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3-Acetyl-5-acylpyridin-2(1*H*)-ones and 3-acetyl-7,8-dihydro-2,5(1*H*,6*H*)-quinolinediones: synthesis, cardiotonic activity and computational studies

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Abstract

A series of milrinone analogues, namely 6-substituted 3-acetyl-5-acylpyridin-2(1*H*)-ones **4a–c**, **e**, **f** and 7-substituted or unsubstituted 3-acetyl-7,8-dihydro-2,5(1*H*,6*H*)-quinolinediones **4g–j**, in which the cyano group was replaced by the acetyl function, was prepared. In a preliminary pharmacological investigation on spontaneously beating atria from reserpine-treated guinea-pigs, all new compounds did not induce any inotropic effect equivalent or higher than that of the milrinone chosen as the reference compound. In order to rationalise how the structure modifications influence the activity and the selectivity of the title compounds, a computational study has been performed. The important role of the substituents in positions-3 and -6 on the pyridone nucleus has been highlighted.

Keywords: Pyridone derivatives; Quinolinediones derivatives; Inotropic activity; Chronotropic activity; Molecular electrostatic potentials (MEPs)

1. Introduction

Many drugs have been developed to enhance the inotropic state of the failing heart as cardiac contractility is impaired in patients with chronic heart failure.

Bipyridine derivatives such as milrinone and amrinone are well-established positive inotropic and vasodilatatory agents [1,2], which increase the concentration of intracellular cyclic AMP in response to the inhibition of a selective cardiac cAMP-phosphodiesterase (PDEase III).

Since the increase in cardiac cyclic AMP concentration has been associated with the risk of arrhytmias, for years we have been searching for compounds which have potent positive inotropic activities without the undesired side effects. Part of our studies in this field reported the synthesis of 6-substituted 5-acyl-1,2-dihydro-2-oxo-3-pyridinecarbonitriles like **1** [3–6] which, as milrinone analogues, were tested for their cardiotonic activity. Among these derivatives, **1a–c** [4,6] (Scheme 1) induced a positive inotropic effect which was equivalent at 10^{-4} M and superior at higher concentrations than that of milrinone, used as reference compound. Furthermore, they showed a much less pronounced chronotropic effect than that induced by milrinone. A pharmacological characterisation of ketone **1a** suggested a primary mechanism of action via PDEase III inhibition, and a secondary via antagonism toward the negative influence exerted by endogenous adenosine on the heart [4,7].

Starting from the preliminary theoretical studies on a pharmacophoric model for positive inotropic activity in the class of milrinone analogues [8,9], we recently performed a 3D-SAR study using distance comparison

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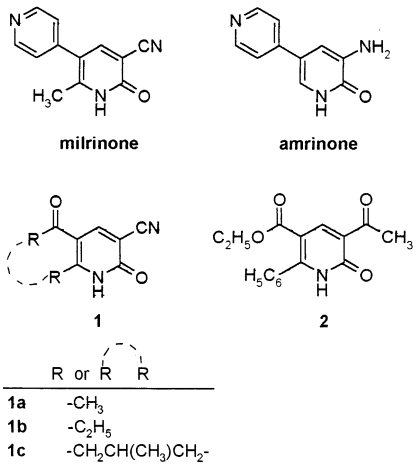
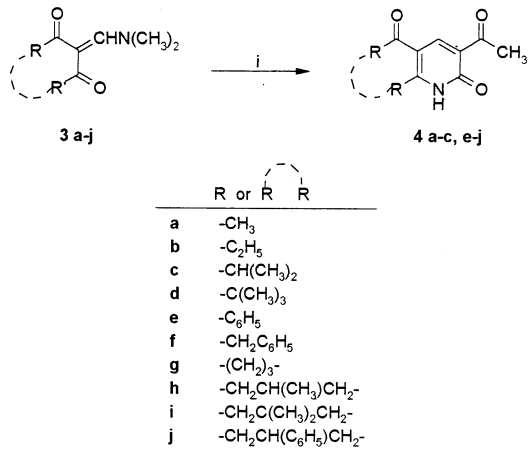


Scheme 1.



i) $CH_3COCH_2CONH_2$, NaH, dry THF.

Scheme 2.

(DISCO) strategy, GRID force-field and principal component analysis (PCA) and confirmed that the salient features strictly necessary for cardiotonic activity via a PDEase III inhibition are: (a) a strong dipole (carbonyl group of the lactam moiety) at one end of the molecule, (b) an adjacent acid proton, (c) a small sized alkyl substituent on the pyridone ring, and (d) an electron rich centre and/or a hydrogen bond acceptor site opposite to the dipole [10].

At the same time we synthesised a series of esters of 2-substituted 5-acetyl-1,6-dihydro-6-oxo-3-pyridinecarboxylic acids, in which all the above-described features have been kept, with the exception of the cyano group, which was replaced by an acetyl group. Among the latter compounds, ester **2** (Scheme 1) showed a potent inotropic effect which was higher, at each assayed concentration (10^{-6}–10^{-3} M), than that induced by milrinone [11].

Surprisingly, the mechanism of action of compound **2** involved both an antagonism toward the negative influence exerted by endogenous adenosine on the heart and an activation of cardiac β-adrenoceptors [12].

Furthermore, to obtain inotropic agents devoid of influence on PDE III activity and, consequently, further information concerning the structure–activity relationship between milrinone analogues, we planned the synthesis of a number of 3-acetyl-5-acylpyridin-2(1*H*)-ones (**4a–c**, **e**, **f**) and 3-acetyl-7,8-dihydro-2,5(1*H*,6*H*)-quinolinediones (**4g–j**) in which the 2-pyridone nucleus was characterised by the presence of the acetyl instead of the cyano group in position-3, as in compound **2**, and the hydrogen bond acceptor group opposite to the lactam dipole was a ketone instead of an ester function, as in compounds **1**, whereas the alkyl substituent in position-6 was varied by homologation, ramification and also aromatic substitution, or included in a second ring, keeping position-4 unsubstituted. The inotropic and chronotropic activities of compounds **4a–c**, **e–j** were also investigated.

Finally, we performed a preliminary computational study on **1a** and **4a** structures to rationalise the effect of the substitution of the cyano with the acetyl group on the biological activity.

2. Chemistry

The synthesis of the newly designed products is described in Scheme 2. The open chain and cyclic *sym*-2-dimethylaminomethylene-1,3-diones (**3**) were prepared according to the reported procedures [6,13]. Similarly to the preparation of 5-acyl-1,2-dihydro-2-oxo-3-pyridinecarbonitriles, in which the sodium cyanoacetamide, a *C*–C–*N* dinucleophile, was employed [3,6], the 6-substituted 3-acetyl-5-acylpyridin-2(1*H*)-ones (**4a–c**, **e**, **f**) and the 7-substituted or unsubstituted 3-acetyl-7,8-dihydro-2,5(1*H*,6*H*)-quinolinediones (**4g–j**) were regiospecifically obtained by the reaction of **3a–j** with another *C*–C–*N* dinucleophile, namely sodium acetoacetamide. Owing to the reduced acidity of the acetoacetamide methylene group in comparison to that of the cyanoacetamide, the reaction performed by Method A (sodium ethoxide in dry ethanol at room temperature (r.t.)) gave poor yields (ranging from 3 to 34%). For this reason it was necessary to generate sodium acetoacetamide using a stronger base, such as sodium hydride, and heating (Method B) so that the desired compounds **4** were obtained in good yield (ranging from 45 to 80%). Only in the case of synthons **3d** did the above reaction not occur because of the strong steric hindrance caused by the *tert*-butyl group.

To our knowledge, between the title compounds, the sole one described in literature was **4a**, obtained in an 83% yield by reaction of ethyl 3-ethoxymethylene-2,4-pentanedione with acetoacetamide in the presence of sodium ethoxide at r.t. [14]. On the contrary, when we

Table 1
Yields, physical and spectroscopic data for compounds **4**

Compd.	R or $\widehat{R\ R}$	Formula [a]	M.p. (°C)	Solvent	Yield (%)	IR (cm^{-1})	^1H NMR (δ)
4a[14]	$-CH_3$	$C_{10}H_{11}NO_3$	258–260	[b]	30 (Method A), 60 (Method B)	3300–2500, 1680, 1658, 1568 [d]	2.48 (s, 3H, CH_3-6), 2.57 (s, 6H, $2CH_3CO$), 8.52 (s, 1H, CH-4), 12.60 (broad s, 1H, NH; disappears with D_2O) [e]
4b	$-C_2H_5$	$C_{12}H_{15}NO_3$	203–205	[c]	14 (Method A), 80 (Method B)	3200–2400, 1675, 1650, 1560 [d]	1.03 (t, $J = 7.2$, 3H, CH_3CH_2-6), 1.15 (t, $J = 7.2$, 3H, CH_3CH_2CO), 2.56 (s, 3H, CH_3CO), 2.50–3.35 (m, 4H, $2CH_2CH_3$), 8.52 (s, 1H, CH-4), 12.65 (broad s, 1H, NH; disappears with D_2O) [e]
4c	$-CH(CH_3)_2$	$C_{14}H_{19}NO_3$	195–197	[c]	10 (Method A), 58 (Method B)	3200–2600, 1678, 1642, 1555 [d]	1.06 (d, $J = 6.6$, 6H, $(CH_3)_2$C-6), 1.25 (d, $J = 6.6$, 6H, $(CH_3)_2CHCO$), 2.58 (s, 3H, CH_3CO), 3.35 (h, $J = 6.6$, 2H, $2CH(CH_3)_2$, 8.36 (s, 1H, CH-4), 12.20 (broad s, 1H, NH; disappears with D_2O) [e]
4e	$-C_6H_5$	$C_{20}H_{15}NO_3$	265–267	[b]	34 (Method A), 78 (Method B)	3200–2500, 1672, 1635, 1555 [d]	2.64 (s, 3H, CH_3CO), 7.41 (m, 10H, $2C_6H_5$), 8.24 (s, 1H, CH-4), 12.80 (broad s, 1H, NH; disappears with D_2O) [e]
4f	$-CH_2C_6H_5$	$C_{22}H_{19}NO_3$	182–184	[c]	19 (Method A), 70 (Method B)	3200–2500, 1680, 1655, 1570 [f]	2.65 (s, 3H, CH_3CO), 4.19 (s, 2H, CH_2), 4.50 (s, 2H, CH_2CO), 7.25–7.40 (m, 10H, $2C_6H_5$), 8.26 (s, 1H, CH-4), 11.20 (broad s, 1H, NH; disappears with D_2O) [g]
4g	$-(CH_2)_3-$	$C_{11}H_{11}NO_3$	240–242	[c]	3 (Method A), 45 (Method B)	3200–2500, 1678, 1642, 1572 [d]	2.03 (q, $J = 6$, 2H, CH_2-7), 2.48 (t, $J = 6$, 2H, CH_2-6), 2.54 (s, 3H, CH_3CO), 2.88 (t, $J = 6$, 2H, CH_2-8), 8.35 (s, 1H, CH-4), 12.65 (broad s, 1H, NH; disappears with D_2O) [e]
4h	$-CH_2CH(CH_3)CH_2-$	$C_{12}H_{13}NO_3$	225–227	[b]	15 (Method A), 58 (Method B)	3200–2500, 1708, 1655, 1562 [d]	1.07 (near d, 3H, CH_3-7), 2.55 (s, 3H, CH_3CO), 2.10–3.00 (m, 5H, CH_2-6 + CH_2-8 + CH-7), 8.40 (s, 1H, CH-4), 12.50 (broad s, 1H, NH; disappears with D_2O) [e]
4i	$-CH_2C(CH_3)_2CH_2-$	$C_{13}H_{15}NO_3$	223–225	[b]	5 (Method A), 45 (Method B)	3200–2500, 1678, 1655, 1572 [d]	1.03 (s, 6H, $(CH_3)_2$C-7), 2.40 (s, 2H, CH_2-6), 2.55 (s, 3H, CH_3CO), 2.80 (s, 2H, CH_2-8), 8.41 (s, 1H, CH-4), 12.50 (broad s, 1H, NH; disappears with D_2O) [e]
4j	$-CH_2CH(C_6H_5)CH_2-$	$C_{17}H_{15}NO_3$	253–255	[b]	9 (Method A), 52 (Method B)	3300–2500, 1688, 1640, 1570 [d]	2.57 (s, 3H, CH_3CO), 1.60–3.80 (m, 5H, CH_2-6 + CH_2-8 + CH-7), 7.42 (s, 5H, C_6H_5), 8.45 (s, 1H, CH-4), 12.90 (broad s, 1H, NH; disappears with D_2O) [e]

[a] Analytical results for C, H, N were within $\pm 0.3\%$ of the calculated values.
[b] From 95% ethanol.
[c] From ethyl acetate.
[d] In KBr.
[e] In DMSO-d_6.
[f] In $CHCl_3$.
[g] In $CDCl_3$.

realised the above reaction, it was complicated by the formation of 2,4-dimethyl-6-hydroxynicotinamide [15], which was difficult to separate from pyridone **4a** and accounted for a smaller yield obtained by us not only in the case of **4a** (30%) but probably also in the case of the whole series of compounds **4** when we used Method A (Table 1).

3. Experimental

3.1. Chemistry

All melting points were determined with Fisher–Johns melting point apparatus and are uncorrected. IR spectra were recorded with a Perkin–Elmer 398 spectrometer and are expressed in cm^{-1}. ^1H NMR spectra were obtained using a Hitachi Perkin–Elmer R-600 (60 MHz) and/or a Varian Gemini 200 (200 MHz) spectrometer; chemical shifts are reported in δ (ppm) units relative to the internal reference tetramethylsilane (TMS). Elemental analyses were performed at the Microanalytical Laboratory of the 'Dipartimento di Scienze Farmaceutiche' at the University of Genoa with a CE Instruments elemental analyser (model EA1110) for C, H, N and the results were within $\pm 0.3\%$ of the theoretical values. Solvents and reagents were of the highest available commercial grade and were used without additional purification.

*3.1.1. General procedure for the preparation of 6-substituted 3-acetyl-5-acylpyridine-2(1H)-ones (**4a–c**, **e**, **f**) and 3-acetyl-7,8-dihydro-2,5(1H,6H)-quinolinediones (**4g–j**)*

3.1.1.1. Method A. The title compounds were prepared starting from both open-chain and cyclic *sym*-2-dimethylaminomethylene-1,3-diones (**3a–j**) following a general procedure previously described [3,6] using sodium acetoacetamide instead of cyanoacetamide.

3.1.1.2. Method B. A solution of **3a–j** (20 mmol) in dry THF (100 ml) was slowly added and stirred at r.t. to a solution of sodium acetoacetamide in the same solvent, prepared by adding acetoacetamide (2.43 g, 24 mmol) to a suspension of sodium hydride (0.60 g of 80% mineral oil dispersion, 20 mmol) in anhydrous THF (60 ml). After the addition, the mixture was refluxed for 24 h. Then the reaction mixture was evaporated under reduced pressure to give the solid residue that was dissolved in water (80 ml). The solution was extracted with diethyl ether. The aqueous phase was then acidified at 0°C with 6 M hydrochloric acid (pH ~ 1) to give a precipitate which was then filtered, washed with water, dried and recrystallised by an appropriate solvent. The melting points, yields, analytical and spectroscopic (IR and ^1H NMR) data are reported in Table 1.

3.2. Pharmacology

Milrinone analogues, namely pyridones **4a–c**, **e**, **f** and quinolinediones **4g–j** (1 μM–1 mM) were tested for their inotropic (Table 2) and chronotropic (Table 3) activities on spontaneously beating atria from reserpine-treated guinea-pigs and were compared to the effects of milrinone (kindly provided by Sterling Winthrop) on the same cardiac activity parameters.

Table 2
Effects of compounds **4** on the contractile force of spontaneously beating atria from reserpine-treated guinea-pigs: comparison with milrinone [a]

Compd.	Developed tension (% variation from the control)						
	10^{-6} M	3×10^{-6} M	10^{-5} M	3×10^{-5} M	10^{-4} M	3×10^{-4} M	10^{-3} M
Milrinone	16.69 ± 0.95	30.24 ± 0.75	38.68 ± 1.25	46.16 ± 0.80	52.70 ± 0.83	52.25 ± 1.13	41.63 ± 1.33
4a	3.01 ± 0.24	9.12 ± 0.05	14.42 ± 0.21	18.71 ± 0.72	24.17 ± 0.56	24.81 ± 0.51	28.98 ± 0.58
4b	0.00 ± 0.00	0.00 ± 0.00	2.83 ± 0.01	6.61 ± 0.07	9.19 ± 0.03	10.21 ± 0.05	n.d.
4c	8.28 ± 0.17	17.14 ± 0.61	24.00 ± 0.42	32.85 ± 1.29	28.00 ± 0.94	9.12 ± 0.72	5.71 ± 0.84
4e	8.58 ± 0.19	7.14 ± 0.58	−0.66 ± 0.16	−3.57 ± 0.81	−4.10 ± 0.64	[b]	
4f	0.00 ± 0.00	0.00 ± 0.00	−6.18 ± 0.02	−15.48 ± 0.08	−24.52 ± 0.10	−26.48 ± 0.18	n.d.
4g	0.00 ± 0.00	0.00 ± 0.00	0.98 ± 0.12	5.44 ± 0.01	10.87 ± 0.71	12.90 ± 0.71	8.44 ± 0.40
4h	2.46 ± 0.77	8.74 ± 0.12	21.67 ± 0.34	22.18 ± 0.25	30.33 ± 0.43	40.44 ± 0.85	60.07 ± 2.47
4i	6.25 ± 0.27	6.49 ± 0.15	6.25 ± 0.17	8.94 ± 0.54	15.15 ± 1.08	21.51 ± 0.78	22.81 ± 0.91
4j	0.00 ± 0.00	−2.85 ± 0.68	−5.71 ± 0.48	−3.58 ± 0.98	−10.28 ± 0.71	−15.89 ± 0.15	[b]

[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 six to ten assays from ten different experiments. Negative values indicate a negative inotropic effect.

[b] Not soluble at this concentration; n.d., not determinate.

Table 3
Effects of compounds **4** on the frequency rate of spontaneously beating atria from reserpine-treated guinea-pigs: comparison with milrinone [a]

Compd.	Frequency (% variation from the control)						
	10^{-6} M	3×10^{-6} M	10^{-5} M	3×10^{-5} M	10^{-4} M	3×10^{-4} M	10^{-3} M
Milrinone	3.01 ± 0.24	9.12 ± 0.05	11.52 ± 0.33	18.22 ± 0.84	24.46 ± 0.75	32.81 ± 1.04	24.71 ± 0.78
4a	−4.56 ± 0.40	−1.23 ± 0.07	1.66 ± 0.26	6.66 ± 0.42	14.13 ± 0.57	23.71 ± 0.61	35.74 ± 0.07
4b	0.00 ± 0.00	0.00 ± 0.00	2.78 ± 0.03	2.78 ± 0.01	5.56 ± 0.01	5.56 ± 0.02	n.d.
4c	3.12 ± 0.43	0.00 ± 0.00	6.25 ± 0.15	6.25 ± 0.15	9.37 ± 0.18	6.25 ± 0.15	3.12 ± 0.43
4e	−3.12 ± 0.14	−6.25 ± 0.38	−3.18 ± 0.17	−6.45 ± 0.28	−9.37 ± 0.16	[b]	
4f	0.00 ± 0.00	0.00 ± 0.00	−1.56 ± 0.01	−1.56 ± 0.03	−7.48 ± 0.04	−8.51 ± 0.06	n.d.
4g	0.00 ± 0.00	0.00 ± 0.00	2.02 ± 0.19	−1.06 ± 0.10	−3.04 ± 0.20	−8.78 ± 0.05	−5.04 ± 0.20
4h	0.00 ± 0.00	−5.13 ± 0.15	−7.38 ± 0.61	−7.38 ± 0.61	−7.38 ± 0.61	−9.68 ± 0.71	−10.95 ± 1.12
4i	−3.12 ± 0.14	−3.12 ± 0.14	−6.78 ± 0.43	−9.73 ± 0.04	−9.37 ± 0.05	−12.98 ± 0.17	−11.58 ± 0.68
4j	−3.45 ± 0.17	−5.26 ± 0.21	−5.26 ± 0.21	−7.89 ± 0.81	−13.07 ± 1.07	−23.49 ± 1.24	[b]

[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 six to ten assays from ten different experiments. Negative values indicate a negative chronotropic effect.

[b] Not soluble at this concentration; n.d., not determinate.

Compounds were solubilised in dimethyl sulfoxide (DMSO). The final concentration of DMSO in the medium did not influence the basal activity of the atrial preparation.

3.2.1. Isolated atria preparations

Reserpine-treated male guinea-pigs (300–500 g) were killed with 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 physiological salt solution of the following composition (mM): NaCl, 120; KCl, 2.7; $MgCl_2$, 0.1; 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 developed tension was recorded isometrically by means of a 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 a 3 ms duration and a voltage higher than the 10–20% 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 the frequency rate.

3.2.2. Inotropic activity

The general procedure of experimental protocol was performed as already described [4]. All the inotropic agents (milrinone, **4a–c**, **e–j**) were added cumulatively and the inotropic effect was recorded for 5 min before adding a higher concentration.

Between newly synthesised compounds, only **4a**, **c** and **h** displayed a positive inotropic activity, though their influence on inotropism was less marked in comparison to that induced by milrinone. In particular, the activity of **4c** greatly decreased at higher concentrations (Table 2). The action on the atria of all the studied compounds was characterised by a decrease in frequency rate (Table 3).

4. Computational chemistry

In order to better rationalise the effect on the biological activity of the acetyl group which represents the new, common chemical feature in position-3 of the pyridone moiety of these compounds in respect to the analogues with a cyano group in the same position, we decided to take into account compounds **1a** and **4a**, having a very similar chemical structure, but a different cardiotonic effect. In fact, **1a** induced an increase of inotropism in spontaneously beating and also in electrically driven left atria from reserpine-treated guinea-pigs, which was greater than that of **4a** at any concentration tested [4].

As previously reported, the mechanism of action of our milrinone analogues was, at first, considered due to a different capability to inhibit selectively the PDEase III [4,7]. Furthermore, according to more recent findings, the electrostatic effects of various PDE III inhibitors seem to be very important in determining the biological activity, confirming the hypothesis of a very open binding site, in which the driving binding force is electrostatic [16]. Moreover, the ligand must be at-

Table 4
Conformational analysis results obtained using molecular mechanics for **1a** and **4a**

M_cf [a]	T_1 [b]	T_2 [c]	E [d]	ΔE [e]
1a_1	24.8 [f]		−67.88	0
1a_2	137.7		−66.56	1.32
4a_1	23.4	−163.1	−58.78	0
4a_2	135.2	−163.0	−56.48	2.3
4a_3	25.2	−22.9	−55.37	3.41
4a_4	139.1	−17.0	−54.49	4.29

[a] **M_cf**: molecule name_conformation number.
[b] T_1: C=C–C=O torsional angle (°).
[c] T_2: C=C–$COCH_3$ torsional angle (°).
[d] E: calculated energies (kcal/mol).
[e] ΔE: relative energies (kcal/mol).
[f] The X-ray structure has this angle equal to 11° [4].

tracted into the binding site from some distance, and long-range attractive forces are primarily of electrostatic nature.

Taking into account all these facts, we decided to investigate and compare the electrostatic features of **1a** and **4a**, to assess which and how much is the effect of an acetyl group respect to a cyano group on the charge distribution on the molecule. Besides amrinone and milrinone have also been taken into account to better rationalise the structure–activity relationships, since similarly to our molecules they have a different chemical group in position-3 on the pyridone moiety and a different biological potency.

A complete geometry optimisation of **1a** and **4a** was obtained by using the MM2* force-field implemented in the package MacroModel [17] and two optimisation methods (subsequently used): PRCG (Polak–Ribiere conjugate gradient) and FMNR (full-matrix Newton–Raphson); the **1a** X-ray data, retrieved from the Cambridge Crystallographic Data Base [4], and the **4a**, designed using the interactive model building of MacroModel, have been used as starting structures.

A systematic conformational analysis in order to determine the energetically stable conformations was carried out using the same molecular mechanics method mentioned above.

Torsional angles and the relative calculated energies of the two energy minima of **1a** and of the four energy minima of **4a** are fully reported in Table 4. These calculations were performed on a Silicon Graphics Indigo 2 workstation.

In order to define the electrostatic similarity or dissimilarity, we used a rigorously defined electrostatic model, which is the electrostatic potential map obtained by calculating the ab initio wave function of each compound.

Ab initio molecular orbital calculations were carried out on the two minima of **1a** and on the four minima of **4a** and, using the PC version of Hyperchem package [18] and the UNIX version of Gaussian-94 package [19], run on an Alpha AXP-3000/500 cluster.

Table 5
Selected geometrical parameters and energies for **1a**, **4a**, amrinone and milrinone obtained using the STO-3G ab initio method

M_cf [a]	T_1 [b]	T_2 [c]	E [d]	ΔE [e]
1a_1	−5.1		−374261.007080400	0.00
1a_2	−151.8		−374257.151802400	3.85
4a_1	0.0	180.0	−411450.266413792	0.00
4a_2	148.5	175.7	−411446.031300000	4.24
4a_3	5.0	−1.7	−411447.731860500	2.53
4a_4	153.0	−7.3	−411444.031300000	6.24
Amrinone	33.8 [f]		−385474.776319322	
Milrinone	51.3 [g]		−432422.142091734	

[a] **M_cf**: molecule name_conformation number.
[b] T_1: C=C–C=O torsional angle (°) for **1a** and **4a** or inter-ring torsion angle (°) for amrinone and milrinone.
[c] T_2: C=C–$COCH_3$ torsional angle (°).
[d] E: calculated energies (kcal/mol).
[e] ΔE: relative energies (kcal/mol).
[f] The X-ray structure has this angle equal to 11.1° [32].
[g] The X-ray structure has this angle equal to 43.6° [20].

The six equilibrium geometries were preliminarily determined at the SCF level by analytic gradient techniques, using a STO-3G basis set, and then optimised, using a 3-21G** basis set.

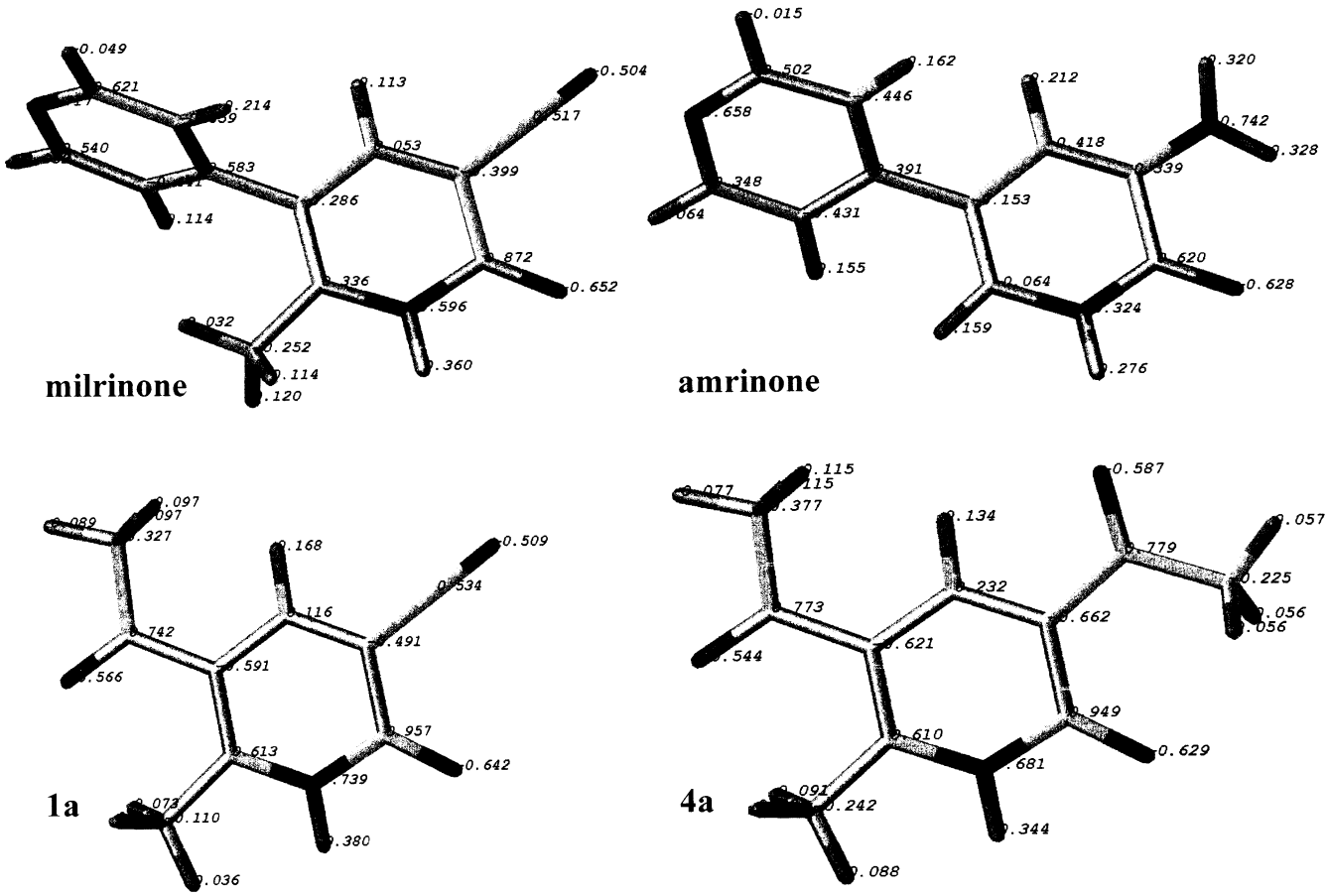
Calculations reported in literature on amrinone and milrinone were performed with a 3-21G basis set; we decided to employ the same split-valence basis set, improving it by adding single first polarisation functions.

Relevant geometrical parameters and relative energies for each conformer are reported in Tables 5 and 6. The electrostatic potential derived charges were calculated on the fully optimised structures using the 3-21G** basis set and the Chelp and the Merz–Kollman–Singh schemes [19]. The same computational procedure was performed on amrinone and

Table 6
Selected geometrical parameters and energies for **1a**, **4a**, amrinone and milrinone obtained using the 3-21G** ab initio method

M_cf [a]	T_1 [b]	T_2 [c]	E [d]	ΔE [e]
1a_1	0.0		−376996.454584500	0.00
1a_2	180.0		−376991.394142000	5.06
4a_1	0.0	180.0	−414474.659545200	0.00
4a_2	180.0	180.0	−414467.718017500	6.94
4a_3	0.0	0.0	−414464.832748900	9.83
4a_4	180.0	0.0	−414460.667104900	13.99
Amrinone	47.9		−388254.809292734	
Milrinone	74.6		−435509.211634430	

[a] **M_cf**: molecule name_conformation number.
[b] T_1: C=C–C=O torsional angle (°) for **1a** and **4a** or inter-ring torsion angle (°) for amrinone and milrinone.
[c] T_2: C=C–$COCH_3$ torsional angle (°).
[d] E: calculated energies (kcal/mol).
[e] ΔE: relative energies (kcal/mol).



Scheme 3.

milrinone X-ray data retrieved from the Cambridge Crystallographic Data Base [20], in order to make a comparison and to relate the obtained results with the different biological activities. Since the Chelp and the Merz–Kollman–Singh schemes provided quite similar results, the atomic charges calculated by the former are fully reported in Scheme 3.

In Figs. 1 and 2, the molecular electrostatic potentials (MEPs) of milrinone, amrinone, **1a** and **4a** have been displayed using different graphic techniques.

5. Results and conclusion

In spite of the unsatisfactory preliminary pharmacological results, it is important to underline that, although all the salient features suggested by the pharmacophoric model discussed in Section 1 are present in the structure of compounds **4**, the cyano group in position-3 of the pyridone nucleus seems to be determinant for good cardiotonic activity.

In examining all the computational data, we can also point out that the acetyl group in position-3 does not greatly modify the charge distribution on the lactam moiety in **4a** compared to the charge distribution on the same group in **1a**; consequently the biological role of the amide function, considered in literature as the primary binding site since it mimics the phosphate group in cAMP, it is not altered.

An electron rich centre which is a pyridine nitrogen in milrinone and amrinone or a carbonyl function in **1a** and **4a** is confirmed as an important binding site opposite to the primary one; we would like to underline the need of a hydrogen bond acceptor atom rather then a generic electron rich substituent in this position [10]. A small sized alkyl substituent on the heterocyclic ring in position-6 is confirmed as an important feature to improve the biological activity and the enzyme selectivity. Amrinone lacks this feature and its activity is lower than that of milrinone [8].

On the other hand, our computational study highlights the importance of the substitution in position-3 on the pyridone ring. In fact, **1a** and milrinone have the same group in this position (–CN) and so the same charge distribution (see the blue area in Fig. 1 representing negative electrostatic potentials), while amrinone, having an $-NH_2$ group in the same position, presents a different electronic behaviour in this region (see the red area in Fig. 1, representing positive electrostatic potentials).

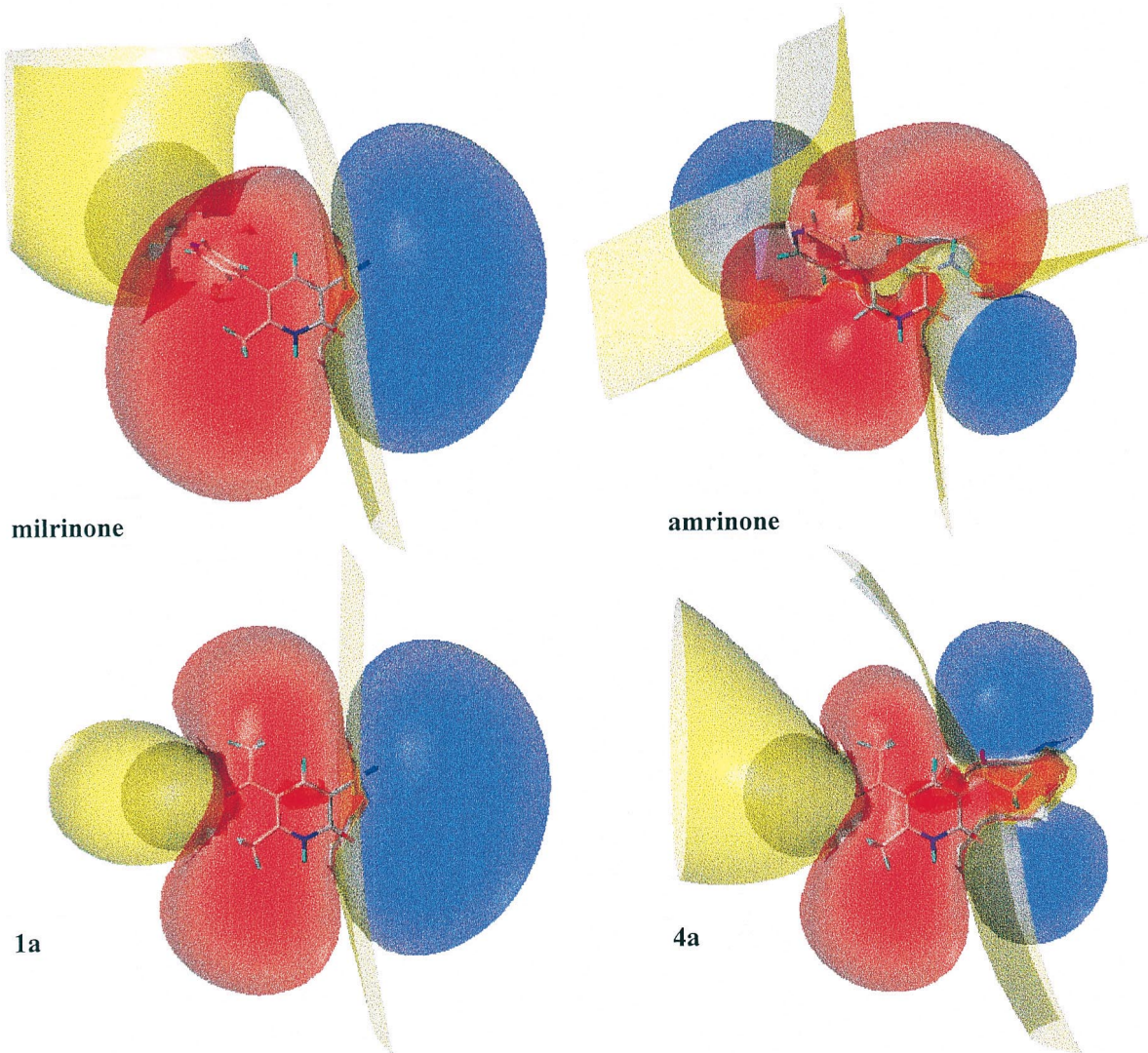


Fig. 1. Visualisation of the MEP (molecular electrostatic potential) of milrinone, amrinone, **1a** and **4a** global minima, displayed as a 3D isocontour map contoured at: -1 kcal mol^{-1} (blue), 0 kcal mol^{-1} (yellow) and $+1$ kcal mol^{-1} (red).

Furthermore, **4a**, which has an acetyl group in position-3, presents in this area, in any energy conformation studied, an electronic density similar to but differently oriented from milrinone and **1a** (see Fig. 2). In addition **4a** could also induce a negative effect on the biological activity for steric bulk.

Since **4a** and amrinone are respectively less active than **1a** and milrinone, in order to improve the cardiotonic activity via PDEase III inhibition, according to our study, in position-3 on the heterocyclic ring it is important to have a small sized electron-rich substituent like a cyano dipole, whose electron cloud has a favourable orientation to fit the biological counterpart.

On the contrary, when position-3 is substituted by a more bulky and differently oriented dipole, the cardiotonic activity decreases. This decrease in potency is probably connected only to the mechanism of action via PDE III inhibition. This hypothesis has been confirmed by the pharmacological study already realised on compound **2** [12]. This compound characterised, like compound **4a**, by a pyridone nucleus, an acetyl instead of a cyano group in position-3, a hydrogen-bond acceptor atom like the carbonyl oxygen of the ester function in position-5, and, differently from compound **4a**, by a bulky, lipophylic substituent in position-6, not only showed good inotropic activity, but also a mechanism of action different from that of compound **1a**, as previously discussed in Section 1 [12].

In conclusion, the present paper suggests some chemical modifications which, if introduced on the pyridone ring, could be useful to obtain agents whose inotropic activity is related to a mechanism of action characterised by no variations in their cellular cyclic AMP content and, consequently, no risk of arrhytmias.

We are developing the synthesis of a new series of analogues to compound **2** and we are searching in

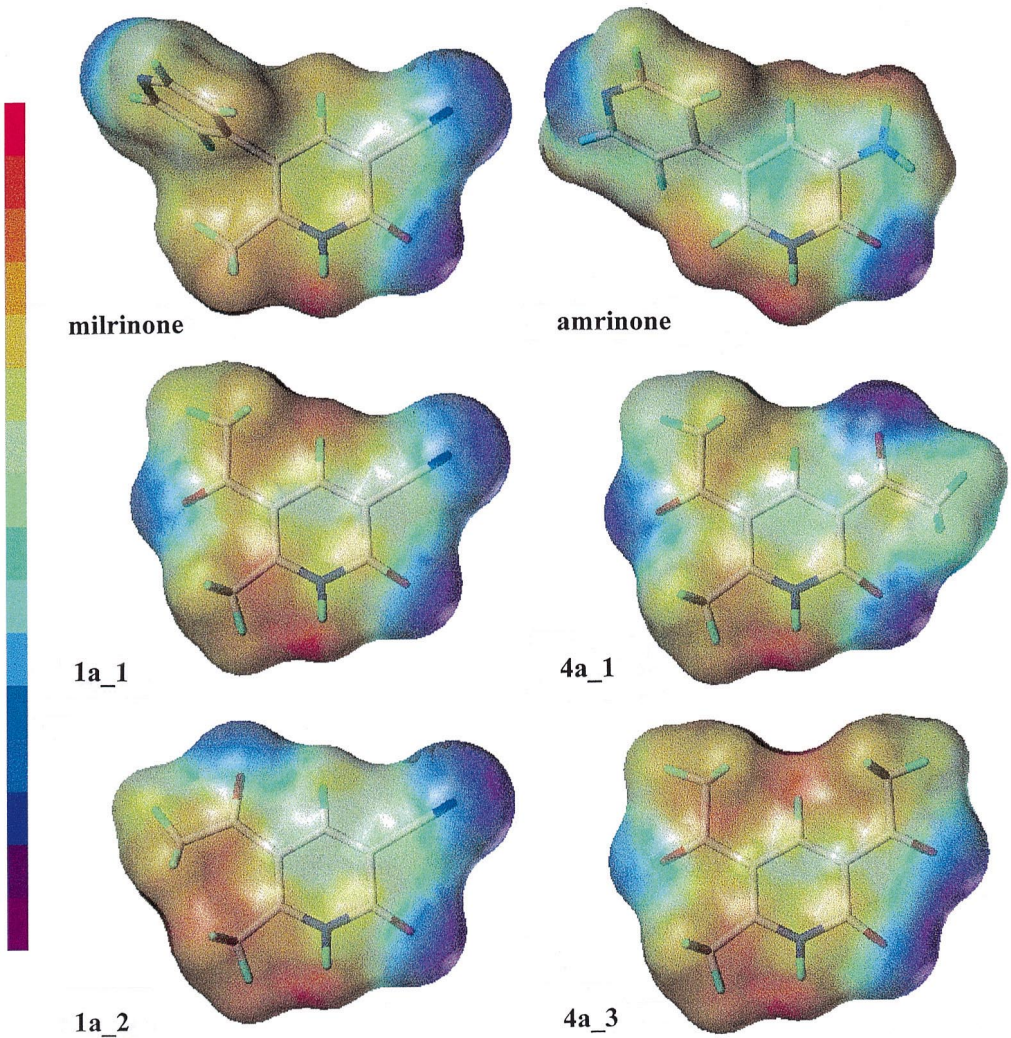


Fig. 2. The MEPs for milrinone, amrinone, **1a_1** and **4a_1** global minima and for **1a_2** and **4a_3** local minima have been mapped onto the Connolly surfaces using the MOLCAD module of Sybyl 6.3 package [21]. The colour code for EP (the electrostatic potentials) ranges from red (most positive values) to purple–blue (most negative values) as one can see on the colour ramp.

depth into the biological behaviour of these new classes of cardiotonic agents.

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