Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry 12 (2004) 6287–6299
Bioorganic & Medicinal Chemistry

Synthesis and topoisomerase I inhibitory properties of luotonin A analogues

Ali Cagir, Brian M. Eisenhauer, Rong Gao, Shannon J. Thomas and Sidney M. Hecht*

Department of Chemistry and Department of Biology, University of Virginia, Charlottesville, VA 22901, USA

Received 28 June 2004; accepted 18 August 2004
Available online 30 September 2004

Abstract—Luotonin A, a naturally occurring pyrroloquinazolinoquinoline alkaloid, has been previously demonstrated to be a topoisomerase I poison. A number of luotonin A derivatives have now been prepared through the condensation of anthranilic acid derivatives and 1,2-dihydropyrrolo[3,4-*b*]quinoline-3-one in the presence of phosphorus oxychloride. When dichloromethane was used as solvent the reaction proceeded to a single product. In contrast when the reaction was carried out in tetrahydrofuran or in phosphorus oxychloride, an additional isomeric product was obtained. The luotonin A analogues were evaluated for their ability to effect stabilization of the covalent binary complex formed between human topoisomerase I and DNA, and for cytotoxicity toward a yeast strain expressing the human topoisomerase I.

1. Introduction

Camptothecin (CPT, **1**) is a cytotoxic alkaloid first isolated from *Camptotheca acuminata* in 1966 by Wall et al. (Fig. 1).[1] Two semi-synthetic camptothecin analogues, topotecan, and irinotecan, are now used clinically for the treatment of ovarian small cell lung and colon cancers.[2] The success of these analogues in the clinic has encouraged studies to identify additional topoisomerase I poisons.[3] CPT and its analogues are believed to function primarily at the locus of the topoisomerase I–DNA covalent binary complex.

Topoisomerase I is an enzyme[4] that catalyzes the relaxation of supercoiled chromosomal DNA during replication and transcription.[5] This process is initiated by (reversible) covalent attachment of topoisomerase I to the DNA backbone, creating a transient single strand break in the DNA duplex.[5] The formed topoisomerase I–DNA covalent binary complex normally undergoes religation to afford an intact duplex following DNA relaxation, but can be stabilized by camptothecin via formation of a ternary complex.[6] Persistence of the stabilized DNA break leads to cell death.[7]

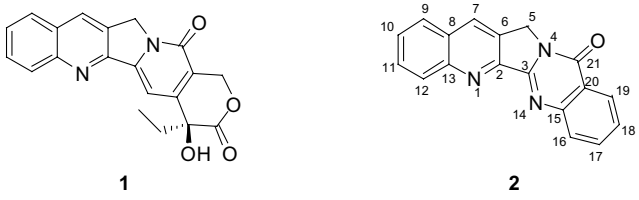


Figure 1. Structures of CPT (**1**) and luotonin A (**2**).

The nature of the CPT–topoisomerase I–DNA ternary complex has been studied by molecular modeling[8] and X-ray crystallographic analysis.[9] While the detailed models resulting from these studies differ significantly, two types of interactions appear to contribute to the binding of CPT to the topoisomerase I–DNA covalent binary complex. These include H-bonding interactions with the (*S*)-α-hydroxylactone (E-ring) and stacking of CPT between adjacent base pairs in the DNA substrate at the site of reversible DNA cleavage. The importance of the 20(*S*)-α-hydroxylactone has also been defined by preparing E-ring modified analogues. While 20-chloro, bromo, and amino derivatives of CPT could stabilize the topoisomerase I–DNA covalent binary complex, 20(*R*)-OH CPT, and 20-deoxy CPT were essentially incapable of stabilizing the binary complex.[10,11]

While the relative contributions of hydrogen bonding and π-stacking interactions to stabilization of the CPT–topoisomerase I–DNA ternary complex are not yet clear, the importance of π-stacking interactions

Keywords: Camptothecin; Luotonin A; Topoisomerase I–DNA inhibition; Structure–activity relationships.
* Corresponding author. Tel.: +1 434 924 3906; fax: +1 434 924 7856; e-mail: sidhecht@virginia.edu

0968-0896/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2004.08.052

was reinforced by the finding that luotonin A (**2**) is a topoisomerase I poison.[11] Luotonin A (**2**) is a pyrroloquinazolinoquinoline alkaloid, originally isolated from *Peganum nigellastrum* (Fig. 1).[12] The structure of **2** is similar to that of CPT. The 20(*S*)-α-hydroxylactone ring in CPT is replaced by a benzene ring in luotonin A and the (14)-CH moiety of CPT is replaced by a nitrogen atom in luotonin A. In addition to their structural similarities, we have also reported that topoisomerase I-mediated DNA cleavage exhibits the same pattern in the presence of both **1** and **2**, consistent with the possibility that CPT and luotonin A interact with the topoisomerase I–DNA binary complex in a similar fashion. One notable difference between the compounds is the potency of luotonin A, which is significantly less efficient than CPT in stabilizing the topoisomerase I–DNA covalent binary complex and approximately 10-fold less cytotoxic than camptothecin toward a yeast cell line expressing human topoisomerase I.[11,13] This difference might well be due to lack of the (*S*)-α-hydroxylactone ring of CPT and consequent absence of hydrogen bonds between the drug and the topoisomerase I–DNA binary complex. We have shown previously that the potency of luotonin A may be altered by substitution in ring E.[14]

Presently, we have explored further alterations of the E-ring of luotonin A to define the effects of potential π-stacking and H-bonding interactions on the ability of luotonin A derivatives to function as topoisomerase I poisons.

2. Results

Since the initial report of the isolation of luotonin A,[12] eleven different synthetic routes have been reported.[15] Five of these routes have utilized a coupling of the pyrroloquinoline precursor **4**[16] and anthranilic acid derivatives as the final step.[15a–e] We envisioned the preparation of luotonin A derivatives by the condensation of anthranilic acid methyl esters with imino chloride **3**,[15a] the latter of which can be obtained in situ by treatment of pyrroloquinoline **4** with phosphorus oxychloride (Figs. 2 and 3). While Lee et al. reported that the chlorination of **4** with phosphorus oxychloride followed by treatment with methyl anthranilate gave either low yields or side products,[15a] we found that the condensation of **4** with methyl anthranilate in the presence of phosphorus oxychloride at 40 °C prevented the formation of side products.[14] As shown in Figure 3, eight new luotonin A derivatives modified in ring E (**5a**–**g**) were prepared by this method, as well as 16,17,18,19-tetrahydroluotonin A (**6**) (Fig. 3). The yields of products **5** varied from 11% to 62%. Compound **7**, prepared from **5d** by demethylation with hydrogen bromide at reflux, has a phenolic OH substitutent oriented roughly in the same position as the 20(*S*)-OH group of CPT (cf. Figs. 1 and 4). Luotonin B (**8**) (Fig. 4), another natural alkaloid isolated from *Peganum nigellastrum*,[12] was prepared from luotonin A in two steps. First the methylene carbon was mono-brominated with *N*-bromosuccinimide, and then the bromine was replaced with OH by treatment with silver oxide at reflux.[15j]

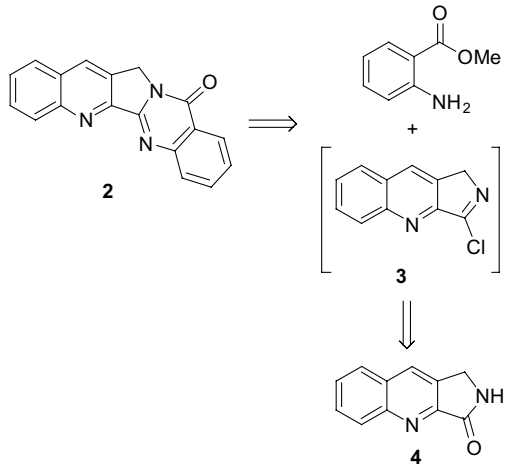
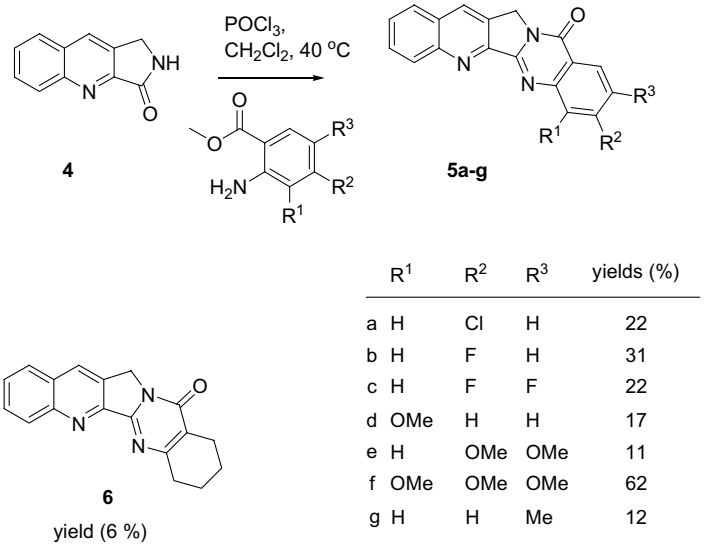


Figure 2. Retrosynthetic analysis of luotonin A.



	R^1	R^2	R^3	yields (%)
a	H	Cl	H	22
b	H	F	H	31
c	H	F	F	22
d	OMe	H	H	17
e	H	OMe	OMe	11
f	OMe	OMe	OMe	62
g	H	H	Me	12

Figure 3. Synthesis of luotonin A derivatives **5a**–**g** and **6**.

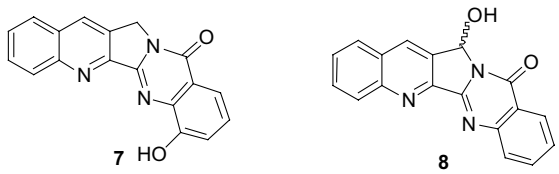


Figure 4. Structures of 16-hydroxyluotonin A (**7**) and luotonin B (**8**).

An interesting rearrangement has been reported previously for **4**. Treatment of **4** with phosphorus pentasulfide gave 78% of **9** and 6% of isomeric **10**. Slow conversion of **9** to **10** was also observed in the presence of phosphorus pentasulfide at 85 °C or hydrogen sulfide at 120 °C in the presence of 1% aqueous pyridine (Fig. 5).[17]

Similarly, the formation of a side product has been reported for the condensation of **4** with methyl anthranilate.[15a] We were able to separate this side product from luotonin A. Treatment of a mixture of **4** and methyl anthranilate with phosphorus oxychloride in tetrahydrofuran at 40 °C gave 12% luotonin A and 4% of the side product. The same result was obtained when the reaction was performed neat. In contrast, when the sequence of addition was changed the ratio of the products also changed. Treatment of **4** with phosphorous oxychloride at 40 °C in tetrahydrofuran for 1 h followed by addition of methyl anthranilate reversed the ratio of products, giving 4% luotonin A and 22% of the side product (Fig. 6). Mass spectrometric analysis of the side product indicated that it had the same molecular weight as luotonin A. ^{1}H NMR analysis showed that the proton attached to C-7 of luotonin A (Fig. 1) was shifted downfield approximately 0.5 ppm, in principle consistent with the interpretation that the adjacent methylene group was substituted. However, the resonance for the C-5 methylene group was still present in the ^{1}H NMR spectrum of the side product. Additionally, while the NOESY spectrum of **2** showed a crosspeak between the protons attached to C-7 and C-5, this crosspeak was absent in the side product.

The structure of the side product was assigned as **11** (Fig. 6) based on the rearrangement reported by Sugasawa et al.[17] To support this assignment the isomeric pyrroloquinoline precursor **12**[18] was prepared and treated with methyl anthranilate under the same conditions. Luotonin A was produced as the minor product (3%) while isoluotonin A (**11**) was obtained in somewhat greater (6%) yield (Fig. 6).

As shown in Table 1, a number of E-ring substituted luotonin A derivatives (**14a**–**20a**) and isoluotonin A derivatives (**13, 14b**–**20b**) were prepared and characterized. Additionally different luotonin A and isoluotonin

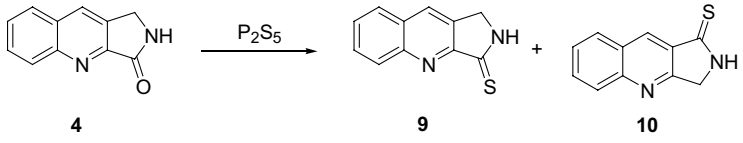


Figure 5. Synthesis of thiolactam **9** and formation of rearranged product **10**.

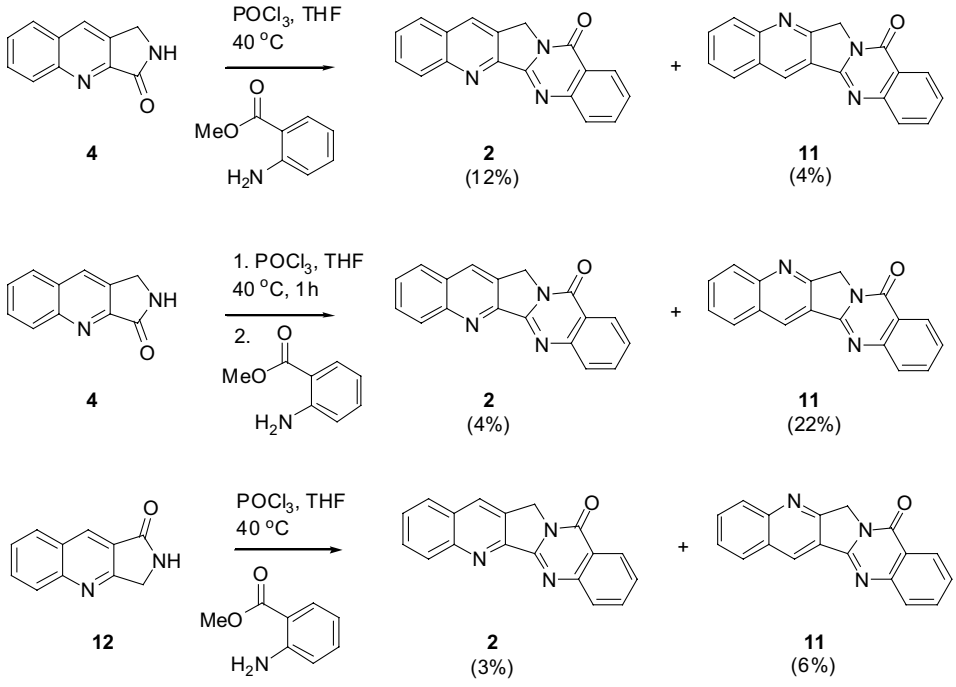
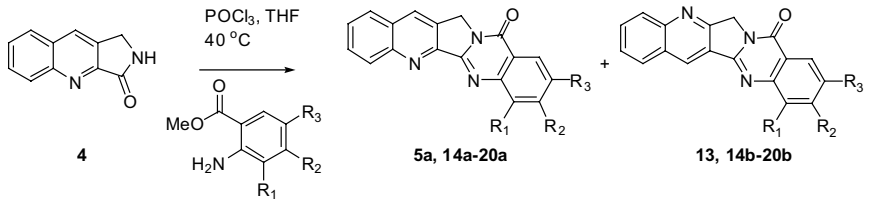


Figure 6. Synthesis of luotonin A (**2**) and formation of rearranged product isoluotonin A (**11**).

Table 1. Synthesis of E-ring modified luotonin A derivatives



	R_1	R_2	R_3	Yield (%)	Yield (%)
5a, **13**	H	Cl	H	37	9
14a, **14b**	H	CO_2Me	H	13	<1
15a, **15b**	H	CO_2Bn	H	24	15
16a, **16b**	H	Ph	H	19	9
17a, **17b**	H	CN	H	15	12
18a, **18b**	H	H	Br	14	2
19a, **19b**[a]	H	NO_2	H	9	9
20a, **20b**[a]	CF_3	H	H	14	2

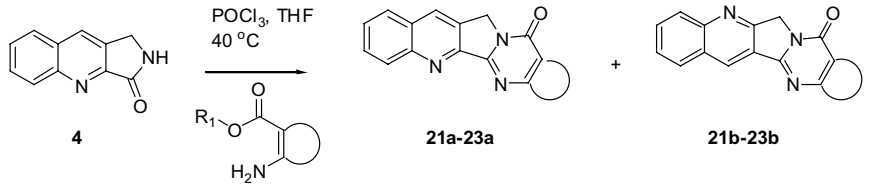
[a] Prepared using the anthranilic acid derivative, rather than its methyl ester, at reflux for 1 h.

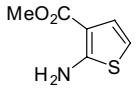
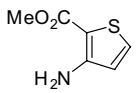
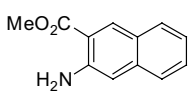
A derivatives, having different aromatic 'E-ring' systems such as thiophene and naphthalene (**21a**–**23a**, and **21b**–**23b**), were prepared using the same method (Table 2).

Several of the luotonin A and isoluotonin A derivatives obtained were also converted to new analogues that may either contribute to π-stacking interaction with DNA bases or form hydrogen bonds with the topoisomerase I–DNA covalent binary complex (Fig. 7). Compound **18a** was transformed to **24** by the use of a Suzuki coupling reaction in 7% yield. The nitro group of **19a** was reduced using tin(II)chloride in acidic medium to afford **25** in 40% yield. The methyl ester derivatives of luotonin A (**14a**) and isoluotonin A (**14b**) were reduced to their alcohols **26** and **27** in 21% and 32% yields, respectively. Finally, the isomer of 17-fluoroluotonin A (**28**) was prepared analogously starting from precursor **12**.

In addition to luotonin A itself,[11] we have recently described the ability of several luotonin A analogues to effect the stabilization of the human topoisomerase I–DNA covalent binary.[14] The derivatives tested included **5a**–**g**, **6**, and **7**. Of these, compounds **5a**,**b**, **6**, and **7** were found to exhibit reasonable stabilization of the binary complex. In the present study, preliminary assays indicated that many of the compounds stabilized the enzyme–DNA covalent binary complex weakly, if at all. Accordingly, the assay was carried out initially at a high concentration of the inhibitors (625 μM) to facilitate the detection of those compounds exhibiting any activity as topoisomerase I poisons. The data are summarized in Table 3, which quantified the extent of cleavage at each of three sites relative to the cleavage observed in the presence of 50 μM CPT.[19] Those luotonin A derivatives which exhibited reasonable activity in the initial assay

Table 2. Synthesis of luotonin A derivatives having different aromatic E-rings



	Entry	Yield (%)	Yield (%)
21a, **21b**		22	16
22a, **22b**		33	3
23a, **23b**		20	4

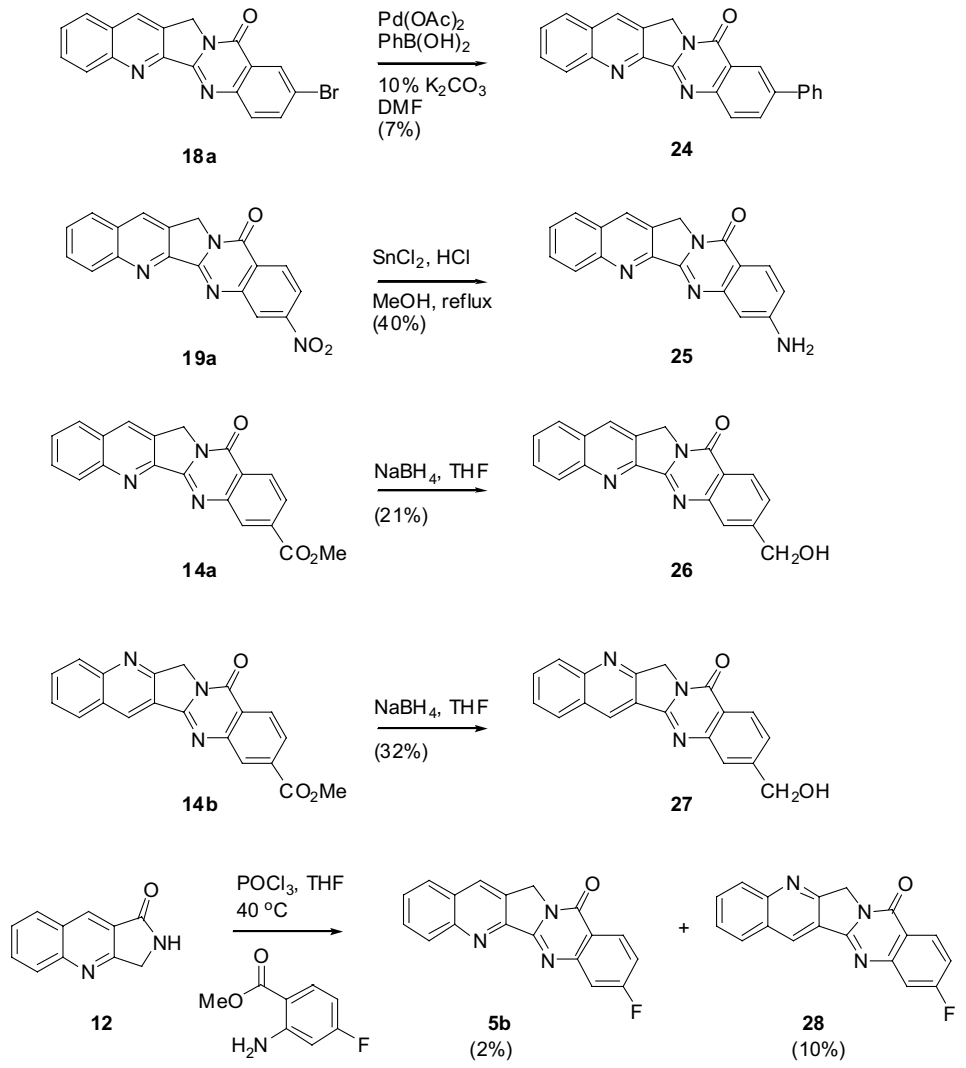


Figure 7. Conversion of luotonin A and isoluotonin A intermediates to new analogues.

were run again at 50 μM concentration in direct comparison with CPT and luotonin A (Fig. 8). As shown in the figure, derivatives exhibiting reasonable activity as topoisomerase I poisons included isoluotonin A (**11**), 17-cyanoluotonin A (**17a**), and 17-cyanoisoluotonin A (**17b**), thiophene analogues **22a** and **22b**, as well as 17-hydroxymethylluotonin A (**26**). Also found to be quite active as a topoisomerase I poison was 17-aminoluotonin A (**25**). It is interesting that the results obtained with the corresponding luotonin A and isoluotonin A derivatives were generally comparable. Thus both 17-chloroluotonin A (**5a**) and 17-chloroisoluotonin A (**13**) afforded reasonably strong stabilization of the enzyme–DNA covalent binary complex. This was also true for the 17-fluoro derivatives of luotonin A (**5b**) and isoluotonin A (**28**), which were both found to afford reasonable stabilization of the covalent binary complex.

Those compounds that mediated reasonable stabilization of the topoisomerase I–DNA covalent binary complex (Table 3 and Fig. 8) were tested for cytotoxicity in a yeast strain that lacked the homologous topoisomerase I, but harbored a plasmid containing the human topoisomerase I gene under the control of a galactose promoter.[13] This yeast strain was also rendered permeable to exogenous agents by virtue of a mutation in the gene for an export pump. The topoisomerase I-dependent cytotoxicity was determined by comparing the results when this yeast strain was grown in raffinose or galactose. In addition to those luotonin A derivatives previously shown to exhibit topoisomerase I-dependent cytotoxicity (i.e., **5b**,**g**, and **6**), two additional derivatives (**11** and **25**) were found to exhibit reasonably strong topoisomerase I-dependent cytotoxic activity. Also fairly strongly cytotoxic were thiophene derivatives **21a**, **22a** and **22b**, although none was selectively cytotoxic to yeast expressing topoisomerase I.

3. Discussion

The synthesis of luotonin A derivatives was accomplished using what has become a fairly common strategy[15a–e] involving the condensation of pyrroloquinoline **4** with anthranilic acid derivatives. While the yields of products varied significantly, and were

Table 3. Stabilization of human topoisomerase I mediated DNA cleavage at three sites in the presence of luotonin A analogues at 625 μM concentration[a]

Compound	Site 1	Site 2	Site 3
5a	5.7	6.0	2.4
5b	11.0	9.2	2.5
11	8.0	2.9	2.6
13	2.6	1.2	0.9
14a	1.0	1.2	0.9
15a	0.7	1.0	0.3
15b	0.7	0.8	0.3
16a	1.7	1.9	0.6
16b	2.6	3.5	1.5
17a	5.4	5.6	2.4
17b	7.6	7.5	4.0
19a	2.3	2.8	1.0
19b	5.2	5.4	2.7
20a	0.8	0.9	0.2
20b	1.7	2.0	0.8
21a	5.7	3.4	2.6
21b	0.9	0	0
22a	21.1	14.4	7.8
22b	15.0	6.4	4.4
23a	3.1	2.6	1.0
23b	0.3	0	0.3
24	1.3	1.7	0.2
25	13.6	13.8	5.0
26	13.4	10.7	8.4
27	6.5	5.4	3.2
28	14.9	9.6	3.6

[a] Relative to the intensity of the band at each site produced by 50 μM CPT.

sometimes rather low, it was possible to obtain sufficient material in each case for the requisite biochemical and biological assays. One interesting facet of the synthesis was the formation of an isomer of luotonin A in which the N atom in ring B was present at position-7 (luotonin A numbering, Fig. 1), rather than position-1. Support for this assignment was obtained in an experiment that involved changing the order of addition of reagents, and another that started with precursor **12**, an isomer of **4** that should lead unambiguously to the 7-aza, 1-deaza analogue of luotonin A (Fig. 6). An NOE experiment, demonstrating the lack of a crosspeak between the protons at positions 5 and 7, also supported the assigned structure **11**.

The initial biochemical assays, involving stabilization of topoisomerase I-mediated DNA cleavage of a 222-base pair DNA restriction fragment, indicated rather modest stabilization of cleavage for most of the luotonin A analogues not studied previously. To assure that the lack of stabilization was not due to unusual dose–response profiles, all of the new analogues were assayed at very high concentration (Table 3). Those which afforded reasonable stabilization of the topoisomerase I–DNA covalent binary complex were tested at 50 μM concentration in direct comparison with luotonin A (Fig. 8). Surprisingly, little difference in cleavage stabilization was observed at the lower concentration, quite possibly reflecting the fact that most of the analogues were poorly soluble in aqueous solution.

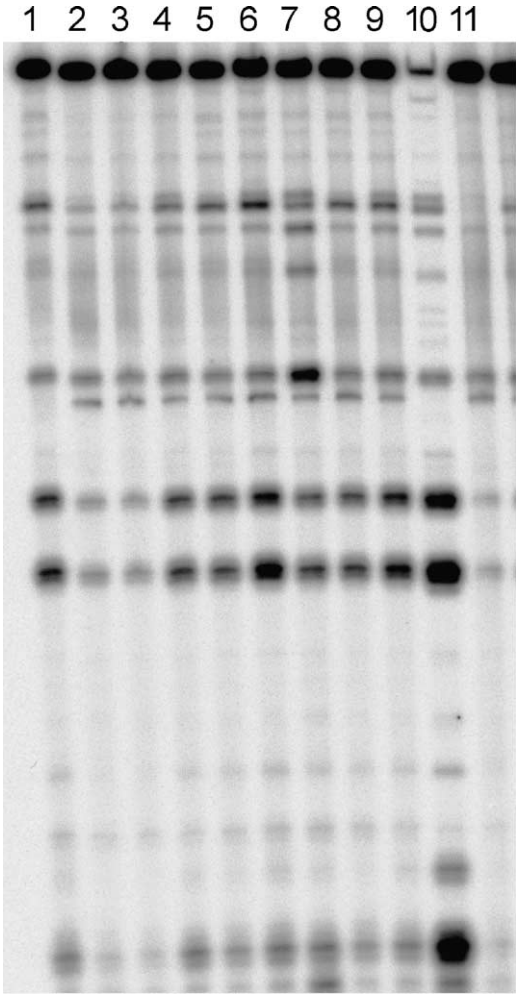


Figure 8. Autoradiogram of a 10% denaturing gel showing the effect of luotonin A analogues on human topoisomerase I-mediated cleavage of a 222-base pair DNA restriction fragment 3′-^{32}P end labeled on the scissile strand. The individual incubation mixtures contained 36 ng of topoisomerase I and 50 μM luotonin A analogues as indicated. Following incubation at 37 °C for 30 min, the samples were digested with proteinase K prior to polyacrylamide gel analysis. Lanes 1–10, 50 μM compounds **11**, **17a**, **17b**, **22a**, **22b**, **25**, **26**, **28**, **2**, and **1**, respectively. Lane 11, DNA + topoisomerase I.

Luotonin A analogues exhibiting good activity in the stabilization of the enzyme–DNA covalent binary complex included isoluotonin A (**11**), 17-aminoluotonin A (**25**), 17-hydroxymethylluotonin A (**26**), and 17-fluoroisoluotonin A (**28**). Previously, luotonin A (**2**) and 17 fluoroluotonin A (**5b**) have been shown to exhibit good stabilization of the covalent binary complex,[11,14] indicating that the position of the ring B N-atom does not significantly affect stabilization of topoisomerase I-mediated DNA cleavage.

Several luotonin A analogues were used to determine their ability to mediate topoisomerase I-dependent cytotoxicity in a yeast strain that lacks the homologous topoisomerase I, but expresses human topoisomerase I when grown on galactose. As shown in Table 4, isoluotonin A (**11**) exhibited topoisomerase I-dependent cytotoxicity and had an IC_{50} value (11.8 μM) quite similar

Table 4. Human topoisomerase I-dependent cytotoxicity of CPT (**1**) and luotonin A analogues toward *S. cerevisiae*

Compound	Concentration (μM)	% Inhibition on growth medium		IC_{50} (μM) for galactose
		Raffinose	Galactose	
1	15	21	74	0.86
	1.5	23	59	
	0.75	6	51	
	0.39	26	38	
	0.19	19	35	
	0.03	40	22	
2	25	68	81	9.58
	10	35	56	
	1	0	36	
	0.5	0	23	
5b	100	25	71	36.3
	50	14	73	
	25	0	40	
	10	5	0	
	5	4	0	
	1	0	0	
5c	50	44	0	—[a]
	25	41	0	
	10	53	0	
	5	10	0	
	1	26	0	
5d	50	16	0	—[a]
	25	47	2	
	10	36	0	
	5	35	0	
	1	41	0	
5g	10	39	49	—[b]
	1	8	14	
6	100	18	51	91.4
	50	3	46	
	25	23	0	
	10	34	0	
	1	32	0	
7	100	7	13	>100
	50	0	9	
	25	0	19	
	10	36	3	
	5	16	15	
	1	0	4	
8	100	27	18	—[c]
	50	27	5	
	25	18	0	
	10	19	10	
	1	28	10	
11	100	69	83	11.8
	50	41	80	
	25	7	72	
	12.5	7	53	
	6.25	0	32	
21a	100	31	57	—[c]
	50	51	62	
	25	62	66	
	12.5	60	64	
	6.25	57	67	
21b	100	65	57	177
	50	19	28	
	25	4	5	
	12.5	5	7	
	6.25	3	19	
22a	100	63	81	—[c]
	50	81	74	
	25	94	86	
	12.5	97	90	
	6.25	97	93	
22b	100	88	68	—[c]
	50	80	72	
	25	65	54	
	12.5	31	59	
	6.25	49	36	
25	100	0	63	15.7
	50	4	39	
	25	7	16	
	12.5	0	0	
26	100	44	18	—[c]
	50	28	0	
	25	0	0	
	12.5	0	0	
27	100	14	0	—[c]
	50	0	0	
	25	0	0	
	12.5	0	0	
28	100	35	3	—[c]
	50	2	0	
	25	0	0	
	12.5	0	0	

[a] Essentially no activity in TOP1-gal.
[b] Not determined.
[c] Cytotoxicity was not topoisomerase I-dependent.

to that of luotonin A (**2**) itself (9.58 μM). Also found to be cytotoxic in this assay was 17-aminoluotonin A (**25**) (IC_{50} 15.7 μM), consistent with its strong activity in stabilizing the topoisomerase I–DNA covalent binary complex. In comparison, some analogues found to stabilize the binary complex were found to be essentially non-cytotoxic in the yeast strain grown on galactose; examples included 17-hydroxymethylluotonin A (**26**). Given the poor aqueous solubility of many of the analogues, the lack of cytotoxicity may simply reflect inadequate concentrations of the analogues in solution, or poor uptake by yeast cells.

One facet of the yeast testing results worthy of mention is illustrated by thiophene analogues **22a** and **22b**. Both of these analogues effected quite reasonable stabilization of the enzyme–DNA covalent binary complex (Table 3 and Fig. 8), and both were fairly strongly cytotoxic (Table 4). However, the cytotoxicity for both was at least as strong when the yeast strain was grown on raffinose as when it was grown on galactose, indicating that the predominant cytotoxic response was not due to the activity of the compounds as topoisomerase I poisons. Because potency of covalent binary complex formation and mammalian cell toxicity have been used to guide the

structure modification of CPT lead compounds,[20] it seems essentially to also assure that the cytotoxicity is mediated at the level of topoisomerase I.

Most of the luotonin A and isoluotonin A derivatives that act as topoisomerase I poisons have substituents in the 17-position, a position that is roughly spatially analogous to the position of the 20(*S*)-OH group in CPT. In common with CPT, in which the introduction of 20-Cl, Br, and NH_2 substituents in lieu of the 20-OH group supported covalent complex stabilization and topoisomerase I-dependent cytotoxicity,[10b] 17-chloro, 17-fluoro, and 17-aminoluotonin A all supported stabilization of the enzyme–DNA covalent binary complex.[10b] However, while all of the 20-substituted CPTs were cytotoxic in the yeast strain in the presence of galactose, 17-chloroluotonin A (**5a**) lacked cytotoxicity. Further, while 20-amino CPT was much less cytotoxic than 20-chloro or 20-bromo CPT,[10b] 17-aminoluotonin A (**25**) was the most cytotoxic of the 17-substituted derivatives, having an IC_{50} value (15.7 μM) not much different than luotonin A itself (9.58 μM). Other cytotoxic luotonin A derivatives (e.g., **6**)[14] also differed in their behavior from the corresponding CPT analogues (e.g., 20-deoxy CPT which does not stabilize the covalent binary complex), underscoring our earlier conclusion[11,14] that the binding of luotonin A to the topoisomerase I–DNA binary complex must differ somewhat from the binding of CPT in spite of the obvious structural similarities between the two.

4. Experimental

4.1. General methods

High resolution mass spectra were obtained by the mass spectrometry facility at Michigan State University. All ^1H and ^{13}C NMR spectra were recorded on a General Electric QE-300 MHz or Varian Unity 300 MHz NMR spectrophotometer using residual solvent peaks at 2.50 ppm for DMSO-d_6 or 3.31 ppm for methanol-d_4 for calibration. TLC separations were carried out on silica gel 60 F254 analytical TLC plates purchased from EM Science. TLC plates were visualized under 254 and 365 nm UV light. SiO_2 (230–400 mesh) was used for column chromatography (Silicycle Chemicals). Dichloromethane was distilled from calcium hydride prior to use. THF was distilled from sodium benzophenone ketal. All reactions were carried out under a nitrogen or argon atmosphere. Methyl 4-phenylanthranilate was prepared as described.[21] Methyl 2-cyanoanthranilate was prepared from methyl 4-aminoterephthalate by sequential treatment with thionyl chloride, concentrated ammonia, and phosphorous oxychloride. Purchased anthranilic acids were converted their esters by previously reported procedures.

4.1.1. General procedure for preparation of luotonin A derivatives in dichloromethane (5a–g). To a suspension of 1 equiv of 1,2-dihydropyrrolo[3,4-*b*]quinoline-3-one (**4**) and 1–4 equiv of methyl anthranilate in 5 mL of anhydrous dichloromethane was added dropwise at room temperature 0.50 mL (820 mg, 5.35 mmol) of phosphorus oxychloride. The resulting suspension was warmed to 40 °C and stirred overnight. The reaction mixture was poured onto ice, and then concentrated ammonium hydroxide was added to make the solution basic. The reaction mixture was extracted with three 75 mL portions of chloroform. The combined organic phase was washed with 75 mL of water containing 10 mL of concentrated ammonium hydroxide, dried over magnesium sulfate, and concentrated under diminished pressure. The crude product was dissolved in chloroform and applied to a silica gel column. The column was washed as described below.

4.1.2. 17-Chloroluotonin A (5a). Elution with 19:1 methylene chloride–methanol afforded **5a** as a colorless solid: yield 11 mg (22%); mp 263 °C; silica gel TLC R_f 0.63 (ethyl acetate); ^1H NMR ($CDCl_3$) δ 5.34 (s, 2H), 7.53 (dd, 1H, $J = 8.8$ and 1.9 Hz), 7.71 (dd, 1H, $J = 8.2$ and 6.9 Hz), 7.87 (dd, 1H, $J = 8.2$ and 6.9 Hz), 7.97 (d, 1H, $J = 8.2$ Hz), 8.09 (d, 1H, $J = 1.9$ Hz), 8.36 (d, 1H, $J = 8.8$ Hz) and 8.40–8.55 (m, 2H); mass spectrum (FAB), *m/z* 320.0592 ($C_{18}H_{11}ClN_3O$ requires 320.0591).

4.1.3. 17-Fluoroluotonin A (5b). Elution with 19:1 ethyl acetate–methanol gave **5b** as a colorless solid: yield 16 mg (31%); silica gel TLC R_f 0.43 (ethyl acetate); ^1H NMR ($CDCl_3$) δ 5.34 (s, 2H), 7.30–7.33 (m, 1H), 7.68–7.77 (m, 2H), 7.83–7.90 (m, 1H), 7.95–8.00 (m, 1H), and 8.42–8.50 (m, 3H); mass spectrum (FAB), *m/z* 304.0887 $(M + H)^+$ ($C_{18}H_{11}FN_3O$ requires 304.0886).

4.1.4. 17,18-Difluoroluotonin A (5c). Elution with 1:2 ethyl acetate–methylene chloride afforded **5c** as a colorless solid: yield 14 mg (22%); mp 229 °C (dec); silica gel TLC R_f 0.58 (1:1 ethyl acetate–methylene chloride); ^1H NMR ($CDCl_3$) δ 5.35 (s, 2H), 7.68–7.76 (m, 1H), 7.82–8.00 (m, 3H), 8.13–8.23 (m, 1H), and 8.40–8.52 (m, 2H); mass spectrum (FAB), *m/z* 322.0791 $(M + H)^+$ ($C_{18}H_{10}F_2N_3O$ requires 322.0792).

4.1.5. 16-Methoxyluotonin A (5d). Elution with 1:1 ethyl acetate–methylene chloride afforded **5d** as a colorless solid: yield 10 mg (17%) silica gel TLC R_f 0.29 (1:3 ethyl acetate–methylene chloride); ^1H NMR ($CDCl_3$) δ 4.09 (s, 3H), 5.34 (s, 2H), 7.28 (dd, 1H, $J = 8.0$ and 1.2 Hz), 7.51 (dd, 1H, $J = 7.9$ and 7.9 Hz), 7.66 (ddd, 1H, $J = 7.5$, 7.5, and 1.2 Hz), 7.82 (ddd, 1H, $J = 7.9$, 7.7, and 1.5 Hz), 7.93 (dd, 1H, $J = 8.1$ and 1.3 Hz), 7.98 (dd, 1H, $J = 8.0$ and 1.2 Hz), and 8.38–8.46 (m, 2H); mass spectrum (FAB), *m/z* 316.1086 $(M + H)^+$ ($C_{19}H_{14}N_3O_2$ requires 316.1086).

4.1.6. 17,18-Dimethoxyluotonin A (5e). Elution with ethyl acetate afforded **5e** as a colorless solid: yield 7 mg (11%); mp 282 °C (dec); silica gel TLC R_f 0.30 (ethyl acetate); ^1H NMR ($CDCl_3$) δ 4.06 (s, 3H); 4.07 (s, 3H), 5.36 (s, 2H), 7.56 (s, 1H), 7.70 (dd, 1H, $J = 8.1$ and 7.0 Hz), 7.76 (s, 1H), 7.86 (dd, 1H, $J = 8.1$ and 7.0 Hz), 7.88 (d, 1H, $J = 8.1$ Hz), and 8.42–8.45 (m, 2H); mass spectrum (FAB), *m/z* 346.1191 $(M + H)^+$ ($C_{20}H_{16}N_3O_3$ requires 346.1191).

4.1.7. 17,18,19-Trimethoxyluotonin A (5f). Elution with ethyl acetate afforded **5f** as a colorless solid: yield 37 mg (62%); mp 285 °C (dec); silica gel TLC R_f 0.42 (ethyl acetate); ^{1}H NMR ($CDCl_3$) δ 4.04 (s, 3H), 4.09 (s, 3H), 4.27 (s, 3H), 5.33 (s, 2H), 7.54–7.63 (m, 1H), 7.64–7.73 (m, 1H), 7.74–8.00 (m, 2H), and 8.40–8.46 (m, 2H); mass spectrum (FAB), *m/z* 376.1298 $(M + H)^+$ ($C_{21}H_{18}N_3O_4$ requires 376.1297).

4.1.8. 18-Methylluotonin A (5g). Elution with 1:2 ethyl acetate–hexanes afforded **5g** as a colorless solid: yield 20 mg (12%); mp 270 °C (dec); silica gel TLC R_f 0.60 (ethyl acetate); ^{1}H NMR ($CDCl_3$) δ 2.57 (s, 3H), 5.36 (s, 2H), 7.63–7.74 (m, 2H), 7.86 (ddd, 1H, J = 6.8, 6.8, and 1.3 Hz), 7.97 (d, 1H, J = 8.4 Hz), 8.03 (d, 1H, J = 8.4 Hz), 8.23 (s, 1H), and 8.45–8.50 (m, 2H); mass spectrum (FAB), *m/z* 300.1137 $(M + H)^+$ ($C_{19}H_{14}N_3O$ requires 300.1137).

4.1.9. 16,17,18,19-Tetrahydroluotonin A (6). Elution with 1:1 ethyl acetate–methylene chloride afforded **6** as a colorless solid: yield 4.4 mg (6%); mp 275 °C (dec); silica gel TLC R_f 0.29 (1:1 ethyl acetate–methylene chloride); ^{1}H NMR ($CDCl_3$) δ 1.76–1.97 (m, 4H), 2.60–2.74 (m, 2H), 2.84–3.00 (m, 2H), 5.22 (s, 2H), 7.69 (ddd, 1H, J = 7.5, 7.5, and 1.3 Hz), 7.84 (ddd, 1H, J = 8.2, 7.5, and 1.3 Hz), 7.95 (d, 1H, J = 8.2 Hz), and 8.38–8.51 (m, 2H); mass spectrum (FAB), *m/z* 290.1293 $(M + H)^+$ ($C_{18}H_{16}N_3O$ requires 290.1293).

4.1.10. 16-Hydroxyluotonin A (7). A solution of 14 mg (0.04 mmol) of 16-methoxyluotonin A (**5d**) in 10 mL of 48% aq HBr was heated at reflux overnight. The resulting solution was neutralized with saturated sodium bicarbonate and extracted with three 30 mL portions of ethyl acetate. The combined organic phase was washed with water and dried over magnesium sulfate. After removal of the solvent under diminished pressure, chromatographic purification was performed on an Alltech Alltima C_{18} reversed-phase HPLC column (150 × 4.6 mm) using a gradient of water and acetonitrile. A linear gradient was employed (90:10 H_2O–CH_3CN → 10:90 H_2O–CH_3CN over a period of 40 min at a flow rate of 1 mL/min). Fractions containing the desired product were collected, frozen and lyophilized to afford a colorless solid: yield 1.1 mg (10%); ^{1}H NMR (DMSO-d_6) δ 5.27 (s, 2H), 7.27 (d, 1H, J = 7.6 Hz), 7.40 (dd, 1H, J = 8.2 and 7.6 Hz), 7.66–7.75 (m, 2H), 7.87 (dd, 1H, J = 6.9 and 6.9 Hz), 8.13 (d, 1H, J = 8.2 Hz), 8.22 (d, 1H, J = 8.2 Hz), 8.72 (s, 1H), and 9.95 (s, 1H); mass spectrum (FAB), *m/z* 302.0930 $(M + H)^+$ ($C_{18}H_{12}N_3O_2$ requires 302.0930).

4.1.11. General procedure for preparation of luotonin A and isoluotonin A derivatives in THF. To a suspension of 1 equiv of 1,2-dihydropyrrolo[3,4-*b*]quinoline-3-one (**4**) and 1–5 equiv of anthranilic acid derivative in 5 mL of dry THF was added 1 mL of $POCl_3$. The reaction mixture was heated at 40 °C overnight. The resulting suspension was poured into ice and the solution was made basic by addition of concentrated sodium carbonate (or 1 M sodium hydroxide). The mixture was extracted with three 100 mL portions of ethyl acetate. The combined organic phase was washed with two 50 mL portions of saturated sodium carbonate (or 1 M sodium hydroxide), dried over magnesium sulfate, and concentrated under diminished pressure. The crude product was purified by silica gel column chromatography.

4.1.12. Luotonin A (2) and isoluotonin A (11). Elution of the silica gel column with 1:4 ethyl acetate–methylene chloride afforded **2** and **11** as colorless solids. For **2**: yield 5 mg (12%); silica gel TLC R_f 0.40 (1:4 ethyl acetate–methylene chloride); ^{1}H NMR was the same as for the previously published data.[12,15] For **11**: yield 2 mg (4%); silica gel TLC R_f 0.14 (1:4 ethyl acetate–methylene chloride); ^{1}H NMR ($CDCl_3$) δ 5.34 (s, 2H), 7.54 (ddd, 1H, J = 7.9, 6.3, and 1.9 Hz), 7.68 (dd, 1H, J = 8.1 and 6.9 Hz), 7.78–7.92 (m, 3H), 8.05 (d, 1H, J = 8.1 Hz), 8.21 (d, 1H, J = 8.5 Hz), 8.42 (d, 1H, J = 8.1 Hz), and 8.97 (s, 1H); mass spectrum (FAB), *m/z* 286.0979 $(M + H)^+$ ($C_{18}H_{12}N_3O$ requires 286.0980).

4.1.13. 17-Chloroluotonin A (5a) and 17-chloroisoluotonin A (13). Elution with 1:4 ethyl acetate–dichloromethane afforded **5a** and **13** as colorless solids. For **5a**: yield 130 mg (37%); silica gel TLC R_f 0.50 (1:4 ethyl acetate–dichloromethane); spectroscopic data were agreement with those reported above. For **13**: yield 30 mg (9%); silica gel TLC R_f 0.15 (1:4 ethyl acetate–dichloromethane); ^{1}H NMR ($CDCl_3$) δ 5.35 (s, 2H), 7.50 (dd, 1H, J = 8.5 and 1.9 Hz), 7.71 (dd, 1H, J = 8.2 and 7.5 Hz), 7.86 (d, 1H, J = 1.9 Hz), 7.92 (ddd, 1H, J = 8.5, 6.9, and 1.3 Hz), 8.08 (d, 1H, J = 8.8 Hz), 8.23 (d, 1H, J = 8.2 Hz), 8.36 (d, 1H, J = 8.8 Hz), and 8.98 (s, 1H); mass spectrum (FAB), *m/z* 320.0590 $(M + H)^+$ ($C_{18}H_{11}ClN_3O$ requires 320.0591).

4.1.14. Analogues 14a and 14b. Elution with 1:4 ethyl acetate–methylene chloride afforded **14a** and **14b** as colorless solids. For **14a**: yield 6 mg (13%); silica gel TLC R_f 0.49 (1:4 ethyl acetate–methylene chloride); ^{1}H NMR ($CDCl_3$) δ 4.02 (s, 3H), 5.36 (s, 2H), 7.70 (dd, 1H, J = 8.1 and 6.9 Hz), 7.86 (dd, 1H, J = 8.4 and 6.7 Hz), 7.96 (d, 1H, J = 8.4 Hz), 8.17 (dd, 1H, J = 8.4 and 1.6 Hz), 8.44–8.52 (m, 3H), and 8.78 (s, 1H); mass spectrum (FAB), *m/z* 344.1037 $(M + H)^+$ ($C_{20}H_{14}N_3O_3$ requires 344.1035). For **14b**: yield <1 mg; silica gel TLC R_f 0.12 (1:4 ethyl acetate–methylene chloride);mass spectrum (FAB), *m/z* 344.1036 $(M + H)^+$ ($C_{20}H_{14}N_3O_3$ requires 344.1035).

4.1.15. Analogues of 15a and 15b. Elution with 1:2 ethyl acetate–methylene chloride afforded **15a** and **15b** as colorless solids. For **15a**: yield 16 mg (24%); mp 240 °C (dec); silica gel TLC R_f 0.50 (1:2 ethyl acetate–methylene chloride); ^{1}H NMR ($CDCl_3$) δ 5.37 (s, 2H), 5.44 (s, 2H), 7.37–7.55 (m, 5H), 7.71 (dd, 1H, J = 8.1 and 6.9 Hz), 7.87 (dd, 1H, J = 8.4 and 6.9 Hz), 7.97 (d, 1H, J = 8.3 Hz), 8.21 (dd, 1H, J = 8.3 and 1.5 Hz), 8.43–8.53 (m, 3H), 8.84 (d, 1H, J = 1.5 Hz); mass spectrum (FAB), *m/z* 420.1347 $(M + H)^+$ ($C_{26}H_{18}N_3O_3$ requires 420.1348). For **15b**: yield 10 mg (15%); mp 245 °C (dec); silica gel TLC R_f 0.25 (1:2 ethyl acetate–methylene chloride); ^{1}H NMR ($CDCl_3$) δ 5.36 (s, 2H), 5.44 (s, 2H),

7.36–7.54 (m, 5H), 7.70 (dd, 1H, $J = 8.1$ and 6.9 Hz), 7.91 (dd, 1H, $J = 7.1$ and 7.1 Hz), 8.08 (d, 1H, $J = 8.3$ Hz), 8.14–8.25 (m, 2H), 8.48 (d, 1H, $J = 8.4$ Hz), 8.58 (s, 1H), and 9.00 (s, 1H); mass spectrum (FAB), *m/z* 420.1347 $(M + H)^+$ ($C_{26}H_{18}N_3O_3$ requires 420.1348).

4.1.16. Analogues 16a and 16b. Elution with 1:2 ethyl acetate–methylene chloride afforded **16a** and **16b** as colorless solids. For **16a**: yield 30 mg (19%); silica gel TLC R_f 0.49 (1:2 ethyl acetate–methylene chloride); ^{1}H NMR ($CDCl_3$) δ 5.31 (s, 2H), 7.39–7.55 (m, 3H), 7.63 (ddd, 1H, $J = 8.1$, 6.9, and 1.2 Hz), 7.69–7.90 (m, 5H), 8.32 (d, 1H, $J = 1.7$ Hz) and 8.37–8.46 (m, 3H); mass spectrum (FAB), *m/z* 362.1292 $(M + H)^+$ ($C_{24}H_{16}N_3O$ requires 362.1293). For **16b**: yield 14 mg (9%); mp 272 °C (dec); silica gel TLC R_f 0.23 (1:2 ethyl acetate–methylene chloride); ^{1}H NMR ($CDCl_3$) δ 5.36 (s, 2H), 7.41–7.56 (m, 3H), 7.64–7.80 (m, 4H), 7.84–7.92 (m, 1H), 8.01–8.09 (m, 2H), 8.20 (d, 1H, $J = 8.6$ Hz), 8.45 (d, 1H, $J = 8.3$ Hz), and 8.99 (s, 1H); mass spectrum (FAB), *m/z* 362.1294 $(M + H)^+$ ($C_{24}H_{16}N_3O$ requires 362.1293).

4.1.17. 17-Cyanoluotonin A (17a) and 17-cyanoisoluotonin A (17b). Elution with 1:4 ethyl acetate–dichloromethane afforded **17a** and **17b** as light yellow solids. For **17a**: yield 26 mg (15%); silica gel TLC R_f 0.40 (1:4 ethyl acetate–dichloromethane); ^{1}H NMR ($CDCl_3$) δ 5.39 (s, 2H), 7.72–7.80 (m, 2H), 7.90 (dd, 1H, $J = 8.4$ and 7.1 Hz), 8.00 (d, 1H, $J = 8.4$ Hz), 8.41 (s, 1H), and 8.45–8.56 (m, 3H); mass spectrum (FAB), *m/z* 311.0934 $(M + H)^+$ ($C_{19}H_{11}N_4O$ requires 311.0933). For **17b**: yield 20 mg (12%); silica gel TLC R_f 0.10 (1:4 ethyl acetate–dichloromethane); ^{1}H NMR ($CDCl_3$) δ 5.37 (s, 2H), 7.70–7.77 (m, 2H), 7.94 (dd, 1H, $J = 7.1$ and 7.1 Hz), 8.11 (d, 1H, $J = 7.9$ Hz), 8.17 (d, 1H, $J = 1.5$ Hz), 8.24 (d, 1H, $J = 8.3$ Hz), 8.51 (d, 1H, $J = 8.3$ Hz), and 9.02 (s, 1H); mass spectrum (FAB), *m/z* 311.0934 $(M + H)^+$ ($C_{19}H_{11}N_4O$ requires 311.0933).

4.1.18. Analogues 18a and 18b. Elution with 1:2 ethyl acetate–methylene chloride afforded **18a** and **18b** as yellow solids. For **18a**: yield 97 mg (14%); silica gel TLC R_f 0.57 (1:2 ethyl acetate–methylene chloride); ^{1}H NMR ($CDCl_3$) δ 5.35 (s, 2H), 7.66–7.74 (m, 1H), 7.82–8.00 (m, 4H), 8.44–8.49 (m, 2H), 8.55 (d, 1H, $J = 2.3$ Hz); mass spectrum (FAB), *m/z* 364.0091 $(M + H)^+$ ($C_{18}H_{11}BrN_3O$ requires 364.0085). For **18b**: yield 15 mg (2%); silica gel TLC R_f 0.30 (1:2 ethyl acetate–methylene chloride); ^{1}H NMR ($CDCl_3$) δ 5.36 (s, 2H), 7.65–7.76 (m, 2H), 7.91–7.95 (m, 2H), 8.07 (d, 1H, $J = 7.7$ Hz), 8.22 (d, 1H, $J = 8.3$ Hz), 8.55 (d, 1H, $J = 2.3$ Hz), and 9.00 (s, 1H); mass spectrum (ESI), *m/z* 364.4 $(M + H)^+$ (theoretical 364.0).

4.1.19. Analogues 19a and 19b. Elution with 1:4 ethyl acetate–methylene chloride afforded **19a** and **19b** as colorless solids. For **19a**: yield 6 mg (9%); silica gel TLC R_f 0.50 (1:4 ethyl acetate–methylene chloride). ^{1}H NMR (DMSO-d_6) δ 5.35 (s, 2H), 7.79 (dd, 1H, $J = 7.8$ and 7.1 Hz), 7.93 (dd, 1H, $J = 8.5$ and 6.7 Hz), 8.20 (d, 1H, $J = 7.8$ Hz), 8.24–8.39 (m, 2H), 8.51 (d, 1H, $J = 8.5$ Hz), 8.67 (d, 1H, $J = 2.3$ Hz), and 8.81 (s, 1H); mass spectrum (FAB), *m/z* 331.0833 $(M + H)^+$ ($C_{18}H_{11}N_4O_3$ requires 331.0831). For **19b**: yield 6 mg (9%); silica gel TLC R_f 0.22 (1:4 ethyl acetate–methylene chloride); ^{1}H NMR (DMSO-d_6) δ 5.32 (s, 2H), 7.74 (dd, 1H, $J = 8.4$ and 7.7 Hz), 7.95 (dd, 1H, $J = 8.4$ and 7.1 Hz), 8.16 (d, 1H, $J = 8.4$ Hz), 8.24–8.34 (m, 2H), 8.45–8.54 (m, 2H), and 9.24 (s, 1H); mass spectrum (FAB), *m/z* 331.0833 $(M + H)^+$ ($C_{18}H_{11}N_4O_3$ requires 331.0831).

4.1.20. Analogues 20a and 20b. Elution with 1:4 ethyl acetate–methylene chloride afforded **20a** and **20b** as colorless solids. For **20a**: yield 8 mg (14%); silica gel TLC R_f 0.56 (1:4 ethyl acetate–methylene chloride); ^{1}H NMR ($CDCl_3$) δ 5.35 (s, 2H), 7.58–7.74 (m, 2H), 7.86 (m, 1H), 7.95 (d, 1H, $J = 8.3$ Hz), 8.20 (d, 1H, $J = 7.3$ Hz), 8.46–8.54 (m, 2H), and 8.64 (d, 1H, $J = 7.9$ Hz); mass spectrum (FAB), *m/z* 354.0853 $(M + H)^+$ ($C_{19}H_{11}F_3N_3O$ requires 354.0854). For **20b**: yield 1 mg (2%); silica gel TLC R_f 0.31 (1:4 ethyl acetate–methylene chloride); ^{1}H NMR ($CDCl_3$) δ 5.38 (s, 2H), 7.61 (dd, 1H, $J = 8.2$ and 7.9 Hz), 7.72 (dd, 1H, $J = 7.1$ and 6.9 Hz), 7.93 (ddd, 1H, $J = 8.2$, 7.1, and 1.2 Hz), 8.08–8.20 (m, 2H), 8.25 (d, 1H, $J = 9.2$ Hz), 8.63 (d, 1H, $J = 8.0$ Hz), and 9.05 (s, 1H); mass spectrum (FAB), *m/z* 354.0854 $(M + H)^+$ ($C_{19}H_{11}F_3N_3O$ requires 354.0854).

4.1.21. Analogues 21a and 21b. Elution with 1:3 ethyl acetate–dichloromethane afforded luotonin A derivatives **21a** and **22b** as light yellow solids. For **21a**: yield 7 mg (22%); silica gel TLC R_f 0.30 (1:3 ethyl acetate–dichloromethane); ^{1}H NMR ($CDCl_3$) δ 5.33 (s, 2H), 7.39 (d, 1H, $J = 5.7$ Hz), 7.63 (d, 1H, $J = 5.7$ Hz), 7.70 (ddd, 1H, $J = 8.1$, 6.9, and 1.3 Hz), 7.86 (ddd, 1H, $J = 8.1$, 6.9, and 1.3 Hz), 7.96 (d, 1H, $J = 8.2$ Hz), 8.43 (d, 1H, $J = 9.4$ Hz), and 8.46 (s, 1H); mass spectrum (FAB), *m/z* 292.0543 $(M + H)^+$ ($C_{16}H_{10}N_3OS$ requires 292.0545). For **21b**: yield 5 mg (16%); silica gel TLC R_f 0.16 (1:3 ethyl acetate–dichloromethane); ^{1}H NMR ($CDCl_3$) δ 5.37 (s, 2H), 7.34 (d, 1H, $J = 5.7$ Hz), 7.63 (d, 1H, $J = 5.7$ Hz), 7.72 (m, 1H), 7.92 (m, 1H), 8.08 (d, 1H, $J = 7.6$ Hz), 8.27 (d, 1H, $J = 8.2$ Hz), and 8.94 (s, 1H); mass spectrum (FAB), *m/z* 292.0543 $(M + H)^+$ ($C_{16}H_{10}N_3OS$ requires 292.0545).

4.1.22. Analogues 22a and 22b. Elution with 1:4 ethyl acetate–dichloromethane afforded luotonin A derivatives **22a** and **22b** as light yellow solids. For **22a**: yield 20 mg (33%), silica gel TLC R_f 0.34 (1:4 ethyl acetate–dichloromethane); ^{1}H NMR ($CDCl_3$) δ 5.34 (s, 2H), 7.63 (d, 1H, $J = 5.7$ Hz), 7.69 (dd, 1H, $J = 8.2$ and 7.6 Hz), 7.82–7.90 (m, 2H), 7.95 (d, 1H, $J = 8.2$ Hz), 8.43–8.46 (m, 2H); mass spectrum (FAB), *m/z* 292.0543 $(M + H)^+$ ($C_{16}H_{10}N_3OS$ requires 292.0545). For **22b**: yield 2 mg (3%); mp 280 °C (dec); silica gel TLC R_f 0.17 (1:4 ethyl acetate–dichloromethane); ^{1}H NMR ($CDCl_3$) δ 5.37 (s, 2H), 7.49 (d, 1H, $J = 5.7$ Hz), 7.70 (dd, 1H, $J = 8.2$, and 7.6 Hz), 7.88–7.93 (m, 2H), 8.07 (d, 1H, $J = 7.6$ Hz), 8.23 (d, 1H, $J = 8.8$ Hz), and 8.96 (s, 1H); mass spectrum (FAB), *m/z* 292.0543 $(M + H)^+$ ($C_{16}H_{10}N_3OS$ requires 292.0545).

4.1.23. Analogues 23a and 23b. Elution with 1:3 ethyl acetate–methylene chloride afforded **23a** and **23b** as yel-

low solids. For **23a**: yield 23 mg (20%); silica gel TLC R_f 0.49 (1:3 ethyl acetate–methylene chloride); ^{1}H NMR ($CDCl_3$) δ 5.42 (s, 2H), 7.56–7.74 (m, 3H), 7.83–7.90 (m, 1H), 7.97 (d, 1H, J = 8.1 Hz), 8.07–8.14 (m, 2H), 8.47 (s, 1H), 8.51 (d, 1H, J = 8.6 Hz), 8.65 (s, 1H), and 9.04 (s, 1H); mass spectrum (FAB), *m/z* 336.1138 $(M + H)^+$ ($C_{22}H_{14}N_3O$ requires 336.1137). For **23b**: yield 5 mg (4%); silica gel TLC R_f 0.20 (1:3 ethyl acetate–methylene chloride); ^{1}H NMR ($CDCl_3$) δ 5.39 (s, 2H), 7.53–7.72 (m, 3H), 7.89 (ddd, 1H, J = 8.4, 6.8, and 1.5 Hz), 8.00–8.12 (m, 3H), 8.21 (d, 1H, J = 8.2 Hz), 8.34 (s, 1H), 9.01 (s, 1H), and 9.02 (s, 1H); mass spectrum (FAB), *m/z* 336.1138 $(M + H)^+$ ($C_{22}H_{14}N_3O$ requires 336.1137).

4.1.24. Analogue 24. Nitrogen gas was passed through a suspension of 75 mg (0.21 mmol) of 18-bromoluotonin A (**18a**) and 28 mg (0.23 mmol) of phenyl boronic acid in a 1:1 mixture of 10% potassium carbonate–DMF for 40 min. Then 5 mg (0.02 mmol) of palladium(II)acetate was added and the mixture was heated to reflux for 4 h. The resulting solution was poured into 50 mL of water and extracted with three 50 mL portions of ethyl acetate. The combined organic phase was washed with 50 mL portions of water and brine. After drying over magnesium sulfate, the solution was concentrated under diminished pressure and purified by silica gel column chromatography. Elution with 1:9 ethyl acetate–methylene chloride afforded **24** as a colorless solid: yield 4 mg (7%); silica gel TLC R_f 0.30 (1:9 ethyl acetate–methylene chloride); ^{1}H NMR ($CDCl_3$) δ 5.40 (s, 2H), 7.39–7.55 (m, 3H), 7.68–7.79 (m, 3H), 7.87 (ddd, 1H, J = 8.5, 7.1, and 1.4 Hz), 7.98 (d, 1H, J = 8.3 Hz), 8.12 (dd, 1H, J = 8.5 and 2.2 Hz), 8.21 (d, 1H, J = 8.4 Hz), 8.47–8.53 (m, 2H), and 8.67 (d, 1H, J = 2.3 Hz); mass spectrum (FAB), *m/z* 362.1294 $(M + H)^+$ ($C_{24}H_{16}N_3O$ requires 362.1293).

4.1.25. Analogue 25. To a suspension of 6 mg (0.018 mmol) of 17-nitroluotonin A (**19a**) in 10 mL of methanol, was added 34 mg (0.18 mmol) of tin(II)chloride. The reaction mixture was heated and stirred at reflux for 2 h. Then 1 mL of concentrated aq HCl was added dropwise and stirring was continued for an additional 2 h. The resulting solution was poured onto ice and the pH of the solution was adjusted to 12 by addition of 1 M sodium hydroxide. The solution was extracted with three 50 mL portions of chloroform and three 50 mL portions of ethyl acetate. The combined organic phase was dried over magnesium sulfate and concentrated under diminished pressure. The product was purified by silica gel column chromatography. Elution with 5% methanol in ethyl acetate afforded **25** as a yellow solid: yield 2 mg (40%); silica gel TLC R_f 0.38 (5% methanol in ethyl acetate); ^{1}H NMR (DMSO-d_6) δ 5.24 (s, 2H), 6.22 (br s, 2H), 6.85 (dd, 1H, J = 8.5 and 2.2 Hz), 6.90 (s, 1H), 7.71 (dd, 1H, J = 7.8 and 7.1 Hz), 7.86-7.97 (m, 2H), 8.18 (d, 1H, J = 8.3 Hz), 8.27 (d, 1H, J = 8.1 Hz), and 8.74 (s, 1H); mass spectrum (FAB), *m/z* 301.1088 $(M + H)^+$ ($C_{18}H_{13}N_4O$ requires 301.1089).

4.1.26. Analogue 26. To a suspension of 30 mg (0.09 mmol) of ester **14a** in 30 mL of dry THF was added 150 mg (3.97 mmol) of sodium borohydride. The reaction mixture was heated to reflux for 8 h. Then 5 mL of methanol was added and the reaction mixture was heated to reflux for an additional 10 min. The resulting yellow solution was poured into ice and extracted with three 75 mL portions of ethyl acetate. The combined organic phase was extracted with brine, dried over magnesium sulfate, and concentrated under diminished pressure. The product was purified by silica gel column chromatography. Elution with 9:9:1 ethyl acetate–dichloromethane–methanol afforded **26** as a yellow solid: yield 9 mg (21%); silica gel TLC R_f 0.13 (9:9:1 ethyl acetate–dichloromethane–methanol); ^{1}H NMR 4:1 $CDCl_3$–CD_3OD δ 4.71 (s, 2H), 5.22 (s, 2H), 7.48 (d, 1H, J = 8.1 Hz), 7.56–7.62 (m, 1H), 7.69–7.75 (m, 1H), 7.84–7.88 (m, 2H), 8.20–8.26 (m, 2H), and 8.40 (s, 1H); mass spectrum (FAB), *m/z* 316.1086 $(M + H)^+$ ($C_{19}H_{14}N_3O_2$ requires 316.1086).

4.1.27. Analogue 27. This compound was prepared as described for **26** starting from **14b**. Elution with 9:9:1 ethyl acetate–dichloromethane–methanol afforded **27** as a yellow solid: yield 3 mg (32%); silica gel TLC R_f 0.13 (9:9:1 ethyl acetate–dichloromethane–methanol); ^{1}H NMR (4:1 $CDCl_3$–CD_3OD) δ 4.78 (s, 2H), 5.35 (s, 2H), 7.54–7.71 (m, 2H), 7.88–7.93 (m, 1H), 7.96 (s, 1H), 8.11–8.16 (m, 2H), 8.30 (d, 1H, J = 8.1 Hz), and 9.41 (s, 1H); mass spectrum (ESI), *m/z* 316.1086 $(M + H)^+$ ($C_{19}H_{14}N_3O_2$ requires 316.1086).

4.1.28. 17-Fluoroluotonin A (5b) and 17-fluoroisoluotonin A (28). To a suspension of 50 mg (0.27 mmol) of 2,3-dihydro-1*H*-pyrrolo[3,4-*b*]quinolin-1-one (**12**) and 50 mg (0.30 mmol) of methyl 4-fluoroanthranilate in 6 mL of dry THF was added 1 mL of phosphorus oxychloride. The reaction mixture was stirred overnight at 40 °C. The resulting suspension was poured onto ice and the resulting solution was made basic by the addition of 1 M NaOH. The resulting solution was extracted with three 75 mL portions of ethyl acetate. The combined organic phase was washed with 50 mL of 1N NaOH, dried over magnesium sulfate, and concentrated under diminished pressure. The crude product was purified by silica gel column chromatography. Elution with 1:2 ethyl acetate–dichloromethane afforded 17-fluoroluotonin A (**5b**) and 17-fluoroisoluotonin A (**28**) as light yellow solids. For **5b**: yield 1.4 mg (2%); silica gel TLC R_f 0.41 (1:2 ethyl acetate–dichloromethane); For **28**: yield 8.1 mg (10%); silica gel TLC R_f 0.19 (1:2 ethyl acetate–dichloromethane); ^{1}H NMR ($CDCl_3$) δ 5.35 (s, 2H), 7.24–7.30 (m, 1H), 7.53 (dd, 1H, J = 9.7 and 2.4 Hz), 7.71 (m, 1H), 7.92 (ddd, 1H, J = 8.4, 7.1, and 1.4 Hz), 8.09 (d, 1H, J = 8.3 Hz), 8.23 (d, 1H, J = 8.4 Hz), 8.44 (dd, 1H, J = 8.8 and 6.1 Hz), and 9.02 (s, 1H); mass spectrum (FAB), *m/z* 304.0885 $(M + H)^+$ ($C_{18}H_{11}FN_3O$ requires 304.0885).

4.1.29. Topoisomerase I-mediated DNA cleavage. The topoisomerase I-mediated DNA cleavage reaction was carried out (37 °C, 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 (*Hin*dIII-*Pvu*II fragment from pSP64 DNA),

and 36 ng of human DNA topoisomerase I. CPT analogues were employed at 50 μM concentration. The reactions were terminated by SDS-proteinase K treatment. After extraction with phenol and chloroform, the DNA was recovered by ethanol precipitation. The DNA was dissolved in 80% formamide loading buffer (10 mM NaOH, 1 mM EDTA, 0.1% xylene cyanol, and bromophenol blue), and analyzed on a 10% denaturing gel.

4.1.30. Yeast strain growth. A transformed strain of *S. cerevisiae*, RS321Nph-TOP1, had the 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*). This 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), which contained 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).

4.1.31. Yeast cytotoxicity assay. The exponentially growing yeast strain was diluted to OD_{595} 0.015 with minimal media having 3% galactose or raffinose. Samples to be assayed were dissolved in DMSO to a concentrations of 50 mM, then diluted in the yeast incubation to the appropriate concentrations; the final DMSO concentration were less than 1% and the assays were carried out in 96-well microtiter plates. Camptothecin was used as a positive control. The plates were incubated in a high humidity chamber (30 °C, 48 h) and the optical density of cells in each well was measured (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, which was defined as the concentration of a compound at which yeast growth was inhibited by 50%.

Acknowledgements

We thank Dr. Mary-Ann Bjornsti, St. Jude Children's Hospital, for the yeast strain used for evaluation of luotonin derivatives. This work was supported by NIH Research Grant CA78415, awarded by the National Cancer Institute.

References and notes

1. 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.
2. (a) Takimoto, C. H.; Wright, J.; Arbuck, S. G. *Biochim. Biophys. Acta* **1998**, *1400*, 107; (b) O'Leary, J.; Muggia, F. M. *Eur. J. Cancer* **1998**, *34*, 1500; (c) Saltz, L. B.; Cox, J. V.; Blanke, C.; Rosen, L. S.; Fechrenbacher, L.; Moore, M. J.; Maroun, J. A.; Ackland, S. P.; Locker, P. K.; Pirotta, N.; Elfring, G. L.; Miller, L. L. *New Engl. J. Med.* **2000**, *343*, 905; (d) Ozols, R. F. *Int. J. Gynecol. Cancer* **2000**, *10*, 33; (e) Vanhoefer, U.; Harstrick, A.; Achterrath, W.; Cao, S.; Seeber, S.; Rustum, Y. M. *J. Clin. Oncol.* **2001**, *19*, 1501; (f) Ulukan, H.; Swaan, P. W. *Drugs* **2002**, *62*, 2039; (g) Garcia-Carbenero, R.; Supko, J. G. *Clin. Cancer Res.* **2002**, *8*, 641.
3. (a) 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; (b) Yaegashi, T.; Sawada, S.; Nagata, H.; Furuta, T.; Yokokura, T.; Miyasaka, T. *Chem. Pharm. Bull.* **1994**, *42*, 2518; (c) Lackey, K.; Besterman, J. M.; Fletcher, W.; Leitner, P.; Morton, B.; Sternbach, D. D. *J. Med. Chem.* **1995**, *38*, 906; (d) Luzzio, M. J.; Besterman, J. M.; Emerson, D. L.; Evans, M. G.; Lackey, K.; Leitner, P. L.; McIntyre, G.; Morton, B.; Myers, P. L.; Peel, M.; Sisco, J. M.; Sternbach, D. D.; Tong, W.-Q.; Truesdale, A.; Uehling, D. E.; Vuong, A.; Yates, J. *J. Med. Chem.* **1995**, *38*, 395; (e) Lackey, K.; Sternbach, D. D.; Croom, D. K.; Emerson, D. L.; Evans, M. G.; Leitner, P. L.; Luzzio, M. J.; McIntyre, G.; Vuong, A.; Yates, J.; Besterman, J. M. *J. Med. Chem.* **1996**, *39*, 713; (f) Lavergne, O.; Lesueur-Ginot, L.; PlaRodas, F.; Bigg, D. C. H. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 2235; (g) Lavergne, O.; Lesueur-Ginot, L.; PlaRodas, F.; Kasprzyk, P. G.; Pommier, J.; Demarquay, D.; Prevost, G.; Ulibarri, G.; Rolland, A.; Schiano-Liberatore, A.-M.; Harnett, J.; Pons, D.; Camara, J.; Bigg, D. C. H. *J. Med. Chem.* **1998**, *41*, 5410; (h) Garbarda, A. E.; Du, W.; Isarno, T.; Tangirala, R. S.; Curran, D. P. *Tetrahedron* **2002**, *58*, 6329.
4. (a) Wang, J. C. *Annu. Rev. Biochem.* **1996**, *65*, 635; (b) Wang, J. C. Q. *Rev. Biophys.* **1998**, *31*, 107; (c) Pommier, Y. *Biochimie* **1998**, *80*, 255; (d) Champoux, J. *J. Annu. Rev. Biochem.* **2002**, *70*, 369.
5. (a) Pommier, Y.; Pourquier, P.; Fan, Y.; Strumberg, D. *Biochim. Biophys. Acta* **1998**, *1400*, 83; (b) Redinbo, M. R.; Champoux, J. J.; Hol, W. G. J. *Biochemistry* **2000**, *39*, 6832.
6. (a) Hsiang, Y.-H.; Hertzberg, R.; Hecht, S.; Liu, L. F. *J. Biol. Chem.* **1985**, *260*, 14873; (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; (c) Covey, J. M.; Jaxel, C.; Kohn, K. W.; Pommier, Y. *Cancer Res.* **1989**, *49*, 5016.
7. Hsiang, Y.-H.; Lihou, M. G.; Liu, L. F. *Cancer Res.* **1989**, *49*, 5077.
8. (a) Fan, Y.; Weinstein, J. N.; Kohn, K. W.; Shi, L. M.; Pommmier, Y. *J. Med. Chem.* **1998**, *41*, 2216; (b) Kerrigan, J. E.; Pilch, D. S. *Biochemistry* **2001**, *40*, 9792; (c) Laco, G. S.; Collins, J. R.; Luke, B. T.; Kroth, H.; Sayer, J. M.; Jerina, D. M.; Pommier, Y. *Biochemistry* **2002**, *41*, 1428.
9. (a) Redinbo, M. R.; Stewart, L.; Kuhn, P.; Champoux, J. J.; Hol, W. G. J. *Science* **1998**, *279*, 1504; (b) Staker, B. L.; Hjerrild, K.; Feese, M. D.; Behnke, C. A.; Burgin, A. B., Jr.; Stewart, L. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 15387; (c) Stewart, L.; Redinbo, M. R.; Qui, X.; Hol, W. G.; Champoux, J. J. *Science* **1998**, *279*, 1534.
10. (a) Jaxel, C.; Kohn, K. W.; Wani, M. C.; Wall, M. E.; Pommier, Y. *Cancer Res.* **1989**, *49*, 1465; (b) Wang, X.; Zhou, X.; Hecht, S. M. *Biochemistry* **1999**, *38*, 4374; (c) Hertzberg, R. P.; Caranfa, M. J.; Hecht, S. M. *Biochemistry* **1989**, *28*, 4629.
11. Cagir, A.; Jones, S. H.; Gao, R.; Eisenhauer, B. M.; Hecht, S. M. *J. Am. Chem. Soc.* **2003**, *125*, 13628.
12. (a) Xiao, X.-H.; Qou, G.-L.; Wang, H.-L.; Lui, L.-S.; Zheng, Y.-L.; Jia, Z.-J.; Deng, Z.-B. *Chin. J. Pharmacol. Toxicol.* **1988**, 232; (b) Ma, Z. Z.; Hano, Y.; Nomura, T.; Chen, Y. J. *Heterocycles* **1997**, *46*, 541.
13. Bjornsti, M. A.; Benedetti, P.; Viglianti, G. A.; Wang, J. C. *Cancer Res.* **1989**, *49*, 6318.

14. Cagir, A.; Jones, S. H.; Eisenhauer, B. M.; Gao, R.; Hecht, S. M. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 2051.
15. (a) Lee, E. S.; Park, J.-G.; Jahng, Y. *Tetrahedron Lett.* **2003**, *44*, 1883; (b) Osborne, D.; Stevenson, P. J. *Tetrahedron Lett.* **2002**, *43*, 5469; (c) Dallavalle, S.; Merlini, L. *Tetrahedron Lett.* **2002**, *43*, 1835; (d) Yadav, J. S.; Reddy, B. V. S. *Tetrahedron Lett.* **2002**, *43*, 1905; (e) Wang, H.; Ganesan, A. *Tetrahedron Lett.* **1998**, *39*, 9097; (f) Ma, Z.-Z.; Hano, Y.; Nomura, T.; Chen, Y.-J. *Heterocycles* **1999**, *51*, 1593; (g) Kelly, T. R.; Chamberland, S. Silva, R. A. *Tetrahedron Lett.* **1999**, *40*, 2723; (h) Molina, P.; Tarraga, A.; Gonzalez-Tejero, A. *Synthesis-Stuttgart* **2000**, *11*, 1523; (i) Toyota, M.; Komori, C.; Ihara, M. *Heterocycles* **2002**, *56*, 101; (j) Harayama, T.; Morikami, Y.; Shigeta, Y.; Abe, H.; Takeuchi, Y. *Synlett* **2003**, *6*, 847; (k) Mhaske, S. B.; Argade, N. P. *J. Org. Chem.* **2004**, *69*, 14563.
16. Danishefsky, S.; Bryson, T. A.; Puthenpurayil, J. *J. Org. Chem.* **1975**, *40*, 796.
17. Sugasawa, T.; Toyota, T.; Sasakura, K.; Hidaka, T. *Chem. Pharm. Bull.* **1971**, *19*, 1971.
18. Tanaka, T.; Mashimo, K.; Wagatsuma, M. *Tetrahedron Lett.* **1971**, *30*, 2803.
19. Repetition of these experiments at 50 μM concentration indicated surprisingly little difference, presumably reflecting the limited aqueous solubility of the luotonin A analogues.
20. (a) Wall, M. E.; Wani, M. C.; Nicholas, A. W.; Manikumar, G.; Tele, G.; Moore, L.; Truesdale, A.; Leitner, P.; Besterman, J. M. *J. Med. Chem.* **1993**, *36*, 2689; (b) 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.
21. Quan, M. L.; Dominguez, W. C.; Duncia, J. V.; Kettner, C. A.; Ellis, C. D.; Liauw, A. Y.; Park, J. M.; Santella, J. B.; Knabb, R. M.; Thoolen, M. J.; Weber, P. C.; Wexler, R. R. *Bioorg. Med. Chem. Lett.* **1997**, *13*, 1595.