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Synthesis and Cytotoxic Activity of Pyridazino[1′,6′:1,2] pyrido[3,4-*b*]indol-5-inium Derivatives as Anti-Cancer Agents

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Abstract—Several new pyridazino[1′,6′:1,2]pyrido[3,4-*b*]indol-5-inium derivatives were synthesised from β-carboline derivatives and their cytotoxic activity and effect on the cell cycle were evaluated against L1210 cancer cells.

The term intercalator is applied to those compounds having a planar chromophore capable of stacking between the DNA base pairs.[1,2] Intercalation provokes conformational changes in the double helix, with consequences on the normal mechanisms of DNA replication, transcription, and repair.[3] Interferences with these functions usually results in non-specific cell killing.[4] Thus, potential applications can be devised in the field of antitumor drugs.[5–7]

A class of DNA intercalating agents is constituted by charged molecules, with azinium[8] and quinolizinium[9–13] salts being the most representative examples of this kind of polycyclic cations (Fig. 1). Our interest in the field focussed our studies on DNA intercalators having a bridgehead quaternary nitrogen, and led us to prepare some benzimidazolium[9,10] and γ-carbolinium[11–13] cations, which showed intercalating properties[10–13] and in vitro antiproliferative activity.[9]

The very related pyridazino[1′,6′:1,2]pyrido[3,4-*b*]indol-5-inium system has been also previously prepared in our laboratory by condensation of a β-carbolinium salt with 1,2-dicarbonyl[14] derivatives (Westphal reaction). The method was applied to the synthesis of the naturally occurring alkaloid flavocorylene and related zwitterionic indolo[2,3-*a*]quinolizinium compounds.[15]

Continuing our work in the field, we describe in this communication the synthesis of several new pyridazino[1′,6′:1,2]pyrido[3,4-*b*]indol-5-inium derivatives and their in vitro antiproliferative activity against L1210 leukaemia cell line. Starting from commercially available β-carboline derivatives harmane (**1**), harmol (**2**) or harmine (**3**), a series of compounds **4** (harmane series), **5** (harmol series) and **6** (harmine series) were prepared, with variations at the domains 1 (bromo, nitro, amino, alkoxy or hydroxy), 2 (carbamate, ester, amide, aliphatic saturated or unsaturated) and 3 (aliphatic saturated, unsaturated or aromatic) (Fig. 2).

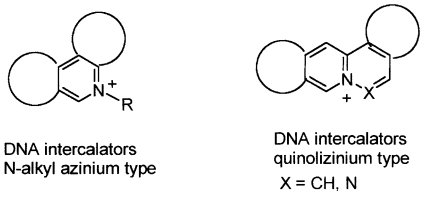

Figure 1. General structure for azinium and quinolizinium DNA intercalating agents.

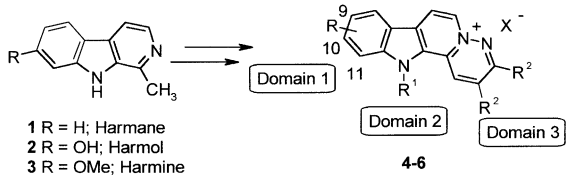

Figure 2. Starting materials **1**–**3** and general structure and domains explored for compounds **4**–**6**.

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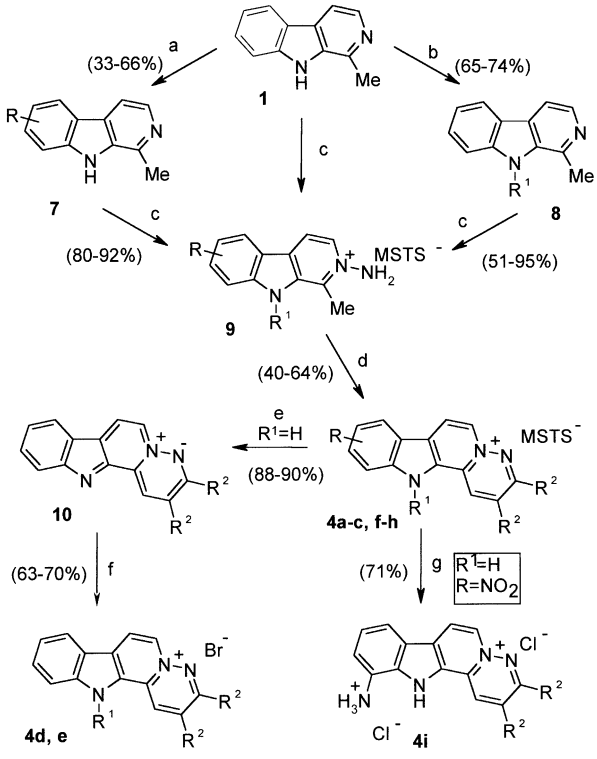
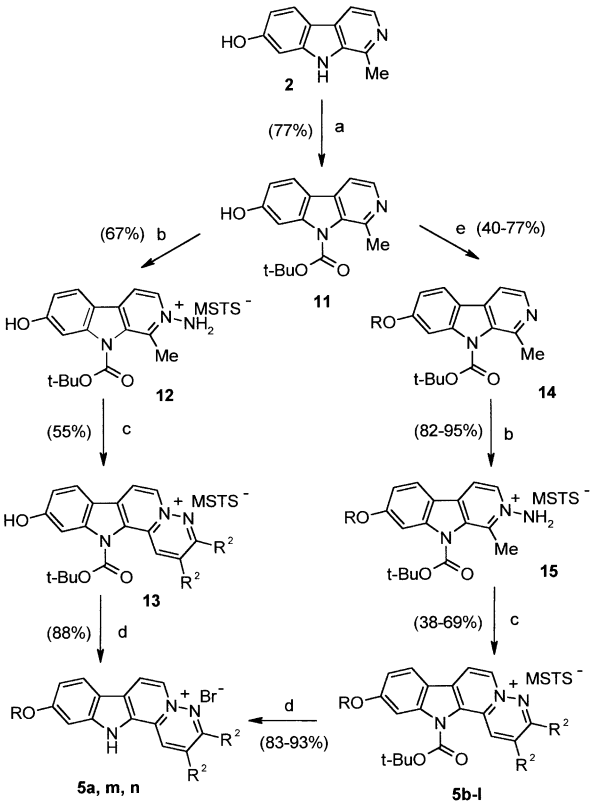
From **1**, bromo or nitro derivatives **7** were prepared.[16,17] On the other hand, **1** was alkylated at the indolic nitrogen to give **8**. Amination of **7** and **8** with *O*-(mesitylsulfonyl)hydroxylamine (MSH)[18,19] gave *N*-amino-β-carbolinium derivatives **9**, which then were reacted with 1,2-diketones through a Westphal reaction, to give **4a–c** and **4f–h**. Some **4** derivatives, unsubstituted at the indolic position, were then treated with base to give the ylide **10**, which was then alkylated to give **4d–e**. Finally, for **4h**, reduction with stannous chloride yielded **4i** (Scheme 1 and Table 1).

From **2**, indolic nitrogen was first protected as *terc*-butoxy carbamate **11**. Amination with MSH yielded **12**, which was then reacted with 3,4-hexanedione under Westphal process to give **13**. Carbamate was deprotected in acidic media to yield **5a**. On the other hand, **11** was reacted through a Williamson reaction with several alkyl halides to give **14**. Further reaction with MSH to give **15** and then, Westphal reaction with several 1,2-diketones led to **5b–l**. For compounds **5k** and **5l**, treatment with hydrobromic acid yielded **5m** and **5n** (Scheme 2 and Table 1).

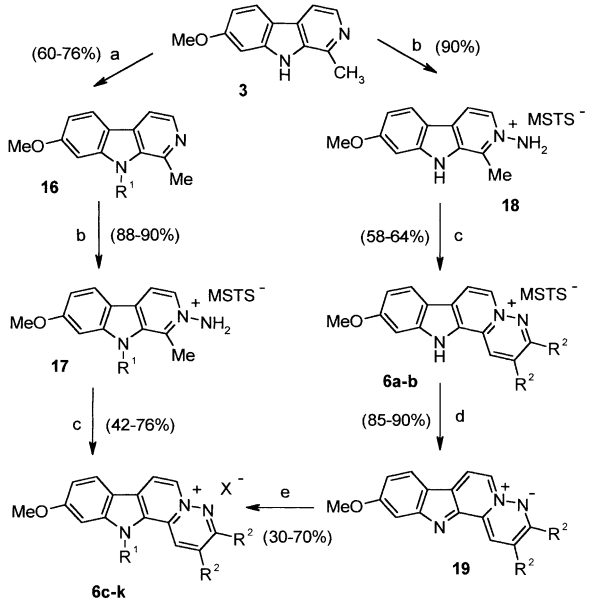
The last series were prepared from **3**. First, alkylation at the indolic nitrogen gave **16**, which was then reacted with MSH to give **17**. On the other hand, **3** was aminated by MSH to yield **18**.[15] Then, Westphal reaction was performed on **17** and **18** to give **6c–g** and **6a**,**b** respectively. Finally, compounds **6a** and **6b** were treated with base to give **19** which was then alkylated to yield **6h–k**. When the indolic nitrogen in **6** was substituted with an aliphatic chain bearing an ester group (**6i–k**) then, alkylation must be carried out after Westphal reaction, otherwise this reaction would completely fail. (Scheme 3 and Table 1).



Scheme 1. Reagents and conditions: (a) Br_2, THF or HNO_3; (b) RX, KOH, K_2CO_3, MeCN; (c) MSH, CH_2Cl_2; (d) 1,2-diketone, Et_3N, EtOH, reflux; (e) Et_3N, H_2O; (f) R^1X, μw, 10 min, 300 watt, two runs or R^1X, CH_2Cl_2; (g) $SnCl_2$, HCl, reflux.



Scheme 2. Reagents and conditions: (a) Boc_2O, Et_3N, DMAP, CH_2Cl_2; (b) MSH, CH_2Cl_2; (c) 1,2-diketone, Et_3N, EtOH, reflux; (d) HBr, acetone, reflux; (e) RX, KOH, K_2CO_3, MeCN.



Scheme 3. Reagents and conditions: (a) RX, KOH, K_2CO_3, MeCN; (b) MSH, CH_2Cl_2; (c) 1,2-diketone, Et_3N, EtOH, reflux; (d) Et_3N, H_2O; (e) R^1X, μw, 10 min, 300 watt, two runs or RX, CH_2Cl_2.

Table 1. Cytotoxicity and cell cycle effects against L1210 leukaemia for compound **1**

Compd	R	R^1	R^2	IC_{50} (μM)	Cell cycle[a]		Concn (μM)
					G_1[b]	G_2M[c]	
4a	H	$(CH_2)_3CCH$	1,8-Dinaphthyl	>4	+		1[d]
4b	H	$(CH_2)_3CCH$	Et	6.4	+		25[d]
4c	H	CH_2CO_2Et	1,8-Dinaphthyl	1.2	NS[e]	NS	10[d]
4d	H	$CH_2CONHCH_2CCH$	1,8-Dinaphthyl	10.4	NS	NS	50[d]
4e	H	$(CH_2)_3Br$	Et	1.9	NS	NS	20[d]
4f	9-Br	H	Et	2.3	NS	NS	10[d]
4g	9-NO_2	H	Et	15.3	NS	NS	50
4h	11-NO_2	H	Et	1.5	NS	NS	5[f]
4i	11-NH_2	H	Et	1.0	NS	NS	5[f]
5a	10-OH	H	Et	10.0	NS	NS	10
5b	10-OC_2H_5	Boc	Me	6.7	56		50
5c	10-O-C_2H_5	Boc	Et	0.5	64		5
5d	10-O-nC_3H_7	Boc	Et	0.46	NS	NS	5
5e	10-O-nC_4H_9	Boc	Me	1.3	NS	NS	10
5f	10-O-nC_4H_9	Boc	Et	0.36	+		5
5g	10-O-nC_5H_{11}	Boc	Me	0.23	NS	NS	5
5h	10-O-nC_5H_{11}	Boc	Et	0.28	+		2
5i	10-O-nC_7H_{15}	Boc	Et	0.13	62		2
5j	10-O-$nC_{10}H_{21}$	Boc	Et	0.17	63		0.5
5k	10-OBn	Boc	Me	2.3	NS	NS	10
5l	10-OBn	Boc	Et	0.9	59		2.5
5m	10-OBn	H	Me	5.2	NS	NS	10
5n	10-OBn	H	Et	1.9	+		20
6a	10-OMe	H	Me	1.3		38	5
6b	10-OMe	H	Et	2.0		35	5
6c	10-OMe	Me	$(i\text{-}Pr)_3SiCCH$	2.3	NE[g]		
6d	10-OMe	nC_5H_{11}	Me	0.048	57		1
6e	10-OMe	nC_5H_{11}	Et	0.065	60		1
6f	10-OMe	$(CH_2)_3CCH$	2-Furyl	1.3	+		5[d]
6g	10-OMe	$CH_2CONH\ CH_2CCH$	Et	72.9	NE		
6h	10-OMe	Me	Et	1.1	+		10[d]
6i	10-OMe	CH_2CO_2Et	Me	5.0	NS	NS	50
6j	10-OMe	CH_2CO_2Et	Et	1.1		++	5
6k	10-OMe	$(CH_2)_3CO_2Et$	Et	2.0	NS	NS	10
Adriamycin				0.025		81	0.1
Camptothecin				0.03		80	0.05

[a]Percent of untreated control L1210 cells in the phases of the cell cycle: 41% (G_1), 24% (G_2M).
[b]Percent of treated cells in the G_1 phase: (+) 54–64%; (++) 65–75%.
[c]Percent of treated cells in the G_2M phase: (+) 35–45%; (++) 46–66%.
[d]Toxic.
[e]NS, not significant.
[f]30% apoptosis (10 μM).
[g]NE, not evaluated.

Cytotoxic activity

Compounds **4**–**6** were tested in vitro against L1210 leukaemia and for their effect on the L1210 cell cycle.[20,21] Results are reported in Table 1.

The activity data show that compounds **5i** and **5j** were the most active in the harmol series. In the harmine series compounds **6d** and **6e** were the most active prepared so far. They showed good activity as compared to adriamycin and camptothecin.[22] From cytotoxicity data it can be deduced than a lypophillic side chain, more than seven atoms long at C10-position is preferred in domain 1 (**5i**, **5j**). For domain 2, lypophillic chains at least five atoms long give better activities (**6d**, **6e**). In domain 3, aliphatic groups are preferred. On the other hand, it is interesting to notice than the most cytotoxic compounds have effect in the G_1 phase of the cell cycle while the reference compounds exert their effect in the G_2M phase.

As a result, a series of new pyridazino[1′,6′:1,2]pyrido[3,4-*b*]indol-5-inium derivatives have been prepared and their cytotoxic activity tested in vitro against L1210 leukaemia and for their effect on the L1210 cell cycle. Two compounds, **6d** and **6e** showed activity at the same level than adriamycin and camptothecin, and exert their effect in the G_1 phase of the cell cycle.

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22. **6d**: ^{1}H NMR (300 MHz, $DCCl_3$) δ 8.98 (d, 1H, $J=7.0$ Hz); 8.65 (s, 1H); 8.56 (d, 1H, $J=7.0$ Hz); 8.19 (d, 1H, $J=8.7$ Hz); 7.23 (s, 1H); 7.04 (d, 1H, $J=8.7$ Hz); 6.71 (s, 2H); 4.85–4.62 (m, 2H); 3.97 (s, 3H); 2.74 (s, 3H); 2.67 (s, 3H); 2.49 (s, 6H); 2.11 (s, 3H); 1.89–1.86 (m, 2H); 1.42–1.25 (m, 4H); 0.84 (t, 3H, $J=6.8$ Hz). Anal. calcd for $C_{31}H_{37}N_3O_4S$, C: 61.88; H: 7.20; N: 6.98; found C: 61.74; H: 7.37; N: 7.07. **6e**: ^{1}H NMR (300 MHz, $DCCl_3$) δ 9.13 (d, 1H, $J=7.2$ Hz); 8.67–8.65 (m, 2H); 8.31 (d, 1H, $J=8.7$ Hz); 7.37 (s, 1H); 7.16 (d, 1H, $J=8.7$ Hz); 6.78 (s, 2H); 4.96–4.93 (m, 2H); 4.04 (s, 3H); 3.23 (c, 2H, $J=7.0$ Hz); 3.07 (c, 2H, 7.2 Hz); 2.55 (s, 6H); 2.18 (s, 3H); 2.05–1.99 (m, 2H); 1.57–1.48 (m, 10H); 0.93 (t, 3H, $J=7.2$ Hz). Anal. calcd for $C_{33}H_{41}N_3O_4S$. $2H_2O$, C: 66.75; H: 7.30; N: 7.08; found C: 66.97; H: 7.31; N: 6.85.