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TARGETING DNA TOPOISOMERASE II WITH PODOPHYLLOTOXIN AZA-ANALOGUE

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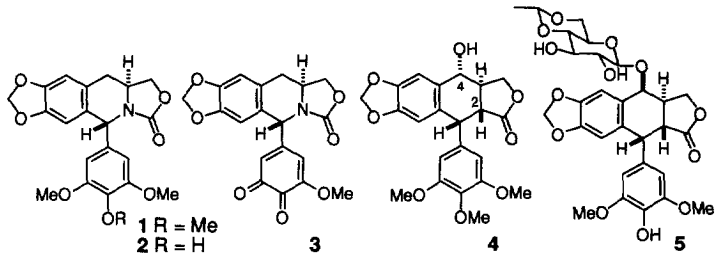
Abstract: Oxidation of a cytotoxic podophyllotoxin aza-analogue **2** into the quinone **3** succeeded in acquiring DNA topoisomerase II inhibitory activity at a concentration of 25 μM due to stabilization of the cleavable complex. © 1997 Elsevier Science Ltd.

DNA topoisomerases (Topo) are ubiquitous enzymes that alter the topological state of DNA, thus playing crucial roles in several biological processes.[1] Inhibitors of these enzymes are of special interest since these can be promising candidates for antitumor drugs.[2] In our continuing studies towards antitumor compounds,[3] we have already succeeded in design and synthesis of the podophyllotoxin aza-analogues[4] **1** and **2** based on microtubule assembly inhibitor podophyllotoxin (**4**)[5] and Topo II inhibitor etoposide (**5**).[6,7]



Aza-analogue **1** exhibited cytotoxicity due to inhibition of microtubule assembly, while **1** did not inhibit Topo II activity. Furthermore, **2** exhibited no Topo II inhibitory activity in spite of the monophenolic form, which is essential for Topo II inhibition and DNA breakage by **5**.[8] The same lack of activity of aza-etoposide has been reported.[9] By chemical modification of the phenolic moiety of **2**, it became apparent that the quinone **3** and the corresponding catechol congener do exhibit *in vitro* Topo II inhibition. We describe herein inhibitory effect of **3** on human Topo II.

A solution of racemic **2** in $CHCl_3$ was treated with 6M HNO_3 at rt for 30 min. Usual workup and purification through SiO_2 column chromatography ($CHCl_3/Me_2CO$ = 6/1) gave **3** in 99% yield.[10]

Topo II inhibition by **3** was observed through the conversion of catenated kinetoplast DNA (kDNA) to minicircle monomers.[11] The quinone **3** inhibited the catalytic Topo II activity in a dose-dependent manner where decatenation of kDNA was completely blocked at a concentration of 25 μM and more than 50% even at 12.5 μM as shown in Fig 1. Incubation of **3** with pUC19 DNA in assay buffer (37 °C, 30 min, no enzyme) did not break, bind to or intercalate to DNA over the range 50 – 400 μM.[6] Furthermore, DNA cleavage assay afforded linear DNA that generated *via* double-strand DNA breaks.[11] These behaviors strongly suggest that **3**

stabilizes the cleavable complex of DNA and Topo II as does **5** (not shown). On the other hand, **3** did not affect relaxation of pUC19 supercoiled DNA induced by Topo I at a concentration of less than 400 μM. Accordingly, the quinone **3** is a novel non-intercalative Topo II specific inhibitor,[12] which suggests that quinone or catechol structure of etoposide **5** might be a form to stabilize the cleavable complex.

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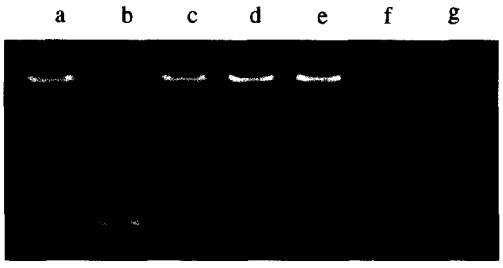

Figure 1. Effect of **3** on the decatenation activity of human Topo II.
a, catenated kDNA alone; *b*, decatenated minicircle control; *c* to *g*, decatenation in the presence of 100, 50, 25, 12.5 and 6,25 μM of **3**, respectively.

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10. The catechol congener was obtained as the minor product in transformation of **1** to **2**[4] or $Na_2S_2O_4$ reduction of **2**. Satisfactory spectroscopic and analytical data of **2** and **3** were obtained.
11. The standard reaction mixture contained 50 mM Tris-HCl (pH 8.0), 120 mM KCl, 10 mM $MgCl_2$, 0.5 mM ATP, 0.5 mM DTT, kDNA (200 ng), 2 μl of sample solution (10% DMSO) and 1 unit of human placenta topoisomerase IIα (170 KDa) in a total volume of 20 μl. The reaction was incubated at 37 °C for 30 min and terminated with 2 μl of stop buffer (5% sarkosyl, 0.0025% bromophenol blue, 25% glycerol). Reaction products were electrophoresed on a 1% agarose gel in TAE (Tris-acetate-EDTA) running buffer. Both agarose gel and running buffer contained 0.5 μg/ml ethidium bromide.
    For cleavage assay, reactions (100 μM of sample, 8 units of the enzyme and 200 ng of pUC19 plasmid DNA) were incubated in cleavage buffer (30 mM Tris-HCl (pH 7.6), 60 mM NaCl, 8 mM $MgCl_2$, 3 mM ATP, 15 mM mercaptoethanol), followed by digestion with proteinase K prior to loading to the gel.
12. We have observed that the aza-etoposide quinone congener also inhibited Topo II activity.

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