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Synthesis and Biological Evaluation of 10,11-Methylenedioxy-14-azacamptothecin

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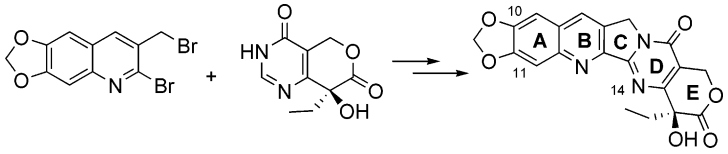
ABSTRACT



10,11-Methylenedioxy-14-azacamptothecin, a potent analogue of the antitumor agent camptothecin (CPT), has been prepared via a key condensation between AB and DE ring precursors. The biological testing of this compound validated a strategy for modulation of the off-rate of camptothecin analogues from the topoisomerase–DNA–CPT ternary complex via structural modification.

Camptothecin (CPT, **1**) (Figure 1) is an alkaloid first isolated from extracts of *Camptotheca acuminata*;[1] the compound exhibits potent antitumor activity but has a complicated history of clinical development due to its poor aqueous solubility.[2] Mechanistically, CPT has been shown to function by stabilizing the covalent binary complex between type I DNA topoisomerase and its DNA substrate.[3] These initial findings led to a flurry of activity which has resulted in a number of interesting CPT analogues, two of which are marketed as anticancer agents and several of which are in clinical trials.[4]

Biochemical studies of CPT revealed that the E ring was essential for stabilization of the topoisomerase I−DNA covalent binary complex.[5] Moreover, removal of the C20−OH group or inversion of the stereochemistry at that center proved detrimental to the activity of CPT as a topoisomerase I poison.[6] Accordingly, it was surprising to find that luotonin A (**2**) (Figure 1), which contains nitrogen at position 14 of the D ring and lacks the E ring lactone altogether, also acted as a topoisomerase I poison.[7] Accordingly, our laboratory prepared and tested luotonin A, CPT, and rosettacin (structural homologue of luotinin A with a CH at position 14, **3**). Although we were able to confirm the previously reported weak ability of rosettacin to act as a topoisomerase I poison,[8] it was found that the cytotoxicity of rosettacin was *not* topoisomerase I dependent.[9] Thus, the presence of a CH group rather than a nitrogen at position 14 in luotonin A

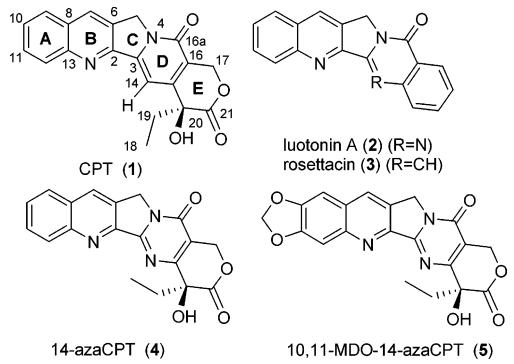


Figure 1. Structures of CPT and related analogues.

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actually eliminated its activity as a topoisomerase I-dependent cytotoxin. A potential rationalization for these results is possible based on a crystal structure of a topoisomerase I−DNA complex containing a CPT analogue.[10] In this structure, a hydrogen bond network exists between residues Asp533 and Arg364 of topoisomerase I and the C20−OH group of CPT, thus creating a possible steric clash between the clustered C14−H and C20−OH/ethyl groups.

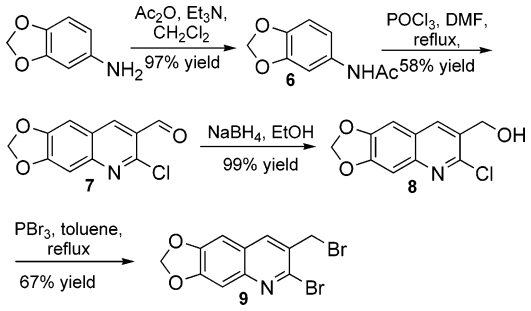
To test this hypothesis, the synthesis and biochemical evaluation of the water-soluble 14-azaCPT (**4**) was carried out.[9,11] It was found that **4** stabilized the topoisomerase I−DNA complex at the same sites as CPT and was cytotoxic with a similar but somewhat greater IC_{50} value. Further, it was also shown that 14-azaCPT mediated inhibition of DNA relaxation more effectively than CPT. Critically, **4** possessed a faster off-rate from the ternary complex than CPT. It appears that replacing the C14−H group with N does improve the ability to form the ternary complex while concomitantly reducing the lifetime of the formed complex, thus reducing the cytotoxic effects of the resulting analogue.

Previously, it was found that the key event leading to cell death was the persistence of the ternary complex, which can be converted to a double-strand break in DNA as a consequence of interaction with a moving replication fork.[12] Numerous studies now support the thesis that failure to resolve the formed protein−DNA complex, i.e., persistence of the complex, is correlated to the development of a cytotoxic response.[13] Although the concentration of topoisomerase I−DNA−CPT ternary complexes formed at equilibrium logically constitutes the source of the cytotoxic response resulting from treatment with CPT (analogues), it has been shown convincingly that CPT analogues that promote ternary complex formation to comparable extents can nonetheless differ in the cytotoxic response that they promote.[14] The available data argue that much of the apparent discrepancy is due to differences in the off-rates of individual CPTs from the formed ternary complexes; those CPT analogues having slower off-rates tend to be more cytotoxic.

Given these interesting biological results, we hypothesized that altering structural features in different regions of the molecule could provide a way to modulate the off-rate. Previously, it had been shown that the 10,11-methylenedioxy (MDO) group increases the stability of the topoisomerase I−DNA complex, thus diminishing the off-rate.[14a] By incorporating this structural feature into the 14-aza core, it seemed that it might be possible to maintain the stabilization and inhibitory properties of CPT while decreasing the off-rate, thus making a more effective topoisomerase I poison and anticancer therapeutic candidate. The synthesis and initial biological testing of 10,11-MDO-14-azaCPT (**5**) are presented herein.

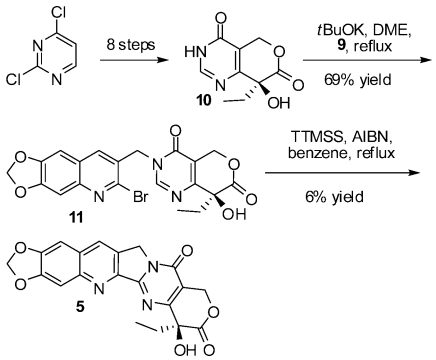
The synthesis of 10,11-MDO-14-azaCPT is outlined in Schemes 1 and 2. The required dibromide **9** was prepared

Scheme 1. Synthesis of Dibromide **9**



in four steps from commercially available 3,4-(methylenedioxy)aniline which was initially acetylated to afford **6** in

Scheme 2. Completion of the Synthesis of 10,11-MDO-14-azaCPT (**5**)



97% yield. Following the procedure of Lavergne et al.,[15] we subjected the acetylated amine to Vilsmeier conditions to

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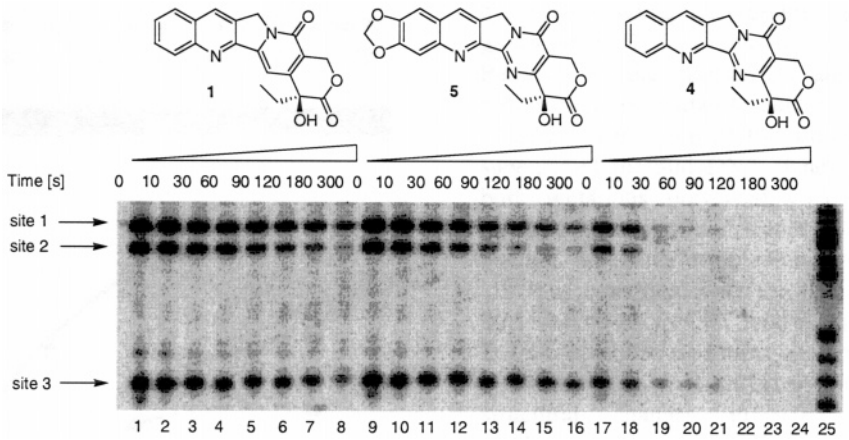

Figure 2. Salt-induced dissociation of the CPT−DNA−topoisomerase I ternary complex. Autoradiogram of the sequencing gel showing the religation of the cleavage complex after addition of NaCl to a concentration of 0.35 M. At the times listed, the reactions were stopped by the addition of SDS and proteinase K. The reaction shown in lanes 1−8 was carried out in the presence of 50 μM **1**. Lanes 9−16 were in the presence of 50 μM **5**. Lanes 17−24 were in the presence of 50 μM **4**. Lane 25 is a Maxam−Gilbert G-lane.

afford aldehyde **7** in 58% yield. Reduction of **7** to alcohol **8** with $NaBH_4$ in EtOH proceeded nearly quantitatively. Bromination of **8** in toluene with PBr_3 at reflux for 18 h gave a mixture of mono- and dibromides, which were subjected to bromination conditions again to give key intermediate **9** in 67% yield.

Pyrimidone **10** was prepared in eight steps from the commercially available 2,4-dichloropyrimidine according to a published procedure (Scheme 2).[11,16] Pyrimidone **10** was then condensed with **9** using *t*BuOK in DME at reflux to afford key intermediate **11** in 69% yield. Radical-mediated cyclization of **11** using tris(trimethylsilyl)silane and AIBN as the radical initiator in benzene at reflux afforded 10,11-MDO-14-azaCPT (**5**) as a colorless solid in 6% yield after purification by column chromatography.

As shown in Figure 2, CPT analogue **5** possessed potency comparable to that of CPT and produced stabilization at the same sites in DNA. The salt-induced dissociation of the CPT−DNA−topoisomerase I ternary complex revealed that incorporation of the 10,11-MDO group actually did lead to an increased persistence of the formed complex (Table 1).

Table 1. Topoisomerase I-Dependent Cytotoxicity of **1**, **4**, and **5** and First-Order Rate Constants for Their Dissociation from the CPT−Topo I−DNA Ternary Complex

compound	rate constant k ($\times 10^{-3}$ s^{-1})[a]	IC_{50} (μM)
1	18.3	0.15
4	115	>10
5	21.5	1.28

[a] Determined using 50 μM **1**, **4**, and **5** at cleavage site 2.

Specifically, the dissociation rate constant increased to 115 from 18.3 when the C14−H group was replaced with N. When the 10,11-MDO group was additionally incorporated, the rate constant returned to 21.5, similar to that of CPT.

The cytotoxic effects of the analogues tested confirm this trend. The IC_{50} values toward A549 cells were determined using an MTT assay, as shown in Table 1. Compound **4** was found to have an IC_{50} value >10 μM, whereas CPT itself had an IC_{50} value of 0.15 μM. The increased persistence of the ternary complex resulting from the introduction of the 10,11-MDO group in **5** resulted in an IC_{50} value of 1.28 μM.

Finally, the ability of CPT and 10,11-MDO-14-azaCPT to inhibit the relaxation[16] of supercoiled plasmid DNA by topoisomerase I was compared (Figure 3). These results

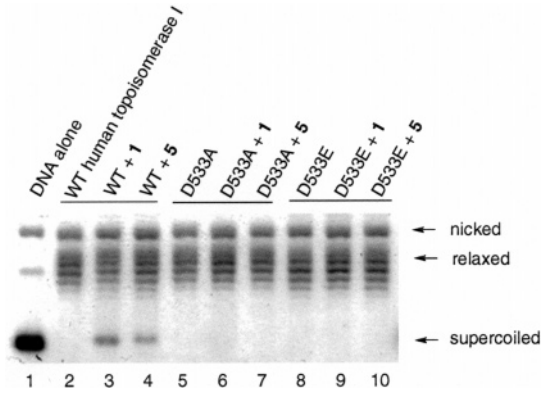

Figure 3. Plasmid relaxation assay comparison of **1** and **5** (80 μM) against wild-type (WT) topoisomerase I and mutants D533A and D533E.

indicate that the two were comparable, although **5** possessed a slightly weaker ability to inhibit topoisomerase I-mediated

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(16) Experimental details and full characterization of all intermediates are available in the Supporting Information.

DNA relaxation. A direct comparison of the time course of relaxation in the presence of 500 μM **1** and **5** gave similar results, albeit with slightly greater inhibition in the presence of **5** (Figure 4). More important, however, was the comparison of the effects of CPTs **1** and **5** on topoisomerase I mutants. Specifically, we prepared two modified topoisomerases I[17] (D533A and D533E). Asp533, as indicated above, is thought to be involved in hydrogen bonding interactions with the C20 hydroxyl group of CPT and thus is a useful site to probe. Removal of the carboxylate altogether (D533A) and introduction of glutamic acid in lieu of aspartic acid (D533E) still afforded topoisomerases I able to relax supercoiled plasmid DNA (lanes 5 and 8). However, both mutations prevented CPT from inhibiting this relaxation, as shown in lanes 6 and 9. In a similar manner, **5** also no longer inhibits relaxation (lanes 7 and 10) which is consistent with the thesis that both CPT and 10,11-MDO-14-azaCPT bind in the same manner to the DNA−topoisomerase I binary complex.

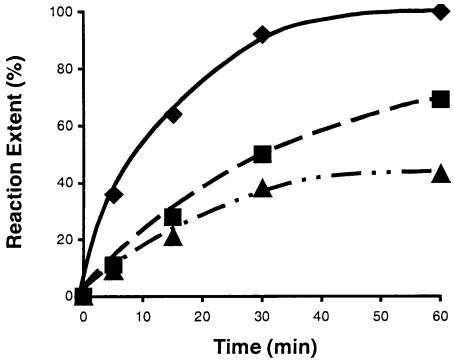

Figure 4. Kinetics of the relaxation of supercoiled pSP64 plasmid DNA in the presence of CPT and 10,11-MDO-14-azaCPT. The supercoiled DNA was incubated at 37 °C with 0.05 ng of human topoisomerase I alone (diamonds) or in the presence of 500 μM CPT (squares) or 500 μM 10,11-MDO-14-azaCPT (triangles). Aliquots were treated with loading buffer containing 1% SDS at predetermined times and analyzed by 1% agarose gel electrophoresis.

These results confirm the hypothesis that appropriate structural changes in CPT distant from the mechanistically critical D and E rings can correct detrimental changes accompanying modification of the D/E rings. Specifically, it is possible to modulate the off-rate resulting from introduction of an aza group into the 14-position of CPT by introducing additional structural features far from the 14-position. Thus, 10,11-MDO-14-azaCPT represents another important step toward the development of a CPT analogue with useful therapeutic properties.

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Supporting Information Available: Experimental procedures and full characterization for all compounds. Protocols for the cytotoxicity assay. This material is available free of charge via the Internet at http://pubs.acs.org.

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