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Cyanine Dyes, New Potent Antitumor Agents

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Antitumor agents of a new structural type are reported. A number of cyanines with mono-, di- and tricyclic nuclei, merocyanines and oxonols have been *i.p.* screened for antitumor activity against P388 leukemia and B16 melanoma. Among these compounds, monomethin-, trimethin- and pentamethincyanines having naphthothiazole, naphthoxazole and benzindole nuclei resulted in a significant prolongation of the survival time of tumor-bearing mice. Replacement of the conjugated chain system between the terminal two nuclei with a saturated aliphatic chain produced a marked decrease in the antitumor activity. Structure–activity relationships are discussed.

Keywords——cyanine dyes; antitumor activity; structure–activity relationship; benzthiacyanines; benzoxacyanines; benzindocyanines; anthrathiazolocyanine; quaternization reaction; P388 leukemia; B16 melanoma

Cyanines, used mainly as photographic sensitizers, include thousands of compounds with structures intimately related to each other. Studies on their possible use in areas other than photography have been pursued for a long time. One approach has been to determine their possible use in chemotherapy. Previous work on this approach has dealt almost exclusively with their use as antiseptic,[1] antitripanosomal,[1d-h] antimalarial,[2] antifilarial[3] and antiparasitic agents.[3a,4] Little attention has yet been paid to the application of cyanines in cancer chemotherapy.

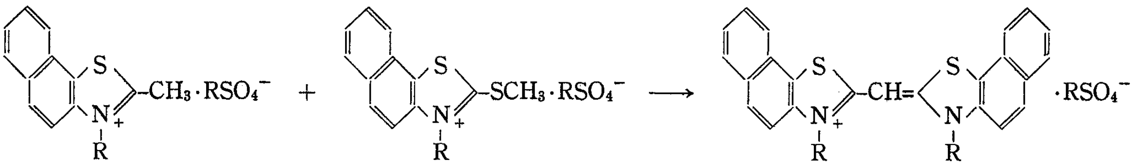
It has been reported that several dyes such as hematoporphyrin,[5] fluorescein,[6] tetracycline,[7] acridine orange[8] and berberine[9] accumulate significantly in tumor tissues in preference to surrounding normal tissue. Selective retention in tumor tissues could provide a drug with the important advantage of selective tumor destruction. With these considerations in mind, we attempted to screen a number of cyanines and related dyes for antitumor activity in two *in vivo* systems and found that certain cyanines showed a marked prolongation effect on the survival time of mice bearing experimental tumors.

Chemistry

The structural characteristic of cyanines is the amidinium ion system, in which a positively charged quaternary nitrogen is linked to a tertiary nitrogen by a conjugated chain of odd carbon number. The cyanine molecules generally appear to be planar with the conjugated chain in the extended form.[10] The methods used in cyanine syntheses have been extensively described.[11] A general approach consists of the construction of the intermediate heteroaromatic nuclei and their subsequent connection with a conjugated methine chain of variable length.

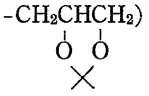
Our concern is with the cyanines having tricyclic (**3a**—**i**, **5a**—**g**) and tetracyclic heteroaromatic nuclei (**30**—**35**) from the viewpoint of structure–activity relationships (SAR). The benzthiacyanines (**3a**, **c**—**i**) which have a tricyclic monomethine structure were synthesized by condensing a 2-methyl-3-alkylnaphthothiazolium salt (**1a**, **c**—**i**) with the corresponding 2-methylthio analogs (**2a**, **c**—**i**) (Chart 1). The monomethincyanines with tetracyclic nuclei, *e.g.* monomethinanthrathiazolocyanine (**30**) and monomethinanthraquinonothiazolocyanine

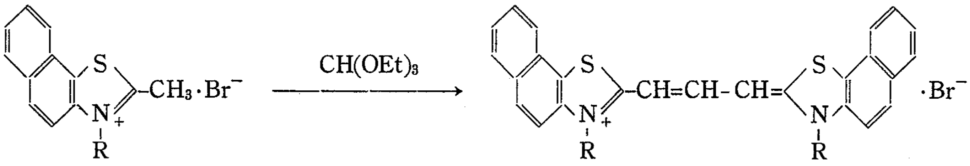
(**31**), were prepared individually from β-aminoanthracene and β-aminoanthraquinone by several steps; cyclization by the known methods[12,13] to the corresponding 2-methylthiothiazoles (**22**, **26**) and 2-methylthiazoles,[14,15] followed by quaternization and subsequent condensation of the 2-methylthiothiazolium salts (**23**, **27**) with the corresponding 2-methyl congeners (**24**,[14] **28a**[15]), which are shown in Chart 2. The trimethincyanines having tricyclic (**5c**—**g**) and tetracyclic nuclei (**32**, **34**) were prepared by treating the appropriate 2-methylthiazolium compounds (**1j**—**n**, **28a**,[15] **29**) with ethyl orthoformate. Similarly, the heptamethincyanines (**33**, **35**) with tetracyclic nuclei were synthesized by treating the corresponding 2-methylthiazolium compounds (**28b**,[15] **29**) with 1-anilino-5-anilo-1,3-pentadiene.[16]


1a,c—i **2a,c—i** **3a,c—i**

R_2SO_4 **4a,c—i**

1—4: **a**, $R=CH_3$; **c**, $R=C_3H_7$; **d**, $R=C_4H_9$; **e**, $R=C_7H_{15}$; **f**, $R=C_{12}H_{25}$; **g**, $R=C_{18}H_{37}$; **h**, $R=C_{22}H_{45}$; **i**, $R=-CH_2\underset{OH}{CH}CH_2OH$ (**1**, **2**, **4**: **i**, R=


$-CH_2CHCH_2)$


1j—n **5c—g**

1, **5**: **j**, **c**, $R=-CH_2CH_2OH$; **k**, **d**, $R=-CH_2CH(OH)CH_2OH$; **l**, **e**, $R=-CH_2COCH_3$; **m**, **f**, $R=-CH_2COPh$; **n**, **g**, $R=-CH_2Ph$

Chart 1

In order to enhance the water solubility of cyanines, compounds (**3i**, **5c**, **5d**) with hydrophilic substituents on the R group attached to the naphthothiazole nitrogen were synthesized. Similarly, by introducing a long chain alkyl group into the nucleic nitrogen, cyanines (**3c**—**h**) with increased lipophilicity were synthesized (Table VIII).

The intermediary naphtho[2,1-*d*]thiazolium salts (**1a**, **c**—**i**, **2a**, **c**—**i**) were prepared by treating the corresponding thiazoles with alkylating agents. In the quaternization of 2-methylthionaphtho[2,1-*d*]thiazole, the choice of alkylating agents is most important. The use of ethyl halide and its congeners gave a complex mixture owing to the occurrence of undesirable interchange of alkyl groups at positions 2 and 3 and to the decomposition of the product.[17] With dialkyl sulfates (**4a**, **c**—**i**), the reaction progressed smoothly and gave the desired quaternary salts (**2a**, **c**—**i**) in good yields. To the best of our knowledge, the majority of these intermediates (**1a**, **c**—**i**, **2a**, **c**—**i**) and the corresponding cyanines (**3a**, **c**—**i**) prepared from them, except the *N*-methyl and *N*-ethyl congeners, have not previously been reported. Their physical properties are listed in Tables VI, VII and VIII in the experimental section.

In order to examine the structural requirement for antitumor activity of cyanines, a chemical modification was attempted which involved replacing the conjugated chain system connecting the two nuclei with a saturated group. Thus, 1,3-bis(naphtho[2,1-*d*]thiazolyl)-

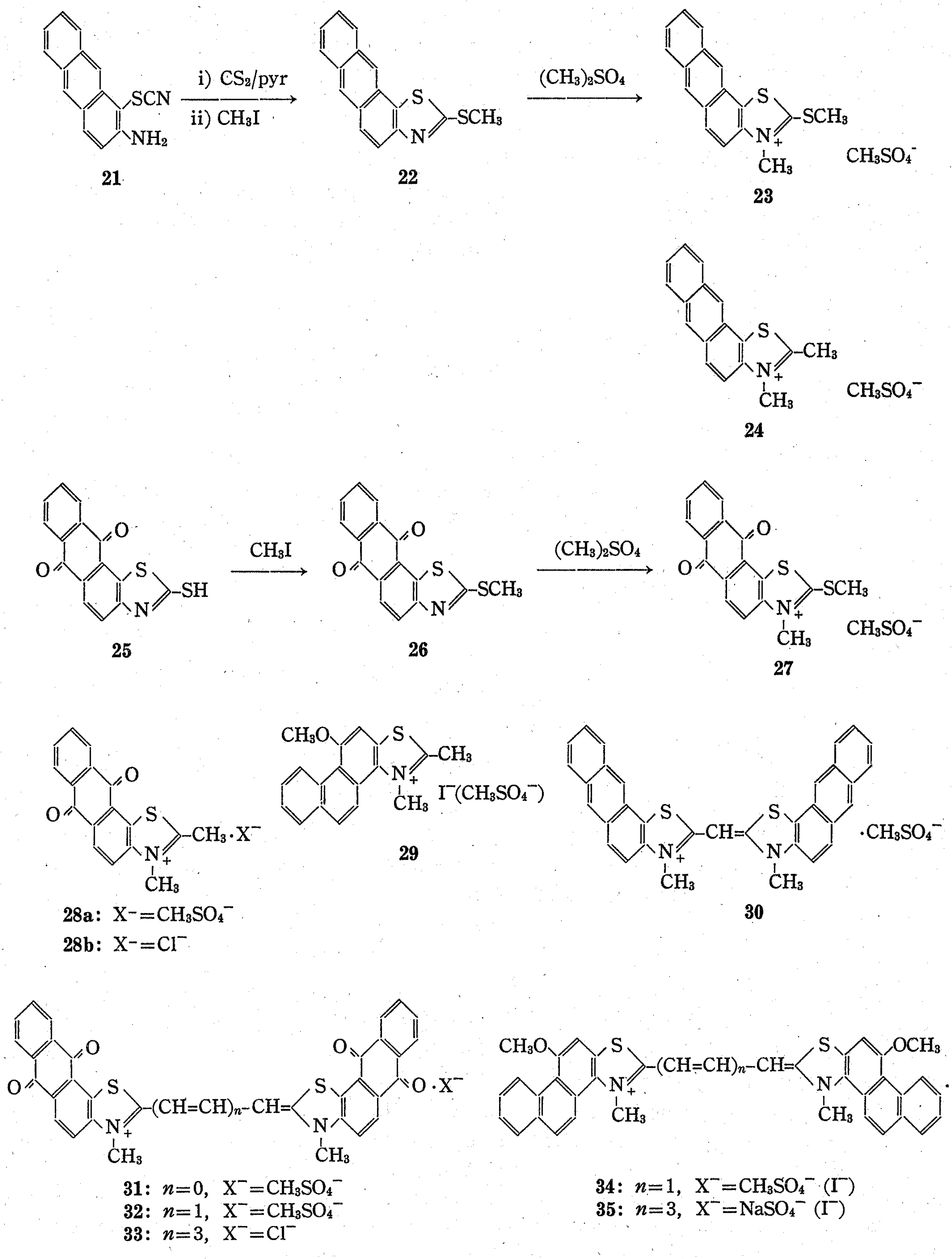


Chart 2

propane (**38**) and the corresponding bis(quaternary)salt (**39**) were prepared as shown in Chart 3. The intermediate *N*,*N'*-bis(*β*-naphthyl)glutaramide (**36**), obtained from *β*-naphthylamine, was converted to the thioamide (**37**) which, on treatment with potassium ferricyanide, cyclized to the *α*-position of the naphthalene ring[18] yielding naphtho[2,1-*d*]thiazole **38**. The desired bis(quaternary)salt (**39**) was synthesized in high yield by treating **38** with dimethyl sulfate.

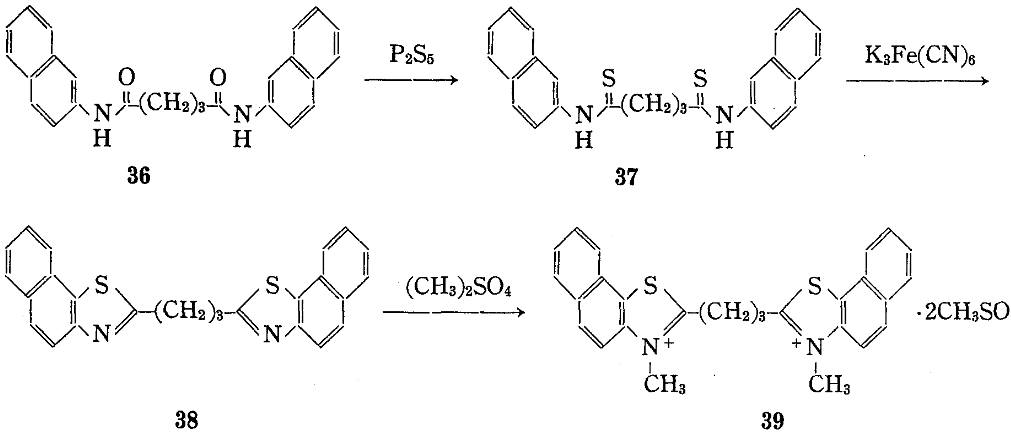


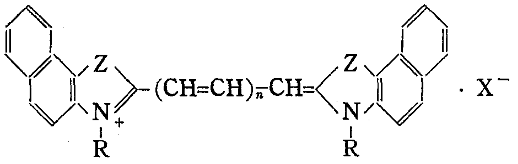
Chart 3

Structure and Activity Relationships

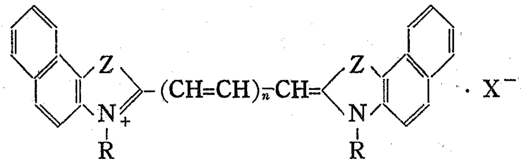
In vivo antitumor activities of cyanine dyes are shown in Tables I—IV. The first point of interest concerns the influence of structural change of the cyanines on their antitumor activity. A number of cyanines with a variety of chemical structures were tested on experimental tumors in mice (P388 leukemia and B16 melanoma). Among the compounds studied so far, the great majority of cyanines with monocyclic and bicyclic nuclei, for example thiazole, benzothiazole, quinoline or isoquinoline, were shown to be less active against P388 leukemia. In contrast, many of the cyanines with naphthothiazole nuclei (**3b**, **5a**, **6**, **7a**—**c**, **12a**, **13a,b**, **15**, **16**, **17a,b**, **18**, **19**, **20**) showed much more encouraging results (Tables I—III). The isosteric replacement of S by O or C, that is, the compounds with naphthoxazole (**8**, **9**, **14**) and benzindole nuclei (**11**), retained activity comparable to that of the naphthothiazoles. A relatively wide effective dose range was observed with those cyanines (**3b**, **12a**, **13a,b**, **15**, **19**) which have tricyclic nuclei in the molecule (Tables I—III).

In this class of cyanines, structural isomers exist with respect to the arrangement of the two benzene rings and one five-membered ring in the tricyclic nuclei. As shown in Tables I, II and III, the difference in activity between each pair of isomers, *e.g.* cyanines with naphtho[2,1-*d*]thiazole (**5a**, **7a,b**, **19**) and those with naphtho [1,2-*d*]thiazole (**12a**, **13a,b**, **20**), was modest and therefore the effect of the geometrical change of the cyanine nucleus was not obvious.

Table I. Antitumor Activity against P388 Leukemia[a]

 Compd.	n	R	Z	X$^-$	Dose, (mg/kg)	T/C,[b] (%)	Average body wt. change, $T-C$, (g)	Survival at 5th day
3a	0	CH$_3$	S	CH$_3$SO$_4^-$	50	123	+0.2	6/6
					25	122	−0.5	6/6
					12.5	123	−0.3	6/6
3b	0	C$_2$H$_5$	S	I$^-$	10	182	−4.0	6/6
					5	176	−2.9	6/6
					2.5	157	−1.6	6/6
3c	0	C$_3$H$_7$	S	C$_3$H$_7$SO$_4^-$	10	146	−3.7	6/6
					5	145	−1.7	6/6
					1.25	128	−1.4	6/6

(continued)



Compd.	*n*	R	Z	X$^-$	Dose, (mg/kg)	*T*/*C*,[b] (%)	Average body wt. change, *T*−*C*, (*g*)	Survival at 5th day
3d	0	C_4H_9	S	$C_4H_9SO_4^-$	10	128	−4. 4	6/6
					2. 5	122	−1. 6	6/6
					1. 25	120	−0. 7	6/6
3e	0	C_7H_{15}	S	$C_7H_{15}SO_4^-$	10	141	−2. 0	6/6
					5	129	−1. 2	6/6
					2. 5	127	−1. 1	6/6
3f	0	$C_{12}H_{25}$	S	$C_{12}H_{25}SO_4^-$	200[c]	103	−0. 8	6/6
3h	0	$C_{22}H_{45}$	S	$C_{22}H_{45}SO_4^-$	200	95	−1. 8	6/6
3i	0	CH_2- CHOH CH_2OH	S	$CH_2SO_4^-$ CHOH CH_2OH	200	111	−3. 7	5/6
5a	1	C_2H_5	S	I^-	1[d]	142	−1. 2	3/3
					0. 5	135	−1. 3	3/3
5b	1	C_8H_{17}	S	ClO_4^-	400	103	−4. 7	6/6
5c	1	CH_2CH_2OH	S	Br^-	200	109	−2. 8	6/6
5d	1	CH_2- CHOH CH_2OH	S	Br^-	200	90	−2. 1	5/6
5e	1	CH_2COCH_3	S	Br^-	100	99	−3. 4	4/6
5f	1	CH_2COPh	S	Br^-	200	108	−3. 0	6/6
5g	1	CH_2Ph	S	Br^-	100	135	−3. 7	6/6
					50	129	−3. 5	6/6
					25	142	−1. 9	6/6
6	2	C_2H_5	S	Br^-	6. 25	146	−4. 7	6/6
					3. 12	141	−2. 7	6/6
					1. 56	145	−0. 8	6/6
7a	3	CH_3	S	I^-	50	132	−3. 0	6/6
					25	145	−0. 8	6/6
					12. 5	131	−1. 1	6/6
7b	3	C_2H_5	S	I^-	10[d]	114	−2. 5	3/3
					5	154	−2. 8	3/3
7c	3	C_2H_5	S	$C_2H_5SO_4^-$	50	137	−4. 3	6/6
					25	140	−3. 5	6/6
					12. 5	141	−3. 1	6/6
8	2	C_2H_5	O	I^-	6. 25	148	−3. 7	6/6
					3. 12	151	−2. 3	6/6
					1. 56	145	−1. 0	6/6
9	3	C_2H_5	O	Br^-	25	144	−4. 0	6/6
					12. 5	150	−3. 8	6/6
					6. 25	145	−2. 6	6/6
10	2	$(CH_2)_4SO_3^-$ $(CH_2)_4SO_3Na$	CH_3CH_3 C	—	400	105	−1. 5	6/6
11	3	CH_3	CH_3CH_3 C	ClO_4^-	1. 25	151	−1. 0	5/6
					0. 62	149	−1. 1	6/6
					0. 31	132	−1. 0	6/6

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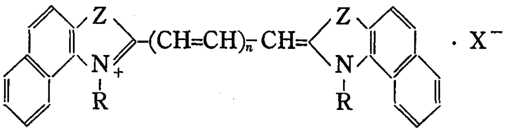
a) Intraperitoneally administered to CDF_1 mice (6 mice/group) once at a given dose on days 1 and 5 after inoculation of the animals with P388 leukemia cells (10^6 cell/mice) unless otherwise mentioned.

b) *T*/*C* represents the ratio of the median survival time of drug-treated to control, untreated tumor-bearing mice, expressed as a percentage.

c) For inactive compounds (*T*/*C* $<$120), data are presented for the protocol using the highest nontoxic dose tested.

d) Intraperitoneally administered to P388-bearing mice (3 mice/group) once daily on days 1—9 after inoculation of the animals with tumor cells (10^6 cell/mice).

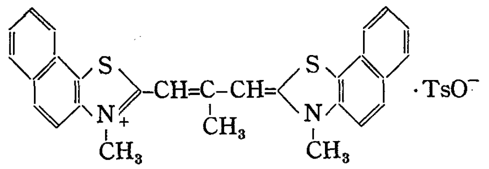
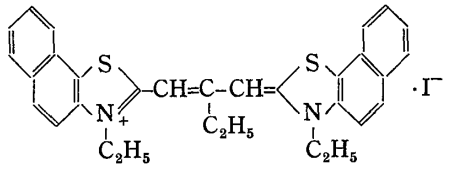
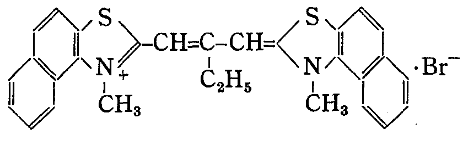
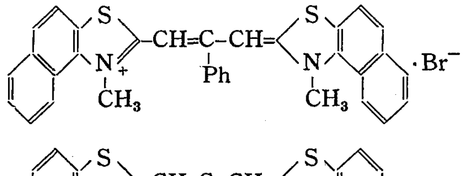
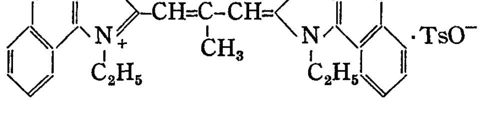
TABLE II. Antitumor Activity against P388 Leukemia[a]



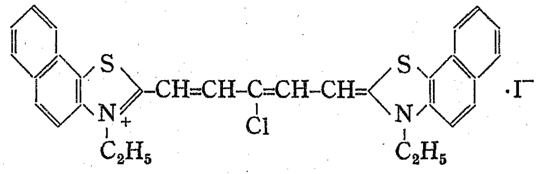
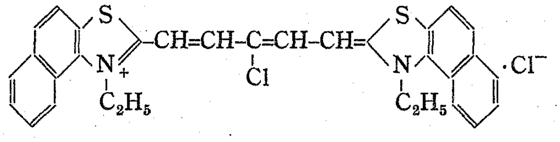
Compd.	n	R	Z	X^-	Dose, (mg/kg)	T/C,[b] (%)	Average body wt. change, $T-C$, (g)	Survival at 5th day
12a	1	C_2H_5	S	I^-	100	166	−3.5	6/6
					50	155	−2.8	6/6
					12.5	138	−1.3	6/6
12b	1	$(CH_2)_3SO_4^-$ C_2H_5	S	—	400[c]	100	−1.3	6/6
13a	3	CH_3	S	I^-	50	141	−2.2	6/6
					12.5	161	−1.0	6/6
					3.12	138	−1.5	6/6
13b	3	C_2H_5	S	I^-	25	157	−3.6	6/6
					6.25	171	−2.6	6/6
					1.56	168	−1.7	6/6
14	1	C_2H_5	O	I^-	12.5	145	−4.7	6/6
					6.25	148	−2.8	6/6
					3.12	130	−1.5	6/6

[a—c] See corresponding footnotes in Table I.

TABLE III. Antitumor Activity against P388 Leukemia[a]

Compd.		Dose, (mg/kg)	T/C,[b] (%)	Average body wt. change, $T-C$, (g)	Survival at 5th day
15		6.25	171	−3.6	6/6
		3.12	166	−2.1	6/6
		1.56	141	−1.3	6/6
16		6.25	156	−1.1	6/6
		3.12	141	−1.1	6/6
		1.56	140	−0.9	6/6
17a		0.78	163	−1.3	6/6
		0.39	148	−1.0	6/6
17b		0.78	160	−3.2	5/6
		0.39	142	−1.9	6/6
18		1.25	141	−3.0	5/6
		0.62	144	−2.4	5/6

(continued)

Compd.		Dose, (mg/kg)	T/C,[b] (%)	Average body wt. change, $T-C$, (g)	Survival at 5th day
19		12.5	178	−1.6	6/6
		3.12	169	−1.3	6/6
		1.56	157	−1.0	6/6
20		25	137	−5.4	5/6
		12.5	159	−3.4	6/6

a, b) See corresponding footnotes in Table I.

In comparing the activities of cyanines with mono-, di- and tricyclic nuclei, we considered, at first, that the antitumor activity might increase with increase in the number of benzene rings in the planar fused-aromatic nuclei of the molecule and postulated that the antitumor cyanines might exert their chemotherapeutic effect as bifunctional deoxyribonucleic acid (DNA) intercalating agents. In order to examine this possibility, we attempted the synthesis of cyanines with tetracyclic nuclei (**30**—**35**), as mentioned above. The biological tests showed that all of these tetracyclic analogs were less effective in the prolongation of the survival time of mice bearing P388 leukemia, though the toxicities were very low (acute toxicity: dose >200 mg/kg, mice, *i.p.*).

In addition, antitumor cyanines (**3b**, **7b**, **12a**) were negative in the Ames[19] and rec-assay test[20] and therefore, are probably not mutagenic. Thus, their interaction with the DNA chain seems to be unlikely.

For given nuclei, the length of the conjugated chain located between the two heterocycles showed a profound influence on the antimelanoma activity and a modest effect on the anti-

Table IV. Antitumor Activity against B16 Melanoma[a]

Compd.	Dose, (mg/kg)	T/C,[b] (%)	Average body wt. change, $T-C$, (g)	Survival at 5th day
3b	2.4	237	−1.9	5/5
	1.2	194	−0.4	5/5
	0.6	174	−0.1	5/5
7b	5[c]	107	−0.6	5/5
12a	10	166	−2.7	5/5
	5	136	−0.9	5/5
13b	2.5	121	−0.5	5/5
	1.25	107	+0.2	5/5
14	4	162	−1.4	5/5
	2	131	−0.6	5/5
	1	126	+0.2	5/5
20	5	143	−0.1	5/5
	2.5	129	−0.2	5/5
	1.25	124	0	5/5
38	100	99	−0.1	5/5
39	100	111	−3.6	5/5

a) Intraperitoneally administered to BDF_1 mice (5 mice/group) once daily on days 1—9 after inoculation of the animals with B16 melanomacells (0.5 ml of 1: 4 tumor homogenates of B16 in 0.9% NaCl solution) as described by Geran *et al.*[26]

b, c) See corresponding footnotes in Table I.

leukemic activity. Tables I, II and IV show that the monomethin- (**3b**) and trimethincyanine (**12a**) containing naphthothiazole nuclei were active against both P388 leukemia and B16 melanoma in mice, while the corresponding heptamethincyanines (**7b**, **13b**) were active against P388 leukemia and less active against B16 melanoma. The trimethinbenzoxacyanine (**14**) and pentamethinbenzthiacyanine (**20**) were moderately active against B16 melanoma in mice.

Tests were carried out on trimethin- (**15**, **16**, **17a**,**b**, **18**) and pentamethincyanines (**19**, **20**) with a substituent, *e.g.* methyl, ethyl, phenyl or halogen, introduced at a conjugated chain carbon (Table III). Comparison of the *T*/*C* values of **5a** and **16**, **12a** and **18**, and **6** and **19** showed that introduction of such a substituent seems to be favorable for antitumor activity, though the effect is not marked.

The relation between antitumor activity and the structure of the substituent on heteroaromatic nitrogen in the molecule was studied. Tricyclic cyanines possessing a methyl or ethyl group attached to the naphthothiazole nitrogen generally showed a potent antitumor activity, and **3b** gave the highest *T*/*C* values in P388 leukemia and B16 melanoma in mice (Table I—IV). However, the problem of poor solvent solubilities, which generally occurs with cyanines, must be resolved. The cyanine molecule comprises two nitrogen-containing heteroaromatic nuclei, each of which has a lipophilic alkyl moiety attached to the nitrogen. One of the nitrogens is positively charged and functions as a hydrophilic center. It was considered that an analog with more desirable physicochemical properties but with the same or better activity might be synthesized by chemical modification of the substituent attached to the naphthothiazole nitrogen. In fact, introduction of a long chain alkyl (C_3H_7, C_4H_9, C_7H_{15}, C_8H_{17}, $C_{12}H_{25}$, $C_{18}H_{37}$, $C_{22}H_{45}$) or benzyl group at the naphthothiazole nitrogen gave analogs with high lipophilicity (**3c**—**h**, **5b**,**g**). However, these products showed a tendency for decreased activity with increasing size of the substituent (Table I). The introduction of a substituent containing a hydrophilic group gave analogs (hydroxyl: **3i**, **5c**,**d**; carbonyl: **5e**,**f**; sulfo: **10**, **12b**) with improved hydrophilicity; for instance, **5c** showed good water solubility. However unfortunately, these products showed a marked decrease in activity. In these compounds, the tolerated doses in mice were much higher than those of the active compounds (Tables I and II). Lack of effectiveness and low toxicity of this class of compounds may be attributable to diminished transport to or affinity for normal and malignant tissues. However, this remains to be clarified.

The results obtained so far by this type of chemical modification have not been favorable. However, the possibility of obtaining a useful compound by placing the lipophilic or hydrophilic substituents elsewhere in the molecule, *e.g.*, on the naphthalene ring, may still remain and a study along this line is in progress.

Another question to be clarified concerns the apparent role of the conjugation system in cyanines. To investigate this problem, trimethylene bis(naphthothiazole) (**38**) and the corresponding bis(naphthothiazolium) salt (**39**) were synthesized. In these compounds the conjugation system of cyanines located between the terminal nuclei was disrupted by replacement with a trimethylene chain. In contrast to the corresponding trimethincyanine (**5a**), these trimethylene compounds (**38**, **39**) did not show any activity against P388 leukemia or B16 melanoma. This suggests that the conjugated chain system is essential for antitumor activity.

The screening test was carried out on other types of dyes, *e.g.*, merocyanines (non ionic dyes) and oxonols (anionic dyes). None of these compounds tested so far shown any appreciable activity against P388 leukemia in mice.

On the basis of the present results, one of the requirements for antitumor activity is the structure peculiar to cyanines, that is, the existence of the conjugation system involving two terminal nitrogens, one being cationic. The additional requirements for activity are the existence of tricyclic nuclei (naphthothiazole, naphthoxazole and benzindole) and of a lower alkyl group attached to the heterocyclic nitrogen.

Tests of acute toxicity in mice were carried out on monomethin- (**3b**), trimethin- (**12a**) and pentamethincyanine (**19**) (Table IX). Among these compounds, pentamethincyanine (**19**) appears to possess a better therapeutic index than monomethin- (**3b**) and trimethincyanine (**12a**).

The mechanism of action of antitumor cyanine dyes is a challenging theme for study. Recent publications[21] have shown that there is a correlation between reduction potentials and the ability to inhibit cell division in fertilized sea urchin eggs, and inhibition of the respiratory chain reactions on the mitochondrial membrane is a possible mode of action for cyanine dyes. However, we could not find any definite correlation between the antitumor activity of cyanine dyes and their reduction potentials (the literature values). The mode of action of the antitumor cyanines remains to be clarified.

The tumors studied were P388 leukemia and B16 melanoma, which grow much faster than most human tumors. For efficient prediction of experimental results in clinical situations, the use of tumor systems with low growth rates is considered to be desirable.[22] With this in mind, studies on the antitumor cyanines should be carried out on slow growing solid tumors, even though these have been shown to be more refractory to antitumor agents. Studies along this line are in progress.

Experimental

Melting points were determined in open capillary tubes using a Yamato MP-1 melting point apparatus, unless otherwise mentioned, and are uncorrected. Infrared (IR) spectra were measured with a Hitachi EPI-S_2 spectrometer, and nuclear magnetic resonance (NMR) spectra with a Varian T-60 spectrometer using tetramethylsilane TMS as an internal reference. Chemical shifts are given as δ values.

Cyanines——Compounds **3b**, **5a**, **5b**, **6**, **7a**—**c**, **8**, **9**, **10**, **11**, **12a**, **12b**, **13a**, **13b**, **14**, **15**, **16**, **17a**, **17b**, **18**, **19** and **20** were purchased from Nippon Kankoh-Shikiso Kenkyusho, Okayama, Japan.

Di-*n*-alkyl Sulfates (4e—i)——These compounds (**4e**—**h**) were synthesized by the reaction of the corresponding dialkyl sulfites with SO_2Cl_2.[23] Bis(2,3-*O*-isopropylidenepropyl) sulfate (**4i**) was synthesized from 1,2-*O*-isopropylideneglycerol.[24] The yields, solvents for crystallization, and physical data are shown in Table V.

TABLE V. Di-*n*-alkyl Sulfates

Compd.	mp (°C)	Crystn. solvent	Yield[a] (%)	Formula	Analysis (%) Calcd (Found) C	H	S
4e	—[b]	—[c]	85	$C_{14}H_{30}O_4S$	57.11 (57.12	10.27 10.48	10.89 10.93)
4f	40—41	*n*-Hexane	65	$C_{24}H_{50}O_4S$	66.31 (66.49	11.59 11.85	7.38 7.47)
4g	65—66	*n*-Hexane	80	$C_{36}H_{74}O_4S$	71.70 (71.53	12.37 12.40	5.32 5.46)
4h	79—80	*n*-Hexane	90	$C_{44}H_{90}O_4S$	73.89 (74.19	12.68 13.04	4.48 4.62)
4i	64—66	*n*-Hexane	20	$C_{12}H_{22}O_8S$	44.16 (44.04	6.79 6.81	9.82 9.83)

a) Based on dialkyl sulfites.
b) Purified by column-chromatography on silica gel with *n*-hexane as the eluent.
c) Colorless liquid.

3-Alkyl-2-methylnaptho[2,1-*d*]thiazolium Alkylsulfates (1c—h)——General Procedure: A mixture of 2-methylnaphtho[2,1-*d*]thiazole (1 mmol) and dialkyl sulfate (1.5—3 mmol) was warmed at 120°C for 10—25 h. The reaction mixture was chromatographed on a column of silica gel with $CHCl_3$–MeOH (97 : 3) as the eluent. The eluate was concentrated and the residue was crystallized from the solvent listed at room temperature to give the desired product (**1c**—**g**) as colorless needles. In the case of **1h**, the product was isolated as colorless needles after $CHCl_3$ washing of the reaction mixture and subsequent crystallization from the solvent listed at room temperature. The yields, solvents for crystallization, and physical data are shown in Table VI.

TABLE VI. 3-Alkyl-2-methylnaphtho[2,1-*d*]thiazolium Alkylsulfates

Compd.	R	Reaction conditions		mp (°C) Crystn. solvent	NMR δ ($CDCl_3$)			Formula	Analysis (%) Calcd (Found)			
		Time (h)	Yield (%)		N^+-CH_2-	-$CH_2SO_4^-$	C-2 CH_3		C	H	N	S
1c	C_3H_7	10	90	146—149 $CHCl_3$-C_6H_6	4.97	3.92	3.41	$C_{15}H_{16}NS\cdot C_3H_7SO_4\cdot 0.6H_2O$	55.11 (54.82	6.22 5.96	3.57 3.96	16.35 16.63)
1d	C_4H_9	10	90	183—184 $CHCl_3$-C_6H_6	4.98	3.93	3.43	$C_{16}H_{18}NS\cdot C_4H_9SO_4$	58.65 (58.51	6.64 6.65	3.42 3.47	15.66 15.62)
1e	C_7H_{15}	13	80	164—166 $CHCl_3$-C_6H_6	5.00	3.86	3.42	$C_{19}H_{24}NS\cdot C_7H_{15}SO_4$	63.25 (63.33	7.96 7.86	2.84 3.03	12.99 12.89)
1f	$C_{12}H_{25}$	25	60	155—158 $CHCl_3$-AcOEt	4.91	3.84	3.38	$C_{24}H_{34}NS\cdot C_{12}H_{25}SO_4$	68.20 (68.05	9.38 9.40	2.21 2.23	10.12 10.05)
1g	$C_{18}H_{37}$	25	60	140—143 $CHCl_3$-AcOEt	4.93	3.86	3.40	$C_{30}H_{46}NS\cdot C_{18}H_{37}SO_4$	71.86 (71.85	10.43 10.71	1.75 1.69	7.99 7.94)
1h	$C_{22}H_{45}$	25	65	142—145 $CHCl_3$-MeOH-AcOEt	5.06	3.95	3.47	$C_{34}H_{54}NS\cdot C_{22}H_{45}SO_4$	73.55 (73.52	10.91 11.21	1.53 1.52	7.01 7.02)

3-Alkyl-2-methylthionaptho[2,1-*d*]thiazolium Alkylsulfates (2c—h)——General Procedure: A mixture of 2-methylthionaphtho[2,1-*d*]thiazole (1 mmol) and dialkyl sulfate (3 mmol) was warmed at 120°C for 5—25 h. Purification of the reaction mixture by column chromatography on silica gel with $CHCl_3$–MeOH (97: 3) as the eluent followed by crystallization at room temperature gave **2c**—**h** as colorless crystals. The yields, solvents for crystallization, and physical data of the products are shown in Table VII.

TABLE VII. 3-Alykl-2-methylthionaphtho[2,1-*d*]thiazolium Alkylsulfates

Compd.	R	Reaction conditions		mp (°C) Crystn. solvent	NMR δ ($CDCl_3$)			Formula	Analysis (%) Calcd (Found)			
		Time (h)	Yield (%)		N^+-CH_2-	-$CH_2SO_4^-$	C-2 SCH_3		C	H	N	S
2c	C_3H_7	5	55	143—145 $CHCl_3$-AcOEt	4.70	4.01	3.26	$C_{15}H_{16}NS_2\cdot C_3H_7SO_4$	52.27 (52.48	5.61 5.82	3.39 3.52	23.26 23.12)
2d	C_4H_9	7	50	118—121 $CHCl_3$-AcOEt	4.72	4.04	3.26	$C_{16}H_{18}NS_2\cdot C_4H_9SO_4\cdot H_2O\cdot 0.2CHCl_3$	50.18 (50.15	6.09 5.99	2.90 3.02	19.89 20.28)
2e	C_7H_{15}	8	40	114—116 $CHCl_3$-AcOEt	4.65	4.00	3.27	$C_{19}H_{24}NS_2\cdot C_7H_{15}SO_4\cdot 0.3H_2O$	58.79 (58.76	7.51 7.39	2.64 2.87	18.11 18.13)
2f	$C_{12}H_{25}$	22	55	130—132 $CHCl_3$-AcOEt	4.67	4.03	3.27	$C_{24}H_{34}NS_2\cdot C_{12}H_{25}SO_4\cdot H_2O$	63.21 (63.17	8.99 8.86	2.05 2.31	14.06 14.02)
2g	$C_{18}H_{37}$	25	55	126—130 $CHCl_3$-AcOEt	4.72	3.97	3.29	$C_{30}H_{46}NS_2\cdot C_{18}H_{37}SO_4$	69.10 (69.16	10.03 10.08	1.68 1.69	11.53 11.30)
2h	$C_{22}H_{45}$	25	50	106—110 $CHCl_3$-AcOEt	4.77	3.98	3.30	$C_{34}H_{54}NS_2\cdot C_{22}H_{45}SO_4\cdot 1.5H_2O$	69.08 (69.07	10.56 10.37	1.44 1.62	9.88 9.84)

Monomethinbenzthiacyanines (**3a**, **c**—**h**)——General Procedure: A mixture of 3-alkyl-2-methylnaphtho[2,1-*d*]thiazolium alkylsulfates (**1a**, **c**—**h**) (1 mmol), 3-alkyl-2-methylthionaphtho[2,1-*d*]thiazolium alkylsulfates (**2a**, **c**—**h**) (1 mmol) and pyridine (5—10 ml) was warmed at 125°C for 1—4 h, then evaporated to dryness. The residue in the case of **3a** was recrystallized to yield the desired crystalline product. In the case of **3c**—**h**, each residue was purified by column chromatography on silica gel with $CHCl_3$–MeOH (98: 2) as the eluent followed by crystallization to yield the desired product as yellow crystals. The yields, solvents for crystallization, and physical data of **3a**,**c**—**h** are shown in Table VIII.

3-(2,3-Dihydroxypropyl)-2-[[3-(2,3-dihydroxypropyl)naphtho[2,1-*d*]thiazol-2(3*H*)-ylidene]methyl]naphtho[2,1-*d*]thiazolium 2,3-Dihydroxypropylsulfate (3i)——i) 3-(2,3-*O*-Isopropylidenepropyl)-2-methylnaphtho[2,1-*d*]thiazolium salt (**1i**) was prepared by warming a mixture of 2-methylnaphtho[2,1-*d*]thiazole (0.2 g, 1 mmol) and **4i** (0.33 g, 1 mmol) at 120°C for 2.5 h.

ii) 3-(2,3-*O*-Isopropylidenepropyl)-2-methylthionaphtho[2,1-*d*]thiazolium salt (**2i**) was prepared by warming a mixture of 2-methylthionaphtho[2,1-*d*]thiazole (0.23 g, 1 mmol) and **4i** (0.33 g, 1 mmol) at 120°C for 2.5 h.

iii) A mixture of **1i** (0.53 g, 1 mmol), **2i** (0.56 g, 1 mmol) and pyridine (5 ml) was stirred overnight at room temperature and then warmed at 40°C for 4 h and evaporated to dryness *in vacuo*. The residue was taken up in 70% acetic acid (10 ml) and the mixture was warmed at 40°C for 1.5 h, then concentrated. The residue was washed successively with water, $CHCl_3$ and Et_2O, and dried *in vacuo*. The desired product was obtained as greenish-yellow needles (0.35 g, 50%). The yield and physical data are shown in Table VIII.

TABLE VIII. Monomethinbenzthiacyanines

Compd.	Yield (%)	mp (°C)	Crystn. solvent	IR $\nu_{\max}^{\text{KBr}}$ cm^{-1}	Formula	Analysis (%) Calcd (Found) C	H	N	S
3a	80	>300	CH_3OH	1530, 1500, 1270,	$C_{25}H_{19}N_2S_2\cdot$	59.75	4.24	5.36	18.40
				1245, 1220	CH_3SO_4	(59.54	4.16	5.28	18.28)
3c	75	255—258	$CHCl_3$-C_6H_6	2970, 1530, 1505,	$C_{29}H_{27}N_2S_2\cdot$	62.41	5.73	4.55	15.62
				1225	$C_3H_7SO_4\cdot H_2O$	(62.35	5.56	4.47	51.81)
3d	84	247—249	$CHCl_3$-C_6H_6	2950, 1525, 1505,	$C_{31}H_{31}N_2S_2\cdot$	64.78	6.21	4.32	14.82
				1210	$C_4H_9SO_4$	(64.55	5.98	4.58	14.80)
3e	73	228—231	C_6H_6	2950, 1525, 1500,	$C_{37}H_{43}N_2S_2\cdot$	68.18	7.54	3.61	12.41
				1230	$C_7H_{15}SO_4$	(67.90	7.34	3.56	12.47)
3f	76	223—225	C_6H_6	2920, 2850, 1525,	$C_{47}H_{63}N_2S_2\cdot$	71.90	9.00	2.84	9.76
				1500, 1250	$C_{12}H_{25}SO_4$	(72.19	8.74	2.82	9.76)
3g	72	188—190	C_6H_6	2925, 2850, 1528,	$C_{59}H_{87}N_2S_2\cdot$	74.70	10.10	2.26	7.77
				1505, 1255	$C_{18}H_{37}SO_4$	(74.51	10.28	2.52	7.75)
3h	45	175—177	C_6H_6	2925, 2850, 1525,	$C_{67}H_{103}N_2S_2\cdot$	76.01	10.61	1.99	6.84
				1500, 1250	$C_{22}H_{45}SO_4$	(76.19	10.68	2.08	6.82)
3i	50	276—279	…	1528, 1503, 1265	$C_{29}H_{27}N_2S_2\cdot$	54.68	4.88	3.99	13.69
					$C_3H_7O_6S$	(54.89	4.59	4.13	14.09)

3-(2-Hydroxyethyl)-2-[3-[3-(2-hydroxyethyl)naphtho[2,1-*d*]thiazol-2(3*H*)-ylidene]-1-propenyl]naphtho[2,1-*d*]thiazolium Bromide (5c)——A mixture of 2-methylnaphtho[2,1-*d*]thiazole (1 g, 5 mmol) and 2-bromoethanol (0.94 g, 7.5 mmol) was warmed at 120°C for 27 h. After addition of $CHCl_3$, the mixture was stirred overnight at room temperature. The precipitate was collected by filtration and dissolved in water. Lyophilization gave 1.1 g (67%) of 3-(2-hydroxyethyl)-2-methylnaphtho[2,1-*d*]thiazolium bromide (**1j**) as a pale brown powder: *Anal.* Calcd for $C_{14}H_{14}BrNOS$: C, 51.86; H, 4.35; N, 4.32; S, 9.89. Found: C, 51.65; H, 4.05; N, 4.14; S, 9.85. This was combined with ethyl orthoformate (1.5 g, 9.9 mmol) in pyridine (11 ml) and the mixture was warmed at 130°C for 4 h, then concentrated. The residue was triturated with $CHCl_3$ and filtered. The product was washed successively with $CHCl_3$, H_2O, and Et_2O to give 0.55 g (56%) of **5c** as a bluish-purple powder: IR $\nu_{\max}^{\text{KBr}}$ cm^{-1}: 1575, 1553, 1430, 1200. *Anal.* Calcd for $C_{29}H_{25}BrN_2O_2S_2\cdot 1.2H_2O$: C, 58.13; H, 4.61; Br, 13.34; N, 4.68; S, 10.70. Found: C, 58.07; H, 4.31; Br, 12.95; N, 4.79; S, 10.30.

3-(2,3-Dihydroxypropyl)-2-[3-[3-(2,3-dihydroxypropyl)naphtho[2,1-*d*]thiazol-2(3*H*)ylidene]-1-propenyl]naphtho[2,1-*d*]thiazolium Bromide (5d)——A mixture of 2-methylnaphtho[2,1-*d*]thiazole (2 g, 10 mmol) and α-bromopropylene glycol (2.32 g, 15 mmol) was warmed at 120°C for 27 h. After addition of $CHCl_3$, the mixture was stirred overnight at room temperature. The precipitate was collected by filtration and dissolved in water. Filtration and lyophilization gave 1.7 g (49%) of 3-(2,3-dihydroxypropyl)-2-methylnaphtho[2,1-*d*]thiazolium bromide (**1k**) as a pale brown powder: *Anal.* Calcd for $C_{15}H_{16}BrNO_2S\cdot 0.75H_2O$: C, 48.99; H, 4.79; Br, 21.72; N, 3.81; S, 8.71. Found: C, 49.22; H, 4.76; Br, 21.88; N, 4.07; S, 8.41. This was combined with ethyl orthoformate (2.1 g, 14 mmol) in pyridine (14 ml) and the mixture was warmed at 130°C for 4 h, then concentrated. The residue was triturated with $CHCl_3$ and filtered. The product was treated with 28% aqueous HBr (10 ml) at room temperature for 2 h with stirring. Filtration, followed by washing with H_2O and Et_2O gave 1.2 g (78%) of **5d**. Crystallization from methanol gave bluish-purple prisms: mp 255—256.5°C (dec.). IR $\nu_{\max}^{\text{KBr}}$ cm^{-1}: 1572, 1550, 1442, 1428, 1190. *Anal.* Calcd for $C_{31}H_{29}BrN_2O_4S_2 \cdot H_2O$: C, 56.79; H, 4.77; Br, 12.19; N, 4.27; S, 9.78. Found: C, 56.79; H, 4.81; Br, 12.57; N, 4.34; S, 9.52.

3-Acetonyl-2-[3-(3-acetonylnaphtho[2,1-*d*]thiazol-2(3*H*)-ylidene)-1-propenyl]naphtho[2,1-*d*]thiazolium Bromide (5e)——A mixture of 2-methylnaphtho[2,1-*d*]thiazole (1 g, 5 mmol) and acetonyl bromide (0.68 g, 5 mmol) was warmed at 70°C for 4 h. After addition of $CHCl_3$, the mixture was stirred overnight at room temperature. The precipitate was collected by filtration to give 1.2 g (70%) of 3-acetonyl-2-methylnaphtho-[2,1-*d*]thiazolium bromide (**11**). Crystallization from $CHCl_3$–MeOH–C_6H_6 (20: 1: 60) at room temperature gave colorless needles: mp 205—208°C (dec.). *Anal.* Calcd for $C_{15}H_{14}BrNOS \cdot 0.3H_2O$: C, 52.73; H, 4.31; Br, 23.39; N, 4.10; S, 9.38. Found: C, 52.55; H, 4.60; Br, 23.58; N, 4.10; S, 9.32. This product was combined with ethyl orthoformate (2.6 g, 17.5 mmol) in pyridine (10 ml) and the mixture was warmed at 130°C for 3 h. Work-up in the manner described for **5e** gave 0.61 g (58%) as a bluish-purple powder: IR $\nu_{\max}^{\text{KBr}}$ cm^{-1}: 1727, 1547, 1443, 1413, 1205. *Anal.* Calcd for $C_{31}H_{25}BrN_2O_2S_2 \cdot 1.4H_2O$: C, 59.40; H, 4.47; Br, 12.75; N, 4.47; S, 10.23. Found: C, 59.59; H, 4.36; Br, 12.50; N, 4.56; S, 10.16.

3-Phenacyl-2-[3-(3-phenacylnaphtho[2,1-*d*]thiazol-2(3*H*)-ylidene)-1-propenyl]naphtho[2,1-*d*]thiazolium Bromide (5f)——A mixture of 2-methylnaphtho[2,1-*d*]thiazole (1 g, 5 mmol) and phenacyl bromide (1 g, 5 mmol) was warmed at 100°C for 3 h. Recrystallization of the product from $CHCl_3$–MeOH–C_6H_6 (20: 1: 60) at room temperature gave 1.5 g (75%) of **1m** as colorless needles: mp 167—169°C. *Anal.* Calcd for $C_{20}H_{16}BrNOS$: C, 60.31; H, 4.05; Br, 20.06; N, 3.52; S, 8.05. Found: C, 60.05; H, 4.25; Br, 20.22; N, 3.55; S, 8.03. This was combined with ethyl orthoformate (1.5 g, 10 mmol) in pyridine (6 ml) and the mixture was warmed at 130°C for 2 h. Work-up in the manner described for **5c** gave 0.41 g (30%) of **5f** as a bluish-purple powder: IR $\nu_{\max}^{\text{KBr}}$ cm^{-1}: 1690, 1548, 1445, 1410, 1195. *Anal.* Calcd for $C_{41}H_{29}BrN_2O_2S_2 \cdot H_2O$: C, 66.21; H, 4.20; Br, 10.74; N, 3.77; S, 8.62. Found: C, 66.30; H, 4.18; Br, 10.22; N, 3.84; S, 8.70.

3-Benzyl-2-[3-(3-benzylnaphtho[2,1-*d*]thiazol-2(3*H*)-ylidene)-1-propenyl]naphtho[2,1-*d*]thiazolium Bromide (5g)——A mixture of 2-methylnaphtho[2,1-*d*]thiazole (1 g, 5 mmol) and benzyl bromide (1.28 g, 7.5 mmol) was warmed at 120°C for 6 h. After addition of $CHCl_3$, the mixture was stirred overnight at room temperature. The precipitate was collected by filtration to give 1.75 g (95%) of 3-benzyl-2-methylnaphtho-[2,1-*d*]thiazolium bromide (**1n**). Crystallization from $CHCl_3$–MeOH–C_6H_6 gave colorless needles: mp >260 °C (subl). *Anal.* Calcd for $C_{19}H_{16}BrNS \cdot 0.4H_2O$: C, 60.45; H, 4.49; Br, 21.17; N, 3.71; S, 8.49. Found: C, 60.34; H, 4.39; Br, 21.56; N, 3.74; S, 8.63. This was combined with ethyl orthoformate (1.9 g, 13 mmol) in pyridine (19 ml) and the mixture was warmed at 130°C for 3 h. Work-up in the manner described above gave 1.15 g (78%) of **5g** as a bluish-purple powder: IR $\nu_{\max}^{\text{KBr}}$ cm^{-1}: 1550, 1410, 1190. *Anal.* Calcd for $C_{39}H_{29}BrN_2S_2$: C, 69.94; H, 4.36; Br, 11.94; N, 4.18; S, 9.58. Found: C, 69.77; H, 4.48; Br, 12.43; N, 4.38; S, 9.54.

2-Amino-1-thiocyanatoanthracene (21)——Cupric thiocyanate (3.6 g, 20 mmol) was added to an ice-cooled solution of 2-aminoanthracene (1.93 g, 10 mmol) in ethyl acetate (200 ml). The mixture was stirred overnight at room temperature. After filtration, the filtrate was shaken with 2.5% aqueous $NaHCO_3$ (0.84 g, 10 mmol) and concentrated *in vacuo*. Filtration and washing with Et_2O gave 1 g (40%) of **21** as yellow prisms: IR $\nu_{\max}^{\text{KBr}}$ cm^{-1}: 3450 (NH), 3350 (NH), 2135 (SCN), 1625, 874, 740.

2-Methylthioanthra[2,1-*d*]thiazole (22)——A mixture of **21** (2.2 g, 8.8 mmol), carbon disulfide (12 ml) and pyridine (12 ml) was warmed at 60°C for 5 h, then evaporated to dryness. Trituration followed by washing with H_2O gave 2.1 g of anthra[2,1-*d*]thiazole-2-thiol as a yellow solid: IR $\nu_{\max}^{\text{KBr}}$ cm^{-1}: 1310 (CSNH), 1080. This was treated with CH_3I (3.4 g, 24 mmol) and 30% aqueous NaOH (2.1 ml) in EtOH (21 ml) at room temperature for 3 h with stirring, then concentrated. The residue was extracted with $CHCl_3$. The extract, after being dried over anhydrous Na_2SO_4, was evaporated to dryness *in vacuo*. Column chromatography on silica gel (45 g) with benzene as the eluent gave 1.5 g (61%) of **22**. Crystallization from EtOH gave yellow needles: mp 156—157°C. IR $\nu_{\max}^{\text{KBr}}$ cm^{-1}: 1420, 1061, 867, 739. NMR ($CDCl_3$) δ: 2.85 (s, 3H, SCH_3). *Anal.* Calcd for $C_{16}H_{11}NS_2$: C, 68.29; H, 3.94; N, 4.98; S, 22.79. Found: C, 68.00; H, 3.89; N, 4.84; S, 22.79.

3-Methylnaphtho[2,1-*d*]thiazoline-2(3*H*)-thione (Isomer of **22**): mp 243—245°C. NMR ($CDCl_3$) δ: 3.98 (s, 3H, NCH_3).

3-Methyl-2-methylthioanthra[2,1-*d*]thiazolium Methylsulfate (23)——A mixture of **22** (0.56 g, 2 mmol) and dimethyl sulfate (0.76 g, 6 mmol) was warmed at 120°C for 4 h, then cooled. The precipitate was triturated with benzene and filtered. Thorough washing with $CHCl_3$ and Et_2O gave 0.8 g (98%) of **23** as a pale brown powder: IR $\nu_{\max}^{\text{KBr}}$ cm^{-1}: 1244, 1226, 1010, 758, 740. *Anal.* Calcd for $C_{17}H_{14}NS_2 \cdot CH_3SO_4 \cdot 0.9H_2O$: C, 51.02; H, 4.47; N, 3.31; S, 22.70. Found: C, 50.78; H, 4.23; N, 3.29; S, 22.95.

2,3-Dimethylanthra[2,1-*d*]thiazolium Methylsulfate (24)——A mixture of 2-methylanthra[2,1-*d*]thiazole[14] (0.45 g, 1.8 mmol) and dimethyl sulfate (0.68 g, 5.4 mmol) was warmed at 120°C for 3 h. Work-up in the manner described for **23** gave 0.57 g (84%) of **24** as a pale brown powder: IR $\nu_{\max}^{\text{KBr}}$ cm^{-1}: 1255, 1210, 1000, 761. *Anal.* Calcd for $C_{17}H_{14}NS \cdot CH_3SO_4 \cdot 0.5H_2O$: C, 56.23; H, 4.72; N, 3.64; S, 16.68. Found: C, 56.23; H, 4.48; N, 3.53; S, 16.97.

3-Methyl-2-[(3-methylanthra[2,1-*d*]thiazol-2(3*H*)-ylidene)methyl]anthra[2,1-*d*]thiazolium Methylsulfate (30)——A mixture of **23** (0.3 g, 0.75 mmol) and **24** (0.28 g, 0.75 mmol) in pyridine (3 ml) was warmed at 125°C for 1 h, then evaporated to dryness *in vacuo*. The residue was triturated with MeOH and filtered.

Successive washing with $CHCl_3$, H_2O and Et_2O gave 0.45 g (97%) of **30** as a yellow powder: IR ν_{max}^{KBr} cm^{-1}: 1517, 1274, 1243, 1223. *Anal.* Calcd for $C_{33}H_{23}N_2S_2 \cdot CH_3SO_4 \cdot 0.75H_2O$: C, 64.18; H, 4.36; N, 4.40; S, 15.12. Found: C, 63.89; H, 4.17; N, 4.49; S, 15.47.

Anthraquinono[2,1-*d*]thiazole-2-thiol (25)——A solution of 2-amino-1-mercaptoanthraquinone (1 g, 4 mmol) and carbon disulfide (0.6 g, 8 mmol) in pyridine (3 ml) was warmed at 55°C for 3 h with stirring. After cooling, the reaction mixture was diluted with ethanol and the resulting precipitate was collected by filtration and washed successively with $CHCl_3$ and Et_2O to give 0.73 g (61%) of **25** as an orange powder. Crystallization from $CHCl_3$–MeOH (1: 1) gave orange needles: mp >270°C. IR ν_{max}^{KBr} cm^{-1}: 3210 (NH), 1668 (1,4-quinone) 1570, 1280, 710. *Anal.* Calcd for $C_{15}H_7NO_2S$: C, 60.59; H, 2.37; N, 4.71. Found: C, 60.56; H, 2.34; N, 4.68.

2-Methylthioanthraquinono[2,1-*d*]thiazole (26)——To an ice-cooled solution of **25** (0.24 g, 0.8 mmol) and 30% aqueous NaOH (0.2 ml) in EtOH (2 ml) was added CH_3I (0.34 g, 2.4 mmol). The mixture was stirred overnight at room temperature. The resulting precipitate was collected by filtration and washed successively with EtOH, H_2O and Et_2O to give 0.23 g (92%) of **26** as a yellow powder. Crystallization from $CHCl_3$–MeOH (1: 1) gave yellow needles: mp 252—253°C. IR ν_{max}^{KBr} cm^{-1}: 1663 (1,4-quinone), 1288, 707. NMR ($CDCl_3$) δ: 3.01 (s, 3H, SCH_3). *Anal.* Calcd for $C_{16}H_9NO_2S_2$: C, 61.72; H, 2.91; N, 4.50; S, 20.60. Found: C, 61.77; H, 2.80; N, 4.52; S, 20.66.

3-Methyl-2-methylthioanthraquinono[2,1-*d*]thiazolium Methylsulfate (27)——A mixture of **26** (1.44 g, 4.6 mmol) and dimethyl sulfate (1.75 g, 14 mmol) was heated at 160°C for 40 min. Work-up in the manner described for **23** gave 1.8 g (90%) of **27** as a yellow powder: IR ν_{max}^{KBr} cm^{-1}: 1665 (1,4-quinone), 1575, 1290, 1250, 1210, 1023, 757, 720.

3-Methyl-2-[(3-methylanthraquinono[2,1-*d*]thiazol-2(3*H*)-ylidene)methyl]anthraquinono[2,1-*d*]thiazolium Methylsulfate (31)——A mixture of 2,3-dimethylanthraquinono[2,1-*d*]thiazolium methylsulfate (**28a**)[15] (0.4 g, 1 mmol) and **27** (0.44 g, 1 mmol) in pyridine (4 ml) was stirred at 130°C for 45 min. Work-up in the manner described for **30** gave 0.52 g (76%) of **31** as a reddish-purple powder: IR ν_{max}^{KBr} cm^{-1}: 1665 (1,4-quinone), 1510, 1260. *Anal.* Calcd for $C_{33}H_{19}N_2O_4S_2 \cdot CH_3SO_4 \cdot 2H_2O$: C, 56.81; H, 3.65; N, 3.90; S, 13.38. Found: C, 56.71; H, 3.54; N, 3.95; S, 13.37.

3-Methyl-2-[3-(3-methylanthraquinono[2,1-*d*]thiazol-2(3*H*)-ylidene)-1-propenyl]anthraquinono[2,1-*d*]thiazolium Methylsulfate (32)——A mixture of **28a**[15] (0.4 g, 1 mmol), ethyl orthoformate (0.45 g, 3 mmol) and pyridine (5 ml) was warmed at 130°C for 4 h. Work-up in the manner described for **30** gave 0.27 g (75%) of **32** as a bluish-purple powder: IR ν_{max}^{KBr} cm^{-1}: 1663 (1,4-quinone), 1543, 1390, 1290, 1195. *Anal.* Calcd for $C_{35}H_{21}N_2O_4S_2 \cdot CH_3SO_4 \cdot H_2O$: C, 59.99; H, 3.54; N, 3.89; S, 13.35. Found: C, 60.10; H, 3.66; N, 3.81; S, 12.99.

3-Methyl-2-[7-(3-methylanthraquinono[2,1-*d*]thiazol-2(3*H*)-ylidene)-1,3,5-heptatrienyl]anthraquinono-[2,1-*d*]thiazolium Chloride (33)——A mixture of 2,3-dimethylanthraquinono[2,1-*d*]thiazolium chloride (**28b**)[15] (0.33 g, 1 mmol), 1-anilino-5-anilo-1,3-pentadiene[16] (0.125 g, 0.5 mmol) and acetic anhydride (2 ml) was warmed at 125°C for 45 min. Work-up in the manner described for **30** gave 0.125 g (35%) of **33** as a deep blue powder: IR ν_{max}^{KBr} cm^{-1}: 1662 (1,4-quinone), 1510, 1447, 1385, 1285, 1040—1080 (br). *Anal.* Calcd for $C_{39}H_{25} \cdot ClN_2O_4S_2 \cdot 2.5H_2O$: C, 64.14; H, 4.14; Cl, 4.86; N, 3.84; S, 8.78. Found: C, 64.26; H, 4.06; Cl, 4.52; N, 3.88; S, 8.98.

10-Methoxy-2,3-dimethylphenanthro[1,2-*d*]thiazolium Salt (29)——A mixture of 10-methoxy-2,3-dimethylphenanthro[1,2-*d*]thiazole[25] (1.4 g, 5 mmol) and dimethyl sulfate (1.89 g, 15 mmol) was heated at 160°C for 1 h. Work-up in the manner described for **23** gave a yellow powder: IR ν_{max}^{KBr} cm^{-1}: 1580, 1250, 1210, 1060, 1000. This was dissolved in water and the solution was filtered to remove insoluble impurities. KI (8.3 g, 50 mmol) was added to the clear filtrate and the mixture was left to stand with cooling to give 1.53 g (73%) of **29** as a yellow powder: IR ν_{max}^{KBr} cm^{-1}: 1580, 1440, 1248, 1085. *Anal.* Calcd for $C_{18}H_{16}INOS$–$C_{18}H_{16}NOS \cdot CH_3SO_4$ (9: 1)$\cdot 0.3H_2O$: C, 51.14; H, 4.01; I, 26.87; N, 3.29; S, 8.30. Found: C, 51.09; H, 3.82; I, 26.98; N, 3.39; S, 8.48.

10-Methoxy-2-[3-(10-methoxy-3-methylphenanthro[1,2-*d*]thiazol-2(3*H*)-ylidene)-1-propenyl]-3-methylphenanthro[1,2-*d*]thiazolium Salt (34)——A mixture of **29** (1.53 g, 3.6 mmol), ethyl orthoformate (1.62 g, 11 mmol) and pyridine (7.3 ml) was warmed at 130°C for 3 h. Work-up in the manner described for **30** gave 0.4 g (31%) of **34** as a bluish-purple powder: IR ν_{max}^{KBr} cm^{-1}: 1550, 1465, 1405, 1197. *Anal.* Calcd for $C_{37}H_{29}N_2O_2S_2 \cdot CH_3SO_4$–$C_{37}H_{29}IN_2O_2S_2$(6: 4)$\cdot H_2O$: C, 61.59; H, 4.51; N, 3.82; S, 11.37. Found: C, 61.56; H, 4.22; N, 3.86; S, 11.39.

10-Methoxy-2-[7-(10-methoxy-3-methylphenanthro[1,2-*d*]thiazol-2(3*H*)-ylidene)-1,3,5-heptatrienyl]-3-methylphenanthro[1,2-*d*]thiazolium Salt (35)——To a solution of Na (0.08 g, 3.5 mg atom) in absolute ethanol (10 ml) were added 1-anilino-5-anilo-1,3-pentadiene·HCl[16] (0.43 g, 1.5 mmol) and **29** (0.99 g, 3 mmol). The mixture was refluxed for 30 min. Work-up in the manner described for **30** gave 0.15 g (14%) of **35** as a deep purple powder: IR ν_{max}^{KBr} cm^{-1}: 1500, 1433, 1400, 1050—1080 (br). *Anal.* Calcd for $C_{41}H_{33}N_2O_2S_2 \cdot NaSO_4$–$C_{41}H_{33}ClN_2O_2S_2$(8.5: 1.5)$\cdot H_2O$: C, 63.59; H, 4.56; Cl, 0.69; N, 3.62; S, 11.80. Found: C, 63.76; H, 4.37; Cl, 0.83; N, 3.85; S, 11.64.

***N,N′*-Bis(*β*-naphthyl)glutaramide** (36)——A solution of glutaric acid dichloride (0.845 g, 5 mmol) in $CHCl_3$ (5 ml) was added to an ice-cooled solution of *β*-naphthylamine (1.43 g, 10 mmol) in pyridine, and the mixture was stirred at room temperature for 1 h. White crystals that separated were collected by filtration and washed with $CHCl_3$ to give 1.55 g (81%) of **36**: mp 220°C. NMR (Me_2SO-d_6) δ: 2.10 (q, 2H, CH_2), 2.50 (t, 4H, $CH_2CH_2CH_2$), 7.17—8.57 (m, 14H, aromatic), 10.2 (s, 2H, NH × 2).

N,N'**-Bis(*β*-naphthyl)glutarthioamide (37)**——A mixture of **36** (2.5 g, 6.48 mmol) and phosphorus pentasulfide (3.33 g, 15 mmol) in pyridine (100 ml) was refluxed for 1 h, then cooled. The precipitate was filtered off and the filtrate was evaporated to dryness. The residue was washed with ice-water and MeOH to give 2.1 g of **37** as a brown powder.

1,3-Bis(2-naphtho[2,1-*d*]thiazolyl)propane (38)——To a solution of **37** (1.9 g, 4.55 mmol) in ethanol (23 ml) was added dropwise 1 N NaOH (20 ml) at 40°C. The mixture was stirred for 20 min and filtered. The filtrate was added dropwise to an ice-cooled solution of potassium ferricyanide (6 g) in water (30 ml), and the mixture was further stirred overnight. The precipitate was collected by filtration, washed with H_2O and dried. Purification by column chromatography on silica gel with *n*-hexane–AcOEt (2: 1) as the eluent and subsequent recrystallization from ethyl acetate gave 1.0 g (53%) of **38** as a pale brownish powder: IR ν_{max}^{KBr} cm^{-1}: 1517, 1253, 815, 747. NMR ($CDCl_3$) δ: 2.60 (q, $J=8$ Hz, 2H, $CH_2C\underline{H}_2CH_2$), 3.40 (t, $J=8$ Hz, 4H, $C\underline{H}_2CH_2C\underline{H}_2$), 7.17—8.17 (m, 12H, aromatic). *Anal.* Calcd for $C_{25}H_{18}N_2S_2 \cdot 0.25H_2O$: C, 72.34; H, 4.49; N, 6.75; S, 15.45. Found: C, 72.15; H, 4.40; N, 6.75; S, 15.71.

1,3-Bis[2-(3-methylnaphtho[2,1-*d*]thiazolium)propane Bis(methylsulfate) (39)——A mixture of **38** (0.41 g, 1 mmol) and dimethyl sulfate (0.76 g, 6 mmol) was stirred at 120°C for 20 min. After cooling, the reaction mixture was triturated with $CHCl_3$ and filtered. Thorough washing with $CHCl_3$ and Et_2O gave 0.54 g (81%) of **39** as a pale purple powder: IR ν_{max}^{KBr} cm^{-1}: 1512, 1250, 1225, 1007, 805, 735. *Anal.* Calcd for $C_{27}H_{24}N_2S_2 \cdot 2CH_3SO_4 \cdot 0.5H_2O$: C, 51.76; H, 4.79; N, 4.16; S, 19.06. Found: C, 51.47; H, 4.49; N, 4.18; S, 19.23.

Antitumor Activity

Animals——DBA/2 and CDF_1 mice were supplied by Simonsen Labs., Inc., Gilroy, Calif., and Laboratory Supply Co., Inc., Indianapolis, respectively, under the auspices of Drug Research and Development, National Cancer Institute. C57BL/6 and BDF_1 mice were obtained from the Drug Safety Research Laboratories of this Central Research Division. ICR mice were purchased from CLEA Japan. The mice were fed with a pellet diet (CE-2; CLEA Japan, Inc., Tokyo, Japan) with water *ad libitum.*

Tumors——P388 leukemia and B16 melanoma were provided by Drug Research and Development, Division of Cancer Treatment, National Cancer Institute, NIH, Bethesda, Md., and maintained in DBA/2 and C57BL/6 mice, respectively.

Tumor Transplantation——Tumors were transplanted *i.p.* into mice. CDF_1 and BDF_1 mice were inoculated with 1×10^6 P388 cells and with 0.5 ml of 1: 4 tumor homogenates of B16 in 0.9% NaCl solution, respectively, as described by Geran *et al.*[26] The median survival time of tumor-bearing mice was about 10 days for P388 and 17.5 days for B16.

Drug Preparation——Cyanines were suspended in 0.9% NaCl solution and Tween 80 at a ratio of 95: 5 (v/w).

Therapeutic Test——CDF_1 mice (6 mice/group) were given the drug solutions (0.2 ml/20 g body weight) *i.p.* on days 1 and 5, starting 24 h after tumor transplantation. BDF_1 mice (5 mice/group) were given the drug solution (0.2 ml/20 g body weight) *i.p.* daily for 1 to 9 consecutive days starting 24 h after tumor transplantation. Median survival time was calculated, and the antitumor activity of drugs was assessed in terms of $T/C\%$ ($T/C\%$ = treated/control × 100) as described by Geran *et al.*[26] Antitumor activity was considered to be positive when $T/C\%$ was over 120% for P388 and 125% for B16.

TABLE IX. Acute Toxicity in Mice

Compd.	Dose (mg/kg) *i.p.*	No. of mice died / No. of mice	Body weight change (g) (day 0—5)
12a	200	3/3	—
	100	2/3	2.1
	50	1/3	−4.2
	25	0/3	0.1
3b	400	2/3	1.9
	200	3/3	—
	100	3/3	—
	50	2/3	−5.2
19	100	3/3	—
	50	2/3	1.9
	25	0/3	1.7
	12.5	0/3	2.3
Control		0/6	2.8

Acute Toxicity in Mice——Acute toxicity of cyanines was determined on ICR mice (3 mice/group) by single intraperitoneal administration of the drugs. Mice were observed daily over a 14-day period. Bodyweight of mice was measured on days 0 and 5 (Table IX).

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