

## Synthesis, anticancer and cytostatic activity of some 6*H*-indolo[2,3-*b*]quinoxalines

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Various 6-*aralkyl*-9-substituted-6*H*-indolo[2,3-*b*]quinoxalines were synthesized by reaction of 1,5-disubstituted 2,3-dioxo-2,3-dihydroindole and orthophenylene diamine. Appreciable anticancer activity of compounds **5b**, **5d**, **5g** and **5l** at various cell lines among 59 human tumor cell panels was observed. All the synthesized compounds were evaluated for cytostatic activity against human Molt 4/C8 and CEM T-lymphocytes as well as for murine L1210 leukemia cells. Compound **5h** exhibited an  $IC_{50}$  of  $71 \mu\text{mol L}^{-1}$  against Molt 4/C8 and  $117 \mu\text{mol L}^{-1}$  against CEM compared to melphalan  $3.2 \mu\text{mol L}^{-1}$  and  $2.5 \mu\text{mol L}^{-1}$ , respectively. The  $IC_{50}$  for compound **7i** against L1210 was  $7.2 \mu\text{mol L}^{-1}$  compared to melphalan  $2.1 \mu\text{mol L}^{-1}$ .

**Keywords:** indolo[2,3-*b*]quinoxaline, cytostatic activity, anticancer activity

Quinoxaline derivatives seem to have very interesting biological properties (1–3). The plant alkaloid ellipticine (5,11-dimethyl-6*H*-pyrido[4,3-*b*]carbazole) has DNA-intercalating and antitumor activity and is active against the herpes simplex virus (4, 5). Graslund *et al.* (6) have studied ellipticine and the derivatives 2,3-dimethyl-6-(2-dimethyl-aminoethyl)6*H*-indolo[2,3-*b*]quinoxaline and 6-(2-dimethylaminoethyl)6*H*-indolo[2,3-*b*]quinoxaline for their interaction with oligodeoxynucleotide duplexes. They reported that compounds were intercalated in a non-specific fashion and by an AT-specific interaction.

Recently, Sauvain *et al.* (7) reported that 3-(4'-chloro)phenylquinoxaline-2-carbonitrile-1,4-di-*N*-oxide had potent antimalarial activity particularly against a chloroquine-resistant strain of *Plasmodium falciparum*. Moarbess *et al.* (8) examined imidazo[1,2-*a*]quinoxaline, imidazo[1,5-*a*]quinoxaline and pyrazolo[1,5-*a*]quinoxaline derivatives for their *in vitro* and *in vivo* anti-tumoral activities. Toshima *et al.* (9) have designed and evaluated quinoxaline-carbohydrate hybrids as novel and selective photo-induced DNA cleaving and cytotoxic agents.

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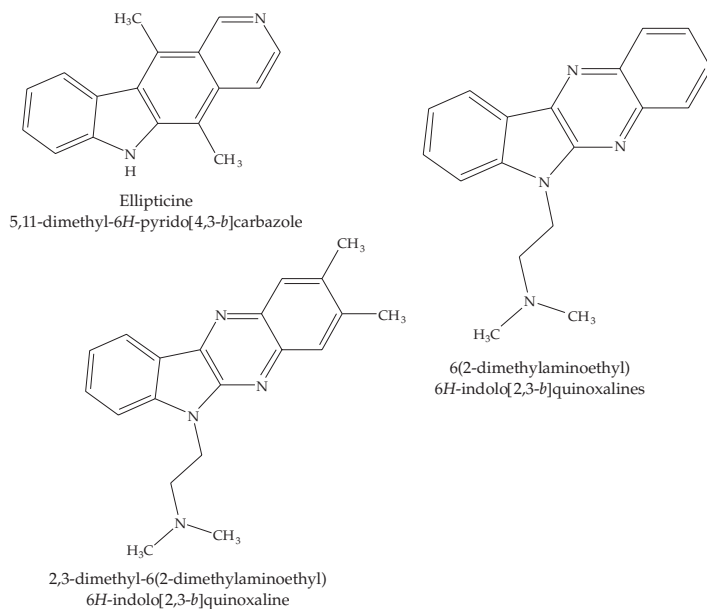


Fig. 1. Structures of ellipticine and other indolo[2,3-*b*]quinoxalines.

Some quinoxalin-2-ones have shown antifungal activity (10, 11) whereas quinoxalin-1-oxides have antibacterial activity (12). 6*H*-indolo[2,3-*b*]quinoxaline can be regarded as an aza analogue of ellipticine. It was therefore of interest to test 6,9-disubstituted-6*H*-indolo[2,3-*b*]quinoxalines for their anticancer and cytostatic activity.

#### EXPERIMENTAL

Melting points were determined in open capillaries and were uncorrected.  $R_f$  values were obtained using silica gel thin layer chromatography plates and a solvent system of chloroform/methanol (9:1). 2,3-Dioxy-2,3-dihydroindoles/5-substituted 2,3-dioxy-2,3-dihydroindoles and 1-(4-substituted)arylmethyl-2,3-dioxy-2,3-dihydroindoles were prepared according to literature (13, 14). The infrared spectra of all compounds were determined by a diffuse reflectance technique using potassium bromide powder on a Jasco 460 FTIR machine (Jasco, Japan).  $^{13}\text{C}$  NMR for **5i** and **5j** and  $^1\text{H}$  NMR spectra (400 MHz) of all compounds were generated in dimethylsulfoxide- $d_6$ /CDCl $_3$  on a Bruker Ultraspec spectrophotometer (Germany). FAB mass spectra for **5c** and **5j** were obtained by using a Jeol SX-102 instrument (Jeol, Japan). CHN for all compounds were generated on an elemental analyzer Vario EL III (Germany).

*General procedure for the synthesis of  
6-*aralkyl*-9-substituted-6*H*-indolo[2,3-*b*]quinoxalines (5a-m)*

A mixture of 1-arylmethyl-2,3-dioxy-2,3-dihydroindole (0.005 mol), orthophenylene diamine (0.005 mol), glacial acetic acid (0.5–1.0 mL) and anhydrous ethanol (100 mL) was heated under reflux until the reaction was complete (4 h). Approximately half of the ethanol was removed in vacuo and the solution was left overnight at room temperature. The solid that precipitated was collected, washed with cold ethanol and recrystallized from suitable solvent. Products obtained were: 9-fluoro-6-(4-fluorobenzyl)-6*H*-indolo[2,3-*b*]quinoxaline (**5a**), 6-benzyl-9-fluoro-6*H*-indolo[2,3-*b*]quinoxaline (**5b**), 9-methyl-6-(4-methylbenzyl)-6*H*-indolo[2,3-*b*]quinoxaline (**5c**), 6-benzyl-9-methyl-6*H*-indolo[2,3-*b*]quinoxaline (**5d**), 9-chloro-6-(4-fluorobenzyl)-6*H*-indolo[2,3-*b*]quinoxaline (**5e**), 6-benzyl-9-chloro-6*H*-indolo[2,3-*b*]quinoxaline (**5f**), 6-(4-fluorobenzyl)-6*H*-indolo[2,3-*b*]quinoxaline (**5g**), 6-(4-methylbenzyl)-6*H*-indolo[2,3-*b*]quinoxaline (**5h**), 9-bromo-6-(4-methylbenzyl)-6*H*-indolo[2,3-*b*]quinoxaline (**5i**), 9-chloro-6-(4-methylbenzyl)-6*H*-indolo[2,3-*b*]quinoxaline (**5j**),

Table I. Physicochemical data of synthesized compounds

Compd.	Yield (%)	M.p. (°C)	Mol. formula ( $M_r$ )	$R_f$ value	Elemental analysis calcd./found (%)		
					C	H	N
<b>5a</b>	65	218–220	C <sub>21</sub> H <sub>13</sub> F <sub>2</sub> N <sub>3</sub> 345.34	0.76	73.04	3.79	12.17
					72.99	3.56	12.25
<b>5b</b>	68	220–222	C <sub>21</sub> H <sub>14</sub> FN <sub>3</sub> 327.35	0.52	77.05	4.31	12.84
					76.88	4.25	13.11
<b>5c</b>	72	220–225	C <sub>23</sub> H <sub>19</sub> N <sub>3</sub> 337.42	0.48	81.87	5.68	12.45
					81.26	5.66	12.75
<b>5d</b>	75	215–218	C <sub>22</sub> H <sub>17</sub> N <sub>3</sub> 323.39	0.55	81.71	5.30	12.99
					81.65	5.22	13.09
<b>5e</b>	75	235–240	C <sub>21</sub> H <sub>13</sub> ClFN <sub>3</sub> 361.80	0.40	69.71	3.62	11.61
					70.00	3.55	11.55
<b>5f</b>	68	198–201	C <sub>21</sub> H <sub>14</sub> ClN <sub>3</sub> 343.81	0.55	73.36	4.10	12.22
					72.98	4.01	12.39
<b>5g</b>	72	162–163	C <sub>21</sub> H <sub>14</sub> FN <sub>3</sub> 327.35	0.70	77.05	4.31	12.84
					77.25	3.99	13.02
<b>5h</b>	80	185–190	C <sub>22</sub> H <sub>17</sub> N <sub>3</sub> 323.39	0.76	81.71	5.30	12.99
					80.98	5.32	13.28
<b>5i</b>	75	193–195	C <sub>22</sub> H <sub>16</sub> BrN <sub>3</sub> 402.29	0.60	65.68	4.01	10.45
					65.01	3.75	11.01
<b>5j</b>	80	192–194	C <sub>22</sub> H <sub>16</sub> ClN <sub>3</sub> 357.84	0.56	73.84	4.51	11.74
					73.01	4.11	12.13
<b>5k</b>	71	212–215	C <sub>21</sub> H <sub>13</sub> BrN <sub>3</sub> F 406.25	0.31	62.09	3.23	10.34
					62.45	3.01	11.01
<b>5l</b>	71	177–180	C <sub>22</sub> H <sub>16</sub> FN <sub>3</sub> 341.38	0.80	77.40	4.72	12.31
					76.97	4.39	12.55
<b>5m</b>	76	223–225	C <sub>22</sub> H <sub>16</sub> FN <sub>3</sub> 341.38	0.60	77.40	4.72	12.31
					76.78	4.51	12.81

9-bromo-6-(4-fluorobenzyl)-6*H*-indolo[2,3-*b*]quinoxaline (**5k**), 9-fluoro-6-(4-methylbenzyl)-6*H*-indolo[2,3-*b*]quinoxaline (**5l**), 6-(4-fluorobenzyl)-9-methyl-6*H*-indolo [2,3-*b*] quinoxaline (**5m**).

Physico-chemical data for synthesized compounds **5a-m** are reported in Table I.

### *Anticancer activity*

Compounds **5b**, **5d**, **5g** and **5l** were submitted to *in vitro* disease-oriented antitumor screen (15). This assay involves determination of a test agent's effect on growth parameters against a panel of approximately 60 human tumor cell lines, derived largely from solid tumors, including non-small cell lung, colon, central nervous system, melanoma, ovarian, prostate and breast cancers, plus a few leukemia and renal cell lines. Compounds were tested at a  $1.0 \times 10^{-5}$  mol L<sup>-1</sup> using DMSO as solvent; a 48-h continuous drug exposure protocol was applied and a sulforhodamine B (SRB) protein assay was used to estimate cell viability or growth. The measured effect of the compound on a cell line was calculated according to one of the following two expressions:

if

$$(A_{\text{test}} - A_{\text{tzero}}) \geq 0,$$

then

$$\text{percentage growth} = 100 (A_{\text{test}} - A_{\text{tzero}}) / (A_{\text{ctrl}} - A_{\text{tzero}})$$

if

$$(A_{\text{test}} - A_{\text{tzero}}) < 0,$$

then

$$\text{percentage growth} = 100 (A_{\text{test}} - A_{\text{tzero}}) / A_{\text{tzero}}$$

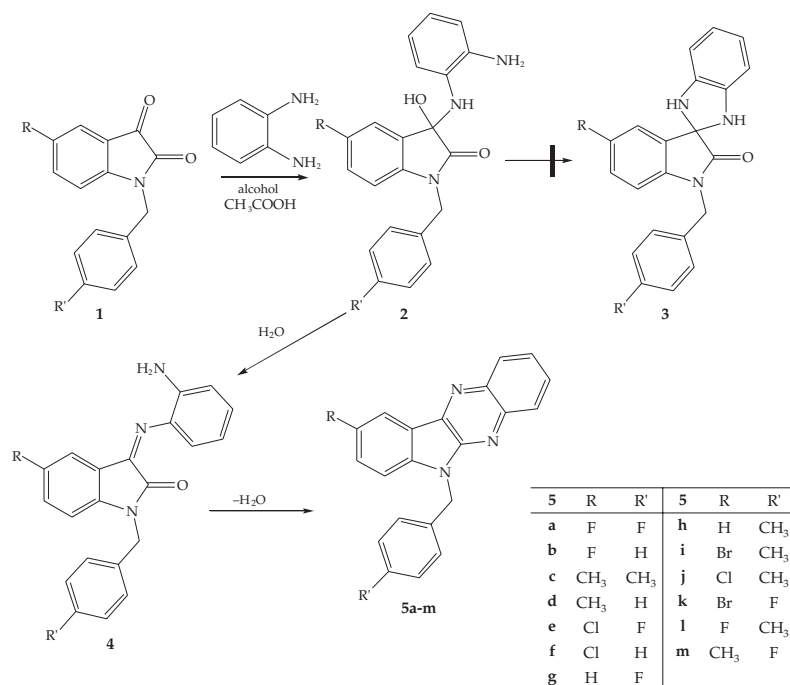
where,  $A_{\text{tzero}}$  is the average of absorbance measurements of SRB-derived color just before exposure of cells to the test compound,  $A_{\text{test}}$  is the average absorbance measurements of SRB-derived color just after 48-h exposure of cells to the test compound,  $A_{\text{ctrl}}$  is the average of absorbance measurements of SRB-derived color just after 48 h with no exposure of cells to the test compound.

### *Cytostatic activity*

The methodology for cytostatic activity assays in Molt 4/C8, CEM and L1210 assays has been published previously (16). In brief, varying concentrations of compounds were incubated at 37 °C with the cells for 72 h (human Molt 4/C8 or CEM T-lymphocytes) or 48 h (murine L1210 cells). After the incubation period cell number was counted by a coulter counter (Harpden Herz, UK).

## RESULTS AND DISCUSSION

We have synthesized a series of thirteen derivatives of 6*H*-indolo[2,3-*b*]quinoxalines (**5a-m**) containing an *N*-aralkyl group at the 6<sup>th</sup> position by reacting *N*-aralkylisatin with



Scheme 1

orthophenylenediamine, as depicted in Scheme I. The reaction of isatin with orthophenylenediamine has been reported to give spirobenzimidazolinone, isatin-3-imine and/or 6*H*-indolo[2,3-*b*]quinoxaline in various kinds of solvents. No spiro compound or Schiff's base

Table II. Spectral data of synthesized compounds

Compd.	IR ( $\nu$ , $\text{cm}^{-1}$ )	$^1\text{H}/^{13}\text{C}$ NMR ( $\delta$ , ppm) (DMSO- $d_6$ )	MS
5a	3100–3030, 2917–2849, 1604, 1584, 1508, 1486	5.73 (s, 2H, $-\text{CH}_2-$ ), 7.02–7.06 (m, 2H, Ar-H), 7.40–7.43 (m, 2H, Ar-H), 7.52–7.57 (m, 1H, Ar-H), 7.62 (d, 1H, $J = 8.0$ , Ar-H), 7.75–7.79 (m, 1H, Ar-H), 7.83–7.87 (m, 1H, Ar-H), 8.19–8.24 (m, 2H, Ar-H), 8.38 (d, 1H, $J = 8.0$ , Ar-H)	
5b	3066–3004, 2904–2849, 1618, 1582, 1511, 1489	5.77 (s, 2H, $-\text{CH}_2-$ ), 7.28–7.31 (m, 4H, Ar-H), 7.32–7.39 (m, 2H, Ar-H), 7.42 (d, 1H, Ar-H), 7.74–7.79 (m, 1H, Ar-H), 7.82–7.86 (m, 1H, Ar-H), 8.19–8.24 (m, 2H, Ar-H), 8.38 (d, 1H, $J = 8.0$ , Ar-H)	
5c	3052–3019, 2920–2851, 1615, 1578, 1513, 1487	2.34 (s, 3H, $\text{CH}_3$ ), 2.59 (s, 3H, $\text{CH}_3$ ), 5.71 (s, 2H, $-\text{CH}_2-$ ), 7.14 (d, 2H, $J = 7.6$ , Ar-H), 7.27 (d, 2H, $J = 8.0$ , Ar-H), 7.30–7.32 (m, 2H, Ar-H), 7.49 (d, 1H, $J = 8.4$ , Ar-H), 7.74 (t, 1H, $J = 14.8$ , Ar-H), 7.81 (t, 1H, $J = 14.8$ , Ar-H), 8.20 (d, 1H, $J = 8.0$ , Ar-H), 8.35–8.37 (m, 1H, Ar-H)	338 (100)

5d	3059–3024, 2966–2850, 1616, 1578, 1508, 1489	2.59 (s, 3H, -CH <sub>3</sub> ), 5.76 (s, 2H, -CH <sub>2</sub> -), 7.29–7.38 (m, 6H, Ar-H), 7.49 (d, 1H, J = 8.4, Ar-H), 7.72–7.76 (m, 1H, Ar-H), 7.79–7.83 (m, 1H, Ar-H), 8.18–8.20 (m, 1H, Ar-H), 8.36–8.38 (m, 2H, Ar-H)	
5e	3066, 2917–2848, 1605, 1581, 1509, 1474	5.73 (s, 2H, -CH <sub>2</sub> -), 7.01–7.37 (m, 4H, Ar-H), 7.61–7.64 (m, 2H, Ar-H), 7.76–7.80 (m, 1H, Ar-H), 7.84–7.88 (m, 1H, Ar-H), 8.22 (d, 1H, J = 8.0, Ar-H), 8.39 (d, 1H, J = 8.0, Ar-H), 8.52–8.53, (m, 1H, Ar-H)	
5f	3062, 2918–2849, 1609, 1581, 1509, 1455	5.77 (s, 2H, -CH <sub>2</sub> -), 7.28–7.35, (m, 6H, Ar-H), 7.57–7.62, (m, 1H, Ar-H), 7.75–7.87 (m, 2H, Ar-H), 8.19–8.22 (m, 1H, Ar-H), 8.36–8.38, (m, 1H, Ar-H), 8.53, (s, 1H, Ar-H)	
5g	3045–3013, 2954–2849, 1610, 1583, 1513, 1469.	5.69 (s, 2H, -CH <sub>2</sub> -), 7.00 (t, 2H, J = 16, Ar-H), 7.31–7.41 (m, 4H, Ar-H), 7.63 (t, 1H, J = 16.0, Ar-H), 7.69–7.73 (m, 1H, Ar-H), 7.76–7.80 (m, 1H, Ar-H), 8.16 (d, 1H, J = 8.8, Ar-H), 8.32–8.34 (m, 1H, Ar-H), 8.51 (d, 1H, J = 7.6, Ar-H)	
5h	3053–3022, 2964–2920, 1612, 1583, 1510, 1471	2.34 (s, 3H, CH <sub>3</sub> ), 5.74 (s, 2H, -CH <sub>2</sub> -), 7.15 (d, 2H, J = 8.0, Ar-H), 7.27–7.34 (m, 2H, Ar-H), 7.44 (d, 2H, J = 8.0, Ar-H), 7.68 (t, 1H, J = 15.6, Ar-H), 7.73–7.77 (m, 1H, Ar-H), 7.83 (t, 1H, J = 15.2, Ar-H), 8.22 (d, 1H, J = 8.4, Ar-H), 8.39 (d, 1H, J = 8.4, Ar-H), 8.55 (d, 1H, J = 7.6, Ar-H)	
5i	3053–3021, 2918–2849, 1609, 579, 1516, 1453	2.29 (s, 3H, CH <sub>3</sub> ), 5.66 (s, 2H, -CH <sub>2</sub> -), 7.10 (d, 2H, J = 8.0, Ar-H), 7.20 (d, 2H, J = 7.6, Ar-H), 7.24 (s, 1H, Ar-H), 7.67–7.73 (m, 2H, Ar-H), 7.79 (t, 1H, J = 13.8, Ar-H), 8.16 (d, 1H, J = 8.0, Ar-H), 8.32 (d, 1H, J = 8.0, Ar-H), 8.61 (s, 1H, Ar-H). 21.17 (s), 29.79 (s), 44.90 (s), 111.72 (s), 114.02 (s), 121.31 (s), 125.40 (s), 126.48 (s), 127.21 (s), 128.01 (s), 129.32 (s), 129.58 (s), 133.09 (s), 133.48 (s), 137.64 (s), 138.71 (s), 139.69 (s), 140.92 (s), 142.73 (s)	
5j	3054–3022, 2972–2849, 1613, 1581, 1516, 1467	2.34 (s, 3H, -CH <sub>3</sub> ), 5.72 (s, 2H, -CH <sub>2</sub> -), 7.15 (d, 2H, J=8, Ar-H), 7.25 (d, 2H, J=8, Ar-H), 7.34–7.36 (m, 1H, Ar-H), 7.59–7.61 (m, 1H, Ar-H), 7.75–7.86 (m, 2H, Ar-H), 8.19–8.22 (m, 1H, Ar-H), 8.35–8.38 (m, 1H, Ar-H), 8.51 (d, 1H, Ar-H). 21.18 (s), 29.80 (s), 44.90 (s), 111.27 (s), 120.80 (s), 122.39 (s), 126.44 (s), 126.80 (s), 127.22 (s), 128.01 (s), 129.29 (s), 129.52 (s), 129.01 (s), 130.81 (s), 133.15 (s), 137.62 (s), 138.84 (s), 139.65 (s), 140.90 (s), 142.35 (s), 145.83 (s).	357 (100)
5k	3066–3024, 2917–2848, 1605, 1578, 1508, 1475	5.67 (s, 2H, -CH <sub>2</sub> -), 7.00 (t, 2H, J=16, Ar-H), 7.23–7.31 (m, 4H, Ar-H), 7.69–7.75 (m, 2H, Ar-H), 7.78–7.82 (m, 1H, Ar-H), 8.16 (d, 1H, J=8.8, Ar-H), 8.33 (d, 1H, J=8, Ar-H).	
5l	3054–3029, 2917–2849, 1613, 585, 1515, 1485	2.30 (s, 3H, CH <sub>3</sub> ), 5.68 (s, 2H, -CH <sub>2</sub> -), 7.12 (d, 2H, Ar-H), 7.22 (d, 2H, Ar-H), 7.29–7.37 (m, 3H, Ar-H), 7.69–7.73 (m, 1H, Ar-H), 7.77–7.81 (m, 1H, Ar-H), 8.15–8.18 (m, 1H, Ar-H), 8.33 (d, 1H, J=8.4, Ar-H).	
5m	3055–3026, 2919–2849, 1603, 579, 1506, 1488	2.55 (s, 3H, CH <sub>3</sub> ), 5.66, (s, 2H, -CH <sub>2</sub> -), 6.95–6.98 (m, 2H, Ar-H), 7.23–7.32 (m, 3H, Ar-H), 7.45 (d, 1H, J=8.4, Ar-H), 7.68–7.71 (m, 1H, Ar-H), 7.75–7.79 (m, 1H, Ar-H), 8.14 (d, 1H, J=8.8, Ar-H), 8.32 (d, 2H, J=6.8, Ar-H).	

were detected. Compounds **5a–m** showed absorption bands ranging from 3052–3000  $\text{cm}^{-1}$  for C-H aromatic stretching, 2960–2900  $\text{cm}^{-1}$  for C-H aliphatic stretching; there were also some bands for C=C and C=N at 1580–1575  $\text{cm}^{-1}$  and 1505–1480  $\text{cm}^{-1}$ , respectively (Table II). In  $^1\text{H}$  NMR spectra, the presence of a singlet between  $\delta$  5.8–5.6 ppm was observed for the methylene group and multiplets were observed between  $\delta$  8.55–7.00 ppm for aromatic protons. In the  $^{13}\text{C}$  NMR spectra, signals from  $\delta$  142.73 to 111.72 ppm were observed for aromatic carbon and  $\delta$  44.90 to 21.12 ppm for alkyl carbons. Fast atomic bombardment (FAB) mass spectra showed accurate molecular ion peaks at  $m/z$  338 and 357 for **5c** and **5j**, respectively (Table II).

The anti-cancer activity of compounds **5b**, **5d**, **5g** and **5l** at concentrations  $1.0 \times 10^{-5}$  mol  $\text{L}^{-1}$  was tested against 59 human tumor cell lines representing leukemia, melanoma and cancers of the lung, colon, brain, ovary, breast, prostate and kidney. The data for anti-cancer activity, in terms of the percent growth of treated cells, is given in Table III.

Table III. Growth of cancer cells (%) when treated with compounds **5b**, **5d**, **5g** and **5l**<sup>a</sup>

Panel/cell line	Percentage of growth (%)			
	<b>5b</b>	<b>5d</b>	<b>5g</b>	<b>5l</b>
Non-small cell lung cancer				
HOP-92	34.69	47.75	–	57.14
Colon cancer				
HCC-2998	55.67	–	–	–
HCT-116	69.24	–	69.57	54.68
Breast cancer	–	–	–	68.94
HS 578T				
Ovarian cancer	69.90	–	44.75	55.85
IGROV1				
Leukemia				
HL-60(TB)	–	–	–	30.11
K-562	–	32.40	–	69.85
MOLT-4	7.31	45.51	–	–
RPMI-8226	50.03	58.90	–	–
SR	–	–	–	18.70
Renal cancer				
UO-31	–	–	66.05	–
Melanoma				
SK-MEL-2	–	–	37.12	–
SK-MEL-5	–	–	59.20	–
Prostate cancer				
PC-3	–	–	–	58.63
CNS cancer				
SF-539	–	–	–	66.09
SNB-75	–	–	–	60.96

<sup>a</sup>  $c = 1.0 \times 10^{-1}$  mol  $\text{L}^{-1}$

Table IV. Cytostatic activity: IC<sub>50</sub> values

Compd.	L1210 <sup>a</sup>	Molt4/C8 <sup>a</sup>	CEM <sup>a</sup>
<b>5a</b>	289 ± 14	381 ± 136	303 ± 191
<b>5b</b>	91 ± 45	> 610	189 ± 52
<b>5c</b>	285 ± 24	272 ± 24	246 ± 59
<b>5d</b>	260 ± 78	389 ± 161	290 ± 22
<b>5e</b>	85 ± 2.8	> 552	≥ 552
<b>5f</b>	75 ± 17	513 ± 23	119 ± 55
<b>5g</b>	199 ± 27 <sup>c</sup>	170 ± 18 <sup>c</sup>	188 ± 40 <sup>c</sup>
<b>5h</b>	117 ± 74	71 ± 31	117 ± 19
<b>5i</b>	7.2 ± 5.3	222 ± 25 <sup>c</sup>	247 ± 28 <sup>c</sup>
<b>5j</b>	32 ± 25	284 ± 8 <sup>c</sup>	279 ± 7 <sup>c</sup>
<b>5k</b>	50 ± 27 <sup>b</sup>	> 500	400 ± 142 <sup>c</sup>
<b>5l</b>	164 ± 2.9 <sup>c</sup>	≥ 1465	764 ± 179 <sup>c</sup>
<b>5m</b>	32 ± 16	424 ± 81 <sup>c</sup>	> 500
Melphalan	2.1 ± 0.02	3.2 ± 0.6	2.5 ± 0.2

DMSO – negative control.

IC<sub>50</sub> – 50 % inhibitory concentration (μmol L<sup>-1</sup>) required to inhibit tumor cell proliferation by 50 %.

<sup>a</sup> Values are mean ± SEM, *n* = 2 to 3.

Significant difference *vs.* melphalan: <sup>b</sup> *p* < 0.05, <sup>c</sup> *p* < 0.01.

Synthesized compounds and melphalan as a standard were also evaluated for their *in vitro* cytostatic activity against human Molt 4/C8 and CEM T-lymphocytes as well as murine leukemia L1210 cells. The results of cytostatic activity are presented in Table IV.

Only melphalan showed IC<sub>50</sub> values consistently lower than 10 μmol L<sup>-1</sup> (IC<sub>50</sub> range 2.1–3.2 μmol L<sup>-1</sup>). The test compounds had IC<sub>50</sub> values ranging between 23 and ≥ 1465 μmol L<sup>-1</sup> (except for **5i** that had and IC<sub>50</sub> of 7.2 μmol L<sup>-1</sup> for L1210 cell proliferation). Compound **5h** emerged as the only compound that consistently inhibited cell proliferation of all three tumor cell lines at an IC<sub>50</sub> ranging between 71 and 117 μmol L<sup>-1</sup>. It is unclear why **5i** is markedly more cytostatic to L1210 cells than to the human lymphocyte cell lines. Also, for **5e**, **5j**, **5k**, **5l** and **5m**, there was a clear trend of higher cytostatic activity against the murine L1210 than against the human lymphocyte cell lines.

### Structure activity relation

Quinoxaline derivatives with substituents like F and CH<sub>3</sub> on benzyl at 4<sup>th</sup> position and F, CH<sub>3</sub>, Cl, Br at 9<sup>th</sup> position exhibited anticancer activity against various cell lines of HOP-92, HCT-116, IGROV1, K-562 Molt-4 and RPMI-8226. The order of potency was found to be **5l** > **5b** > **5d** ≥ **5g** as it is evident from the overall percentage of growth inhibition. In addition, compound **5l** is selective against prostate cancer.

Quinoxaline derivatives with substituents like F and CH<sub>3</sub> on 4-aralkyl at 6<sup>th</sup> position and Cl, F, Br and CH<sub>3</sub> at 9<sup>th</sup> position were studied for cytostatic activity against human



Molt 4/C8 and CEM T-lymphocytes as well as murine L1210 leukemia cells. In most cases, there was a more pronounced cytostatic activity against the murine leukemia L1210 cells than against the human lymphocyte cells. Among the tested compounds **5b**, **5e**, **5f**, **5h**, **5i**, **5j** and **5k** were moderately cytostatic for the leukemia cell line L1210, as it is evident from Table IV ( $IC_{50} < 100 \mu\text{mol L}^{-1}$ ). The order of cytostatic activity for L1210 was **5i** > **5f**  $\geq$  **5b**, therefore it suggests that by placing a halogen like bromine on the ninth position and a methyl on the 4<sup>th</sup> position of aralkyl on the 6<sup>th</sup> position of quinoxaline produced compounds which were less cytostatic than the standard melphalan. By keeping the electron donating groups like methyl on 6 and 9<sup>th</sup> position of aralkyl, there was not any improvement of cytostatic activity (**5c**).

## CONCLUSIONS

The reaction of 5-substituted isatin with orthophenylene diamine provided the corresponding quinoxalines **5a-m** in good yields. Investigations of cytostatic activities revealed better cytostatic activity of several compounds (**5b**, **5e**, **5f**, **5i**, **5j**, **5k**, **5m**) against the leukemia cell line L1210 than the human lymphocytic cell lines (the lowest  $IC_{50}$  of  $7.2 \mu\text{mol L}^{-1}$  was noted for **5i**) compared to melphalan ( $IC_{50}$   $2.1 \mu\text{mol L}^{-1}$ ).

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## S A Ž E T A K

### Sinteza, antitumorsko i citostatsko djelovanje derivata 6*H*-indolo[2,3-*b*]kinoksalina

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Reakcijom 1,5-disupstituiranih 2,3-dioekso-2,3-dihidroindola s ortofenilen diaminom sintetizirani su različiti 6-aralkil-9-supstituirani-6*H*-indolo[2,3-*b*]kinoksalini. Spojevi **5b**, **5d**, **5g** i **5l** pokazali su značajno antitumorsko djelovanje na 59 humanih tumorskih stanica. Svi sintetizirani spojevi ispitani su na citostatsko djelovanje na stanične linije Molt 4/C8 i CEM T-limfocite, te na murin L1210 stanice leukemije.  $IC_{50}$  za spoj **5h** je 71  $\mu\text{mol L}^{-1}$  na staničnu liniju Molt 4/C8 i 117  $\mu\text{mol L}^{-1}$  na CEM/0, dok su vrijednosti za melfalan 3,2, odnosno 2,5  $\mu\text{mol L}^{-1}$ .  $IC_{50}$  spoja **7i** na stanice L1210/0 je 7,2, dok je za melfalan 2,1  $\mu\text{mol L}^{-1}$ .

*Ključne riječi:* indolo[2,3-*b*]kinoksalin, citostatsko djelovanje, antitumorsko djelovanje

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