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INHIBITORS OF FARNESYL PROTEIN TRANSFERASE. SYNTHESIS AND BIOLOGICAL ACTIVITY OF AMIDE AND CYANOGUANIDINE DERIVATIVES CONTAINING A 5,11-DIHYDRO[1]BENZTHIEPIN, BENZOXEPIN, AND BENZAZEPIN [4,3-*b*]PYRIDINE RING SYSTEM.

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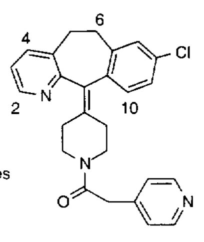
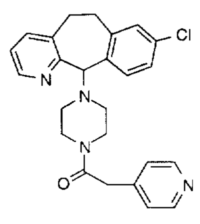
Abstract: Bioisosteric replacement of the C-6 carbon atom in piperidine **I** and piperazine **II** with S, O, and N heteroatoms is described. Amide and cyanoguanidine derivatives of these compounds were evaluated *in vitro* and found to be good inhibitors of farnesyl-protein transferase. An improved method of preparing the 5,11-dihydro-[1]-benzthiepin nucleus **6** was accomplished in high yield and with excellent regioselectivity using an $AlCl_3$ melt protocol.

Introduction: The observation that a large percentage of human cancers carry activating mutations in one of their ras genes[1] has stimulated an intensive research effort over the last decade aimed at identifying and developing small molecules that inhibit oncogenic Ras proteins.[2] Most medicinal chemistry approaches for treating Ras associated tumors have focused on inhibiting the post-translational farnesylation of a cysteine residue located in the CAAX sequence present at the C-terminus of the Ras protein.[3] This prenylation step, which is carried out by the enzyme farnesyl-protein transferase (FPT), enables the Ras protein to localize to the plasma membrane. This particular mechanistic process must occur in order for the cell to acquire transforming ability, and as such is an attractive therapeutic target for the development of antitumor agents.[4] As part of an overall program[5] aimed at improving the potency of our lead FPT inhibitors **I** and **II** (figure 1), we focused our structure–activity relationship (SAR) investigation on the tricyclic nucleus and replacement of the methylene group at C-6 with S, O, and N heteroatoms.

Figure 1



I, Piperidine Series (IC_{50} = 0.25 μM)



II, Piperazine Series (IC_{50} = 0.36 μM)

PII: S0960-894X(98)00439-9

Chemistry: The 5,11-dihydro[1]benzoxepino[4,3-*b*]pyridine-5-one system **5** has been synthesized previously in approximately 50% yield following ring closure of the carboxylic acid **3** with polyphosphoric acid[6] (Figure 2), and served as the key intermediate for compounds **20**, **21**, **34**, **35**, and **54**. The synthesis of the novel benzazepin series will be described in a future publication[7] of which the aza-piperazine **15** served as the precursor to amides **44**–**46** and the cyanoguanidine **49** (Table 1). Previous reports describing the preparation of the 5,11-dihydro-[1]benzothiepino-[4,3-*b*]pyridine-5-one skeleton **6** and **8** have proved disappointing with yields of less than 18% being obtained employing conventional Friedel–Crafts conditions.[8] In stark contrast, replacement of the pyridine ring in **4** with a benzene ring affords the tricyclic ketone **2** in yields ranging from 61–86% under PPA cyclization conditions (Figure 2).[8b]

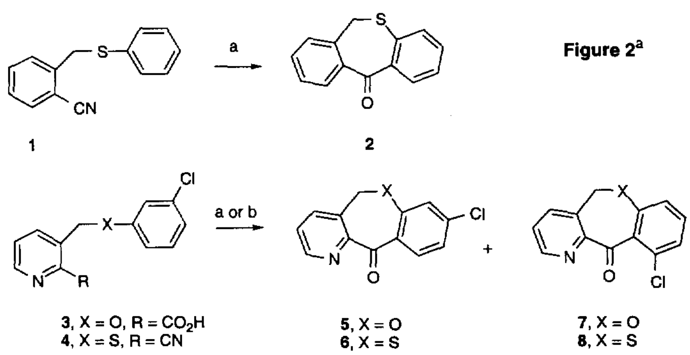


Figure 2[a]

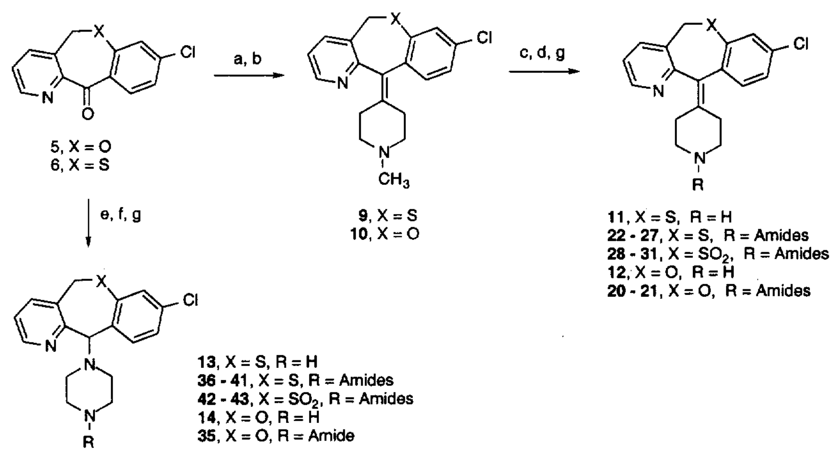
[a]**Reagents**: (a) Polyphosphoric Acid, 195 °C, 15 min. (b) Powdered $AlCl_3$, 170 °C, <30 min.

The fact that the cyclization of **1** to **2** can be obtained in good yield using PPA relative to the pyridyl system **4** suggests that it is not the longer C-S bond lengths[9] in the bridge that hinders the formation of ketones **6** and **8** but the electron deficient pyridine ring, which is deactivated further under typical Friedel–Crafts conditions. Fortunately, we discovered[10] that admixing the nitrile **4**, with 3–5 equivalents of powdered $AlCl_3$ and melting this mixture at 170 °C for <30 min, afforded the tricyclic ketones **6** and **8** in greater than 80% yield. Furthermore, this protocol proved to be highly regioselective, providing the 8-chloro isomer almost exclusively (>97:1, **6**:**8**). Introduction of the central piperidine and piperazine rings was accomplished as illustrated in Scheme 1.[8,11] Standard carbodiimide[12] coupling with the appropriate carboxylic acids afforded the amide derivatives which are listed in Table 1.

The sulfone analogs **28**–**31** and **42**–**43** were most easily obtained by oxidation of the corresponding sulfides with mCPBA in a CH_2Cl_2 solution containing 3-5 equiv of methanesulfonic acid which prevented pyridine N-oxide formation. The three acyl piperazine derivatives in the aza series **44**–**46**, and the three derivatives in the oxa series **20**–**21** and **35** were prepared using standard carbodiimide methodology as noted above.[13] A bromine substituent was introduced at the C-3 position in the oxa series as outlined in Scheme 2. Treatment of **50** with tetrabutylammonium nitrite and trifluoroacetic anhydride afforded the nitro derivative **51** in 50% yield. Reduction of **51** using $Fe/CaCl_2$ produced the amine **52** in 40% yield, which was subjected to diazotization using $NaNO_2/HBr/Br_2$ to give compound **53** in 60% yield. Acid hydrolysis of the carboethoxy group and subsequent carbodiimide coupling with 4-pyridylacetic acid N-oxide provided the amide **54** in 75% yield.

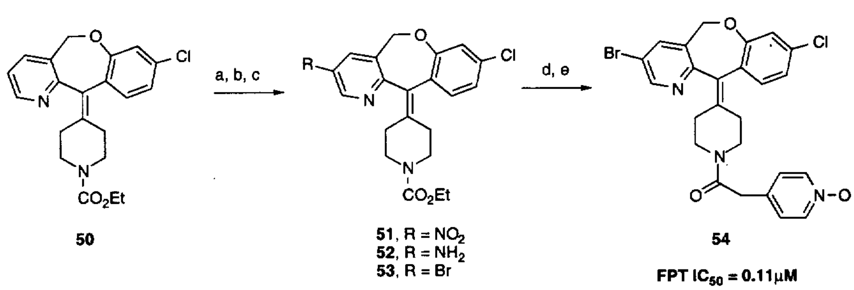
The cyanoguanidine analogs **47–49** and **32–34** were prepared in two steps from the corresponding NH intermediates as illustrated in Scheme 3.[14] Thus, treatment of the piperazines **13** and **15**, and the piperidines **11** and **12** with dimethyl N-cyanodithioiminocarbonate[15] in refluxing CH_3CN afforded the corresponding thiomethylcyanamide intermediates in excellent yield. Displacement of the thiomethyl moiety with 3-, or 4-picolylamine produced the desired cyanoguanidine targets.

Scheme 1[a]



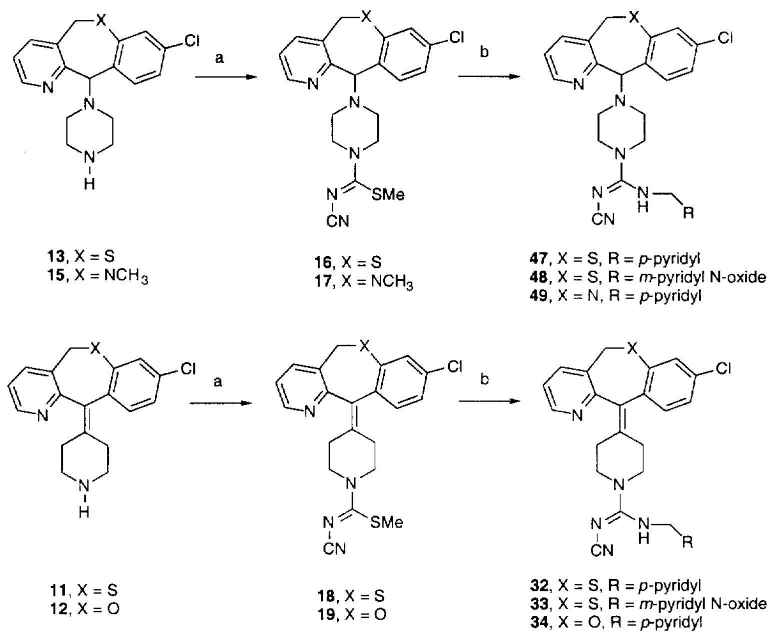
[a]**Conditions and reagents**: (**a**) (N-methylpiperidyl)magnesium chloride, THF >90% Crude, (**b**) HOAc, Ac_2O, AcCl, 100 °C, 20 h, ~90% crude, (**c**) $ClCO_2Et$, $PhCH_3$ ~70%, (**d**) KOH, H_2O, EtOH, 110 °C, 18h, 75% (**e**) $NaBH_4$, MeOH, 40 min, ~85%, (**f**) $SOCl_2$, CH_2Cl_2, 2.5 h, ~85%, then Piperazine, THF, 4.5 h, ~90%, (**g**) appropriate acid, HOBT, EDCI, DMF, Et_3N, 22 °C, 24 h, 65-85%.

Scheme 2[b]



[b]**Conditions and reagents**: (**a**) Bu_4NNO_3, $(CF_3CO)_2O$, CH_2Cl_2, 24 h, 50% (**b**) Fe, $CaCl_2$, $EtOH:H_2O$, 60 °C, 6 h, 40% (**c**) $NaNO_2$, HBr, Br_2, 0 °C ---> 20 °C, 3 h, 60% (**d**) conc. HCl, 80 °C, 24 h, 90% (**e**) ref 6, 75%

Scheme 3[a]



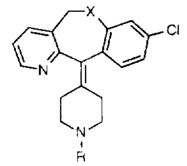
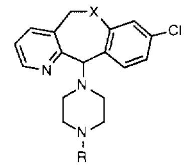
[a]**Conditions and reagents**: (a) NCN=C(SMe)$_2$, CH_3CN, Et_3N, 80 °C, 2.5 h, 85-90%. (b) H_2NCH_2-p-Pyridyl, or H_2NCH_2-m-Pyridyl N-Oxide, CH_3CN, 80 °C, 4 h, 75-83%.

Results and Discussion: In general, FPT potency as determined by the ability to inhibit the transfer of [^{3}H]-farnesyl from farnesyl pyrophosphate to H-Ras-CLVS,[16] increased within the three series of heteroatom isosteres in the following order; S > O > N. Within the sulfur series, the piperidine analogs were found to be only slightly more potent than the corresponding piperazine derivatives, with the 4-pyridinylacetyl moiety imparting the best activity among the types of amides we evaluated. Oxidation of the thioether linkage to a sulfone moiety in both the piperidine and piperazine series, resulted in a notable decrease in FPT activity as demonstrated by compounds **29–31** which showed poor inhibition of FPT even at 1 μM. Compound **28** was the only sulfone which exhibited FPT activity below the 1 μM level. A significant improvement in FPT inhibition was observed when the amide moiety was replaced with a cyanoguanidine group as noted by compounds **32–33** and **47–48**. Additionally, of the seven benzthiepin compounds that were examined for their ability to inhibit the processing of Ras in intact cells, six demonstrated good inhibition of FPT in a COS cell-based assay.[16]

The only direct comparison that could be made from the benzoxepin series indicated that the piperazine and piperidine analogs, **35** and **21** respectively, were essentially equivalent in activity. Interestingly, in contrast to both the benzthiepin and benzazepin series, introduction of a cyanoguanidine group in the benzoepin series did not improve FPT inhibition as evidenced by compound **34**. However, when a 3-bromo substituent was introduced into the pyridine ring of the benzoxepin nucleus, FPT potency improved 14-fold as noted by compound **54**. This observation is consistent with finding that 3,8-dihalo substitution dramatically improves FPT potency.[5]

While synthetic limitations precluded the preparation of piperidine compounds within the benzazepin series, the two piperazine analogs (**44** and **45**) paralleled the results of the amide derivatives in the benzoxepin and benzthiepin series. Similarly, a pronounced improvement in FPT inhibition was observed for the cyanoguanidine compound **49**.

Table 1. *In vitro* FPT and COS activities for analogs containing amide and cyanoguanidine functionalities.

Piperidine Series 					Piperazine series 				
Entry	**R**	**X**	**FPT IC_{50} (μM)**	**COS IC_{50} (μM)**	**Entry**	**R**	**X**	**FPT IC_{50} (μM)**	**COS IC_{50} (μM)**
I	$COCH_2$-*p*-C_5H_4N	C	0.25	1.0	II	$COCH_2$-*p*-C_5H_4N	C	0.36	3.7
21	$COCH_2$-*p*-C_5H_4NO	O	1.52	41(20)[a]	36	$COCH_2C_5H_4N$	S	0.32	
22	$COCH_2$-*p*-C_5H_4N	S	0.19	4.2	37	$COCH_2$-*p*-C_5H_4NO	S	0.79	10
23	$COCH_2$-*p*-C_5H_4NO	S	0.52	10	38	$COCH_2C_5H_9NCH_3$	S	2.1	
24	$COCH_2C_5H_9NCH_3$	S	1.79		39	$COCH_2C_5H_9NH$	S	1.1	
25	$COCH_2C_5H_9NH$	S	0.59		40	$COCH_2$-*p*-C_5H_4N	S	1.0	
26	$COCH_2C_5H_9NCONH_2$	S	0.92	3.8	41	$COCH_2C_5H_9NCONH_2$	S	1.12	
27	$COCH_2S$-*p*-C_5H_4NO	S	0.62	5.0	42	$COCH_2C_5H_4N$	SO_2	1.0	
28	$COCH_2$-*p*-C_5H_4N	SO_2	0.56		43	$COCH_2$-*p*-C_5H_4NO	SO_2	3.4	
29	$COCH_2$-*p*-C_5H_4NO	SO_2	30(1.2)[a]		44	$COCH_2$-*p*-C_5H_4N	NCH_3	0.84	0(20)[a]
30	$COCH_2C_5H_9NH$	SO_2	28(1.2)[a]		45	$COCH_2$-*p*-C_5H_4NO	NCH_3	2.7	20
31	$COCH_2C_5H_9NCONH_2$	SO_2	35(1.1)[a]		46	$COCH_2C_5H_9NH$	NCH_3	10(1.3)[a]	
32	C=(NCN)$NHCH_2$-*p*-C_5H_4N	S	0.043	1.7	47	C=(NCN)$NHCH_2$-*p*-C_5H_4N	S	0.061	
33	C=(NCN)$NHCH_2$-*m*-C_5H_4NO	S	0.038	26(20)[a]	48	C=(NCN)$NHCH_2$-*m*-C_5H_4NO	S	0.140	
34	C=(NCN)$NHCH_2$-*p*-C_5H_4N	O	25(0.042)[a]		49	C=(NCN)$NHCH_2$-*p*-C_5H_4N	NCH_3	0.140	10(20)[a]

(a) IC_{50} values were not determined for compounds that displayed FPT inhibitions of <50% at 1 μM. These values are represented by the notation; %inhibition (conc. of assay).

In conclusion, we demonstrated that while replacement of the C-6 methylene unit in the tricyclic nucleus with N, O, and S heteroatoms afforded compounds with good FPT activity, a marked improvement over the lead carbon analogs **I** and **II** was not realized. However, through this study important SAR information was generated with respect to the tricyclic skeleton which should help in the design of future FPT inhibitors.

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