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# SYNTHESIS AND CYTOTOXICITY OF ANALOGUES OF THE ANTIBIOTIC BE 10988 INHIBITORS OF DNA TOPOISOMERASE II

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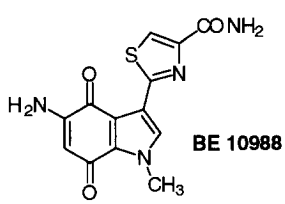
**Abstract:** Indolequinone derivatives of the antitumour antibiotic BE 10988 were synthesized and evaluated for their cytotoxicity and action mechanism. The quinone system is essential to biological activity and the thiazole ring plays a major role in the poisoning of topoisomerase II. 

Indolequinones represent an important series of anticancer agents. In 1998, a large number of 1-methylindole-4,7-diones disubstituted at positions 2 and 5 and bearing a variety of leaving groups at position 3 were developped as bioreductively-activated prodrugs to target a hypoxic sub-population of cells in human tumours.[1,2] This family of compounds, typified by the archetypal bioreductive quinone EO9 and the mitosene antitumour drugs, generally alkylate DNA via highly reactive electrophilic sites formed upon the reduction of the *p*-quinonoid moiety in the presence of reductive enzymes.[2]

In contrast, the indolequinone antibiotic BE 10988 does not bind covalently to DNA but triggers double-stranded cleavage of DNA *via* an inhibition of the ubiquitous enzyme topoisomerase II.[3] This DNA relaxing enzyme is essential to the control of DNA topology in cells and represents the primary target of several clinically used anticancer drugs including etoposide and mitoxantrone.[4] BE 10988 was isolated from the culture broth of a strain of actinomycetes in 1991.[3]




**BE 10988**

This indolequinone substituted by a thiazole ring was shown to be an inhibitor of topoisomerase II endowed with marked cytotoxic activities against a panel of human tumour cell lines.[3] The total synthesis of the antibiotic has been described[5] but the structure-activity relationships (SAR) in this series remain as yet largely unknown.[6] On the basis of our long experience in the synthesis of thiazole-containing antitumour agents[7] and in the development of topoisomerase II poisons,[8] we decided to explore the SAR in this series.

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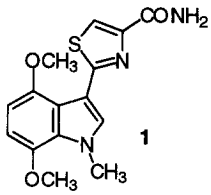
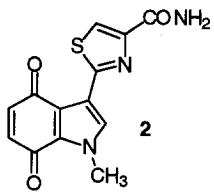
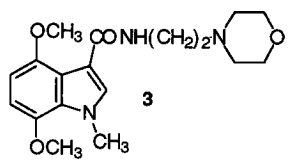
Two pairs of indolequinone derivatives were prepared. Compounds **1** and **2** possess the thiazole ring characteristic of the natural product but lack the 5-amino group. In an effort to test the importance of the thiazole moiety and to increase the solubility of the drugs in aqueous media, the thiazole ring was replaced with a longer side chain containing a morpholino group, and resulting in compounds **3** and **4**. In both cases, we compared the biological activity of indolequinones **2** and **4** with that of the corresponding 4,7-dimethoxyindole derivatives **1** and **3**.



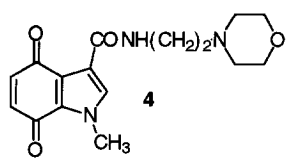

**1**




**2**




**3**



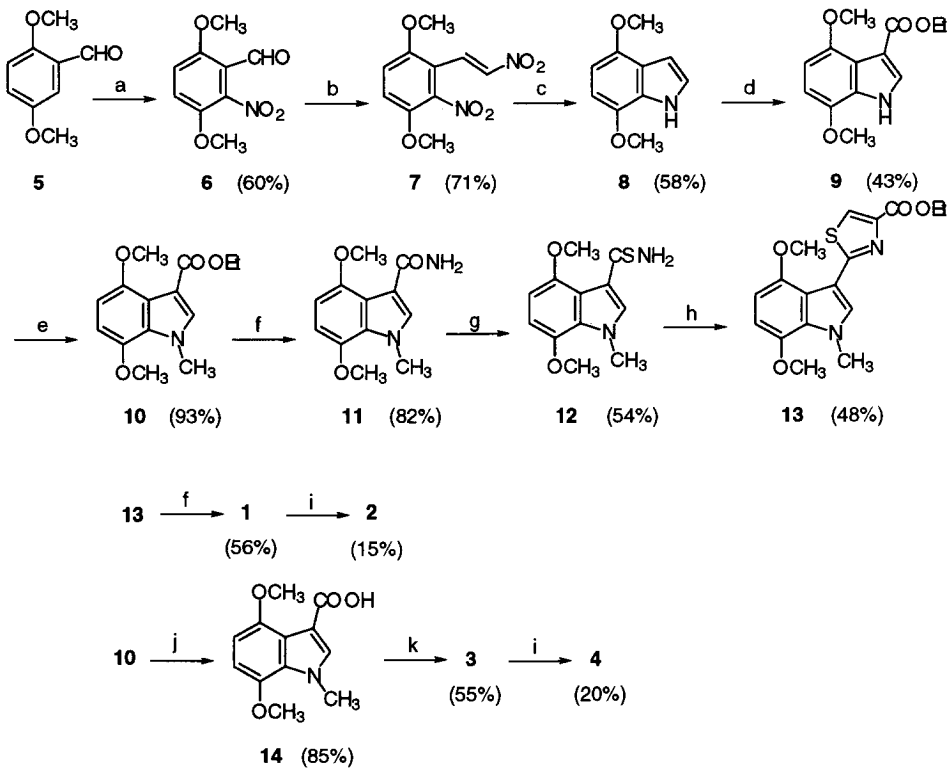

**4**

**Synthesis**. 4,7-Dimethoxyindoles **1**, **3** and 4,7-indolequinones **2**, **4** were prepared from ethyl 3-indolecarboxylate **9**. The indole heterocycle was obtained[9] from 2,5-dimethoxybenzaldehyde **5** by regioselective nitration giving 2-nitrobenzaldehyde **6**, followed by a Perkin type reaction to result in 2-nitro-β-nitrostyrene **7**. Catalytic hydrogenation with $Pd(OH)_2$ favoured cyclisation into indole **8** which was reacted with ethyl chloroformate after formation *in situ* of organomagnesium bromide to create ester **9**. Subsequent N-alkylation (**10**) sequentially followed by amidification with formamide (**11**), thioamidification with Lawesson's reagent (**12**) and cyclisation to thiazole with ethyl bromopyruvate led to 3-thiazoloindole **13**. Carboxamide **1** was obtained by the previously described amidification procedure and further oxidation under Trahanovsky conditions (CAN in aqueous acetic acid) gave indoloquinone **2**. Amide **3** was prepared in two steps from ester **9** by saponification leading to acid **10** followed by treatment with 2-morpholinoethylamine (and formation *in situ* of the corresponding acid chloride). Indoloquinone **4** resulted from the same oxidation procedure as for **2**.

**Cytotoxicity**. The four synthesized compounds were evaluated for their antiproliferative activities using the murine L1210 leukemia cell line.[10] The results expressed as $IC_{50}$ values (concentration reducing by 50% the cell proliferation) are reported in the table 1. It appears that the indolequinone skeleton is essential to the activity since compounds **2** and **4** are cytotoxic toward the L1210 cells whereas the corresponding 4,7-dimethoxy indole derivatives **1** and **3** are devoided of any significant cytotoxicity. Compound **2** which bears the 4-aminocarbonylthiazole moiety of BE10998 is about 8-fold more active than the morpholino derivative **4** suggesting thus that the thiazole ring is also likely to play a determining role for the cytotoxic action.

Table 1: Cytotoxicity

| Cpds | $IC_{50}$ (μM) |
|---|---|
| **1** | >10 |
| **2** | 0.8 |
| **3** | >100 |
| **4** | 6.5 |




**Reagents and conditions**: (a) $HNO_3$/AcOH, 0°C; (b) $CH_3NO_2$, $NH_4OAc$, 80°C; (c) $H_2$, $Pd(OH)_2/C$; (d) EtMgBr, then $ClCO_2Et$; (e) NaH, $CH_3I$; (f) $HCONH_2$, $CH_3ONa$, DMF; (g) Lawesson's reagent/THF; (h) ethyl bromopyruvate/$CH_2Cl_2$; (i) CAN, AcOH/$H_2O$; (j) 2N NaOH; (k) oxalyl chloride/$CH_2Cl_2$, then 4-(2-aminoethyl)morpholine, $K_2CO_3$.

**Binding to DNA**. Figure 1 displays the absorption spectra of **2** and **4** in the absence and presence of DNA. In both cases, a marked hypochromism can be detected attesting that the ligands have significant interaction with DNA. The absorption peaks centered at 258 (**2**) or 274 nm (**4**) decrease to a DNA-phosphate/drug ratio (P/D) of about 20 and then remain constant at higher P/D when the ligand is fully bound to DNA. However, the interaction of the drugs with DNA causes no bathochromic shift and the ligands do not stabilize DNA against heat denaturation. The *T*m values of helix-to-coil transition of calf thymus DNA and poly(dA-dT)•(dA-dT) remained unchanged upon complex formation of DNA with the ligands. It is therefore unlikely that the drugs intercalate into DNA. The binding must be non-specific.

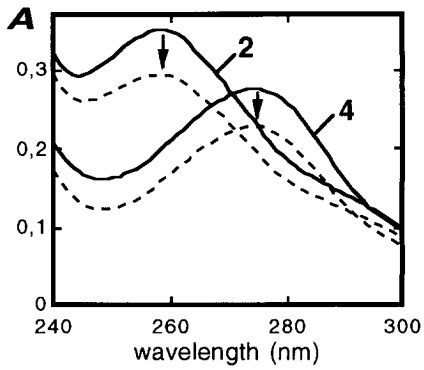



**Fig. 1** Absorption spectra of compounds **2** and **4** (20 μM each) in the absence (full line) and presence (dashed line) of calf thymus DNA (100 μM).

**Topoisomerase II inhibition**. Thiazolylindolequinone **2** is more effective than the piperidino analogue **4** on inhibiting the decatenation of kinetoplast DNA by purified human topoisomerase II. Concentrations of 25 and 80 μM are required to inhibit the decatenation of DNA by 50% with **2** and **4**, respectively (Figure 2). Another experimental approach was used to further investigate the anti-topoisomerase II effects of the drugs. Closed circular DNA was treated with topoisomerase II in the presence of increasing concentrations of the test drugs. Supercoiled plasmid DNA was relaxed by the enzyme in the absence of the drug. In the presence of etoposide, a strong band corresponding to linear DNA (form III) could be seen, proving that this antitumour drug inhibits the religation of DNA once the double helix is cleaved by the enzyme. As shown in Figure 3, the same band can be detected with 50 μM compound **2** but its intensity is much weaker than with etoposide. In this assay, no significant effect was detected with **4**. With **2**, the extent of DNA cleavage is proportional to drug concentration but the band of linear DNA never exceeded 8% of the DNA products even using drug concentrations as high as 100 μM. This set of data confirms that **2** is a weak inhibitor of topoisomerase II.

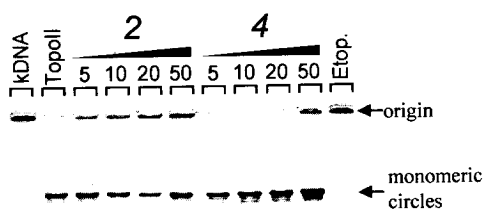



**Figure 2**: Effect of increasing concentrations of **2** and **4** on the decatenation of kinetoplast by topoisomerase II. The DNA (0.5 μg) (lane DNA) was incubated with 4 units topoisomerase II in the absence (lane TopoII) or presence of drug at the indicated concentration (μM). Etoposide (lane Etop.) was used at 50 μM. Reactions were stopped with sodium dodecylsulfate and treatment with proteinase K. DNA samples were separated by electrophoresis on a agarose gels containing ethidium bromide.

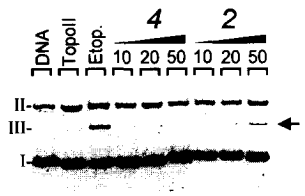



**Figure 3**: Effect of increasing concentrations of **2** and **4** on the relaxation of supercoiled DNA by topoisomerase II. The DNA (0.5 μg) (lane DNA) was incubated with 4 units topoisomerase II in the absence (lane TopoII) or presence of drug at the indicated concentration (μM). Etoposide (lane Etop.) was used at 50 μM. Reactions were stopped with sodium dodecylsulfate and treatment with proteinase K. DNA samples were separated by electrophoresis on a agarose gels containing ethidium bromide. Nck, nicked; Lin, linear; Rel, relaxed; Sc, supercoiled.

In addition, we studied the inhibition of topoisomerase II using a radiolabelled DNA substrate. The 117bp *Eco*RI-*Pvu*II restriction fragment from plasmid pBS was incubated with the drugs prior to cleavage by topoisomerase II. A few cleavage sites were sequenced (Figure 4). Very little effect was seen with **4** and the cutting sites detected with **2** were much weaker than those obtained with etoposide, concording with the results presented in Figure 3. However, there is no doubt that compound **2** stimulates DNA cleavage by the enzyme (arrows in Figure 4). The cleavage patterns observed with **2** are quite similar to those seen with etoposide, the majority of the cleavage sites being identical. Taken together, these various experiments with human topoisomerase II enzyme indicate that thiazolylindole quinone **2** functions as a weak but noticeable topoisomerase II poison stabilizing DNA-topoisomerase II covalent complexes.

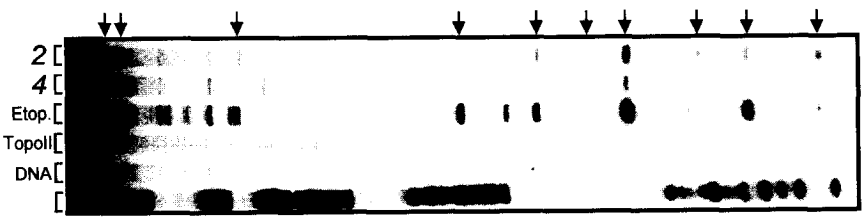



**Figure 4**: Sequence analysis of the topoisomerase II cleavage sites stimulated by indolequinones. The 117-mer DNA fragment from pBS, labelled at the 5'-end, was incubated in the absence (lane TopoII) or presence of the drug at the indicated concentration (μM). Etoposide was used at 50 μM. Topoisomerase II cleavage reactions were analyzed on an 8% denaturing polyacrylamide gel. The arrows point to the cleavage sites stimulated by **2** or etoposide on the sequence.

**Conclusion**. The results show that thiazolylindolequinone analogue **2** acts as a topoisomerase II poison as does the antibiotic BE10988. But enzyme inhibition is observed for drug concentrations well over the $IC_{50}$ value. Therefore, it is likely that the cytotoxicity of molecules is not directly linked to the inhibition of topoisomerase II.[6] The fact that the quinone system is required for cytotoxicity suggests that a bioreductive pathway is involved in the action mechanism.

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10. L1210 murine leukemia cells (provided by the NCI, Frederick, USA) were grown in RPMI 1640 medium supplemented with 10% fetal calf serum, 2 mM glutamine, 100 units/ml penicillin, 100 μg/ml streptomycin, and 10 mM HEPES buffer, pH 7.4. Cytotoxicity was measured by the standard microculture tetrazolium assay. Cells were exposed to graded concentrations of the compounds for 48 h and results expressed as $IC_{50}$ (concentration which reduced by 50% the optical density of treated cells with respect to untreated controls). We are grateful to the Institut de Recherches Servier for performing these tests.