Synthesis and Biological Evaluation of Quinocarcin Derivatives

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Cyanation of quinocarcin readily opened the oxazolidine ring to provide DX-52-1 (2), which was a key compound in the synthesis of quinocarcin derivatives. Various electrophilic reactions toward aromatic ring of DX-52-1 were examined, and 10-substituted (*e.g.*, halogen, nitro, formyl, cyano, hydroxy, *etc.*) analogs were prepared. Dehydrocyanation of the derivatives could be achieved to reproduce the oxazolidine ring upon treatment with HCl or $AgNO_3$. 10-Chloride 10 and 10-bromide 11 were the most promising among the derivatives prepared. Antitumor activity of 10 was extended to B-16 melanoma.

Keywords quinocarcin; 10-halo quinocarcin derivative; DX-52-1; 10-substituted DX-52-1 analog; cyanation; electrophilic substitution; antitumor activity

Quinocarcin (**1**),[1] a novel antitumor antibiotic isolated from the culture broths of *Streptomyces melanovinaceus*,[1a] is active against several experimental tumor systems.[1d,e] It is known to block ribonucleic acid (RNA) synthesis in preference to deoxyribonucleic acid (DNA) and protein synthesis in P388 leukemia cells,[1d] while in *Bacillus subtilis* DNA synthesis is inhibited preferentially.[1c] In spite of little information on the mode of action of quinocarcin, a plausible mechanism is alkylation of DNA by opening of the oxazolidine ring. This functionality could give an iminium ion that would be highly reactive toward nucleophiles on DNA, which has been proposed for saframycin A[2] and naphthyridinomycin.[3] Lack of antitumor activity in quinocarcinol, which has no oxazolidine ring, strengthens this hypothesis. Additionally, it was reported[1c] that quinocarcin cleaved double stranded DNA, however, it was unclear whether this cleavage was responsible for the antitumor activity.

Quinocarcin has significant activity against P388 leukemia and human Xenograft MX-1 in daily administration. Against murine solid tumor, *e.g.* sarcoma-180, its activity is rather low and only marginal toward B-16 melanoma. To increase the antitumor activity and broaden the spectrum, the preparation of quinocarcin analogs was undertaken.

Chemistry Chemical modification of quinocarcin is limited by its instability owing to the presence of the oxazolidine ring. First, we initiated a complementary investigation aimed at the development of masking the oxazolidine ring and its reproduction. Cyanation of quinocarcin with NaCN readily gave DX-52-1 (**2**),[4] which had not only significant antitumor activity but also sufficient stability. In infrared spectrum of **2** no absorption of nitrile was observed, as was true in other 4-cyano analogs. However, it showed a peak at 119.4 ppm in carbon-13 nuclear magnetic resonance (^{13}C-NMR), which was indicative of nitrile. Upon treatment with hydrochloric acid or silver nitrate **2** reproduced quinocarcin moderately. Silver nitrate was particularly effective for acid labile derivatives. Here, we have undertaken chemical modification of **2**, a useful congener of quinocarcin. This report describes the synthesis and the antitumor activity of aromatic ring analogs of quinocarcin.

Electrophilic reaction is the general way to introduce a substituent on an aromatic ring. To enhance chemical reactivity of the aromatic ring toward electrophiles, demethylation of the methoxy group in **2** was employed with BBr_3.[5] Aqueous workup followed by addition of NaCN provided demethyl DX-52-1 (**4**) in good yield. Without

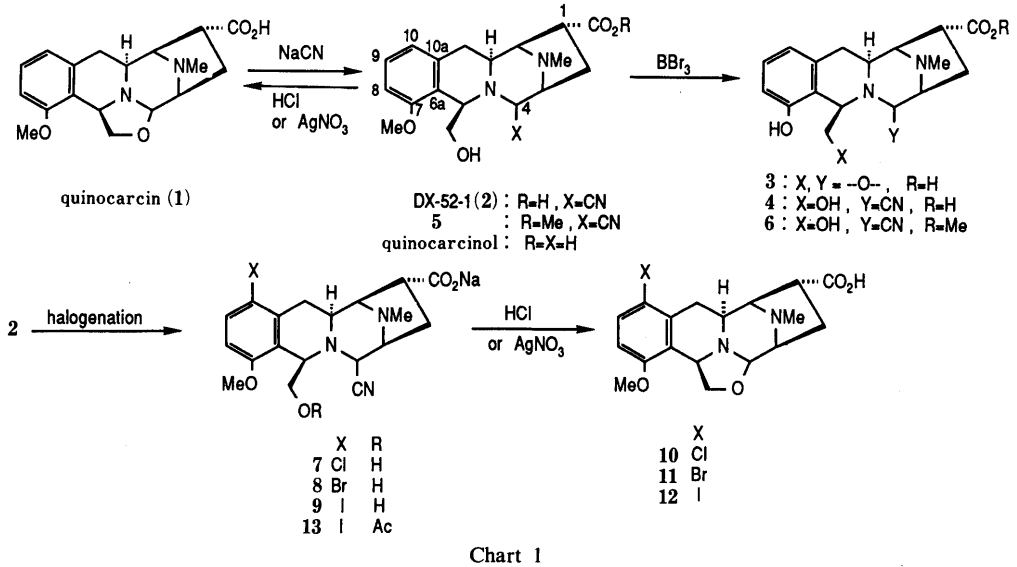
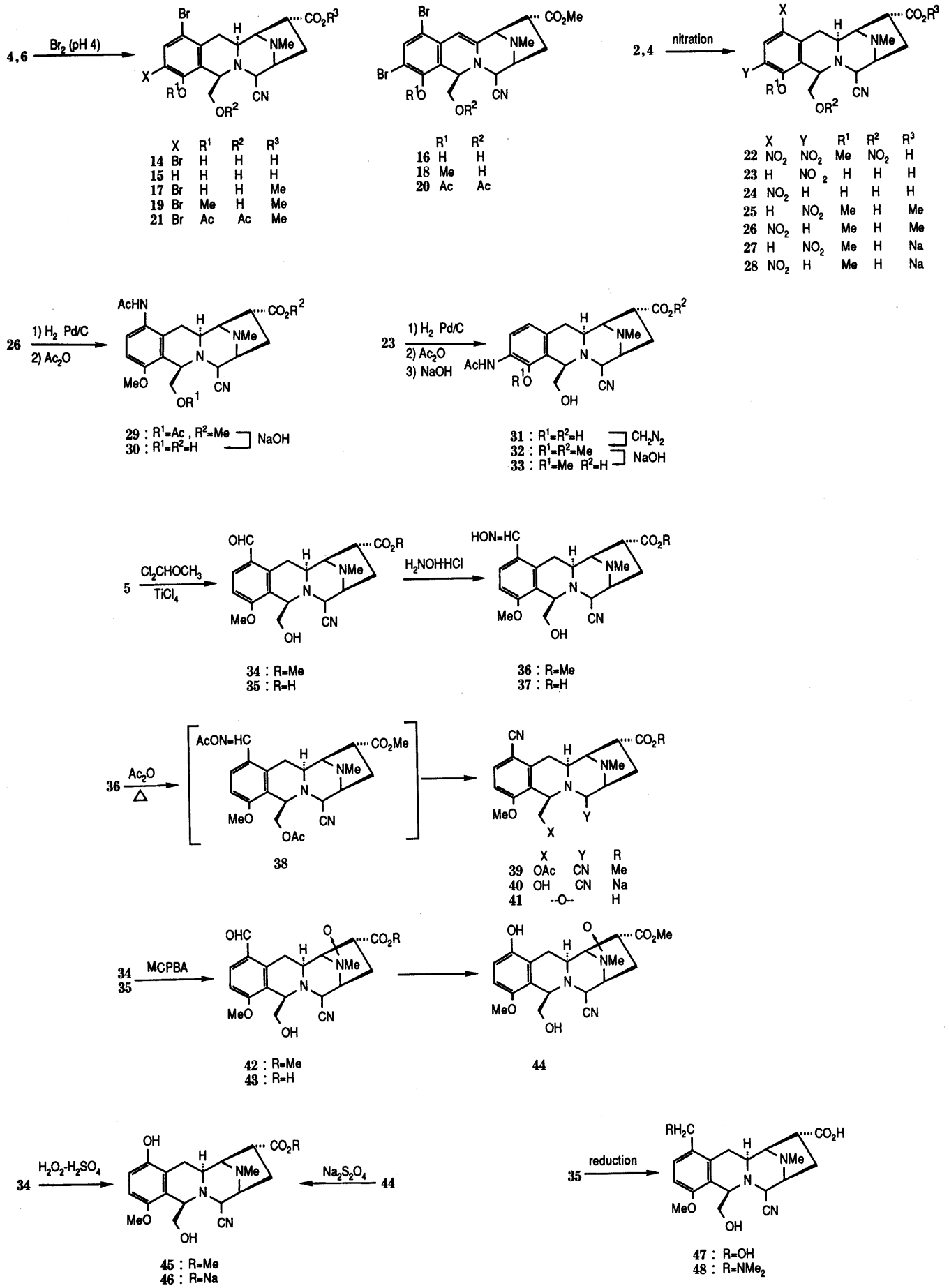


Chart 1



	X	R^1	R^2	R^3
14	Br	H	H	H
15	H	H	H	H
17	Br	H	H	Me
19	Br	Me	H	Me
21	Br	Ac	Ac	Me

	R^1	R^2
16	H	H
18	Me	H
20	Ac	Ac

	X	Y	R^1	R^2	R^3
22	NO_2	NO_2	Me	NO_2	H
23	H	NO_2	H	H	H
24	NO_2	H	H	H	H
25	H	NO_2	Me	H	Me
26	NO_2	H	Me	H	Me
27	H	NO_2	Me	H	Na
28	NO_2	H	Me	H	Na

29 : R^1=Ac , R^2=Me
30 : R^1=R^2=H

31 : R^1=R^2=H
32 : R^1=R^2=Me
33 : R^1=Me R^2=H

34 : R=Me
35 : R=H

36 : R=Me
37 : R=H

38

	X	Y	R
39	OAc	CN	Me
40	OH	CN	Na
41	--O--		H

42 : R=Me
43 : R=H

44

45 : R=Me
46 : R=Na

47 : R=OH
48 : R=NMe_2

Chart 1. (continued)

treatment by NaCN only demethyl quinocarcin (**3**) was obtained. Upon treatment of **2** and **4** with BF_3–Et_2O in MeOH methyl esters **5** and **6** were readily prepared.

Chlorination of **2** was effected with *N*-chlorosuccinimide (NCS) in dimethylformamide (DMF) or Cl_2 in AcOH to afford 10-chloride **7**. Iodination[6] and bromination were also employed in the usual way to give 10-halides **8** and **9**, respectively. Neither 8-halide or 8,10-dihalides were obtained in these halogenations. In contrast, bromination of **4** gave 8,10-dibromide **14** exclusively with 3 eq of bromine. With less than 1 eq of bromine, 10-bromide **15** was a main product along with dibromide **14**. Upon treatment with 5—6 eq of bromine in a mixture of tetrahydrofuran (THF) and pH 4.0 acetate buffer, **6** afforded 8,10-dibromide **17** and, unexpectedly, 11,11a-dehydro-8,10-dibromide **16**, which was rather unstable. In proton nuclear magnetic resonance (^{1}H-NMR) spectrum of **16** vinylic proton (5.61 ppm) appeared, and protons due to 11 and 11a positions diminished. In addition, 6-H was shifted downfield to 4.88 ppm (4.40 in **6**). In ^{13}C-NMR olefinic methine and quartenary carbon appeared at 97.0 and 106.7 ppm, respectively. Treatment of **16** with diazomethane or Ac_2O–$HClO_4$ afforded **18** and **20**, respectively. While the reaction mechanism was ambiguous, 11,11a-dehydro compound **16** might be produced *via* 11-bromide as an intermediate. 10-Halogenated compounds **7**—**9** were converted to the oxazolidine form with HCl or $AgNO_3$ to afford **10**—**12**.

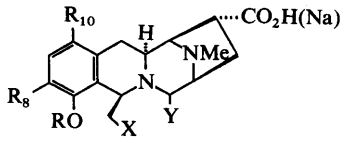
Nitration of **2** with HNO_3–H_2SO_4 afforded 8,10-dinitro-14-nitrate **22** exclusively. Milder nitration with HNO_3 in AcOH resulted in only 14-acetylation without nitration of the aromatic ring. Several conditions examined also gave unsuccessful results. Similar to bromination, nitration of **4** with HNO_3 in AcOH proceeded smoothly to provide 8-nitro **23** and 10-nitro analog **24**, in the ratio of *ca.* 1 : 1, which were readily separated by column chromatography. Nitro compounds **23** and **24** were methylated with diazomethane followed by ester hydrolysis to give 8-nitro **27** and 10-nitro DX-52-1 (**28**), respectively. Acetamides **30** and **33** were obtained from **26** and **23** as shown in Chart 1.

Formyl substituent is of interest, since it can be derivatized to other functionalities. Formylation of **5** was employed with dichloromethylmethylether–$TiCl_4$[7] to give 10-aldehyde **34** in fairly good yield along with a small amount of starting material. Ester of **34** was hydrolyzed to provide corresponding acid **35**. Oximes **36** and **37** were easily obtained from aldehydes **34** and **35** with hydroxylamine hydrochloride. While the *syn–anti* stereochemistry

Table I. Chemical Shifts of Aromatic Carbons

Carbon No.	2 ppm	7 ppm	7 Δppm	8 ppm	8 Δppm	9 ppm	9 Δppm
6a	122.4	124.5	+2.1	124.8	+2.4	124.6	+2.2
7	156.4	155.1	−1.3	155.8	−0.6	156.7	+0.3
8	110.1	111.1	+1.0	111.6	+1.5	112.3	+2.2
9	129.0	128.8	−0.2	132.1	+3.1	138.8	+9.8
10	121.4	125.0	+3.6	114.9	−5.5	89.9	−31.5
10a	138.1	135.4	−2.7	137.1	−1.0	140.4	+2.3

Table II. Antitumor Activities



No.	R	R^8	R^{10}	X	Y	HeLa S_3 IC_{50} (μg/ml)	Dose (mg/kg) ×1	ILS_{max} (%)	P388 i.p.–i.p. (*R*)	Dose ×5	ILS	(*R*)
2	Me	H	H	OH	CN	0.05	20	26	(0.59)	7.5	62	(0.70)
3	H	H	H	–O–		3.03	6.25	14	(0.35)	6.25	22	(0.30)
4	H	H	H	OH	CN	5.32	3.13	18	(0.43)	3.13	22	(0.22)
7	Me	H	Cl	OH	CN	0.042	12.5	23	(0.79)	12.5	88	(0.98)
10	Me	H	Cl	–O–		0.04	12.5	40	(0.93)	6.25	121	(1.00)
8	Me	H	Br	OH	CN	NT	12.5	17	(0.52)	12.5	97	(1.43)
11	Me	H	Br	–O–		0.048	12.5	50	(0.86)	1.56	81	(0.60)
9	Me	H	I	OH	CN	0.11	50	31	(1.15)	25	85	(0.96)
12	Me	H	I	–O–		0.04	25	24	(0.56)	12.5	90	(0.74)
14	H	Br	Br	OH	CN	0.80	25	28	(0.88)	NT		
15	H	H	Br	OH	CN	2.12	NT			NT		
23	H	NO_2	H	OH	CN	0.47	5	17	(0.33)	2.5	30	
27	Me	NO_2	H	OH	CN	0.43	NT			NT		
28	Me	H	NO_2	OH	CN	0.99	100	27	(0.69)	NT		
31	H	NHAc	H	OH	CN	>10	NT			NT		
33	Me	NHAc	H	OH	CN	2.76	NT			NT		
30	Me	H	NHAc	OH	CN	>10	NT			NT		
35	Me	H	CHO	OH	CN	0.56	200	38	(0.95)	NT		
37	Me	H	CH=NOH	OH	CN	1.1	200	38	(0.68)	NT		
40	Me	H	CN	OH	CN	0.30	25	40	(0.74)	22	49	(0.52)
41	Me	H	CN	–O–		0.51	20	22	(0.67)	12.5	75	(0.77)
46	Me	H	OH	OH	CN	1.7	12.5	30	(0.64)	6.25	37	(0.40)
47	Me	H	CH_2OH	OH	CN	>10	NT			NT		
48	Me	H	CH_2NMe_2	OH	CN	>10	NT			NT		
Quinocarcin (**1**)						0.05—0.11	10—20	27—56	(1)	5—10	68—121	(1)

$(R) = ILS_{derivative}/ILS_{quinocarcin}$. NT; not tested.

was not determined, the oximes might be one stereoisomer from their ^{1}H-NMR spectrum. Heating the oxime **36** at 130 °C in Ac_2O afforded the desired nitrile **39** *via* an acetate **38** in good yield. When the reaction was carried out at 60 °C, primarily acetate **38** was obtained. Nitrile **39** was hydrolyzed to give acid **40**, whose treatment with $AgNO_3$ afforded corresponding oxazolidine **41**.

Baeyer–Villiger oxidation of aldehyde **34** was first examined with *m*-chloroperbenzoic acid (MCPBA). With 1 eq of MCPBA *N*-oxide **42** was provided predominantly. Further oxidation of **42** with MCPBA afforded 10-hydroxide **44** *via* formate. The aldehyde was directly converted to **44** with 3 eq of MCPBA in 40% yield. Reduction of **44** with $Na_2S_2O_4$ gave desired 10-hydroxide **45**. On the other hand, oxidation of the aldehyde was preferred with H_2O_2–H_2SO_4[8] to afford **45** without formation of *N*-oxide. Reduction of aldehyde **35** with $NaBH_4$ readily gave alcohol **48**. Reductive amination of **35** with dimethylamine–$NaCNBH_3$ provided **49**.

In the above derivatives, the regiochemistry of the substituent was confirmed by NMR study. 10-Bromide **8** showed significant nuclear Overhauser effect (NOE) between 7-methoxy and 8-H. The other compounds were determined by comparison of ^{13}C-NMR with that of DX-52-1 (**2**). The difference of the chemical shifts of aromatic carbons shown in Table I well agreed with the substituent shielding effect in monosubstituted benzene.[9]

Antitumor Activities Antitumor activities of these derivatives were evaluated as growth inhibition toward HeLa S_3 cells (*in vitro*) and increased life span (ILS) of P388 implanted mice (*in vivo*). DX-52-1 (**2**) retains a significant activity, therefore 4-cyano derivatives were first subjected to evaluation. Subsequently, those derivatives that had considerable activity were converted to oxazolidine form for evaluation.

As shown in Table II, halogen derivatives, even as 4-cyano forms **7**, **8** and **9**, were most promising. They were superior to **2** and their oxazolidine derivatives, chloride **10** and bromide **11** in particular, were more potent than quinocarcin. It was noteworthy that the optimum dose of **11** at daily administration was extremely low, while those of iodides **9** and **12** were rather high.

Demethyl quinocarcin (**3**), demethyl DX-52-1 (**4**) and its bromides **14** and **15** were shown to possess low cytotoxic potency relative to quinocarcin; they might, however, have high toxicity toward experimental animals, as their optimum doses were rather low. The result with nitro derivatives was also disappointing. 10-Nitro DX-52-1 (**28**) showed a considerable effect in a single administration, but a remarkably high dose was necessary; this was also the case for formyl **35** and oxime **37** derivatives. While 10-cyano **40** and **41** exhibited appreciable activity *in vivo*, they could not reach quinocarcin. 10-Hydroxide **46** also had diminished activity, and other electron donating substituents, *e.g.* **30**, **31**, **47** and **48** were all devoid of cytotoxicity. These results suggested that the electron density of aromatic ring was responsible for its antitumor activity. Appropriate electron negativity of a substituent might be desirable. 10-Chloride **10** and 10-bromide **11** were selected for further evaluation. As shown in Table III, both **10** and **11** were active against MX-1. Additionally, **10** was revealed to extend its activity toward B-16 melanoma.

TABLE III. Antitumor Activities toward B-16 and MX-1

Compound No.	B-16 (i.p.–i.p.) Dose (mg/kg)	ILS (%)	MX-1 (s.c.–i.v.) Dose (mg/kg)	*T/C*
10	6.25 × 1	20	3.53 × 5	0.059
	3.13 × 5	80	5.54 × 5	0.0056
11	6.25 × 5	33	6.63 × 5	0.00079
			8.84 × 5	0.0023
1	12.5 × 1	22	5.59 × 5	0.14
	12.5 × 5	36	8.78 × 5	0.045

Experimental

Infrared (IR) spectra were measured with a JASCO IR-810, NMR spectra were measured on Varian EM-390, JEOL FX-100 and Bruker AM-400 spectrometers. Mass spectra (MS) were measured with a Hitachi B-80. For column chromatography, silica gel (SiO_2, Wako C-200) or high porous polymer resin (Mitsubishi Kasei Diaion HP-20 or HP-20SS) were used. Thin layer chromatography (TLC) was performed on Silica gel 60 F_{254} plate (Merck). All organic solvent extracts were dried over anhydrous sodium sulfate. All aqueous fractions after chromatography were freeze dried.

4-Cyano-6-hydroxymethyl-3,12-imino-7-methoxy-13-methyl-1,2,3,4,6,11,-11a,12-octahydroazepino[1,2-*b*]isoquinoline-1-carboxylic Acid (2) Quinocarcin (55 g, 0.156 mol) was dissolved in H_2O (1 l). A saturated $NaHCO_3$ solution was added to adjust the pH to 5.0. To the mixture was added NaCN (11.46 g, 0.234 mol), and the reaction mixture was stirred at room temperature for 2 h. Then, 6 N HCl was added to adjust the pH to 3.5 under ice-cooling. After standing for 3 h at 0 °C, the resulting precipitate was filtered and dried to afford **2** (53.5 g, 90%) as a white solid. An analytical sample was prepared by recrystallization from MeOH–ether. mp 190—195 °C (dec.). *Anal.* Calcd for $C_{19}H_{23}N_3O_4$: C, 63.85; H, 6.49; N, 11.76. Found: C, 64.12; H, 6.75; N, 11.47. ^{1}H-NMR (D_2O, pD = 6.5) ppm: 7.25 (1H, t, J = 7.9 Hz), 6.92 (1H, d, J = 8.1 Hz), 6.86 (1H, d, J = 7.4 Hz), 4.23 (1H, d, J = 3.0 Hz), 4.13 (1H, t, J = 2.3 Hz), 3.82 (3H, s), 3.72 (1H, dd, J = 11.4, 3.1 Hz), 3.66 (1H, dd, J = 11.4, 3.9 Hz), 3.57 (1H, dd, J = 5.8, 3.0 Hz), 3.49 (1H, br s), 3.09 (1H, dd, J = 10.1, 5.5 Hz), 2.79 (1H, m), 2.68 (2H, m), 2.50 (1H, dt, J = 13.2, 6.0 Hz), 2.20 (3H, s), 2.03 (1H, dd, J = 13.1, 10.0 Hz). ^{13}C-NMR (D_2O, pD = 7.2) ppm: 183.7, 156.4, 138.1, 129.0, 122.4, 121.4, 119.4, 110.1, 70.8, 65.5, 65.2, 58.7, 58.4, 58.3, 56.3, 45.1, 41.8, 33.0, 30.0 IR (KBr): 3350, 1739, 1592, 1472, 1389, 1351, 1267 cm^{-1}.

***O*-Demethyl-quinocarcin (3)** Compound **2** (40 mg, 0.105 mmol) was suspended in BBr_3/CH_2Cl_2 (0.5 M, 4 ml). The mixture was stirred at −60 °C to room temperature for 26 h. Ice was added and the aqueous layer was separated, then concentrated *in vacuo*. The residue was purified by column chromatography (Diaion HP-20SS 10 ml, H_2O) to afford **3** (6.8 mg, 17.7%). ^{1}H-NMR (CD_3OD) ppm: 7.00 (1H, m), 6.66 (1H, d, J = 8.1 Hz), 6.63 (1H, d, J = 7.5 Hz), 4.60 (1H, d, J = 3.0 Hz), 4.55 (1H, dd, J = 6.9, 3.1 Hz), 4.14 (1H, br s), 4.13 (1H, m), 3.74 (1H, dd, J = 10.7, 3.1 Hz), 3.54 (1H, dd, J = 10.3, 5.4 Hz), 3.45 (1H, m), 3.44 (1H, dd, J = 10.7, 7.1 Hz), 2.80 (3H, s), 2.75 (1H, m), 2.63 (1H, m), 2.61 (1H, dd, J = 14.5, 2.7 Hz), 2.53 (1H, dd, J = 13.5, 10.3 Hz). SIMS (*m/z*): 317 $(M+1)^+$, 245.

4-Cyano-7-hydroxy-6-hydroxymethyl-3,12-imino-13-methyl-1,2,3,4,6,11,-11a,12-octahydroazepino[1,2-*b*]isoquinoline-1-carboxylic Acid (4) To a suspension of **2** (15 g, 42 mmol) in CH_2Cl_2 (150 ml) was added dropwise 150 ml of BBr_3/CH_2Cl_2 (50 g/200 ml) at −78 °C. The mixture was stirred for 22 h at −78 °C to room temperature, then cooled to −78 °C and another 100 ml of BBr_3/CH_2Cl_2 (25 g/200 ml) was added. After stirring at −78 °C to room temperature for 7 h, ice was added to the reaction mixture and the pH of aqueous layer was adjusted at 7.2 with 5 N NaOH. NaCN (3.87 g) was added and the mixture was stirred for 1 h, then the aqueous layer was separated and concentrated *in vacuo*. The residue was subjected to chromatography (Diaion HP-20, 2 l, H_2O : MeOH = 1 : 0—9 : 1) to give Na salt of **4** (11.17 g). It was dissolved in H_2O (80 ml), and 1 N HCl was added to adjust the pH at 3.5. The precipitate was filtered and dried to obtain **4** (10.4 g, 76.6%) as a white solid. ^{1}H-NMR (D_2O, NaOD. pD = 9.3) ppm: 7.13 (1H, m), 6.75 (2H, m), 4.24 (1H, d, J = 2.7 Hz), 4.13 (1H, m), 3.70 (2H, m), 3.57 (1H, m), 3.51 (1H, br s), 3.10 (1H, dd, J = 10.0, 5.4 Hz), 2.40—2.84 (4H, m), 2.22 (3H, s), 2.03 (1H, dd, J = 10.5, 13.2 Hz). SIMS (*m/z*): 344 $(M+1)^+$, 317. ^{13}C-NMR (D_2O, NaOD, pD = 9.3) ppm: 184.4, 154.6, 138.6, 128.9, 121.9, 120.1, 119.7, 114.8, 70.7, 65.0, 65.0, 58.6, 45.3, 41.9, 33.3, 30.0.

Methyl 4-Cyano-6-hydroxymethyl-3,12-imino-7-methoxy-13-methyl-1,2,3,4,6,11,11a,12-octahydroazepino[1,2-*b*]isoquinoline-1-carboxylate (5) To a solution of Na salt of **2** (500 mg, 1.32 mmol) in MeOH (15 ml) was added $BF_3 \cdot OEt_2$ (1.5 ml). The mixture was stirred at room temperature for 18 h, at 40 °C for 8 h, then at room temperature for a further 15 h. The reaction mixture was concentrated, then AcOEt and saturated $NaHCO_3$ were added. The organic layer was separated, washed with brine, dried and concentrated to afford methyl ester **5** (433 mg, 88.4%) as a white solid. An analytical sample was prepared by recrystallization from ether. mp 179—180.5 °C. *Anal.* Calcd for $C_{20}H_{25}N_3O_4$: C, 64.66; H, 6.80; N, 11.31. Found: C, 64.38; H, 6.76; N, 11.15. ^{1}H-NMR ($CDCl_3$) ppm: 7.15 (1H, m), 6.72 (2H, m), 4.29 (1H, m), 4.02 (1H, d, J=2.7 Hz), 3.81 (3H, s), 3.73 (3H, s), 3.60—3.92 (2H, m), 3.44 (2H, m), 3.16 (1H, dd, J=9.5, 5.4 Hz), 2.50—3.10 (4H, m), 2.33 (3H, s), 1.97 (1H, dd, J=13.2, 9.5 Hz). ^{13}C-NMR ($CDCl_3$) ppm: 175.9, 155.8, 136.2, 127.8, 122.1, 120.4, 117.8, 108.6, 70.5, 65.8, 64.7, 58.0, 57.8, 57.6, 55.3, 52.2, 42.8, 41.9, 33.0, 28.9. SIMS (*m/z*): 372 $(M+1)^+$, 345, 340.

Methyl 4-Cyano-7-hydroxy-6-hydroxymethyl-3,12-imino-13-methyl-1,2,-3,4,6,11,11a,12-octahydroazepino[1,2-*b*]isoquinoline-1-carboxylate (6) To a solution of **4** (500 mg, 1.37 mmol) in MeOH (15 ml) was added $BF_3 \cdot OEt_2$ (1.5 ml), and the mixture was stirred for 23 h at room temperature. After concentration, AcOEt and saturated $NaHCO_3$ were added to the residue, and the organic layer was separated, washed with brine, dried and concentrated to afford **6** (424 mg, 86.7%) as a white solid. An analytical sample was prepared by recrystallization from acetone–ether. mp 180—185 °C (dec.). *Anal.* Calcd for $C_{19}H_{23}N_3O_4$: C, 63.85; H, 6.49; N, 11.76. Found: C, 64.08; H, 6.69; N, 11.57. ^{1}H-NMR ($CDCl_3$–CD_3OD) ppm: 7.04 (1H, m), 6.68 (2H, m), 4.26 (2H, m), 3.76 (3H, s), 3.44—4.08 (4H, m), 3.26 (1H, dd, J=10, 6 Hz), 3.02 (1H, m), 2.48—2.88 (3H, m), 2.30 (3H, s), 2.08 (1H, dd, J=13, 10 Hz). IR (KBr): 3460, 3156, 1718, 1685, 1589, 1466, 1434 cm^{-1}.

Sodium 10-Chloro-4-cyano-6-hydroxymethyl-3,12-imino-7-methoxy-13-methyl-1,2,3,4,6,11,11a,12-octahydroazepino[1,2-*b*]isoquinoline-1-carboxylate (7) To a solution of Na salt of **2** (113 mg, 0.30 mmol) in AcOH (1.5 ml) was added dropwise Cl_2/AcOH (1.3 M, 3.4 ml). The mixture was stirred at room temperature for 1.5 h, then concentrated. The residue was dissolved in H_2O, and aqueous $NaHCO_3$ was added to adjust the pH to 8. This solution was then subjected to chromatography (Diaion HP-20 12 ml, H_2O : MeOH = 1 : 0—0 : 1) to give **7** (87 mg, 70%). ^{1}H-NMR (CD_3OD) ppm: 7.23 (1H, d, J=9 Hz), 6.82 (1H, d, J=9 Hz), 4.28 (1H, d, J=3 Hz), 4.20 (1H, m), 3.83 (3H, s), 3.30—3.73 (4H, m), 2.40—3.20 (5H, m), 2.33 (3H, s), 2.07 (1H, dd, J=13, 9 Hz). ^{13}C-NMR (D_2O) ppm: 184.2, 155.1, 135.4, 128.8, 125.0, 124.5, 119.6, 111.1, 70.5, 65.0, 64.8, 58.6, 58.4, 57.9, 56.5, 45.3, 41.9, 30.7, 30.1. IR (KBr): 3410, 2950, 1650, 1558, 1465, 1394, 1277, 1259, 1075 cm^{-1}. EIMS (*m/z*): (Me ester) 374, 376 (M^+).

Sodium 10-Bromo-4-cyano-6-hydroxymethyl-3,12-imino-7-methoxy-13-methyl-1,2,3,4,6,11,11a,12-octahydroazepino[1,2-*b*]isoquinoline-1-carboxylate (8) Na salt of **2** (700 mg, 1.85 mmol) was dissolved in AcOH (10 ml). Br_2/AcOH (1 M, 2.9 ml) was added in three portions over 5 h with stirring. The mixture was concentrated and the residue was dissolved in aqueous $NaHCO_3$. This solution was subjected to chromatography (Diaion HP-20SS 110 ml, H_2O : MeOH = 1 : 0—1 : 2) to afford **8** (478 mg, 56.5%). ^{1}H-NMR (CD_3OD) ppm: 7.40 (1H, d, J=9 Hz), 6.77 (1H, d, J=9 Hz), 4.30 (1H, d, J=3 Hz), 4.22 (1H, m), 3.38 (3H, s), 3.40—3.80 (4H, m), 2.43—3.20 (5H, m), 2.33 (3H, s), 2.15 (1H, m). ^{13}C-NMR (D_2O) ppm: 184.2, 155.8, 137.1, 132.1, 124.8, 119.6, 114.9, 111.6, 70.4, 65.0, 65.0, 58.6, 58.4, 58.1, 56.4, 45.4, 42.0, 33.6, 30.1. SIMS (*m/z*): 458, 460 $(M+1)^+$.

Sodium 10-Iodo-4-cyano-6-hydroxymethyl-3,12-imino-7-methoxy-13-methyl-1,2,3,4,6,11,11a,12-octahydroazepino[1,2-*b*]isoquinoline-1-carboxylate (9) Na salt of **2** (504 mg, 1.33 mmol) was dissolved in a mixture of AcOH (10 ml), H_2O (2 ml) and H_2SO_4 (0.3 ml). To the solution was added I_2 (337 mg) and H_5IO_6 (151 mg), then the mixture was heated at 65 °C for 1 h 15 min. After cooling, 5 N NaOH (1.9 ml) was added and the mixture was concentrated *in vacuo*. The residue was dissolved in saturated $NaHCO_3$, then 5 N NaOH (4 ml) was added to adjust the pH above 11. After addition of $Na_2S_2O_3$ (2 g), the mixture was subjected to chromatography (Diaion HP-20 100 ml, H_2O : MeOH = 1 : 0—1 : 1) to get **9** (402 mg, 60%) as a white solid. mp 205—210 °C (dec.). *Anal.* Calcd for $C_{19}H_{21}N_3NaO_4 \cdot 2H_2O$: C, 42.16; H, 4.65; N, 7.76. Found: C, 42.27; H, 4.67; N, 7.48. ^{1}H-NMR (CD_3OD) ppm: 7.67 (1H, d, J=8 Hz), 6.63 (1H, d, J=8 Hz), 4.27 (1H, d, J=3 Hz), 4.20 (1H, dd, J=7, 3 Hz), 3.83 (3H, s), 3.33—3.80 (4H, m), 2.50—3.60 (5H, m), 2.34 (3H, s), 2.08 (1H, dd, J=13, 10 Hz). ^{13}C-NMR (D_2O) ppm: 184.2, 156.8, 140.4, 138.8, 124.6, 119.6, 112.3, 89.9, 70.3, 65.0, 65.0, 58.6, 58.4, 56.4, 45.4, 42.0, 38.9, 30.1. IR (KBr): 3420, 2946, 1559, 1461, 1394, 1276, 1258, 1075 cm^{-1}. EIMS (*m/z*): (Me ester) 497 (M^+), 466 $(M\text{-}OMe)^+$.

10-Chloro Quinocarcin (10) Na salt of **7** (366 mg, 0.88 mmol) was heated in conc. HCl (7 ml) at 50 °C for 2.5 h. After cooling, the mixture was subjected to chromatography (Diaion HP-20 40 ml, H_2O : MeOH = 1 : 0—1 : 1) to give **10** (143 mg, 44%). ^{1}H-NMR (CD_3OD) ppm: 7.28 (1H, d, J=8.8 Hz), 6.89 (1H, d, J=8.9 Hz), 4.62 (1H, d, J=2.9 Hz), 4.55 (1H, dd, J=6.1, 2.6 Hz), 4.28 (1H, s), 4.25 (1H, m), 3.84 (3H, s), 3.75 (1H, dd, J=10.4, 5.7 Hz), 3.70 (1H, dd, J=11.0, 2.7 Hz), 3.47 (1H, m), 3.43 (1H, dd, J=11.0, 6.2 Hz), 3.11 (1H, dd, J=15.2, 2.6 Hz), 2.85 (3H, s), 2.70—2.54 (3H, m). ^{13}C-NMR (CD_3OD) ppm: 174.8, 155.9, 134.4, 129.2, 126.2, 125.3, 111.3, 91.5, 71.8, 67.2, 66.6, 56.2, 56.1, 54.9, 40.8, 40.2, 29.7, 27.2. IR (KBr): 3410, 1585, 1470, 1385, 1290, 1265, 1090, 810 cm^{-1}. SIMS (*m/z*): 365, 367 $(M+1)^+$.

10-Bromo Quinocarcin (11) In the same manner as described for **10**, Na salt of **8** (308 mg) gave **11** (107 mg, 37.0%). ^{1}H-NMR (D_2O) ppm: 7.50 (1H, d, J=8.9 Hz), 6.85 (1H, d, J=8.9 Hz), 4.95 (1H, d, J=3.3 Hz), 4.48 (1H, m), 4.27 (1H, br s), 3.98 (1H, m), 3.82 (3H, s), 3.63 (1H, dd, J=11.4, 3.3 Hz), 3.55 (1H, dd, J=9.5, 4.6 Hz), 3.53 (1H, dd, J=11.4, 4.8 Hz), 3.45 (1H, m), 3.09 (1H, dd, J=15.4, 2.5 Hz), 2.81 (3H, s), 2.57 (2H, m), 2.46 (1H, dd, J=14.2, 10.6 Hz). ^{13}C-NMR (D_2O) ppm: 179.4, 156.0, 136.2, 132.2, 125.7, 114.7, 112.1, 81.8, 71.6, 70.0, 65.7, 56.5, 54.5, 53.6, 41.1, 40.6, 32.4, 27.4. SIMS (*m/z*): 411, 409 $(M+1)^+$.

10-Iodo Quinocarcin (12) Na salt of **9** (329 mg, 0.65 mmol) in MeOH (6 ml) and CH_3CN (6 ml) was treated with $AgNO_3$ (121 mg) at room temperature for 2 h. The mixture was concentrated *in vacuo*, and 1 N HCl (4 ml) was added to the residue. The precipitate was filtered off, and the filtrate was subjected to chromatography (Diaion HP-20 40 ml, H_2O : MeOH = 1 : 0—1 : 1) to afford **12** (152 mg, 51%). ^{1}H-NMR (CD_3OD) ppm: 7.70 (1H, d, J=8.7 Hz), 6.69 (1H, d, J=8.7 Hz), 4.59 (1H, d, J=3.1 Hz), 4.54 (1H, dd, J=6.4, 2.7 Hz), 4.22 (1H, s), 4.16 (1H, m), 3.83 (3H, s), 3.66 (1H, dd, J=10.8, 2.7 Hz), 3.55 (1H, m), 3.41 (1H, d, J=12.1 Hz), 3.39 (1H, dd, J=10.9, 6.6 Hz), 2.93 (1H, dd, J=15.1, 2.5 Hz), 2.82 (3H, s), 2.69 (1H, dd, J=15.0, 11.8 Hz), 2.64 (1H, m), 2.51 (1H, dd, J=14.1, 10.5 Hz). ^{13}C-NMR (CD_3OD) ppm: 175.3, 157.5, 139.8, 139.2, 126.3, 112.5, 91.8, 89.5, 72.4, 67.6, 66.7, 56.2, 56.1, 55.6, 41.6, 40.7, 38.3, 27.8. IR (KBr): 3400, 2960, 1725, 1580, 1465, 1280, 1265, 1090, 810 cm^{-1}. SIMS (*m/z*): 457 $(M+1)^+$, 331.

4-Cyano-8,10-dibromo-7-hydroxy-6-hydroxymethyl-3,12-imino-13-methyl-1,2,3,4,6,11,11a,12-octahydroazepino[1,2-*b*]isoquinoline-1-carboxylic Acid (14) In the same manner as described for **8**, Na salt of **4** (300 mg) with 4 eq Br_2 afforded **14** (353 mg. 82.2%) as a pale brown solid. mp 160—165 °C (dec.). ^{1}H-NMR (CD_3OD) ppm: 7.59 (1H, s), 4.28 (2H, m), 3.33—3.90 (4H, m), 2.40—3.17 (4H, m), 2.37 (3H, s), 2.10 (1H, m). ^{13}C-NMR (D_2O) ppm; 176.1, 149.6, 134.9, 134.7, 125.1, 116.6, 114.9, 110.9, 70.6, 66.0, 65.6, 58.5, 56.8, 56.4, 41.2, 40.6, 31.9, 28.7. IR (KBr): 3400, 1710, 1580 cm^{-1}.

10-Bromo-4-cyano-7-hydroxy-6-hydroxymethyl-3,12-imino-13-methyl-1,2,3,4,6,11,11a,12-octahydroazepino[1,2-*b*]isoquinoline-1-carboxylic Acid (15) In the same manner as described for **8**, Na salt of **4** (200 mg) with 0.7 eq Br_2 gave **15** (56.4 mg, 24.4%). ^{1}H-NMR (CD_3OD) ppm: 7.23 (1H, d, J=8 Hz), 6.58 (1H, d, J=8 Hz), 4.57 (1H, m), 4.03—4.37 (3H, m), 3.80 (1H, m), 3.53 (1H, br s), 3.37—3.57 (2H, m), 2.43—3.07 (3H, m), 2.33 (3H, s), 2.10 (1H, m). ^{13}C-NMR (D_2O) ppm: 176.2, 152.9, 135.3, 132.7, 122.9, 122.7, 116.5, 113.8, 70.7, 65.9, 65.3, 58.0, 56.9, 40.5, 32.1, 28.6.

Methyl 4-Cyano-8,10-dibromo-7-hydroxy-6-hydroxymethyl-3,12-imino-13-methyl-1,2,3,4,6,12-hexahydroazepino[1,2-*b*]isoquinoline-1-carboxylate (16) and Methyl 4-Cyano-8,10-dibromo-7-hydroxy-6-hydroxymethyl-3,12-imino-13-methyl-1,2,3,4,6,11,11a,12-octahydroazepino[1,2-*b*]isoquinoline-1-carboxylate (17) Me ester **6** (100 mg, 0.28 mmol) in THF (4 ml) and 0.2 M acetate buffer (pH 4.0, 4 ml) was treated with Br_2 (0.072 ml, 1.40 mmol) for 50 min. The mixture was extracted with AcOEt, washed with aqueous Na_2SO_3 and brine, dried and concentrated to afford a crude product (208 mg). This was purified by column chromatography (SiO_2 10 ml, *n*-hexane : AcOEt = 1 : 1) to give **16** (68.6 mg, 47.8%) as a red purple solid and **17** (46.9 mg, 32.5%). **16**: mp 152—155 °C. ^{1}H-NMR ($CDCl_3$) ppm: 7.49 (1H, s), 5.61 (1H, s), 4.88 (1H, dd, J=8.9, 3.4 Hz), 4.33 (1H, br s), 3.98 (1H, s), 3.74—3.84 (2H, m), 3.76 (3H, s), 3.57 (1H, dd, J=10.7, 3.5 Hz), 3.15 (1H, dd, J=9.8, 5.3 Hz), 2.70 (1H, ddd, J=13.6, 6.9, 5.5 Hz), 2.58 (3H, s), 2.35 (1H, dd, J=13.6, 9.9 Hz). ^{13}C-NMR ($CDCl_3$) ppm: 174.1, 147.2, 141.6, 133.9, 132.6, 118.4, 114.4, 110.2, 106.7, 97.0, 66.6, 64.0, 63.6, 57.3, 52.8, 52.6, 49.0, 38.3, 31.2. EIMS (*m/z*): 515 513 511 (M^+), 484 482 480 $(M-OMe)^+$, 398 396 394, 345 343 341. **17**: ^{1}H-NMR (CD_3OD–DCl) ppm: 7.65 (1H, s), 4.33—4.60 (3H, m), 3.82 (3H, s), 3.40—3.97 (4H, m),

2.93 (3H, s), 2.47—3.13 (4H, m). EIMS (*m/z*): 514 516 518, 482 484 486, 346 348 350, 343 345 347.

Methyl 4-Cyano-8,10-dibromo-6-hydroxymethyl-3,12-imino-7-methoxy-13-methyl-1,2,3,4,6,12-hexahydroazepino[1,2-*b*]isoquinoline-1-carboxylate (18) and Methyl 4-Cyano-8,10-dibromo-6-hydroxymethyl-3,12-imino-7-methoxy-13-methyl-1,2,3,4,6,11,11a,12-octahydroazepino[1,2-*b*]isoquinoline-1-carboxylate (19) In the same manner as described for **16** and **17**, methyl ester **6** (100 mg) afforded a crude mixture of **16** and **17**. This crude product was treated with CH_2N_2/ether. After completion of the reaction, monitored by TLC, the mixture was concentrated and then subjected to chromatography (SiO_2 20 ml, *n*-hexane : AcOEt = 2 : 1) to obtain **18** (22.8 mg, 15.5%) and **19** (60.5 mg, 40.9%). **18**: ^{1}H-NMR ($CDCl_3$) ppm: 7.58 (1H, s), 5.63 (1H, s), 4.72 (1H, dd, *J* = 9, 5 Hz), 4.29 (1H, m), 3.97 (1H, s), 3.88 (3H, s), 3.75 (3H, s), 3.60—3.83 (2H, m), 3.43 (1H, m), 3.12 (1H, dd, *J* = 10, 6 Hz), 2.57 (3H, s), 2.10—2.87 (2H, m). EIMS (*m/z*): 529 527 525 (M^+), 412 410 408, 359 357 355. **19**: ^{1}H-NMR ($CDCl_3$) ppm: 7.68 (1H, s), 4.17 (1H, m), 4.03 (1H, d, *J* = 3 Hz), 3.88 (3H, s), 3.75 (3H, s), 3.37—3.83 (4H, m), 3.18 (1H, dd, *J* = 10, 5 Hz), 1.93—3.10 (5H, m), 2.33 (3H, s). EIMS (*m/z*): 531 529 527 (M^+), 500 498 496, 361 359 357, 180, 140, 121.

Methyl 7-Acetoxy-6-acetoxymethyl-4-cyano-8,10-dibromo-3,12-imino-13-methyl-1,2,3,4,6,12-hexahydroazepino[1,2-*b*]isoquinoline-1-carboxylate (20) and Methyl 7-Acetoxy-6-acetoxymethyl-4-cyano-8,10-dibromo-3,12-imino-13-methyl-1,2,3,4,6,11,11a,12-octahydroazepino[1,2-*b*]isoquinoline-1-carboxylate (21) In the same manner as for **16** and **17**, methyl ester (100 mg) gave a crude mixture of **16** and **17**. This was dissolved in Ac_2O (3 ml), and 70% $HClO_4$ (0.15 ml) was added under ice-cooling. The mixture was stirred at room temperature for 6.5 h, then concentrated. To the residue AcOEt and saturated $NaHCO_3$ were added, and the organic layer was separated. This was washed with brine, dried and concentrated. The residue was purified by column chromatography (SiO_2 20 ml, *n*-hexane : AcOEt = 2 : 1—1 : 1) to give **20** (34.6 mg, 20.7%) and **21** (17.4 mg, 10.4%). **20**: ^{1}H-NMR ($CDCl_3$) ppm: 7.68 (1H, s), 5.81 (1H, s), 4.75 (1H, dd, *J* = 8, 5 Hz), 3.67—4.10 (5H, m), 3.77 (3H, s), 3.08 (1H, dd, *J* = 10, 5 Hz), 2.70 (1H, m), 2.53 (3H, s), 2.42 (3H, s), 2.23 (1H, m), 2.09 (3H, s). EIMS (*m/z*): 600 598 596 $(M+1)^+$, 526 524 522, 484 482 480. **21**: ^{1}H-NMR ($CDCl_3$) ppm: 7.77 (1H, s), 4.00—4.30 (3H, m), 3.67—3.87 (2H, m), 3.75 (3H, s), 3.48 (1H, br s), 3.43 (1H, m), 2.87—3.13 (2H, m), 2.37 (3H, s), 2.32 (3H, s), 2.03 (3H, s), 1.87—2.70 (3H, m). EIMS (*m/z*): 602 600 598 $(M+1)^+$, 528 526 524, 486 484 482, 389 387 385, 347 345 343, 180, 140.

4-Cyano-8,10-dinitro-3,12-imino-7-methoxy-13-methyl-6-nitroxymethyl-1,2,3,4,6,11,11a,12-octahydroazepino[1,2-*b*]isoquinoline-1-carboxylic Acid (22) Compound **2** (100 mg, 0.28 mmol) was added to a mixture of H_2SO_4–HNO_3 (1 : 1, 0.5 ml) and the mixture was stirred for 3 h 20 min under ice-cooling. Ice was added to the mixture and the precipitate was filtered, washed with H_2O, then dried to afford **22** (84.9 mg, 61.6%) as a yellow solid. mp 172—175 °C (dec.). ^{1}H-NMR ($CDCl_3$–CD_3OD) ppm: 8.63 (1H, s), 4.30—4.70 (4H, m), 4.07 (3H, s), 4.00 (1H, m), 3.40—3.70 (overlapped with solvent peak) 2.92 (3H, s), 2.87 (1H, m), 2.45 (1H, m). IR (KBr): 3430, 1726, 1639, 1596, 1532, 1384, 1347, 1278 cm^{-1}. EIMS (*m/z*): (Me ester) 506 $(M)^+$, 460 $(M-NO_2)^+$, 430, 264.

4-Cyano-7-hydroxy-6-hydroxymethyl-3,12-imino-13-methyl-8-nitro-1,2,-3,4,6,11,11a,12-octahydroazepino[1,2-*b*]isoquinoline-1-carboxylic Acid (23) and 4-Cyano-7-hydroxy-6-hydroxymethyl-3,12-imino-13-methyl-10-nitro-1,2,3,4,6,11,11a,12-octahydroazepino[1,2-*b*]isoquinoline-1-carboxylic Acid (24) To a solution of **4** (100 mg, 0.29 mmol) in AcOH (4 ml) was added conc. HNO_3 (0.039 ml), and the mixture was stirred for 2.5 h. Then conc. HNO_3 (0.01 ml) was added, and stirring was continued for an additional 2 h. The reaction mixture was concentrated and subjected to chromatography (Diaion HP-20 10 ml, H_2O : MeOH = 1 : 0—1 : 1) to give **23** (33.2 mg, 31.2%) and **24** (40.3 mg, 37.9%) as a yellow solid. **23**: mp > 160 °C (dec.). ^{1}H-NMR (CD_3OD) ppm: 8.04 (1H, d, *J* = 8.5 Hz), 6.88 (1H, d, *J* = 8.5 Hz), 4.36 (2H, m), 3.92 (1H, dd, *J* = 10, 3 Hz), 3.66 (1H, dd, *J* = 10, 6 Hz), 3.58 (1H, m), 3.56 (1H, br s), 2.48—3.08 (4H, m), 2.34 (3H, s), 2.12 (1H, dd, *J* = 13, 10 Hz). ^{13}C-NMR (D_2O) ppm: 176.2, 151.7, 146.0, 133.1, 124.9, 123.9, 121.1, 116.6, 70.7, 66.0, 64.6, 57.6, 56.7, 56.0, 41.2, 40.5, 32.2, 28.6. EIMS (*m/z*): $(+CH_2N_2)$ 415 $(M-1)^+$, 385, 246, 180, 140. **24**: ^{1}H-NMR (CD_3OD) ppm: 7.90 (1H, d, *J* = 9 Hz), 6.82 (1H, d, *J* = 9 Hz), 4.34 (2H, m), 3.40—3.96 (5H, m), 2.52—3.24 (4H, m), 2.34 (3H, s), 2.14 (1H, m). IR (KBr): 3400, 1588, 1520, 1340, 1303 cm^{-1}. SIMS (*m/z*): 389 $(M+1)^+$.

Methyl 4-Cyano-6-hydroxymethyl-3,12-imino-7-methoxy-13-methyl-8-nitro-1,2,3,4,6,11,11a,12-octahydroazepino[1,2-*b*]isoquinoline-1-carboxylate (25) To a solution of **23** (60 mg) in MeOH (0.6 ml) was gradually added CH_2N_2 in ether until N_2 gas evolution had ceased. Then the reaction mixture was concentrated and subjected to chromatography (SiO_2 10 ml, *n*-hexane : AcOEt = 2 : 1—1 : 1) to obtain **25** (54 mg, 83.9%). ^{1}H-NMR ($CDCl_3$) ppm: 7.78 (1H, d, *J* = 8 Hz), 7.02 (1H, d, *J* = 8 Hz), 4.28 (1H, m), 4.12 (1H, m), 3.94 (3H, s), 3.84 (1H, m), 3.76 (3H, s), 3.52 (1H, br s), 3.44—3.64 (2H, m), 2.96—3.28 (2H, m), 2.48—2.92 (3H, m), 2.32 (3H, s), 2.02 (1H, dd, *J* = 13, 9 Hz). IR (KBr): 3400—3550, 1728, 1594, 1524, 1460, 1348, 1247, 1177, 1069 cm^{-1}.

Methyl 4-Cyano-6-hydroxymethyl-3,12-imino-7-methoxy-13-methyl-10-nitro-1,2,3,4,6,11,11a,12-octahydroazepino[1,2-*b*]isoquinoline-1-carboxylate (26) In the same manner as for **25**, **24** (200 mg) afforded **26** (162 mg, 75.5%). ^{1}H-NMR ($CDCl_3$) ppm: 7.98 (1H, d, *J* = 9 Hz), 6.82 (1H, d, *J* = 9 Hz), 4.30 (1H, m), 4.03 (1H, d, *J* = 3 Hz), 3.92 (3H, s), 3.79 (3H, s), 3.40—3.87 (3H, m), 3.50 (1H, br s), 2.50—3.30 (5H, m), 2.33 (3H, s), 2.00 (1H, dd, *J* = 14, 10 Hz). ^{13}C-NMR ($CDCl_3$) ppm: 175.6, 159.4, 142.3, 133.5, 125.9, 124.5, 117.5, 108.3, 70.0, 65.0, 64.6, 58.0, 57.5, 56.8, 56.2, 52.4, 42.8, 41.9, 30.4, 29.1. IR (KBr): 3400, 1726, 1603, 1584, 1514, 1472, 1443, 1346, 1297, 1275 cm^{-1}. EIMS (*m/z*): 416 (M^+), 385 $(M-OMe)^+$.

Sodium 4-Cyano-6-hydroxymethyl-3,12-imino-7-methoxy-13-methyl-8-nitro-1,2,3,4,6,11,11a,12-octahydroazepino[1,2-*b*]isoquinoline-1-carboxylate (27) Methyl ester **25** (50 mg, 0.12 mmol) in THF (1.5 ml) and MeOH (0.3 ml) was treated with 0.5 N NaOH (1.0 ml) for 1.5 h. The reaction mixture was concentrated and the residue was chromatographed (Diaion HP20 10 ml, H_2O : MeOH = 1 : 0—4 : 1) to afford **27** (36.8 mg, 72.2%). ^{1}H-NMR (CD_3OD) ppm: 7.78 (1H, d, *J* = 8 Hz), 7.12 (1H, d, *J* = 8 Hz), 4.30 (2H, m), 3.96 (3H, s), 3.40—3.88 (4H, m), 2.48—3.16 (5H, m), 2.36 (3H, s), 2.08 (1H, m). IR (KBr): 1636, 1593, 1557, 1541, 1522, 1464, 1394, 1348, 1244, 1135, 1067, 1030 cm^{-1}.

Sodium 4-Cyano-6-hydroxymethyl-3,12-imino-7-methoxy-13-methyl-10-nitro-1,2,3,4,6,11,11a,12-octahydroazepino[1,2-*b*]isoquinoline-1-carboxylate (28) In the same manner as for **27**, methyl ester **26** (134 mg) gave **28** (100 mg, 73.8%). ^{1}H-NMR (CD_3OD) ppm: 8.00 (1H, d, *J* = 8 Hz), 7.00 (1H, d, *J* = 8 Hz), 4.30 (2H, m), 3.98 (3H, s), 3.32—3.88 (4H, m), 2.44—3.20 (5H, m), 2.34 (3H, s), 2.08 (1H, m). IR (KBr): 3400—3450, 1647, 1586, 1559, 1512, 1471, 1395, 1348, 1272 cm^{-1}. SIMS (*m/z*): 425 $(M+1)^+$.

Methyl 10-Acetamido-4-cyano-6-hydroxymethyl-3,12-imino-7-methoxy-13-methyl-1,2,3,4,6,11,11a,12-octahydroazepino[1,2-*b*]isoquinoline-1-carboxylate (29) To a solution of **26** (257 mg, 0.62 mmol) in MeOH (5 ml) and 1 N HCl (1.5 ml) was added 5% Pd–C (80 mg). The mixture was stirred under H_2 stream for 4.5 h. The catalyst was filtered off, and the filtrate was concentrated. To the residue was added Ac_2O (4 ml), and the mixture was stirred for 15 h, then concentrated. Aqueous $NaHCO_3$ was then added, and the mixture was extracted with AcOEt, washed with brine, dried and evaporated. The residue was purified by column chromatography (SiO_2 30 ml, *n*-hexane : AcOEt = 1 : 2—0 : 1) to afford **29** (75 mg, 25.8%) as a white solid. mp 125.5—126.5 °C. ^{1}H-NMR ($CDCl_3$) ppm: 7.18 (1H, d, *J* = 9 Hz), 6.70 (1H, d, *J* = 9 Hz), 4.25—4.47 (2H, m), 3.90—4.07 (2H, m), 3.81 (3H, s), 3.73 (3H, s), 3.45 (2H, m), 2.83—3.17 (2H, m), 2.40—2.80 (3H, m), 2.31 (3H, s), 2.15 (3H, s), 2.01 (3H, s), 1.99 (1H, m). IR (KBr): 1736, 1731, 1664, 1602, 1486, 1266, 1231 cm^{-1}. EIMS (*m/z*): 470 (M^+), 439 $(M-OMe)^+$, 397, 291, 258, 231, 180, 140, 121. High-resolution EIMS (HREIMS): Calcd for $C_{24}H_3N_4O_6$ 470.2164. Found: 470.2169.

10-Acetamido-4-cyano-6-hydroxymethyl-3,12-imino-7-methoxy-13-methyl-1,2,3,4,6,11,11a,12-octahydroazepino[1,2-*b*]isoquinoline-1-carboxylic Acid (30) A solution of **29** (74 mg, 0.16 mmol) in MeOH (2 ml) and H_2O (2 ml) was treated with 1 N NaOH (0.89 ml) for 3 h. 1 N HCl was added to acidify the mixture, and that was concentrated. Purification on column chromatography (Diaion HP-20 10 ml, H_2O : MeOH = 1 : 0—1 : 1) gave **30** (44.7 mg, 68.6%) as a white solid. mp > 180 °C (dec.). *Anal.* Calcd for $C_{21}H_{26}N_4O_5 \cdot H_2O$: C, 58.32; H, 6.53; N, 12.95. Found: C, 58.31; H, 6.42; N, 12.56. ^{1}H-NMR (D_2O) ppm: 7.17 (1H, d, *J* = 8.8 Hz), 6.96 (1H, d, *J* = 8.8 Hz), 4.72 (1H, d, *J* = 2.5 Hz), 4.38 (1H, m), 4.31 (1H, dd, *J* = 4.9, 2.9 Hz), 4.23 (1H, br s), 3.85 (3H, s), 3.76 (1H, dd, *J* = 11.6, 2.9 Hz), 3.65 (1H, dd, *J* = 11.6, 5.0 Hz), 3.47 (1H, dd, *J* = 10.5, 5.5 Hz), 3.14 (1H, m), 2.83 (3H, s), 2.81 (1H, m), 2.75 (1H, m), 2.53 (1H, dd, *J* = 15.3, 11.4 Hz), 2.46 (1H, dd, *J* = 14.4, 10.6 Hz), 2.18 (3H, s). ^{13}C-NMR (D_2O) ppm: 179.7, 174.9, 155.6, 133.4, 127.8, 126.9, 122.7, 116.9, 110.7, 71.8, 66.1, 65.3, 57.8, 57.1, 56.6, 56.5, 42.7, 41.0, 29.4, 27.9, 22.6. SIMS (*m/z*): 415 $(M+1)^+$.

8-Acetamido-4-cyano-7-hydroxy-6-hydroxymethyl-3,12-imino-13-methyl-1,2,3,4,6,11,11a,12-octahydroazepino[1,2-*b*]isoquinoline-1-carboxylic Acid (31) To a solution of **23** (250 mg, 0.64 mmol) in a mixture of MeOH (4 ml), H_2O (2 ml) and 1 N HCl (2 ml) was added 5% Pd–C (80 mg). The mixture was stirred under H_2 stream for 10 h. The catalyst was filtered off, and the filtrate was concentrated. The residue was treated with Ac_2O (4 ml) and pyridine (1 ml), then allowed to stand for 5 h. After concentration 1 N NaOH (6 ml) was added, and the mixture was stirred for 2 h. Then 1 N NaOH (2 ml) was added and stirring was continued for 1 h 40 min. 1 N HCl

was added to adjust the pH at 3—4, then the mixture was concentrated. The residue was purified by column chromatography (Diaion HP-20 30 ml, H_2O : MeOH = 1 : 0—5 : 1) to afford **31** (143 mg, 55.5%). ^{1}H-NMR (CD_3OD) ppm: 7.03 (1H, d, J = 8 Hz), 6.63 (1H, d, J = 8 Hz), 4.27 (2H, m), 3.37—3.93 (3H, m), 3.50 (1H, br s), 3.20 (1H, m), 2.40—3.00 (4H, m), 2.32 (3H, s), 2.17 (3H, s), 2.07 (1H, m). IR (KBr): 3200—3500, 1701, 1650, 1545, 1495, 1455, 1385 cm^{-1}.

Methyl 8-Acetamido-4-cyano-6-hydroxymethyl-3,12-imino-7-methoxy-13-methyl-1,2,3,4,6,11,11a,12-octahydroazepino[1,2-*b*]isoquinoline-1-carboxylate (32) A solution of CH_2N_2 in ether was added slowly to a solution of **31** (50 mg, 0.125 mmol) in MeOH (2 ml) until evolution of N_2 had ceased. The mixture was evaporated and the residue was subjected to chromatography (SiO_2 10 ml, *n*-hexane : AcOEt = 1 : 2—1 : 3) to give **32** (28.5 mg, 53.3%) as a pale orange solid. mp 108.5—110 °C. ^{1}H-NMR ($CDCl_3$) ppm: 8.05 (1H, d, J = 8 Hz), 7.57 (1H, br), 6.85 (1H, d, J = 8 Hz), 4.13 (1H, m), 4.00 (1H, d, J = 3 Hz), 3.77 (3H, s), 3.72 (3H, s), 3.33—3.70 (4H, m), 2.87—3.23 (2H, m), 2.43—2.80 (3H, m), 2.30 (3H, s), 2.17 (3H, s), 1.98 (1H, m). IR (KBr): 3300—3500, 1728, 1670, 1654, 1521, 1453, 1383 cm^{-1}. EIMS (*m/z*): 429 $(M+1)^+$, 398 $(M+1-OMe)^+$, 345, 261.

8-Acetamido-4-cyano-6-hydroxymethyl-3,12-imino-7-methoxy-13-methyl-1,2,3,4,6,11,11a,12-octahydroazepino[1,2-*b*]isoquinoline-1-carboxylic Acid (33) To a solution of **32** (48 mg, 0.112 mmol) in a mixture of MeOH (1.5 ml), H_2O (2 ml) and dioxane (0.3 ml) was added 1 N NaOH (0.45 ml), and the mixture was stirred for 2 h. Additional 1 N NaOH (0.1 ml) was added and stirring was continued for 3 h 20 min. The pH of the solution was adjusted to 4 with 1 N HCl. Then it was concentrated, followed by chromatography (Diaion HP-20 10 ml, H_2O : MeOH = 1 : 0—1 : 1) to give **33** (36.3 mg, 78.2%) as a white solid. mp > 185 °C (dec.). *Anal.* Calcd for $C_{21}H_{26}N_4O_5 \cdot H_2O$: C, 58.32; H, 6.53; N, 12.95. Found: C, 58.56; H, 6.39; N, 12.71. ^{1}H-NMR (D_2O) ppm: 7.35 (1H, d, J = 8.1 Hz), 7.06 (1H, d, J = 8.1 Hz), 4.71 (1H, d, J = 2.5 Hz), 4.37 (1H, br d, J = 6.6 Hz), 4.27 (2H, m), 3.76 (3H, s), 3.71 (1H, dd, J = 11.5, 3.3 Hz), 3.57 (1H, dd, J = 11.5, 5.7 Hz), 3.46 (1H, dd, J = 10.5, 5.5 Hz), 3.20 (1H, m), 2.83 (3H, s), 2.72—2.82 (3H, m), 2.47 (1H, dd, J = 14.4, 10.5 Hz), 2.21 (3H, s). ^{13}C-NMR (D_2O) ppm: 179.8, 174.4, 151.1, 135.7, 128.6, 127.4, 126.7, 124.9, 117.0, 71.8, 66.5, 65.9, 61.8, 58.3, 57.5, 57.4, 42.8, 41.0, 31.9, 29.4, 23.1. IR (KBr): 3400, 1660, 1593, 1526, 1455, 1385 cm^{-1}.

Methyl 4-Cyano-10-formyl-6-hydroxymethyl-3,12-imino-7-methoxy-13-methyl-1,2,3,4,6,11,11a,12-octahydroazepino[1,2-*b*]isoquinoline-1-carboxylate (34) A solution of $TiCl_4$ (0.74 ml) in anhydrous CH_2Cl_2 (1 ml) was added to a solution of **5** (500 mg, 1.35 mmol) in anhydrous CH_2Cl_2 (10 ml) under ice-cooling. After stirring for 10 min a solution of CH_3OCHCl_2 (0.25 ml) in CH_2Cl_2 (1 ml) was added to the mixture. It was stirred under ice-cooling for 2 h 10 min and at room temperature for 2 h 10 min. Ice water was added and the mixture was stirred for a few minutes. An aqueous layer was separated and neutralized with 5 N NaOH. It was extracted with AcOEt, washed with brine, dried and evaporated. The residue was chromatographed (SiO_2 *n*-hexane : AcOEt = 2 : 1—1 : 1) to obtain aldehyde **34** (418 mg, 77.7%) as a pale yellow solid along with the starting material **5** (98 mg, 19%). mp 173—175 °C. *Anal.* Calcd for $C_{21}H_{25}N_3O_5$: C, 63.13; H, 6.32; N, 10.52. Found: C, 62.88; H, 6.44; N, 10.16. ^{1}H-NMR ($CDCl_3$) ppm: 10.02 (1H, s), 7.70 (1H, d, J = 8.6 Hz), 6.90 (1H, d, J = 8.6 Hz), 4.31 (1H, t, J = 3.6 Hz), 4.04 (1H, d, J = 2.9 Hz), 3.91 (3H, s), 3.76 (1H, m), 3.76 (3H, s), 3.69 (1H, dd, J = 16.6, 2.5 Hz), 3.58 (1H, dd, J = 11.0, 4.4 Hz), 3.54 (1H, br s), 3.50 (1H, m), 3.18 (1H, dd, J = 9.7, 5.6 Hz), 2.99 (1H, br d, J = 11.7 Hz), 2.69 (1H, m), 2.65 (1H, dd, J = 16.5, 11.8 Hz), 2.35 (3H, s), 2.03 (1H, dd, J = 13.5, 9.7 Hz). ^{13}C-NMR ($CDCl_3$) ppm: 191.6, 175.7, 159.9, 139.3, 135.5, 126.6, 123.7, 117.6, 108.3, 70.1, 65.0, 64.5, 57.9, 57.4, 56.9, 55.8, 52.3, 42.8, 41.9, 29.1, 28.9. IR (KBr): 3480, 1728, 1683, 1592, 1580, 1483, 1451, 1269 cm^{-1}. EIMS (*m/z*): 399 (M^+), 368 $(M-OMe)^+$, 229, 202, 180, 140. HREIMS: Calcd for $C_{21}H_{25}N_3O_5$ 399.1792. Found 399.1768.

4-Cyano-10-formyl-6-hydroxymethyl-3,12-imino-7-methoxy-13-methyl-1,2,3,4,6,11,11a,12-octahydroazepino[1,2-*b*]isoquinoline-1-carboxylic Acid (35) In the some manner as described for **30**, **34** (301 mg) afforded **35** (252 mg, 86.7%) as a pale yellow solid. mp 170—173 °C. *Anal.* Calcd for $C_{20}H_{23}N_3O_5 \cdot 3/4H_2O$: C, 60.22; H, 6.19; N, 10.53. Found: C, 59.96; H, 6.07; N, 10.20. ^{1}H-NMR (CD_3OD) ppm: 10.04 (1H, s), 7.77 (1H, d, J = 8 Hz), 7.03 (1H, d, J = 8 Hz), 4.28 (1H, d, J = 3 Hz), 4.23 (1H, m), 3.99 (3H, s), 3.40—3.87 (4H, m), 3.53 (1H, br s), 2.43—2.97 (4H, m), 2.34 (3H, s), 2.12 (1H, dd, J = 14, 10 Hz). SIMS (*m/z*): 386 $(M+1)^+$.

Methyl 4-Cyano-10-hydroxyiminomethyl-6-hydroxymethyl-3,12-imino-7-methoxy-13-methyl-1,2,3,4,6,11,11a,12-octahydroazepino[1,2-*b*]isoquinoline-1-carboxylate (36) To a solution of aldehyde **34** (253 mg, 0.63 mmol) in MeOH (7 ml) was added $H_2NOH \cdot HCl$ (88 mg). The mixture was stirred for 2 h, then concentrated. Aqueous $NaHCO_3$ was added, then it was extracted with AcOEt, washed with brine, dried and evaporated to give **36** (253 mg, 96.4%) as a white solid. mp 205—208 °C (dec.). ^{1}H-NMR ($CDCl_3$) ppm: 8.31 (1H, s), 7.51 (1H, d, J = 8 Hz), 6.76 (1H, d, J = 8 Hz), 4.30 (1H, m), 4.03 (1H, d, J = 3 Hz), 3.82 (3H, s), 3.75 (3H, s), 3.10—3.80 (3H, m), 3.49 (1H, br s), 3.18 (1H, dd, J = 10, 6 Hz), 2.47—3.13 (4H, m), 2.34 (3H, s), 2.00 (1H, dd, J = 14, 10 Hz). EIMS (*m/z*): 414 (M^+), 383 $(M-OMe)^+$, 365, 244, 226, 180, 140. HREIMS: Calcd for $C_{21}H_{26}N_4O_5$ 414.1901. Found 414.1948.

4-Cyano-10-hydroxyiminomethyl-6-hydroxymethyl-3,12-imino-7-methoxy-13-methyl-1,2,3,4,6,11,11a,12-octahydroazepino[1,2-*b*]isoquinoline-1-carboxylic Acid (37) To a solution of **35** (90 mg, 0.23 mmol) in MeOH (2 ml) was added $H_2NOH \cdot HCl$ (81 mg). The mixture was stirred for 40 min, then concentrated. The residue was chromatographed (Diaion HP-20, 10 ml H_2O : MeOH = 1 : 0—3 : 2) to afford **37** (78.3 mg, 83.7%) as a pale yellow solid. mp 185—190 °C (dec.). *Anal.* Calcd for $C_{20}H_{24}N_4O_5 \cdot 2/3H_2O$: C, 58.24; H, 6.19; N, 13.58. Found: C, 58.50; H, 6.24; N, 13.27. ^{1}H-NMR (D_2O) ppm: 8.32 (1H, s), 7.50 (1H, d, J = 8.7 Hz), 6.92 (1H, d, J = 8.7 Hz), 4.79 (1H, d, J = 2.5 Hz), 4.49 (1H, m), 4.43 (1H, br s), 4.33 (1H, dd, J = 5.4, 2.8 Hz), 3.83 (3H, s), 3.73 (1H, m), 3.72 (1H, dd, J = 11.5, 2.8 Hz), 3.55 (1H, dd, J = 11.5, 5.5 Hz), 3.26 (1H, m), 3.13 (1H, dd, J = 15.6, 2.5 Hz), 2.92 (3H, s), 2.83 (1H, m), 2.69 (1H, dd, J = 15.5, 11.5 Hz), 2.58 (1H, dd, J = 14.6, 10.6 Hz). IR (KBr): 3380, 1708, 1599, 1490, 1462, 1268 cm^{-1}. ^{13}C-NMR (D_2O) ppm: 177.1, 157.8, 150.5, 135.2, 128.5, 122.41, 122.38, 116.6, 110.6, 71.1, 66.0, 65.7, 57.6, 57.1, 56.5, 56.4, 41.1, 28.8, 28.6. SIMS (*m/z*): 401 $(M+1)^+$, 374.

Methyl 4,10-Dicyano-6-hydroxymethyl-3,12-imino-7-methoxy-13-methyl-1,2,3,4,6,11,11a,12-octahydroazepino[1,2-*b*]isoquinoline-1-carboxylate (39) A solution of oxime **36** (200 mg, 0.48 mmol) in Ac_2O (4 ml) was heated at 130 °C for 4 h. The reaction mixture was concentrated and dissolved in AcOEt. It was washed with saturated $NaHCO_3$ and brine, dried, then evaporated. Purification by column chromatography (SiO_2 15 ml, *n*-hexane : AcOEt = 2 : 1) gave **39** (186 mg, 88.0%). ^{1}H-NMR ($CDCl_3$) ppm: 7.57 (1H, d, J = 8 Hz), 6.82 (1H, d, J = 8 Hz), 4.30—4.50 (2H, m), 3.85—4.07 (2H, m), 3.89 (3H, s), 3.78 (3H, s), 3.47 (1H, br s), 3.43 (1H, m), 2.90—3.15 (3H, m), 2.43—2.80 (2H, m), 2.33 (3H, s), 1.98 (3H, s), 1.92 (1H, m). EIMS (*m/z*): 438 (M^+), 407 $(M-OMe)^+$, 379, 365, 226, 180, 140.

Sodium 4,10-Dicyano-6-hydroxymethyl-3,12-imino-7-methoxy-13-methyl-1,2,3,4,6,11,11a,12-octahydroazepino[1,2-*b*]isoquinoline-1-carboxylate (40) A solution of **39** (489 mg, 1.12 mmol) in MeOH (10 ml) and H_2O (5 ml) was treated with 1 N NaOH (4.5 ml) for 18 h. The mixture was concentrated and the residue was chromatographed (Diaion HP-20, 60 ml, H_2O : MeOH = 1 : 0—9 : 1) to afford **40** (350 mg, 77%) as a white solid. *Anal.* Calcd for $C_{20}H_{21}N_4NaO_4 \cdot 2.5H_2O$: C, 53.45; H, 5.83; N, 12.47. Found: C, 53.57; H, 5.64; N, 12.15. ^{1}H-NMR (D_2O) ppm: 7.63 (1H, d, J = 8.7 Hz), 6.94 (1H, d, J = 8.7 Hz), 4.27 (1H, d, J = 2.8 Hz), 4.16 (1H, m), 3.87 (3H, s), 3.72 (1H, dd, J = 11.7, 2.8 Hz), 3.67 (1H, dd, J = 11.7, 3.9 Hz), 3.61 (1H, m), 3.60 (1H, br s), 3.12 (1H, dd, J = 10.0, 5.4 Hz), 3.00 (1H, dd, J = 15.3, 2.2 Hz), 2.90 (1H, br d, J = 11.5 Hz), 2.74 (1H, dd, J = 15.3, 11.5 Hz), 2.55 (1H, m), 2.26 (3H, s), 2.06 (1H, dd, J = 13.5, 10.0 Hz). ^{13}C-NMR (D_2O) ppm: 184.2, 160.3, 142.0, 134.6, 124.1, 119.8, 119.7, 110.5, 103.0, 70.5, 65.1, 64.1, 58.5, 58.2, 57.8, 56.7, 45.4, 42.0, 31.9, 30.2. IR (KBr): 3420, 2224, 1652, 1598, 1559, 1482, 1393, 1282, 1076 cm^{-1}. SIMS (*m/z*): 405 $(M+1)^+$, 383, 356.

10-Cyano Quinocarcin (41) To a solution of **40** (100 mg, 0.25 mmol) in CH_3CN (3 ml) was added $AgNO_3$ (46 mg). The mixture was stirred for 1 h. Additional $AgNO_3$ (25 mg) was added and stirring was continued for 1 h. Acetate buffer (pH 4.0) was added, and the resulting precipitate was filtered off. The filtrate was concentrated to subject chromatography (HP-20 15 ml, H_2O : MeOH = 1 : 0—2 : 1) to obtain **41** (70.8 mg, 80.6%). ^{1}H-NMR (D_2O) ppm: 7.64 (1H, d, J = 8.7 Hz), 6.99 (1H, d, J = 8.7 Hz), 4.95 (1H, d, J = 3.3 Hz), 4.48 (1H, m), 4.26 (1H, br s), 3.98 (1H, m), 3.89 (3H, s), 3.68 (1H, dd, J = 11.5, 3.2 Hz), 3.59 (1H, dd, J = 11.5, 4.4 Hz), 3.51 (1H, br d, J = 11.3 Hz), 3.43 (1H, dd, J = 10.5, 5.3 Hz), 3.02 (1H, dd, J = 15.3, 2.4 Hz), 2.81 (3H, s), 2.75 (1H, dd, J = 15.2, 11.9 Hz), 2.57 (1H, m), 2.43 (1H, dd, J = 14.2, 10.6 Hz). ^{13}C-NMR (D_2O) ppm: 180.3, 160.3, 140.8, 134.5, 124.8, 119.6, 110.7, 102.8, 81.7, 71.7, 69.9, 65.0, 56.6, 54.3, 53.1, 41.7, 40.5, 30.7, 27.7. IR (KBr): 3410, 2222, 1597, 1482, 1381, 1295, 1276 cm^{-1}. SIMS (*m/z*): 356 $(M+1)^+$.

Methyl 4-Cyano-10-formyl-6-hydroxymethyl-3,12-imino-7-methoxy-13-methyl-13-oxy-1,2,3,4,6,11,11a,12-octahydroazepino[1,2-*b*]isoquinoline-1-carboxylate (42) To a solution of **34** (50 mg, 0.125 mmol) in CH_2Cl_2 (2.5 ml) was added MCPBA (24 mg, 0.138 mmol). The mixture was stirred for 30 min. It was washed with H_2O, dried and evaporated. The residue

was subjected to chromatography (SiO_2 10 ml, $CHCl_3$: MeOH = 100 : 1—10 : 1) to give **42** (32.3 mg, 62.1%). ^{1}H-NMR ($CDCl_3$–CD_3OD) ppm: 10.02 (1H, s), 7.75 (1H, d, J=9 Hz), 6.97 (1H, d, J=9 Hz), 4.83 (1H, d, J=2.3 Hz), 4.52 (1H, dd, J=7.5, 3.8 Hz), 4.13 (1H, br s), 3.97 (3H, s), 3.77 (3H, s), 3.54 (3H, s), 3.10—4.00 (7H, m), 2.70 (1H, dd, J=16, 11 Hz), 2.27 (1H, dd, J=14, 11 Hz). IR (KBr): 3400, 1729, 1681, 1595, 1581, 1486, 1451, 1293, 1272, 1227, 1054, 1030 cm^{-1}. EIMS (*m/z*): 398, 368, 308, 232, 188, 140, 121.

4-Cyano-10-formyl-6-hydroxymethyl-3,12-imino-7-methoxy-13-methyl-13-oxy-1,2,3,4,6,11,11a,12-octahydroazepino[1,2-*b*]isoquinoline-1-carboxylic Acid (43) To a solution of **35** (40 mg, 0.104 mmol) in MeOH (1.5 ml) was added MCPBA (21 mg, 0.125 mmol). The mixture was stirred for 1.5 h. Additional MCPBA (5 mg, 0.03 mmol) was added and stirring was continued for 3 h. The resulting precipitate was filtered and dried to afford **43** (16.6 mg, 39.8%) as a white solid. mp 165—170 °C (dec.). ^{1}H-NMR (D_2O) ppm: 9.83 (1H, s), 7.76 (1H, d, J=8.7 Hz), 7.04 (1H, d, J=8.8 Hz), 5.00 (1H, d, J=2.2 Hz), 4.79 (1H, br d, J=6.7 Hz), 4.75 (1H, br s), 4.44 (1H, dd, J=5.4, 2.7 Hz), 3.91 (3H, s), 3.89 (3H, s), 3.71—3.81 (3H, m), 3.59 (1H, br d, J=10.2 Hz), 3.54 (1H, dd, J=11.6, 5.5 Hz), 3.00 (1H, ddd, J=13.4, 6.9, 4.6 Hz), 2.78 (1H, dd, J=15.8, 11.1 Hz), 2.50 (1H, dd, J=13.4, 10.4 Hz). ^{13}C-NMR (D_2O) ppm: 195.2, 176.2, 161.4, 138.5, 136.6, 126.3, 123.3, 117.3, 110.3, 80.0, 74.7, 65.3, 56.8, 56.5, 55.1, 53.0, 48.4, 41.5, 30.4, 28.4.

Methyl 4-Cyano-10-hydroxy-6-hydroxymethyl-3,12-imino-7-methoxy-13-methyl-13-oxy-1,2,3,4,6,11,11a,12-octahydroazepino[1,2-*b*]isoquinoline-1-carboxylate (44) To a stirred solution of **34** (30 mg, 0.075 mmol) in a mixture of MeOH (1 ml) and CH_2Cl_2 (0.5 ml) was added portionwise MCPBA (43 mg, 0.25 mmol) in 10 h at 0 °C—room temperature. $NaHCO_3$ sol was added, then MeOH and CH_2Cl_2 were distilled off. The resultant solution was extracted AcOEt–THF three times. The combined extracts were dried, then concentrated. The residue was chromatographed (SiO_2 7 ml, $CHCl_3$: MeOH = 50 : 1—5 : 1) to give **44** (12.3 mg, 40.6%) as a pale yellow solid. mp 185—190 °C (dec.). ^{1}H-NMR (DMSO-d_6) ppm: 9.18 (1H, s), 6.68 (1H, d, J=8.8 Hz), 6.67 (1H, d, J=8.8 Hz), 4.90 (1H, dd, J=6.4, 4.1 Hz), 4.84 (1H, d, J=2.2 Hz), 4.18 (1H, dd, J=7.3, 2.4 Hz), 3.90 (1H, br s), 3.83 (1H, br d, J=7.1 Hz), 3.72 (3H, s), 3.62 (1H, m), 3.61 (3H, s), 3.35 (3H, s), 3.27 (1H, m), 3.19 (1H, br d, J=9.7 Hz), 2.99 (1H, m), 2.91 (1H, dd, J=15.0, 2.4 Hz), 2.30 (1H, dd, J=15.0, 11.3 Hz), 2.05 (1H, dd, J=11.5, 9.7 Hz). SIMS (*m/z*): 404 $(M+1)^+$.

Methyl 4-Cyano-10-hydroxy-6-hydroxymethyl-3,12-imino-7-methoxy-13-methyl-1,2,3,4,6,11,11a,12-octahydroazepino[1,2-*b*]isoquinoline-1-carboxylate (45) Thirty percent H_2O_2 (1.28 ml) and conc. H_2SO_4 (0.5 ml) were added to a solution of **34** (3.0 g, 7.5 mmol) in MeOH (50 ml). The mixture was stirred at room temperature for 7 h, then neutralized with saturated $NaHCO_3$. MeOH was evaporated off, and the residue was extracted with AcOEt three times. The combined extracts were washed with brine, dried and concentrated. The residue was purified by column chromatography (SiO_2 600 ml, $CHCl_3$: EtOH = 1 : 0—50 : 1) to give **45** (2.40 g, 82.5%) as a white solid along with the starting material **34** (329 mg, 11%). mp 120—121.5 °C. *Anal.* Calcd for $C_{20}H_{25}N_3O_5 \cdot 1/4H_2O$: C, 61.29; H, 6.56; N, 10.72. Found: C, 61.29; H, 6.60; N, 10.35. ^{1}H-NMR ($CDCl_3$) ppm: 6.55 (2H, s), 4.21 (1H, m), 4.01 (1H, d, J=3 Hz), 3.72 (6H, s), 3.63—3.80 (2H, m), 3.47 (2H, m), 2.23—3.30 (5H, m), 2.31 (3H, s), 1.98 (1H, dd, J=13, 9 Hz). EIMS (*m/z*): 387 (M^+), 356 $(M-OMe)^+$.

Sodium 4-Cyano-10-hydroxy-6-hydroxymethyl-3,12-imino-7-methoxy-13-methyl-1,2,3,4,6,11,11a,12-octahydroazepino[1,2-*b*]isoquinoline-1-carboxylate (46) In the same manner as described for **40**, ester **45** (200 mg, 0.52 mmol) afforded **46** (145 mg, 73.2%). ^{1}H-NMR (D_2O) ppm: 6.82 (2H, s), 4.24 (1H, d, J=2.9 Hz), 4.12 (1H, m), 3.78 (3H, s), 3.70 (1H, dd, J=11.5, 3.0 Hz), 3.62 (1H, dd, J=11.5, 4.5 Hz), 3.58 (1H, m), 3.54 (1H, br s), 3.13 (1H, dd, J=9.9, 5.6 Hz), 2.89 (1H, dd, J=15.4, 2.5 Hz), 2.77 (1H, br d, J=11.1 Hz), 2.53 (1H, m), 2.35 (1H, dd, J=15.3, 11.5 Hz), 2.23 (3H, s), 2.05 (1H, dd, J=13.4, 10.0 Hz). ^{13}C-NMR (D_2O) ppm: 184.5, 150.4, 147.1, 125.8, 124.3, 119.7, 115.2, 111.3, 70.8, 65.2, 65.1, 58.8, 58.5, 58.3, 57.1, 45.4, 41.9, 30.1, 27.0. SIMS (*m/z*): 396 $(M+1)^+$, 374, 347.

4-Cyano-6,10-dihydroxymethyl-3,12-imino-7-methoxy-13-methyl-1,2,3,4,6,11,11a,12-octahydroazepino[1,2-*b*]isoquinoline-1-carboxylic Acid (47) To a solution of **35** (75 mg, 0.195 mmol) in MeOH (3 ml) was added $NaBH_4$ (7.4 mg, 0.195 mmol) under ice-cooling. The mixture was stirred at 0 °C for 45 min and at room temperature for 1 h. $NaBH_4$ (10 mg) was added and stirring was continued for 1 h 15 min. The mixture was acidified with 1 N HCl, then concentrated. The residue was subjected to chromatography (Diaion HP-20 10 ml, H_2O : MeOH ≒ 1 : 0—2 : 1) to afford **47** (48.5 mg, 64.3%) as a white solid. mp > 190 °C (dec.). ^{1}H-NMR (CD_3OD) ppm: 7.20 (1H, d, J=9 Hz), 6.77 (1H, d, J=9 Hz), 4.57 (2H, s), 4.30 (1H, d, J=3 Hz), 4.22 (1H, m), 3.82 (3H, s), 3.73 (1H, m), 3.17—3.57 (4H, m), 2.43—3.07 (4H, m), 2.32 (3H, s), 2.13 (1H, dd, J=13, 10 Hz). IR (KBr): 3400, 1698, 1592, 1488, 1391, 1267 cm^{-1}.

4-Cyano-10-dimethylaminomethyl-6-hydroxymethyl-3,12-imino-7-methoxy-13-methyl-1,2,3,4,6,11,11a,12-octahydroazepino[1,2-*b*]isoquinoline-1-carboxylic Acid (48) $NaCNBH_3$ (9.8 mg, 0.156 mmol) was added to a mixture of **35** (30 mg, 0.078 mmol) and $Me_2NH \cdot HCl$ (38 mg, 0.47 mmol) in MeOH (1.5 ml). The mixture was stirred for 23 h. $NaCNBH_3$ (6 mg) and 5 N HCl/MeOH (0.015 ml) were added and stirring was continued for a further 24 h. The mixture was concentrated, then chromatographed (Diaion HP-20 10 ml, H_2O : MeOH = 1 : 0—9 : 1) to give **48** (20.2 mg, 62.6%). ^{1}H-NMR (CD_3OD) ppm: 7.30 (1H, d, J=9 Hz), 6.87 (1H, d, J=9 Hz), 4.25 (2H, m), 3.85 (3H, s), 3.62—4.13 (4H, m), 3.53 (1H, br s), 3.43 (1H, m), 2.43—3.20 (5H, m), 2.68 (6H, s), 2.30 (3H, s), 2.05 (1H, m).

Evaluation of Antitumor Activity HeLa S_3 cells (5×10^4) were seeded in plastic tubes or dishes containing 1 ml of growth medium. Grated concentrations of drugs were added 24 h after the cells were seeded. After 72 h drug exposure, the tumor cells were counted and the IC_{50} value was determined.

P388 (1×10^6) cells were implanted intraperitoneally (i.p.) into $CD2F_1$ mice and i.p. administration of drugs was started the day after tumor implantation. B-16 melanoma was introduced into $B6D2F_1$ mice by i.p. administration of 0.5 ml of a 10% homogenate of tumor brei and drug administration was started the following day. Antitumor efficacy was expressed as an increased life span (ILS).

BALB/c-*nu*/*nu* mice were given subcutaneously (s.c.) tumor fragment equivalent to 8 mm^3 of MX-1 tumor passaged in nude mouse. When tumor volume reached 100—300 mm^3, the mice were pair matched in groups of 6 each and drug and vehicle was administered intravenously (i.v.). Tumor volume was calculated and drug efficacy was expressed as the percentage of the control group.

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