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Short communication

Synthesis and potential coanthracyclinic activity of pyridylmethylene and indolylmethylene lactams[1]

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Abstract – The synthesis of pyridylmethylene lactams and indolylmethylene-2-indolinones is reported. These compounds were tested for their cytotoxic and positive inotropic activity. The presence of both the effects (coanthracyclinic activity) was confirmed in some of the indolylmethylene-2-indolinones. © Elsevier, Paris

lactams / 2-indolinone / positive inotropic activity / cytotoxic activity / coanthracyclinic activity

1. Introduction

In previous papers we reported the positive inotropic activity of 2-indolinones [2–5] and other lactams [6, 7]. In one of these papers [5] the term 'coanthracyclinic activity' was introduced to indicate the pharmacological behavior of a molecule endowed with both antitumor activity (in order to reduce the anthracycline toxicity by reducing its dosage) and positive inotropic activity (in order to counteract the heart depression induced by anthracyclines).

As a continuation of this project we wish to collect here the results obtained in the effort of improving the coanthracyclinic activity of two series of compounds reported in two different papers. The first one, published in 1993 [6], described the synthesis and positive inotropic activity of one pyridylmethylene (**1**, see *figure 1*) and several methoxybenzylidene lactams. Since **1** was among the most active compounds, we planned the synthesis of analogous pyridylmethylene derivatives with the substitution of pyrrolidinone with other lactams (**2**, **3**). The second paper, published three years later [8], reported the synthesis and cytotoxic activity of 3-indolylmethylene-2-indolinones **4a–d** and the by-product **5**. We wish now to describe the potential coanthracyclinic activity of these compounds in comparison to a newly synthesized series (**4e–j**) with the introduction of a methoxy group at the 5 position or of a phenyl group in position 1.

2. Chemistry

The pyridylmethylene lactams (5-pyridin-4-ylmethylene-imidazolidine-2,4-dione **2a**, 5-pyridin-4-ylmethylene-thiazolidine-2,4-dione **2b**, 2-imino-5-pyridin-4-ylmethylene-thiazolidin-4-one **2c**) were prepared from 4-pyridinecarboxaldehyde in water, in the presence of glycine and sodium carbonate. Under the same experimental conditions, barbituric acid did not react to give the expected derivative **3** (5-pyridin-4-ylmethylene-pyrimidine-2,4,6-trione), therefore the reaction was performed in methanol and piperidine. According to our previous report [6], the desired compound was obtained as a salt ($\mathbf{3}\cdot C_5H_{11}N$) which, by treatment with hydrochloric acid, gave the free acid **3**. Both **3** and its salt were subjected to the pharmacological tests. The 3-indolylmethylene-2-indolinones were prepared under the same experimental conditions (methanol/piperidine). The IR

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[1]See [1]

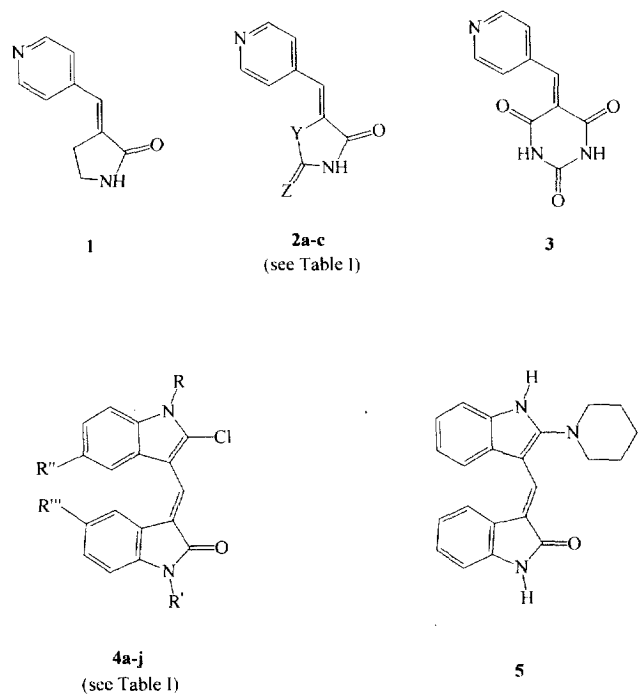

Figure 1.

(*table I*) and ^{1}H-NMR spectra (*table II*) of the new compounds are in agreement with the assigned structures.

As far as the geometrical isomerism is concerned, compound **3** is symmetric and the other pyridylmethylene lactams **2a–c** (one isomer only was isolated) belong to the *Z*-configuration (see *figure 1*) according to the reasons we reported for the analogous dimethoxybenzylidene lactams [6]. Also in the case of the substituted 3-(2-chloro-3-indolylmethylene)1,3-dihydroindol-2-ones we isolated one isomer only, except for **4g**: it gave a mixture of both the isomers which were separated by means of fractional crystallization. Since one of these isomers was unstable in solution and the peaks of the ^{1}H-NMR-spectra were too near for significant NOE experiments, we choose compound **4h** for this purpose. First of all, by means of a COSY experiment, we assigned all the signals to the different protons. Then the peak at 6.55 ppm (indole H-4) was irradiated and NOE was observed at 3.56 ppm (methoxy group at the 5 position), at 6.83 ppm (oxindole H-4) and at 7.73 ppm (methine group). On the basis of these results we believe that compound **4h** has a twisted *E*-conformation, as we observed for the unsubstituted analog [8], and therefore the other compound isolated, whose solution in DMSO was unstable, is the *Z*-isomer. All the other compounds **4**, which gave only one stable isomer with spectroscopic features similar to *E*-**4h**, should belong to the *E*-configuration.

3. Pharmacological results

The potential coanthracyclinic activity of compounds **1–5** is reported in *table III*. In the series of pyridylmethylene lactams (**1–3**) only compound **2c** showed borderline cytotoxic activity but it was devoid of positive inotropic effect.

Table I. Pyridylmethylene lactams **2–3** and 3-indolylmethylene-2-indolinones **4–5**.

Compound	Y		Z		Formula (m.w.)	M.p., °C	ν_{max}, cm^{-1}
2a	NH		O		$C_9H_7N_3O_2$ (189.2)	335–338 dec	3200, 1735, 1660, 1600, 875
2b	S		O		$C_9H_6N_2O_2S$ (206.2)	275–280 dec	3440, 1675, 1305, 1200, 805
2c	S		NH		$C_9H_7N_3OS$ (205.2)	310–313 dec	3470, 1670, 1600, 1530, 1150
3	–		–		$C_{10}H_7N_3O_3$ (217.2)	325–328 dec	3110, 1680, 1605, 1295, 1240
	R	R′	R″	R‴			
4a	H	H	H	H	[8]		
4b	H	CH_3	H	H	[8]		
4c	CH_3	H	H	H	[8]		
4d	CH_3	CH_3	H	H	[8]		
5	–	–	H	H	[8]		
4e	H	H	H	OCH_3	$C_{18}H_{13}ClN_2O_2$ (324.8)	204–208 dec	3180, 1670, 1585, 1190, 1090
4f	H	H	OCH_3	H	$C_{18}H_{13}ClN_2O_2$ (324.8)	230–233 dec	3180, 1680, 1605, 1215, 745
E-**4g**	CH_3	H	H	OCH_3	$C_{19}H_{15}ClN_2O_2$ (338.8)	188–192 dec	3140, 1685, 1600, 1290, 1090
Z-**4g**	CH_3	H	H	OCH_3	$C_{19}H_{15}ClN_2O_2$ (338.8)	178–182 dec	3140, 1685, 1600, 1260, 1190
4h	H	CH_3	OCH_3	H	$C_{19}H_{15}ClN_2O_2$ (338.8)	190–192 dec	3180, 1685, 1600, 1205, 735
4i	H	H	OCH_3	OCH_3	$C_{19}H_{15}ClN_2O_3$ (354.8)	195–200 dec	3180, 1675, 1595, 1090, 835
4j	C_6H_5	C_6H_5	H	H	$C_{29}H_{19}ClN_2O$ (446.9)	125–128 dec	1700, 1590, 1495, 735

Table II. ^{1}H-NMR of compounds **2a–c**, **3**, **4e–j**.

Compound	^{1}H-NMR: δ, ppm in DMSO-d_6 [a]
2a	6.14 (1H, s, –CH=); 7.58 (2H, dd, py); 8.50 (2H, dd, py); 11.20 (2H, s, NH)
2b	7.24 (1H, s, –CH=); 7.45 (2H, dd, py); 8.58 (2H, dd, py)
2c	7.51 (2H, dd, py); 7.55 (1H, s, –CH=); 8.69 (2H, dd, py); 9.49 (2H, s, NH)
3	6.12 (1H, s, –CH=); 7.44 (2H, dd, py); 8.53 (2H, dd, py); 10.22 (2H, s, NH)
4e	3.40 (3H, s, OCH_3); 6.38 (1H, s, ind); 6.78 (2H, s, ind); 7.16 (2H, m, ind); 7.27 (1H, m, ind); 7.47 (1H, d, ind); 7.63 (1H, s, –CH=); 10.43 (1H, s, NH); 12.84 (1H, s, NH)
4f	3.59 (3H, s, OCH_3); 6.58 (1H, s, ind); 6.83 (1H, t, ind); 6.86–6.92 (3H, m, ind); 7.18 (1H, t, ind); 7.38 (1H, d, ind); 7.66 (1H, s, –CH=); 10.62 (1H, s, NH); 12.68 (1H, s, NH)
E-**4g**	3.39 (3H, s, CH_3); 3.90 (3H, s, OCH_3); 6.38 (1H, s, ind); 6.79 (2H, s, ind); 7.17–7.26 (2H, m, ind); 7.36 (1H, t, ind); 7.65 (1H, s, –CH=); 7.69 (1H, d, ind); 10.43 (1H, s, NH)
Z-**4g**	3.78 (3H, s, CH_3); 3.86 (3H, s, OCH_3); 6.77 (2H, m, ind); 7.19 (1H, m, ind); 7.28 (1H, m, ind); 7.47 (2H, m, ind); 7.57 (1H, m, ind); 7.83 (1H, s, –CH=); 10.19 (1H, s, NH)
4h	3.26 (3H, s, CH_3); 3.56 (3H, s, OCH_3); 6.55 (1H, d, H-4 ind); 6.83 (1H, d, H-4 ox); 6.89 (1H, dd, H-6 ind); 6.94 (1H, t, H-5 ox); 7.06 (1H, d, H-7 ox); 7.29 (1H, t, H-6 ox); 7.37 (1H, d, H-7 ind); 7.73 (1H, s, –CH=); 12.75 (1H, s, –NH)
4i	3.46 (3H, s, OCH_3); 3.60 (3H, s, OCH_3); 6.42 (1H, s, ind); 6.58 (1H, d, ind); 6.81 (2H, s, ind); 6.92 (1H, dd, ind); 7.39 (1H, d, ind); 7.66 (1H, s, –CH=); 10.44 (1H, s, –NH); 12.79 (1H, s, –NH)
4j	6.82 (1H, d, ind); 6.97 (1H, m, ind); 7.07 (1H, d, ind); 7.12–7.40 (5H, m, ind); 7.45–7.74 (10H, m, ar); 7.89 (1H, s, –CH=)

[a] Abbreviations: py = pyridine; ar = aromatic; ind = indole or oxindole (only for **4h** ind = indole, ox = oxindole).

Table III. Potential coanthracyclinic activity of the pyridylmethylene lactams **1–3** and 3-indolylmethylene-2-indolinones **4–5**.

Compound	Positive inotropic activity				Cytotoxic activity	
	%Δ from baseline value = 100 [c]	Concentration to obtain E_{max} (μg/mL)	EC_{50} (μg/mL)	Δ rate (%)	50% inhibition (μg/mL)	100% inhibition (μg/mL)
1 [a]	173 ± 20	400	55	–7	> 10	–
2a	Ns	–	–	–	> 10	–
2b	123 ± 10	4	1.2	–24	> 10	–
2c	Ns	–	–	–	4	10
3	Ns	–	–	–	> 10	–
3·$C_5H_{11}N$	121 ± 6	15	5	–10	> 10	–
4a [b]	176 ± 8	20	2.7	–13	0.6	1
4b [b]	151 ± 11	80	16.2	–31	0.7	1
4c [b]	126 ± 1	80	40.3	–47	2	3
4d [b]	132 ± 1	80	24.5	+1	3	10
5 [b]	162 ± 13	80	15.3	–13	6	12
4e	Ns	–	–	–16	7	10
4f	136 ± 5	50	1.3	–30	0.2	0.3
4g	132 ± 3	50	9.5	–10	2	3
4h	Ns	–	–	+4	0.3	1
4i	Ns	–	–	–6	0.6	1
4j	Ns	–	–	–2	> 10	–
5-Fluorouracil	–	–	–	–	6	–
Doxorubicin	–	–	–	–	0.005	–
Sulmazole	163 ± 9	100	6.2	–	–	–

[a] Positive inotropic activity taken from [6]; [b] cytotoxic activity taken from [8]; [c] initial contractile force: 0.6 ± 0.2 g; Ns = not significant.

A much more interesting pharmacological behavior was observed in the series of 3-indolylmethylene-2-indolinones (**4–5**). Compounds **4a–d** and **5**, which were previously tested only as potential antitumor agents [8], showed cardiotonic activity too: taking into account the chronotropic effect, a potential coanthracyclinic activity is evident mostly for compound **4a**. The pharmacological data obtained from the newly synthesized compounds (**4e–j**), compared to those of **4a**, show that the introduction of a phenyl ring at the 1 positions (**4j**) is detrimental for both cytotoxic and cardiotonic activity. A bulky substituent could be allowed only in one of the two indole rings. This aspect should be verified within our next series of compounds.

Also the introduction of a methoxy group at the 5 position of the indolinone ring (**4e,g**) is not useful for the coanthracyclinic activity whereas the same group at the same position of the chloroindole ring, brings to the greatest cytotoxic activity with a concomitant reduction (**4f**) or disappearance (**4h**) of the cardiotonic effect.

4. Experimental protocols

4.1. Chemistry

The melting points are uncorrected. Analyses (C, H, N) were within ±0.4% of the theoretical values. TLC was performed on Bakerflex plates (Silica gel IB2-F) and Kieselgel 60 (Merck) was used for column chromatography: the eluent was a mixture of petroleum ether/acetone in various proportions. The IR spectra (*table I*) were recorded in nujol on a Perkin-Elmer 683. The ^{1}H-NMR spectra (*table II*) were recorded on a Varian Gemini (300 MHz), and were referenced to solvent signals. The mass spectra were recorded on a VG 7070E.

4-Pyridinecarboxaldehyde, imidazolidine-2,4-dione (hydantoin), thiazolidine-2,4-dione, 2-iminothiazolidine-4-one (pseudothiohydantoin), pyrimidine-2,4,6-trione (barbituric acid) and indolin-2-one are commercially available. The other indolinones and the chloroaldehydes were prepared according to the literature: 5-methoxy-2-indolinone [9], 1-methyl-2-indolinone [10], 1-phenyl-2-indolinone [10], 2-chloroindole-3-carboxaldehyde [11], 2-chloro-5-methoxyindole-3-carboxaldehyde [12, 13], 1-methyl-2-chloroindole-3-carboxaldeyde [14], 1-phenyl-2-chloroindole-3-carboxaldehyde [15].

4.1.1. Synthesis of compounds 2a–c

4-Pyridinecarboxaldehyde (10 mmol) was added to a solution of glycine (10 mmol) and Na_2CO_3 (5 mmol) in water (10 mL). The appropriate lactam (hydantoin, thiazolidine-2,4-dione, pseudothiohydantoin) was added to the mixture which was refluxed for 5 h. The resulting precipitate was separated by filtration and purified by means of column chromatography with a yield of 30–35%.

4.1.2. Synthesis of compounds 3–5

The appropriate lactam (5 mmol) was dissolved in methanol (30 mL) and treated with 5 mmol of the appropriate aldehyde and 2 mL of piperidine. The reaction mixture was refluxed for 5 h and the resulting precipitate was collected by filtration and purified by column chromatography (fractional crystallization for compound **4g**) with a yield of 60–70%.

Mass spectra were recorded for compound **2b** (NH group undetectable in the ^{1}H-NMR spectrum) and **3** (low ^{1}H-NMR signals due to low solubility).

2b: 206 (M^+, 12), 174 (31), 135 (23), 103 (31), 60 (100).

3: 217 (M^+, 100), 216 (53), 173 (13), 128 (7).

4.2. Pharmacology

4.2.1. Positive inotropic activity

The experiments were carried out on spontaneously beating guinea pig (400–600 g body weight) atria. The preparation was suspended at 37 °C in a 20 mL bath of Tyrode solution (composition in g/L: NaCl 8.0, $NaHCO_3$ 1.0, KCl 0.2, NaH_2PO_4 0.005, $MgCl_2$ 0.1, $CaCl_2$ 0.2, glucose 1.0). An initial tension of 1 g was applied to the preparation. Isometric contractions were recorded by a strain gauge transducer connected to a recording microdynamometer. After taking basal responses, the test compounds were added to the preparation on a cumulative basis (in the range of 1–100 μg/mL) and the responses were recorded. The contact time for each dose was 5 min. Concentrations producing 50% of the maximal effect (EC_{50}) were calculated from concentration–response curves [16].

4.2.2. Cytotoxic activity

Stock cultures of HeLa cells were plated on Falcon plastic dishes (150 cells/plate) in MEM (Minimum Essential Medium: Whittaker-M.A. Bioproducts) and incubated at 37 °C in 5% CO_2. The compounds under test, dissolved in DMSO, were added directly to the growth medium after 48 h; the amount of DMSO, previously used in analogous experiments, did not affect cell growth. At the end of the drug exposure period (48 h) the growth medium was removed and a new medium was added. Colonies that contained more than 50 cells were counted after 7 days of incubation and IC_{50} were calculated.

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