0.05 mmHg, 10 h): R_f by TLC (EtOAc/Me_2CO/MeOH/H_2O 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 11100), 242 nm (ε 10800), ~265 nm (br sh); IR (KBr) 3450–3000 (br), 2920 (sh), 2850 (sh), 1750 (br), 1610 cm^{-1}; ^1H NMR (D_2O) 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. ($C_{10}H_{11}N_3O_7 \cdot 0.5H_2O$) 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 IC_{50} 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 S_3 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 xenographted 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[1e,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

† Tokyo Research Laboratories.
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(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.

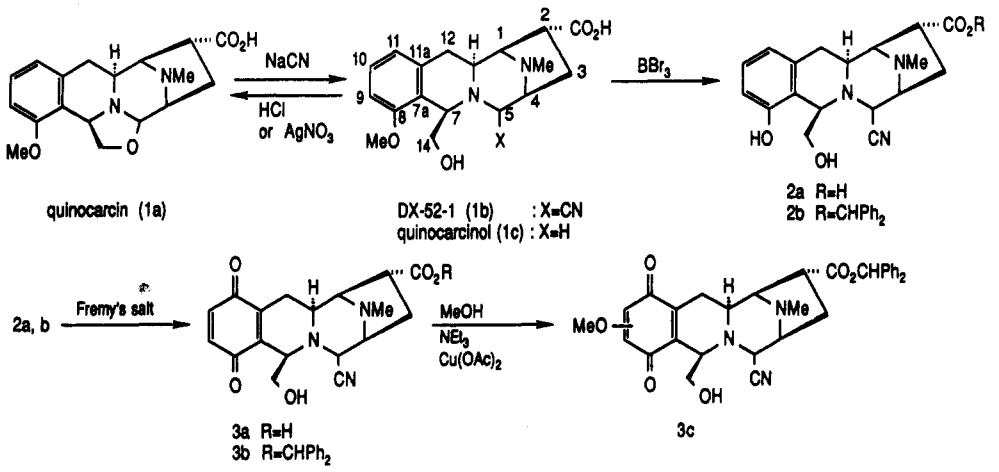
(2) (a) Saito, H.; Kobayashi, S.; Uosaki, Y.; Sato, A.; Fujimoto, K.; Miyoshi, K.; Ashizawa, T.; Morimoto, M.; Hirata, T. *Chem. Pharm. Bull.* **1990**, *38*, 1278. (b) Saito, H.; Sato, A.; Ashizawa, T.; Morimoto, M.; Hirata, T. *Ibid.* **1990**, *38*, 3202. (c) In the infrared spectrum of **1b**, no absorption corresponding to a nitrile was observed; however, in ^{13}C 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.

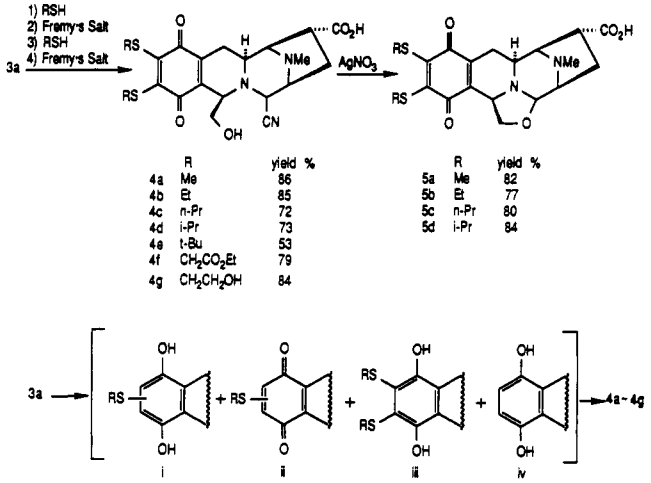
(4) (a) Carter, S. K.; Crooke, S. T. *Mytomycin C. Current Status and New Developments*; Academic Press: New York, 1979. (b) Remers, W. A. *The Chemistry of Antitumor Antibiotics*; John Wiley and Sons: New York, 1979; Vol. I. (c) Zein, N.; Kohn, H. *J. Am. Chem. Soc.* **1987**, *109*, 1576.

0022-2623/91/1834-1959$02.50/0

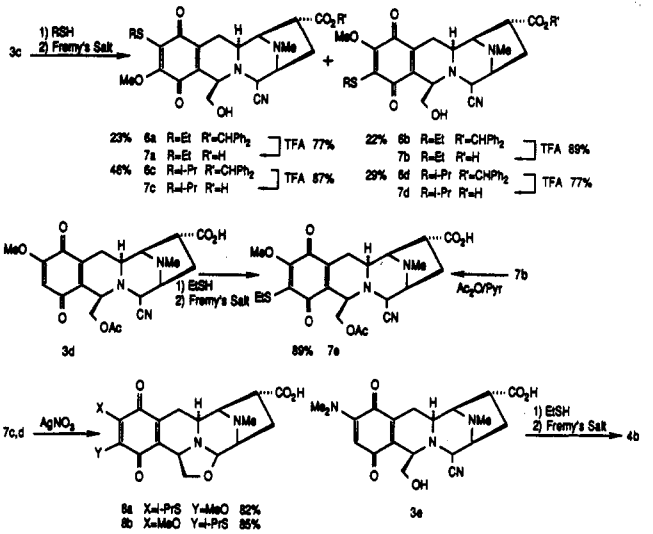
Scheme I



Scheme II



Scheme III



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 encouraging 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.

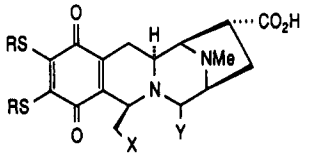
Chemistry

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 salt[10] yielded the desired quinones **3a** and **3b** effectively (Scheme I).

Addition of mercaptan[11] to **3a** initially gave a complex mixture on thin-layer chromatograhy, 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

(5) (a) Arcamone, F. *Doxorubicin Anticancer Antibiotics*; Academic Press: New York, 1981. (b) Frederick, C. A.; Williams, L. D.; Ughetto, G.; Vandermarel, G. A.; Vanboom, J. H.; Rich, A.; Wang, A. H. J. *Biochemistry* **1990**, *29*, 2538.
(6) (a) Zee-Cheng, R. K.; Cheng, C. C. *J. Med. Chem.* **1978**, *21*, 291. (b) Murdock, K. C.; Child, R. G.; Fabio, P. F.; Angier, R. B.; Wallace, R. E.; Durr, F. E.; Citarella, R. V. *Ibid.* **1979**, *22*, 1024. (c) Taylor, S. A.; Fleming, T.; Vonhoff, D. D.; Mccracken, J. D.; Bukowski, R. M.; Talley, R. W.; Natale, R. B.; Guy, J. T.; Samlowski, W. E.; Constanzi, J. H. *Invest. New Drugs* **1990**, *8*, 77. (d) Wheeler, H.; Woods, R. L.; Page, J.; Levi, J. *Ibid.* **1990**, *8*, 109.
(7) (a) McBride, J. J.; Oleson, T. J.; Wolff, D. *Cancer Res.* **1966**, *26*, 727. (b) White, H. L.; White, J. R. *Mol. Pharmacol.* **1968**, *4*, 549. (c) Shaikh, I. A.; Johnson, F.; Grollman, A. P. *J. Med. Chem.* **1986**, *29*, 1329.
(8) (a) Arai, T.; Takahashi, K.; Kubo, A. *J. Antibiot.* **1977**, *30*, 1015. (b) Arai, T.; Takahashi, K.; Ishiguro, K.; Yazawa, K. *Ibid.* **1980**, *33*, 951. (c) Ishiguro, K.; Takahashi, K.; Yazawa, K.; Sakiyama, S.; Arai, T. *J. Biol. Chem.* **1981**, *256*, 2162. (d) Lown, J. W.; Joshua, A. V.; Lee, J. S. *Biochemistry* **1982**, *21*, 419.
(9) (a) Kluepfel, D.; Baker, H. A.; Piattoni, G.; Sehgal, S. N.; Sidorowicz, A.; Singh, K.; Vezina, C. *J. Antibiot.* **1975**, *28*, 497. (b) Sygusch, J.; Brisse, F.; Hanessian, S. *Tetrahedron Lett.* **1974**, 4021. (c) Zmijewski, M. J., Jr.; Miller-Hatch, K.; Mikolajczak, M. *Chem.-Biol. Interact.* **1985**, *52*, 361.
(10) Zimmer, H.; Lankin, D. C.; Horgan, S. W. *Chem. Rev.* **1971**, *71*, 229.
(11) Patai, S. *The Chemistry of the Quinonoid Compounds*; John Wiley and Sons: London, New York, 1974; Part 2, p 877.

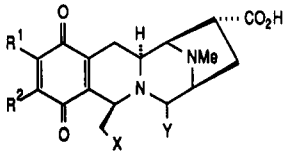
Table I. Bis(alkylthio)quinones



no.	R	X	Y	HeLa S_3 IC_{50},[a] μg/mL	P 388 ip–ip[b] optimal dose × 1	% ILS[c]	R[d]	optimal dose × 5	% ILS	R	formula[e]	mp, °C
4a	Me	OH	CN	0.13	12.5	53	1.23	6.25	71	0.59	$C_{20}H_{23}N_3O_5S_2$	150–151
4b	Et	OH	CN	0.11	12.5	50	1.16	6.25	51	0.42	$C_{22}H_{27}N_3O_5S_2$	168–169
4c	*n*-Pr	OH	CN	0.05	25	56	1.17	6.25	42	0.48	$C_{24}H_{31}N_3O_5S_2$	110–111
4d	*i*-Pr	OH	CN	0.012	25	65	1.48	12.5	119	1.35	$C_{24}H_{31}N_3O_5S_2$	166–167
4e	*t*-Bu	OH	CN	0.004	25	48	1.26	3.13	46	0.50	$C_{26}H_{35}N_3O_5S_2$	187–188
4f	EtO_2CCH_2	OH	CN	9.96				50	30		$C_{26}H_{31}N_3O_9S_2 \cdot 0.5H_2O$	88–89
4g	$HOCH_2CH_2$	OH	CN	2.47	12.5	26	0.62	6.25	20		$C_{22}H_{27}N_3O_7S_2 \cdot H_2O$	137–140 dec
5a	Me	–O–		0.019	6.25	48	1.85	3.13	91	0.88	$C_{19}H_{22}N_2O_5S_2 \cdot 1.5H_2O$	167–172 dec
5b	Et	–O–		0.08	6.25	64	1.33	3.13	82	0.93	$C_{21}H_{26}N_2O_5S_2 \cdot 1.5H_2O$	160–163 dec
5c	*n*-Pr	–O–		0.03	12.5	58	1.21	6.25	86	0.98	$C_{23}H_{30}N_2O_5S_2 \cdot 1.2H_2O$	140–145 dec
5d	*i*-Pr	–O–		0.0019	6.25	69	1.57	3.13	98	1.11	$C_{23}H_{30}N_2O_5S_2 \cdot 0.5H_2O$	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 S_3 cells by 50%. [b] $CD2F_1$ mice (5 mice/group) were implanted intraperitoneally (ip) with 10^6 cells, and drug was dosed (mg/kg) ip on day 1 and days 1–5. [c] Percent increase in life span, calculated $(T/C - 1) \times 100$, 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 optimal dose mg/kg, × 1	% ILS	R	optimal dose, mg/kg, × 5	% ILS	R	formula	mp, °C
7a	EtS	MeO	OH	CN	2.42	6.25	29	0.67	6.25	51	0.65	$C_{21}H_{25}N_3O_6S \cdot H_2O$	131–132
7b	MeO	EtS	OH	CN	2.88	25	31	0.72	6.25	59	0.76	$C_{21}H_{25}N_3O_6S \cdot 0.2H_2O$	160–165 dec
7c	*i*-PrS	MeO	OH	CN	1.12	12.5	21	0.58	12.5	51	0.68	$C_{22}H_{27}N_3O_6S \cdot 0.5H_2O$	139–140
7d	MeO	*i*-PrS	OH	CN	0.56	12.5	17		12.5	75	1.00	$C_{22}H_{27}N_3O_6S \cdot 0.5H_2O$	136–137
8a	*i*-PrS	MeO	–O–		0.79	6.25	31	1.29	6.25	81	1.00	$C_{21}H_{26}N_2O_6S \cdot 2H_2O$	130–135 dec
8b	MeO	*i*-PrS	–O–		2.37	6.25	30	1.25	1.56	51	0.63	$C_{21}H_{26}N_2O_6S \cdot 1.5H_2O$	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–g**[12] 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.

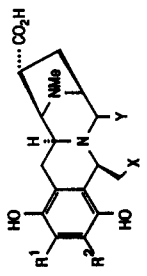
In contrast to methoxyquinone, the addition of ethyl mercaptan to (dimethylamino)quinone **3e**,[2b] which was prepared from **3a**, by the addition of dimethylamine, resulted in replacement of the substituent to give bis(ethylthio)quinone **4b** (Scheme III).

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]

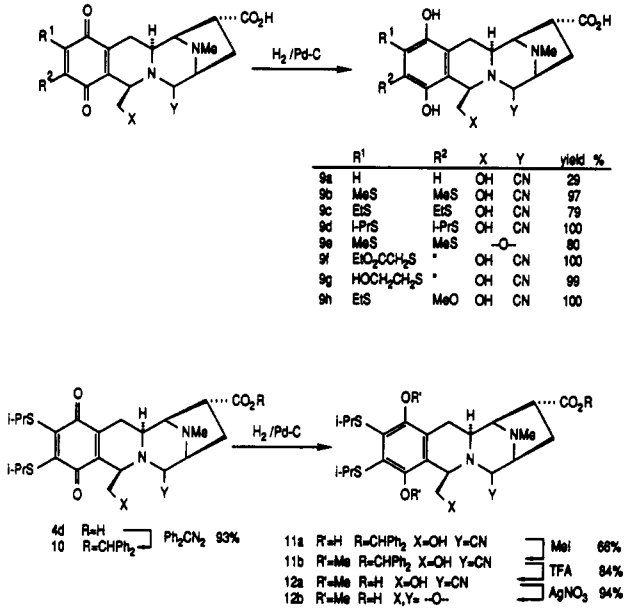
(13) Ishihara, Y.; Shirahata, K.; Sano, H. *J. Antibiot.* **1989**, *42*, 49.

Table III. Hydroquinones



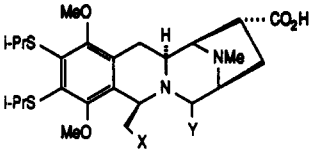
no.	R^1	R^2	X	Y	HeLa S_3 IC_{50}, μg/mL	P 388 ip–ip optimal dose, mg/kg, × 1	% ILS	R	optimal dose mg/kg, × 5	% ILS	R	formula	mp, °C
3a	H	H	OH	CN	6.10	12.5	23		6.25	23		$C_{19}H_{21}N_3O_3 \cdot H_2O$	135–140 dec
3b	MeS	MeS	OH	CN	0.09	6.25	47	1.09	3.13	92	0.86	$C_{20}H_{23}N_3O_3S_2 \cdot H_2O$	170–175 dec
3c	EtS	EtS	OH	CN	<0.03							$C_{22}H_{29}N_3O_3S_2 \cdot 0.5H_2O$	140–141
3d	*i*-PrS	*i*-PrS	OH	CN	<0.03	12.5	51	1.00	6.25	102	1.09	$C_{24}H_{33}N_3O_3S_2 \cdot 0.5H_2O$	136–137
3e	MeS	MeS	–O–		0.13	12.5	65	1.51	6.25	59	0.57	$C_{19}H_{24}N_2O_3S_2 \cdot H_2O$	165–170 dec
3f	EtO_2CCH_2S	EtO_2CCH_2S	OH	CN	>10	200	37	0.90				$C_{26}H_{33}N_3O_7S_2 \cdot 0.5H_2O$	103–104
3g	$HOCH_2CH_2S$	$HOCH_2CH_2S$	OH	CN	3.24	6.25	18	0.44	6.25	41	0.39	$C_{22}H_{29}N_3O_5S_2 \cdot 0.7H_2O$	149–150
9h	EtS	MeO	OH	CN	1.18	12.5	28	0.65	6.25	58	0.79	$C_{21}H_{27}N_3O_6S \cdot 2.2H_2O$	144–147 dec
1a	quinocarcin				0.05–0.11	20	41–51	1	5–10	73–104	1		

Scheme IV



	R^1	R^2	X	Y	yield %
9a	H	H	OH	CN	29
9b	MeS	MeS	OH	CN	97
9c	EtS	EtS	OH	CN	79
9d	i-PrS	i-PrS	OH	CN	100
9e	MeS	MeS	–O–		80
9f	EtO_2CCH_2S	"	OH	CN	100
9g	$HOCH_2CH_2S$	"	OH	CN	99
9h	EtS	MeO	OH	CN	100

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 S_3 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**, IC_{50} = 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**, IC_{50} = 9.96 μg/mL; **4g**, IC_{50} = 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** (IC_{50} = 0.019 μg/mL)

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

	S-180 sc-iv[a]		MX-1 sc-iv		LC-06 sc-iv		St-4 sc-iv		Co-3 sc-iv	
no.	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 μg/mL), showed higher cytotoxicities than corresponding 4-cyano forms **4a** (IC_{50} = 0.13 μg/mL) and **4d** (IC_{50} = 0.012 μg/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 μg/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 μg/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 μg/mL) and **12b** (IC_{50} = 0.05 μg/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 ^{13}C 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 (SiO_2, 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 F_{254} 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 CH_3CN (10 mL) and H_2O (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 H_2O (5 mL) were added portionwise in a 2-h period. Excess thiol and CH_3CN 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 (SiO_2, $CHCl_3$–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, H_2O–MeOH 1:0–1:1) to yield **4g**. **4b**: 85% yield; red solid; mp 168–169 °C; ^1H NMR ($CDCl_3$–CD_3OD) 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, SCH_2Me × 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, NCH_3), 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, SCH_2CH_3), 1.296 (3 H, t, J = 7.4 Hz, SCH_2CH_3); ^{13}C NMR ($CDCl_3$–CD_3OD) 179.2 (8-C), 179.1 (11-C), 177.5 (CO_2H), 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 (SCH_2), 29.15 (SCH_2), 28.6 (3-C), 25.5 (12-C), 15.3 (SCH_2CH_3) 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. ($C_{22}H_{27}N_3O_5S_2$) C, H, N. **4c**: 72% yield; mp 110–111 °C. Anal. ($C_{24}H_{31}N_3O_5S_2$) C, H, N. **4d**: 73% yield; mp 166–167 °C. Anal. ($C_{24}H_{31}N_3O_5S_2$) C, H, N. **4e**: 53% yield; mp 187–188 °C. Anal. ($C_{26}H_{35}N_3O_5S_2$) C, H, N. **4f**: 79% yield; mp 88–89 °C. Anal. ($C_{26}H_{31}N_3O_9S_2 \cdot 0.5H_2O$) C, H, N. **4g**: 84% yield; mp 137–140 °C dec. Anal. ($C_{22}H_{27}N_3O_7S_2 \cdot H_2O$) 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 CH_3SNa in H_2O (4.5 mL) was added to a solution of **3a** (3.0 g, 8.4 mmol) in CH_3CN (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 H_2O (140 mL) were added. After 40 min acetate buffer (20 mL) and 15% solution of CH_3SNa (3.0 mL) were added and stirring was continued for 50 min. Fremy's salt (4.3 g) and H_2O (50 mL) were added, then the mixture was stirred for a further 1 h. Excess CH_3SH and CH_3CN 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 (SiO_2, 450 mL, $CHCl_3$–MeOH 1:0–20:1) to give **4a** (3.22 g, 85.5%) as a red solid: mp 150–151 °C; ^1H NMR ($CDCl_3$–CD_3OD) 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, SCH_3), 2.61 (3 H, s, SCH_3), 2.36 (3 H, s, NCH_3), 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; ^{13}C NMR ($CDCl_3$–CD_3OD) 178.9 (8-C), 178.8 (11-C), 177.3 (CO_2H), 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 (NCH_3), 28.7 (3-C), 25.4 (12-C), 18.2 (SCH_3), 18.1 (SCH_3) 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. ($C_{20}H_{23}N_3O_5S_2$) C, H, N.

Conversion of 5-Cyanoquinones 4a–d to Oxazolidines 5a–d. A 5-cyanoquinone (**4a–d** 1 mmol) was dissolved in CH_3CN (6 mL) and MeOH (3 mL), and $AgNO_3$ (2–5 mmol) was added. The mixture was stirred for 0.6–2 h. Then H_2O (10 mL) was added and the mixture was filtered. The filtrate was concentrated and the residue was chromatographed (HP-20, H_2O–MeOH 1:0–3:7) to give **5a–d**. **5a**: 82% yield; mp 167–172 °C dec, red solid; ^1H NMR (CD_3OD) 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, NCH_3), 2.62 (1 H, m, 3-H), 2.57 (3 H, s, SCH_3), 2.55 (3 H, s, SCH_3), 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; ^{13}C NMR (CD_3OD) 180.7 (8-C), 180.2 (11-C), 179.0 (CO_2H), 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 (NCH_3), 28.5 (3-C), 26.2 (12-C), 18.5 (SCH_3 × 2); IR (KBr) 3400, 2926, 1650, 1587, 1493, 1422, 1377, 1263, 1170 cm^{-1}; SIMS m/z 425 (M + 3)$^+$. Anal. ($C_{19}H_{22}N_2O_5S_2 \cdot 1.5H_2O$) C, H, N. **5b**: 77% yield; mp 160–163 °C dec. Anal. ($C_{21}H_{26}N_2O_5S_2 \cdot 1.5H_2O$) C, H, N. **5c**: 80% yield; mp 140–145 °C dec. Anal. ($C_{23}H_{30}N_2O_5S_2 \cdot 1.2H_2O$) C, H, N. **5d**: 84% yield; mp 152–157 °C dec. Anal. ($C_{23}H_{30}N_2O_5S_2 \cdot 0.5H_2O$) 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 CH_3CN (10 mL), H_2O (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 H_2O (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 (SiO_2, 100mL, *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 ($CDCl_3$) 7.28–7.39 (10 H, m, Ph_2), 6.90 (1 H, s, $CHPh_2$), 4.06 (3 H, s, OCH_3), 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, SCH_2CH_3), 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, NCH_3), 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, SCH_2CH_3) 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. ($C_{34}H_{35}N_3O_6S$) C, H, N. **6b**: mp 94–95 °C; ^1H NMR ($CDCl_3$) 7.28–7.40 (10 H, m, Ph_2), 6.91 (1 H, s, $CHPh_2$), 4.08 (3 H, s, OCH_3), 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, SCH_2CH_3), 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, NCH_3), 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, SCH_2CH_3) 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. ($C_{34}H_{35}N_3O_6S$) 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. ($C_{35}H_{37}N_3O_6S \cdot 0.2H_2O$) C, H, N. **6d**: mp 93–94 °C; ^1H NMR ($CDCl_3$) 7.28–7.40 (10 H, m, Ph_2), 6.91 (1 H, s, $CHPh_2$), 4.10 (3 H, s, OCH_3), 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, $SCHMe_2$), 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, NCH_3), 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, $SCH(CH_3)_2$), 1.27 (3 H, d, J = 6.7 Hz, $SCH(CH_3)_2$) 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. ($C_{35}H_{37}N_3O_6S \cdot 0.2H_2O$) C, H, N.

Ester Cleavage of 6a–d for Preparing 7a–d. A solution of ester (**6a–d**, ca. 300 mg) in CH_2Cl_2 (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, H_2O–MeOH 1:0–3:7) to give **7a–d**. **7a**: red solid; 77% yield; mp 131–132 °C; ^1H NMR (CD_3OD) 4.55 (1 H, d, J = 2.7 Hz, 5-H), 4.03 (3 H, s, OCH_3), 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, SCH_2CH_3), 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, NCH_3), 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, SCH_2CH_3) ppm; ^{13}C NMR (CD_3OD) 184.1 (8-C), 180.4 (11-C), 177.0 (CO_2H), 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 (OCH_3), 58.7 (7-C), 57.3 (5-C), 56.6 (12a-C), 42.2 (2-C), 41.7 (NCH_3), 29.2 (SCH_2), 28.0 (3-C), 25.9 (12-C), 15.6

(SCH_2CH_3) 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. ($C_{21}H_{25}N_3O_6S \cdot H_2O$) C, H, N. **7b**: 89% yield; mp 160–165 °C dec. Anal. ($C_{21}H_{25}N_3O_6S \cdot 0.2H_2O$) C, H, N. **7c**: 87% yield; mp 139–140 °C. Anal. ($C_{22}H_{27}N_3O_6S \cdot 0.5H_2O$) C, H, N. **7d**: 77% yield; mp 136–137 °C. Anal. ($C_{22}H_{27}N_3O_6S \cdot 0.5H_2O$) 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 CH_3CN (3 mL) and H_2O (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 CH_3CN 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 (SiO_2, 20 mL, $CHCl_3$–MeOH 1:0–100:1) to afford **7e** (152 mg, 89.0%) as a red solid: mp 94–95 °C; ^1H NMR (CD_3OD) 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, OCH_3), 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, SCH_2CH_3), 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, NCH_3), 2.04 (1 H, ddd, J = 16.9, 10.3, 2.4 Hz, 12-H), 1.99 (3 H, s, $COCH_3$), 1.94 (1 H, dd, J = 13.4, 9.7 Hz, 3-H), 1.24 (3 H, t, J = 7.4 Hz, SCH_2CH_3) ppm; ^{13}C NMR ($CDCl_3$) 182.6 (11-C), 180.2 (8-C), 179.0 (CO_2H), 170.6 ($COCH_3$), 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 (OCH_3), 56.0 (7-C), 55.9 (5-C), 55.7 (12a-C), 42.5 (2-C), 41.7 (NCH_3), 28.2 (SCH_2), 27.5 (3-C), 24.7 (12-C), 20.9 ($COCH_3$), 15.1 (SCH_2CH_3) 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. ($C_{23}H_{27}N_3O_7S \cdot 1.5H_2O$) 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 (CD_3OD) 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, OCH_3), 3.88 (1 H, heptet, J = 6.7 Hz, $SCH(CH_3)_2$), 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, NCH_3), 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, $SCH(CH_3)_2$), 1.24 (3 H, d, J = 6.7 Hz, $SCH(CH_3)_2$) 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. ($C_{21}H_{28}N_2O_6S \cdot 2H_2O$) C, H, N. **8b**: 85% yield; mp 132–137 °C dec; ^1H NMR (CD_3OD) 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, OCH_3), 3.84 (1 H, heptet, J = 6.7 Hz, $SCH(CH_3)_2$), 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, NCH_3), 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, $SCH(CH_3)_2$), 1.23 (3 H, d, J = 6.6 Hz, $SCH(CH_3)_2$) ppm; IR (KBr) 3420, 2958, 1710, 1649, 1587, 1540, 1450, 1383, 1282, 1231, 1109, 1005 cm^{-1}; SIMS m/z 437 $(M + 3)^+$. Anal. ($C_{21}H_{28}N_2O_6S \cdot 1.5H_2O$) 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 H_2 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, H_2O–MeOH 1:0–1:1). **9a**: pale brown solid; 29% yield; mp 135–140 °C dec; ^1H NMR (CD_3OD) 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, NCH_3), 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. ($C_{18}H_{21}N_3O_5S_2 \cdot H_2O$) C, H, N. **9b**: pale pink solid; 97% yield; mp 170–175 °C dec; ^1H NMR ($CDCl_3$) 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, $SCH_3 \times 2$), 2.347 (3 H, s, NCH_3), 2.04 (1 H, dd, J = 13.2, 9.8 Hz, 3-H) ppm; ^{13}C NMR ($CDCl_3$–CD_3OD) 178.2 (CO_2H), 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 (NCH_3), 28.6 (3-C), 26.7 (12-C), 19.7 (SCH_3), 19.6 (SCH_3) 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. ($C_{20}H_{25}N_3O_5S_2 \cdot H_2O$) C, H, N. **9c**: 79% yield; mp 140–141 °C. Anal. ($C_{22}H_{29}N_3O_5S_2 \cdot 0.5H_2O$) C, H, N. **9d**: 100% yield; mp 136–137 °C. Anal. ($C_{24}H_{33}N_3O_5S_2 \cdot 0.5H_2O$) C, H, N. **9e**: 80% yield; mp 165–170 °C dec; pale pink solid; ^1H NMR (CD_3OD) 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, NCH_3), 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, SCH_3), 2.32 (3 H, s, SCH_3) ppm; ^{13}C NMR (CD_3OD) 179.3 (CO_2H), 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 (NCH_3), 28.2 (3-C), 27.0 (12-C), 20.0 (SCH_3), 19.8 (SCH_3) ppm; IR (KBr) 3380, 2924, 1718, 1589, 1410, 1255, 1206, 1077, 1010, 972, 946, 857 cm^{-1}; SIMS m/z 425 $(M + 1)^+$. Anal. ($C_{19}H_{24}N_2O_5S_2 \cdot H_2O$) C, H, N. **9f**: 100% yield; mp 103–104 °C. Anal. ($C_{26}H_{33}N_3O_5S_2 \cdot 0.5H_2O$) C, H, N. **9g**: 99% yield; mp 149–150 °C. Anal. ($C_{22}H_{29}N_3O_7S_2 \cdot 0.7H_2O$) C, H, N. **9h**: pale orange solid; 100% yield; mp 144–147 °C dec; ^1H NMR (CD_3OD) 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, OCH_3), 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, SCH_2CH_3), 2.65 (1 H, dt, J = 13.4, 6.3 Hz, 3-H), 2.46 (3 H, s, NCH_3), 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, SCH_2CH_3) ppm; ^{13}C NMR (CD_3OD) 178.4 (CO_2H), 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 (OCH_3), 59.5 (7-C), 59.4 (5-C), 58.8 (12a-C), 43.2 (2-C), 42.0 (NCH_3), 30.3 (SCH_2), 29.3 (3-C), 27.3 (12-C), 15.2 (SCH_2CH_3) 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. ($C_{21}H_{27}N_3O_6S \cdot 2.2H_2O$) 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 $CHCl_3$ (15 mL) was added Ph_2CN_2 (230 mg, 1.19 mmol) in $CHCl_3$ (1 mL). After stirring for 50 min, Ph_2CN_2 (100 mg) in $CHCl_3$ (0.6 mL) was added and stirring was continued for 45 min. AcOH was added to decompose the excess Ph_2CN_2 and the mixture was concentrated. The residue was partitioned between AcOEt and H_2O. The organic layer was separated and washed with 5% aqueous $NaHCO_3$ and brine, dried, and concentrated. The residue was chromatographed (SiO_2, 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 ($CDCl_3$) 7.28–7.40 (10 H, m, Ph_2), 6.91 (1 H, s, $CHPh_2$), 4.05–4.15 (2 H, m, $SCHMe_2 \times 2$), 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, NCH_3), 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, $SCH(CH_3)_2 \times 2$) 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. ($C_{37}H_{41}N_3O_5S_2$) 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 H_2. The catalyst was filtered off and washed with AcOEt and $CHCl_3$. 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; ^{1}H NMR ($CDCl_3$) 7.28–7.38 (10 H, m, Ph_2), 6.99 (1 H, s, O*H*), 6.91 (1 H, s, C*H*Ph_2), 6.83 (1 H, s, O*H*), 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, SC*H*Me_2 × 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, NCH_3), 2.17 (1 H, m, 3-H), 1.22–1.29 (12 H, m, SCH(CH_3)$_2$ × 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. ($C_{37}H_{43}N_3O_5S_2 \cdot 2H_2O$) 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 K_2CO_3 (148 mg, 1.07 mmol). MeI (0.26 mL, 4.2 mmol) and $NaBH_4$ (11 mg) were added portionwise followed by 25 h of stirring. The reaction mixture was concentrated and partitioned between AcOEt and H_2O. The organic layer was separated and washed with brine, dried, and concentrated. The residue was subjected to chromatography (SiO_2, 70 mL, *n*-hexane–AcOEt 4:1–3:1) to give 11b (414 mg, 66.2%): ^{1}H NMR ($CDCl_3$) 7.30 (10 H, m, Ph_2), 6.87 (1 H, s, C*H*Ph_2), 4.00–4.24 (2 H, m, 5- and 7-H), 3.84 (3 H, s, OCH_3), 3.78 (3 H, s, OCH_3), 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, NCH_3), 1.98 (1 H, m, 3-H), 1.12–1.36 (12 H, m, SCH(CH_3)$_2$ × 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%): ^{1}H NMR (CD_3OD) 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, OCH_3), 3.76 (3 H, s, OCH_3), 3.61–3.71 (2 H, m, 14-H_2), 3.34–3.41 (3 H, m, 1-H and SC*H*Me_2 × 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, NCH_3), 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(CH_3)$_2$ × 2) ppm; SIMS m/z 509 (M + 1)$^+$, 495, 467.

Biological Studies. HeLa S_3 cells (5 × 10^4) 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% CO_2. 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 IC_{50} value (drug concentration required for 50% inhibition of the cell growth) was determined.

Lymphocytic leukemia P388 (1 × 10^6) cells were implanted intraperitoneally (ip) into $CD2F_1$ 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 × 10^6 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

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

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 mm^3 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 mm^3, 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 (^{1}H and ^{13}C 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

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