Communication

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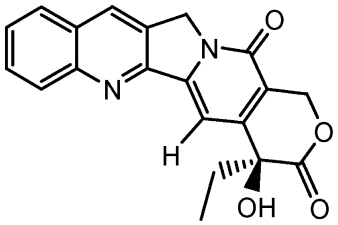

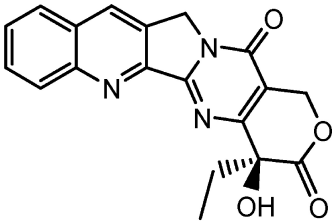

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14-Azacamptothecin: A Potent Water-Soluble Topoisomerase I Poison

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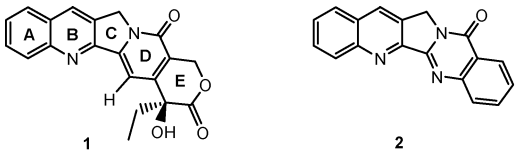
Camptothecin (CPT, **1**) is an antitumor antibiotic[1] believed to exert its cytotoxic effects by binding noncovalently to the covalent binary complex formed transiently between DNA and topoisomerase I during DNA relaxation.[2] Persistent stabilization of the covalent binary complex by CPT (analogues) leads to cell death.[3] While CPT itself is not used clinically due to complications arising from its extreme aqueous insolubility,[4] two CPT analogues are utilized clinically for the treatment of specific cancers; these include ovarian, colon, and small cell lung cancers.[5]

Recently, we reported that the pyrroloquinazolinoquinoline alkaloid luotonin A (**2**) also poisons topoisomerase I.[6] While less potent than CPT, luotonin A produced DNA strand breaks at the same sites (Figure 1) and was also cytotoxic toward a yeast strain expressing human topoisomerase I (Table 1).[6] Structurally, CPT and luotonin A are similar, having identical rings A−C. Ring D of luotonin A is nearly identical with that of CPT, differing only in the presence of a N atom in lieu of the CH in CPT. In contrast, ring E of luotonin A is totally different than that of CPT and lacks structural elements thought to be essential for topoisomerase I-dependent cytotoxicity.[7] Subsequent exploration of structural changes in ring E of luotonin A have better defined those structural elements in ring E that are consistent with binding to the topoisomerase I−DNA covalent binary complex and expression of topoisomerase I-dependent cytotoxicity in yeast, but failed to fully accommodate those structural elements in CPT known to be essential to function as a topoisomerase I poison.[8]



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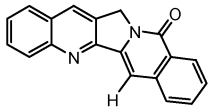

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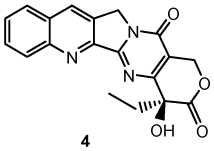

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In an effort to better understand the seemingly inexplicable divergence in behavior of luotonin A relative to CPT as a topoisomerase I poison, we synthesized a previously reported compound[9] that is a hybrid between luotonin A and CPT. Rosettacin (**3**)[10] is identical with CPT (**1**) in rings A, B, C, and D, and identical with luotonin A in rings A, B, C, and E. Rosettacin produced topoisomerase I-dependent DNA cleavage to an extent only about 50% that of luotonin A, consistent with an earlier report that it was a weak poison (Figure 1).[9] Rosettacin was also cytotoxic to a yeast strain that lacked yeast topoisomerase I but harbored a plasmid having human topoisomerase I gene under the control of a galactose promoter.[11] However, the expression of human topoisomerase I in this yeast strain actually *diminished* the cytotoxicity of rosettacin (Table 1). Thus, this compound does not function as a topoisomerase I poison to any significant extent.

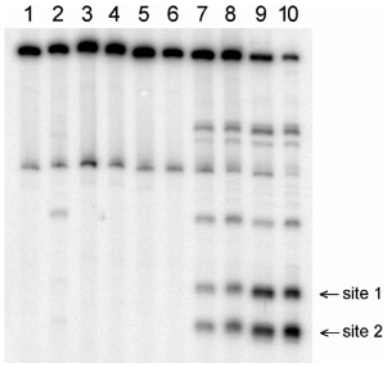

Figure 1. Autoradiogram of a 10% denaturing polyacrylamide gel showing the effect of CPT (**1**), luotonin A (**2**), rosettacin (**3**), and 14-aza CPT (**4**) on human topoisomerase I-mediated cleavage of the *Hin*dIII−*Pvu*II restriction fragment of pSP64 plasmid DNA (3′-^{32}P end labeled on the scissile strand). The cleavage reactions were incubated at 37 °C for 30 min and then digested with proteinase K. Lane 1, DNA alone; lane 2, 36 ng of topoisomerase I; lanes 3−6, **3**, **2**, **4**, and **1** (50 μM each), respectively; lanes 7−10, topoisomerase I + **3**, **2**, **4**, and **1**, (50 μM each), respectively.

Table 1. Human Topoisomerase I-Dependent Cytotoxicity of CPTs **1**−**4** toward *Saccharomyces cerevisiae*[a]

		% inhibition on growth medium	
compound	concn (μM)	raffinose	galactose
CPT (**1**)	1.0	0	74
luotonin A (**2**)	1.0	0	36
	0.5	0	23
rosettacin (**3**)	2.5	58	11
	0.5	32	0
14-aza CPT (**4**)	2.0	0	46

[a] Inhibition of RS321Nph-TOP1 grown in minimal medium containing 3% raffinose or galactose for 2 days at 30 °C.

The foregoing results argue that the CH group at position 14 of rosettacin is actually a *negative determinant* of binding to the topoisomerase I−DNA covalent complex. The X-ray crystal structure of a topoisomerase I−DNA complex containing a bound CPT analogue[12] reveals that two enzyme residues, Asp533 and Arg364, which are H-bonded to the CPT 20-OH group and to each other, could plausibly clash sterically with the clustered 14-CH/20-Et/20-OH substituents in CPT.

To test the hypothesis concerning the effect of the 14-CH group in CPT, we prepared 14-aza CPT (**4**).[13] In contrast to CPT, **4** was water soluble at millimolar concentration; it was tested for its ability to stabilize the topoisomerase I−DNA binary complex. At 50 μM concentration, 14-aza CPT had a potency comparable to that of CPT and produced stabilization at the same sites (Figure 1). Comparable responses were also noted at lower concentrations (Figure S1). When tested for cytotoxicity in the yeast strain

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Table 2. Topoisomerase I-Dependent Cytotoxicity of **1** and **4** and First-Order Rate Constants for their Dissociation from the CPT−Topoisomerase I−DNA Ternary Complex

compound	rate constant k ($\times 10^{-3}$ s^{-1})[a]	IC_{50} (μM)[b]
1	18.3	0.74−0.86
4	115	2.2

[a] Determined using 50 μM **1** and **4** at cleavage site 2 (cf Figure 1), as described in ref 15. [b] Determined as described in ref 15b.

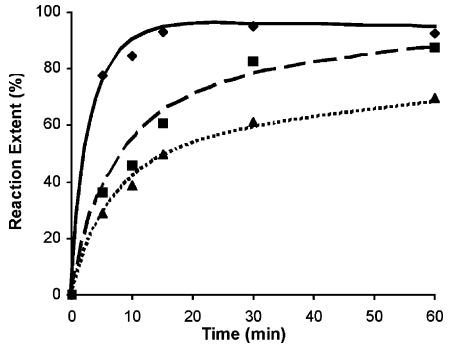

Figure 2. Kinetics of the relaxation of supercoiled pSP64 plasmid DNA in the presence of CPT (**1**) and 14-aza CPT (**4**). The supercoiled DNA was incubated at 37 °C with 0.1 ng of human topoisomerase I alone (diamonds), or in the presence of 500 μM CPT (squares) or 500 μM 14-aza CPT (triangles). Aliquots were treated with loading buffer containing 1% SDS at predetermined times and analyzed by 1% agarose gel electrophoresis.

harboring human DNA−topoisomerase I, 14-aza CPT was found to have an IC_{50} value of 2.2 μM; this compares favorably with the range of IC_{50} values (0.74−0.86 μM) determined for CPT in replicate experiments (Table 2). The cytotoxicity of 14-aza CPT was completely topoisomerase I-dependent. 14-Aza CPT also inhibited the topoisomerase I-mediated relaxation of DNA more effectively than CPT (Figure 2).[14]

It has been shown previously that persistence of the ternary complex formed from topoisomerase I poisons such as CPT is the key event that leads to the expression of cytotoxicity.[3] Logically, topoisomerase poisons having a greater affinity for the enzyme−DNA binary complex should generally produce a stronger cytotoxic response; this is reflected in Figure 1 where those species exhibiting the strongest topoisomerase I-mediated cleavage (i.e., displaying the greatest equilibrium affinity, K_A) were also the most cytotoxic. However, it has also been noted that CPT analogues exhibit a surprising diversity in their dynamics of binding to the topoisomerase I−DNA covalent binary complex. For those poisons having comparable K_A values, the species exhibiting significantly faster off-rates from the enzyme−DNA−CPT ternary complex are generally much less cytotoxic.[15]

In this context, the off-rate of 14-aza CPT is of interest, as it was 6.3, 7.5, and 8.9 times faster than that of CPT at three different DNA cleavage sites (Figure 1 and Table 2). Thus, the comparable cytotoxicities of CPT and 14-aza CPT were realized despite the much greater lability of the ternary complex containing 14-aza CPT. This observation supports the thesis concerning the importance of position 14 in defining the nature of CPT binding to the topoisomerase I−DNA covalent complex.

We have demonstrated previously that structural alterations in different regions of the CPT molecule can affect off-rate from the ternary complex.[15] It seems reasonable to anticipate that alteration of 14-aza CPT at sites remote from position 14 may afford an analogue exhibiting greater persistence of binding to the topoisomerase I−DNA covalent complex, resulting in a stronger cytotoxic response.

In conclusion, we find that substituents at the 14-position of CPT diminish interaction with the enzyme−DNA binary complex. Thus, water-soluble 14-aza CPT represents an attractive core structure for the elaboration of CPTs with improved properties.

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Supporting Information Available: Concentration dependence of DNA cleavage stabilization by CPT and 14-azaCPT. This material is available free of charge via the Internet at http://pubs.acs.org.

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