Synthesis and Antitumor Activity of 9-Acyloxyellipticines

Naoyuki HARADA, Kunihiko OZAKI, Kouji ODA, Noriyuki NAKANISHI, Motoaki OHASHI, Tomiki HASHIYAMA, and Kenji TSUJIHARA*

Lead Optimization Research Laboratory, Tanabe Seiyaku Co., Ltd., 2–2–50 Kawagishi, Toda-shi, Saitama 335, Japan.
Received December 12, 1996; accepted March 3, 1997

Various kinds of water-soluble 9-acyloxyellipticine derivatives were synthesized in a search for compounds with potent antitumor activity. Antitumor activities against several tumors in mice (P388 leukemia, colon 26, Lewis lung carcinoma and B16 melanoma) were evaluated by using intravenous administration. Many compounds exhibited good antitumor activities; in particular, the glutarate derivative (5o) showed potent antitumor activity. This compound (5o) may be converted to 9-hydroxyellipticine (2) by enzyme-catalyzed hydrolysis in the body.

Key words 9-acyloxyellipticine; water-soluble antitumor substance; P388 leukemia; Lewis lung carcinoma; B16 melanoma

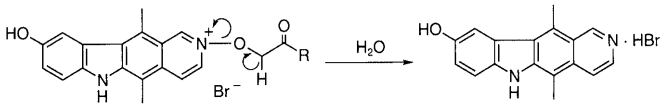
As an ongoing part of our work on water-soluble ellipticine derivatives, we previously reported novel quaternary salts of 9-hydroxyellipticine 2-oxide (**1**) which exhibited low acute toxicity yet potent antitumor activity.[1] However, these compounds were unstable in water, being readily converted to 9-hydroxyellipticine (9-HE) (**2**) (Chart 1).[2] Consequently, we terminated development. However, the above observation suggested the feasibility of designing a prodrug of 9-HE (**2**).

We have reported that 9-HE (**2**) caused selective inhibition of phosphorylation of mutant p53 protein, a tumor suppressor gene product, and there was a good correlation between this inhibition and the cytotoxic activity of 9-HE (**2**). In contrast, 2-*N*-substituted derivatives (**3**[3] and **4**[4]) of 9-HE (**2**) barely inhibited this phosphorylation.[5]

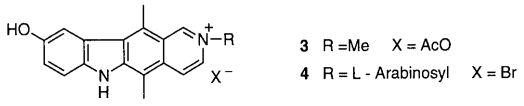
In this paper, we describe the synthesis and antitumor activity of various kinds of 9-acyloxyellipticines[6] as a part of our search for derivatives that have good water solubility and improved pharmacokinetics and tissue distribution.

Chemistry

Several groups have reported the synthesis of 9-HE (**2**) and its derivatives.[6–9] The synthetic methods of **2** can be classified into 2 types as follows: a) demethylation of 9-methoxyellipticine[6,8] and b) regioselective oxidation of ellipticine.[9] Among the numerous papers, Guthrie's method (type a) was considered to be more efficient and adaptable for large-scale production of **2**.[6] However, it has become apparent that a tedious purification procedure is inevitable in order to obtain pure **2** in the final demethylation step using pyridine hydrochloride. Furthermore, other demethylation reagents such as boron tribromide, trimethylsiliyl iodide and 47% aqueous hydrogen bromide proved to be impractical. Therefore, we turned our attention to the use of a pivaloyl protecting group in the early stage of the synthesis, as shown in Chart 2. Oku

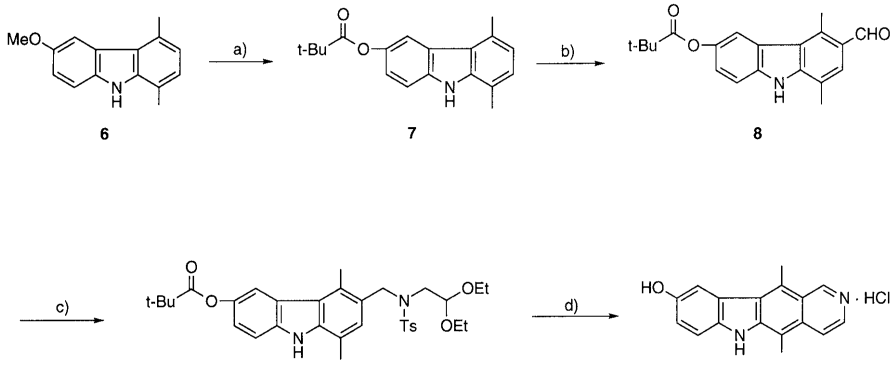


1 **2**·HBr



3 R = Me X = AcO
4 R = L-Arabinosyl X = Br

Chart 1



6 **7** **8**

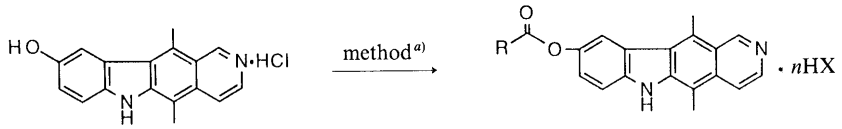
9 **2** · HCl

a) *t*-BuCOCl (1.5 eq), NaI (2 eq), MeCN, reflux, 88%; b) *N*-Methylformanilide (1.5 eq), $POCl_3$ (1.5 eq), toluene, reflux, 96%; c) 1) $H_2NCH_2CH(OEt)_2$ (1.1 eq), $TsOH \cdot H_2O$ (cat.), toluene, reflux, 2) H_2, PtO_2, EtOH, r.t., 3) TsCl (1.1 eq), Et_3N (2 eq), THF, 0 °C ~ r.t. 63% (3 steps); d) 6*N* HCl, dioxane, reflux, 96%

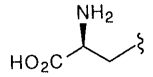
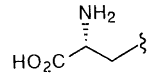
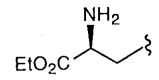
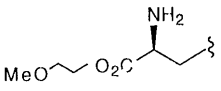
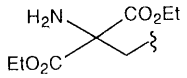
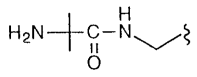
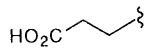
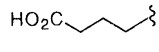
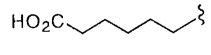
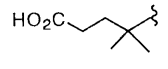
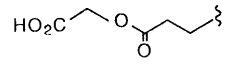
Chart 2

* To whom correspondence should be addressed.

Table 1. Water-soluble 9-Acyloxyellipticine Derivatives (**5**)



2·HCl **5**

5	Method[a)]	R	HX	*n*	Yield (%) (from **2**·HCl)
5a	A	$EtOCH_2$–	MsOH	1	51
5b	A	$MeOCH_2CH_2OCH_2$–	MsOH	1	43
5c	A	iso-$BuOCH_2CH_2OCH_2$–	MsOH	1	70
5d	A	$EtOCH_2CH_2OCH_2CH_2OCH_2CH_2OCH_2$–	HCl	1	29
5e	B		HCl	2	35
5f	B		HCl	2	32
5g	B		HCl	2	66
5h	C		HCl	2	54
5i	C		HCl	2	56
5j	B		HCl	2	66
5k	B		HCl	2	61
5l	B		HCl	2	50
5m	B		HCl	2	17
5n	C		MsOH	1	54
5o	C		HCl	1	50
5p	C		HCl	1	51
5q	C		HCl	1	49
5r	C		MsOH	1	58

a) Method A: R-CO_2H, DCC, HOBT, DMAP (cat.), Et_3N, DMF r.t. then HX; Method B: 1) R′-CO_2H, DCC, HOBT, DMAP (cat.), Et_3N, DMF, r.t. 2) HCl–dioxane, r.t.; Method C: 1) R′-CO_2H, DCC, HOBT, DMAP (cat.), Et_3N, DMF, r.t. 2) H_2, 10%Pd-C, HX, AcOH–H_2O, r.t.

and co-workers reported that alkyl ethers were regioselectively cleaved by the reagent system of sodium iodide and acyl chloride to afford the corresponding acyloxy derivatives.[10] Therefore, we tried to apply the reaction of aryl alkyl ether. 6-Methoxy-1,4-dimethylcarbazole (**6**)[6,8a] was reacted with sodium iodide (2.0 eq) and pivaloyl chloride (1.5 eq) in refluxing acetonitrile to give 1,4-dimethyl-6-pivaloyloxycarbazole (**7**) in 88% yield. This provides the basis for a convenient method to prepare the acyloxyaryl compounds from the aryl alkyl ethers.

Next, we examined regiospecific formylation of the carbazole (**7**). Compound **7** was reacted with the Vilsmeier reagent, prepared from *N*-methylformanilide (1.5 eq) and phosphorus oxychloride (1.5 eq), in refluxing toluene to give the desired 3-formyl derivative (**8**) in excellent yield (96%). In this reaction, a remarkable solvent effect was observed.[11]

The 3-formyl derivative (**8**) was converted to the *N*-tosyl derivative (**9**) by employing Guthrie's method.[6] Compound **8** was allowed to react with aminoacetaldehyde diethyl acetal in toluene in the presence of a catalytic amount of *p*-toluenesulfonic acid, followed by platinum (IV) oxide-catalyzed hydrogenation in ethanol to give the amine, which was treated with tosyl chloride to afford the corresponding *N*-tosyl derivative (**9**) in good yield (63%, 3 steps). Treatment of **9** in refluxing 6 N hydrogen chloride and dioxane achieved ring closure and depivaloylation simultaneously to give pure 9-hydroxyellipticine hydrochloride (**2**·HCl) in excellent yield (96%). This improved method is considered to be adaptable for industrial-scale production of **2**.

Finally, various kinds of water-soluble 9-acyloxyellipticine derivatives (**5**) were synthesized as shown in Table 1. The synthesized 9-acyloxyellipticines (**5**) were classified into 3 types as follows: a) ether type derivatives (**5a**—**d**), b) amine type derivatives (**5e**—**m**) and c) carboxylic acid type derivatives (**5n**—**r**). The ether type derivatives (**5a**—**d**) were prepared from **2**·HCl and the corresponding carboxylic acid in the presence of dicyclohexylcarbodiimide (DCC), 1-hydroxybenzotriazole (HOBT), triethylamine and a catalytic amount of 4-dimethylaminopyridine (DMAP) in *N*,*N*-dimethylformamide (DMF). Similarly, **2**·HCl was reacted with protected amino acid and carboxylic acid derivatives, followed by appropriate deprotection to give the desired amine (**5e**—**m**) and carboxylic acid type derivatives (**5n**—**r**), respectively. The amine type derivatives of **5** were soluble to the extent of more than 40 mg/ml in water, and the ether and the carboxylic acid type derivatives of **5** showed solubility exceeding 10 mg/ml in water.

Antitumor Activity and Discussion

The water-soluble derivatives **5** described above were examined for antitumor activity against P388 leukemia and colon 26 in mice by intravenous (i.v.) administration and elliptinium acetate (**3**) was included for comparison (Tables 2 and 3).

A number of water-soluble derivatives (**5**) showed potent antitumor activities, whereas the elliptinium acetate (**3**) exhibited weak activity. In the case of the ether derivatives (**5a**—**d**), longer side chain compounds were superior to shorter ones in antitumor activity. In addition, the amine derivatives (**5e**—**m**) showed potent antitumor activity. In particular, the aspartate derivative (**5h**), which has a β-amino acid moiety, caused >70% increase in life span (ILS) in mice with P388 leukemia, and a high therapeutic ratio and a 60% cure rate were observed in mice with colon 26 tumor. Modification of the carboxylic acid of the aspartate derivative (**5h**) to esters (**5j**—**l**) tended to reduce the activity. The absolute configuration of the amine derivatives influenced the activity slightly (**5h** *vs.* **5i**). The carboxylic acid type derivatives (**5n**—**r**) showed potent activity as well, except for the branched one (**5q**).

Next, we examined antitumor activity against Lewis lung carcinoma in mice by i.v. administration (Table 4).

Large differences in efficacy were observed among the water-soluble derivatives. The ether derivative (**5b**) and

Table 2. Antitumor Activity against P388 Leukemia

No.	OD (mg/kg)[a]	ILS (%)[b]	TR[c]
5a	40	31.9	1.0
5b	40	59.1	1.8
5c	25	16.5	—
5d	40	71.4	2.4
5e	40	57.0	2.0
5f	40	46.2	1.8
5g	40	62.6	3.1
5h	70	71.1	1.6
5i	70	62.6	1.5
5j	40	60.4	1.7
5k	40	40.7	1.4
5l	50	16.5	—
5m	70	62.6	2.5
5n	40	62.6	1.7
5o	70	78.0	2.3
5p	50	78.9	1.7
5q	12.5	14.9	—
5r	40	42.9	1.3
2[d]	30	64.8	2.0
3	2.5	6.8	—

a) Optimal dose of drug. *b*) Increase in life span of mice when treated at the optimal dose. ILS (%)=(mean survival time of treated group/that of control group−1)×100. *c*) Therapeutic ratio=OD/ILS_{30}. *d*) The methanesulfonate of **2** was used.

Table 3. Antitumor Activity against Colon 26

No.	OD (mg/kg)[a]	Inhibition (%)[b]	TR[c]
5b	20	73.8	1.8
5d	20	69.2	2.1
5e	40	89.2	4.0
5f	20	77.2	2.0
5g	20	81.1	2.4
5h	70	99.3	3.2
5i	70	91.6	3.2
5j	40	99.9	3.1
5k	70	88.0	3.0
5m	40	90.1	3.3
5n	40	95.9	3.8
5o	40	93.8	2.6
5r	40	97.3	1.9
2[a]	40	95.0	3.5
3	1.3	84.2	1.2

a) See Table 2. *b*) Inhibition of tumor growth when treated at the optimal dose. Inhibition (%)=(1−mean tumor weight of treated group/that of control group)×100. *c*) Therapeutic ratio=OD/ED_{50}. ED_{50}, daily dose providing 50% inhibition of the tumor growth compared to the control.

Table 4. Antitumor Activity against Lewis Lung Carcinoma

No.	OD (mg/kg)[a]	ILS (%)[a]	Cure[b]	TR[a]
5b	40	20.8	0/5	—
5e	80	33.7	0/5	1.2
5h	70	212.9	4/5	2.0
5i	70	191.0	3/5	1.5
5j	60	30.3	0/5	1.0
5k	60	30.3	0/5	1.0
5o	40	230.4	4/5	1.9
5r	60	35.9	0/5	1.1
2[a]	30	42.8	0/5	1.4
3	1.6	12.6	0/5	—
ADM[c]	5	66.3	0/5	1.9

a) See Table 2. *b*) Cure was defined as survival for 60 d with no detectable tumor. *c*) Adriamycin.

Table 5. Antitumor Activity against B16 Melanoma

No.	Schedule[a]	OD (mg/kg)[b]	ILS (%)[b]	Cure[c]	TR[b]
5h	A	70	73.3	0/5	1.3
5o	B	150	68.7	2/5	1.5
2[b]	A	25	6.6	0/5	—
	B	75	26.5	0/5	—
3	A	0.78	3.0	0/5	—
	B	5	3.6	0/5	—

a) Drugs were administered A, i.v. on days 1 to 7; B, i.v. on days 1, 5, 9. *b*) See Table 2. *c*) Cure was defined as survival for 90 d with no detectable tumor.

α-amino acid derivative (**5e**) had weak activity. In contrast, aspartate derivatives (**5h** and **5i**) showed potent activity, while their ester derivatives (**5j** and **5k**) had weak activity. Furthermore, the glutarate derivative (**5o**), which has a terminal carboxylic acid, had potent activity. Elliptinium acetate (**3**) had no activity and 9-HE (**2**) had weak activity. Compounds **5h**, **5i** and **5o** gave high ILS values (212.9%, 191.0% and 230.4% respectively), and resulted in 80%, 60% and 80% cure rates, respectively. Finally, selected carboxylates (**5h** and **5o**) were evaluated against subcutaneous (s.c.) implanted B16 melanoma in mice by i.v. administration (Table 5). These compounds displayed good activity (ILS >65%), and in the case of **5o**, a 40% cure rate was observed.

As a preliminary examination of the pharmacokinetics of **5o**, which had good activity, we tested the stability of **5o** in plasma. The period of half decay ($t_{1/2}$) of **5o** in phosphate buffer (pH 7.4) at 37 °C was 2.6 (±0.03) h.[12] However, the $t_{1/2}$ for **5o** in mouse plasma or blood was only 2.7 (±0.35) or 3.5 (±0.11) min, respectively. The metabolite of **5o** was 9-HE (**2**). It is clear that **5o** is readily converted to 9-HE (**2**) by enzyme-catalyzed hydrolysis in the body.

In conclusion, we have synthesized water-soluble 9-acyloxyellipticine derivatives having potent antitumor activity. In particular, **5o** showed strong antitumor activity against P388 leukemia, colon 26, Lewis lung carcinoma and B16 melanoma in mice. A preliminary study of the hydrolysis kinetics suggested that **5o** was converted to 9-HE (**2**) by enzyme-catalyzed hydrolysis in the body. Moreover, 9-HE (**2**) strongly and selectively inhibited mutant p53 protein phosphorylation, suggesting that accumulation of unphosphorylated mutant p53 protein may induce apoptosis. Accordingly, **5o** is a promising anticancer agent, with a new mechanism of action. Among the 9-acyloxyellipticines studied, **5o** was selected as a candidate for further testing.

Experimental

Melting points were determined with a Büchi 535 digital melting point apparatus. All melting points are uncorrected. IR spectra were obtained with an Analect FX-6200 FT-IR spectrophotometer. ^{1}H-NMR spectra were measured with a JEOL JNM-FX-200 spectrometer or a Varian Gemini-300 spectrometer. Mass spectra (MS) were recorded with a Hitachi RMU-6 or a JEOL JMS-HX100 mass spectrometer. Microanalyses were performed on a Perkin–Elmer 240B CHN analyzer. Silica gel 60K-230 (230—430 mesh, Katayama) was used for column chromatography. In general, reactions were carried out in dry solvents under an argon atmosphere unless otherwise mentioned.

1,4-Dimethyl-6-pivaloyloxycarbazole (7) Pivaloyl chloride (209 g, 1.73 mol) and sodium iodide (346 g, 2.31 mol) were added to a solution of 6-methoxy-1,4-dimethylcarbazole (**6**, 260 g, 1.15 mol) in CH_3CN (3 l). The mixture was heated to reflux for 1 h, then concentrated *in vacuo*. The residue was dissolved in ethyl acetate (AcOEt). This solution was washed with water, aqueous sodium thiosulfate, aqueous sodium bicarbonate and brine, and dried. The solvent was removed *in vacuo*, and the residue was washed with isopropanol–water to give **7** (300 g, 88%) as a powder. IR (Nujol): 3400, 1740 cm^{-1}. MS *m/z*: 295 (M^+). ^{1}H-NMR ($CDCl_3$) δ: 1.43 (9H, s), 2,49 (3H, s), 2.74 (3H, s), 6.88 (1H, d, *J*=7 Hz), 7.04 (1H, dd, *J*=2, 9 Hz), 7.10 (1H, d, *J*=7 Hz), 7.34 (1H, d, *J*=9 Hz), 7.72 (1H, d, *J*=2 Hz), 8.05 (1H, br s, D_2O exchangeable (exch.)), *Anal.* Calcd for $C_{19}H_{21}NO_2$: C, 77.26; H, 7.17; N, 4.74. Found: C, 76.97; H, 7.15; N, 4.64.

3-Formyl-1,4-dimethyl-6-pivaloyloxycarbazole (8) A mixture of *N*-methylformanilide (68.6 g, 0.508 mol) and phosphorus oxychloride (77.9 g, 0.508 mol) was stirred at room temperature for 30 min. A solution of **7** (100.0 g, 0.339 mol) in toluene (1.5 l) was added, and the reaction mixture was heated to reflux for 3.5 h, then concentrated *in vacuo*. An aqueous solution of potassium acetate was added, and the whole was stirred for 18 h. The resulting solid was collected by filtration, washed with 1% hydrogen chloride, water and toluene–hexane (1 : 1), and dried *in vacuo* to give **8** (104.7 g, 96%) as a powder, mp >250 °C. IR (Nujol): 1750, 1680 cm^{-1}. MS *m/z*: 323 (M^+). ^{1}H-NMR (DMSO-d_6) δ: 1.37 (9H, s), 2.56 (3H, s), 3.08 (3H, s), 7,18 (1H, dd, *J*=2, 9 Hz), 7.59 (1H, d, *J*=9 Hz), 7.69 (1H, s), 7.90 (1H, d, *J*=2 Hz), 10.36 (1H, s), 11.80 (1H, s, D_2O-exch.). *Anal.* Calcd for $C_{20}H_{21}NO_3$: C, 74.28; H, 6.54; N, 4.33. Found: C, 73.98; H, 6.69; N, 4.18.

1,4-Dimethyl-6-pivaloyloxy-3-(*N*-tosyl-2,2-diethoxyethlyaminomethyl)-9*H*-carbazole (9) A mixture of **8** (104.7 g, 0.324 mol), aminoacetaldehyde diethyl acetal (47.4 g, 0.356 mol) and *p*-toluenesulfonic acid monohydrate (2.03 g, 10.7 mmol) in toluene (1.2 l) was heated to reflux for 1.5 h (azeotropic). The reaction mixture was concentrated *in vacuo*, then the residue was dissolved in ethanol (1.2 l). Platinum (IV) oxide (2.43 g, 10.7 mmol) was added, and the mixture was hydrogenated (atmospheric pressure) at room temperature for 3.5 h. The catalyst was removed by filtration, and the filtrate was concentrated *in vacuo*. The residue was dissolved in tetrahydrofuran (THF) (1.2 l) and the solution was cooled on an ice bath. Triethylamine (65.5 g, 0.647 mol) and tosyl chloride (67.9 g, 0.356 mol) were added to the solution with stirring, then the mixture was allowed to come to ambient temperature, stirred for 18 h, and concentrated *in vacuo*. Aqueous sodium bicarbonate was added to the residue. The mixture was stirred at room temperature for 30 min, then the resulting powder was collected by filtration, washed with water, 5% aqueous citric acid and water, and dried *in vacuo*. The resulting powder was purified by recrystallization to give **9** (121.3 g, 63% from **8**), mp 160—162 °C (AcOEt–hexane). IR (Nujol): 1745 cm^{-1}. FAB-MS (+NaCl) *m/z*: 617 (M^++Na). ^{1}H-NMR ($CDCl_3$) δ: 1.06 (6H, t, *J*=7 Hz), 1.43 (9H, s), 2.37 (3H, s), 2.39 (3H, s), 2.71 (3H, s), 3.19 (2H, d, *J*=6 Hz), 3.3—3.5 (4H, m), 4.43 (1H, t, *J*=6 Hz), 4.65 (2H, s), 6.96 (1H, s), 7.05 (1H, dd, *J*=2, 9 Hz), 7.26 (2H, d, *J*=8 Hz), 7.35 (1H, d, *J*=9 Hz), 7.73 (2H, d, *J*=8 Hz), 7.76 (1H, d, *J*=2 Hz), 8.07 (1H, s, D_2O-exch.). *Anal.* Calcd for $C_{33}H_{42}N_2O_6S$: C, 66.64; H, 7.12; N, 4.71. Found: C, 66.38; H, 7.15; N, 4.52.

9-Hydroxy-5,11-dimethyl-6*H*-pyrido[4,3-*b*]carbazole (9-Hydroxyellipticine) hydrochloride (2·HCl) A suspension of **9** (182.09 g, 0.306 mol) in dioxane (1.4 l) containing 6 N hydrogen chloride (330 ml) was heated

Table 6. Analytical and Physical Data for **5**

No.	IR cm^{-1}	FAB-MS *m/z*	^{1}H-NMR (DMSO-d_6) δ *) (D_2O)	Formula	Analysis (%) Calcd (Found)		
					C	H	N
5a	1770, 1740, 1645	349 (MH^+)	1.23 (3H, t, J=7 Hz), 2.39 (3H, s), 2.78 (3H, s), 3.19 (3H, s), 3.69 (2H, q, J=7 Hz), 4.48 (2H, s), 7.41 (1H, dd, J=2, 9 Hz), 7.61 (1H, d, J=9 Hz), 8.17 (1H, d, J=2 Hz), 8.35 (1H, d, J=7 Hz), 8.41 (1H, d, J=7 Hz), 9.88 (1H, s), 12.1 (1H, s, D_2O-exch.), 15.1 (1H, br s, D_2O-exch.)	$C_{21}H_{20}N_2O_3 \cdot CH_3SO_3H \cdot 0.5H_2O$	58.27 (57.91	5.56 5.52	6.18 6.14)
5b	1760, 1645	379 (MH^+)	2.42 (3H, s), 2.75 (3H, s), 3.17 (3H, s), 3.32 (3H, s), 3.56 (2H, m), 3.78 (2H, m), 4.53 (2H, s), 7.40 (1H, m), 7.60 (1H, d, J=8 Hz), 8.14 (1H, d, J=2 Hz), 8.36 (2H, m), 9.86 (1H, s), 12.1 (1H, s, D_2O-exch.), 15.0 (1H, br s, D_2O-exch.)	$C_{22}H_{22}N_2O_4 \cdot CH_3SO_3H \cdot 1.5H_2O$	55.08 (55.08	5.83 5.71	5.58 5.71)
5c	1765, 1650	421 (MH^+)	0.89 (6H, d, J=7 Hz), 1.83 (1H, m), 2.37 (3H, s), 2.81 (3H, s), 3.22 (2H, d, J=7 Hz), 3.23 (3H, s), 3.60 (2H, m), 3.78 (2H, m), 4.54 (2H, s), 7.43 (1H, dd, J=2, 9 Hz), 7.64 (1H, d, J=9 Hz), 8.20 (1H, d, J=2 Hz), 8.39 (1H, d, J=7 Hz), 8.43 (1H, d, J=7 Hz), 9.90 (1H, s), 12.1 (1H, s, D_2O-exch.), 15.0 (1H, br s, D_2O-exch.)	$C_{25}H_{28}N_2O_4 \cdot CH_3SO_3H \cdot 0.5H_2O$	59.41 (59.38	6.33 6.33	5.33 5.30)
5d	3380, 1765	481 (MH^+)	*) 1.22 (3H, t, J=7 Hz), 1.61 (6H, br s), 3.77 (12H, m), 3.62 (2H, q, J=7 Hz), 4.45 (2H, s), 6.23 (1H, m), 6.49 (1H, d, J=8 Hz), 6.57 (1H, br s), 7.10 (1H, m), 7.37 (1H, m), 8.11 (1H, s)	$C_{27}H_{32}N_2O_6 \cdot HCl \cdot H_2O$	60.61 (61.02	6.59 6.65	5.24 5.28)
5e	3380, 1770	334 (MH^+)	1.69 (3H, d, J=7 Hz), 2.86 (3H, s), 3.28 (3H, s), 4.44 (1H, m), 7.47 (1H, m), 7.72 (1H, m), 8.30 (1H, m), 8.45 (2H, m), 8.86 (3H, br s, D_2O-exch.), 9.94 (1H, s), 12.4 (1H, s, D_2O-exch.), 15.0 (1H, br s, D_2O-exch.)	$C_{20}H_{19}N_3O_2 \cdot 2HCl \cdot H_2O$	56.61 (56.92	5.46 5.54	9.90 10.08)
5f	1760	362 (MH^+)	1.19 (3H, d, J=7 Hz), 1.21 (3H, d, J=7 Hz), 2.44 (1H, m), 2.82 (3H, s), 3.22 (3H, s), 4.18 (1H, d, J=5 Hz), 7.44 (1H, dd, J=2, 9 Hz), 7.67 (1H, d, J=9 Hz), 8.24 (1H, d, J=2 Hz), 8.34 (1H, d, J=7 Hz), 8.42 (1H, d, J=7 Hz), 9.04 (3H, br s, D_2O-exch.), 9.90 (1H, s), 12.4 (1H, s, D_2O-exch.), 15.5 (1H, br s, D_2O-exch.)	$C_{22}H_{23}N_3O_2 \cdot 2HCl \cdot 1.5H_2O$	57.27 (57.73	6.12 6.35	9.11 9.38)
5g	3440, 1700	378 (MH^+)	*) 1.82 (3H, s), 1.90 (3H, s), 3.68 (2H, m), 4.57 (1H, d, J=10 Hz), 4.64 (1H, d, J=10 Hz), 5.15 (1H, t, J=6 Hz), 6.50 (1H, d, J=9 Hz), 6.72 (1H, m), 6.85 (1H, br s), 7.29 (1H, d, J=7 Hz), 7.54 (1H, d, J=7 Hz), 8.38 (1H, s)	$C_{21}H_{19}N_3O_2S \cdot 2HCl \cdot 0.5H_2O$	54.90 (54.53	4.83 4.91	9.15 8.95)
5h	1740, 1600	378 (MH^+)	*) 1.58 (6H, s), 3.19 (1H, dd, J=6, 18 Hz), 3.31 (1H, dd, J=6, 18 Hz), 4.36 (1H, t, J=6 Hz), 6.22 (1H, m), 6.48 (1H, m), 6.54 (1H, s), 7.03 (1H, d, J=7 Hz), 7.31 (1H, m), 8.08 (1H, s)	$C_{21}H_{19}N_3O_4 \cdot 2HCl \cdot H_2O$	53.86 (53.81	4.95 5.05	8.97 8.70)
5i	1750, 1600	378 (MH^+)	*) 2.24 (6H, s), 3.18 (1H, dd, J=6, 18 Hz), 3.30 (1H, dd, J=6, 18 Hz), 4.32 (1H, m), 6.19 (1H, m), 6.45 (1H, m), 6.54 (1H, s), 7.01 (1H, m), 7.31 (1H, m), 8.07 (1H, s)	$C_{21}H_{19}N_3O_4 \cdot 2HCl \cdot H_2O$	53.86 (53.40	4.95 5.12	8.97 8.71)
5j	3380, 1745	406 (MH^+)	*) 1.40 (3H, t, J=7 Hz), 1.94 (3H, s), 2.05 (3H, s), 3.51 (2H, m), 4.46 (2H, m), 4.72 (1H, t, J=5 Hz), 6.68 (1H, d, J=9 Hz), 6.83 (1H, dd, J=2, 9 Hz), 6.96 (1H, br s), 7.43 (1H, d, J=7 Hz), 7.64 (1H, d, J=7 Hz), 8.54 (1H, s)	$C_{23}H_{23}N_3O_4 \cdot 2HCl \cdot 0.8H_2O$	56.06 (56.19	5.44 5.66	8.52 8.32)
5k	3400, 1750	436 (MH^+)	*) 1.96 (3H, s), 2.12 (3H, s), 3.47 (3H, s), 3.54 (2H, m), 3.85 (2H, m), 4.57 (2H, m), 4.77 (1H, m), 6.71 (1H, d, J=9 Hz), 6.84 (1H, dd, J=2, 9 Hz), 7.06 (1H, br s), 7.44 (1H, d, J=7 Hz), 7.65 (1H, d, J=7 Hz), 8.58 (1H, s)	$C_{24}H_{25}N_3O_5 \cdot 2HCl \cdot 1.5H_2O$	53.84 (53.61	5.65 5.89	7.85 7.65)
5l	1750	478 (MH^+)	*) 1.42 (6H, t, J=7 Hz), 1.92 (3H, s), 2.02 (3H, s), 3.78 (2H, s), 4.51 (4H, q, J=7 Hz), 6.68 (1H, d, J=9 Hz), 6.81 (1H, m), 6.91 (1H, m), 7.43 (1H, d, J=7 Hz), 7.65 (1H, d, J=7 Hz), 8.55 (1H, s)	$C_{26}H_{27}N_3O_6 \cdot 2HCl \cdot 0.5H_2O$	55.82 (55.93	5.41 5.55	7.51 7.34)
5m	3380, 1760, 1675	405 (MH^+)	*) 1.77 (6H, s), 1.95 (3H, s), 2.08 (3H, s), 4.40 (2H, s), 6.64 (1H, d, J=8 Hz), 6.82 (1H, dd, J=2, 8 Hz), 7.06 (1H, d, J=2 Hz), 7.43 (1H, d, J=7 Hz), 7.63 (1H, d, J=7 Hz), 8.52 (1H, s)	$C_{23}H_{24}N_4O_3 \cdot 2HCl \cdot 2H_2O$	53.81 (54.05	5.89 6.18	10.91 10.93)
5n	3200, 1760, 1720	363 (MH^+)	2.39 (3H, s), 2.69 (2H, m), 2.78 (3H, s), 2.91 (2H, m), 3.19 (3H, s), 7.36 (1H, dd, J=2, 8 Hz), 7.62 (1H, d, J=8 Hz), 8.09 (1H, d, J=2 Hz), 8.37 (2H, m), 9.89 (1H, s), 12.1 (1H, s, D_2O-exch.), 12.3 (1H, br s, D_2O-exch.), 15.0 (1H, br s, D_2O-exch.)	$C_{21}H_{18}N_2O_4 \cdot CH_3SO_3H \cdot 0.5H_2O$	56.52 (56.62	4.96 5.01	5.99 6.00)
5o	1750, 1700	377 (MH^+)	1.95 (2H, m), 2.44 (2H, t, J=7 Hz), 2.74 (2H, t, J=7 Hz), 2.77 (3H, s), 3.18 (3H, s), 7.36 (1H, dd, J=2, 9 Hz), 7.59 (1H, d, J=9 Hz), 8.09 (1H, d, J=2 Hz), 8.30 (1H, d, J=7 Hz), 8.38 (1H, d, J=7 Hz), 9.84 (1H, s), 12.3 (1H, s, D_2O-exch.), 12.3 (1H, br s, D_2O-exch.), 15.7 (1H, br s, D_2O-exch.)	$C_{22}H_{20}N_2O_4 \cdot HCl \cdot 0.2H_2O$	63.45 (63.42	5.18 5.11	6.73 6.69)

Table 6. (continued)

No.	IR cm^{-1}	FAB-MS *m/z*	^{1}H-NMR (DMSO-d_6) δ *) (D_2O)	Formula	Analysis (%) Calcd (Found) C	H	N
5p	1740, 1730	405 (MH^+)	1.45 (2H, m), 1.61 (2H, m), 1.74 (2H, m), 2.29 (2H, t, J=7 Hz), 2.68 (2H, t, J=7 Hz), 2.77 (3H, s), 3.18 (3H, s), 7.35 (1H, dd, J=2, 9 Hz), 7.59 (1H, d, J=9 Hz), 8.07 (1H, d, J=2 Hz), 8.31 (1H, d, J=7 Hz), 8.38 (1H, d, J=7 Hz), 9.85 (1H, s), 12.1 (1H, br s, D_2O-exch.), 12.3 (1H, s, D_2O-exch.), 15.7 (1H, br s, D_2O-exch.)	$C_{24}H_{24}N_2O_4 \cdot HCl \cdot 0.8H_2O$	63.31 (63.40	5.89 5.83	6.15 6.12)
5q	3170, 1740, 1645	405 (MH^+)	1.37 (6H, s), 2.01 (2H, m), 2.40 (2H, m), 2.81 (3H, s), 3.23 (3H, s), 7.33 (1H, dd, J=2, 9 Hz), 7.63 (1H, d, J=9 Hz), 8.06 (1H, d, J=2 Hz), 8.35 (1H, d, J=7 Hz), 8.40 (1H, d, J=7 Hz), 9.90 (1H, s), 12.3 (2H, br s, D_2O-exch.), 15.7 (1H, br s, D_2O-exch.)	$C_{24}H_{24}N_2O_4 \cdot HCl \cdot H_2O$	62.81 (62.42	5.93 5.74	6.10 5.93)
5r	3200, 1750	421 (MH^+)	2.38 (3H, s), 2.79 (3H, s), 2.88 (2H, m), 2.96 (2H, m), 3.21 (3H, s), 4.66 (2H, s), 7.38 (1H, dd, J=2, 9 Hz), 7.63 (1H, d, J=9 Hz), 8.12 (1H, d, J=2 Hz), 8.37 (1H, d, J=7 Hz), 8.42 (1H, d, J=7 Hz), 9.90 (1H, s), 12.1 (1H, s, D_2O-exch.), 13.0 (1H, br s, D_2O-exch.), 14.5 (1H, br s, D_2O-exch.)	$C_{23}H_{20}N_2O_6 \cdot CH_3SO_3H \cdot H_2O$	53.93 (53.57	4.90 4.80	5.24 5.09)

to reflux for 1.5 h. The reaction mixture was concentrated *in vacuo*, and 1% aqueous sodium chloride was added to the residue. The whole was stirred at room temperature for 1 h. The resulting precipitate was collected by filtration, washed with water, and dried. The solid was washed well with acetone and dried to give **2**·HCl (87.3 g, 96%) as an orange powder, mp >250 °C. IR (Nujol): 3200 cm^{-1}. MS *m/z*: 262(M^+). ^{1}H-NMR (DMSO-d_6) δ: 2.75 (3H, s), 3.17 (3H, s), 7.13 (1H, dd, J=2, 9 Hz), 7.45 (1H, d, J=9 Hz), 7.75 (1H, d, J=2 Hz), 8.29 (1H, d, J=7 Hz), 8.34 (1H, d, J=7 Hz), 9.45 (1H, br s, D_2O-exch.), 9.80 (1H, s), 11.93 (1H, s, D_2O-exch.). *Anal.* Calcd for $C_{17}H_{14}N_2O \cdot HCl \cdot 0.5H_2O$: C, 66.34; H, 5.24; N, 9.10. Found: C, 66.14; H, 5.31; N, 8.89.

General Procedure for the Synthesis of 5 Method A: A mixture of a carboxylic acid derivative (4.2 mmol), DCC (5.0 mmol) and HOBT (4.2 mmol) in DMF (50 ml) was stirred at room temperature for 4 h. **2**·HCl (3.5 mmol), triethylamine (4.2 mmol) and DMAP (0.35 mmol) were added, and the reaction mixture was stirred at room temperature for 18 h, then concentrated *in vacuo*. AcOEt was added to the residue, and insoluble precipitates were removed by filtration. The filtrate was washed with 2% aqueous potassium carbonate and brine, and dried. The solvent was removed *in vacuo*, and the residue was purified by column chromatography on silica gel using chloroform–methanol (20 : 1) as an eluent. The resulting pure solid was treated with hydrogen chloride or methanesulfonic acid to give **5** as an amorphous powder.

Method B: **2**·HCl was reacted with a *tert*-butyloxycarbonyl(Boc)-protected amino acid derivative in the same manner as described for method A. The resulting product was suspended in dioxane (20 ml), then 15% hydrogen chloride in dioxane (20 ml) was added to the suspension with stirring on an ice bath. The mixture was allowed to come to ambient temperature and further stirred for 2—5 h. After addition of diethyl ether (50 ml), the reaction mixture was stirred vigorously for 1 h. The resulting precipitate was collected by filtration, washed with ethanol-acetone, and dried *in vacuo* to give **5** as an amorphous powder.

Method C: **2**·HCl was reacted with a benzyloxycarbonyl (Z) and/or benzyl ester-protected carboxylic acid derivative in the same manner as described for method A. The resulting product was dissolved in acetic acid (50 ml)–water (50 ml). Concentrated hydrogen chloride (or methanesulfonic acid) and 10% palladium on activated carbon (500 mg) were added to the solution, and hydrogenation (atmospheric pressure) was conducted at room temperature for 5 h. The catalyst was removed by filtration, and the filtrate was concentrated *in vacuo*. Acetone was added to the residue, and the mixture was stirred at room temperature for 18 h. The resulting precipitate was collected by filtration, washed with ethanol–acetone, and dried *in vacuo* to give **5** as an amorphous powder.

Analytical and physical data for **5** are summarized in Table 6.

Antitumor Activity against P388 Leukemia Male CDF_1 mice (4 weeks old) were inoculated intraperitoneally with P388 leukemia cells (1×10^6 cells/body) on day 0. Each compound was administered daily i.v. as a single injection (0.2 ml/body), from days 1 to 5. Compounds were dissolved in saline or 10% dimethyl sulfoxide–saline. Each group except the control consisted of five mice; the control group consisted of ten mice. ILS was determined by comparing the mean survival time of the treated group with the mean survival time of the control group.

Antitumor Activity against Colon 26 Male CDF_1 mice (5 weeks old) were inoculated subcutaneously in the left inguinal region with colon 26 cells (1×10^6 cells/body) on day 0. Each compound was administered daily i.v. as a single injection (0.2 ml/body), from days 1 to 7. Compounds were dissolved in saline or 10% dimethyl sulfoxide–saline. Each group except the control consisted of five mice; the control group consisted of ten mice. On day 16, tumors were dissected and weighed. Inhibition of tumor growth was determined by comparing the mean weight of the tumor in the treated group with the mean weight of the tumor in the control group.

Antitumor Activity against Lewis Lung Carcinoma Male BDF_1 mice (5 weeks old) were inoculated subcutaneously in the left inguinal region with Lewis lung carcinoma cells (2.6×10^5 cells/body) on day 0. Each compound was administered daily i.v. as a single injection (0.2 ml/body), from days 1 to 7. Compounds were dissolved in saline or 10% dimethyl sulfoxide–saline. Each group except the control consisted of five mice; the control group consisted of ten mice. ILS was determined by comparing the mean survival time of the treated group with the mean survival time of the control group.

Antitumor Activity against B16 Melanoma Male BDF_1 mice (5 weeks old) were inoculated subcutaneously in the left inguinal region with B16 melanoma cells (20% homogenate/0.15 ml/body) on day 0. Each compound was administered daily i.v. as a single injection (0.2 ml/body), from days 1 to 7 or 1, 5, 9. Compounds were dissolved in saline. Each group except the control consisted of five mice; the control group consisted of ten mice. ILS was determined by comparing the mean survival time of the treated group with the mean survival time of the control group.

References and Notes

1) Harada N., Kawaguchi T., Inoue I., Oda K., Ohashi M., Hashiyama T., Tsujihara K., *Chem. Pharm. Bull.*, **45**, 134—137 (1997).
2) For example, 2-(3-methoxy-2-oxopropanoxy)-9-hydroxy-5,11-dimethyl-6*H*-pyrido[4,3-*b*]carbazolium bromide (**1**, R=$MeOCH_2$) was dissolved in water and the solution was allowed to stand at room temperature for 1 h, affording **2** in 72% yield.
3) Juret P., Tanguy A., Girard A., LeTalaer J. Y., Abbatucci J. S., *Eur. J. Cancer*, **14**, 205—206 (1978).
4) Honda T., Kato M., Inoue M., Shimamoto T., Shima K., Nakanishi T., Yoshida T., Noguchi T., *J. Med. Chem.*, **31**, 1295—1305 (1988).
5) Ohashi M., Sugikawa E., Nakanishi N., *Jpn. J. Cancer Res.*, **86**, 819—827 (1995).
6) Guthrie R. W., Brossi A., Mennona F. A., Mullin J. G., Kierstead R. W., Grunberg E., *J. Med. Chem.*, **18**, 755—760 (1975). They synthesized 9-acetoxy, propoxy, isobutyroxy and caproxy ellipticine

derivatives and tested these compounds for antitumor activity against sarcoma 180.
7) Suffness M., "The Alkaloids," Vol. 25, ed. by Brossi A., Academic Press, Inc., New York, 1985, pp. 89—304; Gribble G. W., *ibid.*, Vol. 39, 307—352 (1990); Kansal V. K., Potier P., *Tetrahedron*, **42**, 2389—2408 (1986); Gouyette A., Reynaud R., Sadet J., Baillarge M., Gansser C., Cros S., Goffic F., Pecq J.-B., Paoletti C., Viel C., *Eur. J. Med. Chem.*, **15**, 503—510 (1980).
8) *a*) Dalton L. K., Demerac S., Elmes B. C., Loder J. W., Swan J. M., Teitei T., *Aust. J. Chem.*, **20**, 2715—2727 (1967); *b*) Griffle G. W., Saulnier M. G., Obaza-Nutaitis J. A., Ketcha D. M., *J. Org. Chem.*, **57**, 5891—5899 (1992); *c*) Anderson W. K., Gopalsamy A., Reddy P. S., *J. Med. Chem.*, **37**, 1955—1963 (1994).
9) Plug J. P. M., Koomen G.-J., Pandit U. K., *Synthesis*, 1221—1222 (1992).
10) Oku A., Harada T., Kita K., *Tetrahedron Lett.*, **23**, 681—684 (1982).
11) *o*-Dichlorobenzene (60%), dichloroethane and fluorobenzene (<50%).
12) Compound **5o** was more stable in phosphate buffer (pH 4.0) at 40 °C (86% remained after 10 h).