Structure−Activity of Novel Rhodacyanine Dyes as Antitumor Agents

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We have previously reported that rhodacyanine dyes, such as **1** and **2**, exhibited a potent inhibitory effect on the growth of several tumor cells and that 4-oxothiazolidine (rhodanine) was an essential moiety for antitumor activity. On the basis of our foregoing work, two types of rhodacyanine dyes, which categorized into class I and II depending on the methine length, were synthesized and evaluated as a novel antitumor agent. Attention was particularly focused on the structure−activity study of two heteroaromatic rings. In class I, where the A rings were conjugated to rhodanine via two methine groups, compounds **1**, **20**, **23**, and **24** were found to be efficacious in tumor-bearing nude mice model study, but they did not have the chemical properties (stability, solubility) suitable for clinical use. In contrast, in class II, where the A rings were directly conjugated to rhodanine, compounds **13** and **25**, which possessed a benzothiazole moiety for the A ring, exhibited the favarable biological and chemical properties. Therefore, we decided to have a benzothiazole moiety as the A ring and introduce various heterocyclic groups for the B ring. As a result, the pyridinium ring was selected as the optimal moiety for the B ring (compound **13**). Further, the variation of counteranion had a profound effect on solubility in water without influence on antitumor activity. Chloride anion was selected as the favorable anion with respect to synthetic method as well as solubilty in water. Our study finally led us to the identification of compound **3** (MKT 077, 1-ethyl-2-[[3-ethyl-5-(methylbenzothiazolin-2-ylidene)-4-oxothiazolidin-2-ylidene]methyl]pyridinium chloride) as the candidate for clinical trials and is currently subjected to further investigation as a potent antitumor agent in phase I clinical trial for the treatment of solid tumors.

Introduction

During the past 20 years, a variety of approaches have been taken for cancer chemotherapy, and many antitumor drugs have been developed for clinical use. In the treatment of solid tumors, however, the conventional approaches have met with only limited success, and cancer still remains as one of the leading causes of human mortality. Current chemotherapeutic antitumor drugs suffer two major drawbacks: adverse effects and drug resistance. Adverse effects associated with conventional antitumor drugs are usually caused by their indiscriminate cytotoxic effect on normal cells. In drug resistance, the use of combination chemotherapy, which is the administration of several drugs with different and complementary mechanisms of action, is regarded as the effective approach. Therefore, to overcome the shortcomings of the present cancer chemotherapy, an antitumor drug with a new mechanism of action, which is capable of discriminating tumor cells from normal proliferative cells and exhibits selective toxicity against cancer, is the subject of our current research.

As a new mechanism of action, we focused our attention on the studies by Chen et al. which reported that some "π electron-delocalized lipophilic cations (denoted as DLCs)" exhibited marked and selective antitumor activity. They rationalized that this effect of DLCs was caused by their selective accumulation in the negatively charged mitochondria in carcinoma cells due to the electrochemical proton gradient.[1−7] However, the compounds described in the studies were still rather toxic to normal cells and have never been developed for clinical use.

In our search for a DLC compound possessing satisfactory chemical and biological properties, we conducted an extensive screening of numerous cationic dye molecules, which were originally developed for silver halide photographic systems in our research laboratories. As a result, we found that rhodacyanine dyes exhibited selective antitumor activity against the human colon carcinoma cell line, CX-1, in comparison to its activity against the indicator cell for normal epithelial cell line, CV-1. In our previous paper,[8] we reported that two classes of rhodacyanine dyes, such as **1** (class I) and **2** (class II) (Chart 1), exhibited a potent inhibitory effect in vitro on the growth of several tumor cell lines and were also efficacious in tumor-bearing nude mice models. Furthermore, our structure−activity study revealed that rhodanine and π electron delocalization were the essential structural requirements and that any slight modification on the moiety resulted in a loss of the antitumor activity.

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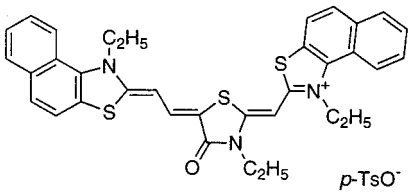
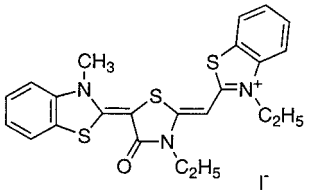
Chart 1



1



2

On the basis of these findings, we designed and synthesized a wide variety of rhodacyanine dyes and evaluated them for biological (in vitro activity, in vivo efficacy, and toxicity) and chemical (stability, water solubility) properties. We placed attention on the structure−activity relationship of two heteroaromatic groups which are conjugated to the rhodanine moiety. This led to the identification of compound **3** (MKT 077), which is currently undergoing evaluation in phase I clinical trial.

Chemistry

Rhodacyanine dye consists of three rings, two heteroaromatic rings (A and B) and a central 4-oxothiazolidine (rhodanine), in which two-dye-conjugate systems, a neutral and a cationic dye unit, are integrated at the rhodanine moiety. The dye molecules discussed in this study are structurally categorized into two classes, class I/class II, depending on the methine length between A ring and the rhodanine moiety. Class I has two methine groups (for general formula, see Table 1) and class II has the A ring conjugated directly to the rhodanine moiety (for general formula, see Table 2). Syntheses of these compounds were accomplished according to reported procedures,[9,10] but with some modifications.

Scheme 1 illustrates a typical synthesis scheme of a class I rhodacyanine dye. 3-Ethyl-4-oxothiazolidine-2-thione (**4**) was reacted with 1,3-diaza-1,3-diphenylpropene and then with acetic anhydride to give compound **5**, which was condensed with 3-ethyl-2-methylnaphtho[1,2-*d*]thiazolinium *p*-toluenesulfonate to give neutral dye **6**. *S*-Methylation of **6** was followed by the reaction of 3-ethyl-2-methylnaphtho[1,2-*d*]thiazolinium *p*-toluenesulfonate in the presence of triethylamine to give the desired rhodacyanine dye **1**. Other class I rhodacyanine dyes, whose structures are summarized in Table 1, were also synthesized in an analogous manner.

In Scheme 2, an example of a typical synthesis of a class II rhodacyanine dye is described. *N*-Methylation of 2-methylthiobenzothiazole (**8**) using methyl *p*-toluenesulfonate was followed by the condensation with 3-ethyl-4-oxothiazolidine-2-thione (**4**) to give neutral dye **10**. Conversion of the intermediate **10** to the target rhodacyanine **12** was accomplished via reactions similar to those in the synthesis of **1** from **6** in Scheme 1. The chloride compound **3** was prepared from *p*-toluenesulfonate salt **12** by passing it through the commercially available strongly basic anion-exchange resin (chloride form), and the acetate compound **13** was obtained from **12** by using weakly basic anion-exchange resin which was converted to acetate form by acetic acid before use. As for the synthesis of a structurally restricted rhodacyanine **17**, the condensation of 2-aminothiophenol and 5-bromovaleryl chloride was followed by an intramolecular N-alkylation to give intermediate **16**, which was converted into the desired compound **17** by the usual methods (Scheme 3). Other class II rhodacyanine dyes, whose structures are summarized in Tables 2 and 3, were also synthesized in an analogous manner.

Class II rhodacyanine dye **3** was subjected to X-ray crystallography analysis. As shown in Figure 1, the study revealed that all three rings (A, B, rhodanine) were almost coplanar, and both of the *N*-ethyl groups on the B ring and rhodanine were situated on the same side of the molecule while the *N*-methyl group on the A ring was sitting on the opposite side. NOE signals between the methine proton and the two *N*-ethyl groups were observed in an NMR study (Figure 2). This indicated that the molecular geometry of Figure 2, at least in the cationic dye unit (rhodanine−B ring), was conserved in a 10:1 solution of methanol-d_4 and chloroform-*d*.

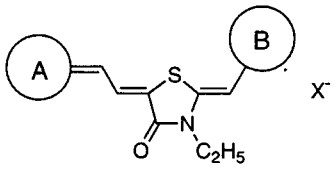
Results and Discussion

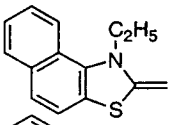
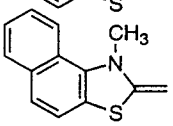
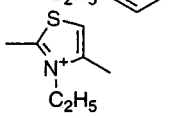
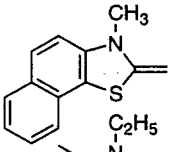
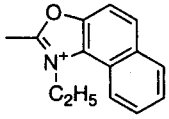
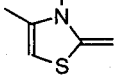
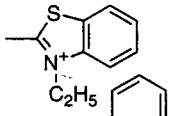
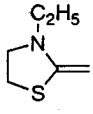
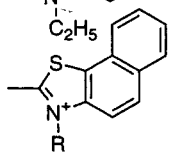
We previously reported that both class I and II rhodacyanine dyes displayed marked and selective antitumor activity and that rhodanine was the essential moiety.[8] In our present study, rhodacyanine dyes having a variety of heteroaromatic groups were evaluated for their in vitro and in vivo antitumor activity. The compounds, which exhibited antitumor activity, were also tested on thier toxicity and chemical properties as well to select an optimized compound for clinical trials.

We first devoted our efforts to the study of class I, the results of which are summarized in Table 1. Most of the compounds in this class, except for **18** and **21e**, exhibited high inhibitory in vitro effect on the growth of human carcinoma, the colon (CX-1) and epidermoid (KB), with IC_{50} values of below 0.5 and 1.0 μM, respectively. These active compounds were further evaluated for their prolongation activities against human melanoma LOX cell lines in nude mice model. After the intraperitoneal (ip) implantation of 2×10^6 human melanoma LOX cells, appropriately determined doses of the compounds were intraperitoneally administered according to schedule. The evaluation of efficacy was determined by the ratio of the survival periods of the treated group against the control group (T/C). Compounds **1**, **20**, **23**, and **24** exhibited modest in vivo efficacy with T/C values of 126, 122, 138, and 143, respectively, among the class I rhodacyanine dyes evaluated.

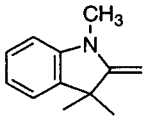
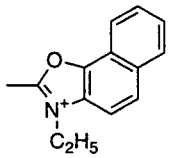
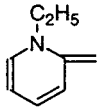
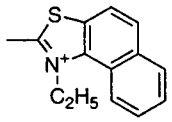
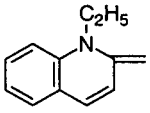
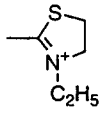
We also examined the in vitro inhibitory effect of N-substituents of the B ring, and the results are summarized in Table 1. The increase in size from the

Table 1. Effect of Heterocycles on Antitumor Activity



Compd.	A	B	X^-	in vitro IC_{50} CX-1 (clonogenic) (μM)	in vitro IC_{50} KB (cytotoxic) (μM)	in vivo T/C[a] (LOX) %
1			*p*-TsO^-	0.04	0.21	126[b] (10 mg/kg/day × 5)
18			AcO^-	>1.80	2.0	
19			*p*-TsO-	0.04	0.07	113 (5×5)
20			AcO^-	0.06	0.25	138 (5 × 3, 10 × 1)
						

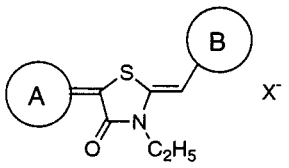
Compd.	R			
21a	CH_3	I^-	0.03	0.49
21b	C_2H_5	I^-	0.02	0.32
21c	*n*-C_4H_9	I^-	0.03	0.46
21d	C_2H_4OH	I^-	0.39	
21e	$C_2H_4CO_2H$	I^-	> 1.50	> 15.0

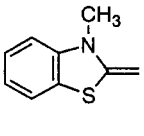
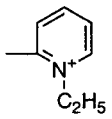
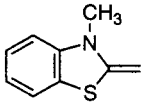
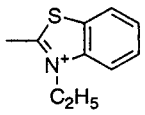
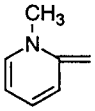
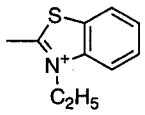
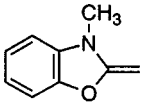
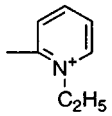
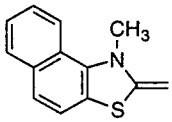
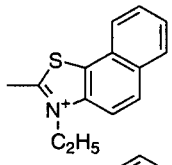
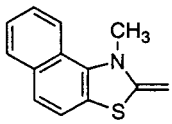
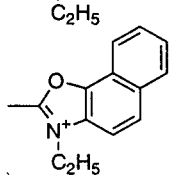
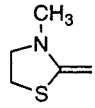
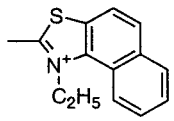
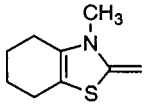
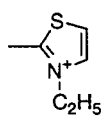
22			*p*-TsO-	0.01	0.27	94 (2.5 × 1, 5 × 3)
23			Cl^-	0.08	0.77	122 (4 × 5)
24			AcO^-	0.16	0.91	143 (10 × 5)

[a] T/C represents the ratio of the median survival time of drug-treated to control, untreated tumor-bearing mice, expressed as a percentage. Each group consisted of five nude mice.
[b] The statistical significance of difference between the control and the drug-treated group was determined by applying the Bartlett's, Dunnett's, and Scheffet's test. * $P<0.01$, ** $P<0.001$.

methyl to the butyl group had little effect (**21a**−**c**), while introduction of the hydrophilic or anionic substituents, such as the hydroxyl (**21d**) or the carboxyl group (**21e**), led to a decrease or even a loss of the in vitro activities. This indicated the importance of the hydrophobic character of the rhodacyanine dyes for antitumor activities, which was, in turn, consistent with the hypothesis of the DLCs.

Table 2. Effect of Heterocycles on Antitumor Activity

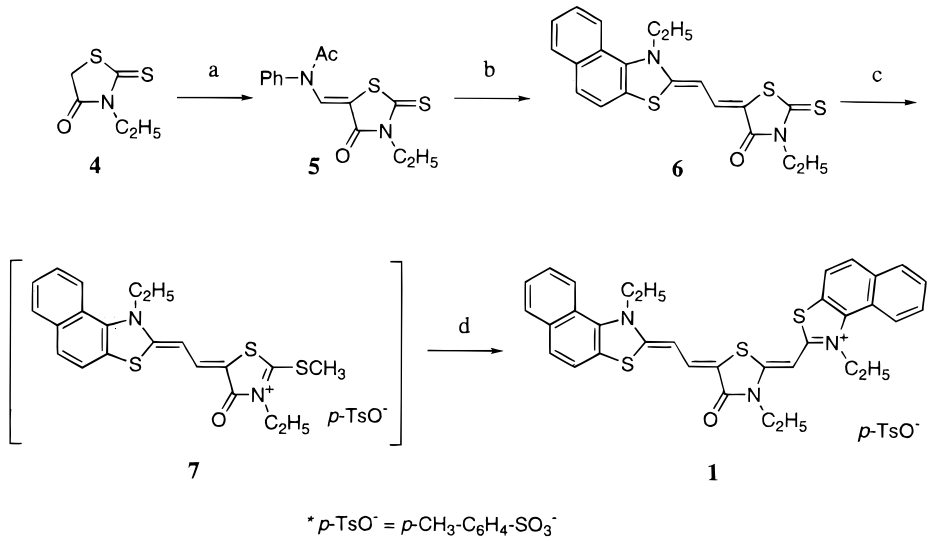


Compd.	A	B	X^-	in vitro IC_{50}		in vivo
				CX-1 (clonogenic)	KB (cytotoxic)	T/C[a] (LOX)
				(μM)	(μM)	%
13			AcO^-	1.10	0.86	> 310%*[b] (5 mg/kg/day × 5)
25			AcO^-	0.06	0.06	163** (4 × 7)
26			*p*-TsO^-	1.76	2.17	
27			Cl^-	> 2.40	3.73	
28			Cl^-	0.05	0.34	112 (7 × 5)
29			AcO^-	0.68	0.08	120 (2 × 5)
30			AcO^-	0.16	0.25	106 (4 × 5)
31			AcO^-	1.71	0.86	118 (3 × 5)

[a] T/C represents the ratio of the median survival time of drug-treated to control, untreated tumor-bearing mice, expressed as a percentage. Each group consisted of five nude mice.
[b] The statistical significance of difference between the control and the drug-treated group was determined by applying the Bartlett's, Dunnett's and Scheffet's test. * $P<0.01$, ** $P<0.001$.

Compounds **1**, **20**, **23**, and **24** which exhibited efficacy in the LOX nude mice model, were further evaluated for chemical stability and water solubility, which are important factors for clinical development. These compounds did not satisfy the criteria. Compound **20** decomposed rapidly in aqueous solution, and compound **24** decomposed in its solid state by 5% at 50 °C in a month. Although compounds **1** and **23** were chemically stable, they were poorly soluble in plasma because of their high hydrophobicity. They were observed to precipitate in several organs, especially in the lung, even when administered at effective doses to mice, and such

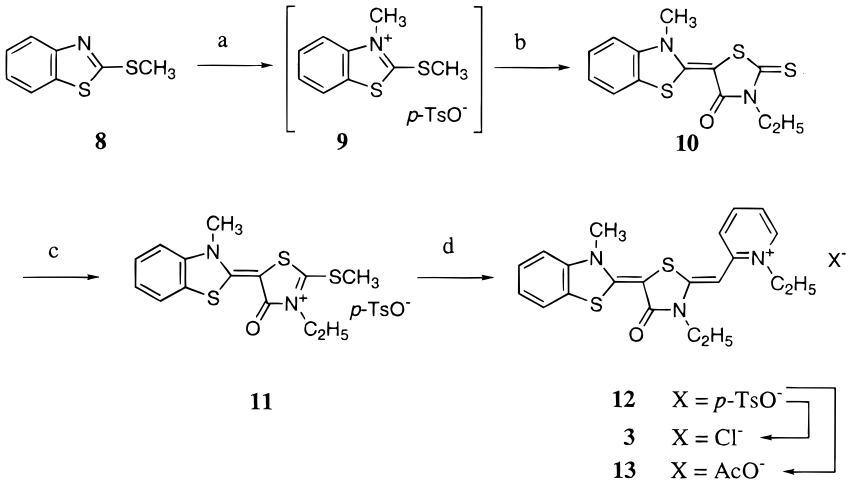
Scheme 1[a]



* *p*-TsO$^-$ = *p*-CH_3-C_6H_4-SO_3^-

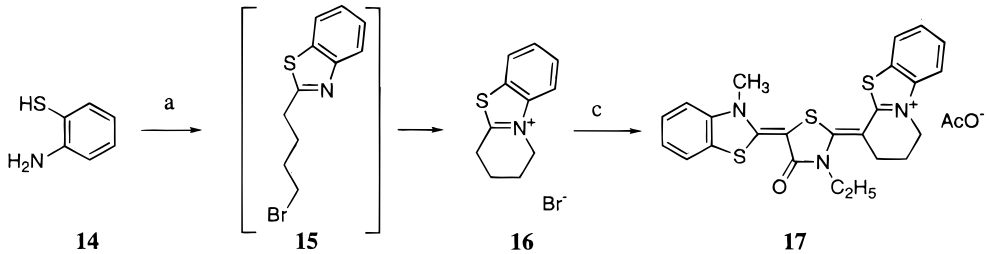
[a] Reagents: (a) (i) 1,3-diaza-1,3-diphenylpropene/ligroin, (ii) Ac_2O/NEt_3; (b) 3-ethyl-2-methylnaphtho[1,2-*d*]thiazolium *p*-toluenesulfonate/Ac_2O/NEt_3/MeCN; (c) methyl *p*-toluenesulfonate/DMF/toluene; (d) 3-ethyl-2-methylnaphtho[2,1-*d*]thiazolium *p*-toluenesulfonate/NEt_3/MeCN.

Scheme 2[a]



[a] Reagents: (a) methyl *p*-toluenesulfonate/anisole; (b) 3-ethyl-4-oxothiazolidine-2-thione (**4**)/NEt_3/MeCN; (c) methyl *p*-toluenesulfonate/DMF; (d) (i) 1-ethyl-2-methylpyridinium *p*-toluenesulfonate/NEt_3/MeCN, (ii) PA-318/MeOH/CH_2Cl_2.

Scheme 3[a]



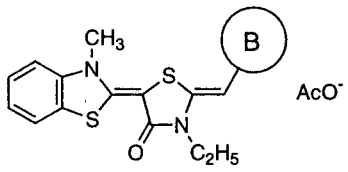
[a] Reagents: (a) 5-bromovaleryl chloride/xylene; (c) (i) **11**/NEt_3/MeCN, (ii) WA-21/MeOH/CH_2Cl_2.

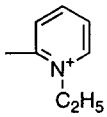
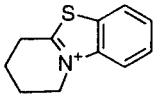
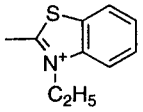
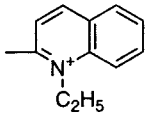
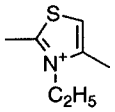
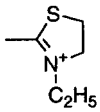
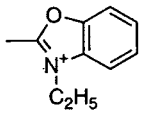
precipitations might be conducive to the development of serious adverse effects.

Being unable to find a compound for further development from class I series, we next worked on the class II series. In this series, on the basis of our previously obtained data in Table 1 (**21a**−**e**: note that a longer alkyl chain had an unfavorable effect on the solubility), the *N*-substituent of the B ring was retained as the constant ethyl group. As summarized in Table 2, class II rhodacyanine dyes, which have the thiazole (including benzo- and naphtho-fused) or thiazoline as their A ring, exhibited significant in vitro antitumor activity, in general. Replacing sulfur with oxygen (**13** vs **27**) led to a decrease in the activity. Reversing the A and the B ring of compound **13** also resulted in a decrease in activity for compound **26**.

On the basis of these findings, class II rhodacyanine dyes having the thiazole or thiazoline as the A ring

Table 3. Effect of Heterocycles on Antitumor Activity and Toxicity



Compd.	B	in vitro IC_{50} CX-1 (clonogenic)	KB (cytotoxic)	CV-1 (cytotoxic)	in vivo T/C[a] (LOX)	in vivo T.I.[b] (CA755)	Toxicity LD_{50} i.v.	Toxicity LD_{50} i.p.
		(μM)	(μM)	(μM)	%	%	(mg/kg)	(mg/kg)
13		1.10	0.86	63.2	> 310*[c] (5 mg/kg/day × 5)	76.5** (3 mg/kg/day × 4)	20	50
17		0.23	0.23	2.9	> 208** (4 × 5)		3	30
25		0.06	0.06	1.4	163** (4 × 7)	59.4 (1 × 4)	5	30
32		0.28	0.28	8.7	124 (2 × 5)		5-10	30
33		0.11	0.95	23.6	> 173* (4 × 5)	45.6 (3 × 4)	20	> 30
34		1.04	6.22	13.2	133 (2 × 5)		15	40
35		0.18	1.13	14.7	140 (5 × 5)		> 10	5-10

[a] T/C represents the ratio of the median survival time of drug-treated to control, untreated tumor-bearing mice, expressed as percentage. Each group consisted of five nude mice. [b] TI represents tumor inhibition ratio, which was evaluated at day 11 and calculated as follows: inhibition ratio (%) = $(A - B)/A \times 100$, where A is the average tumor weight in the control group and B is that in the treated group. Each group consisted of five nude mice. [c] The statistical significance of difference between the control and the drug-treated group was determined by applying the Bartlett's, Dunnett's, and Scheffer's test. *$P < 0.01$, **$P < 0.001$.

shown in Table 2 were further evaluated for their in vivo efficacy. Compound **13** was the most efficacious in prolongation of survival time with T/C = >310%, which implied a cure, followed by compound **25** with T/C = 163%. These two compounds were also found to have satisfactory chemical properties, i.e., good solubility in water (>10 mg/mL) and chemical stability (little decomposition either in solid at 95 °C for 2 weeks or in aqueous solution at 50 °C for 2 weeks).

Compounds **13** and **25**, which exhibited the most favarable biological and chemical properties, both possessed a benzothiazole moiety for the A ring. Therefore, we decided to have a benzothiazole moiety as ring A and introduce various heterocyclic groups for the B ring in the class II series. Most of the compounds thus designed displayed significant in vitro activities against CX-1 and KB, with IC_{50} values of below 1.1 μM (Table 3). Among them, compound **13**, in particular again, was found to have exceptionally low toxic activity against the normal kidney cell line, CV-1 (IC_{50} = 63.2 μM), and consequently it also exhibited selective toxicity against tumor cells. An in vivo study (the LOX ip xenograft model) of the compounds demonstrated that compounds **13**, **17**, **25**, **33**, and **35** displayed marked efficacy, with T/C >140% at doses of 4−5 mg/kg (ip administration). Among the compounds, compound **17** showed a relatively high acute toxicity (single intravenous (iv) administration into ICR mice) with LD_{50} = 3 mg/kg, and compound **35** was chemically unstable because the oxazolium moiety was easily hydrolyzed. These two compounds were, therefore, eliminated from the list of candidates for further study. The remaining compounds **13**, **25**, and **33** were chemically stable. They were further evaluated for their inhibitory effect using

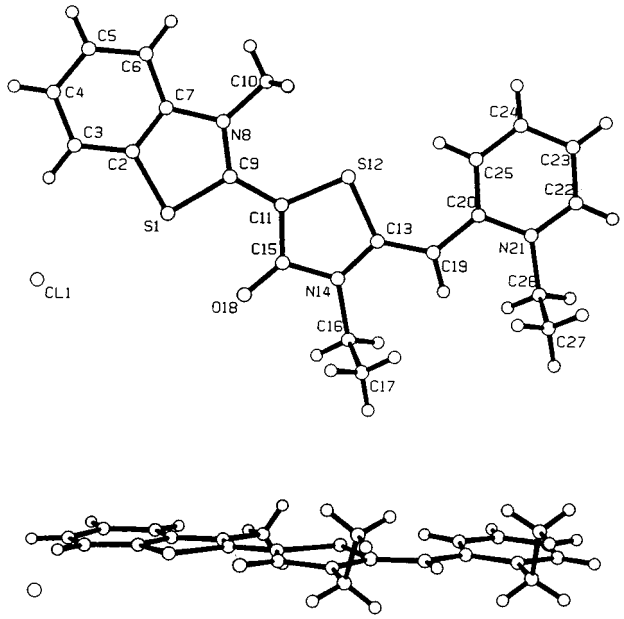


Figure 1. X-ray-determined structure of rhodacyanine dye **3**.

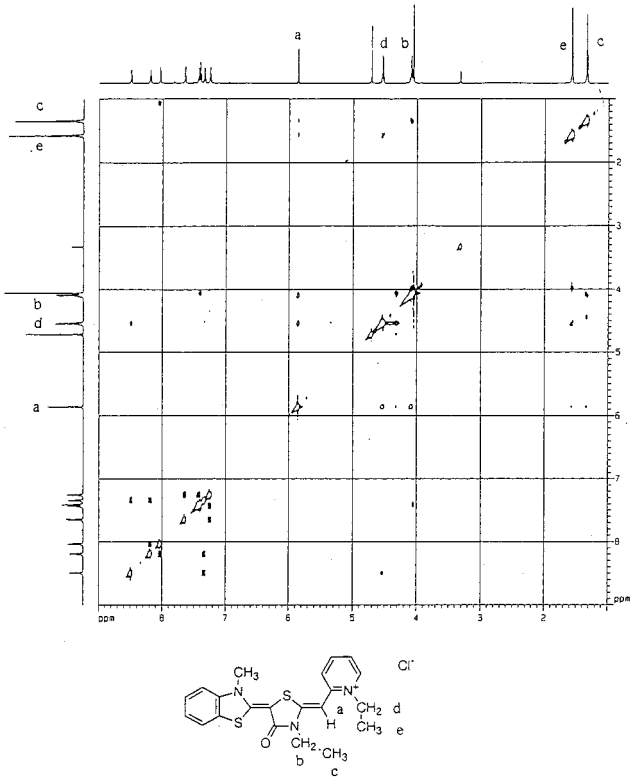


Figure 2. ROESY spectrum of rhodacyanine dye **3** (600 MHz, $DC_3OD/CDCl_3$ (10/1)).

the adenocarcinoma CA755 subcutaneous (sc) allograft model and iv treatment in BDF_1 mice. Compound **13** displayed the highest efficacy with tumor inhibition ratio (TI) = 76.5%, while compounds **25** and **33** were also moderately effective with TI = 59.4 and 45.6%, respectively. An acute toxicity study demonstrated that compound **13** showed the lowest toxicity (LD_{50} = 20 mg/kg (iv), 50 mg/kg (ip)). The pyridinium ring was, therefore, selected as the optimal moiety for the B ring.

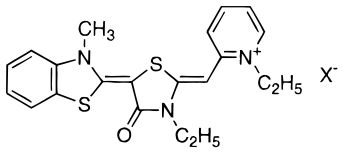
After finding that the best ring combination through our experiments with the class II series was that of A (benzothiazole) and B (pyridine), we focused our final attention on the counteranion, which might affect the chemical (stability, solubility) and the biological (antitumor effect, toxicity) properties. As shown in Table 4, while the iodide **36** was poorly soluble in water and saline, conversion to the chloride and acetate (**3** and **13**) apparently enhanced the solubility by more than 25-fold. However, despite its significant effect on the chemical properties, the variation of the counteranion had little effect on its biological properties. Compounds **3**, **13** and **36** displayed quite similar in vitro activities against KB cells. The former two compounds, whose water solubility were high enough for in vivo studies, exhibited virtually the same in vivo efficacy (against LOX and CA755) and toxicity. While there were no significant differences found in the chemical and the biological properties of the compounds **3** and 1**3**, the former could be more readily prepared from *p*-toluenesulfonate salt (**12** in Scheme 2) by using a commercially available anion-exchange resin (quaternary ammonium chloride form). Thus, we selected chloride as the preferred counteranion.

On the basis of the data obtained from its in vitro activity, in vivo efficacy and toxicity, chemical stability, water solubility, and synthetic availability, we finally selected compound **3** (named MKT 077) as the candidate for clinical trials. Extensive studies on pharmacology, pharmacokinetics, toxicity, and mechanism of action were carried out.[11−13] Mechanistic studies have demonstrated that **3** inhibited respiratory activity in mitochondrial membrane fragments in a dose-dependent manner.[11] Further, our study showed that under the same conditions and without a loss of nuclear DNA, **3** caused a selective loss of mitochondrial DNA in CX-1 cells (carcinoma), but not CV-1 cells (normal epithelial). **3** is the first antitumor agent which selectively inhibits mitochondrial function, and the phase I clinical trials are in progress.

Conclusion

Various class I and II rhodacyanine dyes were synthesized and evaluated for their chemical (water solubility, chemical stability) and biological (in vitro antitumor activity, in vivo efficacy and toxicity) properties. Our attention and effort was particularly focused on the structure−activity study of heteroaromatic rings, which were conjugated to rhodanine moiety. This led to the finding of benzothiazole (A ring) plus pyridinium ring (B ring) as the best combination in class II series. The reversal of these positions resulted in a decrease in activity. The counteranion had a marked effect on solubility in water, while it had virtually no effect on the biological properties. The selected compound **3** (MKT 077) displayed high-level efficacy in both LOX xenograft and CA755 allograft models with low acute toxicity. From this work, compound **3** emerged as a promising antitumor agent and has entered into phase I clinical trials.

Table 4. Effect of Anions on Solubility and in Vitro Activity



		solubility (mg/mL)		IC_{50} (μM)		in vivo		toxicity LD_{50} mg/kg	
compd	X^-	H_2O	0.9% saline	KB cell	CV-1	T/C (%)[a] (LOX)	TI (%)[b] (CA755)	iv	ip
3	Cl^-	>5.0	>5.0	0.81	>69.0	>344**[c] (5 mg/kg/day × 4)	75.5** (3 mg/kg/day × 4)	20	50
13	AcO^-	>5.0	>5.0	0.86	63.2	>310* (3 mg/kg/day × 4)	76.5 (3 mg/kg/day × 4)	20	50
36	I^-	0.2	<0.1	0.80	46.8				

[a] T/C represents the ratio of the median survival time of drug-treated to control, untreated tumor-bearing mice, expressed as percentage. Each group consisted of five nude mice. [b] TI represents tumor inhibition ratio, which was evaluated at day 11 and calculated as follows: inhibition ratio (%) = $(A - B)/A \times 100$, where A is the average tumor weight in the control group and B is that in the treated group. Each group consisted of five nude mice. [c] The statistical significance of difference between the control and the drug-treated group was determined by applying the Bartlett's, Dunnett's, and Scheffer's test. *$P < 0.01$, **$P < 0.001$.

Experimental Section

The ^{1}H NMR spectra were recorded on a Bruker AMX-600, ARX-300, or AC-200 spectrometer with tetramethylsilane as internal standard. Chemical shifts are given in ppm, coupling constants are in hertz, and splitting patterns are designated as follows: s, singlet; brs, broad singlet; d, doublet; dd, doublet of doublets; t, triplet; q, quartet; f, fifthtet; m, multiplet; dd, doublet of doublets. Ultraviolet−visible (UV−vis) absorption spectra were recorded on a Shimadzu UV-260 spectrophotometer. Fast atom bombardment (FAB) mass spectra were determined with a JEOL DX303 mass spectrometer, and high-resolution mass spectrometry (HRMS) was recorded on a JEOL SX-102A mass spectrometer; electron ionization (EI) mass spectra were determined with a JEOL JMS-D300 instrument. Elemental analyses were performed on Yanagimoto MT-3 and Dionex 2000i/SP instruments, and the results (C, H, N) were within ±0.4% of theoretical values unless indicated otherwise. Because of hygroscopicity, correct elemental analyses for most of the compounds could only be obtained by factoring in partial hydration of these organic salts.

5-[(*N*-Acetyl-*N*-phenylamino)methylidene]-3-ethyl-4-oxothiazolidine-2-thione (5). To a solution of 3-ethyl-4-oxothiazolidine-2-thione (**4**) (25.0 g, 155 mmol) in ligroin (145 mL) was added 1,3-diaza-1,3-diphenylpropene (32.5 g, 166 mmol), and the mixture was stirred at 70 °C for 1 h. After the mixture was cooled to room temperature, the precipitate was collected and washed with acetone (100 mL) to give 3-ethyl-4-oxo-5-[(phenylamino)methylidene]thiazoline-2-thione (45.0 g). This product was mixed with acetic anhydride (110 g, 1080 mmol) and triethylamine (0.18 g, 2 mmol), and the mixture was stirred at 110 °C for 30 min. The reaction mixture was concentrated to about half volume under reduced pressure. To this residue was added methanol (225 mL), and the mixture was stirred at 10 °C for 1 h. The precipitate formed was collected and washed with methanol (100 mL) to give **5** (37.0 g, 77.9%) as yellow crystals: ^{1}H NMR (DMSO-d_6) δ 1.08 (t, J = 7.2 Hz, 3H), 2.02 (s, 3H), 3.95 (q, J = 7.2 Hz, 2H), 7.47−7.57 (m, 2H), 7.59−7.72 (m, 3H), 8.47 (s, 1H); MS (EI) m/z 306. Anal. ($C_{14}H_{14}N_2O_2S_2$) C, H, N, S.

3-Ethyl-5-[2-(3-ethylnaphtho[1,2-*d*]thiazolin-2-ylidene)ethylidene]-4-oxothiazolidine-2-thione (6). A mixture of **5** (29.8 g, 97 mmol), 3-ethyl-2-methylnaphtho[1,2-*d*]-thiazolinium *p*-toluenesulfonate (38.8 g, 97 mmol), and acetic anhydride (14.2 g, 140 mmol) in acetonitrile (1000 mL) was stirred at 50 °C for 1 h. To this was added triethylamine (36.3 g, 359 mmol) at 50 °C, and the mixture was stirred for an additional 4 h at 60 °C. After the mixture was cooled to 25 °C, the precipitate formed was collected and washed with acetonitrile (250 mL). The crude product thus obtained was suspended in methanol (750 mL), and the mixture was stirred under reflux for 1 h. After the mixture was cooled to 25 °C, the precipitate was collected to give **6** (27.4 g, 70.7%) as purple-red crystals: ^{1}H NMR (DMSO-d_6) δ 1.16 (t, J = 7.2 Hz, 3H), 1.59 (t, J = 7.2 Hz, 3H), 4.04 (q, J = 7.2 Hz, 2H), 4.66 (q, J = 7.2 Hz, 2H), 5.58 (d, J = 13.2 Hz, 1H), 7.54−7.69 (m, 3H), 7.85−7.93 (m, 2H), 8.08 (d, J = 9.0 Hz, 1H), 8.46 (d, J = 9.0 Hz, 1H); MS (HRMS) for $C_{20}H_{18}N_2OS_3$ calcd 398.0581, found 398.0572. Anal. ($C_{20}H_{18}N_2OS_3$) C, H; N: calcd, 24.13; found, 24.58.

3-Ethyl-2-[[3-ethyl-5-[2-(3-ethylnaphtho[1,2-*d*]thiazolin-2-ylidene)ethylidene]-4-oxothiazolidin-2-ylidene]methyl]naphtho[1,2-*d*]thiazolium *p*-Toluenesulfonate (1). A mixture of **6** (14.5 g, 36 mmol) and methyl *p*-toluenesulfonate (20.2 g, 109 mmol) in *N*,*N*-dimethylformamide (35 mL) and toluene (13 mL) was stirred at 115 °C for 6 h. To the mixture was added 3-ethyl-2-methylnaphtho[1,2-*d*]thiazolinium *p*-toluenesulfonate (14.7 g, 36 mmol) in acetonitrile (1080 mL). To this was added triethylamine (11.0 g, 109 mmol) at 75 °C, and the resulting mixture was stirred at 75 °C for an additional 1 h and then cooled to 30 °C. The precipitate formed was collected and washed with acetonitrile (300 mL). The crude product thus obtained was suspended in methanol (720 mL), and the mixture was stirred under reflux for 1 h. After the mixture was cooled to 25 °C, the precipitate was collected and washed with acetonitrile (300 mL). Compound **1** (20.4 g, 74.1%) was obtained as green crystals: ^{1}H NMR (DMSO-d_6) δ 1.28 (t, J = 7.2 Hz, 3H), 1.65−1.72 (m, 6H), 2.29 (s, 3H), 4.17 (q, J = 7.2 Hz, 2H), 4.72 (q, J = 7.2 Hz, 2H), 4.98 (q, J = 7.2 Hz, 2H), 6.02 (d, J = 12.9 Hz, 1H), 6.62 (s, 1H), 7.05−7.82 (m, 12H), 7.97 (d, J = 8.4 Hz, 1H), 8.03−8.12 (m, 2H), 8.30 (d, J = 8.4 Hz, 1H), 8.52 (d, J = 8.4 Hz, 1H); MS (FAB^+, nitrobenzyl alcohol) for $C_{34}H_{30}N_3OS_3$ m/z 592, (FAB^-, nitrobenzyl alcohol) for $C_7H_7O_3S$ m/z 171; HRMS (FAB^+) for $C_{34}H_{30}N_3OS_3$ calcd 592.1551, found 592.1561. Anal. ($C_{41}H_{37}N_3O_4S_4 \cdot 2.7H_2O$) C, N, S; H: calcd, 5.26; found, 4.84.

3-Ethyl-5-(3-methylbenzothiazolin-2-ylidene)-4-oxothiazolidine-2-thione (10). A mixture of 2-(methylthio)-benzothiazole (**8**) (40.0 g, 220 mmol), methyl *p*-toluenesulfonate (61.5 g, 330 mmol), and anisole (56 mL) was stirred at 120−136 °C for 4 h. After the mixture was cooled to room temperature, 3-ethyl-4-oxothiazolidine-2-thione (**4**) (35.0 g, 220 mmol) and acetonitrile (800 mL) were added. To this mixture was added triethylamine (36.4 g, 360 mmol) dropwise under 15 °C with constant stirring and cooling, and the resulting mixture was stirred at 10 °C for 4 h. The yellow precipitate was collected and washed with acetonitrile (40 mL) and then with methanol (140 mL). The crude product thus obtained was suspended in acetone (210 mL) and methanol (420 mL), and the mixture was stirred under reflux for 15 min. After the mixture was cooled to 25 °C, the precipitate was collected and washed with methanol (140 mL) to give **10** (59.0 g, 87.0%) as yellow crystals: ^{1}H NMR (DMSO-d_6) δ 1.20 (t, J = 7.2 Hz, 3H), 3.98 (s, 3H), 4.10 (q, J = 7.2 Hz, 2H), 7.35 (t, J = 8.0 Hz,

1H), 7.52 (t, J = 8.0 Hz, 1H), 7.78 (d, J = 8.0 Hz, 1H), 7.93 (d, J = 8.0 Hz, 1H); MS (EI) m/z 308. Anal. ($C_{13}H_{12}N_2OS_3$) C, H, N, S.

3-Ethyl-5-(3-methylbenzothiazolin-2-ylidene)-2-(methylthio)-4-oxothiazolinium *p*-Toluenesulfonate (11). A mixture of **10** (58.0 g, 188 mmol), methyl *p*-toluenesulfonate (105.0 g, 564 mmol), and *N*,*N*-dimethylformamide (58 mL) was stirred at 130−145 °C for 2.5 h. After the mixture was cooled to 95 °C, acetone (500 mL) was added. The mixture was further cooled to 25 °C with constant stirring, and the precipitate formed was collected and washed with acetone (150 mL). The crude product thus obtained was suspended in acetone (400 mL) and the mixture was stirred under reflux for 15 min. After being cooled to 25 °C, the precipitate was collected and washed with acetone (150 mL) to give **11** (86.5 g, 93.0%) as orange crystals: ^{1}H NMR (DMSO-d_6) δ 1.33 (t, J = 7.2 Hz, 3H), 2.27 (s, 3H), 3.05 (s, 3H), 4.17 (q, J = 7.2 Hz, 2H), 4.24 (s, 3H), 7.10 (d, J = 8.9 Hz, 2H), 7.46 (d, J = 8.9 Hz, 2H), 7.52 (t, J = 8.0 Hz, 1H), 7.70 (t, J = 8.0 Hz, 1H), 8.00 (d, J = 8.0 Hz, 1H), 8.18 (t, J = 8.0 Hz, 1H); MS (FAB$^+$, glycerine) for $C_{14}H_{15}N_2OS_3$ m/z 323, (FAB$^-$, triethanolamine) for $C_7H_7O_3S$ m/z 171. Anal. ($C_{21}H_{22}N_2O_4S_4\cdot0.3H_2O$) C, H, N, S.

1-Ethyl-2-[[3-ethyl-5-(3-methylbenzothiazolin-2-ylidene)-4-oxothiazolidin-2-ylidene]methyl]pyridinium *p*-Toluenesulfonate (12). To a mixture of **11** (24.7 g, 50 mmol) and 3-ethyl-2-methylpyridinium *p*-toluenesulfonate (14.7 g, 50 mmol) in acetonitrile (250 mL) was added triethylamine (15.2 g, 150 mmol) dropwise at 70 °C, and the mixture was stirred for 1.5 h at the same temperature. To the reaction mixture was added ethyl acetate (250 mL), and the mixture was cooled to 30 °C with constant stirring. The orange precipitate was collected and washed with ethyl acetate (125 mL). The crude product thus obtained was dissolved in methanol (80 mL), and then to this solution was added ethyl acetate (250 mL) at 50 °C with constant stirring. The precipitate was collected and washed with ethyl acetate (110 mL) to give **12** (14.2 g, 50.0%) as orange crystals: ^{1}H NMR (DMSO-d_6) δ 1.24 (t, J = 7.2 Hz, 3H), 1.43 (t, J = 7.2 Hz, 3H), 2.28 (s, 3H), 4.03 (s, 3H), 4.11 (q, J = 7.2 Hz, 2H), 4.60 (q, J = 7.2 Hz, 2H), 5.98 (s, 3H), 7.11 (d, J = 8.9 Hz, 2H), 7.28 (dd, J = 8.0, 8.0 Hz, 1H), 7.47 (dd, J = 8.0, 8.0 Hz, 1H), 7.49 (d, J = 8.9 Hz, 2H), 7.61 (dd, J = 8.0, 8.0 Hz, 1H), 7.88 (dd, J = 8.0, 8.0 Hz, 1H), 8.06 (d, J = 8.0 Hz, 1H), 8.26 (t, J = 8.0 Hz, 1H), 8.70 (d, J = 8.0 Hz, 1H); MS (FAB$^+$, glycerine) for $C_{21}H_{22}N_3OS_2$ m/z 396, (FAB$^-$, triethanolamine) for $C_7H_7SO_3$ m/z 171. Anal. ($C_{28}H_{29}N_3O_4S_3\cdot0.7H_2O$) C, H, N, S.

1-Ethyl-2-[[3-ethyl-5-(3-methylbenzothiazolin-2-ylidene)-4-oxothiazolidin-2-ylidene]methyl]pyridinium Chloride (3, MKT 077). A solution of **12** in the solution (375 mL) of chloroform/methanol (1/2, v/v) was passed through the basic anion-exchange resin (about 350 mL, Diaion PA-318, chloride form), and the resin was washed with a solution (375 mL) of chloroform/methanol (1/2, v/v) and then methanol (250 mL). The eluent was evaporated, and the residue was dissolved in ethanol (275 mL) at 60 °C and then cooled to 45 °C. To this solution was added ethyl acetate (725 mL) at 40−45 °C with constant stirring. The precipitate was collected and washed with ethyl acetate (175 mL) to give **3** (9.0 g, 42.0%) as orange crystals: mp 253−257 °C dec; IR (KBr) 760, 1050, 1160, 1385, 1515, 1550, 1635, 1650, 2900−3000 cm^{-1}; UV−vis λ_{max} = 494.0 (MeOH, ϵ = 4.94 × 10^4), 486 nm (H_2O, ϵ = 4.56 × 10^4); ^{1}H NMR (CD_3OD:$CDCl_3$ = 10:1) δ 1.34 (t, J = 7.2 Hz, 3H), 1.57 (t, J = 7.2 Hz, 3H), 4.05 (s, 3H), 4.09 (q, J = 7.2 Hz, 2H), 4.54 (q, J = 7.2 Hz, 2H), 5.86 (s, 1H), 7.25 (ddd, J = 8.0, 7.0, 1.4 Hz, 1H), 7.34 (ddd, 7.5, 6.6, 1.4 Hz, 1H), 7.40 (dd, J = 8.3, 1.4 Hz, 1H), 7.44 (ddd, J = 8.3, 7.0, 1.4 Hz, 1H), 7.65 (dd, J = 8.0, 1.4 Hz, 1H), 8.04 (dd, J = 8.5, 1.4 Hz), 8.19 (ddd, 8.5, 7.5, 1.9 Hz, 1H), 8.49 (dd, 6.6, 1.9 Hz, 1H); ^{13}C NMR (CD_3OD + $CDCl_3$) δ 12.5, 14.7, 35.2, 39.8, 53.8, 80.2, 84.4, 112.4, 120.5, 122.8, 125.0, 125.1, 127.5, 128.3, 141.4, 143.6, 144.8, 152.0, 153.5, 157.0, 165.7; MS (FAB$^+$, glycerine) for $C_{21}H_{22}N_3OS_2$ m/z 396, (FAB$^-$, triethanolamine) for Cl m/z 35; HRMS (FAB$^+$) for $C_{21}H_{22}N_3OS_2$ calcd 396.1204, found 396.1186. Anal. ($C_{21}H_{22}ClN_3OS_2$) C, H, Cl, N, S.

1-Ethyl-2-[[3-ethyl-5-(3-methylbenzothiazolin-2-ylidene)-4-oxothiazolidin-2-ylidene]methyl]pyridinium Acetate (13). This compound was prepared from *p*-toluenesulfonate salt **(12)** using the anion-exchange resin (Diaion WA-21, acetate form): ^{1}H NMR (DMSO-d_6) δ 1.22 (t, J = 7.2 Hz, 3H), 1.45 (t, J = 7.2 Hz, 3H), 1.74 (s, 3H), 4.03 (s, 3H), 4.10 (q, J = 7.2 Hz, 2H), 4.61 (q, J = 7.2 Hz, 2H), 5.98 (s, 1H), 7.28 (dd, J = 8.0, 8.0 Hz, 1H), 7.43−7.51 (m, 2H), 7.59 (d, J = 8.0 Hz, 1H), 7.86 (d, J = 8.0 Hz, 1H), 8.06 (d, J = 8.0 Hz, 1H), 8.28 (dd, J = 8.0, 8.0 Hz, 1H), 8.71 (d, J = 8.0 Hz, 1H); MS (FAB$^+$, glycerine) for $C_{21}H_{22}N_3OS_2$ m/z 396, (FAB$^-$, triethanolamine) for Cl m/z 35; HRMS (FAB$^+$) for $C_{21}H_{22}N_3OS_2$ calcd 396.1204, found 396.1217.

Tetrahydrobenzo[*b*]benzothiazolium Bromide (16). To a solution of 2-mercaptoaniline (14.9 g, 0.12 mol) in xylene (150 mL) was added 5-bromovaleryl chloride (25.0 g, 0.13 mol) dropwise at room temperature. The mixture was stirred under reflux for 1.5 h. After the mixture was cooled to room temperature, the supernatant was removed, and to the residual oil were added methanol (20 mL) and then ethyl acetate (100 mL). The precipitate formed was collected and washed with ethyl acetate (30 mL) to give **16** (9.7 g, 30.7%) as pale brown crystals: ^{1}H NMR (DMSO-d_6) δ 2.02−2.08 (m, 2H), 2.18−2.23 (m, 2H), 3.58 (t, J = 7.0 Hz, 2H), 4.58 (t, J = 7.0 Hz, 2H), 7.79−7.92 (m, 2H), 8.24 (d, J = 8.4 Hz, 1H), 8.45 (t, J = 8.4 Hz, 1H); MS (FAB$^+$, nitrobenzyl alcohol) for $C_{11}H_{12}NS$ m/z 190, (FAB$^-$, triethanolamine) for Br m/z 79, 81; HRMS (FAB$^+$) for $C_{11}H_{12}NS$ calcd 190.0690, found 190.0699. Anal. ($C_{11}H_{12}BrNS\cdot0.5H_2O$) C, H, Br, N, S.

1-[3-Ethyl-5-(3-methylbenzothiazolin-2-ylidene)-4-oxothiazolidine-2-ylidene]tetrahydrobenz[*b*]benzothiazolium Acetate (17). This compound was synthesized from intermediates **11** and **16**, in a manner analogous to the preparation of rhodacyanine dye **13**: ^{1}H NMR (DMSO-d_6) δ 1.34 (t, J = 7.2 Hz, 3H), 1.56 (s, 3H), 2.20−2.22 (m, 2H), 3.05−3.08 (m, 2H), 4.20 (s, 3H), 4.23 (t, J = 7.2 Hz, 2H), 4.32−4.35 (m, 2H), 7.41 (t, J = 8.4 Hz, 1H), 7.52 (t, J = 8.4 Hz, 1H), 7.57 (t, J = 8.4 Hz, 1H), 7.68 (t, J = 8.4 Hz, 1H), 7.77−7.83 (m, 2H), 8.01 (t, J = 8.4 Hz, 1H), 8.15 (t, J = 8.4 Hz, 1H); MS (FAB$^+$, nitrobenzyl alcohol) for $C_{24}H_{22}N_3OS_3$ m/z 464, (FAB$^-$, triethanolamine) for $C_2H_3O_2$ m/z 59; HRMS (FAB$^+$) for $C_{24}H_{22}N_3OS_3$ calcd 464.0925, found 464.0921. Anal. ($C_{26}H_{25}BrN_3O_3S_3\cdot3.5H_2O$) C, N; S: calcd, 5.50; found, 4.91.

3-Ethyl-2-[[3-ethyl-5-[2-(3-methylnaphtho[1,2-*d*]thiazolin-2-ylidene)ethylidene]-4-oxothiazolidin-2-ylidene]-methyl]-4-methylthiazolium Acetate (18). The *p*-toluenesulfonate salt of the target compound was synthesized from 2,3-dimethylnaphtho[1,2-*d*]thiazolinium *p*-toluenesulfonate, 3-ethyl-4-oxothiazolidine-2-thione **(4)**, and 3-ethyl-2,4-dimethylthiazolium *p*-toluenesulfonate in a manner analogous to the preparation of rhodacyanine dye **1**, and it was converted to acetate salt **(18)** using anion-exchange resin (Diaion WA-21, acetate form): ^{1}H NMR (DMSO-d_6) δ 1.18 (t, J = 7.2 Hz, 3H), 1.29 (t, J = 7.2 Hz, 3H), 1.69 (s, 3H), 2.38 (s, 3H), 4.04 (t, J = 7.2 Hz, 2H), 4.19 (s, 3H), 4.35 (t, J = 7.2 Hz, 2H), 5.86 (d, J = 13.0 Hz, 1H), 6.45 (s, 1H), 7.50−7.65 (m, 4H), 7.78−7.89 (m, 2H), 8.01 (d, J = 8.3 Hz, 1H), 8.58 (d, J = 8.3 Hz, 1H); MS (FAB$^+$, nitrobenzyl alcohol) for $C_{26}H_{26}N_3OS_3$ m/z 492, (FAB$^-$, nitrobenzyl alcohol) for $C_2H_3O_2$ m/z 59; HRMS (FAB$^+$) for $C_{26}H_{26}N_3OS_3$ calcd 492.1238, found 492.1234.

3-Ethyl-2-[[3-ethyl-5-[2-(3-ethylnaphtho[2,1-*d*]thiazolin-2-ylidene)ethylidene]-4-oxothiazolidin-2-ylidene]methyl]naphtho[1,2-*d*]oxazolium *p*-Toluenesulfonate (19). This compound was synthesized from 2,3-dimethylnaphtho[2,1-*d*]-thiazolinium *p*-toluenesulfonate, 3-ethyl-4-oxothiazolidine-2-thione **(4)**, and 3-ethyl-2-methylnaphtho[1,2-*d*]oxazolium *p*-toluenesulfonate in a manner analogous to the preparation of rhodacyanine dye **1**: ^{1}H NMR (DMSO-d_6) δ 1.25 (t, J = 7.2 Hz, 3H), 1.41 (t, J = 7.2 Hz, 3H), 1.58 (t, J = 7.2 Hz, 3H), 2.29 (s, 3H), 4.15 (q, J = 7.2 Hz, 2H), 4.47 (q, J = 7.2 Hz, 2H), 4.92 (q, J = 7.2 Hz, 2H), 6.09 (d, J = 13.1 Hz, 1H), 6.50 (s, 1H), 7.10 (d, J = 7.9 Hz, 2H), 7.43−7.52 (m, 3H), 7.59 (t, J = 8.6 Hz, 1H), 7.70−7.90 (m, 5H), 8.02−8.11 (m, 3H), 8.19 (d, J = 8.6 Hz, 1H), 8.26 (d, J = 8.6 Hz, 1H), 8.48 (d, J = 8.6 Hz, 1H);

MS (FAB$^+$, nitrobenzyl alcohol) for $C_{34}H_{30}N_3O_2S_2$ *m/z* 576, (FAB$^-$, triethanolamine) for $C_7H_7O_3S$ *m/z* 171. Anal. ($C_{41}H_{37}N_3O_5S_3 \cdot 0.5H_2O$) C, H, N, S.

3-Ethyl-2-[[3-ethyl-5-[2-(3-ethyl-4-methylthiazolin-2-ylidene)ethylidene]-4-oxothiazolidin-2-ylidene]methyl]-benzothiazolium Acetate (20). The *p*-toluenesulfonate salt of the target compound was synthesized from 3-ethyl-2,4-dimethylthiazolinium *p*-toluenesulfonate, 3-ethyl-4-oxothiazolidine-2-thione **(4)**, and 3-ethyl-2-methylbenzothiazolium *p*-toluenesulfonate in a manner analogous to the preparation of rhodacyanine dye **1**, and it was converted to acetate salt **20** using anion-exchange resin (Diaion WA-21, acetate form): ^{1}H NMR (DMSO-d_6) δ 1.23 (t, J = 7.2 Hz, 3H), 1.28−1.39 (m, 6H), 1.76 (s, 3H), 2.33 (s, 3H), 4.11−4.24 (m, 4H), 4.69 (q, J = 7.2 Hz, 2H), 6.00 (d, J = 13.5 Hz, 1H), 6.69 (s, 1H), 6.95 (s, 1H), 7.53 (dd, J = 8.4, 8.4 Hz, 3H), 7.71 (dd, J = 8.4, 8.4 Hz, 1H), 7.74 (d, J = 13.5 Hz, 1H), 7.94 (d, J = 8.4 Hz, 1H), 8.20 (d, J = 8.4 Hz, 1H); MS (FAB$^+$, nitrobenzyl alcohol) for $C_{23}H_{26}N_3OS_3$ *m/z* 456, (FAB$^-$, nitrobenzyl alcohol) for $C_2H_3O_2$ *m/z* 59; HRMS (FAB$^+$) for $C_{23}H_{26}N_3OS_3$ calcd 456.1238, found 456.1231.

2-[[3-Ethyl-5-[2-(3-ethylthiazolidin-2-ylidene)ethylidene]-4-oxothiazolidin-2-ylidene]methyl]-3-methylnaphtho[2,1-*d*]thiazolium Iodide (21a). This compound was synthesized from 3-ethyl-2-methylthiazolinium *p*-toluenesulfonate, 3-ethyl-4-oxothiazolidine-2-thione **(4)**, and 2,3-dimethylnaphtho[2,1-*d*]thiazolium iodide in a manner analogous to the preparation of rhodacyanine dye **1**: ^{1}H NMR (DMSO-d_6) δ 1.26 (t, J = 7.2 Hz, 6H), 3.40−3.47 (m, 2H), 3.72 (q, J = 7.2 Hz, 2H), 4.09 (q, J = 7.2 Hz, 2H), 4.16−4.28 (m, 5H), 5.80 (d, J = 13.2 Hz, 1H), 6.83 (s, 1H), 7.62 (d, J = 13.2 Hz, 1H), 7.74 (dd, J = 7.9, 7.9 Hz, 1H), 7.87 (dd, J = 7.9, 7.9 Hz, 1H), 8.18 (d, J = 7.9 Hz, 1H), 8.21−8.28 (m, 2H), 8.32 (d, J = 7.9 Hz, 1H); MS (FAB$^+$, nitrobenzyl alcohol) for $C_{25}H_{26}N_3OS_3$ *m/z* 480, (FAB$^-$, nitrobenzyl alcohol) for I *m/z* 127. Anal. ($C_{25}H_{26}IN_3OS_3 \cdot 0.5H_2O$) C, H, I, N, S.

3-Ethyl-2-[[3-ethyl-5-[2-(3-ethylthiazolidin-2-ylidene)-ethylidene]-4-oxothiazolidin-2-ylidene]methyl]naphtho-[2,1-*d*]thiazolium Iodide (21b). This compound was synthesized from 3-ethyl-2-methylthiazolinium *p*-toluenesulfonate, 3-ethyl-4-oxothiazolidine-2-thione **(4)** and 3-ethyl-2-methylnaphtho[2,1-*d*]thiazolium iodide in a manner analogous to the preparation of rhodacyanine dye **1**: ^{1}H NMR (DMSO-d_6) δ 1.20−1.29 (m, 6H), 1.44 (t, J = 7.2 Hz, 3H), 3.40−3.50 (m, 2H), 3.72 (q, J = 7.2 Hz, 2H), 4.07 (t, J = 7.2 Hz, 2H), 4.19 (q, J = 7.2 Hz, 2H), 4.86 (q, J = 7.2 Hz, 2H), 5.80 (d, J = 13.2 Hz, 1H), 6.82 (s, 1H), 7.62 (d, J = 13.2 Hz, 1H), 7.75 (dd, J = 7.9, 7.9 Hz, 1H), 7.86 (d, J = 7.9, 7.9 Hz, 1H), 8.14−8.36 (m, 3H), 8.33 (d, J = 7.9 Hz, 1H); MS (FAB$^+$, nitrobenzyl alcohol) for $C_{26}H_{28}N_3OS_3$ *m/z* 494, (FAB$^-$, nitrobenzyl alcohol) for I *m/z* 127; HRMS (FAB$^+$) for $C_{26}H_{28}N_3OS_3$ calcd 494.1394, found 494.1396. Anal. ($C_{26}H_{28}IN_3OS_3 \cdot 1.3H_2O$) C, H, I, N, S.

3-Butyl-2-[3-ethyl-5-[2-(3-ethylthiazolidin-2-ylidene)-ethylidene]-4-oxothiazolidin-2-ylidene]methyl]naphtho-[2,1-*d*]thiazolium Iodide (21c). This compound was synthesized from 3-ethyl-2-methylthiazolinium *p*-toluenesulfonate, 3-ethyl-4-oxothiazolidine-2-thione **(4)**, and 3-buthyl-2-methylnaphtho[2,1-*d*]thiazolium iodide in a manner analogous to the preparation of rhodacyanine dye **1**: ^{1}H NMR (DMSO-d_6) δ 0.94 (t, J = 7.2 Hz, 3H), 1.22−1.29 (m, 6H), 1.40−1.50 (m, 2H), 1.83 (t, J = 7.2 Hz, 2H), 3.42 (t, J = 7.2 Hz, 2H), 3.73 (q, J = 7.2 Hz, 2H), 4.08 (t, J = 7.2 Hz, 2H), 4.20 (q, J = 7.2 Hz, 2H), 4.87 (t, J = 7.2 Hz, 2H), 5.80 (d, J = 13.2 Hz, 1H), 6.80 (s, 1H), 7.63 (d, J = 13.2 Hz, 1H), 7.74 (dd, J = 7.9, 7.9 Hz, 1H), 7.87 (dd, J = 7.9, 7.9 Hz, 1H), 8.14−8.36 (m, 4H); MS (FAB$^+$, nitrobenzyl alcohol) for $C_{28}H_{32}N_3OS_3$ *m/z* 522, (FAB$^-$, nitrobenzyl alcohol) for I *m/z* 127; HRMS (FAB$^+$) for $C_{28}H_{32}N_3OS_3$ calcd 522.1707, found 522.1697. Anal. ($C_{28}H_{32}IN_3OS_3 \cdot 0.5H_2O$) C, H, I, N, S.

2-[[3-Ethyl-5-[2-(3-ethylthiazolidin-2-ylidene)ethylidene]-4-oxothiazolidin-2-ylidene]methyl]-3-(2-hydroxyethyl)naphtho[2,1-*d*]thiazolium Iodide (21d). This compound was synthesized from 3-ethyl-2-methylthiazolinium *p*-toluenesulfonate, 3-ethyl-4-oxothiazolidine-2-thione **(4)**, and 3-(2-hydroxyethyl)-2-methylnaphtho[2,1-*d*]thiazolium iodide in a manner analogous to the preparation of rhodacyanine dye **1**: ^{1}H NMR (DMSO-d_6) δ 1.20−1.31 (m, 6H), 3.40−3.45 (m, 2H), 3.72 (q, J = 7.2 Hz, 2H), 3.93 (t, J = 7.2 Hz, 2H), 4.07−4.17 (m, 4H), 4.92 (t, J = 7.2 Hz, 2H), 5.78 (d, J = 13.2 Hz, 1H), 6.94 (s, 1H), 7.61 (d, J = 13.2 Hz, 1H), 7.74 (dd, J = 8.0, 8.0 Hz, 1H), 7.86 (dd, J = 8.0, 8.0 Hz, 1H), 8.14 (d, J = 8.0 Hz, 1H), 8.19−8.31 (m, 3H). MS (FAB$^+$, nitrobenzyl alcohol) for $C_{26}H_{28}N_3O_2S_3$ *m/z* 510, (FAB$^-$, nitrobenzyl alcohol) for I *m/z* 127; HRMS (FAB$^+$) for $C_{26}H_{28}N_3O_2S_3$ calcd 510.1343, found 510.1333. Anal. ($C_{26}H_{28}IN_3O_2S_3 \cdot 2.0H_2O$) C, H, I, N, S.

3-(2-Carboxyethyl)-2-[[3-ethyl-5-[2-(3-ethylthiazolidin-2-ylidene)ethylidene]-4-oxothiazolidin-2-ylidene]methyl]naphtho[2,1-*d*]thiazolium Iodide (21e). This compound was synthesized from 3-ethyl-2-methylthiazolinium *p*-toluenesulfonate, 3-ethyl-4-oxothiazolidine-2-thione **(4)**, and 3-(2-carboxyethyl)-2-methylnaphtho[2,1-*d*]thiazolium iodide in a manner analogous to the preparation of rhodacyanine dye **1**: ^{1}H NMR (DMSO-d_6) δ 1.22−1.30 (m, 6H), 2.90 (t, J = 7.2 Hz, 2H), 3.40−3.46 (m, 2H), 3.72 (q, J = 7.2 Hz, 2H), 4.05−4.13 (m, 4H), 4.97 (t, J = 7.2 Hz, 2H), 5.80 (d, J = 13.2 Hz, 1H), 6.95 (s, 1H), 7.61 (d, J = 13.2 Hz, 1H), 7.73 (dd, J = 8.0, 8.0 Hz, 1H), 7.84 (dd, J = 8.0, 8.0 Hz, 1H), 8.11 (d, J = 8.0 Hz, 1H), 8.16−8.28 (m, 3H); MS (FAB$^+$, nitrobenzyl alcohol) for $C_{27}H_{28}N_3O_3S_3$ *m/z* 538, (FAB$^-$, nitrobenzyl alcohol) for I *m/z* 127; HRMS (FAB$^+$) for $C_{27}H_{28}N_3O_3S_3$ calcd 538.1293, found 538.1290.

3-Ethyl-2-[[3-ethyl-5-[2-(1,3,3-trimethylindolin-2-ylidene)ethylidene]-4-oxothiazolidine-2-ylidene]methyl]-naphtho[2,1-*d*]oxazolium *p*-Toluenesulfonate (22). This compound was synthesized from 1,2,3,3-tetramethylindolinium *p*-toluenesulfonate, 3-ethyl-4-oxothiazolidine-2-thione **(4)**, and 3-ethyl-2-methylnaphtho[1,2-*d*]oxazolium *p*-toluenesulfonate in a manner analogous to the preparation of rhodacyanine dye **1**: ^{1}H NMR (DMSO-d_6) δ 1.27 (t, J = 7.2 Hz, 3H), 1.48 (t, J = 7.2 Hz, 3H), 1.65 (s, 6H), 2.28 (s, 3H), 3.58 (s, 3H), 4.17 (q, J = 7.2 Hz, 2H), 4.65 (q, J = 7.2 Hz, 2H), 5.84 (d, J = 13.5 Hz, 1H), 6.64 (s, 1H), 7.10 (d, J = 7.6 Hz, 2H), 7.11−7.14 (m, 1H), 7.22 (d, J = 7.8 Hz, 1H), 7.30−7.39 (m, 1H), 7.47 (d, J = 7.6 Hz, 2H), 7.49−7.52 (m, 1H), 7.76 (dd, J = 7.9, 7.9 Hz, 1H), 7.92 (dd, J = 7.9, 7.9 Hz, 1H), 8.05 (d, J = 7.8 Hz, 1H), 8.07 (d, J = 13.5 Hz, 1H), 8.21−8.28 (m, 2H), 8.58 (d, J = 7.8 Hz, 1H); MS (FAB$^+$, nitrobenzyl alcohol) for $C_{32}H_{32}N_3O_2S$ *m/z* 522, (FAB$^-$, nitrobenzyl alcohol) for $C_7H_7O_3S$ *m/z* 171. Anal. ($C_{39}H_{39}N_3O_5S_2 \cdot 1.0H_2O$) C, H, N, S.

3-Ethyl-2-[[3-ethyl-5-[2-(1-ethylpyridin-2-ylidene)-ethylidene]-4-oxothiazolidin-2-ylidene]methyl]naphtho-[1,2-*d*]thiazolium Chloride (23). The *p*-toluenesulfonate salt of the target compound was synthesized from 1-ethyl-2-methylpyridinium *p*-toluenesulfonate, 3-ethyl-4-oxothiazolidine-2-thione **(4)**, and 3-ethyl-2-methylnaphtho[1,2-*d*]-thiazolium *p*-toluenesulfonate in a manner analogous to the preparation of rhodacyanine dye **1** to convert to chloride salt **22** using anion-exchange resin (Diaion PA-318, chloride form): ^{1}H NMR (DMSO-d_6) δ 1.27 (t, J = 7.2 Hz, 3H), 1.42 (t, J = 7.2 Hz, 3H), 1.71 (t, J = 7.2 Hz, 3H), 4.23 (q, J = 7.2 Hz, 2H), 4.35 (q, J = 7.2 Hz, 2H), 5.01 (q, J = 7.2 Hz, 2H), 5.64 (d, J = 13.5 Hz, 1H), 6.70 (s, 1H), 6.91−6.98 (m, 1H), 7.62−7.84 (m, 4H), 7.83 (d, J = 7.8 Hz, 1H), 7.96−8.03 (m, 1H), 8.05 (d, J = 13.5 Hz, 1H), 8.12−8.23 (m, 3H), 8.62 (d, J = 7.8 Hz, 1H); MS (FAB$^+$, nitrobenzyl alcohol) for $C_{28}H_{28}N_3OS_2$ *m/z* 486, (FAB$^-$, nitrobenzyl alcohol) for Cl *m/z* 35; HRMS (FAB$^+$) for $C_{28}H_{28}N_3OS_2$ calcd 486.1674; found 486.1671.

3-Ethyl-2-[[3-ethyl-5-[2-(1-ethylquinolin-2-ylidene)-ethylidene]-4-oxothiazolidin-2-ylidene]methyl]thiazolinium Acetate (24). The *p*-toluenesulfonate salt of the target compound was synthesized from 1-ethyl-2-methylquinolinium *p*-toluenesulfonate, 3-ethyl-4-oxothiazolidine-2-thione, and 3-ethyl-2-methylthiazolinium *p*-toluenesulfonate in a manner analogous to the preparation of rhodacyanine dye **1**, and it was converted to acetate salt **24** using anion-exchange resin (Diaion WA-21, acetate form): ^{1}H NMR (DMSO-d_6) δ 1.15 (t, J = 7.2 Hz, 3H), 1.22 (t, J = 7.2 Hz, 3H), 1.37 (t, J = 7.2 Hz, 3H), 1.50 (s, 3H), 3.67 (t, J = 7.2 Hz, 2H), 3.83 (q, J = 7.2 Hz, 2H), 4.09 (q, J = 7.2 Hz, 2H), 4.18 (t, J = 7.2 Hz, 3H),

4.39 (q, J = 7.2 Hz, 2H), 5.62 (d, J = 12.9 Hz, 1H), 6.13 (s, 1H), 7.38 (dd, J = 7.8, 7.8 Hz, 1H), 7.70 (dd, J = 7.8, 7.8 Hz, 1H), 7.73−7.84 (m, 1H), 7.88 (d, J = 7.8 Hz, 1H), 8.26 (d, J = 12.9 Hz, 1H); MS (FAB$^+$, nitrobenzyl alcohol) for $C_{24}H_{28}N_3OS_2$ m/z 438, (FAB$^-$, nitrobenzyl alcohol) for $C_2H_3O_2$ m/z 59; HRMS (FAB$^+$) for $C_{24}H_{28}N_3OS_2$ calcd 438.1674, found 438.1655. Anal. ($C_{26}H_{31}N_3O_3S_2$·4.3H_2O) C, H, N, S.

3-Ethyl-2-[[3-ethyl-5-(3-methylbenzothiazolin-2-ylidene)-4-oxothiazolidin-2-ylidene]methyl]benzothiazolium Acetate (25). This compound was synthesized from 3-ethyl-2-methylbenzothiazolium *p*-toluenesulfonate and 3-ethyl-4-oxothiazolidine-2-thione in a manner analogous to the preparation of rhodacyanine dye **13**: ^{1}H NMR (DMSO-d_6) δ 1.26 (t, J = 7.1 Hz, 3H), 1.37 (t, J = 7.1 Hz, 3H), 1.68 (s, 4.5 H), 4.21 (s, 4.5H), 4.28 (q, J = 7.2 Hz, 2H), 4.70 (q, J = 7.2 Hz, 2H), 6.67 (s, 1H), 7.33 (dd, J = 7.8, 7.8 Hz, 1H), 7.50 (q, J = 7.8 Hz, 2H), 7.71 (q, J = 8.5 Hz, 2H), 7.94 (d, J = 8.1 Hz, 2H), 8.24 (d, J = 8.1 Hz, 1H); MS (FAB$^+$, nitrobenzyl alcohol) for $C_{23}H_{22}N_3OS_3$ m/z 452, (FAB$^-$, nitrobenzyl alcohol) for $C_2H_3O_2$ m/z 59; HRMS (FAB$^+$) for $C_{23}H_{22}N_3OS_3$ calcd 452.0925, found 452.0891.

3-Ethyl-2-[[3-ethyl-5-(1-methylpyridin-2-ylidene)-4-oxothiazolidin-2-ylidene]methyl]benzothiazolium *p*-Toluenesulfonate (26). This compound was synthesized from 1,2-dimethylpyridinium *p*-toluenesulfonate, 3-ethyl-4-oxothiazolidine-2-thione, and 3-ethyl-2-methylbenzothiazolium *p*-toluenesulfonate in a manner analogous to the preparation of rhodacyanine dye **12**: ^{1}H NMR (DMSO-d_6) δ 1.26 (t, J = 7.2 Hz, 3H), 1.35 (t, J = 7.2 Hz, 3H), 2.28 (s, 3H), 4.20 (s, 3H), 4.22 (q, J = 7.2 Hz, 2H), 4.60 (q, J = 7.2 Hz, 2H), 6.60 (s, 1H), 7.10 (d, J = 7.8 Hz, 2H), 7.21 (dd, J = 7.8, 7.8 Hz, 1H), 7.41−7.44 (m, 1H), 7.47 (d, J = 7.8 Hz, 2H), 7.65 (dd, J = 7.8, 7.8 Hz, 1H), 7.82 (d, J = 7.8 Hz, 1H), 7.93 (dd, J = 7.8, 7.8 Hz, 1H), 8.13 (d, J = 7.8 Hz, 1H), 8.37 (d, J = 7.8 Hz, 1H), 8.50 (d, J = 7.8 Hz, 1H); MS (FAB$^+$, nitrobenzyl alcohol) for $C_{21}H_{22}N_3OS_2$ m/z 396, (FAB$^-$, nitrobenzyl alcohol) for $C_7H_7SO_3$ m/z 171. Anal. ($C_{28}H_{29}N_3O_4S_3$) C, H, N, S.

1-Ethyl-2-[[3-ethyl-5-(3-methylbenzoxazol-2-ylidene)-4-oxothiazolidin-2-ylidene]methyl]pyridinium Chloride (27). This compound was synthesized from 2,3-dimethylbenzoxazolium *p*-toluenesulfonate, 3-ethyl-4-oxothiazolidine-2-thione and 1-ethyl-2-methylpyridinium *p*-toluenesulfonate in a manner analogous to the preparation of rhodacyanine dye **3**: ^{1}H NMR (DMSO-d_6) δ 1.15 (t, J = 7.2 Hz, 3H), 1.41 (t, J = 7.2 Hz, 3H), 3.22 (s, 3H), 3.89 (q, J = 7.2 Hz, 2H), 4.70 (q, J = 7.2 Hz, 2H), 6.40 (s, 1H), 6.95 (dd, J = 7.8, 7.8 Hz, 1H), 7.08 (d, J = 7.8 Hz, 1H), 7.23 (dd, J = 7.8, 7.8 Hz, 1H), 7.34 (d, J = 7.8 Hz, 1H), 7.86 (dd, J = 7.8, 7.8 Hz, 1H), 8.13 (d, J = 7.8 Hz, 1H), 8.49 (dd, J = 7.8, 7.8 Hz, 1H), 9.04 (d, J = 7.8 Hz, 1H); MS (FAB$^+$, nitrobenzyl alcohol) for $C_{21}H_{22}N_3O_2S$ m/z 380, (FAB$^-$, nitrobenzyl alcohol) for Cl m/z 35; HRMS (FAB$^+$) for $C_{21}H_{22}N_3O_2S$ calcd 380.1432, found 380.1429. Anal. ($C_{21}H_{22}ClN_3O_2S_2$·2.0H_2O) C, H, Cl, N, S.

3-Ethyl-2-[[3-ethyl-5-(3-methylnaphtho[1,2-*d*]thiazolin-2-ylidene)-4-oxothiazolidin-2-ylidene]methyl]naphtho-[2,1-*d*]thiazolium Chloride (28). This compound was synthesized from 2,3-dimethylnaphtho[1,2-*d*]thiazolium *p*-toluenesulfonate, 3-ethyl-4-oxothiazolidine-2-thione, and 3-ethyl-2-methylnaphtho[2,1-*d*]thiazolium *p*-toluenesulfonate in a manner analogous to the preparation of rhodacyanine dye **3**: ^{1}H NMR (DMSO-d_6) δ 1.23 (t, J = 7.2 Hz, 3H), 1.45 (t, J = 7.2 Hz, 3H), 4.32 (q, J = 7.2 Hz, 2H), 4.63 (s, 3H), 4.83 (q, J = 7.2 Hz, 2H), 6.76 (s, 1H), 7.43 (dd, J = 7.8, 7.8 Hz, 1H), 7.61 (dd, J = 7.8, 7.8 Hz, 1H), 7.71 (dd, J = 7.8, 7.8 Hz, 1H), 7.76−7.87 (m, 2H), 7.94 (d, J = 7.8 Hz, 1H), 8.00 (d, J = 7.8 Hz, 1H), 8.13 (d, J = 7.8 Hz, 1H), 8.16−8.24 (m, 2H), 8.31 (d, J = 7.8 Hz, 1H), 8.53 (d, J = 7.8 Hz, 1H); MS (FAB$^+$, nitrobenzyl alcohol) for $C_{31}H_{26}N_3OS_3$ m/z 552, (FAB$^-$, nitrobenzyl alcohol) for Cl m/z 35; HRMS (FAB$^+$) for $C_{31}H_{26}N_3OS_3$ calcd 552.1238, found 552.1242. Anal. ($C_{31}H_{26}ClN_3OS_3$·3.0H_2O) C, H, Cl, N, S.

3-Ethyl-2-[[3-ethyl-5-(3-methylnaphtho[1,2-*d*]thiazolin-2-ylidene)-4-oxothiazolidin-2-ylidene]methyl]naphtho-[2,1-*d*]oxazolium Acetate (29). This compound was synthesized from 2,3-dimethylnaphtho[1,2-*d*]thiazolium *p*-toluenesulfonate, 3-ethyl-4-oxothiazolidine-2-thione, and 3-ethyl-2-methylnaphtho[2,1-*d*]oxazolium *p*-toluenesulfonate in a manner analogous to the preparation of rhodacyanine dye **13**: ^{1}H NMR (DMSO-d_6) δ 1.32 (t, J = 7.2 Hz, 3H), 1.48 (t, J = 7.2 Hz, 3H), 1.81 (s, 3H), 4.00 (q, J = 7.2 Hz, 2H), 4.60−4.70 (m, 5H), 6.49 (s, 1H), 7.53 (dd, J = 7.8, 7.8 Hz, 2H), 7.63−7.74 (m, 2H), 7.88−8.05 (m, 4H), 8.21 (d, J = 7.8 Hz, 1H), 8.32 (d, J = 7.8 Hz, 1H), 8.21 (d, J = 7.8 Hz, 2H), 8.32 (d, J = 7.8 Hz, 1H), 8.63 (d, J = 7.8 Hz, 1H); MS (FAB$^+$, nitrobenzyl alcohol) for $C_{31}H_{26}N_3O_2S_2$ m/z 536, (FAB$^-$, nitrobenzyl alcohol) for $C_2H_3O_2$ m/z 59; HRMS (FAB$^+$) for $C_{31}H_{26}N_3O_2S_2$ calcd 536.1466, found 536.1464. Anal. ($C_{33}H_{29}N_3O_4S_2$·3.0H_2O) C, H, N, S.

3-Ethyl-2-[[3-ethyl-5-(3-methylthiazolidin-2-ylidene)-4-oxothiazolidin-2-ylidene]methyl]naphtho[1,2-*d*]thiazolium Acetate (30). This compound was synthesized from 3-methyl-2-(methylthio)thiazolidinium *p*-toluenesulfonate, 3-ethyl-4-oxothiazolidine-2-thione **(4)**, and 3-ethyl-2-methylnaphtho[1,2-*d*]thiazolium *p*-toluenesulfonate in a manner analogous to the preparation of rhodacyanine dye **13**: ^{1}H NMR (DMSO-d_6) δ 1.25 (t, J = 7.2 Hz, 3H), 1.66 (s, 3H), 1.71 (t, J = 7.2 Hz, 3H), 3.30−3.41 (m, 2H), 3.57 (s, 3H), 4.01 (q, J = 7.2 Hz, 2H), 4.22 (q, J = 7.2 Hz, 2H), 5.06 (q, J = 7.2 Hz, 2H), 6.70 (s, 1H), 7.78 (dd, J = 7.8, 7.8 Hz, 1H), 7.84 (dd, J = 7.8, 7.8 Hz, 1H), 8.08 (d, J = 7.8 Hz, 1H), 8.22 (d, J = 7.8 Hz, 1H), 8.26 (d, J = 7.8 Hz, 1H), 8.64 (d, J = 7.8 Hz, 1H); MS (FAB$^+$, nitrobenzyl alcohol) for $C_{23}H_{24}N_3OS_3$ m/z 454, (FAB$^-$, nitrobenzyl alcohol) for $C_2H_3O_2$ m/z 59; HRMS (FAB$^+$) for $C_{23}H_{24}N_3OS_3$ calcd 454.1081, found 454.1085. Anal. ($C_{25}H_{27}N_3O_3S_3$·3.0H_2O) C, H, N, S.

3-Ethyl-2-[[3-ethyl-5-(3-methyl-4,5,6,7-tetrahydrobenzothiazolidin-2-ylidene]-4-oxothiazolidine-2-ylidene}-methyl]thiazolinium Acetate (31). This compound was synthesized from 3-methyl-2-(methylthio)-4,5,6,7-tetrabenzothiazolidinium *p*-toluenesulfonate, 3-ethyl-4-oxothiazolidine-2-thione **(4)**, and 3-ethyl-2-methylnaphtho[1,2-*d*]thiazolium *p*-toluenesulfonate in a manner analogous to the preparation of rhodacyanine dye **13**: ^{1}H NMR (DMSO-d_6) δ 1.19 (t, J = 7.2 Hz, 3H), 1.22 (t, J = 7.2 Hz, 3H), 1.57 (s, 3H), 1.70−1.87 (m, 4H), 2.58−2.64 (m, 4H), 3.60 (t, J = 7.2 Hz, 2H), 3.77 (q, J = 7.2 Hz, 2H), 3.84 (s, 3H), 4.08 (t, J = 7.2 Hz, 2H), 4.15 (q, J = 7.2 Hz, 2H), 6.00 (s, 1H); MS (FAB$^+$, nitrobenzyl alcohol) for $C_{19}H_{26}N_3OS_3$ m/z 408, (FAB$^-$, nitrobenzyl alcohol) for $C_2H_3O_2$ m/z 59. Anal. ($C_{21}H_{29}N_3O_3S_3$·2.0H_2O) C, H, N, S.

1-Ethyl-2-[[3-ethyl-5-(3-methylbenzothiaolin-2-ylidene)-4-oxothiazolidin-1-ylidene]methyl]quinolinium Acetate (32). This compound was synthesized from 3-ethyl-2-methylbenzothiazolium *p*-toluenesulfonate, 3-ethyl-4-oxothiazolidine-2-thione, and 1-ethyl-2-methylquinolinium *p*-toluenesulfonate in a manner analogous to the preparation of rhodacyanine dye **13**: ^{1}H NMR (DMSO-d_6) δ 1.29 (t, J = 7.2 Hz, 3H), 1.48 (t, J = 7.2 Hz, 3H), 1.50 (s, 3H), 4.12 (s, 3H), 4.22 (q, J = 7.2 Hz, 2H), 4.78 (q, J = 7.2 Hz, 2H), 6.11 (s, 1H), 7.30 (dd, J = 7.8, 7.8 Hz, 1H), 7.48 (dd, J = 7.8, 7.8 Hz, 1H), 7.68 (d, J = 7.8 Hz, 1H), 7.71 (dd, J = 7.8, 7.8 Hz, 1H), 7.91 (d, J = 7.8 Hz, 1H), 7.98 (dd, J = 7.8, 7.8 Hz, 1H), 8.11−8.18 (m, 2H), 8.23 (d, J = 7.8 Hz, 1H), 8.58 (d, J = 7.8 Hz, 1H); MS (FAB$^+$, nitrobenzyl alcohol) for $C_{25}H_{24}N_3OS_2$ m/z 446, (FAB$^-$, nitrobenzyl alcohol) for $C_2H_3O_2$ m/z 59; HRMS (FAB$^+$) for $C_{25}H_{24}N_3OS_2$ calcd 446.1361, found 446.1366. Anal. ($C_{27}H_{27}N_3O_3S_2$·3.0H_2O) C, H, N, S.

3-Ethyl-2-[[3-ethyl-5-(3-methylbenzothiazolin-2-ylidene)-4-oxothiazolidin-2-ylidene]methyl]-4-methylthiazolium Acetate (33). This compound was synthesized from 3-ethyl-2-methylbenzothiazolium *p*-toluenesulfonate, 3-ethyl-4-oxothiazolidine-2-thione, and 1-ethyl-2-methylquinolinium *p*-toluenesulfonate in a manner analogous to the preparation of rhodacyanine dye **13**: ^{1}H NMR (DMSO-d_6) δ 1.25 (t, J = 7.2 Hz, 3H), 1.32 (t, J = 7.2 Hz, 3H), 1.67 (s, 3H), 2.47 (s, 3H), 4.06 (s, 3H), 4.18 (q, J = 7.2 Hz, 2H), 4.43 (q, J = 7.2 Hz, 2H), 6.55 (s, 1H), 7.34 (dd, J = 7.5, 7.5 Hz, 1H), 7.52 (dd, J = 7.5, 7.5 Hz, 1H), 7.54 (s, 1H), 7.68 (d, J = 7.5 Hz, 1H), 7.92 (d, J = 7.5 Hz, 1H); MS (FAB$^+$, nitrobenzyl alcohol) for $C_{20}H_{22}N_3$-

OS_3 *m/z* 416, (FAB^-, nitrobenzyl alcohol) for $C_2H_3O_2$ *m/z* 59; HRMS (FAB^+) for $C_{20}H_{22}N_3OS_3$ calcd 416.0925, found 416.0923.

3-Ethyl-2-[[3-ethyl-5-(3-methylbenzothiazolin-2-ylidene)-4-oxothiazolidin-2-ylidene]methyl]thiazolinium Acetate (34). This compound was synthesized from 3-ethyl-2-methylbenzothiazolium *p*-toluenesulfonate, 3-ethyl-4-oxothiazolidine-2-thione, and 3-ethyl-2-methylbenzoxazolium *p*-toluenesulfonate in a manner analogous to the preparation of rhodacyanine dye **13**: ^{1}H NMR (DMSO-d_6) δ 1.20 (t, J = 7.2 Hz, 3H), 1.25 (t, J = 7.2 Hz, 3H), 1.57 (s, 3H), 3.66 (t, J = 7.2 Hz, 2H), 3.82 (q, J = 7.2 Hz, 2H), 4.09 (s, 3H), 4.12 (t, J = 7.2 Hz, 2H), 4.19 (q, J = 7.2 Hz, 2H), 6.06 (s, 1H), 7.37 (dd, J = 7.5, 7.5 Hz, 1H), 7.55 (dd, J = 7.5, 7.5 Hz, 1H), 7.72 (d, J = 7.5 Hz, 1H), 7.96 (d, J = 7.5 Hz, 1H); MS (FAB^+, nitrobenzyl alcohol) for $C_{19}H_{22}N_3OS_3$ *m/z* 404, (FAB^-, nitrobenzyl alcohol) for $C_2H_3O_2$ *m/z* 59; HRMS (FAB^+) for $C_{19}H_{22}N_3OS_3$ calcd 404.0925, found 404.0923. Anal. ($C_{21}H_{25}N_3O_3S_3 \cdot 3.1H_2O$) C, H, N, S.

3-Ethyl-2-[[3-ethyl-5-(3-methylbenzothiazolin-2-ylidene)-4-oxothiazolidin-2-ylidene]methyl]benzoxazolium Acetate (35). This compound was synthesized from 3-ethyl-2-methylbenzothiazolium *p*-toluenesulfonate, 3-ethyl-4-oxothiazolidine-2-thione, and 3-methyl-2-(methylthio)benzoxazolium *p*-toluenesulfonate in a manner analogous to the preparation of rhodacyanine dye **13**: ^{1}H NMR (DMSO-d_6) δ 1.27 (t, J = 7.2 Hz, 3H), 1.39 (t, J = 7.2 Hz, 3H), 1.77 (s, 3H), 4.18 (s, 3H), 4.23 (q, J = 7.2 Hz, 2H), 4.48 (q, J = 7.2 Hz, 2H), 6.37 (s, 1H), 7.38 (d, J = 7.5 Hz, 2H), 7.48−7.58 (m, 2H), 7.80 (dd, J = 7.5, 7.5 Hz, 2H), 7.96 (d, J = 7.5 Hz, 1H), 8.01 (d, J = 7.5 Hz, 1H); MS (FAB^+, nitrobenzyl alcohol) for $C_{23}H_{22}N_3O_2S_2$ *m/z* 436, (FAB^-, nitrobenzyl alcohol) for $C_2H_3O_2$ *m/z* 59; HRMS (FAB^+) for $C_{23}H_{22}N_3O_2S_2$ calcd 436.1153, found 436.1159. Anal. ($C_{25}H_{25}N_3O_4S_2 \cdot 2.4H_2O$) C, H, N, S.

1-Ethyl-2-[[3-ethyl-5-(3-methylbenzothiazolin-2-ylidene)-4-oxothiazolidin-2-ylidene]methyl]pyridinium Iodide (36). This compound was synthesized from 3-ethyl-2-methylbenzothiazolium *p*-toluenesulfonate, 3-ethyl-4-oxothiazolidine-2-thione, and 1-ethyl-2-methylpyridinium iodide in a manner analogous to the preparation of rhodacyanine dye **12**: ^{1}H NMR (DMSO-d_6) δ 1.25 (t, J = 7.2 Hz, 3H), 1.45 (t, J = 7.2 Hz, 3H), 4.03 (s, 3H), 4.11 (q, J = 7.2 Hz, 2H), 4.60 (q, J = 7.2 Hz, 2H), 5.98 (s, 1H), 7.29 (dd, J = 8.0, 8.0 Hz, 1H), 7.42−7.50 (m, 2H), 7.59 (d, J = 8.0 Hz, 1H), 7.86 (d, J = 8.0 Hz, 1H), 8.06 (d, J = 8.0 Hz, 1H), 8.27 (dd, 8.0, 7.0 Hz, 1H), 8.79 (d, J = 8.0 Hz, 1H); MS (FAB^+, nitrobenzyl alcohol) for $C_{21}H_{22}N_3OS_2$ *m/z* 396, (FAB^-, triethanolamine) for I *m/z* 127. Anal. ($C_{21}H_{22}IN_3OS_2 \cdot 1.5H_2O$) C, H, I, N, S.

X-ray Structure Analysis of 3. Compounds **3** was crystallized from a 1:3 solution of methanol and ethyl acetate. X-ray diffraction data was measured by a Rigaku AFC-5R using a Cu Kα radiation and graphite monochromator. Structures were determined by a direct method SHELXS86 and succesive Fourier syntheses and refined by a full-matrix least-squares method. Full crystallographic details are available as Supporting Information.

***In Vitro* Clonogenic Assay.** CX-1 cell line was grown in a 50:50 mix of Dulbecco's modified Eagle's (DMEM) and RPMI 1640 medium (GIBCO Laboratories, Grand Island, NY) supplemented with 10% calf serum (Hyclone Laboratories Inc., Logan, UT) and antibiotics at 37 °C under 5% CO_2, 95% air, and 100% humidity. CX-1 (human colon carcinoma) was obtained from Dr. M. Wolpert (National Cancer Institute).

For the clonogenic assay, cells were seeded at 1500 cells/wells for CX-1 in 96-well plates (Becton Dickinson Labware, Lincoln Park, NJ). The assay was performed in duplicate. The drug are first dissolved in dimethyl sulfoxide, to prepare 10 mg/mL stock solution. The final drug solution was made by mixing 100 μL of this stock solution with 10 mL of 5% CS DME media solution. On the following day, cells were treated with test compounds at varied concentrations and cultured precisely for 24 h in the media. After rinsing, cells were incubated in drug-free medium for 2 weeks. Colonies were stained with 2% crystal violet in 70% ehtanol and counted by an automated colony counter (Artek counter model 880, Dynatech Lab. Inc., Chantilly, VA).

In Vitro Cytotoxic Assay. For the cytotoxic assay, cells were seeded at 4×10^4 cells/20 mm plate and incubated at 37 °C in 5% CO_2. On the following day, cells were treated with each compound at varying concentrations and then incubated for 48 h (KB-cell) or 1 week (CV-1). Cells were stained with crystal violet, and the number of viable cells were determined by monocellater.

In Vivo Human Tumor Xenografts in Nude Mice and Tumor Allografts in BDF1 Mice. Male Swiss nu/nu mice (about 5-weeks old) were obtained from Taconic Farm, Inc. (Germantown, NY). Group housing (five/cage) was provided in polycarbonate cages with wire top and filters. Mice were allowed to acclimate for 1-week prior to experiments. Only normal, healthy mice were used. Human melanoma LOX cells used in this model were first grown sc in nude mice. On the day of ip implantation, tumors were excised, and a single cell suspension was prepared. RBCs were lysed by ammonium chloride. Each mouse received 2×10^6 LOX cells (trypan blue negative) in 0.2 mL of PBS by ip injection. On the day after ip implantation, test compounds (0.2 mL/20 g mouse body weight, 45% Encapsin HPB (hydroxypropyl β-cyclodextrin (American Maise-Products Co.) in water) were administered ip for LOX tumor, bearing with appropriately determined doses and schedules.

Inbred 5-week-old male BDF1 mice were obtained from Charles River Japan Inc. and were allowed to acclimate for approximately 1 week prior to use. Each mouse received 5×10^5 CA755 cells by sc injection. Test compounds (0.2 mL/20 g mouse body weight, 0.9% saline) were administered iv for CA755 tumor, bearing with appropriately determined doses and schedules. For both in vivo evaluations, the statistical significance of difference between the control and the drug-treated groups was determined by applying the Bartlett's, Dunnett's, and Scheffet's test.[14−16]

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Supporting Information Available: X-ray crystallographic data for compound **3** (7 pages). Ordering information is given on any current masthead page.

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