Synthesis and Cardiotonic Activity of Novel Pyrimidine Derivatives: Crystallographic and Quantum Chemical Studies

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The synthesis of ethyl or methyl 4-substituted or unsubstituted 2-(dimethylamino)-5-pyrimidinecarboxylates **10**−**20**, which is mainly carried out by reaction of ethyl or methyl 2-[(dimethylamino)methylene]-3-oxoalkanoates with 1,1-dimethylguanidine, is described. The above esters were hydrolyzed to the relative carboxylic acids **21**−**30**, which were decarboxylated to the corresponding 2,4-disubstituted pyrimidines **31**−**40**. All the new synthesized pyrimidines were evaluated in spontaneously beating and electrically driven atria from reserpine-treated guinea pigs. Their effects were compared to those induced by milrinone in both atria preparations. Compound **28** (4-benzyl-2-(dimethylamino)-5-pyrimidinecarboxylic acid) was the most effective positive inotropic agent, while the corresponding methyl ester **17** reduced both the contractile force and the frequency of guinea pig atria. An antagonism toward the negative influence exerted by endogenous adenosine on the heart seems to be involved in the contractile activity of compound **28**. By contrast, compound **17** might be partial agonist at the purinergic inhibitory (A1) receptor. X-ray analysis carried out on **17** and **28** and molecular modeling investigations extended also to related derivatives allowed a possible rationalization between structure and inotropic activity for this series of compounds.

Introduction

Antagonism of endogenous adenosine at A1 inhibitory receptors in the heart represents an investigational area which could offer a creative possibility in the treatment of heart failure.[1] Adenosine, which is released in high concentrations during cardiac heart failure,[2] may further damage the heart by slowing conduction in the sinoatrial and AV nodes and by reducing atrial contractility.[3−5] The negative effects exerted by adenosine on the heart appear to be related to a reduction of the duration of the action potential in response to a direct inhibition of Ca^{2+} channels[6−9] and/or to a reduction in Ca^{2+} flux into the cell due to an increased K^+ conductance and hyperpolarization of cell membrane.[7,10,11] Thus, an antagonism toward endogenous adenosine may indirectly increase intracellular Ca^{2+} concentration and activate the contractile system without increases in cyclic AMP levels and without the related risk of dangerous arrhythmias.[12] In fact, all the phosphodiesterase III (PDE III) inhibitors, by increasing intracellular cAMP content, exert favorable hemodynamic effects in patients with cardiac failure, but most of them may exacerbate ventricular arrhythmias, provoke myocardial ischemia, accelerate the progression of the underlying disease, and increase the mortality rate.[13,14]

Although classified as PDE III inhibitors, the bipyridine derivatives, such as amrinone and milrinone, at elevated concentrations, displace endogenous adenosine from its cardiac receptors.[15−19] In order to obtain pure adenosine antagonists, devoid of inhibitory influence on PDE III, we had already synthesized a number of milrinone analogues in which the 1,6-dihydro-6-oxopyridine moiety of the parent drug has been variously functionalized.[20−22] Inotropic activity of some of these compounds in the guinea pig atria was greater than that of milrinone, and an inhibition of the negative influence exerted by endogenous adenosine seems to be involved in their contractile activity.[23−25] Moreover, an X-ray structural characterization and quantum chemical analysis had been carried out on the above compounds.[24,26,27] Theoretical data indicate that one of the most critical factors for the different inotropic activities (positive or negative) seems to be related to the steric and electronic requirements of the substituent at position 2 in the 1,6-dihydro-6-oxopyridine nucleus. On the other hand, pyridinyl-2-pyrimidinamines were reported to be active as cardiotonics by a Lesher U.S. patent.[28] Since the pyrimidine moiety represents an integral part of the purine nucleoside adenosine, we utilized synthons **1**−**9** and synthesized a series of compounds (**10**−**40**) characterized by the pyrimidine nucleus bearing the electronic donor dimethylamino group at position 2 and by a variety of functionalizations at positions 4 and 5 (Scheme 1).

The cardiac activity of these new compounds was investigated by determining their effects on the contractile force and the frequency rate of spontaneously beating atria isolated from guinea pigs. The most

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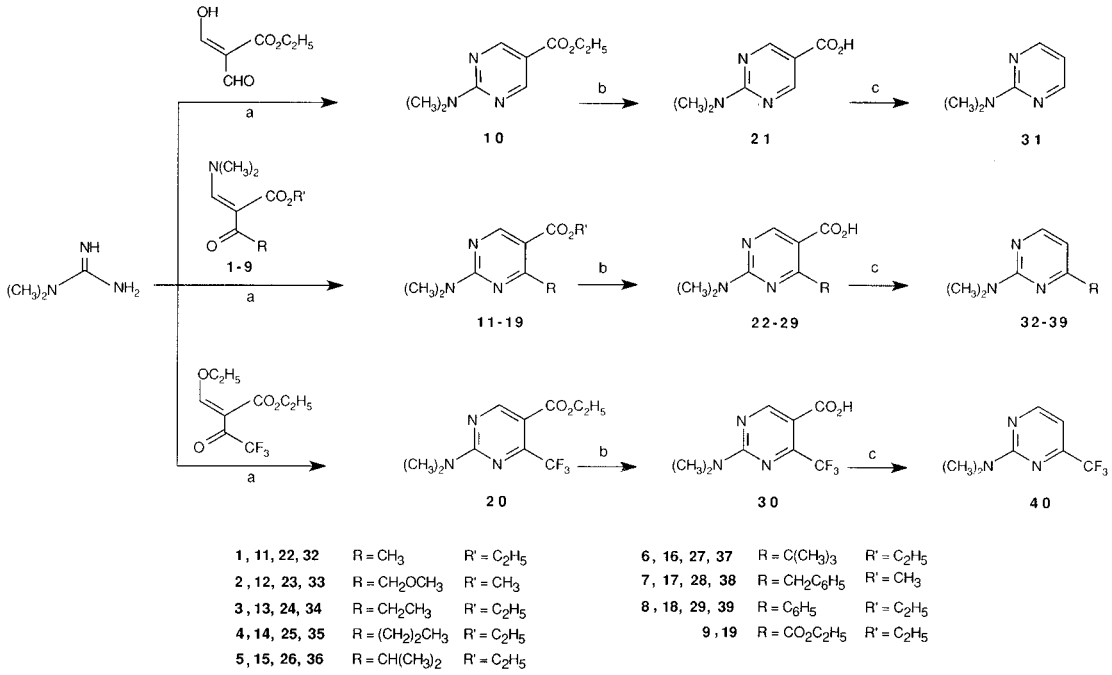
Scheme 1[a]



1, 11, 22, 32	R = CH_3	R' = C_2H_5
2, 12, 23, 33	R = CH_2OCH_3	R' = CH_3
3, 13, 24, 34	R = CH_2CH_3	R' = C_2H_5
4, 14, 25, 35	R = $(CH_2)_2CH_3$	R' = C_2H_5
5, 15, 26, 36	R = $CH(CH_3)_2$	R' = C_2H_5
6, 16, 27, 37	R = $C(CH_3)_3$	R' = C_2H_5
7, 17, 28, 38	R = $CH_2C_6H_5$	R' = CH_3
8, 18, 29, 39	R = C_6H_5	R' = C_2H_5
9, 19	R = $CO_2C_2H_5$	R' = C_2H_5

[a] (a) NaOEt/EtOH or NaOMe/MeOH at room temperature; (b) (1) KOH/EtOH or KOH/MeOH, reflux, (2) 6 M HCl; (c) heating over their melting points.

interesting agents were further studied in electrically driven left atrium. X-ray analysis and quantum chemical methods were also applied in order to gain additional information on the possible structure−activity relationships. The results have been related to the pharmacological data.

Chemistry

In recent papers[29,30] we described the efficient reaction of ethyl or methyl 2-[(dimethylamino)methylene]-3-oxoalcanoates **1**−**9** with N−C−N dinucleophiles such as guanidine, acetamidine, benzamidine, or 2-methylisothiourea. This synthetic pathway afforded the relative esters of 4-substituted 2-amino-, 2-methyl, 2-phenyl-, or 2-(methylthio)-5-pyrimidinecarboxylic acids, respectively, in high yields.

Using our method, the methyl or ethyl esters of 4-substituted or unsubstituted 2-(dimethylamino)-5-pyrimidinecarboxylic acids **10**−**20** (Scheme 1, Table 1) were synthesized by reacting ethyl 2,2-diformylacetate,[31] synthons **1**−**9**,[32,33] and ethyl-2-(ethoxymethylene)-4,4,4-trifluoro-3-oxobutanoate,[34] respectively, with 2,2-dimethylguanidine sulfate in the presence of sodium ethoxide or methoxide. Esters **10**−**20** were converted, generally in high yields, to the corresponding 4-substituted or unsubstituted 2-(dimethylamino)-5-pyrimidinecarboxylic acids **21**−**30** by the usual saponification procedure (potassium hydroxide in boiling ethanol or methanol) followed by acidification. Only in the case of ester **19** did the above reaction afford a mixture of dicarboxylic and monocarboxylic derivatives which proved difficult to separate. Finally, decarboxylation of acids **21**−**30** by simply heating at temperatures above their melting points led to 4-substituted or unsubstituted 2-(dimethylamino)pyrimidines **31**−**40** in high yields.

Some of these pyrimidines are already known, although prepared by other routes. Therefore, the identity in general of their physical constants with those of our compounds (Table 1), as well as spectral data, unequivocally established the structure of pyrimidines **31**, **32**, **34**, and **37**[35−37] and consequently that of starting esters **10**, **11**, **13**, and **16**.

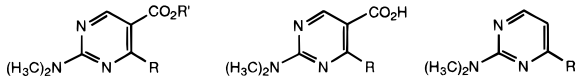
Biological Activity: Isolated Atria Preparations

All these new pyrimidine derivatives (1 μM−1 mM) exerted qualitatively and/or quantitatively different effects on both contractile force (Table 2) and frequency (Table 3) of spontaneously beating atria from reserpine-treated guinea pigs. Among the tested compounds, only **28** induced a marked increase in inotropism (Table 2). The positive inotropic effect induced by **28** at the highest concentration tested (1 mM), expressed as a percentage variation with respect to the control, was greater than that induced by higher active concentration of milrinone (0.3 mM). Moreover the respective EC_{50} was not determined since **28**, in the range of concentrations tested, did not reach its maximum inotropic effect. In contrast to milrinone, **28** did not increase the beating rate of the heart preparation (Table 3). Thus the acid appears to be a more "force" than "frequency" specific agent with a more advantageous pharmacological profile. The positive inotropic activity of **28** was also confirmed in electrically driven left atria (Table 4). On the contrary, the related methyl ester, compound **17**, after a slight initial increase in the developed tension, evoked marked negative inotropic and chronotropic effects (Table 2 and 3).[1] EC_{50} values[4] for its negative inotropic and chronotropic effect[2] of 0.30 and 0.88 mM, respectively, were calculated. The negative inotropic activity of **17** was also confirmed in electrically driven left atria (Table 4).

Mechanism of Action

The electrically driven left atrium was used to study the mechanism of action of **17** and **28**. Release of endogenous catecholamines is not involved in the posi-

Table 1. Physical Data of Compounds **10-40**



10-20 **21-30** **31-40**

compd	R	R′	heating time (h)	mp (°C) or bp (°C, mmHg)	solvent[a]	yield	formula
10	H	C_2H_5		60−61	A	59	$C_9H_{13}N_3O_2$
11	CH_3	C_2H_5		56−58	B	87	$C_{10}H_{15}N_3O_2$
12	CH_2OCH_3	CH_3		102−104	A	70	$C_{10}H_{15}N_3O_3$
13	CH_2CH_3	C_2H_5		43−45	A	85	$C_{11}H_{17}N_3O_2$
14	$(CH_2)_2CH_3$	C_2H_5		36−38	B	86	$C_{12}H_{19}N_3O_2$
15	$CH(CH_3)_2$	C_2H_5		95−100 (0.4)		82	$C_{12}H_{19}N_3O_2$
16	$C(CH_3)_3$	C_2H_5		115−120 (0.9)		72	$C_{13}H_{21}N_3O_2$
17	$CH_2C_6H_5$	CH_3		63−65	A	41	$C_{15}H_{17}N_3O_2$
18	C_6H_5	C_2H_5		37−39	B	86	$C_{15}H_{17}N_3O_2$
19	$CO_2C_2H_5$	C_2H_5		67−69	B	41	$C_{12}H_{17}N_3O_4$
20	CF_3	C_2H_5		95−100 (0.3)		70	$C_{10}H_{12}F_3N_3O_2$
21	H			263−264	C	70	$C_7H_9N_3O_2$
22	CH_3			193−195	D	66	$C_8H_{11}N_3O_2$
23	CH_2OCH_3			190−192	D	77	$C_9H_{13}N_3O_2$
24	CH_2CH_3			178−180	D	99	$C_9H_{13}N_3O_2$
25	$(CH_2)_2CH_3$			162−164	D	89	$C_{10}H_{15}N_3O_2$
26	$CH(CH_3)_2$			152−154	D	96	$C_{10}H_{15}N_3O_2$
27	$C(CH_3)_3$			167−169	D	78	$C_{11}H_{17}N_3O_2$
28	$CH_2C_6H_5$			183−185	D	95	$C_{14}H_{15}N_3O_2$
29	C_6H_5			236−238	C	99	$C_{13}H_{13}N_3O_2$
30	CF_3			188−190	E	82	$C_8H_8F_3N_3O_2$
31	H		1.5	85−90 (20) [b]		95	$C_6H_9N_3$
32	CH_3		5	85−90 (13) [c]		73	$C_7H_{11}N_3$
33	CH_2OCH_3		5	102−106 (12)		83	$C_8H_{13}N_3O$
34	CH_2CH_3		5	91−92 (15) [d]		89	$C_8H_{13}N_3$
35	$(CH_2)_2CH_3$		7	110−120 (13)		83	$C_9H_{15}N_3$
36	$CH(CH_3)_2$		7	105−110 (15)		86	$C_9H_{15}N_3$
37	$C(CH_3)_3$		5	115−118 (14) [e]		81	$C_{10}H_7N_3$
38	$CH_2C_6H_5$		20	120−125 (0.4)		62	$C_{13}H_{15}N_3$
39	C_6H_5		5	48−49	A	90	$C_{12}H_{13}N_3$
40	CF_3		20	85−90 (12)		88	$C_7H_8F_3N_3$

[a] Recrystallization solvents: A = diethyl ether, B = petroleum ether (bp 40−70 °C), C = 95% ethanol, D = ethyl acetate, E = petroleum ether−diethyl ether, 1:1. [b] Lit.[35] bp 85−86 °C (28); 88% yield. [c] Lit.[36] bp 103−106 °C (40); 80% yield. [d] Lit.[36] bp 91−92 °C (15); 50% yield. [e] Lit.[37] bp 50−55 °C (0.1); 42% yield.

tive inotropic effect of **28** since the effect is evident in atria isolated from reserpine-treated animals and thus depleted in catecholamines. The involvement of endogenous adenosine in the action of the new compounds was tested by treating left atria with adenosine deaminase, the enzyme which inactivates adenosine by metabolizing it to inosine. In these conditions an increase in developed tension occurred, which declined within 15−20 min, and the force of contraction reached a new steady state which was 15% higher than the control. In the presence of adenosine deaminase, the positive inotropic activity of **28** was drastically reduced (Table 5). The positive inotropic effect of **28**, not modified by adenosine deaminase, was insensitive to β-blocker propranolol (0.1 μM), α-blocker prazosine (5 nM), and histamine antagonists pyrilamine (0.1 μM) and ranitidine (10 μM) (data not shown). Thus the activation of a catecholamine or histamine receptor is not involved in the cardiac activity of **28**, although an antagonism toward endogenous adenosine seems to be present. However, the nature of the component of the inotropic effect not related to the adenosine antagonism remains to be clarified.

In order to confirm the antagonism toward adenosine, the effect of **28** on the left atrium was evaluated in the presence of the potent agonist at A1 adenosine receptor, (*R*)-PIA (N^6-[(*R*)-phenylisopropyl]adenosine). Figure 1 shows that the concentration−effect curves for the

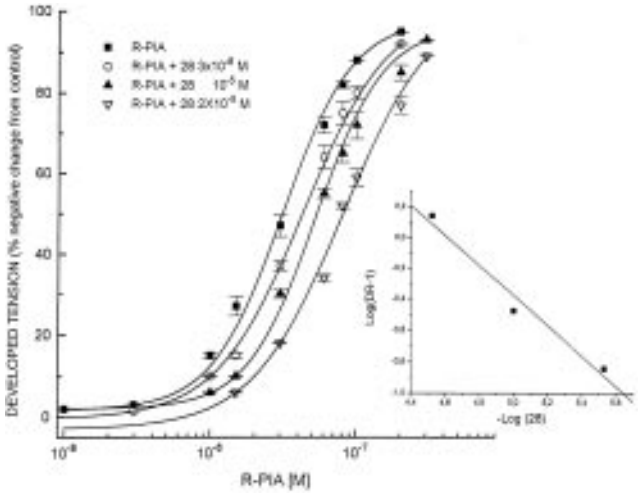


Figure 1. Cumulative concentration−effect curves for negative inotropic effect of (*R*)-PIA in the absence and presence of increasing concentrations of compound **28**. Schild regression analysis is shown in the inset: (■) (*R*)-PIA, (○) (*R*)-PIA plus 3 × 10^{-6} M compound **28**, (▲) (*R*)-PIA plus 10^{-5} M compound **28**, (▽) (*R*)-PIA plus 3 × 10^{-5} M compound **28**. Each point is the mean ± SEM of four to six assays from six different experiments.

negative inotropic effect exerted by (*R*)-PIA were shifted parallel by increasing concentrations of **28**, suggesting a competitive antagonism between **28** and (*R*)-PIA. This was confirmed by the analysis of the data according to

Table 2. Effect of Compounds **10**−**40** on the Contractile Force of Spontaneously Beating Atria from Reserpine-Treated Guinea Pigs: Comparison with Amrinone and Milrinone[a]

	developed tension (% variation from the control)						
compd	10^{-6} M	3×10^{-6} M	10^{-5} M	3×10^{-5} M	10^{-4} M	3×10^{-4} M	10^{-3} M
amrinone	0.00 ± 0.00	0.00 ± 0.00	0.52 ± 0.01	4.50 ± 0.41	10.22 ± 0.85	19.03 ± 0.72	23.12 ± 1.42
milrinone	0.00 ± 0.00	0.00 ± 0.00	16.31 ± 0.49	30.32 ± 0.31	37.77 ± 0.12	45.62 ± 0.48	31.09 ± 0.31
10	1.19 ± 0.06	4.76 ± 0.03	5.55 ± 0.14	11.04 ± 0.41	9.03 ± 0.04	11.71 ± 0.08	−59.61 ± 0.98
11	2.79 ± 0.15	7.12 ± 0.12	9.91 ± 0.80	15.88 ± 0.31	−21.47 ± 0.51	−25.87 ± 0.09	−49.52 ± 0.42
12	3.04 ± 0.21	6.93 ± 0.15	11.14 ± 0.34	14.95 ± 0.67	16.95 ± 0.59	18.95 ± 0.45	25.41 ± 0.83
13	0.00 ± 0.00	0.00 ± 0.00	−39.54 ± 0.91	−67.44 ± 0.73	−100 ± 0.00		
14	0.00 ± 0.00	−5.29 ± 0.08	−57.89 ± 0.65	−100 ± 0.00			
15	0.00 ± 0.00	3.19 ± 0.13	1.06 ± 0.07	−34.22 ± 0.21	−57.53 ± 0.47	−100 ± 0.00	
16	0.00 ± 0.00	2.08 ± 0.01	−12.52 ± 0.12	−50.08 ± 0.34	−100 ± 0.00		
17	0.00 ± 0.00	5.83 ± 0.09	8.97 ± 0.57	−6.54 ± 0.69	−13.54 ± 0.98	−55.28 ± 0.07	−100 ± 0.00
18	1.73 ± 0.13	10.24 ± 0.09	12.05 ± 0.67	2.25 ± 0.18	−40.74 ± 0.87	−70.37 ± 0.95	−100 ± 0.00
19	0.00 ± 0.00	2.18 ± 0.03	0.54 ± 0.06	−5.33 ± 0.13	−48.31 ± 0.96	−76.47 ± 0.89	−100 ± 0.00
20	7.15 ± 0.15	11.26 ± 0.06	10.67 ± 0.78	−3.61 ± 0.11	−30.35 ± 0.14	−47.85 ± 0.78	−60.44 ± 0.45
21	3.76 ± 0.12	6.36 ± 0.06	7.54 ± 0.13	10.48 ± 0.78	9.39 ± 0.04	8.34 ± 0.15	9.64 ± 0.08
22	0.00 ± 0.00	3.77 ± 0.13	5.18 ± 0.2	8.62 ± 0.56	10.82 ± 0.67	15.77 ± 0.17	21.96 ± 0.95
23	5.46 ± 0.17	9.16 ± 0.15	9.99 ± 0.53	9.72 ± 0.42	8.24 ± 0.15	8.19 ± 0.17	5.92 ± 0.12
24	0.00 ± 0.00	2.44 ± 0.16	4.47 ± 0.18	6.17 ± 0.31	6.67 ± 0.21	14.69 ± 0.67	22.03 ± 0.15
25	5.38 ± 0.02	8.66 ± 0.71	11.21 ± 0.15	15.72 ± 0.67	18.35 ± 0.56	19.73 ± 0.51	23.61 ± 0.86
26	1.79 ± 0.06	4.06 ± 0.04	7.13 ± 0.14	7.79 ± 0.19	11.77 ± 0.43	16.41 ± 0.59	23.17 ± 0.74
27	3.51 ± 0.14	7.58 ± 0.81	9.67 ± 0.09	12.51 ± 0.65	13.23 ± 0.76	14.21 ± 0.45	18.64 ± 0.32
28	3.52 ± 0.24	9.37 ± 0.24	15.24 ± 0.76	22.89 ± 0.87	35.26 ± 0.93	67.75 ± 0.98	88.05 ± 0.99
29	6.62 ± 0.81	10.63 ± 0.71	11.47 ± 0.79	12.11 ± 0.82	12.75 ± 0.74	14.22 ± 0.97	18.20 ± 0.54
30	2.25 ± 0.11	6.15 ± 0.50	9.45 ± 0.18	13.44 ± 0.44	14.03 ± 0.75	14.96 ± 0.81	9.52 ± 0.09
31	0.00 ± 0.00	4.92 ± 0.15	9.17 ± 0.14	11.63 ± 0.56	15.08 ± 0.78	37.73 ± 0.71	41.66 ± 0.96
32	0.00 ± 0.00	2.27 ± 0.12	8.90 ± 0.07	16.76 ± 0.74	19.04 ± 0.18	22.47 ± 0.07	−36.67 ± 0.99
33	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	−8.02 ± 0.05	−9.03 ± 0.07	−11.89 ± 0.09	−20.93 ± 0.41
34	5.39 ± 0.15	9.91 ± 0.16	15.05 ± 0.28	9.21 ± 0.07	−10.54 ± 0.66	−43.95 ± 0.24	−84.45 ± 0.62
35	0.00 ± 0.00	7.18 ± 0.13	3.15 ± 0.06	−10.63 ± 0.21	−64.06 ± 0.69	−73.58 ± 0.71	−91.86 ± 0.41
36	0.00 ± 0.00	0.00 ± 0.00	−8.84 ± 0.07	−28.95 ± 0.79	−68.77 ± 0.97	−86.36 ± 0.99	−100 ± 0.00
37	0.00 ± 0.00	1.13 ± 0.09	−5.56 ± 0.04	−24.35 ± 0.27	−37.31 ± 0.77	−34.94 ± 0.61	−35.14 ± 0.91
38	6.43 ± 0.08	10.71 ± 0.15	9.18 ± 0.09	3.17 ± 0.06	−32.51 ± 0.73	−46.99 ± 0.42	−57.09 ± 0.49
39	0.00 ± 0.00	4.67 ± 0.08	7.52 ± 0.06	5.57 ± 0.04	−12.16 ± 0.85	−23.76 ± 0.44	−100 ± 0.00
40	0.00 ± 0.00	2.47 ± 0.08	−12.62 ± 0.11	−30.94 ± 0.78	−67.95 ± 0.64	−83.03 ± 0.89	−100 ± 0.00

[a] The effect of compound was defined by the difference between the force of contraction before and after its addition to the bathing fluid and is expressed as percentage variation with respect to the basal force of contraction. In the absence of compounds, the force of contraction of spontaneously beating atria was 3.3 ± 0.4 mN. Each value is the mean ± SEM of 6−10 assays from 10 different experiments. Negative values indicate a negative inotropic effect.

Schild (insert of Figure 1), from which a slope of −0.98 and pA_2 of 5.62 were calculated.

Taking into consideration all the above, these results underline the ability of **28** to displace adenosine from its A1 cardiac receptor. To substantiate this mechanism of action, the influence of **28** on [^{3}H]CHA (N^6-cyclohexyl-[^{3}H]adenosine) binding to A1 receptor in the guinea pig heart was studied. In the concentration range used to evoke inotropic responses, compound **28** inhibited [^{3}H]-CHA binding to A1 adenosine receptors in guinea pig atria showing an IC_{50} of 583.30 ± 16.7 μM (data not shown). This concentration corresponds to the concentration of compound **28** suitable in inducing 50% of the maximum positive inotropic effect reached under our experimental conditions.

Cardiac activity of compound **28** was studied in the presence of carbachol to exclude any participation of cAMP in its mechanism of action. This agent, in fact, is known to abolish selectively the increase in heart contractility induced either by adenylate cyclase stimulation or by PDE III inhibition in different preparations.[38−40] Carbachol, at a concentration (0.3 μM) which almost completely inhibited the spontaneous contractility and the inotropic response of the atria to isoprenaline,[24] enhanced the inotropic activity of **28** (Figure 2). While the latter effect may, in part, be due to a suppressed base line, the lack of the antagonism by carbachol suggests that an elevation of cAMP is unlikely to mediate the cardiac effect of the pyrimidine derivative. According to this observation, compound **28**

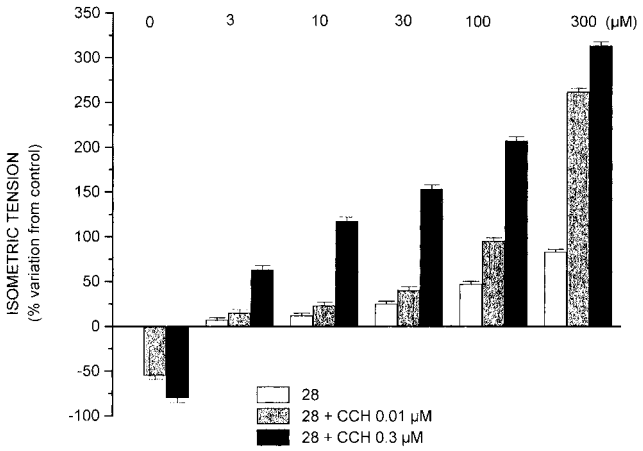


Figure 2. Effect of carbachol on **28**-induced inotropism in electrically driven left atrium from reserpine-treated guinea pigs. Each data point is the mean ± SEM of five assays from five different experiments. *P* values were calculated versus respective control (atrium incubated without carbachol). The statistical significance of the changes induced by carbachol was calculated by the Student's test. *P < 0.001; CCH = carbachol.

significantly inhibited cardiac PDE III activity only at the highest concentration tested (0.1−1 mM) (Figure 3). These results confirm our previous observation that a lack of correlation exists between inhibition of PDE III and an increase in inotropism induced in guinea pig atria by amrinone,[15] milrinone, and milrinone analogues.[23−25] This can be explained by the fact that

Table 3. Effect of Compounds **10**−**40** on the Frequency Rate of Spontaneously Beating Atria from Reserpine-Treated Guinea Pigs: Comparison with Amrinone and Milrinone[a]

	frequency (% variation from the control)						
compd	10^{-6} M	3×10^{-6} M	10^{-5} M	3×10^{-5} M	10^{-4} M	3×10^{-4} M	10^{-3} M
amrinone	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	1.50 ± 0.50	6.25 ± 1.35	7.20 ± 1.46	9.87 ± 0.46
milrinone	0.00 ± 0.00	0.00 ± 0.00	15.22 ± 0.50	27.09 ± 0.24	34.16 ± 0.25	42.85 ± 0.33	37.20 ± 0.85
10	0.00 ± 0.00	0.00 ± 0.00	3.71 ± 0.04	7.43 ± 0.11	11.11 ± 0.14	14.81 ± 0.08	18.51 ± 0.38
11	−3.57 ± 0.05	0.00 ± 0.00	0.00 ± 0.00	3.57 ± 0.21	7.14 ± 0.01	10.71 ± 0.12	−17.85 ± 0.22
12	0.00 ± 0.00	0.00 ± 0.00	2.85 ± 0.04	3.57 ± 0.07	7.14 ± 0.19	7.14 ± 0.19	11.43 ± 0.18
13	−5.88 ± 0.20	−6.25 ± 0.07	−9.37 ± 0.21	−14.76 ± 0.53	−100 ± 0.00		
14	0.00 ± 0.00	−6.06 ± 0.08	−9.37 ± 0.17	−100 ± 0.00			
15	−3.85 ± 0.14	−2.94 ± 0.13	−3.51 ± 0.08	−0.91 ± 0.01	−2.94 ± 0.07	−100 ± 0.00	
16	0.00 ± 0.00	0.00 ± 0.00	4.76 ± 0.11	−7.64 ± 0.14	−100 ± 0.00		
17	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	−8.03 ± 0.09	−16.06 ± 0.08	−55.15 ± 0.17	−100 ± 0.00
18	0.00 ± 0.00	0.00 ± 0.00	3.70 ± 0.17	7.40 ± 0.08	−3.70 ± 0.07	−14.81 ± 0.15	−100 ± 0.00
19	0.00 ± 0.00	0.00 ± 0.00	−4.76 ± 0.06	−7.41 ± 0.12	−40.77 ± 0.16	−55.55 ± 0.59	−100 ± 0.00
20	0.00 ± 0.00	0.00 ± 0.00	−3.23 ± 0.08	−3.61 ± 0.11	−6.66 ± 0.14	−6.66 ± 0.14	−19.35 ± 0.25
21	0.00 ± 0.00	0.00 ± 0.00	3.12 ± 0.13	3.44 ± 0.08	3.44 ± 0.08	3.44 ± 0.08	6.25 ± 0.09
22	0.00 ± 0.00	6.45 ± 0.11	9.67 ± 0.21	3.22 ± 0.06	3.22 ± 0.06	6.45 ± 0.17	−3.12 ± 0.05
23	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	−3.12 ± 0.12	−6.25 ± 0.15	−6.25 ± 0.15	−9.37 ± 0.12
24	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	1.11 ± 0.01	−4.79 ± 0.07	−7.69 ± 0.15
25	0.00 ± 0.00	0.00 ± 0.00	−2.85 ± 0.15	−2.85 ± 0.15	−2.85 ± 0.15	−5.71 ± 0.21	−4.28 ± 0.06
26	0.00 ± 0.00	−3.13 ± 0.13	−3.13 ± 0.13	−10.71 ± 0.19	−14.28 ± 0.23	−17.58 ± 0.39	−28.57 ± 0.64
27	0.00 ± 0.00	3.33 ± 0.15	3.33 ± 0.15	3.33 ± 0.15	3.71 ± 0.16	7.42 ± 0.25	11.11 ± 0.12
28	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	−3.85 ± 0.06	−5.02 ± 0.07	−5.69 ± 0.08	−10.25 ± 0.36
29	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	−3.12 ± 0.04	−3.12 ± 0.04	−6.25 ± 0.14
30	0.00 ± 0.00	0.00 ± 0.00	−2.85 ± 0.18	−3.03 ± 0.04	−3.03± 0.04	−9.09 ± 0.21	−14.28 ± 0.09
31	0.00 ± 0.00	0.00 ± 0.00	1.33 ± 0.14	3.72 ± 0.06	9.72 ± 0.06	17.44 ± 0.21	25.17 ± 0.96
32	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	−6.25 ± 0.06	−17.34 ± 0.29
33	0.00 ± 0.00	0.00 ± 0.00	−1.33 ± 0.18	−4.00 ± 0.05	−4.00 ± 0.05	4.00 ± 0.09	−9.33 ± 0.11
34	0.00 ± 0.00	0.00 ± 0.00	−3.03 ± 0.08	−4.77 ± 0.07	−14.95 ± 0.36	−28.91 ± 0.24	−42.41 ± 0.72
35	0.00 ± 0.00	−5.29 ± 0.13	−26.31 ± 0.16	−31.58 ± 0.21	−52.63 ± 0.65	−52.63 ± 0.65	−52.63 ± 0.65
36	0.00 ± 0.00	−5.15 ± 0.11	−5.15 ± 0.11	−17.26 ± 0.09	−41.66 ± 0.37	−41.66 ± 0.37	−100 ± 0.00
37	0.00 ± 0.00	0.00 ± 0.00	−6.66 ± 0.04	−13.33 ± 0.17	−16.66 ± 0.07	−30.01 ± 0.61	−40.00 ± 0.95
38	0.00 ± 0.00	0.00 ± 0.00	3.03 ± 0.09	3.03 ± 0.09	−3.03 ± 0.03	−9.09 ± 0.18	−12.12 ± 0.09
39	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	−13.33 ± 0.14	−100 ± 0.00
40	0.00 ± 0.00	0.00 ± 0.00	−3.23 ± 0.01	−3.68 ± 0.08	−22.58 ± 0.64	−32.26 ± 0.39	−100 ± 0.00

[a] The effect of compound was defined by the difference between the frequency of atria before and after its addition to the bathing fluid and is expressed as percentage variation with respect to the spontaneous frequency of the atria. In the absence of compounds, the heart rate was 161 ± 7 beats/min. Each value is the mean ± SEM of 6−10 assays from 10 different experiments. Negative values indicate a negative chronotropic effect.

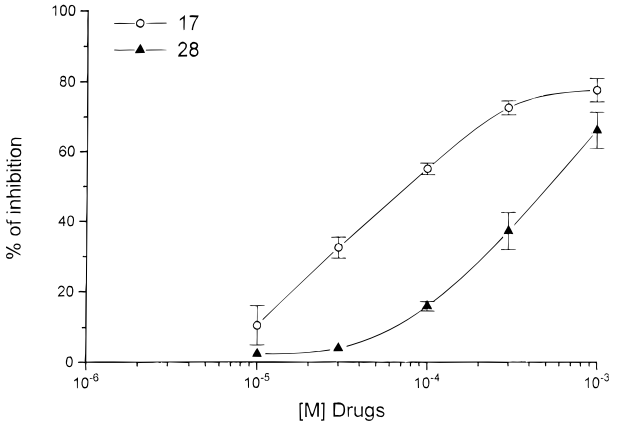


Figure 3. Concentration−effect curves for inhibition of soluble PDE III isolated from calf heart by free **17** and **28**. Each point is the mean ± SEM from four duplicate experiments. PDE III activity in the absence of **17** or **28** was 0.77 ± 0.01 mmol/mg of protein/min.

PDEase III, the isoenzyme that is selectively inhibited by amrinone and milrinone, in the guinea pig heart may be either biochemically uncoupled from myocardial contractile proteins or compartmentalized in the cytosol so that increases in cAMP concentration do not necessarily cause increases in contractility.[41,42] The lack of correlation between PDE III inhibition and increase in cardiac contractility is further substained by present results showing that compound **17**, at a concentration suitable to reduce spontaneous contractile activity in the guinea pig atria, significantly inhibited cardiac PDE III activity (Figure 3).

The negative influence exerted by **17** on heart contractility was insensitive to the presence of atropine (1 μM) in the medium (data not shown), suggesting that an acetylcholine-like action at the specific muscarinic receptors must be excluded. In contrast, an action on A1 adenosine receptors might be involved. Compound **17** reduced the positive inotropic effect induced by the adenosine antagonist 8-phenyltheophylline (30 μM−0.1 mM) (Table 6). Unfortunately, because of its low solubility, higher concentrations of xanthine were not tested so that the nature of the inhibition exerted by **17** remains to be defined. However, in the concentration range suitable to evoke negative influence of the atria contractility, **17** inhibited [^{3}H]CHA binding to A1 adenosine receptors in the guinea pig heart showing an IC_{50} of 21.30 ± 1.30 μM (data not shown). From the comparison of the EC_{50}s, compound **17** appears to be more potent in inhibiting [^{3}H]CHA binding to adenosine receptors than in reducing cardiac inotropism, but the difference might be due to the variable permeability of intact tissue in comparison to isolate receptor preparation. Thus, an adenosine-like action at the specific purinergic (A1) receptor could be involved in the negative inotropic effect of **17**, suggesting that it behaves as partial agonist. If this is true, we can conclude that the esterification of the molecule is sufficient to modify the biological activity of the compound from antagonist to

Table 4. Inotropic Effect of **17** and **28** in Electrically Driven Left Atrium from Reserpine-Treated Guinea Pigs: Comparison with Milrinone[a]

	developed tension (% variation from the control)				
drug conctn (M)	milrinone	**17**		**28**	
10^{-6}	0.00 ± 0.00	0.00 ± 0.00	ns	3.23 ± 0.10	$P < 0.02$
3 × 10^{-6}	9.31 ± 0.17	4.85 ± 0.07	$P < 0.01$	9.70 ± 0.21	ns
10^{-5}	13.25 ± 0.21	7.45 ± 0.76	$P < 0.01$	16.51 ± 0.66	$P < 0.02$
3 × 10^{-5}	22.81 ± 0.44	−5.21 ± 0.37	$P < 0.01$	24.74 ± 0.44	$P < 0.05$
10^{-4}	35.61 ± 0.34	−14.21 ± 0.89	$P < 0.001$	36.30 ± 0.83	ns
3 × 10^{-4}	45.61 ± 0.40	−48.34 ± 0.09	$P < 0.001$	52.95 ± 2.44	$P < 0.001$
10^{-3}	42.89 ± 0.33	−100.00 ± 0.00	$P < 0.001$	78.63 ± 1.77	$P < 0.001$

[a] The effect of compound was defined by the difference between the force of contraction before and after its addition to the bathing fluid and is expressed as percentage variation with respect to the basal force of contraction. In the absence of compounds, the force of concentration of electrically driven left atrium was 5.5 ± 0.6 mN. Each value is the mean ± SEM of six assays from six different experiments. *P* values were determined versus milrinone-treated preparations. The statistical significance of the changes induced by **17** and **28** was calculated by the Student's *t*-test.

Table 5. Effect of Adenosine Deaminase on Inotropic Response to **28** in Electrically Driven Left Atrium from Reserpine-Treated Guinea Pigs[a]

	developed tension (% increase over the control)		
28 (M)	−ADA	+ADA (2 U/mL)	
3 × 10^{-6}	11.21 ± 0.22	0.00 ± 0.00	$P < 0.001$
10^{-5}	27.41 ± 0.31	4.76 ± 0.09	$P < 0.001$
3 × 10^{-5}	33.61 ± 0.86	9.52 ± 0.08	$P < 0.001$
10^{-4}	44.95 ± 0.95	19.05 ± 0.18	$P < 0.001$
3 × 10^{-4}	66.21 ± 0.99	33.33 ± 0.21	$P < 0.001$
10^{-3}	88.35 ± 1.21	57.24 ± 0.32	$P < 0.001$

[a] The effect of **28** was defined by the difference in force of contraction before and after its addition to the bathing fluid and is expressed as percentage variation with respect to the basal force of contraction. In the experiments performed in the presence of ADA, the basal force of concentration was that registered after the addition of ADA itself. Each value is the mean ± SEM of six assays from six different experiments. *P* values were determined versus control (atrium incubated without adenosine deaminase). The statistical significance of the changes induced by adenosine deaminase was calculated by Student's *t*-test. ADA = adenosine deaminase.

agonist, probably in view of a higher lipophilicity of the ester, which could permit a different interaction with the binding site within the receptor cavity.[43]

The present results indicate that compounds containing a pyrimidine ring behave as effective inotropic agents, whose cardiac effects are not related to PDE III inhibition. However, the search for a pure adenosine antagonist, not affecting the cyclic AMP system, remains to be pursued.

Crystallographic Studies

The molecular structures **17** are shown in Figure 4 as an ORTEP view with the atom-numbering scheme used. Significant bond distances and angles are reported in Table 7 together with the corresponding value for **28** (see below). In the crystallographic cell are present two molecules per asymmetric unit (the second indicated by the letter A), which present some conformational differences due mainly to the reciprocal orientation of the pyrimidine and phenyl rings. The dihedral angle between the respective mean planes is 74.3(1)° for one molecule and 70.6(1)° for the second (A). The COOMe group is about coplanar with the pyrimidine ring in both molecules, the dihedral angle between the respective mean planes having 1.3(1)° for one molecule and 2.1(2)° for molecule A, with the same *cisoid* conformation in both molecules (the carbonyl oxygen O(9) is oriented in the direction of the methylene group C(11)). The heterocyclic moieties present a range of the atom deviations from the best mean plane calculated for the ring from −0.015(4) to 0.012(3) Å in one molecule and from −0.009(4) to 0.007(3) Å in A, both rings being nearly planar. In particular, molecular stacking is observed between the centrosymmetric related pyrimidine rings of the A molecule with a distance of about 3.6 Å, in the *b* axis direction.

The N(3)−C(4)−C(11)−C(12) (τ_2) torsion angle is −92.2(4)° (−90.1(4)° in A), and C(4)−C(11)−C(12)−C(13) (τ_3) is −67.1(4)° (69.7(4)° in A). However, the intramolecular contact distance between H(13) and O(9) is the same (2.60(1) Å) in both molecules as well the H(110)···O(9) contact of 2.30(1) and 2.28(1) Å in A. All of the other corresponding bond distances and angles are equal in both molecules. In compound **28**, which differs from **17** by having the acid group unsubstituted, the molecular conformation is different. An ORTEP view with the atom-numbering scheme used is reported in Figure 5. The proton on O(10) forms a strong hydrogen bond interaction with the molecule centrosymmetric related O(10)···O(9)′ (2.631(2) Å), O(10)−H···O(9)′ (1.815(2) Å), and O(10)−H···O(9)′ (173.5(2)°) (prime at $-x,-y,-z$)

Table 6. Effect of Compound **17** on Inotropic Response to 8-Phenyltheophylline in Electrically Driven Left Atrium from Reserpine-Treated Guinea Pigs[a]

	developed tension (% variation from the control)					
8-Ph (M)	−	3 × 10^{-5} M **17**		10^{-4} M **17**		3 × 10^{-4} M **17**	
−	−	−6.66 ± 0.15		−9.37 ± 0.75		−33.52 ± 2.15	
3 × 10^{-6}	11.86 ± 0.97	8.79 ± 0.25	$P < 0.05$	−4.34 ± 0.18	$P < 0.001$	−28.86 ± 1.17	$P < 0.001$
10^{-5}	14.08 ± 1.04	10.96 ± 0.89	$P < 0.05$	−3.33 ± 0.14	$P < 0.001$	−22.17 ± 1.08	$P < 0.001$
3 × 10^{-5}	19.15 ± 0.75	14.88 ± 0.78	$P < 0.05$	2.72 ± 1.15	$P < 0.001$	−11.03 ± 1.06	$P < 0.001$
10^{-4}	29.87 ± 1.17	20.22 ± 1.25	$P < 0.05$	19.02 ± 1.28	$P < 0.05$	−6.66 ± 0.89	$P < 0.001$

[a] Cumulative concentration−effect curves for 8-phenyltheophylline were obtained in the absence and presence of different concentrations of compound **17**. The inotropic effect was determined as indicated under Table 4. Each data point is the mean ± SEM of four assays from four different experiments. *P* values were determined versus control (atrium incubated with 8-Ph). The statistical significance of the changes induced by compound **17** was calculated by Student's *t*-test. 8-Ph = 8-phenyltheophylline.

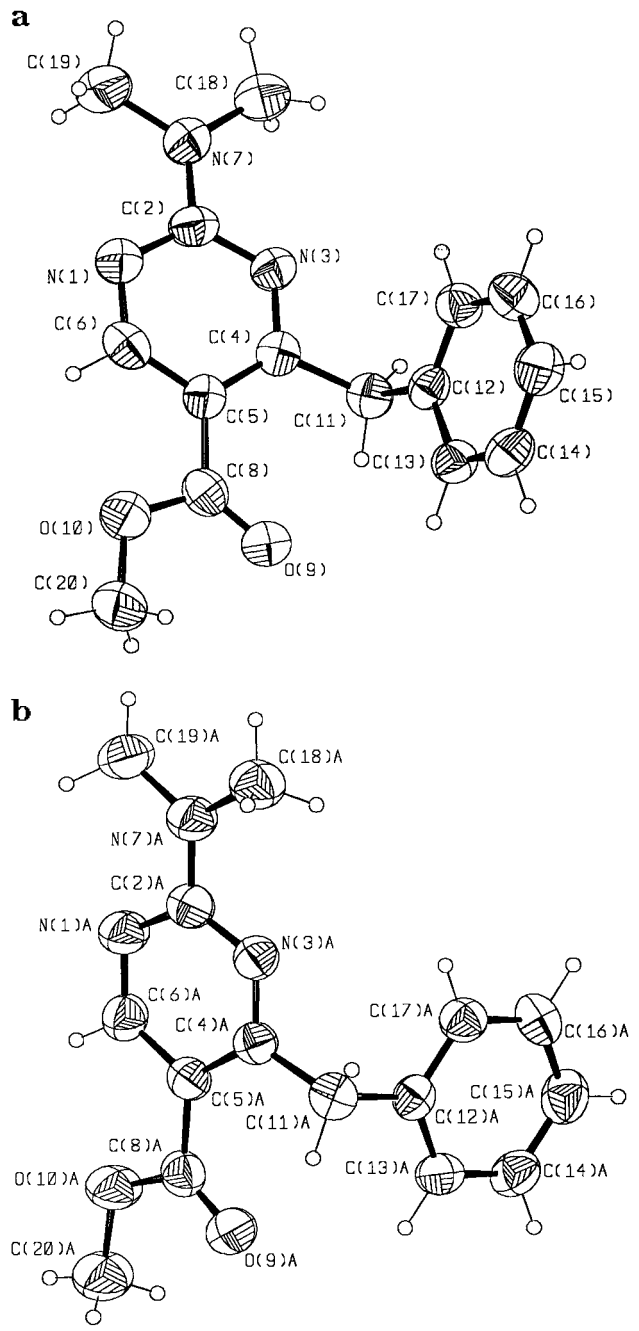


Figure 4. Molecular conformation of the two asymmetric units of **17** (thermal ellipsoids are drawn at the 40% probability level).

which indicates dimer formation. The τ_2 and τ_3 torsion angles are substantially changed with respect to the corresponding methyl ester with values for τ_2 of 101.4(2)° and for τ_3 of 164.5(2)°. The dihedral angle between the mean planes calculated on the pyrimidine and phenyl rings is 89.15(7)°, while the acid group remains coplanar with the pyrimidine moiety with the same *cisoid* conformation as in **17**. The H(110)···O(9) contact of 2.38(1) Å is longer than in **28,** and no significant contacts are present between the phenyl and the heterocyclic moiety due to the orthogonal position of the phenyl with respect to the heterocyclic moiety, which is practically planar also in this molecule (the range of deviations is −0.008(2) → 0.008(2) Å). Bond distances and angles are in agreement with those of **17**. The molecular packing is again characterized by molecular stacking with the centrosymmetric pyrimidine rings

Table 7. Sected Bond Lengths (Å) and Angles (Deg)

	17		
	molecule 1	1A	**28**
N(1)−C(6)	1.314(5)	1.315(5)	1.321(3)
N(1)−C(2)	1.349(5)	1.350(5)	1.354(2)
N(3)−C(4)	1.336(5)	1.331(5)	1.333(3)
N(3)−C(2)	1.353(5)	1.354(5)	1.351(3)
N(7)−C(2)	1.342(5)	1.343(5)	1.341(3)
N(7)−C(18)	1.452(5)	1.442(5)	1.445(3)
N(7)−C(19)	1.455(5)	1.459(5)	1.455(3)
O(9)−C(8)	1.200(5)	1.196(5)	1.229(2)
O(10)−C(8)	1.327(5)	1.340(5)	1.309(2)
O(10)−C(20)	1.430(5)	1.426(5)	
C(4)−C(5)	1.404(5)	1.405(5)	1.403(3)
C(4)−C(11)	1.503(5)	1.501(5)	1.501(3)
C(5)−C(6)	1.396(5)	1.385(5)	1.391(3)
C(5)−C(8)	1.472(5)	1.457(5)	1.457(3)
C(11)−C(12)	1.518(5)	1.520(5)	1.510(3)
C(12)−C(17)	1.382(5)	1.383(5)	1.379(3)
C(12)−C(13)	1.379(5)	1.377(5)	1.381(4)
C(17)−C(16)	1.385(5)	1.379(5)	1.384(5)
C(16)−C(15)	1.370(6)	1.371(6)	1.360(5)
C(15)−C(14)	1.378(6)	1.368(6)	1.358(5)
C(14)−C(13)	1.390(5)	1.380(5)	1.387(5)
N(3)−C(4)−C(11)	114.8(3)	114.6(3)	114.7(2)
N(3)−C(4)−C(5)	121.0(3)	121.2(3)	120.5(2)
N(3)−C(2)−N(7)	117.4(3)	117.5(4)	117.5(2)
C(4)−N(3)−C(2)	117.1(3)	117.7(3)	117.9(2)
N(1)−C(6)−C(5)	125.1(4)	126.0(4)	124.3(3)
N(1)−C(2)−N(7)	116.1(4)	116.8(3)	116.8(2)
N(1)−C(2)−N(3)	126.5(3)	125.7(4)	125.8(2)
C(4)−C(5)−C(8)	124.4(3)	124.0(3)	124.0(2)
C(4)−C(5)−C(6)	115.8(3)	115.1(3)	116.5(2)
C(4)−C(11)−C(12)	111.7(3)	112.3(3)	115.6(2)
C(5)−C(4)−C(11)	124.1(3)	124.2(3)	124.8(2)
C(6)−C(5)−C(8)	119.8(3)	120.9(3)	119.5(3)
C(6)−N(1)−C(2)	114.5(4)	114.4(3)	115.0(2)
C(2)−N(7)−C(19)	122.0(3)	121.1(3)	120.5(2)
C(2)−N(7)−C(18)	120.6(3)	121.1(3)	121.3(2)
C(18)−N(7)−C(19)	117.4(3)	117.8(3)	118.2(2)
C(8)−O(10)−C(20)	115.6(3)	116.6(3)	
O(10)−C(8)−C(5)	112.2(3)	127.5(4)	121.8(2)
O(9)−C(8)−C(5)	125.7(4)	112.1(3)	114.2(2)
O(9)−C(8)−O(10)	122.1(4)	120.4(4)	121.8(2)
C(11)−C(12)−C(13)	121.9(3)	121.6(3)	123.6(2)
C(11)−C(12)−C(17)	119.8(3)	120.1(3)	119.0(2)

parallel, separated by about 3.4 Å, and shorter than in the methyl-substituted **17**.

Molecular Modeling Study

In the present study the following procedure has been used. (a) The structures reported in Figure 6 were built from X-ray data when available (**17** and **28**) or using a general 3D-builder.[50] (b) The Monte Carlo−Metropolis algorithm as implemented in MAD[50] was used to analyze the rotational space and obtain a conformational minimum which was further optimized using the AM1 Hamiltonian[51] as implemented in MOPAC.[52] (c) Starting from the AM1-optimized conformations, the dihedral angles τ_1, τ_2, and τ_3 were rotated from 0° to 360° by a 10° increment while relaxing all the other geometrical parameters. AM1 was used to evaluate energies and charge distributions. The results summarized in Tables 9 and 10 illustrate some molecular features of compounds listed in Figure 6. The prevously reported positive inotropic agent 7,8-dihydro-7-methyl-2,5-(1*H*,6*H*)-quinolinedione (**SF40**)[24] has been included as a reference compound.

In the most stable conformations of the series **11**−**30** (Figure 6, Table 9), when R = methyl, benzyl, trifluoromethyl, the COR_1 group (R_1 = H, C_2H_5) is coplanar

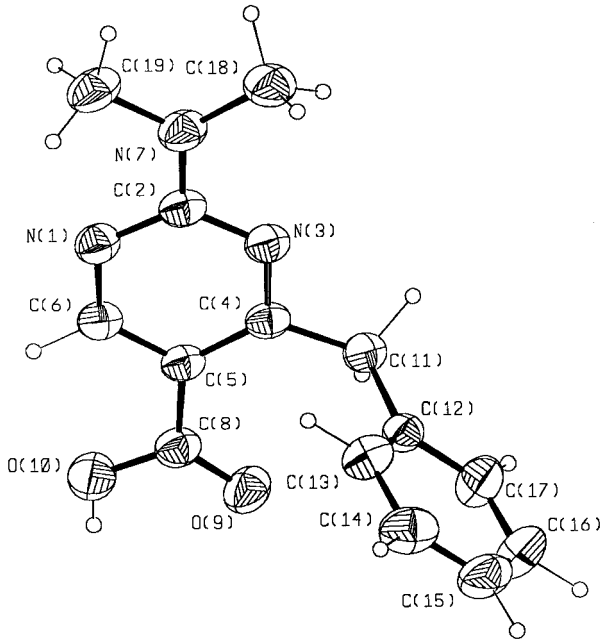


Figure 5. Molecular conformation of **28** (thermal ellipsoids are drawn at the 40% probability level).

Table 8. Experimental Data for the Crystallographic Analyses

	17	**28**
formula	$C_{15}H_{17}N_3O_2$	$C_{14}H_{15}N_3O_2$
mol wt	271.3	257.3
crystal size, mm	0.22 × 0.18 × 0.16	0.24 × 0.20 × 0.32
crystal system	monoclinic	triclinic
space group	$P2_1/c$	$P\bar{1}$
a, Å	9.235(3)	6.258(2)
b, Å	14.989(3)	9.023(2)
c, Å	20.481(4)	11.901(3)
α, deg		104.09(2)
β, deg	91.14(3)	98.89(2)
γ, deg		91.13(2)
V, Å^3	2835(1)	642.8(3)
Z	8	2
D_c, g cm^{-3}	1.272	1.329
$F(000)$	1152	272
2θ range, deg	4−46	4−50
radiation (λ, Å)	Mo Kα (0.71069)	Mo Kα (0.71069)
μ, cm^{-1}	0.87	0.92
no. reflections collected	3802	1876
no. observed $[I \geq 2.5\sigma(I)]$	1695	1771
weighting scheme w	$[\sigma^2(F_o^2) + (0.0902P^*)^2 + 2.10P]^{-1}$	$[\sigma^2(F_o^2) + (0.0938P^*)^2 + 0.17P]^{-1}$
$R(F)$	0.037	0.049
$R_w(F^2)$	0.101	0.140
goodness of fit	0.86	1.05

[a] $P = \max(F_o^2 + 2F_c^2)/3$.

or nearly coplanar with respect to the C(4)−C(5) bond. A *cisoid* conformation is preferred in **17** (R = benzyl, R_1 = methyl), **22** (R = methyl, R_1 = H), **23** (R = methoxymethyl, R_1 = H), and **28** (R = benzyl, R_1= H), whereas a *transoid* one is more stable in **20**, **30** and, to a very light extent, **11**. In all cases, however, the energy difference between the *cisoid* and *transoid* conformations is <1.8 kcal/mol and the $COOR_1$ rotational barrier between $\tau_1 = 0°$ and $\tau_2 = 180°$ ranges from 1.8 (**20**) to 4.19 kcal/mol (**23**). It is therefore reasonable to assume that, in solution, sufficient energy can be found to accommodate the $COOR_1$ group in a more convenient conformation for the binding to the enzyme. In compounds **16** and **17,** the bulky *tert*-butyl substituent

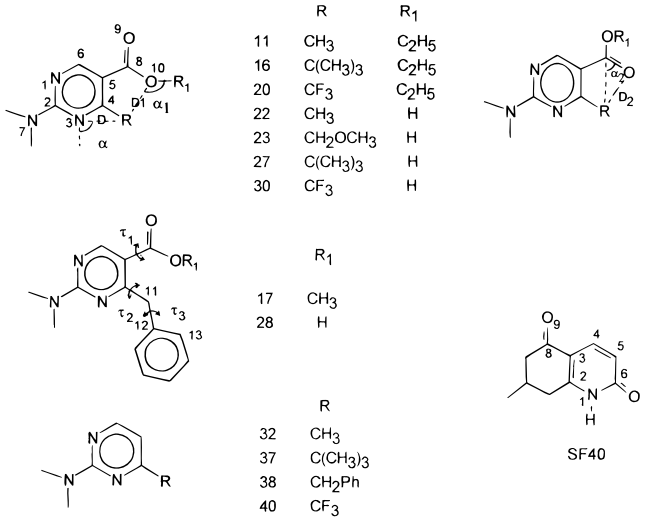


Figure 6. Chemical structures of the compounds studied by quantum chemical methods.

prevents a coplanar conformation so that the $COOR_1$ group is rotated ≈60° and ≈−84°, respectively, from the pyrimidine ring. On the basis of charge distribution analysis (Table 10), three sets of compounds can be considered. The first set includes **22**, **23**, **27**, **28**, and **30** and the reference compound **SF40**. This set is characterized by two areas with high electron density, at 4−6 Å distance, illustrated as areas A and B in Figure 7a for compounds **SF40** and **28**. Both areas include a variable number (from one to three) of heteroatoms, which can function simply as electron rich centers (also with metal-complexing properties) (Lewis bases) or as hydrogen bond acceptors (Brönsted bases). Area B is represented by the aminic or the pyrimidinic nitrogens in **22**−**30** and by the carbonyl oxygen at C(8) in **SF40**. Area B is represented by the amidic oxygen of the pyridone ring in **SF40** and by the carbonyl oxygen of the carboalkoxy residue in **22**−**30**. In addition to areas A and B, a third area, herein defined as area C, consists of a hydrogen donor moiety (Brönsted acid) adjacent to area B**,** the NH of the pyridone ring in **SF40**, and the OH of the carboxy group in **22**−**30**.

The second set includes compounds **11**, **16**, **17**, **20**, and **40**. It is characterized by areas A and B only. The former is represented by the aminic or pyrimidinic nitrogens, the latter by the fluorine atoms in **20** and **40** or the carboalkoxy residue in **11**, **16**, and **20**. In these compounds the hydrogen donor moiety (area C) is missing.

The third set includes compounds **32**, **37**, and **38**. Only area A is present, which can be further divided in three subareas, at ≈2.5 Å distance, which can function as hydrogen bond acceptors.

A further differentiation for compound listed in Figure 6 is represented by the steric and electronic features of the substituent (R) at position 4. Its nature can produce different effects: (a) merely prevent a nearly coplanar conformation of the COR_1 residue and the pyrimidine ring (as in **16** and **27**), (b) create a lipophilic area, (c) introduce an additional area with high electron density which can act as a Lewis or Brönsted base (as in **23** and **30**), and (d) reduce the access to a certain part of the molecule, in particular to area C (as indicated by D_1, α_1 values in Table 9). (A limited access to the NH

Table 9. Energy and Relevant Geometric Parameters for AM1 Selected Conformations of **11**−**30** and Reference Compound **SF40**

compd	τ_1[a] (deg)	τ_2[a] (deg)	τ_3[a] (deg)	ΔE[b] (kcal/mol)	D[c] (Å)	α[d] (deg)	D_1[c] (Å)	α_1[d] (deg)	D_2[c] (Å)	α_2[d] (deg)
11	180.00			0.00	2.43	86.31				
	0.00			0.13	2.44	93.19				
16	60.01			0.00	2.74	51.96				
	180.00			3.99	2.70	49.35				
	0.00			2.10	2.70	49.31				
17	19.10	−87.16	76.98	0.00	3.32	49.68				
	180.00	−72.80	−93.90	0.92	3.32	55.71				
20	180.00			0.00	2.82	63.48				
	0.00			1.65	2.82	63.48				
22	0.01			0.00	2.45	93.23	2.66	158.73	4.28	176.3
	180.00			0.69	2.43	93.23	4.31	113.49	2.80	71.12
23	0.00			0.00	3.24	47.20	4.28	112.78	2.75	70.13
	180.00			0.97	3.18	41.95	3.97	150.80	6.54	172.4
27	−84.82			0.00	2.74	51.41	3.29	75.24	3.25	77.29
	180.00			7.40	2.72	57.80	3.29	41.50	5.92	16.29
	0.00			6.00	2.64	48.73	6.05	102.60	2.97	60.43
28	0.06	86.75	105.92	0.00	3.36	62.67	5.98	93.31	4.42	54.94
	180.00	82.75	100.01	1.70	3.35	58.88	4.46	115.10	4.88	131.3
30	160.00			0.00	2.78	57.45	2.72	149.34	4.27	162.3
	180.00			0.11	2.76	56.68	2.70	156.20	4.34	175.7
	0.00			1.85	2.83	63.93	4.52	116.46	3.09	75.73
SF40	177.00				3.41	54.47				

[a] The τ_1, τ_2, and τ_3 torsion angles are defined as follows: τ_1 = O(9)−C(8)−C(5)−C(4); τ_2 = C(12)−C(11)−C(4)−N(3); τ_3 = C(13)−C(12)−C(11)−C(4). [b] ΔE is calculated with respect to the minimum energy conformation. [c] D, D_1, and D_2 are defined as depicted in Figure 6 and are the distances corresponding to the minimum value of the α, α_1, and α_2 angles, respectively. [d] The angles α, α_1, and α_2 are defined as depicted in Figure 6 and are calculated with respect to each heavy atom of the R substituent.

Table 10. Atomic Charges for Selected Atoms of **11**−**30** and Reference Compound **SF40**

compd	N(1)	N(3)	N(7)	O(9)	O(10)	F[a]
11	−0.25	−0.24	−0.24	−0.28	−0.37	
	−0.25	−0.24	−0.25	−0.29	−0.37	
16	−0.25	−0.23	−0.25	−0.28	−0.35	
	−0.26	−0.23	−0.25	−0.27	−0.38	
	−0.26	−0.24	−0.25	−0.30	−0.36	
17	−0.25	−0.24	−0.25	−0.29	−0.37	
	−0.25	−0.23	−0.25	−0.28	−0.37	
	−0.25	−0.24	−0.25	−0.29	−0.37	
20	−0.21	−0.22	−0.23	−0.29	−0.33	−0.15
	−0.22	−0.22	−0.23	−0.29	−0.33	−0.15
22	−0.24	−0.25	−0.24	−0.33	−0.38	
	−0.24	−0.25	−0.24	−0.32	−0.38	
27	−0.23	−0.25	−0.25	−0.29	−0.29	
	−0.23	−0.25	−0.24	−0.29	−0.32	
	−0.23	−0.26	−0.24	−0.30	−0.31	
28	−0.24	−0.25	−0.24	−0.33	−0.38	
	−0.23	−0.25	−0.24	−0.32	−0.38	
30	−0.22	−0.21	−0.24	−0.31	−0.36	−0.15
	−0.22	−0.21	−0.24	−0.31	−0.37	−0.15
	−0.22	−0.22	−0.24	−0.33	−0.34	−0.15
32	−0.15	−0.19	−0.21			
37	−0.22	−0.20	−0.24			
38	−0.14	−0.19	−0.22			
40	−0.12	−0.14	−0.21			−0.15
SF40		0.28		−0.30	−0.35	

[a] Average value.

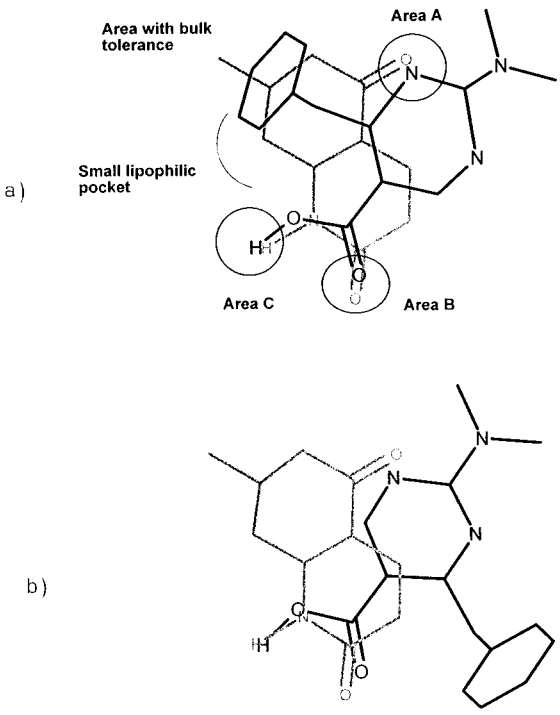


Figure 7. (a) Overlay (manual) of the lowest energy conformation of **SF40** (gray lines) with the *transoid* conformation (τ_1 = 180°) of compound **28**. (b) Overlay (manual) of the lowest energy conformation of **SF40** (gray lines) with the *cisoid* conformation (τ_1 = 0.06°) of compound **28**.

moiety in 2-benzyl-α-pyridone derivatives was considered to depress the positive inotropic activity.[24]) This obviously occurs when the R steric requirements are too demanding.

A previously suggested model for the positive inotropic activity of **SF40** and related compounds was based on the following requirements: (a) the presence of a dipole and a nearby weakly acidic proton donor (the HNCO group) (herein defined as areas B and C), (b) the presence of a hydrogen bond acceptor represented by the carbonyl oxygen (O(9), herein defined as area A), (c) an overall planar topography containing areas A−C, and (d) an alkyl substituent at C(2) which could fit to a small lipophilic pocket.

Analysis of the compounds listed in Figure 6 reveals that the first and the second of the requirements proposed above for positive inotropic activity are fulfilled in compounds **22**, **23**, **27**, **28**, and **30**. Among these compounds, however, **27** does not fulfill the third requirement. Therefore, it should be expected to be the least active. Furthermore, the R residue in **23** and **30** contains from one to three electron rich heteroatoms. This could be at variance with the requirement of a substituent which must fit to a lipophilic pocket (point

4) and could therefore depress the positive inotropic activity.

The analysis of the distance between heteroatoms and of their charge (sign and magnitude) suggests a reasonable fitting of the pyrimidinic derivatives **22** and **28** to the model proposed for **SF40**. Possible overlays of the lowest energy conformation of **SF40** with selected conformations of **28** are illustrated in Figures 7 and 8.

In Figure 7a a rigid manual overlay of the minimum energy conformation of **SF40** (Table 9) and the *transoid* conformation of **28** (Table 9) is reported.[53] The α-pyridone ring of **SF40** and the pyrimidinic ring of **28** are kept on parallel planes; **28** has been moved as a rigid group on top of **SF40** and oriented in such a way as to bring areas A−C as near as possible. In Figure 7b overlay of the minimum energy conformation of **SF40** and the *cisoid* conformation of **28** (Table 9) is reported.

In Figure 7, the areas A−C of the two compounds do not fit exactly but are somewhat displaced. This, however, would imply only a slight different conformation of enzyme groups which define the active site. In Figure 7a, the benzyl group is directed toward the same portion of space which accommodates the methylcyclohexane residue of **SF40** but has, however, a greater steric hindrance below the methylcyclohexane plane. A lipophilic area with bulk tolerance should, therefore, be expected in this part of the receptor.

The benzyl group is two carbons displaced from the hydroxyl residue of the COOH group (area C) and does not limit the access to it. In the more stable *cisoid* conformation (Figure 7b), two lipophilic pockets should be invoked on each side of the HXCO (X = O, N) dipolar moiety: one to accommodate the R substituent in **SF40** and related compounds, the other to accommodate the benzylic group of **28**. In Figure 8 alternate, computer-generated[54] alignments of the heteroatoms have been represented. These, however, do not provide the same level of match as that shown in Figure 7.

In these cases only two of the three different areas (A−C) suggested above for positive inotropic activity fit. In Figure 8a, areas B and C fit but not area A, which is displaced by ≈2.81 Å. In Figure 8b there is a perfect fit of one of the pyrimidine nitrogens in **28** with the oxygen at C(8) in **SF40** (area A) and the OH of the carboxy residue in **28** with the NH in **SF40** (area C). However, area A does not overlay but is symmetrically located with respect to the hydrogen bond donor (OH in **28** or NH in **SF40**). In addition the CO residue of the COOH group in **28** would point where a lipophilic pocket has been invoked for **SF40**, a further feature which disfavors this situation with respect to the other overlays illustrated in Figures 7 and 8.

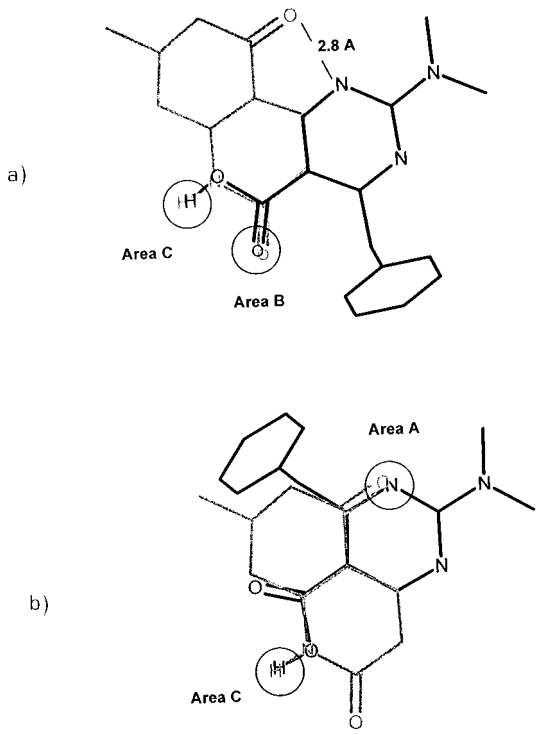


Figure 8. Computer-generated fit (MAD) of **SF40** lowest energy conformation (gray lines) and *cisoid* conformation of **28** (black lines). (a) N(1), C(6), C(5), and C(4) atoms for **SF40** and O(10), C(8), C(5), and C(6) atoms for **28** were included in the fitting procedure. (b) N(1), C(2), C(3), and C(8) atoms for **SF40** and O(10), C(8), C(5), and C(4) atoms for **28** were included in the fitting procedure.

Conclusion

A series of novel 2-(dimethylamino)-5-pyrımidines variously functionalized at positions 4 and 5 was synthesized. The synthesis took advantage of the use of unsymmetrical 2-[(dimethylamino)methylene]1,3-diones to obtain the pyrimidine derivatives through a convenient way.

Cardiac effects of acids **21**−**30** tested on spontaneously beating and electrically driven atria from reserpine-treated guinea pigs showed the most significant positive inotropic activity for **28** which was greater than that of milrinone. In contrast, the esters **10**−**20** and pyrimidines **32**−**40** displayed generally negative inotropic activity. The pyrimidine **31** showed a moderate positive inotropic activity but only at high concentrations.

Ester **17** and its related acid **28** (respectively negative and positive inotropes), were selected for the study of mechanism of action. An antagonism toward endogenous adenosine without variations in the cellular cyclic AMP seems to be involved in the contractile activity of the acid **28**. The corresponding methyl ester **17,** which reduced both the contractile force and the frequency rate of the atria, could be a partial agonist at the purinergic inhibitor A1 receptor in the heart.

Crystallographic studies on **17** and **28** and molecular modeling investigations performed on selected compounds (Figure 6) added support to the model previously proposed for positive inotropic activity founded on the following requirements: (a) the presence of a dipole (area B) and a nearby acidic proton donor (area C), the COOH group, (b) the presence of a hydrogen bond acceptor (area A), the pyrimidinic or aminic hydrogens, and (c) an overall planar topography of areas A−C.

Among the examined compounds, only **22** and **28** fulfill the above stated requirements. The positive inotropic activity of **28,** higher than that of **22** but comparable to that of **SF40**, suggests the importance of a properly sized lipophilic group like a phenyl (**28**) or the substituted cyclohexane moiety (**SF40**) to fit lipophilic pocket with bulk tolerance in the receptor site. Previous studies[27] indicate the presence of another small pocket in the proximity of area B. The two lipophilic areas would be located on the opposite sites with respect to the pyrimidine **28** or the pyridone ring **SF40**. The

methylated ester **17** in relation to the similarities with the acid derivative **28** is missing the C area and the acid proton which is responsible for hydrogen bond interactions. This change could direct the molecule to a different receptor site or simply produce a different mode of binding, with respect to the positive inotropic one, within the receptor cavity.

Experimental Section

Chemistry. All melting points were determined on a Fisher-Johns melting point apparatus and are uncorrected. Microanalyses were performed with a Carlo Erba elemental analyzer (model 1106) for C, H, N, and the results are within ±0.4% of theoretical values. The IR spectra were recorded with a Perkin-Elmer 398 spectrophotometer and are expressed in cm^{-1}. The ^{1}H-NMR spectra were obtained with a Hitachi Perkin-Elmer R-600 instrument (60 MHz); chemical shifts are reported in part per million (ppm) on the δ scale downfield relative to tetramethylsilane (TMS) as internal standard; coupling constants (J) are reported in hertz (Hz). The following abbreviations are used, s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad, and ar = aromatic protons. The physical data of the newly synthesized compounds are shown in Table 1.

Materials. Besides the commercially available starting materials, the following products were prepared according to reported methods: ethyl 2,2-diformylacetate[31] and 2-(ethoxymethylene)-4,4,4-trifluoro-3-oxobutanoate.[34] The compounds used in the biological experiments (and their sources of supply) were as follows: milrinone (Sterling Winthrop), amrinone (Schiapparelli), adenosine deaminase, carbachol, propranolol, prazosine, pyrilamine, ranitidine, 8-phenyltheophylline, tyramine, cAMP, cGMP, DOWEX 1 × 2, DEAE cellulose, 5′-nucleotidase from Crotalus Atrox (Sigma), (*R*)-PIA (RBI), [^{3}H]CHA (NEN Research Products), Instagel (Packard), and 8-[^{3}H]cAMP (Amersham). All the other chemicals and reagents were of analytic grade.

General Procedure for Ethyl 2-(Dimethylamino)-5-pyrimidinecarboxylate (10) and Esters of 4-Substituted 2-(Dimethylamino)-5-pyrimidinecarboxylic Acids 11−20. 1,1-Dimethylguanidine sulfate (1.4 g, 5 mmol) at room temperature was added to a solution of sodium ethoxide or methoxide (in the case of **12**, **17**) in ethanol or methanol, prepared from sodium (0.23 g, 10 mmol) and anhydrous ethanol or methanol, respectively (20 mL), followed by a solution of ethyl 2,2-diformylacetate (**10**; 1.44 g, 10 mmol), ethyl or methyl 2-[(dimethylamino)methylene]-3-oxoalcanoates **1**−**9** (**11**−**19**; 10 mmol), or 2-(ethoxymethylene)-4,4,4-trifluoro-3-oxobutanoate (**20**; 2.40 g, 10 mmol). The mixture was boiled for 1 (**11**−**20**) or 24 (**10**) h and evaporated under reduced pressure. The residue was treated with water (100 mL). In the case of **10**, **12**, and **17**, a crystalline precipitate separated and was filtered and recrystallized from a suitable solvent. In all other cases, the resulting mixture was extracted thoroughly with diethyl ether. The extracts were dried ($MgSO_4$) and evaporated to give a residue which was recrystallized from a suitable solvent or purified by bulb-to-bulb distillation *in vacuo*. Compounds **17** and **19** displayed the following spectral data.

17: ^{1}H-NMR ($CHCl_3$) δ 3.20 [s, 6 H, $(CH_3)_2N$], 3.81 (s, 3 H, CH_3O), 4.41 (s, 2 H, CH_2), 7.27 (m, 5 H, ar, C_6H_5), 8.84 (s, 1 H, ar, H-6); IR ($CHCl_3$) 1707, 1587, 1555, 1408 cm^{-1}.

19: ^{1}H-NMR ($CHCl_3$) δ 1.33 (t, J = 7.2, 3 H, CH_3), 1.38 (t, J = 7.2, 3 H, CH_3), 3.25 [s, 6 H, $(CH_3)_2N$], 4.32 (q, J = 7.2, 2 H, CH_2), 4.44 (q, J = 7.2, 2 H, CH_2), 8.89 (s, 1 H, ar, H-6); IR ($CHCl_3$) 1736, 1707, 1586, 1564, 1410 cm^{-1}.

General Procedure for 2-(Dimethylamino)-5-pyrimidinecarboxylic Acid (21) and 4-Substituted 2-(Dimethylamino)-5-pyrimidinecarboxylic Acids 22−30. Potassium hydroxide (1.68 g, 30 mmol) dissolved in methanol or 95% ethanol (20 mL) was added to a solution of the corresponding ester **10** or **11**−**20** (10 mmol) in the same solvent (30 mL). The resulting solution was boiled for 5 h, the solvent was evaporated under reduced pressure, and the residue was dissolved with water (50 mL). The solution was washed with diethyl ether and acidified with 6 N HCl (pH ∼1), and the white solid which separated was filtered, washed with water, dried in a vacuum oven at 100 °C, and purified by recrystallization from a suitable solvent. The hydrolysis of ester **19** with the above procedure gave a mixture of dicarboxylic and monocarboxylic derivatives which proved difficult to separate.

Compound **28** displayed the following spectral data: ^{1}H-NMR (DMSO-d_6): δ 3.17 [s, 6 H, $(CH_3)_2N$], 4.41 (s, 2 H, CH_2), 7.29 (s, 5 H, ar, C_6H_5), 8.81 (s, 1 H , ar, H-6), 12.70 (br s, 1 H, CO_2H, exchangeable proton); IR (KBr) 2900−2500, 1672, 1586, 1555, 1398, cm^{-1}.

General Procedure for *N*,*N*-Dimethyl-2-pyrimidinamine (31) and 4-Substituted *N*,*N*-Dimethyl-2-pyrimidinamines 32−40. Acids **21**−**29** and **30** (about 10 mmol) were decarboxylated by heating for a certain time at temperatures above their melting points (Table 1) until evolution of carbon dioxide subsided. The products were purified by distillation *in vacuo* or recrystallization from a suitable solvent. Compound **33** displayed the following spectral data: ^{1}H-NMR ($CHCl_3$) δ 3.17 [s, 6 H, $(CH_3)_2N$], 3.45 (s, 3 H, CH_3O), 4.35 (s, 2 H, CH_2), 6.70 (d, J = 5, 1 H, ar, H-5), 8.32 (d, J = 5, 1 H, ar, H-6); IR ($CHCl_3$) 1583, 1560, 1407, cm^{-1}.

Pharmacology. Isolated Atria Preparation. Reserpine-treated male guinea pigs (300−500 g) were killed by a blow to the head followed by exanguination, and the atria were separated from the ventricles and suspended vertically in a bath containing 30 mL of physiologic salt solution of the following composition (mM): NaCl, 120; KCl, 2.7; $MgCl_2$, 0.09; NaH_2PO_4 0.4; $CaCl_2$, 1.37; $NaHCO_3$, 11.9; and glucose, 5.5. The solution was maintained at 29 °C and treated vigorously with a mixture of 95% O_2 and 5% CO_2 which produced a pH of 7.5.

The resting tension was adjusted at 10 g, and the developed tension was recorded isometrically by means of high-sensitivity transducers (Basile type DYO for isolated auricles) and registered by a writing oscillograph (Basile, Unirecord System, model 7050). The basal developed tension ranged from 4.8 to 5.3 mN. Where indicated, the left atrium was mounted on punctate electrodes with a load of 0.5 g and stimulated at a frequency of 1.5 Hz by square-wave electrical pulse of 3 ms duration and a voltage 10−20% greater than the threshold value by a Grass Stimulator (model 24KR). The devel oped tension was 3.28 ± 0.5 mN. The electrical stimulation was performed in order to eliminate any influence on contractile activity due to variations in frequency.

Inotropic Activity. The experiments were performed on spontaneously beating atria or an electrically driven left atrium obtained from reserpine-treated guinea pigs. Reserpine (2 mg/kg, ip) was given 48 and 24 h before the animals were killed in order to eliminate the influence of noradrenaline which might be released from sympathetic nerve terminals.[44] Noradrenaline depletion was determined by exposing isolated atria to a single dose of tyramine (2 μg/mL) before starting the experiments. Experiments were performed only in preparations not responding to tyramine. The drugs were added to the perfusion fluid after 90 min of equilibration. All the inotropic agents (amrinone, milrinone, and newly synthesized compounds) were added cumulatively, and the inotropic effect was recorded for 5 min before adding a higher concentration. Where indicated, carbachol, propranolol, prazosine, pyrilamine, and ranitidine were added to the perfusion medium 15 min before the inotropic agents. In the presence of adenosine deaminase, the preincubation lasted 20 min. The basal and stimulated contractile activity was determined by measuring the amplitude of the developed tension. The data are expressed as percent variation from control (atria incubated without drugs). Compounds **17** and **28** were dissolved in dimethyl sulfoxide (DMSO). The final concentration of DMSO in the medium did not influence the basal activity of the atrial preparation.

Preparation of Soluble PDE III from Calf Heart. PDE III was isolated from calf hearts using the stepwise isolation procedure described by Thompson et al.[45] For each preparation, 10−20 g of bovine heart was placed in 10 vol of ice-cold water and then minced and homogenized using a kinematic PT-300 Polytron instrument with dispersion-aggregated PT-

DA 3020/2P (three bursts of 10 s duration at 10 peripheral speed) (m/s). The homogenate was then sonicated (30 s/mL of homogenate) and centrifuged at 30000*g* for 20 min. The resulting supernatant fraction was filtered through four layers of gauze and applied to a DEAE cellulose column (30 × 3 cm) prepared by cycling with 0.25 N NaOH, 0.25 N HCl, and 0.25 N NaOH and equilibrated with freshly prepared 70 mM sodium-acetate and 5 mM 2-mercaptoethanol (pH 6.5). The column was then washed with 2 bed vol of sodium acetate−mercaptoethanol, after which the PDEs were eluted from the column with step gradients of 220−350 and 700 mM sodium acetate and 5 mM mercaptoethanol (pH 6.5), collecting fractions of 7 mL each. Flow rate was 0.5−1 mL/min. The eluted fractions containing proteins were pooled, dialyzed overnight against 200 vol of ice-cold water, and assayed for protein content according to Lowry et al.[46] and for PDE activity. The fraction eluting with 700 mM sodium acetate contained PDE III. The PDE III from calf heart had K_m for cyclic AMP of 0.67 ± 0.40 μM and V_{max} of 0.73 ± 0.07 nmol/mg of protein/min, respectively. When assayed at 0.4 μM cyclic AMP, the activity was inhibited by 67 ± 7% by 4 μM cyclic GMP. No significant changes in PDE III activity were observed with rolipram. Aliquots of the fraction were iced and stored at −40 °C for several weeks without loss of activity.

PDE Activity Assay. PDE activity was measured as described by Thompson et al.[47] in 0.4 mL of medium containing 40 mM Tris-HCl (pH 8.0), 5 mM $MgCl_2$, 1 mM EGTA-HCl (pH 8.0), 500 μg of bovine serum albumine, and 0.4 μM [^{3}H]cyclic AMP. Incubation time was 10 min at 30 °C, and the enzyme levels did not utilize more than 15% of the substrate during the incubation period. The reaction was stopped by freezing the samples in liquid nitrogen (30 s) and then thawing them in boiling water (90 s). The 5′-nucleotides were converted to [^{3}H]nucleosides with 0.3 U of 5′-nucleotidase. Radioactive nucleosides were separated from labeled cyclic nucleotides using Dowex 1 × 2 anion exchange resin in 2 vol of water. The suspensions were centrifuged at 15000*g* for 10 min, and the radioactivity was determined in aliquots of supernatant by liquid scintillation spectroscopy.

***N*^{6}-Cyclohexyl[^{3}H]adenosine ([^{3}H]CHA) Binding Assay.** For receptor binding assays, male guinea pigs (300−500 g) were killed by a blow to the head followed by exanguination, and the hearts were homogenized in a Polytron (instrument setting) in 20 vol of 50 mM Tris-HCl, pH 7.4. The homogenate was centrifuged at 48000*g* for 10 min, and the pellet was suspended in buffer, centrifuged, and resuspended in Tris-HCl containing 2 IU of adenosine deaminase/mL. After 30 min incubation at 37 °C, membranes were centrifuged, and the pellet was stored at −70 °C. Binding experiments with [^{3}H]-CHA (13.5 Ci mmol^{-1})[48] were performed in 1 mL of buffer which contained 1 nM [^{3}H]CHA, membranes from 15 mg (wet weight) of tissue, and the compounds to be tested. After 120 min of incubation at 23 °C, separation of bound from free ligands was performed by rapid filtration through Whatman GF/B filters which were washed three times with ice-cold buffer, dried, and counted in 5 mL of acidified Instagel. Nonspecific binding was defined as binding in the presence of 10 μL of N^6-[(*R*)-phenylisopropyl]adenosine ((*R*)-PIA) and proved to be <10% of total binding. Six different concentrations of the test compound were assayed in triplicate, and the IC_{50} was calculated by probit analysis.

Calculations. Data are shown as mean ± standard error of the mean. Statistical significance of the differences between means were calculated by Student's test for paired data. Values were considered to be statistically different when *P* was lower than 0.05. The pA_2 value for compound **28** was calculated by Schild regression analysis.[49]

X-ray Measurements and Structure Determination. Crystal data, collected reflections, and parameters of the final refinement for **17** and **28** are reported in Table 8. Reflections were collected using a Philips PW1100 (Febo System) diffractometer with graphite-monochromated Mo Kα radiation. The orientation matrix and cell dimensions were determined by least-squares refinement of the angular positions of 25 reflections. Two standard reflections were monitored every 2 h, and no significant decay was observed during data collection. Because of the low absorption coefficients, no absorption correction was applied to the intensity data. The structures were solved by using direct methods.[55] Refinement was carried out by full-matrix least-squares; the function minimized was $\sum w(F_o^2 - F_c^2)^2$. All non-hydrogen atoms were refined with anisotropic thermal parameters. The H atoms were placed in calculated positions with fixed, isotropic thermal parameters (1.2 U_{equiv} of the parent carbon atom). Structure refinement was carried out with the SHELXL-93[56] program and the scattering factors enclosed therein; the drawings were produced with ORTEP II .[57]

Molecular Modeling Studies. The structural modeling was performed by using the semiempirical computer program AM1[51] as implemented in the MOPAC[52] package. The MAD[50] molecular modeling system was used for molecular graphics studies. Both quantum chemical and molecular graphics investigations were performed on a IBM Risc System/6000 (model 520).

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Supporting Information Available: Tables of atomic coordinates and anisotropic thermal parameters (11 pages); observed and calculated structure factors for compounds **17** and **28** (16 pages). Ordering information is given on any current masthead page.

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