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Antitumor activity and COMPARE analysis of bis-indole derivatives ☆

Aldo Andreani [a,*], Silvia Burnelli [a], Massimiliano Granaiola [a], Alberto Leoni [a], Alessandra Locatelli [a], Rita Morigi [a], Mirella Rambaldi [a], Lucilla Varoli [a], Laura Landi [b], Cecilia Prata [b], Francesco Vieceli Dalla Sega [b], Cristiana Caliceti [b], Robert H. Shoemaker [c]

[a] Dipartimento di Scienze Farmaceutiche, Università di Bologna, Via Belmeloro 6, 40126 Bologna, Italy
[b] Dipartimento di Biochimica 'G. Moruzzi', Università di Bologna, Via Irnerio 48, 40126 Bologna, Italy
[c] Screening Technologies Branch, Developmental Therapeutics Program, Division of Cancer Treatment and Diagnosis, National Cancer Institute at Frederick, Frederick, MD 21702, USA

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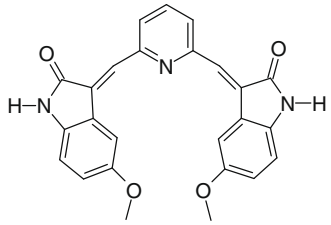
ABSTRACT

This paper reports the synthesis of new derivatives (formed by two indole systems separated by a central moiety) analogous of potent antitumor agents previously described. The activity of the bis-indoles bearing a pyridine core confirms the good result described in the previous paper and compound **4c** was chosen for the first in vivo experiment (Hollow Fiber Assay). COMPARE analysis and structure–activity relationships were also considered. Contrary to data reported by other Authors, no correlations were found between antitumor activity and NQO1 induction.

1. Introduction

In the first paper of this series[2] we described the potent antitumor activity of bis-indole derivatives where the core was formed by a 2,6-disubstituted pyridine ring and the wings by a couple of 2-indolinones bearing different substituents at the benzene ring. The most interesting compound was NSC 724440 which showed GI_{50} 1.2 μM and was tested in the Hollow Fiber Assay.



NSC 724440

Here, we report the synthesis of new analogs with different substituents in the indolinones, with heterocycles different from indolinone in the wings and with cores different from pyridine. The antitumor activity of all the new compounds was evaluated according to the protocols available at the National Cancer Institute, Bethesda, MD (NCI). COMPARE analysis and structure–activity relationships were also considered. Furthermore, since a correlation has been very recently suggested between NQO1 (NAD(P)H-quinone oxidoreductase 1) induction and antiproliferative activity in a series of benzylidene-indolin-2-ones,[3] we studied the effect of some of these antitumor compounds on NQO1 activity and viability in leukemia cell lines.

2. Results and discussion

2.1. Chemistry

This paper describes the design of new analogs (Schemes 1 and 2) of the most active bis-indoles previously described,[2] with the following rationale:

(1) introduction of new substituents in the benzene rings of the indolinone systems (compounds **4a–h**).
(2) Substitution of the indolinone systems with different heterocycles while maintaining the pyridine ring in the core (compounds **4i** and **6**). In the course of the synthesis of compound **4i**, the monosubstituted derivative **5** was also isolated and its antitumor activity was evaluated.

☆ See Ref. 1.

* Corresponding author. Tel.: +39 051 2099714; fax: +39 051 2099734.
E-mail address: aldo.andreani@unibo.it (A. Andreani).

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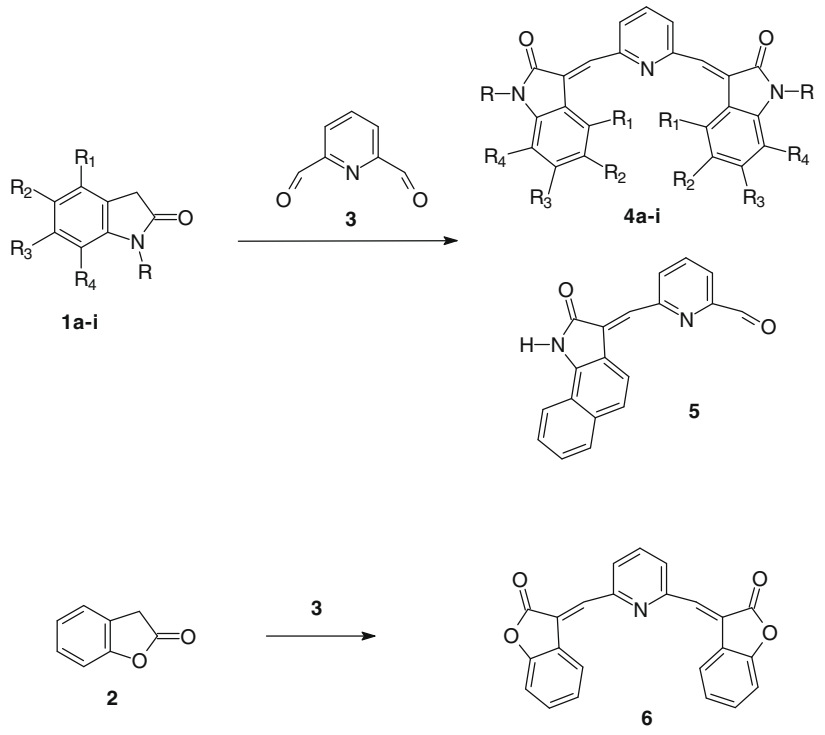


Comp.	Starting material	R	R_1	R_2	R_3	R_4
4a	**1a**	H	I	H	H	H
4b	**1b**	H	F	H	H	H
4c	**1c**	H	OCH_3	OCH_3	OCH_3	H
4d	**1d**	H	OCH_3	H	H	OCH_3
4e	**1e**	H	H	H	OCF_3	H
4f	**1f**	Benzyl	H	H	H	H
4g	**1g**	H	H	SO_2NH_2	H	H
4h	**1h**	H	H	A	H	H
4i	**1i**	H	H	H	B	

A = SO_2-NH-*p*-C_6H_4-SO_3H
B = condensed benzene ring

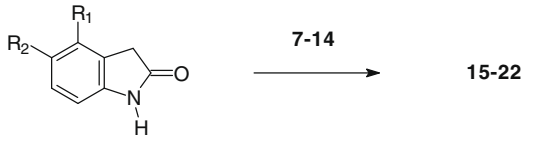
Scheme 1.

(3) Substitution of the core with systems different from pyridine while maintaining the indolinone wings with substituents which gave the best results in the previous paper, that is, halogens and methoxy group.[2] In details the core substitution concerns the introduction of:

- benzene ring: **15j**, **16j**, **17j** and **16k**
- substituted benzene ring: **18j** and **18k**
- phenanthroline: **19j,k**
- pyrrole: **20j,l**
- thiophene: **21j**
- biphenyl: **22j**.

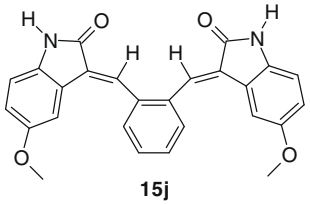
Most of the new derivatives (Schemes 1 and 2) have been prepared with the previously published procedure:[4,5,2] the appropriate indolinone **1** (the 2-coumaranone **2** for compound **6**), in methanol has been treated with pyridine-2,6-dicarbaldehyde **3** or benzene-1,3-dicarbaldehyde **8**, benzene-1,4-dicarbaldehyde **9**, 4-*tert*-butyl-2,6-diformylphenol **10**, 1,10-phenanthroline-2,9-dicarbaldehyde **11**, 2,5-dimethylpyrrole-3,4-dicarbaldehyde **12**, 2,5-thiophenedicarbaldehyde **13**, 6,6′-dihydroxy-5,5′-dimethoxy-[1,1′-biphenyl]-3,3′-dicarbaldehyde **14** in the presence of piperidine or a mixture of acetic acid/hydrochloric acid (see Schemes 1 and 2 and Table 1, methods 1 and 3). Only compound **4c** has been prepared in a mixture of acetic acid and anhydrous sodium acetate (method 2), whereas compound **15j** has been obtained from the indolinone **1j** and benzene-1,2-dicarbaldehyde **7** in the presence of 4-toluenesulfonic acid (method 4).

For the synthesis of compound **4e**, 6-trifluoromethoxyindolin-2-one **1e** was the starting indolinone and it was prepared by treating 6-trifluoromethoxyisatine with 98% hydrazine hydrate.

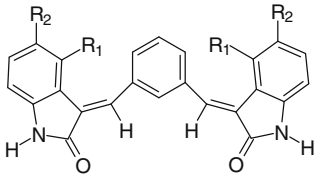


1j-l

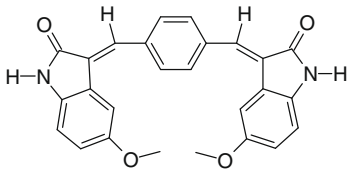
j $R_1 = H$ $R_2 = OCH_3$ **k** $R_1 = Cl$ $R_2 = H$ **l** $R_1 = H$ $R_2 = F$



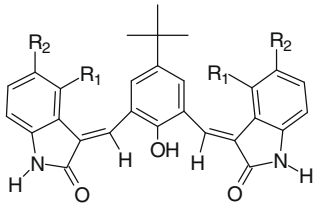
15j



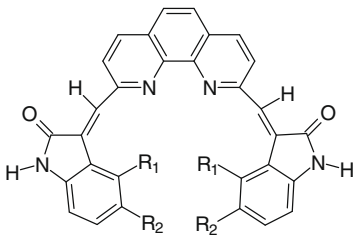
16j-k



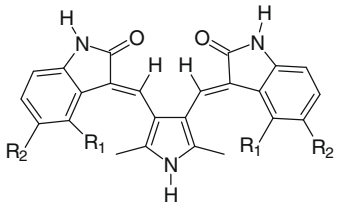
17j



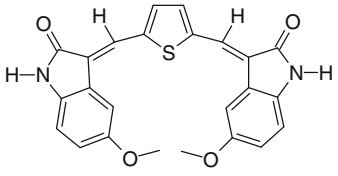
18j-k



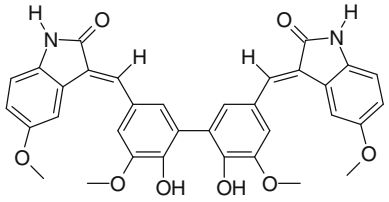
19j-k



20j, l



21j



22j

Scheme 2.

For the synthesis of compound **4h**, 4-{[(2-oxo-2,3-dihydro-1*H*-indol-5-yl)sulfonyl]amino}benzenesulfonic acid **1h** was the starting indolinone and it was prepared by treating 2-oxoindoline-5-sulfonyl chloride with 4-aminobenzenesulfonic acid in toluene.

According to the ^{1}H NMR spectra, all the compounds were obtained as pure *E* isomers, except **4b**, **4i** and **21j** which contain also a small amount of the *Z* isomer. The configuration was assigned by means of NOE experiments: the compounds selected for this purpose were **16j**, **18j** and **19j**.

The spectrum of compound **16j** shows that the geometrical configuration of the two centers is the same, since the couple of NH groups and the other couples of indole protons/substituents (ind-4, ind-6, ind-7, OCH_3-5) give a single signal. The irradiation of ind-4 (6.99 ppm) produced NOE at OCH_3 (3.52 ppm) and at the aromatic protons (singlet 7.95 and doublet 7.81 ppm), whereas NOE was not observed at the –CH= proton; the irradiation of the aromatic singlet at 7.95 produced NOE at 7.67 (–CH=) and at 6.99 ppm (ind-4). All these data are in agreement with the *E* configuration.

Table 1

Compd	Formula	M_w	Method	Mp
4a	$C_{23}H_{13}I_2N_3O_2$	617.18	1	263–265 dec
4b	$C_{23}H_{13}F_2N_3O_2$	401.37	1	310–313 dec
4c	$C_{29}H_{27}N_3O_8$	545.54	2	237–240 dec
4d	$C_{27}H_{23}N_3O_6$	485.49	1	298–307
4e	$C_{23}H_{13}F_6N_3O_4$	509.36	1	325–328
4f	$C_{37}H_{27}N_3O_2$	545.63	1	195–200
4g	$C_{23}H_{17}N_5O_6S_2$	523.55	3	310–314 dec
4h	$C_{35}H_{25}N_5O_{12}S_4.CH_3COOH$	895.92	3	>330
4i	$C_{31}H_{19}N_3O_2$	465.50	1	>330
5	$C_{19}H_{12}N_2O_2$	300.31	1	250–255 dec
6	$C_{23}H_{13}NO_4$	367.36	1	220–222
15j	$C_{26}H_{20}N_2O_4$	424.45	4	258–260 dec
16j	$C_{26}H_{20}N_2O_4$	424.45	1	259–262
16k	$C_{24}H_{14}Cl_2N_2O_2$	433.29	1	>330
17j	$C_{26}H_{20}N_2O_4$	424.45	1	327–330
18j	$C_{30}H_{28}N_2O_5$	496.56	1	220–224
18k	$C_{28}H_{22}Cl_2N_2O_3$	505.39	3	310–315
19j	$C_{32}H_{22}N_4O_4$	526.54	1	250–253
19k	$C_{30}H_{16}Cl_2N_4O_2$	535.38	1	>330
20j	$C_{26}H_{23}N_3O_4$	441.48	1	240–245 dec
20l	$C_{24}H_{17}F_2N_3O_2$	417.41	3	244–247
21j	$C_{24}H_{18}N_2O_4S$	430.48	1	>330
22j	$C_{34}H_{28}N_2O_8$	592.60	1	195–199

Also the spectra of compounds **18j** and **19j** show that the geometrical configuration of the two centers is the same. For compound **18j**, the first NOE analysis was devoted to the identification of the aromatic and –CH= protons. The irradiation of the $C(CH_3)_3$ groups (1.31 ppm) produced NOE at 7.71 ppm (peak which was assigned to the aromatic hydrogens of the core). NOE was produced also at 6.99 ppm (ind-4) and at 3.58 ppm (OCH_3). The irradiation of ind-4 at 6.99 ppm produced the expected NOE at the aromatic hydrogens (7.71 ppm) but not at the methine bridge, whereas the irradiation at 9.87 ppm (OH) gave NOE at 7.72 ppm (–CH=). These measures are in agreement with the *E* configuration.

In compound **19j** the peak at 8.99 ppm (ind-4) was irradiated and only the expected NOE at 3.35 (OCH_3) was observed. The lack of any other effect (in particular with the –CH= group) demonstrates that even compound **19j** belongs to the *E* configuration.

2.2. Effects in cultured human tumor cell lines

As a preliminary test, the compounds were tested at a single high concentration (10^{-5} M) in the full NCI 60 cell panel (NCI 60 cell one-concentration screen). This panel is organized into subpanels representing leukemia, melanoma and cancers of lung, colon, kidney, ovary, breast, prostate and central nervous system. Only compounds with pre-determined threshold inhibition criteria in a minimum number of cell lines progress to the full five-concentration assay. These criteria were selected to efficiently capture compounds with anti-proliferative activity based on careful analysis of historical DTP screening data. The One-concentration data is a mean graph of the percent growth of treated cells (unpublished results).

All but two of the compounds tested were subjected to the full five-concentration assay. They were dissolved in DMSO and evaluated using five concentrations at 10-fold dilutions, the highest being 10^{-4} M. Table 2 shows the results obtained (vincristine is reported for comparison purposes), expressed at three assay endpoints: the 50% growth inhibitory power (GI_{50}), the cytostatic effect (TGI = total growth inhibition) and the cytotoxic effect (LC_{50}). The compounds showing GI_{50} >20 μM (**6**, **17j**, **20j** and **21j**) are not reported. For some derivatives the five-concentration test was repeated and no significant differences were found; in this case the data reported in Table 2 are the mean values between the two experiments.

The activity of the bis-indoles bearing a pyridine core confirms the good result described in the previous paper.[2] Six of the eleven compounds prepared (**4a–f**) were retested and submitted to BEC (Biological Evaluation Committee) for a possible future development. Further studies continued at the NCI where the maximum tolerated dose (MTD) was determined for compounds **4c**, and it

Table 2
Sixty cell panel (growth inhibition, cytostatic and cytotoxic activity of the selected compounds expressed as micromolar concentration)

Comp [a]	Modes	Leukemia	NSCLC	Colon	CNS	Melanoma	Ovarian	Renal	Prostate	Breast	MGMID[b]
4a	GI_{50}	0.9	1.3	0.5	1.2	1.0	1.1	0.9	0.6	0.8	0.9
	TGI	30.9	5.0	1.9	4.0	3.2	5.6	2.6	2.2	4.4	4.4
	LC_{50}	–	21.9	7.6	13.8	13.5	22.4	8.3	24.0	21.9	17.4
4b	GI_{50}	0.8	2.0	0.7	1.1	0.9	1.6	1.1	1.4	0.8	1.1
	TGI	9.8	10.2	3.3	5.7	2.8	8.5	3.8	12.9	10.0	6.2
	LC_{50}	–	41.7	13.8	32.4	8.3	44.7	14.8	36.3	34.7	27.5
4c	GI_{50}	0.2	0.3	0.2	0.3	0.2	0.3	0.3	0.3	0.2	0.2
	TGI	2.8	1.5	0.5	0.8	0.5	0.8	0.7	1.0	1.5	1.0
	LC_{50}	67.6	33.1	7.2	3.2	2.7	5.7	4.5	16.6	24.6	11.0
4d	GI_{50}	0.4	0.9	0.5	0.7	0.7	0.8	0.7	0.5	0.7	0.7
	TGI	8.7	18.6	1.9	3.6	2.2	11.2	2.5	3.4	17.4	5.7
	LC_{50}	64.6	85.1	22.4	21.9	13.5	27.5	18.2	25.1	60.3	32.4
4e	GI_{50}	0.2	0.4	0.2	0.3	0.4	0.4	0.3	0.1	0.3	0.3
	TGI	8.9	1.9	0.5	0.8	1.1	2.5	0.7	0.3	2.0	1.3
	LC_{50}	–	15.5	2.0	2.6	5.7	12.3	2.9	3.2	15.8	7.6
4f	GI_{50}	0.3	6.3	0.6	1.9	0.7	1.8	1.4	1.4	0.7	1.3
	TGI	5.4	43.6	5.5	7.6	5.2	13.8	11.2	19.5	11.5	12.3
	LC_{50}	–	74.1	18.6	35.5	20.9	33.1	44.7	–	79.4	45.7
4i	GI_{50}	1.5	24.0	14.4	15.8	13.8	19.0	17.0	21.9	17.8	14.1
	TGI	10.0	57.5	44.7	49.0	38.0	56.2	41.7	81.3	69.2	43.6
	LC_{50}	77.6	89.1	83.2	85.1	77.6	91.2	79.4	–	97.7	85.1
5	GI_{50}	2.5	14.4	9.5	11.2	11.5	8.7	10.7	5.7	6.2	9.1
	TGI	7.6	37.1	44.7	33.9	35.5	30.9	26.9	33.1	45.7	31.6
	LC_{50}	–	85.1	83.2	91.2	72.4	72.4	74.1	97.7	79.4	81.3
15j	GI_{50}	5.4	14.1	7.8	14.1	13.8	14.1	21.4	14.4	7.8	11.7
	TGI	–	87.1	87.1	72.4	91.2	–	79.4	58.9	–	87.1
16j	GI_{50}	1.0	3.0	1.7	1.4	1.2	2.1	2.5	1.7	1.2	1.7
	TGI	11.5	11.0	7.4	4.3	2.9	6.3	6.2	4.1	5.0	6.0
	LC_{50}	–	85.1	83.2	29.5	30.2	95.5	79.4	25.7	–	64.6

(*continued on next page*)

Table 2 (*continued*)

Comp [a]	Modes	Leukemia	NSCLC	Colon	CNS	Melanoma	Ovarian	Renal	Prostate	Breast	MGMID[b]
16k	GI_{50}	1.3	4.2	2.6	1.7	3.2	2.7	2.3	1.5	2.4	2.4
	TGI	9.8	20.4	14.8	13.5	16.6	14.4	9.3	16.2	13.2	13.8
	LC_{50}	27.5	42.7	33.1	38.9	34.7	41.7	36.3	50.1	42.7	37.1
18j	GI_{50}	3.9	8.7	6.3	4.8	4.4	5.9	7.2	4.8	5.5	5.7
	TGI	43.6	32.4	19.9	21.4	13.2	25.1	22.4	24.5	31.6	24.5
	LC_{50}	–	69.2	53.7	66.1	39.8	61.7	61.7	72.4	63.1	63.1
18k	GI_{50}	2.8	3.5	2.2	2.3	2.9	2.2	3.6	2.2	2.4	2.7
	TGI	46.8	15.1	6.2	11.0	9.3	15.8	15.8	10.2	11.5	13.2
	LC_{50}	–	64.6	21.9	58.9	36.3	44.7	56.2	72.4	67.6	52.5
19j	GI_{50}	3.0	3.2	2.8	3.8	3.2	5.5	3.3	3.7	3.9	3.5
	TGI	60.3	28.2	16.2	–	11.5	58.9	19.5	36.3	75.9	32.4
	LC_{50}	–	75.9	–	–	74.1	–	97.7	–		91.2
19k	GI_{50}	1.1	25.7	3.9	7.4	13.2	6.2	9.5	3.8	14.4	8.1
	TGI	30.2	79.4	30.9	34.7	42.7	30.9	58.9	31.6	70.8	46.8
	LC_{50}	–	–	79.4	77.6	61.7	70.8	97.7	79.4	93.3	85.1
20l	GI_{50}	2.4	7.8	5.0	3.4	5.1	8.1	9.1	7.9	4.9	5.6
	TGI	9.1	25.1	17.0	13.2	18.2	25.7	32.4	20.4	22.4	19.9
	LC_{50}	37.1	74.1	53.7	46.8	52.5	72.4	75.9	44.7	70.8	58.9
22j	GI_{50}	6.9	21.9	10.5	6.8	10.7	15.1	20.9	10.5	13.8	12.6
	TGI	89.1	95.5	75.9	87.1	93.3	–	95.5	–	–	93.3
Vincristine sulfate[c]	GI_{50}	0.1	0.2	0.1	0.1	0.2	0.3	0.3	0.1	0.3	0.2
	TGI	15.8	15.8	4.0	6.3	7.9	19.9	19.9	6.3	7.9	10.0

[a] Highest concn = 100 μM except **16k** (50.1 μM): only modes showing a value <100 μM are reported.
[b] Mean graph midpoint, that is, the mean concentration for all cell lines.
[c] Highest concn = 10^{-3} M.

was found 100 mg/kg. Compound **4c** was then chosen for the first in vivo experiment, that is, the Hollow Fiber Assay.[6] It was subjected to four experiments against a panel of three tumor cell lines each consisting of the breast, non-small cell lung, colon, ovarian, CNS and melanoma cell lines. Under the experimental conditions employed, the activity of compound **4c** was not considered high enough for the subsequent preclinical studies.

2.3. Structure–activity relationships

2.3.1. Wing modification

The introduction of a benzyl group in the indolinone NH group gave the derivative **4f** (mean GI_{50} = 1.3 μM) which was not more active than its parent compound described in the previous paper[2] showing mean GI_{50} = 0.5 μM but **4f** was less toxic and more selective towards leukemias.

The substitution of indolinone with coumaranone (**6**) led to loss of activity. The same happened with benzoindolinone (**4i**) even though the selectivity for leukemias was maintained.

As far as the substituents at the indolinone system are concerned, the introduction of sulfur containing groups (**4g**,**h**) was detrimental whereas for the halogens the activity increased with the following pattern: F, I, Cl: mean GI_{50} values were 1.1 (**4b**), 0.9 (**4a**) and 0.5 μM[2], respectively.

Increase of activity seems also connected to the number of methoxy groups: compounds with one, two and three methoxy groups were prepared and mean GI_{50} values were 1.2,[2] 0.7, 0.2 μM: the compound bearing three methoxy groups (**4c**) is the most active of all the compounds so far prepared, including those described in the previous paper.[2]

Compound **4e** bearing a trifluoromethoxy group, showed a high level of activity (mean GI_{50} 0.3 μM) and was selective towards prostate tumors (GI_{50} 0.1 μM).

2.3.2. Core modification

The substitution of the pyridine core with a thiophene ring (**21j**) led to loss of activity; better results were obtained with the introduction of a 2,5-dimethylpyrrole ring (**20j**,**l**): in particular the compound bearing a fluorine atom on the indolinone wings (**20l**) was more active than the analog bearing a methoxy group (**20j**).

Good results were obtained also with other structures: when the core is a benzene ring, the compound with the wings in the meta position (**16j**) was more active than the analogs ortho (**15j**) and para (**17j**). Between the two derivatives where the benzene ring is substituted (**18j**,**k**), compound **18k** was the most active of this subset of core-modified derivatives and was submitted to BEC.

A particular mention is due to the phenanthroline core (**19j**,**k**) considering potency and toxicity; in fact they showed a great difference between GI_{50} and LC_{50}. Compound **19k**, which was also selective towards leukemias, was retested and then submitted to BEC.

2.4. COMPARE analysis

First of all we defined the level of effect (GI_{50}, TGI, LC_{50}) most useful for COMPARE analysis. LC_{50} was selected since it was achieved in many cell lines and seemed to provide the most consistent patterns of activity. MATRIX COMPARE identifies compounds with most closely related patterns in the NCI-60 (Table S4). The LC_{50} data provided the most useful information and defined a sub-set of seven compounds with a highly coherent pattern of cell killing: **4a**, **4b**, **4c**, **4d**, **4f**, **18k**, **19k** (Table S5).

Then we selected a prototype compound to run MOLECULAR TARGETS COMPARE (on basal expression array data and other characterized potential targets) to probe mechanism. Compound **4b** was selected based on strength of association with the other top six compounds in this series and with a closely related analog (with Cl in place of F) previously shown to have in vivo activity in the hollow fiber tumor model.[2]

No very strong mechanistic leads were apparent for **4b** from the expression array COMPAREs. The strongest negative COMPARE result indicated that CLK4a (cyclin dependent kinase-like 4) expression is inversely correlated, that is, cell lines with low levels are highly sensitive to the cell killing effects of the compound and vice versa. The other highest ranked array results did not seem to complement this result. No compelling correlations or patterns were apparent from COMPARE against other molecular targets. The same analyses were also run using the average pattern for **4a**. Once again, strong COMPAREs were not observed against the basal expression arrays. The CLK4 negative COMPARE result was not

replicated with this compound's data. However, CCND1 (cyclin D1) molecular target levels were the top negative correlation in the characterized target database, supporting the notion that down-regulation of CCND1 sensitizes cells to the killing effect of the drug.

2.5. NQO1 activation in leukemia cell lines

Compounds **4c**, **4e** and **4f** were among the most active of the series in leukemia cells (mean GI_{50} 0.2, 0.2 and 0.3 μM, respectively). Taking into account that a correlation has been recently suggested between antitumor activity and NQO1 induction in a series of benzylidene-indolin-2-ones[3] we decided to investigate whether NQO1 activation might be a possible mechanism of action for compounds **4c**, **4e** and **4f**. Therefore, three human leukemia cell lines (M07e, B1647 and HEL) and non transformed cells (HUVEC) were incubated with these compounds at different concentrations (0.1–10 μM) or with the known NQO1 inducer sulforaphane,[7] as positive control, for 24–48 h. Then, NQO1 activity was assayed.[8] Meanwhile, the effect of compounds on cell proliferation was evaluated with the 2-day MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay, which was also previously used to determine IC_{50} values for each cell line. The MTT assay confirmed the antiproliferative activity of **4c** (IC_{50} = 1–2 μM), **4e** (IC_{50} = 3–5 μM), and **4f** (IC_{50} = 3–10 μM) in leukemia cells. Figure S1 shows NQO1 activation in M07e cells, representative of the three human leukemia cell lines under investigation. Cells were pre-treated with 5 μM sulforaphane and the three compounds at different concentrations. Compounds **4c** and **4e** resulted to be toxic also in HUVEC cells (IC_{50} <5 μM). Sulforaphane induced NQO1 activity in both normal and leukemia cells. On the other hand compounds **4c**, **4e** and **4f** caused a slight NQO1 activation in HUVEC in a concentration range up to 5 μM (not shown). Interestingly in leukemia cells, compound **4f** at 0.5 μM concentration caused a strong activation of NQO1 and behaved as antiproliferative in a dose dependent manner. Conversely it was less toxic in HUVEC cells (IC_{50} >20 μM).

These results suggest that NQO1 activation by compound **4f** may contribute to its antiproliferative effect in some leukemia cells but they do not support the reported assumption.[3]

COMPARE analysis did not show any connection between NQO1 expression levels and the pattern of activity in the NCI 60 cell lines.[9,10]

3. Experimental section

3.1. General

The melting points are uncorrected. All the compounds prepared have a purity of at least 95% as determined by combustion analysis (Table S2). Bakerflex plates (silica gel IB2-F) were used for TLC: the eluent was petroleum ether/acetone in various proportions. Kieselgel 60 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 Nicolet Avatar 320 E.S.P.; ν_{max} is expressed in cm^{-1}. The ^1H NMR spectra were recorded in $(CD_3)_2SO$ on a Varian Gemini (300 MHz); the chemical shift (referenced to solvent signal) is expressed in δ (ppm) and J in hertz; abbreviations: ar = aromatic, ind = indole, py = pyridine, pyr = pyrrole, tio = thiophene.

All the starting indolinones **1**, except 6-trifluoromethoxyindolin-2-one (**1e**) and 4-{[(2-oxo-2,3-dihydro-1*H*-indol-5-yl)sulfonyl]amino}benzenesulfonic acid (**1h**), are described in the literature: **1a**,[11] **1b**,[12] **1c**,[13] **1d**,[14] **1f**,[15] **1g**,[16] **1i**,[17] **1j**,[18] **1k**[19] and **1l**.[20]

2-Coumaranone **2**, pyridine-2,6-dicarbaldehyde **3**, benzene-1,2-dicarbaldehyde (phthaldialdehyde) **7**, benzene-1,3-dicarbaldehyde (isophthaldehyde) **8**, benzene-1,4-dicarbaldehyde (terephthalaldehyde) **9**, 4-*tert*-butyl-2,6-diformylphenol **10**, 1,10-phenanthroline-2,9-dicarbaldehyde **11**,[21] 2,5-dimethylpyrrole-3,4-dicarbaldehyde **12**,[22] 2,5-thiophenedicarbaldehyde **13**, 6,6′-dihydroxy-5,5′-dimethoxy-[1,1′-biphenyl]-3,3′-dicarbaldehyde **14** are commercially available or have been prepared as described in the literature.

3.1.1. Synthesis of 6-trifluoromethoxyindolin-2-one (1e)

6-Trifluoromethoxyisatine (20 mmol), prepared as described in the literature,[23] was treated with 98% hydrazine hydrate (10 mL). After 1 h reflux, water was added (50 mL) and reflux was maintained for one additional hour. The reaction mixture was then cooled and acidified with 2 N hydrochloric acid. The expected indolinone was crystallized from ethanol with a yield of 60%.

M_w 217.15; mp 140 °C; IR: 3135, 1721, 1634, 902; ^1H NMR: 3.49 (2H, s, CH_2), 6.74 (1H, s, ind-7), 6.88 (1H, d, ind-4/5, J = 8.1), 7.29 (1H, d, ind-4/5, J = 8.1), 10.56 (1H, s, NH). Anal. ($C_9H_6F_3NO_2$) C, H, N.

3.1.2. Synthesis of 4-{[(2-oxo-2,3-dihydro-1*H*-indol-5-yl)sulfonyl]amino}benzenesulfonic acid (1h)

2-Oxoindoline-5-sulfonyl chloride[24] (40 mmol) was dissolved in toluene (50 mL) and refluxed for 6 h with 40 mmol of 4-aminobenzenesulfonic acid. After cooling, the resulting precipitate was collected by filtration with a yield of 98% and used as such without further purification.

M_w 368.39; mp 260 °C; dec IR: 3385, 2646, 1713, 1613, 1034. ^1H NMR: 3.50 (2H, s, CH_2), 6.77 (1H, dd, ind-6, J = 7.5, J = 1.5), 7.34 (2H, dd, ar, J = 6.6, J = 2), 7.40 (2H, br, $NHSO_2$+SO_3H), 7.45 (1H, d, ind-7, J = 7.5), 7.46 (1H, d, ind-4, J = 1.5), 7.71 (2H, dd, ar, J = 6.6, J = 2), 10.46 (1H, s, NH). Anal. ($C_{14}H_{12}N_2O_6S_2$) C, H, N.

3.1.3. Synthesis of compounds 4a–i, 5, 6 and 15–22

Four different methods were employed according to the substituents in the indole system (Table 1).

3.1.3.1. Method 1[2] (compounds 4a,b,d–f,i, 5, 6, 16j,k, 17j, 18j, 19j,k, 20j, 21j and 22j). Compound **1** (or **2**, 10 mmol) was dissolved in methanol and treated with the appropriate aldehyde (5 mmol) and piperidine (2 mL). The reaction mixture was refluxed for 3–5 h (according to a TLC test), cooled and, if necessary, concentrated at reduced pressure. The yellow to orange precipitate thus formed was collected by filtration with a yield of 20% for compounds **5**, **18j** and **22j**, 70–80% for compounds **6**, **16j**,**k**, **17j**, **19k** and **21j**, and 40–50% for the others. The compounds were subjected to biological tests after crystallization from ethanol.

Data for **4a**. IR: 3232, 1701, 1163, 1117, 764. ^1H NMR: 6.89 (2H, d, ind-7, J = 7.6), 7.00 (2H, t, ind-6, J = 7.6), 7.52 (2H, d, ind-5, J = 7.6), 7.89 (1H, t, py-4, J = 8), 8.41 (2H, d, py-3+5, J = 8), 8.72 (2H, s, –CH═), 10.79 (2H, broad, NH). Anal. ($C_{23}H_{13}I_2N_3O_2$) C, H, N.

Data for **4b**. IR: 3175, 1716, 1623, 1142, 1086. ^1H NMR: 6.26 (2H, td, ind-6, J = 9, J = 2.4), 6.61 (2H, dd, ind-5, J = 9, J = 2.4), 7.69 (2H, s, –CH═), 7.92 (2H, d, py-3+5, J = 7.8), 8.09 (1H, t, py-4, J = 7.8), 8.20 (2H, dd, ind-7, J = 9, J = 9), 10.81 (2H, broad, NH). Anal. ($C_{23}H_{13}F_2N_3O_2$) C, H, N.

Data for **4d**. IR: 3176, 1701, 1271, 1168, 779. ^1H NMR: 3.78 (6H, s, OCH_3), 3.92 (6H, s, OCH_3), 6.64 (2H, d, ind, J = 9), 6.97 (2H, d, ind, J = 9), 7.88 (1H, t, py-4, J = 7.4), 8.05 (2H, s, –CH═), 8.65 (2H, d, py-3+5, J = 7.4), 10.75 (2H, s, NH). Anal. ($C_{27}H_{23}N_3O_6$) C, H, N.

Data for **4e**. IR: 2919, 1716, 1619, 1143, 794. ^1H NMR: 6.30 (2H, dd, ind-5, J = 8.4, J = 2.4), 6.69 (2H, d, ind-7, J = 2.4), 7.77 (2H, s, –CH═), 7.97 (2H, d, py-3+5, J = 7.5), 8.13 (1H, t, py-4, J = 7.5), 8.19 (2H, d, ind-4, J = 8.4), 10.82 (2H, s, NH). Anal. ($C_{23}H_{13}F_6N_3O_4$) C, H, N.

Data for **4f**. IR: 3334, 1696, 1609, 1096, 748. ^{1}H NMR: 5.02 (4H, s, CH_2), 6.52 (2H, t, ind-5/6, J = 7.5), 6.89 (2H, d, ind-4/7, J = 7.5), 7.10 (2H, t, ind-5/6, J = 7.5), 7.31 (10H, m, ar), 7.90 (2H, s, –CH=), 8.10 (2H, d, ind-4/7, J = 7.5), 8.16 (1H, t, py-4, J = 7.8), 8.24 (2H, d, py-3+5, J = 7.8). Anal. ($C_{37}H_{27}N_3O_2$) C, H, N.

Data for **4i**. IR: 3165, 3052, 1706, 1619, 811. ^{1}H NMR: 7.07 (2H, d, ar, J = 8.7), 7.55 (6H, m, ar), 7.81 (2H, s, –CH=), 7.94 (2H, m, ar), 8.13 (3H, m, ar), 8.40 (2H, d, ar, J = 8.7), 11.43 (2H, s, NH). Anal. ($C_{31}H_{19}N_3O_2$) C, H, N.

Data for **5**. IR: 3158, 1707, 1619, 1224, 814. ^{1}H NMR: 7.54 (3H, m, ar), 7.71 (1H, s, –CH=), 7.93 (1H, m, ar), 8.02 (1H, m, ar), 8.19 (3H, m, py), 9.25 (1H, d, ar, J = 8.4), 10.24 (1H, s, CHO), 11.44 (1H, s, NH). Anal. ($C_{19}H_{12}N_2O_2$) C, H, N.

Data for **6**. IR: 1787, 1609, 1230, 1076, 738. ^{1}H NMR: 6.71 (2H, td, ar, J = 8, J = 1.7), 7.20 (2H, dd, ar, J = 8, J = 1.7), 7.27 (2H, td, ar, J = 8, J = 1.7), 8.00 (2H, s, –CH=), 8.09 (2H, dd, ar, J = 8, J = 1.7), 8.21 (3H, m, py-3,4,5). Anal. ($C_{23}H_{13}NO_4$) C, H, N.

Data for **16j**. IR: 3189, 1714, 1203, 1037, 810. ^{1}H NMR: 3.52 (6H, s, OCH_3), 6.78 (2H, d, ind-7, J = 8.8), 6.84 (2H, dd, ind-6, J = 8.8, J = 1.9), 6.99 (2H, d, ind-4, J = 1.9), 7.67 (2H, s, –CH=), 7.70 (1H, t, ar, J = 7.8), 7.81 (2H, d, ar, J = 7.8), 7.95 (1H, s, ar), 10.43 (2H, s, NH). Anal. ($C_{26}H_{20}N_2O_4$) C, H, N.

Data for **16k**. IR: 3170, 3073, 1716, 1173, 753. ^{1}H NMR: 6.83 (2H, d, ind-7, J = 7.4), 7.04 (2H, d, ind-5, J = 7.4), 7.25 (2H, t, ind-6, J = 7.4), 7.51 (1H, t, ar, J = 7.2), 8.29 (2H, d, ar, J = 7.2), 8.45 (2H, s, –CH=), 8.56 (1H, s, ar), 10. 87 (2H, s, NH). Anal. ($C_{24}H_{14}Cl_2N_2O_2$) C, H, N.

Data for **17j**. IR: 3411, 3176, 1685, 1609, 840. ^{1}H NMR: 3.64 (6H, s, OCH_3), 6.80 (2H, d, ind-7, J = 8.5), 6.87 (2H, dd, ind-6, J = 8.5, J = 2), 7.17 (2H, d, ind-4, J = 2), 7.66 (2H, s, –CH=), 7.88 (4H, s, ar), 10.45 (2H, s, NH). Anal. ($C_{26}H_{20}N_2O_4$) C, H, N.

Data for **18j**. IR: 3600–3100, 1701, 1619, 1209, 723. ^{1}H NMR: 1.31 (9H, s, $C(CH_3)_3$), 3.58 (6H, s, OCH_3), 6.79 (2H, d, ind-7, J = 8.5), 6.85 (2H, dd, ind-6, J = 8.5, J = 2.5), 6.99 (2H, d, ind-4, J = 2.5), 7.71 (2H, s, ar), 7.72 (2H, s, –CH=), 9.87 (1H, s, OH), 10.40 (2H, s, NH). Anal. ($C_{30}H_{28}N_2O_5$) C, H, N.

Data for **19j**. IR: 3160, 1712, 1209, 1035, 851. ^{1}H NMR: 3.35 (6H, s, OCH_3), 6.50 (2H, d, ind-7, J = 8.4), 6.54 (2H, dd, ind-6, J = 8.4, J = 2.4), 7.82 (2H, s, –CH=), 8.07 (2H, s, ar), 8.20 (2H, d, ar, J = 8.4), 8.61 (2H, d, ar, J = 8.4), 8.99 (2H, d, ind-4, J = 2.4), 10.29 (2H, s, NH). Anal. ($C_{32}H_{22}N_4O_4$) C, H, N.

Data for **19k**. IR: 3170, 1706, 1235, 1163, 758. ^{1}H NMR: 6.86 (2H, d, ind-5/7, J = 7.8), 7.08 (2H, d, ind-5/7, J = 7.8), 7.30 (2H, t, ind-6, J = 7.8), 8.06 (2H, s, –CH=), 8.50 (2H, d, ar, J = 8.5), 8.61 (2H, s, ar), 8.66 (2H, d, ar, J = 8.5), 10.95 (2H, s, NH). Anal. ($C_{30}H_{16}Cl_2N_4O_2$) C, H, N.

Data for **20j**. IR: 3237, 1686, 1614, 1199, 1030. ^{1}H NMR: 2.15 (6H, s, CH_3), 3.46 (6H, s, OCH_3), 6.69 (6H, m, ind), 7.36 (2H, s, –CH=), 10.19 (2H, s, NH-ind), 11.68 (1H, s, NH-pyr). Anal. ($C_{26}H_{23}N_3O_4$) C, H, N.

Data for **21j**. IR: 3200, 1711, 1593, 1153, 723. ^{1}H NMR: 3.77 (6H, s, OCH_3), 6.79 (4H, m, ind-4+7), 7.42 (2H, dd, ind-6, J = 11.8, J = 2.2), 7.84 (1H, d, tio, J = 4.6), 7.90 (1H, d, tio, J = 4.6), 8.21 (2H, s, –CH=), 10.45 (2H, s, NH). Anal. ($C_{24}H_{18}N_2O_4S$) C, H, N.

Data for **22j**. IR: 3334, 1690, 1593, 1045, 815. ^{1}H NMR: 3.52 (3H, s, OCH_3), 3.75 (3H, s, OCH_3), 3.90 (6H, s, OCH_3), 6.77 (2H, s, ar), 7.29 (2H, m, ar), 7.37 (2H, dd, ind-6, J = 5, J = 1.8), 7.59 (2H, d, ind-7, J = 5), 7.74 (2H, s, –CH=), 8.80 (2H, d, ind-4, J = 1.8), 9.35 (2H, broad, OH), 10.31 (2H, s, NH). Anal. ($C_{34}H_{28}N_2O_8$) C, H, N.

3.1.3.2. Method 2 (compound 4c). Pyridine-2,6-dicarbaldehyde **3** (15 mmol) was treated with 30 mmol of the indolinone **1c**, 30 mmol of anhydrous sodium acetate and 130 mL of acetic acid. The reaction mixture was refluxed for 3–6 h (according to a TLC test), acetic acid was removed under reduced pressure and the residue was treated with ice water. The resulting precipitate was collected by filtration and purified by column chromatography with a yield of 20%.

Data for **4c**. IR: 3247, 1706, 1629, 1137, 994. ^{1}H NMR: 3.72 (6H, s, OCH_3), 3.83 (6H, s, OCH_3), 3.99 (6H, s, OCH_3), 6.29 (2H, s, ind-7), 7.86 (3H, t, py-4+CH=, J = 8), 8.59 (2H, d, py-3+5, J = 8), 10.58 (2H, s, NH). Anal. ($C_{29}H_{27}N_3O_8$) C, H, N.

3.1.3.3. Method 3^2 (compounds 4g,h, 18k and 20l). The appropriate aldehyde (10 mmol) was dissolved in acetic acid (50 mL) and treated with the appropriate indolinone **1** (20 mmol) and 37% hydrochloric acid (1 mL). The reaction mixture was refluxed for 3–5 h (according to a TLC test) and cooled. The yellow to orange precipitates thus formed were collected by filtration with a yield of 40%. Compound **4h** was isolated as acetate. The compounds were subjected to biological tests after purification by column chromatography (yield 18–25%).

Data for **4g**. IR: 3329, 1721, 1609, 1153, 784. ^{1}H NMR: 6.80 (4H, broad, NH_2), 6.87 (2H, d, ind-7, J = 8.2), 7.52 (2H, dd, ind-6, J = 8.2, J = 1.7), 7.78 (2H, s, –CH=), 7.96 (2H, d, py-3+5, J = 8), 8.12 (1H, t, py-4, J = 8), 8.71 (2H, d, ind-4, J = 1.7), 10.91 (2H, s, NH). Anal. ($C_{23}H_{17}N_5O_6S_2$) C, H, N.

Data for **4h**. IR: 3200–2600, 1711, 1603, 1030, 830. ^{1}H NMR: 1.90 (3H, s, CH_3COO), 5.00 (5H, br, SO_3H+$NHSO_2$+ex), 6.91 (2H, d, ind-7, J = 8.1), 7.30 (4H, d, ar, J = 8.4), 7.67 (2H, dd, ind-6, J = 8.1, J = 1.5), 7.70 (4H, d, ar, J = 8.4), 8.11 (2H, d, ind-4, J = 1.5), 8.24 (2H, s, –CH=), 8.56 (2H, d, py-3+5, J = 8), 8.76 (1H, t, py-4, J = 8), 11.48 (2H, s, NH). Anal. ($C_{37}H_{29}N_5O_{14}S_4$) C, H, N.

Data for **18k**. IR: 3165, 1690, 1573, 1240, 717. ^{1}H NMR: 1.31 (9H, s, $C(CH_3)_3$), 6.85 (2H, d, ind-5/7, J = 7.8), 7.03 (2H, d, ind-5/7, J = 7.8), 7.22 (2H, t, ind-6, J = 7.8), 8.39 (2H, s, ar), 8.70 (2H, s, –CH=), 10.00 (1H, s, OH), 10.80 (2H, s, NH). Anal. ($C_{28}H_{22}Cl_2N_2O_3$) C, H, N.

Data for **20l**. IR: 3211, 1701, 1609, 1173, 779. ^{1}H NMR: 2.18 (6H, s, CH_3), 6.74 (2H, dd, ind-6, J = 9, J = 4.6), 6.80 (2H, dd, ind-4, J = 9, J = 2.7), 6.90 (2H, dt, ind-5, J = 9, J = 2.7), 7.39 (2H, s, –CH=), 10.40 (2H, s, NH-ind), 11.86 (1H, s, NH-pyr). Anal. ($C_{24}H_{17}F_2N_3O_2$) C, H, N.

3.1.3.4. Method 4 (compound 15j). Benzene-1,2-dicarbaldehyde **7** (10 mmol) was dissolved in toluene (5 mL) and treated with the indolinone **1j** (20 mmol) in the presence of 4-toluenesulfonic acid (0.5 mmol). The reaction mixture was refluxed for 3–6 h (according to a TLC test) and after cooling the solvent was removed under reduced pressure. The residue was crystallized from ethanol with a yield of 30%.

Data for **15j**. IR: 3500–3100, 1716, 1697, 1204. ^{1}H NMR: 3.51 (6H, s, OCH_3), 6.65 (2H, d, ind-4, J = 2.4), 6.73 (2H, d, ind-7, J = 8.6), 6.79 (2H, dd, ind-6, J = 8.6, J = 2.4), 7.62 (2H, s, –CH=), 7.66 (2H, m, ar), 7.78 (2H, m, ar), 10.42 (2H, s, NH). Anal. ($C_{26}H_{20}N_2O_4$) C, H, N.

3.2. Biology

3.2.1. In vitro growth inhibition and cytotoxicity

The screening is a two-stage process,[25] beginning with the evaluation of all compounds against the 60 cell lines at a single concentration of 10^{-5} M. Compounds which exhibit significant growth inhibition are evaluated against the 60 cell panel at five concentration levels by the NCI according to standard procedures (http://dtp.nci.nih.gov/branches/btb/ivclsp.html).

3.2.2. Methodology of acute toxicity and hollow fiber assay

See Supplementary data.

3.2.3. Compare

COMPARE analyses[26] were performed using publically available programs at the Developmental Therapeutics Program website (http://dtp.nci.nih.gov).

3.2.4. NQO1 activity and MTT cell proliferation assays

NQO1 activity was determined essentially as described in the literature.[8] In brief, cells were pretreated for 24–48 h with compound dissolved in DMSO (<0.1% final concentration), plated in 96-well plates and incubated for 20 min with a solution containing 0.8% digitonin and 2 mM EDTA (pH 7.8) prior to adding the reaction mixture (0.025 mM Tris–HCl, 0.67 mg/mL bovin serum albumin, 0.01% Tween-20, 5 μM FAD, 1 mM glucose 6-phosphate, 30 μM $NADP^+$, 2 U/mL yeast glucose 6-phosphate dehydrogenase, 0.3 mg/mL MTT, 50 μM menadione). The reaction was arrested after 5 min by the addition of a stop solution (0.3 mM dicumarol in 0.5% DMSO and 5 mM potassium phosphate, pH 7.4) and the blue color generated by the reaction was measured at 595 nm in a multiwell plate reader (Wallac Victor2, Perkin–Elmer). Absorbance values were normalized on protein concentration. MTT reduction was measured following incubation of cells with each compound for 24–48 h.

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Supplementary data

Synthesis of the compounds not reported here. NSC numbers (Table S1), analytical data (elemental analyses) Table S2, LC_{50} matrix COMPARE results (Table S3), LC_{50} matrix COMPARE results for the most closely related compounds (Table S4), methodology of acute toxicity, methodology of the Hollow Fiber Assay, effect of sulforaphane and compounds **4c**, **4e** and **4f** on NQO1 activity in M07e leukemia cell line (Fig. S1). Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2010.03.063.

References and notes

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