Synthesis and Antitumor Activity of Quaternary Salts of 2-(2′-Oxoalkoxy)-9-hydroxyellipticines

Naoyuki HARADA, Takayuki KAWAGUCHI, Isao INOUE, Motoaki OHASHI, Kouji ODA, Tomiki HASHIYAMA, and Kenji TSUJIHARA*

Lead Optimization Research Laboratory, Tanabe Seiyaku Co., Ltd., 2–2–50 Kawagishi, Toda-shi, Saitama 335, Japan.
Received July 18, 1996; accepted September 17, 1996

Various kinds of water-soluble quaternary salts of 2-(2′-oxoalkoxy)-9-hydroxyellipticines were synthesized in a search for compounds with potent antitumor activity and low toxicity. Some compounds exhibited more potent antitumor activities than elliptinium (1) and SUN 4599 (3). In particular, 2-(3′-methoxy-2′-oxopropanoxy)-9-hydroxy-5,11-dimethyl-6*H*-pyrido[4,3-*b*]carbazolium bromide (4d) showed potent antitumor activities against P388 leukemia, colon 26, and Lewis lung carcinoma.

Key words 9-hydroxyellipticine 2-oxide; water-soluble; P388 leukemia; colon 26; Lewis lung carcinoma

Since the synthesis and antitumor activity of ellipticine and its analogues were reported by Dalton *et al.*,[1] a number of attempts to design and prepare new analogues have been reported.[2] The usefulness of early ellipticines was hindered by problems of low water solubility and cardiovascular side effects.[2] To overcome these problems many quaternary salts, such as 9-hydroxy-2-methylellipticium acetate (elliptinium, **1**),[3] 2-[2-(diethylamino)-ethyl]-9-hydroxy-ellipticium chloride (datelliptium, **2**),[4] 2-α-L-arabinopyranosyl-9-hydoxyellipticium bromide (SUN 4599, **3**),[5] *etc.* have been synthesized and evaluated. However, to date, none has been particularly impressive in clinical trials.

In order to develop more potent, water-soluble ellipticine analogues, we designed and synthesized novel quaternary salts of 9-hydroxyellipticine 2-oxide derivatives (**4**), as new lead compounds having potent antitumor activity and low toxicity.

Chemistry

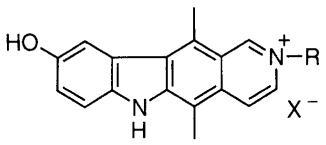
2-(2′-Oxoalkoxy)-9-hydroxyellipticines (**4**) were synthesized by the routes shown in Chart 2. We first aimed to prepare the 9-methoxyellipticine 2-oxide (**7**) as a key compound. As 9-methoxyellipticine (**6**) and **7** are insoluble in usual organic solvents, preparation of **7** is difficult. However, Rivalle and Bisagni have recently reported that the reaction of **6** with *m*-chloroperbenzoic acid (MCPBA) under homogeneous conditions employing boiling chloroform ($CHCl_3$)/ethanol afforded **7** in moderate yield (64%).[7] We have now found a more efficient method to convert **6** into the N-oxide (**7**) under heterogeneous conditions. Namely, treatment of **6** with MCPBA in acetone at room temperature, and purification simply by filtration, provided **7** in excellent yield (95%). Demethylation of **7** was performed with boron tribromide (BBr_3) in dichloromethane followed by column chromatography on activated carbon with hot *N*,*N*-dimethylformamide (DMF)–methanol–water to afford pure 9-hydroxyellipticine 2-oxide (**8**) in 51% yield.[8] In contrast, the reaction of 9-hydroxyellipticine (**9**) or 9-acetoxyellipticine (**10**) with MCPBA in acetone failed, and the N-oxide (**8** or **11**) was not obtained at all.

Finally, 2-(2′-oxoalkoxy)-9-hydroxyellipticines (**4a—k**) were synthesized (Chart 2, Table 1). The reaction of **8** with some α-bromoketones in DMF under a heterogeneous condition gave the corresponding 2-(2′-oxoalkoxy)-9-hydroxyellipticines (**4a—k**) in good yields. These quaternary salts had good solubility in water (1—10 mg/ml).

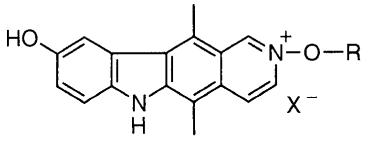
Antitumor Activity and Discussion

The antitumor activity of the prepared water-soluble quaternary salts of 9-hydroxyellipticine 2-oxide derivatives (**4a—k**) against P388 leukemia in mice was evaluated after intravenous (i.v.) administration (Table 2). Compounds **4a**, **4b**, **4d** and **4f** exhibited potent activities comparable to that of SUN 4599 (**3**). Elliptinium acetate (**1**), **4c**, **4e**, **4g**, **4h**, **4i**, **4j** and **4k** were inactive. Compound **4a** showed good activity, while the introduction of a methyl group on C-1′ (**4c**) resulted in weak activity. Replacement of the methoxy group (**4d**) with a hydroxy group (**4e**) also resulted in weak activity. The benzoyl compound (**4f**) exhibited good activity, while the introduction of a chloro substituent (**4g**) or a heterocyclic structure (**4h** and **4i**) decreased the activity.

Compounds **4a**, **4b**, **4d** and **4f** were evaluated for antitumor activity against solid tumor colon 26 in mice by i.v. administration (Table 3). The values of the therapeutic ratio (*TR*) of **4a**, **4b**, **4d** and **4f** were very high (2.7—4.0)



1 R = Me X = AcO
2 R = $Et_2NCH_2CH_2$ X = Cl
3 R = L - Arabinosyl X = Br



4

Chart 1

* To whom correspondence should be addressed.

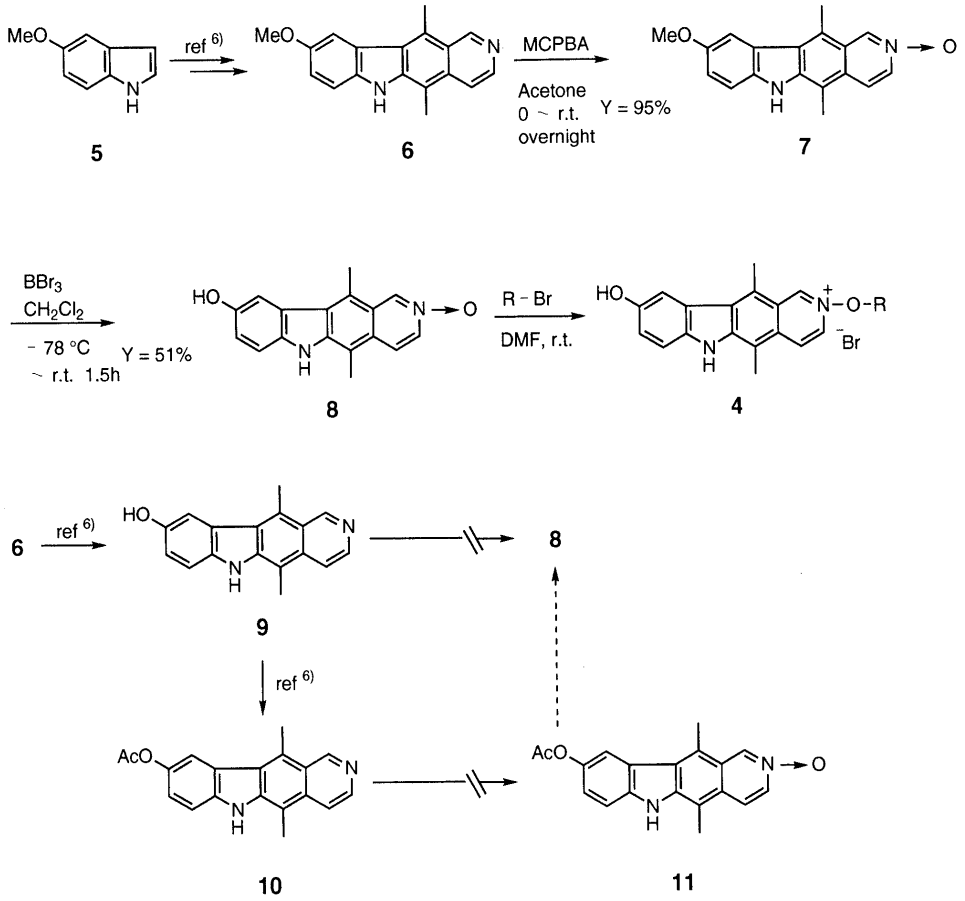


Chart 2

Table 1. Water-soluble 2-(2′-Oxoalkoxy)-9-hydroxyellipticines (**4**)

No.	R	Yield (%)
4a	$-CH_2-\overset{O}{\overset{\|\|}{C}}-Me$	91
4b	$-CH_2-\overset{O}{\overset{\|\|}{C}}-Et$	78
4c	$-\underset{Me}{\underset{\|}{CH}}-\overset{O}{\overset{\|\|}{C}}-Me$	73
4d	$-CH_2-\overset{O}{\overset{\|\|}{C}}-CH_2OMe$	51
4e	$-CH_2-\overset{O}{\overset{\|\|}{C}}-CH_2OH$	61
4f	$-CH_2-\overset{O}{\overset{\|\|}{C}}-Ph$	81
4g	$-CH_2-\overset{O}{\overset{\|\|}{C}}-C_6H_4-Cl$	60
4h	$-CH_2-\overset{O}{\overset{\|\|}{C}}-$(2-thienyl)	65
4i	$-CH_2-\overset{O}{\overset{\|\|}{C}}-$(2-furyl)	63
4j	$-CH_2-\overset{O}{\overset{\|\|}{C}}-OCH_2CH_2OMe$	49
4k	$-CH_2-\overset{O}{\overset{\|\|}{C}}-OCH_2CH_2OCH_2CH_2OMe$	61

Table 2. Antitumor Activity against P388 Leukemia

No.	OD (mg/kg)[a]	*ILS* (%)[b]	*TR*[c]
4a	40	57.8	2.4
4b	40	51.1	1.7
4c	20	20.0	—
4d	40	69.2	1.9
4e	40	27.1	—
4f	40	53.3	1.7
4g	20	26.7	—
4h	40	25.3	—
4i	40	27.5	—
4j	40	9.9	—
4k	40	5.5	—
1	2.5	6.8	—
3	20	60.0	1.7

a) Optimal dose of drug. *b*) Increase of 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}.

Table 3. Antitumor Activity against Colon 26

No.	OD (mg/kg)[a]	Inhibition (%)[b]	*TR*[c]
4a	30	99.0	2.7
4b	40	99.6	3.8
4d	40	94.1	3.4
4f	40	100.0	4.0
1	1.3	84.2	1.2
3	20	93.4	1.9

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]	*TR*[a]
4a	20	27.3	—
4b	20	23.1	—
4d	40	45.1	1.6
4f	20	25.6	—
1	1.6	12.6	—
3	20	35.0	1.0

a) See Table 2.

as compared with those of control compounds **1** and **3** (1.2 and 1.9 respectively). Hence, **4a**, **4b**, **4d** and **4f** were further evaluated for antitumor activity against solid tumor Lewis lung carcinoma in mice. The results are summarized in Table 4. Only **4d** and **3** were effective (increase of life span (*ILS*) of more than 30%). In particular, **4d** had the highest ILS (45.1%) and the largest TR (1.6) of all the tested compounds against Lewis lung carcinoma.

Ellipticine derivatives are known to have a cardio-

Table 5. Analytical and Physical Data for **4**[a]

No.	IR cm^{-1}	FAB-MS *m/z*	^{1}H-NMR (DMSO-d_6) δ	Formula	Analysis (%) Calcd (Found)		
					C	H	N
4a	3180, 1730	335 (M^+)	2.18 (3H, s), 2.79 (3H, s), 3.21 (3H, s), 5.64 (2H, s), 7.14 (1H, dd, J=2, 9 Hz), 7.47 (1H, d, J=9 Hz), 7.75 (1H, d, J=2 Hz), 8.43 (1H, d, J=8 Hz), 8.79 (1H, dd, J=2, 8 Hz), 9.40 (1H, br s, D_2O exch.), 10.31 (1H, d, J=2 Hz), 11.99 (1H, s, D_2O exch.)	$C_{20}H_{19}N_2O_3 \cdot Br \cdot 0.5H_2O$	56.62 (56.84	4.75 4.85	6.60 6.80)
4b	3160, 1730	349 (M^+)	1.01 (3H, t, J=7 Hz), 2.4—2.6 (2H, m), 2.78 (3H, s), 3.21 (3H, s), 5.66 (2H, s), 7.13 (1H, dd, J=2, 9 Hz), 7.47 (1H, d, J=9 Hz), 7.74 (1H, d, J=2 Hz), 8.42 (1H, d, J=9 Hz), 8.72 (1H, dd, J=2, 9 Hz), 9.40 (1H, s, D_2O exch.), 10.30 (1H, d, J=2 Hz), 11.98 (1H, s, D_2O exch.)	$C_{21}H_{21}N_2O_3 \cdot Br$	58.75 (58.65	4.93 4.93	6.53 6.51)
4c	3180, 1725	349 (M^+)	1.66 (3H, d, J=7 Hz), 2.28 (3H, s), 2.77 (3H, s), 3.21 (3H, s), 5.76 (1H, q, J=7 Hz), 7.11 (1H, dd, J=2, 9 Hz), 7.46 (1H, d, J=9 Hz), 7.74 (1H, d, J=2 Hz), 8.37 (1H, d, J=8 Hz), 8.75 (1H, dd, J=2, 8 Hz), 9.34 (1H, br s, D_2O exch.), 10.23 (1H, d, J=2 Hz), 11.95 (1H, s, D_2O exch.)	$C_{21}H_{21}N_2O_3 \cdot Br$	58.75 (58.46	4.93 4.73	6.53 6.50)
4d	3180, 1730	365 (M^+)	2.82 (3H, s), 3.25 (3H, s), 3.35 (3H, s), 4.23 (2H, s), 5.73 (2H, s), 7.16 (1H, d, J=8 Hz), 7.51 (1H, d, J=8 Hz), 7.79 (1H, s), 8.46 (1H, d, J=7 Hz), 8.80 (1H, d, J=8 Hz), 9.42 (1H, s, D_2O exch.), 10.34 (1H, s), 12.03 (1H, s, D_2O exch.)	$C_{21}H_{21}N_2O_4 \cdot Br$	56.64 (56.58	4.75 4.61	6.29 6.22)
4e	3380, 3190, 1735	351 (M^+)	2.78 (3H, s), 3.21 (3H, s), 4.24 (2H, s), 5.46 (1H, br s, D_2O exch.), 5.77 (2H, s), 7.14 (1H, dd, J=2, 8 Hz), 7.47 (1H, d, J=8 Hz), 7.75 (1H, d, J=2 Hz), 8.43 (1H, d, J=8 Hz), 8.81 (1H, dd, J=2, 8 Hz), 9.40 (1H, br s, D_2O exch.), 10.32 (1H, d, J=2 Hz), 11.98 (1H, br s, D_2O exch.)	$C_{20}H_{19}N_2O_4 \cdot Br \cdot 0.5H_2O$	54.56 (54.36	4.58 4.46	6.36 6.46)
4f	3380, 3150, 1690	397 (M^+)	2.82 (3H, s), 3.26 (3H, s), 6.43 (2H, s), 7.16 (1H, d, J=9 Hz), 7.50 (1H, d, J=9 Hz), 7.60 (2H, m), 7.72 (1H, d, J=7 Hz), 7.78 (1H, s), 7.97 (2H, m), 8.49 (1H, d, J=8 Hz), 8.89 (1H, d, J=8 Hz), 9.43 (1H, s, D_2O exch.), 10.42 (1H, s), 12.03 (1H, s, D_2O exch.)	$C_{25}H_{21}N_2O_3 \cdot Br \cdot 0.5H_2O$	61.74 (61.48	4.56 4.36	5.76 5.90)
4g	3160, 1700	431 (M^+)	2.82 (3H, s), 3.26 (3H, s), 6.41 (2H, s), 7.16 (1H, dd, J=2, 9 Hz), 7.50 (1H, d, J=9 Hz), 7.69 (2H, d, J=9 Hz), 7.79 (1H, d, J=2 Hz), 7.98 (2H, d, J=9 Hz), 8.48 (1H, d, J=8 Hz), 8.87 (1H, dd, J=2, 8 Hz), 9.42 (1H, s, D_2O exch.), 10.41 (1H, d, J=2 Hz), 12.03 (1H, s, D_2O exch.)	$C_{25}H_{20}ClN_2O_3 \cdot Br$	58.67 (58.56	3.94 3.97	5.47 5.46)
4h	3140, 1670, 1655	403 (M^+)	2.82 (3H, s), 3.26 (3H, s), 6.31 (2H, s), 7.16 (1H, d, J=9 Hz), 7.34 (1H, dd, J=3, 5 Hz), 7.50 (1H, d, J=9 Hz), 7.79 (1H, s), 8.02 (1H, d, J=3 Hz), 8.17 (1H, d, J=5 Hz), 8.48 (1H, d, J=8 Hz), 8.87 (1H, d, J=8 Hz), 9.43 (1H, s, D_2O exch.), 10.42 (1H, s), 12.03 (1H, s, D_2O exch.)	$C_{23}H_{19}N_2O_3S \cdot Br$	57.15 (56.85	3.96 4.26	5.80 5.98)
4i	3140, 1680, 1655	387 (M^+)	2.81 (3H, s), 3.25 (3H, s), 6.15 (2H, s), 6.82 (1H, m), 7.16 (1H, d, J=9 Hz), 7.50 (1H, d, J=9 Hz), 7.59 (1H, d, J=3 Hz), 7.78 (1H, s), 8.13 (1H, s), 8.47 (1H, d, J=8 Hz), 8.86 (1H, d, J=8 Hz), 9.42 (1H, s, D_2O exch.), 10.40 (1H, s), 12.02 (1H, s, D_2O exch.)	$C_{23}H_{19}N_2O_4 \cdot Br$	59.11 (58.87	4.10 4.39	5.99 6.10)
4j	3180, 1750	395 (M^+)	2.81 (3H, s), 3.25 (3H, s), 3.36 (3H, s), 3.57 (2H, m), 4.31 (2H, m), 5.61 (2H, s), 7.18 (1H, d, J=9 Hz), 7.48 (1H, d, J=9 Hz), 7.78 (1H, s), 8.46 (1H, d, J=9 Hz), 8.79 (1H, d, J=9 Hz), 9.41 (1H, s, D_2O exch.), 10.34 (1H, s), 12.03 (1H, s, D_2O exch.)	$C_{22}H_{23}N_2O_5 \cdot Br$	55.59 (55.78	4.88 4.74	5.89 5.84)
4k	1750	439 (M^+)	2.82 (3H, s), 3.20 (3H, s), 3.26 (3H, s), 3.2—3.8 (6H, m), 4.30 (2H, m), 5.61 (2H, s), 7.16 (1H, d, J=9 Hz), 7.51 (1H, d, J=9 Hz), 7.79 (1H, s), 8.46 (1H, d, J=8 Hz), 8.81 (1H, d, J=8 Hz), 9.42 (1H, s, D_2O exch.), 10.36 (1H, s), 12.05 (1H, s, D_2O exch.)	$C_{24}H_{27}N_2O_6 \cdot Br$	55.50 (55.36	5.24 4.97	5.39 5.44)

a) All compounds had melting points >300 °C.

vascular side effect.[2] So, we performed a preliminary cardiovascular side effect study in mice by i.v. administration (1 mg/kg). Compounds **1** and **3** considerably increased the heart rate in rat ($n=3$) (70 beat/min and 49 beat/min, respectively). Compound **4d**, however, showed only weak effect (15 beat/min) on the heart rate in rat even at 10 mg/kg.

In conclusion, we have synthesized novel water-soluble quaternary salts of 2-(2′-oxoalkoxy)-9-hydroxyellipticines and have found several compounds with potent antitumor activities. In particular, **4d** showed potent antitumor activities against P388 leukemia, colon 26 and Lewis lung carcinoma, and had good water-solubility (10 mg/ml). We selected **4d** for further pharmacological evaluation.

Experimental

Melting points were determined on 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. 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.

9-Methoxy-5,11-dimethyl-6*H*-pyrido[4,3-*b*]carbazole 2-Oxide (9-Methoxyellipticine 2-Oxide) 7 A cold suspension of 9-methoxyellipticine **6** (42.0 g, 0.15 mol) in acetone (1.4 l) was treated with MCPBA (65.5 g, 0.30 mol) on an ice bath, then the mixture was allowed to come to ambient temperature and stirring was continued overnight. After addition of iso-Pr_2O (1 l), the resulting precipitate was collected by filtration, and washed with acetone–iso-Pr_2O (1 : 1, 1 l) to give **7** (42.4 g, yield = 95%) as a yellow powder. mp > 300 °C. IR (Nujol): 3180 cm^{-1}. MS *m/z*: 292 (M^+). ^{1}H-NMR (DMSO-d_6) δ: 2.76 (3H, s), 3.07 (3H, s), 3.90 (3H, s), 7.20 (1H, dd, $J=2$, 9 Hz), 7.48 (1H, d, $J=9$Hz), 7.82 (1H, d, $J=2$ Hz), 8.02 (1H, d, $J=7$ Hz), 8.05 (1H, d, $J=7$ Hz), 9.12 (1H, s), 11.3 (1H, br s, D_2O exchangeable (exch.)). *Anal.* Calcd for $C_{18}H_{16}N_2O_2$: C, 73.95; H, 5.52; N, 9.58. Found: C, 73.65; H, 5.40; N, 9.33.

9-Hydroxy-5,11-dimethyl-6*H*-pyrido[4,3-*b*]carbazole 2-Oxide (9-Hydroxyellipticine 2-Oxide) 8 A suspension of **7** (32.0 g, 0.11 mmol) in dry CH_2Cl_2 (800 ml) was cooled, and BBr_3 (68.4 g, 0.27 mmol) in dry CH_2Cl_2 (250 ml) was added dropwise to it, under an argon atmosphere with stirring on a dry ice-acetone bath. The mixture was allowed to come to ambient temperature and stirred for 2 h. After addition of MeOH (120 ml), the reaction mixture was concentrated *in vacuo*. The residue was taken up in MeOH (500 ml). The cold mixture was treated with CH_3COOK (*ca.* 80 g) on an ice bath, then allowed to come to ambient temperature, and stirring was continued for 30 min. The mixture was concentrated *in vacuo*, and the residue was purified by column chromatography on activated carbon using warmed (*ca.* 60 °C) DMF–MeOH–water (10 : 10 : 1) as an eluent. The resulting powder was washed with EtOH and MeOH to give **8** (16.2 g, yield = 51%) as an orange powder. mp > 300 °C. IR (Nujol): 3230 cm^{-1}. FAB-MS *m/z*: 279 (MH^+). ^{1}H-NMR (DMSO-d_6) δ: 2.73 (3H, s), 2.89 (3H, s), 7.05 (1H, dd, $J=2$, 9 Hz), 7.39 (1H, d, $J=9$ Hz), 7.73 (1H, d, $J=2$ Hz), 8.03 (2H, m), 9.10 (1H, d, $J=2$ Hz), 9.14 (1H, br s, D_2O exch.), 11.12 (1H, br s, D_2O exch.). *Anal.* Calcd for $C_{17}H_{14}N_2O_2$: C, 73.37; H, 5.07; N, 10.07. Found: C, 73.15; H, 4.98; N, 9.88.

General Procedure for the Synthesis of 4 An α-bromoketone (6.0—20.0 mmol) was added to a suspension of **8** (2.0 mmol) in dry DMF (10 ml) under an argon atmosphere with stirring. The mixture was stirred at room temperature for 2—24 h. After addition of dry CH_2Cl_2 (20 ml), the reaction mixture was stirred vigorously for 10 min. The resulting precipitate was collected by filtration, and washed with DMF–CH_2Cl_2, CH_2Cl_2 and Et_2O to give **4** as an orange powder.

Analytical and physical data for **4** are summarized in Table 5.

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% DMSO–saline. Each group except the control consisted of five mice; the control group consisted of ten mice. Increase of life span 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 Colon 26 cells (1×10^6 cells/body) were inoculated subcutaneously into the left inguinal region of male CDF_1 mice (5 weeks old) on day 0. Each compound was administed daily i.v. as a single injection (0.2 ml/body), from days 1 to 7. Compounds were dissolved in saline or 10% DMSO–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 Lewis lung carcinoma cells (2.6×10^5 cells/body) were inoculated subcutaneously into the left inguinal region of male BDF_1 mice (5 weeks old) 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% DMSO–saline. Each group except the control consisted of five mice; the control group consisted of ten mice. Increase of life span 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) Dalton L. K., Demerac S., Elmes B. C., Loder J. W., Swan J. M., *Aust. J. Chem.*, **20**, 2715—2727 (1967).
2) Suffness M., "The Alkaloids," Vol. 25, Brossi A., (ed.), Academic Press, Inc., New York, 1985, pp. 89—304; Gribble G. W., *ibid.*, **39**, 307—352 (1990); Majima H., Niitani H., Taguchi T., Furue H., Abstracts of Papers, 15th International Cancer Congress, Hamburg, Germany, A4, A4-119-17, 1990.
3) Juret P., Tanguy A., Girard A., LeTalaer J. Y., Abbatucci J. S., *Eur. J. Cancer*, **14**, 205—206 (1978).
4) Auclair C., Pierre A., Voisin E., Pepin O., Cros S., Colas C., Saucier J.-M., Verschuere B., Gros P., Paoletti C., *Cancer Res.*, **47**, 6254—6261 (1987).
5) Honda T., Kato M., Inoue M., Shimamoto T., Shima K., Nakanishi T., Yoshida T., Noguchi T., *J. Med. Chem.*, **31**, 1295—1305 (1988).
6) Guthrie R. W., Brossi A., Mennona F. A., Mullin J. G., Kierstead R. W., *J. Med. Chem.*, **18**, 755—760 (1975).
7) Rivalle C., Bisagni E., *Heterocycles*, **38**, 391—397 (1994).
8) Crude **8** could not be purified by usual column chromatography on silica gel or by recrystallization, because it was hardly soluble in usual organic solvents.