Communication

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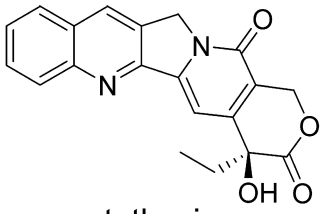
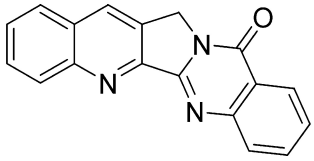


camptothecin



luotonin A

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Luotonin A. A Naturally Occurring Human DNA Topoisomerase I Poison

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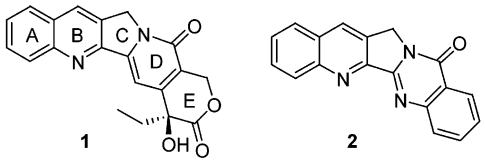
The demonstrated clinical utility of two camptothecin analogues as antitumor agents[1] has prompted intensive efforts to identify additional clinical candidates in this class.[2] Accordingly, study of the mechanism of action of the camptothecins has been of great interest, as it may afford insights leading to improved therapeutic agents. A key biochemical target for CPT (**1**) is the covalent binary complex formed between DNA and topoisomerase I during DNA relaxation; stabilization of this complex by CPT is believed to lead to cell death.[3]



Luotonin A (**2**) is a pyrroloquinazolinoquinoline alkaloid extracted from the Chinese medicinal plant *Peganum nigellastrum*.[4] Luotonin A is cytotoxic toward the murine leukemia P-388 cell line (IC_{50} 1.8 μg/mL), although the mechanism is unknown.[4a,5] There are obvious structural similarities between CPT (**1**) and luotonin A, notably in identical rings A−C. The greatest differences are in ring E, which is known to be critical for CPT function as a topoisomerase I inhibitor and antineoplastic agent.[6] Alterations in the lactone ring or 20-OH group typically render CPT dysfunctional, although a few exceptions have been reported.[2g,7] The lactone of CPT has long been known to be quite electrophilic,[8] and most structure−activity studies have supported a relationship between lactone electrophilicity and the ability of CPT to stabilize the topoisomerase I−DNA covalent binary complex.[6] It has been suggested that this putative relationship may reflect the transient covalent attachment of CPT to the topoisomerase I−DNA complex.[3b,9] In this context, the lack of functionality in the E-ring of luotonin A argues against its ability to function in the same fashion as CPT.

Presently, we demonstrate that despite the lack of E-ring functionality, luotonin A stabilizes the human DNA topoisomerase I−DNA covalent binary complex and mediates topoisomerase I-dependent cytotoxicity in intact cells. Shown in Figure 1 is the effect of CPT and luotonin A on the stabilization of the topoisomerase I−DNA binary complexes in a ^{32}P-end labeled 222 bp DNA duplex. In common with CPT, luotonin A[10] effected concentration-dependent stabilization of the enzyme−DNA binary complex. While luotonin A was less potent than CPT, stabilization was observed at the same sites for both; the identical effect, as judged by electrophoretic analysis, argues that the chemistry of cleavage was the same for both. Neither **1** nor **2** had any measurable effect on DNA in the absence of topoisomerase I.

While not thought to contribute to its antitumor activity, CPT also inhibits topoisomerase I-mediated relaxation of supercoiled DNA when present at high concentrations. This is illustrated in Figure 2 at 100−500 μM CPT concentrations in the presence of

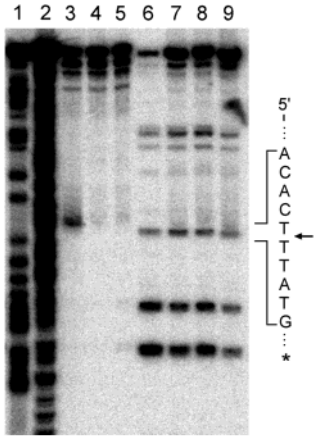


Figure 1. Autoradiogram of a 10% denaturing polyacrylamide gel showing the effect of luotonin A on human topoisomerase I-mediated cleavage of the *Hin*dIII-*Pvu*II restriction fragment of pSP64 plasmid DNA. The DNA substrate was 3′-^{32}P end labeled on the scissile strand. Human topoisomerase I-mediated cleavage reactions were incubated at 37 °C for 1 h and then digested with proteinase K. Lanes 1 and 2, Maxam−Gilbert sequencing reactions; G, and C + T, respectively. Lane 3, DNA alone; lane 4, 50 μM CPT; lane 5, 50 μM luotonin A; lane 6, topoisomerase I and 50 μM CPT; lane 7, topoisomerase I and 50 μM luotonin A; lane 8, topoisomerase I and 10 μM luotonin A; lane 9, topoisomerase I and 2 μM luotonin A. No significant DNA cleavage was observed in the presence of topoisomerase I + DNA alone. In a parallel experiment, cleavage at the site marked by an arrow was stabilized to the extents of 58%, 14%, and 1% at 50, 10, and 2 μM luotonin A, respectively, relative to that achieved with 50 μM CPT.

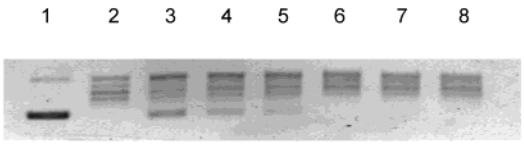


Figure 2. Effect of luotonin A on human topoisomerase I-mediated DNA relaxation. Supercoiled pSP64 plasmid DNA was incubated at 37 °C for 10 min as indicated. Lane 1, supercoiled pSP64 plasmid DNA alone; lane 2, DNA + 0.1 ng of topoisomerase I; lanes 3−5, DNA + topoisomerase I + 500, 200, and 100 μM of camptothecin, respectively; lanes 6−8, DNA + topoisomerase I + 500, 200, and 100 μM of luotonin A, respectively.

0.1 ng of human topoisomerase I. As shown in the Figure, luotonin A (**2**) had no effect on DNA relaxation under the same conditions, although **2** did weakly inhibit plasmid DNA relaxation by calf thymus DNA topoisomerase I (not shown).

To evaluate the possible cytotoxic effects resulting from stabilization of the enzyme−DNA binary complex, luotonin A was evaluated in a strain of *Saccharomyces cerevisiae* lacking yeast topoisomerase I, but harboring a plasmid having the human topoisomerase I gene under the control of a galactose promoter.[11] As shown in Table 1, 1 μM CPT had no effect when this yeast strain was grown on raffinose. However, 1 μM CPT caused 74% inhibition after 2 days when the same yeast strain was grown on galactose, resulting in topoisomerase I expression. Luotonin A produced 36% inhibition of growth when employed at 1 μM

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Table 1. Human Topoisomerase I-Dependent Cytotoxicity of CPT (**1**) and Luotonin A (**2**) toward *S. cerevisiae*[a]

compound	concentration (μM)	% inhibition on growth medium: raffinose	% inhibition on growth medium: galactose
CPT (**1**)	1.0	0	74
luotonin A (**2**)	1.0	0	36
	0.5	0	23

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

concentration in the presence of galactose.[12] In replicate experiments, lutonin A exhibited IC_{50} values from 5.7 to 12.6 μM in the presence of galactose. The comparable values for CPT were 0.74−0.86 μM.

The closely analogous effects of CPT and luotonin A on stabilization of the topoisomerase I−DNA binary complex, and on the production of human topoisomerase I-dependent cytotoxicity in yeast, suggest that the two agents likely function in the same fashion. This conclusion identifies a putative biochemical locus for the cytotoxic action of luotonin A and has important implications both for the mechanism of inhibition of topoisomerase I function by CPT and for the design of new CPT analogues.

At a mechanistic level, it seems clear that no electrophilic E-ring lactone is needed for stabilization of the topoisomerase I−DNA covalent binary complex. Despite the apparent correlation between E-ring lactone electrophilicity and topoisomerase I inhibitory activity,[6] the present findings add to the weight of evidence that argues against the covalent attachment of CPT to the enzyme−DNA binary complex.

In terms of inhibitor design, recent X-ray crystallographic studies[13] and computational models[14] suggest a role for the 20(*S*)-OH group in interaction with DNA topoisomerase I, possibly through hydrogen bonding to the enzyme. This interaction is further supported by the lack of activity of 20(*R*) CPT,[15,16] as well as 20-deoxyCPT.[16,17] The 20-chloro, bromo, and amino derivatives of CPT have been shown to stabilize the topoisomerase I−DNA binary complex and to produce human topoisomerase I-dependent cytotoxicity in yeast, albeit with somewhat reduced potency.[16] While it seems likely that functional groups properly oriented at the 20-position of CPT can contribute to the stability of the ternary complex formed with topoisomerase I and DNA, the present results obtained with luotonin A suggest that, even in the absence of any functional group at the 20-position, an aromatic E-ring can confer reasonable stability to the formed ternary complex. This may reflect a stacking interaction first posited by Kohn, Pommier and co-workers[14a,18] and now present in many models of the ternary complex.[13] It seems reasonable to suggest that the presence of appropriate functional groups on the E-ring might further modulate the interaction of luotonin A with the topoisomerase I−DNA binary complex. In any case, the present results make it clear that an electrophilic E-ring is not essential for stabilization of the topoisomerase I−DNA covalent binary complex.

At a practical level, it may be noted that elaboration of the E-ring of CPT is perhaps the most challenging aspect of the synthesis of this natural product. The several concise syntheses already reported for luotonin A[5,10,19] suggest that the preparation of luotonin A analogues should be straightforward.

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