SYNTHESIS AND BIOLOGICAL ACTIVITY OF NOVEL BENZIMIDAZOLYL AND BENZOTHIAZOLYL SUBSTITUTED ISOINDOLINES

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At the present time, a great challenge for medicinal chemistry is to find new and more selective anticancer agents and therefore there is tremendous growth in the number and types of new compounds which exhibit antitumor activity. Isoindoline moiety has proven its functional propensity in number of natural and synthetic bioactive compounds.[1] As a part of our continuous research to develop new nitrogen containing heterocycles as potential anticancer agents,[2,3] we present here the synthesis, DNA binding, topoisiomerases inhibition and biological activities of novel benzimidazolyl and benzothiazolyl substituted isoindolines.

1. Target compounds were prepared by condensation of *o*-phthalaldehyde and heterocyclic amines in acidic conditions (Scheme 1).
2. The antiproliferative activity was assessed on a panel of tumor cell lines and normal human fibroblasts (Table 1). For selected compounds, further mechanistic studies involving analysis of cell cycle and cell death mechanisms were pursued on (Table 2).
3. The interaction with DNA was then addressed for selected compounds using DNA melting temperature experiment (Table 3). The orientation of the compound relatively to the DNA helix was evaluated using circular dichroism showing groove binding for some derivatives (Figure 1) and using topoisomerase I-induced DNA relaxation evidencing DNA intercalation. Poisoning activities were evidenced on topoisomerase I using 3a (Figure 2) and on toposomerase II using 4a and 4b (Figure 3). All evaluated compounds enter the cell and locates partially or totally in the nucleus as evidenced using fluorescence cell microscopy (Figure 4).

Scheme 1.

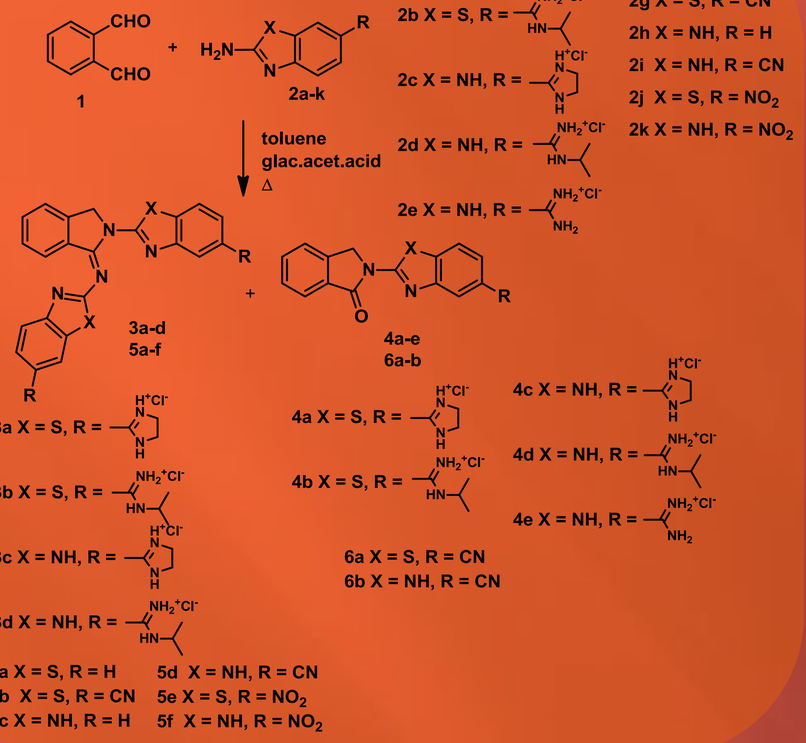


Table 1. The inhibition effects of compounds 3a-6b on the growth of tumour cells and normal fibroblasts *in vitro*.

IC_{50}[a] (μM)

Substance No.	Cell lines				
	MCF-7	SW620	HepG2	HeLa	BJ
3a	1.48	>100	35.02	74.53	31.82
3b	90.09	49.47	68.80	52.93	4.72
3c	23.35	34.98	9.01	15.42	22.94
3d	66.14	>100	84.76	96.78	>100
4a	0.70	0.74	0.78	1.00	0.20
4b	0.36	0.15	0.34	0.32	0.05
4c	2.90	8.67	16.65	6.12	4.71
4d	0.93	70.97	>100	84.30	5.72
4e	>100	>100	91.08	52.18	46.94
5a	0.08	6.98	0.82	4.68	4.87
5c	7.47	>100	7.85	9.32	22.37
5d	0.02	69.95	17.93	75.72	>100
6a	9.93	>100	61.46	54.74	44.50
6b	5.06	>100	10.04	24.05	15.79

Compounds 4a and 4b were the most active compounds in this series

Table 2. Percentage of cells in the subG1 phase upon treatment of SW620 and HeLa cells with compounds 4a and 4b for 48 hours.

	Treatment	SubG1 cell percentage	
		HeLa	SW620
4a	Control	17.0	4.1
	1 μM	29.1	34.4
	5 μM	70.7	85.9
4b	Control	20.1	11.3
	1 μM	38.1	59.9
	0.5 μM	45.7	33.9

Table 3. DNA binding ability is evaluated using DNA melting temperature experiment for the following selected compounds.

Compound	ΔTm (°C) Drug/DNA ratio		
	R=0.25	R=0.5	R=1
3a	6.8	10.5	12.2
3b	12.7	18.0	na
3c	9.3	18.5	na
3d	7.0	12.9	na
4a	1.8	3.1	6.2
4b	1.2	3.2	4.4
5a	nd	-1.3	-2.2
5b	nd	-1.1	-0.7
5d	nd	-0.4	-0.5

Figure 1. Circular dichroism spectra evidenced groove binding for compounds 3a, 3c-d and 4a-b as part of their DNA binding mode.

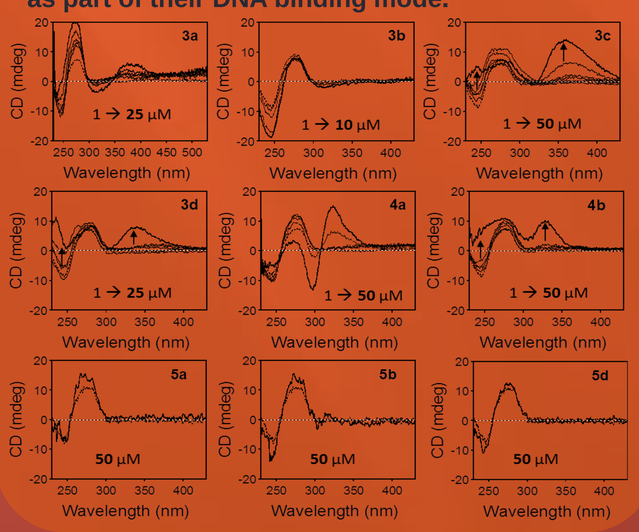


Figure 2. Topoisomerase I-induced DNA relaxation evidenced DNA intercalation as part of the mechanism of action.

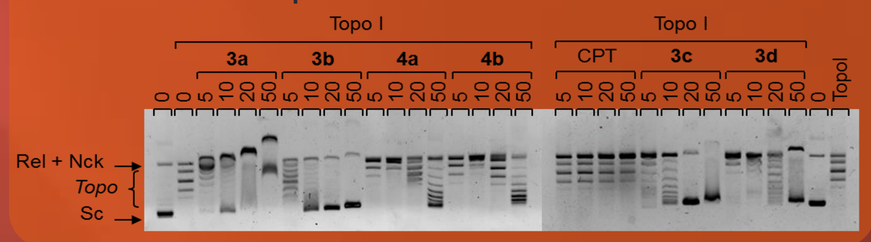
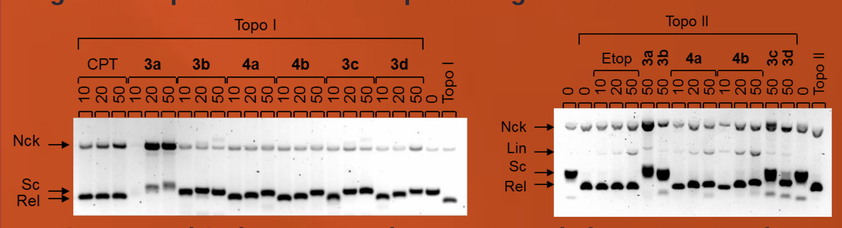
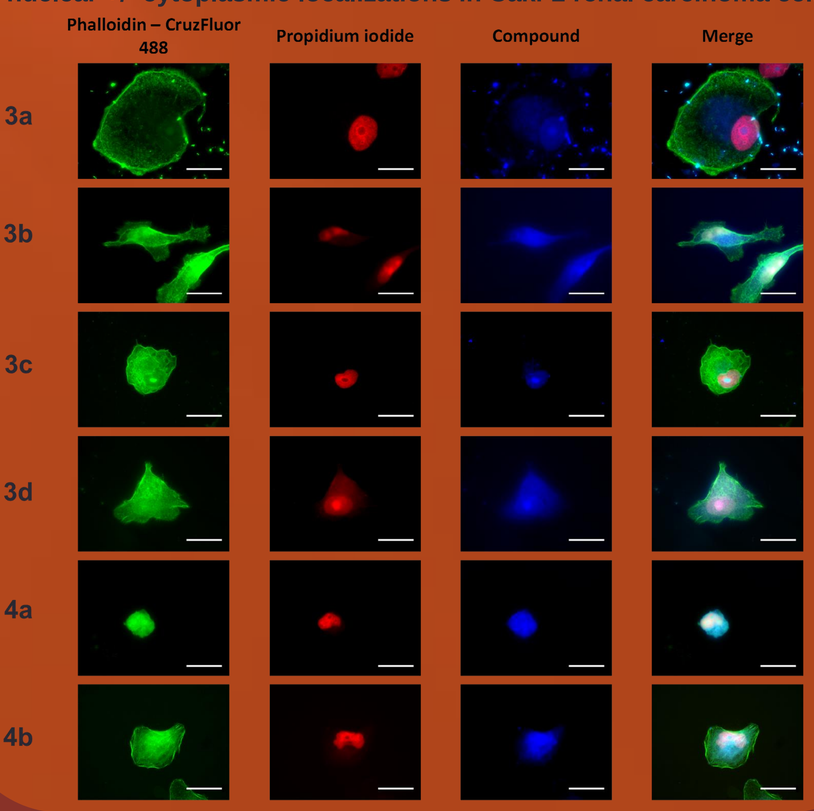


Figure 3. Topoisomerase I or II poisoning effects.



Compound 3a is a topol poison. 4a and 4b are topoII poisons.

Figure 4. Fluorescence microscopy determined the sub-sellular localization of the DNA binding compounds. Based on their intrinsic fluorescence, all evaluated coumpounds enter the cells and evidenced nuclear +/- cytoplasmic localizations in Caki-2 renal carcinoma cells.



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2. Sović, I.; Kraljević Pavelić, S.; Markova-Car, E.; Ilić, N.; Nhili, R.; Depauw, S.; David-Cordonnier, M.-H.; Karminski-Zamola, G. *Eur. J. Med. Chem.* 87 (2014) 372-385.
3. Sović, I.; Stilinović, V.; Kaitner, B.; Kraljević Pavelić, S.; Bujak, M.; Čuljak, K.; Novak, P.; Karminski-Zamola, G. *J. Mol. Struct.* 1006 (2011) 259-265.

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