Novel cAMP PDE III Inhibitors: Imidazo[4,5-*b*]pyridin-2(3*H*)-ones and Thiazolo[4,5-*b*]pyridin-2(3*H*)-ones and Their Analogs

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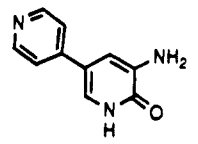
The transformation of milrinone to 1,3-dihydro-5-methyl-6-(4-pyridinyl)-2*H*-imidazo[4,5-*b*]pyridin-2-one (**13a**), 5-methyl-6-(4-pyridinyl)thiazolo[4,5-*b*]pyridin-2(3*H*)-one (**51**), and 7-methyl-6-(4-pyridinyl)-1,8-naphthyridin-2(1*H*)-one (**22**) resulted in very potent cAMP PDE III inhibitors with *in vitro* activity in the nanomolar range. 1,3-Dihydro-2*H*-imidazo[4,5-*b*]pyridin-2-ones **13** were prepared from 2-aminopyridine-3-carboxylic acids (**7**, **10**) via Curtius rearrangement. 1,8-Naphthyridin-2(1*H*)-one **22** and the corresponding 3,4-dihydro derivative **28** were prepared from 5-bromo-2-methyl[3,4′-bipyridin]-6-amine (**21**) and 5-bromo-2-methyl[3,4-bipyridin]-6(1*H*)-one (**24**), respectively, via Heck reaction. Thiazolo[4,5-*b*]pyridin-2(3*H*)-ones **35** were prepared from 6-bromo[3,4′-bipyridin]-6-amines **30** and **32** via a four-step sequence. Treatment of 6-amino-2-methyl[3,4′-bipyridine]-5-thiol (**59**) with ethyl bromoacetate and ethyl bromodifluoroacetate gave pyridothiazinones **60** and **61**, respectively.

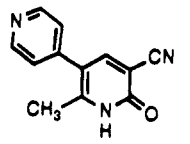
Introduction

A search for novel cardiotonic agents in our laboratory culminated in the successful development of two clinically useful agents, amrinone[1] (**1**) and milrinone[2] (**2**), for the treatment of heart failure. The positive inotropic and vasodilatory actions of amrinone and milrinone are directly related to the inhibition of adenosine 3′,5′-cyclic phosphate phosphodiesterase III (cAMP PDE III).[3] During the SAR studies on amrinone, it was found that 5-(4-pyridinyl)-benzoxazol-2(3*H*)-one (**3**)[4] was about 11 times more potent than amrinone *in vitro* against cAMP PDE III. This discovery showed that a free amino group was not necessary for *in vitro* cAMP PDE III activity and prompted the design and synthesis of analogs of **3**. The synthesis of compounds **3**, **44**, **47**, and **49–56** was reported earlier.[4-9] This paper describes the synthesis of additional analogs of **3** and the *in vitro* cAMP PDE III inhibitory activities of the new compounds and those reported previously.[4-9]



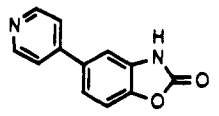
1

cAMP PDE III IC_{50} = 28 μM



2

cAMP PDE III IC_{50} = 0.36 μM



3

cAMP PDE III IC_{50} = 2.5 μM

Chemistry

The synthesis of imidazo[4,5-*b*]pyridinones **13** is depicted in Scheme 1. The superior pharmacological properties[10] exhibited by **13a** *in vivo* led to several modifications of this molecule. Treatment of chlorobipyridine **4** with ammonia and 4-methoxybenzylamine gave nitriles **5** and **6**, respectively, which were hydrolyzed with aqueous sodium hydroxide to the corresponding acids **7a** and **7b**. Chloropyridine carbonitrile **9** prepared from pyridinone **8**[11] was converted in one step to aminopyridinecarboxylic acid **10** by the action of aqueous ammonia in an autoclave at 170–175 °C. Treatment of aminopyridinecarboxylic acids **7a**, **7b**, and **10** with diphenyl phosphorazidate in the presence of triethylamine resulted in the formation of imidazo[4,5-*b*]pyridinones **13**. The Curtius[12] rearrangement of acyl azides **11** generated *in situ* produced isocyanates **12** which underwent intramolecular cyclization with *o*-amino group to form **13**. Oxidation of **13a** with *m*-chloroperbenzoic acid resulted in a mixture of monoxide **14** and dioxide **15** which were separated by repeated fractional crystallizations in low yields. Treatment of monoxide **14** with boiling acetic anhydride[13] furnished pyridinone derivative **16**. Wolff–Kishner[14] reduction of **13c** provided compound **18** with a methylene spacer between the pyridine and imidazopyridinone moieties. The chemoselective synthesis of **20**, the 1-methyl analog of **13a**, was achieved by using a 4-methoxybenzyl protecting group.[15]

Heck reaction[16] of 6-amino-5-bromobipyridine **21** with ethyl acrylate gave 1,8-naphthyridinone **22** along with a small quantity of uncyclized compound **23** (Scheme 2). Heck reaction was also employed in the preparation of **28** which is the 3,4-dihydro derivative of **22**. The ethyl acrylate derivative **25** was reduced catalytically to ethyl propionate derivative **26** which upon treatment with phosphorus oxychloride followed by ammonia gave amide **27** instead of the expected cyclized product **28**. Cyclization of **27** to **28** was accomplished by using NaH in DMF.

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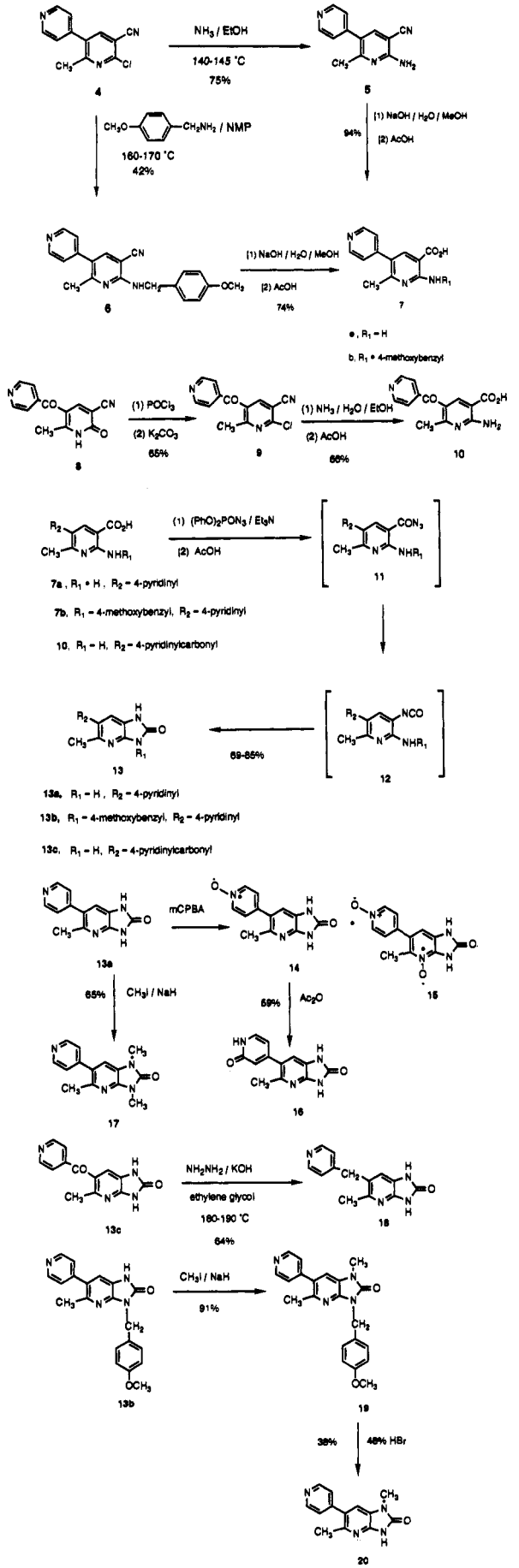
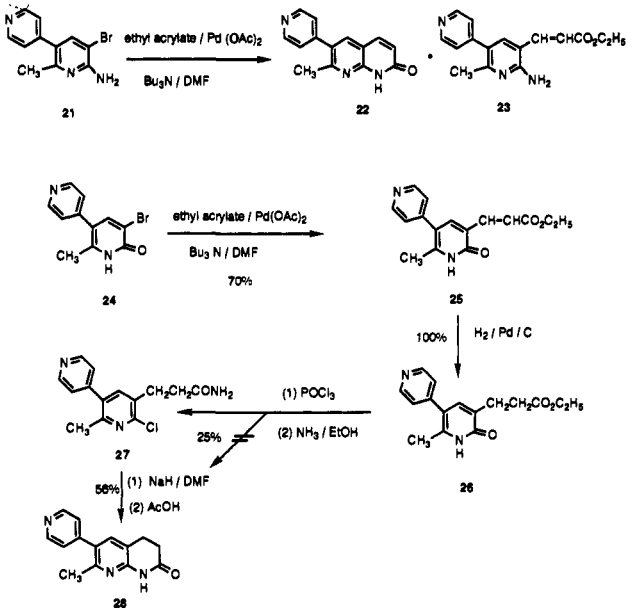
Scheme 1

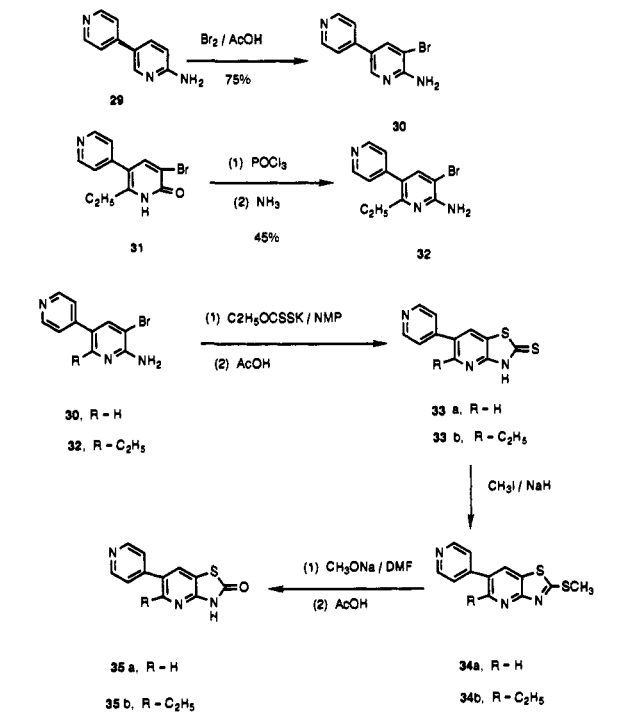


The synthesis of thiazolo[4,5-*b*]pyridinones **35** is illustrated in Scheme 3. These compounds were prepared in four steps by our recently published[5] procedure.

Scheme 2



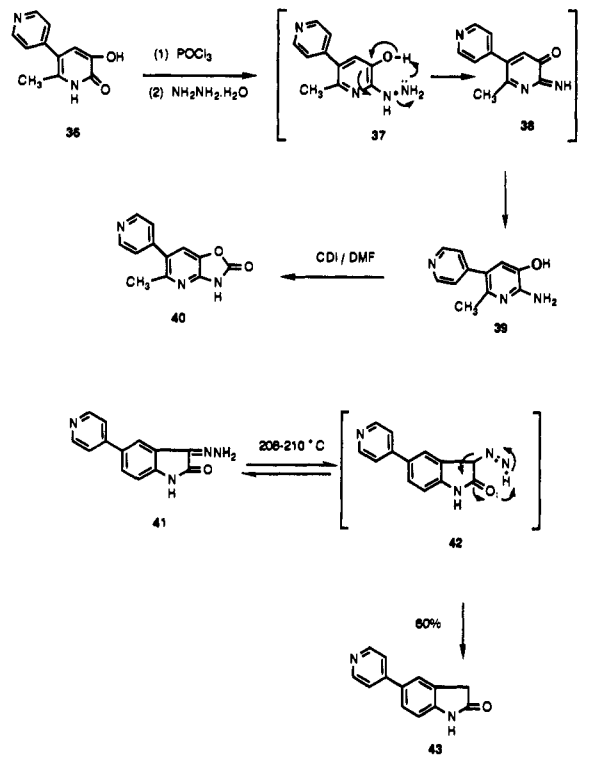
Scheme 3



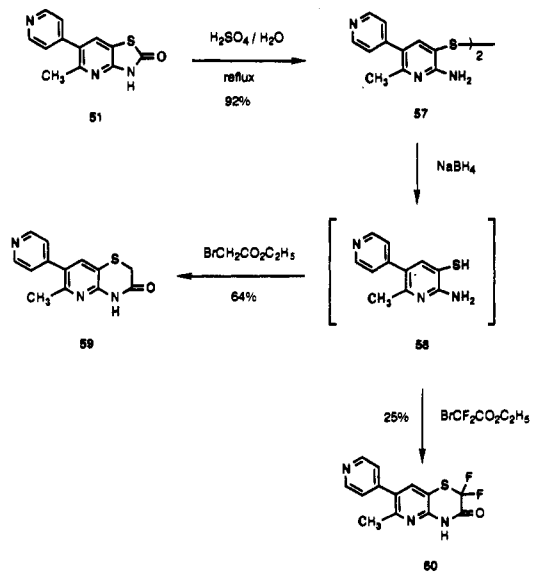
The synthesis of oxazolo[4,5-*b*]pyridinone **40** and indolone **43** is described in Scheme 4. Treatment of hydroxypyridinone **36**[17] with phosphorus oxychloride followed by hydrazine gave a complex mixture from which crude 6-amino-5-hydroxypyridine **39** was isolated in low yield [MS: 202 (MH^+)]. Due to its instability it was converted to **40** by treatment with 1,1′-carbonyldiimidazole without further characterization. The transformation of **37**, the hydrazine derivative formed *in situ*, may involve intermediate **38** which is reduced to **39**. Heteroaryl hydrazines have been reported to undergo similar reactions.[18] The thermal conversion of hydrazone **41**[7] to indolone **43** is probably facilitated via intermediate **42**.

Hydrolysis of thiazolone **51** with aqueous sulfuric acid gave disulfide **58** (Scheme 5). Reduction of **58** with sodium borohydride gave thiol **59** which was reacted *in situ* with ethyl bromoacetate and ethyl difluoroacetate to produce pyridothiazinones **60** and **61**, respectively.

Scheme 4



Scheme 5



Results and Discussion

It has been demonstrated by modeling studies[3,19] that cAMP PDE III inhibitors bear a striking resemblance to cAMP itself. A common feature of cAMP PDE III inhibitors is the presence of a cyclic amide function[3,19] (NHC=O) which has been proposed to occupy the same binding site (primary site) on the enzyme occupied by the phosphate moiety of cAMP. Furthermore, overall planar topography[3,19] is very important for selective and potent cAMP PDE III inhibition. Additionally, introduction of a small hydrophobic moiety[3,19] the size of a methyl group has been shown to dramatically increase the *in vitro* potency, and this group has been proposed to interact at a secondary binding site occupied by a portion of the sugar moiety of cAMP. The *in vitro* cAMP PDE III inhibitory activity is shown in Table 1, and the SAR is discussed in light of the above postulates.

The reverse analog of **3** (**44**) was about 4 times less active than **3**. The isatin **45** was similar in activity. However, isatin **46** was only 3 times less active than **3**. This marginal increase in activity (**46** vs **45**) was evidently caused by the methyl group in position 6. The replacement of C=O (**45**) by CH_2 (**43**) improved the potency. Benzimidazole **47** was similar in activity to **3** whereas imidazopyridinone **48** derived from amrinone (**1**) was 4 times as active as **3**. The presence of a basic nitrogen in **48** (proton acceptor or hydrogen bonding) at position 4 adjacent to the lactam moiety may have led to an increase in binding strength at the primary site. Benzothiazolone **49** showed equal activity. However, its reverse analog (**50**) suffered a 4-fold loss of activity. Thiazolopyridone **35a** derived from amrinone was similar in potency to **49**. Thiazolopyridinone **51** derived from milrinone (**2**) was 20-fold more potent than **35a**. This clearly demonstrated the importance of the methyl group which occupies the secondary site for cAMP PDE III inhibitory activity. Incorporation of a methylene group next to sulfur (**60**) led to an 8-fold drop in potency. This may be due to puckering of the larger thiazinone ring. Replacement of methylene by difluoromethylene (**61**) gave an inactive compound which may be due to the hydration of the carbonyl group. Replacement of the methyl group by an ethyl group (**52**) resulted in diminished activity. Similar results were reported[19] for milrinone and its ethyl analog. The reverse analog **53** was essentially inactive in comparison to **51**. The substitution of CH_2 for sulfur in **51** (**54**) resulted in a 6-fold loss of potency whereas **55** was inactive. Replacement of sulfur by ethylene group (**22**) led to 4-fold decrease in enzyme activity whereas the 3,4-dihydro derivative of **22** (**28**) was about 14-fold less active than **51**. The much diminished activity of **28** was probably due to loss of planar topography. Substitution of oxygen for sulfur in **51** resulted in **40**, which was essentially inactive. However, replacement of sulfur by NH group (**13a**) gave an equipotent compound. Replacement of CH_3 by CF_3 of **13a** had no effect on potency, but the homolog **57** was 50 times less active than **13a**. Monomethylation at N(1) (**20**) resulted in a severe loss of activity, and dimethylation (**17**) caused further loss of activity. Incorporation of CH_2 and CO spacers between the 4-pyridinyl group and imidazopyridinone moiety gave compounds **18** and **13c** which are 80- and 305-fold less potent than **13a**, respectively. The 1′-oxide derivative **14** was 9 times less active than **13a**, whereas the pyridinone derivative **16** was equipotent.

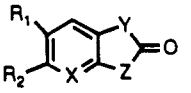
Conclusion

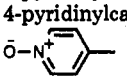
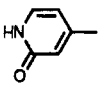
Transformation of milrinone (**2**) to imidazo[4,5-*b*]pyridin-2(3*H*)-one **13a**, thiazolo[4,5-*b*]pyridin-2(3*H*)-one **51**, and 1,8-naphthyridin-2(1*H*)-one **22** resulted in some of the most potent inhibitors of cAMP PDE III with *in vitro* activity in the nanomolar range. The marked difference in the activity of these compounds over that of milrinone is the presence of basic nitrogen N(4) adjacent to the lactam moiety which binds to the primary site on the enzyme. N(4) can strengthen this binding via H-bonding or ionic interaction (by acting as a proton acceptor) which results in increased *in vitro* cAMP PDE III potency.

Experimental Section

Melting points were determined in open capillaries in an oil bath and are uncorrected. The ^1H NMR spectra were obtained on a General Electric QE-300 spectrometer using tetramethylsilane as an internal standard, and chemical shifts are reported

Table 1. In Vitro cAMP PDE III Activity



compd	R1	R2	X	Y	Z	cAMP PDE III IC50 (μM)[a]
3[4]	4-pyridinyl	H	CH	NH	O	2.5 (2.4–2.7)
44[4]	4-pyridinyl	H	CH	O	NH	9.8 (9.1–10.7)
45[7]	4-pyridinyl	H	CH	C=O	NH	16 (15.2–17.8)
46[7]	4-pyridinyl	CH_3	CH	C=O	NH	7.3 (6.4–8.5)
43	4-pyridinyl	H	CH	CH_2	NH	5.3 (4.9–5.7)
47[4]	4-pyridinyl	H	CH	NH	NH	1.3 (0.97–1.6)
48[8]	4-pyridinyl	H	N	NH	NH	0.63 (0.45–0.90)
49[5]	4-pyridinyl	H	CH	S	NH	0.54 (0.46–0.64)
50[5]	4-pyridinyl	H	CH	NH	S	2.0 (1.8–2.3)
35a	4-pyridinyl	H	N	S	NH	0.35 (0.29–0.43)
51[5]	4-pyridinyl	CH_3	N	S	NH	0.024 (0.020–0.030)
60	4-pyridinyl	CH_3	N	SCH_2	NH	0.14 (0.12–0.16)
61	4-pyridinyl	CH_3	N	SCF_2	NH	>3000
52[5]	4-pyridinyl	C_2H_5	N	S	NH	0.078 (0.057–0.11)
53[5]	4-pyridinyl	CH_3	N	NH	S	8.6 (7.2–10.3)
54[6]	4-pyridinyl	CH_3	N	CH_2	NH	0.10 (0.087–0.11)
55[6]	4-pyridinyl	CH_3	N	$CHCH_3$	NH	0.56 (0.51–0.60)
22	4-pyridinyl	CH_3	N	CH=CH	NH	0.069 (0.060–0.080)
28	4-pyridinyl	CH_3	N	CH_2CH_2	NH	0.23 (0.19–0.28)
40	4-pyridinyl	CH_3	N	O	NH	4.2 (3.7–4.7)
13a	4-pyridinyl	CH_3	N	NH	NH	0.020 (0.017–0.024)
56[9]	4-pyridinyl	CF_3	N	NH	NH	0.018 (0.014–0.022)
57[9]	4-pyridinyl	C_2F_5	N	NH	NH	0.85 (0.69–1.0)
20	4-pyridinyl	CH_3	N	NCH_3	NH	1.4 (1.2–1.7)
17	4-pyridinyl	CH_3	N	NCH_3	NCH_3	28 (25–32)
18	4-pyridinylmethyl	CH_3	N	NH	NH	1.6 (1.4–1.9)
13c	4-pyridinylcarbonyl	CH_3	N	NH	NH	6.1 (5.6–6.5)
14		CH_3	N	NH	NH	0.18 (0.13–0.23)
16		CH_3	N	NH	NH	0.023 (0.018–0.028)

[a] Mean of three determinations (the 95% confidence interval is given in parentheses).

in parts per million and given in δ units. All the compounds gave ^{1}H NMR spectra consistent with the structures. Elemental analyses were performed by Galbraith Laboratories, Inc., Knoxville, TN. Each compound gave elemental analysis with ±0.45% of the theoretical values for C, H, and N.

6-Amino-2-methyl[3,4′-bipyridine]-5-carbonitrile (5). A mixture of 6-chloro-2-methyl[3,4′-bipyridine]-5-carbonitrile (4)[20] (50 g, 0.22 mol), concentrated aqueous ammonia (200 mL), and EtOH (190 mL) was heated in an autoclave at 140–145 °C for 24 h. After cooling to room temperature, the contents were transferred to a 1-L round-bottom flask and concentrated on a rotary evaporator. The resulting brown solid residue was treated with 10% aqueous K_2CO_3 (200 mL). The insoluble product was collected, washed with water, air-dried, and recrystallized from DMF to afford 34.4 g (75%) of tan crystals: mp 290–292 °C; MS *m/e* 211 (MH^+); ^{1}H NMR (CF_3COOD) δ 9.10, 8.31 (A_2B_2, J = 6.7 Hz, 4H, C_5H_4N), 8.75 (s, 1H, 4-H), 2.81 (s, 3H, CH_3). Anal. ($C_{12}H_{10}N_4$) C, H, N.

6-[[(4-Methoxyphenyl)methyl]amino]-2-methyl[3,4′-bipyridine]-3-carbonitrile (6). A stirred mixture of **4**[20] (115 g, 0.5 mol), 4-methoxybenzylamine (82.4 g, 0.6 mol), milled anhydrous K_2CO_3 (70 g, 0.5 mol), and NMP (200 mL) was heated at 160–170 °C in an oil bath for 7 h and then cooled to room temperature. To the brown thick oily mixture was added H_2O (500 mL) to dissolve inorganic salts. The mixture was manually stirred, whereupon the product solidified. It was collected, washed with water, dried, and recrystallized from 2-PrOH after decolorizing with charcoal to give 70.1 g (42%) of shiny brown plates: mp 126–128 °C; MS *m/e* 331 (MH^+); ^{1}H NMR (CF_3COOD) δ 9.09, 8.29 (A_2B_2, J = 6.5 Hz, 4H, C_5H_4N), 8.56 (s, 1H, 4-H), 7.48, 7.19 (A_2B_2, J = 8.6 Hz, 4H, C_6H_4), 4.99 (s, 2H, CH_2), 4.07 (s, 3H, OCH_3), 2.82 (s, 3H, CH_3). Anal. ($C_{20}H_{18}N_4O$) C, H, N.

6-Amino-2-methyl[3,4′-bipyridine]-5-carboxylic Acid (7a). A mixture of nitrile **5** (34.9 g, 0.17 mol), 35% aqueous NaOH (84 mL, 0.7 mol), H_2O (200 mL), and MeOH (600 mL) was heated under reflux for 23 h. The resulting light brown solution was treated with charcoal and filtered. The filtrate was concentrated on a rotary evaporator, and the yellow residue was dissolved in water (100 mL) and acidified with AcOH. The resulting yellow precipitate was collected, washed with water, and recrystallized from MeOH to yield 28.4 g (74%) of a yellow powder: mp 278–279 °C; MS *m/e* 230 (MH^+). Anal. ($C_{12}H_{11}N_3O_2$) C, H, N.

6-[[(4-Methoxyphenyl)methyl]amino]-2-methyl[3,4′-bipyridine]-3-carboxylic Acid (7b). A stirred mixture of **6** (47 g, 0.14 mol), 35% aqueous NaOH (50 mL), H_2O (50 mL), and EtOH (300 mL) was heated under reflux for 24 h, and then ethanol was removed on a rotary evaporator. The residual brown mixture was dissolved in boiling H_2O (400 mL), treated with charcoal, and filtered. The filtrate was acidified with acetic acid, and the resulting beige precipitate was collected and washed successively with water and EtOH to afford 46.8 g (94%) of **7b**: mp 198–200 °C dec; MS *m/e* 350 (MH^+). Anal. ($C_{20}H_{19}N_3O_3$) C, H, N.

2-Chloro-6-methyl-5-(4-pyridinylcarbonyl)pyridine-3-carbonitrile (9). A stirred mixture of pyridinone **8**[11] (25.3 g, 0.11 mol) and $POCl_3$ (250 mL) was heated under reflux for 5 h to give a dark brown solution. After cooling to room temperature, most of the unreacted $POCl_3$ was removed under reduced pressure, and the residue was slurried in $CHCl_3$ (200 mL) and poured on ice. The resulting mixture was neutralized by treating with K_2CO_3, and the product was extracted with $CHCl_3$ (200 mL). The $CHCl_3$ extract was dried ($MgSO_4$) and concentrated on a rotary evaporator, and the brown solid residue was recrystallized from 2-PrOH·Et_2O to provide 17.8 g (65%) of light pink crystals of **8**: mp 126–128 °C; MS *m/e* 258 (MH^+); ^{1}H NMR ($CDCl_3$) δ 8.91, 7.57 (A_2B_2, J = 5.6 Hz, 4H, C_5H_4N), 7.96 (s, 1H, 4-H), 2.67 (s, 3H, CH_3). Anal. ($C_{13}H_8ClN_3O$) C, H, N.

2-Amino-6-methyl-5-(4-pyridinylcarbonyl)pyridine-3-carboxylic Acid (10). A mixture of nitrile **9** (20.5 g, 0.08 mol), conconcentrated aqueous ammonia (150 mL), and EtOH (100 mL) was heated in an autoclave at 170–175 °C for 40 h and then cooled to room temperature. The greenish mixture was dissolved by adding water (300 mL), heated on a steam bath for 20 min, treated with charcoal, and then filtered. The filtrate was concentrated on a rotary evaporator, and the residue was dissolved

in 10% aqueous K_2CO_3 (100 mL) and filtered through a Celite pad to remove colloidal impurities. The filtrate was acidified with acetic acid, and the resulting yellow precipitate was collected, washed with water, and dried to yield 13.6 g (66%) of **10**: mp 255–257 °C dec. Anal. ($C_{13}H_{11}N_3O_3 \cdot {}^1/_4H_2O$) C, H, N.

1,3-Dihydro-5-methyl-6-(4-pyridinyl)-2*H*-imidazo[4,5-*b*]pyridin-2-one (13a). To a stirred mixture of acid **7a** (16 g, 69.8 mmol), Et_3N (18.7 mL), and 1,4-dioxane (375 mL) was added diphenyl phosphorazidate (17.1 mL, 88.4 mmol). The resulting mixture was heated under reflux for 3.5 h and then concentrated under reduced pressure. The brown residue was slurried in water (75 mL) and treated with AcOH (12 mL). The yellow precipitate was collected, washed with ether, and recrystallized from DMF to give 13.5 g (85%) of a tan solid: mp >300 °C; MS *m/e* 227 (MH^+); ^1H NMR (CF_3COOD) δ 9.18, 8.29 (A_2B_2, J = 5.8 Hz, 4H, C_5H_4N), 8.25 (s, 1H, 7-H), 2.86 (s, 3H, CH_3). Anal. ($C_{12}H_{10}N_4O$) C, H, N.

1,3-Dihydro-3-[(4-methoxyphenyl)methyl]-5-methyl-6-(4-pyridinyl)-2*H*-imidazo[4,5-*b*]pyridin-2-one (13b). To a stirred slurry of **7b** (17.5 g, 50 mmol), Et_3N (21 mL, 150 mmol), and 1,4-dioxane (300 mL) was added diphenyl phosphorazidate (10.6 mL, 50 mmol). The resulting mixture was stirred at ambient temperature for 1 h and then heated on a steam bath for 6 h. Next, the solvent was removed under reduced pressure, and the residue was slurried in Et_2O (100 mL). The brown solid product was collected and recrystallized from 2-PrOH to yield 11.9 g (69%) of a light yellow solid: mp 239–241 °C; MS *m/e* 347 (MH^+); ^1H NMR (CF_3COOD) δ 9.12, 8.25 (A_2B_2, J = 6.5 Hz, 4H, C_5H_4N), 7.43 (s, 1H, 7-H), 7.43, 7.12 (A_2B_2, J = 8.5 Hz, 4H, $-C_6H_4-$), 5.34 (s, 2H, $-CH_2-$), 4.03 (s, 3H, OCH_3), 2.84 (s, 3H, CH_3). Anal. ($C_{20}H_{18}N_4O_2$) C, H, N.

1,3-Dihydro-5-methyl-6-[(4-pyridinyl)carbonyl]-2*H*-imidazo[4,5-*b*]pyridin-2-one (13c). Via the procedure for the preparation of **13a**, **13c** was prepared in 84% yield as fine yellow crystals: mp >300 °C; MS *m/e* 255 (MH^+); ^1H NMR (CF_3COOD) δ 9.21, 8.52 (A_2B_2, J = 6.5 Hz, 4H, C_5H_4N), 8.35 (s, 1H, 7-H), 3.06 (s, 3H, CH_3). Anal. ($C_{13}H_{10}N_4O_2$): C, H, N.

1,3-Dihydro-5-methyl-6-(4-pyridinyl)-2*H*-imidazo[4,5-*b*]pyridin-2-one 4,1′-Dioxide (15) and 1,3-Dihydro-5-methyl-6-(4-pyridinyl)-2*H*-imidazo[4,5-*b*]pyridin-2-one 1′-Oxide (14). A mixture of **13a** (10 g, 44 mmol), 85% *m*-chloroperbenzoic acid (12.5 g, 72 mmol), $CHCl_3$ (900 mL), and MeOH (60 mL) was stirred at room temperature for 4 days. The resulting yellow solid was collected (10 g) and was found to be a mixture of two components by thin-layer chromatography (silica gel, AcOH/MeOH/EtOAc, 5:15:80). Repeated crystallizations (three times) from DMF yielded 2.9 g (25%) of the more polar compound **15** as a cream-colored powder: mp >300 °C; MS 259 (MH^+). Anal. ($C_{12}H_{10}N_4O_3$) C, H, N.

The mother liquors from above were combined and concentrated under reduced pressure and the resulting solid was recrystallized from water to afford 4.8 g (45%) of the less polar compund **14** as a white powder: mp >300 °C; MS *m/e* 243 (MH^+). Anal. ($C_{12}H_{10}N_4O_2$) C, H, N.

1,3-Dihydro-5-methyl-6-(1′,2′-dihydro-2′-oxo-4′-pyridinyl)-2*H*-imidazo[4,5-*b*]pyridin-2-one (16). A mixture of **14** (340 mg, 1.2 mmol) and Ac_2O (10 mL) was heated under reflux for 48 h and then concentrated to dryness on a rotary evaporator. The resulting brown solid was recrystallized from water to afford 0.2 g (59%) of a tan powder: MS *m/e* 243 (MH^+); ^1H NMR (CF_3COOD) δ 8.25–7.33 (m, 4H, aromatic), 2.80 (s, 3H, CH_3). Anal. ($C_{12}H_{10}N_4O_2$) C, H, N.

1,3-Dihydro-1,3,5-trimethyl-6-(4-pyridinyl)-2*H*-imidazo-[4,5-*b*]pyridin-2-one (17). A mixture of **13a** (3.8 g, 17 mmol), 60% NaH/oil dispersion (1.5 g, 36 mmol), and DMF (50 mL) was stirred at ambient temperature until all the NaH had reacted (15 min) and then was treated with methyl iodide (2.4 mL, 38 mmol) over a 15-min period. The resulting mixture was further stirred for 30 min and then concentrated on a rotary evaporator. The resulting brown semisolid was first washed with hexanes and then slurried in water (25 mL). The resulting light brown solid was collected and recrystallized from 2-PrOH to yield 2.8 g (65%) of white prisms: mp 224–226 °C; MS *m/e* 255 (MH^+); ^1H NMR (CF_3COOD) δ 9.08, 8.30 (A_2B_2, J = 6.5 Hz, 4H, C_5H_4N), 8.06 (s, 1H, 7-H), 3.85 (s, 1H, NCH_3), 3.70 (s, 3H, NCH_3), 2.83 (s, 3H, CH_3). Anal. ($C_{14}H_{14}N_4O$) C, H, N.

1,3-Dihydro-5-methyl-6-[(4-pyridinyl)methyl]-2*H*-imidazo[4,5-*b*]pyridin-2-one (18). A stirred mixture of **13c** (2 g, 7.8 mmol), KOH (1.1 g, 19 mmol), $NH_2NH_2 \cdot H_2O$ (5 mL, 100 mmol), and ethylene glycol (6 mL) was heated in an oil bath at 180–190 °C for 3 h, cooled to room temperature, and treated with AcOH (6 mL). The resulting mixture was diluted with H_2O (15 mL), and the white product was collected, washed with water, dried, and recrystallized from EtOH to yield 1.2 g (64%) of white flakes: mp 254–256 °C; MS *m/e* 241 (MH^+); ^1H NMR (CF_3COOD) δ 8.85, 8.02 (A_2B_2, J = 6.5 Hz, 4H, C_5H_4N), 8.20 (s, 1H, 7-H), 4.70 (s, 2H, CH_2); 2.81 (s, 3H, CH_3). Anal. ($C_{13}H_{12}N_4O$) C, H, N.

1,3-Dihydro-3-[(4-methoxyphenyl)methyl]-1-methyl-6-(4-pyridinyl)-2*H*-imidazo[4,5-*b*]pyridin-2-one (19). A mixture of **13b** (11.2 g, 32 mmol), 60% NaH/oil dispersion (1.4 g, 34 mmol), and DMF (100 mL) was stirred until most of the NaH had reacted (15 min). To the resulting solution was added a solution of methyl iodide (2 mL, 32 mmol) in DMF (10 mL) over a 20-min period. The resulting mixture was further stirred for 1 h and then concentrated under reduced pressure. The solid residue was slurried in hexanes (100 mL) and collected. After washing with water and drying, the product was recrystallized from 2-PrOH to afford 10.4 g (91%) of a bright yellow solid: mp 215–217 °C; MS *m/e* 361 (MH^+). Anal. ($C_{21}H_{20}N_4O_2$) C, H, N.

1,3-Dihydro-1,5-dimethyl-6-(4-pyridinyl)-2*H*-imidazo[4,5-*b*]pyridin-2-one (20). A solution of **19** (2 g, 5.5 mmol) and 48% aqueous hydrobromic acid (20 mL) was heated under reflux for 2 h and then concentrated under reduced pressure. The residue was first quenched with 10% aqueous K_2CO_3 and then acidified with acetic acid. The brown gummy solid was collected and recrystallized from 2-PrOH after treating with charcoal to furnish 0.5 g (38%) of shiny light yellow crystals: mp 271–273 °C; MS *m/e* 240 (MH^+); ^1H NMR (CF_3COOD) δ 9.15, 8.34 (A_2B_2, J = 5.2 Hz, 4H, C_5H_4N), 8.15 (s, 1H, 7-H), 3.73 (s, 3H, NCH_3), 2.88 (s, 3H, CH_3). Anal. ($C_{13}H_{11}N_4O$) C, H, N.

7-Methyl-6-(4-pyridinyl)-1,8-naphthyridin-2(1*H*)-one (22) and Ethyl 3-[6-Amino-2-methyl[3,4′-bipyridin]-5-yl]-2-propenoate (23). A stirred mixture of 5-bromo-2-methyl[3,4′-bipyridin]-6-amine[5] (**21**) (1.5 g, 5.7 mmol), ethyl acrylate (0.74 mL, 6.8 mmol), Bu_3N (1.6 mL, 6.8 mmol), DMF (5 mL), and palladium acetate (30 mg) was heated at 120–130 °C in an oil bath for 32 h under nitrogen, cooled to room temperature, and treated with 10% aqueous K_2CO_3 (50 mL). The resulting mixture was concentrated on a rotary evaporator to give a black residue which was dissolved in boiling $CHCl_3$ (200 mL), treated with charcoal, and filtered. The filtrate was concentrated on a rotary evaporator, and the resulting residue was separated by column chromatography (SiO_2 100 g, Et_2O–5% MeOH in Et_2O). The more polar component was recrystallized from 2-PrOH to afford 0.57 g (42%) of **22** as a bright yellow powder: mp 288–290 °C dec; MS *m/e* 238 (MH^+); ^1H NMR (CF_3COOD) δ 9.18, 8.43 (A_2B_2, J = 6.5 Hz, 4H, C_5H_4N), 8.86 (s, 1H, 5-H), 8.32 (d, $J_{3,4}$ = 9.7 Hz, 1H, 4-H), 7.29 (d, $J_{3,4}$ = 9.7 Hz, 1H, 3-H), 3.04 (s, 3H, CH_3). Anal. ($C_{14}H_{11}N_3O$) C, H, N.

The less polar fraction was recrystallized from 2-PrOH/hexanes to yield 48 mg (3%) of **23** as a cream-colored solid: mp 204–205 °C; MS *m/e* 284 (MH^+). Anal. ($C_{16}H_{17}N_3O_2$) C, H, N.

Ethyl 3-[1,6-Dihydro-2-methyl-6-oxo[3,4′-bipyridin]-5-yl]-2-propenoate (25). A stirred mixture of 5-bromo-2-methyl[3,4′-bipyridin]-6(1*H*)-one (**24**)[21] (100 g, 0.38 mol), ethyl acrylate (37.7 g, 0.38 mol), Bu_3N (180 mL), DMF (175 mL), and palladium acetate (4.4 g, 19.5 mmol) was heated under nitrogen at 130–140 °C for 6 h and then concentrated under reduced pressure to give a greenish solid residue which was treated with H_2O (200 mL). The product was collected, washed with water, dried, and dissolved in boiling $CHCl_3$ (1.2 L). The solution was treated with charcoal and filtered. The filtrate was concentrated under reduced pressure to give a greenish yellow solid which was digested with 2-PrOH (200 mL) and filtered off to yield 70 g (70%) of a pale yellow powder: mp 244–246 °C; MS *m/e* 285 (MH^+); ^1H NMR (CF_3COOD) δ 9.0, 8.26 (A_2B_2, J = 5.8 Hz, 4H, C_6H_4N), 8.20 (s, 1H, 4′-H), 7.96 (d, J = 15.0 Hz, 1H, 3-H), 7.10 (d, J = 15.0 Hz, 2-H), 4.45 (q, J = 7.1 Hz, OCH_2CH_3), 2.67 (s, 3H, CH_3), 1.48 (t, J = 7.1 Hz, OCH_2CH_3). Anal. ($C_{16}H_{16}N_2O_3$) C, H, N.

Ethyl 3-[6-Chloro-2-methyl[3,4′-bipyridin]-5-yl]propionamide (27). A mixture of **25** (24.6 g, 86 mmol), DMF (200 mL), and 10% Pd/C (1.1 g) was reduced on a Parr hydrogenator until

the required amount of H_2 was absorbed. The catalyst was filtered off on a Celite pad, and the filtrate was concentrated under reduced pressure to give 25 g (100%) of a cream-colored solid, mp 164–166 °C. This solid was added to $POCl_3$ (218 mL), and the resulting mixture was heated under reflux for 5 h to give a light brown solution. After cooling to room temperature, most of the $POCl_3$ was removed under reduced pressure. The dark brown residue was dissolved in $CHCl_3$ (500 mL) and poured into a vigorously stirred mixture of ice, water, and K_2CO_3 (300 g). The temperature of the reaction mixture was kept below 10 °C by adding more ice, and more K_2CO_3 was added to keep the reaction mixture basic. After the reaction was complete, the organic phase was separated and the aqueous phase was extracted with $CHCl_3$ (500 mL). The combined $CHCl_3$ extracts were dried ($MgSO_4$) and concentrated under reduced pressure to give 26.4 g of a brown oil. This oil was added to a solution of liquid ammonia (75 mL) and EtOH (300 mL) cooled in dry ice/2-PrOH bath. The resulting mixture was heated in an autoclave at 135–140 °C for 20 h, cooled to room temperature, and concentrated under reduced pressure to give a brown solid which was slurried in 10% aqueous $NaHCO_3$ (100 mL). The brown solid product was filtered off, washed with water, dried, and recrystallized from 2-PrOH to furnish 6.1 g (25%) of tan prisms of **27**: mp 179–180 °C; MS *m/e* 275 (MH^+). Anal. ($C_{14}H_{13}ClN_3O$) C, H, N.

3,4-Dihydro-7-methyl-6-(4-pyridinyl)-1,8-naphthyridin-2(1*H*)-one (28). A mixture of **27** (2.1 g, 7.2 mmol), DMF (20 mL), and 60% NaH/oil dispersion (0.29 g, 7.2 mmol) was stirred and heated under reflux for 28 h, cooled to room temperature, and treated with acetic AcOH (2.5 mL). The resulting mixture was concentrated to dryness under reduced pressure to give a brown residue which was slurried in water (50 mL). The product was collected, washed with water and hexanes, and recrystallized from MeOH to afford 1 g (58%) of a tan solid: mp 218–219 °C; MS *m/e* 287 (MH^+); ^1H NMR (CF_3COOD) δ 9.13, 8.38 (A_2B_2, J = 6.7 Hz, 4H, C_6H_4N), 8.42 (s, 1H, 5-H), 3.45 (t, J = 7.7 Hz, 2H, 2 × 3-H), 3.11 (t, J = 7.7 Hz, 2H, 2 × 4-H), 2.85 (s, 3H, CH_3).

5-Bromo[3,4′-bipyridin]-6-amine (30). To a stirred mixture of 3,4′-bipyridin-6-amine (**29**)[8] (22 g, 0.13 mol) and AcOH (250 mL) was added Br_2 (7.3 mL, 0.14 mol) over 30 min. The resulting orange mixture was heated under reflux for 1.5 h, cooled to room temperature, and diluted with Et_2O (200 mL). The orange precipitate was collected, washed with ether, and slurried in 10% aqueous NaOH (200 mL). The product was collected, washed with water, dried, and recrystallized from 2-PrOH to provide 24.4 g (75%) of a yellow powder: mp 192–193 °C; MS *m/e* 250 (MH^+). Anal. ($C_{10}H_8BrN_2$) C, H, N.

5-Bromo-2-ethyl[3,4′-bipyridin]-6-amine (32). A stirred mixture of pyridinone[21] **31** (29.7 g, 0.1 mol) and $POCl_3$ (200 mL) was heated under reflux for 7 h, and the resulting light brown solution was concentrated under reduced pressure. The oily residue was poured into a vigorously stirred mixture of water, ice, and concentrated aqueous ammonia. The light brown solid was collected, washed with water, and added to a solution of EtOH (100 mL) and concentrated aqueous ammonia (200 mL). The resulting mixture was heated in an autoclave at 230–240 °C for 36 h. After cooling to room temperature, the tan product was collected and recrystallized from acetonitrile to furnish 13.5 g (49%) of **32**: mp 170–172 °C; MS *m/e* 278 (MH^+). Anal. ($C_{12}H_{12}N_3Br$) C, H, N.

6-(4-Pyridinyl)thiazolo[4,5-*b*]pyridine-2(3*H*)-thione (33a). A stirred mixture of amino compound **30** (21.8 g, 0.09 mol), ethyl potassium xanthate (28 g, 0.175 mol), and NMP (200 mL) was heated at 170–175 °C in an oil bath for 3.5 h, cooled to room temperature, and diluted with H_2O (200 mL). The resulting mixture was acidified with AcOH, and the precipitate was collected, washed with water and ethanol, and dried to give 16.4 g (75%) of a yellow fluffy powder: mp >300 °C; MS *m/e* 245 (MH^+). Anal. ($C_{11}H_6N_3S_2$) C, H, N.

5-Ethyl-6-(4-pyridinyl)thiazolo[4,5-*b*]pyridine-2(3*H*)-thione (33b). Via the procedure for the preparation of **33a**, **33b** was obtained in 82% yield: mp 286–289 °C; MS *m/e* 274 (MH^+). Anal. ($C_{13}H_{11}N_3S_2$) C, H, N.

2-(Methylthio)-6-(4-pyridinyl)thiazolo[4,5-*b*]pyridine (34a). A stirred mixture of thione **33a** (24.4 g, 0.1 mol), anhydrous milled K_2CO_3 (21 g, 0.15 mol), and DMF (250 mL) was heated on a steam bath for 30 min and then treated with CH_3I (6.5 mL, 0.11 mol) over a period of 20 min. The resulting mixture was further stirred for 30 min and then concentrated under reduced pressure. The residue was slurried in H_2O (100 mL), and the product was collected, washed with water, dried, and recrystallized from CH_3CN to yield 18.4 g (71%) of off-white needles: mp 207–208 °C; MS *m/e* 258 (MH^+). Anal. ($C_{12}H_8N_3S_2$) C, H, N.

5-Ethyl-2-(methylthio)-6-(4-pyridinyl)thiazolo[4,5-*b*]pyridine (34b). Via the procedure for the preparation of **34a**, **34b** was prepared in 69% yield: mp 126–129 °C; MS *m/e* 288 (MH^+). Anal. ($C_{14}H_{13}N_3S_2$) C, H, N.

6-(4-Pyridinyl)thiazolo[4,5-*b*]pyridin-2(3*H*)-one (35a). A mixture of **34a** (12.9 g, 50 mmol), CH_3ONa (5.4 g, 0.1 mol), and DMF (100 mL) was stirred at room temperature for 7 h and then concentrated to dryness on a rotary evaporator. The residue was dissolved in H_2O (100 mL) and acidified with AcOH. The resulting precipitate was collected, washed with water, dried, and recrystallized from DMF to afford 4.3 g (38%) of a yellow powder: mp >300 °C; MS *m/e* 230 (MH^+); ^1H NMR (CF_3COOD) δ 9.10 (s, 1H, 5-H), 8.97, 8.52 (A_2B_2, J = 6.4 Hz, 4H, C_5H_4N), 8.94 (s, 1H, 7-H). Anal. ($C_{11}H_7N_3OS$) C, H, N.

5-Ethyl-6-(4-pyridinyl)thiazolo[4,5-*b*]pyridin-2(3*H*)-one (35b). Via the procedure for the preparation of **35a**, **35b** was prepared in 51% yield: mp 236–238 °C; MS *m/e* 257 (MH^+); ^1H NMR (DMSO-d_6) δ 12.60 (br s, 1H, NH), 8.66, 7.43 (A_2B_2, J = 6.4 Hz, 4H, C_5H_4N), 7.91 (s, 1H, 7-H), 2.65 (q, J = 7.5 Hz, 2H, CH_2CH_3), 1.51 (t, J = 7.5 Hz, 3H, CH_2CH_3). Anal. ($C_{13}H_{11}N_3OS$) C, H, N.

5-Methyl-6-(4-pyridinyl)oxazolo[4,5-*b*]pyridin-2(3*H*)-one (40). A mixture of 5-hydroxy-2-methyl[3,4′-bipyridin]-6(1*H*)-one[17] (**36**) (20.2 g, 0.1 mol) and $POCl_3$ (480 mL) was heated under reflux for 6.5 h and then cooled to room temperature. Most of the $POCl_3$ was removed under reduced pressure, and the residual oil was poured on ice and neutralized by treating with K_2CO_3 (pH = 9). The resulting mixture was extracted with $CHCl_3$ (4 × 300 mL). The combined $CHCl_3$ extracts were dried ($MgSO_4$) and concentrated under pressure to give 8.2 g of a light brown oil. To this oil was added hydrazine hydrate (2.7 mL, 0.11 mol), and the resulting mixture was heated at 120–125 °C in an oil bath for 27 h. After cooling to room temperature, the reaction mixture was treated with 10% aqueous K_2CO_3 (50 mL), and the resulting light orange solid was collected, washed with water, and dried to give 4.5 g of the crude amino compound **3a**: MS *m/e* 202 (MH^+). The amino compound was dissolved in DMF (50 mL) and treated with 1,1′-carbonyldiimidazole (5 g, 30 mmol). The resulting solution was allowed to stir at room temperature for 5 h and then concentrated under reduced pressure. The residue was slurried in H_2O (25 mL), and the pale orange product was collected, washed with water, dried, and recrystallized from 2-PrOH to give 2.4 g of **40**: mp 297–298 °C; MS *m/e* 228 (MH^+); ^1H NMR (CF_3COOD) δ 9.12, 8.29 (A_2B_2, J = 6.7 Hz, 4H, C_5H_4N), 8.10 (s, 1H, 7-H), 2.71 (s, 3H, CH_3). Anal. ($C_{12}H_9N_3O_2$) C, H, N.

2,3-Dihydro-5-(4-pyridinyl)-1*H*-indol-2-one (43). Hydrazone[7] **41** (3.2 g, 13.5 mmol) was heated in an oil bath at 208–210 °C for 5 min and then cooled to room temperature. The resulting light brown solid was recrystallized from CH_3CN after decolorizing with charcoal to afford 1.7 g (60%) of a beige powder: mp 238–240 °C; MS *m/e* 211 (MH^+); ^1H NMR (DMSO-d_6) δ 10.48 (br s, 1H, NH), 8.58, 6.93 (A_2B_2, J = 6.3 Hz, 4H, C_5H_4N), 7.63 (m, 3H, aromatic), 3.56 (s, 2H, CH_2). Anal. ($C_{13}H_{10}N_2O$) C, H, N.

5,5′-Thiobis(2-methyl[3,4′-bipyridin]-6-amine) (58). To a suspension of thiazolone **51** (10 g, 41 mmol) in H_2O (65 mL) was added concentrated H_2SO_4 (100 mL). The resulting solution was heated under reflux for 24 h, cooled in an ice bath, and neutralized by treating with concentrated aqueous ammonia. The resulting precipitate was collected, washed with water, and dried to yield 8.24 g (92%) of crude **57**. An analytical sample was prepared by recrystallization from $CHCl_3$: mp 264–268 °C; MS *m/e* 433 (MH^+). Anal. ($C_{22}H_{20}N_6S_2$) C, H, N.

6-Methyl-7-(4-pyridinyl)-2*H*-pyrido[3,2-*b*]-1,4-thiazin-3(4*H*)-one (59). To a stirred suspension of **58** (1 g, 2.3 mmol) in 2-PrOH (25 mL) heated to 70–75 °C was added $NaBH_4$ (220 mg, 5.2 mmol). After 30 min, ethyl bromoacetate (0.6 mL, 5.2 mmol) was added, and the reaction was allowed to proceed for 1 h and then concentrated to dryness on a rotary evaporator. The residue was slurried in H_2O (25 mL) and collected. Recrystallization from EtOH gave 0.38 g (64%) of **59**: mp 223–228 °C; MS *m/e* 258 (MH^+); ^1H NMR (DMSO-d_6) δ 11.05 (s, 1H, NH), 8.65,

7.47 (A_2B_2, J = 6.1 Hz, 4H, C_5H_4N), 7.75 (s, 1H, 8-H), 3.57 (s, 2H, CH_2), 2.37 (s, 3H, CH_3). Anal. ($C_{13}H_{11}N_3OS$) C, H, N.

2,2-Difluoro-6-methyl-7-(4-pyridinyl)-2*H*-pyrido[3,2-*b*]-1,4-thiazin-3(4*H*)-one (60). Via the procedure for the preparation of **59**, **60** was prepared in 25% yield from **58** and ethyl bromodifluoroacetate: mp 257–259 °C; MS *m/e* 294 (MH^+); ^1H NMR (DMSO-d_6) δ 11.05 (s, 1H, NH), 8.73, 7.30 (A_2B_2, J = 6.0 Hz, 4H, C_5H_4N), 7.55 (s, 1H, 8-H), 2.49 (s, 3H, CH_3). Anal. ($C_{13}H_9F_2N_3OS$) C, H, N.

***In Vitro* Activity. Isolation of PDE Isozymes.** Slight modifications of the methods of Thompson et al.[22] and Weishaar et al.[23] were used to separate the isozymes of PDE from dog aorta. Thoracic aortae, fresh or previously frozen (stored at −70 °C under nitrogen), were cleaned of adhering connective tissue (similar results were obtained with fresh or frozen tissue). The aorta was minced with fine scissors and immediately homogenized in 10 volumes of a buffer containing 10 mM Tris-acetate, pH 7.5, 2 mM $MgCl_2$, 1 mM dithiothreitol (DTT), and 2000 units/mL of aprotinin. This and subsequent procedures were performed at 0–4 °C. The tissue was homogenized with a Brinkmann PT-20 Polytron and sonicated to release both particulate and soluble phosphodiesterases. The homogenate was then centrifuged at 48000*g* for 30 min, and the supernatant was applied to a (diethylamino)ethyl (DEAE)-cellulose column, equilibrated with 70 mM sodium acetate/1 mM DTT (pH 6.5). PDE isozymes were eluted with a linear gradient of sodium acetate from 70 mM to 1.0 M (total volume of 400 mL). Fractions (4–6 mL each) were collected and assayed for PDE activity with cAMP and cGMP as substrate. The fractions corresponding to cAMP PDE III activity were pooled, dialyzed against 70 mM sodium acetate/0.5 mM DTT (pH 6.5), and then concentrated. Ethylene glycol was added to 50% (v/v), and the enzyme preparation was stored at −20 °C. No significant changes in hydrolysis or sensitivity to inhibitors have been noted with storage up to at least two months.

Phosphodiesterase Assay. PDE activity was measured at 30 °C in 500 μL of a reaction mixture containing 40 mM Tris-acetate (pH 8.0), 5 mM $MgCl_2$, 1 mM DTT, 0.1 μCi of [^3H]cAMP, and inhibitors or vehicle. The total concentration of substrate (cAMP) was equal to the K_m, 0.2 μM. The dilution of enzyme was adjusted to yield less than 20% hydrolysis of the substrate. Inhibitors were preincubated with enzyme in the reaction mixture for 5 min at 30 °C, and then the reaction was initiated by the addition of substrate. After 10 min, the assay was terminated by boiling. The 5′-AMP produced as the result of PDE activity was quantitatively converted to adenosine by the addition of snake venom (*Ophiophagus hannah* venom, 1 mg/mL) containing 5′-nucleotidase. Methanol (1.5 mL) was added, and the mixture was applied to a Dowex-1 anion-exchange column. The effluent was collected along with a 2.0-mL methanol wash, and radioactivity was determined by liquid scintillation counting.

Percent inhibition values were determined in triplicate and were calculated as the difference between the activity in the absence and presence of drug (10 μM), divided by the control activity (after subtraction of the appropriate blank), times 100%. IC_{50} values were calculated by linear regression analysis of the linear portion of the plot of percent inhibition vs log dose, determined at six concentrations of drug (each in triplicate). Thus, the IC_{50} value is a better indicator of a drug's potency than percent inhibition at a single concentration.

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