Synthesis and Biological Evaluation of Novel 2,6-Diaminobenz[*cd*]indole Inhibitors of Thymidylate Synthase Using the Protein Structure as a Guide

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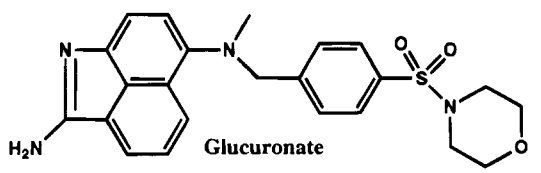
The design, synthesis, and biochemical and biological evaluations of a novel series of 2,6-diaminobenz[*cd*]indole-containing inhibitors of human thymidylate synthase (TS) are described. The compounds are characterized by having either a pyridine or pyridazine ring in place of the (phenylsulfonyl)morpholinyl group of the known inhibitor N^6-[4-(morpholinosulfonyl)benzyl]-N^6-methyl-2,6-diaminobenz[*cd*]indole glucuronate (**i**). Active compounds from this series showed human TS inhibition constants below the 10 nM level and were potent, selective submicromolar antitumor agents in cell culture. The compounds were synthesized by reductive alkylation of a substituted 6-aminobenz[*cd*]indole or reductive cyclization of a substituted 1-cyano-8-nitronaphthalene.

Introduction

The enzyme thymidylate synthase (TS) (EC 2.1.1.45) is the rate-limiting step in the *de novo* synthesis of thymidylate from deoxyuridylate. In rapidly proliferating cell populations, adequate supplies of thymidylate are critical to DNA synthesis, and as a result, inhibition of TS has proven to be an interesting target for anticancer therapy.[1] The essential cofactor 5,10-methylenetetrahydrofolate is the source of the one-carbon unit used in this transformation, and the folate cofactor binding pocket of TS has proven useful in the discovery of a diverse group of structurally distinct antitumor compounds.[2–11]

We have recently reported on the protein structure-based design, synthesis, and biological evaluation of a novel family of benz[*cd*]indole-containing TS inhibitors which has resulted in the discovery of the investigational drug N^6-[4-(morpholinosulfonyl)benzyl]-N^6-methyl-2,6-diaminobenz[*cd*]indole glucuronate (**i**)[5,12] currently undergoing clinical study. As part of our ongoing



i (AG-331)

effort to use the crystal structural information provided by the *Escherichia coli* (*E. coli*) enzyme[13] to discover novel lipophilic inhibitors of TS,[4b] we now report on a potent class of benz[*cd*]indole-containing TS inhibitors in which the (morpholinosulfonyl)phenyl group of the lead compound **i** has been replaced with a number of simple derivatives of pyridine represented in Figure 1. We also report on novel general methods for preparing the 2,6-diamino-substituted benz[*cd*]indole ring system.

Design

The starting point for this work was the X-ray crystal structure of the complex of compound **i** bound in the *E. coli* TS as shown in Figure 2.[14] Our design strategy consisted of removing the phenyl-SO_2-morpholinyl group of AG-331 and replacing it with various substituted pyridine rings using the structure as a guide.[15] Earlier work reported from our group and others[4] had shown there to be a dipole effect on the aromatic ring that occupied this region of the active site as evidenced by increased activity from compounds with strong electron-withdrawing groups. It has long been known experimentally that pyridine rings have dipoles across the aromatic ring similar to benzene rings substituted with electron-withdrawing groups.[16] In addition, it was shown in the quinazoline-containing compounds[7] that 4-thiopyridines were suitable replacements for the *p*-aminobenzoyl glutamate of the cofactor analogues.

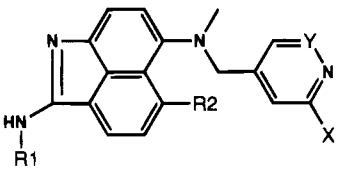


Figure 1. General structure of 2,6-diaminobenz[*cd*]indole-containing inhibitors. The regions designated R_1, R_2, X, and Y represent areas on which structure–activity studies were performed.

To test this simple hypothesis for the benz[*cd*]indole type inhibitors, we prepared compound **3**. As can be seen from Table 1, compound **3** showed modest inhibition (K_i = 1.8 μM) of *E. coli* TS and potent inhibition (K_i = 14 nM) of human TS. In addition, compound **3** showed modest cell growth inhibition against a number of standard cell lines. The striking differences, roughly a factor of 100–1000, seen in the inhibition between the human and bacterial enzymes were also observed in the previous series of benz[*cd*]indole type inhibitors. The explanation for this is believed to be related to the manner in which the benzindole portion of the molecule sits in the deep part of the active site. In this region of the *E. coli* enzyme, the benzindole portion packs tightly against the side chain of Trp 80. In the human enzyme, this residue is replaced by the smaller amino acid asparagine resulting in a larger, more spacious binding pocket. The result is that the benzindole is not as well accommodated by the *E. coli* enzyme and therefore binds

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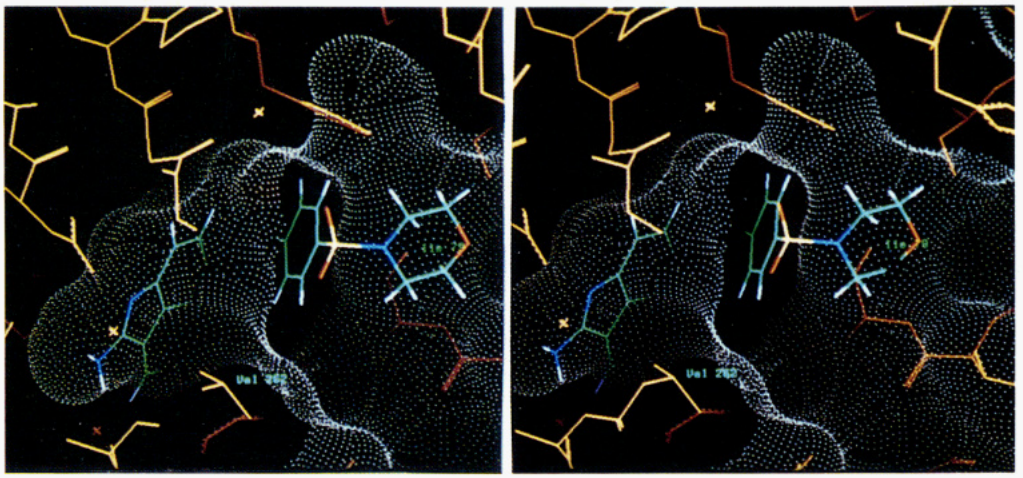


Figure 2. Stereodrawing showing the X-ray structure of compound **i** complexed with *E. coli* TS. The (phenylsulfonyl)morpholinyl portion of the inhibitor is shown exiting the active site. The morpholinyl group is shown resting against the hydrophobic wall created by the Ile 79 side chain.

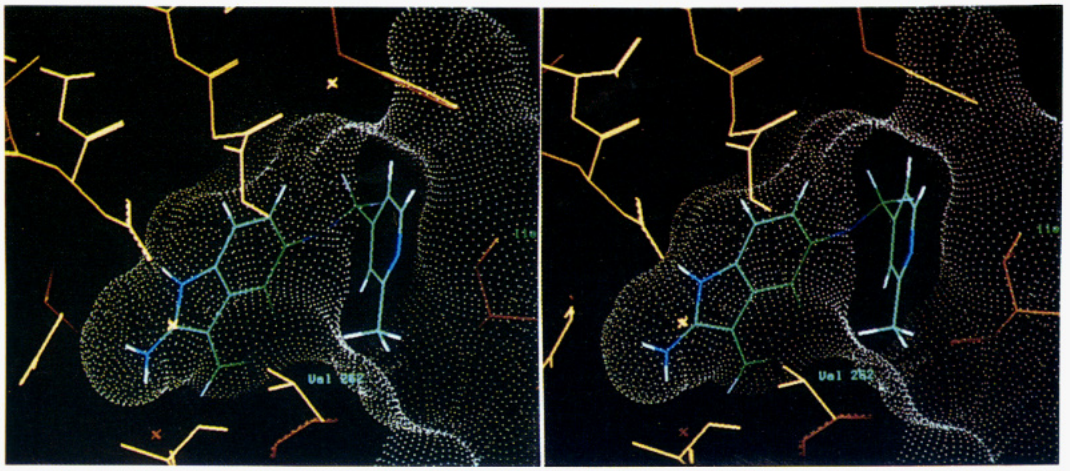


Figure 3. Stereodrawing showing the X-ray structure of compound **3** complexed with *E. coli* TS. The 2-methylpyridine portion of the inhibitor is shown exiting the active site. The 2-methyl group is shown occupying the hydrophobic groove created by the Val 262 side chain.

less favorably. The crystal structure of compound **3** complexed with the *E. coli* TS was solved and is shown in Figure 3. Analysis of the structure of compound **3** allowed us to draw a number of conclusions. Firstly, and most importantly, replacement of the phenyl-SO_2-morpholinyl group of compound **i** does not alter the way in which the benz[*cd*]indole ring sits in the deep hydrophobic pocket. This conclusion was important because it allowed us to reduce the number of substituent changes we would make on this portion of the molecule to the small subset of active ones found in the series represented by compound **i**. Secondly, the structure of compound **3** revealed that the pyridine nitrogen and its adjacent carbon atoms point out of the active site toward bulk solvent and are therefore not desolvated while bound. Lastly, the methyl substituent on the pyridine ring of compound **3** occupies a small open-ended hydrophobic groove, which is created by the side chain of Val 262, in a manner similar to that seen with the *p*-aminobenzoate portion of our quinazoline inhibitors.[4b]

Using the structure of compound **3**, we proceeded to test both the structural and electronic requirements of the protein. Since our interest was in finding active cytotoxic agents, we focused primarily on the human TS inhibition data as our primary guide. We first attempted to increase the dipole of the aromatic ring by preparing pyridazine compound **2**. Compared to its isostere, compound **1** (human, K_i = 17 nM), the additional nitrogen had essentially no effect on inhibition (compound **2**, K_i = 15 nM). In addition, the 2-(trifluoromethyl)pyridine compound **4** showed a factor of 3 weaker inhibition (K_i = 45 nM) than its 2-methyl homologue, compound **3**.

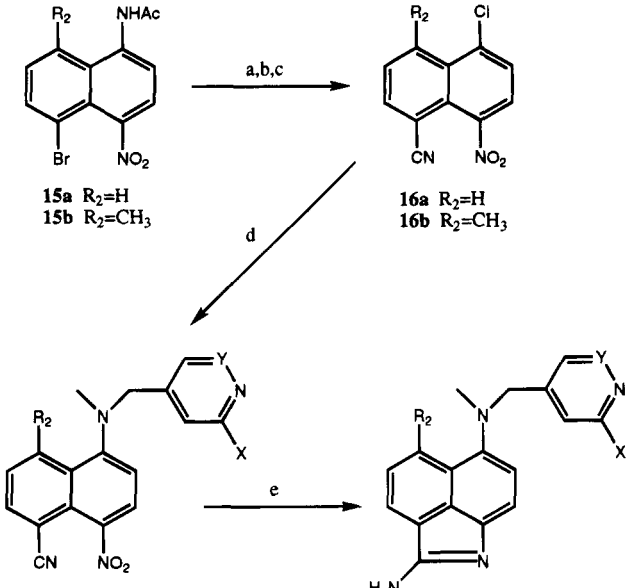
In order to test the steric requirements of the small hydrophobic groove accessible from the 2-position of the pyridine ring, we prepared the 2-substituted compounds **5**–**7**. In general these changes had little effect on the inhibition.

Finally, optimization of the substituent pattern on the benzindole portion of the inhibitors consisted of placing combinations of methyl substituents at either the 2-nitrogen or the 5-position of the tricyclic ring system. A number of these changes resulted in potent inhibitors such as compounds **9** (K_i = 6 nM) and **11** (K_i = 4 nM) with inhibition constants below 10 nM.

Chemistry

In our previous synthesis of compound **i** and its analogues, the right-hand (benzylsulfonyl)morpholinyl portion of the molecule was attached by alkylation of

Scheme 1. General Synthesis of 2,6-Diaminobenz[*cd*]indole, Method A[a]



15a R_2=H
15b R_2=CH_3

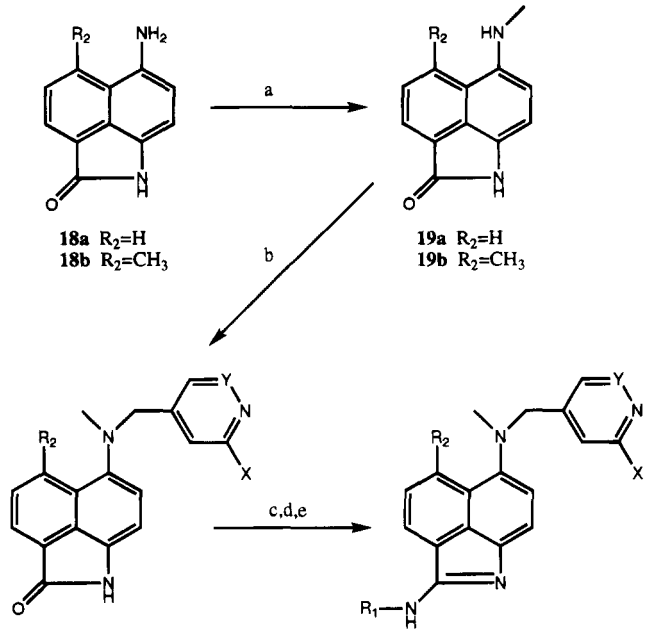
16a R_2=H
16b R_2=CH_3

17a R_2=H, X=H, Y=CH
17b R_2=H, X=H, Y=N
17c R_2=H, X=CH_3, Y=CH
17d R_2=H, X=CF_3, Y=CH
17e R_2=H, X=Et, Y=CH
17f R_2=H, X=CH_2OH, Y=CH
17g R_2=CH_3, X=H, Y=CH
17h R_2=CH_3, X=CH_3, Y=CH

1 R_2=H, X=H, Y=CH
2 R_2=H, X=H, Y=N
3 R_2=H, X=CH_3, Y=CH
4 R_2=H, X=CF_3, Y=CH
5 R_2=H, X=Et, Y=CH
7 R_2=H, X=CH_2OH, Y=CH
9 R_2=CH_3, X=H, Y=CH
11 R_2=CH_3, X=CH_3, Y=CH

[a] (a) CuCN, DMF; (b) NaOH, MeOH; (c) $CuCl_2$, *t*-$BuNO_2$, CH_3CN; (d) amine, DMSO, DIEA, Δ; (e) $SnCl_2 \cdot 2H_2O$, EtOH, Δ.

Scheme 2. General Synthesis of 2,6-Diaminobenz[*cd*]indole, Method B[a]



18a R_2=H
18b R_2=CH_3

19a R_2=H
19b R_2=CH_3

20a R_2=H, X=H, Y=CH
20b R_2=CH_3, X=H, Y=CH
20c R_2=CH_3, X=H, Y=N
20d R_2=H, X=H, Y=N

8 R_1=CH_3, R_2=H, X=H, Y=CH
10 R_1=CH_3, R_2=CH_3, X=H, Y=CH
12 R_1=H, R_2=CH_3, X=H, Y=N
13 R_1=CH_3, R_2=H, X=H, Y=N
14 R_1=CH_3, R_2=CH_3, X=H, Y=N

[a] (a) MeI, DIEA, DMF; (b) aldehyde, $NaBH_3Cn$, HCl, MeOH; (c) Lawesson's reagent, THF, Δ; (d) MeI, NaOH, THF; (e) ammonia or CH_3NH_2, MeOH, Δ.

6-aminobenz[*cd*]indole with the corresponding benzyl bromide.[5] For the pyridine-containing compounds, this type of alkylation approach was not viable due to polymerization of the halomethylpyridine reagent. As a result, we devised two alternate routes to the disubstituted 2,6-diaminobenz[*cd*]indole ring system. The first, shown in Scheme 1, involved delaying construction of the indole portion until the last step of the synthesis. This allowed us to use the nitrogen of the indole, in the form of a nitro group, as an activating group for displacement of a chloride at the 4-position of a substituted naphthalene. Therefore, the disubstituted 6-nitrogen, containing both the alkyl group and the pyridine, could be introduced into the molecule in a single transformation. The second general approach to the final compounds, shown in Scheme 2, involved the attachment of the pyridine rings, in the form of aldehydes, to the 6-aminobenz[*cd*]indole by reductive alkylation.

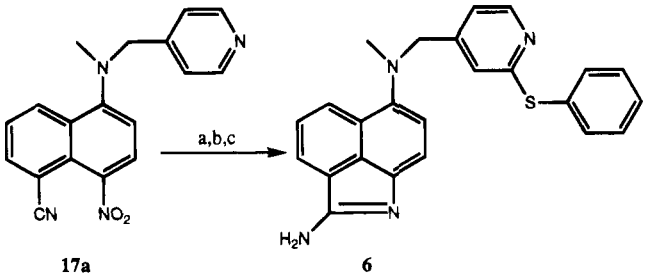
The synthesis of the amidines **1**–**5**, **7**, **9**, and **11** is shown in Scheme 1. The starting bromide **15a** was prepared as described.[17] Replacement of the bromine with cyano proceeded under standard conditions using CuCN.[18] Removal of the acetyl group under basic conditions provided the amine which when treated with *tert*-butylnitrite in the presence of $CuCl_2$[19] gave the key trisubstituted naphthlene **16a**. The more highly substituted starting nathphalene **15b** was prepared from 1-methyl-8-nitro-naphthalene[20] using the same sequence of reactions used to prepare **15a**.[21] Displacement of the chlorine atom with the disubstituted amines **22a**–**d** proceeded under basic conditions in DMSO to give the coupled products in variable yields ranging from 4% to 53%. In general, the displacements in which R_2 was a hydrogen went in significantly higher yields (24–53%) than when R_2 was a methyl (4–8%). Completion of the syntheses consisted of reducing the nitro group using $SnCl_2$ and allowing the intermediate amine to spontaneously cyclize under the acidic conditions of the reaction.

Shown in Scheme 2 is the reductive alkylation method used in the synthesis of compounds **8**, **10**, and **12**–**14**. The synthesis begins with the methyl iodide alkylation of the previously described 6-aminobenz[*cd*]indoles **18a**,**b**.[5] Reductive alkylation was effected with the appropriate pyridine aldehyde and $NaBH_3CN$. Key to the success of these reactions was the prior preparation of the HCl salt of the starting amine. We attribute this observation to the increased acidity of HCl over the more commonly used acetic acid.[22] Conversion of the lactam to the substituted or unsubstituted amidines was accomplished using the procedure previously described.[5]

The synthesis of the 2-(phenylthio)pyridine compound **6** is shown in Scheme 3. Introduction of the thiophenyl group was accomplished by first sulfonylating the corresponding *N*-oxide using benzenesulfonyl chloride followed by displacement with thiophenol generated in situ using (trimethylsilyl)thiophenol.[23,24] We found that in the displacement step the TMS-thiophenol gave higher yields than thiophenol itself. The synthesis of **6** was completed using the method described in Scheme 1.

In Scheme 4 is shown the general method employed for the preparation of the pyridyl and pyridazylmethyl amines **22a**–**d**. The 4-hydroxymethyl starting materials were prepared using the general method of Katz.[25] Synthesis of the disubstituted amines was accomplished

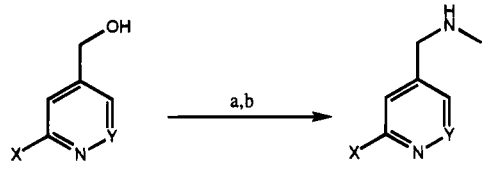
Scheme 3. Synthesis of Compound **6**[a]



17a **6**

[a] (a) MCPBA, CH2Cl2, Δ (76%); (b) $PhSO_2Cl$, $(CH_3)_3SiSPh$, CH_2Cl_2 (22%); (c) $SnCl_2 \cdot 2H_2O$, EtOH, Δ (46%).

Scheme 4. Synthesis of Aromatic Methylamine Intermediates[a]



21a X=CH_3, Y=CH
21b X=CF_3, Y=CH
21c X=H, Y=N
21d X=Et, Y=CH

22a X=CH_3, Y=CH
22b X=CF_3, Y=CH
22c X=H, Y=N
22d X=Et, Y=CH

[a] (a) $SOCl_2$, CH_2Cl_2 or 48% HBr(aq); (b) MeNH2(aq), THF.

with either thionyl chloride or aqueous HBr to give the intermediate halides which were allowed to react with an excess of aqueous methylamine.

Biology and Biochemistry

The compounds shown in Table 1 were evaluated primarily for their inhibition of purified recombinant human thymidylate synthase. Selected compounds were measured against the *E. coli* enzyme. As discussed previously,[5] the inhibition patterns relative to the cofactor 5,10-methylenetetrahydrofolate of these benz[*cd*]indole-containing inhibitors were not strictly competitive, an observation we have seen with a number of other structural classes of TS inhibitors and which still remains unexplained. The values reported in Table 1 are the intercept inhibition constants determined under steady-state conditions.[21]

The compounds in Table 1 were also screened for their cell growth inhibition against three tumor cell lines: L1210 murine leukemia; CCRF-CEM, a human lymphoblastic leukemia line of T-cell origin; and GC_3/M TK^-, a human thymidine kinase deficient adenocarcinoma line. Results are reported as IC_{50} values in micromolar.[21]

Also reported in Table 1 is a thymidine shift value. Since cells can salvage exogenous thymidine to circumvent a TS block, differences in the cell growth inhibition with and without added thymidine are a measure of the specificity of the compound for TS. The numbers in this column are the ratio of the IC_{50} of the compound in the presence of 10 μM thymidine versus the IC_{50} of the compound using no added thymidine. A shift of the IC_{50} to a larger number is indicative that the cell growth inhibition of the compound is due to TS inhibition. The larger the number the more selective the compound is for TS. As was seen with other TS inhibitors,[5] many of the more potent inhibitors did show good protection from cell growth inhibition (thymidine shifts of 4–12). In the cases where no shift in the IC_{50} was observed, the identities of the alternate cellular targets are not yet known.

Discussion

In this study we used the previously designed TS inhibitor compound **i** as a starting point for the design of a series of non-(phenylsulfonyl)morpholinyl-containing pyridine derivatives. The the rationale for this type of substitution had its basis in the fact that the enzyme had previously shown a preference for compounds with large dipoles in this binding region[4] and that pyridines have dipoles of a similar magnitude and direction as that seen with benzene rings substituted with strong electron-withdrawing groups. The synthesis of compound **3** (*E. coli* TS, K_i = 1.8 μM; human TS, K_i = 14 nM) served to validate the initial design concept, and the crystal structure of compound **3** bound in the *E. coli* protein demonstrated that replacement of the (phenylsulfonyl)morpholinyl group with a simple pyridine ring did not drastically alter the binding mode of the benz[*cd*]indole portion of the inhibitor.

Investigation of the electronic requirements of this area of the active site was accomplished by adding an additional nitrogen to the pyridine ring to give the pyridazine derivatives **2** and **12–14**. The additional nitrogen and presumably the corresponding increased dipole had essentially no effect on the energy of binding. Since this portion of the molecule binds at the edge of bulk solvent, and is therefore not desolvated upon binding, the lack of increased activity is somewhat difficult to explain.

Analysis of the crystal structure of compound **3** reveals that the 2-methyl group of the pyridine binds in a small hydrophobic groove at the opening of the active site created by the side chain of Val 262 (see Figure 3). Compounds **4–7** were prepared to investigate the steric requirements of this pocket. Substitution of one of the hydrogens for a hydroxy group gave compound **7** (human TS, K_i = 18 nM) which was equipotent with compound **3**. We believe this is probably due to the likelihood that the hydroxy group points out toward solvent and therefore does not interact with protein in any substantial way. Increasing the bulk of the 2-substituent generally gave compounds with weaker activity.

The most active compounds of the series (**9**, **11**, and **12**) were the result of combining the best benz[*cd*]indole fragments (as determined from the previous series) with the simple 2-methylpyridine or unsubstituted pyridine ring system. In addition to being potent inhibitors of human TS, these compounds showed potent submicromolar cell growth inhibition against both L1210 and CCRF-CEM cells in culture. As was seen with the previous series of benzindole-containing inhibitors,[5] not all compounds showed good intracellular TS selectivity as evidenced by low thymidine shifts. The alternate targets of these compounds are as yet unknown. However, compound **12** was of particular interest because of its potent in vitro cell growth inhibition and high TS selectivity as determined from the thymidine shift ratio of 12.1.

Conclusion

We have described as series of novel benz[*cd*]indole-containing inhibitors of human TS in which the (phen-

Table 1. Effect of Changing Substituents in Regions R_1, R_2, X, and Y on TS Inhibition and Cell Growth

					TS inhibition K_i, μM[a]		cell growth inhibition IC_{50}, μM[b]			
no.	X	Y	R_1	R_2	*E. coli*	human	L1210	CCRF-CEM	GC_3/M TK$^-$	thymidine shift[c]
1	H	CH	H	H	6.6	0.017	0.095	2.4	4.8	4.9
2	H	N	H	H	18	0.015	0.90	4.0	5.0	7.7
3	CH_3	CH	H	H	1.8	0.014	1.6	2.3	7.9	2.1
4	CF_3	CH	H	H	ND	0.045	2.1	5.9	12.0	4.2
5	C_2H_5	CH	H	H	ND	0.025	1.0	3.0	6.5	2.8
6	SPh	CH	H	H	ND	0.052	0.66	2.0	11.5	1.8
7	CH_2OH	CH	H	H	ND	0.018	1.3	3.0	11.0	6.9
8	H	CH	CH_3	H	ND	0.27	1.9	5.1	23.0	2.6
9	H	CH	H	CH_3	0.6	0.006	0.16	0.44	1.1	2.3
10	H	CH	CH_3	CH_3	ND	0.014	0.21	1.1	3.6	4.1
11	CH_3	CH	H	CH_3	ND	0.004	0.17	0.45	0.6	4.1
12	H	N	H	CH_3	ND	0.010	0.45	0.78	2.1	12.1
13	H	N	CH_3	H	ND	0.31	6.9	9.0	26.0	1.9
14	H	N	CH_3	CH_3	ND	0.03	0.8	3.7	9.9	2.6
i	NA	NA	NA	NA	5.4	0.015	0.49	0.46	0.9	6.7

[a] TS activity was assayed by the tritium release method of Lomax and Greenberg[26] with some modifications. Reported K_i values have an average standard deviation of 33%. See the Experimental Section for a detailed description. [b] Inhibition of cellular growth was measured with a modification[28] of the MTT[29] colorimetric assay of Mosemann.[30] See the Experimental Section for a detailed description. [c] Expressed as the ratio of the IC_{50} in the presence of 10 μM thymidine divided by the IC_{50} with no thymidine added. See the Experimental Section for a detailed description.

ylsulfonyl)morpholinyl portion of the known compound **i** has been replaced with substituted and unsubstituted pyridine and pyridazine rings. A number of compounds from this series showed TS inhibition constants below the 10 nM level and were potent, selective antitumor agents in cell culture.

Experimental Section

Proton magnetic resonance spectra were determined using a General Electric QE-300 spectrometer operating at a field strength of 300 MHz. Chemical shifts are reported in parts per million (δ) and setting the references such that in $CDCl_3$ the $CHCl_3$ is at 7.26 ppm and in DMSO-d_6 the DMSO is at 2.49 ppm. Standard and peak multiplicities are designated as follows: s, singlet; d, doublet; dd, doublet of doublets; t, triplet; brs, broad singlet; brd, broad doublet; br, broad signal; m, multiplet. Mass spectra were determined at either the University of California Riverside or the University of California Berkeley Mass Spectrometry Centers. Infrared absorption spectra were taken on either a Perkin-Elmer 457 spectrometer or a MIDAC Corp. FTIR. Elemental microanalyses were performed by Atlantic Microlab Inc., Norcross, GA, or MHW Laboratories, Phoenix, AZ, and gave results for the elements stated with $\pm 0.4\%$ of the theoretical values. *N,N*-Dimethylformamide (DMF) was dried over activated (250°) 4-Å molecular sieves; *N,N*-dimethylacetamide (DMA) (Aldrich Gold Label grade) was similarly dried. Tetrahydrofuran (THF) was distilled from sodium benzophenone ketyl under nitrogen. Ether refers to diethyl ether and DIEA refers to diisopropylethylamine. Pet. ether refers to petroleum ether of bp 36–53 °C. Flash chromatography was performed using silica gel 60 (Merck Art 9385). Thin layer chromatographs (TLC) were performed on precoated sheets of silica gel 60 F_{254} (Merck Art 5719). Melting points were determined on a Mel-Temp apparatus and are uncorrected.

N^6-Methyl-N^6-(pyridin-4-ylmethyl)benz[*cd*]indole-2,6-diamine (1). A stirred solution of 0.21g (0.66 mmol) of **17a** and 0.30 g (0.13 mmol) of tin(II) chloride dihydrate in 15 mL of EtOH was heated at 70 °C for 45 min. The reaction mixture was diluted with EtOAc and washed with 1 N NaOH. The aqueous layer was extracted repeatedly with EtOAc until the aqueous layer was nearly colorless. The combined organic layers were dried ($MgSO_4$) and the solvent removed under reduced pressure. The residue was flash chromatographed on silica gel eluting with CH_2Cl_2/NH_3-saturated MeOH (9:1) to give 77 mg (41%) of the desired amidine as an orange solid: mp 168–170 °C; IR (KBr) 3345, 3183, 1665, 1607, 1535, 1462, 1362, 1248 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.86 (s, 3H), 4.20 (brs, 2H), 4.40 (s, 2H), 6.87 (d, 1H, J = 7.5 Hz), 7.05 (d, 1H, J =7.5 Hz), 7.40 (d, 2H, J = 5.7 Hz), 7.58 (t, 1H, J = 7.6 Hz), 7.79 (d, 1H, J = 7.0 Hz), 8.10 (d, 1H, J = 8.1 Hz), 8.61 (d, 2H, J = 5.9 Hz); HRMS calcd for $C_{18}H_{16}N_4$ MH$^+$ 289.1453, found 289.1444. Anal. ($C_{18}H_{16}N_4$·1.20H_2O) C, H, N.

N^2,N^6-Dimethyl-N^6-(pyridin-4-ylmethyl)benz[*cd*]indole-2,6-diamine (8). A pressure tube containing 315 mg (0.99 mmol) of *N*-[2-(methylthio)benz[*cd*]indol-6-yl]-*N*-(pyridin-4-ylmethyl)methylamine, 10 mL of MeOH, and approximately 1 mL of condensed, anhydrous methylamine gas was sealed and heated at 95 °C for 16 h. The reaction mixture was then cooled. The contents of the tube were transferred to an RB flask, and the volatiles were evaporated under reduced pressure. The residue was flash chromatographed on silica gel eluting with CH_2Cl_2/NH_3-saturated MeOH (12:1) to give 250 mg (84%) of the desired product as an orange solid: mp 194–195 °C; IR (KBr) 3225, 2949, 1634, 1604, 1582, 1445, 1362, 1244 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 2.77 (s, 3H), 3.06 (s, 3H), 4.36 (s, 2H), 6.84 (d, 1H, J = 7.5 Hz), 6.90 (d, 1H, J = 7.5 Hz), 7.40 (d, 2H, J = 5.8 Hz), 7.61 (t, 1H, J = 7.9 Hz), 7.98 (d, 1H, J = 7.0 Hz), 8.03 (d, 1H, J = 8.1 Hz), 8.52 (d, 2H, J = 5.9 Hz). Anal. ($C_{19}H_{18}N_4$·0.25H_2O) C, H, N.

5-[Methyl(pyridin-4-ylmethyl)amino]-8-nitronaphthalene-1-carbonitrile (17a). A solution of 10 mL of DMSO, 0.51 g (2.21 mmol) of chloronaphthalene **16a**, 0.57 g (4.67 mmol) of methyl(pyridin-4-ylmethyl)amine, and 0.80 mL (4.59 mmol) of DIEA was heated at 85 °C for 6 h. The reaction mixture was cooled, poured into H_2O, and extracted three times with EtOAc. The combined organic layers were dried ($MgSO_4$), and after removal of the solvent under reduced pressure, the residue was flash chromatographed on silica gel eluting with CH_2Cl_2/EtOAc (1:1) to give 0.26 g (37%) of the desired product as a yellow solid: mp 153–156 °C; IR (KBr) 2222, 1570, 1505, 1518, 1412, 1323, 1304, 1206 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.94 (s, 3H), 4.41 (s, 2H), 7.15 (d, 1H, J = 8.4 Hz), 7.32 (d, 2H, J = 6.0 Hz), 7.66 (t, 1H, J = 7.3 Hz), 8.03 (d, 1H, J = 8.4 Hz), 8.13 (d, 1H, J = 7.2 Hz), 8.55 (d, 1H, J = 8.6 Hz), 8.64 (d, 1H, J = 6.0 Hz). Anal. ($C_{18}H_{14}N_4O_2$·0.50H_2O) C, H, N.

6-[Methyl(pyridin-4-ylmethyl)amino]-1*H*-benz[*cd*]indol-2-one (20a). A solution of HCl gas in MeOH was added to a suspension of 1.09 g (5.50 mmol) of aniline **19a** in MeOH until a homogeneous solution resulted. After 10 min, ether was added and the precipitate collected, washed with ether, dried, and resuspended in 10 mL of MeOH. To the HCl salt were added 0.70 g (6.60 mmol) of 4-pyridinecarboxaldehyde and 0.35 g (5.50 mmol) of $NaBH_3CN$. After 1 h at room temperature, the temperature was raised to 70 °C for 2 h. The cooled reaction mixture was poured into EtOAc and washed with a saturated $NaHCO_3$ solution (1×) and saturated NaCl (2×). The organic layer was dried ($MgSO_4$), and after removal of the solvent under reduced pressure, the residue was flash chromatographed eluting with CH_2Cl_2/EtOAc (1:1) to give 0.42 g

of starting aniline **19a** and 0.55 g (34%) of the desired product as an orange solid: mp 193–194 °C; IR (KBr) 3026, 2839, 2783, 1701, 1641, 1605, 1472, 1418, 1078 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.86 (s, 3H), 4.38 (s, 2H), 6.89 (AB system, 2H, J = 7.6 Hz), 7.39 (d, 2H, J = 5.9 Hz), 7.71 (t, 1H, J = 7.6 Hz), 8.10 (d, 1H, J = 7.0 Hz), 8.23 (d, 1H, J = 8.2 Hz), 8.46 (brs, 1H), 8.61 (d, 2H, J = 6.0 Hz). Anal. ($C_{18}H_{15}N_3O$) C, H, N.

Methyl[[2-(trifluoromethyl)pyridin-4-yl]methyl]-amine (22b). To an ice cold solution of 3.2 mL (43.84 mmol) of thionyl chloride was added dropwise a solution of 1.56 g (8.81 mmol) of alcohol **21a** in 5 mL of CH_2Cl_2. After 10 min at 0 °C, the volatiles were removed and the oily residue was dissolved in 10 mL of THF and added dropwise to 45 mL of 40% (by wt) aqueous methylamine. After 10 min at room temperature, the volatiles were removed and the residue was diluted with EtOAc, washed with saturated $NaHCO_3$ and then saturated NaCl solution, and dried ($MgSO_4$). The solvent was removed under reduced pressure to give 1.37 g (82%) of the desired product as an oil: IR (neat) 3314, 2853 (br), 1738, 1611, 1431, 1327, 1180, 1138 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.47 (s, 3H), 3.86 (s, 2H), 7.47 (d, 1H, J = 4.9 Hz), 7.69 (s, 1H), 8.66 (d, 1H, J = 4.9 Hz). Anal. ($C_8H_9F_3N_2 \cdot 0.20H_2O$) C, H, N.

***N*-[2-(Methylthio)benz[*cd*]indol-6-yl]-*N*-(pyridin-4-ylmethyl)methylamine.** To a stirred suspension of 296 mg (1.02 mmol) of benzindole **20a** in 10 mL of THF was added 434 mg (1.07 mmol) of Lawesson's reagent. After heating for 90 min at 60 °C, the reaction mixture was cooled, diluted with EtOAc, and washed with saturated $NaHCO_3$ solution. The layers were separated. The aqueous layer was re-extracted with EtOAc. The combined organic layers were washed with saturated NaCl and dried ($MgSO_4$), and the solvent was removed under reduced pressure. To a solution of this crude thio lactam in 25 mL of THF were added 2.05 mL of 1 N NaOH and 0.064 mL (1.02 mmol) of iodomethane. After stirring for 1 h at room temperature, the reaction mixture was diluted with EtOAc and washed with water. The organic layer was washed with saturated NaCl and dried ($MgSO_4$) and the solvent removed under reduced pressure. The residue was flash chromatographed on silica gel eluting with CH_2Cl_2/EtOAc (1:1) and then CH_2Cl_2/EtOAc/MeOH (1:1:0.1) to give 328 mg (~100%) of the desired product as a dark purple foam: ^1H NMR ($CDCl_3$) δ 2.87 (s, 3H), 2.96 (s, 3H), 4.57 (s, 2H), 6.87 (d, 1H, J = 7.7 Hz), 7.41 (d, 2H, J = 5.8 Hz), 7.55 (m, 2H), 7.89 (d, 1H, J = 7.0 Hz), 8.07 (d, 1H, J = 8.1 Hz), 8.64 (d, 2H, J = 6.0 Hz).

***N*-(5-Cyano-4-nitronaphthalen-1-yl)acetamide.** A stirred solution of 3.83 g (12.39 mmol) of bromo compound **15a**[17] and 1.28 g (14.29 mmol) of copper(I) cyanide in 40 mL of DMF was heated at reflux for 1 h. The cooled reaction mixture was poured into a 30% aqueous ethylenediamine solution and extracted repeatedly with EtOAc. The combined organic layers were washed with 10% NaCN solution and then saturated NaCl solution and dried (Na_2SO_4), and the solvent was removed under reduced pressure. The wet solid which resulted was triturated with Et_2O, filtered, and dried (2.28 g). The filtrate was concentrated and the residue flash chromatographed on silica gel eluting with CH_2Cl_2/EtOAc (1:1) to give 0.13 g of product, overall, 2.41 g (72%) as a yellow solid: mp 267–268 °C dec; IR (KBr) 3270, 2230, 1670, 1520, 1342, 1270 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.24 (s, 3H), 7.89 (t, 1H, J = 8.7 Hz), 8.10 (d, 1H, J = 8.5 Hz), 8.30 (d, 1H, J = 8.5 Hz), 8.42 (d, 1H, J = 7.2 Hz), 8.70 (d, 1H, J = 8.7 Hz), 10.50 (brs, 1H). Anal. ($C_{13}H_9N_3O_3 \cdot 0.35H_2O$) C, H, N.

5-Amino-8-nitronaphthalene-1-carbonitrile. A solution of 2.34 g (9.18 mmol) of *N*-(5-cyano-4-nitronaphthalen-1-yl)-acetamide and 9 mL of 2 N NaOH solution in 50 mL of MeOH was heated at reflux for 30 min. The reaction mixture was cooled, and the precipitate which formed was collected, washed with H_2O and then cold MeOH, and dried to give 1.75 g (89%) of the desired aniline as an orange solid: mp 255–260 °C dec; IR (KBr) 3495, 3380, 3260, 2220, 1690, 1575, 1525, 1482, 1295 cm^{-1}; ^1H NMR (DMSO-d_6) δ 6.73 (d, 1H, J = 8.8 Hz), 7.64 (s, 2H), 7.68 (t, 1H, J = 8.5 Hz), 8.17 (d, 1H, J = 8.8 Hz), 8.26 (d, 1H, J = 7.3 Hz), 8.61 (d, 1H, J = 8.5 Hz). Anal. ($C_{11}H_7N_3O_2$) C, H, N.

5-Chloro-8-nitronaphthalene-1-carbonitrile (16a). To a partially dissolved solution of 1.35 g (10.04 mmol) of copper-(II) chloride and 1.70 mL (12.86 mmol) of *tert*-butyl nitrite in 100 mL of CH_3CN was added a suspension of 1.79 g (8.40 mmol) of 5-amino-8-nitro-naphthalene-1-carbonitrile in 50 mL of CH_3CN. After all the aniline had been added, the reaction mixture was stirred for another 30 min at room temperature and then poured into 0.5 N HCl and extracted twice with EtOAc. The combined organic layer was washed with saturated NaCl solution and dried ($MgSO_4$) and the solvent removed under reduced pressure. The residue was recrystallized from EtOH to give 1.47 g of product as light brown needles: mp 210–212 °C. Another 0.20 g of product was obtained after flash chromatography of the concentrated mother liquors eluting with hexanes/CH_2Cl_2 (1:1), overall, 1.67 g (86%): IR (KBr) 3100, 2225, 1560 1530, 1360, 1210, 1050 cm^{-1}; ^1H NMR (DMSO-d_6) δ 8.02 (t, 1H, J = 7.4 Hz), 8.10 (d, 1H, J = 8.2 Hz), 8.32 (d, 1H, J = 8.2 Hz), 8.53 (d, 1H, J = 7.3 Hz), 8.74 (d, 1H, J = 8.7 Hz). Anal. ($C_{11}H_{15}ClN_2O_2$) C, H, N, Cl.

5-Bromo-8-methyl-1-nitronaphthalene. To a stirred solution of 10.60 g (56.32 mmol) of 8-methyl-1-nitronaphthalene[20] and 0.45 g (2.77 mmol) of iron(III) chloride in 150 mL of CCl_4 heated to 60 °C was added dropwise 3.0 mL (58.23 mmol) of bromine. After 1 h, the reaction mixture was poured into saturated $NaHCO_3$ solution, and the layers were separated. The aqueous layer was re-extracted with CH_2Cl_2. The combined organic layers were dried ($MgSO_4$), and the solvent was removed under reduced pressure. The crude residue was recrystallized from EtOH, and the mother liquors were concentrated and flash chromatographed on silica gel eluting with hexanes/EtOAc (12:1). The faster moving 5-bromo isomer was isolated in 84% overall yield as a brown solid: mp 101.5–103.5 °C; IR (KBr) 1524, 1443, 1360, 824 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.51 (s, 3H), 7.30 (d, 1H, J = 7.9 Hz), 7.60 (t, 1H, J = 8.2 Hz), 7.72 (d, 1H, J = 7.3 Hz), 7.82 (d, 1H, J = 7.9 Hz), 8.54 (d, 1H, J = 8.5 Hz). Anal. ($C_{11}H_8BrNO_2 \cdot 0.2H_2O$) C, H, Br, N. The slower moving 7-bromo isomer was isolated in 5% yield.

5-Bromo-8-methylaminonaphthalene. To a stirred solution of 12.54 g (47.13 mmol) of 5-bromo-8-methyl-1-nitronaphthalene in 210 mL of benzene containing 10 mL of MeOH was added 25.48 g (50.60 mmol) of triiron dodecacarbonyl (5% MeOH). The reaction mixture was heated at reflux for 5.5 h, cooled to room temperature, and then filtered through Celite. The solvent was removed under reduced pressure to give 11.40 g of a purple oil which was dissolved in 150 mL of CH_2Cl_2 and filtered through a pad of silica gel to remove residual Fe residues. The CH_2Cl_2 was removed under reduced pressure to give 10.40 g (93%) of the desired product which was used without further purification: ^1H NMR ($CDCl_3$) δ 2.96 (s, 3H), 4.39 (brs, 2H), 6.75 (d, 1H, J = 7.5 Hz), 6.96 (d, 1H, J = 7.7 Hz), 7.32 (t, 1H, J = 8.0 Hz), 7.58 (d, 1H, J = 7.6 Hz), 7.73 (d, 1H, J = 7.73 Hz).

***N*-(5-Bromo-8-methylnapthalen-1-yl)acetamide.** To a stirred solution of 6.68 g (28.29 mmol) of 5-bromo-8-methylaminonaphthalene and 5.90 mL (33.87 mmol) of DIEA in 200 mL of CH_2Cl_2 at 0 °C was added dropwise 2.20 mL (30.94 mmol) of acetyl chloride. After 30 min at 0 °C, the thick precipitate that formed was filtered, washed with H_2O, and dried in vacuo. The filtrate was partitioned between 0.5 N HCl and CH_2Cl_2. The organic layer was concentrated to about 20 mL and the solid collected by filtration and washed with cold CH_2Cl_2. The solids were combined to give 7.20 g (91%) of the desired product as a tan solid: mp 227–229 °C; IR (KBr) 3231, 1644, 1535, 1366, 1294, 806 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.08 (s, 3H), 2.73 (s, 3H), 7.21 (d, 1H, J = 7.7 Hz), 7.42 (d, 1H, J = 7.1 Hz), 7.63 (t, 1H, J = 8.4 Hz), 7.76 (d, 1H, J = 7.7 Hz), 8.14 (d, 1H, J = 8.4 Hz), 9.91 (s, 1H). Anal. ($C_{13}H_{12}BrNO$) C, H, Br, N.

***N*-(5-Bromo-8-methyl-4-nitronapthalen-1-yl)acetamide (15b).** To a cold (−5 °C) suspension of 4.45 g (16.00 mmol) of *N*-(5-bromo-8-methylnapthalen-1-yl)acetamide in 25 mL of CH_3CN was added dropwise a suspension of 2.60 g (16.64 mmol) of nitroniumtetrafluoroborate in 5 mL of CH_3CN. After 10 min, the homogeneous, light yellow reaction

mixture was poured into H_2O and extracted twice with EtOAc. The combined organic layers were washed with saturated NaCl solution and dried ($MgSO_4$), and the solvent was removed under reduced pressure. The crude residue was recrystallized from MeOH to give 1.2 g of the desired product as a light yellow solid: mp 216–217 °C. Another 0.80 g of product was obtained from the concentrated mother liquors, after flash chromatography on silica gel eluting with hexanes/EtOAc (1:1), overall, 2.00 g (39%): IR (KBr) 3275, 1667, 1532, 1373, 1312, 1271 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.29 (brs, 3H), 2.87 (s, 3H), 7.23 (d, 1H, J = 7.9 Hz), 7.65 (brs, 1H), 7.79 (m, 3H). Anal. ($C_{13}H_{11}BrN_2O_3$) C, H, Br, N.

N-(5-Cyano-8-methyl-4-nitronapthalen-1-yl)acetamide. The general procedure used to prepare *N*-(5-cyano-4-nitronapthalen-1-yl)acetamide was employed using bromo compound **15b**. The product (89%) was isolated as a yellow solid by slurrying the crude product in hot CH_2Cl_2, cooling, and collecting the solid by filtration: mp 208–211 °C; IR (KBr) 3283, 2226, 1661, 1535, 1510, 1352, 1269 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.14 (s, 3H), 2.88 (s, 3H), 7.66 (d, 1H, J = 7.6 Hz), 7.73 (d, 1H, J = 8.2 Hz), 8.25 (d, 1H, J = 7.5 Hz), 8.26 (d, 1H, J = 8.25 Hz), 10.23 (s, 1H). Anal. ($C_{14}H_{11}N_3O_3$) C, H, N.

5-Amino-4-methyl-8-nitronaphthalene-1-carbonitrile. The general conditions used to prepare 5-amino-8-nitronaphthalene-1-carbonitrile were employed using *N*-(5-cyano-8-methyl-4-nitronapthalen-1-yl)acetamide. The product (81%) was isolated as a yellow solid: mp 174 °C dec; IR (KBr) 3507, 3353, 3252, 2224, 1644, 1582, 1493, 1474, 1296, 1271 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.96 (s, 3H), 6.81 (d, 1H, J = 8.9 Hz), 7.07 (brs, 2H), 7.40 (d, 1H, J = 7.6 Hz), 8.05 (d, 1H, J = 7.5 Hz), 8.09 (d, 1H, J = 8.9 Hz). Anal. ($C_{12}H_9N_3O_2$) C, H, N.

5-Chloro-4-methyl-8-nitronaphthalene-1-carbonitrile (16b). A suspension of 1.31 g (5.77 mmol) of 5-amino-4-methyl-8-nitronaphthalene-1-carbonitrile in 15 mL of CH_3CN was slowly added to a stirred solution of 1.15 mL (8.79 mmol) of *tert*-Butyl nitrite and 0.93 g (6.92 mmol) of copper(II) chloride in 50 mL of CH_3CN at room temperaturee. After 45 min, the reaction mixture was poured into 0.5 N HCl solution and extracted twice with EtOAc. The combined organic layers were washed with saturated $NaHCO_3$ solution and then saturated NaCl solution and dried ($MgSO_4$), and the solvent was removed at reduced pressure. The residue was refluxed in CH_2Cl_2, cooled, and filtered to give 0.55 g of the desired product. The mother liquors were concentrated and flash chromatographed on silica gel eluting with hexanes:CH_2Cl_2 (1:1) to give another 0.62 g of product, overall, 1.17 g (82%) as a tan solid: mp 179–180 °C; IR (KBr) 3106, 2228, 1572, 1532, 1387, 1364, 1055 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.18 (s, 3H), 7.56 (d, 1H, J = 7.6 Hz), 7.76 (d, 1H, J = 8.2 Hz), 7.84 (d, 1H, J = 8.2 Hz), 7.99 (d, 1H, J = 7.6 Hz). Anal. ($C_{12}H_7ClN_2O_2$) C, H, Cl, N.

Methyl(pyridin-4-ylmethyl)amine. A pressure tube was charged with 2.00 g (14.00 mmol) of 4-pyridinecarboxaldehyde and ca. 25 g of 3-Å molecular sieves. After saturating the aldehyde with anhydrous methylamine, the tube was sealed and heated at 100 °C for 6 h. The contents of the tube plus 0.23 g of 5% Pd on carbon were placed under 40 psi of H_2 for 18 h. The catalyst was removed by filtering through Celite and the filtrate concentrated under reduced pressure. The residue was flash chromatographed on silica gel eluting with CH_2Cl_2/NH_3-saturated MeOH (15:1) to give 2.10 g (92%) of the desired product as a colorless liquid: IR (neat) 3293 (br), 2797, 1605, 1561, 1416, 1362 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.46 (s, 3H), 3.78 (s, 2H), 7.26 (d, 2H, J = 6.2 Hz), 8.54 (d, 2H, J = 5.9 Hz). Anal. ($C_7H_{10}N_2O \cdot 0.15H_2O$) C, H, N.

[2-(Trifluoromethyl)pyridin-4-yl]methanol (21b). The general procedure of Katz[25] was used to prepare the title compound in 12% yield as an oil after flash chromatography on silica gel eluting with hexanes/EtOAc (2:1): IR (neat) 2872, 1615, 1431, 1329, 1184, 1138 cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.78 (s, 2H), 7.46 (d, 1H, J = 5.0 Hz), 7.67 (s, 1H), 8.58 (d, 1H, J = 5.0 Hz). Anal. ($C_7H_6F_3NO \cdot 0.10H_2O$) C, H, N.

Methyl[2-methylpyridin-4-yl)methyl]amine (22a). A stirred solution of 0.86 g (6.98 mmol) of 2-methyl-4-(hydroxymethyl)pyridine (**21a**)[25] in 48% aqueous HBr was heated at reflux for 4 days. The reaction mixute was cooled and added dropwise to 20 mL of 40% aqueous methylamine. The volatiles were removed under reduced pressure, and the residue was slurried in CH_2Cl_2 and filtered. The filter cake was washed with CH_2Cl_2, EtOAc, CH_3CN, and finally 10% MeOH in CH_3CN. The filtrate was concentrated and the residue flash chromatographed on silica gel eluting with CH_2Cl_2/NH_3-saturated MeOH (9:1) to give 0.65 g (68%) of the desired product as a colorless liquid: IR (neat) 3368 (br), 1638, 1609, 1562, 1451, 1406 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.45 (s, 3H), 2.54 (s, 3H), 3.73 (s, 2H), 7.05 (d, 1H, J = 5.1 Hz), 7.13 (s, 1H), 8.42 (d, 1H, J = 5.1 Hz); HRMS calcd for $C_8H_{12}N_2$ M^+ 136.1000, Found: 136.0998. Anal. ($C_8H_{12}N_2 \cdot 0.45H_2O$) C, H, N.

Methyl(pyridazin-4-ylmethyl)amine (22c). The general conditions used to prepare **22b** were employed using 5-(hydroxymethyl)pyridazine (**21c**).[25] The product (86%) was isolated by flash chromatography on silica gel eluting with CH_2Cl_2/NH_3-saturated MeOH (9:1) as an oil: IR (neat) 3300 (br), 1661, 1591, 1451, 1379, 1134 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.47 (s, 3H), 3.83 (s, 2H), 7.49 (d, with fine splitting, 1H, J = 5.2 Hz), 9.13 (d, with fine splitting, 1H, J = 5.3 Hz), 9.18 (s, 1H). Anal. ($C_6H_9N_3 \cdot 0.5H_2O$) C, H, N.

2-Ethyl-4-(hydroxymethyl)pyridine (21d). This compound was prepared via the method described by Katz[25] using 10.0 g (81.2 mmol) of 2-ethylpyridine *N*-oxide. The crude product was purified on gravity silica gel using 20% THF in CH_2Cl_2 and then a gradient of 0–10% MeOH in CH_2Cl_2 as eluent. The material was subjected to a second chromatography on gravity silica gel using a gradient of 0–10% MeOH in CH_2Cl_2 as eluent to give 1.5 g of impure material. The material was then passed through a C_{18} column using H_2O as eluent. The oily product was taken up in a mixture of acetone/THF/acetonitrile and filtered to remove any insolubles. The filtrate was reduced to minimum volume and the residue suspended in NH_4OH/MeOH (10:90). A white precipitate was removed by filtration and the filtrate reduced to minimum volume to obtain 80 mg (1%) of clean product as an oil: IR (NaCl, neat) 3250 (br), 2971, 1611, 1560, 1460, 1414 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.31 (t, 3H, J = 7.63 Hz), 2.82 (q, 2H, J = 7.61 Hz), 4.73 (s, 2H), 7.10 (d, 1H, J = 5.08 Hz), 7.18 (s, 1H), 8.46 (d, 1H, J = 5.08 Hz); HRMS calcd for $C_3H_{11}NO$ 137.0840, found 137.0838.

Methyl[(2-ethylpyridin-4-yl)methyl]amine (22d). Compound **22d** was prepared from 170 mg (11.2 mmol) of 4-(hydroxymethyl)-2-ethylpyridine (**21d**) employing the general conditions used to prepare **22b**. The crude product was flash chromatographed on silica gel using a gradient of CH_2Cl_2/MeOH/NH_4OH (100:0:0–90:9:1) as eluent to obtain 128 mg (69%) of the desired product as an oil: IR (NaCl, neat) 2971, 2924, 2851, 1640, 1607 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.31 (t, 3H, J = 7.62 Hz), 2.48 (s, 3H), 2.82 (q, 2H, J = 7.62 Hz), 3.78 (s, 2H), 7.08 (d, 1H, J = 5.13 Hz), 7.15 (s, 1H), 8.47 (d, 1H, J = 5.04 Hz); HRMS calcd for $C_9H_{14}N_2$ 150.1157, found 150.1162.

***N*-(*tert*-Butoxycarbonyl)-*N*-methyl(pyridin-4-ylmethyl)amine.** To a stirred suspension of 6.2 g (51.5 mmol) of methyl(pyridin-4-ylmethyl)amine in 50 mL of CH_2Cl_2 at room temperature was added dropwise a solution of di-*tert*-butyl dicarbonate in 50 mL of CH_2Cl_2 over 35 min. After an additional 22 h, the mixture was poured into 90 mL of 1 N NaOH. The aqueous layer was extracted with CH_2Cl_2 (3 × 250 mL) and EtOAC (3 × 120 mL). The combined organics were dried (Na_2SO_4). The residue was flash chromatographed on silica gel using a gradient of 35–50% EtOAC/CH_2Cl_2 to give 8.96 g (78%) of the desired product as a tan oil: IR (NaCl, neat) 2974, 2934, 1698, 1481, 1393, 1366 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.43–1.50 (2s, 9H), 2.84, 2.90 (2s, 3H), 4.43 (s, 2H), 7.13 (d, 2H, J = 4.45 Hz), 8.56 (d, 2H, J = 5.69 Hz). Anal. ($C_{12}H_{18}N_2O_2$) C, H, N.

***N*-(*tert*-Butoxycarbonyl)-*N*-methyl[(pyridin-4-yl *N*-oxide)methyl]amine.** To a stirred solution of 1.28 g (5.76 mmol) of *N*-(*tert*-butoxycarbonyl)-*N*-methyl(pyridin-4-ylmethyl)amine in 30 mL CH_2Cl_2 at reflux was added dropwise a solution of 1.05 g (6.1 mmol) of MCPBA in 20 mL of CH_2Cl_2. After 3 h, the solvent was removed under reduced pressure and the crude product was flashed chromatographed on silica gel using a gradient of CH_2Cl_2/MeOH (100:0–90:10) to give 1.3 g (95%) of the desired product as an oil: IR (NaCl, neat) 3400 (br),

2974, 2934, 1688, 1483, 1449, 1393, 1368 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.48 (br s, 9H), 2.86 (br s, 3H), 4.39 (br s, 2H), 7.15 (br d, 2H), 8.18 (d, 2H, J = 6.72 Hz). Anal. ($C_{12}H_{18}N_2O_3 \cdot 1 H_2O$) C, H, N.

***N*-(*tert*-Butoxycarbonyl)-*N*-methyl[[2-(hydroxymethyl)-pyridin-4-yl]methyl]amine.** This compound was prepared from 1.3 g (5.45 mmol) of the corresponding pyridine *N*-oxide employing the general conditions described by Katz.[25] When the reaction was complete, the heat was removed and the mixture diluted with MeOH/NH_4OH (90:10) and stirred at room temperature overnight. The mixture was filtered and the filtrate reduced to minimum volume. The residue was azeotroped to dryness using EtOH. The residue was flash chromatographed on silica gel using a gradient of CH_2Cl_2/MeOH (100:0–92:8) to give 414 mg (30%) of the desired product as an oil: IR (NaCl, neat) 3390 (br) 2974, 1690, 1481, 1393 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.43–1.50 (2 br s, 9H), 2.85–2.89 (2 br s, 3H), 4.43 (s, 2H), 7.05–7.08 (m, 2H), 8.50 (d, 1H, J = 5.00 Hz). Anal. ($C_{13}H_{20}N_2O_3 \cdot 0.25 H_2O \cdot 0.1 CH_2Cl_2 \cdot 0.3$ MeOH) C, H, N.

***N*-(*tert*-Butoxycarbonyl)-*N*-methyl[[2-[(*tert*-butyldiphenylsiloxy)methyl]pyridin-4-yl]methyl]amine.** To a stirring mixture of 6.25 mL (24 mmol) of *tert*-butyldiphenylchlorosilane, 3.65 mL (26.1 mmol) of triethylamine, and 50 mg of DMAP in 50 mL of CH_2Cl_2 at 0 °C was added dropwise a solution of 5.5 g (21.8 mmol) of the alcohol prepared above in 50 mL of CH_2Cl_2 over 50 min. The mixture was allowed to warm to room temperature and after 16 h was poured into 1 N NaOH (100 mL). The aqueous layer was extracted with CH_2Cl_2 (2 × 200 mL), and the combined organics were dried (Na_2SO_4). The solvent was removed under reduced pressure, and the crude residue was chromatographed on gravity silica gel using a gradient of CH_2Cl_2/MeOH (100:0–96:4) to give 9.92 g (93%) of the desired product as an oil: IR (NaCl, neat) 2961, 2930, 2859, 1699, 1605, 1474, 1427, 1391, 1366 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.13 (s, 9H), 1.31–1.26 (2 brs, 9H), 2.88–2.92 (2 brs, 3H), 4.45–4.50 (2 brs, 2H), 4.87 (s, 2H), 7.00 (brd, 1H, J = 4.45 Hz), 7.34–7.43 (m, 6H), 7.52 (brs, 1H), 7.68 (d, 4H, J = 7.74 Hz), 8.42 (brd, 1H, J = 4.49 Hz). Anal. ($C_{29}H_{38}N_2O_3Si \cdot 0.2H_2O$) C, H, N.

***N*-Methyl[[2-[(*tert*-butyldiphenylsiloxy)methyl]pyridin-4-yl]methyl]amine.** A solution of 320 mg (0.65 mmol) of the protected Boc-amine prepared above in 6 mL of TFA/CH_2Cl_2 (1:4) was stirred at room temperature for 1 h. The reaction mixture was poured into 1 N NaOH and extracted with 1 N NaOH (3 × 10 mL). The combined aqueous washes were then extracted with CH_2Cl_2 (5 mL). The combined organics were dried (Na_2SO_4), and after the solvent was removed under reduced pressure, the crude residue was flash chromatographed on silica gel using a gradient of CH_2Cl_2/MeOH (100:0–92:8) to give 218 mg (86%) of the desired product as a pink oil: IR (NaCl, neat) 3071, 2930, 2857, 2793, 1605, 1562, 1472, 1427 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.13 (s, 9H), 2.48 (s, 3H), 3.81 (s, 2H), 4.88 (s, 2H), 7.14 (d, 1H, J = 4.9 Hz), 7.34–7.42 (m, 6H), 7.52 (s, 1H), 7.69 (d, 4H, J = 6.16 Hz), 8.42 (d, 1H, J = 5.04 Hz). Anal. ($C_{24}H_{30}N_2OSi$) C, H, N.

5-[Methyl[[2-[(*tert*-butyldiphenylsiloxy)methyl]pyridin-4-yl]methyl]-amino-8-nitronaphthalene-1-carbonitrile. This compound was prepared from 300 mg (1.26 mmol) of the chloronaphthalene **16a** and 1.2 g (13.07 mmol) of the *N*-methyl[[2-[(*tert*-butyldiphenylsiloxy)methyl]pyridin-4-yl]methyl]-amine employing the general conditions used to prepare **17a**. The crude product was flash chromatographed on silica gel using a gradient of CH_2Cl_2/EtOAc (100:0–92:3) to give 345 mg (47%) of the desired product as a yellow solid in 47% yield: mp 60–65 °C; IR (KBr) 2930, 2855, 2224, 1732, 1605, 1572, 1520, 1331, 1115 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.10 (s, 9H), 2.95 (s, 3H), 4.44 (s, 2H), 4.93 (s, 2H), 7.16–7.19 (m, 2H), 7.31–7.42 (m, 6H), 7.39 (dd, 1H, J = 8.40 Hz, J_2 = 7.44 Hz), 7.65 (d, 4H, J = 7.86 Hz), 7.78 (s, 1H), 8.02 (d, 1H, J = 8.27 Hz), 8.09 (d, 1H, J = 6.56 Hz), 8.50 (d, 1H, J = 5.01 Hz), 8.54 (d, 1H, J = 8.8 Hz).

5-[Methyl[[2-(hydroxymethyl)pyridin-4-yl]methyl]amino]-8-nitronaphthalene-1-carbonitrile (17f). To a stirring solution of 350 mg (0.6 mmol) of the above silyl alcohol in 10 mL of THF was added 6 mL (0.6 mmol) of a 1 M solution of tetrabutylammonium fluoride in THF. The mixture was stirred at room temperature for 10 min and then poured into 100 mL of H_2O. The pH of the aqueous layer was made alkaline by the dropwise addition of 1 N NaOH. The aqueous layer was then extracted with EtOAc (2 × 30 mL) and CH_2Cl_2 (2 × 30 mL). The combined organic layers were dried (Na_2SO_4), and after the solvent was removed under reduced pressure, the crude residue was flash chromatographed on silica gel using a gradient of CH_2Cl_2/MeOH (100:0–94:6) to yield 125 mg (60%) of the desired deprotected alcohol as a yellow crystalline solid: mp 154–155 °C; IR (KBr) 2220, 1609, 1572, 1529, 1343 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.92 (s, 3H), 4.53–4.56 (m, 4H), 5.41 (t, 1H, J = 5.74 Hz), 7.24 (d, 1H, J = 5.02 Hz), 7.31 (d, 1H, J = 8.57 Hz), 7.47 (s, 1H), 7.81 (dd, 1H, J = 8.43 Hz, J_2 = 7.48 Hz), 8.23 (d, 1H, J = 8.38 Hz), 8.37 (d, 1H, J = 7.27 Hz), 8.44 (d, 1H, J = 5.0 Hz), 8.60 (d, 1H, J = 8.75 Hz). Anal. ($C_{19}H_{16}N_4O_3$) C, H, N.

***N*^{6}-Methyl-*N*^{6}-[[2-(hydroxymethyl)pyridin-4-yl]methyl]-benz[*cd*]indole-2,6-diamine (7).** Compound **7** was prepared from 109 mg (0.31 mmol) of the nitronaphthalene **17f** employing the general conditions used to prepare **1**. The reaction mixture was diluted with MeOH/NH_4OH (90:10) and stirred at room temperature for 5 min. After the insoluble material was filtered off, the solvent was removed from the filtrate and the crude residue was flash chromatographed on silica gel using a gradient of CH_2Cl_2/MeOH/NH_4OH (100:0:0–90:9:1) to give 30 mg (30%) of the desired amidine **7** as a red solid: mp >170 °C dec; IR (KBr) 3310 (br), 3160 (br), 1667, 1609, 1534, 1466 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.78 (s, 3H), 4.38 (s, 2H), 4.55 (d, 2H, J = 5.65 Hz), 5.41 (t, 1H, J = 5.78 Hz), 6.83 (d, 1H, J = 7.50 Hz), 6.90 (d, 1H, J = 7.44 Hz), 7.27 (d, 1H, J = 5.41 Hz), 7.55 (s, 1H), 7.64 (t, 1H, J = 7.54 Hz), 8.04–8.08 (m, 2H), 8.43 (d, 1H, J = 4.95 Hz). Anal. ($C_{19}H_{18}N_4O \cdot 0.55H_2O$) C, H, N.

5-[Methyl[(2-ethylpyridin-4-yl)methylamino]-8-nitronaphthalene-1-carbonitrile (17e). This compound was prepared from 81 mg (0.34 mmol) of the chloronaphthalene **16a** and 128 mg (0.85 mmol) of the amine **22d** employing the general conditions used for compound **17a**. The product was isolated by flash chromatography on silica gel using a gradient of MeOH/CH_2Cl_2 (0:100–3:97) to give 62 mg (62%) of the desired compound **17e** as an amorphous yellow solid in 53% yield: IR (KBr) 2222, 1605, 1572, 1518 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.32 (t, 3H, J = 7.62 Hz), 2.85 (q, 2H, J = 7.60 Hz), 2.93 (s, 3H), 4.37 (s, 2H), 7.13–7.16 (m, 3H), 7.65 (dd, 1H, J_1 = 8.62 Hz, J_2 = 7.28 Hz), 8.04 (d, 1H, J = 8.37 Hz), 8.13 (d, 1H, J = 7.25 Hz), 8.52–8.56 (m,3H); HRMS calcd for $C_{20}H_{18}N_4O_2$ 346.1430, found 346.1451.

***N*^{6}-Methyl-*N*^{6}-[(2-ethylpyridin-4-yl)methyl]benz[*cd*]indole-2,6-diamine (5).** This compound was prepared from 80 mg (0.23 mmol) of the nitronaphthalene **17e** employing the general conditions used to prepare **1**. The crude product was flash chromatographed on silica gel using a gradient of CH_2Cl_2/MeOH/NH_4OH (100:0:0–90:9:1) to give 30 mg (41%) of the desired amidine **5** as a brown solid: mp >80 °C dec; IR (KBr) 2930, 1645, 1605, 1537, 1462 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.32 (t, 3H, J = 7.60 Hz), 2.81–2.89 (m, 5H), 4.38 (s, 2H), 6.87 (d, 1H, J = 7.58 Hz), 7.07 (d, 1H, J = 7.53 Hz), 7.22–7.24 (m, 2H), 7.60 (t, 1H, J = 7.62 Hz), 7.87 (d, 1H, J = 7.03 Hz), 8.13 (d, 1H, J = 8.15 Hz), 8.52 (d, 1H, J = 5.65 Hz); HRMS calcd for $C_{20}H_{20}N_4$ 316.1688, found 316.1704. Anal. ($C_{20}H_{20}N_4 \cdot 1H_2O \cdot 0.6$hexane) C, H, N.

5-[Methyl[(pyridine-4-yl *N*-oxide)methyl]amino]-8-nitronaphthalene-1-carbonitrile. A stirring suspension of 1.5 g (4.7 mmol) of the pyridine **17a** in 30 mL of CH_2Cl_2 was heated to reflux. To the mixture was added dropwise a solution of 0.86 g (5.0 mmol) of *m*-chloroperbenzoic acid (MCPBA) in 15 mL of CH_2Cl_2. After stirring at reflux for 1.25 h, another portion of MCPBA solution was added (0.1 equiv in 1 mL of CH_2Cl_2). After refluxing a further 1.5 h, a third portion of MCPBA solution (0.1 equiv in 1 mL of CH_2Cl_2) was added. Heating continued for 1.25 h after the last addition. After cooling, the solvent was removed under reduced pressure and the crude residue was flash chromatographed on silica gel using a gradient of CH_2Cl_2/MeOH (100:0–95:5) to give 1.2 g (76%) of the desired pyridine *N*-oxide as a yellow foam: mp

160–166 °C dec; IR (KBr) 2224, 1572, 1516, 1483, 1242, 1175 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.92 (s, 3H), 4.38 (s, 2H), 7.18 (d, 1H, J = 8.36 Hz), 7.32 (s,2H, J = 6.95 Hz), 7.71 (t, 1H, J = 7.95 Hz), 8.02 (d, 1H, J = 8.32 Hz), 8.15 (d, 1H, J = 7.21 Hz), 8.28 (d, 2H, J = 7.00 Hz), 8.53 (d, 1H, J = 8.68 Hz). Anal. ($C_{18}H_{14}N_4O_3$) C, H, N.

5-[Methyl[[2-(phenylthio)pyridin-4-yl]methyl]amino]-8-nitronaphthalene-1-carbonitrile. To a solution of 300 mg (0.9 mmol) of the pyridine *N*-oxide prepared above in 6 mL of CH_2Cl_2 at room temperature was added 0.115 mL (0.9 mmol) of benzenesulfonyl chloride. After the mixture had stirred at room temperature for 30 min, 0.190 mL (1.0 mmol) of (phenylthio)trimethylsilane was added. After stirring at room temperature for a further 2 h 20 min, the mixture was poured into 1 N NaOH (40 mL). The layers were separated, and the aqueous layer was extracted with CH_2Cl_2 (3 × 10 mL). The combined organics were dried over (Na_2SO_4), and the solvent was removed under reduced pressure. The crude residue was flash chromatographed on silica gel using a gradient of CH_2Cl_2/MeOH (100:0–95:5) to give 84 mg (22%) of the desired phenylthio ether as a yellow solid: mp 125–126 °C; IR (KBr) 2222, 1570, 1516, 1385, 1329 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.83 (s, 3H), a4.26 (s, 2H), 6.78 (s, 1H), 7.00 (t, 2H, J = 6.64 Hz), 7.37–7.40 (m, 3H), 7.54–7.64 (m, 3H), 7.97 (d, 1H, J = 8.31 Hz), 8.12 (d, 1H, J = 7.28 Hz), 8.32 (d, 1H, J = 8.68 Hz), 8.43 (d, 1H, J = 5.06 Hz). Anal. ($C_{24}H_{18}N_4O_2S$) C, H, N, S.

***N*⁶-Methyl-*N*⁶-[[2-(phenylthio)pyridin-4-yl]methyl]benz[*cd*]indole-2,6-diamine (6).** This compound was prepared from 87 mg (0.2 mmol) of the nitronaphthalene prepared above employing the general conditions used to prepare **1**. The crude product was flash chromatographed on silica gel using a gradient of CH_2Cl_2/MeOH/NH_4OH (100:0:0–90:9:1) to give 35 mg (44%) of the desired amidine **6** as a rust-colored solid: mp >75 °C dec; IR (KBr) 1676, 1641, 1589, 1462, 1385 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.79 (s, 3H), 4.27 (s, 2H), 6.78 (d, 1H, J = 7.58 Hz), 6.94 (s, 1H), 7.08 (t, 2H, J = 5.70 Hz), 7.35–7.39 (m, 3H), 7.54–7.58 (m, 2H), 7.64 (t, 1H, J = 7.67 Hz), 8.02 (d, 1H, J = 8.19 Hz), 8.25 (d, 1H, J = 7.17 Hz), 8.42 (d, 1H, J = 5.08 Hz); HRMS calcd for $C_{24}H_{20}N_4S$ 396.1409, found 396.1414. Anal. ($C_{22}H_{20}N_4S$·0.25CCl_4·1H_2O) C, H, N, S, Cl.

5-[Methyl(pyridazin-4-ylmethyl)amino]-8-nitronaphthalene-1-carbonitrile (17b). The general conditions used to prepare **17a** were employed using **16a** and the amine **22c**. The product (24%) was isolated by flash chromatography on silica gel eluting with CH_2Cl_2/MeOH (25:1) as a yellow foam: IR (KBr) 2224, 1572, 1520, 1346, 1235, 1121cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.94 (s, 3H), 4.44 (s, 2H), 7.21 (d, 1H, J = 8.3 Hz), 7.50 (d, with fine splitting, 1H, J = 5.2 Hz), 7.71 (t, 1H, J = 7.3 Hz), 8.02 (d, 1H, J = 8.3 Hz), 8.16 (d, 1H, J = 7.3 Hz), 8.56 (d, 1H, J = 7.4 Hz), 9.22 (d, with fine splitting, 1H, J = 5.3 Hz), 9.29 (s, 1H); HRMS calcd for $C_{17}H_{13}N_5O_2$ M^+ 319.1069, found 319.1072.

***N*⁶-Methyl-*N*⁶-(pyridazin-4-ylmethyl)benz[*cd*]indole-2,6-diamine (2).** The general conditions used to prepare **1** were employed using cyano nitro compound **17b**. The product (23%) was isolated by flash chromatography on silica gel eluting with CH_2Cl_2/NH_3-saturated MeOH (10:1) as a red-orange solid: mp 177–180 °C; IR (KBr) 3308 (br), 3067 (br), 1672, 1537, 1460, 1250 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.88 (s, 3H), 3.00–4.00 (brs, 2H), 4.42 (s, 2H), 6.89 (d, 1H, J = 7.5 Hz), 7.03 (d, 1H, J = 7.5 Hz), 7.55 (d, with fine splitting,1H, J = 5.2 Hz), 7.62 (t, 1H, J = 7.1 Hz), 7.81 (d, 1H, J = 7.0 Hz), 8.09 (d, 1H, J = 8.2 Hz), 9.17 (d, with fine splitting, 1H, J = 5.2 Hz), 9.30 (s, 1H); HRMS calcd for $C_{17}H_{15}N_5$ M^+ 289.1327, found 289.1318. Anal. ($C_{17}H_{15}N_5$) C, H, N.

5-[Methyl[[2-(trifluoromethyl)pyridin-4-yl]methyl]amino]-8-nitronaphthalene-1-carbonitrile (17d). The general conditions used to prepare **17a** were employed using chloronaphthalene **16a** and amine **22b**. The product (38%) was isolated by flash chromatography eluting with CH_2Cl_2 as a brittle yellow foam: mp 104–106 °C; IR (KBr) 2872, 2224, 1574, 1520, 1327, 1180, 1136, 1114 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.94 (s, 3H), 4.47 (s, 2H), 7.21 (d, 1H, J = 8.3 Hz), 7.55 (d, 1H, J = 5.0 Hz), 7.68 (d, 1H, J = 7.3 Hz), 7.71 (d, 1H, J = 7.3 Hz), 7.74 (s, 1H), 8.03 (d, 1H, J = 8.3 Hz), 8.15 (d, 1H, J = 7.2 Hz), 8.53 (d, 1H, J = 8.6 Hz), 8.77 (d, 1H, J = 5.0 Hz). Anal. ($C_{19}H_{13}F_3N_4O_2$) C, H, N.

***N*⁶-Methyl-*N*⁶-[[2-(trifluoromethyl)pyridin-4-yl]methyl]benz[*cd*]indole-2,6-diamine (4).** The general conditions used to prepare **1** were employed using cyanonitronaphthalene **17d**. The product (52%) was isolated by flash chromatography eluting with CH_2Cl_2/NH_3-saturated MeOH (12:1) as a red solid: mp 76–80 °C; IR (KBr) 3154 (br), 1644, 1613, 1530, 1464, 1427, 1327 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.86 (s, 3H), 4.44 (s, 2H), 5.4 (br s, 2H), 6.89 (d, 1H, J = 6.9 Hz), 7.03 (d, 1H, J = 7.5 Hz), 7.59 (m, 2H), 7.79 (s, 1H), 7.82 (d, 1H, J = 7.0 Hz), 8.05 (d, 1H, J = 8.1 Hz), 8.71 (d, 1H, J = 4.9 Hz); HRMS calcd for $C_{19}H_{16}F_3N_4$ M + H 357.1327, found 357.1323. Anal. ($C_{19}H_{15}F_3N_4$) C, H, N.

5-[Methyl[(2-methylpyridin-4-yl)methyl]amino]-8-nitronaphthalene-1-carbonitrile (17c). The general conditions used to prepare **17a** were employed using chloronaphthalene **16a** and amine **22a**. The product (24%) was isolated by flash chromatography on silica gel eluting with CH_2Cl_2/EtOAc (1:1) as a yellow-brown solid: mp 121–123 °C; IR (KBr) 2226, 1641, 1572, 1522, 1449, 1414, 1335 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.59 (s, 3H), 2.93 (s, 3H), 4.36 (s, 2H), 7.16 (m, 3H), 7.66 (t, 1H, J = 7.9 Hz), 8.03 (d, 1H, J = 8.4 Hz), 8.12 (d, 1H, J = 7.2 Hz), 8.51 (m, 2H); HRMS calcd for $C_{19}H_{17}N_4O_2$ M^+ 333.1351, found 333.1357.

***N*⁶-Methyl-*N*⁶-[(2-methylpyridin-4-yl)methyl]benz[*cd*]indole-2,6-diamine (3).** The general conditions used to prepare **1** were employed using cyanonitronaphthalene **17c**. The product (62%) was isolated by flash chromatography on silica gel eluting with CH_2Cl_2/NH_3-saturated MeOH (10:1) as a dark red solid: mp 147–150 °C dec; IR (KBr) 3140 (br), 1650, 1605, 1530, 1460, 1445 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.58 (s, 3H), 2.84 (s, 3H), 2.86 (br s, 2H), 4.36 (s, 2H), 6.87 (d, 1H, J = 7.5 Hz), 7.06 (d, 1H, J = 7.5 Hz), 7.21 (d, 1H, J = 5.1 Hz), 7.26 (s, 1H), 7.59 (t, 1H, J = 7.9 Hz), 7.81 (d, 1H, J = 7.0 Hz), 8.10 (d, 1H, J = 8.1 Hz), 8.49 (d, 1H, J = 5.1 Hz); HRMS calcd for $C_{19}H_{18}N_4$ M^+ 302.1531, found 302.1520. Anal. ($C_{19}H_{18}N_4$·0.5H_2O·0.10Et_2O) C, H, N.

4-Methyl-5-[methyl[(2-methylpyridin-4-yl)methyl]amino]-8-nitronaphthalene-1-carbonitrile (17h). The general conditions used to prepare **17a** were employed using chloronaphthalene **16b** and amine **22a**. The product (4%) was isolated by flash chromatography eluting with CH_2Cl_2/EtOAc (1:1) as a yellow solid: ^1H NMR ($CDCl_3$) δ 2.52 (s, 3H), 2.73 (s, 3H), 3.04 (s, 3H), 4.16 and 4.34 (AB system, 2H, J = 14.2 Hz), 6.78 (d, 1H, J = 5.1 Hz), 6.85 (s, 1H), 7.05 (d, 1H, J = 8.5 Hz), 7.45 (d, 1H, J = 7.7 Hz), 7.94 (d, 1H, J = 8.4 Hz), 7.96 (d, 1H, J = 7.5 Hz), 8.43 (d, 1H, J = 5.1 Hz); HRMS calcd for $C_{20}H_{18}N_4O_2$ M^+ 346.1430, found 346.1424. This material was used without further characterization in the next step.

***5*,*N*⁶-Dimethyl-*N*⁶-[(2-methylpyridin-4-yl)methyl]benz[*cd*]indole-2,6-diamine (11).** The general conditions used to prepare **1** were employed using cyanonitronaphthalene **17h**. The product (50%) was isolated by flash chromatography eluting with CH_2Cl_2/NH_3-saturated MeOH (10:1) as an orange-brown solid: mp 180 °C dec; IR (thin film) 3173, 1651, 1607, 1537, 1462, 1447, 1404 cm^{-1}; ^1H NMR (CD_3OD) δ 2.46 (s, 3H), 2.71 (s, 3H), 3.16 (s, 3H), 4.17 and 4.39 (AB system, 2H, J = 14.4 Hz), 7.03 (d, 1H, J = 7.5 Hz), 7.13 (d, 1H, J = 7.6 Hz), 7.21 (d, 1H, J = 4.8 Hz), 7.26 (s, 1H), 7.51 (d, 1H, J = 7.3 Hz), 7.99 (d, 1H, J = 7.3 Hz), 8.27 (d, 1H, J = 5.2 Hz); HRMS calcd for $C_{20}H_{21}N_4$ M + H 317.1766, found 317.1780.

4-Methyl-5-[methyl(pyridin-4-ylmethyl)amino]-8-nitronaphthalene-1-carbonitrile (17g). The general conditions used to prepare **17a** were employed using chloronaphthalene **16b** and methyl(pyridin-4-ylmethyl)amine. The product (8%) was isolated by flash chromatography on silica gel eluting with CH_2Cl_2/EtOAc (1:1) as a yellow solid: mp 157–158 °C; IR (KBr) 2928, 2222, 1597, 1568, 1512, 1329, 1310 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.73 (s, 3H), 3.05 (s, 3H), 4.20 and 4.39 (AB system, 2H, J = 14.2 Hz), 6.99 (d, 2H, J = 6.0 Hz), 7.05 (d, 1H, J = 8.4 Hz), 7.46 (d, 1H, J = 7.5 Hz), 7.94 (d, 1H, J = 8.3 Hz), 7.96 (d, 1H, J = 6.5 Hz), 8.56 (d, 1H, J = 6.0 Hz); HRMS calcd for $C_{19}H_{17}N_4O_2$ M + H 333.1352, found 33.1350.

***5*,*N*⁶-Dimethyl-*N*⁶-(pyridin-4-ylmethyl)benz[*cd*]indole-2,6-diamine (9).** The general conditions used to prepare **1**

were employed using cyanonitronaphthalene **17g**. The product (63%) was isolated by flash chromatography by eluting with $CHCl_3$/MeOH/HOAc (85:10:5). Appropriate fractions were concentrated, redissolved in EtOAc, washed with saturated $NaHCO_3$ solution, and dried (MgSO4), and the solvent was removed under reduced pressure to give the free base as a rust-colored solid: mp 144–146 °C; IR (KBr) 3202 (br), 1657, 1605, 1532, 1462, 1447, 1418 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.73 (s, 3H), 3.18 (s, 3H), 4.00 (brs, 2H), 4.15 and 4.44 (AB system, 2H, J = 14.2 Hz), 7.12 (AB system, 2H, J = 7.3 Hz), 7.30 (d, 2H, J = 5.2 Hz), 7.47 (d, 1H, J = 7.0 Hz), 7.75 (d, 1H, J = 6.9 Hz), 8.60 (d, 1H, J = 5.2 Hz); HRMS calcd for $C_{19}H_{19}N_4$ M + H 303.1610, found 303.1622.

***N*-Methyl-6-aminobenz[*cd*]indol-2(1*H*)-one (19a).** To a rapidly stirred solution of 690 mg (3.13 mmol) of 6-aminobenz-[*cd*]indol-2(1*H*)-one hydrochloride (**18a**)[5] and 1.25 mL (7.20 mmol) of DIEA in 5 mL of DMF at 70 °C was added 0.2 mL (3.44 mmol) of methyl iodide. After 2 h, the mixture was poured into H_2O/saturated $NaHCO_3$ (1:1), and the aqueous layer was extracted with EtOAc (3 × 100 mL). The combined organic layers were dried (anhydrous Na_2SO_4), and the solvent was removed under reduced pressure. The crude residue was chromatographed on flash silica gel (50 g) with MeOH/CH_2Cl_2 (2.98) to give 232 mg (37%) of the desired material as a red solid: mp 237–240 °C (EtOAc/MeOH, 2:1); IR (KBr) 3180, 1610, 1520, 1450, 1380, 1270, 770, 745 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.81 (brs, 3H), 6.19 (d, 1H, J = 7.7 Hz), 6.44 (m, 1H, -NH-), 6.78 (d, 1H, J = 7.7 Hz), 7.67 (t, 1H, J = 7.2 Hz), 7.93 (d, 1H, J = 7.0 Hz), 8.35 (d, 1H, J = 8.2 Hz), 10.38 (s, 1H, -NHC=O). Anal. ($C_{12}H_{10}N_2O$) C, H, N.

5,*N*⁶-Dimethyl-6-aminobenz[*cd*]indol-2(1*H*)-one (19b). A stirred solution of 1.50 g (7.57 mmol) of aniline **18b**[5] in 20 mL of DMF containing 0.71 mL (11.40 mmol) of methyl iodide and 2.00 mL (11.48 mmol) of DIEA was heated at 50 °C for 5 h. The reaction mixture was poured into saturated $NaHCO_3$ solution and extracted with EtOAc (3×). The combined organic layers were washed with saturated NaCl solution, dried ($MgSO_4$), and concentrated. The residue was slurried in hot EtOH, cooled, filtered, and dried to give 0.97 g (60%) of the desired methylated aniline as a dark red solid: mp 260–262 °C (turns dark at 242 °C); IR (KBr) 3460, 3173, 3032, 1661, 1634, 1466, 1325, 1279 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.79 (s, 3H), 2.94 (s, 3H), 5.45 (br, 1H, NH), 6.34 (d, 1H, J = 7.8Hz), 6.82 (d, 1H, J = 7.7 Hz), 7.42 (d, 1H, J = 7.3 Hz), 7.81 (d,1H, J = 7.2 Hz), 10.39 (s,1H). Anal. ($C_{13}H_{12}N_2O$·0.1H_2O) C, H, N.

5-Methyl-6-[methyl(pyridin-4-ylmethyl)amino]-1*H*-benz-[*cd*]indol-2-one (20b). The general conditions used to prepare **20a** were employed using aniline **19b** and 4-pyridinecarboxaldehyde. The product (14%) was isolated by flash chromatography on silica gel eluting with CH_2Cl_2/MeOH (16:1) as a yellow solid: mp 210–213 °C; IR (KBr) 3030 (br), 1703, 1634, 1607, 1466, 1425, 1074 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.62 (9 s,3H), 3.10 (s, 3H), 4.16 and 4.36 (AB system, 2H, J = 14.0 Hz), 6.84 (d, 1H, J = 7.6 Hz), 7.17 (d, 1H, J = 7.6 Hz), 7.32 (d, 2H, J = 5.8 Hz), 7.56 (d, 1H, J = 7.3 Hz), 7.86 (d, 1H, J = 7.2 Hz), 8.47 (d, 2H, J = 5.8 Hz), 10.59 (s, 1H). Anal. ($C_{19}H_{17}N_3O$·0.3H_2O) C, H, N.

5-Methyl-6-[methyl(pyridazin-4-ylmethyl)amino]-1*H*-benz[*cd*]indol-2-one (20c). The general conditions used to prepare **20a** were employed, using aniline **19b** and 4-pyridazinecarboxaldehyde. The product (10%) was isolated by flash chromatography on silica gel eluting with CH_2Cl_2/MeOH (20:1) as an orange-brown solid: mp 172 °C dec; IR (KBr) 3161, 3046 (br), 1695, 1634, 1460, 1369, 1103, 1079 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.73 (s, 3H), 3.15 (s, 3H), 4.18 and 4.42 (AB system, 2H, J = 14.74 Hz), 6.85 (d, 1H, J = 7.6 Hz), 7.05 (d, 1H, J = 7.6 Hz), 7.39 (d, d, 1H, J = 5.2, 2.1 Hz), 7.55 (d, 1H, J = 7.2 Hz), 7.71 (brs, 1H), 8.00 (d, 1H, J = 7.2 Hz), 9.12 (d, 1H, J = 5.2 Hz), 9.20 (s, 1H); HRMS calcd for $C_{18}H_{16}N_4O$ M^+ 304.1324, found 304.1338.

6-[Methyl(pyridazin-4-ylmethyl)amino]-1*H*-benz[*cd*]-indol-2-one (20d). The general conditions used to prepare **20a** were employed using aniline **19a** and 4-pyridazinecarboxaldehyde. The product (10%) was isolated by flash chromatography on silica gel eluting with CH_2Cl_2/MeOH (20:1) as a red-brown solid: mp 165–168 °C; IR (KBr) 3187 (br), 1690, 1638, 1474, 1379, 1078 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.89 (s, 3H), 4.41 (s, 2H), 6.85 and 6.92 (AB system, 2H, J = 7.6 Hz), 7.54 (m, 1H), 7.75 (t, 1H, J = 7.1 Hz), 7.90 (brs, 1H), 8.11 (d, 1H, J = 6.9 Hz), 8.22 (d, 1H, J = 8.2 Hz), 9.17 (d, with fine splitting, 1H, J = 5.2 Hz), 9.28 (s, with fine splitting,1H). Anal. ($C_{17}H_{14}N_4O$·0.25H_2O) C, H, N.

***N*-[5-Methyl-2-(methylthio)benzo[*cd*]indol-6-yl]-*N*-(pyridazin-4-ylmethyl)methylamine.** The general conditions used to prepare compound *N*-[2-(methylthio)benz[*cd*]indol-6-yl]-*N*-(pyridin-4-ylmethyl)methylamine were employed using benzindole **20c**. The product (34%) was isolated and used in the next step without purification: ^1H NMR ($CDCl_3$) δ 2.76 (s, 3H), 2.86 (s, 3H), 3.12 (s, 3H), 4.21 and 4.48 (AB system, 2H, J = 15.0 Hz), 7.07 (d, 1H, J = 7.6 Hz), 7.35 (d, with fine splitting, 1H, J = 5.3 Hz), 7.45 (d, 1H, J = 7.2 Hz), 7.54 (d, 1H, J = 7.1 Hz), 7.80 (d, 1H, J = 7.1 Hz), 9.11 (d, with five splitting, 1H, J = 5.3 Hz), 9.22 (s, 1H).

***N*-[5-Methyl-2-(methylamino)benz[*cd*]indol-6-yl]-*N*-(pyridazin-4-ylmethyl)methylamine (12).** The general conditions used to prepare compound **8** were employed using the methylthio prepared immediately above. The product (58%) was isolated as an orange-yellow solid by flash chromatography on silica gel eluting with CH_2Cl_2/NH_3-saturated MeOH (15:1): mp 204–205 °C; IR (KBr) 3268, 2928, 2837, 1578, 1449, 1310, 1136 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.71 (s, 3H), 3.09 (s, 3H), 3.30 (s, 3H), 4.15 and 4.42 (AB system, 2H, J = 14.7 Hz), 5.60 (br s, 1H), 7.04 (d, 1H, J = 7.5 Hz), 7.17 (d, 1H, J = 7.5 Hz), 7.37 (m, 2H), 7.63 (d, 1H, J = 7.2 Hz), 9.09 (d, 1H, J = 5.2 Hz), 9.22 (s, 1H). Anal. ($C_{19}H_{19}N_5$·0.50H_2O) C, H, N.

***N*-[2-Amino-5-methylbenz[*cd*]indol-6-yl]-*N*-(pyridazin-4-ylmethyl)methylamine (14).** The general conditions used to prepare compound **8** were employed using *N*-[5-methyl-2-(methylthio)benz[*cd*]indol-6-yl]-*N*-(pyridazin-4-ylmethyl)methylamine and anhydrous ammonia gas. The product (41%) was isolated as a solid by flash chromatography on silica gel eluting with CH_2Cl_2/NH_3-saturated MeOH (10:1): mp 167–169 °C; IR (KBr) 3326, 3162, 1649, 1532, 1447, 1325 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.70 (s, 3H), 3.08 (s, 3H), 4.00 (br s, 2H), 4.15 and 4.41 (AB system, 2H, J = 14.7 Hz), 7.02 (d, 1H, J = 7.6 Hz), 7.08 (d, 1H, J = 7.5 Hz), 7.39 (m, 2H), 7.79 (d, 1H, J = 7.2 Hz), 9.09 (d, 1H, J = 5.2 Hz), 9.20 (s, 1H). Anal. ($C_{18}H_{17}N_5$·0.50H_2O) C, H, N.

***N*-[2-(Methylthio)benz[*cd*]indol-6-yl]-*N*-(pyridazin-4-ylmethyl)methylamine.** The general conditions used to prepare compound *N*-[2-(methylthio)benz[*cd*]indol-6-yl]-*N*-(pyridin-4-ylmethyl)methylamine were employed using benzindole **20d**. The product (45%) was isolated as a dark foam by flash chromatography on silica gel eluting with CH_2Cl_2/MeOH (20:1): ^1H NMR ($CDCl_3$) δ 2.85 (s, 3H), 2.95 (s, 3H), 4.54 (s, 2H), 6.89 (d, 1H, J = 7.6 Hz), 7.51 (d, 1H, J = 7.6 Hz), 7.57 (m, 2H), 7.88 (d, 1H, J = 7.0 Hz), 8.03 (d, 1H, J = 8.1 Hz), 9.18 (d, with fine splitting, 1H, J = 5.2 Hz), 9.29 (s, 1H); HRMS calcd for $C_{18}H_{16}N_4S$ 320.1096, found 320.1100.

N²,N⁶-Dimethyl-*N*⁶-(pyridazin-4-ylmethyl)benz[*cd*]indole-2,6-diamine (13). The general conditions used to prepare compound **8** were employed using the methylthio compound prepared immediately above. The product (76%) was isolated as an orange brittle foam by flash chromatography on silica gel eluting with CH_2Cl_2/NH_3-saturated MeOH (11:1) and subsequent trituration with hexanes: mp 113–115 °C; IR (KBr) 3279, 2955, 1634, 1578, 1456, 1360 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.87 (s, 3H), 3.32 (s, 3H), 4.40 (s, 2H), 6.89 (d, 1H, J = 7.5 Hz), 7.13 (d, 1H, J = 7.4 Hz), 7.56 (m, 2H), 7.76 (d, 1H, J = 6.9 Hz), 8.05 (d, 1H, J = 8.1 Hz), 9.15 (d, 1H, J = 5.2 Hz), 9.29 (s, 1H). HRMS calcd for $C_{18}H_{17}N_5$ 303.1484, found 303.1482. Anal. ($C_{18}H_{17}N_5$·0.1hexanes·0.3H_2O) C, H, N.

***N*-[5-Methyl-2-(methylthio)benz[*cd*]indol-6-yl]-*N*-(pyridin-4-ylmethyl)methylamine.** The general conditions used to prepare *N*-[2-(methylthio)benz[*cd*]indol-6-yl]-*N*-(pyridin-4-ylmethyl)methylamine were employed using benzindole **20b**. The product (79%) was isolated as a dark purple foam by flash chromatography on silica gel eluting with a gradient of 50–100% EtOAc in CH_2Cl_2: ^1H NMR ($CDCl_3$) δ 2.75 (s, 3H), 2.87 (s, 3H), 3.12 (s, 3H), 4.18 and 4.40 (AB system, 2H, J = 15.0 Hz), 7.05 (d, 1H, J = 7.6 Hz), 7.22 (d, 2H, J = 5.9 Hz),

7.42 (d, 1H, J = 7.6 Hz), 7.55 (d, 1H J = 7.5 Hz), 8.55 (d, 2H, J = 5.9 Hz).

***N*-[5-Methyl-2-(methylamino)benz[*cd*]indol-6-yl]-*N*-(pyridin-4-ylmethyl)methylamine (10).** The general conditions used to prepare compound **8** were employed using the above methyl sulfide. The product (72%) was isolated as a solid by flash chromatography on silica gel eluting with CH_2Cl_2/NH_3-saturated MeOH (15:1): mp 225–227 °C; IR (KBr) 3221, 2932, 1647, 1601, 1576, 1443, 1418, 1312 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.61 (s, 3H), 3.05 (s, 6H), 4.11 and 4.37 (AB system, 2H, J = 14.7 Hz), 6.93 (d, 1H, J = 7.4 Hz), 7.07 (d, 1H, J = 7.5 Hz), 7.32 (d, 2H, J = 5.5 Hz), 7.42 (d, 1H, J = 7.1 Hz), 7.88 (d, 1H, J = 7.1 Hz), 8.46 (d, 2H, J = 5.6 Hz). Anal. ($C_{20}H_{20}N_4 \cdot 0.35H_2O$) C, H, N.

Biochemical Assays. TS activity was assayed by a modified procedure of the tritium release method of Lomax and Greenberg.[26] Inhibition constants were determined by steady-state analysis against the cofactor 5,10-methylenetetrahydrofolate as the variable substrate under conditions of saturating dUMP. Reaction conditions in 0.1 mL were 50 mM Tris, pH 7.6, 10 mM dithiothreitol, 1 mM ethylenediaminetetraacetic acid, 25 mM $MgCl_2$, 15 mM formaldehyde, 25 μM dUMP ([5-^3H], specific activity $\cong 2 \times 10^8$cpm/μmol), and tetrahydrofolate (eight concentrations ranging from 5 to 150 μM). Bovine serum albumin at up to 100 μg/mL was present when human TS was assayed. These reactions were either in the absence of inhibitor or in the presence of inhibitor at concentrations ranging, at a minimum, between 0.5 $\times$ K_i and 2.0 $\times$ K_i except when the solubility of the inhibitor was limiting. Reactions were run at room temperature by initiating with the addition of enzyme. After 5 min, the reactions were quenched by the addition of charcoal, the mixture centrifuged to remove unreacted dUMP, and the supernatant counted to determine the release of tritium from the 5-position of dUMP. Experimental results were analyzed by a nonlinear regression analysis program[27] which fits the data to a mixed noncompetitive inhibition scheme.

Measurement of Tissue Culture IC_{50}'s. IC_{50} values for the inhibition of cellular growth were assessed using a modification[28] of the MTT[29] colorimetric assay of Mosmann[30] using mouse (L1210) and human (CCRF-CEM) leukema lines (ATCC) and a human adenocarcinoma (GC_3/M TK^-) deficient in thymidine kinase.[31] Cells were seeded at 1000 (L1210) or 10 000 (CCRF-CEM, GC_3/M TK^-) cells/well in 96-well plates, and growth was assessed over a range of nine 2-fold serial dilutions of each compound. Culture medium (RPM1-1640) contained 5% (L1210, CCRF-CEM) or 10% (GC_3/M TK^-) fetal calf serum and 0.5% DMSO. Following a 3- (L1210) or 5-day (CCRF-CEM, GC_3/M TK^-) incubation and a 4-h treatment with MTT, cells were harvested and growth was measured spectrophotometrically after dissolution of the deposited formazan in DMSO. IC_{50} values were determined from semilogarithmic plots of compound concentration vs the mean of the four growth assesments made at each serial dilution of the agent relative to the growth of control cultures.

Measurement of IC_{50} Shift Due to Thymidine. The ability of thymidine to reverse growth inhibition was assessed by comparing the IC_{50} measured under standard conditions (RPM1-1640 medium containing 5% fetal calf serum) with that obtained in the presence of 10 μM thymidine which was replenished daily during the 3 days of growth. The magnitude of the ratio of the IC_{50} measured in the presence of thymidine to that measured without added nucleoside was used to reflect the extent to which the inhibition of growth could be attributed to intracellular inhibition of thymidylate synthase. A value of 1.0 under these conditions would reflect a probable locus of action other than TS, while larger values probably reflect a direct relationship between growth inhibition and TS targeting.

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