Antitumor Agents. V.[1] Synthesis and Antileukemic Activity of E-Ring-Modified (*RS*)-Camptothecin Analogues

Akio EJIMA,* Hirofumi TERASAWA, Masamichi SUGIMORI, Satoru OHSUKI, Kensuke MATSUMOTO, Yasuyoshi KAWATO, Megumi YASUOKA, and Hiroaki TAGAWA

Exploratory Research Laboratories I, Daiichi Pharmaceutical Co., Ltd., 16–13, Kitakasai 1-chome, Edogawa-ku, Tokyo 134, Japan.
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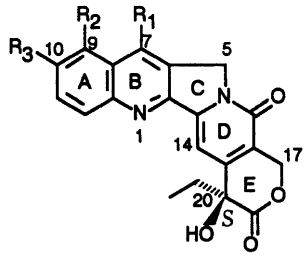
Several E-ring-modified analogues of (*RS*)-camptothecin were synthesized by total synthesis *via* Friedländer condensation and evaluated for cytotoxicity and antitumor activity against P388 mouse leukemia cells. Among them, (*RS*)-20-deoxyamino-7-ethyl-10-methoxycamptothecin (25c) was found to be more active than (*RS*)-camptothecin (1) in the *in vivo* assay.

Keywords camptothecin; aminocamptothecin; Friedländer condensation; antitumor activity

(*S*)-Camptothecin (**1**), an alkaloid originally isolated from *Camptotheca acuminata* (Nyssaceae) by Wall *et al.* in 1966, showed significant antileukemic and antitumor activities.[2] Although unfortunately (*S*)-**1** had a highly toxic effect on both animals and humans,[3] clinical trials of 9-dimethylaminomethyl-10-hydroxycamptothecin (**2**)[4] and a derivative of 7-ethyl-10-hydroxycamptothecin (**3**)[5] as semisynthetic camptothecin analogues have recently commenced in the United States and Japan, respectively (Chart 1). In the process of studies to define structure–activity relationships, it was shown that the hydroxy group at the C-20 position of camptothecin is indispensable for its antitumor activity, since 20-chlorocamptothecin and 20-deoxycamptothecin reduced the antitumor activity.[6] In continuation of our total-synthesis study of natural (*S*)-camptothecin,[7] we have synthesized some camptothecin analogues to investigate further chemotherapeutic possibilities of modifications of the E-ring. This paper describes the synthesis and antileukemic activity of E-ring-modified (*RS*)-camptothecin analogues.

Chemistry The camptothecin analogues were prepared by total synthesis. The indolizine derivatives **4**,[7a] **11**,[7b] and **15**,[8] obtained in the process of the total synthesis of camptothecin, were utilized for the synthesis of E-ring-modified analogues. For the modification of the hydroxy group at the C-20 position, compound **4** was treated with methyl iodide in the presence of sodium hydride in dimethylformamide (DMF) to give compound **5**. Mesylation of **4** in pyridine with methanesulfonyl chloride afforded methanesulfonate **6**, which was treated with *N*-hydroxyphthalimide or NaN_3 to produce **7** or **8**, respectively. Azide **8** was hydrogenated over 10% Pd–C to give **9**, which was deketalized with 80% aqueous trifluoroacetic acid (TFA) to afford **10** as a TFA salt. Furthermore, in order to examine the necessity of the 20-ethyl group of camptothecin for antitumor activity, 20-deethyl or 20-allyl compound was prepared from **11** or **15**, respectively, as follows. Reduction of **11** in the presence of Raney Ni in a mixture of Ac_2O and AcOH gave amide **12**, which was then treated with $NaNO_2$ in the same solvent, and heated in carbon tetrachloride (CCl_4) to afford triester **13**. This triester was treated with 4 N H_2SO_4 to yield **14** by simultaneous cyclization and deketalization. Allylation of **15** with allyl bromide gave **16**, which was treated with $NaNO_2$ in AcOH to afford **17**. This was hydrolyzed and lactonized, producing ketal **18**, which was oxidized with oxygen in the presence of *tert*-BuOK and $(EtO)_3P$, and was deketalized to give tricyclic ketone **20** (Chart 2).



1 : $R_1 = R_2 = R_3 = H$

2 : $R_1 = H$, $R_2 = CH_2NMe_2$, $R_3 = OH \cdot HCl$

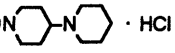
3 : $R_1 = Et$, $R_2 = H$, $R_3 = OCON$  $\cdot HCl$

Chart 1

Construction of a pentacyclic ring system was achieved by Friedländer condensation in the presence of *p*-TsOH in toluene. Thus, compound **7** was deketalized with 80% TFA, and condensed with the protected aldehyde **21**[9] to give **22a**. The deethyl analogue **22b** was obtained from condensation with **21** and **14** in the presence of *p*-TsOH. Compound **22a** was treated with $NH_2NH_2 \cdot H_2O$ to furnish **23**. Miyasaka *et al.* had reported that (*S*)-7-ethyl-10-hydroxycamptothecin ($R_1 = Et$, $R_2 = H$, $R_3 = OH$ in Chart 1), the deprotected compound of **3**, showed stronger antitumor activity than the parent compound **1**.[5b] Then, compound **24**,[10] employed instead of **21**, was condensed with **5**, **8**, **10**, and **20** to give **25a—d**. Compound **25b** was hydrogenated over PtO_2 to give **25c**, whose *Rf* value on thin layer chromatography (TLC) and proton-nuclear magnetic resonance (^1H-NMR) spectrum were identical with those of the compound obtained directly from the condensation of **10** with **24**. Demethylation of **25c** in refluxing hydrobromic acid gave **26**. Demethylation of **25d** to **29** was unsuccessful because of the decomposition of the allyl group of **25d**. Compound **28**, derived from **27**[11] and 2-*tert*-butoxycarbonyloxyimino-2-phenylacetonitrile (BOC-ON), was therefore condensed with **20** in the presence of *p*-TsOH in AcOH. During the condensation, the BOC group was gradually deprotected by *p*-TsOH and AcOH to produce deprotected **29** directly.

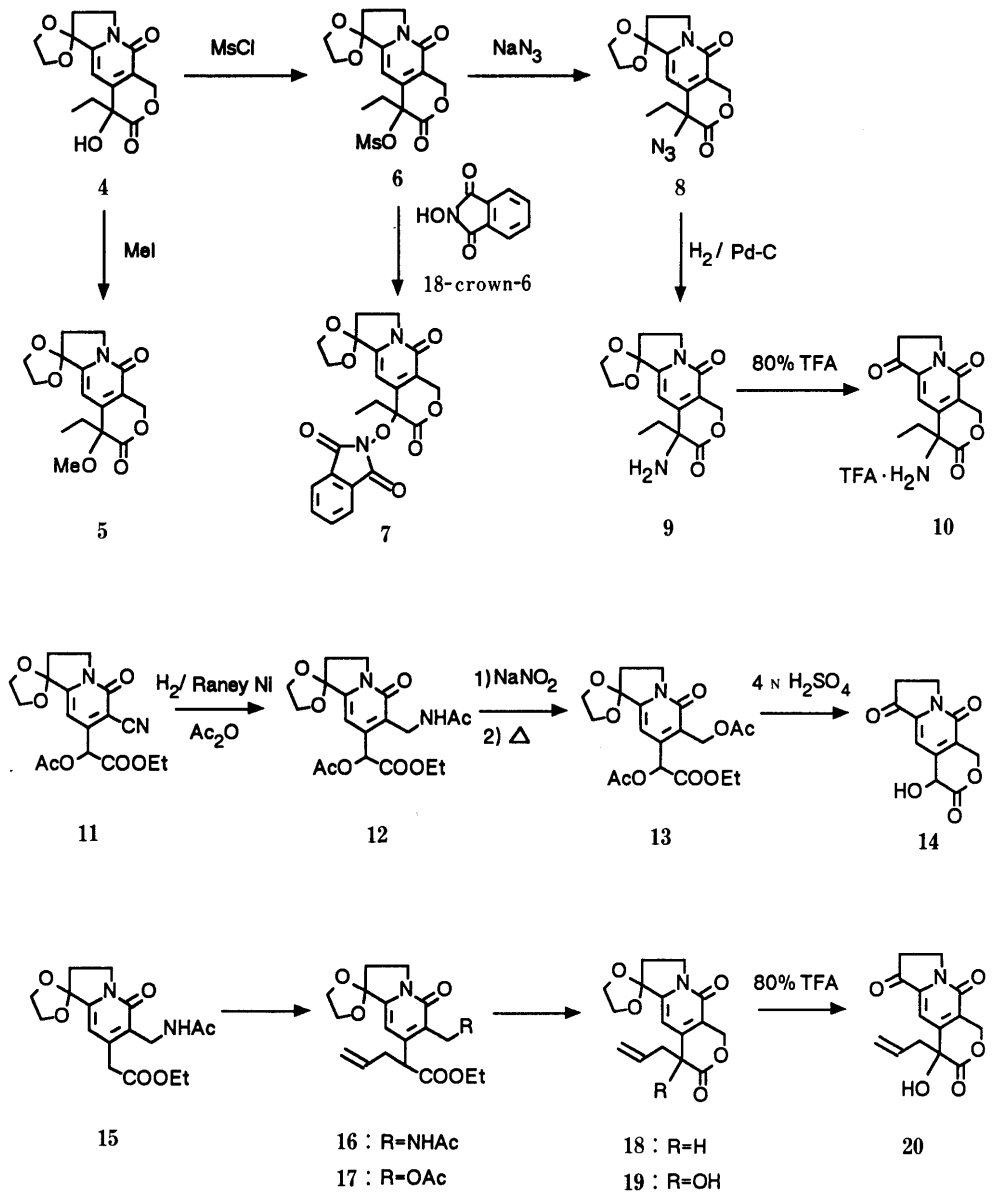


Chart 2

Biological Results and Discussion

The results of the cytotoxicity and antitumor activity of camptothecin analogues against P388 mouse leukemia cells are shown in Table I. For comparison, similar data for (*RS*)-**1**[7b] are presented.

It is interesting that the deethyl compound **22b**, in which the ethyl group at the C-20 position is replaced by hydrogen, was inactive. It appears that bulkiness such as the ethyl group is necessary for cytotoxicity. The aminooxy analogue **23** was about 30 times less active than (*RS*)-**1**. On the other hand, Miyasaka *et al.* reported that a 7-ethyl-10-hydroxy or 7-methyl-10-ethoxy group increases the antitumor activity.[5b] As expected, the cytotoxicity of 7-ethyl-10-methoxy-20-allyl analogue **25d** increased about twice that of (*RS*)-**1** although **29** showed the same degree of activity as (*RS*)-**1**. With regard to the modification of the hydroxy group of the C-20 position, while methoxy **25a** and azide **25b** were inactive, the amino analogues **25c** and **26** retained about one-eighth the cytotoxicity of (*RS*)-**1**.

Compounds **25c—d**, **26**, and **29**, having the cytotoxicity, were tested in the *in vivo* assay. The 7-ethyl-10-hydroxy-20-allyl analogue **29** was toxic at doses of more than 240 mg/kg and was inactive at lower dose levels. However, the 7-ethyl-10-methoxy-20-allyl analogue **25d** was more active at doses of 30—240 mg/kg than (*RS*)-**1**. On the other hand, in spite of its relatively weak cytotoxicity, **25c** exhibited higher activity than (*RS*)-**1** *in vivo*. One out of 6 mice treated with 120 mg of **25c** per kg and 3 out of 6 with 240 mg/kg survived from death by leukemia for more than 40 d. The 7-ethyl-10-hydroxy-20-amino analogue **26** was as active as the allyl analogue **25d**. The maximum tolerated doses (MTD) of **25c** and **26** were 240 mg/kg and more than 480 mg/kg, and their therapeutic ratios (TR) were 14.1 and more than 22.6, respectively, against >3.7 in (*RS*)-**1**.

Thus, it was suggested that the introduction of the amino group at the C-20 position leads to the interesting *in vivo* activity, although the hydroxy group at the C-20 position has been reported to be an absolute requirement[6] for the antitumor activity so far. However, the amino analogues **25c** and **26** were unfortunately not potential chemotherapeutic agents because of their poor water-solubilities. Therefore, further investigations of the relation between

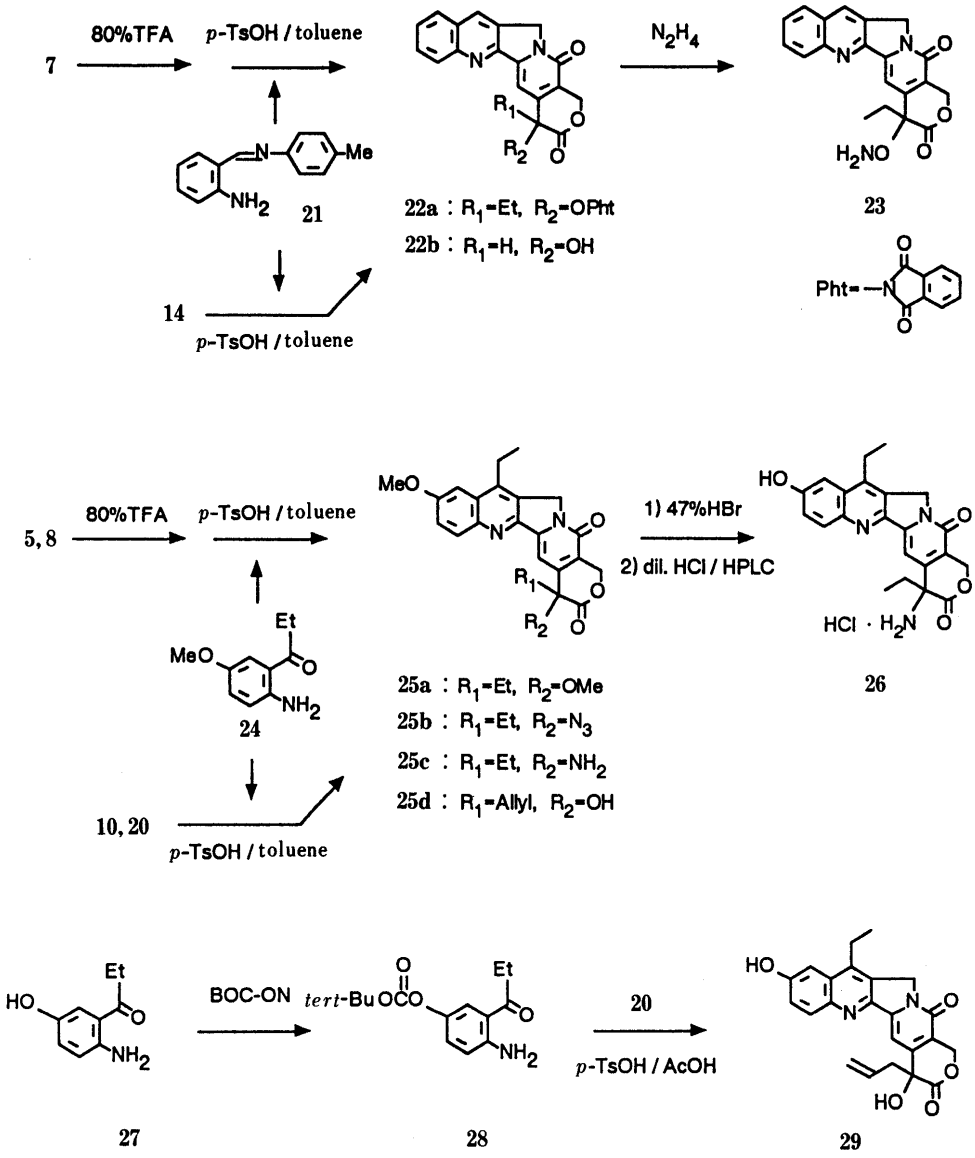


Chart 3

TABLE I. Cytotoxicity and Antitumor Activity of Camptothecin Analogues on P388 Leukemia Cells

Compd.	IC_{50}[b] (nM)	T/C (%)[a] Dose (mg/kg) 480	240	120	60	30	15	TR value[c]
(*RS*)-1[d]	63		163	148	128	128	114	>3.7
22b	>3125			(Not tested)				—
23	1802			(Not tested)				—
25a	>714			(Not tested)				—
25b	>696			(Not tested)				—
25c[e]	432	115	>402(3/6)	270(1/6)	210	166		14.1
25d[d]	26		189	172	151	135		>9.6
26[e]	504	186	179	153	151(1/6)			>22.6
29[d]	62	38.2	38.2	115	111	122		—

a) P388 cells (10^6) were transplanted intraperitoneally (i.p.) into CDF_1 mice on day 0 and the compounds were administered i.p. on day 1. *b*) Concentration inducing 50% inhibition of P388 cell growth. *c*) TR value = MTD/ILS_{30} (MTD: maximum tolerated dose, ILS_{30}: amount required to give a T/C of 130). *d*) Injected as an aqueous solution of the sodium salt. *e*) Injected as a suspension in H_2O containing 0.9% NaCl, 0.9% benzyl alcohol, 0.4% Tween 80 and 0.5% carboxymethyl cellulose.

modifications of camptothecin and its antitumor activity are in progress. The synthesis and antitumor activity of these analogues will be discussed in a forthcoming publication.

Experimental

Melting points were determined on a Yanagimoto apparatus and are uncorrected. Infrared (IR) spectra were recorded on a Hitachi 260-30 or a 270-30 spectrophotometer. ^{1}H-NMR spectra were recorded on a Hitachi R-40 or a JEOL JNM-FX90Q (90 MHz) instrument. Coupling constants are reported in hertz and chemical shifts in ppm (δ units) downfield from internal tetramethylsilane. Mass spectra (MS) were recorded on a JEOL JMS-01SG-2 or a JMS-D300 mass spectrometer. Elemental analyses were performed by a Heraeus instrument. All solvents and reagents were of commercial grade and were used without further purification. DMF and toluene were dried over molecular sieves (4 Å) (Wako Chemicals). Column chromatography was performed with Silica gel 60 F_{254} (70—230 mesh) (Merck). Sodium sulfate was employed as a drying agent.

4-Ethyl-6,6-ethylenedioxy-7,8-dihydro-4-methoxy-1*H*-pyrano[3,4-*f*]-indolizine-3,10(4*H*)-dione (5) Sodium hydride (44 mg, 1.0 mmol, 60% in oil) was added to a solution of 4[7a] (307 mg, 1.0 mmol) in DMF (3 ml) and the resulting mixture was stirred at room temperature for 10 min. Methyl iodide (0.3 ml) was added and the mixture was stirred at room temperature for 3 h. After the solvent was removed *in vacuo*, the residue was diluted with $CHCl_3$ (100 ml), and the organic layer was washed with H_2O, dried, and evaporated *in vacuo*. The residue was chromatographed on silica gel ($CHCl_3$–MeOH, 100:1) to give **5** (240 mg, 74.8%) as crystals, mp 153—157 °C ($CHCl_3$–Et_2O). IR (KBr): 1746, 1668, 1620 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.91 (3H, t, J=7 Hz, CH_3), 1.6—2.6 (4H, m, CH_2, H-7), 3.25 (3H, s, CH_3), 3.9—4.3 (6H, m, ketal, H-8), 5.15, 5.49 (2H, ABq, J=17 Hz,

H-1), 6.36 (1H, s, H-5). MS *m/z*: 321 (M^+). *Anal.* Calcd for $C_{16}H_{19}NO_6 \cdot 1/4H_2O$: C, 58.98; H, 6.03; N, 4.30. Found: C, 58.94; H, 5.88; N, 4.39.

4-Ethyl-6,6-ethylenedioxy-7,8-dihydro-4-methanesulfonyloxy-1*H*-pyrano[3,4-*f*]indolizine-3,10(4*H*)-dione (6) Methanesulfonyl chloride (0.30 ml, 3.89 mmol) was added to a solution of **4** (307 mg, 1.0 mmol) in pyridine (3 ml) at 0 °C. After being stirred for 2 h, the solution was diluted with $CHCl_3$ (100 ml) and washed with 10% aqueous citric acid, 5% aqueous $NaHCO_3$ and brine. After being dried and evaporated *in vacuo*, the residue was chromatographed on silica gel ($CHCl_3$–MeOH, 50:1) to give **6** (150 mg, 38.9%) as a crystalline powder, mp 148—152 °C (dec.) (Et_2O–hexane). IR (KBr): 1746, 1671, 1614 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.96 (3H, t, J=7 Hz, CH_3), 1.7—2.6 (4H, m, CH_2, H-7), 3.30 (3H, s, SCH_3), 3.9—4.5 (6H, m, ketal, H-8), 5.22, 5.54 (2H, ABq, J=17 Hz, H-1), 6.55 (1H, s, H-5). MS *m/z*: 385 (M^+). *Anal.* Calcd for $C_{16}H_{19}NO_8S \cdot 1/2H_2O$: C, 48.73; H, 5.11; N, 3.55. Found: C, 48.45; H, 4.67; N, 3.56.

4-Ethyl-6,6-ethylenedioxy-7,8-dihydro-4-phthalimidoxy-1*H*-pyrano-[3,4-*f*]indolizine-3,10(4*H*)-dione (7) Sodium hydride (56 mg, 1.4 mmol, 60% in oil) was added to a solution of *N*-hydroxyphthalimide (424 mg, 2.6 mmol) and 18-crown-6 (300 mg) in DMF (6 ml) and the resulting mixture was stirred at room temperature for 10 min. To the mixture were added compound **6** (503 mg, 1.31 mmol) and NaI (194 mg, 1.4 mmol), and the solution was stirred at 60 °C for 15 h. After the solvent was removed *in vacuo*, the residue was dissolved in $CHCl_3$ (100 ml), and the organic layer was washed with 5% aqueous $NaHCO_3$ and H_2O, dried, and evaporated *in vacuo*. The residue was chromatographed on silica gel ($CHCl_3$–MeOH, 50:1) to afford **7** (360 mg, 60.7%) as an oily substance. ^{1}H-NMR ($CDCl_3$) δ: 0.83 (3H, t, J=7 Hz, CH_3), 1.6—2.8 (2H, m, CH_2), 2.39 (2H, t, J=7 Hz, H-7), 3.7—4.4 (6H, m, ketal, H-8), 5.01, 5.57 (2H, ABq, J=16 Hz, H-1), 6.57 (1H, s, H-5), 7.76 (4H, s, C_6H_4). MS *m/z*: 452 (M^+).

4-Azido-4-ethyl-6,6-ethylenedioxy-7,8-dihydro-1*H*-pyrano[3,4-*f*]indolizine-3,10-(4*H*)-dione (8) Sodium azide (24 mg, 0.37 mmol) was added to a solution of **6** (130 mg, 0.34 mmol) in DMF (1.3 ml) and the resulting mixture was stirred at 60 °C for 3 h. After the reaction mixture was diluted with $CHCl_3$ (100 ml), the organic layer was washed, dried, and evaporated *in vacuo* to give **8** (113 mg, 100%) as an oil. IR (neat, film): 2120, 1746, 1666 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.97 (3H, t, J=7 Hz, CH_3), 2.06 (2H, q, J=7 Hz, CH_2), 2.42 (2H, t, J=7 Hz, H-7), 4.0—4.5 (6H, m, ketal, H-8), 5.19, 5.55 (2H, ABq, J=17 Hz, H-1), 6.42 (1H, s, H-5).

4-Amino-4-ethyl-6,6-ethylenedioxy-7,8-dihydro-1*H*-pyrano[3,4-*f*]-indolizine-3,10(4*H*)-dione (9) A solution of **8** (390 mg) in a mixture of EtOH (20 ml) and dioxane (20 ml) was hydrogenated in the presence of 10% Pd–C (150 mg) for 3 h. After the catalyst was removed by filtration, the solvent was removed *in vacuo* and the residue was chromatographed on silica gel ($CHCl_3$–MeOH, 30:1) to afford **9** (190 mg, 52.7%) as a colorless powder, mp 165—175 °C ($CHCl_3$–Et_2O). IR (KBr): 1737, 1668, 1608 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.92 (3H, t, J=7 Hz, CH_3), 1.6—2.6 (4H, m, CH_2, H-7), 3.9—4.2 (6H, m, ketal, H-8), 5.18, 5.53 (2H, ABq, J=17 Hz, H-1), 6.70 (1H, s, H-5). *Anal.* Calcd for $C_{15}H_{18}N_2O_5S \cdot 1/2H_2O$: C, 57.14; H, 6.07; N, 8.88. Found: C, 57.23; H, 5.85; N, 8.69.

4-Amino-4-ethyl-7,8-dihydro-1*H*-pyrano[3,4-*f*]indolizine-3,6,10(4*H*)-trione·CF_3CO_2H (10) A solution of **9** (300 mg, 0.98 mmol) in 80% aqueous TFA (9.8 ml, 102 mmol) was stirred at room temperature for 1 h in a nitrogen atmosphere. After the solvent was removed *in vacuo*, the residue was diluted with Et_2O (30 ml). Then, the precipitates were collected by filtration, and washed with Et_2O to give **10** (370 mg, 100%) as a colorless powder, mp 160—170 °C (dec.) ($CHCl_3$–Et_2O). IR (KBr): 1752, 1662, 1584 cm^{-1}. ^{1}H-NMR ($CDCl_3$+CD_3OD) δ: 1.01 (3H, t, J=7 Hz, CH_3), 1.9—2.4 (2H, m, CH_3), 3.00 (2H, t, J=7 Hz, H-7), 5.37, 5.65 (2H, ABq, J=17 Hz, H-1), 7.17 (1H, s, H-5). MS *m/z*: 262 (M^+).

Ethyl α-Acetoxy-6-acetylaminomethyl-1,1-ethylenedioxy-5-oxo-1,2,3,5-tetrahydroindolizine-7-acetate (12) A solution of **11**[7b] (1.95 g, 5.39 mmol) in a mixture of AcOH (20 ml) and Ac_2O (50 ml, 529 mmol) was hydrogenated in the presence of Raney Ni (6 ml; prewashed with AcOH) at atmospheric pressure during 3 h. The catalyst was removed by filtration, and the solvent was removed *in vacuo* to give a crude oil, which was chromatographed on silica gel ($CHCl_3$–MeOH, 30:1) to afford **12** (1.96 g, 89.2%) as an oily substance. IR (KBr): 1745, 1650, 1582 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.23 (3H, t, J=7 Hz, CH_3), 1.92 (3H, s, Ac), 2.20 (3H, s, Ac), 2.39 (2H, t, J=7 Hz, H-2), 3.9—5.5 (10H, m, ketal, H-3, CH_2×2), 6.45 (1H, s, CHCO), 6.6—7.1 (1H, br, NH), 6.73 (1H, s, H-8). MS *m/z*: 408 (M^+). *Anal.* Calcd for $C_{19}H_{24}N_2O_8 \cdot AcOH \cdot 1/2H_2O$: C, 52.83; H, 6.12; N, 5.87. Found: C, 52.65; H, 5.87; N, 6.24.

Ethyl α-Acetoxy-6-acetoxymethyl-1,1-ethylenedioxy-5-oxo-1,2,3,5-tetrahydroindolizine-7-acetate (13) $NaNO_2$ (1.66 g, 24 mmol) was added to a solution of **12** (1.96 g, 4.81 mmol) in a mixture of Ac_2O (30 ml, 318 mmol) and AcOH (9 ml) at 0 °C and the reaction mixture was stirred for 4 h. The inorganic salt was removed by filtration and the solvent was evaporated *in vacuo* at room temperature to afford a colorless oil. Carbon tetrachloride (120 ml) was added to this oil and the solution was heated under reflux for 5 h. The organic layer was washed, dried, and evaporated *in vacuo* to give an oil, which was chromatographed on silica gel (benzene–EtOAc, 3:1) to afford **13** (1.71 g, 86.6%) as colorless crystals, mp 117—118 °C ($CHCl_3$–Et_2O). IR (KBr): 1730, 1650, 1600 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.23 (3H, t, J=7 Hz, CH_3), 2.05 (3H, s, Ac), 2.20 (3H, s, Ac), 2.38 (2H, t, J=7 Hz, H-2), 4.0—5.6 (10H, m, ketal, H-3, CH_2×2), 6.34 (1H, s, CHCO or H-8), 6.37 (1H, s, H-8 or CHCO). MS *m/z*: 409 (M^+). *Anal.* Calcd for $C_{19}H_{23}NO_9$: C, 55.74; H, 5.66; N, 3.42. Found: C, 55.53; H, 5.54; N, 3.50.

7,8-Dihydro-4-hydroxy-1*H*-pyrano[3,4-*f*]indolizine-3,6,10(4*H*)-trione (14) Compound **13** (1.31 g, 2.98 mmol) was dissolved in dimethoxyethane (6.6 ml) and 4 N H_2SO_4 (9.8 ml, 19.6 mmol), and the solution was stirred at 60 °C for 4 h. Brine (10 ml) was added, and the mixture was then extracted with CH_2Cl_2. The organic layer was dried, and evaporated *in vacuo* to give **14** (350 mg, 50.0%) as a colorless powder, mp 136—139 °C (dec.) ($CHCl_3$–Et_2O). ^{1}H-NMR ($CDCl_3$) δ: 2.98 (2H, t, J=7 Hz, H-7), 4.35 (2H, t, J=7 Hz, H-8), 4.9—5.9 (2H, m, H-1), 7.28 (1H, s, H-5). MS *m/z*: 235 (M^+).

Ethyl α-Allyl-6-acetylaminomethyl-1,1-ethylenedioxy-5-oxo-1,2,3,5-tetrahydroindolizine-7-acetate (16) Sodium hydride (263 mg, 6.58 mmol, 60% in oil) was added to a solution of **15**[8] (2.0 g, 5.71 mmol) in DMF (20 ml) and the resulting mixture was stirred at 0 °C for 20 min. After being cooled at −40 °C, allyl bromide (2.07 g, 17.1 mmol) was added. After 1.5 h, saturated NH_4Cl solution (4 ml), H_2O (50 ml), and CH_2Cl_2 (100 ml) were added. The separated organic layer was washed, dried, and evaporated *in vacuo* to give an oil, which was chromatographed on silica gel ($CHCl_3$–MeOH, 100:1) to afford **16** (1.76 g, 79.0%) as a yellow solid, mp 131—132 °C (Et_2O–hexane). IR (KBr): 3064, 1732, 1658 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.21 (3H, t, J=7 Hz, CH_3), 1.92 (3H, s, Ac), 2.38 (2H, t, J=7 Hz, H-2), 2.1—3.0 (2H, m, CH_2), 3.9—4.5 (10H, m, ketal, H-3, CH_2×2), 4.62 (1H, dd, J=7, 14 Hz, CHCO), 4.9—5.3 (2H, m, CH_2), 5.5—5.9 (1H, m, CH), 6.32 (1H, s, H-8), 6.6—6.7 (1H, br, NH). MS *m/z*: 390 (M^+). *Anal.* Calcd for $C_{20}H_{26}N_2O_6$: C, 61.53; H, 6.71; N, 7.17. Found: C, 61.55; H, 6.89; N, 7.18.

Ethyl α-Allyl-6-acetoxymethyl-1,1-ethylenedioxy-5-oxo-1,2,3,5-tetrahydroindolizine-7-acetate (17) In the same manner as for the preparation of **13** from **12**, the title compound was obtained from **16** in a quantitative yield as an oil. IR (film): 1738, 1662, 1610 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.21 (3H, t, J=9 Hz, CH_3), 2.05 (3H, s, Ac), 2.37 (2H, t, J=7 Hz, H-2), 2.1—3.0 (2H, m, CH_2), 3.8—4.4 (9H, m, ketal, H-3, CH_2, CHCO), 5.0—5.3 (2H, m, CH_2), 5.24 (2H, s, CH_2O), 5.5—5.9 (1H, m, CH), 6.33 (1H, s, H-8). MS *m/z*: 391 (M^+).

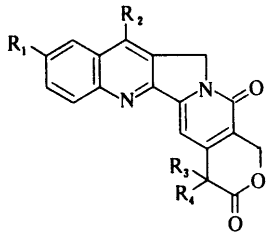
4-Allyl-6,6-ethylenedioxy-7,8-dihydro-1*H*-pyrano[3,4-*f*]indolizine-3,10(4*H*)-dione (18) To a solution of **17** (1.37 g, 3.51 mmol) in a mixture of MeOH (30 ml) and H_2O (10 ml) was added $LiOH \cdot H_2O$ (760 mg, 18 mmol), and the solution was stirred at room temperature for 2 h. Most of the MeOH was removed, and to the residue was added cold H_2O (20 ml) and AcOH (3 ml). After 22 h of stirring at room temperature, the organic material was extracted with CH_2Cl_2, and the organic layer was washed, dried, and removed *in vacuo* to give **18** as a powder (763 mg, 71.7%), mp 96.5—97.5 °C ($CHCl_3$–Et_2O). IR (KBr): 1730, 1660, 1600 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 2.40 (2H, t, J=7 Hz, H-2), 2.72 (2H, dd, J=6.1, 6.4 Hz, CH_2), 3.57 (1H, t, J=6.1 Hz, CHCO), 4.0—4.2 (6H, m, ketal, H-3), 5.0—5.4 (4H, m, H-1, CH_2), 5.5—6.0 (1H, m, CH), 6.14 (1H, s, H-5). MS *m/z*: 303 (M^+). *Anal.* Calcd for $C_{16}H_{17}NO_5$: C, 63.36; H, 5.65; N, 4.62. Found: C, 63.31; H, 5.49; N, 4.71.

4-Allyl-6,6-ethylenedioxy-7,8-dihydro-4-hydroxy-1*H*-pyrano[3,4-*f*]-indolizine-3,10(4*H*)-dione (19) Triethyl phosphite (0.4 ml) was added to a solution of **18** (200 mg, 0.66 mmol) in DMF (15 ml) containing *tert*-BuOK (111 mg, 0.99 mmol) at −40 °C and dry oxygen was bubbled through the solution at the same temperature for 1.5 h. After concentrated HCl (0.15 ml) was added, the solution was stirred for 10 min and poured into CH_2Cl_2 (50 ml). The organic layer was separated, washed, dried, and removed *in vacuo* to give **19** (190 mg, 90.4%) as colorless needles, mp 163.5—164.5 °C (EtOAc). IR (KBr): 1740, 1640, 1580 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 2.41 (2H, t, J=7 Hz, H-2), 2.51 (2H, d, J=7 Hz, CH_2), 2.80 (1H, br s, OH), 4.0—4.3 (6H, m, ketal, H-3), 5.0—6.0 (3H, m, CH_2=CH), 5.20, 5.62 (2H, ABq, J=16 Hz, H-1), 6.56 (1H, s, H-5). MS *m/z*: 319 (M^+). *Anal.* Calcd

for $C_{16}H_{17}NO_6$: C, 60.18; H, 5.37; N, 4.39. Found: C, 60.23; H, 5.39; N, 4.35.

4-Allyl-7,8-dihydro-4-hydroxy-1*H*-pyrano[3,4-*f*]indolizine-3,6,10(4*H*)-trione (20) A solution of **19** (105 mg, 0.33 mmol) in 80% aqueous TFA (3.0 ml, 31.2 mmol) was stirred at room temperature for 1 h in a nitrogen atmosphere. After the solvent was removed *in vacuo*, the residue was diluted with $CHCl_3$ (50 ml). The organic layer was then washed, dried, and removed *in vacuo* to afford **20** (72 mg, 79.3%) as colorless needles, mp 175.5—177 °C (EtOAc–hexane). IR (KBr): 1752, 1737, 1652, 1592 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 2.53 (2H, d, J=7 Hz, CH_2), 2.95 (2H, t, J=7 Hz, H-7), 3.76 (1H, s, OH), 4.34 (2H, t, J=7 Hz, H-8), 4.9—6.0 (3H, m, CH_2=CH), 5.26, 5.66 (2H, ABq, J=17 Hz, H-1), 7.20 (1H, s, H-5). *Anal.* Calcd for $C_{14}H_{13}NO_5$: C, 61.09; H, 4.76; N, 5.09. Found: C, 61.15; H, 4.63; N, 5.14.

TABLE II. Physicochemical Data for Camptothecin Analogues



Compd.	R_1	R_2	R_3	R_4	Starting materials	Yield (%)	mp (°C)	Formula	FD-MS (M^+)	Analysis (%) Calcd (Found)		
										C	H	N
22a	H	H	Et	OPht	**7**+**21**	31	225—235 (dec.)	$C_{28}H_{19}N_3O_6 \cdot 5/4H_2O$	493	65.18 (64.98)	4.20 (4.06)	8.14 (7.81)
22b	H	H	H	OH	**14**+**21**	12	225—230 (dec.)	$C_{18}H_{12}N_2O_4 \cdot H_2O$	320	63.90 (64.12)	4.17 (3.91)	8.28 (8.33)
23	H	H	Et	ONH_2	**22a**	30	255—265 (dec.)	$C_{20}H_{17}N_3O_4 \cdot 3/2H_2O$	363	61.53 (61.31)	5.16 (4.64)	10.76 (10.32)
25a	OMe	Et	Et	OMe	**5**+**24**	74	>300	$C_{24}H_{24}N_2O_5 \cdot 1/2H_2O$	420	67.12 (66.90)	5.87 (5.71)	6.52 (6.39)
25b	OMe	Et	Et	N_3	**8**+**24**	63	230—245 (dec.)	$C_{23}H_{21}N_5O_4 \cdot 1/4H_2O$	431	63.37 (63.07)	4.97 (4.90)	16.07 (15.78)
25c	OMe	Et	Et	NH_2	**10**+**24** **25b**	47 45	285—300 (dec.)	$C_{23}H_{23}N_3O_4 \cdot 3/4H_2O$	405	65.94 (65.81)	5.89 (5.56)	10.03 (10.01)
25d	OMe	Et	Allyl	OH	**20**+**24**	65	270—280 (dec.)	$C_{24}H_{22}N_2O_5 \cdot 1/2H_2O$	418	67.28 (67.44)	5.29 (5.42)	6.36 (6.55)
26	OH	Et	Et	$NH_2 \cdot HCl$	**18d**	45	200—220 (dec.)	$C_{22}H_{21}N_3O_4 \cdot HCl \cdot 11/4H_2O$	391	55.46 (55.57)	5.82 (5.71)	8.82 (8.42)
29	OH	Et	Allyl	OH	**20**+**27**	78	250—260 (dec.)	$C_{23}H_{20}N_2O_5 \cdot 2/3H_2O$	404	66.17 (66.33)	4.96 (5.16)	6.53 (6.73)

TABLE III. Spectral Data for Camptothecin Analogues

Compd.	IR	^{1}H-NMR	
	KBr, cm^{-1}	Solvent	δ: ppm
22a	1743, 1656, 1608	$CDCl_3$	0.96 (3H, t, J=7 Hz, $CH_2C\underline{H}_3$), 2.0—3.0 (2H, m, $C\underline{H}_2CH_3$), 5.40 (2H, s, H-5), 5.72 (2H, s, H-17), 7.4—8.7 (6H, m, Ar$\underline{H}$), 7.89 (4H, s, Ph)
22b	1756, 1662, 1598	DMSO-d_6	4.9—5.8 (5H, m, H-5, H-17, H-20), 7.38 (1H, s, H-14), 7.5—8.7 (5H, m, Ar$\underline{H}$)
23	1746, 1656, 1602	$CDCl_3$+CD_3OD[a]	0.98 (3H, t, J=8 Hz, $CH_2C\underline{H}_3$), 1.8—2.3 (2H, m, $C\underline{H}_2CH_3$), 5.33 (2H, s, H-5), 5.31, 5.67 (2H, ABq, J=18 Hz, H-17), 7.5—8.6 (6H, m, Ar$\underline{H}$)
25a	1755, 1665, 1608	$CDCl_3$+CD_3OD[a]	0.98 (3H, t, J=7 Hz, $CH_2C\underline{H}_3$), 1.44 (3H, t, J=8 Hz, $CH_2C\underline{H}_3$), 1.6—2.3 (2H, m, $C\underline{H}_2CH_3$), 3.0—3.4 (2H, m, $C\underline{H}_2CH_3$), 3.36 (3H, s, OMe), 4.03 (3H, s, OMe), 5.29 (2H, s, H-5), 5.32, 5.63 (2H, ABq, J=17 Hz, H-17), 7.3—8.2 (4H, m, Ar$\underline{H}$)
25b	2122, 1752, 1662	$CDCl_3$+CD_3OD[a]	1.03 (3H, t, J=7 Hz, $CH_2C\underline{H}_3$), 1.42 (3H, t, J=8 Hz, $CH_2C\underline{H}_3$), 2.0—2.4 (2H, m, $C\underline{H}_2CH_3$), 2.9—3.5 (2H, m, $C\underline{H}_2CH_3$), 4.02 (3H, s, OMe), 5.26 (2H, s, H-5), 5.35, 5.65 (2H, ABq, J=18 Hz, H-17), 7.3—8.2 (4H, m, Ar$\underline{H}$)
25c	1730, 1662, 1602	$CDCl_3$+CD_3OD[a]	1.00 (3H, t, J=7 Hz, $CH_2C\underline{H}_3$), 1.44 (3H, t, J=8 Hz, $CH_2C\underline{H}_3$), 1.8—2.2 (2H, m, $C\underline{H}_2CH_3$), 3.0—3.6 (2H, m, $C\underline{H}_2CH_3$), 4.03 (3H, s, OMe), 5.28 (2H, s, H-5), 5.37 5.67 (2H, ABq, J=18 Hz, H-17), 7.3—8.2 (4H, m, Ar$\underline{H}$)
25d	1740, 1650, 1580	$CDCl_3$	1.41 (3H, t, J=8 Hz, $CH_2C\underline{H}_3$), 2.61 (2H, d, J=7 Hz, $C\underline{H}_2CH$=CH_2), 3.16 (2H, q, J=8 Hz, $C\underline{H}_2CH_3$), 4.00 (3H, s, OCH_3), 5.0—5.4 (2H, m, CH_2CH=$C\underline{H}_2$), 5.23 (2H, s, H-5), 5.34, 5.75 (2H, ABq, J=16 Hz, H-17), 7.2—8.2 (4H, m, Ar$\underline{H}$)
26	1735, 1655, 1600	D_2O	1.16 (3H, t, J=7 Hz, $CH_2C\underline{H}_3$ × 2), 2.1—3.0 (4H, m, $C\underline{H}_2CH_3$ × 2), 4.4—4.9 (2H, br s, H-5), 5.4—6.0 (2H, br s, H-17), 6.8—7.8 (4H, m, Ar$\underline{H}$)
29	1752, 1652, 1572	DMSO-d_6	1.32 (3H, t, J=8 Hz, $CH_2C\underline{H}_3$), 2.63 (2H, d, J=8 Hz, $C\underline{H}_2CH$=CH_2), 3.09 (2H, q, J=8 Hz, $C\underline{H}_2CH_3$), 5.26 (2H, s, H-5), 4.9—5.3 (2H, m, CH_2CH=$C\underline{H}_2$), 5.43 (2H, s, H-17), 5.5—6.1 (1H, m, $CH_2C\underline{H}$=CH_2), 7.2—8.2 (4H, m, Ar$\underline{H}$)

a) $CDCl_3$:CD_3OD=4:1.

20-*O*-Phthalimidocamptothecin (22a) A solution of **7** (450 mg, 1.0 mmol) in 80% aqueous TFA (5.0 ml, 51.9 mmol) was stirred at room temperature for 1 h in a nitrogen atmosphere. After the solvent was removed *in vacuo*, the residue was diluted with $CHCl_3$ (100 ml). The organic layer was then washed, dried, and evaporated *in vacuo* to give the corresponding deketalized tricyclic ketone. To this tricyclic ketone were added amino compound **21**[9] (240 mg, 1.0 mmol) and toluene (10 ml), and the resulting mixture was heated to reflux in a nitrogen atmosphere. After 0.5 h, *p*-TsOH·H_2O (10 mg) was added and the reaction mixture was heated under reflux for another 3 h using a Dean–Stark trap. The precipitate obtained after cooling was filtered and chromatographed on silica gel ($CHCl_3$–MeOH, 50:1) to afford **22a** as a colorless solid.

The yields, melting points, field desorption (FD)-mass and elemental analytical data of **22a** and the other camptothecin analogues described below are listed in Table II; IR and ^{1}H-NMR data listed in Table III.

20-Deethylcamptothecin (22b) *p*-TsOH·H_2O (10 mg) was added to a solution of **21** (240 mg, 1.0 mmol) and **14** (275 mg, 1.0 mmol) in toluene (10 ml), and the resulting mixture was heated under reflux for 4 h using a Dean–Stark trap. The precipitate obtained after cooling was filtered and chromatographed on silica gel ($CHCl_3$–MeOH, 50:1) to afford **22b** as a colorless solid.

20-*O*-Aminocamptothecin (23) $NH_2NH_2 \cdot H_2O$ (6 mg, 0.12 mmol) was added to a solution of **22a** (55 mg, 0.11 mmol) in a mixture of EtOH (3 ml) and $CHCl_3$ (1 ml), and the solution was stirred at room temperature for 3 h. After evaporation of the solvent, the residue was chromatographed on silica gel ($CHCl_3$–MeOH, 30:1) to afford **23** (12 mg) as a solid.

20-*O*-Methyl-7-ethyl-10-methoxycamptothecin (25a) and 20-Deoxyazido-7-ethyl-10-methoxycamptothecin (25b) In the same manner as for the preparation of **22a**, compounds **5** and **8** were deketalized, and condensed with **24**[10] to afford **25a** and **25b**, respectively.

20-Deoxyamino-7-ethyl-10-methoxycamptothecin (25c) and 20-Deethyl-allyl-7-ethyl-10-methoxycamptothecin (25d) In the same manner as for the preparation of **22b**, compounds **10** and **20** were condensed with **24** to give **25c** and **25d**, respectively.

20-Deoxyamino-7-ethyl-10-methoxycamptothecin (25c) from 25b A solution of **25b** (430 mg) in a mixture of EtOH (100 ml) and dioxane (100 ml) was hydrogenated in the presence of $PtO_2 \cdot H_2O$ (150 mg) for 6 h. During the reaction, the flask was irradiated with a 100 W tungsten lamp. After the catalyst was removed by filtration, the solvent was concentrated *in vacuo* and the residue was chromatographed on silica gel ($CHCl_3$–MeOH, 30:1) to afford **25c** (190 mg, 46.9%), whose *Rf* value on TLC and ^{1}H-NMR spectrum were identical with those of the compound obtained from **10** and **24**.

20-Deoxyamino-7-ethyl-10-hydroxycamptothecin·HCl (26) A solution of **25c** (81 mg) in 47% aqueous HBr solution (2.5 ml) was heated under reflux for 3 h. After the solvent was removed *in vacuo*, the residue was diluted with $CHCl_3$–Et_2O. The precipitates were collected by filtration and subjected to reversed-phase high performance liquid chromatography (HPLC) using Develosil ODS-5 (Nomura Kagaku Co., Ltd.), and developed with a mixture of MeOH–H_2O (2:3) adjusted to pH 3 with dilute HCl to afford **26** (38 mg) as a solid.

2′-Amino-5′-*tert*-butoxycarbonyloxypropiophenone (28) To a solution of 2′-amino-5′-hydroxypropiophenone **27**[11] (1.0 g, 6.1 mmol) in a mixture of dioxane (26 ml), H_2O (10 ml) and Et_3N (6.2 ml, 44.8 mmol) was added BOC-ON (14.7 g, 60.5 mmol), and the resulting mixture was stirred at room temperature for 24 h. The solution was diluted with CH_2Cl_2 (100 ml), and the organic layer was washed with 10% aqueous citric acid, 5% aqueous $NaHCO_3$ and H_2O. After being dried, the solution was removed *in vacuo* and the residue was chromatographed on silica gel (benzene–$CHCl_3$, 2:1) to give **28** (896 mg, 55.9%) as yellow needles, mp 84.5—85 °C (Et_2O–hexane). IR (KBr): 3360, 1748, 1656 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.19 (3H, t, J=7 Hz, CH_3), 1.56 (9H, s, BOC), 2.94 (2H, q, J=7 Hz, CH_2), 6.10 (1H, br s, NH), 6.63 (1H, d, J=9 Hz, H-3), 7.11 (1H, dd, J=3, 9 Hz, H-4), 7.52 (1H, d, J=3 Hz, H-6). MS (fast atom bombardment) *m/z*: 266 ($M^+ + 1$). *Anal.* Calcd for $C_{14}H_{19}NO_4$: C, 63.38; H, 7.22; N, 5.28. Found: C, 63.37; H, 7.02; N, 5.41.

20-Deethylallyl-7-ethyl-10-hydroxycamptothecin (29) The compound **28** (558 mg, 2.10 mmol) was added to a solution of **20** (280 mg, 1.0 mmol) and AcOH (6 ml), and the mixture was warmed at 60 °C in a nitrogen atmosphere. After 0.5 h, *p*-TsOH·H_2O (30 mg) was added and the reaction mixture was stirred at 60 °C for 15 h. To this mixture was added H_2O (2 ml) and the resulting mixture was stirred at 80 °C for 5 h. After H_2O (40 ml) was added, the precipitates were collected by filtration and washed with H_2O and Et_2O to afford a pale brown solid, which was recrystallized from $CHCl_3$–MeOH to give **29** (315 mg).

Antileukemic Activity For the cytotoxicitic assay, P388 mouse leukemia cells (2×10^4) were cultured with each test compound at 37 °C for 72 h in RPMI 1640 medium containing 10% fetal calf serum and 60 μg/ml kanamycin in a humidified atmosphere of 5% CO_2 in air, and were counted with a Model ZBI Coulter counter. In the control cultures, P388 cells grew exponentially during the incubation period. IC_{50} was determined by plotting the logarithm of the drug concentration *vs.* the growth rate (percentage of control) of the treated cells.

For the *in vivo* assay, P388 cells (10^6) were transplanted intraperitoneally (i.p.) into CDF_1 mice (six mice per group) on day 0 and the compounds were administered i.p. on day 1. Antitumor activity (T/C) was calculated according to the following formula: T/C = MST of the treated group/MST of control group × 100 (%), where MST represents the median survival time. T/C 130% was adopted for the criteria of therapeutic efficacy and those surviving longer than 40 d were considered to be cured.

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