Homocamptothecins: Synthesis and Antitumor Activity of Novel E-Ring-Modified Camptothecin Analogues

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Homocamptothecins: Synthesis and Antitumor Activity of Novel E-Ring-Modified Camptothecin Analogues

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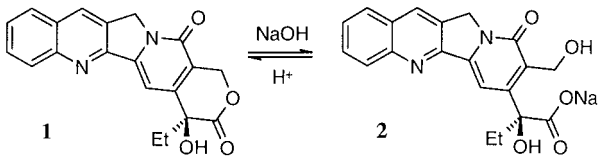
Homocamptothecin (hCPT), a camptothecin (CPT) analogue with a seven membered β-hydroxylactone which combines enhanced plasma stability and potent topoisomerase I (Topo I)-mediated activity, is an attractive template for the elaboration of new anticancer agents. Like CPT, hCPT carries an asymmetric tertiary alcohol and displays stereoselective inhibition of Topo I. The preparation and biological screening of racemic hCPT analogues are described. The 10 hCPTs tested were better Topo I inhibitors than CPT. Fluorinated hCPTs **23c**,**d**,**f**,**g** were found to have potent cytotoxic activity on A427 and PC-3 tumor cell lines. Their cytotoxicity remained high on the K562*adr* and MCF7*mdr* cell lines, which overexpress a functionally active P-glycoprotein. Fluorinated hCPTs were more efficacious in vivo than CPT on HT-29 xenografts. In this model, a tumor growth delay of 25 days was reached with hCPT **23g** at a daily dose of 0.32 mg/kg, compared to 4 days with CPT at 0.625 mg/kg. Thus difluorinated hCPT **23g** warrants further investigation as a novel Topo I inhibitor with high cytotoxicity toward tumor cells and promising in vivo efficacy.

Introduction

Camptothecin (CPT, **1**), an alkaloid isolated from the Chinese tree *Camptotheca acuminata* by Wani and Wall in 1966, was found to possess potent antiproliferative activity which generated high expectations.[1] Clinical trials of the water-soluble sodium salt **2**, resulting from the hydrolysis of **1** (Scheme 1), however, were discontinued because of severe and unpredictable toxicity, in particular hemorrhagic cystitis.[2,3] The potential interest of CPT and its analogues as anticancer agents was revived around 1985, when it was discovered that the cytotoxic activity of CPT was due to a novel mechanism of action involving the nuclear enzyme topoisomerase I (Topo I).[4]

Topo I is a ubiquitous protein which has the capacity to relax supercoiled DNA during transcription or replication.[5] It is an appealing target since Topo I levels are higher in various solid tumors than in normal tissues, a feature raising the possibility of selective chemotherapy.[6] The mechanism of action of CPT is intriguing since the drug does not interact with Topo I alone,[7] nor does it bind to DNA:[8,9] its biological activity stems from its capacity to stabilize the "cleavable complex", a transient molecular species where a tyrosine residue of Topo I binds covalently to DNA via its phosphodiester backbone and generates a single-strand break. The formation of a ternary complex between CPT, Topo I, and the cleaved DNA leads to inhibition of DNA and RNA syntheses, and prolonged exposure

Scheme 1



to the drug results in irreversible DNA damage, triggering cell death.[10] Although the exact structure of the ternary complex is still a subject of investigation,[11,12] this mechanism accounts for the good correlation found between the number of stabilized cleavable complexes and the cytotoxicity of various CPT analogues.[13]

Subsequently, CPT became a valuable lead molecule for a family of anticancer compounds with a novel mechanism of action, potent antiproliferative activity on a wide spectrum of tumor cells including multidrug-resistant lines, and impressive activity in xenograft models.[14] The efforts of a number of research groups, focusing on obtaining water-soluble molecules, resulted in the launching of the prodrug irinotecan[15] (Camptosar or Campto, **3**; Chart 1) and topotecan[16] (Hycamtin, **5**) and the clinical evaluation of GG-211[17] (**4**) and DX-8951f[18] (**6**). The development of non-water-soluble CPT analogues was also undertaken, with clinical trials of oral CPT (**1**) and 9-nitrocamptothecin[19] (9-NC, **7**) and intravenous 9-aminocamptothecin (9-AC, **8**).[20]

Most CPT analogues share the pentacyclic skeleton, and all include the highly electrophilic six-membered α-hydroxylactone ring. The intrinsic instability of CPT analogues arises from the rapid hydrolysis of this ring in basic or neutral media, to give the open carboxylate form (Scheme 1), which is essentially inactive. The reaction is reversible, and the lactone form predomi-

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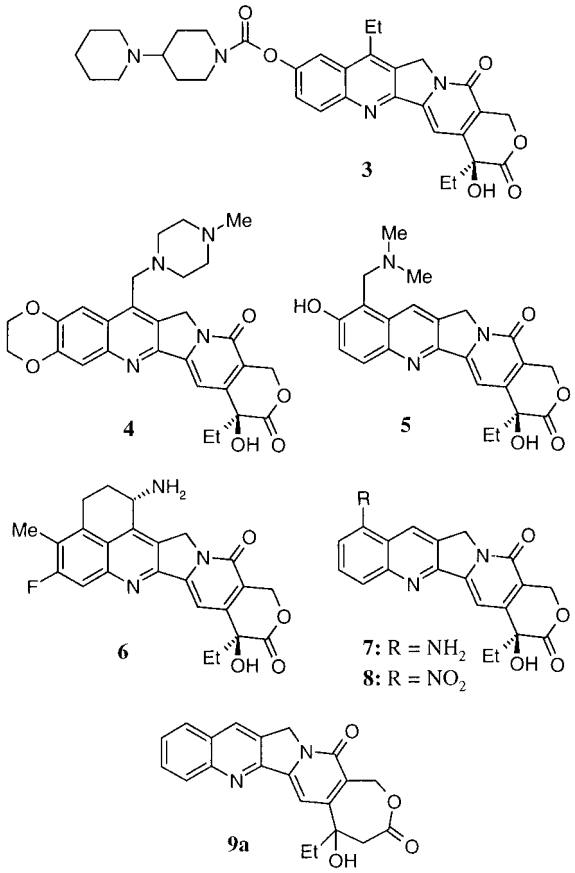
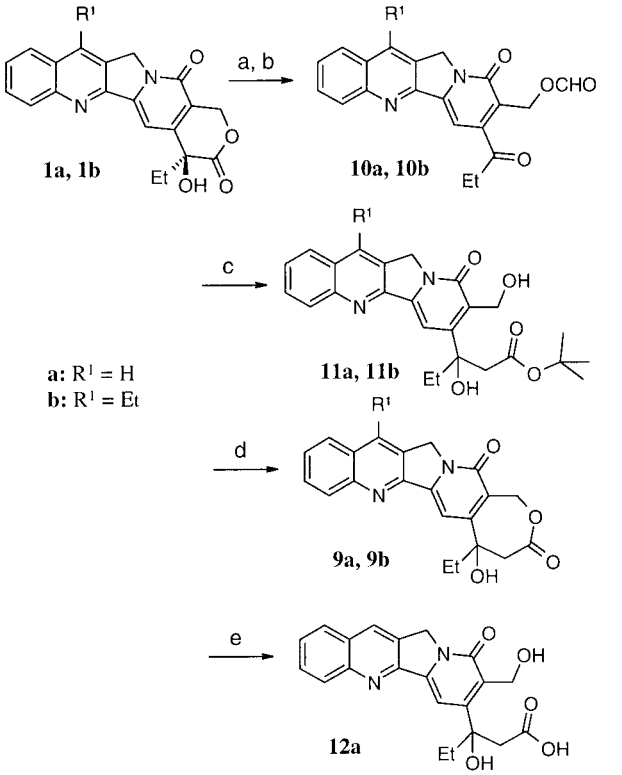
Chart 1



Scheme 2[a]



[a] Reagents: (a) $NaBH_4$, MeOH, rt; (b) $NaIO_4$, AcOH, rt; (c) Zn, $BrCH_2COO$-*t*-Bu, THF, reflux; (d) TFA, rt; (e) 0.1 N KOH, rt, then pH 3.5.

nates only at acidic pH. Pharmacokinetic studies have shown this pH-dependent hydrolytic equilibrium to be shifted toward the carboxylate form in plasma, in a species-dependent manner which is less favorable in man than in rodents.[21] This latter point has been invoked to explain the diminished efficacy of various CPT analogues in the clinic compared to the spectacular results often obtained with xenograft models.[22] Thus, producing a CPT analogue with a prolonged biological life in its active, lactonic form is an important goal.[23] Unfortunately, previously reported modifications of the lactone ring failed to preserve both cytotoxic and Topo I inhibitory activities, [24−26] leading to the general acceptance of an intact α-hydroxylactone moiety as an indispensable structural feature for both in vitro and in vivo anticancer activity.[27]

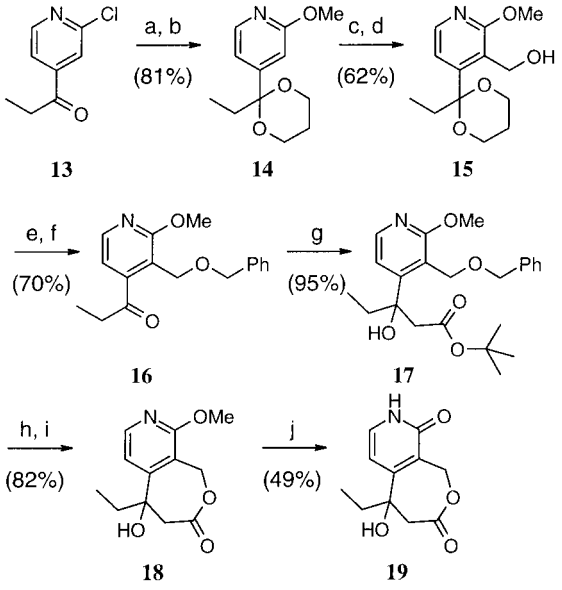
Recently, we decribed a modification of the CPT lactone ring which retains Topo I-mediated activity.[28] The resulting racemic compound, homologous to CPT and named homocamptothecin (*dl*-hCPT, **9a**; Chart 1), was found to display enhanced stability in buffer solutions,[28] due to the reduced electrophilicity of its seven-membered *β*-hydroxylactone. With its modified lactone, hCPT represents an interesting template for the generation of novel anticancer agents, as well as a valuable probe to further explore the mechanism of cleavable complex stabilization. We now report the preparation of a number of hCPT analogues and the biological results obtained for this novel series of compounds.

Chemistry

The semisynthetic sequence which allowed the isolation of hCPT is depicted in Scheme 2. Sodium borohydride reduction of CPT (**1a**) provided the corresponding 1,2-diol which was oxidatively cleaved with periodic acid to give formyloxy-mappicine ketone (**10a**).[29] A Reformatsky reaction of **10a** with *tert*-butyl bromoacetate gave, after workup, *β*-hydroxy ester **11a**, which on treatment with trifluoroacetic acid afforded hCPT (**9a**). This semisynthetic approach could in principle be applied to any substituted CPT, provided that the substituents are compatible with the various reaction conditions, as exemplified for substituted hCPT **9b** prepared from 7-ethyl-CPT (**1b**).[30]

In practice, the number of substituted CPTs available from the natural product is limited, and in order to obtain substituent diversity, hCPT analogues were prepared by total synthesis. An appropriate route, among the various possibilities,[31] was based on the coupling of a DE heterobicycle with an AB quinoline, followed by cyclization under Heck reaction conditions to form the C ring of the pentacycle.[32] Due to its convergent character and the ready access to the required substituted quinolines,[33] this synthesis seemed easily amenable to an analogue generation program. The only lengthy multistep synthetic sequence is limited to the DE heterobicycle, a common precursor to all the envisioned analogues. The preparation of this new seven-membered *β*-hydroxylactone fused to a pyridone was derived from our experience with hCPT semisynthesis. We adopted a pathway where the *β*-hydroxycarboxylic moiety could be obtained by Reformatsky reaction on a 4-propionylpyridine with an adjacent protected hydroxymethyl group, available by ortholithiation. The resulting preparation of the DE heterobicycle is depicted in Scheme 3. The ketone function of chloropyridine **13**[34]

Scheme 3[a]

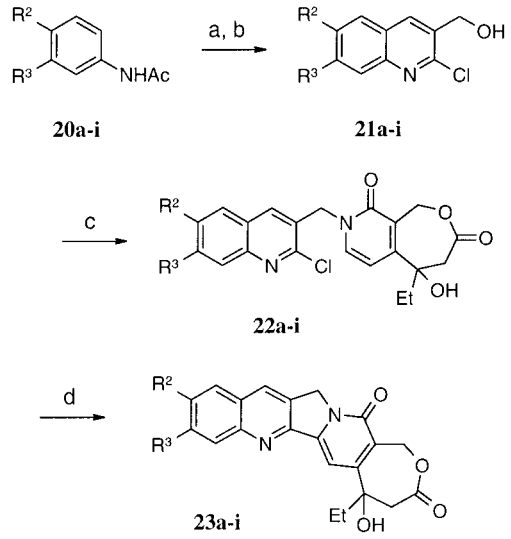


[a] Reagents and conditions: (a) $HO(CH_2)_3OH$, pTSA (cat.), toluene, reflux; (b) MeONa, MeCN, reflux; (c) MesLi, THF, −78 °C to rt, then DMF, −78 °C to rt; (d) $NaBH_4$, MeOH, rt; (e) NaH, $PhCH_2Br$, THF, rt; (f) TFA, 100 °C; (g) Zn, $BrCH_2COO$-*t*-Bu, THF, reflux; (h) H_2 (1 atm), Pd(C), EtOH, rt; (i) TFA, rt; (j) 1 N HCl, reflux.

was protected as a ketal and the chlorine atom was exchanged with a methoxy group to give methoxypyridine **14**. Lithiation of **14** with mesityllithium,[35] followed by trapping with DMF and hydrolysis, gave the corresponding aldehyde which was reduced to alcohol **15**. The hydroxyl group of **15** was protected as the benzyl ether and the ketone deprotected using trifluoroacetic acid. A Reformatsky reaction on ketone **16** provided *β*-hydroxy ester **17** which was debenzylated by catalytic hydrogenolysis. Lactonization to **18** proceeded upon treatment with trifluoroacetic acid, and demethylation in hydrochloric acid gave the desired DE heterobicycle **19**.

The preparative sequence employed to complete the hCPT synthesis is shown in Scheme 4. Acetanilides **20a**−**i** were submitted to Vilsmeier conditions to give intermediate 2-chloro-3-quinolinecarboxaldehydes which were reduced with sodium borohydride to complete the preparation of AB quinolines **21a**−**i**. A Mitsunobu coupling[36] of **21a**−**i** to DE heterobicyle **19**, followed by a Heck reaction to form the C ring of the pentacycle, gave the desired hCPTs **23a**−**i**. This synthesis turned out to be suitable for racemic hCPTs bearing alkyl, alkoxy, and halogen substituents at the 9 and 10 positions. Although Topo I inhibition by CPT is known to be stereoselective,[37] and the asymmetric carbinol responsible for this stereochemical outcome is still present in hCPTs, a racemic process was considered more convenient at this stage of the screening procedure. To study the effect of stereochemistry on hCPT biological activity, a chemical resolution was, however, undertaken on hydroxy acid **12a**, the hydrolytic product of **9a**. Fractional crystallizations of the diastereomeric salts of **12a** with optically pure α-methylbenzylamine gave the separate enantiomers which, upon lactonization, provided both *d* and *l* forms of **9a** in high enantiomeric excess.

Scheme 4[a,b]



[a] See Table 1 for substituent specification. [b] Reagents: (a) DMF, $POCl_3$, 0−80 °C; (b) $NaBH_4$, MeOH, rt; (c) **19**, DEAD, PPh_3, DMF; (d) $Pd(OAc)_2$, PPh_3, KOAc, TBAB, MeCN, reflux.

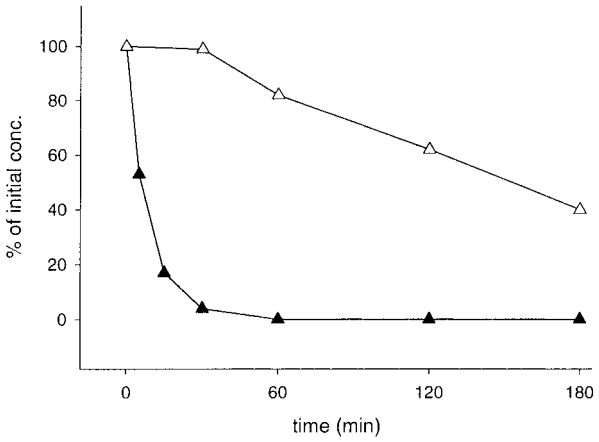
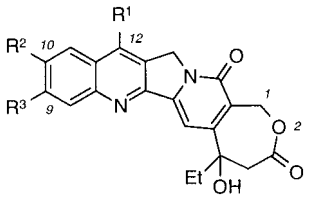


Figure 1. Stability in human plasma at 37 °C of CPT (▲) and *dl*-hCPT (**9a**) (△). Each data point represents the percent of initial concentration (10^{-4} M) of lactone.

Results and Discussion

The previously reported[28] stability study in buffer solutions showed the *β*-hydroxylactonic analogue hCPT (**9a**) to be considerably more stable than the α-hydroxylactonic natural product. Thus, after 24 h at pH 7.4, 87% of hCPT (**9a**) remained in its intact lactone form, in sharp contrast with the values reported for CPT,[38] which reached an equilibrium of ca. 20% lactone within 1 h. Substantial stability enhancement is also found in plasma, for hCPT (**9a**) versus CPT, as shown in Figure 1. After 30 min of incubation in human plasma at 37 °C, CPT levels fell to less than 10% of the initial value, whereas more than 80% of **9a** was still present. Even after 3 h under these conditions, more than 40% of **9a** remained in the lactone form. It was also demonstrated that hCPT hydrolysis product **12a** did not revert to the lactone either in plasma or under the HPLC conditions employed. This observation is in marked contrast to the rapid equilibrium found for CPT and may moderate adverse events such as hemorrhagic cystitis, as reversal of the carboxylate form to the biologically active, and toxic, lactone during excretion is less prone to occur for hCPTs.

Table 1. Topoisomerase I Inhibitory Activities



	substituents			
compd[a]	R^1	R^2	R^3	% relaxed DNA[b]
9a				60 ± 9
d-**9a**				45 ± 7
l-**9a**				100 ± 3
9b	Et			34 ± 12
23a		Cl		57 ± 6
23b		F		47 ± 4
23c		OMe	F	26 ± 2
23d		Me	F	18 ± 3
23e		Me	Cl	59 ± 16
23f		F	Cl	61 ± 13
23g		F	F	56 ± 6
23h		$-OCH_2CH_2O-$		50 ± 11
23i		$-OCH_2O-$		58 ± 13
CPT				64 ± 6

[a] Unless otherwise specified, all the compounds tested were racemic. [b] As measured by calf thymus Topo I-mediated relaxation of supercoiled pUC19 plasmid DNA with 100 μM test compound.

Interestingly, the differences in lactone reactivity between CPT and hCPT do not compromise Topo I-mediated activity. The ability of hCPTs to inhibit Topo I was evaluated using Topo I-induced relaxation of supercoiled pUC19 plasmid DNA, a test known to correlate well with the stabilization of single-strand cleavable complexes.[13] The compounds were assayed at concentrations ranging from 5 to 500 μM and gave dose-dependent responses. For simplification, only the values for 100 μM are reported in Table 1, in terms of percent relaxed DNA with respect to total DNA. Of the two resolved enantiomers of hCPT (**9a**), only *d*-**9a** demonstrated Topo I inhibitory activity. This stereospecificity of Topo I inhibition, which has been previously observed for CPT and some of its analogues,[37] further suggests a similar mechanism of action for hCPT and CPT, although the absolute configuration of *d*-**9a** requires elucidation. All the other hCPTs, tested as racemic mixtures, were found to be as active as native CPT, and three compounds (**9b**, **23c**,**d**) displayed elevated Topo I inhibition in this assay, with less than 40% relaxed DNA.

To verify that Topo I inhibitory activities of hCPTs translate into cytotoxicity, tumor cell antiproliferative assays were performed in comparison with the clinically relevant compounds CPT, TPT, and SN38 (the active metabolite of irinotecan), as shown in Table 2. The solid tumor cell lines, A427 human lung carcinoma[39] and PC-3 human prostate adenocarcinoma,[40] were chosen for their ability to discriminate between compounds, giving IC_{50} values which were spread over several log units of concentration. Interestingly, A427 and PC-3 have a mutant *p53* tumor suppressor gene,[41,42] while the MCF7 cell line, for instance, which expresses a wild-type *p53* gene,[43] gave IC_{50} values grouped within 2 log units of concentration (data not shown). Several compounds showed very high potency; in particular, fluorinated hCPTs **23c**,**d**,**g** and methylenedioxy-hCPT (**23i**) gave subnanomolar IC_{50} values on the PC-3 cell line.

Table 2. Antiproliferative Activities

	IC_{50} (nM)[a]			
	cell line			
compd	A427	PC-3	K562*adr*	MCF7*mdr*
9a	12	60	47	21
9b		28	33	21
23a	0.36	18	2.6	1.3
23b	7.7	0.58	63	
23c	0.0083	<0.001	18	2.2
23d	0.00081	<0.001	14	0.010
23e	0.031	3.6	2.9	
23f	0.023	12	20	1.7
23g	0.10	0.0010	66	
23i	0.62	<0.001	37	0.55
CPT	24	57	57	3.1
TPT	49	160	1100	420
SN38	13	11	320	90
ADR		390	15000	135000
VP16		2300		67000

[a] Mean values of 50% inhibitory concentration determined from three experiments.

Bearing in mind the cross-resistant character often found in tumors of pretreated patients, it appeared judicious to check compounds for their activities on cells resistant to established anticancer drugs. Therefore antiproliferative assays were performed on K562*adr*,[44] a line resulting from prolonged exposure of K562 leukemia[45] cells to adriamycin (ADR), and on MCF7*mdr* cells,[46] obtained by transfection of MCF7 breast pleural effusion[47] with the *mdr* gene. Both cell lines overexpress a functionally active P-glycoprotein and display a high degree of cross-resistance against other drugs such as, for example, etoposide (VP16). The IC_{50} values obtained for K562*adr* and MCF7*mdr* are shown in Table 2. TPT and, to a lesser extent, SN38, which are hydroxylated CPTs, performed rather poorly in this assay, giving IC_{50} values of 1 or more log units greater than that of native CPT. In contrast, several hCPTs (**23a**,**c**−**f**) gave nanomolar and subnanomolar IC_{50} values on these two resistant cell lines. Overall, these antiproliferative assays further confirm our previous observations that homologation of the CPT lactone conserves the cytotoxicity toward tumor cells and does not lead to recognition by the mechanisms of cross-resistance.

It has been reported that some CPT analogues may exert potent in vitro Topo I inhibition and high activity in proliferative cellular assays, while being devoid of in vivo antitumor activity.[17] This is not the case for hCPTs which were tested in vivo in a HT-29 human colon adenocarcinoma model.[48,49] Xenografted athymic mice with established tumors were injected ip with the test compounds following a dosing schedule of 4 days on/3 days off repeated three times. This protocol does not always give tumor regression, which can usually be accomplished with more time-consuming schedules, but it is sufficient for screening purposes. The range of tested doses typically follows a geometrical progression with a factor of 2, until an approximate MTD is reached. The results, reported in Table 3, show that longer tumor growth delays were obtained with fluoro-hCPTs **23c**,**d**,**f**,**g** than with CPT, which gave a tumor growth delay of 4 days at a daily dose of 0.625 mg/kg. The difluoro-hCPT **23g** was particularly efficacious in this model, giving an impressive tumor growth delay of 25 days at a daily dose of 0.32 mg/kg. At this dosage tumor regression was

Table 3. Antitumor Activities on HT-29 Xenografts

	tumor growth delay (days)				
	ip dose (mg/kg)				
compd	2.5	1.25	0.625	0.32	0.16
23c			td[a]	12	4
23d			td	7	4
23f	td	7	2	1	
23g			td	25	8
CPT		td	4	nd[b]	

[a] td, toxic dose; more than half the animals died before the end of the experiment. [b] nd, not determined.

indeed observed, while one out of five animals died indicating that the MTD had been reached.

In conclusion, the data presented here demonstrate that hCPT provides a valuable template for the preparation of new Topo I inhibitors with high cytotoxicity toward tumor cell lines, including those with cross-resistance, along with in vivo activity in the HT-29 xenograft model. These results, combined with their improved plasma stability, make hCPTs a promising new class of anticancer agents worthy of further investigation. Our screening strategy designates difluorinated hCPT **23g**, in particular, as a candidate for pharmacological development. Preclinical evaluation of its enantiopure form is underway and will be published in due course.

Experimental Section

Caution: *Camptothecin and all structurally related compounds must be considered potential mutagens and potential reproductive hazards for both males and females. Appropriate precautions (use of respirators, gloves, fumehood) must be taken when handling these compounds and any waste streams generated from their use.*

Chemistry. Anhydrous tetrahydrofuran (THF), *N*,*N*-dimethylformamide (DMF), diethyl ether, and acetonitrile were purchased in septum-sealed bottles, and all other solvents were of reagent grade and used as received from various commercial sources. Analytical thin-layer chromatography was performed on silica gel 60 F_{254}-coated glass plates, visualized under UV light. Melting points were determined using a Büchi 535 apparatus and are uncorrected. Fourier transformed infrared spectra were recorded on a Brücker IFS spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Gemini 200 or a Brüker ARX 400 spectrometer. Chemical shifts are expressed relative to tetramethylsilane. High-resolution mass spectra (FAB-HRMS) were recorded on a Micromass-VG Organic Zabspec/T apparatus. Elemental analyses were performed on a Fisons EA 1108 apparatus. The purity of all biologically tested compounds was greater than 97% as monitored by HPLC, using a 5-μm Nucleosil C18 column (4.6 × 150 mm) at 30 °C, a Waters 996 diode array detector, and Millennium 2010 V2.15 data-processing software. Enantiomeric and diastereomeric purities (ee and de) were determined by HPLC on chiral stationary phase, using a Chiral-AGP column (4 × 100 mm; Chromtech, Stockholm, Sweden) and 2% acetonitrile in 10 mM (pH 6.9) phosphate buffer as mobile phase.

***tert*-Butyl 3-Hydroxy-3-(3-hydroxymethyl-4-oxo-4,6-dihydroindolizino[1,2-*b*]quinolin-2-yl)pentanoate (11a)**. A suspension of zinc (6.5 g, 100 mmol) magnetically stirred in anhydrous diethyl ether (50 mL) under argon was activated by dropwise addition of chlorotrimethylsilane (0.75 mL, 5.7 mmol), stirred 15 min further at room temperature, and then heated to reflux. The heating bath was removed, and *tert*-butyl bromoacetate (15 mL, 100 mmol) was added dropwise at a reflux-maintaining rate. External heating was resumed and continued 1 h further. The resulting ethereal solution of Reformatsky reagent was allowed to cool to room temperature and transferred by means of a cannula to a suspension of formyloxy-mappicine ketone[29] (**10a**; 1.6 g, 4.7 mmol) in anhydrous THF (40 mL) under argon. The reaction mixture was stirred at reflux 1 h, allowed to cool to room temperature, quenched with saturated ammonium chloride (100 mL), and extracted with chloroform (3 × 100 mL). The combined chloroform extracts were dried over sodium sulfate and concentrated under reduced pressure, and the residue was purified by medium-pressure chromatography over silica gel (1−2% MeOH/CH_2Cl_2) to give ester **11a** (0.64 g, 31% yield) as a pale-yellow solid: mp 146−149 °C; IR (KBr) 764, 1016, 1157, 1580, 1651, 1726 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 0.80 (t, 3H), 1.22 (s, 9H), 1.97 (m, 2H), 2.91 (dd, 2H), 4.79 (s, 1H), 4.87 (dd, 2H), 5.27 (s, 2H), 5.72 (s, 1H), 7.35 (s, 1H), 7.70 (t, 1H), 7.86 (t,1H), 8.12 (d, 1H), 8.16 (d, 1H), 8.67 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 7.91, 27.52, 34.81, 47.91, 50.00, 55.59, 76.74, 79.64, 100.18, 127.31, 128.11, 128.39, 128.52, 128.77, 128.89, 130.16, 131.42, 142.22, 147.92, 152.90, 155.62, 160.99, 169.44. Anal. ($C_{25}H_{28}N_2O_5$) C, H, N.

5-Ethyl-1,4,5,13-tetrahydro-5-hydroxy-3*H*,15*H*-oxepino-[3′,4′:6,7]indolizino[1,2-*b*]quinoline-3,15-dione (9a). Ester **11a** (1.45 g, 3.32 mmol) was dissolved in anhydrous dichloromethane (25 mL) and treated with a saturated solution of hydrogen chloride in dichloromethane (100 mL). The resulting mixture was stored at −20 °C for 16 h. The precipitate was filtered, washed with methanol, and dried under reduced pressure to give hCPT (**9a**) (662 mg, 55% yield) as a yellow solid: mp >300 °C; IR (KBr) 1285, 1580, 1653, 1757 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 0.87 (t, 3H), 1.86 (m, 2H), 3.27 (dd, 2H), 5.27 (s, 2H), 5.47 (dd, 2H), 6.04 (s, 1H), 7.42 (s, 1H), 7.71 (t, 1H), 7.87 (t, 1H), 8.13 (d, 1H), 8.16 (d, 1H), 8.69 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 8.45, 36.48, 42.54, 50.68, 61.44, 73.34, 99.78, 122.71, 127.83, 128.15, 128.75, 129.08, 130.07, 130.61, 131.81, 144.66, 148.04, 152.80, 155.91, 159.26, 172.08. Anal. ($C_{21}H_{18}N_2O_4$) C, H, N.

3-Hydroxy-3-(3-hydroxymethyl-4-oxo-4,6-dihydroindolizino[1,2-*b*]quinolin-2-yl)pentanoic Acid (12a). A suspension of **9a** (500 mg, 1.38 mmol) in aqueous potassium hydroxide (0.1 N, 30 mL) was stirred at room temperature for 16 h and then filtered. The resulting filtrate was treated with 1 N HCl until a pH of 3.5 was reached. The resulting precipitate was recovered by filtration, washed with water and acetone, and dried to give hydroxy acid **12a** (415 mg, 79% yield) as a white solid: mp 165−167 °C; IR (KBr) 1020, 1188, 1586, 1651, 1694 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 0.82 (t, 3H), 2.10 (m, 2H), 2.99 (dd, 2H), 3.25 (s, 1H), 4.81 (s, 2H), 5.26 (s, 2H), 5.76 (s, 1H), 7.38 (s, 1H), 7.71 (t, 1H), 7.84 (t, 1H), 8.10 (d, 1H), 8.18 (d, 1H), 8.34 (s,1H), 12.15 (br,1H); ^{13}C NMR (DMSO-d_6) δ 8.16, 34.80, 46.71, 50.36, 55.73, 76.53, 100.17, 127.50, 128.00, 128.26, 128.69, 129.06, 130.01, 130.45, 131.63, 142.57, 148.09, 153.19, 156.07, 161.22, 172.27. Anal. ($C_{21}H_{20}N_2O_5$·$1H_2O$) C, H, N.

Resolution of hCPT (9a). A mixture of **12a** (1.95 g, 5.1 mmol) and L-(−)-α-methylbenzylamine (1.21 g, 10 mmol) was treated with boiling absolute ethanol (100 mL), filtered while hot, and allowed to stand at room temperature for 68 h. The resulting precipitate was recovered by filtration, washed with ethanol and diethyl ether, and dried to give a white solid (980 mg, 52% de). This compound was recrystallized from 90% ethanol (35 mL) to give a crop of white crystals (450 mg, 82% de). Two additional recrystallization steps from 50% ethanol gave the desired diastereomerically enriched salt (165 mg, de ≥98%) which was dissolved in water (2 mL) and treated with acetic acid (0.35 mL). The resulting precipitate was recovered by filtration, washed with water, ethanol, and ether, and dried under reduced pressure at 80 °C to give enantiomerically enriched **12a** (110 mg), which lactonized upon treatment with concentrated HCl (11.5 N, 1.1 mL) in absolute ethanol (5.5 mL) for 68 h at room temperature to give *d*-**9a** (77 mg, ee ≥98%): mp >300 °C; $[\alpha]_{20}^{D} = +91 \pm 10$ (*c* 0.101, chloroform/methanol, 80/20). Anal. ($C_{21}H_{18}N_2O_4$·$0.25H_2O$) C, H, N. Enantiomer *l*-**9a** was obtained similarly, using D-(+)-α-methylbenzylamine as a resolving agent: mp >300 °C; $[\alpha]_{20}^{D} = -78 \pm 8$ (*c* 0.101, chloroform/methanol, 80/20). Anal. ($C_{21}H_{18}N_2O_4$) C, H, N.

5,12-Diethyl-1,4,5,13-tetrahydro-5-hydroxy-3*H*,15*H*-oxepino[3′,4′:6,7]indolizino[1,2-*b*]quinoline-3,15-dione (9b). This compound was prepared from 7-ethylcamptothecin[30] using a procedure similar to that described for **9a**, to give a deep-yellow solid: mp >270 °C; IR (KBr) 1283, 1595, 1653, 1745 cm^{-1}; ^1H NMR (DMSO-d_6) δ 0.86 (t, 3H), 1.32 (t, 3H), 1.86 (q, 2H), 3.23 (dd, 2H), 3.27 (dd, 2H), 5.32 (s, 2H), 5.47 (dd, 2H), 6.04 (s, 1H), 7.40 (s, 1H), 7.74 (t, 1H), 7.86 (t, 1H), 8.16 (d, 1H), 8.30 (d, 1H); ^{13}C NMR (DMSO-d_6) δ 8.46, 14.15, 22.42, 36.50, 42.54, 49.95, 61.45, 73.35, 99.68, 122.61, 124.27, 126.76, 127.70, 128.27, 129.92, 130.18, 145.17, 145.82, 148.57, 152.15, 155.89, 159.26, 172.08. Anal. ($C_{23}H_{22}N_2O_4$) C, H, N.

4-(2-Ethyl-1,3-dioxan-2-yl)-2-methoxypyridine (14). Water was distilled azeotropically with a Dean−Stark apparatus from a mixture of 1-(2-chloro-4-pyridyl)-1-propanone[34] (**13**; 10 g, 59 mmol), 1,3-propanediol (20 mL), and *p*-toluenesulfonic acid (250 mg) in toluene (150 mL). The solvent was then removed under reduced pressure, the acid neutralized with saturated aqueous sodium bicarbonate (100 mL), and the product extracted with diethyl ether. Combined ethereal extracts were washed with brine, dried over sodium sulfate, and concentrated under reduced pressure to give the crude carbonyl-protected product which was refluxed with 3 equiv of sodium methoxide in acetonitrile until completion of the reaction (as monitored by TLC: SiO_2, *tert*-butyl methyl ether/hexane). The acetonitrile solution was then filtered and concentrated under reduced pressure. The residue was taken up in diethyl ether, washed with water and brine, dried over sodium sulfate, and concentrated under reduced pressure to give a brown oil which was distilled (70−75 °C, 0.04 mbar) to give the pyridine **14** (10.7 g, 81% overall yield) as a clear oil: IR (KBr) 842, 986, 1316, 1388, 1478, 1560, 1608 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.78 (t, 3H), 1.1−1.3 (m, 1H), 1.69 (q, 2H), 1.9−2.2 (m, 1H), 3.6−4.0 (m, 7H) 6.75 (s, 1H), 6.90 (d, 1H), 8.18 (d, 1H); ^{13}C NMR ($CDCl_3$) δ 7.09, 25.36, 36.72, 53.45, 61.42, 101.20, 109.74, 115.83, 147.15, 152.25, 164.86. Anal. ($C_{12}H_{17}NO_3$) C, H, N.

4-(2-Ethyl-1,3-dioxan-2-yl)-3-hydroxymethyl-2-methoxypyridine (15). To a solution of bromomesitylene (13 mL, 85 mmol) in dry THF (300 mL) at −78 °C and under argon was added dropwise, by means of a cannula, *tert*-butyllithium (1.7 M in pentane, 100 mL, 170 mmol). The resulting white precipitate was stirred at −78 °C for 1 h, then **14** (10 g, 44.8 mmol) was added, and the reaction mixture was stirred 15 min at −78 °C, 1 h at 0 °C, and 1 h at room temperature. The mixture was then recooled to −78 °C, dry DMF (100 mmol) was added, and the reaction mixture was allowed to warm to room temperature and stirred 16 h, at which point TLC analysis (SiO_2, *tert*-butyl methyl ether/hexane, 50/50) showed complete consumption of starting material. The reaction mixture was quenched with saturated ammonium chloride and extracted with diethyl ether. The combined extracts were dried over sodium sulfate and concentrated under reduced pressure to give a yellow oil which was purified by medium-pressure chromatography (SiO_2, *tert*-butyl methyl ether/hexane, 0/100 to 5/95 to elute mesitylene derivatives then 20/80 to 50/50 to elute the intermediate aldehyde). The aldehyde was dissolved in methanol (100 mL) and treated with sodium borohydride (5 g, 132 mmol). The resulting mixture was stirred for 1 h. The solvent was evaporated under reduced pressure, the residue taken up in diethyl ether, washed with water and brine, and dried, and the solvent evaporated under reduced pressure. Medium-pressure chromatography (SiO_2, *tert*-butyl methyl ether/hexane, 10/90 to 50/50) of the residue gave compound **15** (7 g, 62% overall yield) as a yellow oil: IR (KBr) 990, 1002, 1079, 1151, 1309, 1388 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.85 (t, 3H), 1.30 (d, 1H), 1.81 (q, 2H), 2.11 (m, 1H), 2.84 (t, 1H), 3.77 (m, 2H), 3.93 (m, 2H), 4.04 (s, 3H), 4.93 (dd, 2H), 7.01 (d, 1H), 8.11 (d, 1H); ^{13}C NMR ($CDCl_3$) δ 7.56, 25.57, 36.68, 54.19, 57.21, 61.94, 102.62, 117.98, 122.36, 145.83, 149.04, 164.12; HRMS (FAB) calcd for $C_{13}H_{19}NO_4H$ 228.0028, found 228.0025 (MH^+).

1-[2-Methoxy-3-[(phenylmethoxy)methyl]-4-pyridinyl]-1-propanone (16). To a suspension of sodium hydride (80% in mineral oil, 1.85 g, 61 mmol) in dry THF (100 mL) was added dropwise a solution of **15** (7 g, 27.6 mmol) and benzyl chloride (5 mL, 45 mmol) in dry THF (50 mL), and the reaction mixture refluxed 16 h. The reaction mixture was allowed to cool to room temperature and quenched with water (50 mL), and the volatiles were evaporated under reduced pressure. The residue was dissolved in diethyl ether (150 mL), washed with water and brine, dried, and concentrated under reduced pressure. The residue was treated with trifluoroacetic acid (10 mL) and water (5 mL) at a bath temperature of 120 °C for 3 h. The reaction mixture was concentrated under reduced pressure, and the residual trace of acid was neutralized by the addition of saturated aqueous sodium bicarbonate. Extraction with diethyl ether followed by medium-pressure chromatography (SiO_2, *tert*-butyl methyl ether/hexane, 10/90) gave **16** (5.8 g, 70% yield) as a clear oil: IR (KBr) 739, 1071, 1388, 1453, 1595, 1708 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.04 (t, 3H), 2.72 (q, 2H), 3.93 (s, 3H), 4.49 (s, 2H), 4.62 (s, 2H), 6.73 (d, 1H), 7.30 (s, 5H), 8.12 (d, 1H); ^{13}C NMR ($CDCl_3$) δ 7.42, 36.13, 53.74, 63.54, 73.31, 113.58, 116.91, 127.68, 127.88, 128.27, 137.60, 146.26, 150.56, 161.66, 205.33. Anal. ($C_{17}H_{19}NO_3$) C, H, N.

***tert*-Butyl *β*-Ethyl-*β*-hydroxy-2-methoxy-3-[(phenylmethoxy)methyl]-4-pyridinepropionate (17).** To a suspension of zinc (5.3 g, 80 mmol, activated by treatment with 6 N HCl for 10 s, then washed successively with water until neutral pH, acetone, and diethyl ether) in dry THF (60 mL) at reflux was added dropwise *tert*-butyl bromoacetate (13 mL, 80 mmol). The reaction medium was maintained at reflux for an additional 10 min after the addition was completed. Then a solution of **16** (5.8 g, 20 mmol) in dry THF (20 mL) was added dropwise, and the reaction mixture was stirred under reflux 1 h further. The reaction was quenched at 0 °C with saturated aqueous ammonium chloride (100 mL) and extracted with diethyl ether. Combined extracts were dried over sodium sulfate and evaporated to give a yellow oil which was purified by medium-pressure chromatography (SiO_2, *tert*-butyl methyl ether/hexane, 5/95 to 10/90) to give *tert*-butyl ester **17** (7 g, 95% yield) as a clear oil: IR (KBr) 1068, 1155, 1384, 1556, 1593, 1723 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.76 (t, 3H), 1.28 (s, 9H), 1.83 (q, 2H), 2.82 (dd, 2H), 3.91 (s, 3H), 4.60 (s, 2H), 4.87 (s, 2H), 5.05 (s, 1H), 6.79 (d, 1H), 7.15−7.40 (m, 5H), 8.03 (d, 1H); ^{13}C NMR ($CDCl_3$) δ 7.85, 27.73, 35.72, 47.02, 53.68, 63.21, 73.56, 77.29, 81.23, 115.57, 117.87, 127.43, 127.10, 128.27, 138.09, 145.56, 156.14, 163.73, 170.97. Anal. ($C_{23}H_{31}NO_5$) C, H, N.

5-Ethyl-4,5-dihydro-5-hydroxy-9-methoxyoxepino[3,4-*c*]pyridin-3(1*H*)-one (18). Benzyl-protected compound **17** (7 g, 17.5 mmol) was hydrogenolyzed at atmospheric pressure and room temperature using 5% palladium on charcoal as catalyst (350 mg) and absolute ethanol as solvent (70 mL). Upon completion of the reaction (6 h), the catalyst was removed by filtration and the solvent was evaporated to give the debenzylated product which was treated with trifluoroacetic acid (30 mL) for 3 h at room temperature. The volatiles were evaporated under reduced pressure, and the residue was purified by medium-pressure chromatography (SiO_2, CH_2Cl_2/MeOH, 100/0 to 98/2) to give a clear oil which upon treatment with toluene gave lactonic compound **18** (3.4 g, 82% yield) as white crystals: mp 97−98 °C; IR (KBr) 839, 1049, 1269, 1291, 1375, 1568, 1600, 1732 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.88 (t, 3H), 1.91 (q, 2H), 3.25 (dd, 2H), 4.05 (s, 3H), 5.35 (dd, 2H), 7.24 (d, 1H), 7.53 (br, 1H), 8.20 (d, 1H); ^{13}C NMR ($CDCl_3$) δ 8.23, 37.19, 42.50, 55.65, 61.86, 73.75, 116.78, 116.95, 144.51, 156.21, 160.07, 172.34. Anal. ($C_{12}H_{15}NO_4$) C, H, N.

5-Ethyl-1,4,5,8-tetrahydro-5-hydroxyoxepino[3,4-*c*]pyridine-3,9-dione (19). Compound **18** (3.4 g, 14.3 mmol) was heated at reflux for 9 h in 1 N HCl (150 mL). The reaction mixture was concentrated under reduced pressure, and the residue was further dried by adding and evaporating toluene twice and then left overnight under reduced pressure in the presence of phosphorus pentoxide. The resulting oil was dissolved in dry acetonitrile (5 mL) and stirred under argon for 24 h. The precipitate was filtered and dried to give the pyridone **19** (1.56 g, 49% yield) as a white solid: mp 118−119

°C; IR (KBr) 812, 1041, 1181, 1284, 1530, 1563, 1625, 1747 cm^{-1}; ^1H NMR (DMSO-d_6) δ 0.77 (t, 3H), 1.68 (m, 2H), 3.14 (dd, 2H), 5.26 (dd, 2H), 5.74 (s, 1H), 6.32 (d, 1H), 7.33 (d, 1H), 11.69 (br, 1H); ^{13}C NMR (DMSO-d_6) δ 8.28, 35.91, 42.43, 61.16, 72.87, 105.05, 122.75, 133.91, 155.80, 161.33, 172.10. Anal. ($C_{11}H_{13}NO_4$) C, H, N.

2-Chloro-6,7-difluoro-3-quinolinemethanol (21g). To a Vilsmeier reagent (obtained by dropwise addition, under an argon atmosphere, of phosphorus oxychloride (103 mL, 1.1 mol) onto anhydrous DMF (34 mL, 440 mmol) cooled with an ice water bath and further stirred during 0.5 h and then allowed to reach room temperature) was added in one portion 3,4-difluoroacetanilide (**20g**; 32 g, 220 mmol). The resulting mixture was stirred at 70 °C for 16 h. After cooling to room temperature, the reaction mixture was added dropwise to ice water (400 mL) and stirred 2 h. The resulting precipitate was filtered and washed sequentially with water, ethanol, and diethyl ether to give the desired quinolinecarboxaldehyde (9 g, 18% yield) as a yellow solid: mp 226.5−229 °C; IR (KBr) 888, 1061, 1262, 1507, 1691 cm^{-1}; ^1H NMR (DMSO-d_6) δ 8.17 (dd, 1H), 8.39 (dd, 1H), 8.97 (d, 1H), 10.34 (d, 1H). This intermediate aldehyde was suspended in methanol (400 mL) and treated with sodium borohydride (2 g, 53 mmol) at room temperature during 0.5 h. Excess reagent was destroyed with acetic acid (2 mL), and the volatiles were removed under reduced pressure. The residue was dissolved in ethyl acetate (500 mL), and the resulting solution was washed sequentially with dilute sodium bicarbonate, water, and brine and then dried over sodium sulfate and concentrated under reduced pressure. The residue was recrystallized with 1,2-dichloroethane to give the alcohol **21g** (8 g, 89% yield) as a beige solid: mp 166.5−167.1 °C; IR (KBr) 871, 1038, 1253, 1513 cm^{-1}; ^1H NMR (DMSO-d_6) δ 4.67 (d, 2H), 5.80 (t, 1H), 8.01 (dd, 1H), 8.22 (dd, 1H), 8.48 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 59.92, 113.94, 114.37, 124.74, 134.57, 135.41, 143.11, 149.05, 149.30, 151.79; HRMS (FAB) calcd for $C_{10}H_6ClF_2NOH$ 230.0184, found 230.0203 (MH^+).

8-[(2-Chloro-6,7-difluoro-3-quinolyl)methyl]-5-ethyl-1,4,5,8-tetrahydro-5-hydroxyoxepino[3,4-*c*]pyridine-3,9-dione (22g). An anhydrous DMF solution (a sufficient volume to dissolve all the reagents, ca. 17 mL) of heterobicycle **19** (892 mg, 4 mmol), alcohol **21g** (918 mg, 4 mmol), and tributylphosphine (1 mL, 4 mmol) was cooled with an ice−water bath and treated under argon by dropwise addition of diethyl azodicarboxylate (630 μL, 4 mmol), and the resulting mixture was allowed to stir 3 h further. The reaction mixture was then concentrated under reduced pressure to near dryness. The residue was taken up in diethyl ether (100 mL) and kept overnight at 4 °C. The resulting precipitate was filtered and washed with diethyl ether to give the coupled product **22g** (580 mg, 33% yield) as an off-white solid: mp 268 °C; IR (KBr) 871, 1054, 1361, 1508, 1583, 1648, 1741 cm^{-1}; ^1H NMR (DMSO-d_6) δ 0.84 (t, 3H), 1.74 (q, 2H), 2.9−3.4 (dd, 2H), 5.25 (m, 4H), 5.81 (s, 1H), 6.52 (d, 1H), 7.82 (d, 1H), 8.06 (m, 2H), 8.20 (t, 1H); ^{13}C NMR (DMSO-d_6) δ 8.35, 35.90, 42.31, 50.32, 61.57, 72.91, 105.79, 114.31, 114.61, 122.66, 124.46, 128.97, 137.53, 138.16, 143.65, 149.60, 149.75, 152.26, 155.37, 160.58, 172.04; HRMS (FAB) calcd for $C_{21}H_{17}ClF_2N_2O_4H$ 435.0923, found 435.0987 (MH^+).

5-Ethyl-9,10-difluoro-1,4,5,13-tetrahydro-5-hydroxy-3*H*,15*H*-oxepino[3′,4′:6,7]indolizino[1,2-*b*]quinoline-3,15-dione (23g). A mixture of **22g** (1.21 g, 2.78 mmol), tetrabutylammonium bromide (984 mg, 3 mmol), sodium acetate (300 mg, 3 mmol), triphenylphosphine (364 mg, 1.39 mmol), and palladium(II) acetate (62 mg, 0.28 mmol) was stirred under argon in anhydrous acetonitrile (60 mL) at reflux temperature for 36 h. After cooling to room temperature the resulting white precipitate was filtered and washed sequentially with acetonitrile, water, ethanol, and diethyl ether to give hCPT **23g** (530 mg, 48% yield) as an off-white solid: mp >250 °C; IR (KBr) 1266, 1512, 1581, 1618, 1751 cm^{-1}; ^1H NMR (DMSO-d_6) δ 0.91 (t, 3H), 1.87 (m, 2H), 3.08 (d, 1H), 3.51 (d, 1H), 5.19 (s, 2H), 5.47 (dd, 2H), 6.02 (br, 1H), 7.33 (s, 1H), 7.54 (s, 1H), 7.55 (s, 1H), 8.43 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 8.43, 36.47, 42.54, 50.52, 61.43, 64.43 (2C), 73.31, 99.07, 112.27, 113.14, 122.00, 124.24, 128.18, 129.74, 144.59, 145.01, 145.33, 147.63, 150.88, 155.88, 159.23, 172.07; HRMS (FAB) calcd for $C_{21}H_{16}F_2N_2O_4H$ 399.1156, found 399.1145 (MH^+). Anal. ($C_{21}H_{16}F_2N_2O_4 \cdot 0.25H_2O$) C, H, N.

10-Chloro-5-ethyl-1,4,5,13-tetrahydro-5-hydroxy-3*H*,15*H*-oxepino[3′,4′:6,7]indolizino[1,2-*b*]quinoline-3,15-dione (23a). This compound was prepared from the appropriate aniline using a procedure similar to that described for **23g**, to give a yellow solid: mp > 250 °C; IR (KBr) 1069, 1483, 1606, 1741 cm^{-1}; ^1H NMR (DMSO-d_6) δ 0.85 (t, 3H), 1.85 (q, 2H), 3.07 (d, 1H), 3.47 (d, 1H), 5.25 (s, 2H), 5.39 (d, 1H), 5.51 (d, 1H), 6.05 (s, 1H), 7.39 (s, 1H), 7.89 (d, 1H), 8.19 (d, 1H), 8.29 (s, 1H), 8.67 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 8.40, 36.46, 42.47, 50.70, 61.42, 73.31, 100.00, 122.96, 127.31, 127.42, 128.87, 131.11, 132.12, 144.34, 146.53, 153.38, 155.88, 159.20, 172.04; HRMS (FAB) calcd for $C_{21}H_{17}ClN_2O_4H$ 397.0655, found 397.0957 (MH^+). Anal. ($C_{21}H_{17}ClN_2O_4 \cdot 0.25H_2O$) C, H, N.

5-Ethyl-10-fluoro-1,4,5,13-tetrahydro-5-hydroxy-3*H*,-15*H*-oxepino[3′,4′:6,7]indolizino[1,2-*b*]quinoline-3,15-dione (23b). This compound was prepared from the appropriate aniline using a procedure similar to that described for **23g**, to give a white solid: mp > 250 °C; IR (KBr) 1209, 1589, 1659, 1739 cm^{-1}; ^1H NMR (DMSO-d_6) δ 0.85 (t, 3H), 1.85 (q, 2H), 3.07 (d, 1H), 3.45 (d, 1H), 5.29 (s, 2H), 5.39 (d, 1H), 5.55 (d, 1H), 6.30 (s, 1H), 7.39 (s, 1H), 7.80 (q, 1H), 7.99 (q, 1H), 8.23 (q, 1H), 8.68 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 8.40, 36.46, 42.48, 50.66, 61.41, 73.31, 99.68, 111.83, 122.75, 128.93, 130.93, 131.22, 131.93, 144.46, 145.27, 152.60, 155.89, 159.21, 172.04; HRMS (FAB) calcd for $C_{21}H_{17}FN_2O_4H$ 381.1251, found 381.1252 (MH^+). Anal. ($C_{21}H_{17}FN_2O_4 \cdot 0.75H_2O$) C, H, N.

5-Ethyl-9-fluoro-1,4,5,13-tetrahydro-5-hydroxy-10-methoxy-3*H*,15*H*-oxepino[3′,4′:6,7]indolizino[1,2-*b*]quinoline-3,15-dione (23c). This compound was prepared from the appropriate aniline using a procedure similar to that described for **23g**, to give a yellow solid: mp > 250 °C; IR (KBr) 1259, 1503, 1602, 1737 cm^{-1}; ^1H NMR (DMSO-d_6) δ 0.89 (t, 3H), 1.85 (q, 2H), 3.08 (d, 1H), 3.49 (d, 1H), 4.00 (s, 3H), 5.25 (s, 2H), 5.39 (d, 1H), 5.51 (d, 1H), 6.00 (s, 1H), 7.32 (s, 1H), 7.72 (d, 1H), 7.91 (d, 1H), 8.58 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 8.43, 36.48, 42.51, 50.68, 56.60, 61.42, 73.29, 99.25, 108.68, 113.52, 122.23, 126.33, 129.99, 130.30, 143.79, 144.70, 148.42, 151.18, 153.19, 155.81, 159.20, 172.06; HRMS (FAB) calcd for $C_{22}H_{19}FN_2O_5H$ 411.1356, found 411.1363 (MH^+). Anal. ($C_{22}H_{19}FN_2O_5$) C, H; N: calcd, 6.83; found, 7.33.

5-Ethyl-9-fluoro-1,4,5,13-tetrahydro-5-hydroxy-10-methyl-3*H*,15*H*-oxepino[3′,4′:6,7]indolizino[1,2-*b*]quinoline-3,15-dione (23d). This compound was prepared from the appropriate aniline using a procedure similar to that described for **23g**, to give a yellow solid: mp > 250 °C; IR (KBr) 1054, 1580, 1651, 1760 cm^{-1}; ^1H NMR (DMSO-d_6) δ 0.89 (t, 3H), 1.85 (q, 2H), 2.49 (s, 3H), 3.08 (d, 1H), 3.49 (d, 1H), 5.21 (s, 2H), 5.39 (d, 1H), 5.51 (d, 1H), 6.05 (s, 1H), 7.39 (s, 1H), 7.87 (d, 1H), 8.05 (d, 1H), 8.61 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 8.40, 15.14, 36.45, 42.52, 50.60, 61.41, 73.28, 99.71, 112.00, 122.66, 125.38, 127.66, 129.59, 130.28, 144.49, 147.88, 152.88, 155.85, 159.18, 162.25, 172.02; HRMS (FAB) calcd for $C_{22}H_{19}FN_2O_4H$ 395.1407, found 395.1412 (MH^+). Anal. ($C_{22}H_{19}FN_2O_4$) C, H, N.

9-Chloro-5-ethyl-1,4,5,13-tetrahydro-5-hydroxy-10-methyl-3*H*,15*H*-oxepino[3′,4′:6,7]indolizino[1,2-*b*]quinoline-3,15-dione (23e). This compound was prepared from the appropriate aniline using a procedure similar to that described for **23g**, to give a yellow solid: mp > 250 °C; IR (KBr) 1208, 1479, 1606, 1656, 1724 cm^{-1}; ^1H NMR (DMSO-d_6) δ 0.85 (t, 3H), 1.85 (q, 2H), 2.55 (s, 3H), 3.07 (d, 1H), 3.45 (d, 1H), 5.25 (s, 2H), 5.39 (d, 1H), 5.51 (d, 1H), 6.05 (s, 1H), 7.39 (s, 1H), 8.10 (s, 1H), 8.20 (s, 1H), 8.60 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 8.43, 20.20, 36.47, 42.49, 50.67, 61.41, 73.28, 99.87, 122.82, 126.98, 127.99, 129.60, 130.53, 131.08, 135.64, 136.56, 144.39, 147.11, 153.10, 155.85, 159.18, 172.03; HRMS (FAB) calcd for $C_{22}H_{19}ClN_2O_4H$ 411.1112, found 411.1109 (MH^+). Anal. ($C_{22}H_{19}ClN_2O_4 \cdot 0.5H_2O$) C, H, N.

9-Chloro-5-ethyl-10-fluoro-1,4,5,13-tetrahydro-5-hydroxy-3*H*,15*H*-oxepino[3′,4′:6,7]indolizino[1,2-*b*]quinoline-3,15-dione (23f). This compound was prepared from the appropriate aniline using a procedure similar to that described for **23g**, to give a yellow solid: mp > 250 °C; IR (KBr) 1488, 1583, 1655, 1743 cm^{-1}; ^1H NMR (DMSO-d_6) δ 0.85 (t, 3H), 1.85 (q, 2H), 3.07 (d, 1H), 3.45 (d, 1H), 5.25 (s, 2H), 5.39 (d, 1H), 5.51 (d, 1H), 6.05 (s, 1H), 7.40 (s, 1H), 8.20 (d, 1H), 8.40 (d, 1H), 8.68 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 8.38, 36.47, 42.58, 50.71, 61.40, 73.26, 99.99, 113.59, 123.09, 124.28, 127.74, 130.64, 131.31, 144.13, 145.08, 153.57, 154.13, 155.84, 156.61, 159.14, 172.00; HRMS (FAB) calcd for $C_{21}H_{16}ClFN_2O_4H$ 415.0861, found 415.0853 (MH^+). Anal. ($C_{21}H_{16}ClFN_2O_4 \cdot 0.25H_2O$) C, H, N.

8-Ethyl-2,3,8,9,12,15-hexahydro-8-hydroxy-10*H*,13*H*-1,4-dioxino[2,3-*g*]oxepino[3′,4′:6,7]indolizino[1,2-*b*]quinoline-10,13-dione (23 h). This compound was prepared from the appropriate aniline using a procedure similar to that described for **23g**, to give a light-yellow solid: mp > 250 °C; IR (KBr) 1065, 1237, 1287, 1501, 1576, 1653, 1752 cm^{-1}; ^1H NMR (DMSO-d_6) δ 0.91 (t, 3H), 1.87 (m, 2H), 3.08 (d, 1H), 3.51 (d, 1H), 4.45 (s, 4H), 5.19 (s, 2H), 5.47 (dd, 2H), 6.02 (s, 1H), 7.33 (s, 1H), 7.54 (s, 1H), 7.55 (s, 1H), 8.43 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 8.43, 36.47, 42.54, 50.52, 61.43, 64.43 (2C), 73.31, 99.07, 112.27, 113.14, 122.00, 124.24, 128.18, 129.74, 144.59, 145.01, 145.33, 147.63, 150.88, 155.88, 159.23, 172.07. Anal. ($C_{23}H_{20}N_2O_6 \cdot 0.5H_2O$) C, H, N.

7-Ethyl-7,8,11,14-tetrahydro-7-hydroxy-9*H*,12*H*-1,3-dioxolo[4.5-*g*]oxepino[3′,4′:6,7]indolizino[1,2-*b*]quinoline-9,12-dione (23i). This compound was prepared from the appropriate aniline using a procedure similar to that described for **23g**, to give a yellow solid: mp > 150 °C; IR (KBr) 1248, 1459, 1606, 1731 cm^{-1}; ^1H NMR (DMSO-d_6) δ 0.85 (t, 3H), 1.85 (q, 2H), 3.07 (d, 1H), 3.45 (d, 1H), 5.20 (s, 2H), 5.39 (d, 1H), 5.51 (d, 1H), 6.00 (s, 1H), 6.30 (s, 2H), 7.30 (s, 1H), 7.49 (d, 2H), 8.45 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 8.43, 36.49, 42.56, 50.58, 61.42, 73.31, 98.87, 102.75, 103.33, 104.92, 121.76, 125.74, 128.59, 130.33, 145.08, 146.69, 148.78, 150.19, 151.49, 155.90, 159.24, 172.08. Anal. ($C_{22}H_{18}N_2O_6 \cdot 1H_2O$) C, H, N.

Biology. Camptothecin (CPT), adriamycin (ADR), and etoposide (VP16) were purchased from Sigma (l'Isle d'Abeau, France), and the reported procedures were employed for the preparation of topotecan (TPT)[16] and SN38.[15]

Drug Stability in Human Plasma. A method established by others for CPT[38] was adapted as follows for the determination of *dl*-hCPT (**9a**). To 500-μL fractions of pooled human plasma (TSEF, Rungis, France), distributed in polystyrene tubes and preincubated at 37 °C for 5 min, was added a 5-μL solution of the drug in dimethyl sulfoxide (DMSO, 10 mM), and the samples were incubated at 37 °C. At defined times the plasma proteins were precipitated by the addition of 2 mL of cold methanol at −50 °C, the resulting mixture was centrifuged (2000*g*) at 4 °C for 5 min, and the supernatant was analyzed immediately in order to avoid further chemical transformation. Samples were run on a 5-μm Nucleosil C18 column (4.6 × 150 mm) at 30 °C with a flow rate of 1 mL/min of an isocratic eluent composed of 1 M tetrabutylammonium dihydrogen phosphate/acetonitrile/75 mM, pH 6.9, ammonium acetate buffer (5/125/870, v/v/v), and the eluted analytes were detected at 220 nm.

DNA Relaxation Assay. All reactions were performed in 20 μL of reaction buffer as previously described.[13] The reaction buffer contained 50 mM Tris-HCl at pH 7.5, 50 mM KCl, 0.5 mM dithiothreitol, 10 mM $MgCl_2$, 0.1 mM EDTA, 30 μg/mL bovine serum albumin, and 300 ng of pUC19 supercoiled plasmid DNA (Pharmacia Biotech, Orsay, France) and the test drug, first dissolved in DMSO (10^{-2} M) and further diluted with water to defined final concentrations. The reactions, initiated by the addition of purified calf thymus Topo I (Life technologies, Paisley, U.K.), were carried out for 15 min at 37 °C and quenched by adding 3 μL of a solution containing 1% sodium dodecyl sulfate, 20 mM EDTA, and 500 μg/mL proteinase K (Boehringer Mannheim, Meylan, France). After an additional 30-min incubation period at 37 °C, 2 μL of a loading buffer containing 10 mM Na_2HPO_4, 0.03% bromophenol blue, and 16% Ficoll was added to the samples which were run on electrophoresis in 1.2% agarose gels at 1 V/cm for 20 h in a buffer containing 36 mM Tris-HCl, pH 7.8, 30 mM NaH_2PO_4, 1 mM EDTA, and 2 μg/mL chloroquine. The gels were stained with 2 μg/mL ethidium bromide, and densitometric analysis under UV light at 312 nm with a CCD camera (BioProfil, Vilber Lourmat, Lyon France) gave percent relaxed DNA as well as mean standard errors.

Cell Growth Assay. The A427 (lung carcinoma) and PC-3 (prostatic adenocarcinoma) cell lines were obtained from ATCC (Rockville, MD). The MCF7*mdr* (breast adenocarcinoma) and K562*adr* (leukemia) cell lines were obtained from A.-M. Faussat (Hôpital Hôtel-Dieu, Paris, France). They were derived from sensitive cell lines by prolonged exposure to adriamycin and have been shown by flow cytometry to overexpress a functionally active P-glycoprotein. Cells (3000 for A427, MCF7*mdr*, and K562*adr* and 1500 for PC-3, in 80 μL of Dulbecco's modified Eagle medium at 4.5 g/L glucose supplemented by 10% heat-inactivated fetal calf serum, 50 units/ml penicillin, 50 μg/mL streptomycin, and 2 mM glutamine; all from Gibco-BRL, Cergy-Pontoise, France) were seeded on a microtiter plate (tissue culture grade, 96 wells, flat bottom) 24 h prior to drug treatment. The cells were treated with the drugs, first dissolved in DMSO (0.1% of final volume) and further diluted to 20 μL with culture medium, at final concentrations ranging from 5×10^{-13} to 1×10^{-6} M. At the end of a 72-h incubation period, the quantification of cell viability was evaluated by a colorimetric assay based on the reduction of the tetrazolium salt WST-1[50] (Boehringer Mannheim, Meylan, France) by mitochondrial dehydrogenases of living cells leading to the formation of soluble formazan. These experiments were performed twice with eight determinations per tested concentration. For each drug, the data points included in the linear part of the sigmoid were selected by linear regression analysis (linearity, deviation from linearity, and difference between experiments) to estimate the 50% inhibitory concentration (IC_{50}).

HT-29 Xenograft Model. Tumors were established by sc injection of tissue culture-derived tumor cells (5×10^6 cells/animal on the left dorsal surface) in 4−6-week-old NCr *nu*/*nu* female athymic nude mice (NCI, Frederick, MD). Tumor volume (mm^3) was calculated as (w^2l)/2, where w is the width and l is the length of the tumor as measured with calipers. The animals were monitored until day 10 postimplant, when the tumors reached an average size of approximately 175 mm^3. The range of tumor volumes was then tallied, and the animals were randomized into appropriate groups (5 animals/treatment and 10 animals for the control group) so that the average tumor volumes were approximately the same for each group at the onset of dosing. The drugs were first dissolved in DMSO (3% total injection volume) and then diluted with Tween 20 (2% total injection volume), and further dilutions were made with 0.9% NaCl solution. Animals were treated ip daily for 4 consecutive days, followed by 3 days without treatment. This 4 days on /3 days off cycle was repeated twice more for a total of 12 injections over the course of 3 weeks. The mice of the control group were administered the vehicle, following the schedule of the treated mice. Three times weekly, tumor sizes and body weights were recorded for all animals. The tumor growth delay (T−C index, measured in days) is calculated as the difference in time required for the mean tumor volume to reach 250% of the tumor average volume at the onset of dosing, for the treated group (T) and the control group (C). Animal care was in accordance with institutional guidelines.

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