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New benzo[5,6]pyrrolizino[1,2-*b*]quinolines as cytotoxic agents

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Abstract—An assessment of structure–activity relationships associated with the new benzo[5,6]pyrrolizino[1,2-*b*]quinoline system displaying potent in vitro cytotoxic activity against the MCF7 cell line is described.

The quinoline pattern is found in a large number of natural products and drug-like compounds known as antitumoural agents, such as camptothecin,[1] luotonin,[2] ascididemin,[3] TAS-103,[4] cryptolepin,[5] indolo[2,3-*b*]quinolines[6]... Similarly, the alkoxyindole nucleus is often seen in various compounds (Fig. 1) known for their cytotoxic activity such as rebeccamycin[7] or azatoxin derivatives,[8] retelliptine[9] or voacangine.[10]

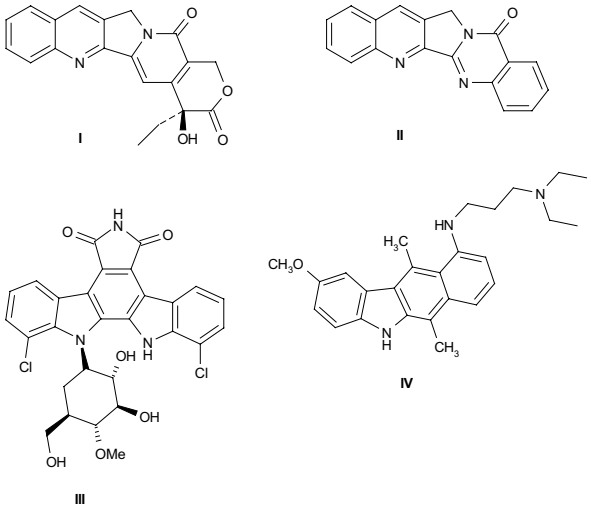

Figure 1. Structures of camptothecin (**I**), luotonin (**II**), rebeccamycin (**III**) and retelliptine (**IV**).

Our approach was to link these two bicyclic skeletons by a pyrrolidine ring, as is found in camptothecin (CPT), leading to a new condensed pentacyclic skeleton, benzo[5,6]pyrrolizino[1,2-*b*]quinoline. The corresponding compounds retain their originality as to the arrangement of the quinoline, pyrrolidine and indole moieties giving the molecule a more extended and linear geometry than camptothecin offers and, in this way, improving their DNA-binding activity.

We report here the synthesis and cytotoxic properties of some substituted benzo[5,6]pyrrolizino[1,2-*b*]quinolines.

In order to establish significant structure–activity relationships for this class of compounds (Fig. 2), different substituents were investigated on positions 2, 8 and 13: a hydrogen -donor/-acceptor bond (hydroxyl and/or methoxy) on the lateral homocycles and a positively charged chain able to engage electrostatic bonds with DNA phosphates (piperidinoethoxy fragment) on carbons 2 and 8 and a methyl or an ethyl group on carbon 13 of quinoline.

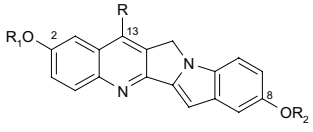

Figure 2. Structure of benzo[5,6]pyrrolizino[1,2-*b*]quinolines.

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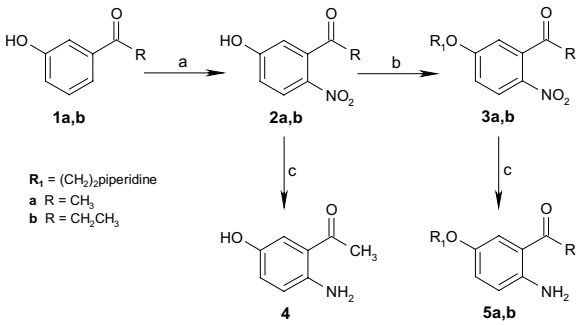
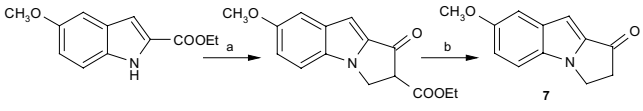


Scheme 1. Synthesis of *o*-aminoketones **4**, **5**. Reagents and conditions: (a) (i) HNO_3–AcOH, 70 °C; (ii) column chromatography CH_2Cl_2–AcOEt, 30–35%; (b) $Cl(CH_2)_2$piperidine, K_2CO_3, DMF, 80 °C, 3 h, 66–92%; (c) Fe, HCl, EtOH–AcOH–H_2O, reflux, 15 min, 66–88%.

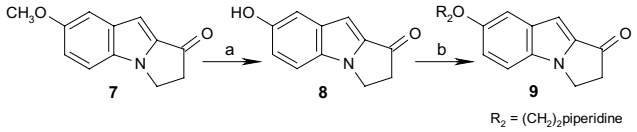
The benzo[5,6]pyrrolizino[1,2-*b*]quinoline scaffold was synthesised on the basis of the Friedländer method for the quinoline preparation, using the appropriate *o*-aminoketone and the enolisable pyrroloindole. *O*-aminoacetophenones **4** and **5a** were obtained (Scheme 1) by nitrating[11] 3-hydroxyacetophenone **1a** followed by the O-alkylation of the phenol, or Bechamp reduction of the nitro group.

O-Aminopropiophenone **5b** was prepared from 3-hydroxypropiophenone[12] (**1b**) whose nitration (HNO_3–CH_3COOH) gave the 6- and 4- (**2b**) regioisomers; after separation by column chromatography (SiO_2, CH_2Cl_2–AcOEt 9/1) the phenol group of **2b** was alkylated and the nitro group reduced. Pyrroloindole **7** (Scheme 2) was obtained according to the pathway described[13] for the analogue devoid of methoxy substituent.

Boron tribromide enabled O-demethylation (Scheme 3), before O-alkylation (Williamson conditions) to give pyrroloindole **9**.



Scheme 2. Synthesis of pyrroloindole **7**. Reagents and conditions: (a) *t*-BuOK, CH_2=CHCOOEt, toluene, reflux, 4 d, 90%; (b) AcOH, reflux, 24 h, 80%.



Scheme 3. Synthesis of pyrroloindoles **8**, **9**. Reagents and conditions: (a) BBr_3, CH_2Cl_2, rt, 3 h, 55%; (b) $Cl(CH_2)_2$piperidine, DMF, 80 °C, 4 h, 52%.

Finally, the Friedländer reaction between *o*-acylanilines and pyrroloindoles (Scheme 4) was carried out in the previous conditions,[14] namely butan-1-ol with pyridinium *p*-toluenesulfonate as catalyst and azeotropic distillation of water.

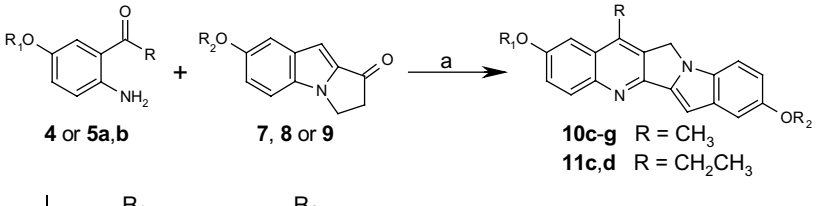
All the compounds, except **11d**, were tested for in vitro cytotoxic activity against the mouse leukemic cell line L1210,[15] the human breast cancer cell line MCF7 and the prostatic one PC3.[16] The results are shown in Table 1.

If compounds with a hydroxyl group on the quinoline moiety show a slight antiproliferative activity (**10f**,**g**), their analogues **10c**–**e** with a side chain on this scaffold exhibit a higher cytotoxic effect. The piperidinoethoxy group, borne by the quinoline moiety, seems to be crucial for cytotoxic activity, as we have demonstrated in a previous study on indolizino[1,2-*b*]quinolines[17] whose condensed tetracyclic structure is derived from camptothecin. Comparison of cytotoxic activity against the

Table 1. In vitro cytotoxicity activities of benzopyrrolizinoquinoline derivatives **10**, **11**

Compound	IC_{50} (μM)		
	Mouse leukemia L1210	Human breast MCF7	Human prostate PC3
10c	0.55	0.55	4.94
10d	3.93	5.30	5.50
10e	0.55	4.60	5.30
10f	3.40	>50	>10
10g	0.60	12.0	>50
11c	0.55	6.30	4.80
11d	—[a]	—[a]	—[a]
CPT	0.06	0.32	0.31

[a] Relative insolubility in culture medium (DMSO ≤ 1‰).



	R_1	R_2
c	$(CH_2)_2$piperidine	H
d	$(CH_2)_2$piperidine	CH_3
e	$(CH_2)_2$piperidine	$(CH_2)_2$piperidine
f	H	H
g	H	CH_3

Scheme 4. Synthesis of benzo[5,6]pyrrolizino[1,2-*b*]quinolines **10**, **11**. Reagents and conditions: (a) PPTS, butan-1-ol, reflux, 2 h, 38–57%.

MCF7 cell line of **10c**,**d** with **11c**,**d** revealed that a methyl substituent on position 13 is better than an ethyl one as in Irinotecan. Analogue **10c** with a hydroxyl group on the indole pattern is more active than those with a piperidine side chain (**10e**) or a methoxy group (**10d**). Moreover, it seems that a group acting as a hydrogen bond donor and acceptor is essential on the indole pattern, as indicated by the better activity of **10c** (bearing a hydroxyl group) compared to **10e** (with a piperidine side chain) and to **10d** (bearing a methoxy group). If the piperidine side chain on the quinoline moiety is important for cytotoxic activity, a methyl substituent is better than an ethyl one, probably due to the lack of water solubility or to steric constraint. Position 8 is best substituted by a hydroxyl group, hydrogen bond donor and acceptor.

We have therefore demonstrated that the benzo[5,6]-pyrrolizino[1,2-*b*]quinoline skeleton exhibits cytotoxicity in the micromolar range against several cancer cell lines. Our data indicates that this original polycyclic structure, which is quite linear, induces good cytotoxicity. Studies directed to identifying the mode of biological action of these analogues are underway.

Acknowledgements

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15. Cytotoxic assay: Leukemic L1210 cells were maintained in RPMI 1640 culture medium supplemented with 10% FCS. For growth assay, the cells were seeded onto 24-well plates at a density approximatively 10^5 cells/well. The tested compounds were added to the culture medium and incubation was performed for 72 h. Cell growth was assessed by numeration on Nageotte hemacytometer.
16. Cytotoxic assay: Human breast cancer MCF7 and prostate cancer PC3 cells were, respectively, maintained in MEM and RPMI culture medium supplemented with 10% FCS. For growth assay, the cells were seeded onto 24-well plates at a density of, respectively, 3×10^5 cells/well and 2×10^5 cells/well. After 24 h, the tested compounds were added to the culture medium for 72 h. Cell growth was assessed by the colorimetric MTT test.
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