Synthesis and Antitumor Activity of 20(*S*)-Camptothecin Derivatives. A-Ring-Substituted 7-Ethylcamptothecins and Their E-Ring-Modified Water-Soluble Derivatives

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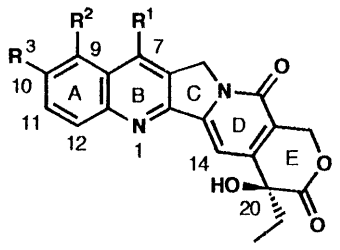
Twenty-six novel A-ring-modified 7-ethylcamptothecins (6) were synthesized by Friedländer's condensation of the chiral tricyclic ketone (5) with aminopropiophenones (4). The compounds substituted with fluorine at the 11 position showed strong cytotoxicity to KB and L1210 cells. The 11-fluoro derivatives also exhibited strong inhibitory activity on DNA topoisomerase I. Nine compounds 6 with four to ten times stronger cytotoxicity than that of camptothecin were selected and converted into water-soluble 17-*O*-acyl amide derivatives (8). Compounds 8e (10-Me, *O*-$COCH_2CH_2SCH_3$) and 8f (11-F, *O*-COC_2H_5) showed activity towards Meth A in mice that was comparable to that of CPT-11, at lower doses than CPT-11.

Keywords camptothecin; A-ring-substituted 7-ethylcamptothecin; DNA topoisomerase I; 17-*O*-acylated amide derivative; L1210; Meth A

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 using the water-soluble, E-lactone ring-opened sodium salt of **1a**, however, severe toxicities restricted its usefulness as a therapeutic agent.[2] Recently two derivatives, irinotecan (**2**)[3] and topotecan (**3**),[4] have been synthesized as anticancer agents of a new type, which acts by inhibiting DNA topoisomerase I.[5] These compounds have solubilizing functional groups in the A-ring, while the E-lactone ring, considered to be essential for the activity, is intact.[1]

We have already reported the synthesis of E-ring-modified water-soluble derivatives of 7-ethylcamptothecin (**1b**) and their remarkable antitumor activities.[6] The solubilizing method described therein is applicable to



1a: R^1=R^2=R^3=H, camptothecin

1b: R^1=Et, R^2=R^3=H

1c: R^1=Et, R^2=H, R^3=OH, SN-38

2: R^1=C_2H_5, R^2=H, R^3=OCON⟨⟩N⟨⟩, HCl, $3H_2O$, irinotecan

3: R^1=H, R^2=CH_2NMe_2, HCl, R^3=OH, topotecan

Fig. 1

camptothecins, which do not have any moiety such as a hydroxyl or an amino group in the A-ring. In this paper, we wish to report the synthesis of A-ring-modified 7-ethylcamptothecins (**6**) and their *in vitro* cytotoxicity as well as their inhibitory activity towards calf thymus DNA topoisomerase I. Selected derivatives of **6** were converted into E-ring-modified water-soluble compounds (**8**), and these compounds were evaluated in leukemia (L1210) and solid tumor (Meth A) models in mice.

Chemistry

In our early studies on the structure–activity relationships of modified camptothecins, the activity was lowered or diminished by introduction of a substituent at the 1, 12 or 14 position, adjacent to the 20-hydroxyl group, whereas the activity was heightened when the 9, 10 or 11 position was substituted.[7] Introduction of an ethyl group at the 7 position was recently reported to stabilize the active E-lactone form in the equilibrium between the lactone and the corresponding, rather inactive, hydroxy acid form under physiological conditions.[8]

The 26 novel A-ring-modified 7-ethylcamptothecins (**6**) presented in Table I were obtained in good yield by Friedländer's condensation of aminopropiophenones (**4**) with a known chiral tricyclic ketone (**5**)[9] (Chart 1).

The aminopropiophenones **4** were prepared by *ortho*-acylation of anilines with propionitrile in the presence of boron trichloride (Table V).[10] The compounds having a methylthio (**4e**), alkyl (**4h, o**), or methoxyl group (**4l**), and the 3,5- or 4,5-disubstituted aminopropiophenones (**4q**, **4s**—**v**) were obtained in satisfactory yields. On the other hand, in the acylation of chloro- or bromoanilines and *p*-phenylenediamines, the yields were poor (**4f, g, n**), and in the case of *m*-phenylenediamine, a mixture of 3-amino (**4a**, 5% yield) and 5-amino compounds (**4m**, 17% yield) was obtained.

The aminopropiophenones (**4**) were heated with the ketone **5** in toluene in the presence of TsOH as a catalyst

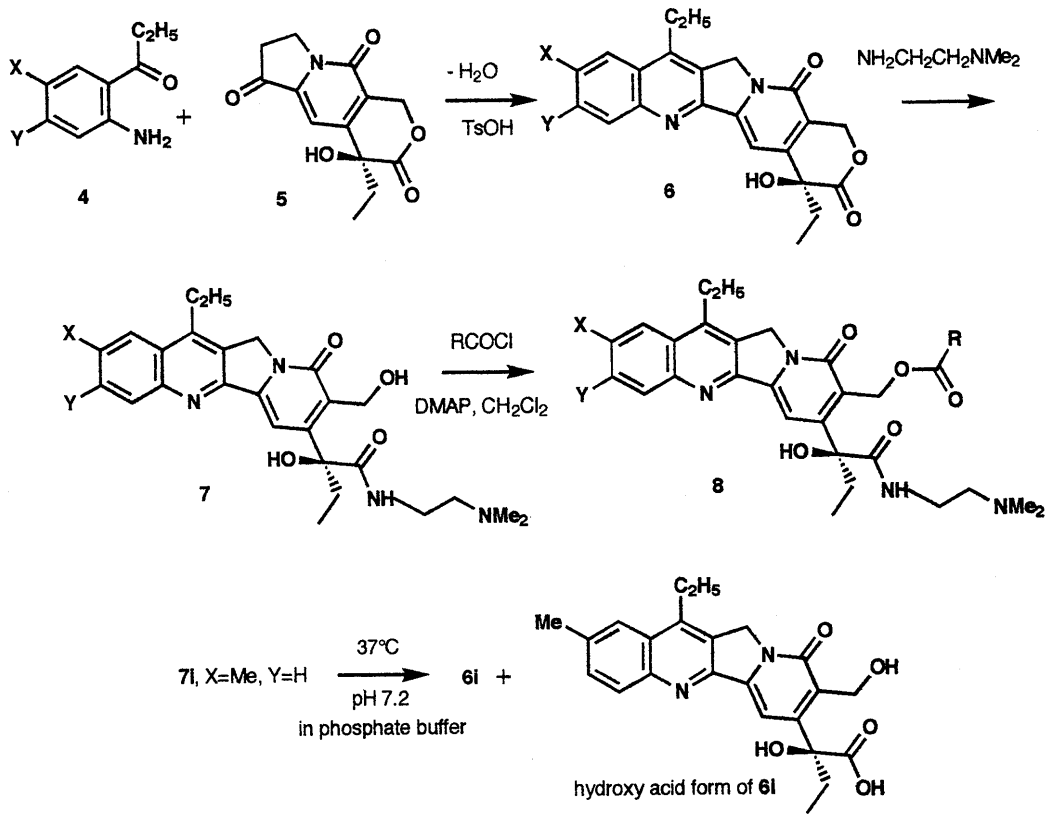


Chart 1

to give the 9- (**6a**), 10- (**6c—i**) and 11-mono- (**6j—r**) and 9,11- (**6w**) or 10,11-disubstituted 7-ethylcamptothecins (**6s—v, y**). The acetamido compounds **6b** and the hydroxylic compounds **6m**, **6x** and **6z** were obtained in the usual manner from the amino derivative **6a** and methoxyl derivatives **6n**, **6y** and **6u**, respectively.

An essential feature of the 17-*O*-acylated amide (**8**), as described in our previous paper,[6] is that the acyl moiety of the amide **8** was enzymatically hydrolyzed to the hydroxy amide **7** and the compound was spontaneously converted to the active principle **6**. For example, the hydroxy amide **7i** was completely hydrolyzed at 37 °C within 30 min in a pH 7.2 phosphate buffer solution, yielding a mixture of **6i** (lactone form) and the hydroxy-acid (E-ring-opened form) in a ratio of about 2 : 1 (Chart 1, Table IX). The lactone form gradually decreased, and in the mixture incubated for 6 and 24 h, both solutions consisted of about a 1 : 4 ratio of the lactone and the acid. The change of the ratio suggested that the initial product is the lactone, and the mixture reached at equilibrium after 6 h.

The acyl amide **8e** was stable for at least 24 h in aqueous solution. The free hydroxyl group at the 17 position affords anchimeric assistance in the facile hydrolysis of the amide functionality. The *O*-acyl group endows the derivatives with stability and lipophilicity and an ester-hydrolyzing enzyme seems to effect rapid recovery of the 17-hydroxyl function.

Nine compounds **6** were converted into the 17-*O*-acylated amide derivatives (**8**). Aminolysis of **6** at about 50 °C with *N*,*N*-dimethylethylenediamine gave the E-lactone-opened hydroxy amide (**7**). Compound **7** was acylated with propionyl, butyryl or 3-methylthiopropionyl chloride in dichloromethane in the presence of 4-*N*,*N*-dimethylaminopyridine (DMAP) to furnish **8** in modest to good yields (Tables II, VII). The acyl amides (**8**) were converted to the corresponding hydrochlorides in the conventional manner, and they were fairly soluble in water (about 40 mg/ml).

Results and Discussion

The cytotoxicity of each of the A-ring-modified 7-ethyl-camptothecins (**6**) synthesized in this work is given in Table I. Many compounds showed slightly higher activity in the L1210 assay than in the KB cell assay. Outstanding activity was shown by the 11- (**6j**) and 10-substituted 11-fluoro compounds (**6u, v**) in both assay systems, both compounds being about 10-fold more active than camptothecin (**1a**). Among the 10-substituted derivatives, the chloro (**6d**), bromo (**6e**) and methyl compounds (**6i**), and 11-chloro (**6k**) and 10,11-difluoro compounds also showed good activity. On the other hand, the compounds having a bulky substituent (**6p**; $-NMe_2$, **6q**; –Et, **6r**; –SMe) at the 11 position and the dimethoxyl (**6v, w**) or dihydroxyl compounds (**6x**) showed diminished activity.

Table III shows the inhibitory activity of four compounds **6** towards DNA topoisomerase I compared with those of **1a** and **1c**. The 11-fluoro compound (**6j**), which exhibited remarkable cytotoxicity, was confirmed to show inhibitory activity as potent as that of SN-38 (**1c**), the active principle of CPT-11.

Compounds **8** were derived from nine compounds **6**

TABLE I. A-Ring-Modified 7-Ethylcamptothecins (**6**)

Compd. No.	Substituent 9-	10-	11-	(%)	mp (dec.) Solvent[a]	$[\alpha]_D^{25}$ (°) (%)[b]	Formula	*Anal.* Calcd (Found) C	H	N	ED_{50}[c] KB	L1210
6a	NH_2	H	H	60	229—231 A	+48.5 (0.2)	$C_{22}H_{21}N_3O_4$	67.51 (67.34)	5.41 5.48	10.74 10.44)	0.9	2.6
6b	NHAc	H	H	9[d]	205—210 A	+48.0 (0.1)	$C_{24}H_{23}N_3O_5 \cdot 1/2H_2O$	65.15 (64.86)	5.47 5.25	9.50 9.39)	6.6	76.0
6c	H	F	H	91	240—242 A	+36.5 (0.2)	$C_{22}H_{19}FN_2O_4$	67.00 (66.89)	4.86 4.83	7.10 7.16)	1.0	2.2
6d	H	Cl	H	83	238—239 A	+40.0 (0.2)	$C_{22}H_{19}ClN_2O_4$	64.31 (64.27)	4.66 4.66	6.82 6.86)	0.4	1.1
6e	H	Br	H	92	241—243 A	+37.5 (0.2)	$C_{22}H_{19}BrN_2O_4$	58.04 (58.00)	4.21 4.30	6.15 6.20)	0.4	1.0
6f	H	SMe	H	99	259—261 B	+25.0 (0.04)	$C_{23}H_{22}N_2O_4S$	65.38 (65.20)	5.25 5.20	6.63 6.39)	1.4	2.1
6g	H	NH_2	H	34	215—217 A	+19.5 (0.2)	$C_{22}H_{21}N_3O_4 \cdot H_2O$	64.54 (64.57)	5.66 5.26	10.26 10.27)	0.3	1.1
6h	H	NMe_2	H	90	270—271.5 B	+7.0 (0.1)	$C_{24}H_{25}N_3O_4$	68.72 (68.40)	6.01 5.99	10.02 10.06)	1.2	2.1
6i	H	Me	H	95	245—247 A	+40.5 (0.2)	$C_{23}H_{22}N_2O_4$	70.75 (70.80)	5.68 5.67	7.17 7.04)	0.5	1.4
6j	H	H	F	85	196—198 A	+46.0 (0.2)	$C_{22}H_{19}FN_2O_4$	67.00 (67.10)	4.86 4.91	7.10 7.28)	0.2	0.4
6k	H	H	Cl	87	205—209 A	+33.0 (0.2)	$C_{22}H_{19}ClN_2O_4 \cdot 1/4H_2O$	63.62 (63.55)	4.73 4.73	6.74 6.70)	0.5	1.1
6l	H	H	Br	80	202—204 A	+27.5 (0.2)	$C_{22}H_{19}BrN_2O_4 \cdot 1/4H_2O$	57.47 (57.39)	4.27 4.28	6.09 6.06)	1.1	1.8
6m	H	H	OH	75[e]	>320 A	+36.0 (0.2)	$C_{22}H_{20}N_2O_5 \cdot 1/2H_2O$	65.83 (66.15)	5.27 5.08	6.98 6.88)	0.4	3.1
6n	H	H	OMe	95	228—230 A	+33.5 (0.2)	$C_{23}H_{22}N_2O_5$	67.97 (68.13)	5.46 5.33	6.89 6.86)	1.3	3.9
6o	H	H	NH_2	20	276—279 A	+20.0 (0.05)	$C_{22}H_{21}N_3O_4 \cdot H_2O$	64.54 (64.91)	5.66 5.25	10.26 10.31)	1.1	4.3
6p	H	H	NMe_2	69	211—212 A	+19.0 (0.2)	$C_{24}H_{25}N_3O_4 \cdot 1/4H_2O$	67.99 (68.14)	6.06 5.99	9.91 9.89)	360.0	810.0
6q	H	H	Et	91	224—227 A	+34.0 (0.2)	$C_{24}H_{24}N_2O_4$	71.27 (71.21)	5.98 5.88	6.93 6.95)	72.0	190.0
6r	H	H	SMe	90	222—225.5 A	+16.0 (0.2)	$C_{23}H_{22}N_2O_4S$	65.38 (65.63)	5.25 5.12	6.63 6.75)	4.0	11.0
6s	H	F	F	82	233—236 A	+41.5 (0.2)	$C_{22}H_{18}F_2N_2O_4$	64.08 (64.00)	4.40 4.42	6.79 6.97)	0.3	0.6
6t	H	Cl	Cl	72	258—262 A	+27.0 (0.2)	$C_{22}H_{18}Cl_2N_2O_4$	59.34 (59.21)	4.07 4.13	6.29 6.33)	0.5	0.6
6u	H	OMe	F	94	254—256 A	+41.0 (0.2)	$C_{23}H_{21}FN_2O_5$	65.09 (65.01)	4.99 4.92	6.60 6.80)	0.1	0.4
6v	H	Me	F	92	218—221 A	+41.5 (0.2)	$C_{23}H_{21}FN_2O_4 \cdot 2H_2O$	62.15 (61.81)	5.67 5.65	6.30 6.42)	0.2	0.4
6w	OMe	H	OMe	66	238—242 A	+28.5 (0.2)	$C_{24}H_{24}N_2O_6$	66.05 (65.72)	5.54 5.50	6.42 6.24)	3.0	6.6
6x	H	OH	OH	38[f]	>320 A	+16.3 (0.08)	$C_{22}H_{20}N_2O_6 \cdot H_2O$	61.97 (61.74)	5.20 5.04	6.57 6.52)	13.0	54.0
6y	H	OMe	OMe	59	265—269 A	+27.0 (0.2)	$C_{24}H_{24}N_2O_6$	66.05 (65.88)	5.54 5.50	6.42 6.34)	150.0	620.0
6z	H	OH	F	85[g]	201—204 A	+32.5 (0.2)	[h]				0.3	2.2

a) A: EtOH, B: $CHCl_3$–MeOH–*n*-C_6H_{14}. *b*) In MeOH–$CHCl_3$ (1:4). *c*) ED_{50}: 50% effective dose (ng/ml); **1a**: 1.5±0.1 ng/ml, 4.5±0.3 ng/ml against KB and L1210 cells, respectively. Mean±S.D. on 8 experiments. *d*) Yield from **6a**. *e*) Yield from **6n**. *f*) Yield from **6y**. *g*) Yield from **6u**. *h*) HR-MS *m/z* 410.1276 [M^+] for $C_{22}H_{19}FN_2O_5$=410.1242.

which showed cytotoxicity four to ten times higher than that of **1a**. The antitumor activities of the hydrochlorides **8** are presented in Table II. All the samples were dissolved in distilled water, and the resulting clear solutions were administered to mice. All the tested compounds showed activity (T/C%>125), and especially significant activity was observed with compounds **8e** and **8f**. These compounds were further examined using a solid tumor system. Table IV summarizes the activity towards Meth A compared with CPT-11. In this assay, compounds **8e** and **8f** exhibited activity comparable to that of CPT-11, at lower doses than CPT-11.[3]

The inhibitory activity (ED_{50}) of **8e**, **8f**, and **8t** towards L1210 and KB cell *in vitro* could not be determined because the compounds were hydrolyzed in the test medium to yield the corresponding active lactones. But

TABLE II. Antitumor Activity of the Water-Soluble Amide Derivatives (**8**) against L1210 in Mice

Compd. No.	Substituent			Total dose (mg/kg)							
	X	Y	R	1.56	3.13	6.25	12.5	25	50	100	200
8a	Cl	H	Et	136[a]	193	257 (1)[b]	176	93	110	100	43
8b	Cl	H	Pr	148	198	280 (1)	98	133	110	100	29
8c	Cl	H	MTE	150	217	295 (3)	117	131	160	107	36
8d	Br	H	MTE	159	223	183 (1)	82	82	89	77	36
8e	Me	H	MTE	120	123	145	191	310 (2)	341 (2)	368 (5)	100
8f	H	F	Et	126	140	236	364 (4)	86	83	69	62
8g	H	F	Pr	126	148	236	293 (2)	81	200	150	55
8h	H	F	MTE	121	148	202	240 (1)	74 (1)	105	140	45
8i	H	Cl	MTE	125	127	148	198	281 (1)	104 (1)	116	89
8j	F	F	Et	214	143 (1)	86	86	121	64	48	29
8k	F	F	Pr	257 (1)	124	86	90	100	93	60	29
8l	F	F	MTE	260	240	86	86	100	95	52	29
8m	Cl	Cl	Et	124	167	286 (2)	107	95	98	71	29
8n	Cl	Cl	Pr	143	225	218	90	90	90	35	30
8o	Cl	Cl	MTE	155	198	98	85	90	90	35	30
8p	Me	F	Et	131	167	217	317	131	100	114	48
8q	Me	F	Pr	143	183	217	318 (2)	326 (1)	205	148	60
8r	Me	F	MTE	148	150	205	300 (1)	252 (1)	100	210	15
8s	OMe	F	Et	190	312	88	90	90	88	60	15
8t	OMe	F	Pr	220	265	75	215	90	85	83	15
8u	OMe	F	MTE	228	158	100	90	143	98	38	15

a) T/C% = (mean survival time of the tested animals)/(mean survival time of the control animals) × 100. *b*) Number of cured mice in a tested group (6 mice). MTE = $MeSCH_2CH_2$–.

TABLE III. DNA Topoisomerase I-Inhibitory Activity of Compounds **6**

Compound No.	*MIC*[a] (μg/ml)
1a	30
1c	<1
6d	10
6i	1
6j	<1
6k	30

a) Minimum inhibitory concentration (see Experimental).

the water-soluble 17-*O*-acyl amides (**8**) were thought to be prodrugs, as described above. We are currently evaluating the antitumor activities of **8e** and **8f** in other animals, as well as their metabolic processes, and the findings will be presented in the near future.

Experimental

The melting points are uncorrected. ^{1}H-NMR spectra were recorded on a JEOL GX-400 (400 MHz) spectrometer with tetramethylsilane as an internal standard. MS were recorded on a Hitachi M 80B spectrometer. Optical rotation was determined with a DIP-360 at 25 °C with a 100-mm cell (Japan Spectroscopic Co., Ltd.).

General Procedure for the Preparation of *o*-Aminopropiophenones (4) (See Table V). Propionitrile (21.1 g, 0.38 mol) and $AlCl_3$ (28.4 g, 0.21 mol) were successively added to a solution, which was prepared by adding BCl_3 (25 g, 0.21 mol) to a substituted aniline (0.19 mol) dissolved or suspended in benzene, under an N_2 gas stream. The mixture was refluxed for 8 h with stirring. After cooling in an ice bath, the reaction was quenched by adding 2 N HCl (200 ml). The mixture was heated at 80 °C for 1 h with stirring. After standing at room temperature, the mixture was diluted with H_2O (200 ml), and the benzene layer was separated. The aqueous solution was extracted with benzene (200 ml × 2), and the combined benzene extracts were washed with H_2O, dried over anhydrous Na_2SO_4, filtered and evaporated to dryness under reduced pressure. The residue was purified by silica gel column chromatography with toluene–AcOEt (5 : 1) as the eluent.

When phenylenediamine was used as the starting material, the reaction mixture after the HCl work-up described above was neutralized with 10% NaOH, and then benzene extraction was conducted.

General Procedure for the Preparation of A-Ring-Modified 7-Ethylcamptothecins (6) (See Tables I and VI). The tricyclic ketone [**5**, 1.0 g, 3.8 mmol, mp 174—176 °C (dec.), $[\alpha]_D^{25}$ +121.6° (c = 0.56 in $CHCl_3$), lit: mp 176—177 °C (dec.), $[\alpha]_D^{23}$ +117.6° (c = 0.56 in $CHCl_3$)], an aminopropiophenone (4.18 mmol) and TsOH (240 mg) were dissolved in toluene (200 ml). The mixture was refluxed for 18 to 24 h with a Dean–Stark dehydration funnel. The mixture was evaporated to dryness *in vacuo* and the residue was purified by silica gel column chromatography with 2% MeOH–$CHCl_3$ as the eluent.

In the syntheses of amino- (**6g, o**) and *N,N*-dimethylaminocamptothecins (**6h, p**) and **6y**, an excess amount of TsOH (0.8—1.2 g) was used. Compound **6y** was precipitated as its TsOH salt in the reaction mixture. The residue was neutralized with 7% $NaHCO_3$, and the free base was isolated.

6b: Acetyl chloride (60 mg, 0.76 mmol) was added dropwise to a solution of **6a** (100 mg, 0.26 mmol) in CH_2Cl_2 (30 ml) in the presence of Et_3N (1 ml) with stirring. The stirring was continued at room temperature for 24 h. The mixture was evaporated to dryness under reduced pressure, and the residue was purified by silica gel column chromatography with 2% MeOH–$CHCl_3$ as the eluent.

Hydrolysis of Methoxycamptothecins. Preparation of 6m, 6x and 6z Methoxy compounds (**6n, 6y, 6u**, 3.0 g) were refluxed in 47% HBr (120 ml) for 2.5 h under an N_2 atmosphere. The solution was evaporated to dryness under reduced pressure and the residue was purified by silica gel column chromatography with 2% MeOH–$CHCl_3$ as the eluent.

General Procedure for the Preparation of 17-*O*-Acyl Amides (8) (See Tables VII and VIII). Compound **6** (3.0 g) was heated at 50 °C in *N,N*-dimethylethylenediamine (15 ml) for 1.5 h with stirring under an N_2 atmosphere. The mixture was evaporated to dryness under reduced pressure to give a residue, which was dissolved in CH_2Cl_2 (15 ml). *n*-C_6H_{14} (500 ml) was poured into this solution with stirring. The precipitate was collected on a filter paper under suction. The solid, *i.e.*, the hydroxy amide (**7**), was washed with *n*-C_6H_{14} and dried *in vacuo*. Next, **7** (1.0 g) was dissolved in CH_2Cl_2 (20 ml) in the presence of DMAP (100 mg), and an acylating agent (1.2 eq) was added dropwise on an ice bath with stirring. The mixture was stirred at room temperature overnight, washed with 7% $NaHCO_3$ and brine, dried over Na_2SO_4, filtered, and then evaporated to dryness *in vacuo*. The residue was purified by silica gel column chromatography with 10% MeOH–$CHCl_3$ as the

TABLE IV. Antitumor Activity of Selected Derivatives (**8e**, **8f**) against Meth A in Mice

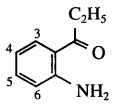
Compd. No.	Total dose (mg/kg)								
	1.56	3.13	6.25	12.5	25	50	100	200	400
CPT-11	—	—	—	—	58.0[b]	50.7	57.1	88.6 (2)[c]	95.2 (2)
8v[a]	—	—	—	—	−10.6	11.7	42.1	72.7	99.3 (3)
8w[a]	—	—	—	—	23.0	39.5	42.2	86.4 (2)	95.3 (1)
8x[a]	—	—	—	—	45.1	58.6	60.9	80.1	97.3 (2)
8e	—	—	—	24.9	43.9	73.1	79.8	92.2 (2)	—
8f	39.1	17.8	47.7	73.5	89.1	—	—	—	—

a) Preceding paper, ref. 1. **8v**; X=Y=H, R=Et, **8w**; X=Y=H, R=Pr, **8x**; X=Y=H, MTE. *b*) Inhibition rate (I.R.)%=(1−mean tumor weight of the tested animals/mean tumor weight of the control animals)×100. *c*) Number of cured mice in the tested group (6 mice). —: not tested.

TABLE V. Physical Constants of Compounds **4a**—**v**

Compound No.	Substituent(s)[a]	Yield (%)	mp (°C)	Solvent[b]	Formula	*m/z* [M$^+$]
4a	3-NH_2	5	65—66.5	A	$C_9H_{12}N_2O$	164
4b	4-F	22	70—72	B	$C_9H_{10}FNO$	167
4c[11a]	4-Cl	10	76—78	B	$C_9H_{10}ClNO$	183
4d	4-Br	9	78—79	B	$C_9H_{10}BrNO$	227
4e	4-SMe	56	64.5—65.5	C	$C_{10}H_{13}NOS$	195
4f	4-NH_2	24	132—135	C	$C_9H_{12}N_2O$	164
4g	4-NMe_2	13	98.5—99.5	D	$C_{11}H_{16}N_2O$	192
4h	4-Me	60	75.5—76.5	B	$C_{10}H_{13}NO$	163
4i	5-F	62	58—61	D	$C_9H_{10}FNO$	167
4j	5-Cl	45	73—75	B	$C_9H_{10}ClNO$	183
4k[11b]	5-Br	46	86—88	B	$C_9H_{10}BrNO$	227
4l	5-OMe	59	54—56	D	$C_{10}H_{13}NO_2$	179
4m	5-NH_2	17	155—157	C	$C_9H_{12}N_2O$	164
4n	5-NMe_2	12	106—107.5	D	$C_{11}H_{16}N_2O$	192
4o	5-Et	67	(Oil)	—	$C_{11}H_{15}NO$	177
4p	5-SMe	76	75—76.5	D	$C_{10}H_{13}NOS$	195
4q	4-F, 5-F	49	71—73	B	$C_9H_9F_2NO$	185
4r	4-Cl, 5-Cl	4	88—89	B	$C_9H_9Cl_2NO$	217
4s	4-OMe, 5-F	70	96.5—97.5	C	$C_{10}H_{12}FNO_2$	197
4t	4-Me, 5-F	86	105—107	D	$C_{10}H_{12}FNO$	181
4u	3-OMe, 5-OMe	92	63—66	C	$C_{11}H_{15}NO_3$	209
4v[11c]	4-OMe, 5-OMe	60	128—129	C	$C_{11}H_{15}NO_3$	209

a) Numberings were as follows:



b) Recrystallized from A: Et_2O, B: *n*-C_6H_{14}, C: *n*-C_6H_{14}–$CHCl_3$, D: *n*-C_6H_{14}–Et_2O.

TABLE VI. ^{1}H-NMR Spectral Data for Compounds **6a**—**z** (in DMSO-d_6, 400 MHz)

6a	0.88 (3H, t, *J*=7 Hz), 1.36 (3H, t, *J*=8 Hz), 1.81—1.91 (2H, m), 3.31 (2H, q, *J*=8 Hz), 5.29 (2H, s), 5.42 (2H, s), 6.48 (1H, s, 20-OH), 6.97 (1H, d, *J*=8 Hz, 10-H), 7.26 (1H, s), 7.43 (1H, d, *J*=8 Hz, 12-H), 7.50 (1H, dd, *J*=8, 8 Hz, 11-H)
6b	0.89 (3H, t, *J*=7 Hz), 1.22 (3H, t, *J*=8 Hz), 1.83—1.93 (2H, m), 2.14 (3H, s, $COCH_3$), 3.28 (2H, q, *J*=8 Hz), 5.32 (2H, s), 5.43 (2H, s), 6.52 (1H, s, 20-OH), 7.36 (1H, s), 7.49 (1H, d, *J*=8 Hz, 10-H), 7.81 (1H, dd, *J*=8, 8 Hz, 11-H), 8.11 (1H, d, *J*=8 Hz, 12-H), 10.11 (1H, s, NHCO)
6c	0.89 (3H, t, *J*=7 Hz), 1.29 (3H, t, *J*=8 Hz), 1.79—1.95 (2H, m), 3.18 (2H, q, *J*=8 Hz), 5.29 (2H, s), 5.43 (2H, s), 6.51 (1H, s, 20-OH), 7.31 (1H, s), 7.75 (1H, ddd, *J*=3, 9, 11 Hz, 11-H), 8.02 (1H, dd, *J*=3, 11 Hz, 9-H), 8.21 (1H, dd, *J*=6, 9 Hz, 12-H)
6d	0.88 (3H, t, *J*=7 Hz), 1.30 (3H, t, *J*=8 Hz), 1.78—1.97 (2H, m), 3.21 (2H, q, *J*=8 Hz), 5.30 (2H, s), 5.43 (2H, s), 6.52 (1H, s, 20-OH), 7.32 (1H, s), 7.85 (1H, dd, *J*=2, 9 Hz, 11-H), 8.16 (1H, d, *J*=9 Hz, 12-H), 8.31 (1H, d, *J*=2 Hz, 9-H)
6e	0.88 (3H, t, *J*=7 Hz), 1.30 (3H, t, *J*=8 Hz), 1.78—1.97 (2H, m), 3.21 (2H, q, *J*=8 Hz), 5.32 (2H, s), 5.43 (2H, s), 6.52 (1H, s, 20-OH), 7.33 (1H, s), 7.96 (1H, dd, *J*=2, 9 Hz, 11-H), 8.09 (1H, d, *J*=9 Hz, 12-H), 8.46 (1H, d, *J*=2 Hz, 9-H)
6f	0.93 (3H, t, *J*=7 Hz), 1.38 (3H, t, *J*=8 Hz), 1.83—1.94 (2H, m), 2.67 (3H, s, SCH_3), 3.22 (2H, q, *J*=8 Hz), 5.28 (2H, s), 5.34 (1H, d, *J*=16 Hz), 5.49 (1H, d, *J*=16 Hz), 6.40 (1H, s, 20-OH), 7.38 (1H, s), 7.68 (1H, dd, *J*=2, 9 Hz, 11-H), 7.83 (1H, d, *J*=2 Hz, 9-H), 8.06 (1H, d, *J*=9 Hz, 12-H)
6g	0.88 (3H, t, *J*=7 Hz), 1.29 (3H, t, *J*=8 Hz), 1.79—1.91 (2H, m), 3.01 (2H, q, *J*=8 Hz), 5.22 (2H, s), 5.38 (1H, d, *J*=16 Hz), 5.42 (1H, d, *J*=16 Hz), 5.93 (2H, s, NH_2), 6.44 (1H, s, 20-OH), 7.07 (1H, d, *J*=3 Hz, 9-H), 7.18 (1H, s), 7.24 (1H, dd, *J*=3, 9 Hz, 11-H), 7.84 (1H, d, *J*=9 Hz, 12-H)
6h	0.88 (3H, t, *J*=7 Hz), 1.31 (3H, t, *J*=8 Hz), 1.81—1.92 (2H, m), 3.05—3.18 (2H, m), 3.12 (6H, s, $N(CH_3)_2$), 5.22 (2H, s), 5.39 (1H, d, *J*=16 Hz), 5.43 (1H, d, *J*=16 Hz), 6.45 (1H, s, 20-OH), 6.96 (1H, d, *J*=3 Hz, 9-H), 7.20 (1H, s), 7.53 (1H, dd, *J*=3, 9 Hz, 11-H), 7.92 (1H, d, *J*=9 Hz, 12-H)

TABLE VI. (continued)

6i	0.89 (3H, t, J=7 Hz), 1.31 (3H, t, J=8 Hz), 1.81—1.93 (2H, m), 2.58 (3H, s, CH_3), 3.19 (2H, q, J=8 Hz), 5.28 (2H, s), 5.43 (2H, s), 6.50 (1H, s, 20-OH), 7.30 (1H, s), 7.68 (1H, dd, J=2, 9 Hz, 11-H), 8.03 (1H, d, J=2 Hz, 9-H), 8.05 (1H, d, J=9 Hz, 12-H)
6j	0.88 (3H, t, J=7 Hz), 1.32 (3H, t, J=8 Hz), 1.78—1.95 (2H, m), 3.23 (2H, q, J=8 Hz), 5.31 (2H, s), 5.44 (2H, s), 6.52 (1H, br s, 20-OH), 7.33 (1H, s), 7.65 (1H, ddd, J=3, 9, 11 Hz, 10-H), 7.90 (1H, dd, J=3, 10 Hz, 12-H), 8.37 (1H, dd, J=6, 9 Hz, 9-H)
6k	0.89 (3H, t, J=7 Hz), 1.31 (3H, t, J=8 Hz), 1.78—1.96 (2H, m), 3.22 (2H, q, J=8 Hz), 5.30 (2H, s), 5.43 (2H, s), 6.51 (1H, s, 20-OH), 7.32 (1H, s), 7.72 (1H, dd, J=2, 9 Hz, 10-H), 8.19 (1H, d, J=2 Hz, 12-H), 8.30 (1H, d, J=9 Hz, 9-H)
6l	0.88 (3H, t, J=7 Hz), 1.31 (3H, t, J=8 Hz), 1.79—1.94 (2H, m), 3.21 (2H, q, J=8 Hz), 5.30 (2H, s), 5.44 (2H, s), 6.52 (1H, s, 20-OH), 7.32 (1H, s), 7.84 (1H, dd, J=2, 9 Hz, 10-H), 8.23 (1H, d, J=9 Hz, 9-H), 8.36 (1H, d, J=2 Hz, 12-H)
6m	0.89 (3H, t, J=7 Hz), 1.29 (3H, t, J=8 Hz), 1.78—1.95 (2H, m), 3.14 (2H, q, J=8 Hz), 5.23 (2H, s), 5.43 (2H, s), 6.49 (1H, s, 20-OH), 7.26 (1H, dd, J=2, 9 Hz, 10-H), 7.27 (1H, s), 7.37 (1H, d, J=2 Hz, 12-H), 8.11 (1H, d, J=9 Hz, 9-H), 10.35 (1H, s, 11-OH)
6n	0.88 (3H, t, J=7 Hz), 1.30 (3H, t, J=8 Hz), 1.78—1.96 (2H, m), 3.18 (2H, q, J=8 Hz), 3.96 (3H, s, OCH_3), 5.26 (2H, s), 5.43 (2H, s), 6.49 (1H, s, 20-OH), 7.30 (1H, s), 7.35 (1H, dd, J=3, 9 Hz, 10-H), 7.55 (1H, d, J=3 Hz, 12-H), 8.17 (1H, d, J=9 Hz, 9-H)
6o	0.86 (3H, t, J=7 Hz), 1.27 (3H, t, J=8 Hz), 1.81—1.92 (2H, m), 3.07 (2H, q, J=8 Hz), 5.17 (2H, s), 5.38 (1H, d, J=16 Hz), 5.44 (1H, d, J=16 Hz), 5.92 (2H, s, NH_2), 6.46 (1H, s, 20-OH), 7.04 (1H, d, J=2 Hz, 12-H), 7.11 (1H, dd, J=2, 9 Hz, 10-H), 7.22 (1H, s), 7.93 (1H, d, J=9 Hz, 9-H)
6p	0.88 (3H, t, J=7 Hz), 1.29 (3H, t, J=8 Hz), 1.80—1.92 (2H, m), 3.09 (6H, s, $N(CH_3)_2$), 3.13 (2H, q, J=8 Hz), 5.20 (2H, s), 5.42 (2H, s), 6.47 (1H, s, 20-OH), 7.12 (1H, d, J=3 Hz, 12-H), 7.25 (1H, s), 7.38 (1H, dd, J=3, 10 Hz, 10-H), 8.05 (1H, d, J=10 Hz, 9-H)
6q	0.89 (3H, t, J=7 Hz), 1.31 (3H, t, J=8 Hz), 1.33 (3H, t, J=8 Hz), 1.83—1.91 (2H, m), 2.87 (2H, q, J=8 Hz), 3.15 (2H, q, J=8 Hz), 5.30 (2H, s), 5.43 (2H, s), 6.51 (1H, s, 20-OH), 7.32 (1H, s), 7.61 (1H, dd, J=2, 9 Hz, 10-H), 7.97 (1H, d, J=2 Hz, 12-H), 8.20 (1H, d, J=9 Hz, 9-H)
6r	0.88 (3H, t, J=7 Hz), 1.30 (3H, t, J=8 Hz), 1.81—1.93 (2H, m), 2.65 (3H, s, SCH_3), 3.18 (2H, q, J=8 Hz), 5.28 (2H, s), 5.43 (2H, s), 6.52 (1H, s, 20-OH), 7.30 (1H, s), 7.56 (1H, dd, J=2, 9 Hz, 10-H), 7.85 (1H, d, J=2 Hz, 12-H), 8.15 (1H, d, J=9 Hz, 9-H)
6s	0.89 (3H, t, J=7 Hz), 1.29 (3H, t, J=8 Hz), 1.75—1.97 (2H, m), 3.19 (2H, q, J=8 Hz), 5.27 (2H, s), 5.42 (2H, s), 6.50 (1H, s, 20-OH), 7.30 (1H, s), 8.12 (1H, dd, J=8, 11 Hz), 8.30 (1H, dd, J=9, 12 Hz)
6t	0.88 (3H, t, J=7 Hz), 1.29 (3H, t, J=8 Hz), 1.78—1.97 (2H, m), 3.22 (2H, q, J=8 Hz), 5.31 (2H, s), 5.44 (2H, s), 6.53 (1H, s, 20-OH), 7.31 (1H, s), 8.42 (1H, s), 8.54 (1H, s)
6u	0.88 (3H, t, J=7 Hz), 1.32 (3H, t, J=8 Hz), 1.75—1.97 (2H, m), 3.22 (2H, q, J=8 Hz), 4.08 (3H, s, OCH_3), 5.29 (2H, s), 5.43 (2H, s), 6.50 (1H, s, 20-OH), 7.27 (1H, s), 7.65 (1H, d, J=9 Hz, 9-H), 7.95 (1H, d, J=12 Hz, 12-H)
6v	0.88 (3H, t, J=7 Hz), 1.31 (3H, t, J=8 Hz), 1.74—1.97 (2H, m), 2.50 (3H, s, CH_3), 3.18 (2H, q, J=8 Hz), 5.24 (2H, s), 5.43 (2H, s), 6.52 (1H, s, 20-OH), 7.29 (1H, s), 7.82 (1H, d, J=11 Hz, 12-H), 8.19 (1H, d, J=8 Hz, 9-H)
6w	0.88 (3H, t, J=7 Hz), 1.25 (3H, t, J=8 Hz), 1.76—1.96 (2H, m), 3.12—3.28 (2H, m), 3.92 (3H, s, OCH_3), 3.95 (3H, s, OCH_3), 5.10 (1H, d, J=18 Hz), 5.16 (1H, d, J=18 Hz), 5.42 (2H, s), 6.48 (1H, s, 20-OH), 6.71 (1H, d, J=3 Hz, 10-H), 7.10 (1H, d, J=3 Hz, 12-H), 7.23 (1H, s)
6x	0.88 (3H, t, J=7 Hz), 1.29 (3H, t, J=8 Hz), 1.78—1.94 (2H, m), 3.05 (2H, q, J=8 Hz), 5.22 (2H, s), 5.41 (2H, s), 6.20—6.76 (1H, br, 20-OH), 7.22 (1H, s), 7.38 (1H, s), 7.39 (1H, s), 10.13 (1H, br s, 10- or 11-OH), 10.38 (1H, br s, 10- or 11-OH)
6y	0.88 (3H, t, J=7 Hz), 1.32 (3H, t, J=8 Hz), 1.77—1.95 (2H, m), 3.17 (2H, q, J=8 Hz), 3.97 (3H, s, OCH_3), 4.00 (3H, s, OCH_3), 5.25 (2H, s), 5.42 (2H, s), 6.39—6.55 (1H, br, 20-OH), 7.25 (1H, s), 7.43 (1H, s), 7.53 (1H, s)
6z	0.88 (3H, t, J=7 Hz), 1.30 (3H, t, J=8 Hz), 1.78—1.96 (2H, m), 3.08 (2H, q, J=8 Hz), 5.25 (2H, s), 5.39 (1H, d, J=16 Hz), 5.44 (1H, d, J=16 Hz), 6.47 (1H, s, 20-OH), 7.26 (1H, s), 7.57 (1H, d, J=10 Hz, 9-H), 7.87 (1H, d, J=12 Hz, 12-H), 10.74—10.98 (1H, br, 10-OH)

TABLE VII. Physical Constants of Compounds **8a**—**u**

Compd. No.	Yield (%)	mp °C (dec.)[a]	$[\alpha]_D^{25}$ [b]	$[M+H]^+$ [c]	Formula	Analysis (%) Calcd			Found		
						C	H	N	C	H	N
8a	51	130—133	+27.5	555	$C_{29}H_{35}ClN_4O_5 \cdot 1/2H_2O$	61.75	6.43	9.93	61.74	6.43	9.66
8b	50	136—138	+26.5	569	$C_{30}H_{37}ClN_4O_5 \cdot 1/2H_2O$	62.33	6.63	9.69	62.69	6.58	9.73
8c	47	139—144	+20.5	601	$C_{30}H_{37}ClN_4O_5S \cdot 1/2H_2O$	59.05	6.28	9.18	59.28	6.31	9.01
8d	47	149—151	+18.5	645	$C_{30}H_{37}BrN_4O_5S$	55.81	5.78	8.68	55.95	5.70	8.50
8e	43	130—134	+21.5	581	$C_{31}H_{40}N_4O_5S$	64.11	6.94	9.65	63.88	7.02	9.43
8f	71	164—166	+24.0	539	$C_{29}H_{35}FN_4O_5 \cdot 2H_2O$	60.61	6.84	9.75	60.24	6.80	9.84
8g	75	134—137	+22.0	553	$C_{30}H_{37}FN_4O_5 \cdot 3/2H_2O$	62.16	6.96	9.67	62.06	6.85	9.70
8h	72	135—138	+16.5	585	$C_{30}H_{37}FN_4O_5S \cdot 3/2H_2O$	58.90	6.59	9.16	58.74	6.48	9.21
8i	30	131—133	+25.5	601	$C_{30}H_{37}ClN_4O_5S \cdot H_2O$	58.20	6.35	9.05	57.80	6.05	8.91
8j	56	168—170	+24.0	557	$C_{29}H_{34}F_2N_4O_5 \cdot H_2O$	60.62	6.31	9.75	60.91	6.22	9.75
8k	58	160—162	+21.5	571	$C_{30}H_{36}F_2N_4O_5 \cdot 1/2H_2O$	62.16	6.43	9.67	61.73	6.53	9.75
8l	42	119—125	+22.0	603	$C_{30}H_{36}F_2N_4O_5S \cdot H_2O$	58.05	6.17	9.03	57.83	6.03	9.08
8m	59	153—156	+34.5	589	$C_{29}H_{34}Cl_2N_4O_5 \cdot 1/2H_2O$	58.20	5.89	9.36	58.12	5.74	9.35
8n	60	142—147	+31.5	603	$C_{30}H_{36}Cl_2N_4O_5 \cdot 1/2H_2O$	58.82	6.09	9.15	58.97	5.95	9.06
8o	56	139—142	+27.5	635	$C_{30}H_{36}Cl_2N_4O_5S \cdot 1/2H_2O$	55.90	5.79	8.69	55.94	5.69	8.79
8p	84	164—165	+24.5	553	$C_{30}H_{37}FN_4O_5 \cdot 3/2H_2O$	62.16	6.96	9.67	61.88	6.85	9.71
8q	82	148—154	+24.0	567	$C_{31}H_{39}FN_4O_5 \cdot 1/2H_2O$	64.68	7.00	9.73	64.28	7.07	9.65
8r	80	107—121	+54.0	599	$C_{31}H_{39}FN_4O_5S \cdot 2H_2O$	60.55	6.83	8.83	60.75	6.67	9.15
8s	70	112—117	+28.5	569	$C_{30}H_{37}FN_4O_6 \cdot 3/2H_2O$	60.49	6.77	9.41	60.15	6.78	9.40
8t	67	109—113	+29.5	583	$C_{31}H_{39}FN_4O_6 \cdot 2H_2O$	60.18	7.01	9.06	60.09	6.99	9.29
8u	65	125—129	+22.0	615	$C_{31}H_{39}FN_4O_6S \cdot 3/2H_2O$	59.89	6.60	8.73	59.82	6.40	9.02

a) Recrystallized from n-C_6H_{14}–$CHCl_3$. *b*) 0.2% in MeOH. *c*) MS (SI-MS) m/z.

TABLE VIII. ^{1}H-NMR Spectral Data for Compounds **8a**—**u** (in $CDCl_3$, 400 MHz)

8a	1.11 (3H, t, J=7 Hz), 1.12 (3H, t, J=7 Hz), 1.34 (3H, t, J=8 Hz), 2.20—2.59 (6H, m), 2.26 (6H, s), 2.92—3.10 (2H, m), 3.21—3.35 (1H, m), 3.38—3.51 (1H, m), 4.99 (1H, d, J=19 Hz), 5.10 (1H, d, J=19 Hz), 5.26—5.60 (1H, br), 5.47 (1H, d, J=12 Hz), 5.50 (1H, d, J=12 Hz), 7.43 (1H, br t, J=5 Hz), 7.49 (1H, s), 7.62 (1H, dd, J=2, 9 Hz), 7.73 (1H, d, J=2 Hz), 7.92 (1H, d, J=9 Hz)
8b	0.92 (3H, t, J=7 Hz), 1.12 (3H, t, J=7 Hz), 1.33 (3H, t, J=8 Hz), 1.64 (2H, sextet, J=7 Hz), 2.16—2.35 (3H, m), 2.25 (6H, s), 2.38—2.59 (3H, m), 2.90—3.07 (2H, m), 3.21—3.32 (1H, m), 3.39—3.50 (1H, m), 4.96 (1H, d, J=19 Hz), 5.07 (1H, d, J=19 Hz), 5.31—5.60 (1H, br), 5.45 (1H, d, J=12 Hz), 5.50 (1H, d, J=12 Hz), 7.46 (1H, br t, J=5 Hz), 7.47 (1H, s), 7.60 (1H, dd, J=2, 9 Hz), 7.68 (1H, d, J=2 Hz), 7.89 (1H, d, J=9 Hz)
8c	1.10 (3H, t, J=7 Hz), 1.34 (3H, t, J=8 Hz), 2.09 (3H, s), 2.23—2.35 (1H, m), 2.31 (6H, s), 2.44—2.68 (5H, m), 2.70—2.80 (2H, m), 2.93—3.09 (2H, m), 3.24—3.35 (1H, m), 3.43—3.57 (1H, m), 5.00 (1H, d, J=19 Hz), 5.09 (1H, d, J=19 Hz), 5.51 (2H, s), 7.51 (1H, s), 7.52 (1H, br t, J=5 Hz), 7.62 (1H, dd, J=2, 9 Hz), 7.74 (1H, d, J=2 Hz), 7.93 (1H, d, J=9 Hz)
8d	1.09 (3H, t, J=7 Hz), 1.36 (3H, t, J=8 Hz), 2.10 (3H, s), 2.22—2.35 (1H, m), 2.25 (6H, s), 2.38—2.55 (3H, m), 2.57—2.68 (2H, m), 2.71—2.81 (2H, m), 2.97—3.10 (2H, m), 3.24—3.34 (1H, m), 3.38—3.50 (1H, m), 4.98—5.34 (1H, br), 5.05 (1H, d, J=19 Hz), 5.13 (1H, d, J=19 Hz), 5.49 (1H, d, J=12 Hz), 5.57 (1H, d, J=12 Hz), 7.40 (1H, br t, J=6 Hz), 7.53 (1H, s), 7.77 (1H, dd, J=2, 9 Hz), 7.90 (1H, d, J=9 Hz), 8.00 (1H, d, J=2 Hz)
8e	1.09 (3H, t, J=7 Hz), 1.32 (3H, t, J=8 Hz), 2.09 (3H, s), 2.20—2.36 (1H, m), 2.26 (6H, s), 2.41—2.55 (3H, m), 2.54 (3H, s), 2.57—2.67 (2H, m), 2.71—2.81 (2H, m), 2.93—3.09 (2H, m), 3.25—3.35 (1H, m), 3.41—3.52 (1H, m), 4.99 (1H, d, J=19 Hz), 5.07 (1H, d, J=19 Hz), 5.50 (1H, d, J=12 Hz), 5.54 (1H, d, J=12 Hz), 7.44 (1H, br t, J=6 Hz), 7.51 (1H, dd, J=2, 8 Hz), 7.52 (1H, s), 7.55 (1H, br s), 7.94 (1H, d, J=8 Hz)
8f	1.08 (3H, t, J=7 Hz), 1.13 (3H, t, J=7 Hz), 1.36 (3H, t, J=8 Hz), 2.24—2.63 (6H, m), 2.30 (6H, s), 3.02—3.20 (2H, m), 3.25—3.55 (2H, m), 5.07 (1H, d, J=19 Hz), 5.14 (1H, d, J=19 Hz), 5.21—5.41 (1H, br), 5.51 (2H, br s), 7.31 (1H, ddd, J=2, 9, 10 Hz), 7.41 (1H, br t, J=5 Hz), 7.55 (1H, s), 7.69 (1H, dd, J=2, 10 Hz), 7.93 (1H, dd, J=6, 9 Hz)
8g	0.93 (3H, t, J=7 Hz), 1.09 (3H, t, J=7 Hz), 1.36 (3H, t, J=8 Hz), 1.65 (2H, sextet, J=7 Hz), 2.20—2.36 (3H, m), 2.25 (6H, s), 2.39—2.53 (3H, m), 3.02—3.17 (2H, m), 3.25—3.35 (1H, m), 3.38—3.49 (1H, m), 5.05 (1H, d, J=19 Hz), 5.12 (1H, d, J=19 Hz), 5.22—5.40 (1H, br), 5.47 (1H, d, J=12 Hz), 5.53 (1H, d, J=12 Hz), 7.30 (1H, ddd, J=3, 9, 10 Hz), 7.38 (1H, br t, J=5 Hz), 7.53 (1H, s), 7.67 (1H, dd, J=3, 10 Hz), 7.91 (1H, dd, J=6, 9 Hz)
8h	1.09 (3H, t, J=7 Hz), 1.36 (3H, t, J=8 Hz), 2.10 (3H, s), 2.20—2.36 (1H, m), 2.25 (6H, s), 2.40—2.54 (3H, m), 2.58—2.68 (2H, m), 2.72—2.82 (2H, m), 3.04—3.18 (2H, m), 3.25—3.36 (1H, m), 3.39—3.50 (1H, m), 4.99—5.31 (1H, br), 5.06 (1H, d, J=19 Hz), 5.14 (1H, d, J=19 Hz), 5.49 (1H, d, J=12 Hz), 5.58 (1H, d, J=12 Hz), 7.31 (1H, ddd, J=3, 9, 10 Hz), 7.39 (1H, br t, J=5 Hz), 7.55 (1H, s), 7.68 (1H, dd, J=3, 10 Hz), 7.93 (1H, dd, J=6, 9 Hz)
8i	1.09 (3H, t, J=7 Hz), 1.37 (3H, t, J=8 Hz), 2.10 (3H, s), 2.22—2.36 (1H, m), 2.26 (6H, s), 2.40—2.54 (3H, m), 2.56—2.68 (2H, m), 2.70—2.81 (2H, m), 3.02—3.18 (2H, m), 3.25—3.35 (1H, m), 3.40—3.52 (1H, m), 4.97—5.35 (1H, br), 5.05 (1H, d, J=19 Hz), 5.13 (1H, d, J=19 Hz), 5.49 (1H, d, J=11 Hz), 5.57 (1H, d, J=11 Hz), 7.42 (1H, br t, J=6 Hz), 7.46 (1H, dd, J=2, 9 Hz), 7.54 (1H, s), 7.85 (1H, d, J=9 Hz), 8.02 (1H, d, J=2 Hz)
8j	1.07 (3H, t, J=7 Hz), 1.13 (3H, t, J=7 Hz), 1.36 (3H, t, J=8 Hz), 2.18—2.52 (6H, m), 2.24 (6H, s), 3.07 (2H, q, J=8 Hz), 3.25—3.36 (1H, m), 3.38—3.49 (1H, m), 5.06—5.38 (1H, br), 5.10 (1H, d, J=19 Hz), 5.16 (1H, d, J=19 Hz), 5.48 (1H, d, J=12 Hz), 5.56 (1H, d, J=12 Hz), 7.36 (1H, br t, J=5 Hz), 7.55 (1H, s), 7.69 (1H, d, J=8, 11 Hz), 7.86 (1H, d, J=8, 11 Hz)
8k	0.93 (3H, t, J=7 Hz), 1.07 (3H, t, J=7 Hz), 1.36 (3H, t, J=8 Hz), 1.65 (2H, sextet, J=7 Hz), 2.16—2.36 (3H, m), 2.24 (6H, s), 2.38—2.52 (3H, m), 3.07 (2H, q, J=8 Hz), 3.25—3.36 (1H, m), 3.37—3.48 (1H, m), 5.05—5.34 (1H, br), 5.10 (1H, d, J=19 Hz), 5.16 (1H, d, J=19 Hz), 5.47 (1H, d, J=12 Hz), 5.57 (1H, d, J=12 Hz), 7.35 (1H, br t, J=5 Hz), 7.55 (1H, s), 7.70 (1H, dd, J=8, 11 Hz), 7.86 (1H, dd, J=8, 11 Hz)
8l	1.08 (3H, t, J=7 Hz), 1.36 (3H, t, J=8 Hz), 2.10 (3H, s), 2.20—2.34 (1H, m), 2.25 (6H, s), 2.40—2.54 (3H, m), 2.58—2.70 (2H, m), 2.71—2.83 (2H, m), 3.07 (2H, q, J=8 Hz), 3.26—3.37 (1H, m), 3.40—3.52 (1H, m), 5.08 (1H, d, J=19 Hz), 5.15 (1H, d, J=19 Hz), 5.49 (1H, d, J=12 Hz), 5.60 (1H, d, J=12 Hz), 7.43 (1H, br t, J=5 Hz), 7.55 (1H, s), 7.67 (1H, dd, J=8, 11 Hz), 7.83 (1H, dd, J=8, 11 Hz)
8m	1.11 (3H, t, J=7 Hz), 1.12 (3H, t, J=7 Hz), 1.38 (3H, t, J=8 Hz), 2.19—2.38 (3H, m), 2.27 (6H, s), 2.40—2.58 (3H, m), 2.96—3.14 (2H, m), 3.21—3.32 (1H, m), 3.40—3.51 (1H, m), 4.99 (1H, d, J=19 Hz), 5.08 (1H, d, J=19 Hz), 5.45 (1H, d, J=12 Hz), 5.49 (1H, d, J=12 Hz), 7.46 (1H, s), 7.49 (1H, br t, J=5 Hz), 7.89 (1H, s), 8.05 (1H, s)
8n	0.92 (3H, t, J=7 Hz), 1.11 (3H, t, J=7 Hz), 1.38 (3H, t, J=8 Hz), 1.64 (2H, sextet, J=7 Hz), 2.18—2.35 (3H, m), 2.26 (6H, s), 2.39—2.56 (3H, m), 2.98—3.14 (2H, m), 3.21—3.32 (1H, m), 3.38—3.52 (1H, m), 5.00 (1H, d, J=19 Hz), 5.09 (1H, d, J=19 Hz), 5.48 (2H, s), 7.45 (1H, br t, J=5 Hz), 7.47 (1H, s), 7.92 (1H, s), 8.07 (1H, s)
8o	1.09 (3H, t, J=7 Hz), 1.38 (3H, t, J=8 Hz), 2.09 (3H, s), 2.21—2.37 (1H, m), 2.29 (6H, s), 2.43—2.67 (5H, m), 2.70—2.81 (2H, m), 2.99—3.16 (2H, m), 3.24—3.35 (1H, m), 3.42—3.55 (1H, m), 5.03 (1H, d, J=19 Hz), 5.11 (1H, d, J=19 Hz), 5.49 (1H, d, J=12 Hz), 5.54 (1H, d, J=12 Hz), 7.49 (1H, br t, J=6 Hz), 7.50 (1H, s), 7.96 (1H, s), 8.11 (1H, s)
8p	1.09 (3H, t, J=7 Hz), 1.13 (3H, t, J=7 Hz), 1.36 (3H, t, J=8 Hz), 2.19—2.57 (6H, m), 2.25 (6H, s), 2.48 (3H, s), 2.99—3.16 (2H, m), 3.23—3.34 (1H, m), 3.38—3.49 (1H, m), 5.02 (1H, d, J=19 Hz), 5.10 (1H, d, J=19 Hz), 5.49 (2H, s), 7.38 (1H, br t, J=5 Hz), 7.49 (1H, s), 7.58 (1H, d, J=11 Hz), 7.67 (1H, d, J=8 Hz)
8q	0.93 (3H, t, J=7 Hz), 1.09 (3H, t, J=7 Hz), 1.36 (3H, t, J=8 Hz), 1.64 (2H, sextet, J=7 Hz), 2.19—2.35 (3H, m), 2.24 (6H, s), 2.38—2.54 (3H, m), 2.48 (3H, s), 2.99—3.16 (2H, m), 3.23—3.34 (1H, m), 3.38—3.50 (1H, m), 5.02 (1H, d, J=19 Hz), 5.10 (1H, d, J=19 Hz), 5.21—5.42 (1H, br), 5.47 (1H, d, J=12 Hz), 5.51 (1H, d, J=12 Hz), 7.38 (1H, br t, J=5 Hz), 7.49 (1H, s), 7.58 (1H, d, J=11 Hz), 7.68 (1H, d, J=8 Hz)
8r	1.10 (3H, t, J=7 Hz), 1.36 (3H, t, J=8 Hz), 2.09 (3H, s), 2.22—2.35 (1H, m), 2.28 (6H, s), 2.40—2.66 (5H, m), 2.46 (3H, s), 2.72—2.81 (2H, m), 2.97—3.14 (2H, m), 3.24—3.34 (1H, m), 3.43—3.54 (1H, m), 4.98 (1H, d, J=19 Hz), 5.07 (1H, d, J=19 Hz), 5.51 (2H, s), 7.47 (1H, s), 7.48 (1H, br t, J=5 Hz), 7.54 (1H, d, J=11 Hz), 7.63 (1H, d, J=8 Hz)
8s	1.12 (3H, t, J=7 Hz), 1.13 (3H, t, J=7 Hz), 1.31 (3H, t, J=8 Hz), 2.21—2.63 (6H, m), 2.28 (6H, s), 2.88—3.08 (2H, m), 3.18—3.30 (1H, m), 3.40—3.52 (1H, m), 4.00 (3H, s), 4.89 (1H, d, J=19 Hz), 5.02 (1H, d, J=19 Hz), 5.41 (1H, d, J=12 Hz), 5.50 (1H, d, J=12 Hz), 6.89 (1H, d, J=9 Hz), 7.40 (1H, s), 7.46 (1H, br t, J=6 Hz), 7.57 (1H, d, J=12 Hz)
8t	0.92 (3H, t, J=7 Hz), 1.13 (3H, t, J=7 Hz), 1.31 (3H, t, J=8 Hz), 1.63 (2H, sextet, J=7 Hz), 2.18—2.37 (3H, m), 2.28 (6H, s), 2.39—2.63 (3H, m), 2.87—3.07 (2H, m), 3.17—3.30 (1H, m), 3.41—3.53 (1H, m), 4.00 (3H, s), 4.89 (1H, d, J=19 Hz), 5.02 (1H, d, J=19 Hz), 5.34—5.57 (1H, br), 5.40 (1H, d, J=12 Hz), 5.49 (1H, d, J=12 Hz), 6.89 (1H, d, J=9 Hz), 7.40 (1H, s), 7.46 (1H, br t, J=6 Hz), 7.57 (1H, d, J=12 Hz)
8u	1.11 (3H, t, J=7 Hz), 1.33 (3H, t, J=8 Hz), 2.09 (3H, s), 2.22—2.36 (1H, m), 2.26 (6H, s), 2.39—2.69 (5H, m), 2.71—2.80 (2H, m), 2.94—3.10 (2H, m), 3.21—3.34 (1H, m), 3.39—3.51 (1H, m), 4.03 (3H, s), 4.93—5.38 (1H, br), 4.97 (1H, d, J=19 Hz), 5.07 (1H, d, J=19 Hz), 5.50 (2H, s), 7.01 (1H, d, J=9 Hz), 7.38 (1H, br t, J=6 Hz), 7.44 (1H, s), 7.64 (1H, d, J=12 Hz)

TABLE IX. Hydrolysis of Amides **7i** and **9** at 37 °C in Phosphate Buffer (pH 7.2)

Time (h)	Ratio (mol %)				Ratio (mol %)			
	7i[a]	Lactone	Acid	Total[b]	**9**[a]	Lactone	Acid	Total
0	44.5	32.2	—	76.7	100.0	—	—	100.0
0.5	—	54.7	32.5	87.2	100.0	—	—	100.0
3	—	18.7	69.2	87.9	94.5	0.3	0.6	95.4
6	—	15.8	69.4	85.2	93.9	0.1	5.2	99.2
24	—	16.2	69.4	85.6	77.8	1.8	20.5	100.1

a) Initial concentration of **7i**: 0.26 nmol/ml. Initial concentration of **9**: 0.22 nmol/ml. *b*) The total peak area was reduced due to the precipitation of **6i** in the sample solution. —: not detected.

eluent. The combined fractions containing the title compound were evaporated to dryness under reduced pressure. The residue was recrystallized from n-C_6H_{14}–$CHCl_3$.

Compound 9 (Isopropylamide of 6i) A mixture of **6i** (40 mg, 0.10 mmol), isopropylamine (4 ml) and CH_2Cl_2 (4 ml) was refluxed for 5 h under a stream of N_2 gas. The mixture was evaporated to dryness under reduced pressure. The residue was purified through silica gel column chromatography with 3% MeOH–$CHCl_3$. The combined fractions containing the title compound were concentrated *in vacuo* and the residue was recrystallized from n-C_6H_{14}–$CHCl_3$. **9**: Yield 42 mg, 91%. mp 249—251 °C (dec.). IR (KBr)ν: 1665, 1645, 1580, 1560, 1510 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.09 (3H, t, J=7 Hz), 1.17 (3H, t, J=8 Hz), 1.20 (3H, d, J=7 Hz), 1.21 (3H, d, J=7 Hz), 2.18—2.30 (1H, m), 2.39—2.52 (1H, m), 2.55 (3H, s), 2.58—2.63 (2H, m), 4.01—4.16 (1H, m), 4.75—5.35 (1H, br), 4.85 (1H, d, J=19 Hz), 4.86 (1H, d, J=13 Hz), 4.91 (1H, d, J=19 Hz), 4.98 (1H, d, J=13 Hz), 6.06 (1H, br s), 6.92 (1H, d, J=8 Hz), 7.36 (1H, s), 7.44 (1H, s), 7.53 (1H, dd, J=2, 9 Hz), 7.93 (1H, d, J=9 Hz). SI-MS m/z: 500 $(M+H)^+$. *Anal.* Calcd for $C_{26}H_{31}N_3O_4 \cdot 1/4H_2O$: C, 68.78; H, 6.99; N, 9.25. Found: C, 68.76; H, 6.98; N, 9.25.

Hydrolysis of Amides 7i and 9 under Physiological Conditions (Table IX).

Preparation of Sample Solutions Compound **7i** or **9** (1.0 mg) was dissolved in MeOH (100 ml) and 1 ml of the solution was diluted with 0.01 M phosphate buffer (pH 7.2) to make a volume of 100 ml. The solution was pipetted into tubes and the tubes were screwed up tight and shaken in a bath at about 37 °C. A part of the solution was taken for HPLC analysis at 0.5, 3, 6 and 24 h.

Calibration Compound **6i** (1.0 mg) was dissolved in MeOH (100 ml) and 1 ml of the solution was diluted with a mixture of water (100 ml) and 0.1 N HCl (5 ml) to make a volume of 100 ml. The solution was used for calibration of the lactone form. Compound **9** (1.0 mg) was dissolved in MeOH (100 ml) and 1 ml of the solution was diluted with 0.01 M phosphate buffer (pH 7.2) to make a volume of 100 ml. The solution was used for the calibration of the amides (**7i, 9**) and the hydroxy-acid form.

HPLC Operating Conditions Column: InertSil ODS 2 (5 × 150 mm). Mobile phase: 0.01 M KH_2PO_4–MeOH (1 : 1). Detection: fluorescence, Ex. (375 nm), Em. (425 nm). Injection volume: 20 μl.

Cytotoxicity KB and L1210 cells were grown at 37 °C in Eagle's minimal essential medium and RPMI medium, respectively, with 10% fetal calf serum under a humidified atmosphere containing 5% CO_2. About 1.5×10^4 cells were exposed to media containing the test samples at various concentrations for 3 d. The treated cells were counted with a Coulter Counter Model ZBI (Coulter Electronics Inc.; Hialeah, FL). The ED_{50} values were calculated from the dose–response curves by interpolation.

Inhibition of DNA Topoisomerase I The compounds listed in Table III were dissolved in dimethylsulfoxide (DMSO, 10 mg/ml) and diluted appropriately with DMSO. To a mixed buffer (1 mM EDTA, pH 7.5 10 mM Tris–HCl buffer, 100 mM NaCl), 0.5 μg of the substrate (plasmid pBR322 DNA), the DMSO sample solution (2 μl) and 0.5 unit of topoisomerase I (purchased from TopoGEN, Inc.; Columbus, Ohio, U.S.A.) were added successively. The test solution (20 μl) was incubated at 37 °C for 30 min. Then 2 μl of a terminating solution (5% sodium dodecyl sulfate (SDS), 70% glycerol, 0.025% bromophenol blue and 0.025% xylene cyanol) was added to the mixture and 10 μl of the resulting mixture was electrophoresed using 1% agarose gel at 100 V for 40 min in TAE buffer (pH 8.1 30 mM Tris–acetate, 2 mM EDTA). The gel was stained with ethidium bromide (0.5 μg/ml) for 30 min, and the dyed gel was photographed while being irradiated with UV light (312 nm). The inhibitory concentration (μg/ml) means the minimum sample concentration which revealed a band corresponding to supercoiled DNA on the photograph. The blank solution had no effect on the substrate DNA or the enzyme.

Antitumor Activity L1210 leukemia cells (10^5) were implanted intraperitoneally (i.p.) into 7-week-old BDF_1 female mice on day 0. Meth A cells (10^5) were inoculated subcutaneously into 7-week-old BALB/c female mice on day 0. Six mice were used for each dose. The sample was dissolved in distilled water and administered i.p. (L1210) or i.v. (Meth A) on days 1, 5 and 9. Control mice were injected with distilled water, and surviving mice were counted on day 40 (L1210) or on day 21 (Meth A).

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