Synthesis of 2,4-Disubstituted Thiazoles and Selenazoles as Potential Antifilarial and Antitumor Agents. 2. 2-Arylamido and 2-Alkylamido Derivatives of 2-Amino-4-(isothiocyanatomethyl)thiazole and 2-Amino-4-(isothiocyanatomethyl)selenazole

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*Received June 1, 1993**

The synthesis of a series of 2-arylamido and 2-alkylamido derivatives of 2-amino-4-(isothiocyanatomethyl)thiazole and 2-amino-4-(isothiocyanatomethyl)selenazole is described. *In vitro* antiproliferative evaluations were carried out using L1210 cells. The 2-(alkylamido)thiazole derivatives were moderately antiproliferative, with IC_{50}'s of 4-8 μM. A significant increase in activity was obtained for the arylamido derivatives, with IC_{50}'s of 0.2-1 μM. The results obtained for the selenazoles were similar to those for the thiazoles. 2-Benzamido-4-(isothiocyanatomethyl)-thiazole (19) was found to be a potent inhibitor of GMP synthetase. None of the compounds prepared in this study demonstrated antifilarial activity.

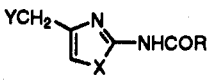
Introduction

In our previous paper in this series, we described the synthesis and evaluation of 2-[(methoxycarbonyl)amino]-4-(isothiocyanatomethyl)thiazole and selenazole.[1] These compounds exhibited significant antiproliferative activity against L1210 leukemic cells. Cell growth inhibition by 2-[(methoxycarbonyl)amino]-4-(isothiocyanatomethyl)-thiazole was associated with the cells being arrested in the mitotic phase of the cell cycle. Various alkyl carbamates have been reported to block cells in mitosis by disrupting microtubule organization.[2-5] Therefore, the antiproliferative mechanism of 2-[(methoxycarbonyl)amino]-4-(isothiocyanatomethyl)thiazole was considered likely to be inhibition of microtubule assembly. To determine whether the 2-[(methoxycarbonyl)amino] moiety was necessary for cytotoxic activity, we then prepared the 2-acetamido derivative of 2-amino-4-(isothiocyanatomethyl)thiazole. Unexpectedly, this compound also demonstrated significant cytotoxic activity. As a result of this finding, we undertook the synthesis and cytotoxic and antifilarial evaluation of a series of 2-amido derivatives of 2-amino-4-(isothiocyanatomethyl)thiazole and 2-amino-4-(isothiocyanatomethyl)selenazole.

Chemistry

Compounds 2-31, shown in Table I, were synthesized in a straightforward manner according to the Scheme I. Treatment of 2-amino-4-(chloromethyl)thiazole hydrochloride (1a)[6] with the appropriate anhydride gave the amides 2-8. The selenazole amides 9-12 were obtained in a similar manner from 2-amino-4-(chloromethyl)-selenazole hydrochloride (1b).[1] Synthesis of the isothiocyanates 14-31 was achieved by a general procedure which involved the treatment of the corresponding chloro derivatives 2-12 with potassium thiocyanate in the presence of potassium iodide. When potassium iodide was not added to the reaction, reduced yields were realized. Compound 6, when treated with potassium thiocyanate and potassium iodide in methanol, gave the deacylated derivative 13a instead of the expected isothiocyanate 18.

Table I. Some 2,4-Disubstituted Thiazoles and Selenazoles



compd	X	Y	R	mp (°C)	yield (%)	crystn[b] solvent	formula
2[a]	S	Cl	CH_3	166-167	32	B	$C_6H_7ClN_2OS$
3	S	Cl	C_2H_5	116-118	73	A	$C_7H_9ClN_2OS$
4	S	Cl	$CH(CH_3)_2$	79-81	93	C	$C_8H_{11}ClN_2OS$
5	S	Cl	$C(CH_3)_3$	147-149	64	A	$C_9H_{13}ClN_2O_2S$
6	S	Cl	CF_3	105-107	98	A	$C_6H_4ClF_3N_2OS \cdot H_2O$
7	S	Cl	C_6H_5	136-138	65	A	$C_{11}H_9ClN_2OS$
8	S	Cl	$4\text{-}CH_3OC_6H_4$	134-136	35	C	$C_{12}H_{11}ClN_2O_2S$
9	Se	Cl	CH_3	166-167	15	C	$C_6H_7ClN_2OSe$
10	Se	Cl	C_2H_5	125-126	62	A	$C_7H_9ClN_2OSe$
11	Se	Cl	$C(CH_3)_3$	122-123	92	C	$C_9H_{13}ClN_2OSe$
12	Se	Cl	C_6H_5	162-164	74	C	$C_{11}H_9ClN_2OSe$
14	S	NCS	CH_3	167-169	11	D	$C_7H_7N_3OS_2$
15	S	NCS	C_2H_5	136-138	67	A	$C_8H_9N_3OS_2$
16	S	NCS	$CH(CH_3)_2$	90-92	71	A	$C_9H_{11}N_3OS_2$
17	S	NCS	$C(CH_3)_3$	104-105	76	A	$C_{10}H_{13}N_3OS_2$
18	S	NCS	CF_3	126-128	40	E	$C_7H_4F_3N_3OS_2$
19	S	NCS	C_6H_5	129-130	73	E	$C_{12}H_9N_3OS_2$
20	S	NCS	$4\text{-}CH_3OC_6H_4$	95-97	78	D	$C_{13}H_{11}N_3O_2S_2$
21	S	NCS	$4\text{-}ClC_6H_4$	144-145	37	C	$C_{12}H_8ClN_3OS_2$
22	S	NCS	$3,4\text{-}Cl_2C_6H_3$	136-138	60	D	$C_{12}H_7Cl_2N_3OS_2$
23	S	NCS	$4\text{-}FC_6H_4$	162-164	38	D	$C_{12}H_8FN_3OS_2$
24	S	NCS	$4\text{-}CH_3C_6H_4$	133-135	46	D	$C_{13}H_{11}N_3OS_2$
25	S	NCS	$4\text{-}CF_3C_6H_4$	105-107	25	C	$C_{13}H_8F_3N_3OS_2$
26	S	NCS	$4\text{-t-}BuC_6H_4$	67-69	26	C	$C_{16}H_{17}N_3OS_2$
27	Se	NCS	CH_3	171-173	61	D	$C_7H_7N_3OSSe$
28	Se	NCS	C_2H_5	124-126	85	E	$C_8H_9N_3OSSe$
29	Se	NCS	$CH(CH_3)_2$	82-84	40	C	$C_9H_{11}N_3OSSe$
30	Se	NCS	$C(CH_3)_3$	101-103	45	A	$C_{16}H_{13}N_3OSSe$
31	Se	NCS	C_6H_5	154-155	79	E	$C_{12}H_9N_3OSSe$

[a] Compound is reported in the literature,[2] but no melting point is given. [b] A = benzene/hexane, B = toluene, C = purified on silica column, D = ethanol, E = benzene.

Treatment of 13a with trifluoroacetic anhydride gave 18. The benzamido derivatives 19-26 were prepared by the reaction of 13a with the appropriately substituted benzoic anhydride. The selenazole benzamido derivative 31 was prepared by treatment of 13b with benzoic anhydride.

Biological Activity

Antitumor Structure-Activity Relationships. The antitumor potential of this series of compounds was studied

* Abstract published in *Advance ACS Abstracts*, October 1, 1993.

0022-2623/93/1836-3849$04.00/0

Scheme I

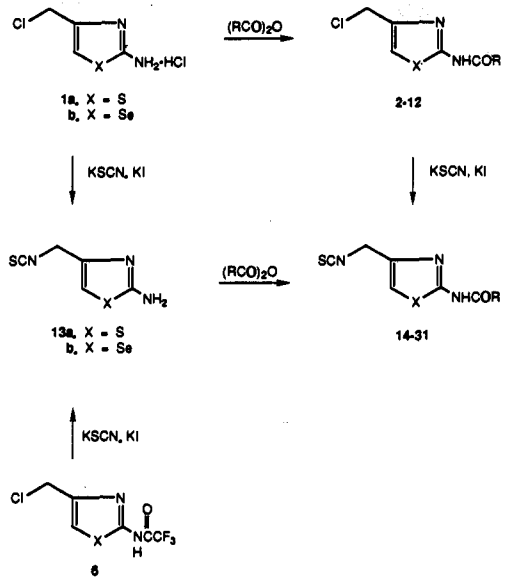
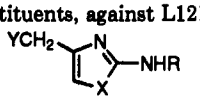


Table II. Antiproliferative Activity of 4-(Isothiocyanatomethyl)- and 4-(Chloromethyl)thiazoles and -selenazoles with 2-Amino and Various 2-Amide Substituents, against L1210 Cells *in Vitro*



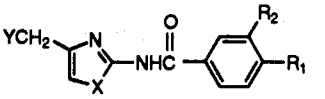
compd	X	Y	R	screen GR[a] (% of control)	IC_{50}[b] (μM)
A[1]	S	NCS	$COOCH_3$		3.2
13a	S	NCS	H	0	26
13b	Se	NCS	H	0	21
14	S	NCS	$COCH_3$	1	5.2
27	Se	NCS	$COCH_3$	14	30
18	S	NCS	$COCF_3$	20	ND[c]
2	S	Cl	$COCH_3$	75	>100
9	Se	Cl	$COCH_3$	65	>100
15	S	NCS	$COCH_2CH_3$	0	6.8
28	Se	NCS	$COCH_2CH_3$	0	3.5
16	S	NCS	$COCH(CH_3)_2$	0	8.2
29	Se	NCS	$COCH(CH_3)_2$	0	28
17	S	NCS	$COC(CH_3)_3$	28	4.3
30	Se	NCS	$COC(CH_3)_3$	0	18

[a] Screen GR, growth rate in the presence of the test compound at 100 μM. See Experimental Section for definition. [b] IC_{50}, concentration required to decrease growth rate to 50% of control. [c] ND, not determined.

by assaying their antiproliferative activity against L1210 cells *in vitro*. 2-[(Methoxycarbonyl)amino]-4-(isothiocyanatomethyl)thiazole had previously been found to be moderately active with an IC_{50} of 3.2 μM. (A in Table II)[1] In the present investigation the structural requirements at the 2 position of 2-amino-4-(isothiocyanatomethyl)-thiazoles and 2-amino-4-(isothiocyanatomethyl)selenazoles were studied. The absence of the 2-[(methoxycarbonyl)amino] substituent rendered the compound significantly less active, as shown by the underivatized 2-amino-4-(isothiocyanatomethyl)thiazole (**13a**) having an IC_{50} about 10-fold higher than A (Table II). In contrast, the antiproliferative activity was retained when a 2-acetamido group (**14**) replaced the 2-[(methoxycarbonyl)-amino] (A) (Table II). Compound 14 and three additional alkyl amido derivatives (**15–17**) had similar activity, with IC_{50}'s ranging from 4.3 to 8.2 μM (Table II).

A dramatic increase in antiproliferative activity was obtained with the benzamido derivative **19**, which had an IC_{50} of 0.2 μM (Table III). To explore the possibility of further enhancing the activity of this lead compound, a

Table III. Antiproliferative Activity of 4-(Isothiocyanatomethyl)- and 4-(Chloromethyl)thiazoles and -selenazoles, with Variously Substituted 2-Benzamido Substituents, against L1210 Cells *in Vitro*



compd	X	Y	R_1	R_2	screen GR[a] (% of control)	IC_{50}[b] (μM)
19	S	NCS	H	H	0	0.20
31	Se	NCS	H	H	13	0.90
7	S	Cl	H	H	100	c
12	Se	Cl	H	H	101	c
20	S	NCS	OCH_3	H	0	0.44
21	S	NCS	Cl	H	0	0.22
23	S	NCS	F	H	0	1.1
22	S	NCS	Cl	Cl	0	0.82
24	S	NCS	CH_3	H	25	0.48
25	S	NCS	CF_3	H	0	0.50
26	S	NCS	$C(CH_3)_3$	H	72	>100

[a] Screen GR, growth rate in the presence of the test compound at 100 μM. See Experimental Section for definition. [b] IC_{50}, concentration required to decrease growth rate to 50% of control. [c] No significant inhibition in the screen.

variety of substituents, both electron-withdrawing and electron-donating, were introduced on the benzene ring (**20–25**). None of these compounds showed an increase in activity. The 3,4-dichloro derivative **22** and the 4-fluoro derivative **23** were slightly less active than **19** (Table III). Since these derivatives represented various branches of the Topliss tree[7] and did not result in any major changes in antiproliferative activity, it appeared that electronic influences in the benzene ring were not an important determinant of antiproliferative activity for these compounds. On the other hand, there did appear to be a limit on the steric bulk that could be tolerated, since the 4-(*tert*-butylbenzamido) derivative **26** was virtually inactive (Table III).

The importance of the 4-(isothiocyanatomethyl) substituent in this series of compounds was evaluated by replacing it with a 4-chloromethyl moiety on 2-acetamidothiazole (**2**) and the 2-benzamidothiazole (**7**). Both of these compounds were virtually inactive (Tables II and III). Therefore, it appeared that the isothiocyanatomethyl functionality was important for the antiproliferative activity of this series of compounds. The same requirement had been found in the previous study, in which all derivatives of 2-[(methylcarbonyl)amino]thiazole with 4-substituents other than 4-(isothiocyanatomethyl) were inactive.[1]

The structure–activity relationships of the selenazole analogs were similar to those of the thiazoles (Tables II and III). In particular, the increase in antiproliferative activity with introduction of the benzamido moiety (**31** cf. **27**), and the decrease with conversion of the isothiocyanato functionality to a chloro (**9** and **12**), were found to be virtually identical to the results obtained with the thiazoles. Thus, it appeared likely that the selenazoles acted by a mechanism similar to that of the thiazoles, and since the selenazoles were slightly less active, they were not studied further. An exception to the IC_{50}'s of the 2-amido-4-(isothiocyanatomethyl)selenazoles being generally higher than those of the 2-amido-4-(isothiocyanatomethyl)thiazoles was the propionamido derivative **28**. Its IC_{50} was similar to that of the corresponding 2-propionamido-4-(isothiocyanatomethyl)thiazole (**15**) and about 10-fold lower than the other alkyl amido derivatives of selenazole

(29, 30). The explanation for this apparent anomaly has not been investigated.

Mechanism of Action. 2-[(Methoxycarbonyl)amino]-4-(isothiocyanatomethyl)thiazole (A) was shown previously[1] to cause accumulation of L1210 cells in mitosis. Thus, it appeared to exert its cytotoxic effect by interfering with completion of mitosis, as do many other alkyl carbamates.[2-5] The unexpected activity of the 2-acetamido derivative 14 (Table II) indicated that the 2-[(methoxycarbonyl)amino] function of A could be modified to this extent without losing cytotoxic activity. To determine whether the mechanism of cytotoxicity was changed by this modification, the potential of 14 to cause accumulation of L1210 cells in mitosis was examined in an experiment directly comparing the fraction of the cells in mitosis after various times of treatment with A and 14. The two compounds caused a similar time course of mitotic accumulation. Thus, the acetamide derivative 14 retained the antimitotic activity exhibited by the 2-[(methoxycarbonyl)amino] derivative A.

The mechanism of antiproliferative activity of 19, on the other hand, was different from those of 14 and A. While the latter compounds blocked the progression of cells in mitosis,[1] 19 did not cause mitotic accumulation. Initial experiments using flow cytotmetry showed that during treatment with 10 μM 19, the G_1 peak of L1210 cell population became broader and that the cells accumulated in S phase (Figure 1). There was no indication of accumulation of cells in the G_2–M region of the DNA histogram, as would be expected if the cells were blocked in mitosis. This result was confirmed by microscopic determination of the mitotic index. After L1210 cells were treated with 100 μM 19 for 24 h, the mitotic index was 3.0 ± 0.5% (SEM, n = 10), the same as the control value of 3.0 ± 0.25% (SEM, n = 10). 2-Benzamido-4-(isothiocyanatomethyl)selenazole (31) also did not cause significant mitotic accumulation. L1210 cells treated for 24 h with 100 μM 31 had a mitotic index of 4.3 ± 0.62% (SEM, n = 5). In contrast, 16% of L1210 cells treated for 24 h with 100 μM 2-[(methoxycarbonyl)amino]-4-(isothiocyanatomethyl)thiazole (A) were microscopically recognizable as being in mitosis,[1] as were 24% of L1210 cells treated for 21 h with colcemid (0.04 μg/mL), a known mitotic blocker.

Studies on the effects of 19 on nucleotide metabolism and nucleic acid and protein synthesis were initiated, to obtain an indication of what the mechanism of its antiproliferative activity might be. These studies showed that 19 inhibited synthesis of guanine nucleotides from hypoxanthine in intact L1210 cells and strongly inhibited partially purified GMP synthetase, IC_{50} = 350 nM.[8]

Antifilarial Activity. The antifilarial activity of these compounds was evaluated against the adult worms of *B. pahangi* and *A. viteae* in jirds.[10] In this screen a compound is considered active if the number of implanted live adult worms at necropsy is decreased more than 65%. All new compounds were evaluated at a dosage of 100 mg/kg × 5 days administered subcutaneously. None of the compounds demonstrated antifilarial activity at this dosage.

Experimental Section

Chemistry. Melting points were determined with a Thomas-Hoover capillary melting point apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer Model 281 spectrophotometer. ^{1}H NMR spectra were obtained on Varian 60-MHz and Brucker 270-MHz spectrometers and chemical shift values (δ) are reported in parts per million downfield from the internal standard Me_4Si (s = singlet; d = doublet; m = multiplet, br = broad). Column chromatography was carried out on silica gel (60–200 mesh) and kieslgel 60F_{254} (70–230 mesh). Thin-layer chromatography (TLC) was performed using Analtech silica gel GF plates, and spots were visualized either by UV or iodine. Microanalyses (C, H, N) were all within ±0.4% of the required values.

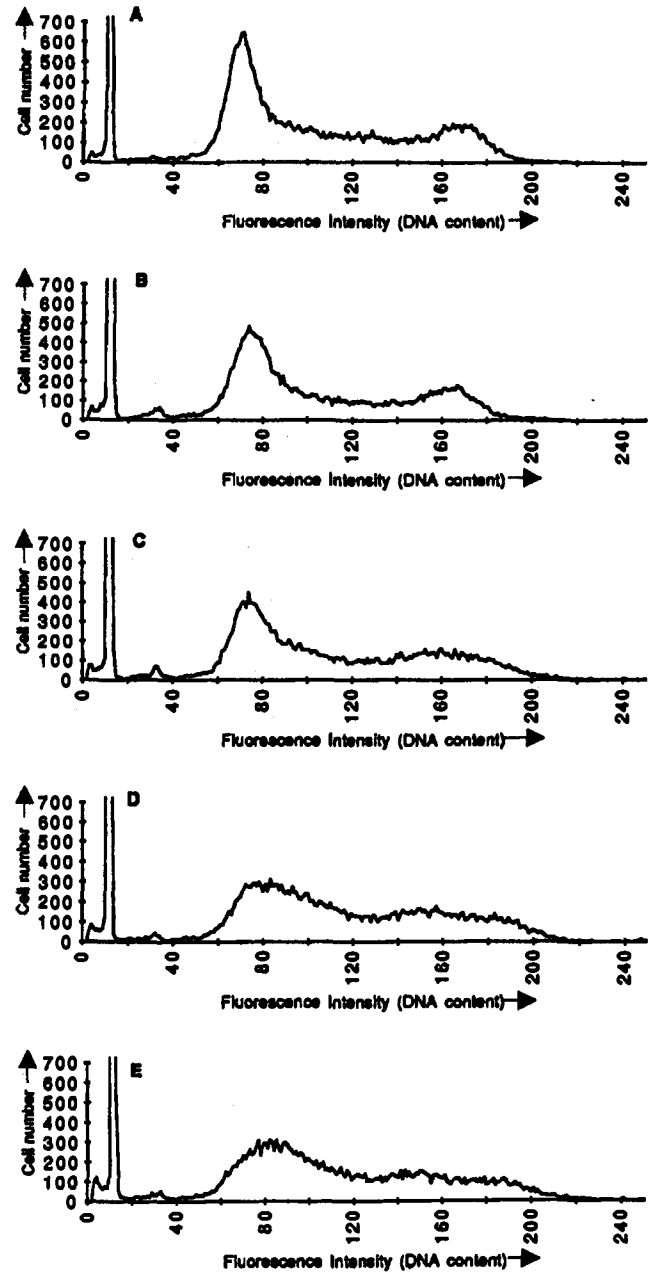


Figure 1. The effect of 10 μM 19 on the cell cycle distribution of L1210 cells. After various times of incubation with 19 (6 h, B; 12 h, C; 18 h, D; 24 h, E; control, A), the cells were fixed and stained as described in the Experimental Section for flow cytometric analysis of the DNA content of individual cells. The number of cells (ordinate) is plotted against the relative DNA content (abscissa), and the same total number of cells is represented in each histogram. The results shown are from a single experiment, and a second experiment showed virtually identical results.

General Procedure for the Synthesis of 2-Substituted 4-(Chloromethyl)thiazoles and -selenazoles (2–12). If the anhydride was a liquid, then a mixture of 1a[2] or 1b (5 mmol) and the appropriate anhydride (15–20 mL) was heated under reflux for 4–6 h. If the anhydride was a solid, then a mixture of 1a or 1b (5 mmol) and the anhydride (5 mmol) in toluene (20 mL) was

heated under reflux for 4 to 6 h. The excess of anhydride or solvent was removed *in vacuo*. Compounds 2–12, were isolated from the residue in pure form either by crystallization from the appropriate solvent or by column chromatography on a silica column. The physical data are given in Table I.

2-Amino-4-(isothiocyanatomethyl)thiazole (13a). A mixture of 2-(trifluoroacetamido)-4-(chloromethyl)thiazole (6, 0.90 g, 4.36 mmol), potassium thiocyanate (0.50 g, 5.15 mmol), and potassium iodide (0.40 g, 2.2 mmol) was heated at reflux in methanol (20 mL) for 6 h. The solvent was evaporated and water (30 mL) was added to the residue. The product was extracted from the water with methylene chloride (3 × 25 mL). The organic extracts were combined, dried (Na_2SO_4), filtered, and evaporated. The residue was crystallized from benzene to give 0.50 g (67%) of 13a: mp 105–106 °C (lit.[1] mp 105–107 °C).

General Procedure for the Synthesis of 2-Substituted-4-(isothiocyanatomethyl)thiazoles and -selenazoles (14–18, 27–31). A mixture of 2-substituted-4-(chloromethyl)thiazole or -selenazole (2–12, 4 mmol), potassium thiocyanate (4:3 mmol), and potassium iodide (2:0 mmol) in methanol (20 mL) was heated at reflux for 3 h. The solvent was removed *in vacuo* and water (30 mL) was added to the crude product. The mixture was extracted with methylene chloride (3 × 30 mL). The organic layer was dried (Na_2SO_4) and concentrated to give the crude product. On crystallization from the appropriate solvent, compounds 14–31 were isolated in pure form.

2-Substituted Benzamido-4-(isothiocyanatomethyl)thiazole (19–26). A mixture of compound 13a[1] (5 mmol) and the appropriately substituted benzoic anhydride (5.2 mmol) was heated at reflux in toluene (50 mL) for 10 h. After cooling, the solvent was washed with 10% $NaHCO_3$ (3 × 30 mL). The solvent layer was dried (Na_2SO_4) and filtered, and the solvent was evaporated under reduced pressure. Compounds 19–26 were obtained in pure form either by crystallization or by column chromatography using a silica column.

Antitumor Studies. The *in vitro* cytotoxicity against L1210 was evaluated as described previously.[9] L1210 cells were grown in static suspension culture at 38 °C using Fischer's medium for leukemic cells of mice, and the growth rate over a 3-day period was determined in the continuous presence of various concentrations of the test compound. Growth rate was defined as the slope of the semilogarithmic plot of cell number against time for the treated culture, as a percent of the slope for the control culture. Experimentally this parameter was determined by calculating the ratio of the population doubling time of control cells to the population doubling time of treated cells. When the growth rate slowed during the experiment, the rate used was the final rate attained at the end of the 3-day period. The IC_{50} was defined as the concentration required to decrease the growth rate to 50% of the control.

For flow cytometric analysis of DNA content, L1210 cells (10^5/mL) were treated with compounds as described in the Results and Discussion, fixed in ethanol, and stained with propidium iodide, as described in detail previously.[1] The cells were prepared for microscopic mitotic index determination as also described previously.[1] When $n = 10$, the values are the averages of results from two independent experiments, five slides per experiment, counting 1000 cells/slide. When $n = 5$, five slides in a single experiment were evaluated, counting 1000 cells/slide.

Antifilarial Studies. All new compounds were evaluated as described previously.[10]

Acknowledgment. This investigation was supported in part by the Filariasis Component of the World Bank/UNDP/WHO Special Program for Research and Training in Tropical Diseases (I.D. 800134), in part by Grant No. DHP-36 from the American Cancer Society, and in part by the University of Michigan Comprehensive Cancer Center Core Grant No. 2P30 CA46592 from the National Cancer Institute, DHHS. We also thank Ms. Tina Powell for expert technical assistance in the preparation of this manuscript, Julie A. Jones, Clare E. Rogers, and Kristine L. Weber for technical assistance with the flow cytometry, and Thomas J. Franks and Camille Horn for technical assistance with the other antitumor studies.

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