summary of SAR data.

acid derivatives **184** and **186** showed only modest FPT activity. The analogues **187**−**189** in which the carbonyl group of the cysteine had been replaced by a methylene group were devoid of FPT activity.

Replacement of the central piperazine ring with an ethylenediamine moiety as in **192** resulted in a loss of FPT activity. Similarly, removal of the ethano bridge as in **196** also gave an inactive compound. The position isomer **201** and the chlorobenzoyl analogue, lacking the pyridyl moiety, were also inactive.

The recognition of the importance of the amide carbonyl together with the adjacent methylene group led us to synthesize the carbamoyl and carboxamido derivatives in which the CH_2 had been replaced with an O or NH spacer moiety, respectively (Table 1). The carboxamido derivatives would be expected to be metabolically stable. The arylcarbamoyl derivatives **207** and **208** were inactive, while **209** and **218** were active as FPT inhibitors. The corresponding 3-pyridyl analogues **211**−**213** exhibited FPT activity similar to that of the corresponding amides described earlier. The *N*-oxides **214a**,**b** were again slightly less active than the free pyridyl analogues. The *N*-methylpiperidinylcarbamates **216a**,**b** and **217a**,**b** were also active as FPT inhibitors. The imidazole derivative **219a** was essentially devoid of FPT activity, while the 3-bromo analogue **219b** was moderately active. The benzylcarboxamide **220** was again less active than the phenylcarboxamide **221** in keeping with what had been observed in the amide series. The pyridylcarboxamide analogues **227**, **228a**,**b**−**231** were all active FPT inhibitors, but were less potent than their amide counterparts described earlier. Methylation of the carboxamide NH as in **232** and **233** resulted in a considerable loss of potency. The *N*-oxides **249a**,**b** and **250a**,**b** were also active FPT inhibitors, having slightly lower potency than their corresponding amide analogues described earlier. In contrast the *N*-methylpiperidinylcarboxamides **251a**,**b** and **252a**,**b** were more potent than their corresponding amide analogues.The carboxamide byproducts **253** and **254** were inactive, as was the piperazinylcarboxamide **255**.

The SAR data for three key members of this class of FPT inhibitors, namely **83b**, **135b**, and **160b**, are summarized in Figure 1. The recent elucidation of the X-ray crystal structure of mammalian farnesyl transferase[37,38] afforded for the first time a detailed picture of the binding pocket. It is clear that the large binding pocket present in farnesyl transferase can readily accommodate these novel tricyclic inhibitors.

In view of the fact that Ras farnesylation is an intracellular event, the effect of these new tricyclic inhibitors in whole cells was examined in Cos-7 monkey kidney cells transiently expressing either H-*ras*-[Val^{12}]-CVLS, or H-*ras*-[Val^{12}]CVLL as described earlier.[7] The Cos cell assay is used to measure processing of the H-*ras* protein in cells in the presence of compounds that exert a biochemical inhibition of farnesyl transferase. This assay measures the ability of a given compound to cross cellular membranes and inhibit farnesyl transferase in cells. These tricyclic FPT inhibitors inhibit fanesylation of Ras-CVLS in cells but do not inhibit Ras-CVLL processing or morphology,[7] indicating that the in vitro specificity observed with these inhibitors carries over into the cell-based processing assay. In contrast, Lovistatin is equally effective at blocking processing and morphological changes induced by Ras-CVLS and Ras-CVLL. The IC_{50} values are given in Table 1. In general the Cos IC_{50s} for Ras farnesylation ranged from 0.7 to

Table 2. Pharmacokinetic Parameters[a]

no.	dose (mg/kg)	AUC (μg·h/mL) iv	AUC (μg·h/mL) po	C_{max} (μM) po	$t_{1/2}$ (h) iv	% oral bioavailability	time above IC_{50} (h) po
			Nude Mice				
45a[b,c]	25	1.8[d]	0.24[d]	0.9	0.2	13	1
45b[b]	25	5.3[e]	1.1[e]	0.9	0.74	20.8	6
50a[b]	25	1.1[e]	0.4[e]	3.9	0.2	36	0.3
80a[b]	25	12.3[d]	11[d]	12	0.6	89	4
80b	25	24.0[e]	34.8[e]	45.4	1.0	100	6
83b	25	23.4[e]	19.6[e]	10	1.2	84	6+
84a	25	11.3[e]	6.0[e]	3	1.1	53	2
84b	25	27.1[e]	31.9[e]	25	1.18	100	7
85a	25	21.5[e]	15.5[e]	10	0.8	72	4
85b	25	26.1[e]	35.3[e]	24.6	1.9	100	7
93b	25	25.0[d]	11.7[d]	11	0.6	47	4
115	25	91.4[e]	43.2[e]	11.5	2.0	47	7+
116	25		19.9	9			7
135a[b]	25	4.1[d]	1.3[d]	1.4	>1	32	4
135b	25	40.6[e]	18.4[e]	4	8.3	45	24+
135b[b]	25	32.9[e]	39.0[e]	30	8.5	100	24+
138b	25	14.9[e]	17[e]	2	5.7	100	24
144b	25	9.51[e]	2.74[e]	3	0.37	28.8	2
150b	25	27.2[e]	17.6[e]	3	2	65	24
160b	25	33.9[e]	70.8[e]	38.8	1.2	100	8+
160b[f]	25	57.2[e]	55.2[e]	71.1	1.5	96.6	7+
167b	25	9.9[e]	33.3[e]	80	0.5	100	4
			Cynomolgus Monkeys				
50a	25[g]	48.6[h]	1.07[h]	0.29	1.5	2.2	
50a	25[b]	48.6[h]	0.45[h]	0.11	1.5	1.4	
83b	5	24.4[i]	9.4 ± 2.3[i]	1.2	2.6	38.5 ± 9.6	7
83b	10		35.6 ± 9.5[i]	5.7			24
83b	10[j]		16.7 ± 1.5	3			24
84b	10		9.1 ± 1.5[i]	1.8			12+
85b	10		58.1 ± 11.7[i]	8			24+
101	10		11.7 ± 1.1[i]	1.9			31
135b	25[b]		37.2[i]	1.5			72
160b	5	12.3[i]	4.27 ± 1.09[i]	0.5	2.4	35	7
160b	10		12.7 ± 6.3[i]	2			24

[a] Animals were dosed with solutions of the hydrochloride salts of the compounds indicated in the table in 20% HPβCD, unless otherwise noted. [b] Administered as the hydrochloride salt in saline solution. [c] ICR mice. [d] AUC (0−4 h). [e] AUC (0−24 h). [f] Administered as the micronized free base in 20% HPβCD. [g] Dosed orally as the micronized free base in corn oil; dosed iv as the hydrochloride salt in saline solution. [h] AUC (0−8 h). [i] AUC (0−48 h). [j] Dosed po as the hydrochloride salt in 0.4% methyl cellulose.

49 times greater than in the corresponding enzyme inhibition assays. This is in sharp contrast to many of the pepidomimetics that have been described in the literature which, despite their higher intrinsic potency in some cases and universally poor pharmacokinetics, exhibit IC_{50} values that are 200−2000 times greater than their IC_{50}s in enzymatic assays. The Cos IC_{50} values seen in these tricyclic inhibitors may well be a result of their favorable metabolic stability and excellent cell penetration.

The effects of two of the most important compounds, namely **83b** and **85b**, on the anchorage-independent growth of NIH-H, NIH-K, and DLD-1 tumor cell lines in soft agar[39,40] were determined. The soft agar cloning assays are used to examine the potential therapeutic effects of a given farnesyl transferase inhibitor. Compounds that are active in the Cos assay, and therefore are shown to cross the cell membrane, are then examined for their potential antitumor activity in the soft agar cloning assays. Compounds that are inhibitors of soft agar cloning are candidates for in vivo analysis. The tumor cell line expressing the H-*ras* oncogene (NIH-H) was found to be more sensitive to **83b** and **85b** than the cell lines expressing the K-*ras* oncogene (NIH-K and DLD-1).

Pharmacokinetic Studies. The pharmacokinetic parameters for a selection of the inhibitors prepared in this group were determined in nude mice, and the results are given in Table 2. The early pyridylacetyl lead structures **45a** and **50a** were shown to be heavily metabolized, and the low AUCs that were observed for these compounds reflect this fact. However, it was encouraging to note that they were both orally bioavailable. The 3-bromo analogue **45b** showed a slight improvement over **45a** in both AUC, serum half-life, and duration of action. The introduction of an *N*-oxide on the pyridylacetyl moiety in **80a**,**b** and **83b** which effectively blocked metabolism at this site resulted in a dramatic increase in the AUCs of these compounds relative to their pyridylacetyl counterparts. The compounds exhibited good oral absorption in mice with serum half-lives of 0.6−1.2 h and 84−100% oral bioavailability. The pharmacokinetics of the pure 11-enantiomers **84a**,**b** and **85a**,**b** were also studied, and in the 3-unsubstituted series the 11*S*(−)-enantiomer **85a** was found to exhibit superior oral absorption characteristics to the 11*R*(+)-enantiomer **84a.** In the 3-bromo series, on the other hand, the enantiomers **84b** and **85b** exhibited similar AUCs. Thus **85b** showed a po AUC of 35.3 μg·h/mL, showed a po C_{max} of 24.6 μM, and was 100% orally bioavailability. The serum concentrations also remained above the IC_{50} for 7 h when dosed orally. The α,α-dimethyl analogue **93b** showed poorer oral absorption characteristics than the unsubstituted compound **80b**. The introduction of a 2*S*-*n*-butyl substituent in the piperazine ring as in **115**

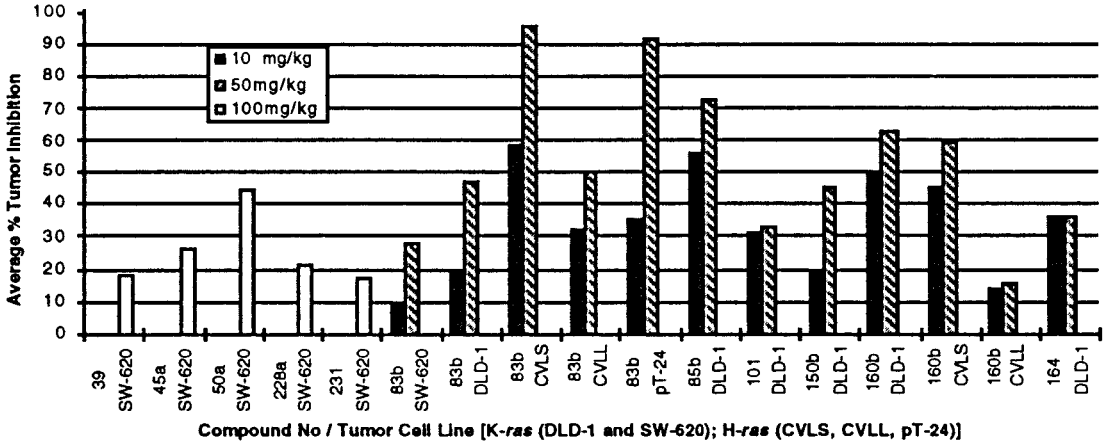


Figure 2. In vivo antitumor efficacy in mice.

resulted in a higher po AUC, but the oral bioavailability was lower than that of **83b**. The introduction of a 2*S*-methoxyethyl substituent in the piperazine ring as in **116** gave a compound with an oral AUC similar to that of **83b**.

The *N*-methyl-4-piperidinylacetyl analogue **135a** exhibited better serum levels and bioavailability than the 4-pyridylacetyl analogue **45a**. The corresponding 3-bromo analogue **135b** showed a comparable po AUC to that of **83b** with a lower C_{max}, a longer serum half-life, and duration above the IC_{50} but a lower oral bioavailability. The oral absorption of **135b** was significantly higher when the compound was dosed in saline solution. The *N*-methyl-3-piperidinylacetyl derivative **138b** also exhibited excellent oral absorption and pharmacokinetics. In general the *N*-methylpiperidine analogues exhibited muscarinic activity (Table 7, Supporting Information). The *N*-acetyl-4-piperidinylacetyl analogue **144b** on the other hand exhibited poor oral absorption, while the N-unsubstituted analogue **150b** showed good oral absorption characteristics. The introduction of an *N*-carboxamido substituent as in **160b** gave a compound with excellent oral pharmacokinetics (Table 2) and negligible muscarinic activity (Table 7). The po AUC was 70.8 μg·h/mL with a po C_{max} of 38.8 μM and a serum half-life of 1.2 h. The compound exhibited 100% oral bioavailability and was present in serum at concentrations above the IC_{50} for 8+ h. When the free base was used, a slightly lower AUC was observed than with the hydrochloride salt. The po AUC of the racemic **160b** was greater than that of the racemic **83b** and than that of the pure 11*S*(−)-enantiomer **85b**. The *N*-methylcarboxamido analogue **167b** also showed good oral absorption, with a po AUC about half that of **160b** and a higher C_{max}. It was also present in serum at concentrations above the IC_{50} for 4 h.

The pharmacokinetics of the more interesting compounds from the above studies in mice were then determined in cynomolgus monkeys, and the results are given in Table 2. As was observed in mice, the 3-pyridylacetyl derivative **50a** showed poor oral bioavailability due to metabolism. On the other hand, the 4-pyridylacetyl *N*-oxides **83b**, **84b**, and **85b** all exhibited excellent oral absorption characteristics with the 11*S*(−)-enantiomer **85b** being superior to either the racemate **83b** or the 11*R*(+)-enantiomer **84b**. Introduction of a 5,6-ene, as in **101**, gave a compound with a lower po AUC than that of the ethano analogue **83b**. Both the *N*-methyl-4-piperidinylacetyl derivative **135b** and the *N*-carboxamido-4-piperidinyl derivative **160b** exhibited excellent oral pharmacokinetics.

In Vivo Antitumor Efficacy in Mice. Nude mice carrying tumors grown from tumor cell lines containing mutated K-*ras* (DLD-1 and SW-620) and mutated H-*ras* oncogenes (CVLS, CVLL and pT-24) were dosed with selected inhibitors prepared in this study, and the results are given in Figure 2. The tumor models include CVLS (NIH3T3 cells transfected with oncogenic H-*ras*) and pT-24 (BALB c/3T3 cells transfected with oncogenic H-*ras*) cells, each of which have activated H-*ras* containing its native CVLS farnesylation sequence. Tumor models using human colon adenocarcinoma DLD-1 and SW-620 cells, which express activated K-*ras*, were also used. It should be noted that the SW-620 model is less sensitive to farnesyl transferase inhibitors than the DLD-1 model. On the other hand, the model using CVLL (NIH3T3 cells transfected with oncogenic H-*ras*) cells in which the terminal sequence of the activated H-*ras* is altered to CVLL was used as a control to direct the prenylation of the protein toward geranylgeranylation rather than farnesylation. As anticipated for selective FPT inhibitors, the percent tumor inhibition in the control CVLL model was considerably lower than that observed in either the CVLS, or pT-24 models suggesting that these tricyclics were specific Ras farnesylation inhibitors.[41,42] However, the observation that efficacy was also seen at higher doses (although significantly reduced) against tumors derived from cells transformed with a geranylgeranylated form of H-*ras* suggests that inhibition of Ras farnesylation may not be solely responsible for the observed antitumor effect.[42] Recent reports have demonstrated that the K- and N-isoforms of the Ras protein are geranylgeranylated in cells treated with FPT inhibitors,[39,43−45] which may also be the case in the in vivo setting although this has not been demonstrated. Whether it is a geranylgeranylated form of the Ras protein that is driving the transformation or whether there is a distinct farnesylated protein lying downstream of Ras in the transformation pathway that is responsible for the observed activity in the CVLL model is still unclear.[42] K- and N-*ras* proteins are more frequently mutated in human cancers than H-*ras*. These results suggest a potential mechanism for resistance of tumor cells to FPT inhibitors. However, it has

been shown[39,40] that 60−70% of human tumor cell lines tested were sensitive to growth inhibition by FPT inhibitors. The mechanism for this sensitivity is not understood. The effects of FPT inhibitors could be mediated through Ras if the geranylgeranylated forms of the protein do not signal as efficiently as the farnesylated forms of the proteins. It is also possible that the effects of FPT inhibitors may be mediated through farnesylated proteins other than Ras, such as rho B.[46] Even though the identity of the proteins mediating the antitumor effects of FPT inhibitors remains an important unanswered question, the effects observed in human tumor cell lines with several different classes of these inhibitors suggest broad utility as cancer chemotherapeutic agents.

The amides **39**, **45a**, and **50a** all inhibited tumor growth to a moderate degree. The racemic 4-pyridylacetyl *N*-oxide **83b** (Sch 59228)[41,42] was considerably more effective in reducing the extent of tumor growth in all of the cell lines tested. The 11*S*(−)-enantiomer **85b** (Sch 61129) was nearly twice as effective as **83b** at comparable doses. The 5,6-ene analogue **101** was slightly less effective in reducing tumor volume than the corresponding ethano analogue **83b**. The 4-piperidinylacetyl derivative **150b** showed inhibition of tumor growth similar to that observed with **83b**, but it also showed some toxicity at the higher dose. The racemic *N*-carboxamidopiperidinylacetyl derivative **160b** was also almost as effective as the 11*S*(−)-enantiomer of the 4-pyridylacetyl *N*-oxide derivative **85b**. The 5,6-ene analogue **164** was once again less effective in reducing tumor growth than the corresponding ethano analogue **160b**. The 3-pyridylcarboxamide **228a** was about half as effective in reducing tumor volume as the corresponding amide **50a**. The 2-pyridylcarboxamide **231** showed efficacy similar to that of **228a**.

These results clearly indicate that these new FPT inhibitors possess in vivo antitumor activity upon oral dosing in tumor models expressing activated *ras* oncogenes. Particularly noteworthy were the compounds **83b** (Sch 59228), **85b** (Sch 61129), and **160b** (Sch 60677) which showed excellent in vivo efficacy in mice suggesting broad utility as potential cancer chemotherapeutic agents.

Conclusions

A novel group of tricyclicpiperazinyl derivatives has been discovered which are selective Ras farnesylation inhibitors. They are selective for FPT over GGPT, are nonpeptidic in nature, and do not contain sulfhydryl groups. In the piperazine series an extensive SAR has been developed within the group of compounds having amido substituents on the piperazine ring and this was used to evaluate the merits of the carbamoyl and carboxamido analogues. All three series gave potent FPT inhibitors in vitro; however, the amides proved to be the superior group based on their cellular activity. Three compounds, namely the racemic 4-pyridylacetyl *N*-oxide **83b** (Sch 59228), its 11*S*(−)-enantiomer **85b** (Sch 61129), and the racemic *N*-carboxamido-4-piperidinylacetyl derivative **160b** (Sch 60677) were clearly superior in view of their excellent pharmacokinetic profiles and oral activity and were evaluated[41,42] along with additional tricyclic derivatives from these laboratories, as potential orally active antitumor agents.

Experimental Section

^{13}C NMR spectra were obtained at 300 MHz on a Varian Gemini 300 instrument. Chemical shifts are reported in ppm (δ_C) using Me_4Si as standard. Chemical ionization mass spectra (CIMS) were obtained on a Hewlett-Packard MS-engine mass spectrometer using methane as a reagent gas. The samples were introduced through a particle-beam interface and the source temperature was kept at 250 °C. Fast atom bombardment mass spectra (FABMS) and liquid secondary ion mass spectra (LSIMS) were carried out on double-focusing mass spectrometers (JEOL JMS-HX-110a, VG-ZAB-SE, MAT-90) operating at 5−10 kV accelerating voltage. Samples were introduced into the mass spectrometer in the condensed phase and desorbed and ionized by bombardment with fast moving atoms (FAB) or ions (LSIMS). In the case of FABMS, xenon atoms at 8 kV energy were used, while for LSIMS, high-energy Cs^+ ions at 25−30 kV were applied. Typically, 1−2 μg of the sample dissolved in 1−2 μL of DMSO was deposited onto a stainless steel probe tip containing 1−2 μL of matrix (3-NBA, or glycerol/thioglycerol mixture, 1:1), which was then introduced onto the mass spectrometer.

Rotations were recorded on a Perkin-Elmer 243B polarimeter. Microanalyses for C, H, N, and S were determined on a Fisons EA1108 elemental analyzer, while Br and Cl were run by Robertson Microlit. All solvents used for reactions were anhydrous. Products were purified by column chromatography on 63−200 mesh silica gel (Selecto Scientific). Reactions and purity of samples were monitored on 250 μm silica gel GF thin layer plates (Analtech). In general the solvent systems used in chromatography consisted of the appropriate percentage (ca. 0.25−10%) of a 10% concentrated ammonium hydroxide solution in methanol, in dichloromethane. Yields are unoptimized. All reactions were carried out under dry argon.

1-(3-Bromo-8-chloro-6,11-dihydro-5*H*-benzo[5,6]-cyclohepta[1,2-*b*]pyridin-11-yl)piperazine (5b) (Scheme 1). Using essentially similar procedures to those described earlier,[11] 3-bromo-8-chloro-11*H*-benzo[5,6]cyclohepta[1,2-*b*]pyridin-11-one **(2b)**[11,12] was reduced to the 11-ol **3b** (92%): CIMS *m*/*z* 324 (MH^+); ^{13}C NMR δ_C (CH_2) 31.3, 29.9; (CH) 145.8, 141.6, 128.6, 127.1, 125.4, 68.8; (C) 154.4, 140.6, 138.6, 134.2, 133.2, 119.6. Anal. ($C_{14}H_{11}BrClNO$) C, H, Br, Cl, N. The 11-ol **3b** was reacted with thionyl chloride to afford the 11-chloro derivative **4b** which was in turn reacted with an excess of piperazine to give **5b** (Table 1).

(+)-1-(3-Bromo-8-chloro-6,11-dihydro-5*H*-benzo[5,6]-cyclohepta[1,2-*b*]pyridin-11(*R*)-yl)piperazine (46b) and (−)-1-(3-Bromo-8-chloro-6,11-dihydro-5*H*-benzo[5,6]-cyclohepta[1,2-*b*]pyridin-11(S)-yl)piperazine (48b). Racemic **5b** (29.92 g) was resolved by Chiral Technologies on a CHIRALPAK AD (10 × 50 cm) HPLC column using hexane−2-propanol−diethylamine (60−40−0.1) as the eluant at 40 °C using UV 254 nm detection to give **46b** (12.9 g, 86.2% recovery, 97.6% ee, 99+% chemical purity) [CIMS *m*/*z* 392 (MH^+); ^{13}C NMR δ_C essentially identical to that reported for **5b** (Table 1); $[\alpha]_D^{25}$ +25.8° (*c* 0.423, MeOH). Anal. ($C_{18}H_{19}BrClN_3$) C, H, Cl, N; Br: calcd, 20.35; found, 19.22] followed by **48b** (8.5 g, 57.1% recovery, 99.7% ee, 97.7% chemical purity): CIMS *m*/*z* 392 (MH^+); ^{13}C NMR δ_C essentially identical to that reported for **5b** (Table 1); $[\alpha]_D^{25}$ −27.9° (*c* 0.445, MeOH). Anal. ($C_{18}H_{19}BrClN_3$) C, H, Cl, N; Br: calcd, 20.35; found, 19.37.

Method A: General Procedure for the Synthesis of the Amides in Table 1. 1-(3-Bromo-8-chloro-6,11-dihydro-5*H*-benzo[5,6]cyclohepta[1,2-*b*]pyridin-11-yl)-4-(4-pyridylacetyl)piperazine N4-oxide (83b). The piperazine derivative **5b** (2.1 g, 5.35 mmol), 4-pyridylacetic acid *N*-oxide **70** (1.23 g, 8.0 mmol), 1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide hydrochloride (1.54 g, 8.0 mmol), 1-hydroxybenzotriazole (1.08 g, 8.0 mmol), and 4-methylmorpholine (1.18 mL, 8.0 mmol) were dissolved in DMF (50 mL), and the mixture was stirred at 25 °C for 18 h. The solution was evaporated to dryness, and the residue was taken up in CH_2Cl_2, washed with

saturated aqueous $NaHCO_3$ and water, and dried (Na_2SO_4). The product was chromatographed on a silica gel column using 2.5% (10% concentrated NH_4OH in MeOH)−CH_2Cl_2 to give **83b** (Table 1).

Using the above procedure, the various intermediate piperazines **5a**,**b**, **46a**,**b**, **48a**,**b**, **90**, **98a**,**b**, **106**, **112**, and **114**, were reacted with the appropriate acids to give the products shown in Table 1 where method A is indicated.

Method B: 1-(3-Bromo-8-chloro-6,11-dihydro-5*H*-benzo-[5,6]cyclohepta[1,2-*b*]pyridin-11-yl)-4-[(4-piperidinyl)-acetyl]piperazine (150b). The *N*-Boc derivative **148b** (4.61 g, 7.5 mmol) was dissolved in MeOH (40 mL), and a 10% (v/v) concentrated H_2SO_4 in dioxane solution (100 mL) was added. The mixture was stirred at 25 °C for 2 h and then basified with concentrated aqueous NaOH. The usual workup followed by chromatography on silica gel using 10% (10% concentrated NH_4OH in MeOH)−CH_2Cl_2 gave **150b** (Table 1).

In Table 1 where method B is indicated, the appropriate *N*-Boc derivative prepared by method A above was deprotected as above, to give the corresponding free amine.

Method C: Methyl 4-[[[4-(8-chloro-6,11-dihydro-5*H*-benzo[5,6]cyclohepta[1,2-*b*]pyridin-11-yl)-1-piperazinyl]-carbonyl]methyl]-1-piperidinecarboxylate (157). The amine **150a** (500 mg, 1.1 mmol) was dissolved in dry THF (5 mL), and MeOCOCl (107.6 mg/0.088 mL, 1.1 mmol) was added. The mixture was stirred at 25 °C for 1 h and then evaporated to dryness. The product was worked up in the usual way and chromatographed on silica gel using 1.5% (10% concentrated NH_4OH in MeOH)−CH_2Cl_2 as the eluant to give **157** (Table 1).

In Table 1 where method C is indicated, the appropriate amine was reacted the chloroformate to give the corresponding carbamoyl derivative.

Method D: 1-(3-Bromo-8-chloro-6,11-dihydro-5*H*-benzo-[5,6]cyclohepta[1,2-*b*]pyridin-11-yl)-4-[(1-acetyl-4-piperidinyl)acetyl]piperazine (144b). The amine **150b** (500 mg, 1.0 mmol) and Ac_2O (591.4 mg/0.546 mL, 6 mmol) were dissolved in MeOH (5 mL), and the mixture was stirred at 25 °C for 19 h. The usual workup followed by chromatography on silica gel using 2% (10% concentrated NH_4OH in MeOH)−CH_2Cl_2 gave **144b** (Table 1).

Method E: Phenyl 4-[2-[4-(3-bromo-8-chloro-6,11-dihydro-5*H*-benzo[5,6]cyclohepta[1,2-*b*]pyridin-11-yl)-1-piperazinyl]-2-oxoethyl]-1-piperidinecarboxylate (156). The amine **150b** (500 mg, 0.1 mmol) and $(C_6H_5O)_2CO$ (1.02 g, 0.2 mmol) were dissolved in 2-propanol (20 mL), and the mixture was heated at 87 °C for 52.5 h. The usual workup followed by chromatography on silica gel using EtOAc as the eluant gave **156** (Table 1).

Method F: 1-(8-Chloro-6,11-dihydro-5*H*-benzo[5,6]-cyclohepta[1,2-*b*]pyridin-11-yl)-4-[(1-ethyl-4-piperidinyl)acetyl]piperazine (159). The amine **150a** (500 mg, 1.0 mmol) was dissolved in 0.6 N HCl in CH_2Cl_2 (10 mL) and stirred for 5 min. Evaporation to dryness gave the hydrochloride which was dissolved in MeOH (20 mL). Acetaldehyde (200.6 mg/0.254 mL, 4.0 mmol), $NaBH_3CN$ (85.9 mg, 1.2 mmol) and 3 Å molecular sieves (500 mg) were added, and the mixture was heated at 40 °C for 115 h. The usual workup, followed by chromatography on silica gel using 8% (10% concentrated NH_4OH in MeOH)−CH_2Cl_2 as the eluant gave **159** (Table 1).

Method G: 4-[2-[4-(3-Bromo-8-chloro-6,11-dihydro-5*H*-benzo[5,6]cyclohepta[1,2-*b*]pyridin-11-yl)-1-piperazinyl]-2-oxoethyl]-1-piperidinecarboxamide (160b). The amine **150b** (11 g, 21.2 mmol) was dissolved in CH_2Cl_2 (110 mL), and TMSNCO (14.7 g/17.25 mL, 127.2 mmol) was added dropwise to the solution. The mixture was stirred at 25 °C for 47 h. Additional TMSNCO (7.35 g/8.63 mL, 63.6 mmol) was added, and the mixture was stirred for a total of 166 h. The mixture was washed with saturated aqueous $NaHCO_3$ and water, dried (Na_2SO_4), and evaporated to dryness. Chromatography on silica gel using 3% (10% concentrated NH_4OH in MeOH)−CH_2Cl_2 as the eluant gave **160b** (Table 1).

In Table 1 where method G is indicated, the appropriate amine was reacted with TMSNCO as described above to give the carboxamide.

Method H: 4-[2-[4-(3-Bromo-8-chloro-6,11-dihydro-5*H*-benzo[5,6]cyclohepta[1,2-*b*]pyridin-11-yl)-1-piperazinyl]-2-oxoethyl]-*N*-methyl-1-piperidinecarboxamide (167b). The amine **150b** (500 mg, 1.0 mmol) and MeNCO (220.3 mg/0.228 mL, 4 mmol) were dissolved in CH_2Cl_2 (5 mL), and the mixture was stirred at 25 °C for 47 h. Additional MeNCO (110.2 mg/0.114 mL, 2.0 mmol) was added, and the mixture was stirred at 25 °C for a total of 144 h and at 74 °C for an additional 5 h. After an additional 24 h at 25 °C, the reaction was evaporated to dryness and chromatographed on silica gel using 2% (10% concentrated NH_4OH in MeOH)−CH_2Cl_2 as the eluant to give **167b** (Table 1).

In Table 1 where method H is indicated, the appropriate amine was reacted with the isocyanate as described above to give the N-substituted carboxamide.

MethodI: 4-[2-[4-(8-Chloro-6,11-dihydro-5*H*-benzo[5,6]-cyclohepta[1,2-*b*]pyridin-11-yl)-1-piperazinyl]-2-oxoethyl]-*N*-methyl-1-piperidinecarbothioamide (172). The amine **150a** (500 mg, 1.1 mmol) and MeNCS (166.6 mg/0.156 mL, 2.2 mmol) were dissolved in CH_2Cl_2 (5 mL), and the mixture was stirred at 25 °C for 24 h. Additional MeNCS (166.6 mg/0.156 mL, 2.2 mmol) was added, and the reaction was allowed to run for a total of 52 h. The usual workup followed by chromatography on silica gel using 3% −10% concentrated NH_4OH in MeOH)−CH_2Cl_2 as the eluant gave **172** (Table 1).

Method J: 1-[[1-(2(*S*)-Amino-3-hydroxy-1-oxopropyl)-4-piperidinyl]acetyl]-4-(3-bromo-8-chloro-6,11-dihydro-5*H*-benzo[5,6]cyclohepta[1,2-*b*]pyridin-11-yl)piperazine (175b). The amine **150b** (500 mg, 1.0 mmol), *N*-Boc-L-serine (297.2 mg, 1.5 mmol), 1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide hydrochloride (277.6 mg, 1.5 mmol), 1-hydroxybenzotriazole (195.7 mg, 1.5 mmol), and 4-methylmorpholine (146.5 mg/0.1593 mL, 1.5 mmol) were dissolved in DMF (25 mL), and the mixture was stirred at 25 °C for 24 h. The product was worked up as described in method A above, and the resulting *N*-Boc derivative was taken up in MeOH (5 mL), and 10% (v/v) concentrated H_2SO_4 in dioxane (10 mL) was added. The mixture was stirred at 25 °C for 2 h. BioRad AG1-X8(OH^-) resin was added, and the resin was filtered off and washed with MeOH. The combined filtrates were evaporated to dryness and chromatographed on silica gel using 5% (10% concentrated NH_4OH in MeOH)−CH_2Cl_2 as the eluant to give **175b** (Table 1).

In Table 1 when method J is indicated, the appropriate amine was reacted as described above with the *N*-Boc amino acid and then deprotected to give the desired compound.

Method K: 1-[[1-(2(R)-Amino-3-mercapto-1-oxopropyl)-4-piperidinyl]acetyl]-4-(3-bromo-8-chloro-6,11-dihydro-5*H*-benzo[5,6]cyclohepta[1,2-*b*]pyridin-11-yl)piperazine Hydrochloride (177). The amine **150b** (1 g, 1.9 mmol), *N*-Boc-*S*-trityl-L-cysteine (1.34 g, 2.85 mmol), 1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide hydrochloride (555.3 mg, 2.85 mmol), 1-hydroxybenzotriazole (391.4 mg, 2.85 mmol), and 4-methylmorpholine (293 mg/0.319 mL, 2.85 mmol) were dissolved in DMF (50 mL), and the mixture was stirred at 25 °C for 24 h. The product was worked up as described in method A above and chromatographed on silica gel using 0.4−0.8% (10% concentrated NH_4OH in MeOH)−CH_2Cl_2 as the eluant to give the *N*-Boc-*S*-trityl derivative (1.66 g, 89%): CIMS *m*/*z* 962.9 (MH^+).

Method L. The *N*-Boc-*S*-trityl derivative (1.56 g, 1.6 mmol) was dissolved in CH_2Cl_2 (15 mL). Et_3Si (753 mg/1.05 mL, 6.4 mmol) and TFA (7.5 mL) were added, and the mixture was stirred at 25 °C for 1 h. The solution was evaporated to dryness, and the residue was partitioned between hexane and water. The aqueous layer was again extracted with hexane (500 mL). The aqueous layer was then passed over a bed of BioRad AG3 × 4(Cl^-) resin (300 mL), and the resin was eluted with water (800 mL). The aqueous solution was lyophilized to give **177** hydrochloride (Table 1).

In Table 1 when method K is indicated, the appropriate amine was reacted as described above with the *N*-Boc-*S*-trityl amino acid and then deprotected to give the desired compound.

Method M: 3-Pyridyl 4-(8-Chloro-6,11-dihydro-5*H*-benzo[5,6]cyclohepta[1,2-*b*]pyridin-11-yl)-1-piperazinecarboxylate N1-Oxide (214a). The piperazine **5a** (500 mg, 1.6 mmol) and pyridine (0.515 mL, 6.4 mmol) in CH_2Cl_2 (12 mL) were added dropwise to a stirred solution of 1.93 M phosgene in toluene (20%) (12.6 mL, 24 mmol) in CH_2Cl_2 (12 mL) at 0 °C over 45 min. The solution was allowed to warm to 25 °C over 30 min. Excess phosgene was removed with a stream of argon, and the solution was evaporated to dryness to give **206** which was used without purification in the next step.

The crude **206** (1.6 mmol) above was dissolved in DMF (10 mL) containing pyridine (0.515 mL, 6.4 mmol), and 3-hydroxypyridine N1-oxide (885 mg, 7.97 mmol) was added. The mixture was stirred at 25 °C for 18 h and worked up in the usual way. Chromatography on silica gel using 1.5% (10% concentrated NH_4OH in MeOH)−CH_2Cl_2 as the eluant gave **214a** (Table 1).

Method N: 3-Pyridyl 4-(8-Chloro-6,11-dihydro-5*H*-benzo[5,6]cyclohepta[1,2-*b*]pyridin-11-yl)-1-piperazinecarboxylate (211). A 1.93 M solution of phosgene in toluene (20%) (173.5 mL, 334.5 mmol) was added to CH_2Cl_2 (175 mL), and the solution was cooled to 0 °C. A solution of 3-hydroxypyridine (7.27 g, 66.9 mmol) and pyridine (7.05 g/7.22 mL, 89.2 mmol) in CH_2Cl_2 (175 mL) was added dropwise at 0 °C over 1 h. The mixture was stirred and allowed to warm to 25 °C over 2 h. Excess phosgene was removed using a stream of argon with suitable trapping of the waste stream. The remaining solution was evaporated to dryness to give **210**. The residue was dissolved in CH_2Cl_2 (53 mL) and pyridine (83 mL), and the piperazine **5a** (7 g, 1222.3 mmol) was added. The mixture was stirred at 25 °C for 24 h and worked up in the usual way, and the product was chromatographed on silica gel using 1% (10% concentrated NH_4OH in MeOH)−CH_2Cl_2 as the eluant to give **211** (Table 1).

Method O: 1-(8-Chloro-6,11-dihydro-5*H*-benzo[5,6]cyclohepta[1,2-*b*]pyridin-11-yl)-4-(1*H*-imidazol-1-ylcarbonyl)piperazine (219a). The piperazine **5a** (10 g, 31.9 mmol) was dissolved in CH_2Cl_2 (100 mL) and added to a stirred solution of carbonyldiimidazole (5.17 g, 31.9 mmol) in CH_2Cl_2 (150 mL) at 0 °C over 45 min. The mixture was stirred at 0 °C for a total of 2 h and then worked up in the usual way. Chromatography on silica gel using 2% (10% concentrated NH_4OH in MeOH)−CH_2Cl_2 as the eluant gave **219a** (Table 1).

Similarly **5b** was reacted as above to give **219b** (Table 1).

Method P: 1-Methyl-3-piperidinyl 4-(3-Bromo-8-chloro-6,11-dihydro-5*H*-benzo[5,6]cyclohepta[1,2-*b*]pyridin-11-yl)-1-piperazinecarboxylate (217b). The imidazole derivative **219b** (670.8 mg, 1.6 mmol), $ZnBr_2$ (1.24 g, 6.4 mmol), and 3-hydroxy-*N*-methylpiperidine (634.9 mg, 6.4 mmol) were dissolved in DMF (20 mL), and the mixture was heated at 90 °C for 72 h. The mixture was evaporated to dryness and worked up in the usual way. The product was chromatographed on silica gel using 1.5% (10% concentrated NH_4OH in MeOH)−CH_2Cl_2 as the eluant to give **217b** (Table 1).

In Table 1 when method P is indicated, the appropriate imidazole derivative was reacted as described above to afford the desired compound.

Method Q: 1-Methyl-3-piperidinyl 4-(8-Chloro-6,11-dihydro-5*H*-benzo[5,6]cyclohepta[1,2-*b*]pyridin-11-yl)-1-piperazinecarboxylate (217a). 3-Hydroxy-*N*-methylpiperidine (550 mg, 4.68 mmol) and a 60% dispersion of NaH in mineral oil (110.5 mg, 3.6 mmol) were added to DMF (10 mL), and the mixture was stirred at 25 °C for 15 min. The imidazole derivative **219a** (500 mg, 1.2 mmol) was added, and the mixture was stirred at 90 °C for 96 h. The reaction was worked up in the usual way and chromatographed on silica gel using 1.5% (10% concentrated NH_4OH in MeOH)−CH_2Cl_2 as the eluant to give **217a** (Table 1).

Method R: *N*-Benzyl-4-(8-chloro-6,11-dihydro-5*H*-benzo[5,6]cyclohepta[1,2-*b*]pyridin-11-yl)-1-piperazinecarboxamide (220). Benzyl isocyanate (424.3 mg/0.394 mL, 3.19 mmol) was dissolved in CH_2Cl_2 (1 mL), and a solution of the piperazine **5a** (1 g, 3.19 mmol) was added dropwise at 25 °C over 20 min. The mixture was stirred at 25 °C for 3 h and then worked up in the usual way. The product was chromatographed on silica gel using 1% (10% concentrated NH_4OH in MeOH)−CH_2Cl_2 as the eluant to give **220** (Table 1).

In Table 1 when method R is cited, the appropriate amine was reacted with the appropriate isocyanate as described above to give the desired compound.

Method S: 4-(8-Chloro-6,11-dihydro-5*H*-benzo[5,6]cyclohepta[1,2-*b*]pyridin-11-yl)-*N*-(1-methyl-3-piperidinyl)-1-piperazinecarboxamide (252a). The piperazine **5a** (500 mg, 1.6 mmol) and 3-(methoxycarbonylamino)-*N*-methylpiperidine (1.098 g, 6.4 mmol) were fused at 160 °C in a sealed pressure reaction vessel for 17 h. The product was chromatographed on silica gel using 1.5% (10% concentrated NH_4OH in MeOH)−CH_2Cl_2 as the eluant to give **252a** (Table 1).

In Table 1 when method S is cited, the appropriate amine was reacted with the appropriate carbamate as described above to give the desired compound.

Method T: 4-(8-Chloro-6,11-dihydro-5*H*-benzo[5,6]cyclohepta[1,2-*b*]pyridin-11-yl)-*N*-methyl-*N*-(3-pyridinyl)-1-piperazinecarboxamide (233). The urea **228a** (10.03 g, 23.1 mmol) was dissolved in DMSO (37.6 mL), and powdered KOH (2.62 g, 23.1 mmol) in DMSO (25.1 mL) was added to the stirred solution at 25 °C over 3 min. MeI (3.31 g/1.4518 mL, 23.1 mmol) was added and the mixture was stirred at 25 °C for 15 min. The usual workup, followed by chromatography on silica gel using 3−5% (10% concentrated NH_4OH in MeOH)−CH_2Cl_2 as the eluant afforded **233** (Table 1).

Method U: 4-(3-Bromo-8-chloro-6,11-dihydro-5*H*-benzo[5,6]cyclohepta[1,2-*b*]pyridin-11-yl)-*N*-(1-methyl-4-piperidinyl)-1-piperazinecarboxamide (251b). The acyl azide **241** (Scheme 14) (1.92 g, 11.7 mmol) in toluene (100 mL) was heated at 110 °C for 1 h. The piperazine **5b** (500 mg, 1.3 mmol) in CH_2Cl_2 (100 mL) was added to the cooled solution at 25 °C, and the mixture was stirred for 85 h. The reaction was worked up in the usual way, and the product was chromatographed on silica gel using 2−3% (10% concentrated NH_4OH in MeOH)−CH_2Cl_2 as the eluant to give **251b** (Table 1).

In Table 1 when method U is cited, the appropriate amine was reacted as described above to give the desired compound.

Method V: 4-(8-Chloro-6,11-dihydro-5*H*-benzo[5,6]cyclohepta[1,2-*b*]pyridin-11-yl)-*N*-(4-pyridinyl)-1-piperazinecarboxamide N1-Oxide (249a). 4-Aminopyridine N1-oxide **248** (prepared as described in Scheme 15) (87.7 mg, 0.8 mmol) was dissolved in dioxane (6 mL), and a 60% dispersion of NaH in mineral oil (19.1 mg, 0.6 mmol) was added. The mixture was stirred at 25 °C for 15 min, and the imidazole **219a** (250 mg, 0.61 mmol) was added. The solution was stirred at 25 °C for 18 h and then worked up in the usual way. The product was chromatographed on silica gel using 3−5.5% (10% concentrated NH_4OH in MeOH)−CH_2Cl_2 as the eluant to give **249a** (Table 1).

Method W: 4-(3-Bromo-8-chloro-6,11-dihydro-5*H*-benzo[5,6]cyclohepta[1,2-*b*]pyridin-11-yl)-*N*-(1-methyl-4-piperidinyl)-1-piperazinecarboxamide (251b). 4-(Ethoxycarbonylamino)-*N*-methylpiperidine (848.5 mg, 4.6 mmol) and Et_3N (553.2 mg/0.762 mL, 5.59 mmol) were dissolved in toluene (10 mL), and the mixture was heated at 110 °C for 5min. The solution was cooled, *β*-chlorocatecholborane (843.5 mg, 5.59 mmol) was added, and the mixture was heated at 110 °C for 5 min. The solution was cooled, and a solution of the piperazine **5b** (500 mg, 1.3 mmol) in CH_2Cl_2 (2 mL) was added. The mixture was stirred at 25 °C for 65 h and then worked up in the usual way. The product was chromatographed on silica gel using 3% (10% concentrated NH_4OH in MeOH)−CH_2Cl_2 as the eluant to give **251b** (Table 1).

In Table 1 when method W is cited, the appropriate amine was reacted as described above to give the desired compound.

1-(8-Chloro-6,11-dihydro-5*H*-benzo[5,6]cyclohepta[1,2-*b*]pyridin-11-yl)-4-[2-(3-pyridinyl)ethyl]piperazine (53). The amide **50a** (500 mg, 1.15 mmol) in THF (2 mL) was added over 3 min to a 1 M $LiAlH_4$ solution in THF (1.154 mL, 1.15 mmol), and the mixture was heated under reflux for 15min. The mixture was heated at 66 °C for 3 h and then worked up in the usual way. The product was chromatographed on silica gel using 3−5.5% (10% concentrated NH_4OH in MeOH)−CH_2Cl_2 as the eluant to give **53** (278.6 mg, 58%) (Table 1).

***N*-[2-(8-Chloro-6,11-dihydro-5*H*-benzo[5,6]cyclohepta-[1,2-*b*]pyridin-11-yl)amino]acetamide (190) (Scheme 2).** Mono-*N*-acetylethylenediamine (25 g, 244.8 mmol) was dissolved in MeOH (250 mL) (Al_2O_3 dried), and 28.8% (w/w) HCl in EtOH (10.35 g, 81.6 mmol) and 3 Å molecular sieves were added. The ketone **2a** (9.94 g, 40.8 mmol) and $NaBH_3CN$ (3.08 g, 40.8 mmol) were added, and the mixture was heated at 40 °C for 68 h and at 60 °C for a total of 236 h. The reaction was filtered through Celite and worked up in the usual way. The product was chromatographed on silica gel using 3−10% (10% concentrated NH_4OH in MeOH)−CH_2Cl_2 as the eluant to give **190** (2.48 g, 25%) (Table 6).

***N*-(8-Chloro-6,11-dihydro-5*H*-benzo[5,6]cyclohepta[1,2-*b*]pyridin-11-yl)-1,2-ethanediamine (191) (Scheme 2).** The N-acetate **190** (2.1 g, 6.4 mmol) was dissolved in MeOH (50 mL), and 85% $NH_2NH_2 \cdot H_2O$ (50 mL) was added. The mixture was heated at 90 °C for 13 days. The mixture was evaporated to dryness and azeotroped with toluene. The product was chromatographed on silica gel using 4% (10% concentrated NH_4OH in MeOH)−CH_2Cl_2 as the eluant to give **191** (2 g, 59%) (Table 6).

***N*-[2-(8-Chloro-6,11-dihydro-5*H*-benzo[5,6]cyclohepta-[1,2-*b*]pyridin-11-yl)aminoethyl]-4-pyridineacetamide (192) (Scheme 2).** The amine **191** (935 mg, 3.2 mmol) was reacted with 4-pyridylacetic acid (445.5 mg, 3.2 mmol) as described in method A above to give **192** (661 mg, 50%) (Table 1).

1-[(4-Chlorophenyl)-2-pyridinylmethyl]-4-(3-pyridinylacetyl)piperazine (196) (Scheme 3). The carbinol **193** (3 g, 13.7 mmol) was reacted with thionyl chloride (1.846 mL, 25.3 mmol) under essentially the same conditions as described earlier[11] to give the crude chloro derivative **194**. The material was dissolved in THF (33 mL), and piperazine (5.7 g, 66.4 mmol) in THF (33 mL) was added. The mixture was stirred at 25 °C for 18 h and then worked up in the usual way. Chromatography on silica gel using 1−7% (10% concentrated NH_4OH in MeOH)−CH_2Cl_2 as the eluant gave **195** (252.1 mg, 6%): CIMS *m*/*z* 288 (MH^+); ^{13}C NMR δ_C (CH_2) 52.8, 52.8, 45.8, 45.8; (CH) 149.2, 136.6, 129.4, 129.4, 128.5, 128.5, 122.0, 122.0, 77.5; (C) 161.2, 139.3, 132.8; HRFABMS calcd for $C_{16}H_{19}ClN_3$ M_r 288.1268 (MH^+), found 288.1265.

The piperazine **195** (250 mg, 0.869 mmol) was reacted with 3-pyridylacetic acid (119 mg, 0.869 mmol) as described in method A above. The product was chromatographed on silica gel using 0.25% (10% concentrated NH_4OH in MeOH)−CH_2Cl_2 as the eluant to give **196** (261.1 mg, 74%) (Table 1).

1-[(4-Chlorophenyl)-4-pyridinylmethyl]-4-(3-pyridinylacetyl)piperazine (201) (Scheme 4). Using essentially the same procedures as described earlier,[11] the ketone **197** (15 g, 68.9 mmol) was reduced to give the carbinol **198** (13.85 g, 91%): CIMS *m*/*z* 220 (MH^+); δ_C (CH) 149.1, 149.1, 128.9, 128.9, 128.2, 128.2, 121.7, 121.7, 73.6; (C) 154.0, 141.6, 133.8. Anal. ($C_{12}H_{10}ClNO$) C, H, Cl, N. The carbinol **198** (3 g, 13.7 mmol) was reacted with thionyl chloride (1.84 mL, 25.3 mmol) to give the chloro derivative **199**. The latter was dissolved in THF (30 mL) and CH_2Cl_2 (10 mL) and reacted with piperazine (3.53 g, 41.1 mmol) to give, after chromatography on silica gel using 15% EtOAc in hexane as the eluant, the piperazine derivative **200** (679.6 mg, 17%): CIMS *m*/*z* 288.25 (MH^+); δ_C (CH_2) 52.4, 52.4, 45.8, 45.8; (CH) 150.5, 150.5, 129.6, 129.6, 129.2, 129.2, 123.1, 123.1, 74.8; (C) 151.3, 139.3, 133.7; HRFABMS calcd for $C_{16}H_{19}ClN_3$ M_r 288.1268 (MH^+), found 288.1265.

The piperazine **200** (160.7 mg, 0.56 mmol) was reacted with 3-pyridylacetic acid (76.5 mg, 0.56 mmol) as described in method A above. The product was chromatographed on silica gel using 2.5% (10% concentrated NH_4OH in MeOH)−CH_2Cl_2 as the eluant to give **201** (182.5 mg, 80%) (Table 1).

1-(4-Chlorobenzoyl)-4-(4-pyridinylacetyl)piperazine (205) (Scheme 5). 1-*N*-Boc-piperazine **202** (1 g, 5.37 mmol) was reacted with 4-pyridylacetic acid (735.5 mg, 5.37 mmol) as described in method A above. The product was chromatographed on silica gel using 2% (10% concentrated NH_4OH in MeOH)−CH_2Cl_2 as the eluant to give **203** (1.28 g, 80%): CIMS *m*/*z* 306 (MH^+); δ_C (CH_3) 28.1; (CH_2) 45.7, 45.7, 43.4, 41.5, 39.9; (CH) 150.4, 150.4, 124.2, 124.2; (C) 168.3, 154.7, 144.0, 80.4. Anal. ($C_{16}H_{23}N_3O_3$) C, H, N.

Using the same procedure as described above for **112**, the *N*-Boc derivative **203** (1.23 g, 0.4 mmol) was deprotected, and the product was chromatographed on silica gel using 3−4% (10% concentrated NH_4OH in MeOH)−CH_2Cl_2 as the eluant to give **204** (322.1 mg, 39%): CIMS *m*/*z* 206 (MH^+); δ_C (CH_2) 47.1, 46.0, 45.5, 42.8, 39.8; (CH) 150.2, 150.2, 124.3, 124.3; (C) 168.1, 144.4. Anal. ($C_{11}H_{15}N_3O \cdot 0.3H_2O$) C, H, N.

The piperazine **204** (90 mg, 0.4 mmol) was dissolved in THF (3 mL), and 4-chlorobenzoyl chloride (0.0557 mL, 0.4 mmol) was added. The mixture was stirred at 25 °C for 1 h and then worked up in the usual way. Chromatography on silica gel using 1−3% (10% concentrated NH_4OH in MeOH)−CH_2Cl_2 as the eluant gave **205** (134.6 mg, 89%) (Table 1).

1,1-Dimethylethyl 2-[1-(3-Bromo-8-chloro-6,11-dihydro-5*H*-benzo[5,6]cyclohepta[1,2-*b*]pyridin-11-yl)-4-piperazinyl]-1(*R*)-[[(triphenylmethyl)thio]methyl]ethylcarbamate Isomers (187 and 188). The piperazine **5b** (300 mg, 0.76 mmol), powdered 3 Å molecular sieves (200 mg), and $NaBH(OAc)_3$ (655 mg, 3.04 mmol) were dissolved in dichloroethane (7.8 mL), and the mixture was cooled to 0 °C. 2(*R*)-(*N*-*tert*-butoxycarbonylamino)-3-triphenylmethylthiopropanal (514.5 mg, 1.15 mmol) in dichloroethane (7.8 mL) was added dropwise to the stirred solution, and the mixture was allowed to warm to 25 °C, the reaction being allowed to run for 21 h. The usual workup followed by chromatography on silica gel using 3−3.5% acetone in CH_2Cl_2 gave the less polar isomer **187** (210 mg, 33%) (Table 6) and the more polar isomer **188** (210 mg, 33%) (Table 6).

4-(3-Bromo-8-chloro-6,11-dihydro-5*H*-benzo[5,6]-cyclohepta[1,2-*b*]pyridin-11-yl)-α(*R*)-(mercaptomethyl)-1-piperazineethanamine Hydrochloride (189). The combined isomers **187** (384.1 mg) and **188** (640 mg) (total: 1.02 g, 1.2 mmol) were dissolved in CH_2Cl_2 (10.24 mL), and Et_3SiH (589 mg/0.809 mL, 5.1 mmol) and TFA (1.544 g/1.043 mL, 13.5 mmol) were added.[22] The mixture was stirred at 25 °C for 1 h and then evaporated to dryness. The solid was partitioned between water and hexane, the aqueous layer was passed over BioRad AG3-X4(Cl^-) resin, and the eluate was lyophilized to give the hydrochloride **189** (530.3 mg, 74%) (Table 1).

H-Ras Processing in Cos Monkey Kidney Cells. Processing of H-*ras* in Cos cells was determined as described previously.[7] Briefly, the mammalian expression vector pSV-Sport (BRL-GIBCO) containing a cDNA encoding the Val^{12}-activated form of H-Ras was electroporated into Cos-7 monkey kidney cells. Cells were then plated into six-well tissue culture dishes containing 1.5 mL of Dulbecco's modified Eagle's Medium (DMEM) supplemented with 10% fetal calf serum and the appropriate FPT inhibitors. After 24 h, media was removed, and fresh media containing the appropriate drugs was re-added. After an additional 24 h incubation, cells were washed, harvested, and lysed by homogenization. Debris was removed by centrifugation. Protein was precipitated using cold trichloroacetic acid, redissolved in SDS−polyacrylamide gel electrophoresis sample buffer and electrophoresed on 14% polyacrylamide gels (Novex, Inc.). Resolved proteins were electroblotted onto nitrocellulose membranes. Nonspecific binding to membranes was blocked by preincubation in buffer containing 2.5% dried milk and 0.5% Tween-20. Ras was detected by incubation with a Ras-specific monoclonal antibody (Y13-259; Oncogene Science) in buffer containing 1% fetal calf serum for 1 h at room temperature. Membranes were then incubated for 1 h with a 1:5000 dilution of rabbit anti-rat IgG conjugated to horseradish peroxidase in buffer containing 1%

fetal calf serum. Processed and unprocessed Ras proteins, which migrate with distinct electrophoretic mobility, were visualized using a colorimetric peroxidase reagent.

Anchorage-Independent Soft Agar Growth Assays. Logarithmically growing H- or K-*ras*-transformed mouse NIH-3T3 fibroblasts were trypsinized and plated in the top layer of 0.35% low-melting point agarose (LMP) at 10 000 cells/well of a six-well cluster dish in DMEM (GIBCO) containing 10% fetal calf serum (FBS) and the appropriate amount of FPT inhibitor. Bottom layers (0.6% LMP) contained the same concentration of inhibitor. After 14 days, the clones were stained with MTT (1 mg/mL in PBS) and then photographed.The number of soft agar colonies was counted and inhibition was calculated relative to the number of colonies in vehicle-treated control wells. When more than one experiment was run, both values are given. The results are summarized below.

	IC_{50} (μM)		
compound	NIH-H	NIH-K	DLD-1
83b (micronized free base)	1.2	4	
85b (micronized free base)	1.5	6.5	8/8
85b (hydrochloride salt)			3/7

In Vivo Antitumor Efficacy in Mice (Figure 2). Nude mice were injected sc with the appropriate tumor cells ($m \times 10^6$: K-*ras* DLD-1, $m = 5$; K-*ras* SW-620, $m = 7$; H-*ras* CVLS, $m = 2$; H-*ras* CVLL, $m = 2$; H-*ras* pT-24, $m = 5$) containing a mutated *ras* oncogene. Drug treatment was initiated and the animals (10 each per control and treatment group) were dosed with the compounds indicated in Figure 2 at 50 and 10 mg/kg, po, qid for 7 days a week using a solution of the hydrochloride salt in 20% HPβCD, except for **39**, **45a**, **50a**, **228a**, and **231**, which were administered to animals (five each per control and treatment group) at a dose of 100 mg/kg, po, qid for 5 days a week using a suspension of the micronized free base in corn oil. Primary tumor measurements were determined twice a week for a period of 3−4 weeks. Tumor volume = $^1/_2$length × width × height. Efficacy is indicated by comparing median tumor size of the treated group with that of the vehicle control group. In the experiment with **150b** at 50 mg/kg using DLD-1 tumor cells, 3/10 mice died.

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Supporting Information Available: Additional experimental procedures, structures, Schemes 6−15, Tables 3−7, physical chemical data (yields, analytical data, mass spectral data, CMR data, specific rotations), antitumor activity, and statistics (for in vitro and in vivo efficacy studies) for all synthetic intermediates and final products described in this study (27 pages). Ordering information is given on any current masthead page.

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