ment. The LD_{50} values, 95% confidence interval, and a test of significance ($p \leq 0.05$) were calculated by the method of Litchfield and Wilcoxon.[21] A protective ratio was determined as the quotient of the LD_{50} value of the treated groups over the LD_{50} of the nerve agent alone.

Acknowledgment. We thank Col. J. von Bredow and Dr. C. J. Cavallito for many helpful discussions and C. K. Dietrich, N. Einhorn, and D. A. Ferrick for excellent technical assistance. This work was supported by Contract No. DAMD17-82-C-2167 from the Army Medical Research and Development Command. The views, opinions, and findings contained in this report are those of the authors and should not be construed as an official Department of the Army position.

Chemistry and Positive Inotropic Effect of Pelrinone and Related Derivatives. A Novel Class of 2-Methylpyrimidones as Inotropic Agents

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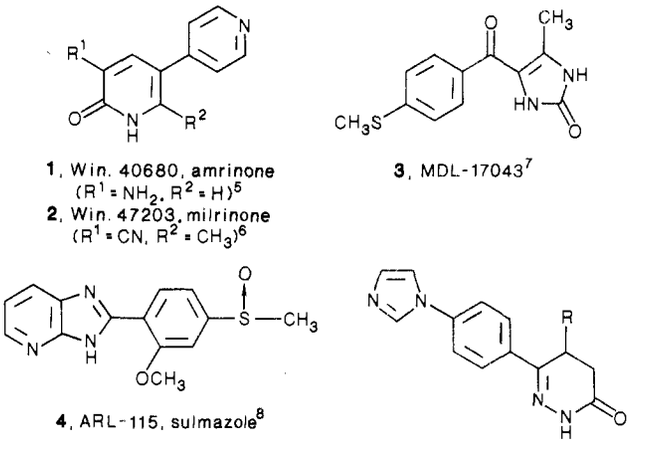
A novel series of pyrimidine derivatives was synthesized and evaluated for positive inotropic activity. Inotropic and chronotropic effects were determined in vitro in cat papillary muscle and right atrium, respectively. Selected compounds were then evaluated in vivo in a dog heart failure model. Changes in ventricular dP/dt, heart rate, and blood pressure were monitored. Several of these agents produced relatively minor changes in heart rate. This class of agents demonstrated a varying degree of vasodilator effects concomitant with increases in ventricular contractility. The most potent analogues, **9**, **48**, and **49**, were evaluated orally in conscious dogs with implanted Konisberg pressure transducers, and their effect on left ventricular dP/dt was compared with that of milrinone. Mechanistically, the agents of this novel class appear not to mediate their effect either via β-receptors or inhibition of Na^+/K^+-ATPase. A major component of their inotropic effect is mediated by the inhibition of cardiac phosphodiesterase (PDE)-Fr. III. This was clearly demonstrated by **9**, **48**, and **49**. Compound **48** was found to be the most potent inhibitor of PDE-Fr. III from among the compounds tested in this assay.

There are several classes of compounds known to exert positive inotropic effect. Of these, there are two that have found utility in the treatment of congestive heart failure in humans, namely, the cardiac glycosides and the catecholamines, both of which suffer from serious disadvantages. The cardiac glycosides, in spite of their long-standing reputation in therapy, have a very narrow therapeutic ratio, are potentially arrhythmogenic, and can cause digitalis intoxication.[1] Serious limitations of the catecholamines[2] include the potential to cause tachycardia concomitant with an increase in contractility, which can lead to cardiac arrhythmia and increased myocardial oxygen consumption. Furthermore, the catacholamines have a short duration of action and often cannot be administered orally.[3]

In recent years, attention has been directed toward the development of orally active, nonsteroidal, noncatechol cardiotonic agents. An ideal agent of this kind would exert direct positive inotropic effect on the heart, without increasing heart rate or myocardial oxygen consumption and without causing vasodilation in the capacitance vessels; moreover, it would possess a high toxic to therapeutic ratio. The attention of several groups has been directed toward generating agents that modulate intracellular levels of cyclic nucleotides. This has led to a variety of phosphodiesterase inhibitors,[4] some of which are shown in Chart I.

The pyrimidine moiety represents an integral part of a number of phosphodiesterase inhibitors.[4] We report herein on some novel derivatives of pyrimidine, identified as potent, positive inotropic agents, which also possess potent phosphodiesterase inhibitory activity.

Chart I



Chemistry. Appropriately functionalized ketene acetals have been reported[10] to yield a variety of heterocyclic

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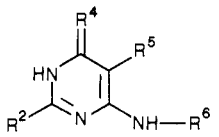
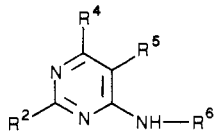
(1) Mason, D. T.; Amsterdam, E. A.; Lee, G. *Congestive Heart Failure*; Dun-Donnelly: New York, 1976; p 332.
(2) Sonnenblick, E. H.; Frishman, W. H.; LeJemtel, T. H. *N. Engl. J. Med.* **1979**, *300*, 17.
(3) Goldberg, L. I. *Am. J. Cardiol.* **1968**, *22*, 177.
(4) Weishaar, R. E.; Cain, M. E.; Bristol, J. A. *J. Med. Chem.* **1985**, *28*, 537.
(5) Alousi, A. A.; Farah, A. E. *Trends Pharmacol. Sci.* **1980**, *2*, 143.
(6) Alousi, A. A.; Helsfosky, A.; Montenaro, M. J.; Cicero, F. *Fed. Proc., Fed. Am. Soc. Exp. Biol.* 1981, *40*, (abstract) 2478.
(7) Dage, R. C.; Roebel, E. L.; Hsieh, C. P.; Weiner, D. L.; Wooward, J. K. *J. Pharmacol.* **1982**, *4*, 500.
(8) Diederen, W.; Kadatz, R. *Arzneim.-Forsch.* **1981**, *31*, 141.
(9) (a) Sircar, I.; Duell, B. L.; Bobowski, G.; Bristol, J. A.; Evans, D. B. *J. Med. Chem.* **1985**, *28*, 1405. (b) Sircar, I.; Duell, B. L.; Cain, M. H.; Burke, S. E.; Bristol, J. A. *J. Med. Chem.* **1986**, *29*, 2142.

0022-2623/88/1831-0814$01.50/0

Table I. Effect of Varying Substitution Patterns on the in Vitro Inotropic and Chronotropic Activity and Physical Properties of Pyrimidone Derivatives



9–26, 33–50



28–32

no.	R^2	R^4	R^5	R^6	% yield	method	formula (anal.)	crystn[c] solv	mp, °C	EC_{50}[f] pap mus (n)	EC_{50}[f] rt atria (n)[h]	C/I[g]
9	CH_3	O	CN	CH_2-3-pyridinyl[e]	76.8	A	$C_{12}H_{11}N_5O$ (C, H, N)	D	>260	5.9 ± 0.8 (8)	212 ± 37 (6)	36
10	CH_3	O	CN	CH_2-2-pyridinyl	80	A	$C_{12}H_{11}N_5O$ (C, H, N)	D/E	>250	43 ± 2 (3)	180 (2)	4
11	CH_3	O	CN	CH_2-4-pyridinyl	64	A	$C_{12}H_{11}N_5O$·HCl (C, H, N)	M/W	>250	4.4 ± 0.8 (4)	250 ± 100 (5)	57
12	CH_3	O	CN	$(CH_2)_2$-2-pyridinyl	48.3	A	$C_{13}H_{13}N_5O$ (C, H, N)	M	268-270	37 ± 2 (3)	71 ± 4 (3)	2
13[a]	CH_3	O	CN	$(CH_2)_2$-3-pyridinyl	90	A	$C_{13}H_{13}N_5O$ (C, H, N)[a]	D	>250	230 ± 30 (3)	300 ± 90 (3)	1.3
14	CH_3	O	CN	$(CH_2)_2$-4-pyridinyl	52.8	A	$C_{13}H_{13}N_5O$ (C, H, N)	D	>250	6.5 ± 3 (4)	396 ± 216 (4)	61
15	CH_3	O	CN	$(CH_2)_2$-phenyl	39.5	A	$C_{14}H_{14}N_4O$ (C, H, N)	M	272-274	20 ± 1.8 (3)	>1000 (4)	>50
16	CH_3	O	CN	CH_2-α-furyl	58	A	$C_{11}H_{10}N_4O_2$ (C, H, N)	M	297-300	10 ± 0.5 (3)	88 ± 2 (4)	8.8
17	CH_3	O	CN	CH_2CH_3	42	B	$C_8H_{10}N_4O$ (C, H, N)	M	>290	2.6 ± 0.3 (6)	300 ± 90 (6)	115
18	CH_3	O	CN	$CH_2CH_2CH_3$	31.5	B	$C_9H_{12}N_4O$ (C, H, N)	D/E	>270	1.9 ± 0.8 (4)	17 ± 5 (3)	9
20	CH_2CH_3	O	CN	CH_2-3-pyridinyl	49	A	$C_{13}H_{13}N_5O$·HCl	M/W	>265	5.5 ± 2 (3)	86 ± 30 (3)	16
21	$CH(CH_3)_2$	O	CN	CH_2-3-pyridinyl	44	A	$C_{14}H_{15}N_5O$ (C, H, N)	M/W	>260	33 ± 10 (3)	10 ± 40 (3)	3
22	$C(CH_3)_3$	O	CN	CH_2-3-pyridinyl	18	A	$C_{15}H_{17}N_5O$·HCl	M/W	>260	55 ± 20 (4)	110 ± 40 (3)	2
23	H	O	CN	CH_2-3-pyridinyl	27.4	A	$C_{11}H_9N_5O$ (C, H, N)	M	236-245	inactive[d]		
24[a]	C_6H_5	O	CN	CH_2-3-pyridinyl	46.3	A	$C_{17}H_{13}N_5O$ (C, H, N)	D	>250	inactive[d]		
25	$CH_2C_6H_5$	O	CN	CH_2-3-pyridinyl	19	A	$C_{16}H_{15}N_5O$ (C, H, N)	A	>260	72 ± 40 (3)	~1000 (3)	14
26	OH	O	CN	CH_2-3-pyridinyl	38	C	$C_{11}H_9N_5O_2$ (C, H, N)	M/D	>300	inactive[d]		
28[a]	CH_3	Cl	CN	CH_2-3-pyridinyl	71.6[b]		$C_{12}H_{10}ClN_5$ (C, H, N)[a]	C/H	165-167	inactive[d]		
29	CH_3	$NHCH_3$	CN	CH_2-3-pyridinyl	56[b]		$C_{12}H_{10}ClN_5$ (C, H, N)[a]	C/H	165-167	inactive[d]		
30	CH_3	OCH_3	CN	CH_2-3-pyridinyl	78.2[b]		$C_{13}H_{14}N_6$ (C, H, N)	M	218-221	inactive[d]		
31[a]	CH_3	SH	CN	CH_2-3-pyridinyl	32.46[b]		$C_{12}H_{11}N_5S$ (C, H, N)[a]	D	>310	14 ± 4 (4)	80 ± 40 (5)	6
32	CH_3	SCH_3	CN	CH_2-3-pyridinyl	51.47	D	$C_{13}H_{13}N_5S$ (C, H, N)	C/H	155-156	inactive[d]		
33	CH_3	O	$CONH_2$	CH_2-3-pyridinyl	85.3[b]		$C_{12}H_{13}N_5O_2$ (C, H, N)	D	264-266	inactive[d]		
37	CH_3	O	Cl	$CH_2CH_2C_6H_5$	51	D	$C_{13}H_{14}ClN_3O$ (C, H, N)	M	230-232	1.2 ± 0.3 (5)	>1000 (6)	>833
43	CH_3	O	NO_2	CH_2CH_3	75	E	$C_7H_{10}N_4O_3$ (C, H, N)	M	279-280	25 ± 1 (6)	230 ± 70 (5)	9
44	CH_3	O	NO_2	CH_2-3-pyridinyl	64	E	$C_{11}H_{11}N_5O_3$ (C, H, N)	M	279-280	5.3 ± 2 (5)	>1000 (3)	>189
45[a]	CH_3	O	$COCH_3$	$CH_{24}CH_3$	26	F	$C_9H_{13}N_3O_2$ (C, H, N)[a]	M	241-242	inactive[d]		
46[a]	CH_3	O	$COCH_3$	CH_2-3-pyridinyl	48	F	$C_{13}H_{14}N_4O_2$ (C, H, N)[a]	C/H	235-236	inactive[d]		
48	CH_3	O	Br	CH_2-3-pyridinyl	76	D	$C_{11}H_{11}BrN_4O$ (C, H, N)	M	258-259	3.9 ± 1.7 (7)	380 ± 70 (5)	97
49	CH_3	O	Br	CH_2CH_3	66[b]		$C_7H_{10}BrN_3O$ (C, H, N)	Ea	201-202	4.1 ± 1.1 (8)	420 ± 70 (5)	102
50	CH_3	O	Br	$CH_2CH_2C_6H_5$	67	D	$C_{13}H_{14}BrN_3O$ (C, H, N)	M/C	237-238	6.1 ± 2.2 (7)	>1000 (7)	>164
milrinone										7.7 ± 1.6 (6)	~300 (4)	~39
MDL-17043										90 ± 20 (5)	200 (2)	2.2

[a] For compounds 13 C: calcd 61.62, found 60.62; 24 C: calcd 67.31, found 66.70; 28, N: calcd 26.96, found 26.5; 31, C, N: calcd 56.01, 27.22, found 55.48, 26.68; 45 C: calcd 55.83, found 55.25; 46, C: calcd 60.47, found 59.99. [b] For the method of preparation, see the Experimental Section. [c] D = dimethylformamide, E = ether, M = methanol, W = water, A = acetone, C = chloroform, H = hexane. [d] Compound showed <50% increase in contractility at a concentration of 100 μmol/L. [e] Pelrinone. [f] Dose in μmol/L that caused 50% increase over baseline contractility (pap mus) or spontaneous beating rate (rt atria). [g] Ratio of chronotropic EC_{50} to inotropic EC_{50}. [h] n represents the number of determinations.

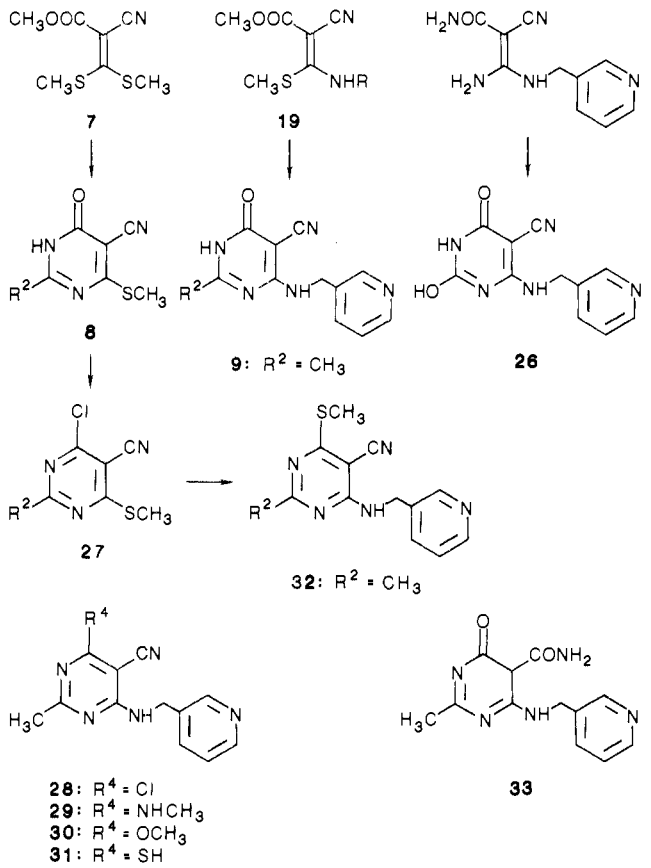
systems useful as intermediates for potential drug molecules. For example, reaction of methyl 2-cyano-3,3-bis(methylthio)acrylate[11] (7) with acetamidine hydrochloride has been reported.[12] Although this reaction was not reproducible in our hands, we were able to carry out the reaction with NaH–DMF at room temperature to yield

(10) For a detailed list of references, see: Tominaga, Y.; Kohra, S.; Okuda, H.; Ushirogochi, A.; Matsuda, Y.; Kobayashi, G. *Chem. Pharm. Bull.* **1984**, *32*, 122.

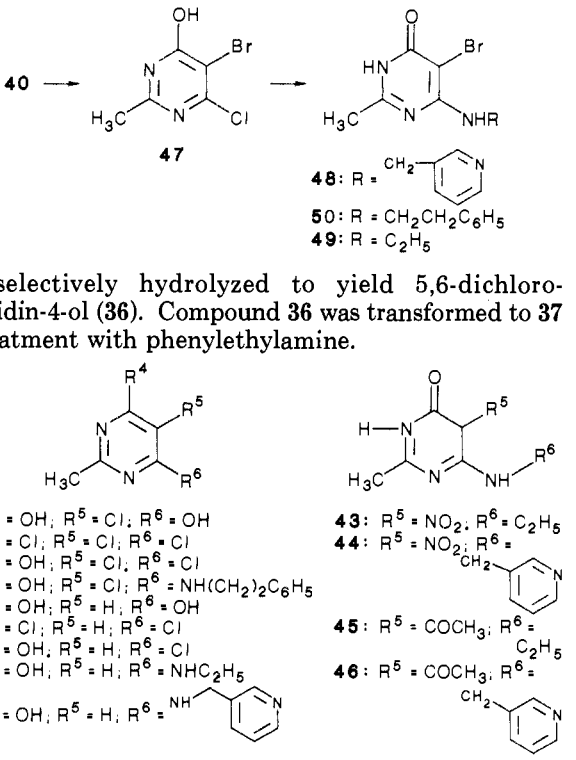
(11) Gomper, R.; Topfl, W. *Chem. Ber.* **1962**, *95*, 2865 and 2871.

(12) Kisaki, S.; Tominaga, Y.; matsuda, Y.; Kobayashi, G. *Chem. Pharm. Bull.* **1974**, *22*, 2246.

Scheme I



Scheme II



1,4-dihydro-2-methyl-6-(methylthio)-4-oxo-5-pyrimidinecarbonitrile (8) (Scheme I). The reaction of 8 with suitable amines in refluxing dimethoxyethane led to 6-substituted amino analogues (method A, Table I) 9–18. Alternatively, these compounds can be prepared by treatment of 7, first with the appropriate amine to obtain 19, followed by condensation with acetamidine hydrochloride (method B) to yield some of the products listed in Table I.

The various amidines required to change the substituents at C-2 of the pyrimidines were synthesized as reported before.[13] Their condensation by method A or B led to the synthesis of compounds 20–25. Compound 26 was synthesized as follows. The methylthio groups of bis(methylthio)ketene acetal obtained from cyanoacetamide[11] were successively displaced with 3-picolylamine, followed by ammonia, to yield β-amino-β-[(3-pyridinylmethyl)amino]-α-cyanoacrylamide. This was reacted with carbonyldiimidazole to yield 26.

To vary substituents at position C-4 of the pyrimidine, compound 8 was treated with phosphorus oxychloride to yield the desired chloro derivative 27. Treatment of 27 with (aminomethyl)pyridine yielded 32. Reaction of sodium salt of compound 9 in the presence of phosphorus oxychloride yielded the chloro analogue 28. This compound, when reacted with suitable nucleophilic agents, led to products 29–31 (Scheme I, Table I).

To examine the effect of varying substituents at C-5, the following reactions were performed. Hydration of cyano group of 9 with concentrated sulfuric acid yielded the amide 33. Condensation of diethyl 2-chloromalonate with acetamidine hydrochloride yielded 5-chloro derivative 34. This was transformed to the 4,5,6-trichloro-2-methylpyrimidine (35) as described.[14] The resulting compound was selectively hydrolyzed to yield 5,6-dichloropyrimidin-4-ol (36). Compound 36 was transformed to 37 by treatment with phenylethylamine.

The C-5 unsubstituted derivatives needed were synthesized by reported methodology.[14] Diol 38 was transformed to dichloro derivative 39 with phosphorus oxychloride. Compound 39 was selectively hydrolyzed with sulfuric acid to yield 40, which, upon reaction with appropriate amines, yielded products 41 and 42. Treatment of unsubstituted amines with fuming nitric acid yielded 43 and 44. Reaction of 41 and 42 in the presence of phosphorus oxychloride and acetic acid[15] led to the isolation of 45 and 46. Bromination of 6-chloro derivative 40 with bromine in chloroform–acetic acid yielded the 5-bromo-6-chloro analogue 47 (Scheme II). Treatment of 47 with appropriate amines led to 48 and 50. Compound 49 was synthesized by bromination of 41.

Biology

The inotropic activity of the compounds described above was first assayed in vitro in cat papillary muscle.[16] The chronotropic effect was assayed in vitro in the isolated right atrium of the cat. Compounds demonstrating an EC_{50} of 1×10^{-4} M or lower in the inotropic assay were considered active and were further investigated. The primary in vivo screen was performed in a dog model of heart failure, in which cardiac failure was induced with pentobarbital. The dose required to increase dP/dt by 50% (ED_{50}) was determined iv and id. The heart rate and blood pressure were also recorded in this model as was the dose needed to drop blood pressure by 20% (ED_{20}) in selected cases. The oral activity of selected compounds was determined in conscious Konigsberg-implanted dogs,[17] and cardiotonic effects were compared with those of nonsteroidal, noncatechol inotropic agents.

(13) Dox, A. W. *Organic Syntheses*; Wiley: New York, 1941; Collect. Vol. I, p 5.
(14) Borwn, D. J. *J. Chem. Soc.* **1964**, 3204.
(15) Vul'fson, N. S.; Zhurin, R. B. *Zh. Obshch. Khim.* **1961**, *31*, 281; *Chem. Abstr.* **1961**, *55*, 24759.
(16) Cattell, McK.; Gold, H. *J. Pharmacol. Exp. Ther.* **1938**, *62*, 116.
(17) Dormer, K. J. *Denshi Igaku* **1980**, 69.
(18) Davis, P. W.; Brody, T. M. *Biochem. Pharmacol.* **1966**, *15*, 703.
(19) Krikovetz, W.; Poch, G. *Adv. Cyclic Nucleotide Res.* **1972**, *1*, 261.
(20) Scholz, H.; Meyer, W. *Circulation* **1986**, *73* (Suppl III), 99.

Selected compounds of this series were tested for their ability to inhibit bovine heart phosphodiesterase (PDE). These studies were done with crude PDE as well as with purified fraction III-PDE.[21]

Structure-Activity Relationship

The data listed in Table I describes the EC_{50} of the variously substituted analogues for their effect on cat papillary muscle as a measure of their inotropic activity. In contrast, cat right atria were utilized to measure their effect on heart rate. Importantly, a drug that is clinically useful for the treatment of congestive heart failure should have minimal effect on heart rate at doses with optimal inotropic effect. For this reason, the higher the ratio of EC_{50}(chronotropy) to EC_{50}(inotropy) (C/I), the better the potential of the compound as a cardiotonic agent.

The pyridinylmethylamines (**9**–**14**, Table I) demonstrated marked differences in their C/I ratio, depending upon the position of pyridine nitrogen. The 2-pyridinyl analogues **10** and **12** were particularly poor in the selectivity of effect on papillary muscle and right atria. In contrast, 4-pyridinyl derivatives **11** and **14** showed consistently better selectivity for ventricular contractility versus tachycardia. The 3-pyridinyl analogue (**9**, pelrinone) was particularly interesting. In vitro, it showed a good separation of inotropic and chronotropic activity (C/I ratio of 36), and demonstrated in vivo, a duration of action superior to those of 4-pyridinyl analogues **11** and **14** (data not shown). Extending the methylene bridge of **9** by one carbon atom (**13**) resulted in a marked drop in inotropic as well as chronotropic activity. When the pyridine moiety was replaced with a phenyl or furyl, a significant degree of inotropic activity was retained; however, only phenyl analogue **15** retained good selectivity toward papillary muscle, whereas furyl derivative **16** showed very poor selectivity. Alkylamine derivatives **17** and **18** retained potent inotropic activity. The selectivity for papillary muscle in the case of **18** dropped strikingly relative to that of **17**. The above results suggest that the substituents at C-6 can be broadly varied and that this area of the molecule is perhaps not directly involved in its interaction with the receptor site. It is, however, possible that this portion may interact with the secondary hydrophobic region of the site involved in producing the inotropic and chronotropic responses. Such hydrophobic binding has been demonstrated for pyrimidines substituted at the 6-position in other enzyme binding studies.[22]

The substituent at position C-2 portrays an interestingly diverse activity. When the carbon load is increased from methyl (**9**) → ethyl (**20**) → isopropyl (**21**) → *tert*-butyl (**22**), there is a gradual but gentle drop in the inotropic activity, with concomitant loss in selectivity as noted by a low C/I ratio. Interestingly, unsubstituted analogue **23** was completely devoid of any activity, suggesting that perhaps the C-2 methyl substituent might block a metabolic change to which the unsubstituted molecule may be exposed. The most common ubiquitous enzyme to which the proton of a formamidine moiety can be a substrate is xanthine oxidase.[23] To test this hypothesis, compounds **9** and **23** were subjected to incubation with xanthine oxidase. It was noted that compound **23** rapidly disappeared from the incubation medium, suggesting it to be a substrate. Furthermore, compound **23**, at a concentration of 10^{-4} M, inhibited xanthine oxidase to the extent of 60–70%. In contrast, compound **9** did not have any inhibitory activity toward the enzyme. The potential metabolite **26** was synthesized and was found to be completely devoid of inotropic activity. These results support the notion that **23** was perhaps rapidly metabolized to the inactive hydroxy derivative **26**. The data of compounds **24** and **25** present another feature that may be important for the cardiotonic activity. The phenyl ring at C-2 in conjugation with **24** must produce enough perturbation in the electronic character of the pyrimidine ring to wipe out the biological activity. This electronic change must be, at least partially, neutralized by the introduction of a methylene bridge in compound **25**, so as to regenerate a significant degree of biological activity.

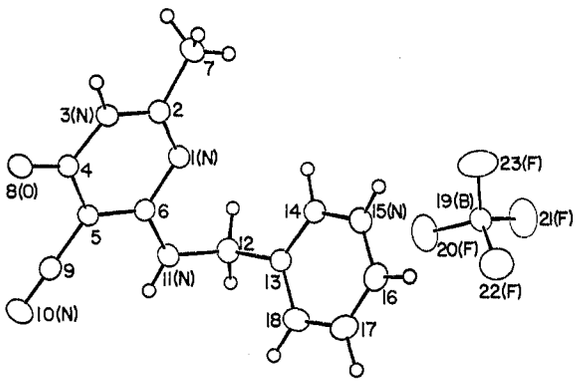


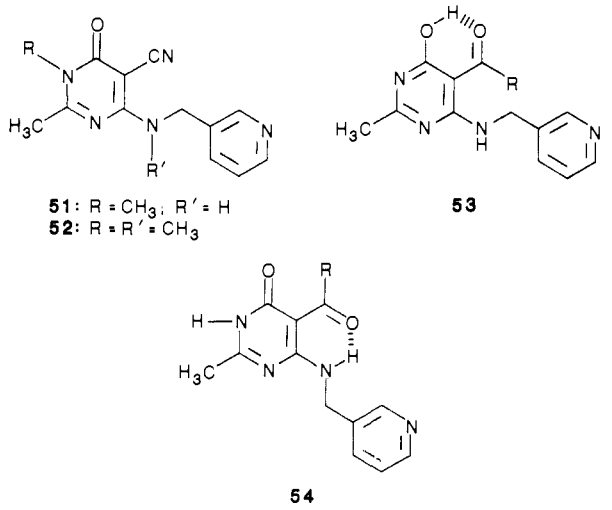
Figure 1. Single-crystal X-ray structure of pelrinone **9** tetrafluoroborate.

Compounds **28**–**32** demonstrate what appears to be the key feature needed for inotropic activity—the presence of an acidic proton. It is important to realize that compounds **28**–**30** and **32** are devoid of an acidic proton and are locked into an aromatic conformation that cannot tautomerize to their conjugated amidic conformation. These compounds were completely devoid of biological activity. The only compound that retained inotropic activity was, not unexpectedly, the thio analogue **31**, which, like pelrinone (**9**), retains the ability to tautomerize between pyrimidinol and the corresponding pyrimidone conformers. The importance of the presence of this acidic hydrogen was further confirmed by the fact that N-methylated analogues **51** and **52** also showed negligible activity (5% and 10% increase in contractility at 100 μM) relative to their desmethyl counterparts. The acidic proton throughout the formula scheme, as well as the *N*-alkyl group in compounds **51** and **52**, are tentatively assigned at 3-nitrogen. This assignment of proton at N-3 is based on analogy with the single-crystal X-ray structure of pelrinone (**9**) tetrafluoroborate salt (Figure 1).

Compounds **33**, **45**, and **46** have a carbonyl group located at the C-5 position. All these compounds were found to have little or no inotropic activity. It is conceivable that in these compounds hydrogen bonding, either through aryl form, as in **53**, or through exocyclic nitrogen, as in **54**, can cause major changes in electron distribution (Chart II). These changes may be sufficient to cause a disruption at the active site of interaction with the drug, resulting in the loss of activity. The nitro analogue **44** of pelrinone (**9**) was essentially equipotent as the inotropic agent; however, its EC_{50} for chronotropic activity exceeded 1000 μM/L, thus enhancing the ratio of C/I from 36 for **9** to >189 for **44**. A similar augmentation was not observed for the corresponding *N*-ethyl analogue **17** (C-5 CN) to **43** (C-5 NO_2). The C-5 halogenated compounds demonstrated enhanced specificity toward papillary muscle. Thus, C-5 bromo

(21) Thompson, W. J.; Terasaki, W. L.; Epstein, P. M.; Strada, S. J. *Adv. Cyclic Nucleotide Res.* **1979**, *10*, 69.
(22) Baker, B. R.; Kawazu, M. *J. Med. Chem.* **1967**, *10*, 311.
(23) Armstrong, F. B. *Biochemistry*, 2nd ed.; Oxford University: New York, 1983; p 424.

Chart II



analogue **48** had a C/I ratio of 97 compared to 36 for pelrinone (**9**). In the C-6 phenethylamine analogue, introduction of a halogen atom at C-5 chlorine (**37**) or bromine (**50**) in place of the cyano group (**15**) also enhanced the inotropic activity. Their chronotropic activity remained essentially unchanged. Because of their weak chronotropic activity however, their C/I ratio could not be determined conclusively.

Biological Results and Discussion

A major consequence of congestive heart failure is reduced cardiac output, which often causes the stimulation of neurohormonal mechanisms such as the sympathetic and the renin–angiotensin systems. These changes can lead to increased systemic vascular resistance, resulting in peripheral vasoconstriction. In these situations, vasodilators are often used as adjunct therapy concomitant with inotropes. It is therefore of interest to identify inotropic agents having intrinsic vasodilator activity.

We have noted a wide range of combinations of intrinsic inotropic and vasodilator properties in the pyrimidone derivatives studied. Several compounds were tested in the dog cardiac failure model, and their inotropic (ED_{50}) and vasodilator (ED_{20}) activities were recorded (see Table II). The ED_{50} represents a dose that causes a 50% increase in ventricular contractility (peak + dP/dt); ED_{20} refers to a dose that reduces mean arterial blood pressure (BP) by 20%. The proximity of the ratio ED_{20}/ED_{50} to 1 suggests the ability of the compound at the dose employed to increase the ventricular contractility and to lower blood pressure by 50% and 20%, respectively. Agents with ratios (ED_{20}/ED_{50}) close to 1 may represent the most efficient intervention in the palliation of congestive heart failure. The data, presented in Table II, suggest that pelrinone (**9**) is about 4-fold better in its inotropic effect relative to its vasodilatory action when administered intravenously. The specificity for the cardiac muscle is enhanced to more than 15-fold when the compound is administered intraduodenally. Replacing the C-5 nitrile function by bromine, as in **48**, appears to alter the lipophilicity of the molecule markedly, making the compound about 10-fold more potent in cardiac tissue and more than 30-fold more potent as a vasodilator when administered intraduodenally, as compared with **9**. Substitution of an ethyl group in place of the pyridinylmethyl moiety as in **17** produces an agent with an ED_{20}/ED_{50} ratio of 1.4 intravenously and 1.2 intraduodenally. Replacement of nitrile by a bromine atom in **17** generates compound **49**, which was found to be the most potent vasodilator in this model. At less than 3

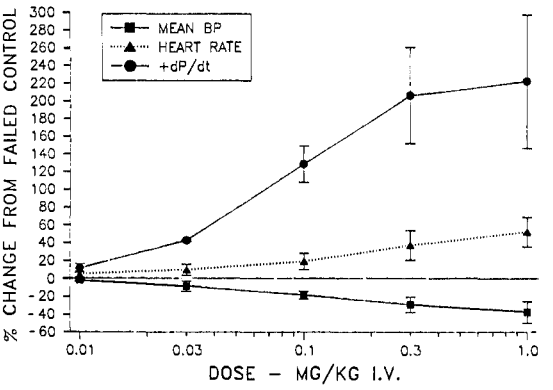


Figure 2. Effects of serial iv dosing with **9** in dogs with pentobarbital-induced cardiac failure. Points represent the mean ± SEM obtained in three dogs.

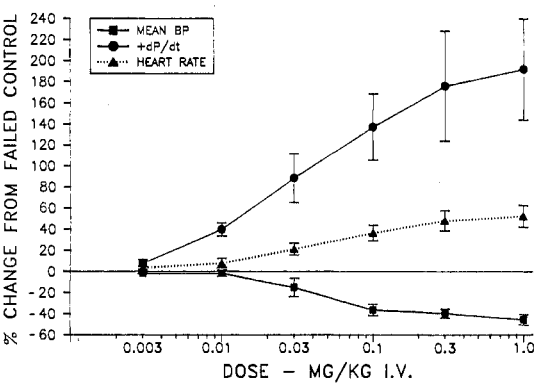


Figure 3. Effects of serial iv dosing with **48** in dogs with pentobarbital-induced cardiac failure. Points represent the mean ± SEM obtained in three dogs.

μg/kg, the compound lowered the mean arterial blood pressure by about 20%. Intraduodenally, however, the compound retained an ED_{20}/ED_{50} ratio of 1.6. Interestingly, in the pyridinylmethyl analogues (**9** and **48**), changing from nitrile to bromine introduces about 3-fold increase in the vasodilatory parameter and about 2-fold increase in the ventricular contractility upon intravenous administration. In contrast, with an ethyl side chain, going from **17** to **49**, there is a greater than 12-fold increase in vasodilatory activity as compared to ~2.7-fold increase in inotropic activity. The picture is quite different, however, when the results of intraduodenal administration are analyzed. In this case, the pyridinylmethyl analogues demonstrate a 10-fold improvement in ventricular contractility and a surprising increase (better than 30-fold) in vasodilatory potency.

In the case of phenethyl side chain represented by compounds **15** and **50**, upon intravenous administration, there is a marked increase in vasodilatory potency as well as in the inotropic effect. Rather unexpectedly, this relationship abruptly disappears when the compounds are administered intraduodenally. Thus, the cyano analogue **15** retains reasonable inotropic and vasodilatory potency id; in contrast, the C-5 bromo derivative **50** is only weakly active both as an inotrope and as a vasodilator.

Compounds **9**, **48**, and **49** represent the most interesting analogues in this series and were investigated extensively. Their cardiovascular profiles iv in anesthetized dogs are shown in Figures 2–4, respectively. In acutely instrumented anesthetized dogs, all three compounds showed a dose-related increase in cardiac contractile force. This inotropic effect was associated with slight changes in the heart rate. In the case of compound **9**, doses of 10 μg/kg to 1 mg/kg produced a dose-related improvement in

Table II. Results of Selected Pyrimidone Derivatives on the Iv and Id Inotropic Effect and Mean Arterial Blood Pressure in Dogs with Pentobarbital-Induced Heart Failure

no.	inotropic ED_{50}[a]	iv BP dec: ED_{20}[b]	$ED_{20}/ED_{50}(n)$	inotropic ED_{50}	id[d] BP dec: ED_{20}	ED_{20}/ED_{50}
9	0.033 ± 0.002	0.12 ± 0.06	4 (3)	0.2	>3	>15
15	0.07 ± 0.05	0.027 ± 0.01	0.4 (3)	0.22	0.3	1.36
17	0.027 ± 0.01	0.37 ± 0.017	1.4 (3)	0.06	0.07	1.2
48[c]	0.018 ± 0.007	0.041 ± 0.017	2.3 (3)	0.022	0.1	4.5
49[c]	0.01 ± 0.002	<0.003	<0.3 (3)	0.03	0.05	1.6
50	0.029 ± 0.005	<0.01	<0.3 (3)	>1	>1	
milrinone	0.016 ± 0.001	0.048 ± 0.014	3 (3)	0.04	0.1	2.5

[a] All ED are in mg/kg. [b] The dose that lowered blood pressure by 20%. [c] Compounds **48** and **49** have code numbers AY-30330 and AY-30468, respectively. [d] For id testing, each dog received only one dose of compound with three to four dogs tested at each dose. ED values were obtained by interpolation from the resulting dose–response curves. [e] *n* represents the number of animals.

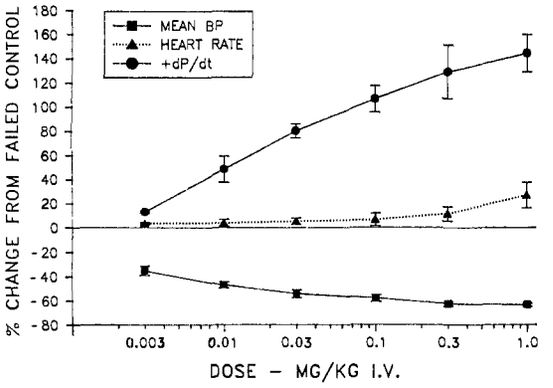


Figure 4. Effects of serial iv dosing with **49** in dogs with pentobarbital-induced cardiac failure. Points represent the mean ± SEM obtained in three dogs.

myocardial contractility (dP/dt max) from 11 ± 5% to 222 ± 76%. Compounds **48** and **49**, when administered in doses from 3 μg/kg to 1.0 mg/kg, demonstrated from 8 ± 3% to 191 ± 48% and from 13 ± 1% to 144 ± 15% increases in cardiac contractility, respectively.

The above changes were accompanied by relatively smaller changes in heart rate. The attendant increase in heart rate in the case of **9** was from 4 ± 3% to 52 ± 17% and, in the case of **49**, from 3 ± 1% to 27 ± 11%. In the case of compound **48**, these changes varied from 3 ± 1% to 52 ± 10%. Reduction in mean aortic blood pressure (MABP) was monitored simultaneously. In the case of compound **9**, these changes varied from 0 at the lowest dose employed to 38 ± 12% at the highest. Compound **48** did not have any effect on blood pressure below 10 μg/kg; however, above this dose and up to 1.0 mg/kg, a gradual dose-dependent drop in MABP was observed up to 46 ± 5%. In contrast, compound **49** produced about a 38% drop in blood pressure at 3 μg/kg, with an attendant decrease of up to 64 ± 3% at 1 mg/kg.

In conscious Konigsberg implanted dogs, the effect of compound **9**, **48**, and **49** by oral administration was evaluated by monitoring the changes in dP/dt max, heart rate, and the blood pressure. These parameters were further compared with those of milrinone, as shown in Figure 5.

It was noted that compound **9** at 2 mg/kg had a slightly more rapid onset of action and demonstrated a comparatively longer duration of action relative to that of **49** and milrinone. In contrast, **48** at 0.5 mg/kg produced an effect of longer duration than that of either of the other two drugs (see Figure 5).

Milrinone, **9**, and **48** all produced increases in heart rate at these doses; however, the effect of **48** appeared to be smaller in magnitude. In contrast, **49** was the only compound that did not increase the heart rate.

Milrinone showed very little effect on blood pressure in this model. Compound **49** produced a transient increase during the first hour, followed by no effect during the

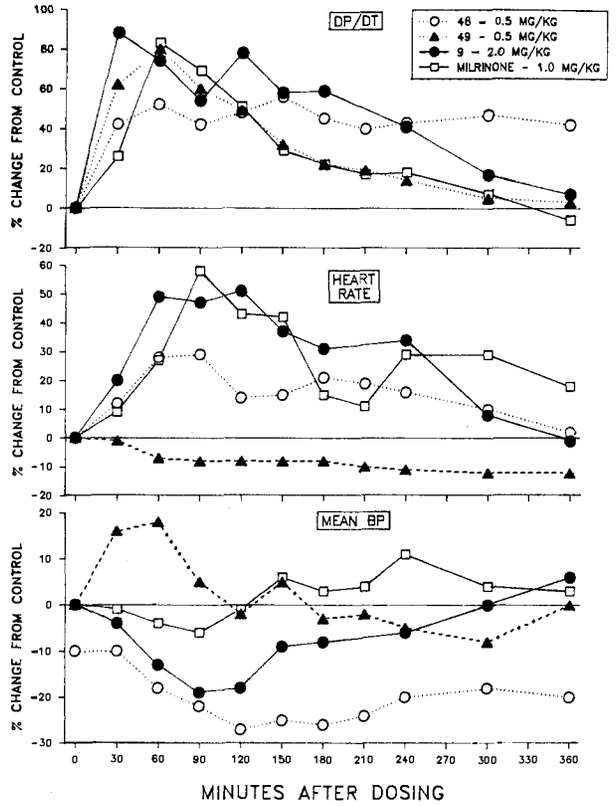


Figure 5. Comparison of the effects of **9**, **48**, **49**, and milrinone on dp/dt, heart rate, and mean blood pressure in conscious dogs with Konigsburg pressure transducers chronically implanted in the left ventricle. Points represent the mean values obtained in three dogs with **9**, six dogs with **48**, and four dogs with **49**.

remaining 6 h monitored. Compounds **9** and **48** both decreased the blood pressure. In the case of **9**, this effect lasted during the first 2.5 h, whereas with **48** the decrease in blood pressure was observed during the entire 6 h of the recording period.

Mechanism of Action. The inotropic effect produced by representative members of the pyrimidone series under discussion was not blocked by a dose of propranol that inhibited the inotropic effect of a dose of isoproterenol, producing a 75–125% increase in basal contractility both in vivo and in vitro (data not shown). This clearly suggested that the inotropic effect produced by the pyrimidones was not mediated by β-adrenergic receptors. Furthermore, preliminary experiments with microsomal Na^+/K^+-activated ATPase of the rat brain using the described methodology[18] indicated that pelrinone (**9**) did not mediate its effect by inhibiting this enzyme in the heart.

It is well documented that cAMP plays a major role in modulating cardiac contractility.[19] The cyclic nucleotide phosphodiesterases are the enzymes primarily responsible for the hydrolysis of cAMP → AMP. In recent years,

Table III. Comparison of Positive Inotropic Effect in Papillary Muscle (in Vitro, ED_{50}) and in Anesthetized Dogs (in Vivo, ED_{50}) and IC_{50} Values for Crude PDE and Fraction III of Bovine Cardiac PDE Enzyme of Selected Pyrimidone Analogues

no.	papillary muscle: EC_{50}	anesthetized dog: ED_{50}, mg/kg	crude PDE: IC_{50}, μM[a]	PDE-Fr. III: IC_{50}, μM
9	5×10^{-6} M	0.03	76	36
48	2.5×10^{-6} M	0.012	35	15
49	2.8×10^{-6} M	0.01		38
milrinone	6.5×10^{-6} M	0.02	192	28
CI-914	7×10^{-6} M	0.08	145	94

[a] These values were average of three determinations in triplicates.

several structurally unrelated cardiotonic agents have been demonstrated[20] to mediate their effect, at least partly, by the inhibition of cAMP phosphodiesterase.

We have examined the inhibition of cAMP phosphodiesterase by several of the cardiotonic drugs in the pyrimidone series and found them to be potent. The experiments were first carried out with crude bovine cardiac phosphodiesterase. Then, via the method of Thompson and co-workers,[21] different molecular forms of the enzyme were isolated. The inhibition studies of the fraction III enzyme by selected compounds of our series were carried out. The results are shown in Table III, together with the in vitro and in vivo inotropic effects of compounds **9**, **48**, and **49**, as compared with milrinone and CI-914. The results indicate that these compounds are specific potent inhibitors of bovine cardiac phosphodiesterase fraction III. This is clearly suggested by the lower IC_{50} values for the purified fraction, compared to those obtained with the crude enzyme preparation. A comparison of in vitro enzyme inhibition studies and in vivo cardiotonic effects suggests that this enzymatic pathway may be a component of the positive inotropic effect of agents of this class. It must be pointed out that the phosphodiesterase used in the biochemical study is a commercial bovine cardiac preparation, whereas the in vivo cardiotonic study was performed in dogs. Presently, the effect of these agents on the canine phosphodiesterase are under examination.

In conclusion, we report in this paper a novel class of 2-methylpyrimidones as positive inotropic agents. Among these, pelrinone (**9**), AY-30 330 (**48**), and AY-30 468 (**49**) are highly potent cardiotonic agents having a wide range of vasodilatory effects. Pelrinone (**9**) is, at the present time, undergoing phase II clinical evaluation. Compound **48** (AY-30 330), represents the most potent nonsympathomometic, noncardenolide cardiotonic agent among the pyrimidone derivatives studied. A component of the cardiotonic effect of these agents appears to be associated with the inhibition of fraction III cardiac phosphodiesterase.

Experimental Section

The infrared spectra were recorded on a Perkin-Elmer diffraction grating or on a Perkin-Elmer 784 spectrophotometer. The ultraviolet spectra were recorded on a Zeiss-DMR-21 spectrometer. The melting points were taken on a Thomas-Hoover apparatus and are uncorrected. The NMR spectra were recorded on either a CFT-20 or a Brucker AM-400 machine. The mass spectra were recorded on a LKB-9000S or a Finigan 8230 high-resolution mass spectrometer. Organic extracts were dried over magnesium sulfate, and the solvents were always removed under vacuum. Merck silica gel 60 (70–230 mesh) was used for column chromatography.

3,4-Dihydro-2-methyl-6-(methylthio)-4-oxo-5-pyrimidinecarbonitrile (8). To a suspension of hexane-washed NaH (1.46 g, 50% in oil, 1.9 equiv) in DMF (1.5 mL) was added dropwise a solution of acetamidine hydrochloride (1.66 g, 1.1 equiv) in DMF (7 mL). The reaction mixture was stirred at room temperature for 1 h. A solution of compound **7** (3.24 g, 1 equiv) in DMF (5 mL, prepared by warming) was added dropwise. The reaction mixture was allowed to stir at room temperature for 4 h. The reaction was then diluted with water (13 mL). Acidification with concentrated HCl (1.5 mL) precipitated the product, which was filtered and dried. The product was crystallized from DMF/ether, to yield pure sample (1.7 g): mp >280 °C; IR (Nujol) 2800, 2220 cm^{-1}; UV (MeOH) λ_{max} 312 (7530), 240 (18 600) nm; ^1H NMR (Me_2SO-d_6) δ 9.88 (1 H, br, NH), 2.55 (3 H, s, SCH_3), 2.35 (3 H, s, N=CCH_3).

The general procedure for the preparation of compounds **9–16** is examplified by the preparation of **3,4-dihydro-2-methyl-4-oxo-6-[(2-pyridin-2-ylethyl)amino]-5-pyrimidinecarbonitrile (12). Method A.** To a suspension of methylthio compound **8** (0.543 g, 1 equiv) in dimethoxyethane (1 mL) was added a solution of 2-pyridinylethylamine (1.1 g, 3 equiv) in dimethoxyethane (1 mL). The mixture was heated at 90 °C for 6 h, cooled to 23 °C, diluted with ether–methanol, and the chilled to 0 °C. The precipitate was isolated by filtration to give a solid (0.4 g). Crystallization from methanol (40 mL) afforded pure product (0.37 g, 48.3%): mp 268–270 °C; IR (Nujol) 3310, 2850, 2220, 1673 cm^{-1}; UV (MeOH) λ_{max} 290 (7000), 268 (8700), 262 (8700), 227 (39 900) nm; ^1H NMR (Me_2SO-d_6) δ 12.0 (1 H, br, NH), 8.45 (1 H, d, Ar), 7.85 (1 H, t, N*H*), 7.65 (1 H, m, Ar), 7.2 (2 H, m, Ar), 3.7 (2 H, m, NCH_2), 2.95 (2 H, t, CCH_2), 2.2 (3 H, s, CCH_3). Anal. ($C_{13}H_{13}N_5O$) C, H, N.

3,4-Dihydro-6-(ethylamino)-2-methyl-4-oxo-5-pyrimidinecarbonitrile (17). Method B. To a suspension of **7** (8.12 g, 1 equiv) in dimethoxyethane (14 mL) was added a solution of ethylamine (2.16 g, 1.2 equiv) in dimethoxyethane (6.5 mL). The suspension turned to a solution, and then a white solid began to precipitate. After about 10 min, the solvent was removed under nitrogen and the residue was filtered with ether to yield a product (7.2 g, 90%), mp 86–88 °C. The spectral analyses (IR and NMR) were compatible with the expected structure **19** (R = C_2H_5). The product was used as such for the next step.

To a solution of acetamidine hydrochloride (0.83 g, 1.1 equiv) in DMF (5.5 mL) was added potassium carbonate (1.22 g, 2.2 equiv). The mixture was stirred at room temperature for about 10 min. The cyano ester **19** (R = C_2H_5, 1.6 g, 1 equiv) was added, and the mixture was heated at 90 °C overnight. The reaction mixture was cooled, diluted with water, and filtered to give a solid (0.3 g). Further concentration to dryness followed by filtration of residue with a small amount of water gave more solid (0.8 g). The two solids were pooled and crystallized from methanol to give pure product (0.6 g, 42%): mp >290 °C; IR (Nujol) 3300, 2800, 2220, 1650 cm^{-1}; UV (MeOH) λ_{max} 289 (6140), 368 (5240), 226 (40 100) nm; ^1H NMR (Me_2SO-d_6) δ 7.75 (1 H, t, NH), 3.4 (2 H, m, CH_2N), 2.23 (3 H, s, N=CCH_3), 1.1 (3 H, s, CCH_3). Anal. ($C_8H_{10}N_4O$) C, H, N.

3,4-Dihydro-2-(1-methylethyl)-4-oxo-6-[(3-pyridinylmethyl)amino]-5-pyrimidinecarbonitrile (21). To a solution of 1,4-dihydro-2-(1-methylethyl)-6-(methylthio)-4-oxo-5-pyrimidinecarbonitrile (**8**, R^2 = $CH(CH_3)_2$; 6 g, 1 equiv) in dimethoxyethane (50 mL) was added 3-(aminomethyl)pyridine (16 mL, 5 equiv), and the mixture was refluxed overnight. The solvent was evaporated, and the residue was put on a silica gel column in chloroform–methanol (97:3). The product was eluted with the same solvent mixture and crystallized from methanol–water to yield pure sample 3.4 g (44%): mp >260 °C; IR (Nujol), 3335, 2900, 2210, 1660 cm^{-1}; UV (MeOH) λ_{max} 290 (7400), 268 (8100), 263 (8100), 227 (39 400) nm; ^1H NMR (Me_2SO-d_6) δ 11.5 (2 H, br, NH), 7.3–8.4 (4 H, m, Ar), 4.55 (2 H, m, NCH_2), 2.7 (1 H, m, C=C*H*), 1.1 (6 H, d, 2 CH_3). Anal. ($C_{14}H_{15}N_5O$) C, H, N.

3,4-Dihydro-4-oxo-6-[(3-pyridinylmethyl)amino]-5-pyrimidinecarbonitrile (23). To a solution of bis(methylthio) ester **7** (24.4 g, 1 equiv) in dimethylformamide (50 mL) was added formamidine acetate (12.48 g, 1 equiv), and the mixture was heated to 120 °C for 4 h. The mixture was cooled and filtered. The residue was washed with very cold methanol–ether and dried to yield crude product (18.0 g). Crystallization from hot methanol gave the product (12.6 g). A crystallization from dimethylformamide followed by another one from methanol yielded the product (3.9 g): mp 233–237 °C; IR (Nujol) 2900, 2220 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 8.4 (1 H, s, Ar), 2.6 (3 H, s, SCH_3). The above

spectral data coupled with elemental analysis was compatible with 3,4-dihydro-4-oxo-6-(methylthio)-5-pyrimidinecarbonitrile.

To a suspension of the above pyrimidone (2.16 g, 1 equiv) in dimethoxyethane (4 mL) was added 3-(aminomethyl)pyridine (5.3 mL, 4 equiv). The mixture was heated to 110 °C for 20 h. The reaction mixture was cooled and diluted with ether, and the product was allowed to crystallize. The precipitate was filtered to yield crude product (1.5 g), which was further purified by passing it through a column of silica gel (200 g) in 5% methanol–ethyl acetate. The product (0.8 g) was eluted with the same solvent. Recrystallization from methanol gave pure product (0.42 g, 27.4%): mp 236–245 °C; IR (Nujol) 3440, 3330, 3150, 2210, 2180, 1665 cm^{-1}; UV (MeOH) λ_{max} 293 (5770), 268 (7760), 262 (8000), 226 (39<<μtc700) nm; ^1H NMR (Me_2SO-d_6) δ 8.45 (1 H, m, Ar), 8.07 (1 H, s, N=CH), 7.3–7.65 (2 H, m, Ar), 4.59 (2 H, d, N-CH_2). Anal. ($C_{11}H_9N_5O$) C, H, N.

1,2,3,4-Tetrahydro-2,4-dioxo-6-[(3-pyridinylmethyl)amino]-5-pyrimidinecarbonitrile (26). Method C. To a solution of bis(methylthio) compound[11] (38.3 g, 1 equiv) in dimethoxyethane (200 mL) was added 3-(aminomethyl)pyridine (22.3 g, 1.1 equiv). The mixture was stirred at room temperature for 2 h. A white precipitate formed, which was isolated by filtration and purified by passing through silica gel. The product was eluted with 4% methanol–chloroform to yield a white solid (41.7 g, 83%). Through a cooled solution of the above product (5.2 g) in methanol (100 mL) was passed ammonia gas for 15 min. The mixture was closed in a pressure bottle and was heated at 85 °C overnight. The mixture was cooled, and the precipitate was filtered to yield the pure product (2.1 g, 46%). The IR and NMR spectra were compatible with the expected β-amino-β-[(3-pyridinylmethyl)amino]-α-cyanoacrylamide.

The amide (2.6 g, 1 equiv) obtained above was mixed with 1,1′-carbonyldiimidazole (3.89 g, 2 equiv) and suspended in dimethylformamide (4.5 mL). The reaction mixture was heated with stirring at 110 °C for 6 h. At the end of this time, most of the solvent was removed under vacuum. Methanol (10 mL) was added to the residue until a solid appeared. Then, ether (10 mL) was added, and the mixture was allowed to crystallize in the refrigerator overnight. The solid was removed by filtration and washed with ether. Recrystallization from methanol–DMF gave the product (1.1 g): mp <300 °C; IR (KBr) 3240, 2010, 1710, 1660, 1600 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 8.55 (2 H, m, Ar), 7.66 (3 H, m, Ar), 5.73 (2 H, d, N-CH_2). Anal. ($C_{11}H_9N_5O_2$) C, H, N.

4-Chloro-2-methyl-6-[(3-pyridinylmethyl)amino]-5-pyrimidinecarbonitrile (28). To phosphorus oxychloride (42.9 g, or 26.1 mL, 8 equiv) preheated to 100 °C (bath temperature) was added the sodium salt of compound **9** (9.2 g, 1 equiv). The reaction mixture was heated for 2 h during which time the suspension became largely solution. It was cooled and poured into ice water (~900 mL) with stirring. The resulting solution was basified with sodium hydroxide (50%, 100 mL). Some ethyl acetate was added, and the resulting precipitate was filtered and dried to yield the product (4.7 g). The aqueous filtrate was extracted with ethyl acetate and dried. The solvent was removed to yield a further quantity of product (1.8 g). The total yield was 71.5%. A sample was crystallized from chloroform–hexane (two times) to yield pure product: mp 165–167 °C; IR ($CHCl_3$) 3410, 3330, 2215, 1570 cm^{-1}; UV (MeOH) λ_{max} 305 (4130), 254 (18100) nm; ^1H NMR ($CDCl_3$) δ 8.05 (2 H, m, Ar), 7.64 (1 H, m, Ar), 7.25 (1 H, m, Ar), 6.3 (1 H, br, NH), 4.72 (2 H, d, NCH_2), 2.54 (3 H, s, CCH_3). Anal. Calcd for $C_{12}H_{10}N_5Cl$: C, 55.48; H, 3.88; N, 26.96. Found: C, 55.00; H, 3.89; N, 26.50.

2-Methyl-4-(methylamino)-6-[(3-pyridinylmethyl)amino]-5-pyrimidinecarbonitrile (29). Monomethylamine was passed for 7 min into a solution of chloro compound **28** (1.1 g, 1 equiv) in methanol (10 mL). The color turned bright yellow and slowly decolorized to pale yellow. The reaction mixture was stirred in a closed container overnight at room temperature. The solvent was removed, the mixture was diluted with water, and the precipitate was filtered to give crude product (0.98 g). A crystallization from hot methanol gave pure sample (0.6 g): mp 219–221 °C; IR (Nujol) 3430, 2190 cm^{-1}; UV (MeOH) λ_{max} 283 (6960, m), 369 (7260, m), 262 (7390), 236 (45800) nm; ^1H NMR (Me_2SO-d_6) δ 8.5 (2 H, m, Ar), 7.6 (1 H, m, Ar), 7.2 (1 H, m, Ar), 5.4 (1 H, d, NH), 5.1 (1 H, t, NH), 4.67 (2 H, d, NCH_2), 3.02 (3 H, d, NCH_3), 2.37 (3 H, s, CCH_3). Anal. ($C_{13}H_{14}N_6$) C, H, N.

4-Methoxy-2-methyl-6-[(3-pyridinylmethyl)amino]-5-pyrimidinecarbonitrile (30). To a suspension of chloro compound **28** (0.65 g) in methanol (3 mL) was added a solution of sodium in methanol (2 N, 2.3 mL). The mixture turned dark yellow and slowly decolorized to off-white. A TLC showed no starting material. The mixture was cooled, the solvent was removed, and the residue was diluted with half-saturated sodium chloride. The resulting precipitate (0.6 g) was filtered. A crystallization from hot methanol–ether gave a pure sample (0.5 g, 78.2%): mp 172–174 °C; IR ($CHCl_3$) 3420, 3350, 2220, 1580 cm^{-1}; UV (MeOH) λ_{max} 289 (5110), 267 (5280), 248 (10400), 222 (3800); ^1H NMR ($CDCl_3$) δ 8.5 (2 H, m, Ar), 7.6 (1 H, m, Ar), 7.2 (1 H, m, Ar), 6.82 (1 H, t, NH), 4.7 (2 H, d, NCH_2), 3.97 (3 H, s, OCH_3) 2.45 (3 H, s, CCH_3). Anal. ($C_{13}H_{13}N_5O$) C, H, N.

1,4-Dihydro-2-methyl-6-[(3-pyridinylmethyl)amino]-4-thioxo-5-pyrimidinecarbonitrile (31). To ethanol (5 mL) was added a solution of potassium hydroxide (3.0 mL, 4 M in H_2O–EtOH; 1:9). The mixture was cooled in an ice bath. Hydrogen sulfide was passed through the solution for 30 min. The chloro compound **28** (0.78 g, 1 equiv) in ethanol (7 mL) was added to the solution. The mixture was stirred at room temperature overnight. The reaction mixture was filtered; the precipitate was washed with some ethanol to yield crude product (0.7 g) and crystallized twice from DMF to yield pure product (0.25 g, 32.5%): mp 310–312 °C; IR (Nujol) 3320, 2800, 2220, 1600, 1080 cm^{-1}, UV (MeOH) λ_{max} 312 (22600), 273 (24600), 242 (12700); ^1H NMR (Me_2SO-d_6) δ 13.2 (1 H, br, NH), 8.8 (1 H, br, NH), 8.5 (2 H, m, Ar), 7.5 (2 H, m, Ar), 4.6 (2 H, d, NCH_2), 2.3 (3 H, s, CCH_3), MS, m/e 257 $(M)^+$, 224 $(M - SH)^+$. Anal. Calcd for $C_{12}H_{11}N_5S$: C, 56.01; H, 4.31; N, 27.22. Found: C, 55.48; H, 4.49; N, 26.68.

3,4-Dihydro-2-methyl-4-oxo-6-[(3-pyridinylmethyl)amino]-5-pyrimidinecarboxamide (33). Sulfuric acid (4 mL) was added to the pyrimidinecarbonitrile **9** (1.2 g). The mixture was heated to 70 °C for 4 h. After 1 h, all the solid went into solution. The reaction was cooled to room temperature and carefully poured on crushed ice (~75 mL). The resulting solution was neutralized with sodium hydroxide (50% aqueous, 13.0 mL) to pH 8, at which point a precipitate appeared. The solid was filtered, dried, and crystallized from boiling DMF to yield pure product (1.1 g, 85.27%): mp 264–266 °C; IR (Nujol) 3760, 3000, 1645, 1585 cm^{-1}; UV (MeOH) λ_{max} 289 (7530), 268 (7530), 263 (5820), 228 (35200) nm; NMR (Me_2SO-d_6) δ 8.5 (1 H, m, Ar), 7.55 (1 H, m, Ar), 7.25 (1 H, m, Ar), 9.15 (1 H, m, NH), 4.7 (2 H, d, NCH_2), 2.22 (3 H, s, CCH_3).

6-(Ethylamino)-2-methylpyrimidin-4-ol (41). The chloro compound **40** (1.45 g, 1 equiv) was suspended in tetrahydrofuran. Ethylamine (70% in water, 1.9 mL, 3 equiv) was added, and the reaction mixture was heated to 90 °C in a sealed tube for 24 h. Additional ethylamine (1.3 mL, 2 equiv) was added, and the reaction mixture was heated for another 18 h. The reaction mixture was cooled, the solvent was removed, and the solid residue was triturated with water and filtered to yield crude product (0.943 g). Saturating the aqueous filtrate with sodium chloride and chilling it yielded additional product (0.16 g). This was crystallized from methanol–ether to give pure sample (0.9 g): mp 224–228 °C; IR (KBr) 3200–3500, 2970, 2860, 1600 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 12.5 (1 H, br, acidic), 6.78 (1 H, t, NH), 48 (1 H, s, vinylic), 3.0 (2 H, m, NCH_2), 2.12 (3 H, s, N=CCH_3), 1.05 (3 H, t, CCH_3). Anal. ($C_7H_{11}N_3O$) C, H, N.

3,4-Dihydro-2-methyl-6-[(3-pyridinylmethyl)amino]pyrimidin-4-one (42). The chloro compound **40** (1.16 g, 1 equiv) was suspended in dry tetrahydrofuran (5 mL), and 3-(methylamino)pyridine (1.9 g, 2.1 equiv) was added to it. The mixture was refluxed for 22 h, cooled to room temperature, and filtered. The filtrate was evaporated. The residue and the solid were combined and triturated with water and then filtered and dried to yield the crude product (1.13 g). Crystallization from methanol–ether gave pure product (0.73 g): mp 220–222 °C; IR (KBr) 3450, 3200, 1630, 1600, 795, 715 cm^{-1}; UV (MeOH) λ_{max} 262 (11750), 221 (30600) nm; ^1H NMR (Me_2SO-d_6) δ 11.5 (1 H, s, acidic), 8.4–8.7 (2 H, m, Ar), 7.2–7.9 (3 H, m, Ar), 4.4 (2 H, d, NCH_2), 2.2 (3 H, s, N=CCH_3). Anal. ($C_{11}H_{12}N_4O$) C, H, N.

5-Chloro-2-methyl-6-[(2-phenylethyl)amino]-4-pyrimidinol (37). Method D. To a suspension of the dichloro compound **36** (2.68 g, 1 equiv) in DME was added 2-phenylethylamine (5.45 g, 3 equiv). The reaction mixture was heated to reflux for 3 h.

The solvent was then removed under vacuum, and the residue was triturated with a small amount of water and filtered to give crude product (2.2 g). The crude product was recrystallized from methanol to give pure material (2.0 g, 51%): mp 230–232 °C; IR (KBr) 3410, 3100–2500, 1665, 1600, 1500 cm^{-1}; UV (MeOH) λ_{max} 274.5 (9781), 223.5 (26 245) nm; ^1H NMR (Me_2SO-d_6) δ 11.9 (1 H, s, OH), 7.25 (5 H, m, Ar), 6.8 (1 H, t, NH), 3.55 (2 H, m, $PhCH_2$), 2.8 (2 H, t, NCH_2), 2.2 (3 H, s, N=CCH_3). Anal. ($C_{13}H_{14}ClN_3O$) C, H, N.

3,4-Dihydro-6-(ethylamino)-2-methyl-5-nitro-4-oxopyrimidine (43). Method E. Pyrimidinol 41 (0.612 g, 4 mmol) was dissolved in concentrated sulfuric acid (1.6 mL). The solution was cooled to 0 °C in an ice–water bath. Fuming nitric acid (0.6 mL) was added, and the reaction mixture was stirred for 20 min. The reaction mixture was then poured on ice and filtered to give crude product (0.6 g). This was recrystallizeed from methanol to yield pure sample (0.45 g, 75%): mp 279–280 °C; IR (Nujol) 3300–2700, 1665, 1600, 1370 cm^{-1}; UV (MeOH) 338.5 (7785), 286 (4005), 233 (14 263), 211.5 (14079) nm; ^1H NMR (Me_2SO-d_6) δ 12.2 (1 H, br, NH), 9.68 (1 H, br, NH), 3.63 (2 H, m, CCH_2), 2.29 (3 H, s, N=CCH_3), 1.2 (3 H, t, CCH_3).

3,4-Dihydro-2-methyl-6-[(3-pyridinylmethyl)amino]-5-nitropyrimidin-4-one (44). This analogue was prepared in a similar manner as above from corresponding desnitro derivative 42: IR (Nujol) 3300, 1675, 1600, 1350 cm^{-1}; UV (MeOH) λ_{max} 234.5 (17 750), 285 (5250), 336.5 (8900); ^1H NMR (Me_2SO-d_6) δ 10.1 (1 H, t, NH), 8.7 (1 H, m, Ar), 7.9 (1 H, m, Ar), 7.45 (1 H, m, Ar), 4.95 (2 H, d, N-CH_2), 2.3 (3 H, s, N=$COCH_3$). Anal. ($C_{11}H_{11}H_5O_3$) C, H, N.

5-Acetyl-3,4-dihydro-6-(ethylamino)-2-methylpyrimidin-4-one (45). Method F. Pyrimidine derivative 41 (1.8 g, 1 equiv) was suspended in acetic acid (8.4 mL). Phosphorus oxychloride (3.76 mL, 3.3 equiv) was added, and the reaction mixture was heated at 70 °C for 21 h. The mixture was cooled to room temperature, diluted with ice water, and filtered to yield crude product (0.42 g). Extraction of filtrate with chloroform gave additional product (0.15 g). Crystallization from methanol yielded (0.43 g, 16.5%) of pure product: mp 241–242 °C; IR (KBr) 3440, 1640, 1600 cm^{-1}; UV (MeOH) λ_{max} 310 (7373), 275.5 (5078), 228.5 (29 064); ^1H NMR (CF_3COOD) δ 3.75 (2 H, q, NCH_2), 2.98 (3 H, s, CH_3), 2.82 (3 H, s, CH_3), 1.5 (3 H, t, C-CH_3). Anal. ($C_9H_{13}N_3O_2$) H, N; C: calcd, 55.83; found, 55.25.

5-Acetyl-3,4-dihydro-6-[(pyridin-3-ylmethyl)amino]-2-methylpyrimidin-4-one (46) was synthesized from 42 in a similar manner: mp 235–236 °C; IR (KBr) 3460, 1670, 1600 cm^{-1}; UV (MeOH) λ_{max} 308 (7612), 269.5 (6601), 229.5 (33 850) nm; ^1H NMR ($CDCl_3$) δ 5.12 (2 H, d, N-CH_2), 2.8 (3 H, s, $COCH_3$), 2.4 (3 H, s, N=CCH_3). Anal. ($C_{13}H_{14}N_4O_2$) H, N; C: calcd, 60.47; found, 59.99.

5-Bromo-3,4-dihydro-6-[(3-pyridinylmethyl)amino]-2-methylpyrimidin-4-one (48). Pyrimidinol (40; 61.5 g, 1 equiv) was dissolved in glacial acetic acid (150 mL) and chloroform (600 mL). To the solution was added bromine (96.4 g, 1.3 equiv) dropwise, and a precipitate appeared. The mixture was stirred at room temperature overnight. The precipitate was filtered, and the filtrate was concentrated under vacuum. The solid was combined with the residue and crystallized from water to yield 73.3 g (47; 83%) pure product: IR (KBr) 2700–3100, 1660, 1590 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 12.6 (1 H, br, NH), 2.14 (3 H, s, N=CCH_3).

A stirred suspension of 47 (5.11 g, 1 equiv) and 3-(aminomethyl)pyridine (7.97 g, 3 equiv) in dimethoxyethane (15 mL) was heated to 70 °C for 21 h in a dry atmosphere. The mixture was cooled, water (9 mL) was added, and the hydrated mixture was stirred for 15 min. The solid was filtered, washed with ether (5 mL) and water (5 mL), dried, and recrystallized from methanol to yield 5.54 g (48; 76%): IR (KBr) 3260, 3170, 1660, 1600 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 12.0 (1 H, br, N*H*), 8.42 (1 H, m, Ar), 7.65 (1 H, m, Ar), 7.3 (2 H, m, Ar), 4.55 (2 H, d, C*H*N), 2.15 (3 H, s, N=CCH_3); MS, m/e 295 $(M)^+$, 107 $(C_6H_7N_2)^+$. Anal. ($C_{11}H_{11}BrN_4O$) C, H, N.

5-Bromo-3,4-dihydro-6-(ethylamino)-2-methylpyrimidin-4-one (49). A stirred homogeneous solution of 41 (4.1 g, 1 equiv) in chloroform (50 mL) and acetic acid (15 mL) was treated with bromine (5.56 g, 1.3 equiv). The mixture was stirred for 63 h at room temperature and then concentrated under vacuum. The resulting residue was treated with refluxing water (400 mL), cooled, and filtered. The solid was crystallized from ether–methylene chloride to give analytically pure product (1.97 g, 32%). Mother liquor was concentrated; and the residue was recrystallized from ethyl acetate to give more pure product (2.15 g, 34%): IR (KBr) 3420, 2600–3000, 1640, 1605, 1580 cm^{-1}; UV (MeOH) λ_{max} 275.5 (8340) nm; ^1H NMR (Me_2SO-d_6) δ 11.94 (1 H, br, acidic) 6.62 (1 H, t, NH), 3.38 (2 H, m, NCH_2), 2.18 (3 H, s, N=CCH_3), 1.1 (3 H, t, CCH_3). Anal. ($C_7H_{10}BrN_3O$) C, H, N.

5-Bromo-3,4-dihydro-6-[(2-phenylethyl)amino]-4-pyrimidinol (50). To a stirred suspension of 47 (3.35 g, 1 equiv) in dimethoxyethane (20 mL) was added 2-phenylethylamine (9.4 mL, 5 equiv). The mixture was heated to reflux for 1 h. The solvent was removed under vacuum, and the residue was triturated with water (40 mL) and filtered to give crude product (3.65 g). Crystallization from methanol–chloroform yielded pure product (3.37 g, 67%): IR (KBr) 3400, 1660, 1600–1500 cm^{-1}; UV (MeOH) λ_{max} 277.5 (9279) nm; ^1H NMR (Me_2SO-d_6) δ 7.4–7.15 (4 H, m, Ar), 6.6 (1 H, m, NH), 3.6 (2 H, q, NCH_2), 2.8 (2 H, m, CH_2Ph), 2.2 (3 H, s, N=CCH_3). Anal. ($C_{13}H_{14}BrN_3O$) C, H, N.

3,4-Dihydro-2,3-dimethyl-6-[methyl(3-pyridinylmethyl)-amino]-4-oxo-5-pyrimidinecarbonitrile (52). To a suspension of washed sodium hydride (1.06 g, 50% suspension, 2.2 equiv) in DMF (10 mL) was added 9 (2.41 g, 1 equiv) in portions. The mixture was stirred at room temperature for 0.5 h. Dimethyl sulfate (3.78 g, 6 equiv) in DMF (25 mL) was added dropwise. The reaction mixture was stirred at room temperature for 3 h. The mixture was then diltued with water (100 mL) and extracted with chloroform. The organic liquor dried, and the solvent was removed to yield the crude residue (2.2 g). The residue was filtered through a column of silica gel (100 g), and the product was eluted with methanol–EtOAc (1:9). Removal of the solvent gave more pure sample (1.1 g). This was crystallized from methanol–ether to give analytically pure product (0.9 g): mp 100–104 °C; IR ($CHCl_3$) 2210, 1660, 1590, 1545 cm^{-1}; UV (MeOH) λ_{max} 277 (10 090), 270 (10 030), 234 (32 100) nm; ^1H NMR ($CDCl_3$) δ 7.25–8.5 (4 H, m, Ar), 4.91 (2 H, s, NCH_2), 3.45 (3 H, s, NCH_3), 3.34 (3 H, s, NCH_3), 2.93 (3 H, s, CCH_3). Anal. ($C_{14}H_{15}N_5O$) C, H, N.

3,4-Dihydro-2,3-dimethyl-4-oxo-6-[(3-pyridinylmethyl)-amino]-5-pyrimidinecarbonitrile (51). To a suspension of hexane-washed sodium hydride (50% in oil, 0.158 g) in DMF (35 mL) was added compound 8 (5.43 g, 1 equiv) in portions. The mixture was stirred at room temperature. After 0.5 h, dimethyl sulfate (5.67 g, 3 equiv) was added dropwise. The solid went into solution, and the mixture turned orange. The reaction was stirred at room temperature for 2 h, diluted with water, and extracted with chloroform. The organic liquor was washed and dried, and the solvent was removed. The residue was triturated with methanol–ether and filtered to give 2,3-dimethyl-4-oxo-6-(methylthio)pyrimidinecarbonitrile (4.16 g). The residue from the filtrate was chromatographed on silica gel (50 g) in ethyl acetate–hexane (1:1) to yield more product (0.1 g): 174–178 °C; IR ($CHCl_3$) 2220, 1675 cm^{-1}; UV (MeOH) λ_{max} 313 (7250), 284 (10 550), 237 (17 750) nm; ^1H NMR ($CDCl_3$) δ 3.5 (3 H, s, NCH_3), 2.63 (3 H, s, SCH_3), 2.6 (3 H, s, CCH_2).

To a suspension of the NCH_3 derivative obtained above (1.95 g, 1 equiv) in dimethoxyethane (5 mL) was added 3-(aminomethyl)pyridine (2.7 g, 2.5 equiv). The reaction was heated to 85 °C for 18 h. The reaction mixtured cooled, and the precipitate was filtered, washed with ether–ethyl acetate, and dried to give crude product (1.9 g). Recrystallization from methanol–ether yielded pure product (1.5 g): mp 224–228 °C; IR (Nujol) 3280, 3150, 2200, 1655 cm^{-1}; UV (MeOH) λ_{max} 290 (6940), 269 (9490), 264 (8900), 227 (41 050) nm; ^1H NMR (Me_2SO-d_6) δ 8.4–7.3 (4 H, m, Ar) 4.56 (2 H, d, NCH_2), 3.3 (3 H, s, NCH_3), 2.43 (3 H, s, CCH_3). Anal. ($C_{13}H_{13}N_5O$) C, H, N.

X-ray Crystallographic Analyses. The tetrafluoroborate salt of compound 9 was prepared by exchanging the hydrochloride salt with sodium tetrafluoroborate and was crystallized from methanol. Molecular formula, $C_{12}H_{12}BF_4N_5O$; mol wt, 329.07; triclinic; a = 4.86 (1) Å, b = 8.952 (1) Å, c = 16.426 (2) Å, α = 77.56 (1)°, β = 87.14 (1)°, γ = 87.09 (1)°; V = 696.4 (1) $Å^3$; space group $P\bar{1}$; Z = 2; ρ_{calcd} = 1.569 g cm^{-3}; T = 293 ± 1 K; λ = 0.71069 Å; λ(Mo Kα) = 1.600 cm^{-1}.

Intensity data were collected on a crystal of dimensions 0.24 × 0.25 × 0.31 mm with 0.20 scans ($3.2 < 2\theta \leq 50°$) with a variable

scan speed of 2.93–29.3° min^{-1} and a scan width of 0.8° below $K\alpha_1$ to 0.8° above $K\alpha_2$ on a Syntex $P2_1$ diffractometer. From a total of 2464 measured reflections, 1671 had $I \geq 3\sigma(I)$ and were used in the structure solution and refinement. Two standard reflection (133, 117) monitored after every 100 measurements showed no change in intensity over the course of the data collection. Data were correct for Lorentz and polarization effects.

The structure was solved by direct methods (MULTAN80) and refined with anisotropic thermal parameters to an R of 0.076 R $= \sum||F_o| - |F_c||/\sum|F_o|$). A difference Fourier synthesis at this stage allowed location of all hydrogen atoms, which were included in subsequent refinements. The structure converged at $R = 0.0465$ with $R_w = 0.053$ ($R_w = [\sum w|F_o| - |F_c|)^2/\sum w|F_o|^2]^{1/2}$, $w^{-1} = 1.61 + 0.081|F_o|^2$). A final difference Fourier was featureless.

Biological Methodology. The method used to obtain right ventricular papillary muscle preparations is similar to that described by Cattell and Gold.[16] Briefly, cats of either sex weighing 1–2 kg were anesthetized with sodium pentobarbital, 25–30 mg/kg iv, and the hearts were rapidly removed and placed in cool Tyrode's solution gassed with 95% O_2–5% CO_2. Suitably sized right ventricular papillary muscles were removed, affixed to tissue holders incorporating a pair of platinum point electrodes, and suspended in tissue baths containing Tyrode's solution at 37 °C, bubbled with the gas mixture described above. Force displacement transducers (Gould UC-2) were used to record twitch tension on a Beckman R511 chart recorder. The preparations were stimulated to contract with rectangular pulses, 2–4 ms in duration and 10% above threshold voltage, at a rate of 0.5 Hz. The muscles were gently stretched until maximal twitch tension was attained. For chronotropic studies, right atria were suspended in the same medium and allowed to beat spontaneously. An equilibration period of at least 1 was allowed prior to administration of test substances. Cumulative concentration–response curves were obtained by increasing the concentration of a compound in the bath in half-log units until the maximal effect was obtained or the total concentration reached 1×10^{-3} M. The EC_{50} was defined as the concentration associated with a 50% increase over the pretreatment baseline in tension developed in the papillary muscles (inotropic) or spontaneous beating rate in the atria (chronotropic). At least four preparations, each obtained from a different animal, were used to obtain EC_{50} values.

Pentobarbital-Induced Cardiac Failure in the Dog. Dogs unselected for breed or sex weighing 10–20 kg were anesthetized with sodium pentobarbital, 30–35 mg/kg iv, intubated, and artificially respired with room air (20 strokes/min, 15 cm^3 air/kg per stroke). Blood pressure was obtained by a cannula inserted into a femoral artery and attached to a Beckman transducer. A Millar pressure-tip catheter (PC 350, size 5f) was inserted into the other femoral artery and advanced into the left ventricle to measure left ventricular pressure and dP/dt. Subdermal needle electrodes were used to obtain a lead(II) ECG and to determine heart rate. Both femoral veins were cannulated, one to deliver pentobarbital, the other for the administration of test substances. A cannula was also placed into the duodenum for those experiments in which assessment of potential oral activity was desired. Following a stabilization period of at least 30 min, induction of heart failure was begun by the iv infusion of sodium pentobarbital, 0.75 mg/kg per min in 0.2 mL of saline/min, until a 40–60% decrease in peak positive dP/dt was obtained. The level of failure was maintained throughout the experiment by continuous infusion of pentobarbital, 0.11–0.15 mg/kg per min, or as required in a particular animal. Once the maintenance infusion was started, at least 15 min was allowed to elapse before administration of test substances was begun. Heart rate, blood pressure, intraventricular pressure, and ECG were recorded on a Beckman R611 physiograph. Serial (i.e., noncumulative) dose–response curves were obtained iv by giving increasingly higher doses in half-log units at half-hour intervals. In the lower dose range (0.003–0.10 mg/kg iv) in which the ED_{50} values were obtained, responses had typically returned to within 10% of pretreatment values before the next higher dose was administered. At doses greater than 0.1 mg/kg iv, a residual effect on one or more parameters was often present when the next dose was given. Single doses only were given intraduodenally. At least three dogs were used for each determination. For both iv and id administration, compounds were suspended in normal saline to which dilute hydrochloric acid was added in a dropwise manner, to form a clear solution (final pH 4–6).

The ED_{50} value was defined as the dose associated with a 50% increase over baseline values for peak positive dP/dt.

Konigsberg-Implanted Conscious Dogs. Dogs unselected for breed or sex were anesthetized and respired as described above. A left thoracotomy was performed through the fourth intercostal space, and a Konigsberg pressure transducer (Model P6-S) was inserted into the left ventricle by apical puncture, as described by Dormer.[17] In some animals, an indwelling cannula was placed in an internal thoracic artery for later measurement of blood pressure. The pericardium was replaced, leaving a hole approximately 1 cm in length to accomodate the transducer lead and to prevent tamponade. The lead was exteriorized near the nape of the neck, the thoracotomy was repaired, and the animals were allowed to recover for a period of 2 weeks before experiments were begun. On the day of an experiment, standard limb leads were applied to obtain a lead(II) ECG, and intraventricular pressure, dP/dt, heart rate, and blood pressure were recorded on a Beckman physiograph. Only one dose of a compound was administered during each experiment. Compounds were administered po without excipients in a gelatin capsule.

Biochemistry. The entire procedure for phosphodiesterase fractionation into its isoenzymes and the assay for measuring the enzyme activity were performed as described by Thompson and co-workers.[21] The bovine heart phosphodiesterase preparation was purchased from Sigma Chemical Co. (St. Louis, MO) and was reconstituted as specified. The enzyme preparation was further diluted with 120 mM Tris·HCl buffer (pH 7.8), containing 5 mM mercaptoethanol and 15 mM $MgCl_2$, to give approximately 0.04 mg of protein/mL. This crude enzyme preparation was also fractionated on a DEAE-cellulose column eluted stepwise with 200, 350, and 700 mM sodium acetate buffer (pH 6.5) containing 5 mM 2-merpcatoethanol. The enzyme fraction, eluted with the 700 mM buffer was designated as fraction III, which was shown to be specific for cAMP as substrate.[21]

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Supplementary Material Available: The atomic coordinates, isotropic thermal parameters, anisotropic thermal parameters, bond length (angstroms) and bond angles (degrees) for pelrinone **9** (5 pages). Ordering information is given on any current masthead page.