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On the role of E-ring oxygen atoms in the binding of camptothecin to the topoisomerase I–DNA covalent binary complex

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Abstract—A recent X-ray crystallographic analysis of the binding of a water soluble camptothecin analogue to the human topoisomerase I–DNA covalent binary complex has suggested the existence of some novel features in the way that camptothecin is bound to the binary complex. Four additional models based on chemical and biochemical data have also been proposed. Presently we describe S-containing analogues of camptothecin prepared on the basis of these models, and report their ability to form stable ternary complexes with human topoisomerase I, and to mediate cytotoxicity at the locus of topoisomerase I. The results indicate that replacement of the 20-OH group of CPT with a SH functionality results in diminution of the potency of CPT as a topoisomerase I poison, while replacement of the O atoms at positions 20 and 21 with S atoms results in essentially complete loss of topoisomerase I inhibitory activity.

1. Introduction

The antitumor agent 20(*S*)-camptothecin (CPT, **1**) is a pentacyclic alkaloid first isolated from extracts of *Camptotheca acuminata*.[1] The cellular target of CPT is believed to be the covalent binary complex formed between topoisomerase I and DNA.[2] While normally present only as a transient intermediate in the process of DNA unwinding, CPT forms a stabilized ternary complex with topoisomerase I and DNA that leads to cell death.[3] Testing as an antitumor agent in animal tumor models produced promising results and led to the evaluation of CPT in the clinic.[4] The initial trials were complicated by the poor solubility of CPT, which was administrated as the water-soluble sodium salt of the ring-opened carboxylate form of CPT (**2**). Severe and unpredictable toxicity led to suspension of the clinical trials.

While camptothecin itself is not used clinically as an anticancer agent due to its poor water solubility and side effects,[1b] modified CPTs such topotecan (Hycamtin, **3**)[5] and irinotecan (Camptosar, **4**)[6] are used for the treatment, respectively, of small cell lung and ovarian cancers, and colorectal cancer (Fig. 1).[7] While most research has focused on the synthesis of camptothecin derivatives bearing substituents on the A and B rings, some particularly interesting results have been obtained by modification of the E-ring of CPT.[8] The includes homocamptothecin (**5**), and homocamptothecin derivatives which show significant potential as antitumor agents.[9]

The exact mechanism by which CPT stabilizes the DNA–topoisomerase I covalent binary complex is not fully understood in spite of the recent publication of an X-ray crystallographic analysis of a ternary complex formed between topotecan, a topoisomerase I construct and a DNA oligonucleotide substrate for the enzyme.[10] Key features of the crystal structure include the intercalation of topotecan between adjacent base pairs, and a single H-bond between topotecan and the enzyme. The E-ring-opened form of topotecan was also bound to the enzyme in the same proportion in which it was present in the medium in which the crystals were prepared. Four molecular models have also been proposed to explain the interactions between camptothecin and the topoisomerase I–DNA covalent complex.[11] While each of these models also involves intercalation of

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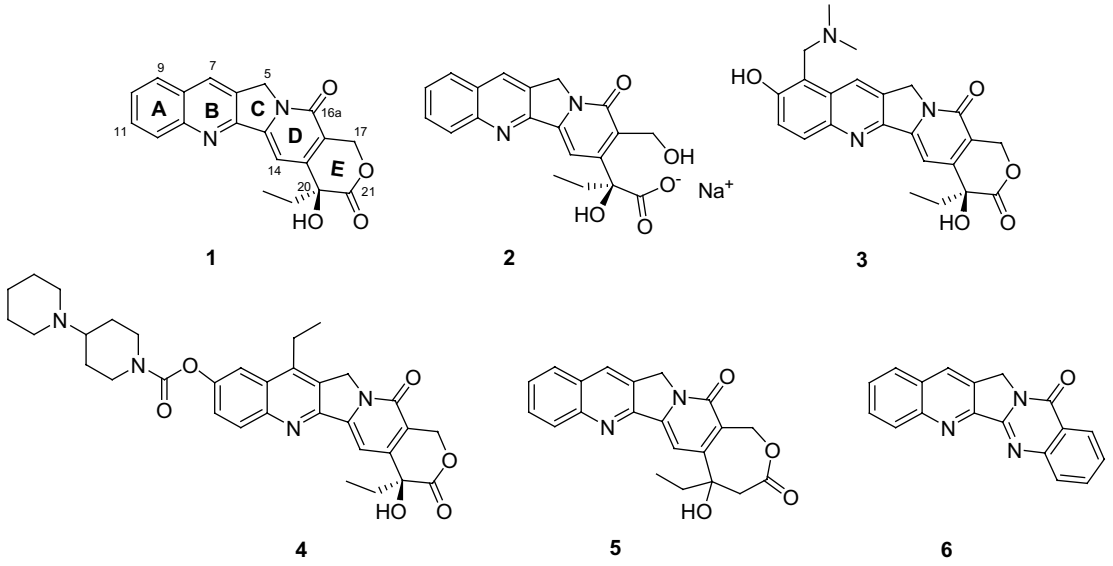


Figure 1. Structure of camptothecin (**1**), its carboxylate form (**2**), topotecan (**3**), irinotecan (**4**), homocamptothecin (**5**), and luotonin A (**6**).

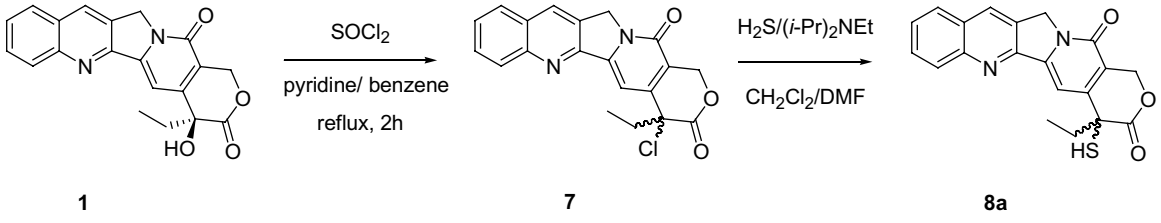
CPT between DNA base pairs, the orientation of the drug is envisioned to be quite different in each case (summarized in Ref. 8). Each of the models also envisions numerous H-bonding interactions between CPT and residues in both the DNA and enzyme within the formed ternary complex, although the proposed H-bonding interactions are not shared in common between the models.

In an effort to define structural elements in CPT essential for binding to the topoisomerase I–DNA covalent binary complex, we have been altering CPT at single sites believed to be critical for function. Presently, we report the effects of replacing the O-atoms attached to C-20 and C-21 with S atoms. All of the published models posit the involvement of at least one of these O atoms in stabilizing the formed ternary complex.

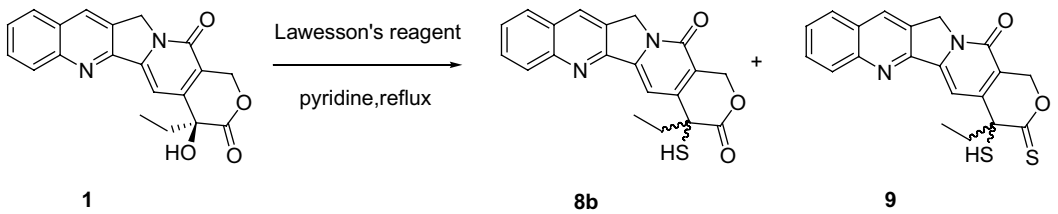
2. Results

20-Mercaptocamptothecin (**8a**) was prepared in 81% yield by treatment of 20-chlorocamptothecin (**7**) with H_2S/diisopropylethylamine in 1:1 methylene chloride–dimethylformamide (Scheme 1). 20-Chlorocamptothecin (**7**) was obtained in 66% yield by treatment of camptothecin with thionyl chloride for 2 h at reflux in benzene (Scheme 1).[1]

Camptothecin was treated with a large excess of Lawesson's reagent in pyridine at reflux to form 20-mercapto derivative **8b** and dimercapto derivative **9** in 3% and 8% yields, respectively (Scheme 2). Also formed were less polar degradation products. Compounds **8b** and **9** were purified by silica gel column chromatography and C_{18} reversed phase HPLC. The structures were determined by ^{1}H NMR, ^{13}C NMR, and mass spectrometry.



Scheme 1.



Scheme 2.

The spectral data for the 20-mercaptocamptothecins **8** synthesized by the two foregoing routes (i.e., **8a** and **8b**) were the same. However, the percentage of the main enantiomer of **8** in the mixture was apparently different for the products prepared by the two synthetic pathways since the product mixtures had [α] values with different signs. It may be noted that it has been shown previously that 20-chloro CPT, formed from CPT by treatment with $SOCl_2$, was nearly racemic.[12]

The ability of the mercaptoCPT derivatives to stabilize the topoisomerase I–DNA covalent binary complex was evaluated using a ^{32}P-end labeled 222 base pair DNA duplex. Figure 2 shows the effect of CPT and CPT analogues **8a**,**b**, and **9** on the stabilization of the topoisomerase I–DNA binary complex formed at individual sites within this DNA duplex. In common with CPT, 20-mercaptocamptothecin (**8a** and **8b**) effected stabilization of the enzyme–DNA covalent binary complex. While the monomercapto derivatives **8a** and **8b** were less potent than CPT, stabilization was observed at the same sites as for CPT itself. In contrast dimercapto derivative **9** had only a very slight effect on the stabilization of the topoisomerase I–DNA complex. Neither CPT nor the mercapto derivatives had any effect on DNA in the absence of topoisomerase I.

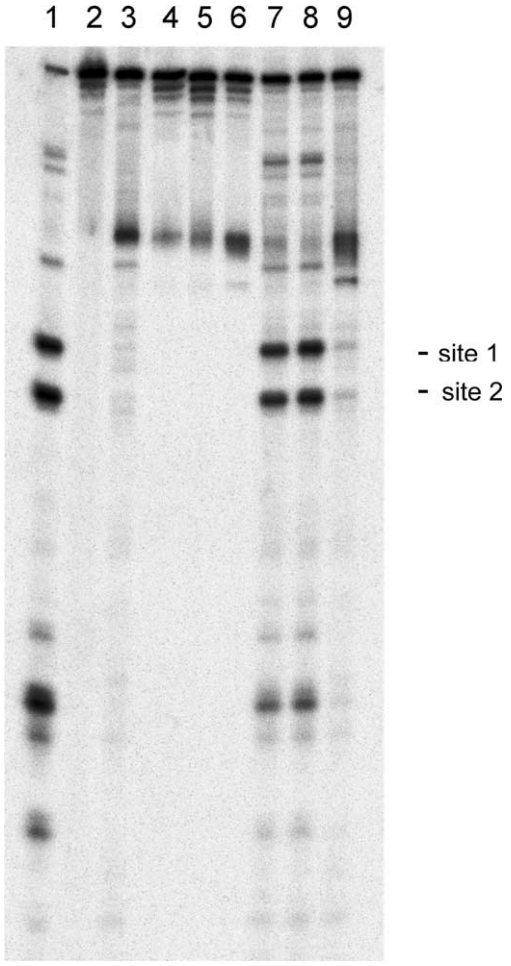


Figure 2. Autoradiogram of a 10% denaturing polyacrylamide gel showing the effects of the CPT derivatives on human topoisomerase I-mediated cleavage of the *Hin*dIII-*Pvu*II DNA restriction fragment of pSP64 plasmid DNA. The DNA was 3′-^{32}P end-labeled on the cleaved strand. The reaction mixtures were incubated at 37 °C for 1 h and then digested with proteinase K prior to gel analysis. Lane 1, 50 μM CPT+36 ng of topoisomerase I; lane 2, DNA alone; lane 3, topoisomerase I alone; lane 4, 50 μM CPT **8a** alone; lane 5, 50 M CPT **8b** alone; lane 6, 50 μM CPT **9** alone; lane 7, 50 μM CPT **8a**+topoisomerase I; lane 8, 50 μ M CPT **8b**+topoisomerase I; lane 9, 50 μM CPT **9**+topoisomerase I.

The religation of the DNA following treatment of the ternary complex with NaCl was measured. As shown in Figure 3, the time course of religation at two sites in the enzyme–DNA complex (cf. Fig. 2) was measured following treatment with NaCl and pseudo first-order dissociation rate constants were calculated using the data obtained at 30 and 60 s following NaCl treatment.[13] The sample containing CPT displayed rate constants for religation of 9.9 and $10.8 \times 10^{-3}\,s^{-1}$ at sites 1 and 2, respectively. The comparable values for CPT derivative **8a** were 18.7 and $39.3 \times 10^{-3}\,s^{-1}$ at sites 1 and 2, respectively. Thus CPT derivative **8a** had an off-rate from the ternary complex 2–4 fold greater than that of CPT itself.

To evaluate the possible cytotoxic effects resulting from stabilization of the topoisomerase I–DNA covalent binary complex, CPT (**1**) and the mercapto CPT derivatives **8a**,**b**, and **9** were evaluated in a strain of *Saccharomyces cerevisiae* lacking yeast topoisomerase I, but harboring a plasmid containing the human topoisomerase I gene under the control of a galactose promoter.[14] As shown in Table 1, 1 μM CPT had only a slight effect when this yeast strain was grown on raffinose. However, when grown on galactose, the same strain showed 88% growth inhibition in the presence of 1 μM CPT, reflecting the expression of DNA topoisomerase I. While CPT was

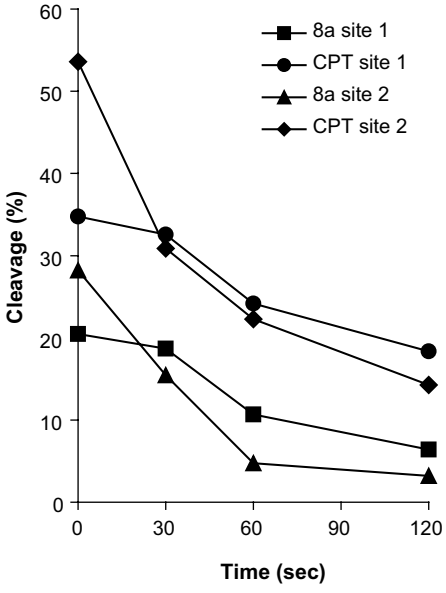


Figure 3. Time course of religation of the complexes formed in the presence of CPTs **1** and **8a**. The DNA substrate was the same one shown in Figure 2. The religation reaction was initiated by addition of NaCl to a final concentration of 0.35 M at 23 °C.

Table 1. Human topoisomerase I-dependent cytotoxicity of camptothecin and two CPT analogues[a]

Compound	Concentration (μM)	% Inhibition on growth medium		
		Raffinose	Galactose	IC_{50}-TOPI-Gal (μM)
CPT (**1**)	2.0	40	88	
	1.0	12	88	0.033
8[b]	50	17	91	
	25	26	56	20.0
	10	30	51	
9	50	0	0	
	25	0	0	No activity
	10	0	0	

[a] Inhibition of RS321 Nph-TOP1 grown in minimal medium containing 3% raffinose or galactose for 2 days at 30 °C.
[b] Inhibition resulting from treatment with CPT **8a**. Compound **8b** gave 55% inhibition in the presence of galactose when employed at 25 μM concentration.

more potent than the monomercapto CPT derivatives **8a** and **8b**, replacement of the alcohol by a thiol moiety still afforded a cytotoxic compound. In comparison, the dimercapto CPT **9** was not cytotoxic in this assay.

3. Discussion

The synthesis of the 20 mercaptoCPT (**8a**) via 20-chloro CPT (**7**) was accomplished in reasonable (53%) overall yield (Scheme 1). The chlorination reaction was reported previously to result in almost complete racemization,[12] so it was anticipated that 20-mercapto CPT would be obtained essentially as a racemate. The low absolute value of the $[\alpha]_D$ obtained for **8a** (−5.5) was consistent with this expectation. Interestingly, **8b** had $[\alpha]_D$ +6.7, suggesting that the other enantiomer was slightly in excess. Because only the (*S*) enantiomer of CPT itself stabilized the topoisomerase I–DNA covalent binary complex,[15] it was anticipated that only 20(*S*)-mercapto CPT might be capable of stabilizing the enzyme–DNA binary complex. Thus the potency of complex stabilization reflected in Figure 2, and cytotoxicity in Table 1, need to be viewed in this context. Nonetheless, the IC_{50} value of **8a** (20 μM for an essentially racemic mixture) was still much greater than that observed for 20(*S*) CPT (**1**) itself (0.033 μM).

It has been shown previously that treatment of incubation mixtures containing a DNA–topoisomerase I–CPT ternary complex with NaCl results in dissociation of the bound CPT, followed by religation of the DNA within the enzyme–DNA covalent binary complex.[13] Because the actual religation reaction is believed to be significantly faster than CPT (analogue) dissociation in most cases, the time course of religation actually reflects the rate of dissociation of the CPT (analogue). A previous study has suggested that persistence of the enzyme–DNA–CPT (analogue) ternary complex may contribute importantly to the expression of cytotoxicity of that individual CPT analogue.[13] The relatively rapid off-rate of **8a** from the complex (Fig. 3) may well contribute to the relatively modest yeast cytotoxicity observed for this (racemic) analogue (Table 1).

In an earlier study, the 20-OH group of CPT was replaced with H, Cl, Br, N_3, and NH_2 substituents.[12] While the 20-deoxy and 20-azido CPTs failed to stabilize the topoisomerase I–DNA covalent binary complex to a significant extent, or to display cytotoxicity in yeast expressing human DNA topoisomerase I, the 20-chloro, 20-bromo, and 20-NH_2 CPT derivatives were active in these assays. When tested as (essentially) racemic mixtures, all three compounds stabilized the topoisomerase I–DNA covalent binary complex with the same sequence selectivity as CPT itself. When employed in the same yeast assay described in Table 1, the IC_{50} values for the 20-chloro, 20-bromo, and 20-NH_2 CPTs were 2.1, 6.7, and 47 μM versus a value of 0.2 μM determined for 20(*S*) CPT. Thus the IC_{50} value of 20 μM obtained in the present study for 20-mercapto CPT **8** does not represent an exceptionally strong cytotoxic response.

It is interesting that while all of the 20-substituted CPT derivatives studied displayed reasonable potency in stabilizing the topoisomerase I–DNA covalent binary complex (Fig. 2 and Ref. 12), the analogues that displayed the strongest topoisomerase I-dependent cytotoxicity in yeast (20-chloro CPT and 20-bromo CPT) lacked any H atom attached to the heteroatom substituent in the 20-position. These two derivatives also displayed off-rates from the enzyme–DNA–inhibitor ternary complexes not dissimilar to that of CPT itself. That neither of these 20-substituted CPTs has a H attached to the substituent at C-20 argues that the donor H-bond, which appears in each of the models of the ternary complex[10,11] cannot be essential to stabilization of the covalent binary complex. This conclusion is reinforced by the activities of the 20-amino and 20-mercapto CPTs, which were no more effective than the 20-halo CPTs in stabilizing the enzyme–DNA covalent binary complex and actually significantly less effective in mediating topoisomerase I-dependent cytotoxicity in the yeast strain expressing the human enzyme.

While the X-ray crystallographic analysis of topotecan bound to a model topoisomerase I–DNA oligonucleotide covalent binary complex[10] does not suggest any role for the C-21 carboxyl moiety of CPT in H-bonding to the binary complex, each of the four models[11] based on (bio)chemical results does posit one or more H-bonding interactions for this O atom. In this sense, the lack of activity of dimercapto CPT **9** tends to support these models, although only two envision interaction with the same structural element in the binary complex, namely Arg 364.[11b,c] Interestingly, both of these models also posit a bidentate interaction of the same amino acid residue with the E-ring lactone O-atom. In fact, it has been shown previously[16] that replacement of the endocyclic lactone O atom with S afforded an analogue that failed to stabilize the topoisomerase I–DNA cleavage binary complex.

In the aggregate, the data presently available are not inconsistent with the involvement of H-bonding interactions between E-ring substituents of CPT with DNA and amino acid residues in the topoisomerase I–DNA binary complex in stabilizing the formed ternary com-

plex. However, it seems clear that a donor H-bond from substituents in the 20-position cannot be essential for ternary complex stabilization and that variations in the mode of binding of individual CPTs may well be involved. In this context it may be noted that CPTs linked covalently to triplex-forming oligonucleotides in distinctly different orientations both strongly facilitated cleavage by topoisomerase I at the site containing the tethered CPT.[17] Thus it seems possible that individual CPTs may bind to the topoisomerase I–DNA ternary complex in orientations that differ somewhat from analogue to analogue. In fact, this would seem to be the most likely explanation for the ability of luotonin A (**6**) to stabilize the enzyme–DNA covalent binary complex in analogy with CPT,[18] since the absence of structural elements in the E-ring of luotonin A found to be essential for the CPTs to function as topoisomerase I poisons[1,5,16] should otherwise render luotonin A dysfunctional as a topoisomerase I poison.

4. Experimental

4.1. General methods

All reaction glassware, transfer syringes, and cannulas were thoroughly dried prior to use. All synthetic transformations were carried out under dry argon. Dry solvents were purchased from Aldrich Chemical Co. Deuterated NMR solvents were purchased from Cambridge Isotope. Flash chromatography was performed using E. Merck silica gel 60 (40–63 mm) and reagent grade solvents. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian spectrometer (300 MHz). Chemical shifts are reported in δ; coupling constants are given in Hz. Melting points are uncorrected.

4.2. 20-Chlorocamptothecin (7)

To a suspension of 100 mg (287 μmol) of camptothecin in 50 mL of benzene was added 210 μL (342 mg, 2.87 mmol) of thionyl chloride and 2 mL (1.96 g, 24.7 mmol) of pyridine. The reaction mixture was stirred at reflux for 2 h, then concentrated under diminished pressure. The residue was purified by flash chromatography on a silica gel column (30 × 4.5 cm). Gradient elution with 0 → 1% methanol in dichloromethane gave **7** as yellow solid: yield 70 mg (66%); silica gel TLC R_f 0.48 (95:5 dichloromethane–methanol); ^{1}H NMR ($CDCl_3$) δ 1.02 (t, 3H, J = 7.4 Hz), 2.56 (m, 1H), 2.73 (m, 1H), 5.32 (s, 2H), 5.55 (s, 2H), 7.55 (s, 1H), 7.69 (td, 1H, J = 7.6 and 1.0 Hz), 7.86 (td, 1H, J = 7.7 and 1.5 Hz), 7.95 (d, 1H, J = 8.1 Hz), 8.26 (d, 1H, J = 8.5 Hz), and 8.42 (s, 1H); mass spectrum (APCI(+)), m/z 330.8 $(M-Cl)^+$ (theoretical 331.1).[1a]

4.3. 20-Mercaptocamptothecin (8a) (route A)

To a solution of 70 mg (190 μmol) of **7** in 8 mL of 1:1 CH_2Cl_2–DMF was added 30 μL (22.1 mg, 171 μmol) of diisopropylethylamine. A stream of hydrogen sulfide was condensed in the solution for 2 h at 25 °C. The reaction mixture was concentrated under diminished pressure. The residue was purified by flash chromatography on a silica gel column (30 × 4.5 cm). Gradient elution with 0 → 1% methanol in dichloromethane gave **8a** as yellow solid: yield 56 mg (81%); silica gel TLC R_f 0.33 (95:5 dichloromethane–methanol); $[\alpha]_D^{24}$ −5.5 (c 0.40, 1:4 methanol–$CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.08 (t, 3H, J = 7.4 Hz), 2.09 (m, 2H), 3.65 (t, 1H, J = 7.6 Hz), 5.28 (s, 2H), 5.38 (d, 1H, J = 16.1 Hz), 5.55 (d, 1H, J = 16.1 Hz), 7.18 (s, 1H), 7.66 (td, 1H, J = 7.5 and 1.1 Hz), 7.83 (td, 1H, J = 7.7 and 1.5 Hz), 7.93 (d, 1H, J = 7.9 Hz), 8.21 (d, 1H, J = 8.3 Hz), and 8.39 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 11.5, 25.5, 45.5, 50.1, 66.2, 100.0, 120.8, 128.2, 128.3, 128.3, 128.7, 129.7, 130.9, 131.4, 146.0, 147.2, 148.9, 152.4, 158.0, and 171.1; mass spectrum (negative ion ESI), m/z 331.4 $(M-1-SH)^-$ (theoretical 331.4).

4.4. 20-Mercaptocamptothecin (8b) and 20,21-bismercaptocamptothecin (9) (route B)

To a solution of 200 mg (574 μmol) of camptothecin in 30 mL of pyridine was added 1.16 g (2.87 mmol) of Lawesson's reagent. The reaction mixture was stirred at reflux for 2 h. To the reaction mixture was added 1.16 g (2.87 mmol) of Lawesson's reagent. The reaction mixture was stirred at reflux for an additional 30 h. To the reaction mixture was added 1.16 g (2.87 mmol) of Lawesson's reagent. The reaction mixture was stirred at reflux for an additional 14 h. The reaction mixture was concentrated under diminished pressure and the residue was purified by flash chromatography on a silica gel column (30 × 7 cm). Gradient elution with 0 → 2% methanol in dichloromethane gave a mixture of compounds having ultraviolet spectra similar to camptothecin. Purification was achieved by semi-preparative (25 × 1 cm column) C_{18} reversed phase HPLC using a linear gradient of H_2O containing increasing amounts of CH_3CN (0 → 40 min, linear gradient from 35% → 60% CH_3CN at a flow rate of 3 mL/min: R_t 17 min for **8b** and R_t 28 min for **9**). This procedure gave **8b** as a yellow solid: yield 6.5 mg (3%) and **9** as an orange solid: yield 17.5 mg (8%). The eluate was monitored at 360 nm.

4.5. Compound 8b

$[\alpha]_D^{24}$ +6.71 (c 0.23, 1:4 methanol–$CHCl_3$; ^{1}H NMR ($CDCl_3$) δ 1.13 (t, 3H, J = 7.5 Hz), 2.11 (m, 2H), 3.65 (t, 1H, J = 7.6 Hz), 5.34 (s, 2H), 5.43 (d, 1H, J = 16.1 Hz), 5.60 (d, 1H, J = 16.1 Hz), 7.29 (s, 1H), 7.71 (td, 1H, J = 7.1 and 1.0 Hz), 7.88 (td, 1H, J = 7.1 and 1.2 Hz), 7.98 (d, 1H, J = 7.8 Hz), 8.27 (d, 1H, J = 8.5 Hz), and 8.44 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 11.5, 25.5, 46.0, 50.1, 66.2, 100.2, 121.0, 128.27, 128.30, 128.33, 128.8, 129.6, 131.0, 131.6, 145.9, 147.3, 148.8, 152.4, 158.0, and 171.0; mass spectrum (negative ion ESI), m/z 363.3 $(M-1)^-$ (theoretical 363.4).

4.6. Compound 9

$[\alpha]_D^{24}$ +22.6 (c 0.20, 1:4 methanol–$CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.10 (t, 3H, J = 7.4 Hz), 1.93 (m, 1H), 2.11 (m, 1H), 3.65 (t, 1H, J = 7.6 Hz), 4.37 (d, 1H, J = 16.1 Hz), 4.60 (d, 1H, J = 16.1 Hz), 5.30 (s, 2H),

7.20 (s, 1H), 7.66 (td, 1H, $J = 7.1$ and 1.0 Hz), 7.83 (td, 1H, $J = 8.3$ and 1.5 Hz), 7.93 (d, 1H, $J = 8.3$ Hz), 8.23 (d, 1H, $J = 8.5$ Hz), and 8.39 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 12.5, 24.2, 26.6, 50.4, 59.8, 102.2, 123.5, 128.1, 128.2, 128.3, 128.9, 129.6, 130.9, 131.5, 144.8, 148.7, 150.8, 152.5, 159.3, and 203.9; mass spectrum (FAB), *m/z* 381.0742 $(M+1)^+$ (theoretical 381.0732).

Topoisomerase I-mediated DNA cleavage: The topoisomerase I-mediated DNA cleavage reaction was carried out at 37 °C for 30 min in a 40 μL (total volume) reaction mixture containing 20 mM Tris–HCl (pH 7.6), 10 mM KCl, 5 mM $MgCl_2$, 0.5 mM EDTA, 0.5 mM DTT, 30 μg/mL BSA, 12 fmol of labeled DNA restriction fragment, and 36 ng of human DNA topoisomerase I. The final concentration of CPT analogues employed was 50 μM. The reactions were terminated by SDS-proteinase K treatment. Following sequential extraction with phenol and chloroform, the DNA was recovered by ethanol precipitation. The DNA was dissolved in a formamide loading buffer (80% formamide, 10 mM NaOH, 1 mM EDTA, and 0.1% xylene cyanol and bromophenol blue), and analyzed on a 10% denaturing gel to determine topoisomerase I cleavage.

Yeast strain growth: A transformed strain of *S. cerevisiae*, RS321Nph-TOP1, had the following genotype: RS321Nph-TOP1 (*Mat a ade2-1 his3-1 leu3,112 trp1-1 ura3-1 can1-100 erg6 rad52::TRP1 top1-8::LEU2 phTOP1::URA*). The yeast strain was grown from a 15% glycerol stock to log phase (OD_{595} 1–3) at 30 °C in minimal media (0.9% yeast nitrogen base without amino acids, 0.025 mg/mL each of adenine and histidine) containing 3% glucose as the carbon source. The yeast was then transferred to the same minimal medium containing 3% raffinose, a neutral carbon source, instead of glucose. Cultures were then grown to log phase (OD_{595}1–3).

Yeast cytotoxicity assay: The exponentially growing yeast strain was diluted to an absorbance of 0.015 at 595 nm with minimal media having 3% galactose or minimal media having 3% raffinose. Samples to be assayed were dissolved in DMSO to 50 mM, then diluted in the yeast incubation to the appropriate concentrations with a final DMSO concentration less than 1% in a 96-well microtiter plate. Camptothecin was used as a positive control for DNA damage activity. The plates were incubated at 30 °C in a high humidity chamber for 48 h and the optical density of cells in each well was measured at 595 nm by using a microplate reader. Data was plotted as OD_{595} versus natural log of concentration. This provided a linear plot for easy analysis of IC_{50} values, defined as the concentration of a compound at which yeast growth was inhibited by 50%.

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References and notes

1. (a) Wall, M. E.; Wani, M. C.; Cook, C. E.; Palmer, K. H.; McPhail, A. T.; Sim, G. A. *J. Am. Chem. Soc.* **1966**, *88*, 3888; (b) Wall, M. E. *Med. Res. Rev.* **1998**, *18*, 299.
2. (a) Hsiang, Y.-H.; Hertzberg, R.; Hecht, S.; Liu, L. F. *J. Biol. Chem.* **1985**, *260*, 14873; (b) Hsiang, Y.-H.; Liu, L. F. *Cancer Res.* **1988**, *48*, 1722; (c) Covey, J. M.; Jaxel, C.; Kohn, K. W.; Pommier, Y. *Cancer Res.* **1989**, *49*, 5016; (d) Jaxel, C.; Kohn, K. W.; Wani, M. C.; Wall, M. E.; Pommier, Y. *Cancer Res.* **1989**, *49*, 1465; (e) Kohn, K. W.; Pommier, Y. *Ann. N.Y. Acad. Sci.* **2000**, *922*, 11; (f) Kim, D.-K.; Lee, N. *Mini. Rev. Med. Chem.* **2002**, *2*, 611.
3. (a) Liu, L. F.; Desai, S. D.; Li, T.-K.; Mao, Y.; Sun, M.; Sim, S.-P. *Ann. N.Y. Acad. Sci.* **2000**, *922*, 1; (b) Ulukan, H.; Swaan, P. W. *Drugs* **2002**, *62*, 2039.
4. (a) Moertel, C. G.; Schutt, A. J.; Reitemeier, R. J.; Hahn, R. G. *Cancer Chemoth. Rep.* **1972**, *56*, 95; (b) Hsiang, Y.-H.; Liu, L. F.; Wall, M. E.; Wani, M. C.; Nicholas, A. W.; Manikumar, G.; Kirschenbaum, S.; Silber, R.; Potmesil, M. *Cancer Res.* **1989**, *49*, 4385.
5. Kingsbury, W. D.; Boehm, J. C.; Jakas, D. R.; Holden, K. G.; Hecht, S. M.; Gallagher, G.; Caranfa, M. J.; McCabe, F. L.; Faucette, L. F.; Johnson, R. K.; Hertzberg, R. P. *J. Med. Chem.* **1991**, *34*, 98.
6. Sawada, S.; Okajima, S.; Aiyama, R.; Nokata, K.-I.; Furuta, T.; Yokokura, T.; Sugino, E.; Yamaguchi, K.; Miyasaka, T. *Chem. Pharm. Bull.* **1991**, *39*, 1446.
7. Garcia-Carbonero, R.; Supko, J. G. *Clini. Cancer Res.* **2002**, *8*, 641.
8. Thomas, C. J.; Rahier, N. J.; Hecht, S. M. *Bioorg. Med. Chem.* **2004**, *12*, 1585.
9. Bailly, C. *Crit. Rev. Oncol./Hemat.* **2003**, *45*, 91.
10. Staker, B. L.; Hjerrild, K.; Feese, M. D.; Behnke, C. A.; Burgin, A. B.; Stewart, L. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 15397.
11. (a) Fan, Y.; Weinstein, J. N.; Kohn, K. W.; Shi, L. M.; Pommier, Y. *J. Med. Chem.* **1998**, *41*, 2216; (b) Redinbo, M. R.; Stewart, L.; Kuhn, P.; Champoux, J. J.; Hol, W. G. J. *Science* **1998**, *279*, 1504; (c) Kerrigan, J. E.; Pilch, D. S. *Biochemistry* **2001**, *40*, 9792; (d) Laco, G. S.; Collins, J. R.; Luke, B. T.; Kroth, H.; Sayer, J. M.; Jerina, D. M.; Pommier, Y. *Biochemistry* **2002**, *41*, 1428.
12. Wang, X.; Zhou, X.; Hecht, S. M. *Biochemistry* **1999**, *38*, 4374.
13. Wang, X.; Wang, L.-K.; Kingsbury, W. D.; Johnson, R. K.; Hecht, S. M. *Biochemistry* **1998**, *37*, 9399.
14. Bjornsti, M. A.; Benedetti, P.; Viglianti, G. A.; Wang, J. C. *Cancer Res.* **1989**, *49*, 6318.
15. Wall, M. E.; Wani, M. C.; Nicholas, A. W.; Manikumar, G.; Tele, C.; Moore, L.; Truesdale, A.; Leitner, P.; Besterman, J. M. *J. Med. Chem.* **1993**, *36*, 2689.
16. Hertzberg, R. P.; Caranfa, M. J.; Holden, K. G.; Jakas, D. R.; Gallagher, G.; Mattern, M. R.; Mong, S.-M.; Bartus, J. O.; Johnson, R. K.; Kingsbury, W. D. *J. Med. Chem.* **1989**, *32*, 715.
17. Arimondo, P. B.; Boutorine, A.; Baldeyrou, B.; Bailly, C.; Kuwahara, M.; Hecht, S. M.; Sun, J.-S.; Garestier, T.; Hélène, C. *J. Biol. Chem.* **2002**, *277*, 3132.
18. (a) Cagir, A.; Jones, S. H.; Gao, R.; Eisenhauer, B. M.; Hecht, S. M. *J. Am. Chem. Soc.* **2003**, *125*, 13628; (b) Cagir, A.; Jones, S. H.; Eisenhauer, B. M.; Gao, R.; Hecht, S. M. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 2051.