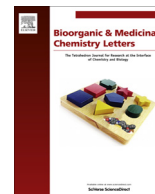




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Antiproliferative activity of 2,3-disubstituted indoles toward apoptosis-resistant cancers cells

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ABSTRACT

Many types of cancer, including glioma, melanoma, NSCLC, among others, are resistant to apoptosis induction and poorly responsive to current therapies with propapptotic agents. We describe a series of 2,3-disubstituted indoles, which display cytostatic rather than cytotoxic effects in cancer cells, and serve as a new chemical scaffold to develop anticancer agents capable of combating apoptosis-resistant cancers associated with dismal prognoses.

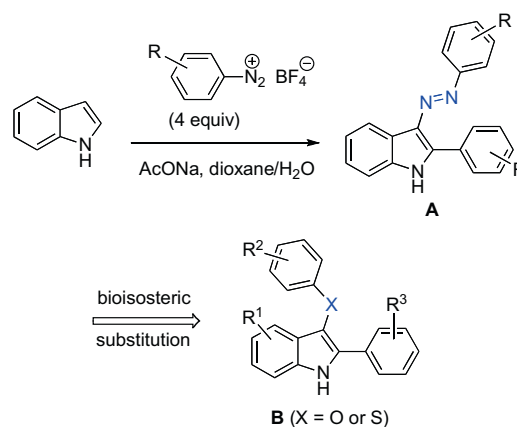
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Apoptosis-resistant cancers represent a major challenge in the clinic as most of the currently available chemotherapeutic agents work through the induction of apoptosis and, therefore, provide very limited therapeutic benefits for the patients affected by these malignancies. Such apoptosis-resistant cancers include the tumors of the lung, liver, stomach, esophagus, pancreas as well as melanomas and gliomas.¹ For example, patients afflicted by a type of gliomas, known as glioblastoma multiforme,^{2,3} have a median survival expectancy of less than 14 months when treated with a standard protocol of surgical resection, radiotherapy and chemotherapy with temozolomide.⁴ Because these glioma cells display resistance to apoptosis, they respond poorly to conventional chemotherapy with proapoptotic agents.^{3,5}

In addition, it must be recalled that 90% of cancer patients die from tumor metastases⁶ and that metastatic cancer cells have acquired resistance to a process termed anoikis, that is, cell death resulting from losing contact with extracellular matrix or neighboring cells.⁶ This phenomenon results in apoptosis resistance by metastatic cells making them unresponsive to a large majority of proapoptotic agents as well.^{3,7–9} One solution to apoptosis resistance entails the complementation of cytotoxic therapeutic regimens

with cytostatic agents and thus a search for novel cytostatic anticancer drugs that can overcome cancer cell resistance to apoptosis is an important pursuit.^{10–13}

Recently we described a series of 2-aryl-3-azoindoles **A** as antibacterial agents active against MRSA (Scheme 1).¹⁴ We showed that the metabolically labile azo group could be bioisosterically replaced by an ether or thioether functionalities leading to structures **B**, which were potent antibacterial agents as well. Unfortunately,



Scheme 1.

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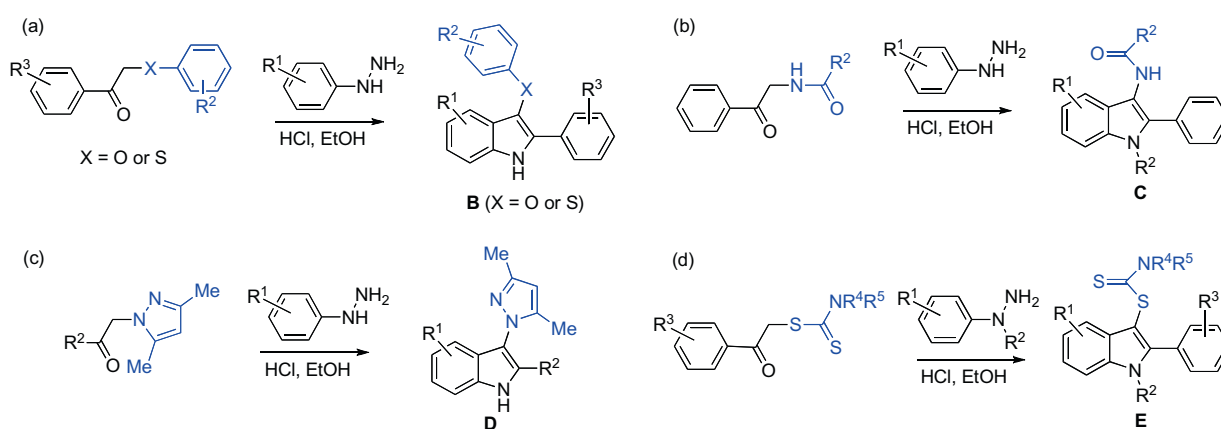
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these compounds were also found to inhibit the growth of human cervical cancer cells HeLa at comparable concentrations and this diminished our interest in pursuing them as novel antibacterials. However, during the antiproliferative assays, we noticed that these compounds do not kill cancer cells at GI_{50} -related concentrations, but rather display cytostatic effects. This observation prompted our efforts to investigate the compounds of this type as a new class of cytostatic agents potentially useful in the treatment of apoptosis-resistant cancers.

Because the antiproliferative effects of indoles **A** and **B** were similar, we surmised that the position C-3 of the indole ring can tolerate structurally diverse substituents. Previously, we developed synthetic pathways based on the Fisher indole reaction, which in addition to C-3 ether and thioether indoles **B** (Scheme 2a) can be used to prepare C-3 amide indoles **C** (Scheme 2b), C-3 pyrazole indoles **D** (Scheme 2c) and C-3 dithiocarbamate indoles

E (Scheme 2d).^{15–18} Using this chemistry, a number of C-3 analogs of each structural type were synthesized and their structures are shown in Table 1.

The synthesized compounds were evaluated for in vitro growth inhibition using the MTT colorimetric assay¹⁹ against a panel of five cancer cell lines including those resistant to proapoptotic stimuli [human U373 glioblastoma,²⁰ human A549 non-small-cell-lung cancer (NSCLC),²¹ and human SKMEL-28 melanoma²²] as well as apoptosis-sensitive tumor models (human Hs683 anaplastic oligodendroglioma²⁰ and mouse B16F10 melanoma²²). Analysis of the data shown in Table 1 reveals that most of the synthesized compounds exhibit antiproliferative properties in the double-digit micromolar region and do not drastically differ in their potencies. Indeed, it appears that the position C-3 of the indole ring tolerates diverse substitution in this type of structure. Yet, C-3 ether and thioether indoles **B** appear to be the most potent, with ether indole **B**₆

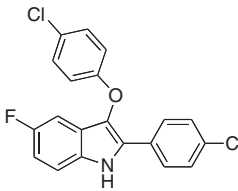
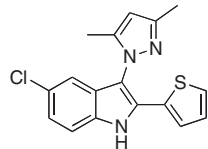
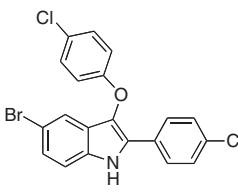
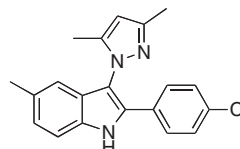
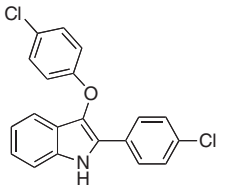
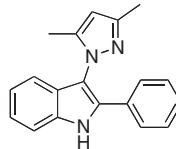
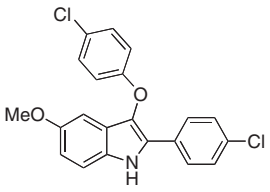
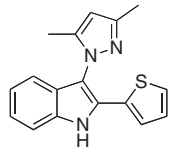
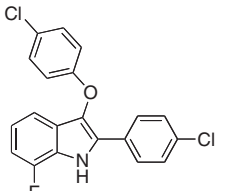
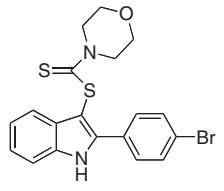
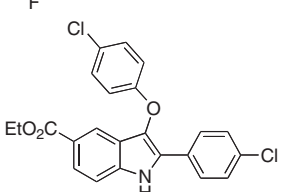
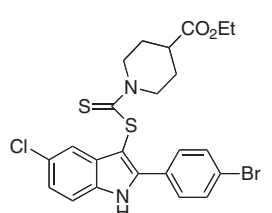
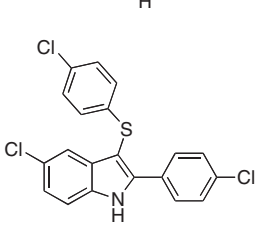
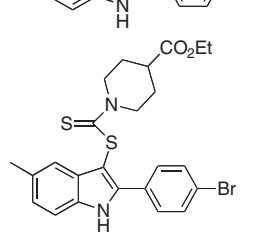
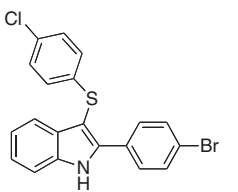
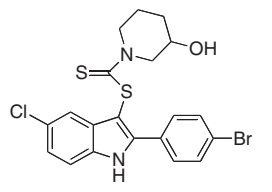


Scheme 2.

Table 1
Structures of C-3 derivatized 2-arylindoles and their antiproliferative activities toward cancer cells

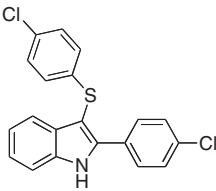
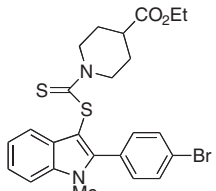
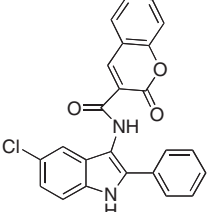
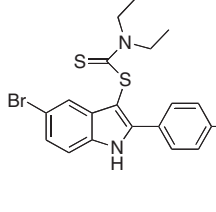
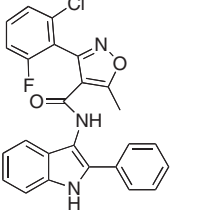
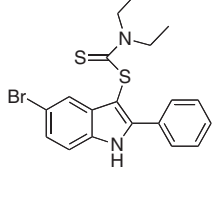
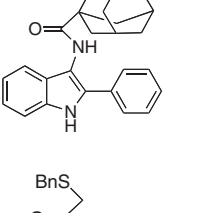
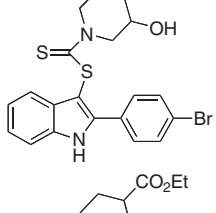
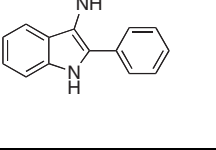
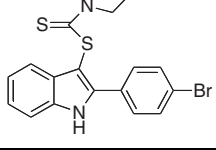
#	Structure	GI_{50} in vitro values ^a (μM)					#	Structure	GI_{50} in vitro values ^a (μM)				
		U373	Hs683	A549	SKMEL	B16			U373	Hs683	A549	SKMEL	B16
A ₁		33	31	26	33	33	C ₅		66	38	37	48	32
A ₂		30	24	23	31	26	D ₁		47	32	29	31	39
B ₁		13	13	10	19	10	D ₂		10	29	26	33	40

Table 1 (continued)

#	Structure	GI ₅₀ in vitro values ^a (μM)					#	Structure	GI ₅₀ in vitro values ^a (μM)				
		U373	Hs683	A549	SKMEL	B16			U373	Hs683	A549	SKMEL	B16
B ₂		20	22	22	24	14	D ₃		32	32	40	35	36
B ₃		35	26	30	28	29	D ₄		43	50	42	46	65
B ₄		25	28	25	28	29	D ₅		84	67	61	84	>100
B ₅		50	54	59	57	31	D ₆		90	58	38	80	73
B ₆		8	7	7	8	8	E ₁		43	32	31	35	20
B ₇		35	24	28	28	20	E ₂		33	36	34	39	21
B ₈		28	31	27	34	28	E ₃		68	34	37	52	37
B ₉		24	25	26	26	35	E ₄		39	31	29	34	37

(continued on next page)

Table 1 (continued)

#	Structure	GI ₅₀ in vitro values ^a (μM)					#	Structure	GI ₅₀ in vitro values ^a (μM)				
		U373	Hs683	A549	SKMEL	B16			U373	Hs683	A549	SKMEL	B16
B₁₀		28	31	27	34	28	E₅		>100	85	72	78	50
C₁		66	45	37	55	27	E₆		9	22	22	29	33
C₂		24	15	9	13	22	E₇		36	26	31	28	40
C₃		38	69	38	67	44	E₈		32	24	24	28	29
C₄		47	33	30	30	35	E₉		35	36	34	36	27

^a U373 (ECACC code 89081403) cell line was cultured in MEM medium supplemented with 5% heat inactivated fetal bovine serum; Hs683 (ATCC code HTB-138), SKMEL-28 (ATCC code HTB-72), A549 (DSMZ code ACC107) and B16F10 (ATCC code CRL-6475) cell lines were cultured in RPMI medium supplemented with 10% heat inactivated fetal bovine serum; MEM and RPMI cell culture media were supplemented with 4 mM glutamine, 100 μg/mL gentamicin and penicillin–streptomycin (200 U/mL and 200 μg/mL).

exhibiting single-digit micromolar GI₅₀ values. Importantly, all synthesized 2,3-disubstituted indoles do not discriminate between the cancer cell lines based on the apoptosis sensitivity criterion and display comparable potencies in both cell types, further indicating that apoptosis induction may not be the primary mechanism responsible for antiproliferative activity in this series of compounds, at least in solid cancers.

We also employed computer-assisted phase-contrast microscopy^{10,22} (quantitative videomicroscopy) to analyze the principal mechanism of action associated with indoles' **B** in vitro growth inhibitory effects, as first revealed by the MTT colorimetric assay. Figure 1 shows that indole **B₁₀** inhibits cancer cell proliferation without inducing cell death when assayed at its GI₅₀ concentrations (Table 1) in SKMEL-28 melanoma and A549 NSCLC cells. Based on the phase contrast pictures obtained by means of quantitative videomicroscopy, we calculated the global growth ratio (GGR), which corresponds to the ratio of the mean number of cells present in a given image captured in the experiment (in this case

after 24, 48 and 72 h) to the number of cells present in the first image (at 0 h). We divided this ratio obtained in the **B₁₀**-treated experiment by the ratio obtained in the control. The GGR values of 0.1 and 0.3 correspondingly in these two cell lines indicate that 10% and 30% of cells grew in the **B₁₀**-treated experiment as compared to the control over a 72 h observation period. Thus, the GGR calculations confirm the MTT colorimetric data in Table 1, that is, 30 μM **B₁₀** exhibits marked growth inhibitory activity in SKMEL-28 and A549 cells, which display resistance to apoptosis induction (Fig. 2).

To confirm that indoles **B** do not induce cell death as suggested by the videomicroscopy experiments, we employed flow cytometric propidium iodide staining, which detects necrotic and late apoptotic cells that have lost the plasma membrane integrity. The experiments performed with apoptosis-resistant A549 NSCLC and SKMEL-28 cells indicate that **B₁₀** at its GI₅₀ concentration of 30 μM does not induce any cell permeabilization even after 72 h of treatment in both cell types. In contrast, 90% of ice-cold ethanol

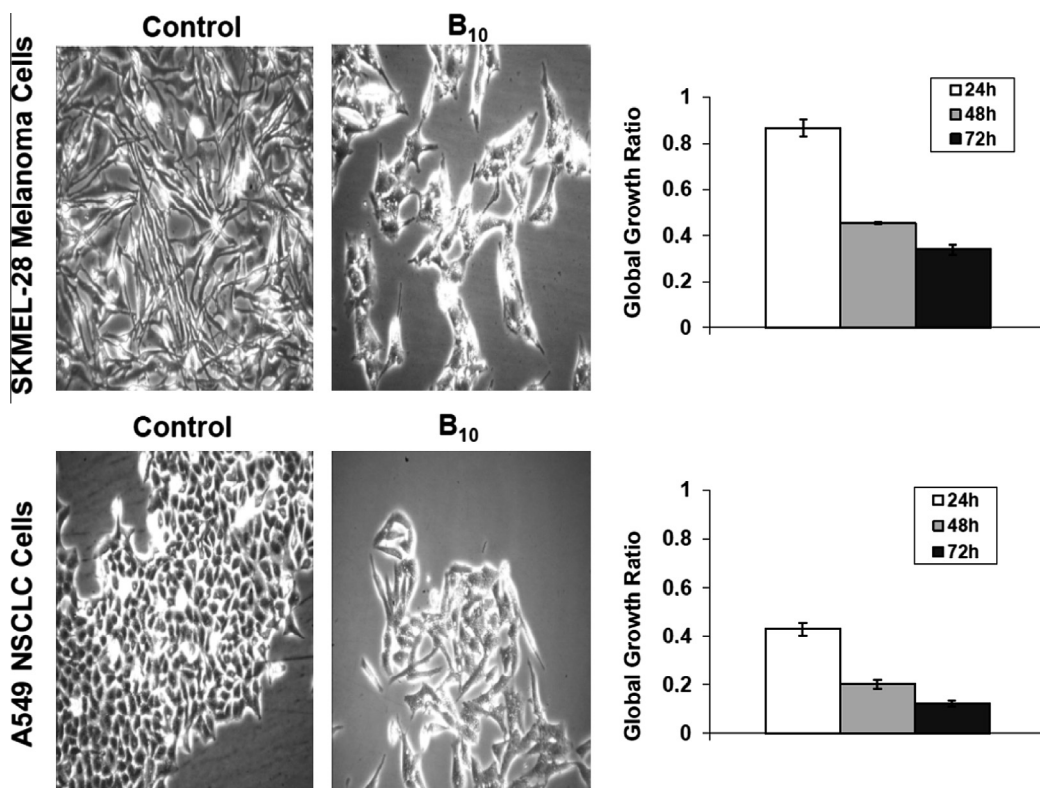


Figure 1. Cellular imaging of **B₁₀** against melanoma SKMEL-28 and NSCLC A549 cells illustrating noncytotoxic, but cytostatic, antiproliferative mechanism at MTT colorimetric assay-related GI_{50} concentrations after 72 h of cell culture with the drug.

fixed and permeabilized cells were positively stained and cisplatin, a pro-apoptotic agent, induced an increase in the percentage of PI positive cells even in these apoptosis-resistant models (increase from 1% to 10% for A549 NSCLC and from 8% to 30% for SKMEL-28 cells).

In conclusion, the anticancer evaluation of C-3 derivatized 2-aryl indoles, accessible by a straightforward synthetic preparation utilizing the Fisher indole reaction, revealed their promising activity against apoptosis-resistant cancers associated with dismal clinical outcomes. The most promising structural type appears to be

the C-3 ether and thioether indoles, which exhibit their antiproliferative effects mainly through cytostatic mechanisms.

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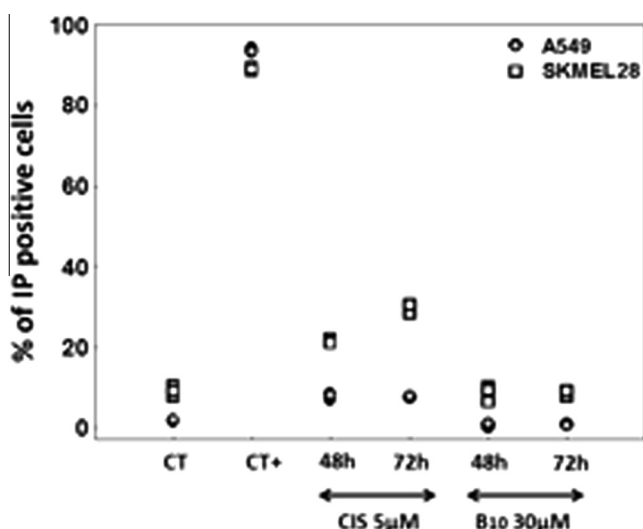


Figure 2. Percentage of cells that lost plasma membrane integrity after treatment with **B₁₀** as assessed by propidium iodide staining. Positive controls correspond to fixed and permeabilized corresponding cells.

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