Novel cAMP PDE III Inhibitors: 1,6-Naphthyridin-2(1H)-ones

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Two series of medorinone (3) analogs were prepared by modifications at C(2) and C(5). The C(2)-series was prepared from 2-chloro-5-methyl-1,6-naphthyridine (4) by replacement of the chloro group with various nucleophiles. The C(5)-series was prepared from 5-acyl-6-[2-(dimethylamino)-ethenyl]-2(1H)-pyridinone (11), 5-bromo-1,6-naphthyridin-2(1H)-one (17), and 1,3-diketones 19 and 27. 1,6-Naphthyridin-2(1H)-ones are novel inhibitors of cAMP PDE III. Modification of the carbonyl group of 3 or N-methylation at N(1) resulted in a dramatic loss of enzyme activity. Absence of the C(5)-methyl group of medorinone (3) or its shift to C(3) or C(7) also resulted in reduced activity. Substitution at C(3) also diminished activity. However, substitution at C(5) by a wide variety of substituents led to improvement of enzyme activity and several C(5)-substituted analogs were more potent than milrinone.

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). The

| 1 | 2 | 3 |
|amrinone|milrinone|medorinone|
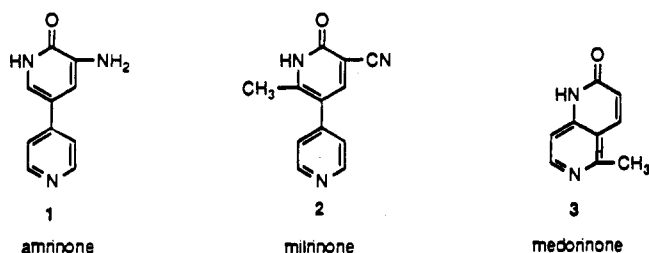
positive inotropic action of amrinone and milrinone is directly related to the inhibition of guanosine 3',5'-cyclic phosphate-inhibitable adenosine 3',5'-cyclic phosphate phosphodiesterase III (cAMP III).[3] SAR studies on 1 and 2 led to the discovery of 1,6-naphthyridin-2(1H)-ones as potent cardiotonic compounds. Medorinone[4] (3), was selected for advanced biological evaluation. The synthesis

of compounds 3 and 33–52 except 45 was reported[5,6] earlier. This paper reports the synthesis of additional analogs of medorinone and the in vitro cAMP PDE III inhibitory activity of the new compounds as well as of those reported[5,6] previously.

The selection of medorinone for advanced biological evaluation prompted several structural modifications of which the following three are included in this paper: (a) replacement of the 2-oxo group by thio, methoxy, and amino groups; (b) modification of the 5-methyl substituent; and (c) replacement of the 5-methyl substituent by a five-membered heteroaryl group.

Chemistry

The preparation of chloro compound 4 by reacting 3 with phosphorus oxychloride or phenylphosphonic dichloride was unfruitful. However, treatment of 3 with 1 equiv of phosphorus pentachloride in phosphorus oxychloride gave 4 in 77% yield (Scheme I). Furthermore, use of 2 equiv of phosphorus pentachloride resulted in the dichlorination of the 5-methyl group to produce 5 in 46% yield. Treatment of 4 with the potassium salt of thiolacetic acid gave thione 6 in 74% yield. Similar reactions of 4 with sodium methoxide and hydrazine yielded the corresponding methoxy compound 7 and hydrazino compound 8 in 79% and 65% yield, respectively. Hydrogenolysis of 8 over Raney Ni afforded amine 9 in 71% yield.

The modification of the 5-alkyl group is depicted in Scheme II. Hydrolysis of 5 with 6 N hydrochloric acid gave naphthyridinone 10 in 67% yield. Reaction of the known enamine 11[5] with hydroxylamine gave the corresponding naphthyridinone 6-oxide 12 in high yields. Reaction of 12a with acetic anhydride[7] at reflux temperature, followed by treatment with aqueous potassium carbonate, gave alcohol 13a in 36% yield. However, treatment of 12b under similar conditions led to an inseparable complex mixture. Modification of the reaction conditions (lower temperature) gave acetate 13c in 39%

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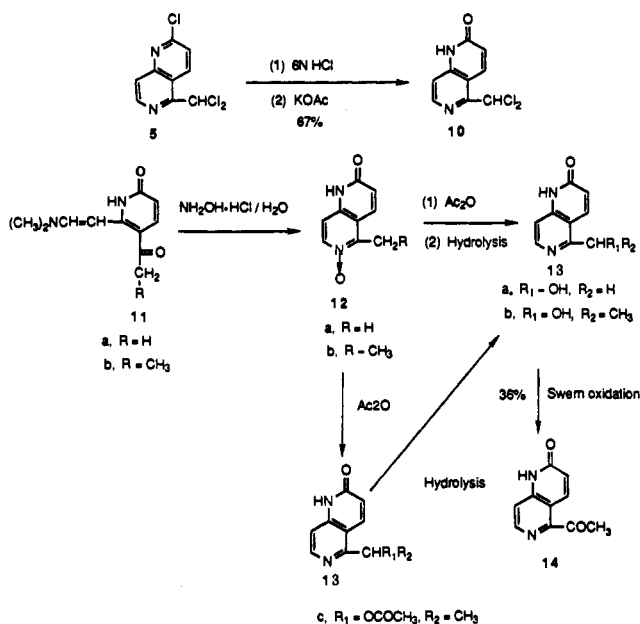(6) Singh, B.; Lesher, G. Y.; Brundage, R. P. Synthesis of 3-Methyl and 7-Methyl Regio Isomers of Medorinone. *Synthesis* 1991, 894–896.
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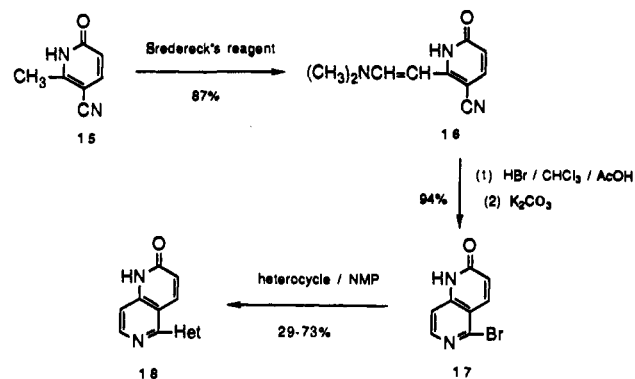
Scheme I
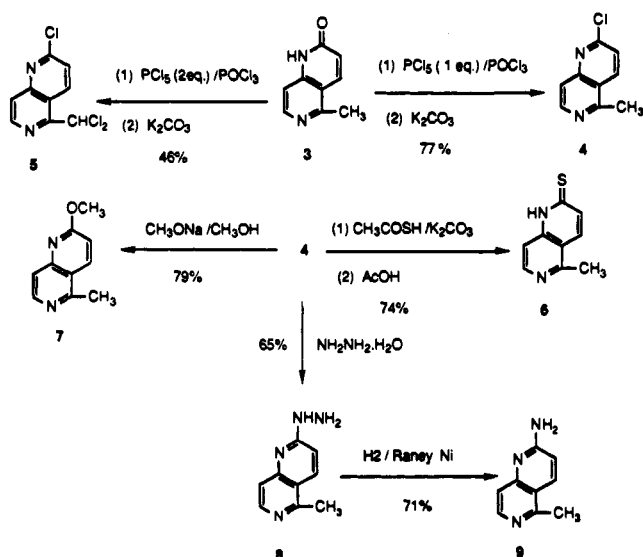
Scheme II

yield. Hydrolysis of the acetate **13c** gave alcohol **13b** in 73% yield, which in turn was converted to ketone **14** by Swern oxidation[8] in 36% yield.

The synthesis of 5-heteroarylnaphthyridinones **18** is shown in Scheme III. Condensation of the activated methyl group of pyridinone **15** with Bredereck's reagent[9] resulted in the formation of enamine **16** in 87% yield. Treatment of **16** with hydrogen bromide resulted in intramolecular cyclization of the nitrile group with the enamine moiety to produce bromonaphthyridinone **17** in 94% yield. Treatment of **17** with excess of heterocyclic bases (imidazoles, pyrazoles, and triazoles) in 1-methyl-2-pyrrolidinone at 150–170 °C gave the corresponding 5-heteroarylnaphthyridinones **18**.

Naphthyridinone **23** was prepared from 1,3-diketone

Scheme III

Scheme IV
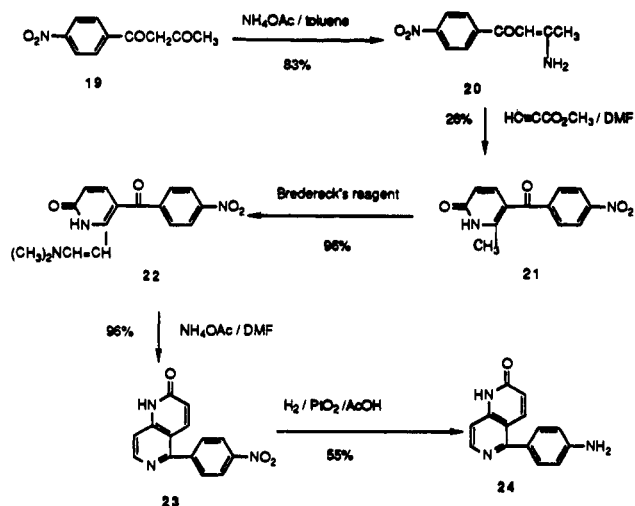
19[10] in 4 steps following our recently published procedure[5] (Scheme IV). However the preparation of naphthyridinone **32** from 1,3-diketone **27**[10] by this procedure was unsuccessful due to the formation of a complex mixture by the reaction of methyl propiolate with 3-amino-1-(4-pyridinyl)-2-buten-1-one[11] prepared from **27**, which in turn was prepared on a large scale by the condensation of acetone with methyl isonicotinate in the presence of sodium methoxide. In an alternative method (Scheme V), **27** was reacted with (CH$_3$)$_2$NCH(OCH$_3$)$_2$ and the resulting intermediate was treated with cyanoacetamide in the presence of NaH to afford a mixture of isomeric pyridones **28** and **29**. Pyridone **28** was converted to naphthyridinone **30** in a two-step one-pot reaction. The acid derivative **31** failed to decarboxylate in boiling 85% sulfuric acid or concentrated phosphoric acid or quinoline in the presence of copper. However, decarboxylation was accomplished by heating the acid neat at 370–375 °C for a short time. Methylation of bromonaphthyridinone **17** followed by reaction with 4-methyl-1H-imidazole gave naphthyridinone **53** and methylation of **44** gave naphthyridinone **45** (Scheme VI).

Results and Discussion

A limited structure–activity relationship (SAR) study was conducted by modifying the C(3)- and C(5)-positions

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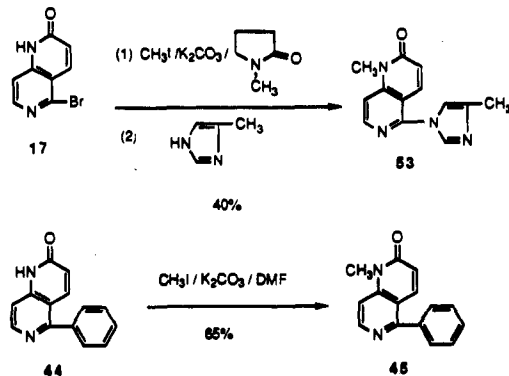
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Scheme V
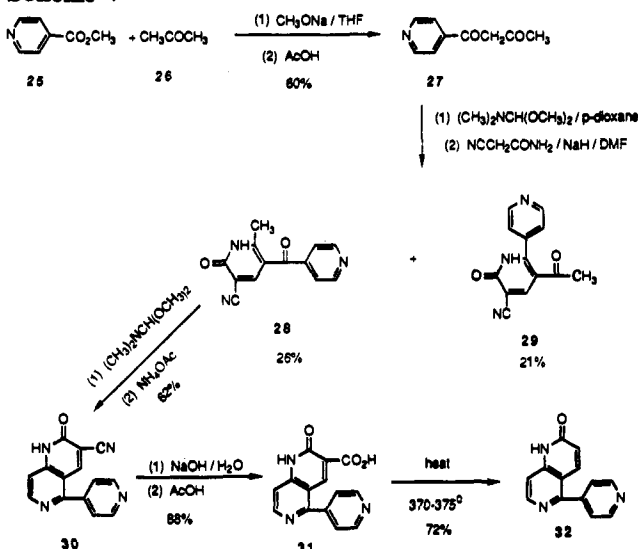
Scheme VI

of medorinone (3). The in vitro cAMP PDE III inhibitory activity of medorinone and its analogs (4–52) is shown in Table I.

The data revealed that the 1-H and C=O group play a key role in determining cAMP PDE III inhibitory activity. The loss of 1-H or modification of the HNC=O moiety resulted in a dramatic fall in activity. For example, N(1)-methylation of medorinone (3) and its analogs 44 and 18i gave compounds 33, 45, and 53, respectively, which are weakly active. Transformation of 3 to the 2-chloro (4), 2-methoxy (7), and 2-amino (9) analogs also resulted in marked reduction in activity. Replacement of the carbonyl oxygen by sulfur (6) lowered the activity by one-half. The shift in the position of the methyl group from C(5) (3) to C(3) (34) or C(7) (35) also reduced the in vitro activity, particularly in the case of 34. Substitution at C(3) by groups like CN, CONH₂, OH, and NH₂ (36–39) also decreased activity. The desmethyl compound 40 was 34 times less active than 3. Conversion of 3 to the 6-oxide derivative 12a and the 5-hydroxymethyl derivative 13a lowered the activity by 6- and 7-fold, respectively. However, replacement of the methyl groups of 3 by CHCl₂ and Br resulted in compounds 10 and 17, which were approximately 3 times more potent than 3. Homologation of the methyl group by one and two carbons increased the activity by 13- and 11-fold, respectively. However, the isobutyl derivative 43 was slightly less active than 3. Transformation of the ethyl group to an acetyl group resulted in a dramatic loss of activity (35-fold). Substitution by an aryl or heteroaryl group at C(5) resulted in potent

compounds. In particular, the 4-chlorophenyl (47) and 4-aminophenyl (24) analogs are approximately twice as potent as medorinone (3). The 2-furanyl (51) and 2-thienyl (52) analogs were equipotent and about 4 times more active than medorinone (3). However, 4-pyridyl substitution at C(3) resulted in a 4-fold loss of activity. Substitution by five-membered heterocycles at C(5) resulted in a weakly active series. The most active member of this series, compound 18i, is 5 times less potent than medorinone.

Conclusion

We synthesized a series of substituted 1,6-naphthyridin-2(1H)-ones and evaluated their in vitro potency against cAMP PDE III. Several analogs were more potent than milrinone. The structure–activity relationship studies revealed that the N(1)-H and the HNC=O moiety of the molecule were critical for in vitro potency. In addition, substitution at the C(3)-position led to a reduction in activity. In contrast, the C(5)-position accommodated a wide variety of substitutents and some produced a dramatic increase in activity.

Experimental Section

Those starting materials which were not available commercially were prepared following published procedures. 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 in parts per million and given in δ units. Elemental analyses were performed by Galbraith Laboratories, Inc., Knoxville, TN.

2-Chloro-5-methyl-1,6-naphthyridine (4). To a stirred mixture of 3 (32 g, 0.2 mol) and POCl₃ (500 mL) was added PCl₅ (42 g, 0.2 mol). The resulting mixture was heated under reflux for 7 h and then most of the POCl₃ was removed under reduced pressure. The dark residue was slurried in CHCl₃ (500 mL) and poured slowly into a vigorously stirred mixture of K₂CO₃ (138 g, 1 mol) and water (300 mL) cooled in an ice bath. The temperature was kept below 10 °C by adding ice to the reaction mixture and more K₂CO₃ was added to keep the reaction mixture on the basic side. The organic layer was separated and dried (MgSO₄). Removal of CHCl₃ gave 37.8 g of a purple solid which was purified by column chromatography (SiO₂ 600 g, 5% MeOH in Et₂O). Recrystallization from Et₂O–hexanes gave 27.5 g (77%) of a pale yellow fluffy solid: mp 98–100 °C; ¹H NMR (CDCl₃) δ 8.59 (d, $J_{7,8}$ = 5.9 Hz, 1 H, 7-H), 8.31 (d, $J_{3,4}$ = 8.7 Hz, 1 H, 4-H), 7.63 (d, $J_{7,8}$ = 5.9 Hz, 1 H, 8-H), 7.43 (d, $J_{3,4}$ = 8.7 Hz, 1 H, 3-H), 2.90 (s, 3 H, CH₃). Anal. (C₉H₇ClN₂) C, H, N.

5-Methyl-1,6-naphthyridin-2(1H)-thione (6). A mixture of thiolacetic acid (7.6 g, 0.1 mol), K₂CO₃ (21 g, 0.15 mol), and MeOH (200 mL) was stirred for 15 min and then 4 (8.9 g, 0.05 mol) was added. The resulting mixture was stirred at room temperature overnight and then concentrated under reduced pressure to give a dark brown syrup. It was dissolved in water (50 mL) and acidified with AcOH whereupon a yellow product precipitated. It was collected, washed with water, and recrystallized from EtOH to afford 6.5 g (74%) of 6: mp 225–228 °C; ¹H NMR (DMSO-d₆) δ 8.34 (d, $J_{7,8}$ = 5.5 Hz, 1 H, 7-H), 7.89 (d, $J_{3,4}$ = 9.5 Hz, 1 H, 4-H), 7.24 (d, $J_{7,8}$ = 5.5 Hz, 1 H, 8-H), 7.23 (d, $J_{3,4}$ = 9.5 Hz, 1 H, 3-H), 2.66 (s, 3 H, CH₃). Anal. (C₉H₈N₂S) C, H, N.

2-Methoxy-5-methyl-1,6-naphthyridine (7). A mixture of 4 (17.8 g, 0.1 mol), CH₃ONa (12 g, 0.22 mol), and MeOH (250 mL) was stirred at room temperature overnight and then concentrated to dryness under reduced pressure. The residue was partitioned between CHCl₃ (400 mL) and water (200 mL). The organic phase was separated, dried (MgSO₄), and concentrated to dryness under reduced pressure to give a solid residue which was recrystallized from Et₂O–hexanes to give 14.1 g (79%) of tan needles: mp 74–77 °C; ¹H NMR (CDCl₃) δ 8.49 (d, $J_{7,8}$ = 6.0 Hz, 1 H, 7-H), 8.17 (d, $J_{3,4}$ = 9.0 Hz, 1 H, 4-H), 7.52 (d, $J_{7,8}$ = 6.0 Hz, 1 H, 8-H), 6.92 (d, $J_{3,4}$ = 9.0 Hz, 1 H, 3-H), 4.08 (s, 3 H, OCH₃), 2.85 (s, 3 H, CH₃). Anal. (C₁₀H₁₀N₂O) C, H, N.

2-Hydrazino-5-methyl-1,6-naphthyridine Dihydrochloride (8). A solution of 4 (8.9 g, 0.05 mol), 85% hydrazine hydrate (6.2 mL, 0.1 mol), and MeOH (200 mL) was stirred at room temperature for 26 h and then concentrated to dryness under reduced pressure. The residue was partitioned between CHCl$_3$ (200 mL) and 5% aqueous K$_2$CO$_3$ (100 mL). The CHCl$_3$ extract was dried (MgSO$_4$) and concentrated to dryness. The residue was dissolved in 6 N HCl (100 mL) and the resulting solution was concentrated to dryness under reduced pressure to give an orange solid which was recrystallized from MeOH to afford 10.4 g (65%) of 8: mp 250–253 °C dec; ^{1}H NMR (CF$_3$COOD) δ 9.17 (d, $J_{7,8}$ = 6.9 Hz, 1 H, 7-H), 9.01 (d, $J_{3,4}$ = 9.8 Hz, 1 H, 4-H), 8.82 (d, $J_{7,8}$ = 6.9 Hz, 1 H, 8-H), 8.10 (d, $J_{3,4}$ = 9.8 Hz, 1 H, 3-H), 3.30 (s, 3 H, CH$_3$). Anal. (C$_9$H$_{10}$N$_4$·2HCl) C, H, N.

5-Methyl-1,6-naphthyridin-2-amine (9). A mixture of 8 (24.4 g, 0.14 mol), Raney Ni (water) (2.5 g), and DMF (200 mL) was hydrogenated at 60–70 °C on a Parr hydrogenator until the required amount of hydrogen was absorbed. After cooling to room temperature, the catalyst was filtered off on a Celite pad and the filtrate was concentrated to dryness on a rotary evaporator. The residue was crystallized from EtOH to give 15.8 g (71%) of an orange solid: mp 218–221 °C; ^{1}H NMR (CF$_3$COOD) δ 8.80 (d, $J_{7,8}$ = 6.9 Hz, 1 H, 7-H), 8.71 (d, $J_{3,4}$ = 9.9 Hz, 1 H, 4-H), 8.23 (d, $J_{7,8}$ = 6.9 Hz, 1 H, 8-H), 7.70 (d, $J_{3,4}$ = 9.9 Hz, 1 H, 3-H), 3.21 (s, 3 H, CH$_3$). Anal. (C$_9$H$_9$N$_3$) C, H, N.

2-Chloro-5-(dichloromethyl)-1,6-naphthyridine (5). To a stirred mixture of 3 (32 g, 0.2 mol) and POCl$_3$ (500 mL) was added PCl$_5$ (84 g, 0.4 mol) over a 10-min period. The resulting mixture was heated under reflux for 10 h and then cooled to room temperature. Most of the POCl$_3$ was removed under reduced pressure. The viscous purple residue was dissolved in CHCl$_3$ (600 mL). The resulting solution was added slowly to a vigorously stirred mixture of K$_2$CO$_3$ (400 g) and water (1 L) cooled in an ice bath. Ice was added to keep the temperature below 20 °C and more K$_2$CO$_3$ was added to keep the reaction mixture basic during the quenching process. The ice bath was removed and the mixture was allowed to come to room temperature. The organic layer was separated and dried (MgSO$_4$). Removal of CHCl$_3$ under vacuum gave a purple sticky solid which was purified by column chromatography (SiO$_2$ 700 g, Et$_2$O). The least polar main component was collected and recrystallized from Et$_2$O–hexanes to give 22.4 g (46%) of white prisms: mp 174–176 °C; ^{1}H NMR (CDCl$_3$) δ 9.04 (d, $J_{3,4}$ = 9.2 Hz, 1 H, 4-H), 8.64 (d, $J_{7,8}$ = 5.8 Hz, 1 H, 7-H), 7.84 (d, $J_{7,8}$ = 5.8 Hz, 1 H, 8-H), 7.59 (d, $J_{3,4}$ = 9.2 Hz, 1 H, 3-H), 7.19 (s, 1 H, CHCl$_2$). Anal. (C$_9$H$_5$Cl$_3$N$_2$) C, H, N.

5-(Dichloromethyl)-1,6-naphthyridin-2(1H)-one (10). A mixture of 5 (10 g, 0.04 mol) and 6 N hydrochloric acid (100 mL) was heated on a steam bath for 1.5 h and then concentrated under reduced pressure. The residue was dissolved in hot water and neutralized by treating with KOAc. The resulting precipitate was collected and recrystallized from 2-PrOH after treatment with charcoal to give 6.2 g (67%) of tan crystals: mp 238–240 °C; ^{1}H NMR (CF$_3$COOD) δ 8.87 (d, $J_{7,8}$ = 5.8 Hz, 1 H, 7-H), 8.73 (d, $J_{3,4}$ = 9.3 Hz, 1 H, 4-H), 8.13 (d, $J_{7,8}$ = 5.8 Hz, 1 H, 8-H), 7.78 (s, 1 H, CHCl$_2$), 7.36 (d, $J_{3,4}$ = 9.3 Hz, 1 H, 3-H). Anal. (C$_9$H$_6$Cl$_2$N$_2$O) C, H, N.

5-Methyl-1,6-naphthyridin-2(1H)-one 6-Oxide (12a). A mixture of enamine 11a[5] (68 g, 0.33 mol), hydroxylamine hydrochloride (68 g, 0.96 mol), and 6 N aqueous HCl (300 mL) was stirred and heated on a steam bath until all the solid dissolved (0.5 h). The resulting solution was allowed to stir at room temperature overnight and then concentrated to dryness under vacuum. The tan solid was recrystallized from MeOH to furnish 56 g (79%) of 12a as white needles: mp 244–245 °C; ^{1}H NMR (CF$_3$COOD) δ 8.75 (d, $J_{7,8}$ = 7.0 Hz, 1 H, 7-H), 8.48 (d, $J_{3,4}$ = 9.9 Hz, 1 H, 4-H), 7.83 (d, $J_{7,8}$ = 7.0 Hz, 1 H, 8-H), 7.30 (d, $J_{3,4}$ = 9.9 Hz, 1 H, 3-H), 3.19 (s, 3 H, CH$_3$). Anal. (C$_9$H$_8$N$_2$O$_2$) C, H, N.

5-(Hydroxymethyl)-1,6-naphthyridin-2(1H)-one (13a). A mixture of 12a (5 g, 24 mmol) and Ac$_2$O (50 mL) was stirred at room temperature for 16 h, heated on a steam bath for 1 h and then stripped to dryness under reduced pressure. The gummy residue was treated with 10% aqueous K$_2$CO$_3$ (25 mL), heated on a steam bath for 1 h, acidified with AcOH and then chilled in ice bath. The white solid which crystallized was collected and recrystallized from MeOH to yield 1.5 g (37%) of 13a: mp 292–

295 °C; ^{1}H NMR (CF$_3$COOD) δ 8.75 (d, $J_{7,8}$ = 6.8 Hz, 1 H, 7-H), 7.98 (d, $J_{3,4}$ = 9.8 Hz, 1 H, 4-H), 8.39 (d, $J_{7,8}$ = 6.8 Hz, 1 H, 8-H), 7.30 (d, $J_{3,4}$ = 9.8 Hz, 1 H, 3-H), 5.70 (s, 2 H, CH$_2$O). Anal. (C$_9$H$_8$N$_2$O$_2$) C, H, N.

5-Ethyl-1,6-naphthyridin-2(1H)-one 6-Oxide (12b). A mixture of enamine 11b[4] (35.0 g, 0.16 mol), hydroxylamine hydrochloride (21.0 g, 0.3 mol), and water (200 mL) was stirred at room temperature overnight. The resulting mixture was concentrated to dryness under reduced pressure. The residue was treated with water (50 mL), and the white shiny crystals were collected and washed with water and EtOH to yield 28.4 g (89%) of 12b: mp 262–264 °C dec; ^{1}H NMR (CF$_3$COOD) δ 8.76 (d, $J_{7,8}$ = 7.4 Hz, 1 H, 7-H), 8.51 (d, $J_{3,4}$ = 10.0 Hz, 1 H, 4-H), 7.80 (d, $J_{7,8}$ = 7.4 Hz, 1 H, 8-H), 7.34 (d, $J_{3,4}$ = 10.0 Hz, 1 H, 3-H), 3.63 (q, J = 7.5 Hz, 2 H, CH$_2$CH$_3$), 1.55 (t, J = 7.5 Hz, 3 H, CH$_2$CH$_3$). Anal. (C$_{10}$H$_{10}$N$_2$O$_2$·^{1}/$_2$H$_2$O) C, H, N.

5-[1-(Acetyloxy)ethyl]-1,6-naphthyridin-2(1H)-one (13c). A mixture of 12b (110.5 g, 0.58 mol) and Ac$_2$O (1.5 L) was stirred and heated on a steam bath for 1.5 h. After 15 min of heating all the solid dissolved forming a light brown solution, and a white solid started separating a few minutes later. This mixture was concentrated to dryness under reduced pressure. To the residue was added CH$_2$Cl$_2$ (1 L) and the resulting mixture was heated under reflux for 25 min and then filtered through a Celite pad. The filtrate was concentrated to dryness and the resulting crude product was purified by column chromatography (SiO$_2$, 1 kg, Et$_2$O–5% MeOH in Et$_2$O) and recrystallized from EtOAc to give 52.4 g (39%) of white fluffy crystals of 13c: mp 182–185 °C; ^{1}H NMR (DMSO-d_6) δ 12.07 (br s, 1 H, NH), 8.38 (d, $J_{7,8}$ = 5.8 Hz, 1 H, 7-H), 8.15 (d, $J_{3,4}$ = 9.8 Hz, 1 H, 4-H), 7.14 (d, $J_{7,8}$ = 5.8 Hz, 1 H, 8-H), 6.59 (d, $J_{3,4}$ = 9.8 Hz, 1 H, 3-H), 6.24 (q, J = 6.5 Hz, 1 H, OCHCH$_3$), 1.99 (s, 3 H, OCOCH$_3$), 1.51 (d, J = 6.5 Hz, 3 H, OCHCH$_3$). Anal. (C$_{12}$H$_{12}$N$_2$O$_3$) C, H, N.

5-(1-Hydroxyethyl)-1,6-naphthyridin-2(1H)-one (13b). A mixture of 13c (34.8 g, 0.15 mol), NaOH (16 g, 0.4 mol), water (50 mL), and MeOH (200 mL) was stirred at room temperature overnight and then treated with AcOH (20 mL). The resulting mixture was concentrated to dryness under reduced pressure and the white solid residue was treated with water (100 mL). The product was collected and recrystallized from 2-PrOH to give 20.2 g (71%) of 13b: mp 213–215 °C; ^{1}H NMR (CF$_3$COOD) δ 8.70 (d, $J_{7,8}$ = 5.8 Hz, 1 H, 7-H), 8.39 (d, $J_{3,4}$ = 9.7 Hz, 1 H, 4-H), 7.92 (d, $J_{7,8}$ = 5.8 Hz, 1 H, 8-H), 7.29 (d, $J_{3,4}$ = 9.7 Hz, 1 H, 3-H), 6.04 (q, J = 6.5 Hz, 1 H, HOCHCH$_3$), 1.87 (d, J = 6.5 Hz, 3 H, HOCHCH$_3$). Anal. (C$_{10}$H$_{10}$N$_2$O$_2$) C, H, N.

5-Acetyl-1,6-naphthyridin-2(1H)-one (14). To a stirred solution of DMSO (27 mL, 0.38 mol) in CHCl$_3$ (250 mL) cooled in dry ice–2-PrOH was added (CF$_3$CO)$_2$O (42 mL, 0.3 mol) below −65 °C over a period of 25 min. The resulting solution was then treated with a slurry of 13b (14.5 g, 0.076 mol) in CHCl$_3$ (300 mL) over a 45-min period while the reaction temperature was maintained below −65 °C, followed by a dropwise addition of Et$_3$N (83 mL, 0.6 mol) over a 50-min period. The reaction mixture was then allowed to come to room temperature and concentrated under reduced pressure. The residual mixture was treated with water (300 mL) and chilled. The product which crystallized was collected and recrystallized from DMF to afford 5.1 g (36%) of tan crystals: mp >300 °C; ^{1}H NMR (DMSO-d_6) δ 12.25 (br s, 1 H, NH), 8.54 (m, 2 H, 7-H, 4-H), 7.39 (d, $J_{7,8}$ = 5.6 Hz, 1 H, 8-H), 6.68 (d, $J_{3,4}$ = 9.6 Hz, 1 H, 3-H), 2.70 (s, 3 H, COCH$_3$). Anal. (C$_{10}$H$_8$N$_2$O$_2$) C, H, N.

2-[2-(Dimethylamino)ethenyl]-1,6-dihydro-6-oxo-3-pyridinecarbonitrile (16). To a stirred mixture of nitrile 15[5] (6.7 g, 50 mmol) and DMF (100 mL) was added in one portion [(CH$_3$)$_2$N]$_2$CHOC(CH$_3$)$_3$ (Bredereck's reagent) (11.5 g, 54 mmol), and the resulting mixture was heated in an oil bath at 110–115 °C for 4 h. After the reaction mixture had cooled to room temperature, the bright yellow solid was filtered off to give 8.2 g (87%) of 16: mp 298–300 °C; ^{1}H NMR (DMSO-d_6) δ 7.92 (d, J = 13.3 Hz, 1 H, (CH$_3$)$_2$NCH=CH], 7.35 (d, J = 9.1 Hz, 1 H, 4-H), 5.74 (d, J = 9.1 Hz, 1 H, 5-H), 4.85 [d, J = 13.3 Hz, 1 H, (CH$_3$)$_2$NCH=CH], 2.92 [s, 6 H, N(CH$_3$)$_2$]. Anal. (C$_{10}$H$_{11}$N$_3$O) C, H, N.

5-Bromo-1,6-naphthyridin-2(1H)-one (17). A stirred suspension of 16 (9.5 g, 50 mmol) in a mixture of CHCl$_3$ (400 mL) and AcOH (150 mL) cooled in an ice bath was saturated with

Table I. In Vitro cAMP PDE III Activity of 1,6-Naphthyridin-2(1H)-ones

compd[a]	R_1	R_2	R_3	R_4	cAMP PDE III	
					% inhibition (10 μM)[b]	IC$_{50}$,[c,d] μM
3	H	CH_3	H	H	67	0.55
33	H	CH_3	H	CH_3	17	
4[e]					18	
7[e]					21	
9[e]					24	
6[e]					81	0.96
34	CH_3	H	H	H	37	
35	H	H	CH_3	H	53	
36	CN	CH_3	H	H	52	
37	$CONH_2$	CH_3	H	H	39	
38	OH	CH_3	H	H	51	
39	NH_2	CH_3	H	H	59	
40	H	H	H	H	55	18.8
12a	H	CH_3	H	H, 6-oxide	65	3.1
13a	H	CH_2OH	H	H	58	3.7
41	H	CH_2CH_3	H	H	87	0.040
42	H	$CH_2CH_2CH_3$	H	H	83	0.048
43	H	$CH_2CH(CH_3)_2$	H	H	81	0.57
14	H	$COCH_3$	H	H	85	1.42
10	H	$CHCl_2$	H	H	83	0.16
17	H	Br	H	H	70	0.17
44	H		H	H	78	1.4
45	H		H	CH_3	36	
46	H		H	H	78	0.94
47	H		H	H	78	0.30
48	H		H	H	55	
49	H		H	H	64	3.2
50	H		H	H	81	0.6
24	H		H	H	91	0.23
51	H		H	H	74	0.14
52	H		H	H	79	0.14
32	H		H	H	78	2.2
18a	H		H	H	18	
18b	H		H	H	34	
18c	H		H	H	43	
18d	H		H	H	13	
18e	H		H	H	46	

Table I. (Continued)

compd[a]	R_1	R_2	R_3	R_4	cAMP PDE III % inhibition $(10 \mu M)$[b]	cAMP PDE III IC_{50},[c,d] μM
18f	H	Br-imidazolyl	H	H	43	
18g	H	$HOCH_2$-imidazolyl	H	H	58	
18h	H	imidazolyl	H	H	56	
18i	H	CH_3-imidazolyl	H	H	72	2.8
53	H	CH_3-imidazolyl	H	CH_3	21	
18j	H	CH_3,CH_3-imidazolyl	H	H	28	
18k	H	triazolyl	H	H	39	
18l	H	CH_3-triazolyl	H	H	30	
18m	H	triazolyl	H	H	50	
18n	H	CH_3-triazolyl	H	H	39	
2	(milrinone)					0.36

[a] Compounds 3 and 33–52 except 45 were reported in refs 5 and 6. [b] Mean of three determinations. [c] Mean of three determinations. [d] Those compounds which caused >55% inhibition at 10 μM and caused <15% change in the right atrial rate[14] from the control were selected for IC_{50} determinations.

HBr gas and then the reaction mixture was removed from the ice bath and stirred at room temperature overnight. Solvent was removed under reduced pressure and the yellow solid residue was treated with ice-cold 10% aqueous K_2CO_3 (300 mL). The resulting light yellow solid was filtered off, washed with water, and dried to furnish 10.5 g (94%) of 17: mp 278–280 °C; ^1H NMR (CF_3COOD) δ 8.41 (d, $J_{7,8}$ = 7.0 Hz, 1 H, 7-H), 8.24 (d, $J_{3,4}$ = 9.9 Hz, 1 H, 4-H), 7.73 (d, $J_{7,8}$ = 7.0 Hz, 1 H, 8-H), 7.10 (d, $J_{3,4}$ = 9.9 Hz, 1 H, 3-H). Anal. ($C_8H_5BrN_2O$) C, H, N.

The General Synthesis of 5-Heteroaryl-1,6-naphthyridin-2(1H)-ones (18). A mixture of 17 (5.6 g, 25 mmol), heterocyclic compound (0.1 mol), and NMP (25 mL) was stirred and heated in an oil bath at 170–180 °C for 6 h and then poured into water (50 mL). The resulting solution was acidified with AcOH. The tan product which crystallized on standing at room temperature was collected and recrystallized (Table II, ^1H NMR of representative examples).

3-Amino-1-(4-nitrophenyl)-2-buten-1-one (20). A mixture of 1-(4-nitrophenyl)-1,3-butanedione (19)[9] (103.5 g, 0.5 mol), NH_4-OAc (96.9 g, 1.26 mol), and toluene (1 L) was heated under reflux for 5 h with azeotropic removal of water and then concentrated to dryness under reduced pressure. The yellow solid residue was recrystallized from Et_2O–hexanes to afford 83.1 g (80%) of pale yellow flakes: mp 175–177 °C. Anal. ($C_{10}H_{10}N_2O_3$) C, H, N.

6-Methyl-5-(4-nitrobenzoyl)-2(1H)-pyridinone (21). To a stirred solution of 20 (76.4 g, 0.37 mol) in DMF (300 mL) was added methyl propiolate (37 mL, 0.4 mol) over a period of 15 min. The resulting solution was heated under reflux for 24 h and then concentrated to near dryness on a rotary evaporator. The brown solid residue was recrystallized from 2-PrOH to give 25

g (26%) of tan crystals: mp 282–284 °C; ^1H NMR (CF_3COOD) δ 8.60–7.10 (m, 6 H, arom), 2.87 (s, 3 H, CH_3). Anal. ($C_{13}H_{10}N_2O_4$) C, H, N.

6-[2-(Dimethylamino)ethenyl]-5-(4-nitrobenzoyl)-2(1H)-pyridinone (22). A stirred mixture of 21 (46 g, 0.18 mol), Bredereck's reagent (41.7 mL, 0.2 mol), and 1,4-dioxane (500 mL) was heated under reflux for 2 h and then cooled to room temperature. The orange crystalline product was collected to afford 53.7 g (96%) of 22: mp 304–305 °C dec. Anal. ($C_{16}H_{15}N_3O_4$) C, H, N.

5-(4-Nitrophenyl)-1,6-naphthyridin-2(1H)-one (23). A stirred mixture of 22 (47.5 g, 0.15 mol), NH_4OAc (24.2 g, 0.31 mol), and DMF (250 mL) was heated under reflux for 4 h and then cooled to room temperature. The resulting brown crystals were collected to yield 39.1 g (96%) of 23: mp >300 °C; ^1H NMR (CF_3COOD) δ 9.00–7.00 (m, 8 H, arom). Anal. ($C_{14}H_{19}N_3O_3$) C, H, N.

5-(4-Aminophenyl)-1,6-naphthyridin-2(1H)-one (24). A mixture of 23 (10.7 g, 40 mmol), PtO_2 (0.3 g), and AcOH (300 mL) was reduced on a Parr hydrogenator until the required amount of H_2 was absorbed (0.12 mol, 1 h). The catalyst was filtered off on a Celite pad and the filtrate was concentrated on a rotary evaporator. The resulting solid residue was recrystallized from MeOH after treatment with charcoal to afford 5.2 g (54%) of dull yellow crystals: mp >300 °C; ^1H NMR (CF_3COOD) δ 8.90–7.10 (m, 8 H, arom). Anal. ($C_{14}H_{11}N_3O$) C, H, N.

1-(4-Pyridinyl)butane-1,3-dione (27). To a vigorously stirred solution of methyl isonicotinate (200 g, 1.45 mol), acetone (350 mL), and THF (1 L) warmed to 35–40 °C was added CH_3ONa (81 g, 1.5 mol) over a period of 1 h. External heat was removed; exothermic reaction started upon the addition of CH_3ONa,

Table II. Physical Properties of 5-Heteroaryl-1,6-naphthyridin-2-(1H)-ones

compd	Het	mp, °C	yield, %	crystn solv	formula[a]	^{1}H NMR[b]
18a	O_2N—imidazole—CH_3	>300	66	DMF	$C_{12}H_9N_5O_3$	8.78 (d, $J_{7,8}$ = 6.4 Hz, 1 H, 7-H), 8.86 (s, 1 H, 4'-H), 8.03 (d, $J_{3,4}$ = 9.6 Hz, 1 H, 4-H), 8.02 (d, $J_{7,8}$ = 6.4 Hz, 1 H, 8-H), 7.27 (d, $J_{3,4}$ = 9.6 Hz, 1 H, 3-H), 2.73 (s, 3 H, 2'-CH₃)
18b	imidazole—CH_3	>300	43	DMF	$C_{12}H_{10}N_4O$	
18c	Ph—imidazole	263–265	32	EtOH	$C_{17}H_{12}N_4O$	
18d	CF_3—imidazole	216–218	35	2-PrOH	$C_{12}H_7F_3N_4O$	
18e	O_2N—imidazole	>300	29	DMF	$C_{11}H_7N_5O_3$	
18f	Br—imidazole	278–280	73	DMF	$C_{11}H_7BrN_4O$	12.42 (br s, 1 H, NH), 8.24 (d, $J_{7,8}$ = 5.6 Hz, 1 H, 7-H), 8.18 (s, 1 H, 2'-H), 7.91 (s, 1 H, 5'-H), 7.78 (d, $J_{3,4}$ = 9.7 Hz, 1 H, 4-H), 7.34 (d, $J_{7,8}$ = 5.6 Hz, 1 H, 8-H), 6.66 (d, $J_{3,4}$ = 9.7 Hz, 1 H, 3-H)
18g	$HOCH_2$—imidazole	259–261	19	EtOH	$C_{12}H_{10}N_4O_2$	
18h	imidazole	290–291	42	DMF	$C_{11}H_8N_4O$	
18i	CH_3—imidazole	263–264	64	2-PrOH	$C_{12}H_{10}N_4O$	9.19 (d, J = 1.0 Hz, 1 H, 2'-H), 8.77 (d, $J_{7,8}$ = 6.1 Hz, 1 H, 7-H), 8.06 (d, $J_{3,4}$ = 9.9 Hz, 1 H, 4-H), 7.92 (d, $J_{7,8}$ = 6.1 Hz, 1 H, 8-H), 7.68 (br s, 1 H, 5'-H), 7.24 (d, $J_{3,4}$ = 9.9 Hz, 1 H, 3-H), 2.59 (s, 3 H, CH₃)
18j	CH_3,CH_3—imidazole	256–258	53	EtOH	$C_{13}H_{12}N_4O$	
18k	pyrazole	>300	52	DMF	$C_{11}H_8N_4O$	
18l	CH_3—pyrazole	>300	50	DMF	$C_{12}H_{10}N_4O$	8.49 (d, $J_{7,8}$ = 6.8 Hz, 1 H, 7-H), 8.38 (d, $J_{3,4}$ = 10.0 Hz, 1 H, 4-H), 8.21 (d, $J_{3',5'}$ = 2.5 Hz, 1 H, 5'-H), 7.70 (d, $J_{7,8}$ = 6.8 Hz, 1 H, 8-H), 7.16 (d, $J_{3,4}$ = 10.0 Hz, 1 H, 3-H), 6.71 (d, $J_{3',5'}$ = 2.5 Hz, 1 H, 3'-H), 2.46 (s, 3 H, CH₃)
18m	triazole	>300	52	DMF	$C_{10}H_7N_5O$	
18n	triazole—CH_3	>300	42	DMF	$C_{11}H_9N_5O$	12.34 (br s, 1 H, NH), 9.12 (s, 1 H, 5'-H), 8.59 (d, $J_{3,4}$ = 10.2 Hz, 1 H, 4-H), 8.37 (d, $J_{7,8}$ = 5.4 Hz, 1 H, 7-H), 7.28 (d, $J_{7,8}$ = 5.4 Hz, 1 H, 8-H), 6.66 (d, $J_{3,4}$ = 10.2 Hz, 1 H, 3-H), 2.47 (s, 3 H, CH₃)

[a] All the compounds analyzed within ±0.45% of the theoretical values for C, H, N. [b] ^{1}H NMR spectra of 18a, 18i, and 18l were recorded in CF₃COOD and those of 18f and 18n in DMSO-d_6.

resulting in a yellow precipitate. The resulting mixture was further stirred at ambient temperature for 30 min and then heated under reflux for 2.5 h. After cooling to room temperature, the yellow solid was collected, washed with Et₂O, and dissolved in water (400 mL). The resulting solution was acidified with AcOH and the resulting oily product was extracted with CHCl₃ (2 × 300 mL). Removal of CHCl₃ on a rotary evaporator gave 164.5 g of a brown liquid which solidified on cooling. Recrystallization from Et₂O–hexanes after treatment with charcoal yielded 142.4 g (60%) of a light yellow granular solid: mp 71–73 °C (lit.[11] mp 72 °C).

1,2-Dihydro-6-methyl-2-oxo-5-(4-pyridinylcarbonyl)-3-pyridinecarbonitrile (28) and 3-Acetyl-1,6-dihydro-6-oxo-

[2,4'-bipyridine]-5-carbonitrile (29). A solution of 27 (62 g, 0.38 mol), (CH₃)₂NCH(OCH₃)₂ (60 mL, 0.45 mol), and 1,4-dioxane (250 mL) was stirred overnight and then concentrated on a rotary evaporator to give a greenish yellow semisolid residue which was dissolved in DMF (500 mL). To the resulting stirred solution was added cyanoacetamide (33.6 g, 0.4 mol) followed by 60% NaH/oil dispersion (16 g, 0.4 mol) over a period of 30 min. The resulting mixture was heated on a steam bath for 3.5 h and then concentrated on a rotary evaporator to give a brown solid residue which was slurried in Et₂O (600 mL). The solid was collected, washed with Et₂O, and dissolved in water (500 mL). The resulting solution was acidified with AcOH whereupon a yellow solid

precipitated. The resulting mixture was stirred for 30 min after the addition of Et_2O (600 mL). The insoluble material was collected, washed with water, and recrystallized from DMF to yield 19.4 g (21%) of the more polar component **29**: mp 253–256 °C; ^{1}H NMR (CF_3COOD) δ 9.14, 8.26 (A_2B_2, $J = 5.7$ Hz, 4 H, C_5H_4N), 9.00 (s, 1 H, 4-H), 2.75 (s, 3 H, CH_3). Anal. ($C_{13}H_9N_3O_2$) C, H, N. The aqueous Et_2O filtrate from above was concentrated on a rotary evaporator to give a yellow solid residue which was slurried in water (100 mL) and then filtered off. Recrystallization from DMF afforded 25.4 g (28%) of the less polar component **28**: mp >300 °C; ^{1}H NMR (CF_3COOD) δ 9.16, 8.45 (A_2B_2, $J = 5.7$ Hz, 4 H, C_5H_4N), 8.35 (s, 1 H, 4-H), 2.95 (s, 3 H, CH_3). Anal. ($C_{13}H_9N_3O_2$) C, H, N.

1,2-Dihydro-2-oxo-5-(4-pyridinyl)-1,6-naphthyridine-3-carbonitrile (30). A mixture of **28** (14 g, 58 mmol), $(CH_3)_2$-$NCH(OCH_3)_2$ (10 mL, 72 mmol), and DMF (100 mL) was heated on a steam bath for 5 h and then treated with NH_4OAc (10.5 g, 0.15 mol). The resulting mixture was heated under reflux for 5 h and then cooled to room temperature. The light brown product which crystallized was collected to yield 8.4 g (62%) of **30**: mp >300 °C; ^{1}H NMR (CF_3COOD) δ 9.31, 8.65 (A_2B_2, $J = 5.8$ Hz, 4 H, C_5H_4N), 8.99 (d, $J_{7,8} = 6.9$ Hz, 1 H, 7-H), 8.59 (s, 1 H, 4-H), 8.29 (d, $J_{7,8} = 6.9$ Hz, 1 H, 8-H). Anal. ($C_{14}H_8N_4O$) C, H, N.

1,2-Dihydro-2-oxo-5-(4-pyridinyl)-1,6-naphthyridine-3-carboxylic Acid (31). A mixture of **30** (10.4 g, 40 mmol), 35% aqueous NaOH (25 mL), and water (200 mL) was heated on a steam bath for 6 h and then treated with charcoal. The filtrate was acidified with AcOH whereupon a yellow solid separated which was collected, washed with water, and dried to yield 9.4 g (88%) of **31**: mp >350 °C. Anal. ($C_{14}H_9N_3O_3$) C, H, N.

5-(4-Pyridinyl)-1,6-naphthyridin-2(1H)-one (32). Finely powdered **31** (9.4 g, 35 mmol) was heated in a high-boiling silicon oil bath at 370–375 °C until all the solid melted (3 min) and then taken out. The resulting dark brown cake was dissolved in hot 5% aqueous NaOH (100 mL) and treated with charcoal. The filtrate was acidified with AcOH and the resulting yellow precipitate was collected and recrystallized from DMF to yield 5.6 g (72%) of **32**: mp >300 °C; ^{1}H NMR (CF_3COOD) δ 9.19, 8.50 (A_2B_2, $J = 6.4$ Hz, 4 H, C_5H_4N), 8.79 (d, $J_{7,8} = 6.8$ Hz, 1 H, 7-H), 8.03 (d, $J_{7,8} = 6.8$ Hz, 1 H, 8-H), 7.95 (d, $J_{3,4} = 10.1$ Hz, 1 H, 4-H), 7.15 (d, $J_{3,4} = 10.1$ Hz, 1 H, 3-H). Anal. ($C_{13}H_9N_3O$) C, H, N.

1-Methyl-5-(4-methyl-1H-imidazol-1-yl)-1,6-naphthyridin-2(1H)-one (53). A stirred mixture of **17** (11.25 g, 50 mmol), anhydrous K_2CO_3 (20.7 g, 0.15 mol), and NMP (75 mL) was heated on a steam bath for 15 min and then treated with methyl iodide (3.3 mL, 50 mmol). The resulting mixture was heated on a steam bath for 30 min and then 4-methyl-1H-imidazole (8.2 g, 0.1 mol) was added. The reaction mixture was then heated at 150–155 °C in an oil bath for 5 h. After cooling to room temperature, most of the solvent was removed under reduced pressure, and the residue was treated with water (100 mL). The dark brown solid product was collected and recrystallized from 2-PrOH after treatment with charcoal to give 4.8 g (40%) of light tan crystals of **53**: mp 225–227 °C; ^{1}H NMR (DMSO-d_6) δ 8.50 (d, $J_{7,8} = 5.8$ Hz, 1 H, 7-H), 7.97 (s, 1 H, 2'-H), 7.74 (d, $J_{3,4} = 10.0$ Hz, 1 H, 4-H), 7.57 (d, $J_{7,8} = 5.8$ Hz, 1 H, 8-H), 7.34 (s, 1 H, 5'-H), 6.71 (d, $J_{3,4} = 10.0$ Hz, 1 H, 3-H), 3.61 (s, 3 H, 1-CH_3), 2.18 (s, 3 H, 4'-CH_3). Anal. ($C_{13}H_{12}N_4O$) C, H, N.

1-Methyl-5-phenyl-1,6-naphthyridin-2(1H)-one (45). A stirred mixture of **44** (22.2 g, 0.1 mol), milled anhydrous K_2CO_3 (13.8 g, 0.1 mol), and DMF (300 mL) was heated on a steam bath for 30 min and then treated with methyl iodide (6.9 mL, 0.11 mol). The resulting mixture was further heated for 20 min and then concentrated on a rotary evaporator. The residue was treated with water (100 mL). The product was collected and recrystallized from MeOH to give 16.4 g (69%) of pale yellow

needles of **45**: mp 190–192 °C; ^{1}H NMR (CF_3COOD) δ 8.90–7.10 (m, 9 H, arom), 4.06 (s, 3 H, NCH_3). Anal. ($C_{15}H_{12}N_2O$) C, H, N.

In Vitro Activity. Separation of Isozymes of PDE. Slight modifications of the methods of Thompson et al.[12] and Weishaar et al.[13] were used to separate the isozymes of PDE from dog aorta. Thoracic aortae were cleaned of adhering connective tissue and either used fresh or frozen in liquid nitrogen and stored at −70 °C (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 48000g for 30 min, and the supernatant was applied to a (diethylamino)ethyl cellulose (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 peak III were separately pooled, dialyzed vs 70 mM sodium acetate/0.5 mM DTT (pH 6.5), and then concentrated. Ethylene glycol was added to 50% (v/v), and the enzymes were stored at −20 °C. No significant changes in hydrolysis or sensitivity to inhibitors have been noted with storage up to at least 2 months.

Phosphodiesterase Assay. PDE activity was measured at 30 °C in 500 mL of a reaction mixture containing 40 mM Tris-acetate (pH 8.0), 5 mM $MgCl_2$, 1 mM DTT, 1 mCi of [3H]cAMP, and inhibitors or vehicle. The total concentration of substrate (cAMP) was equal to the K_m, 0.2 mM. 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 of 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 mM), 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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