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Synthesis and biochemical properties of E-ring modified luotonin A derivatives

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Abstract—Luotonin A is a cytotoxic pyrroloquinazolinoquinoline alkaloid that has been shown to stabilize the human topoisomerase I–DNA covalent binary complex in the same fashion as the antitumor alkaloid camptothecin. A study of the structural elements in luotonin A required for binary complex stabilization has revealed key differences relative to those required for camptothecin.

Camptothecin (CPT, **1**), is a naturally occurring cytotoxic alkaloid first isolated from *Camptotheca acuminata* in 1966 by Wall et al. (Fig. 1).[1] The discovery of two CPT (**1**) analogues having clinically useful antitumor activity[2] has prompted additional efforts to identify analogues with improved activity.[3]

In this context considerable effort has been devoted to studying the mechanism of action of CPT. CPT has been shown to bind to and stabilize the topoisomerase I–DNA covalent binary complex that is an obligatory intermediate in the (reversible) cleavage and unwinding of DNA.[4] It is believed that this stabilization of what is normally a transient intermediate leads to double-strand DNA breakage and cell death.[5] Insight into the nature of the CPT–topoisomerase I–DNA ternary complex has been provided by molecular modeling studies[6] and X-ray crystallographic analysis.[7] In particular, these studies support the belief that the α-hydroxylactone (E-ring) moiety of camptothecin interacts with topoisomerase I in the ternary complex through hydrogen bonds. This interaction was originally suggested by the lack of ability of 20(*R*)-OH CPT,[8] and 20-deoxy CPT[8,9] to support ternary complex formation. The 20-chloro, bromo, and amino derivatives of CPT have also been shown to exhibit reduced potency of ternary complex stabilization, and correspondingly diminished cytotoxic activity.[8b] The α-OH substituent is also believed to contribute to the well known electrophilicity[10] of the E-ring of CPT. Since structure–activity studies of CPT have tended to support a relationship between E-ring electrophilicity and the efficiency of CPT function as a topoisomerase I poison, the 20(*S*)-OH group has been regarded as a critical structural element of CPT.

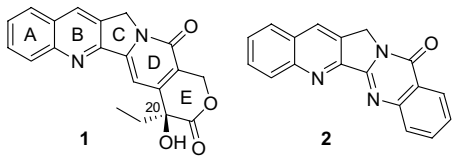

Figure 1. Structures of camptothecin (**1**) and luotonin A (**2**).

Recently, we have reported that the pyrroloquinazolinoquinoline alkaloid luotonin A (**2**) is a topoisomerase I poison.[11] While this may seem unsurprising in light of the close structural relationship between rings A–D of **1** and **2**, luotonin A lacks the hydroxylactone moiety of CPT altogether. Luotonin A is less cytotoxic than CPT, and less potent in stabilizing the topoisomerase I–DNA covalent binary complex.[11] However, the sequence selectivity of DNA cleavage by topoisomerase I in the presence of **1** and **2** is the same, and the identical electrophoretic co-migration of the DNA cleavage products in the presence of these two compounds argues that the cleavage chemistry is also the same. Critically, the E-ring of luotonin A clearly cannot undergo (reversible) covalent attachment to the topoisomerase I–DNA binary complex as has been suggested for CPT.[5b,12] This finding

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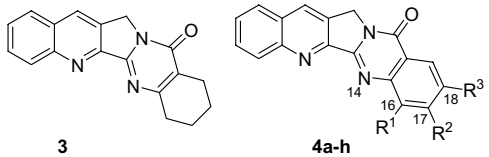
might be thought to make it less likely that CPT binding to the enzyme–DNA binary complex involves transient covalent attachment.

Proposed models for the CPT–topoisomerase I–DNA ternary complex,[6] as well as the X-ray crystallographic structure of a CPT analogue bound to a topoisomerase I construct—DNA oligonucleotide complex,[7b] posit the binding of CPT stacked between adjacent base pairs in the DNA substrate at the site of enzyme cleavage. The aromatic E-ring of luotonin A could clearly contribute to this putative stacking interaction.

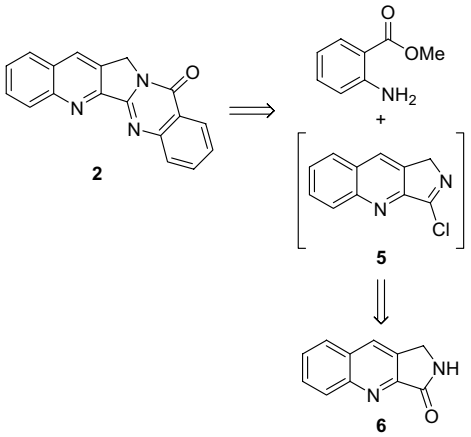
To evaluate the nature of the interaction of luotonin A with the human topoisomerase I–DNA covalent binary complex, and explore possible commonalities with the binding of CPT, we have prepared several analogues of luotonin A (Fig. 2). Several syntheses of luotonin A have been described;[13] these typically rely on the condensation of anthranilic acid derivatives with key intermediate **6** (Fig. 3).[14] In the present study, the best results were obtained when the requisite methyl anthranilate derivatives were treated with **6** in 10:1



3 **4a-h**

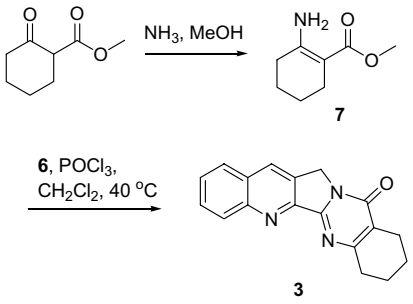
	R^1	R^2	R^3
a	H	Cl	H
b	H	F	H
c	H	F	F
d	OMe	H	H
e	H	OMe	OMe
f	OMe	OMe	OMe
g	H	H	Me
h	OH	H	H

Figure 2. Structures of luotonin A derivatives **3** and **4a**–**h**.



2 **5** **6**

Figure 3. Retrosynthetic analysis of luotonin A.



7 **3**

Scheme 1. Synthesis of 16,17,18,19-tetrahydroluotonin A derivative **3**.

CH_2Cl_2–$POCl_3$ at 40 °C. The condensation reaction presumably proceeded via iminochloride intermediate **5** (Fig. 3). The key 16-hydroxy analogue **4h**, having an OH group in the position analogous to the 20-OH group in CPT, was obtained by demethylation of **4d** in HBr at reflux. The saturated E-ring derivative **3** was synthesized starting from methyl 2-oxocyclohexane carboxylate, which was converted to enamine **7** via a slight modification of a published procedure.[15] Condensation with 1,2-dihydropyrrolo[3,4-*b*]quinoline-3-one (**6**) in the presence of $POCl_3$ then afforded **3** (Scheme 1).

Luotonin A (**2**) and derivatives **3** and **4a**–**h** were evaluated for their ability to stabilize the covalent binary complex formed by human topoisomerase I and a DNA substrate. The latter was a 3′-^{32}P end labeled DNA duplex having 222 base pairs.[16] As shown in Figure 4, these analogues varied significantly in their abilities to stabilize the topoisomerase I–DNA covalent binary complex. The most efficient derivatives in this regard were 17-fluoroluotonin A (**4b**) and 17-chloroluotonin A (**4a**) (lanes 6 and 5, respectively). Weaker stabilization was observed in the presence of the tetrahydro (**3**), bis-fluoro (**4c**), and 16-methoxy (**4d**) and 16-hydroxy (**4h**) derivatives. The remaining derivatives, compounds **4e**–**g** afforded little stabilization.

The luotonin analogues were also evaluated for their cytotoxic effects in a strain of *Saccharomyces cerevisiae* lacking the yeast topoisomerase I, but harboring a plasmid that contained the gene for human topoisomerase I under the control of a galactose promotor.[17] As summarized in Table 1, CPT and luotonin A both exhibited clear concentration dependent inhibition of the yeast strain employed; inhibition was greater at all tested concentrations when the growth medium contained galactose, that is, in the presence of topoisomerase I. Also exhibiting good concentration dependent cytotoxicity in the presence of topoisomerase I was the 17-fluoro derivative of luotonin A (**4b**). Interestingly, the 16-OH derivative (**4h**) containing an OH group in the position analogous to the critical 20-OH group in CPT, exhibited only weak activity at high concentration. The same was observed for the 18-methyl derivative (**4g**). The remaining analogues were either completely inactive (**4a**, **4e**, and **4f**) or else exhibited cytotoxicity that was actually suppressed when topoisomerase I was expressed (**4c** and **4d**).

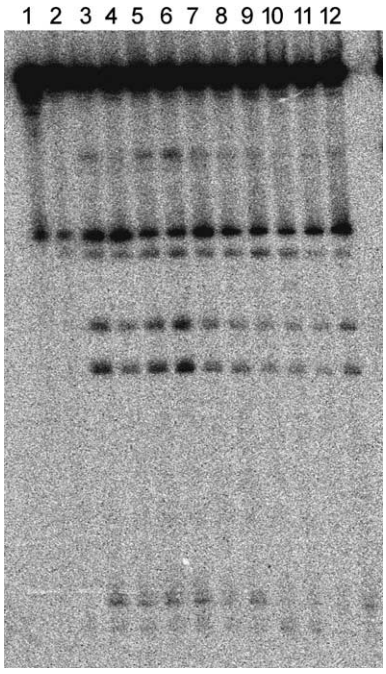


Figure 4. Autoradiogram of a 10% denaturing polyacrylamide gel showing the effects of luotonin A derivatives on human topoisomerase I-mediated cleavage of the 222 base pair *Hin*dIII–*Pvu*II restriction fragment of pSP64 plasmid DNA. The DNA substrate was 3′-^{32}P end labeled on the scissile strand. Human topoisomerase I-mediated cleavage reactions were incubated at 37 °C for 1 h and then digested with proteinase K. All lanes contained DNA + 36 ng of topoisomerase I + 50 μM luotonin A derivatives as indicated. Lane 1, DNA alone; lane 2, DNA + topoisomerase I; lane 3, compound **2**; lane 4, compound **3**; lane 5, compound **4a**; lane 6, compound **4b**; lane 7, compound **4c**; lane 8, compound **4d**; lane 9, compound **4e**; lane 10, compound **4f**; lane 11, compound **4g**; lane 12, compound **4h**.

Although active only at rather high concentration, tetrahydroluotonin A derivative **3** also exhibited good concentration dependent cytotoxicity toward the yeast when topoisomerase I was present. Thus an aromatic E-ring is apparently not essential for targeting the topoisomerase I–DNA covalent binary complex.

In the aggregate, these testing results confirm the validity of luotonin A as a pharmacophore for the development of a new class of topoisomerase I inhibitors. Substitutions in the E-ring of luotonin A produced pronounced changes in the ability of the resulting analogues to stabilize the topoisomerase I–DNA covalent binary complex and mediate topoisomerase I-dependent cytotoxicity. The lesser activity of tetrahydroluotonin A derivative **3** is consistent with the thesis that the E-ring of luotonin A contributes importantly to ternary complex stabilization. The nature of this contribution is not entirely clear at present but may involve an interplay between H bonding and π–π interactions. Additional research will be required to establish this point more definitively.

The dearth of CPT analogues substituted within ring E of CPT makes it difficult to compare the structure–activity results obtained here in the context of analogous changes for CPT. However, the dramatic effect of a 16-OH group in diminishing the activity of luotonin A (cf. compounds **2** and **4h**) contrasts sharply with the requirement for a 20(*S*)-OH group in CPT. Although the reason(s) for this difference is unclear at present, it could plausibly result from intramolecular hydrogen bonding of the 16-OH group in luotonin A to N-14. Alternatively, the introduction of a planar E-ring may have altered the nature of binding to the topoisomerase I–DNA covalent binary complex sufficiently to alter the intermolecular contacts between the enzyme and inhibitor.

Table 1. Human topoisomerase I-dependent cytotoxicity of CPT and luotonin A derivatives toward *S. cerevisiae*[a]

Compound[b]	Concentration (μM)	% Inhibition on growth medium	
		Raffinose	Galactose
CPT (**1**)	15	18	78
	1.5	19	68
	0.75	10	40
Luotonin A (**2**)	10	35	56
	1	0	36
	0.5	0	23
3	100	18	51
	50	3	46
4b	50	14	73
	25	0	40
4c	10	53	0
	1	26	0
4d	25	47	2
	5	35	0
4g	10	39	49
	1	8	14
4h	100	7	13
	50	0	9

[a] Inhibition of RS321Nph-TOP1 grown in minimal medium containing 3% raffinose or galactose.
[b] Minimal activity was observed for **4a**, **4e**, or **4f** at any tested concentration.

Acknowledgements

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