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Synthesis of 4-alkyl (aryl)-6-aryl-3-cyano-2(1*H*)-pyridinones and their 2-imino isosteres as nonsteroidal cardiotonic agents

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Abstract

Thirty new 1,2-dihydropyridine derivatives of the general formula 4-alkyl (aryl)-6-aryl-3-cyano-2(1*H*)-pyridinones (**1–15**) and 4-alkyl (aryl)-6-aryl-3-cyano-2(1*H*)-iminopyridines (**16–30**) were synthesized using one-pot multicomponent reactions of the properly substituted acetophenone, appropriate aldehyde, ammonium acetate and ethyl cyanoacetate (**1–15**) or malononitrile (**16–30**) in ethanol. These target compounds (**1–30**) were evaluated for their cardiotonic activity using the spontaneously beating atria model, from reserpine-treated guinea pigs. The best pharmacological profile was obtained with 3-cyano-6-(3,4-dimethoxyphenyl)-4-(4-hydroxyphenyl)-2(1*H*)-pyridinone (**9**) which displayed selectivity for increasing the force of contraction (108.7 ± 6.7,% change over control) rather than the frequency rate (40.8 ± 5.3,% change over control) at a 5×10^{-4} M concentration. The effects of structural changes upon activity are reported.

Keywords: Cardiotonics; Milrinone analogues; 2(1*H*)-Pyridinones

1. Introduction

Cardiac glycosides (digoxin and digitoxin), discovered in the 18th century, still represent the corner stone of therapy of congestive heart failure (CHF), despite their low therapeutic index and their propensity to cause life-threatening arrhythmia [1–3]. The newer sympathomimetic agents (dobutamine, dopamine) are orally inactive and may lead to tachyphylaxis due to β-receptor down regulation [4,5]. Because of the need for safer and orally effective drugs, a series of nonglycosidic, non-sympathomimetic, cardiotonic agents has been developed. Examples include, amrinone [6] (**A**), milrinone [7] (**B**), loprinone [8] (**C**) and the milrinone analogue [9] (**D**) (see Fig. 1 for structures **A–D**). Many of the reported cardiotonics appear to drive their inotropic effect from inhibiting the phosphodiesterase III isozyme, resulting in an increase of intracellular cAMP [10,11], increasing the myofibrillar Ca^{2+} sensitizing properties [12], antagonizing the negative influence of endogenous adenosine on the heart [9], activating the β-adrenoreceptors [9] or by mixed mechanisms [12,13].

Amrinone was the progenitor of this new class of cardiotonics [6], milrinone is an analogue of amrinone and was reported to be 50 times as active as amrinone and to possess reduced propensity to side effects [14,15]. Structure–activity studies indicated that it is the methyl substituent rather than the amine–nitrile substituent interchange that is responsible for this difference in the inotorpic activities of amrinone and milrinone [14]. However, the milrinone analogue with a phenyl substituent instead of the methyl was found to be inactive [16]. Also, the 3-benzoyl-2-phenyl-6-(1*H*)-pyridinone derivative gave a negative inotropic effect [17]; this was attributed to unfavorable interaction of their side chain at C-2 with steric boundaries at the receptor site [16,17].

Dorigo et al. [9] reported compound **D** (Fig. 1) as a milrinone analogue that is more potent than milrinone as a positive inotropic agent and with weak influence on the frequency rate in isolated guinea pig atria. These findings resulted in a new insight in the SAR of milrinone analogues. Briefly, the presence of a second pyridine nitrogen is not essential for activity, and the replacement of the C-3 cyano by the more lipophilic acetyl function may counteract or even exceed the

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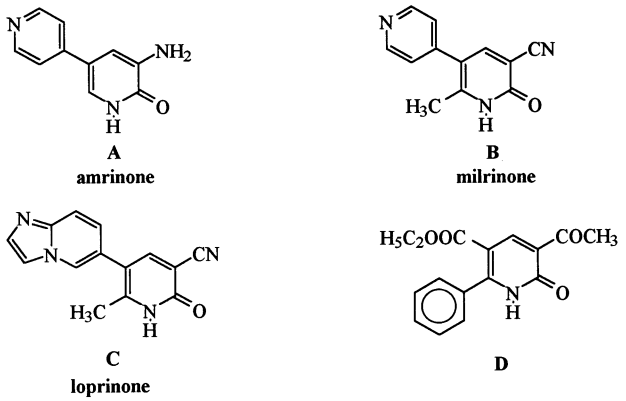


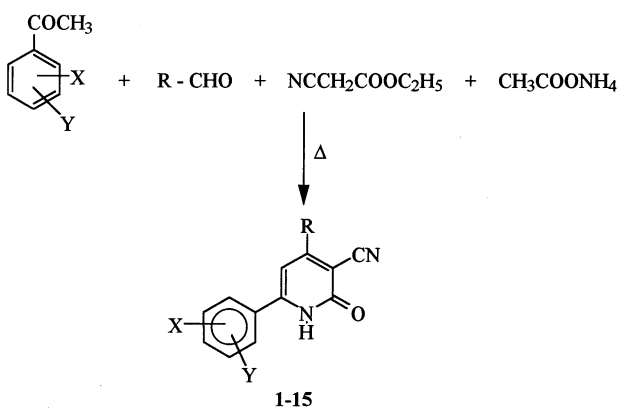
Fig. 1.

deleterious steric effect of the phenyl substituent at C-6. This agrees with the observation that increase in lipophilicity of the pyridine derivative correlates with increase in inotropic activity and with adenosine antagonism but not with increase in chronotropism or with PDE III inhibition [18].

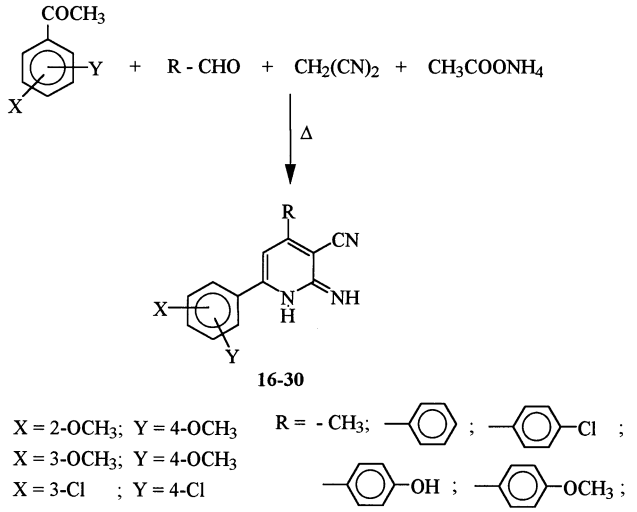
In this work, we present the synthesis, characterization and evaluation of novel milrinone analogues in which position 2 of the pyridine ring is either a carbonyl or its isostere imino group, position 4 is substituted by a methyl or an aryl substituent and position 5 is unsubstituted so as to minimize the steric influence upon position 6 substituent.

2. Chemistry

The preparation of compounds **1–30** was depicted in Schemes 1 and 2. Briefly, 2,4-dimethoxyacetophenone, 3,4-dimethoxyacetophenone or 3,4-dichloroacetophenone was reacted with the appropriate aldehyde, namely acetaldehyde, benzaldehyde, *p*-chlorobenzaldehyde, *p*-hydroxybenzaldehyde, anisaldehyde, and ethyl cyanoacetate in the presence of ammonium acetate and ethanol to give 4-alkyl (aryl)-6-aryl-3-cyano-2(1*H*)-



Scheme 1.



Scheme 2.

pyridinones (**1–15**). The structures of **1–15** were verified by IR, ^{1}H NMR, MS and elementary analyses. The IR spectra of compounds **1–15** showed characteristic absorption bands of the NH group at 3600–3200 cm^{-1}, C≡N at 2220–2200 cm^{-1} and –C=O at 1660–1640 cm^{-1} (Table 2). The mass spectra for **1–15** revealed a molecular ion peak (M^+) which was also the base peak (Table 2); this denotes the relative stability of compounds **1–15** towards electron bombardment. The elemental analyses and ^{1}H NMR of all derivatives (**1–15**) were basically in agreement with their proposed structures (Table 2).

The target compounds 4-alkyl (aryl)-6-aryl-3-cyano-2(1*H*)-iminopyridines (**16–30**) were synthesized using the aforementioned procedure except that ethyl cyanoacetate was replaced by malononitrile (Scheme 2). The IR spectra of **16–30** showed characteristic absorption bands at 3600–3200 cm^{-1} (NH) and 2220–2200 cm^{-1} (C≡N). The mass spectra of **16–30** displayed a molecular ion peak (M^+) which was also the base peak for most of the compounds (Table 2).

From a synthetic point of view, the aforementioned synthetic procedures are considered to be multicomponent reactions (MCRs) where at least three starting materials react together in one pot to give a product incorporating part of all starting materials. This is in contrast to classical multi-step reactions, where only two starting materials react. Whenever a MCR can be applied in chemistry, this is preferred since it is easier to perform, gives higher yields and is less time consuming [19]. The formation of 2-oxo- and 2-iminopyridine derivatives was assumed to proceed via the Michael condensation of ethyl cyanoacetate or malononitrile with α,β-unsaturated ketone, initially formed from the reaction of the aldehyde with acetophenone [20]. Alternatively, the formation of 2-iminopyridines was explained by the initial formation of the arylidene-

malononitrile from the aldehyde and malononitrile followed by its subsequent reaction with acetophenone [20,21].

3. Experimental

3.1. Chemistry

Melting points were determined in open capillaries on electrothermal melting-point apparatus and are uncorrected. IR spectra were recorded on a Perkin–Elmer 727B spectrometer. ^{1}H NMR spectra were recorded in DMSO-d_6 on a Jeol 400 MHz instrument, chemical shifts were recorded in δ (ppm) units, relative to Me_4Si as an internal standard. Microanalytical data (C, H, N) were performed on a Perkin–Elmer 2400 analyzer and were within $\pm 0.4\%$ of the theoretical values. Mass spectra were recorded on a Shimadzu PQ-5000 gas chromatography MS apparatus. TLC was performed on precoated silica gel plates (60-F 254, 0.2 mm) and UV light was used for visualization.

3.2. General procedure for the preparation of 4-alkyl (aryl)-6-aryl-3-cyano-2(1H)-pyridinones (1–15)

A mixture of the appropriate acetophenone (0.01 mol), ethyl cyanoacetate (0.01 mol), the appropriate aldehyde (0.01 mol) and ammonium acetate (0.08 mol) in ethanol (50 ml) was refluxed for 6 h. The reaction mixture was cooled and the formed precipitate was filtered, washed successively with water, dried and crystallized (Tables 1 and 2).

3.3. General procedure for the preparation of 4-alkyl (aryl)-6-aryl-3-cyano-2-(1H)-iminopyridines (16–30)

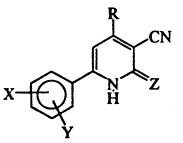
The foregoing method was carried out except that ethyl cyanoacetate was replaced by malononitrile (Tables 1 and 2).

4. Pharmacology

4.1. Preparation of isolated guinea-pig atria

Reserpine-treated guinea pigs (300–500 g) were killed by a blow to the head followed by exsanguination. The chests were opened via a mid-sternal incision, the pericardium and facia around the heart were removed and the heart was exposed. The hearts were rapidly placed in a Petri dish filled with physiological salt solution (PSS) and aerated with 95% O_2 and 5% CO_2 in which the right atrium was separated from the rest of the heart. The atria were then placed in 10 ml organ baths

Table 1
Structural and physical data of compounds **1–30**



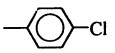
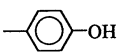
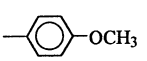
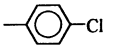
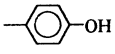
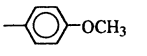
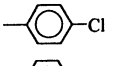
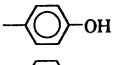
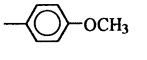
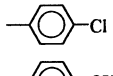
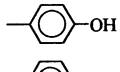
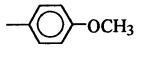
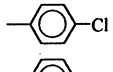
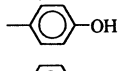
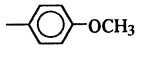
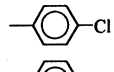
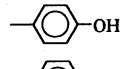
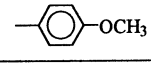
Comp.	X	Y	Z	R	Crystallization solvent	M.p. (°C)	Yield (%)
1	2-OCH_3	4-OCH_3	O	CH_3	MeOH	259-61	40
2	2-OCH_3	4-OCH_3	O		MeOH	168-70	36
3	2-OCH_3	4-OCH_3	O		MeOH	240-42	32
4	2-OCH_3	4-OCH_3	O		MeOH	>300	40
5	2-OCH_3	4-OCH_3	O		MeOH	231-33	31
6	3-OCH_3	4-OCH_3	O	CH_3	MeOH	255-57	37
7	3-OCH_3	4-OCH_3	O		MeOH	287-89	33
8	3-OCH_3	4-OCH_3	O		MeOH	292-94	33
9	3-OCH_3	4-OCH_3	O		MeOH	>300	39
10	3-OCH_3	4-OCH_3	O		MeOH	262-64	38
11	3-Cl	4-Cl	O	CH_3	DMF	>300	33
12	3-Cl	4-Cl	O		DMF	294-96	38
13	3-Cl	4-Cl	O		DMF	>300	40
14	3-Cl	4-Cl	O		DMF	>300	38
15	3-Cl	4-Cl	O		DMF	290-92	33
16	2-OCH_3	4-OCH_3	NH	CH_3	MeOH	175-77	36
17	2-OCH_3	4-OCH_3	NH		MeOH	188-90	38
18	2-OCH_3	4-OCH_3	NH		MeOH	186-88	40
19	2-OCH_3	4-OCH_3	NH		MeOH	225-27	33
20	2-OCH_3	4-OCH_3	NH		MeOH	217-19	31
21	3-OCH_3	4-OCH_3	NH	CH_3	MeOH	190-92	38
22	3-OCH_3	4-OCH_3	NH		MeOH	212-14	33
23	3-OCH_3	4-OCH_3	NH		MeOH	203-7	39
24	3-OCH_3	4-OCH_3	NH		MeOH	205-7	40
25	3-OCH_3	4-OCH_3	NH		MeOH	165-67	38
26	3-Cl	4-Cl	NH	CH_3	DMF	250-52	39
27	3-Cl	4-Cl	NH		DMF	160-62	39
28	3-Cl	4-Cl	NH		DMF	235-37	36
29	3-Cl	4-Cl	NH		DMF	>300	35
30	3-Cl	4-Cl	NH		DMF	180-82	33

Table 2
Analytical data of compounds **1–30**

Comp.	Analysis	IR (KBr, cm^{-1})	MS m/z (%)	^1H NMR (DMSO-d_6)
1	$C_{15}H_{14}N_2O_3$	3450–3250, 2200, 1650	270 (100)	2.35 (s, 3H, CH_3), 3.82 (s, 3H, 2-OCH_3), 3.84 (s, 3H, 4-OCH_3), 6.60–7.75 (m, 4H, aromatic), 12.20 (brs, 1H, NH)
2	$C_{20}H_{16}N_2O_3$	3600–3300, 2200, 1660	332 (100)	3.83 (s, 6H, 2 and 4-OCH_3), 6.51–7.68 (m, 9H, aromatic), 12.4 (brs, 1H, NH)
3	$C_{20}H_{15}ClN_2O_3$	3600–3200, 2210, 1660	366 (100)	3.82 (s, 3H, 2-OCH_3), 3.85 (s, 3H, 4-OCH_3), 6.5–7.72 (m, 8H, aromatic), 12.20 (brs, 1H, NH)
4	$C_{20}H_{16}N_2O_4$	3400–3200, 2220, 1660	348 (100)	3.82 (s, 6H, 2 and 4-OCH_3), 6.45–7.58 (m, 8H, aromatic), 10.4 (s, 1H, OH), 12.4 (brs, 1H, NH)
5	$C_{21}H_{18}N_2O_4$	3500–3400, 2200, 1640	362 (100)	3.83 (s, 9H, 2, 4 and 4-OCH_3), 6.48–7.68 (m, 8H, aromatic), 12.20 (brs, 1H, NH)
6	$C_{15}H_{14}N_2O_3$	3500–3350, 2210, 1660	270 (100)	2.39 (s, 3H, CH_3), 3.81 (s, 3H, 3-OCH_3), 3.84 (s, 3H, 4-OCH_3), 6.75–7.39 (m, 4H, aromatic), 12.4 (brs, 1H, NH)
7	$C_{20}H_{16}N_2O_3$	3600–3300, 2210, 1640	332 (100)	3.82 (s, 3H, 3-OCH_3), 3.85 (s, 3H, 4-OCH_3), 6.84–7.71 (m, 9H, aromatic), 12.4 (brs, 1H, NH)
8	$C_{20}H_{15}ClN_2O_3$	3500–3200, 2210, 1660	366 (100)	3.82 (s, 3H, 3-OCH_3), 3.84 (s, 3H, 4-OCH_3), 6.83–7.74 (m, 8H, aromatic), 12.50 (brs, 1H, NH)
9	$C_{20}H_{16}N_2O_4$	3450–3250, 2200, 1650	348 (100)	3.82 (s, 3H, 3-OCH_3), 3.84 (s, 3H, 4-OCH_3), 6.30–7.90 (m, 8H, aromatic), 10.02 (brs, 1H, OH), 12.20 (brs, 1H, NH)
10	$C_{21}H_{18}N_2O_4$	3600–3350, 2210, 1660	362 (100)	3.82 (s, 3H, 3-OCH_3), 3.84 (s, 6H, 2 and 4-OCH_3), 6.80–6.75 (m, 8H, aromatic), 12.40 (brs, 1H, NH)
11	$C_{13}H_8Cl_2N_2O$	3600–3300, 2210, 1650	278 (100)	2.41 (s, 3H, CH_3), 6.90 (s, 1H, aromatic), 7.78 (s, 2H, aromatic), 8.10 (s, 1H, aromatic), 12.25 (brs, 1H, NH)
12	$C_{18}H_{10}Cl_2N_2O$	3600–3300, 2210, 1660	340 (100)	7.05–8.25 (m, 9H, aromatic), 12.30 (brs, 1H, NH)
13	$C_{18}H_9Cl_3N_2O$	3600–3300, 2210, 1660	374 (100)	7.05–8.24 (m, 8H, aromatic), 12.20 (brs, 1H, NH)
14	$C_{18}H_{10}Cl_2N_2O_2$	3450–3200, 2220, 1660	356 (100)	6.92–8.21 (m, 8H, aromatic), 10.20 (brs, 1H, OH), 12.2 (brs, 1H, NH)
15	$C_{19}H_{12}Cl_2N_2O_2$	3500–3300, 2200, 1650	370 (100)	3.83 (s, 3H, OCH_3), 6.97–8.25 (m, 8H, aromatic), 12.25 (brs, 1H, NH)HHh
16	$C_{15}H_{15}N_3O_2$	3460, 3300, 2210	269 (100)	2.35 (s, 3H, CH_3), 3.83 (s, 6H, 2 and 4-OCH_3), 6.80–8.55 (m, 9H, aromatic and NHs)
17	$C_{20}H_{17}N_3O_2$	3500–3450, 3400–3300, 2200	331 (100)	3.82 (s, 6H, 2 and 4-OCH_3), 6.66–7.84 (m, 11H, aromatic and NHs)
18	$C_{20}H_{16}ClN_3O_2$	3460, 3320, 2200	365 (100)	3.83 (s, 6H, 2 and 4-OCH_3), 6.70–7.95 (m, 10H, aromatic and NHs)
19	$C_{20}H_{17}N_3O_3$	3450, 3350–3250, 2200	347 (100)	3.82 (s, 3H, 2-OCH_3), 3.83 (s, 3H, 4-OCH_3), 6.64–7.81 (m, 10H, aromatic and NHs), 9.93 (brs, 1H, OH)
20	$C_{21}H_{19}N_3O_3$	3450, 3280, 2200	361 (100)	3.81 (s, 3H, 3-OCH_3), 3.83 (s, 6H, 2 and 4-OCH_3), 6.66–7.82 (m, 10H, aromatic and NHs)
21	$C_{15}H_{15}N_3O_2$	3450, 3260, 2200	269 (100)	2.35 (s, 3H, CH_3), 3.83 (s, 3H, 3-OCH_3), 3.85 (s, 3H, 4-OCH_3), 6.74–7.69 (m, 6H, aromatic and NHs)
22	$C_{20}H_{17}N_3O_2$	3520–3440, 3400–3320, 2190	331 (100)	3.82 (s, 3H, 3-OCH_3), 3.84 (s, 3H, 4-OCH_3), 6.95–7.75 (m, 11H, aromatic and NHs)
23	$C_{20}H_{16}ClN_3O_2$	3450, 3320, 2200	365 (100)	3.82 (s, 3H, 3-OCH_3), 3.84 (s, 3H, 4-OCH_3), 6.90–7.90 (m, 10H, aromatic and NHs)
24	$C_{20}H_{17}N_3O_3$	3460, 3360, 2200	347 (100)	3.80 (s, 1H, 3-OCH_3), 3.83 (s, 6H, 2 and 4-OCH_3), 6.87–7.73 (m, 10H, aromatic and NHs)
25	$C_{21}H_{19}N_3O_3$	3470, 3360, 2200	361 (100)	3.80 (s, 3H, 3-OCH_3), 3.83 (s, 6H, 2 and 4-OCH_3), 6.87–7.73 (m, 10H, aromatic and NHs)
26	$C_{13}H_9Cl_2N_3$	3450, 3330, 2210	277 (100)	2.40 (s, 3H, CH_3), 6.83–7.87 (m, 6H, aromatic and NHs)
27	$C_{18}H_{11}Cl_2N_3$	3450, 3350, 2200	339 (100)	6.85–7.94 (m, 11H, aromatic and NHs)
28	$C_{18}H_{10}Cl_3N_3$	3460, 3340, 2200	373 (20) 44 (100)	6.85–8.55 (m, 10H, aromatic and NHs)
29	$C_{18}H_{11}Cl_2N_3O$	3470, 3350, 2200	355 (55) 44 (100)	6.90–8.50 (m, 10H, aromatic and NHs), 10.0 (brs, 1H, OH)
30	$C_{19}H_{13}Cl_2N_3O$	3470, 3330, 2200	369 (100)	3.84 (s, 3H, OCH_3), 7.06–8.51 (m, 10H, aromatic and NHs)

containing PSS of the following composition (in mM): NaCl, 120; KCl, 2.7; $MgCl_2$, 0.9; NaH_2PO_4, 0.4; $CaCl_2$, 1.37; $NaHCO_3$, 11.9 and glucose, 5.5 [9]. The solution was maintained at 34°C and was bubbled vigorously

Table 3
Effect of compounds **1–30** upon contractile activity and frequency rate of spontaneously beating atria from reserpine-treated guinea pigs at 5×10^{-4} M concentration

Comp.	Developed tension (% change overcontrol) [a]	Frequency rate (% change over control) [a]	Comp.	Developed tension (% change overcontrol) [a]	Frequency rate (% change over control) [a]
1	[b]	[b]	**16**	[b]	[b]
2	[b]	[b]	**17**	[b]	[b]
3	[b]	[b]	**18**	[b]	[b]
4	46.1 ± 4.3	42 ± 5.7	**19**	11.9 ± 2.1	4.6 ± 0.2
5	25 ± 3.4	28.7 ± 4.1	**20**	37.6 ± 2.7	34.4 ± 4.1
6	[b]	[b]	**21**	[b]	[b]
7	[b]	[b]	**22**	[b]	[b]
8	21.1 ± 0.9	35 ± 3.1	**23**	[b]	[b]
9	108.7 ± 6.7	40.8 ± 6.7	**24**	[b]	[b]
10	[b]	[b]	**25**	[b]	[b]
11	[b]	[b]	**26**	[b]	[b]
12	[b]	[b]	**27**	19.3 ± 1.5	31.7 ± 1.4
13	[b]	[b]	**28**	[b]	[b]
14	18.3 ± 1.1	15.2 ± 3.6	**29**	[b]	[b]
15	[b]	[b]	**30**	14.2 ± 1.0	[b]
Isoproterenol sulphate	83.7 ± 4.1	76.4 ± 3.8			

[a] Mean ± SEM from four atria.
[b] No inotropic or chronotropic effect.

with a mixture of 95% O_2 and 5% CO_2 which produced pH 7.5. The resting tension was adjusted at 1 g and developed tension was recorded isometrically by means of a Myograph F-60, Narco Bio-System isometric transducer. Signals from the force transducer were continuously recorded on a Narco Bio-System, MK-111 physiograph. After a period of stabilization of 60 min, control measurements of contractile force and frequency were made.

4.2. Effect on isolated atria

The experiments were performed on spontaneously beating atria obtained from reserpine-treated guinea pigs. Reserpine (2 mg/kg, i.p.) was given 24 h before the animals were sacrificed in order to eliminate the influence of noradrenaline, which might be released from the sympathetic nerve terminals. Noradrenaline depletion was determined by exposing the 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. All compounds were added to the perfusion fluid in a final concentration of 5×10^{-4} M. After each addition, the inotropic effect was recorded for 3 min before washing and replacement of the perfusion fluid [9,17].

All compounds were dissolved in DMSO and added to the bath in a volume of 100 μl. The same volume of DMSO did not produce any effect. Isoproterenol sulfate at a concentration of 5×10^{-4} M was used as a reference standard to be able to follow the changes in both the atria contractility and frequency rate.

Data are presented as the mean ± SEM (Table 3), statistical comparisons were made by Student's *t*-test for paired data. Significance was accepted at $P = 0.05$ [22]. The results are shown in Table 3.

4.3. Acute toxicity

Four groups of mice, each consisting of six animals, were used. The compounds were given orally in doses of 1, 10, 100 and 500 mg/kg, respectively. Twenty-four hours later, the % mortality in each group and for each compound was recorded and the LD_{50} was calculated using the method described by Litchfield and Wilcoxon [23].

5. Results and discussion

Biological evaluation of compounds **1–30** as cardiotonic agents using the spontaneously beating atria model from reserpine-treated guinea pigs to eliminate the influence of noradrenaline upon contractility was adopted. Isoproterenol sulfate was used as a positive control. Inotropic and chronotropic activities were expressed as % change in the force of contraction and frequency rate over control (Table 3).

Replacement of the cyclic amide oxygen by an imino group afforded a series of isosteric compounds **1–15** versus **16–30**, respectively. There is no clear direction to the effect of this oxygen-imino isosterism upon inotropic activity, for example **4** > **19**; **8** > **23**; **9** > **24** and **14** > **29**; on the contrary **20** > **5**; **27** > **12** and **30** > **15**

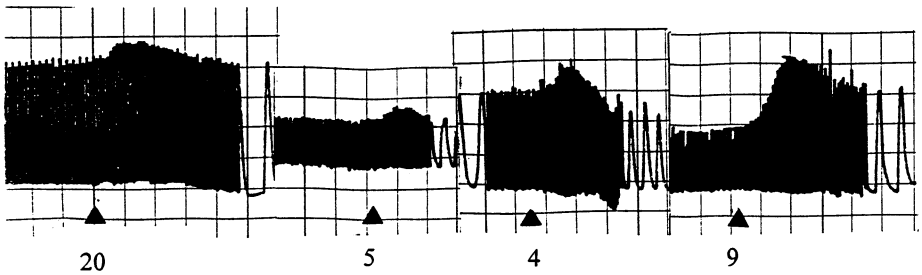


Fig. 2. Effects of compounds **20**, **5**, **4**, and **9** (in a final concentration of 5×10^{-4} M) on the contractile activity of the spontaneously beating guinea-pig atria.

(Table 3). This indicates that a cyclic amide is not a requisite for cardiotonic activity and that the NH isosteric group may replace the lactam oxygen without loss of cardiotonic activity. Regarding the substituent at C-4 of the pyridine ring, in all cases the presence of a methyl substituent leads to inactive compounds (**1**, **6**, **11**, **16**, **21** and **22,** Table 3) regardless of the nature of the other substituents. Meanwhile, the introduction of a phenyl or *p*-chlorophenyl substituent to C-4 of the pyridine ring led to inactive compounds in most cases e.g. **2**, **3**, **7**, **12**, **13**, **17**, **18**, **22**, **23** and **28**. The two exceptions were compounds **8** and **27**; even with the cardiotonic activity of the latter, their effect on the frequency rate exceeded their effect on the force of contraction (approximately 1.6 times in both cases, Table 3). On the other hand, substitution of the pyridine 4 position by *p*-hydroxyphenyl or *p*-methoxyphenyl groups, that are eventually able to form hydrogen bonding produced active cardiotonics as in the case of **4**, **5**, **9**, **14**, **19**, **20** and **30** (Fig. 2) and their cardiotonic effect was accompanied by either no, lower or equal effect upon frequency rate (Table 3).

The obtained data clearly indicate the obvious necessity for an aromatic substituent in position 4 of the pyridine ring for cardiotonic activity and the modulating influence of this substituent upon selectivity (inotropy versus chronotropy). In addition, it should be taken into consideration that substitution of the pyridine ring in position 4 rather than position 5 reduces the additive steric effect that the position 5 substituent may have upon the fitting of position 6 substituent into its receptor. Comparing the activities of the synthesized compounds relative to the electronic, lipophilic and steric parameters of the C-4 aryl substituent [24] signifies the relevance of the electronic properties of this substituent to inotropic activity rather than the lipophilic or steric ones (compounds **4**, **5**, **9** and **20** were the most active of the series). For the pyridine C-6 substituent, it appears that the presence of an aryl substituent at this position, particularly with the absence of C-5 substitution, does not cause loss of cardiotonic activity. The topological model for cardiac cAMP phosphodiesterase receptors, developed by Erhardt et al. [25], recognized four binding sites: one for a resonance dipole moiety similar to acidic amide, a binding site for an electron-rich center similar to a double bond both in one plane, a binding area for π electrons when the latter are turned approximately 20° from the perpendicular to the plane established by the first two sites and this site probably protrudes from the top of the receptor pocket, and a site nearly in the plane of the first two sites that interacts with an electron-rich system. Also, the aforementioned model recognized two steric boundaries one on each side of the first two binding sites. In that respect, our molecules are not compatible with Erhardt's model; thus, mechanisms of action other than the inhibition of PDE III cannot be excluded.

The LD_{50} values of four active compounds, namely **4**, **5**, **9** and **20**, were determined in mice and found to be 258 mg/kg for **9**, while others were found to be safe up to 500 mg/kg.

In conclusion, some of the prepared compounds were found to possess cardiotonic activity in vitro, together with selectivity e.g. **9**, **19** and **30**, and they represent a good model for future derivatization. An investigation into the activity of these compounds using an in vivo model and their exact mechanism of action is currently underway.

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