A Pharmacological, Crystallographic, and Quantum Chemical Study of New Inotropic Agents

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The cardiac activity of a series of milrinone analogues, 2-substituted 3-acyl-1,6-dihydro-6-oxo-5-pyridinecarbonitriles, 1,6,3,2,11,12-hexahydro-6,3-dioxo-5-quinolinecarbonitriles, the correlated carboxylic acids, 2-substituted 3-acyl-6(1*H*)-pyridones, and 7,8-dihydro-2,5(1*H*,6*H*)-quinolinediones, was evaluated in spontaneously beating and in electrically driven atria from reserpine-treated guinea pigs. Their effects were compared with those induced by amrinone and milrinone in both the atria preparations. Compounds SF28 (3-acetyl-1,6-dihydro-2-methyl-6-oxo-5-pyridinecarbonitrile) and SF40 (7,8-dihydro-7-methyl-2,5(1*H*,6*H*)-quinolinedione) were the most effective positive inotropic agents. An inhibition of the negative influence exerted by endogenous adenosine on heart preparations seems to be involved in their contractile activity. SF38 (3-benzoyl-2-phenyl-6(1*H*)-pyridinone), on the contrary, reduced the contractile force and the frequency rate of guinea pig atria with a mechanism not related to an activation of cholinergic or purinergic inhibitory receptors on the heart. X-ray analysis carried out on the three model compounds, SF28, SF40 (positive inotropic agents), and SF38 (negative inotropic agent), and molecular modeling evidenced that the change from phenyl (SF38) to methyl (SF28) or the introduction of a side cyclic aliphatic chain (SF40) results in a variation of conformational preference and topography which may adress the different molecules toward distinct receptor pockets according to the resulting inotropic effect.

Bipyridine derivatives such as amrinone and milrinone are well-established positive inotropic and vasodilatatory agents.[1-3] In relation to their mechanism of action they are classified as selective phosphodiesterase (PDEase III) inhibitors,[2,4-10] but endogenous adenosine also appears to be involved in both the cardiac[11-15] and vascular[16] effects of these drugs. An antagonism toward endogenous adenosine without variations in the cellular cyclic AMP content appears to be the mechanism of action of amrinone and milrinone in guinea pig isolated atria,[11,14] where adenosine receptors are not functionally related to adenylate cyclase[17,18] and where increases in cyclic AMP concentration in response to inhibition of PDEase III do not necessarily cause an increase in contractility.[19] Since increase in cardiac cyclic AMP concentration is associated with the risk of arrhythmias,[20] the search for new inotropic agents that do not affect cyclic AMP levels can be an important target in drug research. These considerations prompted us to study the milrinone analogues shown in Figure 1.

The cardiac activity of the new compounds was investigated by determining their influence on contractile activity and frequency of spontaneously beating atria from reserpine-treated guinea pigs. A comparison with the effects of amrinone and milrinone on the same parameters was also made. The inotropic effect of the most active compounds was confirmed in electrically driven left atrium from reserpine-treated guinea pigs where their specific mechanism of action was also investigated.

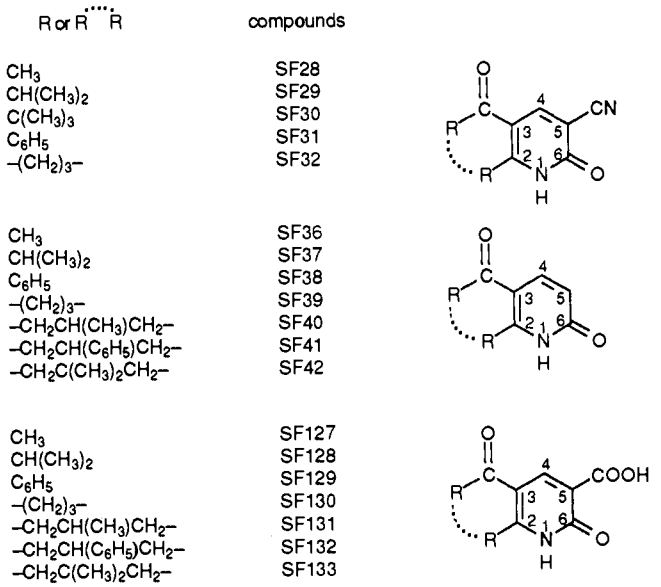

Figure 1. Chemical structure of milrinone analogues.

In addition, three models molecules, SF28, SF38, and SF40 [SF28 = 3-acetyl-1,6-dihydro-2-methyl-6-oxo-5-pyridinecarbonitrile, SF38 = 3-benzoyl-2-phenyl-6(1*H*)-pyridinone, SF40 = 7,8-dihydro-7-methyl-2,5(1*H*,6*H*)-quinolinedione], showing quantitatively or qualitatively different inotropic activities, have been studied by X-ray analysis and by quantum chemical methods in order to obtain some information on the possible structure–activity relationships.

The results have been related to the pharmacological data.

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Table I. Effect of Milrinone Analogues on Contractile Activity of Spontaneously Beating Atria from Reserpine-Treated Guinea Pigs[a]

compounds	developed tension (% increase over control)					
	10^{-5} M	5×10^{-5} M	10^{-4} M	5×10^{-4} M	10^{-3} M	2×10^{-3} M
amrinone	7.08 ± 0.13	11.52 ± 0.27	17.92 ± 0.18	23.92 ± 0.21	28.81 ± 0.35	25.30 ± 0.69
milrinone	18.25 ± 0.20	31.32 ± 0.35	38.76 ± 0.14	46.52 ± 0.50	33.09 ± 0.33	29.31 ± 0.65
SF28	13.90 ± 0.40	27.64 ± 0.46	35.57 ± 0.26	57.22 ± 2.37	65.35 ± 1.77	64.11 ± 1.15
SF29	8.22 ± 0.11	11.62 ± 0.27	1.42 ± 0.26	−34.43 ± 0.57	−67.64 ± 0.53	−86.43 ± 0.30
SF30	7.34 ± 0.89	11.34 ± 1.17	−16.07 ± 0.22	−66.56 ± 0.60	−81.39 ± 0.96	−89.11 ± 0.50
SF31	1.22 ± 0.15	−6.67 ± 0.37	−22.26 ± 0.34	−32.19 ± 0.63	−35.54 ± 0.59	−39.43 ± 0.78
SF32	2.59 ± 0.31	7.87 ± 0.26	16.12 ± 0.24	25.67 ± 0.54	23.30 ± 0.43	13.01 ± 0.27
SF36	5.35 ± 0.22	5.84 ± 0.32	10.35 ± 0.41	9.72 ± 0.40	10.27 ± 0.30	10.35 ± 0.12
SF37	5.34 ± 0.18	11.69 ± 0.56	16.38 ± 0.74	−9.42 ± 0.06	−26.37 ± 0.65	−44.47 ± 2.38
SF38	−2.67 ± 0.59	−7.30 ± 0.05	−24.76 ± 0.30	−38.32 ± 0.19	−43.71 ± 0.16	−46.27 ± 0.79
SF39	0.48 ± 0.20	7.65 ± 0.15	14.70 ± 0.07	23.87 ± 0.17	25.64 ± 0.18	26.56 ± 0.46
SF40	4.28 ± 0.15	18.88 ± 0.22	28.89 ± 0.36	39.48 ± 0.45	46.09 ± 0.09	80.98 ± 0.96
SF41	2.28 ± 0.22	4.52 ± 0.09	1.20 ± 0.07	1.46 ± 0.14	1.73 ± 0.03	3.44 ± 0.13
SF42	0.00 ± 0.00	−3.45 ± 0.13	−3.46 ± 0.17	−5.50 ± 0.19	2.45 ± 0.15	4.71 ± 0.18
SF127	2.63 ± 0.26	5.72 ± 0.11	5.46 ± 0.25	9.55 ± 0.15	11.48 ± 0.14	10.83 ± 0.31
SF128	3.46 ± 0.23	3.21 ± 0.27	−8.88 ± 0.30	−49.63 ± 1.50	−82.26 ± 1.72	−99.99 ± 0.00
SF129	2.45 ± 0.16	−1.52 ± 0.04	−10.02 ± 0.35	−61.56 ± 0.42	−90.39 ± 0.67	−100.0 ± 0.00
SF130	arrhythmias					
SF131	2.55 ± 0.15	3.78 ± 0.08	4.57 ± 0.12	8.53 ± 0.07	8.37 ± 0.02	11.38 ± 0.16
SF132	1.53 ± 0.23	2.21 ± 0.13	−1.21 ± 0.13	−7.59 ± 0.29	−23.77 ± 0.57	−27.77 ± 0.15
SF133	0.51 ± 0.12	2.70 ± 0.14	1.66 ± 0.18	−0.69 ± 0.22	−3.94 ± 0.13	−4.46 ± 0.08

[a] Each value is the mean ± SEM of 6–10 ten assays from different experiments. Negative values indicate a negative inotropic effect.

Table II. Effect of Milrinone Analogues on the Frequency Rate of Spontaneously Beating Atria from Reserpine-Treated Guinea Pigs

compounds	frequency rate (% increase over control)					
	10^{-5} M	5×10^{-5} M	10^{-4} M	5×10^{-4} M	10^{-3} M	2×10^{-3} M
amrinone	4.62 ± 0.24	8.85 ± 0.24	15.68 ± 0.37	24.11 ± 0.49	30.69 ± 0.43	26.30 ± 0.41
milrinone	16.41 ± 0.51	28.10 ± 0.26	35.26 ± 0.21	43.96 ± 0.34	39.20 ± 0.97	31.79 ± 0.74
SF28	0.23 ± 0.04	1.35 ± 0.10	6.62 ± 0.24	11.61 ± 0.72	21.93 ± 0.49	22.98 ± 0.23
SF29	2.33 ± 0.18	5.14 ± 0.09	9.64 ± 0.33	18.85 ± 0.22	22.83 ± 0.08	16.27 ± 0.84
SF30	2.98 ± 0.18	0.28 ± 0.05	−0.44 ± 0.12	−6.73 ± 0.04	−14.41 ± 0.23	−15.71 ± 0.52
SF31	0.50 ± 0.13	−3.01 ± 0.35	−3.36 ± 0.25	−6.26 ± 0.14	−8.29 ± 0.29	−9.96 ± 0.26
SF32	3.02 ± 0.29	−3.08 ± 0.28	−6.06 ± 0.31	−3.85 ± 0.64	−0.57 ± 0.12	−0.79 ± 0.03
SF36	1.79 ± 0.18	4.14 ± 0.10	4.70 ± 0.20	10.85 ± 0.40	10.85 ± 0.40	14.19 ± 0.54
SF37	−3.28 ± 0.20	−5.14 ± 0.06	−0.99 ± 0.06	20.80 ± 0.54	26.33 ± 0.54	32.13 ± 0.54
SF38	−4.26 ± 0.25	−9.30 ± 0.32	−14.92 ± 0.15	−24.37 ± 0.36	−32.89 ± 0.64	−40.57 ± 0.43
SF39	0.00 ± 0.00	0.00 ± 0.00	6.26 ± 0.15	12.44 ± 0.23	15.52 ± 0.26	18.17 ± 0.12
SF40	2.63 ± 0.17	3.21 ± 0.20	3.29 ± 0.23	13.45 ± 0.11	19.68 ± 0.47	26.44 ± 0.49
SF41	2.19 ± 0.13	−3.42 ± 0.17	5.61 ± 0.19	8.85 ± 0.25	12.61 ± 0.69	14.52 ± 0.16
SF42	0.00 ± 0.00	1.27 ± 0.29	1.05 ± 0.14	1.09 ± 0.18	1.06 ± 0.15	2.65 ± 0.16
SF127	−4.20 ± 0.22	−4.31 ± 0.25	−5.67 ± 0.28	−2.31 ± 0.20	−1.43 ± 0.14	−1.39 ± 0.08
SF128	0.58 ± 0.33	0.75 ± 0.16	6.01 ± 0.29	23.17 ± 0.89	16.19 ± 0.56	11.35 ± 0.58
SF129	−2.23 ± 0.22	−3.38 ± 0.28	−5.31 ± 0.13	−14.43 ± 0.29	−25.30 ± 0.68	−35.93 ± 0.09
SF130	arrhythmias					
SF131	1.50 ± 0.16	2.78 ± 0.04	3.58 ± 0.13	6.53 ± 0.06	7.38 ± 0.08	10.36 ± 0.18
SF132	0.58 ± 0.23	1.22 ± 0.18	−1.21 ± 0.13	−4.58 ± 0.39	−13.88 ± 0.87	−27.07 ± 0.16
SF133	0.41 ± 0.18	1.80 ± 0.17	0.88 ± 0.19	−0.69 ± 0.22	−2.94 ± 0.18	−4.76 ± 0.09

[a] Each value is the mean ± SEM of 6–10 assays from 10 different experiments. Negative values indicate a chronotropic inotropic effect.

Results

Biological Activity: Isolated Atria Preparation. Milrinone analogues (10 μM-2 mM) exerted qualitatively and/or quantitatively different effects on both contractile force (Table I) and frequency rate (Table II) of spontaneously beating atria from reserpine-treated guinea pigs. Among the compounds tested, only SF28 and SF40 induced a marked increase of inotropism (Table I). This increase was particularly evident at the highest concentrations tested. Furthermore, the maximum inotropic effect induced by SF28 and SF40, expressed as percent variation from the control, was greater than that of milrinone, but SF28 and SF40 reached their maximum inotropic effect at higher concentrations (1 and 2 mM, respectively) in comparison to milrinone (0.5 mM). Both SF28 and SF40 also increased the frequency rate of the preparation, but their chronotropic effect was less pronounced than that induced by milrinone (Table II).

The inotropic activity of SF28 and SF40 was confirmed also in electrically driven left atrium (Table III), where the regularly induced pulses do not influence the contractile response to the tested drugs.

Table III. Inotropic Effect of SF28 and SF40 in Electrically Driven Left Atrium from Reserpine-Treated Guinea Pigs: Comparison with Amrinone and Milrinone[a]

drug concn (M)	developed tension (% increase over control)			
	milrinone	amrinone	SF28	SF40
10^{-5}	13.25 ± 0.21	4.50 ± 0.27*	12.64 ± 0.13 ns	6.31 ± 0.18*
5×10^{-5}	22.83 ± 0.31	6.49 ± 0.12*	25.17 ± 0.63**	19.17 ± 0.33*
10^{-4}	34.61 ± 0.43	17.52 ± 0.18*	38.06 ± 0.69**	26.62 ± 0.59*
5×10^{-4}	44.72 ± 0.38	22.34 ± 0.24*	48.67 ± 0.39*	37.23 ± 0.19*
10^{-3}	41.96 ± 0.32	27.00 ± 0.15*	58.73 ± 0.23*	48.86 ± 0.16*
2×10^{-3}	37.95 ± 0.51	34.60 ± 0.29*	67.13 ± 0.13*	93.66 ± 1.53*

[a] 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 amrinone, SF-28 and SF-40 was calculated by the Student's t test. ns = not statistically significant ($P > 0.05$). *$P < 0.001$. **$P \leq 0.01$.

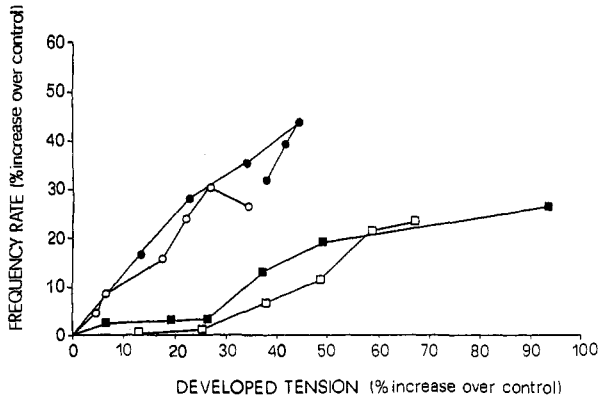


Figure 2. Relation between the inotropic effects on electrically driven left atrium and chronotropic effects on spontaneously beating atria in response to increasing concentrations of amrinone, milrinone, SF28, and SF40 (see Tables II and III). Each data point is mean of 6–10 assays from 10 different experiments: (○) amrinone, (●) milrinone, (□) SF28, (■) SF40.

Figure 2 illustrates the more positive relationship between inotropic and chronotropic effects induced by SF28 and SF40 as compared to both amrinone and milrinone. SF28 and SF40 appear to be more "force-" than "frequency-" specific agents, consequently with more advantageous pharmacological profiles. Contrary to the results for SF28 and SF40, compound SF38 evoked a negative inotropic (Table I) effect, and among the different compounds it was the most active in reducing the frequency rate (Table II).

Mechanism of Action: Isolated Atria Preparation. Electrically driven left atrium was used for studying the mechanism of action of compounds SF28, SF40, and SF38. A release of endogenous catecholamines is not involved in the positive inotropic effect of SF28 and SF40, since catecholamine depletion had been achieved by treating the animals with reserpine. Also, an activation of β-adrenoceptors may be excluded since propranolol (0.1 μM) did not inhibit the increase in inotropism evoked by SF28 or by SF40 (data not shown).

Carbachol, at a concentration (0.3 μM) that inhibited almost completely the spontaneous contractility of the atria, enhanced the inotropic activity of SF28 and SF40 (Figure 3). While the latter effect may in part be due to a suppressed baseline, the lack of an antagonism by carbachol suggests that an elevation of cyclic AMP is unlikely to mediate the cardiac effects of the two compounds. This interpretation is further supported by the results obtained with isoprenaline. In the guinea pig left atrium carbachol (10 nM–0.3 μM) inhibited in a concentration-dependent manner the cyclic AMP-mediated inotropic response to this catecholamine (Figure 4). Charbacol, in fact, is known to selectively abolish the elevation of heart contractility sustained by increases in cyclic AMP levels induced either by adenylate cyclase stimulation or by PDEase III inhibition in different preparations.[10,21,22] Pretreatment of the preparations with a concentration (10 μM) of 8-phenyltheophylline suitable to displace endogenous adenosine from its binding to A_1-purinergic receptors on the heart[23,24] caused a significant inotropic effect (33.82 ± 0.59% increase in developed tension over the control, $P < 0.001$). This increase in contractile force declined in 15–20 min to reach a stabilization at a new level, still higher than the control (+15% increase over the control). At this point the addition of SF28 or SF40 was characterized by a significant reduction in their

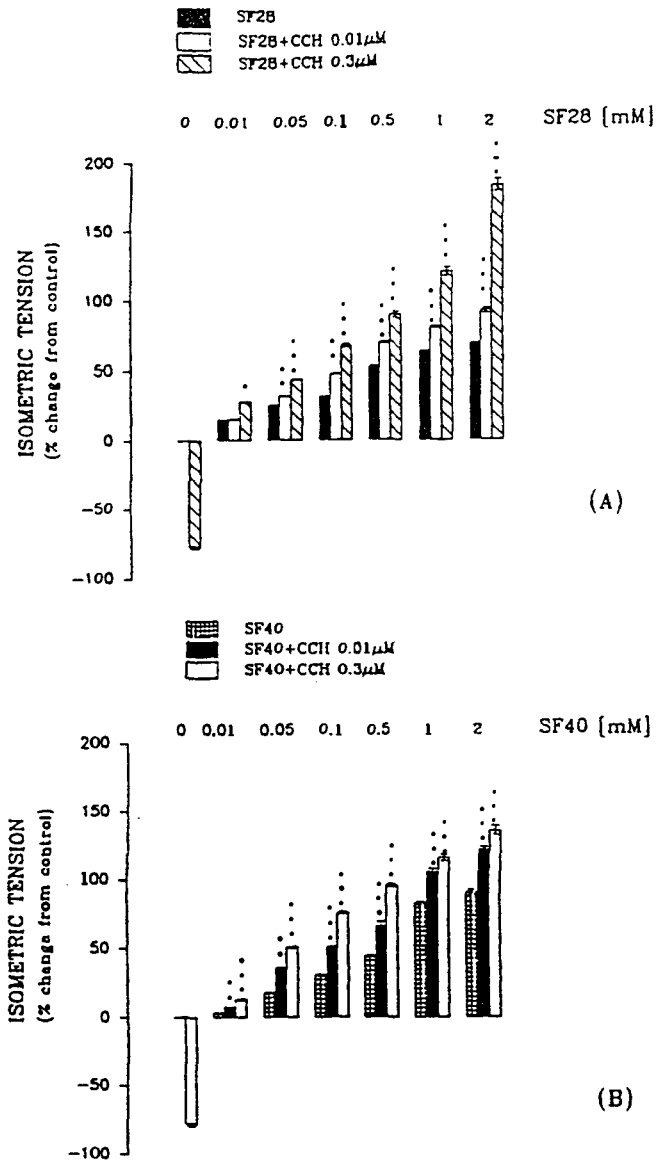


Figure 3. Effect of carbachol on (A) SF28- and (B) SF40-induced inotropism in electrically driven left atrium from reserpine-treated guinea pigs. Each value is the mean ± SEM of five assays from five different experiments. P values were determined versus the respective control (atrium incubated without carbachol). The statistical significance of the changes induced by carbachol was calculated by the Student's t test $^*P < 0.005$; $^{**}P < 0.01$; $^{***}P < 0.001$; CCH = carbachol.

inotropic effect (Table IV), SF40 being the most sensitive to the xanthine. The effect of 8-phenyltheophylline on SF28 was less marked that on SF40, particularly at higher concentrations of the compound, but it was still statistically significant. When endogenous adenosine was removed by treating left atrium preparations with adenosine deaminase, the enzyme that inactivates adenosine by metabolizing it to inosine, an increase in developed tension occurred, similar to that induced by 8-phenyltheophylline (32.28 ± 0.61% over the control, $P < 0.001$). This effect declined within 15–20 min and the force of contraction reached a new steady state that was 15% higher than the control. Also under these conditions, i.e. in the presence of adenosine deaminase, the inotropic activity of SF28 and SF40 was reduced, SF40 being the most sensitive to the effect of the enzyme (Table V).

The influence of 8-phenyltheophylline and of adenosine deaminase on the inotropic action of these compounds suggests that the increase in developed tension in response

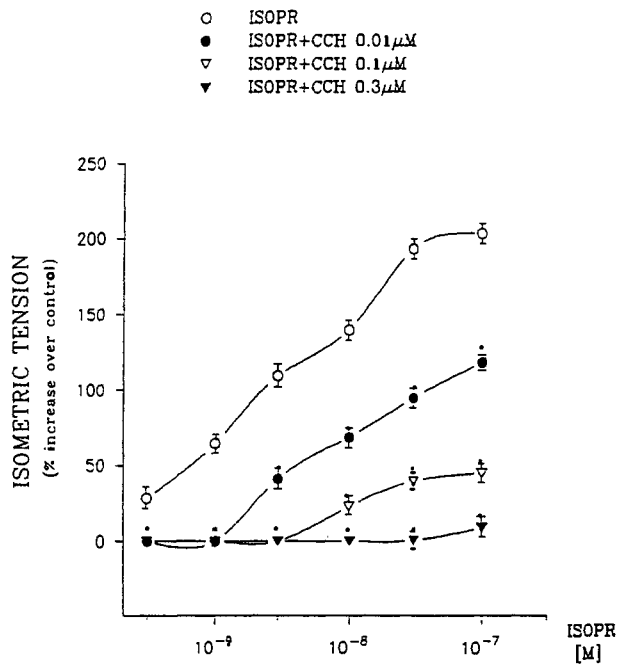


Figure 4. Effect of carbachol on isprenaline-induced inotropism in electrically driven left atrium from reserpine-treated guinea pigs. In the absence of isoprenaline, carbachol at 0.001, 0.1, and 0.3 μM induced an inhibition of the basal contractile activity corresponding to −0.25 ± 0.01, −45.21 ± 1.87, and −79.63 ± 2.31% of the initial level. Each data is mean ± SEM of four assays from four different experiments. *P* values were calculated versus respective control (atria perfused without carbachol). The statistical significance of the changes induced by carbachol was calculated by the Student's *t* test. **P* < 0.001; ISOPR = isoprenaline, CCH = carbachol.

Table IV. Effect of 8-Phenyltheophylline on Inotropic Response to SF28 and SF40 in Electrically Driven Left Atrium from Reserpine-Treated Guinea Pigs[a]

drug concn (M)	developed tension (% increase over control)			
	SF28	SF28 + 10^{-5} M 8-Ph	SF40	SF40 + 10^{-5} M 8-Ph
10^{-5}	12.62 ± 0.36	7.75 ± 0.79* (−39)	4.64 ± 0.44	0.29 ± 0.06* (−94)
5×10^{-5}	17.81 ± 0.43	11.59 ± 0.58* (−35)	18.83 ± 0.38	3.95 ± 0.11* (−79)
10^{-4}	28.87 ± 0.48	23.03 ± 0.69* (−21)	24.77 ± 0.62	8.67 ± 0.33* (−65)
5×10^{-4}	48.18 ± 0.72	40.37 ± 0.59* (−17)	38.88 ± 0.41	15.18 ± 0.73* (−61)
10^{-3}	56.71 ± 0.45	50.44 ± 0.52* (−12)	56.72 ± 0.51	25.45 ± 0.87* (−55)
2×10^{-3}	62.73 ± 0.42	58.97 ± 0.49* (−6)	87.60 ± 0.41	52.56 ± 0.46* (−40)

[a] Each value is the mean ± SEM of six assays from six different experiments. *P* values were determined versus the respective control (atrium incubated without 8-phenyltheophylline). The statistical significance of the changes induced by 8-phenyltheophylline was calculated by the Student's *t* test (**P* < 0.001). 8-Ph = 8-phenyltheophylline. Values in parentheses are the percent inhibition of the contractile response.

to SF28 and SF40 may originate, at least in part, from their ability to prevent the negative influence exerted by endogenous adenosine on the heart.

This mechanism of action was previously found to account for the inotropic effect of amrinone and of milrinone in guinea pig atria.[11,14] In this heart preparation the negative influence of adenosine does not appear to be related to changes in the cyclic AMP content[17,18,25,26] but is likely to be a consequence of the reduction in the action potential duration in response to a direct inhibition of

Table V. Effect of Adenosine Deaminase on Inotropic Response to SF28 and SF40 in Electrically Driven Left Atrium from Reserpine-Treated Guinea Pigs[a]

drug concn (M)	developed tension (% increase over control)			
	SF28	SF28 + ADA (2 units/mL)	SF40	SF40 + ADA (2 units/mL)
10^{-5}	5.26 ± 0.22	3.42 ± 0.19* (−35)	15.78 ± 0.36	2.48 ± 0.30* (−85)
5×10^{-5}	13.32 ± 0.30	8.32 ± 0.27* (−38)	25.00 ± 0.59	5.52 ± 0.37* (−78)
10^{-4}	12.61 ± 0.64	10.08 ± 0.77* (−21)	27.60 ± 0.58	9.12 ± 0.31* (−67)
5×10^{-4}	3.79 ± 0.21	20.37 ± 0.49* (−15)	36.16 ± 0.94	14.35 ± 0.32* (−61)
10^{-3}	42.05 ± 0.61	37.93 ± 0.47* (−10)	46.19 ± 0.49	20.63 ± 0.50* (−55)
2×10^{-3}	84.60 ± 1.38	80.21 ± 0.53* (−5)	67.12 ± 0.58	39.98 ± 0.47* (−49)

[a] Each value is the mean ± SEM of six assays from six different experiments. *P* values were determined versus the respective control (atrium incubated without adenosine deaminase). The statistical significance of the changes induced by adenosine deaminase was calculated by the Student's *t* test (**P* < 0.001). ADA = adenosine deaminase. Values in parentheses are the percent inhibition of the contractile response.

Table VI. Effect of Verapamil on Inotropic Response to SF28 and SF40 in Electrically Driven Left Atrium from Reserpine-Treated Guinea Pigs[a]

drug concn (M)	developed tension (% increase over control)			
	SF28	SF28 + 10^{-7} M VER	SF40	SF40 10^{-7} M + VER
10^{-5}	18.83 ± 0.16	12.63 ± 0.43* (−33)	5.76 ± 0.26	−0.32 ± 0.11* (−100)
5×10^{-5}	28.29 ± 0.36	19.50 ± 0.54* (−32)	11.60 ± 0.43	2.45 ± 0.12* (−79)
10^{-4}	37.34 ± 0.38	28.47 ± 0.21* (−24)	17.96 ± 0.16	2.43 ± 0.12* (−87)
5×10^{-4}	62.96 ± 0.33	55.47 ± 0.24* (−22)	31.40 ± 0.50	14.70 ± 0.23* (−53)
10^{-3}	77.46 ± 0.48	66.40 ± 0.23* (−15)	45.89 ± 0.31	23.78 ± 0.15* (−48)
2×10^{-3}	94.47 ± 0.36	88.84 ± 0.43* (−6)	75.51 ± 0.98	43.80 ± 0.27* (−42)

[a] Each value is the mean ± SEM of seven assays from seven different experiments. *P* values were determined versus the respective control (atrium incubated without inhibitor). The statistical significance of the changes induced by verapamil was calculated by the Student's *t* test (**P* < 0.001). VER = verapamil. Values in parentheses are the percent inhibition of the contractile response.

Ca^{2+} channels[26,27-30] and/or to a reduction in Ca^{2+} flux into the cell due to an increased K^+ conductance and hyperpolarization of the cell membrane.[26,31] Thus an antagonism toward endogenous adenosine may lead to an increased uptake of extracellular Ca^{2+} through voltage-sensitive channels,[26-31] with consequent increase in intracellular Ca^{2+} availability for the contractile machinery. In order to find further support to this hypothesis, the contractile effect of SF28 and SF40 was investigated in the presence of verapamil, a well-known Ca^{2+}-channels blocker. Verapamil, at a concentration sufficiently high (0.1 μM) to block slow Ca^{2+} channels without affecting intracellular Ca^{2+} pools,[32,33] inhibited the contractile response to SF28 and, even more markedly, to SF40 (Table VI). For both compounds the inhibition by verapamil was quantitatively similar to the inhibition caused by adenosine deaminase (Table V) and 8-phenyltheophylline (Table IV). These results are in favor of the hypothesis that SF40 and, to a lesser extent, SF28, by acting as adenosine antagonists, increase Ca^{2+} uptake through slow channels. Very recent data indicate that SF28, in the

Table VII. Lack of Effect of SF38 on Inotropic Response to 8-Phenyltheophylline in Electrically Driven Left Atrium from Reserpine-Treated Guinea Pigs[a]

8-Ph concn (M)	developed tension (% increase over control)		
		5×10^{-5} M SF38	10^{-4} M SF38
10^{-6}	4.27 ± 0.61	5.70 ± 1.58 ns	4.56 ± 2.99 ns
3×10^{-6}	7.33 ± 0.88	8.13 ± 1.43 ns	11.38 ± 2.69 ns
10^{-5}	11.19 ± 1.10	14.44 ± 1.05 ns	18.78 ± 2.15*
3×10^{-5}	14.72 ± 1.04	17.41 ± 1.34 ns	20.17 ± 2.67**
10^{-4}	20.81 ± 1.62	25.91 ± 2.67 ns	27.05 ± 2.13**

[a] SF38 was added to the perfusion medium 10 min before 8-phenyltheophylline (8-Ph). Each value is the mean ± SEM of four assays from four different experiments. P values were determined versus the respective control (atrium incubated in the absence of SF38). The statistical significance of the changes induced by SF-38 was calculated by the Student's t test (ns = not significant, $P > 0.05$; *$P < 0.05$; **$P < 0.01$).

concentration range used to evoke inotropic responses, inhibits adenosine binding to A1 receptors in guinea pig atria.[34,35] The presence of an adenosine deaminase- and 8-phenyltheophylline-resistent component in the inotropic action of these compounds indicates the existence of some additional cellular target site, different from adenosine receptors, that remains to be identified. At the present we can exclude that this site is PDEase III, both because cyclic AMP does not appear to be involved in the action of SF28 and SF40, as discussed above, and because other studies have shown that in guinea pig atria this isoenzyme is either biochemically uncoupled from the myocardial contractile proteins or may be compartmentalized in cytosol, so that increases in cyclic AMP concentrations do not correlate to increases in cardiac inotropism.[18]

Moreover, the positive inotropic effect of a series of pyridine derivatives, including amrinone and milrinone, was recently shown to be directly correlated with their ability to bind to adenosine receptors, but not to their potency as PDEase III inhibitors.[35] Finally, the negative influence exerted by SF38 on heart contractility was insensitive to the presence of atropine (1 μM) in the medium (data not shown). Furthermore SF38 did not influence the positive inotropic effect induced by the adenosine-antagonist 8-phenyltheophylline (1 μM–0.1 mM) (Table VII). Thus an acetylcholine or an adenosine-like action at the specific muscarinic or purinergic (A1) receptors in the cardiac activity of SF38 must be excluded.

Crystallographic Studies. The SF28 molecular conformation as an ORTEP view and the numbering scheme used for the atoms are shown in Figure 5. Significant bond lengths and angles are given in Table VIII. The molecular structure is characterized by a rather planar arrangement of the atoms. The heterocyclic moiety presents a range of the atom deviations, from the best mean plane calculated for the ring, from –0.022(2) to 0.020(2) Å. The COMe group is rotated with respect to the pyridone ring of 11.4(1)° and its conformation is *cisoid* (the O(9) is oriented in the direction of the methyl substituent in position 2). Pairs of centrosymmetric molecules are connected via hydrogen bonds between the NH proton of the pyridone ring and pyridone oxygen of the adjacent molecule (primed position at $2-x, 2-y, -z$) (N(1)···O(7)′, 2.800(2) Å; H(1)···O(7)′, 1.89(2) Å; and N(1)–H(1)···O(7)′, 173(3)°). The dimers bear additional intermolecular interactions having the CN group facing the C(4) proton of the centrosymmetrically related molecule (double primed position at $-x, 1-y, -z$). Significant parameters are C(4)···N(12)″ [3.354(3) Å], H(4)···N(12)″ [2.42(3) Å], and C(4)–H(4)···N(12)″ [161(2)°]. The molecular structure of SF38 is shown in Figure 6, as an ORTEP view, with the atom labeling. Significant bond lengths and angles are in Table IX. The COPh group is staggered with respect to the phenyl substituent in the 2 position. The pyridone ring is rather puckered, the range of the deviations from the best mean plane is from –0.041(3) to 0.027(3) Å. The phenyl ring (C(16)→C(21)) is rotated with respect to the pyridone of 49.9(1)° while with the second phenyl group (C(10)→C(15)) the rotation is 135.5(1)°. The C(10)–C(3)–C(8)–O(9) skeleton makes an angle of 45.2(1)° to the pyridone, of 151.9(1)° to the attached phenyl, and of 56.1(1)° to the phenyl in the 2 position.

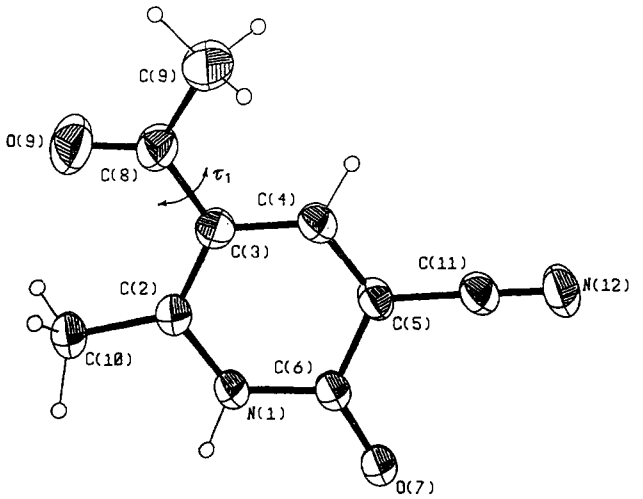


Figure 5. Molecular conformation of SF28 (thermal ellipsoids are drawn at the 50% probability level).

Table VIII. Selected Bond Lengths (Å) and Angles (deg) for Compound SF28

Bond Lengths			
O(7)–C(6)	1.236(2)	O(9)–C(8)	1.203(4)
N(1)–C(6)	1.381(3)	N(1)–C(2)	1.356(3)
C(6)–C(5)	1.440(3)	C(5)–C(4)	1.368(3)
C(5)–C(11)	1.430(3)	C(11)–N(12)	1.150(3)
C(4)–C(3)	1.409(3)	C(3)–C(2)	1.391(3)
C(3)–C(8)	1.495(3)	C(2)–C(10)	1.497(3)
C(8)–C(9)	1.501(4)		
Bond Angles			
C(6)–N(1)–C(2)	127.1(2)	O(7)–C(6)–N(1)	121.0(2)
N(1)–C(6)–C(5)	113.7(2)	O(7)–C(6)–C(5)	125.3(2)
C(6)–C(5)–C(11)	116.7(2)	C(4)–C(5)–C(11)	122.7(2)
C(6)–C(5)–C(4)	120.5(2)	C(5)–C(4)–C(3)	122.4(2)
C(4)–C(3)–C(8)	120.4(2)	C(4)–C(3)–C(2)	117.7(2)
C(2)–C(3)–C(8)	121.8(2)	N(1)–C(2)–C(3)	118.4(2)
C(3)–C(2)–C(10)	126.8(2)	N(1)–C(2)–C(10)	114.8(2)
O(9)–C(8)–C(3)	122.2(3)	C(3)–C(8)–C(9)	118.3(2)
O(9)–C(8)–C(9)	119.5(3)	N(12)–C(11)–C(5)	178.9(3)

Its structure differs from that of SF28 by having the acylic methyl as well as the methyl in the 2 position substituted by phenyls, in addition, the cyano group is absent. The presence of the phenyl groups do not influence the crystal packing as no interactions among the molecules are present, due probably to the high conformational freedom of the system.

The third compound analyzed, SF40, is characterized by the presence of a second aliphatic ring. Its solid-state structure is shown in Figure 7. Significant bond lengths

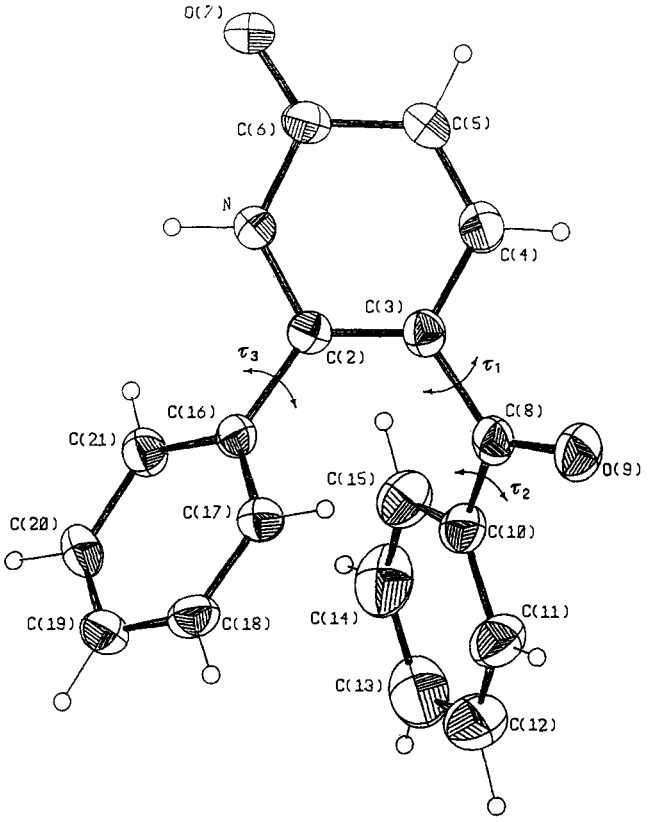


Figure 6. Molecular conformation of SF38 (thermal ellipsoids are drawn at the 50% probability level).

Table IX. Selected Bond Lengths (Å) and Angles (deg) for Compound SF38

N–C(2)	1.372(5)	N–C(6)	1.367(4)
O(7)–C(6)	1.246(5)	O(9)–C(8)	1.219(4)
C(2)–C(3)	1.372(5)	C(2)–C(16)	1.480(4)
C(3)–C(4)	1.411(5)	C(3)–C(8)	1.492(6)
C(4)–C(5)	1.347(6)	C(5)–C(6)	1.427(5)
C(8)–C(10)	1.484(4)		
	Bond Angles		
C(2)–N–C(6)	125.4(3)	N–C(2)–C(16)	116.6(3)
N–C(2)–C(3)	118.6(3)	C(3)–C(2)–C(16)	124.8(3)
C(2)–C(3)–C(8)	124.3(3)	C(2)–C(3)–C(4)	118.2(3)
C(4)–C(3)–C(8)	117.5(3)	C(3)–C(4)–C(5)	121.5(3)
C(4)–C(5)–C(6)	121.1(3)	O(7)–C(6)–C(5)	124.7(3)
N–C(6)–C(5)	114.7(3)	N–C(6)–O(7)	120.5(3)
O(9)–C(8)–C(3)	119.6(3)	C(3)–C(8)–C(10)	120.1(3)
O(9)–C(8)–C(10)	120.1(3)		

and angles are in Table X. As in the previous structures pairs of centrosymmetric molecules are connected *via* hydrogen bonds between the NH proton of the pyridone ring of one molecule and the ring carbonyl oxygen of the second one with the formation of eight-membered rings: N(1)···O(7)′, 2.811(5) Å; H(1)···O(7), 2.02(4) Å; N(1)–H(1)···O(7)′, 172(4)°. In the molecule the conformation of the aliphatic ring is a sofa with ring puckering parameters[36] Q = 0.412(5) Å, θ = 126.2(6)°, and ϕ = 57.7(8)°. The etherocyclic ring is about planar; the deviations of the atoms from their best mean planes are in the range −0.012(4)–0.017(4) Å. The "dimers" do not bear significant interactions among them, being separated by the normal van der Waals distances.

A close examination of the packing of the three compounds shows that significant intermolecular interactions with dimer formation are present in all compounds. In addition in SF28, among the dimers, additional interactions are present between the cyano group and the C(4) proton of the pyridone ring.

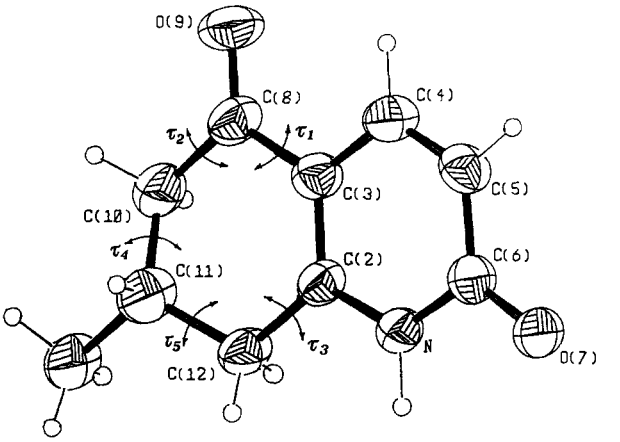


Figure 7. Molecular conformation and numbering of SF40 (thermal ellipsoids are drawn at the 50% probability level).

Table X. Selected Bond Lengths (Å) and Angles (deg) for Compound SF40

N–C(2)	1.362(6)	N–C(6)	1.370(5)
C(2)–C(3)	1.374(6)	C(2)–C(12)	1.498(5)
C(3)–C(4)	1.423(6)	C(3)–C(8)	1.469(6)
C(4)–C(5)	1.349(7)	C(5)–C(6)	1.432(6)
C(6)–O(7)	1.247(6)	C(8)–O(9)	1.212(6)
C(8)–C(10)	1.536(6)	C(10)–C(11)	1.463(7)
C(11)–C(12)	1.503(6)	C(11)–C(13)	1.520(6)
	Bond Angles		
C(2)–N–C(6)	125.3(3)	N–C(2)–C(12)	117.7(3)
N–C(2)–C(3)	119.0(3)	C(3)–C(2)–C(12)	123.3(3)
C(2)–C(3)–C(8)	120.9(3)	C(2)–C(3)–C(4)	118.0(4)
C(4)–C(3)–C(8)	121.0(4)	C(3)–C(4)–C(5)	121.8(4)
C(4)–C(5)–C(6)	120.3(4)	N–C(6)–C(5)	115.5(4)
C(5)–C(6)–O(7)	125.1(4)	N–C(6)–O(7)	119.3(3)
C(3)–C(8)–C(10)	116.0(4)	C(3)–C(8)–O(9)	121.9(4)
O(9)–C(8)–C(10)	122.1(4)	C(8)–C(10)–C(11)	114.8(4)
C(10)–C(11)–C(13)	112.4(4)	C(10)–C(11)–C(12)	113.3(4)
C(12)–C(11)–C(13)	112.2(4)	C(2)–C(12)–C(11)	111.5(3)

Molecular Modeling Studies. The tridimensional coordinates of SF28, SF38, and SF40 obtained from single-crystal X-ray diffraction data have been used as starting geometries for the analysis of their conformational aspects and electronic properties.

The optimum geometry for SF28, SF38, and SF40 was determined with the AMPAC package[37] using the AM1 Hamiltonian.[38] Further refinement has been achieved for SF28 and SF38 by scanning the torsional angles τ_1, τ_2, and τ_3 by 10° increments in the range 0–360° (Figures 5 and 6), while all the other geometric parameters are relaxed. In compound SF40, τ_1 was rotated ±30° around 180°, whereas τ_2, τ_4, and τ_5 were scanned in the range ±60° (Figure 7). The most significant conformations of SF28, SF38, and SF40 are reported in the Table XI: all are characterized by a nearly planar conformation of the heterocyclic ring.

Torsion angles and calculated energies for the conformations observed in the solid state have been reported for comparison and evidence a good agreement between X-ray and molecular modeling results for SF28 and SF40. A difference has been observed for the τ_1 value in SF38; however the energy difference between the solid-state and the free molecule conformation is only 1.6 kcal/mol. For completeness, conformational analysis has been also performed and reported (Table XI) for the reference compounds amrinone and milrinone; for the latter the energy minimum corresponds to a nonplanar conformation (τ_1 = −50.2°) in accord with both solid-state[39] and previous molecular modeling results.[40]

Table XI. Energy and Relevant Geometric Parameters of the Most Significant Conformations of Compounds SF28, SF38, and SF40 and for the Reference Compounds Amrinone and Milrinone[a]

	τ_1	τ_2	τ_3	τ_4	τ_5	ΔE
			SF-28			
AM1	−10.02[b]					0.00
	−3.95[c]					0.06
	0.00					0.06
	180.00					5.09
X-ray	−11.1(4)					0.03
			SF-38			
AM1	−103.05[c]	−166.79	−129.37			0.36
	−79.17[b]	179.83	−124.83			0.00
	0.00	109.82	−108.73			2.70
	99.61	0.00	−126.29			0.10
	180.00	−66.22	−61.72			6.20
X-ray	−139.0(4)	−156.7(4)	−128.3(3)			1.60
			SF-40			
AM1	179.48[b]	29.92	−26.75	−56.13	53.77	0.00
	179.56[c]	29.85	−26.82	−57.10	54.23	0.01
X-ray	−179.2(4)	26.3(6)	−23.1(6)	−50.6(6)	47.9(5)	0.16
			Amrinone			
AM1	143.92					0.00
	−140.01[d]					0.09
X-ray	155.91					0.40
	−169.83					1.35
	−13.56					1.03
	−8.80					1.40
			Milrinone			
AM1	−50.20[e]					0.00
X-ray	−45.26					0.09

[a] The angels are in degree. τ_1 is defined as the torsional angle O(9)–C(8)–C(3)–C(2) (Figures 5–7). In SF38, τ_2 is defined as C_{11}–C_{10}–C_8–C_3 and τ_3 as C_{17}–C_{16}–C_2–N_1 (Figure 6). In SF40, τ_1, τ_2, τ_3, τ_4, and τ_5 are defined as follows: τ_2 = C_{11}–C_{10}–C_8–C_3; τ_3 = C_{11}–C_{12}–C_2–C_3; τ_4 = C_{12}–C_{11}–C_{10}–C_8; τ_5 = C_{10}–C_{11}–C_{12}–C_2 (Figure 7). For amrinone and milrinone, τ_1 refers to the orientation of the pyridine ring with respect to the C_3–C_2 bond where C_3 is the atom bearing the pyridine ring and C_2 the carbon α to the nitrogen atom. ΔE is calculated with respect to the most stable conformation and it is expressed in kcal/mol. [b] Most stable conformation. [c] Fully optimized conformation. [d] The overall planar conformation (τ_1 = 180°) is 1.6 kcal/mol less stable. [e] The overall planar conformation (τ_1 = 180°) is 7.5 kcal/mol less stable.

In the most stable conformation of SF28 the COR group is nearly coplanar and cisoid with respect to the C(3)–C(2) bond as observed in the crystal.

In SF38 the COR group is more perpendicularly oriented with respect to the heterocyclic ring after optimization (τ_1 = −79.2°) than in the crystal (τ_1 = −139.0°). As a result the two phenyl rings occupy a slightly different region of the space. Their orientation with respect to the heterocyclic ring varies from 50° and 135° in the crystal to 77° and 125° in the most stable conformation of the free molecule. In the latter the two phenyl rings are at −79° with respect to each other. A cis coplanar orientation of the COR group with respect to the C(3)–C(2) bond in SF38 is, however, accessible at a low energy cost (≤3 kcal/mol).

The conformations corresponding to a coplanar transoid orientation of the COR group, and the C(3)–C(2) bonds in SF28 and SF38 are the less stable due to the destabilizing steric interactions between the two R substituents. However, according to the AM1 model, the energy barrier to rotation around the C(3)–C(8) and the C(2)–C(11) bonds is low and does not exceed 6.5 kcal/mol. It is therefore reasonable to assume that, in the solution, sufficient energy can be found to rotate the COR group into the most convenient conformation for the binding to the enzyme. As the CO group is forced transoid in SF40, this conformation should be "active", even if it is not the most stable. The substituents H, COOH, and CN at the *cis* position do not influence significantly the conformational behavior.[41]

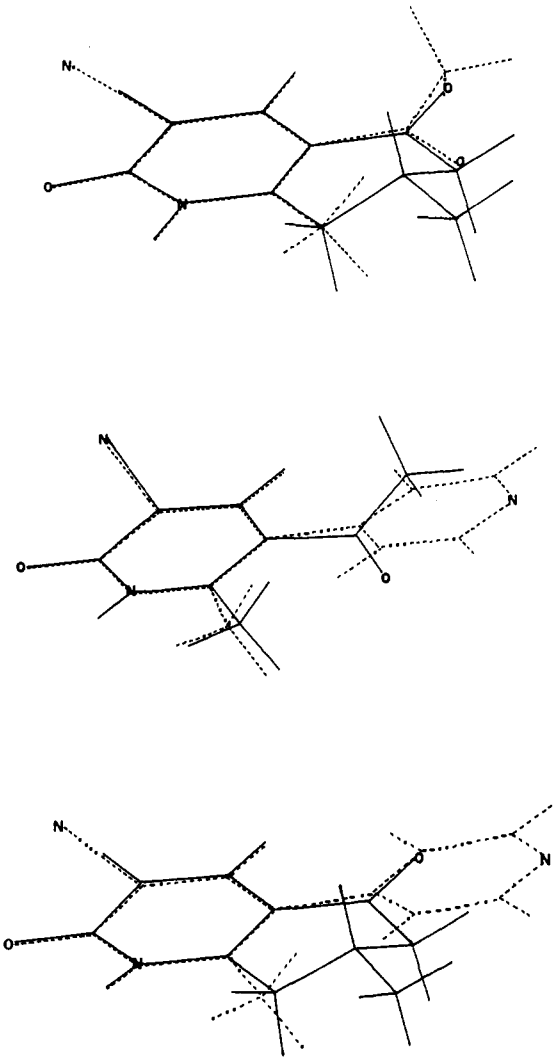


Figure 8. (top) Computer-generated fit (MAD) of the most stable conformations of SF28 (dashed lines) and SF40 (solid lines). (middle) Computer-generated fit of the most stable conformations of SF28 (solid lines) and milrinone (dashed lines). The N(1), C(2), C(3), O(7) atoms were included in the fitting procedure. (bottom) Computer-generated fit of the most stable conformation of SF40 (solid lines) and milrinone (dashed lines). The N(1), C(2), C(3), O(7) atoms were included in the fitting procedure.

Several parameters have been studied in an attempt to correlate the biological activity of SF28, SF38, and SF40 with their structures. The ability to achieve a coplanar conformation of the COR and the heterocyclic ring; the size of the substituent at C(2), the shape and extension of the Connolly's surface,[42] the electrostatic potential maps, and the lipophilicity have been used in the structure–activity relationship.

Figures 8 and 9 show the superimposition of the most stable conformations of SF28, SF38, and SF40 and milrinone by matching the corresponding atoms in the heterocyclic ring. For the most stable conformations of SF28, SF38, and SF40, the Connolly's surfaces and the electrostatic potential maps on these surfaces have been calculated according to the methods implemented in the MAD system.[43] These additional data confirm the difference in the volume occupied by the C(2) substituent, as already evidenced in Figures 8 and 9. In fact, the phenyl ring at C(2) in SF38 occupies an extra region not occupied

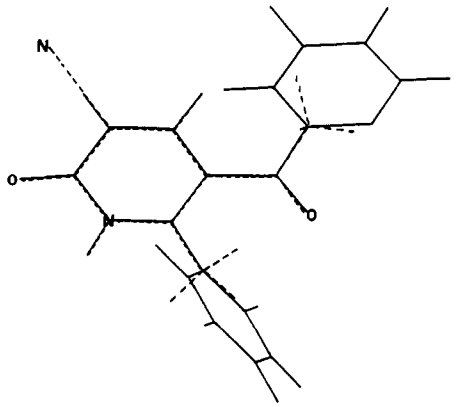
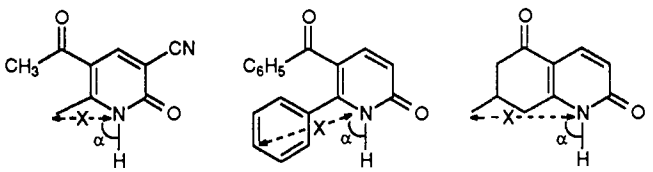


Figure 9. Computer-generated fit (MAD) of the most stable conformations of SF28 (dashed lines) and SF38 (solid lines).

Table XII. Selected Geometric Parameters and Lipofilicity of Compounds SF28, SF38, and SF40, and References Compounds Amrinone and Milrinone



SF28 SF38 SF40

	X^b	α^c	log P^d	lipole^e
SF-28	2.46	87.01	−0.10	1.03
	2.44	85.43^f		
SF38	5.04	69.40	3.82	4.84
	4.94	65.90^f		
SF40	4.93	88.80	0.45	1.48
amrinone	2.11	91.72	2.66	9.25
milrinone	2.46	86.61	2.89	6.94

[a] The most stable conformation was used unless otherwise noted. [b] Distance between the N(1) atom and the farthest carbon atom of the R substituent at C(2). [c] Angle between the N(1)–H vector and the vector connecting N(1) to the farthest carbon atom of the R substituent at C(2). [d] Defined as the sum of the contributions of each atom to the total lipofilicity of the molecule. The contributions are from J. Chem. Inf. Compnt Sci, 1989, 29, 163–173 (Ghose and Crippen). [e] Lipole is like dipole, but using lipofilic contributions instead of charges. It is defined as $-(C_iR_i)$, where C_i is the contribution of the atom i to lipofilicity and R_i is the vector distance between this atom and the center of the molecule. [f] Conformation corresponding to $\tau_1 = 180°$.

by SF28 and SF40. The region representing the difference between the two occupied volumes is most likely the region where a steric repulsion occurs between the receptor wall and the R substituent at C(2) and could be therefore responsible for the different activity of SF28 and SF40 with respect to SF38. Not only the dimension of the exceeding volume in SF38 but also its location and its orientation with respect to the heterocyclic ring could be potential pharmacophore elements to differentiate between positive and negative inotropic activities. These elements have been defined: X, the distance from the N_1 atom and the farthest carbon atom of the substituent at C(2); and α, the angle between the N_1–H vector and the vector connecting N_1 to the farthest carbon atom of the substituent at C(2) (Table XII). Comparison of X and α values in the three compounds evidences in SF38 a reduction of the space available around the α-pyridone amide, the dipolar moiety with hydrogen-bonding ability which is the common pharmacophore element of these compounds. The electrostatic potential maps, calculated with MAD on the Connolly's surface of the most stable conformation of SF28, SF38, and SF40, evidence two negative areas in SF40, at ~6 Å distance, localized on the carbonyl oxygens, whereas the remaining surface is essentially characterized by a positive potential. In SF28, a third negative area is localized on the CN nitrogen at ~3.6 and ~7.2 Å, respectively, from the other two. In SF38, the areas of negative potential are more extended. Lipophilicity, calculated both as log P and as a molecular lipole, according to the algorithms implemented in MAD[43] (Table XII), does not seem to be responsible for the different activities observed: with respect to SF28 and SF40 either log P or the molecular lipole increases significantly in SF38 but also in amrinone and milrinone.[44]

Conclusion

The pharmacological data indicate that an antagonism toward endogenous adenosine without variations in the cellular cyclic AMP content seems to be involved in the contractile effect of SF28 and SF40. On the contrary the negative influence exerted by SF38 on contractile force of the preparation is not related to an activation of adenosine A1 or of acetylcholine receptors on the heart. Both solid-state and theoretical analysis, performed on several compounds to find the structural parameters related to the activity, evidence that the introduction of a phenyl ring with respect to a methyl or to a three carbon connecting chain between C(2) and C(8) determines changes in the conformational preference and topography. In fact, (a) the COR group is forced out of the plane of the heterocyclic ring in the most stable conformation, (b) a steric bulk is introduced which may exceed the receptor's essential volume, (c) further areas of negative potential are introduced, and (d) lipophilicity, expressed as log P and the molecular lipole, increases significantly. Point a should not be discriminant for activity as rotation around the C(3)–C(8) bond in SF38 in order to orient the CO group as in SF40 should be possible in solution. Concerning point c, the presence of a negative electrostatic potential area around the substituent at C_2 in SF38 should not be significant as 2-*tert*-butyl-5-cyano-1,6-dihydro-6-oxopyridinecarboxylate[45] shows similar negative inotropic activity, but has a positive electrostatic potential in that area.

Finally the higher molecular lipophilicity of SF38 and the derivatives SF31 and SF129, as compared to SF28 and SF40 (and their corresponding derivatives SF36, SF127, SF31, SF32) (point d), may not be sufficient to explain the qualitative change in activity, since a recent study shows that, in the lipophilicity range explored, this parameter is directly related to the positive inotropic activity of a series of pyridine derivatives.[35] Point b emphasizes the role of the substituent at C(2) position, whose steric requirements may exceed the receptor essential volume and/or address it toward a different receptor pocket.

Experimental Section

Isolated Atria Preparations. Reserpine-treated male guinea pigs (300–500 g) were killed by a blow to the head followed by exsanguination and the atria were separated from ventricles and suspended vertically in a bath containing 30 mL of physiologic salt solution of the following composition (mM): NaCl, 120; KCl, 2.7; $MgCl_2$, 0.9; NaH_2PO_4, 0.4; $CaCl_2$, 1.37; $NaHCO_3$, 11.9; glucose, 5.5.

The solution was maintained at 29 °C and was bubbled vigorously with a mixture of 95% O_2 and 5% CO_2, which produced pH 7.5. The resting tension was adjusted at 1.0 g and the

Table XIII Crystal Data[a]

	SF28	SF38	SF40
molecular formula	$C_9H_8N_2O_2$	$C_{18}H_{13}NO_2$	$C_{10}H_{10}NO_2$
M	176.17	275.31	176.19
space group	$P\bar{1}$	$P\bar{1}$	$P2_1/c$
cryst syst	triclinic	triclinic	monoclinic
a/Å	5.712(2)	11.552(2)	13.328(3)
b/Å	7.592(2)	10.229(2)	7.848(2)
c/Å	11.056(2)	6.274(2)	8.822(2)
α/deg	71.29(3)	73.93(3)	
β/deg	90.08(3)	104.71(3)	109.41(4)
γ/deg	103.02(3)	100.06(3)	
V/Å^3	441.2(2)	684.3(3)	870.3(4)
Z	2	2	4
D_c/g cm^{-3}	1.33	1.34	1.35
μ(Mo Kα)/cm^{-1}	0.90	0.82	0.88
crystal size/mm	0.41 × 0.16 × 0.24	0.40 × 0.18 × 0.10	0.32 × 0.22 × 0.14
F(000)	184	288	372
$2\theta_{max}$/deg	52	46	50
reflections measured	1820	1909	1775
reflections $I \geq 2.5\sigma(I)$	1144	1263	951
weighting scheme, w	$[\sigma^2(F_o) + 0.002951(F_o)^2]^{-1}$	$[\sigma^2(F_o) + 0.000862(F_o)^2]^{-1}$	$[\sigma^2(F_o) + 0.004035(F_o)^2]^{-1}$
final R	0.048	0.047	0.065
final R'	0.059	0.049	0.081
goodness of fit	1.19	1.30	1.42

[a] For all compounds λ = 0.7107 Å.

developed tension was recorded isometrically by means of high-sensitivity transducer (Basile type DYO for isolated auricles) and registered by a writing oscillograph (Basile, Unirecord System, Model 7050). The basal developed tension ranged from 0.8 to 1.3 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 pulses of 3-ms duration and a voltage 10–20% greater than the threshold value by a Grass stimulator (Model 24 KR). The developed tension ranged from 0.09 to 0.20 mN. The electrical stimulation was performed in order to eliminate any influence on contractile activity due to variations in frequency rate.

Experimental Protocol. The experiments were performed on spontaneously beating atria or on 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.[46] 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 and milrinone and its analogues) were added cumulatively and the inotropic effect was recorded for 5 min before adding a higher concentration. Where indicated, carbachol, propranolol, and verapamil where added to the perfusion medium 10 min before the inotropic agents. In the presence of 8-phenyltheophylline and adenosine deaminase the preincubation lasted 20 min.

Drugs. The compounds used in the experiments (and their sources of supply) were as follows: milrinone (Sterling Winthrop), amrinone (Schiapparelli), isoprenaline, adenosine deaminase, carbachol, propranolol, verapamil hydrochloride, and 8-phenyltheophylline (Sigma). Milrinone analogues were synthetized by Mosti et al.[47] All the other chemicals and reagents were of analytic grade.

Calculations. Data are shown as mean ± standard error of the mean. Statistical significance of the differences between means were calculated by Student's t test. Values were considered to be statistically different when $P < 0.05$.

Crystallographic Measurements. Suitable crystals of the studied complexes were obtained from ethanol solution. Crystal data are given in Table XIII. Unit cell and intensity data for all compounds were obtained by using a Philips PW1100 diffractometer. Reflections were measured by the $\theta/2\theta$ scan method with a scan speed of 1.80° min^{-1} and a scan width of 1.20°. Background counts at both ends of the scan lasted 20 s. Because of the low absorption coefficients, no absorption correction was applied to the intensity data. The structures were solved by using direct methods.[48] For SF28 hydrogen atoms except those of the methyls were introduced at the positions indicated by difference Fourier and refined with isotropic thermal parameters, while the methyl hydrogens were fixed in tetrahedral position at a distance of 1.06 Å with a unique thermal parameter of 0.08 Å^2, which was not refined. The hydrogen atoms in SF38 were introduced at fixed positions d_{C-H} = 0.98 Å and with a unique isotropic thermal parameter of 0.07 Å^2. Those in SF40 were treated as in SF38, except for pyridone hydrogens introduced at the position indicated by difference Fourier map and refined with isotropic thermal parameters. Full-matrix least-squares refinement was used for SF28, SF38, and SF40 structures. The non-hydrogen atoms were refined anisotropically. The final Fourier difference maps showed no significant peaks. In all structures the final shift/error ratio in the refinement was less than 0.01. Calculations were performed using the SHELX 76 program.[49] Puckering parameters were calculated according to ref 50. Atomic scattering factors were from ref 51. The ORTEP program was used for drawing.[52]

Molecular Modeling Studies. The structural modeling was performed by use of the semiempirical computer program AM1[38] as implemented in the AMPAC package.[37] The MAD[43] molecular modeling system was used for molecular graphics studies: molecular fitting, Connolly's surfaces, and molecular electrostatic potential calculations. Both quantum chemical and molecular graphics studies were performed on a IBM Risc System/6000 (Model 520).

Supplementary Material Available: Tables of atomic coordinates, anisotropic thermal parameters (9 pages); observed and calculated structure factors for compounds SF28, SF38, and SF40 (20 pages). Ordering information is given on any current masthead page.

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