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

Baldev Singh,*,† George Y. Lesher,†,‡ Kevin C. Pluncket,† Edward D. Pagani,§ Donald C. Bode,§ Ross G. Bentley,§ Mary J. Connell,§ Linda T. Hamel,§ and Paul J. Silver§

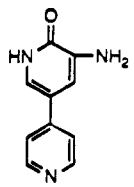
Sterling Winthrop Pharmaceuticals Research Division, Rensselaer, New York 12144

Received June 22, 1992

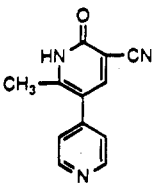
Two series of medorinone (3) analogs were prepared by modifications at C(2) and C(5). The C(2)-series was prepared from 2-chloro-5-methyl-1,6-naphthyridine (4) by replacement of the chloro group with various nucleophiles. The C(5)-series was prepared from 5-acyl-6-[2-(dimethylamino)-ethenyl]-2(1*H*)-pyridinone (11), 5-bromo-1,6-naphthyridin-2(1*H*)-one (17), and 1,3-diketones 19 and 27. 1,6-Naphthyridin-2(1*H*)-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

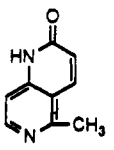
A search for novel cardiotonic agents in our laboratory culminated in the successful development of two clinically useful agents, amrinone[1] (1) and milrinone[2] (2). The



1
amrinone



2
milrinone



3
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(1*H*)-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%

† Department of Medicinal Chemistry.
§ Department of Cardiovascular Pharmacology.
‡ Deceased.

(1) Alousi, A. A.; Edelson, J. Amrinone. In *Pharmacological and Biochemical Properties of Drug Substances*; Goldberg, M. E., Ed.; American Pharmaceutical Association: Washington, DC, 1982; Vol. 3, p 120.

(2) Alousi, A. A.; Stankus, G. P.; Stuart, J. C.; Walton, L. H. Characterization of the Cardiotonic Effects of Milrinone, a New and Potent Cardiac Bipyridine, on Isolated Tissues From Several Animal Species. *J. Cardiovasc. Pharmacol.* **1983**, *5*, 804–811.

(3) Moos, W. H.; Humblet, C. C.; Sircar, I.; Rithner, C.; Weishaar, R. E.; Bristol, J. A.; McPhail, A. T. Cardiotonic Agents. 8. Selective Inhibitors of Adenosine 3′,5′-Cyclic Phosphate Phosphodiesterase III. Elaboration of a Five-Point Model for Positive Inotropic Activity. *J. Med. Chem.* **1987**, *30*, 1963–1977.

(4) Alousi, A. A.; Canter, J.; Roth, G.; Montenaro, M.; Fort, D.; Hamel, D. The Inotropic and Vasodilatory Activity of Medorinone in Dogs. *FASEB J.* **1988**, *2*, A365 (abstract). Silver, P. J.; Hamel, L. T.; Bentley, R. G.; Dillon, K.; Connell, M. J.; O'Connor, B.; Ferrari, R. A.; Pagani, E. D. Inhibition of Cardiovascular Low-Km cAMP Phosphodiesterase Activity by Medorinone. *Drug Dev. Res.* **1990**, *21*, 93–104. Silver, P. J.; Lepore, R. E.; Canniff, P. C.; O'Connor, B.; Lemp, B.; Harris, A. L. Activation of cAMP System by Medorinone Correlates with Positive Inotropy or Vasorelaxation. *Drug Dev. Res.* **1990**, *21*, 105–117. Lee, K. C.; Ezrin, A. M.; Pagani, E. D.; Canniff, P. C.; Hamel, D. W.; Fort, D. J.; Silver, P. J. Cardiovascular Effects of Medorinone in β-Adrenoreceptor-Blocked and Non-Blocked Anesthetized Dog. *Drug. Dev. Res.* **1990**, *21*, 119–133.

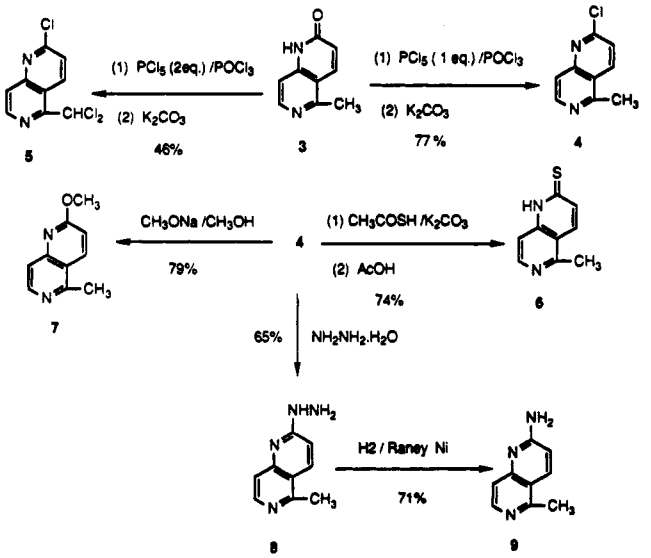
(5) Singh, B.; Lesher, G. Y. A Facile and Novel Synthesis of 1,6-Naphthyridin-2(1H)-ones. *J. Heterocycl. Chem.* **1990**, *27*, 2085–2091.

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

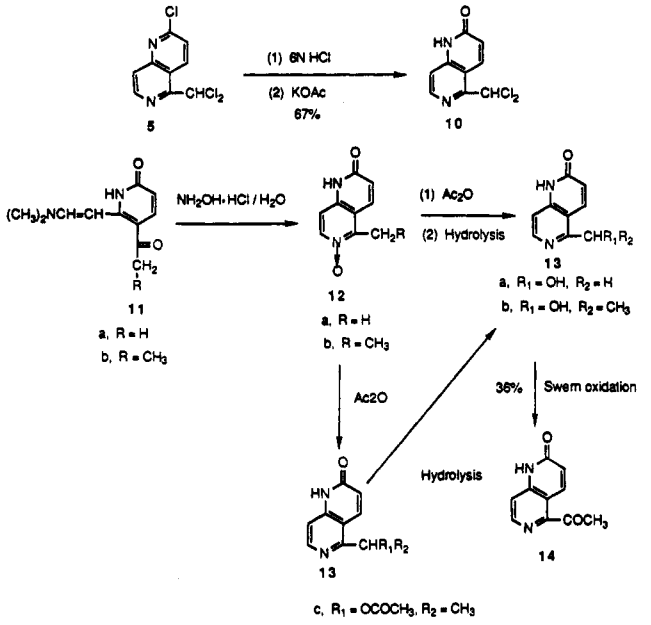
(7) Katada, M. Polarization of Aromatic Heterocyclic Compounds. LIIb. Reaction Between Pyridine 1-Oxide and Acid Anhydrides. *J. Pharm. Soc. Jpn* **1947**, *67*, 51–52; *Chem. Abstr.* **1951**, *45*, 9536*d*.

0022-2623/92/1835-4858$03.00/0

Scheme I



Scheme II

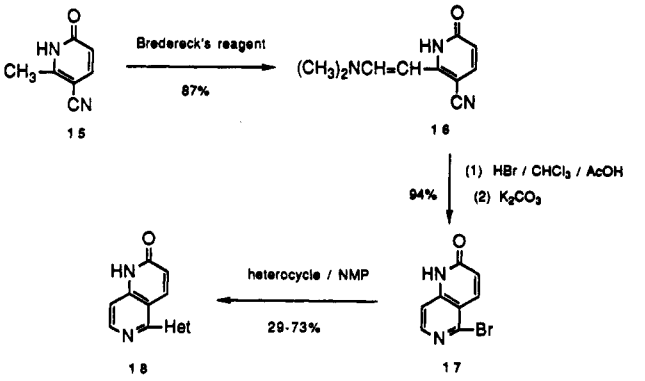


yield. Hydrolysis of the acetate **13c** gave alcohol **13b** in 73% yield, which in turn was converted to ketone **14** by Swern oxidation[8] in 36% yield.

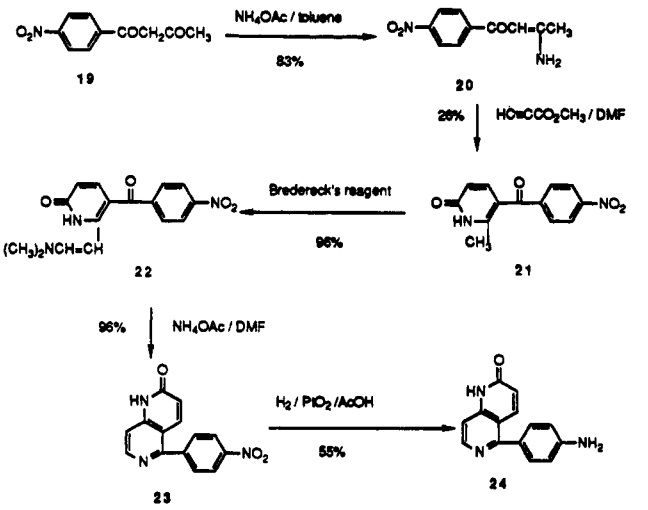
The synthesis of 5-heteroarylnaphthyridinones **18** is shown in Scheme III. Condensation of the activated methyl group of pyridinone **15** with Bredereck's reagent[9] resulted in the formation of enamine **16** in 87% yield. Treatment of **16** with hydrogen bromide resulted in intramolecular cyclization 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 $(CH_3)_2NCH(OCH_3)_2$ 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-1*H*-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

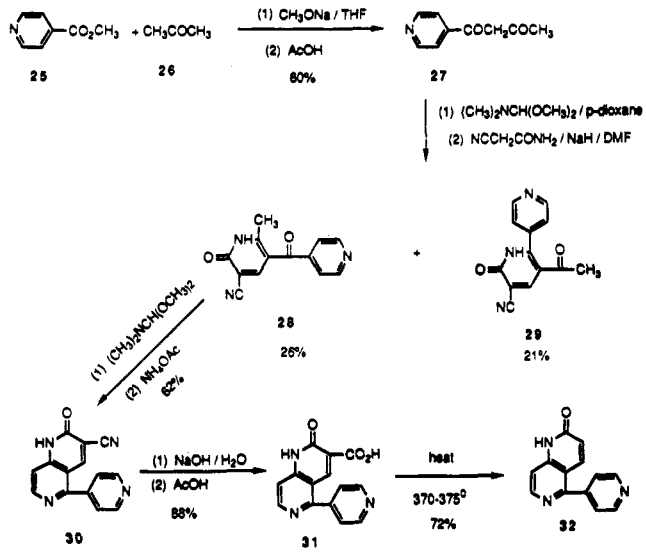
(8) Omura, K.; Sharma, A. K.; Swern, D. Dimethyl Sulfoxide-Trifluoroacetic Acetic Anhydride: A New Reagent for Oxidation of Alcohols to Carbonyls. *J. Org. Chem.* **1976**, *41*, 957–962.

(9) Haeflinger, H.; Knecht, H. Benz[c,d]indoles-I. The Use of tert-Butoxybis(dimethylamino)methane as a Condensation Agent. *Tetrahedron Lett.* **1984**, *25*, 285–288.

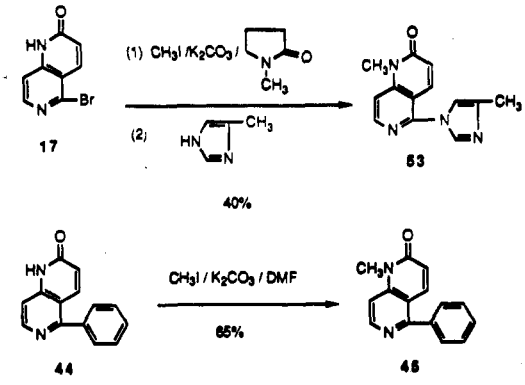
(10) Sardella, D. J.; Heinert, D. H.; Shapiro, B. L. Nuclear Magnetic Resonance Studies of Enol-Enol and Keto-Enol Equilibria in Substituted Benzoylacetones. *J. Org. Chem.* **1969**, *34*, 2817–2821.

(11) Longo, E.; Mugnaioni, M. Two New Derivatives of γ-Pyridylpyrimidine. *Boll. Chim. Farm.* **1961**, *100*, 430–432; *Chem. Abstr.* **1962**, *57*, 8576d.

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, $CONH_2$, OH, and NH_2 (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 $CHCl_2$ 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(1*H*)-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 ^{1}H-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 $POCl_3$ (500 mL) was added PCl_5 (42 g, 0.2 mol). The resulting mixture was heated under reflux for 7 h and then most of the $POCl_3$ was removed under reduced pressure. The dark residue was slurried in $CHCl_3$ (500 mL) and poured slowly into a vigorously stirred mixture of K_2CO_3 (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 K_2CO_3 was added to keep the reaction mixture on the basic side. The organic layer was separated and dried ($MgSO_4$). Removal of $CHCl_3$ gave 37.8 g of a purple solid which was purified by column chromatography (SiO_2 600 g, 5% MeOH in Et_2O). Recrystallization from Et_2O–hexanes gave 27.5 g (77%) of a pale yellow fluffy solid: mp 98–100 °C; ^{1}H NMR ($CDCl_3$) 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, CH_3). Anal. ($C_9H_7ClN_2$) C, H, N.

5-Methyl-1,6-naphthyridin-2(1*H*)-thione (6). A mixture of thiolacetic acid (7.6 g, 0.1 mol), K_2CO_3 (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; ^{1}H NMR (DMSO-d_6) 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, CH_3). Anal. ($C_9H_8N_2S$) C, H, N.

2-Methoxy-5-methyl-1,6-naphthyridine (7). A mixture of 4 (17.8 g, 0.1 mol), CH_3ONa (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 $CHCl_3$ (400 mL) and water (200 mL). The organic phase was separated, dried ($MgSO_4$), and concentrated to dryness under reduced pressure to give a solid residue which was recrystallized from Et_2O–hexanes to give 14.1 g (79%) of tan needles: mp 74–77 °C; ^{1}H NMR ($CDCl_3$) 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, OCH_3), 2.85 (s, 3 H, CH_3). Anal. ($C_{10}H_{10}N_2O$) 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_2CO_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_3COOD) 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. ($C_9H_{10}N_4 \cdot 2HCl$) 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_3COOD) 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. ($C_9H_9N_3$) 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_2CO_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_2CO_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_2O). The least polar main component was collected and recrystallized from Et_2O–hexanes to give 22.4 (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. ($C_9H_5Cl_3N_2$) C, H, N.

5-(Dichloromethyl)-1,6-naphthyridin-2(1*H*)-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_3COOD) 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. ($C_9H_6Cl_2N_2O$) C, H, N.

5-Methyl-1,6-naphthyridin-2(1*H*)-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_3COOD) 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. ($C_9H_8N_2O_2$) C, H, N.

5-(Hydroxymethyl)-1,6-naphthyridin-2(1*H*)-one (13a). A mixture of **12a** (5 g, 24 mmol) and Ac_2O (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_2CO_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_3COOD) 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_2O). Anal. ($C_9H_8N_2O_2$) C, H, N.

5-Ethyl-1,6-naphthyridin-2(1*H*)-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_3COOD) 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_2CH_3), 1.55 (t, J = 7.5 Hz, 3 H, CH_2CH_3). Anal. ($C_{10}H_{10}N_2O_2 \cdot {}^1/_2H_2O$) C, H, N.

5-[1-(Acetyloxy)ethyl]-1,6-naphthyridin-2(1*H*)-one (13c). A mixture of **12b** (110.5 g, 0.58 mol) and Ac_2O (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_2Cl_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_2O–5% MeOH in Et_2O) 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. ($C_{12}H_{12}N_2O_3$) C, H, N.

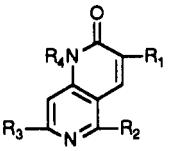
5-(1-Hydroxyethyl)-1,6-naphthyridin-2(1*H*)-one (13b). A mixture of **13c** (34.8 g, 0.15 mol), NaOH (16 g, 0.4 mol), water (50 mL), and MeOH (200 mL) was stirred at room temperature overnight and then treated with AcOH (20 mL). The resulting mixture was concentrated to dryness under reduced pressure 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_3COOD) 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. ($C_{10}H_{10}N_2O_2$) C, H, N.

5-Acetyl-1,6-naphthyridin-2(1*H*)-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 $(CF_3CO)_2O$ (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_3N (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. ($C_{10}H_8N_2O_2$) 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 $[(CH_3)_2N]_2CHOC(CH_3)_3$ (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, $(CH_3)_2NCH$=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, $(CH_3)_2NCH$=CH], 2.92 [s, 6 H, $N(CH_3)_2$]. Anal. ($C_{10}H_{11}N_3O$) C, H, N.

5-Bromo-1,6-naphthyridin-2(1*H*)-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(1*H*)-ones



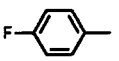
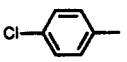
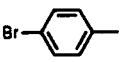
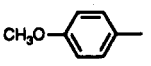
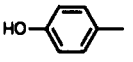
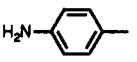
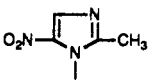
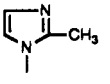
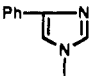
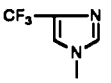
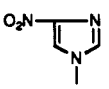
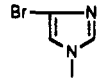
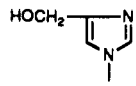
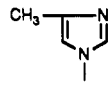
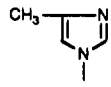
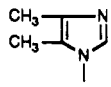
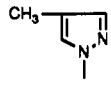
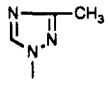
compd[a]	R_1	R_2	R_3	R_4	cAMP PDE III	
					% inhibition (10 μM)[b]	IC_{50},[c,d] μM
3	H	CH_3	H	H	67	0.55
33	H	CH_3	H	CH_3	17	
4[e]					18	
7[e]					21	
9[e]					24	
6[e]					81	0.96
34	CH_3	H	H	H	37	
35	H	H	CH_3	H	53	
36	CN	CH_3	H	H	52	
37	$CONH_2$	CH_3	H	H	39	
38	OH	CH_3	H	H	51	
39	NH_2	CH_3	H	H	59	
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 μM)[b]	cAMP PDE III IC_{50},[c,d] μM
18f	H		H	H	43	
18g	H		H	H	58	
18h	H		H	H	56	
18i	H		H	H	72	2.8
53	H		H	CH_3	21	
18j	H		H	H	28	
18k	H		H	H	39	
18l	H		H	H	30	
18m	H		H	H	50	
18n	H		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; ^{1}H 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(1*H*)-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, ^{1}H 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_4OAc (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(1*H*)-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; ^{1}H 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.

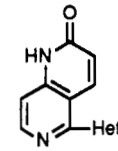
6-[2-(Dimethylamino)ethenyl]-5-(4-nitrobenzoyl)-2(1*H*)-pyridinone (22). A stirred mixture of **21** (46 g, 0.18 mol), Bredereck's reagent (41.7 mL, 0.2 mol), and 1,4-dioxane (500 mL) was heated under reflux for 2 h and then cooled to room temperature. The orange crystalline product was collected to 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(1*H*)-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; ^{1}H NMR (CF_3COOD) δ 9.00–7.00 (m, 8 H, arom). Anal. ($C_{14}H_{19}N_3O_3$) C, H, N.

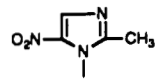
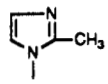
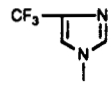
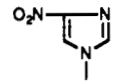
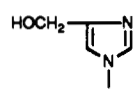
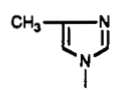
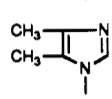
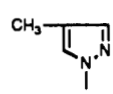
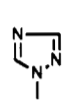
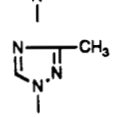
5-(4-Aminophenyl)-1,6-naphthyridin-2(1*H*)-one (24). A mixture of **23** (10.7 g, 40 mmol), PtO_2 (0.3 g), and AcOH (300 mL) was reduced on a Parr hydrogenator until the required amount of H_2 was absorbed (0.12 mol, 1 h). The catalyst was filtered off on a Celite pad and the filtrate was concentrated 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; ^{1}H 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-(1*H*)-ones



18

compd	Het	mp, °C	yield, %	crystn solv	formula[a]	^{1}H NMR[b]
18a		>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′-CH_3)
18b		>300	43	DMF	$C_{12}H_{10}N_4O$	
18c		263–265	32	EtOH	$C_{17}H_{12}N_4O$	
18d		216–218	35	2-PrOH	$C_{12}H_7F_3N_4O$	
18e		>300	29	DMF	$C_{11}H_7N_5O_3$	
18f		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		259–261	19	EtOH	$C_{12}H_{10}N_4O_2$	
18h		290–291	42	DMF	$C_{11}H_8N_4O$	
18i		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, CH_3)
18j		256–258	53	EtOH	$C_{13}H_{12}N_4O$	
18k		>300	52	DMF	$C_{11}H_8N_4O$	
18l		>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, CH_3)
18m		>300	52	DMF	$C_{10}H_7N_5O$	
18n		>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, CH_3)

[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 CF_3COOD 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 Et_2O, and dissolved in water (400 mL). The resulting solution was acidified with AcOH and the resulting oily product was extracted with $CHCl_3$ (2 × 300 mL). Removal of $CHCl_3$ on a rotary evaporator gave 164.5 g of a brown liquid which solidified on cooling. Recrystallization from Et_2O–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), $(CH_3)_2NCH(OCH_3)_2$ (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 Et_2O (600 mL). The solid was collected, washed with Et_2O, 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; ^1H 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; ^1H 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), $(CH_3)_2NCH(OCH_3)_2$ (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; ^1H 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(1*H*)-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; ^1H 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-1*H*-imidazol-1-yl)-1,6-naphthyridin-2(1*H*)-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-1*H*-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; ^1H 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(1*H*)-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; ^1H 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 48000*g* 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.

(12) Thompson, W. J.; Terasaki, W. L.; Epstein, P. M.; Strada, S. J. Assay of Cyclic Nucleotide Phosphodiesterase and Resolution of Multiple Molecular Forms of the Enzyme. *Adv. Cycl. Nucleotide Res.* **1979**, *10*, 69–92.

(13) Weishaar, R. E.; Burrows, S. D.; Kobylarz, D. C.; Quade, M. M.; Evans, D. B. Multiple Molecular Forms of Cyclic Nucleotide Phosphodiesterase in Cardiac and Smooth Muscle and in Platelets. Isolation, Characterization, and Effects of Various Reference Phosphodiesterase Inhibitors Cardiotonic Agents. *Biochem. Pharmacol.* **1986**, *35*, 787–800.

(14) Alousi, A. A.; Farah, A. E.; Lesher, G. Y.; Opalka, C. J., Jr. Cardiotonic Activity of Amrinone-WIN 40680 [5-Amino-3,4′-bipyridin-6(1H)-one]. *Circ. Res.* **1979**, *45*, 666–677.