Synthesis of 2,4-Disubstituted Thiazoles and Selenazoles as Potential Antitumor and Antifilarial Agents: 1. Methyl 4-(Isothiocyanatomethyl)thiazole-2-carbamates, -selenazole-2-carbamates, and Related Derivatives

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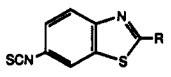
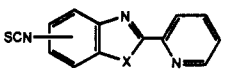
Methyl 4-(isothiocyanatomethyl)thiazole-2-carbamate and methyl 4-(isothiocyanatomethyl)selenazole-2-carbamate have been prepared *via* chemical transformations involving 2-amino-4-(chloromethyl)thiazole (1) and 2-amino-4-(chloromethyl)selenazole (2), respectively, as starting materials. The homoanalog, methyl 4-(2-isothiocyanatoethyl)thiazole-2-carbamate, was prepared from (2-aminothiazol-4-yl)acetic acid. All compounds prepared were evaluated for their ability to inhibit leukemia L1210 cell proliferation. Methyl 4-(isothiocyanatomethyl)thiazole-2-carbamate (7) was the most active compound in this screen, inhibiting the growth of L1210 leukemic cells with an $IC_{50} = 3.2\ \mu M$. Mitotic blocking appears to be its primary mechanism of cytotoxic activity. Compound 7 also was the only compound which demonstrated significant *in vivo* antifilarial activity against the adult worms of *Acanthocheilonema viteae* in experimentally infected jirds. This compound was inactive against *Brugia pahangi* at a dosage of 100 mg/kg × 5 days.

Introduction

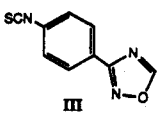
Recent publications[1-3] from these laboratories have described our approach to the design of antifilarial agents which possess macrofilaricidal activity and oral efficacy. The interesting antifilarial activity which has been described for a series of benzothiazoles[4] of the general structure I and the anthelmintic activity which has been demonstrated by 2-(pyridin-2-yl)-5-isothiocyanatobenzimidazole (**IIa**),[5] isothiocyanatobenzoxazoles (**IIb**),[6] isothiocyanatobenzothiazoles (**IIc**),[6] and 3-(4-isothiocyanatophenyl)-1,2,4-oxadiazole (**III**)[7] prompted the synthesis of a series of thiazole derivatives which possess an isothiocyanate functionality. In addition, a 2-methoxycarbamoyl



I



II a X = NH
b X = O
c X = S



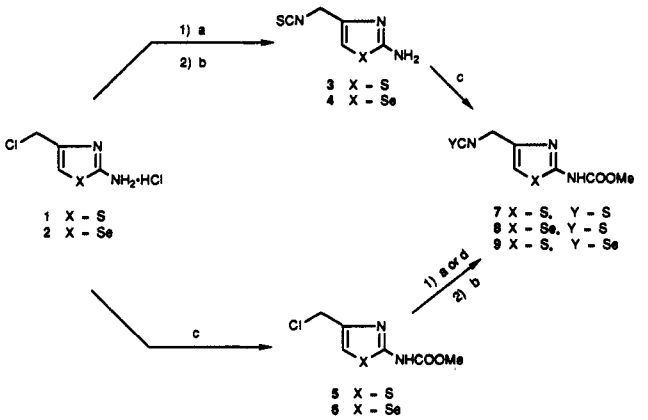
III

group was designed into these compounds to increase the potential for inhibition of microtubule assembly, the predominant mechanism of action of the anthelmintic benzimidazole carbamates.[8] A variety of carbamates have also shown cytotoxic activity against tumor cells and potential as antitumor agents.[9,10] Therefore, each compound was screened for its ability to inhibit growth of L1210 leukemic cells, as well as for antifilarial activity.

Chemistry

As shown in Scheme I, the synthesis of the target compounds could be accomplished by two methods which differ only by the timing of the introduction of the methoxycarbamoyl moiety into the molecule. In our initial approach, the isothiocyanato group was first placed on the molecule by the treatment of 2-amino-4-(chloromethyl)thiazole hydrochloride (1)[11] or 2-amino-4-(chloromethyl)selenazole hydrochloride (2)[11] with potassium thiocyanate in the presence of potassium iodide in

Scheme I[a]



[a] Reagents: (a) KSCN, KI; (b) NH_4OH; (c) $ClCO_2Me$, K_2CO_3; (d) KSeCN, KI.

methanol to afford 2-amino-4-(isothiocyanatomethyl)-thiazole (3) and 2-amino-4-(isothiocyanatomethyl)selenazole (4) in 76% and 53% yields, respectively. The 2-amino derivatives 3 and 4 were then reacted with methyl chloroformate and potassium carbonate to afford the desired carbamates 7 and 8 in 39% and 38% yield, respectively. Alternatively, the compounds 1 and 2 were converted to the respective 2-methoxycarbamoyl derivatives 5 and 6 in 49% and 10% yields. Compound 5 was subsequently reacted with potassium thiocyanate, or with potassium selenocyanate in the presence of potassium iodide, to give methyl 4-(isothiocyanatomethyl)thiazole-2-carbamate (7) and methyl 4-(isoselenocyanatomethyl)-thiazole-2-carbamate (9) in 39% and 36% yield, respectively. Because of the low yield of 6, this method was not used to prepare 8.

To prepare methyl 4-(2-isothiocyanatoethyl)thiazole-2-carbamate (16), (2-aminothiazol-4-yl)acetic acid (10) was first converted to its corresponding ethyl ester, 11, in ethanol at reflux in the presence of concentrated hydrochloric acid (Scheme II). The ester 11 was then treated with methyl chloroformate and potassium carbonate to furnish the 2-methoxycarbamoyl derivative 12. The reduction of 12 with lithium borohydride, followed by acid

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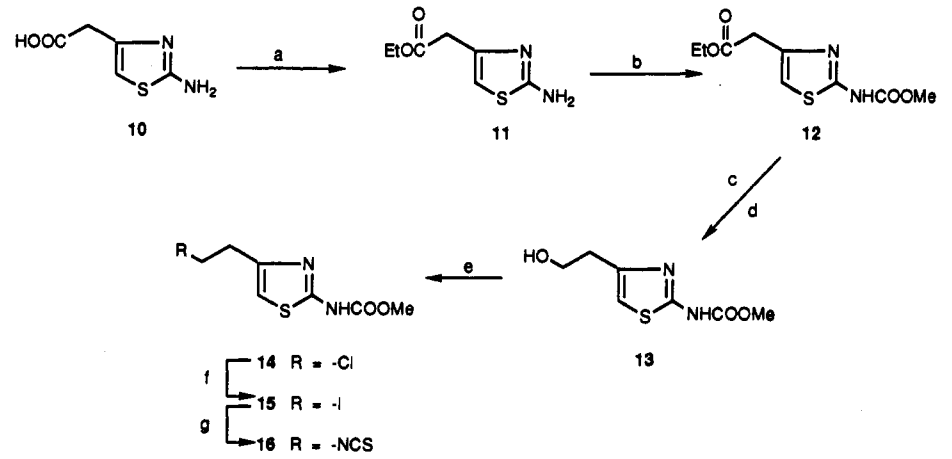
Scheme II[a]



[a] Reagents: (a) EtOH, HCl; (b) $ClCO_2Me$, K_2CO_3; (c) $LiBH_4$; (d) HCl; (e) $SOCl_2$; (f) KI, ethyl methyl ketone; (g) KSCN.

hydrolysis, afforded methyl 4-(2-hydroxyethyl)thiazole-2-carbamate (**13**) in 90% yield. Treatment of **13** with thionyl chloride at 50 °C in benzene for 30 min afforded the chloro derivative **14** in 79% yield. Reactions of **14** with potassium thiocyanate in the presence of potassium iodide in attempts to afford the desired isothiocyanate derivative **16** were unsuccessful. To circumvent this problem, **14** was treated with sodium iodide in ethyl methyl ketone which furnished the corresponding iodo compound **15** in 71% yield. Treatment of **15** with potassium thiocyanate in methanol at reflux furnished **16** in 84% yield.

Biological Results and Discussion

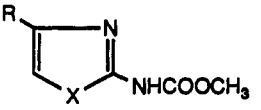
Antifilarial Activity. The antifilarial activity of these compounds was evaluated against the adult worms of *Brugia pahangi* and *Acanthocheilonema viteae* in jirds.[3] In this screen a compound is considered active if the number of implanted live adult worms at necropsy is decreased more than 65%. All new compounds were evaluated by this screen, and only **7** demonstrated antifilarial activity. This compound was 85% effective against *A. viteae* at a dosage of 100 mg/kg × 5 days, but was not active against *B. pahangi* at this dosage.

Antitumor Studies. Structure–Activity Requirements. The antitumor potential of these compounds was evaluated by determining their ability to inhibit proliferation of L1210 cells *in vitro* (Table I). The isothiocyanato or isoselenocyanato moiety at the 4-position was required for antiproliferative activity in this series of compounds. Of these active compounds, methyl 4-(isothiocyanatomethyl)thiazole-2-carbamate (**7**) was the most potent, followed closely by the corresponding 4-isoselenocyanatomethyl derivative **9**. Compound **8**, the selenazole analog of **7**, was less potent, as was compound **16**, the 4-isothiocyanatoethyl derivative. These results indicated that the antiproliferative activity of this series of compounds was optimal with the chemically reactive isothiocyanato 4-substituent and that there was a certain degree of steric constraint on the size of the 4-substituent. These observations suggest that the antiproliferative activity may involve binding and covalent attachment to a specific cellular macromolecule.

Mechanism of Action. The cellular effects of **7** were studied further, with the objective of elucidating the mechanism of its antiproliferative activity. In all the studies 100 μM **7** was used because this was the lowest concentration that caused total inhibition of cell proliferation. The effect of this growth inhibition on the viability of the cells was investigated by determining the fraction of the cells that were able to form colonies after various times of treatment. Cellular viability decreased sharply during the first 6 h to about 20% of control (Figure 1). Thereafter, it did not change significantly for the duration of the 24-h experiment. These results demonstrate that approximately 80% of the cells were irreversibly stopped from dividing within the first 6 h of exposure to **7**.

To obtain an indication of the possible mechanism of this damage, the effect of **7** on the distribution of the L1210 cell population through the cell cycle was studied using flow cytometric analysis of the DNA content of the cells. As shown in Figure 2, after continuous treatment with **7** for one cell cycle time, 12 h, virtually all cells had a DNA content corresponding to that found for cells in G_2 or mitosis, indicating accumulation of the cells in this portion of the cell cycle. To distinguish whether progression of the cells through the cell cycle was blocked in G_2 or in mitosis, the fraction of cells in mitosis after various times of treatment with **7** was determined microscopically. Within the first 6 h of treatment, about 30% of the cells accumulated in mitosis, and more prolonged treatment did not lead to any further increase (Figure 3). Thus, the fraction of the cells in microscopically identifiable mitosis

Table I. Antiproliferative Activity of 4-Substituted Methyl Thiazole-2-carbamates and Selenazole-2-carbamates against L1210 Cells *in Vitro*



compd no.	X	R	Screen: concn, μM	Screen: GR,[a] % of control	IC_{50},[b] μM
5	S	CH_2Cl	100	96	–[c]
6	Se	CH_2Cl	100	103	–
7	S	CH_2NCS	100	0	3.2
8	Se	CH_2NCS	100	31	22
9	S	CH_2NCSe	100	0	7.6
12	S	$CH_2COOCH_2CH_3$	100	104	–
13	S	CH_2CH_2OH	100	102	–
14	S	CH_2CH_2Cl	100	92	–
15	S	CH_2CH_2I	10[d]	92	–
16	S	CH_2CH_2NCS	100	0	26

[a] GR, growth rate. 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. [d] This compound could not be evaluated at 100 μM because of limited solubility.

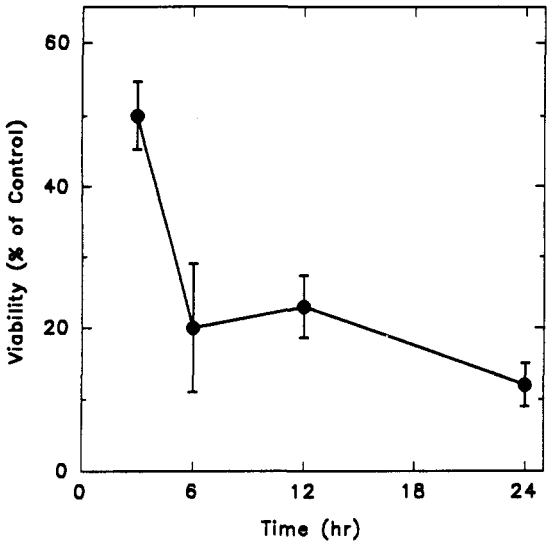


Figure 1. Effect of 100 μM 7 on the viability of L1210 cells. After the indicated times of treatment with 7, the cells were washed free of the compound and suspended in soft agar for colony formation, as described in the Experimental Section. The fraction of the treated cells that formed colonies was expressed as the percentage of the fraction of control cells that formed colonies. The values shown are the averages of three to four determinations (each the average of 10 replicate tubes) in two to four independent experiments, and the bars indicate the SEM. The average fraction of control cells that formed colonies in these experiments was 0.76 ± 0.038, SEM (n = 10).

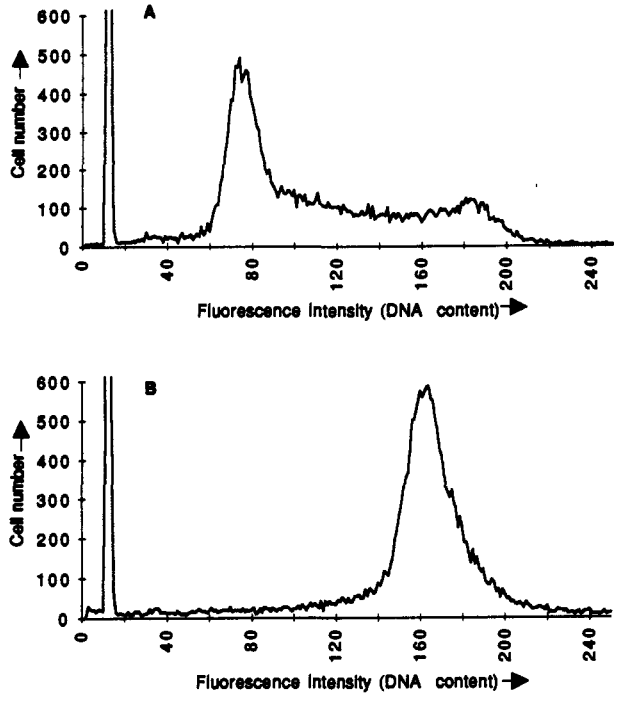


Figure 2. The effect of 100 μM 7 on the cell cycle distribution of L1210 cells. After 12 h of incubation with 7 (B) or as controls (A), the cells were fixed and stained as described in the Experimental Section, for flow cytometric analysis of the DNA content of individual cells. The number of cells (ordinate) is plotted against relative DNA content (abscissa), and the areas of the histograms have been normalized so that the same total number of cells is represented in each of them. The results shown are from a single experiment. A second experiment showed virtually identical results.

(metaphase or later) did not reach the level attained with colcemid, a known mitotic blocker included as a positive control (Figure 3). Considering that on the basis of DNA

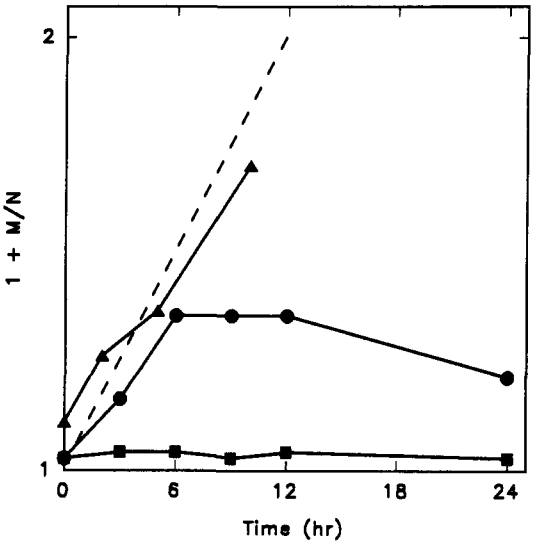


Figure 3. The effect of 100 μM 7 on the mitotic index of L1210 cells. After the indicated times of treatment with colcemid (▲), 7 (●), or as controls (■), the fraction of the cells in mitosis (mitotic index, M/N) was determined by microscopic examination, and (1 + M/N) was plotted semilogarithmically as a function of time. The values are the averages of two to four independent experiments, in each of which three to five slides were evaluated, scoring 500–1000 cells per slide. The dashed line indicates the ideal time course of accumulation of cells in mitosis that would occur if mitosis were blocked and no other effect occurred.

Table II. Effects of 7 on DNA, RNA, and Protein synthesis L1210 Cells[a]

time, h	[^{3}H]dThd	[^{3}H]Urd	[^{3}H]leucine
0	88 (87–89)	74 (61–87)	58 (51–65)
6	56 (52–60)	67 (62–72)	48 (40–56)

[a] The cells were treated simultaneously with 100 μM 7 and ^{3}H-dThd, ^{3}H-Urd or ^{3}H-leucine. Incubations lasted 1 hr and were started at the time of addition of 7 (0 hr) or 6 hr later. Then the incorporation of ^{3}H into the acid-insoluble fraction was determined as described in the Experimental. The values shown are the averages of two determinations, in separate experiments, expressed as % of control, with the range shown in parentheses.

content (flow cytometry) the cells appeared to have accumulated at a single point in the cell cycle (Figure 2), a fraction of the cells may be blocked in G_2 or in prophase (early mitosis). Alternatively, the mitotic figures may have deteriorated with time of treatment with 7 so that they were not microscopically recognizable. This point has not been further investigated. In any case, it is clear that 7 causes mitotic arrest of at least a major fraction of the cells, and it seems likely that this action at least partially accounts for its antiproliferative activity.

The effects of 7 on synthesis of DNA, RNA and protein in L1210 cells were not pronounced (Table II). The moderate inhibition of incorporation of [^{3}H]Leu into acid-insoluble material appeared to represent genuine inhibition of protein synthesis since the incorporation into acid-soluble material was less affected. In the first hour of treatment, it was 73% of control (range, 59–86), and in the seventh hour, 127% (range, 119–135). There also appeared to be a lesser, but progressive inhibition of DNA synthesis, while RNA synthesis was slightly inhibited, to a similar degree in the first and seventh hours of treatment with 7 (Table II). The possibility that 7 might inhibit ribonucleotide reductase was also examined. Over the entire concentration range studied, 25–250 μM, 7 had no effect on the reduction of CDP by highly purified enzyme from Ehrlich ascites tumor cells.[13]

The possible effect of 7 on DNA synthesis was further investigated utilizing the alkaline elution assay to examine the average molecular size of DNA synthesized in the presence of 7. L1210 cells were incubated for 6 h with 100 μM 7 and [^{14}C]dThd added simultaneously. Then alkaline elution of the DNA was performed, and no effect on the retention of the [^{14}C]DNA was detected. In two independent experiments, each having duplicate determinations, the average value for the fraction of the [^{14}C]DNA retained on the filter after elution was 0.92, for both control and treated cells. Thus, at least during the first 6 h of treatment with 7, the newly synthesized DNA was of sufficiently high molecular weight to be retained on the filter to an extent indistinguishable from that of control cells. Taken together with the results described above (Table II), these observations strongly suggested that the initial effect of 7 on L1210 cells did not involve significant inhibition of DNA synthesis.

The possibility that 7 might cause strand breakage in preformed DNA also was examined. L1210 cells were incubated for 12 h with [^{14}C]dThd, followed by 6 h with 100 μM 7, and alkaline elution of the DNA was performed. The retention of [^{14}C]DNA on the filter was not affected by 7. The average value of duplicate determinations in a single experiment was 0.95, for both control and treated cells. These results indicate that 7 did not affect the average molecular size of DNA replicated before the cells were treated. Thus, 7 did not appear to cause DNA strand breakage.

In summary, it appeared that 7 inhibited growth of L1210 cells primarily by acting as a mitotic blocker. The moderate inhibition of DNA, RNA, and protein synthesis is considered likely to be secondary to the arrest of a major fraction of the cells in mitosis.

Experimental Section

Melting points are uncorrected. IR spectra were recorded on a Perkin-Elmer Model 281 spectrometer. ^{1}H NMR spectra were obtained on a Bruker 270-MHz spectrometer, and chemical shift values (δ) are reported in parts per million. Column chromatography was carried out on silica gel, Kieslgel 60 F_{254} (70–230 mesh). TLC was performed on Analtech silica gel GF plates. Elemental analyses are within ±0.4% of the required values.

2-Amino-4-(isothiocyanatomethyl)thiazole (3). A mixture of 1 (1.0 g, 5.41 mmol), potassium thiocyanate (0.6 g, 6.22 mmol), and potassium iodide (0.45 g, 2.73 mmol) in methanol (20 mL) was heated at reflux for 2 h. The solvent was concentrated under reduced pressure, and the resulting residue was diluted with 10% aqueous NH_3 (20 mL). This solution was extracted with methylene chloride (2 × 30 mL). The organic layer was dried (K_2CO_3), the solvent was removed, and the crude product was crystallized from benzene to give 0.7 g (76%) of 3: mp 105–107 °C; ^{1}H NMR (DMSO-d_6) δ 4.15 (s, 2H, CH_2), 6.55 (s, 1H, CH), 7.10 (s, 2H, NH_2). Anal. ($C_5H_5N_3S_2$) C, H, N.

2-Amino-4-(isothiocyanatomethyl)selenazole (4). Compound 4 was prepared from 2 by the same procedure as given for compound 3: yield 53%; mp 113–115 °C; ^{1}H NMR (DMSO-d_6) δ 4.09 (s, 2H, CH_2), 7.00 (s, 1H, CH), 7.32 (s, 2H, NH_2). Anal. ($C_5H_5N_3SSe$) C, H, N.

Methyl 4-(Chloromethyl)thiazole-2-carbamate (5). To a mixture of 1 (2.0 g, 10.8 mmol) and potassium carbonate (2.9 g, 27.36 mmol) in methylene chloride (25 mL) was added methyl chloroformate (0.95 mL, 13.54 mmol) dropwise over a 5 min. The reaction mixture was heated at reflux until the TLC [developed in 95:5 (chloroform–methanol)] (~1 h) showed no starting material. The salts were removed by filtration and washed with methylene chloride. The solvent was removed from the combined filtrate and washed under reduced pressure, and the resulting residue was recrystallized from ethanol to afford 1.10 g (49%) of 5: mp 189–190 °C; ^{1}H NMR (DMSO-d_6) δ 3.76 (s, 3H, CH_3), 4.70 (s, 2H, CH_2), 7.25 (s, 1H, CH), 12.06 (s, 1H, NH). Anal. ($C_6H_7ClN_2O_2S$) C, H, N.

Methyl 4-(Chloromethyl)selenazole-2-carbamate (6). Compound 6 was synthesized from compound 2 by the same method as given for the preparation of 5. The crude product was purified by column chromatography using silica and chloroform–methanol (97:3) as the eluant. The pure compound was recrystallized from ethanol: yield 10%; mp 179–180 °C; ^{1}H NMR (DMSO-d_6) δ 3.75 (s, 3H, CH_3), 4.64 (s, 2H, CH_2), 7.69 (s, 1H, CH), 12.06 (s, 1H, NH). Anal. ($C_6H_7ClN_2O_2Se$) C, H, N.

Methyl 4-(Isothiocyanatomethyl)thiazole-2-carbamate (7). Method 1. A mixture of 5 (2.0 g, 8.73 mmol), potassium thiocyanate (0.94 g, 9.69 mmol), and potassium iodide (0.10 g, 0.60 mmol) was heated at reflux in methanol (40 mL) for 2 h. The solvent was removed, and the residue was taken up in methylene chloride. The salts were removed by filtration, and the filtrate was concentrated *in vacuo*. The crude product was purified on a silica column (2 × 40 cm) using chloroform–methanol (98:2) as the eluant. After removing the solvent from the fractions containing product, the residue was crystallized from ethanol to yield pure 7: yield 0.67 g (30%); mp 165–166 °C; ^{1}H NMR (DMSO-d_6) δ 3.73 (s, 3H, CH_3), 4.32 (s, 2H, CH_2), 7.17 (s, 1H, CH), 11.88 (s, 1H, NH). Anal. ($C_7H_7N_3O_2S_2$) C, H, N.

Method 2. Using the same procedure described for the preparation of 5, compound 7 was prepared from 3 in 39% yield. This material was identical in all respects with 7 prepared in method 1.

Methyl 4-(Isothiocyanatomethyl)selenazole-2-carbamate (8). Compound 8 was prepared from compound 4 using the same procedure as given for the synthesis of compound 7 (method 2). The crude product was purified on a silica column using methylene chloride–methanol (99.5:0.5) as eluant. The material isolated from the column was crystallized from toluene to yield 8 (38%): mp 155–157 °C; ^{1}H NMR (DMSO-d_6) δ 3.75 (s, 3H, OCH_3), 4.29 (s, 2H, CH_2), 7.63 (s, 1H, CH), 12.06 (s, 1H, NH). Anal. ($C_7H_7N_3SO_2Se$) C, H, N.

Methyl 4-(Isoselenocyanatomethyl)thiazole-2-carbamate (9). Compound 9 was prepared from 5 using a procedure similar to that used to obtain 7. Potassium selenocyanate was used in the place of potassium thiocyanate. The crude product was purified on a silica column using chloroform–methanol (97:3) as the eluant. The fractions containing product were pooled and evaporated, and the residue was recrystallized from ethanol to yield 9 (36%); mp 174–175 °C; ^{1}H NMR (DMSO-d_6) δ 3.75 (s, 3H, CH_3), 4.30 (s, 2H, CH_2), 7.15 (s, 1H, CH), 12.10 (s, 1H, NH). Anal. ($C_7H_7N_3O_2SSe$) C, H, N.

Ethyl (2-(Methoxycarbamoyl)thiazol-4-yl)acetate (12). To a suspension of (2-aminothiazol-4-yl)acetic acid (10, 4 g, 15.5 mmol) in ethanol (70 mL) was added concentrated HCl (20 mL). The mixture was heated at reflux for 3 h. The reaction was then cooled to <25 °C, and the solvent was evaporated *in vacuo*. The residue was treated with saturated aqueous sodium carbonate (30 mL). The solid which separated was collected by filtration, washed with water (2 × 25 mL), and dried over P_2O_5 to give 11: 4.3 g (92%); mp 85–87 °C [lit.[12] mp 94–95 °C]. To a mixture of 11 (4.0 g, 21.51 mmol) and potassium carbonate (5.0 g, 36.23 mmol) in methylene chloride (30 mL) was added methyl chloroformate (2.5 g, 26.45 mmol) dropwise over a period of 10 min. The reaction mixture was heated at reflux for 24 h. The solid which separated was collected by filtration and washed with water (15 mL) to afford 1.0 g of unreacted 11. The filtrate was concentrated, and the resulting residue was crystallized from ethanol to give 3.1 g (79%) of 12 (based upon reacted starting material): mp 135–137 °C; ^{1}H NMR (DMSO-d_6) δ 1.17 (t, 3H, CH_3), 3.66 (s, 2H, CH_2), 3.92 (s, 3H, OCH_3), 4.08 (q, 2H, OCH_2), 6.84 (s, 1H, CH). Anal. ($C_9H_{12}N_2O_4S$) C, H, N.

Methyl 4-(2-Hydroxyethyl)thiazole-2-carbamate (13). To a solution of 12 (2.0 g, 8.19 mmol) in methanol (100 mL) was added lithium borohydride (0.80 g, 36.36 mmol) in 5 portions. The mixture was heated at reflux for 48 h. The excess of lithium borohydride was destroyed by adding water (5 mL). The reaction mixture was acidified with 10% hydrochloric acid (20 mL) and heated on a steam bath for 10 min. The water was removed *in vacuo*, and the resulting residue was applied directly onto a silica column (2 × 40 cm). The required compound was eluted by methylene chloride–methanol (97:3) to yield 1.5 g (90%) of 13:

mp 134–136 °C; ^{1}H NMR (DMSO-d_6) δ 2.69 (t, 2H, CH_2), 3.6–3.7 (m, 2H, OCH_2; changed into a triplet after D_2O addition), 4.59 (t, 1H, OH; exchangeable with D_2O), 6.75 (s, 1H, CH), 11.64 (s, 1H, NH, exchangeable with D_2O). Anal. ($C_7H_{10}N_2O_3S$) C, H, N.

Methyl 4-(2-Chloroethyl)thiazole-2-carbamate (14). To a suspension of **13** (1.5 g, 7.43 mmol) in benzene (150 mL) was added thionyl chloride (15 mL) dropwise at room temperature. The reaction mixture was stirred for 30 min at 50 °C. The solvent was then removed *in vacuo*, and the residue was crystallized from ethanol to yield 1.3 g (79%) of **14**: mp 160–161 °C; ^{1}H NMR (DMSO-d_6) δ 3.00 (t, 2H, CH_2), 3.71 (s, 3H, CH_3), 3.85 (t, 2H, CH_2), 6.89 (s, 1H, CH), 11.71 (s, 1H, NH). Anal. ($C_7H_9ClN_2O_2S$) C, H, N.

Methyl 4-(2-Iodoethyl)thiazole-2-carbamate (15). A mixture of **14** (0.8 g, 3.62 mmol) and anhydrous sodium iodide (0.8 g, 5.35 mmol) was refluxed in methyl ethyl ketone (20 mL) for 48 h. The solvent was removed *in vacuo*, and the residue was treated with water. The resulting solid was collected by filtration and recrystallized from ethanol to yield 0.8 g (71%) of **15**: mp 166–167 °C; ^{1}H NMR (DMSO-d_6) δ 3.11 (t, 2H, CH_2), 3.45 (t, 2H, CH_2), 3.71 (s, 3H, CH_3), 6.87 (s, 1H, CH), 11.72 (s, 1H, NH). Anal. ($C_7H_9IN_2O_2S$) C, H, N.

Methyl 4-(2-Isothiocyanatoethyl)thiazole-2-carbamate (16). A mixture of **15** (0.55 g, 1.76 mmol) and potassium thiocyanate (0.21 g, 2.20 mmol) was heated in methanol (50 mL) at reflux for 48 h. The solvent was removed *in vacuo*, and the residue was treated with water. The solid was collected by filtration and crystallized from ethanol to yield 0.36 g (84%) of **16**: mp 152–154 °C; ^{1}H NMR (DMSO-d_6) δ 3.00 (t, 2H, CH_2), 3.33 (t, 2H, CH_2), 3.71 (s, 3H, CH_3), 6.93 (s, 1H, CH), 11.72 (s, 1H, NH). Anal. ($C_6H_9N_3O_2S_2$) C, H, N.

Antifilarial Studies. All compounds were evaluated as described previously.[3]

Antitumor Studies. Cellular Proliferation. The inhibition of L1210 cell growth was evaluated *in vitro* as described previously.[14] L1210 cells were grown in static suspension culture at 38 °C using Fischer's medium for leukemic cells of mice, supplemented with 10% heat-inactivated (56 °C, 30 min) horse serum. 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.

Cellular Viability. Compound-treated and control cells were separated from the medium by centrifugation and cloned in soft agar for colony formation as described previously,[14] except that the cultures contained 0.115% Purified Agar (Difco Laboratories, Detroit, MI). The fraction of the compound-treated cells that formed colonies was determined and is reported as the percent of the fraction of control cells that formed colonies (0.76 ± 0.038, n = 10).

Flow Cytometric Analysis of DNA Content Distribution. Compound-treated and control L1210 cells (10^6/sample) were separated from the medium by centrifugation. The medium was removed by aspiration, and the cells were resuspended using a vortex mixer in Puck's Saline G (according to the formulation in the Gibco Laboratories, Grand Island, NY, catalog). Trout erythrocytes (7 × 10^5/sample) were added, and the combined cells were sedimented by centrifugation. The supernatant was removed by aspiration, and the cells were resuspended as described above, in 1 mL of Puck's Saline G/sample, and chilled on ice. The cells in each sample were fixed in ethanol (final concentration 70%) by adding 2.5 mL of ice-cold ethanol dropwise down the side of the tube while mixing the cell suspension gently using a vortex mixer. The addition of ethanol was performed over a period of 2 min for each sample. The samples were then refrigerated (4 °C) 12 h or longer to complete the fixation. Then the cells were separated from the ethanol by centrifugation, the supernatant was aspirated, and the cells were resuspended using a vortex mixer. Then 5 mL of propidium iodide stain solution was added to each sample, the samples were placed on ice, and the cells were allowed to settle by gravity. Then 4 mL of the stain solution was removed, and the cells were resuspended in the remaining 1 mL for analysis. The composition of the propidium iodide stain solution was as follows: 10 mM Trizma base, 10 mM NaCl, 0.075 mM propidium iodide, 700 units/L RNase, pH 8.0. It was filter sterilized and stored at 4 °C.

The flow cytometric analysis was performed by the Flow Cytometry Core Facility of the University of Michigan Cancer Center using a Coulter Epics V instrument.

Mitotic Index. L1210 cells (5 mL, 10^5/mL) were incubated for the indicated time intervals with 100 μM 7. Then the cells were fixed and stained as described previously[3] for mitotic index determination.

Incorporation of Radiolabeled Precursors. The incorporation of [*methyl*-^{3}H]dThd (20 Ci/mmol, 1 μCi/mL) and [5-^{3}H]-Urd (28.5 Ci/mmol, 1 μCi/mL) into the acid-insoluble fraction of L1210 cells was determined as described previously,[15] except that the liquid scintillation fluid was Cytoscint (ICN Biomedicals, Inc., Irvine, CA).

The incorporation of L-[3,4,5-^{3}H(N)]leucine (143 Ci/mmol, 1 μCi/mL) into the acid-soluble and acid-insoluble fraction of L1210 cells was determined on duplicate 4-mL samples [(1–1.5) × 10^5 cells/mL] diluted at the stated time into 5 mL of ice-cold Puck's Saline G. The cells were sedimented by centrifugation, resuspended in 10 mL of ice-cold Puck's Saline G, and again sedimented by centrifugation. Then the cells were resuspended in 0.5 mL of bovine serum albumin solution (2 mg/mL) in Puck's Saline G, 0.5 mL of 8% perchloric acid was added, and the samples were allowed to precipitate on ice for 30 min. The precipitates were sedimented by centrifugation, and the supernatants (acid-soluble) were transferred to vials for liquid scintillation counting using Ecolite (ICN Biomedicals, Inc., Irvine, CA). The precipitates (acid-insoluble) were solubilized in 0.5 mL of 1 N NaOH and hydrolyzed by incubating 2 h or longer at 37 °C. The resulting solutions were transferred to scintillation counting vials, neutralized with 0.5 mL of 1 N HCl, and counted using Ecolite.

Alkaline Elution. L1210 cells were incubated with [^{14}C]dThd (54 mCi/mmol, 0.02 μCi/mL) and the compound as described in the text. Alkaline elution of the DNA was then performed as described previously.[16]

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