Chemical Modification of an Antitumor Alkaloid, 20(*S*)-Camptothecin: E-Lactone Ring-Modified Water-Soluble Derivatives of 7-Ethylcamptothecin

Seigo SAWADA,*[,a] Takashi YAEGASHI,[a] Tomio FURUTA,[a] Teruo YOKOKURA[a] and Tadashi MIYASAKA[b]

Yakult Central Institute for Microbiological Research,[a] Yaho 1796, Kunitachi-shi, Tokyo 186, Japan and School of Pharmaceutical Sciences, Showa University,[b] Hatanodai 1–5–8, Shinagawa-ku, Tokyo 142, Japan. Received July 30, 1992

7-Ethylcamptothecin (1d), a model which does not have any site on the A-ring for further modification was converted into water-soluble derivatives by opening the E-ring lactone. 1d was heated in *N,N*-dimethylenediamine to yield amide 2a, and this was then acylated to furnish 3a—q, which were soluble in water as their HCl salts. The propionyl (3b), butyryl (3c) and methylthiopropionyl (3h) derivatives showed higher activity than the sodium salt of 1d. The acyl group makes the derivatives more lipophilic, and ease of hydrolysis of amide 2a to 1d is thought to be necessary for significant activity.

Keywords 20(*S*)-camptothecin; 7-ethylcamptothecin; L1210; amide; *N,N*-dimethylethylenediamine; 17-*O*-acyl derivative

Camptothecin (**1a**) is an alkaloid which was first isolated from a Chinese tree, *Camptotheca acuminata* (Nyssaceae), by Wall and co-workers in 1966.[1] It attracted much attention because of its significant antitumor activity in animals. In clinical trials, however, severe toxicities restricted its usefulness as a therapeutic agent.[2] About 20 years after the initial isolation, two derivatives, topotecan (**1b**)[3] and irinotecan (**1c**),[4] are now being developed as novel anticancer agents which act as an inhibitor of DNA topoisomerase I.[5] These compounds have solubilizing functional groups in the A-ring, while the E-lactone ring is intact. The lactone is considered to be an essential moiety for activity.[1]

In spite of many efforts at modification of the E-lactone, no derivative was found to have potential as a therapeutic agent.[7] We recently found that 11-halogenated camptothecins show excellent cytotoxicity *in vitro*, but the sodium salts of the compounds showed rather weak activity *in vivo* (mouse leukemia L1210).

We wish to describe here the synthesis of E-ring-modified water-soluble derivatives and their remarkable antitumor activities.

Chemistry We selected 7-ethylcamptothecin (**1d**) as a starting material because **1d** showed higher activity than camptothecin (**1a**) toward L1210 *in vivo*[6] and like 11-halogenated camptothecins, has no site for introduction of a solubilizing moiety.

Compound **1d** was heated at 50 °C in *N,N*-dimethylethylenediamine to yield amide **2a**. Without further purification, **2a** was acylated in dichloromethane in the presence of *N,N*-dimethylaminopyridine (DMAP) to furnish **3a—q** in good yields (Chart 1, Table I). These 17-*O*-acylated derivatives **3** were converted to the corresponding hydrochlorides, and they were soluble in water (about 20 mg/ml) and stable for at least 24 h in an aqueous solution.

Results and Discussion

The antitumor activities of the hydrochlorides of **3** are compared with the sodium salts (**4a** and **4b**) in Table I. All the samples were dissolved in water, and the resulting clear solutions were administered. All the tested derivatives showed activity (T/C > 125%). The propionyl (**3b**), butyryl (**3c**) and methylthiopropionyl (**3h**) derivatives showed higher activity than **4b**. In the substituted benzoyl series, the derivatives having a fluoro group (**3i** and **3k**) showed higher activity than other derivatives (**3l—q**).

The 17-*O*-acyl isopropylamide derivatives of **1a** showed weaker activity than **4a**,[7a] whereas compounds **3b**, **3c** and

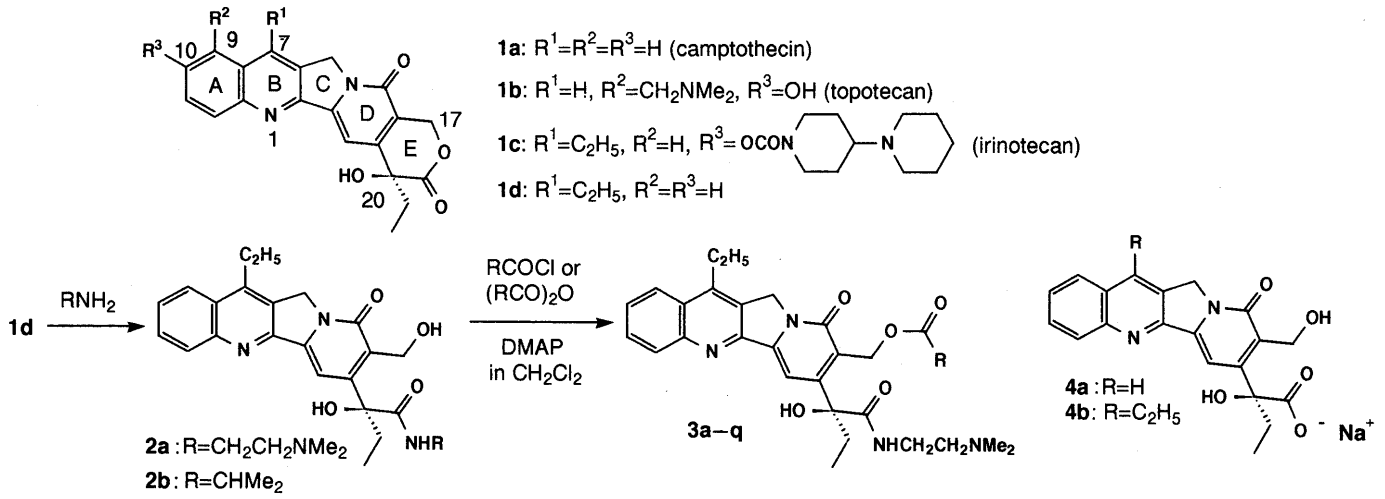

Chart 1

3h showed higher activity than **4a** and **4b**. The marked activity of the derivatives is mainly due to the hydrolytic profile of amide **2a**. Hydroxy-amide **2a** was completely hydrolyzed at 37°C within 30 min in a pH 7.2 phosphate buffer solution, yielding a mixture of **1d** (lactone form) and hydroxy-acid (E-ring open form) in a ratio of about 1 : 2. In contrast, isopropyl amide **2b** remained more than 70% unaltered after standing at 37 °C for 24 h in the buffer solution (Experimental).

When **3** was administered, amide **2a** was formed through enzymatic hydrolysis *in vivo* and spontaneously converted to a mixture of **1d** and the hydroxy-acid, the former or both of which are thought to be the active principle. The acyl group makes the derivatives more lipophilic and the substrate of an ester hydrolyzing-enzyme.[8] This hydrolysis is thought to be necessary for the expression of significant activity.

The derivatization described herein is promising for camptothecin derivatives not having any site on the A-ring for further modification. We will report in the near future on the sythesis and significant antitumor activity of the derivatives, starting from 11-halogenated camptothecins.

TABLE I. Antitumor Activity of **3** (HCl Salt, L1210 in Mice)

Compd. No.	R	T/C%[a] Total dose (mg/kg)						
		3.13	6.25	12.5	25	50	100	200
4a	–	138	171	212	188	199	86	—[b]
4b	–	127	129	131	182	233	328 (1)[c]	213 (2)
3a	$-CH_3$	118	124	131	182	316	189 (1)	138
3b	$-C_2H_5$	116	124	140	184	493 (5)	276 (3)	237 (1)
3c	$-C_3H_7$	122	124	164	196	272 (1)	363 (3)	351 (3)
3d	$-CH(CH_3)_2$	127	131	156	189	260	311 (3)	104
3e	$-C_4H_9$	127	131	140	173	202	300 (2)	87
3f	$-CH=CHCH_3$	120	122	127	138	182	203 (2)	330 (2)
3g	$-C_2H_4OC_2H_5$	116	140	135	165	242	300 (2)	272
3h	$-C_2H_4SCH_3$	135	135	144	193	244	363 (5)	412 (4)
3i	$-C_6H_5$	123	137	130	156	193	248 (2)	86
3j	$-C_6H_4-F(p)$	123	135	137	158	202	312 (4)	70
3k	$-C_6H_4-F(m)$	126	140	147	179	214	265 (4)	53
3l	$-C_6H_4-F(o)$	110	117	119	167	213	238 (2)	63
3m	$-C_6H_4-Cl(p)$	106	115	138	152	209 (2)	117	42
3n	$-C_6H_4-Cl(m)$	108	115	119	135	217	133 (1)	58
3o	$-C_6H_4-Cl(o)$	98	110	117	117	144	242 (3)	54
3p	$-C_6H_4-Br(p)$	113	117	119	156	192	125 (2)	46
3q	$-C_6H_4-OCH_3(p)$	92	98	113	115	117	175	113

a) T/C% = (mean survival time of the tested animals)/(mean survival time of the control animals) × 100. *b*) Not tested. *c*) Number of surviving mice/6 mice tested.

Experimental

The melting points (with decomposition) are uncorrected. ^{1}H-NMR spectra were recorded on a JEOL GX-400 (400 MHz) spectrometer with tetramethylsilane as the internal standard. MS were recorded on a Hitachi M 80B spectrometer. IR spectra were measured with a Shimadzu IR 435 spectrometer. HPLC analysis was conducted with a Shimadzu LC-6A, a spectrophotometer (Shimadzu SPD-6A) and an integrator (Shimadzu

TABLE II. Physical Constants of Compound **3a**—**q**

Compd. No.	Yield (%)	Appearance mp (°C)	Formula $(M+H)^+$	Analysis (%) Calcd			Found		
				C	H	N	C	H	N
3a	89	Pale yellow powder 160—164	$C_{28}H_{34}N_4O_5 \cdot 1/2H_2O$ 507[a]	65.23	6.84	10.87	64.92	6.82	10.61
3b	91	Pale yellow powder 137—154	$C_{29}H_{36}N_4O_5 \cdot H_2O$ 521	64.67	7.11	10.40	64.50	7.05	10.34
3c	79	Pale yellow needles 164.5—166.5	$C_{30}H_{38}N_4O_5 \cdot H_2O$ 535	65.20	7.30	10.14	65.38	7.19	9.88
3d	68	Pale yellow needles 181—182	$C_{30}H_{38}N_4O_5 \cdot H_2O$ 535	65.20	7.30	10.14	65.21	7.08	10.37
3e	81	Pale yellow powder 148—150	$C_{31}H_{40}N_4O_5 \cdot 1/2H_2O$ 549	66.76	7.41	10.05	66.72	7.36	10.00
3f	49	Pale yellow powder 147—148	$C_{30}H_{36}N_4O_5 \cdot H_2O$ 533	65.44	6.96	10.17	65.13	6.93	10.14
3g	77	Yellow powder 119—123	$C_{31}H_{40}N_4O_6 \cdot H_2O$ 565	63.90	7.27	9.62	64.24	7.06	9.63
3h	80	Yellow needles 96—103	$C_{30}H_{38}N_4O_5S \cdot 1/2H_2O$ 567	62.59	6.83	9.73	62.66	6.67	9.51
3i	86	Pale yellow powder 172—176	$C_{33}H_{36}N_4O_5$ 569	69.70	6.38	9.85	69.79	6.42	9.73
3j	76	Pale yellow powder 167—169.5	$C_{33}H_{35}FN_4O_5$ 587	—[b]	—	—	—[b]	—	—
3k	87	Pale yellow powder 132—138	$C_{33}H_{35}FN_4O_5$ 587	—	—	—	—	—	—
3l	84	Pale yellow needles 154—160	$C_{33}H_{35}FN_4O_5$ 587	—	—	—	—	—	—
3m	73	Pale yellow powder 167—188	$C_{33}H_{35}ClN_4O_5 \cdot 1/2H_2O$ 603	64.75	5.93	9.15	64.93	5.78	8.95
3n	87	Pale yellow powder 148—154	$C_{33}H_{35}ClN_4O_5$ 603	65.72	5.85	9.29	65.31	5.97	9.08
3o	85	Colorless needles 195—197	$C_{33}H_{35}ClN_4O_5$ 603	65.72	5.85	9.29	65.75	5.90	8.98
3p	66	Pale yellow powder 176—179.5	$C_{33}H_{35}BrN_4O_5 \cdot 1/2H_2O$ 649	60.37	5.53	8.53	60.46	5.39	8.67
3q	88	Pale yellow powder 176—177.5	$C_{34}H_{38}N_4O_6 \cdot H_2O$ 599	66.22	6.54	9.08	66.57	6.41	8.60

a) MS (SIMS) *m/z*. *b*) Not tested.

TABLE III. ^{1}H-NMR Spectral Data for Compounds **3a**—**q** (in $CDCl_3$, 400 MHz)

3a	1.09 (3H, t, J=7 Hz), 1.35 (3H, t, J=8 Hz), 2.08 (3H, s), 2.23 (6H, s), 2.26—2.54 (4H, m), 3.01—3.18 (2H, m), 3.27—3.48 (2H, m), 5.08 (1H, d, J=19 Hz), 5.15 (1H, d, J=19 Hz), 5.48 (1H, d, J=12 Hz), 5.54 (1H, d, J=12 Hz), 7.23 (1H, br t, J=5 Hz), 7.53 (1H, m), 7.57 (1H, s), 7.72 (1H, m), 7.90 (1H, d, J=8 Hz), 8.11 (1H, d, J=8 Hz)
3b	1.10 (3H, t, J=7 Hz), 1.13 (3H, t, J=8 Hz), 1.33 (3H, t, J=8 Hz), 2.24 (6H, s), 2.26—2.56 (6H, m), 2.96—3.14 (2H, m), 3.24—3.50 (2H, m), 5.02 (1H, d, J=19 Hz), 5.09 (1H, d, J=19 Hz), 5.50 (2H, s), 7.40 (1H, br t, J=6 Hz), 7.47 (1H, m), 7.55 (1H, s), 7.69 (1H, m), 7.81 (1H, d, J=8 Hz), 8.06 (1H, d, J=8 Hz)
3c	0.93 (3H, t, J=7 Hz), 1.09 (3H, t, J=7 Hz), 1.34 (3H, t, J=8 Hz), 1.65 (2H, sextet, J=7 Hz), 2.23 (6H, s), 2.25—2.53 (6H, m), 2.99—3.16 (2H, m), 3.25—3.49 (2H, m), 5.05 (1H, d, J=19 Hz), 5.12 (1H, d, J=19 Hz), 5.48 (1H, d, J=12 Hz), 5.54 (1H, d, J=12 Hz), 7.37 (1H, br t, J=5 Hz), 7.51 (1H, m), 7.56 (1H, s), 7.71 (1H, m), 7.87 (1H, d, J=8 Hz), 8.09 (1H, d, J=8 Hz)
3d	1.08 (3H, t, J=7 Hz), 1.16 (3H, d, J=7 Hz), 1.17 (3H, d, J=7 Hz), 1.34 (3H, t, J=8 Hz), 2.24 (6H, s), 2.25—2.52 (4H, m), 2.57 (1H, heptet, J=7 Hz), 3.00—3.17 (2H, m), 3.26—3.49 (2H, m), 5.07 (1H, d, J=19 Hz), 5.14 (1H, d, J=19 Hz), 5.47 (1H, d, J=12 Hz), 5.53 (1H, d, J=12 Hz), 7.37 (1H, br t, J=5 Hz), 7.53 (1H, m), 7.58 (1H, s), 7.72 (1H, m), 7.91 (1H, d, J=8 Hz), 8.11 (1H, d, J=8 Hz)
3e	0.88 (3H, t, J=7 Hz), 1.10 (3H, t, J=7 Hz), 1.28—1.39 (5H, m), 1.56—1.65 (2H, m), 2.24 (6H, s), 2.25—2.56 (6H, m), 2.95—3.13 (2H, m), 3.24—3.50 (2H, m), 5.00 (1H, d, J=19 Hz), 5.08 (1H, d, J=19 Hz), 5.48 (1H, d, J=12 Hz), 5.51 (1H, d, J=12 Hz), 7.42 (1H, br t, J=6 Hz), 7.47 (1H, m), 7.54 (1H, s), 7.68 (1H, m), 7.81 (1H, d, J=8 Hz), 8.05 (1H, d, J=8 Hz)
3f	1.09 (3H, t, J=7 Hz), 1.33 (3H, t, J=8 Hz), 1.82 (3H, dd, J=1, 7 Hz), 2.23 (6H, s), 2.25—2.56 (4H, m), 2.96—3.15 (2H, m), 3.22—3.49 (2H, m), 5.02 (1H, d, J=19 Hz), 5.11 (1H, d, J=19 Hz), 5.55 (1H, d, J=12 Hz), 5.61 (1H, d, J=12 Hz), 5.84 (1H, dq, J=1, 15 Hz), 6.98 (1H, dq, J=7, 15 Hz), 7.40—7.51 (2H, m), 7.57 (1H, s), 7.69 (1H, m), 7.81 (1H, d, J=8 Hz), 8.06 (1H, d, J=8 Hz)
3g	1.07 (3H, t, J=7 Hz), 1.17 (3H, t, J=7 Hz), 1.36 (3H, t, J=8 Hz), 2.23 (6H, s), 2.24—2.53 (4H, m), 2.61 (2H, t, J=6 Hz), 3.02—3.19 (2H, m), 3.26—3.58 (4H, m), 3.62—3.80 (2H, m), 5.04 (1H, d, J=19 Hz), 5.11 (1H, d, J=19 Hz), 5.50 (1H, d, J=11 Hz), 5.57 (1H, d, J=11 Hz), 7.38 (1H, t, J=6 Hz), 7.56 (1H, m), 7.60 (1H, s), 7.74 (1H, m), 7.96 (1H, d, J=8 Hz), 8.14 (1H, d, J=8 Hz)
3h	1.10 (3H, t, J=7 Hz), 1.33 (3H, t, J=8 Hz), 2.09 (3H, s), 2.24 (6H, s), 2.25—2.83 (8H, m), 2.96—3.14 (2H, m), 3.24—3.52 (2H, m), 5.01 (1H, d, J=19 Hz), 5.08 (1H, d, J=19 Hz), 5.51 (1H, d, J=11 Hz), 5.56 (1H, d, J=11 Hz), 7.43 (1H, t, J=5 Hz), 7.48 (1H, m), 7.55 (1H, s), 7.69 (1H, m), 7.82 (1H, d, J=8 Hz), 8.06 (1H, d, J=8 Hz)
3i	1.11 (3H, t, J=7 Hz), 1.32 (3H, t, J=8 Hz), 2.18 (6H, s), 2.29—2.44 (3H, m), 2.46—2.60 (1H, m), 2.74—3.23 (3H, m), 3.29—3.41 (1H, m), 5.02 (1H, d, J=19 Hz), 5.12 (1H, d, J=19 Hz), 5.78 (1H, d, J=12 Hz), 5.83 (1H, d, J=12 Hz), 7.35 (2H, t, J=8 Hz), 7.42—7.52 (3H, m), 7.59 (1H, s), 7.67 (1H, m), 7.79 (1H, d, J=8 Hz), 8.02 (1H, dd, J=1, 8 Hz), 8.05 (1H, d, J=8 Hz)
3j	1.11 (3H, t, J=7 Hz), 1.29 (3H, t, J=8 Hz), 2.26 (6H, s), 2.28—2.60 (4H, m), 2.87—3.08 (2H, m), 3.12—3.23 (1H, m), 3.37—3.49 (1H, m), 4.95 (1H, d, J=19 Hz), 5.06 (1H, d, J=19 Hz), 5.78 (2H, s), 7.00 (2H, dd, each J=9 Hz), 7.40 (1H, m), 7.56 (1H, s), 7.64 (1H, m), 7.69 (1H, d, J=8 Hz), 7.73 (1H, br t, J=6 Hz), 7.96—8.05 (3H, m)
3k	1.13 (3H, t, J=7 Hz), 1.30 (3H, t, J=8 Hz), 2.19 (6H, s), 2.29—2.47 (3H, m), 2.50—2.64 (1H, m), 2.90—3.09 (2H, m), 3.10—3.22 (1H, m), 3.32—3.43 (1H, m), 4.97 (1H, d, J=19 Hz), 5.08 (1H, d, J=19 Hz), 5.79 (1H, d, J=12 Hz), 5.82 (1H, d, J=12 Hz), 7.16 (1H, m), 7.32 (1H, ddd, J=6, 8, 8 Hz), 7.40 (1H, m), 7.57 (2H, s, br t, J=6 Hz), 7.61—7.72 (3H, m), 7.80 (1H, ddd, J=1, 1, 8 Hz), 7.99 (1H, d, J=8 Hz)
3l	1.11 (3H, t, J=7 Hz), 1.31 (3H, t, J=8 Hz), 2.18 (6H, s), 2.29—2.61 (4H, m), 2.94—3.13 (2H, m), 3.18—3.30 (1H, m), 3.32—3.44 (1H, m), 5.02 (1H, d, J=19 Hz), 5.10 (1H, d, J=19 Hz), 5.75 (1H, d, J=12 Hz), 5.86 (1H, d, J=12 Hz), 7.02—7.15 (2H, m), 7.40—7.52 (3H, m), 7.59 (1H, s), 7.68 (1H, m), 7.81 (1H, d, J=8 Hz), 7.92 (1H, ddd, J=2, 8, 8 Hz), 8.05 (1H, d, J=8 Hz)
3m	1.13 (3H, t, J=7 Hz), 1.30 (3H, t, J=8 Hz), 2.19 (6H, s), 2.30—2.46 (3H, m), 2.52—2.65 (1H, m), 2.92—3.18 (3H, m), 3.30—3.43 (1H, m), 4.98 (1H, d, J=19 Hz), 5.10 (1H, d, J=19 Hz), 5.80 (2H, s), 7.30 (2H, d, J=9 Hz), 7.40 (1H, m), 7.57 (1H, s), 7.59 (1H, br t, J=6 Hz), 7.64 (1H, m), 7.70 (1H, d, J=8 Hz), 7.93 (2H, d, J=9 Hz), 8.00 (1H, d, J=8 Hz)
3n	1.12 (3H, t, J=7 Hz), 1.32 (3H, t, J=8 Hz), 2.18 (6H, s), 2.29—2.45 (3H, m), 2.48—2.52 (1H, m), 2.94—3.23 (3H, m), 3.31—3.42 (1H, m), 5.02 (1H, d, J=19 Hz), 5.11 (1H, d, J=19 Hz), 5.80 (2H, s), 7.29 (1H, dd, each J=8 Hz), 8.41—8.52 (3H, m), 7.59 (1H, s), 7.66 (1H, m), 7.76 (1H, d, J=8 Hz), 7.90 (1H, ddd, J=2, 2, 8 Hz), 7.98 (1H, dd, each J=2 Hz), 8.03 (1H, d, J=8 Hz)
3o	1.11 (3H, t, J=7 Hz), 1.32 (3H, t, J=8 Hz), 2.19 (6H, s), 2.28—2.45 (3H, m), 2.47—2.59 (1H, m), 2.96—3.14 (2H, m), 3.16—3.27 (1H, m), 3.31—3.43 (1H, m), 5.04 (1H, d, J=19 Hz), 5.11 (1H, d, J=19 Hz), 5.75 (1H, d, J=12 Hz), 5.81 (1H, d, J=12 Hz), 7.22 (1H, ddd, J=2, 8, 8 Hz), 7.34 (1H, ddd, J=2, 8, 8 Hz), 7.39 (1H, dd, J=2, 8 Hz), 7.41 (1H, br t, J=5 Hz), 7.48 (1H, m), 7.57 (1H, s), 7.69 (1H, m), 7.82 (1H, d, J=8 Hz), 7.86 (1H, dd, J=2, 8 Hz), 8.06 (1H, d, J=8 Hz)
3p	1.13 (3H, t, J=7 Hz), 1.31 (3H, t, J=8 Hz), 2.18 (6H, s), 2.29—2.45 (3H, m), 2.51—2.64 (1H, m), 2.94—3.19 (3H, m), 3.29—3.41 (1H, m), 5.00 (1H, d, J=19 Hz), 5.10 (1H, d, J=19 Hz), 5.79 (2H, s), 7.42 (1H, m), 7.47 (2H, d, J=8 Hz), 7.52 (1H, br t, J=6 Hz), 7.57 (1H, s), 7.65 (1H, m), 7.73 (1H, d, J=8 Hz), 7.86 (2H, d, J=8 Hz), 8.01 (1H, d, J=8 Hz)
3q	1.13 (3H, t, J=7 Hz), 1.30 (3H, t, J=8 Hz), 2.17 (6H, s), 2.28—2.44 (3H, m), 2.50—2.62 (1H, m), 2.93—3.10 (2H, m), 3.12—3.22 (1H, m), 3.28—3.40 (1H, m), 3.80 (3H, s), 4.99 (1H, d, J=19 Hz), 5.09 (1H, d, J=19 Hz), 5.73 (1H, d, J=12 Hz), 5.81 (1H, d, J=12 Hz), 6.82 (2H, d, J=9 Hz), 7.42 (1H, m), 7.52 (1H, br t, J=5 Hz), 7.57 (1H, s), 7.65 (1H, m), 7.73 (1H, d, J=8 Hz), 7.96 (2H, d, J=9 Hz), 8.02 (1H, d, J=8 Hz)

C-R4A).

2a A mixture of **1d** (5.00 g, 13.3 mmol) in *N*,*N*-dimethylethylenediamine (25 ml) was heated at 50 °C for 1.5 h under an N_2 atmosphere with stirring. The mixture was evaporated *in vacuo*, and the residue was dissolved in CH_2Cl_2. The solution was added to n-C_6H_{14} with stirring. The precipitate was collected by suction and washed with n-C_6H_{14}. **2a**: Yield 71%. Pale yellow powder, mp 195—215 °C (n-C_6H_{14}–CH_2Cl_2). IR(KBr): 1650, 1590 cm^{-1}. NMR($CDCl_3$) δ: 1.10 (3H, t, J=7 Hz), 1.30 (3H, t, J=8 Hz), 2.21—2.31 (1H, m), 2.26 (6H, s), 2.41—2.61 (3H, m), 3.87—3.00 (2H, m), 3.25—3.35 (1H, m), 3.58—3.70 (1H, m), 4.85 (1H, d, J=13 Hz), 4.98 (1H, d, J=19 Hz), 5.04 (1H, d, J=19 Hz), 5.07 (1H, d, J=13 Hz), 7.46—7.58 (3H, m), 7.68 (1H, m), 7.84 (1H, d, J=8 Hz), 8.06 (1H, d, J=9 Hz). MS (SIMS) m/z: 465 $(M+H)^+$.

2b A mixture of **1d** (1.00 g, 2.7 mmol) in isopropylamine (20 ml) was refluxed for 1.5 h under an N_2 atmosphere with stirring. The mixture was evaporated *in vacuo*, and the residue was purified by silica gel column chromatography with 1% MeOH–$CHCl_3$. The combined fractions containing **2b** were evaporated under reduced pressure, and the residue was recrystallized from a mixture of n-C_6H_{14}–$CHCl_3$. **2b**: Yield 86%. Pale yellow needles, mp 243—247 °C. IR(KBr): 1645, 1585 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 0.90 (3H, t, J=7 Hz), 1.06 (3H, d, J=7 Hz), 1.11 (3H, d, J=7 Hz), 1.31 (3H, t, J=8 Hz), 2.07—2.28 (2H, m), 3.21 (2H, q, J=8 Hz), 3.87—3.99 (1H, m), 4.70 (1H, dd, J=6, 12 Hz), 4.85 (1H, dd, J=6, 12 Hz), 5.06 (1H, t, J=6 Hz), 5.26 (2H, s), 6.45 (1H, s), 7.42 (1H, s), 7.68—7.77 (2H, m), 7.83 (1H, m), 8.17 (1H, d, J=8 Hz), 8.26 (1H, d, J=8 Hz). MS (SIMS) m/z: 436 $(M+H)^+$. *Anal.* Calcd for $C_{25}H_{29}N_3O_4 \cdot 2/3CHCl_3$: C, 59.85; H, 5.81; N, 8.16. Found: C, 60.14; H, 6.01; N, 7.95.

General Procedure, Preparation of 3 To a solution of **2a** (1.0 g, 2.15 mmol) in CH_2Cl_2 (20 ml), DMAP (100 mg) and an acylating agent (1.2 eq) were added. The mixture was stirred at room temperature for 1.5 h. The mixture was washed with 7% $NaHCO_3$ and then a saturated aqueous NaCl solution. The organic layer was separated and dried with Na_2SO_4, filtered and evaporated to dryness under reduced pressure. The residue was passed through a silica gel column with 10% MeOH–$CHCl_3$.

TABLE IV. IR (in KBr) Spectral Data for Compounds **3a**—**q**

No.	$\nu_{C=O}$ (ester)	$\nu_{C=O}$ (amide)	$\nu_{C=C}$
3a	1730	1650	1590
3b	1730	1650	1595
3c	1730	1650	1590
3d	1725	1650	1590
3e	1730	1650	1595
3f	1710	1650	1595
3g	1730	1645	1590
3h	1730	1645	1590
3i	1710	1640	1590
3j	1710	1650	1600
3k	1715	1650	1590
3l	1715	1650	1610
3m	1710	1670, 1650	1590
3n	1715	1650	1595
3o	1720	1670, 1650	1595
3p	1710	1670, 1650	1595
3q	1700	1660	1600

TABLE V. Hydrolysis Profiles of Amides **2a** and **2b**

Time (h)	Ratio (mol %)			Total	Ratio (mol %)			Total
	2a[a]	Lactone	Acid		**2b**[a]	Lactone	Acid	
0	20.5	39.1	1.4	61.0[b]	100.0	—[c]	—	100.0
0.5	—	68.5	31.5	100.0	100.0	—	—	100.0
2.5	—	2.4	48.8	51.2[d]	95.6	2.2	2.2	100.0
24	—	17.7	41.9	59.6[d]	76.8	6.6	16.6	100.0

a) Initial concentration: 2.15 μmol/50 ml in 0.1 M phosphate buffer (pH 7.2). Temperature: 37 °C. Retention time: **2a** (9 min), **2b** (30 min), **1d** (23 min), hydroxy-acid of **1d** (12 min). *b*) Amide **2a** was hydrolyzed in the HPLC column during the analysis. The total area was smaller than expected due to the peak of **2a** being broadened. *c*) Not detected. *d*) The total area was reduced due to the precipitation of **1d** in the sample solutions.

The combined fractions containing **3** were evaporated *in vacuo*, and the residue was recrystallized from n-C_6H_{14}–$CHCl_3$.

HPLC Analysis of Hydrolysis Profile of Amides 2a and 2b **a) HPLC Conditions** Column; Inertsil ODS-2, 4 mm × 25 cm (GL Science Co., Ltd.). Temperature: around 40 °C. Detection: 254 nm. Eluent: 0.01 M KH_2PO_4–MeOH (1 : 1).

b) Preparation of Sample Solutions Amides **2** (1.0 mg) was dissolved in 0.1 M phosphate buffer (pH 7.2, 50 ml), and the solution was gently shaken at 37 °C. A part of the solution was taken at 0, 0.5, 2.5 and 24 h for HPLC analysis under the conditions described above.

c) Calibration Curves Accurately weighed 4 mg of **1d** and **2b** were dissolved in DMSO to make exactly 50 ml with DMSO. Five ml of each solution were pipetted and diluted with DMSO to make exactly to 20 ml. Five ml of each resulting solution were taken and exactly adjusted to 20 ml with DMSO. A 20 μl portion of each solution was injected into the chromatography. The peak areas of **1d** and **2b** were used for making the calibration curves. The linear regression equations for the curves were expressed as follows: $y = 1.750E-5x+0.003$ ($r^2=1$, for **1d**), $y = 2.090E-5x+0.004$ ($r^2=1$, for **2b**), here y axis means μg/20 μl, x axis peak area. The concentration of amide **2a** was estimated based on the calibration curves of **2b** and the acid based on **1d**.

Antitumor Activity L1210 leukemia cells (10^5) were implanted intraperitoneally (i.p.) to 7-week-old BDF_1 female mice on day 1. Six mice were used for each dose. The sample was dissolved in distilled water and was administered i.p. on days 1, 5 and 9. Control mice were injected with distilled water, and the surviving mice were counted on day 40.

References and Notes

1) *a*) M. E. Wall, M. C. Wani, C. E. Cook, K. H. Palmer, A. T. McPhail and G. A. Sim, *J. Am. Chem. Soc.*, **88**, 3888 (1966); *b*) Reviews; J.-C. Cai and C. R. Hutchinson, "The Alkaloids," Vol. 21, ed. by A. Brossi, Academic Press, New York, 1983, p. 101; H. Terasawa, A. Ejima and M. Sugimori, *J. Synthetic Org. Chem.*, **49**, 1013 (1991).
2) C. G. Moertel, A. J. Schutt, R. J. Reitemeier and R. G. Hahn, *Cancer Chemother. Rep.*, **56**, 95 (1972); J. A. Gottlieb and J. K. Luce, *ibid.*, **56**, 103 (1972).
3) W. D. Kingsbury, J. C. Boehm, D. R. Jakas, K. G. Holden, S. M. Hecht, G. Gallagher, M. J. Caranfa, F. L. McCabe, L. F. Faucette, R. K. Johnson and R. P. Hertzberg, *J. Med. Chem.*, **34**, 98 (1991) and references cited herein.
4) S. Sawada, S. Okajima, R. Aiyama, K. Nokata, T. Furuta, T. Yokokura, E. Sugino, K. Yamaguchi and T. Miyasaka, *Chem. Pharm. Bull.*, **39** 1446 (1991); K. Nitta, T. Yokokura, S. Sawada, M. Takeuchi, T. Tanaka, N. Uehara, H. Baba, M. Takeuchi, T. Miyasaka and M. Mutai, "Proceeding of the 14th International Congress of Chemotherapy," ed. by J. Ishigami, Tokyo Univ. Press, Tokyo, 1985, p. 29; *idem, Jpn. J. Cancer Chemother.*, **14**, Part II, 850; Y. Wang, S.-C. Chen and M. Ogawa, *ibid.*, Part I, 1264; Phase II trial of irinotecan (Non-small cell lung cancer), M. Fukuoka, S. Negoro, H. Niitani and T. Taguchi, Annual Meeting of the American Society of Clinical Oncology, Washington D. C., May 1990.
5) *a*) Y.-H. Hsiang, R. Hertzberg, S. Hecht and L.-F. liu, *J. Biol. Chem.*, **27**, 14873 (1985); *b*) M. R. Mattern, S.-M. Mong, H. F. Bartus, S. T. Crooke and R. K. Johnson, *Cancer Res.*, **47**, 1793 (1987); *c*) T. Andoh, K. Ishii, Y. Suzuki, Y. Ikegami, Y. Kusunoki, Y. Takamoto and K. Okada, *Proc. Natl. Acad. Sci., U.S.A.*, **84**, 5565 (1987).
6) *a*) S. Sawada, K. Nokata, T. Furuta, T. Yokokura and T. Miyasaka, *Chem. Pharm. Bull.*, **39**, 2574 (1991); T. Kunimoto, K. Nitta, T. Tanaka, N. Uehara, H. Baba, M. Takeuchi, T. Yokokura, S. Sawada, T. Miyasaka and M. Mutai, *J. Pharmacobio-Dyn.*, **10**, 481 (1987); H. Nagata, N. Kaneda, T. Furuta, S. Sawada, T. Yokokura, T. Miyasaka, M. Fukada and K. Nokata, *Cancer Treat. Rep.*, **71**, 341 (1987); *b*) **1b** showed the inhibitory activity as strong as camptothecin (**1a**) against calf thymus DNA topoisomerase, I. H. Nagata, Yakult Inst. for Microbiol. Res., unpublished data.
7) *a*) J. A. Adamovics and C. R. Hutchinson, *J. Med. Chem.*, **22**, 310 (1979); *b*) R. P. Hertzberg, M. J. Caranfa, K. G. Holden, D. R. Jakas, G. Gallagher, M. R. Mattern, S.-M. Mong, J. O. Bartus, R. K. Johnson, and W. D. Kingsbery, *ibid.*, **32**, 715 (1989); *c*) A. W. Nicholas, M. C. Wani, G. Manikumar, M. E. Wall, K. W. Kohn and Y. Pommier, *ibid.*, **33**, 972 (1990).
8) *Cf.* A. A. Sinkula and S. H. Yalkowsky, *J. Pharm. Sci.*, **64**, 181 (1975).