Communication

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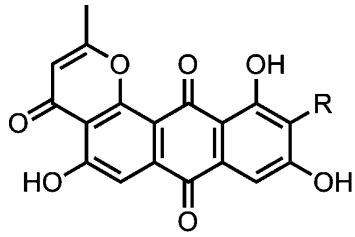
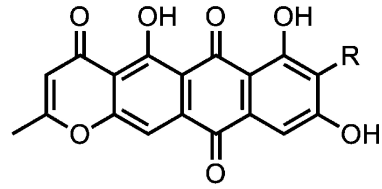
J. Am. Chem. Soc., **2008**, 130 (39), 12888-12889 • DOI: 10.1021/ja805421b • Publication Date (Web): 06 September 2008



topopyrone A R=Cl
topopyrone C R=H



topopyrone B R=Cl
topopyrone D R=H

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Published on Web 09/06/2008

The Topopyrones Poison Human DNA Topoisomerases I and II

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Human DNA topoisomerases are essential for the processes of chromosomal segregation and relaxation of DNA during replication and transcription, and may also be required for recombination.[1] Generally, topoisomerase poisons convert a functional enzyme to a DNA damaging agent by trapping the covalent binary complex formed between a topoisomerase and its DNA substrate.[2] DNA topoisomerase I is a ubiquitous nuclear enzyme which catalyzes the relaxation of superhelical DNA by inducing a transient single strand nick in the duplex through cycles of cleavage and religation.[3] In contrast, DNA topoisomerase II mediates the ATP-dependent induction of coordinated nicks in both strands of the DNA duplex, followed by passage of another double strand DNA through the transiently broken duplex.[4]

Several human topoisomerase II-directed agents are used clinically for antitumor therapy, including etoposide, doxorubicin, amsacrine, and mitoxantrone.[5] In contrast, only a single family of topoisomerase I-targeted agents, the camptothecins,[2b] are used clinically at present.[6] However, several promising new topoisomerase I poisons have been identified, including indolocarbazoles,[7] indenoisoquinolines,[8] terbenzimidazoles,[9] aporphine alkaloids,[10] and the topopyrones.[11]

The topopyrones are planar anthraquinones having a fused 1,4-pyrone ring; they bear structural relationships both to doxorubicin and to some of the pluramycins.[12] Four topopyrones (A− D) (Figure 1) have been isolated from the culture broths of two fungi, *Phoma* sp. BAUA2861 and *Penicillium* sp. BAUA4206.[11] The four topopyrones strongly inhibited the growth of yeast harboring a plasmid for human topoisomerase I and did so only under conditions leading to expression of human topoisomerase I. They were also reported to inhibit DNA relaxation mediated by topoisomerase I, but not by topoisomerase II.[11a] While the study of topopyrones has been limited by their exclusive availability as natural products, recent synthetic efforts have now provided access to all four naturally occurring topopyrones[13] as well as synthetic analogues.[13c] Presently we report that in addition to stabilization of the topoisomerase I−DNA covalent binary complex,[11a] the topopyrones are also strong topoisomerase II poisons.

Shown in Figure 2 is the effect of topopyrones A−D on the topoisomerase I-mediated cleavage of a 23 bp double strand DNA derived from the *Tetrahymena thermophilus* rDNA spacer sequence,[14] which is known to contain a strong topoisomerase I cleavage site.[15] Each of the topopyrones stabilized the enzyme-DNA covalent binary complex at the same site as camptothecin, but did so less efficiently.[16] These results are fully in agreement with the earlier reports for the compounds.[11]

Figure 3 illustrates the effect of topopyrones A−D on the toposomerase IIα[17] mediated cleavage of a 34-bp oligonucleotide derived from the simian virus 40 nuclear matrix associated protein.[18]

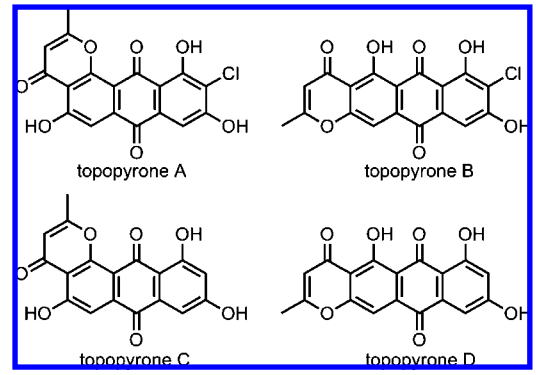

Figure 1. Structures of topopyrones A, B, C, and D.

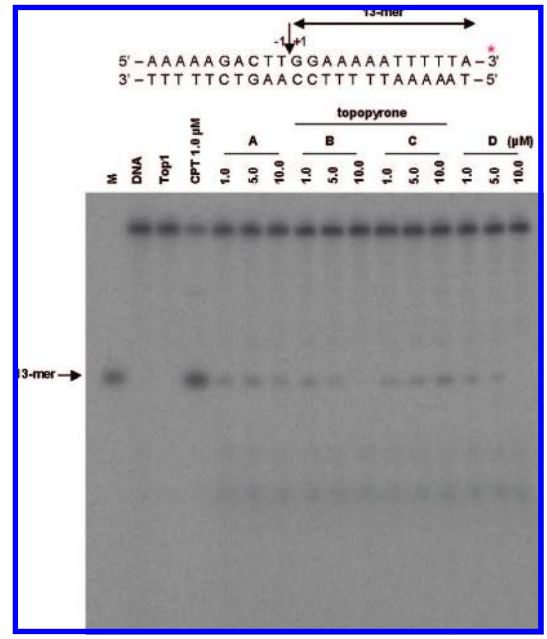

Figure 2. Effect of topopyrones A−D on topoisomerase I-mediated DNA cleavage and religation of a 23-bp DNA substrate. Lane M contained a 13-nucleotide molecular weight standard.

The topoisomerase IIα poison VP-16 stabilizes a cleavage site at position 4265 (upper strand of DNA duplex shown in Figure 3), generating a free 16-nt product observable when the upper strand is [5′-^{32}P]-end labeled. At the concentrations tested, the four topopyrones were as potent as VP-16, a clinically used antitumor agent, in stabilizing the topoisomerase II−DNA covalent binary complex. Assay of topoisomerase IIα-mediated cleavage of the lower strand of the same DNA substrates (Figure 4) indicated precisely the same effect.[19]

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10.1021/ja805421b CCC: $40.75

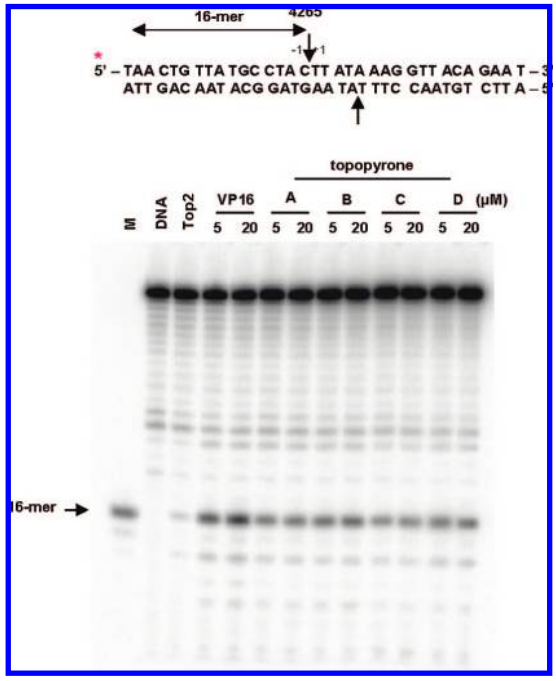

Figure 3. Effect of topopyrones A−D on human topoisomerase IIα-mediated DNA cleavage and religation of a 34-bp DNA substrate radiolabeled at the 5′-end of the upper strand shown.

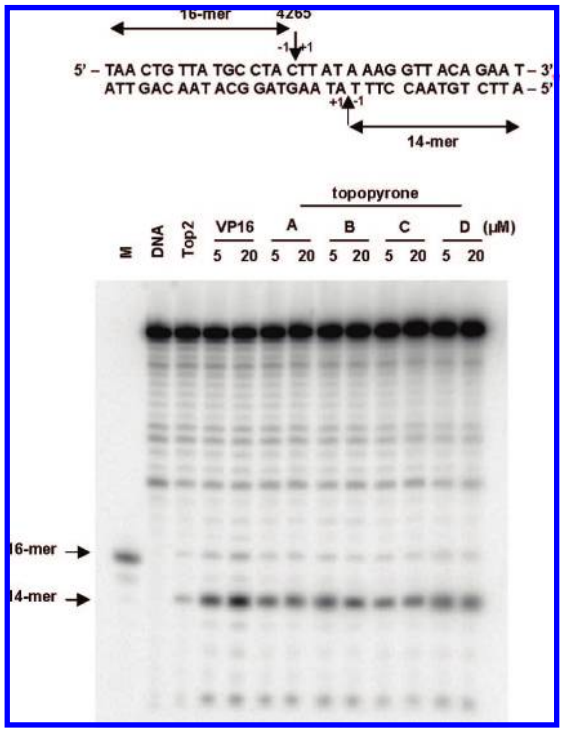

Figure 4. Effect of topopyrones A−D on human topoisomerase IIα-mediated DNA cleavage and religation of a 34-bp DNA substrate radiolabeled at the 5′-end of the lower strand shown. Lane M contained a 5′-^{32}P end labeled 16-mer oligonucleotide standard.

The experiments shown in Figures 2 −4 establish unequivocally that topopyrones A−D act as poisons of both topoisomerase I and topoisomerase IIα, both of which are validated targets for antitumor therapy. The results obtained in comparison with the clinically used agents camptothecin (a specific topoisomerase I poison[2b]) and VP-16 (a specific topoisomerase IIα-poison[5a]) suggest that the actions of topopyrones A−D may be more potent at the locus of topoisomerase IIα-DNA interaction.

The topopyrones thus represent a rare example of molecules capable of interacting effectively with more than one DNA topoisomerase.[20] The exploration of modified topopyrones optimized for interaction at both loci offers an interesting opportunity to enhance the antitumor activity of these interesting dual DNA topoisomerase poisons.

Acknowledgment. This work was supported by a research grant from the Arizona Technology and Research Initiative Fund.

Supporting Information Available: Experimental procedures for oligonucleotide preparation and topoisomerase-mediated DNA oligonucleotide cleavage. This material is available free of charge via the Internet at http://pubs.acs.org.

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JA805421B