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Laboratory note

Synthesis and potential *coanthracyclinic* activity of substituted 3-(5-imidazo[2,1-*b*]thiazolylmethylene)-2-indolinones†

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Summary — A compound endowed with *coanthracyclinic* activity should potentiate the antitumor activity of anthracyclines while counteracting their cardiodepressant effect. We now report the synthesis and configuration of a series of substituted 3-(5-imidazo[2,1-*b*]thiazolylmethylene)-2-indolinones which were tested for both cardiotonic and cytotoxic activity. Several compounds were potent cytotoxic agents and two of them (in particular 3-[6-(2,5-dimethoxyphenyl)-5-imidazo[2,1-*b*]thiazolylmethylene]-2-indolinone), which showed even cardiotonic activity, are potential coanthracyclinic agents.

imidazo[2,1-*b*]thiazolylmethylene-2-indolinones / cytotoxic activity / cardiotonic activity / coanthracyclinic activity

In one of our recent papers [2] we stressed that we believe in the design of a compound endowed with both antitumor and cardiotonic activity which we now suggest to call *coanthracyclinic* activity meaning a pharmacological behavior which cooperates in potentiating the antitumor activity of the anthracyclines while counteracting, by means of a positive inotropic effect, the undesired cardiodepressant activity.

In the previous paper of our series devoted to the search of new cardiotonic agents [3] we described the positive inotropic activity of 6-substituted imidazothiazoles connected, by means of a methine group, to a monocyclic lactam **1** (scheme 1). Due to our interest in the chemistry of indoles since 1973 [4] and more recently in indole cardiotonics [5–7], we planned a new series of 6-substituted imidazothiazoles **5** where the lactam ring is the bicyclic 2-indolinone system. The substituent at the 6 position of imidazo[2,1-*b*]-thiazole **3** (R) and at the 5 position of 2-indolinone **4** (R') were selected according to the results described in the previously mentioned papers [3, 5–7]. Moreover, we prepared the 2,3-dihydro derivative **5e** bearing a 2,5-dimethoxyphenyl group (which in the previous papers resulted in one of the best pharmacophoric groups) in order to compare its activity with that of the analog **5d**. From a preliminary test we found that **5e** resulted as a cardiotonic agent less potent than **5d** and with a stronger undesired bradycardic effect, therefore we did not prepare the whole series of 2,3-dihydro analogs.

One of the 3-(2-chloro-3-indolylmethylene)-2-indolinones **2** we recently published as an antitumor agent [8], related to the previously published 3-(3-indolylmethylene)-2-indolinone [9, 10], showed even cardiotonic activity [11]; since the structure of the 3-(5-imidazo[2,1-*b*]thiazolylmethylene)-2-indolinones **5** is related to that of **2**, we subjected compounds **5** to a cardiotonic and to a cytotoxic test.

Chemistry

The Knoevenagel reaction between the aldehyde **3** and the indolinone **4** (scheme 1) was performed in sodium acetate/acetic acid. Since the yields of the two compounds **5e,h** was too low, a different experimental condition was employed (methanol/piperidine) which gave better results. The compounds prepared are reported in tables I and II.

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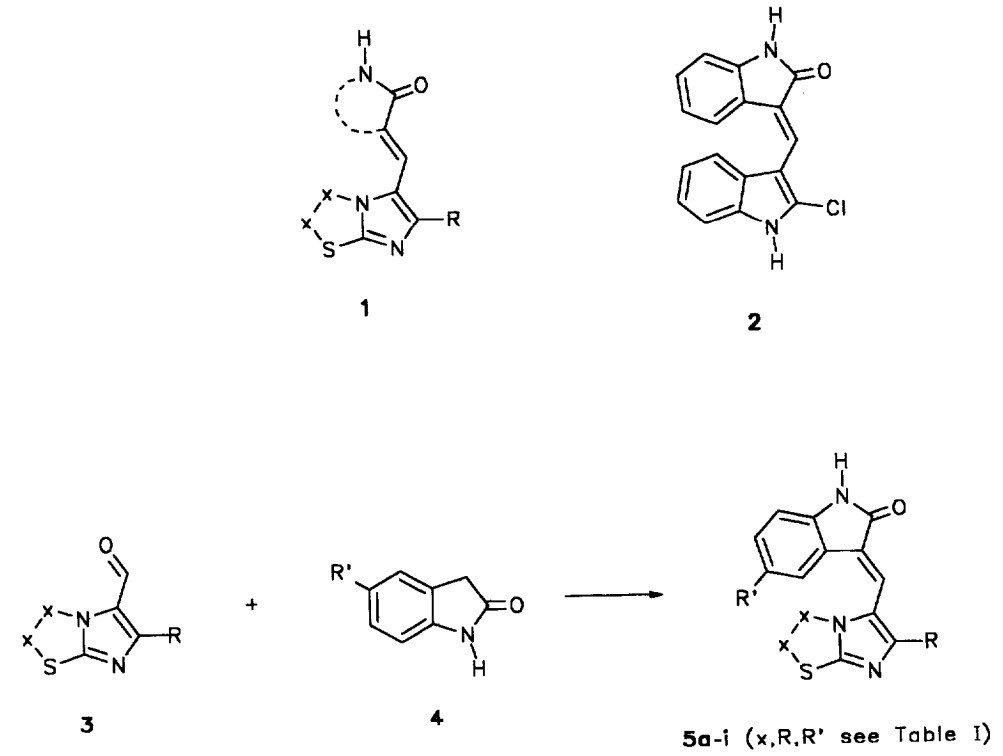
†Presented at the first Italian–Swiss meeting on Medicinal Chemistry (September 23–26, 1997) in Turin, Italy [1].



Scheme 1.

Table I. Compounds **5a–i**.

Compound	*x*	*R*	*R'*	*Formula*	*Mw*	*Method*	*Mp (°C)*	*Solvent*
5a	CH	Cl	H	$C_{14}H_8ClN_3OS$	301.8	1	265–268	EtOH
5b	CH	CH_3	H	$C_{15}H_{11}N_3OS$	281.3	1	285–287	EtOH
5c	CH	C_6H_5	H	$C_{20}H_{13}N_3OS$	343.4	1	280–283	EtOH
5d	CH	$C_6H_3(OCH_3)_2(2,5)$	H	$C_{22}H_{17}N_3O_3S$	403.5	1	260–265 dec	EtOH
5e	CH_2	$C_6H_3(OCH_3)_2(2,5)$	H	$C_{22}H_{19}N_3O_3S$	405.5	2	250–253	EtOH
5f	CH	Cl	OCH_3	$C_{15}H_{10}ClN_3O_2S$	331.8	1	270–272 dec	EtOH
5g	CH	CH_3	OCH_3	$C_{16}H_{13}N_3O_2S$	311.4	1	214–217	Toluene
5h	CH	C_6H_5	OCH_3	$C_{21}H_{15}N_3O_2S$	373.4	2	243–246	EtOH
5i	CH	$C_6H_3(OCH_3)_2(2,5)$	OCH_3	$C_{23}H_{19}N_3O_4S$	433.5	1	226–230 dec	EtOH

Table II. IR and ^{1}H-NMR of compounds **5a–i**.

Compound	*Config*	*IR:*[a] ν_{max}, cm^{-1}	*^{1}H-NMR:*[b] δ, *ppm in DMSO–d_6*
5a[c]	*E/Z*	1700, 1610, 1435, 1240	6.79 (1H, d, ind, J = 7.5), 6.90 (2H, m, ind), 7.25 (1H, dt, ind, J = 7.5, J = 1), 7.51 (1H, s, CH), 7.54 (1H, d, it-2, J = 4.5), 7.81 (1H, d, it-3, J = 4.5), 10.70 (1H, s, NH)
5b	*E*	1695, 1635, 1610, 1295	2.24 (3H, s, CH_3), 6.72 (1H, d, ind-4, J = 7.5), 6.89 (1H, d, ind-7, J = 7.5), 6.90 (1H, t, ind-5, J = 7.5), 7.21 (1H, t, ind-6, J = 7.5), 7.37 (1H, d, it-2, J = 4.3), 7.63 (1H, s, CH), 7.70 (1H, d, it-3, J = 4.3), 10.63 (1H, s, NH)
5c	*E*	1695, 1605, 1455, 1205	6.43 (1H, d, ind, J = 7.5), 6.78 (1H, t, ind, J = 7.5), 6.89 (1H, d, ind, J = 7.5), 7.20 (1H, t, ind, J = 7.5), 7.40 (3H, m, ar), 7.43 (1H, d, it-2, J = 4.4), 7.46 (1H, d, it-3, J = 4.4), 7.63 (1H, s, CH), 7.74 (2H, m, ar), 10.70 (1H, s, NH)
5d	*E*	1690, 1610, 1495, 1210	3.64 (3H, s, OCH_3), 3.73 (3H, s, OCH_3), 6.38 (1H, d, ind, J = 7.5), 6.73 (1H, dt, ind, J = 7.5, J = 1), 6.84 (1H, d, ind, J = 7.5), 6.91 (1H, dd, ar, J = 9, J = 3), 6.98 (1H, d, ar, J = 9), 7.13 (1H, d, ar, J = 3), 7.15 (1H, dt, ind, J = 7.5, J = 1), 7.44 (1H, d, it-2, J = 4.5), 7.55 (1H, d, it-3, J = 4.5), 7.63 (1H, s, CH), 10.60 (1H, s, NH)
5e	*E*	1700, 1600, 1495, 1215	3.54 (3H, s, OCH_3), 3.70 (3H, s, OCH_3), 3.95 (2H, t, it, J = 6.8), 4.12 (2H, t, it, J = 6.8), 6.75 (5H, m, ar), 7.10 (2H, m, ar), 7.43 (1H, s, CH), 10.54 (1H, s, NH)
5f[c]	*E/Z*	1700, 1300, 1230, 1190	3.59 (3H, s, OCH_3), 6.35 (1H, d, ind-4, J = 2), 6.80 (1H, d, ind-7, J = 8), 6.85 (1H, dd, ind-6, J = 8, J = 2), 7.50 (1H, s, CH), 7.56 (1H, d, it-2, J = 4.4), 7.81 (1H, d, it-3, J = 4.4), 10.52 (1H, s, NH)
5f	*Z*	1685, 1610, 1265, 1190	3.77 (3H, s, OCH_3), 6.76 (1H, d, ind-7, J = 8), 6.83 (1H, dd, ind-6, J = 8, J = 2), 7.43 (1H, d, it-2, J = 4.4), 7.48 (1H, d, ind-4, J = 2), 7.64 (1H, d, it-3, J = 4.5), 7.68 (1H, s, CH), 10.42 (1H, s, NH)
5g[c]	*E/Z*	1690, 1610, 1260, 1195	2.27 (3H, s, CH_3), 3.57 (3H, s, OCH_3), 6.24 (1H, s, ind), 6.78 (2H, m, ind), 7.39 (1H, d, it-2, J = 4.4), 7.62 (1H, s, CH), 7.66 (1H, d, it-3, J = 4.4), 10.45 (1H, s, NH)
5h[c]	*E/Z*	1690, 1620, 1605, 1190	3.42 (3H, s, OCH_3), 6.01 (1H, s, ind), 6.80 (2H, m, ind), 7.45 (5H, m: 3H, ar + 2H, it), 7.68 (1H, s, CH), 7.75 (2H, m, ar), 10.53 (1H, s, NH)
5i	*E*	1690, 1615, 1270, 1215	3.46 (3H, s, OCH_3), 3.61 (3H, s, OCH_3), 3.72 (3H, s, OCH_3), 5.91 (1H, s, ind), 6.73 (2H, m, ind), 6.90 (1H, dd, ar, J = 9, J = 3), 6.96 (1H, d, ar, J = 9), 7.17 (1H, d, ar, J = 3), 7.45 (1H, d, it-2, J = 4.5), 7.60 (1H, d, it-3, J = 4.5), 7.63 (1H, s, CH), 10.40 (1H, s, NH)

[a]The NH stretching vibrations are around 3150 cm^{-1}. [b]Abbreviations: it = imidazothiazole, ind = indole, ar = aromatic. [c]The ^{1}H-NMR data of the *E* isomer were extrapolated from the spectrum of the *E/Z* mixture.

According to our previous experience with Knoevenagel adducts, even this time some compounds were obtained as pure stable isomers and others as *E/Z* mixtures. We attempted to separate one of these mixtures (**5f**) by fractional crystallization but the solution of the only pure isomer obtained was unstable and gave again an *E/Z* mixture after some hours; therefore we decided to test the pharmacological activity of these derivatives as *E/Z* mixtures even because, at least for the cardiotonic activity, we did not find a significant difference in a series of analogous derivatives, when we tested the same compound as *E* and *Z* isomer [6].

In order to determine the configuration of these 3-(5-imidazo[2,1-*b*]thiazolylmethylene)-2-indolinones, we started studying compound **5b** which was subjected to a series of NOE experiments. Figure 1 shows that the *Z* configuration ranges from the structures **a** to **b** whereas the *E* configuration ranges from **c** to **d**. First of all the NH group (10.62 ppm) was irradiated in order to confirm which one of the two indole *(ind)* doublets (6.72 and 6.89 ppm) was *ind*-7. Since NOE was observed only at 6.89 ppm, we could establish that the peak at this position was *ind*-7 whereas the peak at 6.72 ppm was *ind*-4. The second NOE experiment was the irradiation of the methine group (7.63 ppm) which gave NOE at the methyl group only (2.24 ppm): this excludes the isomers **a**, **c** and, since NOE was not observed at *ind*-4 (6.72 ppm), excludes the isomer **b** too.

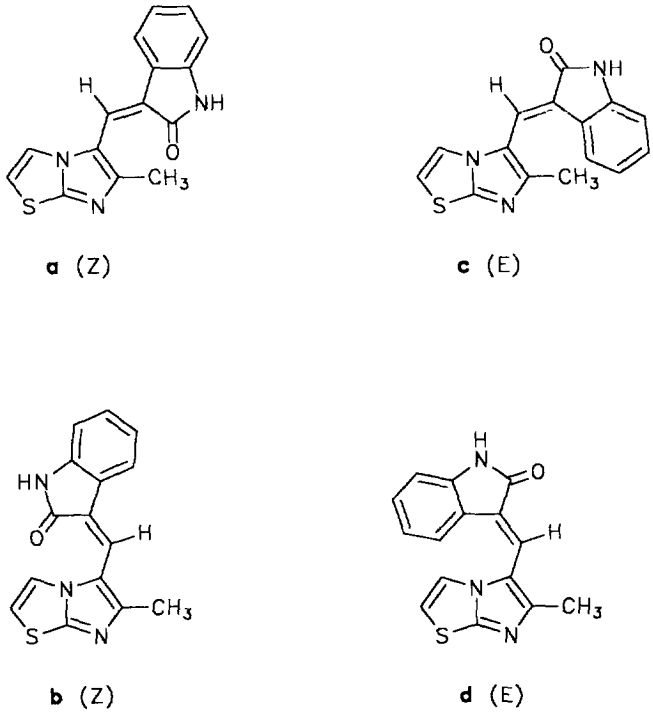


Fig 1. *Z*- and *E*- **5b**.

After these experiments we assigned the *E* configuration to compound **5b** but, since it can range from **c** to **d**, we believe that the compound under investigation corresponds to the intermediate structure reported in figure 2. In fact a third irradiation at 2.24 ppm (CH_3) confirmed the spatial connection of this group with the methine group (NOE at 7.63 ppm) and revealed also a connection between CH_3 and *ind*-4 (6.72 ppm). A fourth irradiation at 6.72 ppm (*ind*-4) confirmed the latter connection and revealed an effect at 6.90 ppm (which allows the assignment of *ind*-5) and at 7.70 ppm, ie, the proton at the 3-position of the imidazothiazole *(it)* system. This connection was confirmed by a final irradiation at 7.70 ppm (*it*-3) which gave NOE at 6.72 ppm and also at 7.37 ppm (*it*-2).

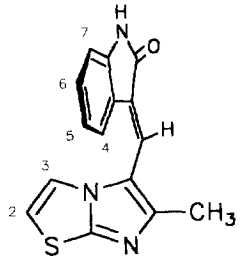


Fig 2. Twisted *E*-**5b**.

The ^{1}H-NMR features of the other derivatives suggest that they belong to the configuration reported in table II.

Pharmacological results

Compounds **5a–f** were tested for cardiotonic (isolated guinea pig atria) and cytotoxic activity (HeLa cells). The result obtained is reported in table III. All the compounds bearing chlorine, methyl or phenyl groups at the 6-position of the imidazothiazole were cytotoxic and those arising from 5-methoxy-2-indolinone (**5f–h**) were more active than the others (**5a–c**) arising from the 5-unsubstituted analog. The introduction of a 2,5-dimethoxyphenyl group produces a drop of activity when the double bond at the 2,3-position of the thiazole ring is lacking (**5e**) and when a methoxy group is present in the indolinone (**5i**): only compound **5d** maintains a good cytotoxic activity.

As we mentioned in the introduction, the presence of a bulky substituent at the 6-position of the imidazothiazole system could be useful for the positive inotropic activity [12] but compound **5e** was only apparently cardiotonic (since a strong bradycardic effect was also observed) and compounds arising from 5-methoxy-2-indolinone (**5h,i**) were inactive. On the other hand the analogs **5c,d**, showing both cytotoxic and cardiotonic effect, might display the coanthracyclinic activity we were looking for.

Experimental protocols

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 were recorded in nujol on a Perkin-Elmer 683. The ^{1}H-NMR spectra were recorded on a Varian Gemini (300 MHz), using TMS as the internal standard; *J* values are reported in Hz.

2-Indolinone is commercially available whereas 5-methoxy-2-indolinone [13] and the starting aldehydes [3] were prepared according to the literature. The Knoevenagel reaction was performed with one of the following methods until the expected compound was isolated. This means that we did not test both methods for all the reactions and the yields could be improved.

Method 1: preparation of ***5a–d,f,g,i***

The aldehyde **3** (15 mmol) was treated with 15 mmol of the 2-indolinone **4**, 30 mmol of anhydrous sodium acetate and

Table III. Cardiotonic and cytotoxic activity of compounds **5a–i**.

Compound	*Positive inotropic activity*				*Cytotoxic activity*	
	%Δ from baseline value = 100[a]	*Concentration to obtain E_{max} (μg/mL)*	*EC_{50} (μg/mL)*	*Δ rate (%)*	*50% inhibition (μg/mL)*	*100% inhibition (μg/mL)*
5a	ns	–	–	–6	0.6	3
5b	ns	–	–	–19	0.8	4
5c	140 ± 15	8	1.6	–20	1.5	5
5d	164 ± 5	16	4.6	–25	0.8	3
5e	157 ± 6	80	31.9	–62	ns	–
5f	ns	–	–	+3	0.1	0.3
5g	ns	–	–	–10	0.2	1
5h	ns	–	–	–19	1.5	3
5i	ns	–	–	–34	ns	–
5-Fluorouracil	–	–	–	–	6	–
Doxorubicin	–	–	–	–	0.005	–
Sulmazole	163 ± 9	100	6.2	–	–	–

[a]Initial contractile force: 0.6 ± 0.2 g; ns = not significant.

30 mL of acetic acid. The reaction mixture was refluxed for 6 h. Acetic acid was removed under reduced pressure and the residue was poured into ice water. The resulting precipitate was recovered by filtration with a yield of 50–60% and it was purified by crystallization (**5a–d,g,i**) or by column chromatography (**5f**).

*Method 2: preparation of **5e,h***

The appropriate 2-indolinone **4** (10 mmol) was dissolved in methanol (100 mL) and treated with the aldehyde **3** (10 mmol) and piperidine (2 mL). The reaction mixture was refluxed for 5 h, cooled and concentrated under reduced pressure. The resulting precipitate was collected by filtration with a yield of 75% and it was purified by crystallization.

Pharmacology

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 [14].

Cytotoxic activity

Stock cultures of HeLa cells were plated on Falcon plastic dishes (150 cells/plate) in MEM (Minimum Essential Medium: Whittaker–MA 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} values were calculated.

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