

0.05 mmHg, 10 h): R_f by TLC (EtOAc/Me₂CO/MeOH/H₂O 7:1:0.5:0.5 v/v/v/v) = 0.45; MS (CI) m/z 286 (MH^+ = 286); UV λ max (pH 1) 231 nm (ϵ 8700), 263 nm (ϵ 5600); (pH 7) 232 nm (ϵ 9000), 260 nm (sh); (pH 11) 226 nm (ϵ 11 100), 242 nm (ϵ 10800), ~265 nm (br sh); IR (KBr) 3450-3000 (br), 2920 (sh), 2850 (sh), 1750 (br), 1610 cm^{-1} ; ¹H NMR (D₂O) δ 4.90 (d, 1, 1'-H, $J_{1'-2}$ = 6.8 Hz), 4.30 (pst, 1, 2'-H), 4.09 (t, 1, 3'-H), 3.95 (q, 1, 4'-H), 3.63 (m, 2, 5'-H and 5''-H). Anal. (C₁₀H₁₁N₃O₇·0.5H₂O) C, H, N.

Antitumor Studies. The *in vitro* cytotoxicity against L1210 was evaluated as described previously.³⁴ L1210 cells were grown in static suspension culture at 38 °C using Fischer's medium for leukemic cells of mice, and the growth rate over a 3-day period was determined in the continuous presence of various concentrations of the test compound. For studies on protection from growth inhibition, the cytotoxic compound and the compound being tested for protective capacity were added to the cultures simultaneously. Both compounds were present continuously during the 3-day period of growth-rate determination. Growth rate was defined as the slope of the semilogarithmic plot of cell number against time for the treated culture, as a percent of the slope for the control culture. Experimentally this parameter was determined by calculating the ratio of the population doubling time of control cells to the population doubling time of treated cells. When the growth rate slowed during the experiment, the

rate used was the final rate attained at the end of the 3-day period. The IC₅₀ was defined as the concentration required to reduce the growth rate to 50% of the control.

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Registry No. 7, 30868-30-5; 8, 133470-82-3; 9, 133470-83-4; 10, 37085-15-7; 11, 133470-84-5; 12, 133470-85-6; 13, 133470-86-7; 14, 133470-87-8; 15, 133470-88-9; 16, 133470-89-0; 17, 133470-90-3; 17 5'-O-acetyl derivative, 133471-02-0; 18, 99298-13-2; 19, 133470-91-4; 20, 133470-92-5; 21, 133470-93-6; 22, 133470-94-7; 23, 133470-95-8; 24, 133470-96-9; 25, 133470-97-0; 27, 133470-98-1; 28, 79264-07-6; 29, 133470-99-2; 30, 57274-26-7; 31 (isomer 1), 133471-00-8; 31 (isomer 2), 133471-03-1; 32 (isomer 1), 133471-01-9; 32 (isomer 2), 133471-04-2; 33, 90524-84-8.

Synthesis and Biological Evaluation of Quinocarcin Derivatives: Thioalkyl-Substituted Quinones and Hydroquinones

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Varieties of thioalkyl-containing quinone and hydroquinone analogues of quinocarcin (1a) were prepared effectively, by addition of mercaptan to 3a-c, which were derived from 1a via DX-52-1 (1b). Antitumor activities of these analogues were preliminarily evaluated by growth inhibition of HeLa S₃ cells (*in vitro*) and increased life span of P388 implanted mice (*in vivo*). Bis(alkylthio)quinones 4a-d and 5a-d, and corresponding hydroquinones 9b-d exhibited high activities both *in vitro* and *in vivo*. They were superior to 1a especially in single administration. Selected compounds 4a, 4d, 5a, 5d, and 9b were subjected to further evaluation, and bis(methylthio)quinone 5a was revealed to possess broad-spectrum activity toward human xenografted carcinomas MX-1, Co-3, St-4, and LC-06.

Quinocarcin (1a)¹ is an antitumor antibiotic isolated from the culture broths of *Streptomyces melanovinaceus*.^{1a} Its structure was elucidated by NMR analysis^{1b} and X-ray crystallography^{1d} of quinocarcinol (1c), which was produced by the same organism. Quinocarcin is active against several experimental tumor systems^{1a,f} and thought to exert its activity via inhibition of DNA^{1c} and RNA^{1e} synthesis. In spite of little information on the mode of action of 1a, a plausible mechanism is alkylation of DNA by opening of the oxazolidine ring, which would give a highly reactive iminium ion susceptible to a nucleophilic moiety of DNA. 1a has a significant activity against P388 leukemia and human xenograft MX-1; however, repeated daily administration is required for high efficacy. To enhance the antitumor activity and to broaden the spectrum of 1a, we attempted the synthetic studies of its analogues.² Among the neoplastic agents, quinone-containing compounds continue to receive considerable attention,³ both for clinical use and under preclinical studies (e.g. mytomycin,⁴

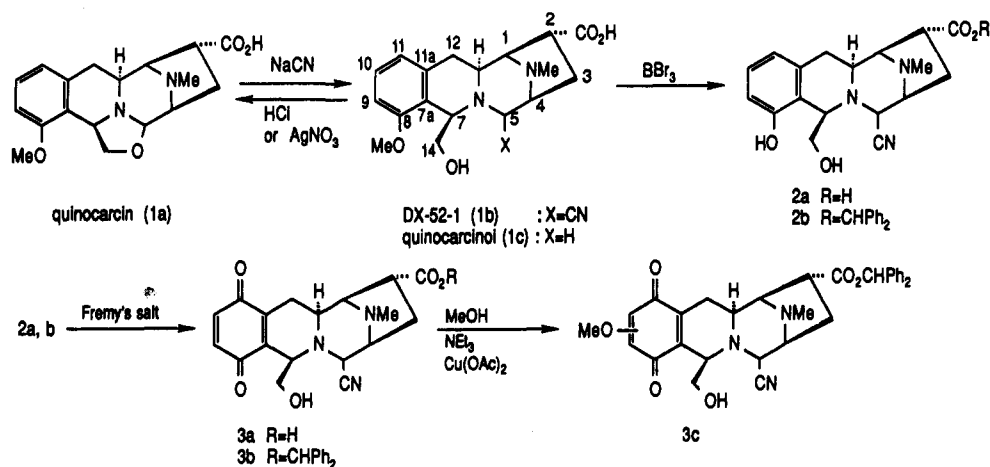
adriamycin,⁵ mitoxantrone,⁶ streptonigrin,⁷ saframycin,⁸ and their derivatives, etc.). Additionally saframycin and

- (1) (a) Tomita, F.; Takahashi, K.; Shimizu, K. *J. Antibiot.* 1983, 36, 463. (b) Takahashi, K.; Tomita, F. *Ibid.* 1983, 36, 468. (c) Tomita, F.; Takahashi, K.; Tamaoki, T. *Ibid.* 1984, 37, 1268. (d) Hirayama, N.; Shirahata, K. *J. Chem. Soc. Perkin Trans 2* 1983, 1705. (e) Fujimoto, K.; Oka, T.; Morimoto, M. *Cancer Res.* 1987, 47, 1516. (f) Inaba, S.; Shimoyama, M. *Ibid.* 1988, 48, 6029.
- (2) (a) Saito, H.; Kobayashi, S.; Uesaki, Y.; Sato, A.; Fujimoto, K.; Miyoshi, K.; Ashizawa, T.; Morimoto, M.; Hirata, T. *Chem. Pharm. Bull.* 1990, 38, 1278. (b) Saito, H.; Sato, A.; Ashizawa, T.; Morimoto, M.; Hirata, T. *Ibid.* 1990, 38, 3202. (c) In the infrared spectrum of 1b, no absorption corresponding to a nitrile was observed; however, in ¹³C NMR the peak at 119.4 ppm was indicative of the presence of nitrile. It was also the case for other 4-cyano analogues.
- (3) Bredy, J. *Quinone and Similar Antibiotics*; CRC Press: Boca Raton, FL, 1980; Vol. III.
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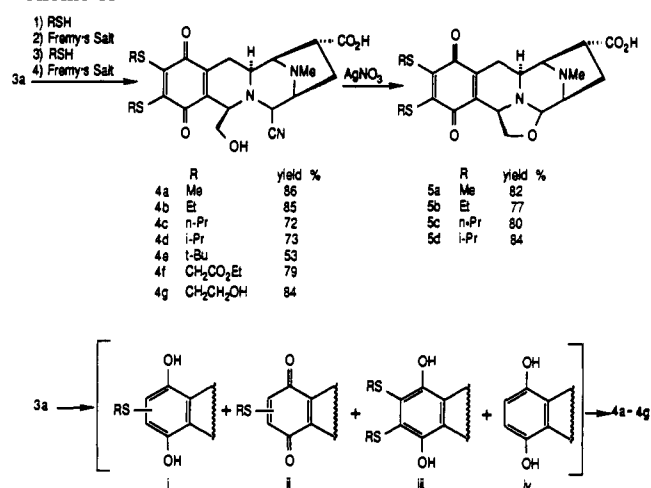
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Scheme I

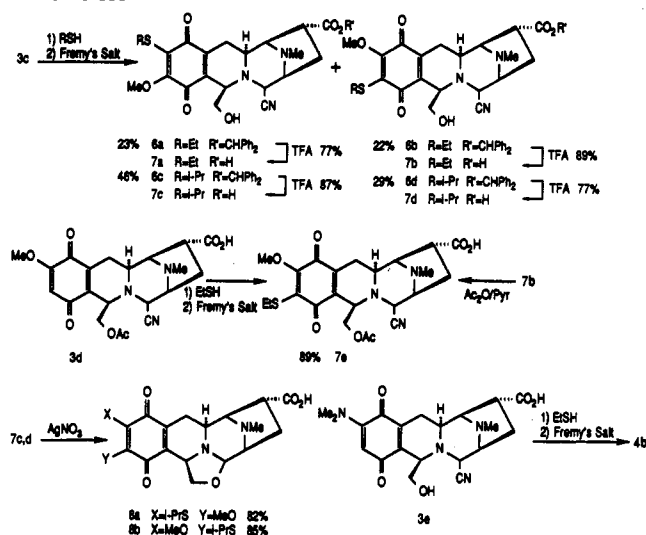


Scheme II



naphthyridinomycin,⁹ both structurally related to 1a, were more potent than 1a. Therefore, we were interested in quinone derivatives of 1a and synthetic efforts were focused on them. Precedingly we had found bis(methylthio)quinone 4a^{2b} to be the most promising among various heteroatom-substituted quinone analogues. This en-

Scheme III



couraging fact was the stimulus for our synthetic research. Now, in this report, further synthesis of thioalkyl-substituted quinone and hydroquinone derivatives related to 4a and the evaluation of their antitumor activities are described.

Chemistry

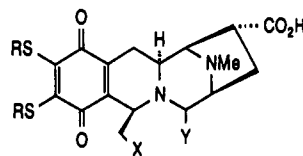
Cyanation of quinocarcin (1a) with NaCN readily gave DX-52-1 (1b)^{2a}, the 4-cyano congener of 1a, which had not only significant antitumor activity but also sufficient stability for chemical manipulation. Conversely 1b regenerated 1a in good yield upon treatment with hydrochloric acid or silver nitrate. This method for masking and reproduction of the oxazolidine ring in 1a played a significant role for analogue synthesis. Silver nitrate was particularly efficient for quinone derivatives. Demethylation of 1b (and esterification) and subsequent oxidation of 2a and 2b with Fremy's salt¹⁰ yielded the desired quinones 3a and 3b effectively (Scheme I).

Addition of mercaptan¹¹ to 3a initially gave a complex mixture on thin-layer chromatography, which may have contained compounds i-iv (Scheme II). Compound iv was produced as a result of action of 3a as an oxidant. By subsequent oxidation with Fremy's salt and repetition of

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- (11) Patai, S. *The Chemistry of the Quinonoid Compounds*; John Wiley and Sons: London, New York, 1974; Part 2, p 877.

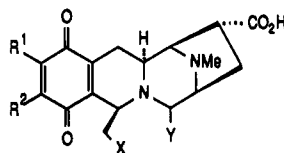
Table I. Bis(alkylthio)quinones



no.	R	X	Y	HeLa S ₃ IC ₅₀ ^a μg/mL	P 388 ip-ip ^b					R	formula ^c	mp, °C
					optimal dose × 1	% ILS ^c	R ^d	optimal dose × 5	% ILS			
4a	Me	OH	CN	0.13	12.5	53	1.23	6.25	71	0.59	C ₂₀ H ₂₂ N ₂ O ₆ S ₂	150–151
4b	Et	OH	CN	0.11	12.5	50	1.16	6.25	51	0.42	C ₂₂ H ₂₇ N ₂ O ₆ S ₂	168–169
4c	<i>n</i> -Pr	OH	CN	0.05	25	56	1.17	6.25	42	0.48	C ₂₄ H ₃₁ N ₂ O ₆ S ₂	110–111
4d	<i>i</i> -Pr	OH	CN	0.012	25	65	1.48	12.5	119	1.35	C ₂₄ H ₃₁ N ₂ O ₆ S ₂	166–167
4e	<i>t</i> -Bu	OH	CN	0.004	25	48	1.26	3.13	46	0.50	C ₂₀ H ₂₂ N ₂ O ₆ S ₂	187–188
4f	EtO ₂ CCH ₂	OH	CN	9.96				50	30		C ₂₀ H ₂₁ N ₂ O ₆ S ₂ ·0.5H ₂ O	88–89
4g	HOCH ₂ CH ₂	OH	CN	2.47	12.5	26	0.62	6.25	20		C ₂₂ H ₂₇ N ₂ O ₆ S ₂ ·H ₂ O	137–140 dec
5a	Me	—O—		0.019	6.25	48	1.85	3.13	91	0.88	C ₁₉ H ₂₂ N ₂ O ₆ S ₂ ·1.5H ₂ O	167–172 dec
5b	Et	—O—		0.08	6.25	64	1.33	3.13	82	0.93	C ₂₁ H ₂₆ N ₂ O ₆ S ₂ ·1.5H ₂ O	160–163 dec
5c	<i>n</i> -Pr	—O—		0.03	12.5	58	1.21	6.25	86	0.98	C ₂₃ H ₃₀ N ₂ O ₆ S ₂ ·1.2H ₂ O	140–145 dec
5d	<i>i</i> -Pr	—O—		0.0019	6.25	69	1.57	3.13	98	1.11	C ₂₃ H ₃₀ N ₂ O ₆ S ₂ ·0.5H ₂ O	152–157 dec
1a	quinocarcin			0.05–0.11	10–20	24–48	1	5–10	70–120	1		

^a Drug concentration required to inhibit the growth of HeLa S₃ cells by 50%. ^b CD2F₁ mice (5 mice/group) were implanted intraperitoneally (ip) with 10⁶ cells, and drug was dosed (mg/kg) ip on day 1 and days 1–5. ^c Percent increase in life span, calculated (T/C - 1) × 100, where T and C are median survival times of treated and control mice, respectively. ^d Ratio of ILS tested compound/quinocarcin. ^e C, H, and N analyses were within ±0.4% of the theoretical values.

Table II. Methoxyalkylthioquinones



no.	R ¹	R ²	X	Y	HeLa S ₃ IC ₅₀ , μg/mL	P 388 ip-ip					R	formula	mp, °C
						optimal dose mg/kg, × 1	% ILS	R	optimal dose, mg/kg, × 5	% ILS			
7a	EtS	MeO	OH	CN	2.42	6.25	29	0.67	6.25	51	0.65	C ₂₁ H ₂₆ N ₂ O ₆ S·H ₂ O	131–132
7b	MeO	EtS	OH	CN	2.88	25	31	0.72	6.25	59	0.76	C ₂₁ H ₂₆ N ₂ O ₆ S·0.2H ₂ O	160–165 dec
7c	<i>i</i> -PrS	MeO	OH	CN	1.12	12.5	21	0.58	12.5	51	0.68	C ₂₃ H ₂₇ N ₂ O ₆ S·0.5H ₂ O	139–140
7d	MeO	<i>i</i> -PrS	OH	CN	0.56	12.5	17		12.5	75	1.00	C ₂₃ H ₂₇ N ₂ O ₆ S·0.5H ₂ O	136–137
8a	<i>i</i> -PrS	MeO	—O—		0.79	6.25	31	1.29	6.25	81	1.00	C ₂₁ H ₂₆ N ₂ O ₆ S·2H ₂ O	130–135 dec
8b	MeO	<i>i</i> -PrS	—O—		2.37	6.25	30	1.25	1.56	51	0.63	C ₂₁ H ₂₆ N ₂ O ₆ S·1.5H ₂ O	132–137 dec
1a	quinocarcin				0.05–0.11	10–20	24–48	1	5–10	64–92	1		

mercaptan addition and oxidation, they converged to bis(alkylthio)quinones 4a–g¹² in good yield. It was difficult to isolate mono(alkylthio)quinones. Conversion of these 4-cyano forms to oxazolidines was accomplished efficiently with silver nitrate, producing 5a–d.

Methoxyquinone (3c)^{2b} was prepared by addition of methanol to 3b as a regioisomer mixture (Scheme I). Subsequently, as shown in Scheme III, ethyl mercaptan addition to 3c, followed by oxidation, gave 9-(ethylthio)-8-methoxyquinone 6a and its isomer 6b in a ratio of ca. 3:2, which could be readily separated by column chromatography. The ratio of 6a:6b reflected the regioisomer ratio of 3c. Esters of 6a and 6b were cleaved with trifluoroacetic acid to give 7a and 7b, respectively. Addition of ethyl mercaptan to 9-methoxy-14-acetoxyquinone (3d)^{2b} which was prepared by an alternative method, proceeded in a similar manner, as described above, to afford 7e. In NMR and HPLC analyses, the acetylation product of 7b coincided with 7e, therefore the regiochemistry of 7a and 7b were confirmed as shown in Scheme III.

In a similar manner, compounds 7c and 7d were obtained, and were transformed to oxazolidine forms 8a and 8b, respectively.

In contrast to methoxyquinone, the addition of ethyl mercaptan to (dimethylamino)quinone 3e,^{2b} which was prepared from 3a, by the addition of dimethylamine, resulted in replacement of the substituent to give bis(ethylthio)quinone 4b (Scheme III).

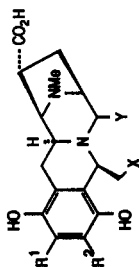
Consequently, the quinone analogues prepared were subjected to hydrogenation to give corresponding hydroquinones 9a–h in high yields, except for 9a in 29% yield (Scheme IV). Their antitumor activities were revealed to be almost equal to those of corresponding quinones (vide infra). Stable under acidic conditions, they gradually oxidized to quinones in a neutral to basic environment. These facts implied that the active form responsible for the antitumor activity might be a quinone. To conform this hypothesis, hydroquinone dimethyl ethers 12a and 12b were synthesized from 4d as shown in Scheme IV. Esterification of 4d and subsequent reduction gave 11a in high yield. It was necessary to carry out the methylation of 11a in the presence of a reducing agent to avoid conversion to quinone 10. Ester cleavage yielded 12a, which was converted to the oxazolidine form 12b. These dimethyl ethers 12a and 12b were considered resistant to

(12) In secondary ion mass spectrum (SIMS) quinone derivatives showed a peak of M + 3, which was conceivable for quinone-containing molecules.¹³

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Table III. Hydroquinones

no.	R ¹	R ²	X	Y	HeLa S ₃ IC ₅₀ , μg/mL	optimal dose, mg/kg, × 1		% ILS	R	formula	mp, °C
						optimal dose, mg/kg, × 1	optimal dose, mg/kg, × 5				
9a	H	H	OH	CN	6.10	12.5	23	23	0.86	C ₁₉ H ₂₁ N ₃ O ₇ H ₂ O	135-140 dec
9b	MeS	MeS	OH	CN	0.09	6.25	47	92		C ₂₀ H ₂₁ N ₃ O ₇ S ₂ H ₂ O	170-175 dec
9c	EiS	EiS	OH	CN	<0.03					C ₂₁ H ₂₃ N ₃ O ₇ S ₂ ·0.5H ₂ O	140-141
9d	i-PrS	i-PrS	OH	CN	<0.03	12.5	51	102	1.09	C ₂₂ H ₂₅ N ₃ O ₇ S ₂ ·0.5H ₂ O	136-137
9e	MeS	MeS	-O-	CN	0.13	12.5	65	59	0.57	C ₁₉ H ₂₁ N ₃ O ₆ S ₂ H ₂ O	165-170 dec
9f	EtO ₂ CCH ₂ S	EtO ₂ CCH ₂ S	OH	CN	>10	200	37	41	0.39	C ₂₃ H ₂₇ N ₃ O ₇ S ₂ ·0.5H ₂ O	103-104
9g	HOCH ₂ CH ₂ S	HOCH ₂ CH ₂ S	OH	CN	3.24	6.25	18	58	0.79	C ₂₂ H ₂₅ N ₃ O ₇ S ₂ ·0.7H ₂ O	149-150
9h	EiS	MeO	OH	CN	1.18	12.5	28	73-104		C ₂₁ H ₂₃ N ₃ O ₆ S ₂ ·2H ₂ O	144-147 dec
1a	quinocarcin				0.05-0.11	20	41-51	1			



P 388 ip-ip

Scheme IV

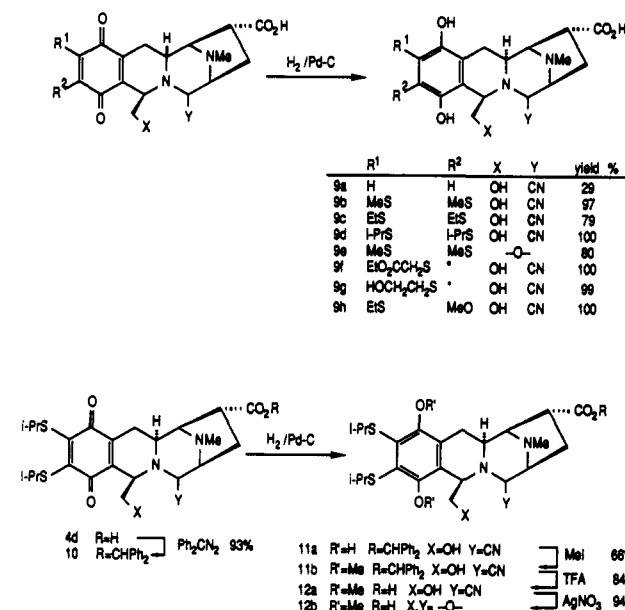


Table IV. Hydroquinone Dimethyl Ether

no.	X	Y	HeLa S ₃ IC ₅₀ , μg/mL	P 388 ip-ip	
				dose, mg/kg, × 1	% ILS
12a	OH	CN	0.17	50	7
12b	-O-		0.05	37.5	9
1a	quinocarcin		0.05-0.11	10-20	24-33

the facile conversion to quinone in a physiological environment.

Biological Results and Discussion

The antitumor activities of these derivatives were evaluated primarily by growth inhibition toward HeLa S₃ cells (in vitro) and the increased life span (ILS) of P388 implanted mice (in vivo), in single and five daily administrations (Tables I-IV). All of the tested bis(alkylthio)quinones caused a significant inhibition of cell growth. The cytotoxicities of bis(alkylthio)quinones (4a-c, IC₅₀ = 0.05-0.13 μg/mL) were comparable, and those of 4d (0.012 μg/mL) and 4e (0.004 μg/mL) were superior to that of quinocarcin (1a, 0.05-0.11 μg/mL). The comparison of 4a-e with each other illustrated the significance of the bulkiness (or lipophilicity) of the thioalkyl group to cytotoxicity, while in vivo experiments showed no such correlation. Both 4f and 4g were inclined to decrease the activity both in vitro and in vivo. Their cytotoxicities (4f, IC₅₀ = 9.96 μg/mL; 4g, IC₅₀ = 2.47 μg/mL) were 50-100 times less potent than that of 1a. Also they showed only marginal activity in vivo (ILS in five daily administrations 4f, 30%; 4g, 20%). It was likely that a hydrophilic substituent on the thioalkyl group was somewhat unsuitable. In vivo experiments with 4a-d revealed these compounds to possess high efficacy in single administration (ILS 50-65%) in comparison with 1a (ILS 24-48%). However, in 5 daily administrations, 4a-c (ILS 42-71%) were less effective than 1a (ILS 70-120%), while 4d exhibited comparable activity (ILS 119%) to 1a. As expected,^{2a} their oxazolidine forms, compounds 5a (IC₅₀ = 0.019 μg/mL)

Table V. Antitumor Activities against Murine and Human Xenografted Solid Tumors

no.	S-180 sc-iv ^a		MX-1 sc-iv		LC-06 sc-iv		St-4 sc-iv		Co-3 sc-iv	
	dose × 5	T/C ^b	dose × 5	T/C	dose × 5	T/C	dose × 5	T/C	dose × 5	T/C
4a	4.41	1.06	4.41	0.67						
5a	5.85	0.86	5.58	0.006 (2/5) ^c	5.58	0.05	9.18	0.20	9.18	0.21
			3.55	0.21	3.55	0.32	5.58	0.56	5.58	0.43
4d	12.5	0.37	9.35	0.22						
			5.96	0.83						
5d	3.13	0.73	4.67	0 (5/5) ^c						
			2.97	0.046						
7a	12.5	0.47								
9b	6.25	0.40	12.7	0 (4/5) ^c						
			8.09	0.067						
1a	10	0.74	8.78	0 (4/4) ^c	5.59	0.23	13.8	0.42	13.8	0.22
			5.59	0.01 (1/5) ^c			8.78	0.55		

^a Mice (five mice/group) were implanted subcutaneously (sc) with tumor cells, and drug was dosed (mg/kg) intravenously (iv) on days 1-5. ^b T and C are mean tumor volume of treated and control mice, respectively. ^c Number of cured mice.

and 5d ($IC_{50} = 0.0019 \mu\text{g/mL}$), showed higher cytotoxicities than corresponding 4-cyano forms 4a ($IC_{50} = 0.13 \mu\text{g/mL}$) and 4d ($IC_{50} = 0.012 \mu\text{g/mL}$), respectively. The in vivo efficacy of 5a-d was comparable to that of 4a-d in a single administration (ILS 48-69%), while superior in five daily administrations (ILS 82-98%). It was noteworthy that the optimal doses of 5a-d (6.25-12.5 mg/kg in a single dose and 3.13-6.25 mg/kg in five daily administrations) were lower than corresponding 4a-d (12.5-25 mg/kg in a single dose and 6.25-12.5 mg/kg in five daily administrations). Bis(isopropylthio)quinone 5d possessed the highest cytotoxicity and exhibited superior effectiveness of life-span extension, both in a single dose (ILS 69%) and five daily administrations (ILS 98%). These analogues seemed to deviate from the schedule-dependent activity of 1a to some degree. It implied the mode of action of these quinone analogues was somewhat different from that of 1a.

As shown in Table II, methoxy(alkylthio)quinones 7a-d were shown to diminish the activity both in vitro ($IC_{50} = 0.56-2.88 \mu\text{g/mL}$) and in vivo (ILS 17-31% in a single administration). The regiochemistry of the substituents on the quinone ring seemed to have no influence on the efficacy (compare 7a with 7b, and 7c with 7d). Even in the oxazolidine forms, compounds 8a and 8b, antitumor potency could not be so enhanced as those of the bis(alkylthio)quinones 5a-d. Only 8a expressed comparable activity (ILS 31% in a single dose and 81% in five daily administrations) to 1a.

Antitumor activities of the hydroquinone derivatives mostly showed good correlation with the corresponding quinone analogues. That is, bis(alkylthio)hydroquinones 9b-d expressed high efficacy ($IC_{50} \leq 0.03-0.09 \mu\text{g/mL}$, ILS 47% (9b) and 51% (9d) in a single dose 92% (9b) and 102% (9d) in five daily administrations) among the hydroquinones. As described above, these hydroquinones were gradually converted to the corresponding quinones in pH 7-8 solution, while the hydroquinone dimethyl ethers (12a,b) were stable under such conditions. Unexpectedly, the cytotoxicity of 12a ($IC_{50} = 0.17 \mu\text{g/mL}$) and 12b ($IC_{50} = 0.05 \mu\text{g/mL}$) were comparable to that of 1a, although inferior to that of 9d. However, they were ineffective in in vivo experiments (ILS 7% (12a) and 9% (12b) in a single administration). These data were not sufficient to conclude a relationship between quinone and hydroquinone; however, it seemed that high efficiency of bis(alkylthio)hydroquinones 9b,d in vivo was responsible for their facile convertibility to quinones.

Quinones (4a, 4d, 5a, 5d, and 7a) and hydroquinone (9b) were selected for further evaluation against solid tumors in five daily administrations. As shown in Table V, efficacy was expressed as T/C, where T and C were mean tumor volume of treated and control mice, respectively. Toward

murine sarcoma 180, 4d ($T/C = 0.37$) and 9b ($T/C = 0.40$) exhibited significant activity, while 1a ($T/C = 0.74$) and other analogues were ineffective (Table V). There was no clear structure-activity relationship. It was an unexpected result that hydroquinone 9b was effective, while its corresponding quinone 4a was not. There might be a considerable difference in pharmacokinetic or pharmacodynamic properties between 9b and 4a, which resulted in different in vivo efficacy. Similarly, against human mammary carcinoma MX-1, 9b exhibited high effectiveness ($T/C = 0$), while 4a was not effective ($T/C = 0.67$). Quinones 5a ($T/C = 0.006$) and 5d ($T/C = 0$) were revealed to possess high efficiency toward MX-1. As shown in Table V, 5a was examined against several human xenografted carcinomas, LC-06, St-4 and Co-3, and showed significant activity with a T/C value of 0.05, 0.20, and 0.21, respectively. It was noteworthy that 5a exhibited antitumor activity superior to that of 1a against most of the human xenografted carcinomas. Further preclinical study of 5a (KT 6104) is underway.

Experimental Section

All melting points were measured on a Yanagimoto micro melting point apparatus and are uncorrected. Infrared spectra (IR) were recorded on a JASCO IR-810; ¹H and ¹³C NMR spectra were measured on a Varian EM-390, JEOL FX-100, and Bruker AM-400 spectrometer. Chemical shifts were reported in parts per million (ppm) downfield from tetramethylsilane. Mass spectra were measured with a Hitachi B-80. For column chromatography, silica gel (SiO₂, Wako C-200) or highly porous polymer resin (Mitsubishi Kasei Diaion HP-20) was used. Analytical thin-layer chromatography (TLC) was performed on silica gel 60 F₂₅₄ plate (Merck). All organic solvent extracts were dried over anhydrous sodium sulfate. All aqueous fractions, after polymer resin chromatography, were freeze-dried.

General Procedure for Preparing Bis(alkylthio)quinones 4b-g. Thiol (1.5 mmol) was added to a solution of 3a^{2b} (1 mmol) in CH₃CN (10 mL) and H₂O (10 mL) and the mixture was stirred for 1 h. Fremy's salt (2 mmol) was added and stirring was continued for 30 min. Thiol (1.5 mmol) was added again, and after 30 min Fremy's salt (4 mmol) and H₂O (5 mL) were added portionwise in a 2-h period. Excess thiol and CH₃CN were distilled off in vacuo, then acetate buffer (pH 4.0) was added and the mixture was extracted with AcOEt twice. The combined extracts were washed with brine, dried, then concentrated. The residue was subjected to column chromatography (SiO₂, CHCl₃-MeOH 1:0-20:1) to give 4b-f. In the case of 4g the reaction mixture was concentrated and the residue was chromatographed directly (HP-20, H₂O-MeOH 1:0-1:1) to yield 4g. 4b: 85% yield; red solid; mp 168-169 °C; ¹H NMR (CDCl₃-CD₃OD) 4.12 (1 H, d, $J = 2.9$ Hz, 5-H), 3.94 (1 H, m, 7-H), 3.79 (1 H, dd, $J = 11.6, 2.7$ Hz, 14-H), 3.61 (1 H, dd, $J = 11.6, 3.8$ Hz, 14-H), 3.53 (1 H, br s, 1-H), 3.51 (1 H, dd, $J = 6.3, 2.4$ Hz, 4-H), 3.16-3.23 (4 H, m, SCH₂Me × 2), 3.08 (1 H, dd, $J = 9.6, 5.8$ Hz, 2-H), 2.89 (1 H, m, 12a-H), 2.82 (1 H, m, 12-H), 2.61 (1 H, dt, $J = 13.3, 6.3$ Hz, 3-H), 2.35 (3 H,

s, NCH₃), 2.13 (1 H, ddd, *J* = 17.8, 10.9, 2.9 Hz, 12-H), 1.97 (1 H, dd, *J* = 13.5, 9.7 Hz, 3-H), 1.302 (3 H, t, *J* = 7.4 Hz, SCH₂CH₃), 1.296 (3 H, t, *J* = 7.4 Hz, SCH₂CH₃); ¹³C NMR (CDCl₃-CD₃OD) 179.2 (8-C), 179.1 (11-C), 177.5 (CO₂H), 145.4 (9-C), 145.0 (10-C), 142.6 (11a-C), 140.2 (7a-C), 117.1 (CN), 69.9 (1-C), 64.7 (4-C), 63.5 (14-C), 58.2 (7-C), 57.0 (5-C), 56.3 (12a-C), 42.6 (2-C), 41.8 (NMe), 29.23 (SCH₂), 29.15 (SCH₂), 28.6 (3-C), 25.5 (12-C), 15.3 (SCH₂CH₃) ppm; IR (KBr) 3430, 2964, 1737, 1654, 1490, 1373, 1256, 1171, 1063, 1020 cm⁻¹; SIMS *m/z* 480 (M + 3)⁺, 453 (M + 3 - HCN)⁺. Anal. (C₂₂H₂₇N₃O₅S₂) C, H, N. 4c: 72% yield; mp 110–111 °C. Anal. (C₂₄H₃₁N₃O₅S₂) C, H, N. 4d: 73% yield; mp 166–167 °C. Anal. (C₂₄H₃₁N₃O₅S₂) C, H, N. 4e: 53% yield; mp 187–188 °C. Anal. (C₂₆H₃₅N₃O₅S₂) C, H, N. 4f: 79% yield; mp 88–89 °C. Anal. (C₂₆H₃₁N₃O₅S₂·0.5H₂O) C, H, N. 4g: 84% yield; mp 137–140 °C dec. Anal. (C₂₂H₂₇N₃O₇S₂·H₂O) C, H, N.

9,10-Bis(methylthio)-5-cyano-8,11-dioxo-7-(hydroxymethyl)-13-methyl-1,2,3,4,5,7,8,11,12,12a-decahydro-1,4-iminoazepino[1,2-*b*]isoquinoline-2-carboxylic Acid (4a). Fifteen percent solution of CH₃SNa in H₂O (4.5 mL) was added to a solution of 3a (3.0 g, 8.4 mmol) in CH₃CN (80 mL) and 0.2 M acetate buffer (pH 4.0, 80 mL) and the mixture was stirred for 1 h. Fremy's salt (4.5 g, 16.8 mmol) and H₂O (140 mL) were added. After 40 min acetate buffer (20 mL) and 15% solution of CH₃SNa (3.0 mL) were added and stirring was continued for 50 min. Fremy's salt (4.3 g) and H₂O (50 mL) were added, then the mixture was stirred for a further 1 h. Excess CH₃SH and CH₃CN were distilled off in vacuo and the resultant solution was extracted with AcOEt twice. The combined extracts were washed with brine, dried, and then concentrated. The residue was subjected to chromatography (SiO₂, 450 mL, CHCl₃-MeOH 1:0–20:1) to give 4a (3.22 g, 85.5%) as a red solid; mp 150–151 °C; ¹H NMR (CDCl₃-CD₃OD) 4.05 (1 H, d, *J* = 2.7 Hz, 5-H), 3.95 (1 H, m, 7-H), 3.79 (1 H, dd, *J* = 11.7, 2.8 Hz, 14-H), 3.65 (1 H, dd, *J* = 11.7, 3.3 Hz, 14-H), 3.53 (1 H, br s, 1-H), 3.52 (1 H, m, 4-H), 3.05 (1 H, dd, *J* = 9.6, 5.8 Hz, 2-H), 2.91 (1 H, br d, *J* = 11.0 Hz, 12a-H), 2.81 (1 H, ddd, *J* = 18.0, 3.0, 1.2 Hz, 12-H), 2.63 (1 H, m, 3-H), 2.63 (3 H, s, SCH₃), 2.61 (3 H, s, SCH₃), 2.36 (3 H, s, NCH₃), 2.12 (1 H, ddd, *J* = 18.0, 11.0, 2.8 Hz, 12-H), 1.95 (1 H, dd, *J* = 13.6, 9.7 Hz, 3-H) ppm; ¹³C NMR (CDCl₃-CD₃OD) 178.9 (8-C), 178.8 (11-C), 177.3 (CO₂H), 145.4 (9-C), 144.9 (10-C), 142.6 (11a-C), 140.0 (7a-C), 116.9 (CN), 69.8 (1-C), 64.6 (4-C), 63.2 (14-C), 58.1 (7-C), 56.8 (5-C), 56.1 (12a-C), 42.5 (2-C), 41.8 (NCH₃), 28.7 (3-C), 25.4 (12-C), 18.2 (SCH₃), 18.1 (SCH₃) ppm; IR (KBr) 3450, 2930, 1711, 1653, 1496, 1419, 1261, 1211, 1174 cm⁻¹; SIMS *m/z* 452 (M + 3)⁺, 425 (M + 3 - HCN)⁺. Anal. (C₂₀H₂₃N₃O₅S₂) C, H, N.

Conversion of 5-Cyanoquinones 4a–d to Oxazolines 5a–d. A 5-cyanoquinone (4a–d 1 mmol) was dissolved in CH₃CN (6 mL) and MeOH (3 mL), and AgNO₃ (2–5 mmol) was added. The mixture was stirred for 0.6–2 h. Then H₂O (10 mL) was added and the mixture was filtered. The filtrate was concentrated and the residue was chromatographed (HP-20, H₂O-MeOH 1:0–3:7) to give 5a–d. 5a: 82% yield; mp 167–172 °C dec, red solid; ¹H NMR (CD₃OD) 4.48 (1 H, d, *J* = 3.2 Hz, 5-H), 4.22 (1 H, m, 7-H), 4.14 (1 H, m, 4-H), 4.12 (1 H, br s, 1-H), 3.79 (1 H, dd, *J* = 11.5, 2.8 Hz, 14-H), 3.56 (1 H, dd, *J* = 11.5, 3.9 Hz, 14-H), 3.27–3.31 (2 H, m, 2- and 12a-H), 2.83 (1 H, dd, *J* = 17.3, 2.3 Hz, 12-H), 2.78 (3 H, s, NCH₃), 2.62 (1 H, m, 3-H), 2.57 (3 H, s, SCH₃), 2.55 (3 H, s, SCH₃), 2.28 (1 H, dd, *J* = 14.0, 10.5 Hz, 3-H), 2.18 (1 H, ddd, *J* = 17.2, 11.2, 2.2 Hz, 12-H) ppm; ¹³C NMR (CD₃OD) 180.7 (8-C), 180.2 (11-C), 179.0 (CO₂H), 146.8 (9-C), 146.7 (10-C), 143.8 (11a-C), 142.7 (7a-C), 90.9 (5-C), 72.7 (1-C), 66.5 (4-C), 64.9 (14-C), 56.2 (7-C), 54.3 (12a-C), 43.1 (2-C), 41.2 (NCH₃), 28.5 (3-C), 26.2 (12-C), 18.5 (SCH₃ × 2); IR (KBr) 3400, 2926, 1650, 1587, 1493, 1422, 1377, 1263, 1170 cm⁻¹; SIMS *m/z* 425 (M + 3)⁺. Anal. (C₁₅H₂₂N₂O₅S₂·1.5H₂O) C, H, N. 5b: 77% yield; mp 160–163 °C dec. Anal. (C₂₁H₂₆N₂O₅S₂·1.5H₂O) C, H, N. 5c: 80% yield; mp 140–145 °C dec. Anal. (C₂₃H₃₀N₂O₅S₂·1.2H₂O) C, H, N. 5d: 84% yield; mp 152–157 °C dec. Anal. (C₂₃H₃₀N₂O₅S₂·0.5H₂O) C, H, N.

Diphenylmethyl 5-Cyano-8,11-dioxo-10-(ethylthio)-7-(hydroxymethyl)-9-methoxy-13-methyl-1,2,3,4,5,7,8,11,12,12a-decahydro-1,4-iminoazepino[1,2-*b*]isoquinoline-2-carboxylate (6a) and Diphenylmethyl 5-Cyano-8,11-dioxo-9-(ethylthio)-7-(hydroxymethyl)-10-methoxy-13-methyl-1,2,3,4,5,7,8,11,12,12a-decahydro-1,4-iminoazepino[1,2-*b*]isoquinoline-2-carboxylate (6b). To a solution of 3c^{2b} (600 mg,

1.08 mmol) in CH₃CN (10 mL), H₂O (10 mL), and 0.2 M acetate buffer (pH 4.0, 3 mL) was added ethanethiol (0.24 mL, 3.2 mmol) gradually over a 2-h period. After stirring for 17 h, Fremy's salt (580 mg, 2.2 mmol) was added and the mixture was stirred for 40 min. Excess ethanethiol was distilled off in vacuo and H₂O (20 mL) was added. The mixture was extracted with AcOEt and washed with pH 7.7 phosphate buffer and brine, then dried and evaporated. The residue was subjected to chromatography (SiO₂, 100 mL, *n*-hexane-AcOEt 4:1–2:1) to afford 6a (221 mg, 33.3%) and 6b (143 mg, 21.5%), both as red solids. 6a: mp 92–93 °C, ¹H NMR (CDCl₃) 7.28–7.39 (10 H, m, Ph₂), 6.90 (1 H, s, CHPh₂), 4.06 (3 H, s, OCH₃), 3.99 (1 H, d, *J* = 2.3 Hz, 5-H), 3.95 (1 H, m, 7-H), 3.82 (1 H, dd, *J* = 11.7, 3.1 Hz, 14-H), 3.69 (1 H, br, 14-H), 3.52 (1 H, br s, 1-H), 3.51 (1 H, m, 4-H), 3.10 (2 H, q, *J* = 7.4 Hz, SCH₂CH₃), 3.10 (1 H, dd, *J* = 9.5, 5.7 Hz, 2-H), 2.95 (1 H, m, 12a-H), 2.85 (1 H, ddd, *J* = 18.0, 3.1, 1.3 Hz, 12-H), 2.70 (1 H, m, 3-H), 2.17 (3 H, s, NCH₃), 2.15 (1 H, ddd, *J* = 18.0, 11.1, 2.9 Hz, 12-H), 1.94 (1 H, dd, *J* = 13.5, 9.5 Hz, 3-H), 1.29 (3 H, t, *J* = 7.4 Hz, SCH₂CH₃) ppm; IR (KBr) 3460, 2950, 1727, 1649, 1559, 1495, 1452, 1305, 1264, 1227, 1170, 1059, 1013, 909, 851, 741, 699 cm⁻¹; SIMS *m/z* 616 (M + 3)⁺, 589 (M + 3 - HCN)⁺. Anal. (C₂₄H₃₅N₃O₆S) C, H, N. 6b: mp 94–95 °C; ¹H NMR (CDCl₃) 7.28–7.40 (10 H, m, Ph₂), 6.91 (1 H, s, CHPh₂), 4.08 (3 H, s, OCH₃), 3.99 (1 H, d, *J* = 2.1 Hz, 5-H), 3.96 (1 H, m, 7-H), 3.81 (1 H, dd, *J* = 11.7, 3.1 Hz, 14-H), 3.67 (1 H, br, 14-H), 3.52 (1 H, br s, 1-H), 3.51 (1 H, m, 4-H), 3.09 (2 H, q, *J* = 7.4 Hz, SCH₂CH₃), 3.09 (1 H, m, 2-H), 2.96 (1 H, br d, *J* = 9.5 Hz, 12a-H), 2.83 (1 H, ddd, *J* = 18.0, 3.1, 1.3 Hz, 12-H), 2.70 (1 H, m, 3-H), 2.17 (3 H, s, NCH₃), 2.13 (1 H, ddd, *J* = 18.0, 11.0, 3.0 Hz, 12-H), 1.95 (1 H, dd, *J* = 13.5, 9.7 Hz, 3-H), 1.28 (3 H, t, *J* = 7.4 Hz, SCH₂CH₃) ppm; IR (KBr) 3470, 2950, 1730, 1654, 1566, 1495, 1453, 1313, 1272, 1222, 1171, 1056, 1010, 741, 699 cm⁻¹; SIMS *m/z* 616 (M + 3)⁺, 589 (M + 3 - HCN)⁺. Anal. (C₃₄H₃₅N₃O₆S) C, H, N.

Diphenylmethyl 5-Cyano-8,11-dioxo-7-(hydroxymethyl)-10-(isopropylthio)-9-methoxy-13-methyl-1,2,3,4,5,7,8,11,12,12a-decahydro-1,4-iminoazepino[1,2-*b*]isoquinoline-2-carboxylate (6c) and Diphenylmethyl 5-Cyano-8,11-dioxo-7-(hydroxymethyl)-9-(isopropylthio)-10-methoxy-13-methyl-1,2,3,4,5,7,8,11,12,12a-decahydro-1,4-iminoazepino[1,2-*b*]isoquinoline-2-carboxylate (6d). With the same procedure as that described for 6a and 6b, except for use of *i*-PrSH in place of EtSH, 3c (1.2 g) gave 6c (629 mg, 46.2%) and 6d (391 mg, 28.7%) both as red solids. 6c: mp 94–95 °C; IR (KBr) 3480, 2952, 1727, 1652, 1566, 1495, 1454, 1423, 1365, 1304, 1262, 1225, 1172, 1053, 1013, 909, 850, 740, 699 cm⁻¹; EIMS *m/z* 627 (M⁺), 629 (M + 2)⁺, 598. Anal. (C₃₆H₃₇N₃O₆S·0.2H₂O) C, H, N. 6d: mp 93–94 °C; ¹H NMR (CDCl₃) 7.28–7.40 (10 H, m, Ph₂), 6.91 (1 H, s, CHPh₂), 4.10 (3 H, s, OCH₃), 3.98 (1 H, d, *J* = 2.7 Hz, 5-H), 3.97 (1 H, m, 7-H), 3.89 (1 H, heptet, *J* = 6.7 Hz, SCHMe₂), 3.80 (1 H, dd, *J* = 11.8, 3.0 Hz, 14-H), 3.65 (1 H, br, 14-H), 3.52 (1 H, br s, 1-H), 3.51 (1 H, m, 4-H), 3.09 (1 H, dd, *J* = 9.6, 5.8 Hz, 2-H), 2.96 (1 H, m, 12a-H), 2.83 (1 H, ddd, *J* = 18.0, 3.2, 1.3 Hz, 12-H), 2.70 (1 H, dt, *J* = 13.4, 6.3 Hz, 3-H), 2.16 (3 H, s, NCH₃), 2.13 (1 H, ddd, *J* = 18.0, 11.0, 2.9 Hz, 12-H), 1.94 (1 H, dd, *J* = 13.6, 9.6 Hz, 3-H), 1.29 (3 H, d, *J* = 6.7 Hz, SCH(CH₃)₂), 1.27 (3 H, d, *J* = 6.7 Hz, SCH(CH₃)₂) ppm; IR (KBr) 3510, 2952, 1727, 1655, 1559, 1495, 1455, 1312, 1272, 1222, 1170, 1054, 1011, 913, 741, 698 cm⁻¹; EIMS *m/z* 627 (M⁺), 629 (M + 2)⁺, 598. Anal. (C₃₆H₃₇N₃O₆S·0.2H₂O) C, H, N.

Ester Cleavage of 6a–d for Preparing 7a–d. A solution of ester (6a–d, ca. 300 mg) in CH₂Cl₂ (10 mL) was treated with TFA (1 mL) and anisole (0.3 mL) for 1–2 h. The mixture was concentrated and the residue was purified by chromatography (HP-20, H₂O-MeOH 1:0–3:7) to give 7a–d. 7a: red solid; 77% yield; mp 131–132 °C; ¹H NMR (CD₃OD) 4.55 (1 H, d, *J* = 2.7 Hz, 5-H), 4.03 (3 H, s, OCH₃), 3.96 (1 H, m, 7-H), 3.91 (1 H, br s, 1-H), 3.83 (1 H, dd, *J* = 11.6, 2.3 Hz, 14-H), 3.64 (1 H, dd, *J* = 11.6, 4.1 Hz, 14-H), 3.43 (1 H, dd, *J* = 10.0, 5.7 Hz, 2-H), 3.07 (2 H, q, *J* = 7.4 Hz, SCH₂CH₃), 2.97 (1 H, m, 12a-H), 2.84 (1 H, dd, *J* = 17.5, 2.1 Hz, 12-H), 2.71 (1 H, m, 3-H), 2.61 (3 H, s, NCH₃), 2.24 (1 H, dd, *J* = 13.8, 10.2 Hz, 3-H), 2.20 (1 H, ddd, *J* = 17.6, 10.9, 2.5 Hz, 12-H), 1.25 (3 H, t, *J* = 7.4 Hz, SCH₂CH₃) ppm; ¹³C NMR (CD₃OD) 184.1 (8-C), 180.4 (11-C), 177.0 (CO₂H), 158.1 (9-C), 142.9 (11a-C), 138.6 (7a-C), 130.9 (10-C), 117.2 (CN), 71.3 (1-C), 66.3 (4-C), 64.1 (14-C), 61.6 (OCH₃), 58.7 (7-C), 57.3 (5-C), 56.6 (12a-C), 42.2 (2-C), 41.7 (NCH₃), 29.2 (SCH₂), 28.0 (3-C), 25.9 (12-C), 15.6

(SCH₂CH₃) ppm; IR (KBr) 3430, 2950, 1719, 1653, 1564, 1457, 1424, 1303, 1272, 1201, 1136, 1060, 1011, 851, 720 cm⁻¹; SIMS *m/z* 450 (M + 3)⁺, 423 (M + 3 - HCN)⁺. Anal. (C₂₁H₂₅N₃O₆S·H₂O) C, H, N. 7b: 89% yield; mp 160–165 °C dec. Anal. (C₂₁H₂₅N₃O₆S·0.2H₂O) C, H, N. 7c: 87% yield; mp 139–140 °C. Anal. (C₂₂H₂₇N₃O₆S·0.5H₂O) C, H, N. 7d: 77% yield; mp 136–137 °C. Anal. (C₂₂H₂₇N₃O₆S·0.5H₂O) C, H, N.

7-(Acetoxymethyl)-5-cyano-8,11-dioxo-9-(ethylthio)-10-methoxy-13-methyl-1,2,3,4,5,7,8,11,12,12a-decahydro-1,4-iminoazepino[1,2-*b*]isoquinoline-2-carboxylic Acid (7e). To a solution of 3d^{2b} (150 mg, 0.35 mmol) in CH₃CN (3 mL) and H₂O (3 mL) was added ethanethiol (0.078 mL, 1.05 mmol) and the mixture was stirred for 23 h. Fremy's salt (188 mg, 0.70 mmol) was added and stirring was continued for 2.6 h. Then excess ethanethiol and CH₃CN were distilled off in vacuo. The mixture was extracted with AcOEt three times. The combined extracts were washed with brine, dried and concentrated. The residue was subjected to chromatography (SiO₂, 20 mL, CHCl₃-MeOH 1:0–100:1) to afford 7e (152 mg, 89.0%) as a red solid; mp 94–95 °C; ¹H NMR (CD₃OD) 4.53 (1 H, dd, *J* = 11.7, 3.2 Hz, 14-H), 4.21 (1 H, d, *J* = 2.9 Hz, 5-H), 4.10 (1 H, dd, *J* = 11.7, 2.7 Hz, 14-H), 4.05 (3 H, s, OCH₃), 3.99 (1 H, m, 7-H), 3.53 (1 H, br d, *J* = 6.3 Hz, 4-H), 3.49 (1 H, br s, 1-H), 3.02–3.11 (2 H, m, SCH₂CH₃), 2.94 (1 H, dd, *J* = 9.7, 5.9 Hz, 2-H), 2.80 (1 H, m, 12a-H), 2.76 (1 H, m, 12-H), 2.54 (1 H, dt, *J* = 13.2, 6.4 Hz, 3-H), 2.32 (3 H, s, NCH₃), 2.04 (1 H, ddd, *J* = 16.9, 10.3, 2.4 Hz, 12-H), 1.99 (3 H, s, COCH₃), 1.94 (1 H, dd, *J* = 13.4, 9.7 Hz, 3-H), 1.24 (3 H, t, *J* = 7.4 Hz, SCH₂CH₃) ppm; ¹³C NMR (CDCl₃) 182.6 (11-C), 180.2 (8-C), 179.0 (CO₂H), 170.6 (COCH₃), 155.9 (10-C), 140.1 (11a-C), 138.5 (7a-C), 131.2 (9-C), 116.6 (CN), 69.8 (1-C), 64.6 (4-C), 62.3 (14-C), 61.2 (OCH₃), 56.0 (7-C), 55.9 (5-C), 55.7 (12a-C), 42.5 (2-C), 41.7 (NCH₃), 28.2 (SCH₂), 27.5 (3-C), 24.7 (12-C), 20.9 (COCH₃), 15.1 (SCH₂CH₃) ppm; IR (KBr) 2954, 1738, 1649, 1565, 1457, 1376, 1315, 1261, 1222, 1179, 1046, 1018 cm⁻¹; SIMS *m/z* 492 (M + 3)⁺, 490 (M + 1)⁺, 465 (M + 3 - HCN)⁺, 463 (M + 1 - HCN)⁺, 418. Anal. (C₂₃H₂₇N₃O₇S·1.5H₂O) C, H, N.

Preparation of 8a and 8b. With the same procedure as that described for 5a–d, 7c and 7d gave 8a and 8b, respectively, both as orange solids. 8a: 82% yield; mp 130–135 °C dec; ¹H NMR (CD₃OD) 4.48 (1 H, d, *J* = 3.2 Hz, 5-H), 4.20 (1 H, m, 7-H), 4.14 (1 H, m, 4-H), 4.11 (1 H, br s, 1-H), 4.04 (3 H, s, OCH₃), 3.88 (1 H, heptet, *J* = 6.7 Hz, SCH(CH₃)₂), 3.82 (1 H, dd, *J* = 11.5, 2.7 Hz, 14-H), 3.59 (1 H, dd, *J* = 11.5, 3.8 Hz, 14-H), 3.27 (1 H, m, 12a-H), 2.84 (1 H, dd, *J* = 17.2, 2.3 Hz, 12-H), 2.78 (3 H, s, NCH₃), 2.62 (1 H, m, 3-H), 2.29 (1 H, dd, *J* = 14.1, 10.5 Hz, 3-H), 2.19 (1 H, ddd, *J* = 17.2, 11.1, 2.6 Hz, 12-H), 1.25 (3 H, d, *J* = 6.7 Hz, SCH(CH₃)₂), 1.24 (3 H, d, *J* = 6.7 Hz, SCH(CH₃)₂) ppm; IR (KBr) 3420, 2962, 1719, 1650, 1580, 1457, 1383, 1260, 1227, 1109, 1054, 1016, 799 cm⁻¹; SIMS *m/z* 437 (M + 3)⁺. Anal. (C₂₁H₂₅N₃O₆S·2H₂O) C, H, N. 8b: 85% yield; mp 132–137 °C dec; ¹H NMR (CD₃OD) 4.49 (1 H, d, *J* = 3.3 Hz, 5-H), 4.21 (1 H, m, 7-H), 4.14 (1 H, m, 4-H), 4.11 (1 H, br s, 1-H), 4.06 (3 H, s, OCH₃), 3.84 (1 H, heptet, *J* = 6.7 Hz, SCH(CH₃)₂), 3.80 (1 H, dd, *J* = 11.5, 2.6 Hz, 14-H), 3.53 (1 H, dd, *J* = 11.5, 3.9 Hz, 14-H), 3.27 (1 H, m, 12a-H), 2.82 (1 H, dd, *J* = 17.1, 2.4 Hz, 12-H), 2.78 (3 H, s, NCH₃), 2.62 (1 H, m, 3-H), 2.30 (1 H, dd, *J* = 14.1, 10.5 Hz, 3-H), 2.18 (1 H, ddd, *J* = 17.1, 11.1, 2.5 Hz, 12-H), 1.25 (3 H, d, *J* = 6.7 Hz, SCH(CH₃)₂), 1.23 (3 H, d, *J* = 6.6 Hz, SCH(CH₃)₂) ppm; IR (KBr) 3420, 2958, 1710, 1649, 1587, 1540, 1450, 1383, 1282, 1231, 1109, 1005 cm⁻¹; SIMS *m/z* 437 (M + 3)⁺. Anal. (C₂₁H₂₅N₃O₆S·1.5H₂O) C, H, N.

Preparation of Hydroquinones 9a–h. A solution of quinone in MeOH in the presence of 5% Pd-C (20–30 wt %) was stirred in an atmosphere of H₂ for 1–3 h. The catalyst was filtered off and the filtrate was concentrated to afford hydroquinone 9a–h. In the case of 9a and 9e the products were not pure. 9a and 9e were then subjected to chromatography (HP-20, H₂O-MeOH 1:0–1:1). 9a: pale brown solid; 29% yield; mp 135–140 °C dec; ¹H NMR (CD₃OD) 6.53 (2 H, s, 9- and 10-H), 4.27 (1 H, d, *J* = 3 Hz, 5-H), 4.17 (1 H, m, 7-H), 3.52 (1 H, br s, 1-H), 3.3–3.9 (3 H, m), 3.13 (1 H, m), 2.4–3.0 (4 H, m), 2.35 (3 H, s, NCH₃), 2.12 (1 H, m, 3-H) ppm; IR (KBr) 3380, 1701, 1652, 1581, 1490, 1464, 1385, 1280, 1154, 1074, 1015, 942, 818 cm⁻¹; SIMS *m/z* 360 (M + 1)⁺, 333 (M + 1 - HCN)⁺. Anal. (C₁₈H₂₁N₃O₂S₂·H₂O) C, H, N. 9b: pale pink solid; 97% yield; mp 170–175 °C dec; ¹H NMR (CDCl₃) 4.21 (1 H, m, 7-H), 4.10 (1 H, d, *J* = 2.6 Hz, 5-H), 3.82

(1 H, dd, *J* = 11.1, 2.8 Hz, 14-H), 3.70 (1 H, dd, *J* = 11.1, 3.5 Hz, 14-H), 3.56 (1 H, s, 1-H), 3.51 (1 H, m, 4-H), 3.21 (1 H, dd, *J* = 9.3, 5.8 Hz, 2-H), 2.98–3.02 (2 H, m, 12- and 12a-H), 2.63 (1 H, dt, *J* = 13.2, 6.6 Hz, 3-H), 2.35–2.40 (1 H, m), 2.354 (6 H, s, SCH₃ × 2), 2.347 (3 H, s, NCH₃), 2.04 (1 H, dd, *J* = 13.2, 9.8 Hz, 3-H) ppm; ¹³C NMR (CDCl₃-CD₃OD) 178.2 (CO₂H), 147.9 (8-C), 147.2 (11-C), 124.5 (11a-C), 123.0 (7a-C), 122.3 (9-C), 122.0 (10-C), 117.6 (CN), 70.1 (1-C), 64.8 (14-C), 64.4 (4-C), 58.3 (7-C), 57.7 (5-C), 56.8 (12a-C), 42.5 (2-C), 41.6 (NCH₃), 28.6 (3-C), 26.7 (12-C), 19.7 (SCH₃), 19.6 (SCH₃) ppm; IR (KBr) 3370, 2926, 1717, 1653, 1593, 1457, 1411, 1388, 1260, 1235, 1182, 1141, 1090, 1066, 889, 858 cm⁻¹; SIMS *m/z* 452 (M + 1)⁺, 425 (M + 1 - HCN)⁺. Anal. (C₂₀H₂₅N₃O₆S₂·H₂O) C, H, N. 9c: 79% yield; mp 140–141 °C. Anal. (C₂₂H₂₉N₃O₆S₂·0.5H₂O) C, H, N. 9d: 100% yield; mp 136–137 °C. Anal. (C₂₄H₃₃N₃O₆S₂·0.5H₂O) C, H, N. 9e: 80% yield; mp 165–170 °C dec; pale pink solid; ¹H NMR (CD₃OD) 4.57 (1 H, d, *J* = 3.1 Hz, 5-H), 4.53 (1 H, dd, *J* = 6.3, 3.0 Hz, 7-H), 4.15 (1 H, br s, 1-H), 4.12 (1 H, m, 4-H), 3.75 (1 H, dd, *J* = 10.9, 3.0 Hz, 14-H), 3.50 (1 H, dd, *J* = 10.9, 6.4 Hz, 14-H), 3.39 (1 H, dd, *J* = 10.5, 5.1 Hz, 2-H), 3.37 (1 H, br d, *J* = 12.3 Hz, 12a-H), 3.08 (1 H, dd, *J* = 15.4, 2.6 Hz, 12-H), 2.80 (3 H, s, NCH₃), 2.63 (1 H, m, 3-H), 2.46 (1 H, dd, *J* = 14.0, 10.6 Hz, 3-H), 2.43 (1 H, dd, *J* = 15.2, 12.0 Hz, 12-H), 2.33 (3 H, s, SCH₃), 2.32 (3 H, s, SCH₃) ppm; ¹³C NMR (CD₃OD) 179.3 (CO₂H), 149.6 (8-C), 149.1 (11-C), 125.9 (11a-C), 125.4 (7a-C), 124.9 (9-C), 124.4 (10-C), 92.1 (5-C), 73.1 (1-C), 67.3 (14-C), 66.7 (4-C), 56.7 (7-C), 55.0 (12a-C), 42.8 (2-C), 40.6 (NCH₃), 28.2 (3-C), 27.0 (12-C), 20.0 (SCH₃), 19.8 (SCH₃) ppm; IR (KBr) 3380, 2924, 1718, 1589, 1410, 1255, 1206, 1077, 1010, 972, 946, 857 cm⁻¹; SIMS *m/z* 425 (M + 1)⁺. Anal. (C₁₉H₂₄N₂O₆S₂·H₂O) C, H, N. 9f: 100% yield; mp 103–104 °C. Anal. (C₂₀H₂₃N₃O₆S₂·0.5H₂O) C, H, N. 9g: 99% yield; mp 149–150 °C. Anal. (C₂₂H₂₉N₃O₇S₂·0.7H₂O) C, H, N. 9h: pale orange solid; 100% yield; mp 144–147 °C dec; ¹H NMR (CD₃OD) 4.44 (1 H, d, *J* = 2.7 Hz, 5-H), 4.22 (1 H, dd, *J* = 6.3, 2.9 Hz, 7-H), 3.83 (1 H, dd, *J* = 10.8, 3.0 Hz, 14-H), 3.82 (3 H, s, OCH₃), 3.70 (1 H, m, 4-H), 3.69 (1 H, br s, 1-H), 3.53 (1 H, dd, *J* = 10.8, 6.4 Hz, 14-H), 3.39 (1 H, dd, *J* = 9.8, 5.7 Hz, 2-H), 2.97 (1 H, dd, *J* = 15.3, 2.6 Hz, 12-H), 2.94 (1 H, br d, *J* = 11.4 Hz, 12a-H), 2.75 (2 H, q, *J* = 7.4 Hz, SCH₂CH₃), 2.65 (1 H, dt, *J* = 13.4, 6.3 Hz, 3-H), 2.46 (3 H, s, NCH₃), 2.35 (1 H, dd, *J* = 15.3, 11.4 Hz, 12-H), 2.22 (1 H, dd, *J* = 13.6, 10.0 Hz, 3-H), 1.16 (3 H, t, *J* = 7.4 Hz, SCH₂CH₃) ppm; ¹³C NMR (CD₃OD) 178.4 (CO₂H), 148.8 (8-C), 148.5 (11-C), 141.0 (9-C), 125.1 (7a-C), 118.6 (11a-C), 118.5 (CN), 112.5 (10-C), 72.1 (1-C), 67.4 (14-C), 66.3 (4-C), 61.5 (OCH₃), 59.5 (7-C), 59.4 (5-C), 58.8 (12a-C), 43.2 (2-C), 42.0 (NCH₃), 30.3 (SCH₂), 29.3 (3-C), 27.3 (12-C), 15.2 (SCH₂CH₃) ppm; IR (KBr) 3400, 2948, 1717, 1653, 1565, 1452, 1424, 1263, 1202, 1138, 1011, 916, 720 cm⁻¹; SIMS *m/z* 450 (M + 1)⁺, 423 (M + 1 - HCN)⁺. Anal. (C₂₁H₂₇N₃O₆S·2.2H₂O) C, H, N.

Diphenylmethyl 9,10-Bis(isopropylthio)-5-cyano-8,11-dioxo-7-(hydroxymethyl)-13-methyl-1,2,3,4,5,7,8,11,12,12a-decahydro-1,4-iminoazepino[1,2-*b*]isoquinoline-2-carboxylate (10). To a solution of 4d (600 mg, 1.19 mmol) in CHCl₃ (15 mL) was added Ph₂CN₂ (230 mg, 1.19 mmol) in CHCl₃ (1 mL). After stirring for 50 min, Ph₂CN₂ (100 mg) in CHCl₃ (0.6 mL) was added and stirring was continued for 45 min. AcOH was added to decompose the excess Ph₂CN₂ and the mixture was concentrated. The residue was partitioned between AcOEt and H₂O. The organic layer was separated and washed with 5% aqueous NaHCO₃ and brine, dried, and concentrated. The residue was chromatographed (SiO₂, 90 mL, *n*-hexane-AcOEt 4:1–3:1) to give 10 (741 mg, 92.9%) as a red solid; mp 90–91 °C; ¹H NMR (CDCl₃) 7.28–7.40 (10 H, m, Ph₂), 6.91 (1 H, s, CHPh₂), 4.05–4.15 (2 H, m, SCHMe₂ × 2), 4.01 (1 H, m, 5-H), 4.00 (1 H, m, 7-H), 3.82 (1 H, dd, *J* = 11.7, 3.0 Hz, 14-H), 3.66 (1 H, br, 14-H), 3.53 (1 H, br s, 1-H), 3.53 (1 H, m, 4-H), 3.11 (1 H, dd, *J* = 9.7, 5.7 Hz, 2-H), 2.99 (1 H, br d, *J* = 11.2 Hz, 12a-H), 2.88 (1 H, ddd, *J* = 18.1, 3.2, 1.4 Hz, 12-H), 2.70 (1 H, m, 3-H), 2.18 (3 H, s, NCH₃), 2.16 (1 H, ddd, *J* = 18.1, 11.0, 2.9 Hz, 12-H), 1.96 (1 H, dd, *J* = 13.5, 9.7 Hz, 3-H), 1.26–1.33 (12 H, m, SCH(CH₃)₂ × 2) ppm; IR (KBr) 3410, 2960, 2928, 2866, 1727, 1653, 1494, 1454, 1365, 1258, 1168, 1048, 741, 698 cm⁻¹; EIMS *m/z* 672 (M + 1)⁺, 643, 354, 167. Anal. (C₃₇H₄₁N₃O₆S₂) C, H, N.

Diphenylmethyl 9,10-Bis(isopropylthio)-5-cyano-8,11-dihydroxy-7-(hydroxymethyl)-13-methyl-1,2,3,4,5,7,12,12a-octahydro-1,4-iminoazepino[1,2-*b*]isoquinoline-2-carboxylate

(11a). A solution of 10 (700 mg, 1.04 mmol) in MeOH (30 mL) in the presence of 5% Pd-C (200 mg) was stirred for 15 min under a stream of H₂. The catalyst was filtered off and washed with AcOEt and CHCl₃. The filtrate and washings were combined and concentrated to give 11a (686 mg, 97.7%) as a pale pink solid: mp 172-178 °C dec; ¹H NMR (CDCl₃) 7.28-7.38 (10 H, m, Ph₂), 6.99 (1 H, s, OH), 6.91 (1 H, s, CHPh₂), 6.83 (1 H, s, OH), 4.25 (1 H, t, *J* = 3.6 Hz, 7-H), 4.07 (1 H, m, 5-H), 3.84 (1 H, dd, *J* = 11.0, 3.3 Hz, 14-H), 3.76 (1 H, br, 14-H), 3.57 (1 H, br s, 1-H), 3.51 (1 H, m, 4-H), 3.31-3.39 (2 H, m, SCHMe₂ × 2), 3.29 (1 H, dd, *J* = 10.3, 5.9 Hz, 2-H), 3.10 (1 H, m, 12a-H), 3.07 (1 H, dd, *J* = 15.8, 2.8 Hz, 12-H), 2.69 (1 H, m, 3-H), 2.45 (1 H, dd, *J* = 15.8, 11.4 Hz, 12-H), 2.19 (3 H, br s, NCH₃), 2.17 (1 H, m, 3-H), 1.22-1.29 (12 H, m, SCH(CH₃)₂ × 2) ppm; IR (KBr) 3426, 3398, 2966, 2924, 2866, 1724, 1593, 1495, 1458, 1447, 1406, 1387, 1363, 1327, 1274, 1223, 1151, 1089, 1065, 1052, 1027, 995, 929, 873, 857, 754, 736, 702 cm⁻¹; EIMS *m/z* 673 (M⁺), 643, 354, 167. Anal. (C₃₇H₄₃N₃O₅S₂·2H₂O) C, H, N.

Diphenylmethyl 9,10-Bis(isopropylthio)-5-cyano-8,11-dimethoxy-7-(hydroxymethyl)-13-methyl-1,2,3,4,5,7,12,12a-octahydro-1,4-iminoazepino[1,2-*b*]isoquinoline-2-carboxylate (11b). To a solution of 11a (600 mg, 1.04 mmol) in DMF (30 mL) was added K₂CO₃ (148 mg, 1.07 mmol). MeI (0.26 mL, 4.2 mmol) and NaBH₄ (11 mg) were added portionwise followed by 25 h of stirring. The reaction mixture was concentrated and partitioned between AcOEt and H₂O. The organic layer was separated and washed with brine, dried, and concentrated. The residue was subjected to chromatography (SiO₂, 70 mL, *n*-hexane-AcOEt 4:1-3:1) to give 11b (414 mg, 66.2%): ¹H NMR (CDCl₃) 7.30 (10 H, m, Ph₂), 6.87 (1 H, s, CHPh₂), 4.00-4.24 (2 H, m, 5- and 7-H), 3.84 (3 H, s, OCH₃), 3.78 (3 H, s, OCH₃), 3.32-3.76 (6 H, m), 3.26 (1 H, dd, *J* = 9, 6 Hz, 2-H), 2.84-3.16 (2 H, m), 2.24-2.80 (2 H, m), 2.13 (3 H, s, NCH₃), 1.98 (1 H, m, 3-H), 1.12-1.36 (12 H, m, SCH(CH₃)₂ × 2) ppm; SIMS *m/z* 702 (M + 1)⁺, 675 (M + 1 - HCN)⁺, 509.

9,10-Bis(isopropylthio)-5-cyano-8,11-dimethoxy-7-(hydroxymethyl)-13-methyl-1,2,3,4,5,7,12,12a-octahydro-1,4-iminoazepino[1,2-*b*]isoquinoline-2-carboxylic Acid (12a). With the same procedure as that for 7a-d, 11b (385 mg) provided 12a (246 mg, 83.7%): SIMS *m/z* 536 (M + 1)⁺, 522, 509 (M + 1 - HCN)⁺, 495.

Conversion of 12a to Oxazolidine Form 12b. With the same procedure as that for 5a-d, 12a (90 mg) gave 12b (80.1 mg, 93.7%): ¹H NMR (CD₃OD) 4.56 (1 H, d, *J* = 3.1 Hz, 5-H), 4.45 (1 H, dd, *J* = 7.2, 3.1 Hz, 7-H), 4.14 (1 H, br s, 1-H), 4.12 (1 H, m, 4-H), 3.83 (3 H, s, OCH₃), 3.76 (3 H, s, OCH₃), 3.61-3.71 (2 H, m, 14-H₂), 3.34-3.41 (3 H, m, 1-H and SCHMe₂ × 2), 3.25 (1 H, m, 12a-H), 3.06 (1 H, dd, *J* = 15.2, 2.5 Hz, 12-H), 2.80 (3 H, s, NCH₃), 2.64 (1 H, m, 3-H), 2.54 (1 H, dd, *J* = 15.2, 12.0 Hz, 12-H), 2.47 (1 H, dd, *J* = 13.7, 10.4 Hz, 3-H), 1.15-1.23 (12 H, m, SCH(CH₃)₂ × 2) ppm; SIMS *m/z* 509 (M + 1)⁺, 495, 467.

Biological Studies. HeLa S₃ cells (5 × 10⁴) were seeded in Eagle's minimum essential medium (Nissui Pharmaceutical Co., Ltd., Tokyo, Japan) containing of 10% fetal bovine serum (Grand Island Biological Co.) and 0.06 mg/mL of kanamycin. Graded concentrations of drugs, appropriately diluted with growth medium, were added 24 h after the cells were seeded. The cultures were incubated at 37 °C in a humidified atmosphere of 5% CO₂. After 72 h of drug exposure, the monolayer cells were washed with phosphate-buffered salts solution (Flow Laboratories) and incubated with 0.05% trypsin (Difco Laboratories, Detroit, MI) and 0.02% EDTA (Wako Pure Chemical Industries Co., Ltd., Osaka, Japan). The cells were counted with a Toa Micro-Cell counter (Toa Medical Electronics Co., Ltd., Kobe, Japan) and the IC₅₀ value (drug concentration required for 50% inhibition of the cell growth) was determined.

Lymphocytic leukemia P388 (1 × 10⁶) cells were implanted intraperitoneally (ip) into CD2F₁ mice (about 22 g weight) divided into groups each consisting of five test mice. Administration of drugs was started the day after tumor implantation. Antitumor efficacy was expressed as an increased life span (ILS), calculated (T/C - 1) × 100, where T and C are median survival times of treated and control mice.

Sarcoma 180 (5 × 10⁶ cells/mouse) was inoculated subcutaneously (sc) at the axillary region in ddY mice divided into groups each consisting of five test mice. Drugs were administered intravenously (iv) starting the day after tumor inoculation. Antitumor efficacy was expressed as T/C, where T and C are mean tumor volume of treated and control mice. Tumor volume was calculated by using the formula for a prolate ellipsoid

$$\text{tumor volume} = L (\text{mm}) \times W^2 (\text{mm}) / 2$$

in which L is the length of the major axis and W is the length of the minor axis.¹⁴

BALB/c-nu/nu mice were administered with a tumor fragment equivalent to 8 mm³ of MX-1 (human mammary carcinoma), Co-3 (human colon carcinoma), LC-06 (human lung carcinoma) or St-4 (human gastric carcinoma) tumor passed in nude mouse. When tumor volume reached 100-300 mm³, the mice were pair matched in groups of five each and the drug was administered intravenously. Antitumor efficacy was expressed as T/C, as described for sarcoma 180.

Supplementary Material Available: Listings of complete analytical data and physicochemical data (¹H and ¹³C NMR, IR and mass spectrum) of new compounds except for those described in the paper (7 pages). Ordering information is given on any current masthead page.

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Persistent Binding of Fatty Acyl Derivatives of Naltrexamine to Opioid Receptors

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A series of fatty acid derivatives of naltrexamine and naltrexhydrazine were synthesized and evaluated for their persistent binding to opioid receptors. Members of this series were found to require greater than five washes for removal from mouse brain membranes when the fatty acyl chain was saturated. The presence of unsaturation in the fatty acyl groups enhanced the persistent binding. In this regard the persistent binding increased as a function of the number of double bonds, with the unsaturated congeners requiring greater than 10 washes for removal of the ligand from the membranes. The results of this study are consistent with the apparently important role of polyunsaturated fatty acids in the binding of ligands to opioid receptors.

Typically, common opioid ligands such as morphine or naloxone readily dissociate from opioid receptors and are nearly completely removed from brain membranes after

a single wash. A notable exception to this behavior was originally noted for opiate hydrazones and azines (e.g., naloxonazine), which required at least two washings for