Topoisomerase I-Mediated Antiproliferative Activity of 10-Substituted and 12-Substituted Homocamptothecins

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Homocamptothecin (hCPT) is an *E*-ring modified camptothecin (CPT) analogue, which showed pronounced inhibitory activity of topoisomerase I. In search of novel hCPT-type anticancer agents, two series of hCPT derivatives were synthesized and evaluated *in vitro* against three human tumor cell lines. The results indicated that the 10-substituted hCPT derivatives had a considerably higher cytotoxic activity than the 12-substituted ones. Among the 10-substituted compounds, **8a**, **8b**, **9b**, and **9i** showed an equivalent or even more potent activity than the positive control drug topotecan against the lung cancer cell line A-549. Moreover, the hCPT analogues **8a** and **8b** exhibited a higher topoisomerase I inhibitory activity than CPT at a concentration of 100 μM.

Introduction. – As part of a *National Cancer Institute* compound screening program, camptothecin (CPT; *Fig. 1*) was first characterized in 1966 from *Camptotheca acuminata*, a plant with impressive activity against a variety of tumors [1]. However, an obvious shortcoming was its poor H_2O solubility. To overcome this solubility problem, numerous derivatives had been optimized concerning their H_2O solubility, to facilitate intravenous drug administration. Now, irinotecan (CPT-11) and topotecan (TPT), as two marketed drugs, are successfully used in clinical practice for colon and ovarian cancer treatment, respectively.

In an attempt to stabilize the closed lactone of CPT, a seven-membered derivative was synthesized by *Lavergne et al.* [2] in 1997, *i.e.*, homocamptothecin (hCPT), a semi-synthetic CPT analog with a modified *E* ring, *i.e.*, the natural α-hydroxy-δ-lactone *E* ring was replaced by a β-hydroxy-ε-lactone ring [2]. In this decade, a number of hCPT derivatives were prepared by total or semi-synthetic strategies, of which two promising compounds, BN80915 (diflomotecan) and BN80927, are so far in clinical phrase II and I trials, respectively.

The high cytotoxic activity of 10-substituted CPT derivatives such as SN-38 (active metabolite of CPT-11), which has been submitted to clinical studies, prompted researchers to investigate the introduction of other moieties at C(10) of CPT and hCPT. In previous studies, we synthesized a series of H_2O-soluble 10-phosphate ester, 10-amino acid ester, and 9-(hetarylmethylidene)amino derivatives of hCPT [3–5]. To discover new compounds with more potent and broad-spectrum antitumor activities,

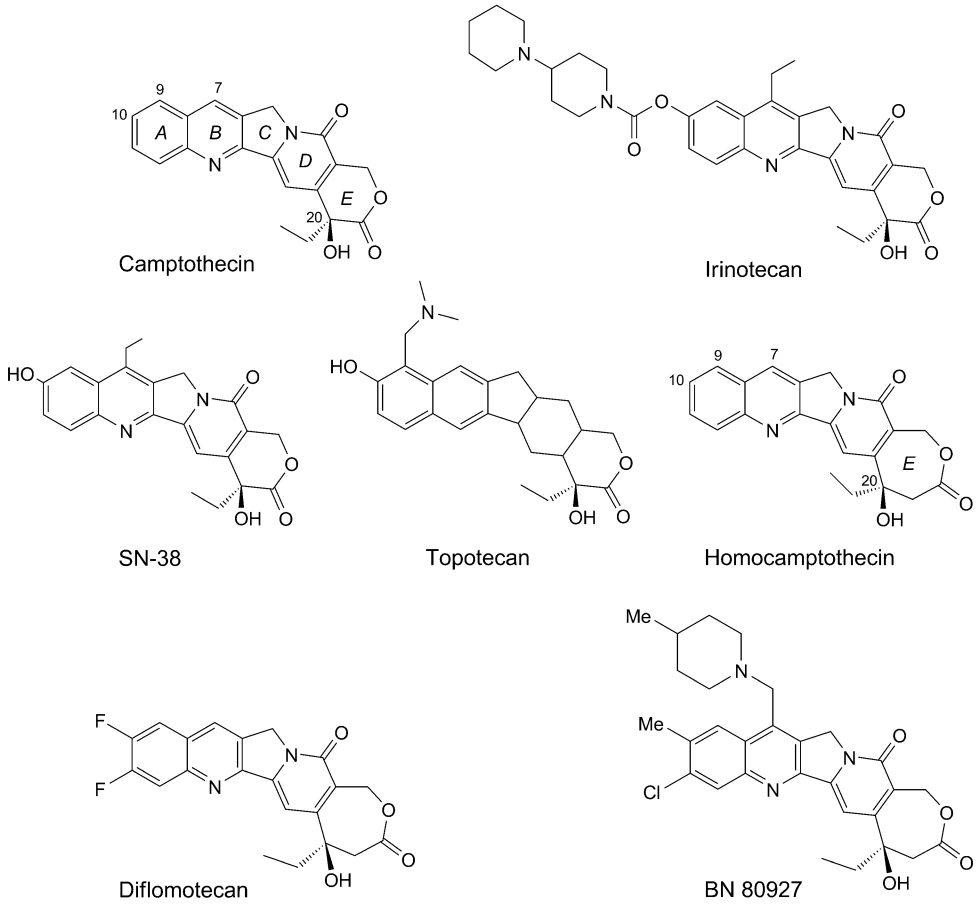
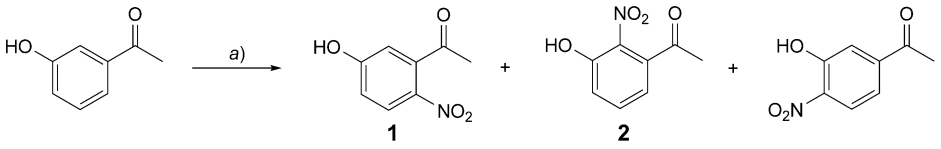


Fig. 1. *Chemical structures of camptothecin analogues*

the design of a series of 10- and 12-alkoxy hCPT derivatives and the study of their structure–activity relationships are reported here.

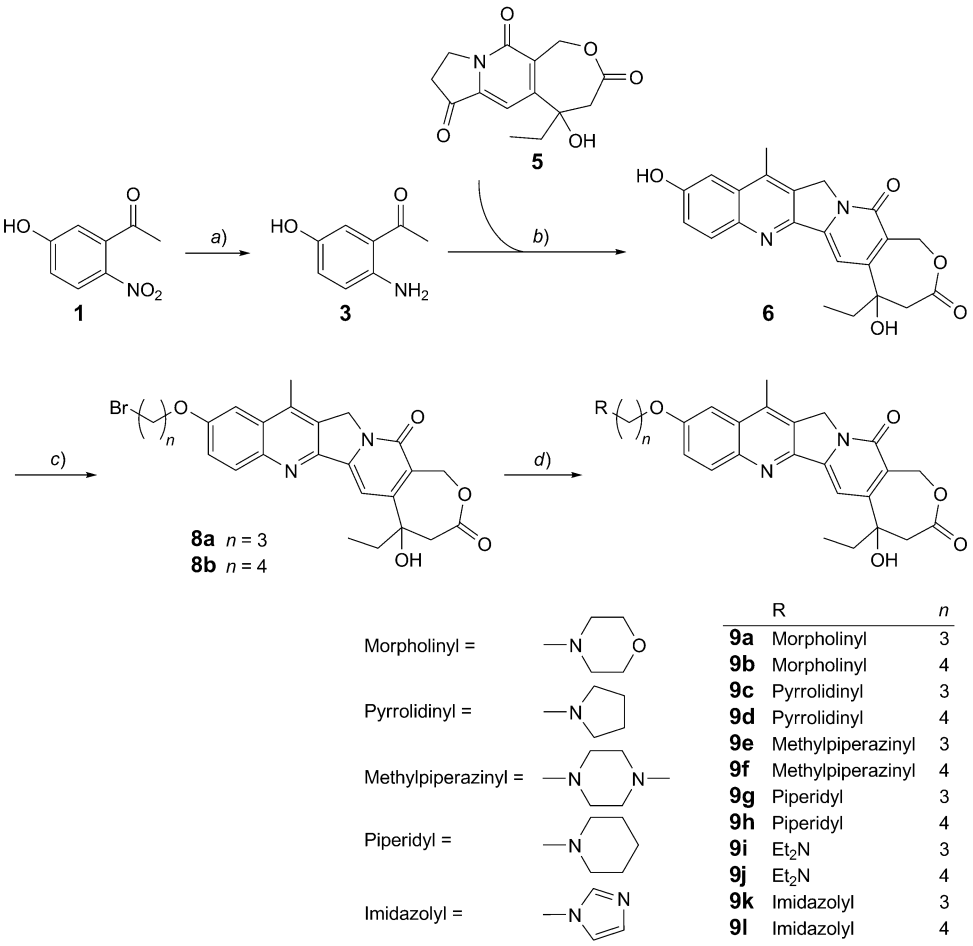
Results and Discussion. – *Synthesis.* Ring *A* of the 10- and 12-alkoxy hCPTs was prepared in two steps, which are illustrated in *Scheme 1*. The isomers **1** and **2** were prepared from 3-hydroxyacetophenone by nitration (KNO_3 and AcOH) [6]. Then, compounds **1** and **2** were hydrogenated in EtOH with Pd/C as catalyst to give compounds **3** and **4**, respectively (*Schemes 2* and *3*). Compound **5**, which is the building block that introduces rings *C*, *D*, and *E* into the final hCPT derivatives, was synthesized according to a reported route of 16 reaction steps, which was optimized in our previous studies [7–9]. The 10-OH- and 12-OH-substituted hCPTs **6** and **7** were prepared *via* a *Friedländer* cyclization of ring *A* with **5** and obtained with a good yield of 85%. When toluene and *p*-toluenesulfonic acid (TsOH) were used for the cyclization reaction, a lower yield of **6** and **7** (40%) was obtained than with DMF and tetramethylsilylchloride

Scheme 1



a) KNO_3, AcOH, 60°, overnight; yield 22% for **1**, 21% for **2**.

Scheme 2



Morpholinyl =

Pyrrolidinyl =

Methylpiperazinyl =

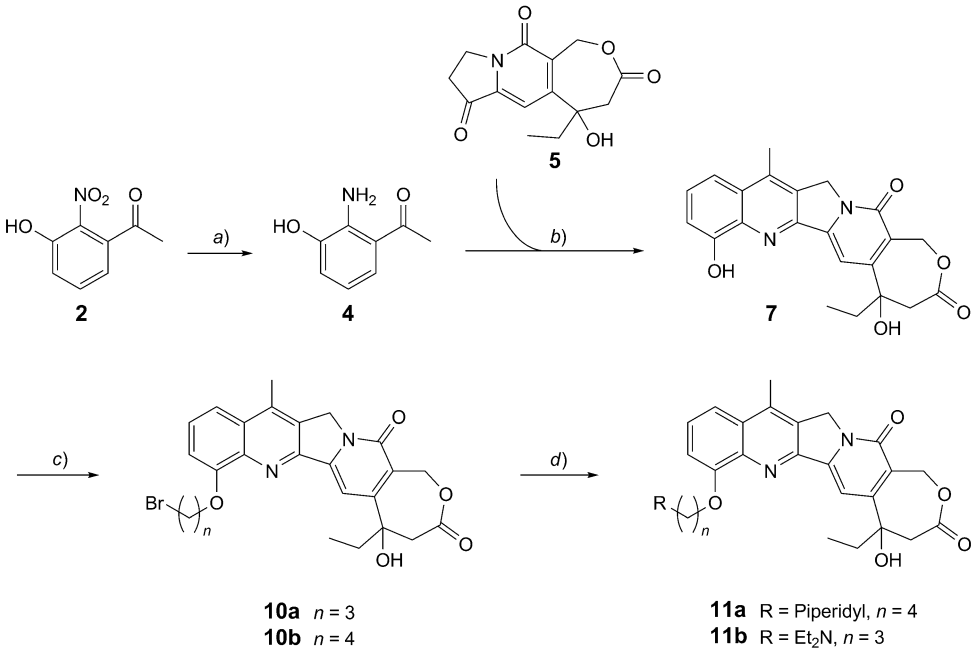
Piperidyl =

Imidazolyl =

	R	*n*
9a	Morpholinyl	3
9b	Morpholinyl	4
9c	Pyrrolidinyl	3
9d	Pyrrolidinyl	4
9e	Methylpiperazinyl	3
9f	Methylpiperazinyl	4
9g	Piperidyl	3
9h	Piperidyl	4
9i	Et_2N	3
9j	Et_2N	4
9k	Imidazolyl	3
9l	Imidazolyl	4

a) H_2, Pd/C, EtOH, 30°, 3 bar, 3 h; yield 91%. *b*) Me_3SiCl (TMSCl), DMF, 2 h, 100°; yield 85%. *c*) 1,3-Dibromopropane or 1,4-dibromobutane, DMF, 60°, overnight; yield 61% for **8a**, 63% for **8b**. *d*) DMF, 60°, N_2, 2 h; yield 37–51%.

Scheme 3



a) H_2, Pd/C, AcOEt, 35°, 3 bar, 2 h; yield 90 %. *b*) TMSCl, DMF, 2 h, 100°; yield 86%. *c*) 1,3-Dibromopropane or 1,4-dibromobutane, DMF, 60°, overnight; yield 60% for **10a**, 64% for **10b**. *d*) DMF, 60°, N_2, 2 h; yield 50% for **11a**, 30% for **11b**.

(TMSCl; 85%). 10-(3-Bromopropoxy)-7-methylhomocamptothecin (**8a**) and 10-(4-bromobutoxy)-7-methylhomocamptothecin (**8b**) were obtained from 1,3-dibromopropane or 1,4-dibromobutane and 10-hydroxy-7-methylhomocamptothecin (**6**) in DMF. Compounds **8a** and **8b** were reacted with different N-containing compounds to afford the 10-alkoxy-hCPTs **9a**–**9l**. The 12-alkoxy-hCPTs **10a**, **10b**, **11a**, and **11b** were obtained by a similar route (*Scheme 3*) than the 10-alkoxy hCPTs.

Cytotoxicity. The hCPT derivatives were evaluated for their cytotoxicity against three cancer cell lines, *i.e.*, A-549 (human lung adenocarcinoma epithelial cell line), MDA-MB-435 (human breast carcinoma cell line), and HCT-116 (human colon carcinoma cell line), using the MTT method *in vitro*. The results are summarized in *Tables 1* and *2*. Most of the 10-substitued hCPTs showed antitumor activities against the three cell lines, especially against the A-549 cell line. Among them, the three compounds **8a**, **8b**, and **9b** exhibited particularly high inhibitory activities against the A-549 cell line, with IC_{50} values lower than 0.6 μM; hence, they were more potent than the reference drug topotecan against this cell line. But the length of the alkane chain between the hCPT and the heterocycle seemed not to be related to the activities. Indeed, compounds **9b** and **9d** showed higher activities than **9a** and **9c**, while compounds **9g**, **9i**, and **9k** showed higher activities than **9h**, **9j**, and **9l**, respectively. However, the 12-subsituted hCPTs displayed lower activities compared with the 10-

Table 1. *Cytotoxicity of the 10-Alkoxy-hCPT Derivatives against Three Human Tumor Cell Lines*

Compounds	IC_{50} [μM][a])		
	A-549	MDA-MB-435	HCT-116
8a	0.58	19.67	9.82
8b	0.20	15.67	5.32
9a	22.62	–[b])	–
9b	0.35	–	56.27
9c	–	76.60	–
9d	19.15	62.88	–
9e	–	–	–
9f	–	–	–
9g	3.00	90.28	27.52
9h	15.03	80.68	56.00
9i	1.57	–	75.19
9j	9.42	–	–
9k	6.16	–	74.28
9l	43.70	–	–
Topotecan	2.44	21.79	4.97

[a]) The IC_{50} values were determined by using the MTT method. [b]) –: $IC_{50} > 100$ μM.

Table 2. *Comparison of the Cytotoxicity of the 12-Alkoxy-hCPTs with that of the Most Active 10-Alkoxy-hCPTs*

Compounds	IC_{50} [μM][a])		
	A-549	MDA-MB-435	HCT-116
10a	–[b])	–	–
10b	–	–	–
11a	88.24	–	–
11b	–	–	–
8a	0.58	19.67	9.82
8b	0.20	15.67	5.32
9b	0.35	–	56.27
9i	1.57	–	75.19
Topotecan	2.44	21.79	4.97

[a]) The IC_{50} values were determined by using the MTT method. [b]) –: $IC_{50} > 100$ μM.

substituted ones. As shown in *Table 2*, the 10-substituted derivatives **9i** and **9b** showed two to seven times higher activities than topotecan, whereas the 12-subsituted hCPTs **11a** and **11b** showed very low or no cytotoxic activity against A-549 cells. The previously published binding mode of hCPT within the active site of topoisomerase I showed that C(12) of hCTP was located on the minor groove side of the intercalation binding pocket and that there was less space for a substituent at this position than at the 10-position [5]. The present results showed that the activities of the derivatives with long substituents at C(12) were drastically decreased, which was in good agreement with the proposed binding mode [5]. The determined cytotoxic effects of the synthesized hCPT

derivatives affirmed that long substituents at C(12) were unfavorable for the activity, which was similar to CPT derivatives.

Topoisomerase I-Mediated DNA Relaxation. The topoisomerase I-inhibitory properties of the selected hCPT derivatives **8a**, **8b**, and **9g** were investigated with the *in vitro* cleavable-complex assay. Negative supercoiled plasmid pBR322 was incubated with human topoisomerase I in the absence and presence of 100 μM hCPT derivatives. A parallel experiment was performed with CPT. The results indicated that compounds **8a** and **8b**, which were found to be highly cytotoxic agents, had higher topoisomerase I-inhibitory properties than CPT (*Fig. 2*), while compound **9g** showed the same lower activity than CPT in the DNA cleavage assay.

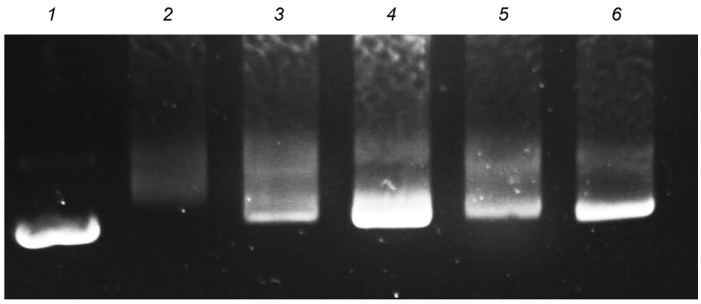


Fig. 2. *Effect of three homocamptothecin derivatives on topoisomerase I-mediated DNA relaxation. Lane 1:* supercoiled DNA pBR322; *Lane 2:* supercoiled DNA and Topoisomerase I; *Lanes 3–6:* supercoiled DNA, Topoisomerase I, and 100 μM of CPT, **8a**, **9g**, or **8b**, respectively.

Conclusions. – In continuation of the work about 10- and 12-alkoxy hCPT derivatives, 18 new hCPT derivatives were synthesized from our previously published route [5][7–9]. Preliminary biological studies of these hCPT analogues including the determination of the inhibitory potential of topoisomerase I-mediated DNA cleavage reactions and of the *in vitro* cytotoxicity exhibited that most 10-substituted derivatives possessed cytotoxic activities. The three compounds **8a**, **8b**, and **9b** showed to be even more efficient against the human lung adenocarcinoma epithelial cell line A-549 than topotecan. Moreover, compounds **8a** and **8b** were found to exhibit a stronger topoisomerase I-dependent cytotoxic activity than CPT at a concentration of 100 μM.

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Experimental Part

General. Commercial solvents were used without any further purification or pretreatment. Column chromatography (CC): silica gel *60 G* (SiO_2; *Qingdao Haiyang Chemical Co. Ltd.*, Qingdao, P. R. China). TLC: precoated GF_{254} silica-gel plates (*Qingdao Haiyang Chemical Co. Ltd.*, Qingdao, P. R. China). ^1H- and ^{13}C-NMR Spectra: *Bruker 500* NMR spectrometer, at 500 and 125 MHz, resp., in (D_6)DMSO; δ in ppm rel. to Me_4Si, J in Hz. ESI-MS: *API-3000* mass spectrometer; in m/z. Elemental analysis: *MOD-1106* instrument; analses were consistent with the theoretical values within $\pm 0.4\%$.

5-Hydroxy-2-nitroacetophenone (=*1-(5-Hydroxy-2-nitrophenyl)ethanone*; **1**). 3-Hydroxyacetophenone (13.6 g, 0.1 mol) was dissolved in AcOH (100 ml) under stirring for 10 min at r.t., and then KNO_3 (25.0 g, 0.2 mol) was added slowly over a period of 1 h. After raising the temp. to 60°, the mixture was stirred overnight. Then, the residue was cooled to r.t., poured into H_2O (500 ml), and extracted with AcOEt. The AcOEt extract was washed successively with sat. aq. $NaHCO_3$ and H_2O, and concentrated *in vacuo* to give an oily residue, which was purified by flash chromatography (FC; SiO_2; hexane/AcOEt 2:1) to afford **1** (4.0 g, 22%). Yellow solid. M.p. 146–147°. ^{1}H-NMR: 2.51 (*s*, Me); 6.84 (*d*, *J*=2.6, H–C(6)); 6.98 (*dd*, *J*=2.6, 9.1, H–C(4)); 8.07 (*d*, *J*=9.1, H–C(3)); 11.34 (*s*, OH). ESI-MS: 180.42 ($[M-H]^-$).

3-Hydroxy-2-nitroacetophenone (=*1-(3-Hydroxy-2-nitrophenyl)ethanone*; **2**). Compound **2** was prepared according to the method described for **1**: 3.8 g (21%). Yellow solid. M.p. 144–146°. ^{1}H-NMR: 2.53 (*s*, Me); 6.85 (*d*, *J*=7.5, H–C(4)); 7.24 (*d*, *J*=8.7, H–C(6)); 7.60 (*t*, *J*=5.7, H–C(5)); 10.53 (*s*, OH). ESI-MS: 180.51 ($[M-H]^-$).

2-Amino-5-hydroxyacetophenone (=*1-(2-Amino-5-hydrophenyl)ethanone*; **3**). To a soln. of **1** (1.3 g, 7.1 mmol) in EtOH (200 ml) Pd-C (0.1 g) was added as catalyst and hydrogenation followed at 3 bar for 2 h at 30°. The resulting suspension was filtered and concentrated under reduced pressure to afford **3** (1.0 g, 91%). Yellow solid. M.p. 162–165°. ^{1}H-NMR: 2.58 (*s*, Me); 6.52 (*t*, *J*=7.5, H–C(3)); 6.89 (*dd*, *J*=2.7, 8.7, H–C(4)); 7.37(*d*, *J*=2.7, H–C(6)); 7.27 (*s*, OH). ESI-MS: 150.41 ($[M-H]^-$).

10-Hydroxy-7-methylhomocamptothecin (=*5-Ethyl-1,4,5,13-tetrahydro-5,10-dihydroxy-12-methyl-3*H,*15*H-*oxepino[3′,4′:6,7]indolizino[1,2-*b*]quinoline-3,15-dione*; **6**). The tricyclic ketone **5** (0.3 g, 1.8 mmol) and **1** (0.5 g, 2.0 mmol) were dissolved in DMF at r.t. TMSCl (0.8 ml, 6.5 mmol) was added drop wise, and then the mixture was refluxing at 100° for 2 h. The mixture was poured into ice/H_2O (100 ml) and filtered. The crude product was washed with acetone (10 ml) and MeOH (10 ml) to give **6** (0.6 g, 85%). Yellow solid. M.p. >300° (dec.). ^{1}H-NMR: 0.87 (*t*, *J*=7.5, Me(18)); 1.86 (*q*, *J*=7.6, CH_2(19)); 2.66 (*s*, Me(7)); 3.47 (*q*, *J*=13.7, CH_2(21)); 5.21 (*s*, CH_2(5)); 5.52 (*q*, *J*=15.1, CH_2(17)); 5.98 (*s*, HO–C(20)); 7.30 (*d*, *J*=2.7, H–C(9)); 7.32(*s*, H–C(14)); 7.42 (*dd*, *J*=2.7, 9.2, H–C(11)); 8.15 (*d*, *J*=9.2, H–C(12)); 10.30 (*s*, HO–C(10)). ESI-MS: 393.10 ($[M-H]^+$).

10-(3-Bromopropoxy)-7-methylhomocamptothecin (=*10-(3-Bromopropoxy)-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*; **8a**). A mixture of **6** (3.0 g, 7.8 mmol), 1,3-dibromopropane (15.9 ml, 156.0 mmol), and anh. K_2CO_3 (5.4 g, 39.0 mmol) in anh. DMF (20 ml) was stirred at 60° under N_2 overnight. The mixture was filtered, and the residue was poured into ice H_2O (200 ml) and extracted with CH_2Cl_2. The CH_2Cl_2 extract was washed successively with H_2O and concentrated *in vacuo* to give an oily residue, which was purified by FC (SiO_2; CH_2Cl_2/MeOH 100:5) to afford **7a** (2.3 g, 60%). Yellow solid. M.p. >250°. ^{1}H-NMR: 0.87 (*t*, *J*=7.4, Me(18)); 1.88 (*q*, *J*=7.5, CH_2(19)); 2.35–2.37 (*m*, CH_2(2′)); 2.73 (*s*, Me(7)); 3.38 (*q*, *J*=13.8, CH_2(21)); 3.74 (*t*, *J*=6.0, CH_2(3′)); 4.32 (*t*, *J*=6.6, CH_2(1′)); 5.23 (*s*, CH_2(5)); 5.52 (*q*, *J*=15.1, CH_2(17)); 6.09 (*s*, HO–C(20)); 7.32 (*s*, H–C(14)); 7.49 (*d*, *J*=2.6, H–C(9)); 7.50 (*dd*, *J*=2.7, 9.1, H–C(11)); 8.03 (*d*, *J*=9.1, H–C(12)). ^{13}C-NMR: 8.7; 15.5; 31.6; 32.3; 36.8; 42.9; 50.5; 61.8; 66.4; 73.6; 99.4; 103.9; 122.2; 122.7; 129.3; 129.4; 131.6; 138.8; 144.4; 145.7; 149.8; 156.3; 157.7; 159.5; 172.4. ESI-MS: 511.38 ($[M-H]^-$). Anal. calc. for $C_{25}H_{25}BrN_2O_5$ (512.09): C 58.49, H 4.91, N 5.46; found: C 58.40, H 4.90, N 5.48.

10-(4-Bromobutoxy)-7-methylhomocamptothecin (=*10-(4-Bromobutoxy)-5-ethyl-1,4,5,13-tetrahydro-5-hydroxy-12-methyl-3*H,*15*H-*oxepino[3′,4′:6,7]indolizino[1,2-*b*]quinoline-3,15-dione*; **8b**). Compound **8b** was prepared according to the method described for **8a** and obtained as a yellow solid (2.6 g, 63%). M.p. >250°. ^{1}H-NMR: 0.87 (*t*, *J*=7.4, Me(18)); 1.86 (*q*, *J*=14.8, CH_2(19)); 1.85–2.04 (*m*, CH_2(2′)); 2.04–2.35 (*m*, CH_2(3′)); 2.74 (*s*, Me(7)); 3.38 (*q*, *J*=15.1, CH_2(21)); 3.67 (*t*, *J*=6.6, CH_2(4′)); 4.25 (*t*, *J*=6.3, CH_2(1′)); 5.27 (*s*, CH_2(5)); 5.52 (*q*, *J*=15.0, CH_2(17)); 6.01 (*s*, HO–C(20)); 7.32 (*s*, H–C(14)); 7.50 (*d*, *J*=2.4, H–C(9)); 7.50 (*dd*, *J*=2.7, 9.1, H–C(11)); 8.04 (*d*, *J*=9.0, H–C(12)). ^{13}C-NMR: 8.6; 15.5; 27.7; 29.5; 35.2; 36.7; 39.5; 42.8; 50.4; 61.7; 67.6; 73.5; 99.2; 103.7; 122.1; 122.7; 129.3; 131.5; 138.6; 144.3; 145.7; 149.6; 156.3; 157.8; 159.5; 172.3. ESI-MS: 525.22 ($[M-H]^-$). Anal. calc. for $C_{26}H_{27}BrN_2O_5$ (526.11): C 59.21, H 5.16, N 5.31; found: C 59.13, H 5.17, N 5.29.

7-Methyl-10-(morpholin-3-ylpropoxy)homocamptothecin (=*5-Ethyl-1,4,5,13-tetrahydro-5-hydroxy-12-methyl-10-[3-(morpholin-4-yl)propoxy]-3*H,*15*H-*oxepino[3′,4′:6,7]indolizino[1,2-*b*]quinoline-3,15-dione*; **9a**). A mixture of **8a** (50 mg, 0.1 mmol) and morpholine (0.2 ml) in anh. DMF (10 ml) was stirred

at 60° for 2 h under N_2 atmosphere. The mixture was poured into ice/H_2O (100 ml) and extracted with CH_2Cl_2. The CH_2Cl_2 extract was washed successively with H_2O and concentrated *in vacuo* to give an oily residue, which was purified by FC (SiO_2; CH_2Cl_2/MeOH 100:5) to afford **9a** (25 mg, 49%). Yellow solid. M.p. >250°. ^{1}H-NMR: 0.87 (*t*, *J* = 7.5, Me(18)); 1.85 (*q*, *J* = 7.5, CH_2(19)); 2.36 (*s*, CH_2(2′)); 2.74 (*s*, Me(7)); 3.07–3.61 (*m*, CH_2(2″), CH_2(3″), CH_2(5″), CH_2(6″), CH_2(21), CH_2(3′)); 4.30 (*t*, *J* = 1.5, CH_2(1′)); 5.27 (*s*, CH_2(5)); 5.52 (*q*, *J* = 15.1, CH_2(17)); 6.07 (*s*, HO–C(20)); 7.33 (*s*, H–C(14)); 7.47 (*d*, *J* = 2.6, H–C(9)); 7.51 (*dd*, *J* = 2.8, 9.2, H–C(11)); 8.05 (*d*, *J* = 9.2, H–C(12)). ESI-MS: 518.27 ([*M* − H]$^-$). Anal. calc. for $C_{29}H_{33}N_3O_6$ (519.24): C 67.04, H 6.40, N 8.09; found: C 67.11, H 6.39, N 8.11.

7-Methyl-10-(morpholin-4-ylbutoxy)homocamptothecin (= *5-Ethyl-1,4,5,13-tetrahydro-5-hydroxy-12-methyl-10-[4-(morpholin-4-yl)butoxy]-3*H,*15*H-*oxepino[3′,4′:6,7]indolizino[1,2-*b*]quinoline-3,15-dione*; **9b**). Compound **9b** was prepared from **8b** and morpholine according to the method described for **9a** and obtained: 20 mg (39%). Yellow solid. M.p. >250°. ^{1}H-NMR: 0.87 (*t*, *J* = 7.4, Me(18)); 1.82 (*q*, *J* = 7.5, CH_2(19)); 1.65–1.85 (*m*, CH_2(2′), CH_2(3′)); 2.36–2.38 (*m*, CH_2(2″), CH_2(6″)); 2.74 (*s*, Me(7)); 3.06 (*q*, *J* = 13.9, CH_2(21)); 3.48–3.58 (*m*, CH_2(4′), CH_2(3″), CH_2(5″)); 4.24 (*t*, *J* = 6.3, CH_2(1′)); 5.26 (*s*, CH_2(5)); 5.50 (*q*, *J* = 14.5, CH_2(17)); 6.00 (*s*, HO–C(20)); 7.33 (*s*, H–C(14)); 7.46 (*d*, *J* = 2.5, H–C(9)); 7.75 (*dd*, *J* = 2.6, 9.1, H–C(11)); 7.76 (*d*, *J* = 9.3, H–C(12)). ESI-MS: 532.46 ([*M* − H]$^-$). Anal. calc. for $C_{30}H_{35}N_3O_6$ (533.25): C 67.52, H 6.61, N 7.87; found: C 67.63, H 6.63, N 7.85.

7-Methyl-10-(pyrrolidin-3-ylpropoxy)homocamptothecin (= *5-Ethyl-1,4,5,13-tetrahydro-5-hydroxy-12-methyl-10-[3-(pyrrolidin-1-yl)propoxy]-3*H,*15*H-*oxepino[3′,4′:6,7]indolizino[1,2-*b*]quinoline-3,15-dione*; **9c**). Compound **9c** was prepared from **8a** and pyrrolidine according to the method described for **9a**: 20 mg (41%). Yellow solid. M.p. >250°. ^{1}H-NMR: 0.79 (*t*, *J* = 7.3, Me(18)); 1.83–1.84 (*m*, CH_2(19)); 1.86–1.89 (*m*, CH_2(3″), CH_2(4″)); 1.90–2.20 (*m*, CH_2(2′)); 2.74 (*s*, Me(7)); 3.15–3.17 (*m*, CH_2(1″), CH_2(4″)); 3.19 (*q*, *J* = 13.7, CH_2(21)); 3.50–3.54 (*m*, CH_2(3′)); 4.30–4.32 (*m*, CH_2(1′)); 4.65 (*s*, CH_2(5)); 4.80 (*q*, *J* = 15.0, CH_2(17)); 6.49 (*s*, HO–C(20)); 7.31 (*s*, H–C(14)); 7.50–7.51 (*m*, H–C(9), H–C(11)); 8.07 (*d*, *J* = 8.3, H–C(12)). ESI-MS: 503.24 ([*M* − H]$^-$). Anal. calc. for $C_{29}H_{33}N_3O_5$ (503.24): C 69.17, H 6.60, N 8.34; found: C 69.03, H 6.58, N 8.36.

7-Methyl-10-(pyrrolidin-4-ylbutoxy)homocamptothecin (= *5-Ethyl-1,4,5,13-tetrahydro-5-hydroxy-12-methyl-10-[4-(pyrrolidin-1-yl)butoxy]-3*H,*15*H-*oxepino[3′,4′:6,7]indolizino[1,2-*b*]quinoline-3,15-dione*; **9d**). Compound **9d** was prepared from **8b** and pyrrolidine according to the method described for **9a**: 25 mg (51%). Yellow solid. M.p. >250°. ^{1}H-NMR: 0.80 (*t*, *J* = 7.4, Me(18)); 1.87 (*q*, *J* = 7.4, CH_2(19)); 1.93–1.98 (*m*, CH_2(2′), CH_2(3′), CH_2(3″), CH_2(4″)); 2.74 (*s*, Me(7)); 3.20–3.22 (*m*, CH_2(2″), CH_2(5″), CH_2(21)); 3.33–3.50 (*m*, CH_2(1′), CH_2(4′)); 4.24 (*s*, CH_2(5)); 4.86 (*q*, *J* = 15.1, CH_2(17)); 6.50 (*s*, HO–C(20)); 7.31 (*s*, H–C(14)); 7.32 (*d*, *J* = 2.2, H–C(9)); 7.63 (*dd*, *J* = 2.4, 9.3, H–C(11)); 7.76 (*d*, *J* = 3.2, H–C(12)). ESI-MS: 516.27 ([*M* − H]$^-$). Anal. calc. for $C_{30}H_{35}N_3O_5$ (517.26): C 69.61, H 6.82, N 8.12; found: C 69.49, H 6.81, N 8.13.

7-Methyl-10-[3-(methylpiperazin-4-yl)propoxy]homocamptothecin (= *5-Ethyl-1,4,5,13-tetrahydro-5-hydroxy-12-methyl-10-[3-(4-methylpiperazin-1-yl)propoxy]-3*H,*15*H-*oxepino[3′,4′:6,7]indolizino[1,2-*b*]quinoline-3,15-dione*; **9e**). Compound **9e** was prepared from **8a** and 4-methylpiperazine according to the method described for **9a**: 19 mg (37%). Yellow solid. M.p. >250°. ^{1}H-NMR: 0.87 (*t*, *J* = 7.4, Me(18)); 1.87 (*q*, *J* = 7.4, CH_2(19)); 1.88–2.23 (*m*, CH_2(2′)); 2.50 (*s*, Me(4″)); 2.64 (*s*, Me(7)); 3.03–3.08 (*m*, CH_2(2″), CH_2(3″), CH_2(5″), CH_2(6″)); 3.34 (*q*, *J* = 13.8, CH_2(21)); 4.25 (*t*, *J* = 6.2, CH_2(3′)); 4.27 (*t*, *J* = 6.3, CH_2(1′)); 5.27 (*s*, CH_2(5)); 5.52 (*q*, *J* = 15.1, CH_2(17)); 6.00 (*s*, HO–C(20)); 7.32 (*s*, H–C(14)); 7.47 (*d*, *J* = 2.6, H–C(9)); 7.51 (*dd*, *J* = 2.6, 9.1, H–C(11)); 8.05 (*d*, *J* = 9.2, H–C(12)). ESI-MS: 531.68 ([*M* − H]$^-$). Anal. calc. for $C_{30}H_{36}N_4O_5$ (532.27): C 67.65, H 6.81, N 10.52; found: C 67.77, H 6.82, N 10.50.

7-Methyl-10-[4-(methylpiperazin-4-yl)butoxy]homocamptothecin (= *5-Ethyl-1,4,5,13-tetrahydro-5-hydroxy-12-methyl-10-[4-(4-methylpiperazin-1-yl)butoxy]-3*H,*15*H-*oxepino[3′,4′:6,7]indolizino[1,2-*b*]quinoline-3,15-dione*; **9f**). Compound **9f** was prepared from **8b** and 4-methylpiperazine according to the method described for **9a**: 20 mg (39%). Yellow solid. M.p. >250°. ^{1}H-NMR: 0.86 (*t*, *J* = 7.4, Me(18)); 1.87 (*q*, *J* = 7.5, CH_2(19)); 1.81–1.86 (*m*, CH_2(2′), CH_2(3′)); 2.30 (*s*, Me(4″)); 2.50–2.72 (*m*, CH_2(2″), CH_2(3″), CH_2(5″), CH_2(6″)); 2.73 (*s*, Me(7)); 3.45–3.48 (*m*, CH_2(4′)); 4.20–4.21 (*m*, CH_2(1′)); 3.37 (*q*, *J* = 13.5, CH_2(21)); 5.26 (*s*, CH_2(5)); 5.53 (*q*, *J* = 15.0, CH_2(17)); 6.01 (*s*, HO–C(20)); 7.32 (*s*, H–C(14)); 7.46–7.50 (*m*, H–C(11)); 8.04 (*d*, *J* = 9.1, H–C(12)). ESI-MS: 545.54 ([*M* − H]$^-$). Anal. calc. for $C_{31}H_{38}N_4O_5$ (546.28): C 68.11, H 7.01, N 10.25; found: C 68.22, H 7.03, N 10.23.

7-Methyl-10-(piperidin-3-ylpropoxy)homocamptothecin (*=5-Ethyl-1,4,5,13-tetrahydro-5-hydroxy-12-methyl-10-[3-(piperidin-1-yl)propoxy]-3*H,*15*H-*oxepino[3′,4′:6,7]indolizino[1,2-*b*]quinoline-3,15-dione*; **9g**). Compound **9g** was prepared from **8a** and piperidine according to the method described for **9a**: 26 mg (51%). Yellow solid. M.p. >250°. ^{1}H-NMR: 0.86 (*t*, *J*=7.4, Me(18)); 2.07–2.43 (*m*, CH_2(3″), CH_2(4″), CH_2(5″), CH_2(19)); 2.43 (*s*, CH_2(2′)); 2.74 (*s*, Me(7)); 3.29–3.60 (*m*, CH_2(2″), CH_2(6″), CH_2(21)); 3.80 (*s*, CH_2(3′)); 4.28 (*s*, CH_2(1′)); 5.26 (s, CH_2(5)); 5.52 (*q*, *J* = 15.1, CH_2(17)); 6.01 (*s*, HO–C(20)); 7.33 (*s*, H–C(14)); 7.37–7.45 (*m*, H–C(9), H–C(11)); 8.05 (*d*, *J*=9.0, H–C(12)). ^{13}C-NMR: 8.6; 15.7; 25.9; 36.8; 41.4; 42.9; 43.4; 50.4; 53.6; 61.8; 63.8; 66.3; 67.4; 73.5; 99.7; 110.7; 116.1; 122.5; 128.1; 129.2; 129.4; 140.0; 140.1; 145.7; 150.5; 155.4; 156.2; 159.5; 172.3. ESI-MS: 516.78 ($[M-H]^-$). Anal. calc. for $C_{30}H_{35}N_3O_5$ (517.26): C 69.61, H 6.82, N 8.12; found: C 69.52, H 6.84, N 8.10.

7-Methyl-10-(piperidin-4-ylbutoxy)homocamptothecin (*=5-Ethyl-1,4,5,13-tetrahydro-5-hydroxy-12-methyl-10-[4-(piperidin-1-yl)butoxy]-3*H,*15*H-*oxepino[3′,4′:6,7]indolizino[1,2-*b*]quinoline-3,15-dione*; **9h**). Compound **9h** was prepared from **8b** and piperidine according to the method described for **9a**: 22 mg (44%). Yellow solid. M.p. >250°. ^{1}H-NMR: 0.80 (*t*, *J*=7.4, Me(18)); 1.23(*q*, *J*=7.5, CH_2(19)); 1.55–1.64 (*m*, CH_2(2′), CH_2(3′)); 1.81–1.87 (*m*, CH_2(3″), CH_2(4″), CH_2(5″)); 2.74 (*s*, Me(7)); 2.87–3.03 (*m*, CH_2(2″), CH_2(6″)); 3.46–3.48 (*m*, CH_2(4′), CH_2(21)); 4.26–4.28 (*m*, CH_2(1′)); 5.27 (*s*, CH_2(5)); 5.55 (*q*, *J* = 14.8, CH_2(17)); 6.00 (*s*, HO–C(20)); 7.28 (*s*, H–C(14)); 7.48–7.52 (*m*, H–C(9), H–C(11)); 8.07 (*d*, *J*= 9.2, H–C(12)). ESI-MS: 530.52 ($[M-H]^-$). Anal. calc. for $C_{31}H_{37}N_3O_5$ (531.27): C 70.03, H 7.01, N 7.90; found: C 70.14, H 7.04, N 7.89.

10-[3-(Diethylamino)propoxy]-7-methylhomocamptothecin (*=10-[3-(Diethylamino)propoxy]-5-ethyl-1,4,5,13-tetrahydro-5-hydroxy-12-methyl-3*H,*15*H-*oxepino[3′,4′:6,7]indolizino[1,2-*b*]quinoline-3,15-dione*; **9i**). Compound **9i** was prepared from **8a** and Et_2NH according to the method described for **9a**: 22 mg (45%). Yellow solid. M.p. >250°. ^{1}H-NMR: 0.87 (*t*, *J*=7.4, Me(18)); 1.16–1.18 (*m*, 2 *Me*CH_2N); 1.86 (*q*, *J*=7.4, CH_2(19)); 2.15–2.18 (*m*, CH_2(2′)); 2.73 (*s*, Me(7)); 3.04–3.07 (*m*, 2 MeC*H*$_2$N); 3.38 (*q*, *J*=13.7, CH_2(21)); 3.46–3.49 (*m*, CH_2(3′)); 4.29–4.31 (*m*, CH_2(1′)); 5.28 (*s*, CH_2(5)); 5.52 (*q*, *J* = 15.1, CH_2(17)); 6.00 (*s*, HO–C(20)); 7.32 (*s*, H–C(14)); 7.47 (*d*, *J*=2.5, H–C(9)); 7.51 (*dd*, *J*=2.7, 9.2, H–C(11)); 7.76 (*d*, *J*=9.2, H–C(12)). ESI-MS: 503.52 ($[M-H]^-$). Anal. calc. for $C_{29}H_{35}N_3O_5$ (505.26): C 68.89, H 6.98, N 8.31; found: C 68.99, H 6.98, N 8.32.

10-[4-(Diethylamino)butoxy]-7-methylhomocamptothecin (*=10-[4-(Diethylamino)butoxy]-5-ethyl-1,4,5,13-tetrahydro-5-hydroxy-12-methyl-3*H,*15*H-*oxepino[3′,4′:6,7]indolizino[1,2-*b*]quinoline-3,15-dione*; **9j**). Compound **9j** was prepared from **8b** and Et_2NH according to the method described for **9a**: 25 mg (51%). Yellow solid. M.p. >250°. ^{1}H-NMR: 0.80 (*t*, *J*=7.4, Me(18)); 1.23 (*q*, *J*=7.5, CH_2(19)); 1.55–1.64 (*m*, CH_2(2′), CH_2(3′)); 1.81–1.87 (*m*, 2 *Me*CH_2N); 2.74 (*s*, Me(7)); 2.87–3.03 (*m*, 2 MeC*H*$_2$N); 3.33–3.48 (*m*, CH_2(4′), CH_2(21)); 4.24–4.26 (*m*, CH_2(1′)); 5.27(*s*, CH_2(5)); 5.55(*q*, *J* = 14.8, CH_2(17)); 6.00 (*s*, HO–C(20)); 7.28 (*s*, H–C(14)); 7.48–7.53 (*m*, H–C(9), H–C(11)); 8.07 (*d*, *J*=9.2, H–C(12)). ESI-MS: 520.95 ($[M+H]^+$). Anal. calc. for $C_{30}H_{37}N_3O_5$ (519.27): C 69.34, H 7.18, N 8.09; found: C 69.44, H 7.19, N 8.05.

10-(Imidazol-3-ylpropoxy)-7-methylhomocamptothecin (*=5-Ethyl-1,4,5,13-tetrahydro-5-hydroxy-10-[3-(1*H*-imidazol-1-yl)propoxy]-12-methyl-3*H,*15*H-*oxepino[3′,4′:6,7]indolizino[1,2-*b*]quinoline-3,15-dione*; **9k**). Compound **9k** was prepared from **8a** and 1*H*-imidazole according to the method described for **9a**: 25 mg (45%). Yellow solid. M.p. >250°. ^{1}H-NMR: 0.87 (*t*, *J*=7.4, Me(18)); 1.86, (*q*, *J*=6.4, CH_2(19)); 2.28 (*m*, CH_2(2′)); 2.71 (*s*, Me(7)); 3.38 (*q*, *J*=13.9, CH_2(21)); 4.17 (*t*, *J*=6.0, CH_2(3′)); 4.22 (*t*, *J*=6.9, CH_2(1′)); 5.22 (*s*, CH_2(5)); 5.52 (*q*, *J* = 14.9, CH_2(17)); 6.00 (*s*, HO–C(20)); 6.94 (*s*, H–C(4″)); 7.30 (*s*, H–C(5″)); 7.33 (*s*, H–C(14)); 7.44 (*s*, H–C(9)); 7.50 (*dd*, *J*=2.6, 9.0, H–C(11)); 7.73 (*s*, H–C(2″)); 8.04 (*d*, *J*=9.0, H–C(12)). ESI-MS: 499.49 ($[M-H]^-$). Anal. calc. for $C_{28}H_{28}N_4O_5$ (500.21): C 67.19, H 5.64, N 11.19; found: C 67.07, H 5.63, N 11.18.

10-(Imidazol-4-ylbutoxy)-7-methylhomocamptothecin (*=5-Ethyl-1,4,5,13-tetrahydro-5-hydroxy-10-[4-(1*H*-imidazol-1-yl)butoxy]-12-methyl-3*H,*15*H-*oxepino[3′,4′:6,7]indolizino[1,2-*b*]quinoline-3,15-dione*; **9l**). Compound **9l** was prepared from **8b** and 1*H*-imidazole according to the method described for **9a**: 25 mg (39%). Yellow solid. M.p. >250°. ^{1}H-NMR: 0.87 (*t*, *J*=7.4, Me(18)); 1.80–1.86 (*m*, CH_2(19), CH_2(3′)); 1.90–1.95 (*m*, CH_2(2′)); 2.76 (*s*, Me(7)); 3.10 (*q*, *J*=13.7, CH_2(21)); 4.19–4.28 (*m*, CH_2(1′), CH_2(4′)); 5.28 (*s*, CH_2(5)); 5.50 (*q*, *J* = 15.0, CH_2(17)); 6.00 (*s*, HO–C(20)); 6.93 (*s*, H–C(4″)); 7.07 (*s*, H–C(5″)); 7.32 (*s*, H–C(14)); 7.45 (*d*, *J*=2.3, H–C(9)); 7.50 (*dd*, *J*=2.6, 9.0, H–C(11)); 7.73 (*s*, H–C(12));

8.05 (*d*, *J* = 9.1, H–C(2″)). ESI-MS: 513.67 ([*M* − H]$^-$). Anal. calc. for $C_{29}H_{30}N_4O_5$ (514.22): C 67.69, H 5.88, N 10.89; found: C 67.76, H 5.89, N 10.87.

2-Amino-3-hydroxyacetophenone (= *1-(2-Amino-3-hydroxyphenyl)ethanone*; **4**). The title compound was prepared according to the method described for **3**: 1.0 g (90%). Yellow solid. M.p. 179–181°. ^{1}H-NMR: 2.50 (*s*, Me); 6.40 (*t*, *J* = 7.9, H–C(5)); 6.69 (*s*, NH_2); 6.80 (*dd*, *J* = 7.8, 1.1, H–C(4)); 7.25 (*d*, *J* = 8.1, H–C(6)); 9.66 (*s*, OH). ESI-MS: 150.30 ([*M* − H]$^-$).

12-Hydroxy-7-methylhomocamptothecin (= *5-Ethyl-1,4,5,13-tetrahydro-5,8-dihydroxy-12-methyl-3*H,*15*H-*oxepino[3′,4′:6,7]indolizino[1,2-*b*]quinoline-3,15-dione*; **7**). Compound **7** was prepared from **4** and **5** according to the method described for **6**: 0.6 g (86%). Yellow solid. M.p. >300° (dec). ^{1}H-NMR: 0.87 (*t*, *J* = 7.2, Me(18)); 1.87 (*q*, *J* = 8.7, CH_2(19)); 2.73 (*s*, Me(7)); 3.47 (*q*, *J* = 14.0, CH_2(21)); 5.28 (*s*, CH_2(5)); 5.52 (*q*, *J* = 15.1, CH_2(17)); 6.04 (*s*, HO–C(20)); 7.18 (*d*, *J* = 7.3, H–C(11)); 7.54 (*m*, H–C(10), H–C(14)); 7.63 (*d*, *J* = 8.3, H–C(9)); 9.95 (*s*, HO–C(12)). ESI-MS: 391.83 ([*M* − H]$^-$).

12-(3-Bromopropoxy)-7-methylhomocamptothecin (= *8-(3-Bromopropoxy)-5-ethyl-1,4,5,13-tetrahydro-5-hydroxy-12-methyl-3*H,*15*H-*oxepino[3′,4′:6,7]indolizino[1,2-*b*]quinoline-3,15-dione*; **10a**). Compound **10a** was prepared from **7** and 1,3-dibromopropane according to the method described for **8a**: 60%. Yellow solid. M.p. >250°. ^{1}H-NMR: 0.87 (*t*, *J* = 7.4, Me(18)); 1.86 (*q*, *J* = 8.7, CH_2(19)); 2.43–2.50 (*m*, CH_2(2′)); 2.75 (*s*, Me(7)); 3.38 (*q*, *J* = 13.7, CH_2(21)); 3.83 (*t*, *J* = 6.5, CH_2(3′)); 4.36–4.40 (*m*, CH_2(1′)); 5.28 (*s*, CH_2(5)); 5.52 (*q*, *J* = 15.0, CH_2(17)); 6.09 (*s*, HO–C(20)); 7.33 (*s*, H–C(14)); 7.37 (*d*, *J* = 7.2, H–C(11)); 7.63 (*t*, *J* = 8.1, H–C(10)); 7.80 (*d*, *J* = 8.4, H–C(9)). ESI-MS: 511.53 ([*M* − H]$^-$). Anal. calc. for $C_{25}H_{25}BrN_2O_5$ (512.09): C 58.49, H 4.91, N 5.46; found: C 58.37, H 4.90, N 5.47.

12-(4-Bromobutoxy)-7-methylhomocamptothecin (= *8-(4-Bromopropoxy)-5-ethyl-1,4,5,13-tetrahydro-5-hydroxy-12-methyl-3*H,*15*H-*oxepino[3′,4′:6,7]indolizino[1,2-*b*]quinoline-3,15-dione*; **10b**). Compound **10b** was prepared from **7** and 1,4-dibromobutane according to the method described for **8a**: 64%. Yellow solid. M.p. >250°. ^{1}H-NMR: 0.87 (*t*, *J* = 7.4, Me(18)); 1.87 (*q*, *J* = 7.2, CH_2(19)); 2.10–2.11 (*m*, CH_2(3′)); 2.35–2.37 (*m*, CH_2(2′)); 2.75 (*s*, Me(7)); 3.38 (*q*, *J* = 13.8, CH_2(21)); 3.78–3.82 (*m*, CH_2(4′)); 4.28–4.31 (*m*, CH_2(1′)); 5.28 (*s*, CH_2(5)); 5.52 (*q*, *J* = 15.1, CH_2(17)); 6.04 (*s*, HO–C(20)); 7.31 (*d*, *J* = 8.0, H–C(11)); 7.34 (*s*, H–C(14));7.63 (*t*, *J* = 8.1, H–C(10)); 7.77 (*d*, *J* = 8.5, H–C(9)). ^{13}C-NMR: 8.7; 15.7; 27.6; 30.2; 35.4; 36.7; 42.9; 50.5; 61.7; 68.6; 73.5; 99.8; 110.5; 116.1; 122.5; 128.1; 129.2; 129.5; 140.0; 140.1; 145.8; 150.6; 155.4; 156.1; 159.5; 172.3. ESI-MS: 527.85 ([*M* + H]$^+$). Anal. calc. for $C_{26}H_{27}BrN_2O_5$ (526.11): C 59.21, H 5.16, N 5.31; found: C 58.33, H 5.18, N 5.29.

7-Methyl-12-(4-piperidylbutoxy)homocamptothecin (= *5-Ethyl-1,4,5,13-tetrahydro-5-hydroxy-12-methyl-8-[4-(piperidin-1-yl)butoxy]-3*H,*15*H-*oxepino[3′,4′:6,7]indolizino[1,2-*b*]quinoline-3,15-dione*; **11a**). Compound **11a** was prepared from **10b** and piperidine according to the method described for **9a**: 25 mg (50%). Yellow solid. M.p. >250°. ^{1}H-NMR: 0.87 (*t*, *J* = 7.4, Me(18)); 1.63–1.66 (*m*, CH_2(3″), CH_2(4″), CH_2(5″), CH_2(19)); 1.84–1.99 (*m*, CH_2(2′), CH_2(3′)); 2.75 (*s*, Me(7)); 2.89–3.39 (*m*, CH_2(2″), CH_2(6″)); 3.10 (*q*, *J* = 14.0, CH_2(21)); 3.49–3.51 (*m*, CH_2(4′)); 4.30 (*m*, CH_2(1′)); 5.23 (*s*, CH_2(5)); 5.55 (*q*, *J* = 15.1, CH_2(17)); 6.10 (*s*, HO–C(20)); 7.32 (*s*, H–C(14)); 7.33 (*d*, *J* = 8.3, H–C(11)); 7.63 (*t*, *J* = 8.1, H–C(10)); 7.77 (*d*, *J* = 8.3, H–C(9)). ESI-MS: 530.86 ([*M* − H]$^-$). Anal. calc. for $C_{31}H_{37}N_3O_5$ (531.27): C 70.03, H 7.01, N 7.90; found: C 70.16, H 7.03, N 7.88.

12-[3-(Diethylamino)propoxy]-7-methylhomocamptothecin (= *8-(3-Diethylamino)propoxy)-5-ethyl-1,4,5,13-tetrahydro-5-hydroxy-12-methyl-3*H,*15*H-*oxepino[3′,4′:6,7]indolizino[1,2-*b*]quinoline-3,15-dione*; **11b**). Compound **11b** was prepared from **10a** and according to the method described for **9a**: 22 mg (30%). Yellow solid. M.p. >250°. ^{1}H-NMR: 0.87 (*t*, *J* = 7.4, Me(18)); 1.18–1.30 (*m*, 2 *Me*CH_2N); 1.86 (*q*, *J* = 7.4, CH_2(19)); 2.47–2.49 (*m*, CH_2(2′)); 2.75 (*s*, Me(7)); 3.08–3.15 (*m*, 2 MeC*H*$_2$N); 3.38 (*q*, *J* = 13.8, CH_2(21)); 4.29–4.34 (*m*, CH_2(1′), CH_2(3′)); 5.27 (*s*, CH_2(5)); 5.52 (*q*, *J* = 15.3, CH_2(17)); 6.05 (*s*, HO–C(20)); 7.29 (*s*, H–C(14)); 7.33 (*d*, *J* = 8.0, H–C(11)); 7.63 (*t*, *J* = 8.0, H–C(10)); 7.76 (*d*, *J* = 8.3, H–C(9)). ESI-MS: 503.52 ([*M* − H]$^-$). Anal. calc. for $C_{29}H_{35}N_3O_5$ (505.26): C 68.89, H 6.98, N 8.31; found: C 68.97, H 6.99, N 8.29.

Cytotoxicity Assay. The prepared compounds were tested for their *in vitro* cytotoxic activities against three cell lines, *i.e.*, A-549 (human lung adenocarcinoma epithelial cell line), MDA-MB-435 (human breast carcinoma cell line), and HCT-116 (human colon carcinoma cell line), using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT)-based assay. The cells were plated in 96-well plates at 1200 cells/well and incubated at 37° for 24 h. Then, the test compounds were added at

different concentrations into triplicate wells, and 0.1% DMSO was used for the control. After 3 d of incubation at 37°, 20 µl of MTT soln. (5 mg/ml) was added to each well, and after shaking for 1 min the plate was incubated for further 4 h. The formazan crystals were dissolved with 100 µl of DMSO. The absorbance (OD) at 570 nm was quantified with a microplate reader (*WellscanMK-2, Labsystems Co., Ltd.*, Finland). Wells containing no test compounds were used as blanks. The survival of the cells was expressed as percentage relative to untreated control cells.

Inhibition of Topoisomerase I-Mediated DNA Relaxation. Camptothecin (CPT) was obtained from *Tianzunzezhong*, P. R. China, and calf thymus topoisomerase I (Topo I), reaction buffer, bovine serum albumin (BSA), loading buffer, and supercoilded DNA pBR322 were purchased from *TaKaRa Biotechnology Co., Ltd.*, P. R. China. To 16 µl dist. H_2O, 2 µl reaction buffer containing 0.25 µg supercoiled DNA and 0.5 U Topo I, 2 µl 0.1% BSA, and 0.02 µl test compound soln. in 1.5-ml sample tube (final concentration 100 µM) were added. The mixture was incubated at 37° for 15 min. The reactions were stopped by adding SDS (0.5% final concentration), and 3.5 µl of 6 × loading buffer (0.1 mM EDTA, 7% glycerol, 0.01% xylene cyanol FF, and 0.01% bromopenol blue) was added to the mixtures, which were electrophoresed in 0.8% agarose gel in TAE buffer (0.4M TRIS, 0.01M EDTA, and 0.2M AcOH, pH 8.5) for 40 min at 120 V. The gels were stained with ethidium bromide at r.t. and photographed with an UV transilluminator (*Tannon 2500*, *Tianneng Co., Ltd.*, P. R. China).

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