Synthesis and Biological Activity of Certain Alkyl 5-(Alkoxycarbonyl)-1*H*-benzimidazole-2-carbamates and Related Derivatives: A New Class of Potential Antineoplastic and Antifilarial Agents[1]

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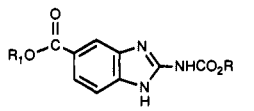
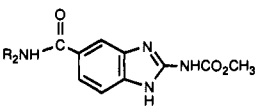
A series of methyl and ethyl 5-(alkoxycarbonyl)-1*H*-benzimidazole-2-carbamates (**7–19**) and methyl 5-carbamoyl-1*H*-benzimidazole-2-carbamates (**24–34**) have been synthesized via the reaction of an appropriate alcohol or amine with the acid chloride derivatives **6a** or **6b** at room temperature. Reaction of an alcohol with acid chloride **6a** at reflux temperature afforded transesterified products **20–23** in good yield. Treatment of methyl 5-amino-1*H*-benzimidazole-2-carbamate with substituted benzoyl chlorides furnished the methyl 5-benzamido-1*H*-benzimidazole-2-carbamates (**36–38**). Compounds **9**, **16**, **20**, and **22** demonstrated significant growth inhibition in L1210 cells with IC_{50}'s < 1 μM. Growth inhibition by this series of compounds appears to be associated with mitotic spindle poisoning. All the compounds tested, **9**, **10**, **19**, **20**, **22**, and **23**, caused significant accumulation of L1210 cells in mitosis. Compounds **7**, **9**, **19**, **25**, **26**, **27**, and **36** showed significant in vivo antifilarial activity against adult worms of *Brugia pahangi*, *Litomosoides carinii*, and *Acanthocheilonema viteae* in experimentally infected jirds.

The parasitic disease filariasis remains a major problem for world health, affecting over 250 million people. Human filarial diseases, i.e., *Wuchereria bancrofti*, *Brugia malayi*, and *Onchocerca volvulus* are responsible for a large number of disabilities including disfigurement and blindness.[2] These extraintestinal nematodes, especially *O. volvulus*, have proved difficult to treat since the adult filarial nematodes (macrofilariae) and their early larval stages (microfilariae) inhabit the body cavities, blood vessels, lymphatics, and subcutaneous tissues of the host. Although the macrocyclic lactone antibiotic ivermectin has recently been used successfully to treat the microfilarial stage of the disease,[3,4] no drug has been found to be effective against the adult worm. Thus, the search for a broad-range macrofilaricidal agent remains urgent.

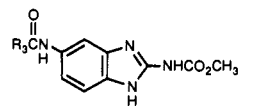
Intestinal parasites, in contrast, can be effectively treated with a number of benzimidazolecarbamates. Recently, several of these have also demonstrated macrofilaricidal activity when administered subcutaneously. However, no oral activity has been observed.[5,6] The lack of oral activity is not unexpected since the known anthelmintic benzimidazolecarbamates were designed to be poorly absorbed orally and, therefore, to be effective against intestinal parasites with minimal host involvement. The anthelmintic activity of this class of compounds appears to be related to their selective antimitotic action, due to the preferential binding of these agents to helmintic tubulin over mammalian (host) tubulin.[7] In a continuation of our studies[8-10] to develop an orally available benzimidazolecarbamate for use in filarial infections, we have prepared several alkyl 5-(substituted)-1*H*-benzimidazole-2-carbamates of the general structures **1–3** for evaluation as antifilarial agents. Since certain benzimidazolecarbamates have also been shown to possess antineoplastic activity,[11] the ability of the compounds to inhibit the growth of tumor cells in vitro was also studied as a preliminary indication of their antineoplastic potential.

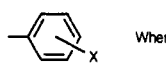


1 R_1 = Alkyl, Alkylaryl, R = Alkyl



2 R_2 = Alkyl, Aryl





3 R_3 = Where X = o, m, p-F

Chemistry

The required precursors (**5a–c**) were prepared by a literature procedure[12] which consisted of a condensation of the appropriate phenylenediamine (**4a,b**) with either N_1-(methoxycarbonyl)-2-methylthiopseudourea or N_1-(ethoxycarbonyl)-2-methylthiopseudourea (Scheme I).

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(1) A part of this work has been presented in abstract form: Wotring, L. L.; Ram, S.; Wise, D. S.; Townsend, L. B. New 2,5-Disubstituted Benzimidazoles as Potential Mitotic Blockers. *Proc. Am. Assoc. Cancer Res.* **1983**, *24*, 286.

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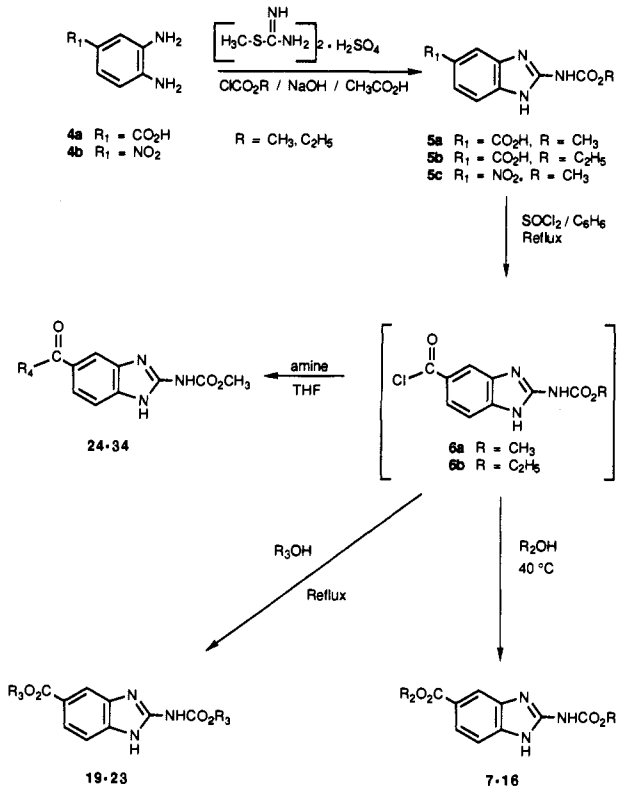
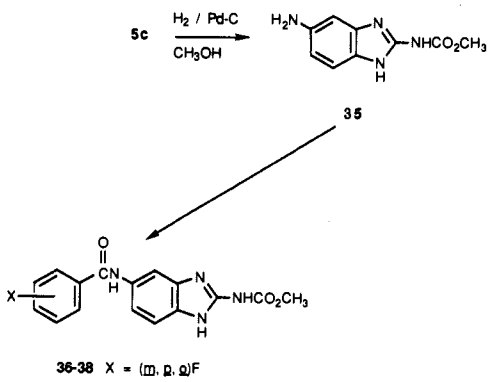
Scheme I



The reaction of compounds **5a** and **5b** with thionyl chloride in benzene at reflux temperature furnished the unstable acid chloride derivatives **6a** and **6b**. Compounds **6a** and **6b** were treated, without isolation, with a large excess of the appropriate alcohol at room temperature to afford the desired 2-(methoxycarbonyl) 5-carboxylates **7–18**. While the sterically hindered alcohol neopentyl alcohol furnished a poor yield of product **11**, other derivatives generally were produced in very good yield. The reaction of **6a** with cyclopropylmethyl alcohol at room temperature gave a mixture of the desired methyl 5-[(cyclopropylmethoxy)-carbonyl]-1*H*-benzimidazole-2-carbamate (**10**) and cyclopropylmethyl 5-[(cyclopropylmethoxy)carbonyl]-1*H*-benzimidazole-2-carbamate (**23**). The formation of **23** was due to the transesterification of the C-2 methyl ester with cyclopropylmethyl alcohol. By using a large excess of cyclopropylmethyl alcohol in the reaction and heating at reflux for 12 h, the bis-cyclopropylmethyl derivative **23** could be isolated in 70% yield. By taking advantage of this transesterification, the acid chloride **6a** was treated directly with various alcohols at reflux temperature to furnish the bis-substituted analogues **19–23** in good yield. Treatment of the carbonyl functionality of the 5-(isopropoxycarbonyl) group of **9** with sodium borohydride in refluxing 2-propanol funished only the bis-isopropyl compound **22** rather than the expected 5-hydroxymethyl derivative.

Scheme II



To prepare the target 5-amido derivatives **24–34**, acid chloride **6a** was treated with a variety of amines in tetrahydrofuran. While the yields of these reactions were generally very good, compound **30** was isolated only in 17% yield.

C-5 amino derivative **37**, prepared by the hydrogenation of methyl 5-nitro-1*H*-benzimidazole-2-carbamate (**5c**)[13] in the presence of 5% palladium on carbon, was reacted with substituted benzoyl chlorides in dimethylformamide/potassium carbonate. This reaction afforded the 5-benzoylamino derivatives **36–38** in good yield (Scheme II). The structures of all new compounds were established by spectroscopic and analytical data.

Biological Activities

Antifilarial Activity. The antifilarial activity of these compounds was evaluated against the filarial worms *B. pahangi*, *L. carinii*, and/or *A. viteae* in jirds. In this screen (Tables I–IV) a compound is considered active if the number of live adult worms at necropsy is decreased more than 60%. We examined the effect of structural modifications of the carbamate (**19–23**) group at C-2 (Table II) and variations of the 5-carboxylates and 5-carboxamides (Tables I and III) to develop a structure–activity relationship. In addition, three fluorinated 5-benzamides were evaluated for their antifilarial activity (Table IV). The series of 5-carboxylate and 5-carbamoyl carbamate compounds which retained a methyl 2-carbamate (Tables I and III) proved to be the most active while compounds **20–23**, which possessed a C-2 ethyl, *n*-propyl, isopropyl, or cyclopropylmethyl carbamate (Table II), showed no macrofilaricidal activity at the maximum dose tested, 25 mg/kg × 5 days. For example, compound **9**, which possesses a methyl 2-carbamate and a 5-isopropoxycarbonyl group (Table I), was curative at a dosage of 12.5 mg/kg × 5 days and 82% effective at a dosage of 6.25 mg/kg × 5 days, while the corresponding isopropyl 2-carbamate, 5-(isopropoxycarbonyl) derivative **22** (Table II) was devoid of activity at 25 mg/kg × 5 days. 5-Carboxylic acid derivative **5a** (Table I), per se, proved to be curative at a dosage of 100 mg/kg × 5 days; however, no activity was evident at lower dosages. The methyl (**19**), ethyl (**7**), and isopropyl

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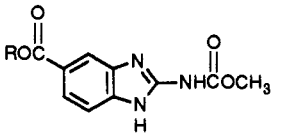
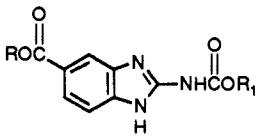
Table I. Inhibition of L1210 and H. Ep. 2 Cell Growth in Vitro and Reduction of *B. pahangi* and *A. viteae* Macrofilariae in Jirds by Alkyl and Aryl Esters of Methyl 5-Carboxy-1*H*-benzimidazole-2-carbamates



			L1210		H. Ep. 2		*B. pahangi*		
compd	R	concn in screens,[a] μM	growth rate, in screen,[b] % of control	IC_{50},[c] μM	growth rate, in screen,[b] % of control	IC_{50},[c] μM	dosage,[d] mg/kg per day	route[e]	macrofilariae at necropsy, % of control
5a	H	10	100	–			100	sc	0
							100	or	100
19[f]	CH_3	10	50	5.0	0	1.1	25	sc	4
							25[g]	sc	53
							12.5	sc	12
							6.2	sc	79
7	CH_2CH_3	100	0	2.8	0	0.24	25	sc	0
							12.5	sc	6
							1.6	sc	100
8	$CH_2CH_2CH_3$	10	38	7.4			100	sc	100
9[h]	$CH(CH_3)_2$	100	0	0.70	0	0.22	25	sc	0
							12.5	sc	0
							6.2	sc	18
							1.6	sc	51
							100	or	100
10	CH_2–cyclopropyl	10	45	6.9			25	sc	69
11	$CH_2CH_2CH(CH_3)_2$	10	60	~10			25	sc	69
12	$CH_2C(CH_3)_3$						25	sc	82
13	$CH_2CH{=}CH_2$	10	75	>10			25	sc	87
14	$CH_2C{\equiv}CH$	10	58	~10			25	sc	79
15	CH_2CH_2N–pyrrolidine	No data. Sol. too low.							
16	$(CH_2)_2CHCH_3CH_2C(CH_3)_3$	10	0	0.74			25	sc	85
17[i]	$CH_2C_6H_4$-*p*-F	No data. Sol. too low.					25	sc	83
18	CH_2–thiophene	10	93	–	0	2.3	25	sc	71

[a] Concentration at which the ability of the compound to inhibit mammalian cell growth was initially determined, in both cell lines where applicable. [b] Growth rate in the presence of the compound at the concentration in screens. [c] Concentration required to decrease the growth rate to half of the control rate. A dash indicates no significant growth inhibition at the concentration in screens. [d] Administered on 5 consecutive days unless otherwise noted. [e] sc = subcutaneous injection; or = oral administration. [f] 0% of control adult *A. viteae* at a sc dosage of 25 mg/kg × 5 days. [g] Administered on day 1 only. [h] 0% of control adult *A. viteae* at a sc dosage of 25 mg/kg × 5 days. [i] 86% of control adult *A. viteae* at a sc dosage of 25 mg/kg × 5 days.

Table II. Inhibition of L1210 and H. Ep. 2 Cell Growth in Vitro and Reduction of *B. pahangi* and *A. viteae* Macrofilariae in Jirds by Alkyl Esters of 5-Carboxy-1*H*-benzimidazole-2-carbamates

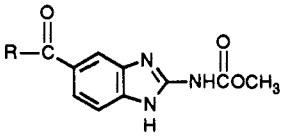


				L1210		H. Ep. 2		*B. pahangi*		
compd	R	R_1	concn in screens,[a] μM	growth rate in screen,[b] % of control	IC_{50},[c] μM	growth rate, in screen,[b] % of control	IC_{50},[c] μM	dosage,[d] mg/kg per day	route[e]	macrofilariae at necropsy, % of control
5b[f]	H	CH_2CH_3	100	100	–			25	sc	90
20	CH_2CH_3	CH_2CH_3	10	0	0.76	0	0.29	25	sc	62
21	$CH_2CH_2CH_3$	$CH_2CH_2CH_3$	10	36	8.2			25	sc	85
22	$CH(CH_3)_2$	$CH(CH_3)_2$	100	0	0.73	0	0.39	25	sc	87
23	CH_2–cyclopropyl	CH_2–cyclopropyl	10	24	2.2			25	sc	72

[a] Concentration at which the ability of the compound to inhibit mammalian cell growth was initially determined, in both cell lines where applicable. [b] Growth rate in the presence of the compound at the concentration in screens. [c] Concentration required to decrease the growth rate to half of the control rate. A dash indicates no significant growth inhibition at the concentration in screens. [d] Administered on 5 consecutive days unless otherwise noted. [e] sc = subcutaneous injection; or = oral administration. [f] 36% of control adult *A. viteae* at a sc dosage of 25 mg/kg × 5 days.

(9) 5-carboxylate methyl 2-carbamates were the most active derivatives prepared in this series of compounds. In contrast, *n*-propyl ester 8 was devoid of activity at 25 mg/kg administered for 5 days. All other esters (10–18) similarly demonstrated no activity at this dosage (Table I).

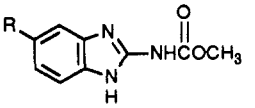
Table III. Inhibition of L1210 and H. Ep. 2 Cell Growth in Vitro and Reduction of *B. pahangi*, *A. viteae*, and *L. carinii* Macrofilariae in Jirds by Alkyl and Aryl 5-Carboxamides of Methyl 1*H*-Benzimidazole-2-carbamates



			L1210		H. Ep. 2		*B. pahangi*		
compd	R	concn in screen,[a] μM	growth rate in screen,[b] % of control	IC_{50},[c] μM	growth rate in screen,[b] % of control	IC_{50},[c] μM	dosage,[d] mg/kg per day	route[e]	macrofilariae of necropsy, % of control
25[f]	$NHCH_2CH_3$	10	41	10	0	6	100	sc	0
							50	sc	0
							25	sc	0
							100	or	89
26[g]	$NHCH(CH_3)_2$	10	0	2.1	0	1.6	25	sc	0
							12.5	sc	0
							6.5	sc	4
							1.6	sc	94
							100	or	110
27[h]	$NHCH_2C(CH_3)_3$	10	48	8.6			100	sc	0
							50	sc	0
							25	sc	50
							100	or	85
28	NHC_6H_4-*p*-F	10	92	–			25	sc	67
29	NHC_6H_4-*p*-CF_3	100	100	–			25	sc	77
24	$N(CH_3)_2$	100	88	–			25	sc	95
30							25	sc	55
31[i]		10	100	–			100	sc	101
32		No data. Sol. too low.					25	sc	94
33[j]		10	100	–			25	sc	100
34		100	0	60					

[a] Concentration at which the ability of the compound to inhibit mammalian cell growth was initially determined, in both cell lines where applicable. [b] Growth rate in the presence of the compound at the concentration in screens. [c] Concentration required to decrease the growth rate to half of the control rate. A dash indicates no significant growth inhibition at the concentration in screens. [d] Administered on 5 consecutive days unless otherwise noted. [e] sc = subcutaneous injection; or = oral administration. [f] 80% of control adult *A. viteae* at an oral dosage of 100 mg/kg × 5 days. [g] 57% of control adult *A. viteae* at a sc dosage of 1.56 mg/kg × 5 days. [h] 0% of control adult *A. viteae* at a sc dosage of 50 mg/kg × 5 days and 54% of control adult *A. viteae* at an oral dosage of 100 mg/kg × 5 days. [i] 49% of control adult *L. carinii* at a sc dosage of 100 mg/kg × 5 days. [j] 54% of control adult *L. carinii* at a sc dosage of 25 mg/kg × 5 days.

Table IV. Inhibition of L1210 and H. Ep. 2 Cell Growth in Vitro and Reduction of *B. pahangi* and *A. viteae* Macrofilariae in Jirds by 5-Substituted Methyl 1*H*-Benzimidazole-2-carbamates



			L1210		H. Ep. 2		*B. pahangi*		
compd	R	concentration in screen,[a] μM	growth rate in screen,[b] % of control	IC_{50},[c] μM	growth rate in screen,[b] % of control	IC_{50},[c] μM	dosage,[d] mg/kg per day	route[e]	macrofilariae at necropsy, % of control
36[f]	$NHCO-C_6H_4$-*m*-F	10	100	–			50	sc	0
37	$NHCO-C_6H_4$-*p*-F						25	sc	133
38	$NHCO-C_6H_4$-*o*-F	100	0	14			25	sc	116
nocodazole		10	0	0.038	0	0.033			

[a] Concentration at which the ability of the compound to inhibit mammalian cell growth was initially determined, in both mammalian cell lines where applicable. [b] Growth rate in the presence of the compound at the concentration in screens. [c] Concentration required to decrease the growth rate to half of the control rate. A dash indicates no significant growth inhibition at the concentration in screens. [d] Administered on 5 consecutive days unless otherwise noted. [e] sc = subcutaneous injections; or = oral administration. [f] 25% of control adult *A. viteae* at a sc dosage of 25 mg/kg × 5 days.

In the series of 5-carboxamides (**24–34**) prepared in this study the smaller alkylamides proved to possess the most macrofilaricidal activity (Table III). The 5-*N*-ethyl and *N*-isopropyl derivatives **25** and **26** were curative at 25 and 6.25 mg/kg × 5 days, respectively. The aromatic carboxamides **28** and **29** were devoid of activity. Dialkylamides (**24**, **30–34**) also demonstrated no antifilarial activity.

Three isomeric fluorobenzamides **36–38** were examined

as congeners of the drug flubendazole. *m*-Fluoro derivative **36** was curative at a dosage of 50 mg/kg × 5 days, while the *p*- and *o*-fluoro derivatives showed no activities at 25 mg/kg × 5 days (Table IV).

Although they are not effective when administered orally, the lead compounds **9** and **26** are undergoing secondary biological evaluation in dogs and these results will be reported elsewhere.

Antitumor Results and Discussion. The potential antitumor activity of these compounds was evaluated by assaying their ability to inhibit the growth of L1210 murine leukemia and H. Ep. 2 human epidermoid carcinoma cells in vitro. A series of esters of the 5-carboxylic acid derivative of methyl 1*H*-benzimidazole-2-carbamate were tested, and all except **13** and **18** showed cytotoxic activity against L1210 cells, with IC_{50}'s ≤ 10 μM (Table I). In this series, esterification of the 5-carboxy function was required for cytotoxic activity as shown by the observation that compound **5a** with the underivatized 5-carboxylic acid was completely inactive. The most potent compounds in this series were the isopropyl (**9**) and $CH_2CH_2CHCH_3CH_2C(CH_3)_3$ (**16**) esters, with IC_{50}'s of less than 1 μM for L1210 cells. These two compounds have branched alkyl R groups in common. This may be a necessary but not sufficient condition for the maximal cytotoxic activity in this series, since several compounds with other branched alkyl R groups, such as **10** and **11**, are much less potent, with IC_{50}'s on the order of 10 μM. Compound **18** (Table I) was of particular interest because of its close resemblance to nocodazole, a compound with demonstrated antitumor activity.[11] H. Ep. 2 cells were moderately sensitive to compound **18** (IC_{50} = 2.3 μM), while L1210 cells were not significantly affected at 10 μM. These results are consistent with the generally greater sensitivity of H. Ep. 2 cells to compounds in this series, as shown by the results with **7**, **9**, and **19**. In contrast, both cell lines were very sensitive to nocodazole (Table IV). This observation confirms the importance of the structural parameters for the 5-substituent in determining the potential cytotoxic activity of this series of compounds.

A more limited series of 5-carboxylic ester benzimidazole-2-carbamates with alkyl groups other than methyl on the 2-carbamate moiety was also evaluated (Table II). The importance of esterification of the carboxylic acid was confirmed in this series. Ethyl 5-carboxy-1*H*-benzimidazole-2-carbamate (**5b**) (like **5a**) was totally devoid of cytotoxic activity, while compounds **20–23** with esterified 5-carboxy functions were cytotoxic. The most cytotoxic compounds in this series were **20** and **22**. These compounds had IC_{50}'s less than 1 μM, comparable to the activity of **9** and **16** (Table I). The activity of **22** was very comparable to that of **9**. Apparently, the activity conferred by the presence of an isopropyl ester substituent at the 5-position cannot be improved upon by changing the methyl 2-carbamate (**9**) to an isopropyl 2-carbamate (**22**). On the other hand, when the 5-substituent was an ethyl ester the activity was improved by converting the methyl 2-carbamate (**7**) to an ethyl 2-carbamate (**20**).

The specificity of the requirement for an esterified 5-carboxy functionality discussed above was investigated in a third series of compounds, the alkyl and aryl 5-carboxamides of methyl benzimidazole-2-carbamates (Table III). Compounds **25** and **26** are analogues of compounds **7** and **9** in Table I in the sense that the ester linkage has been converted to an amide. This change resulted in a 3–4-fold decrease in cytotoxic potency, though the structure–activity relationship for the R group was not altered since the isopropyl moiety conferred greater activity than the ethyl in both cases, **9** vs **7** and **26** vs **25**. In fact, compound **26** was the most cytotoxic of all the amides prepared. When the amide nitrogen was disubstituted, as in compounds **24** and **31–34**, or when the substituent was an aryl function, as in compounds **28** and **29**, cytotoxic activity was drastically decreased (**34**) or abolished.

Table V. Effect of Selected Cytotoxic 2,5-Disubstituted Benzimidazoles on the Colony-Forming Ability of L1210 Cells[a]

compd	colony formation % of control (range) 24 h	48 h
9	5.0 (3.3–7.3)	2.2 (0.67–3.7)
20	3.7 (2.9–4.9)	3.7 (3.3–4.2)
22	4.0 (3.1–5.0)	1.5 (0.64–2.4)

[a] Cells were incubated for 24 or 48 h with the indicated compound (10 μM). Then the fraction of cells which were able to form colonies was determined as described in the Experimental Section. The values are the averages of three determinations, each from an independent experiment in which the average number of colonies in nine replicate tubes was expressed as the percent of the control for the corresponding drug and treatment time. The ranges of the three determinations are shown in parentheses.

Table IV shows the cytotoxicity evaluations of several additional methyl 5-substituted benzimidazole-2-carbamates. Apparently the presence of an aryl moiety in the 5-substituent is not altogether incompatible with cytotoxic activity since compound **38** had significant activity. The position of the fluorine-substituent on the benzene ring appears to be important since *o*-fluoro compound **38** was significantly cytotoxic, while meta isomer **36** was completely inactive. As noted above, compounds **28** and **29** (Table III) with *p*-F and *p*-CF_3, respectively, were also totally inactive. Nocodazole, a methyl 5-substituted benzimidazole-2-carbamate which has antitumor activity,[11] was included in these studies for comparison. It was found to have a lower IC_{50} than any of the new compounds reported here (Table IV).

The effect of L1210 cell viability was investigated for several of the most cytotoxic compounds (**9**, **20**, and **22**) at 10 μM, a concentration that caused complete inhibition of cell growth. Treatment for 24 h with any one of these three compounds killed ≥95% of the cells, as determined by colony formation (Table V). Incubation for an additional 24 h killed a small additional increment of cells, up to 98%. Thus, it is clear that growth inhibition by these compounds can be lethal to the cells, suggesting their potential usefulness as antineoplastic agents.

The mechanism of action for the cytotoxic benzimidazole-2-carbamates reported previously appeared to be a mitotic block caused by binding to tubulin subunits.[7,11] This mechanism of action also may apply to the compounds studied here. Six of the compounds were tested and were found to cause a significant accumulation of cells in mitosis. After exponentially growing L1210 cells were treated for 24 h with 10 μM compound **9**, **10**, **19**, or **23**, the mitotic indices of the population were 0.17, 0.22, 0.14, and 0.12, respectively, while the average control value was 0.02. Furthermore, after treatment with compound **23**, nuclear fragmentation was observed in a significant fraction of the cells. The time course of the accumulation of L1210 cells in mitosis was studied in more detail for compounds **9**, **20**, and **22** at 10 μM (Figure 1). The accumulation deviated from the ideal accumulation expected if a pure mitotic block were the only effect of the compounds, to an extent similar to that observed with colcemid, a known microtubule poison (Figure 1). The cause of this deviation is not known. It could be due to a second block to cell progression being induced at another point, earlier in the cell cycle. Alternatively, it could be due to a portion of the cells

being blocked at an earlier stage in mitosis, i.e., before recognizable metaphase chromosomes were formed. The 5-substituted benzimidazole-2-carbamates reported here further resembled colcemid, in contrast to the vinca alkaloid vincristine, in that during continuous treatment many cells were arrested in microscopically recognizable mitosis (Figure 1), while cells treated continuously with vincristine were arrested in G_2 and entered mitosis only after removal of the drug.[14]

Comparison of the antitumor and antifilarial data in Tables I–III shows that all compounds with antifilarial activity also showed antitumor activity, but not all compounds with antitumor activity showed antifilarial activity. Therefore, for this group of compounds, it appeared that the antifilarial compounds were a subset of the antitumor compounds. This observation is consistent with the hypothesis that both activities may be accounted for by binding to tubulin and preventing the formation of microtubules, as reported for nocodazole.[11] In addition, the antifilarial compounds would be proposed to possess selectivity for binding to filarial tubulin in preference to mammalian tubulin, as reported for other benzimidazoles,[7] and/or to possess pharmacokinetic properties that enable them to exhibit antifilarial activity in vivo. It is interesting to note that only methyl (not other alkyl) carbamates had antifilarial activity (Tables I and II, cf. Table III). This observation suggests that the larger alkyl carbamates do not possess the proposed selectivity of tubulin binding or the favorable pharmacokinetic properties. In contrast, compound **36** (Table IV) has antifilarial activity but not antitumor activity, suggesting that it may possess a different mechanism of antifilarial activity than the compounds in Tables I–III.

Experimental Section

Chemistry. Melting points were determined using a Thomas-Hoover capillary melting point apparatus and are uncorrected. IR spectra were recorded with a Perkin-Elmer Model 281 infrared spectrophotometer and the values are expressed in cm^{-1}. ^1H NMR spectra were recorded with a Varian EM-360, 60-MHz spectrometer, and the chemical shift values are reported in parts per million on the δ scale from internal standard tetramethylsilane. Column chromatography was carried out with silica gel (60–200 mesh) or Kiesel gel 60 F_{254} (70–230 mesh), and $CHCl_3$, $CHCl_3$/MeOH (9:1), or $CHCl_3$/hexane mixtures as eluants. Thin layer chromatography (TLC) was performed on silica gel-GF, Analtech plates and spots were visualized either by UV or by development in an iodine atmosphere. All evaporations were performed with a rotary evaporator under water aspirator reduced pressure or high vacuum at 40 °C, unless otherwise stated. Microanalyses were performed by M.H.W. Laboratories, Phoenix, AZ. All the compounds were analyzed for C, H, and N. Analytical results were within ±0.4% of theoretical values.

Methyl 5-Carboxy-1*H*-benzimidazole-2-carbamate (5a).[12] A 25% aqueous solution of sodium hydroxide was added to an ice-cold stirred mixture of 2-methylthiopseudourea sulfate (2.79 g, 0.01 mmol) and methyl chloroformate (1.89 g, 0.02 mmol) in water (3.5 mL), until the pH of the reaction mixture reached 8.0. Care was taken to keep the temperature below 10–15 °C. The pH of the reaction mixture was then adjusted to 5.0 with glacial acetic acid. To the above suspension was added 3,4-diaminobenzoic acid (1.52 g, 0.01 mmol) followed by addition of water (40 mL). The resulting reaction mixture was stirred at 95 °C for 2 h, then cooled to room temperature. A light grey solid precipitated from the reaction mixture which was collected by filtration, washed with water (50 mL), and air-dried. The solid was resuspended in methanol (100 mL), refluxed for $^1/_2$ h, and then cooled to room temperature and collected by filtration. The solid was air-dried to yield 2.2 g (85%) of **5a**: mp >315 °C; IR (KBr) ν_{max} 3360, 1705, 1685, 1642, 760 cm^{-1}; ^1H NMR (DMSO-d_6) δ 3.83 (s, 3 H, OCH_3), 7.35–8.33 (m, 3 H, Ar-H), 8.00–11.67 (m, 2 H, NH and CO_2H, exchangeable with D_2O). Anal. ($C_{10}H_9N_3O_4$) C, H, N.

Ethyl 5-Carboxy-1*H*-benzimidazole-2-carbamate (5b). A 25% aqueous solution of sodium hydroxide was added dropwise to a 10–15 °C stirred mixture of 2-methyl-2-thiopseudourea sulfate (13.92 g, 0.05 mol) and ethyl chloroformate (10.85 g, 0.1 mol) in H_2O (10 mL) until the pH of the reaction mixture reached 8.0. At this point, the pH of the reaction mixture was then adjusted to 5.0 with glacial acetic acid. To the above reaction mixture was added 3,4-diaminobenzoic acid (7.60 g, 0.05 mol) followed by the addition of H_2O (100 mL). The resulting reaction mixture was stirred and heated at 90 °C for 3 h, then cooled to room temperature, the solid which separated was collected by filtration, washed with water (100 mL), and air-dried. The solid was suspended in methanol (200 mL) and stirred at room temperature for 2 h, and then collected by filtration and dried to yield 11.0 g (89%) of **5b**; mp >300 °C; IR (KBr) ν_{max} 3380, 1715, 765 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.33 (t, 3 H, CH_3), 4.33 (q, 2 H, OCH_2), 7.41–8.33 (m, 3 H, Ar-H), 10.67–13.00 (bm, 2 H, NH and COOH). Anal. ($C_{11}H_{11}N_3O_4$) C, H, N.

General Procedure for the Synthesis of Methyl or Ethyl 5-(Alkoxycarbonyl)-1*H*-benzimidazole-2-carbamates (7–18). Thionyl chloride (6.0 mL) was added to an ice-cold stirred suspension of either **5a** or **5b** (0.0075 mmol) in dry benzene (30 mL). The resulting reaction mixture was stirred at reflux temperature for 3–4 h, and then cooled to room temperature. The solvent was evaporated under vacuum. The last traces of thionyl chloride were removed by coevaporation with benzene (3 × 10 mL). The appropriate alcohol was added to the residue and the resulting reaction mixture was stirred at room temperature for 2–12 h. The reaction mixture was heated at 40 ± 5 °C for 1 h and then cooled to room temperature. The solvent was evaporated under reduced pressure (water aspirator). The residue was purified by either column chromatography over silica gel 60 F_{254} (70–230 mesh) using neat $CHCl_3$, 1:1 $CHCl_3$/MeOH (v/v) or 1:1 $CHCl_3$/hexane (v/v) as the eluant. Several of the crude products were purified by crystallization to furnish the desired product.

Methyl 5-(Ethoxycarbonyl)-1*H*-benzimidazole-2-carbamate (7). This compound was prepared by using the general procedure, in a yield of 81%: mp 297 °C; IR (KBr) ν_{max} 1750, 1720, 762 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.35 (t, 3 H, CH_3), 3.87 (s, 3 H, OCH_3), 4.30 (q, 2 H, OCH_2), 6.46 (bs, 3 H, NH_2, exchangeable with D_2O), 7.78 (dd, 2 H, Ar-H), 8.20 (s, 1 H, Ar-H). Anal. ($C_{12}H_{14}N_3O_4$·HCl) C, H, N.

Methyl 5-(*n*-Propoxycarbonyl)-1*H*-benzimidazole-2-carbamate (8). This compound was prepared by using the general procedure, in a yield of 85%: mp 291–293 °C, recrystallized from *n*-propanol/ether; IR (KBr) ν_{max} 3240–2760, 1750, 1720, 760 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.03 (t, 3 H, CH_3), 1.80 (g, 2 H, CH_2), 3.87 (s, 3 H, OCH_3), 4.23 (t, 2 H, OCH_2), 5.34–7.67 (m, 3 H, H_2O, exchangeable with D_2O), 7.85 (dd, 2 H, Ar-H), 8.25 (s, 1 H, Ar-H). Anal. ($C_{13}H_{16}N_3O_4$·HCl·0.5H_2O) C, H, N.

Methyl 5-(Isopropoxycarbonyl)-1*H*-benzimidazole-2-carbamate (9). This compound was prepared by using the general procedure, in a yield 85%: mp 265–266 °C; IR (KBr) ν_{max} 3400, 2980, 1735, 1715, 765 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.47 (d, 6 H, 2 × CH_3), 3.23 (s, 1 H, NHCO), 3.85 (s, 3 H, OCH_3), 5.20 (q, 1 H, OCH), 7.10–8.30 (m, 3 H, Ar-H), 12.03 (s, 1 H, NH). Anal. ($C_{13}H_{16}N_3O_4$) C, H, N.

Methyl 5-(Neopentoxycarbonyl)-1*H*-benzimidazole-2-carbamate (11). This compound was prepared, using the general procedure, in a yield of 96%: mp 238–240 °C; IR (KBr) ν_{max} 3400, 2960, 2860, 1735, 1710, 765 cm^{-1}; ^1H NMR (DMSO-d_6) δ 0.97 (d, 6 H, 2 × CH_3), 1.33–2.27 (m, 3 H, CH_2CH), 3.86 (s, 3 H, OCH_3), 4.32 (t, 2 H, OCH_2), 7.37–8.38 (m, 3 H, Ar-H), 12.08 (bs, 1 H, NH, exchangeable with D_2O). Anal. ($C_{15}H_{19}N_3O_4$) C, H, N.

Methyl 5-(*sec*-Butoxycarbonyl)-1*H*-benzimidazole-2-carbamate (12). With the general procedure, this compound was prepared in a yield of 15%: mp >300 °C; IR (KBr) ν_{max} 3410–3390, 2960, 1730, 1715, 765 cm^{-1}; ^1H NMR (DMSO-d_6) δ 0.6–1.67 (m, 9 H, 3 × CH_3), 3.84 (s, 3 H, OCH_3), 3.97 (s, 2 H, OCH_2), 7.27–8.23

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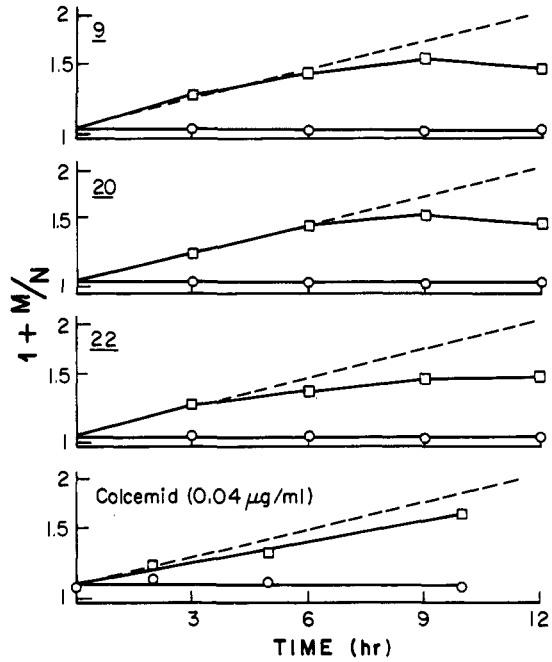

Figure 1. Accumulation of L1210 Cells in mitosis during incubation with 10 μM compounds **9**, **20**, and **22** and 0.04 μg/mL colcemid. At each time point, samples of the treated (□) and control (○) cells were prepared and their mitotic index was determined as described in the Experimental Section. The values shown are the averages of two experiments, each with three slides evaluated for each point. The range of the two values was less than 10% of the mean for all the determinations on treated cells, except 6 h with compound **22**, which was 20%. The ranges of the control values were 8–106% of the means. The dashed line in each figure represents the theoretical result of a pure mitotic block.

(m, 3 H, Ar-H), 12.20 (bs, 1 H, NH). Anal. ($C_{15}H_{19}N_3O_4$) C, H, N.

Methyl 5-[(Propargyloxy)carbonyl]-1*H*-benzimidazole-2-carbamate (14). With the general procedure, this compound was prepared, in a yield of 65%: mp >300 °C; crystallized from ethanol/ether; IR (KBr) 3400, 1725, 1710, 762 cm^{-1}; ^1H NMR (DMSO-d_6) δ 3.43 (s, 1 H, C≡CH), 3.82 (s, 3 H, OCH_3), 4.84 (t, 2 H, OCH_2), 7.28–8.26 (m, 3 H, Ar-H), 8.34–9.85 (m, 3 H, NH_2, NHCO). Anal. ($C_{13}H_{13}N_3O_4 \cdot H_2O$) C, H, N.

Methyl 5-[(2-Pyrrolidinoethoxy)carbonyl]-1*H*-benzimidazole-2-carbamate (15). With the general procedure, this compound was prepared, in a yield of 59%: mp 256–257 °C; IR (KBr) ν_{max} 3420, 2950, 1740–1705, 760 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.80–2.43 (m, 4 H, CH_2CH_2), 2.93–3.77 (m, 6 H, $H_2CNCH_2)_2$), 3.82 (s, 3 H, OCH_2), 4.67 (t, 2 H, OCH_2), 7.30–8.38 (m, 3 H, Ar-H), 12.0 (bs, 1 H, NH). Anal. ($C_{16}H_{22}N_4O_5 \cdot HCl$) C, H, N.

Methyl 5-[[(3,5,5-Trimethylhexyl)oxy]carbonyl]-1*H*-benzimidazole-2-carbamate (16). With the general procedure, this compound was prepared, in a yield of 52%: mp 219–220 °C; IR (KBr) ν_{max} 3400–3360, 2960, 1730–1705, 770, 760 cm^{-1}; ^1H NMR (DMSO-d_6) δ 0.88–2.20 (m, 16 H, $CH_2CH(CH_3)CH_2C(CH_3)_3$), 3.85 (s, 3 H, OCH_3), 4.25 (t, 2 H, OCH_2), 7.33–8.45 (m, 3 H, Ar-H), 12.00 (bs, 1 H, NH). Anal. ($C_{19}H_{27}N_3O_4$) C, H, N.

Methyl 5-[[(*p*-Fluorobenzyl)oxy]carbonyl]-1*H*-benzimidazole-2-carbamate (17). With the general procedure, this compound was prepared, in a yield of 71%: mp >300 °C; IR (KBr) ν_{max} 1750, 1725, 760 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.34 (t, 3 H, CCH_3), 4.33 (g, 2 H, OCH_2), 5.32 (s, 2 H, OCH_2Ar), 6.57–8.43 (m, 10 H, Ar-H, NH_2, NHCO, and H_2O, exchangeable with D_2O). Anal. ($C_{18}H_{17}N_3FO_4 \cdot 0.5H_2O \cdot HCl$) C, H, N.

Methyl 5-[(2-Thienylmethoxy)carbonyl]-1*H*-benzimidazole-2-carbamate (18). With the general procedure, this compound was prepared, in a yield of 86%: mp 268–271 °C; IR (KBr) ν_{max} 1760, 1730–1710, 760, 700 cm^{-1}; ^1H NMR (DMSO-d_6) δ 3.82 (s, 3 H, OCH_3), 5.48 (s, 2 H, OCH_2), 5.63 (bs, NH, NHCO, and H_2O), 6.83–8.20 (m, 6 H, Ar-H). Anal. ($C_{15}H_{15}N_3O_4S$) C, H, N.

Methyl 5-(Methoxycarbonyl)-1*H*-benzimidazole-2-carbamate Hydrochloride Salt (19). Thionyl chloride (6.0 mL) was added to a cold stirred suspension of **5a** (2.0 g, 0.0085 mmol) in dry benzene (40 mL). The mixture was stirred at reflux for 4 h and then cooled to room temperature. The solvent was evaporated under reduced pressure. The last traces of thionyl chloride were removed by coevaporation of the residue with dry benzene (20 mL × 3). The residue, **6a**, was resuspended in absolute methanol (150 mL) and stirred at reflux for 4 h. The purple solution was decolored by treatment with charcoal. The mixture was filtered while hot through a Celite bed which was washed with warm methanol (50 mL). The filtrate on concentration under vacuo afforded **19** as a colorless solid. Recrystallization from methanol yielded 2.1 g (87%) of **19**: mp 283–285 °C; IR (KBr) ν_{max} 3200–2730, 1744, 1715, 760 cm^{-1}; ^1H NMR (DMSO-d_6) δ 3.90 (s, 6 H, 2-OCH_3), 7.64–8.60 (m, 3 H, Ar-H), 9.20–10.30 (m, 3 H, 2 × NH, HCl, exchangeable with D_2O). Anal. ($C_{11}H_{12}N_3ClO_4$) C, H, N.

Ethyl 5-(Ethoxycarbonyl)-1*H*-benzimidazole-2-carbamate (20). With **5b** and ethanol, the same procedure described to prepare **19** was used to prepare **20** in a yield of 91%: mp >300 °C; IR (KBr) ν_{max} 3400–3360, 2980, 1735–1700, 770–760 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.37 (t, 6 H, 2 × CH_3), 4.36 (q, 4 H, 2 × OCH_2), 6.12 (bs, NH_2, NHCO, exchangeable with D_2O), 7.83 (dd, 2 H, Ar-H), 8.38 (s, 1 H, Ar-H). Anal. ($C_{13}H_{16}N_3O_4 \cdot 0.5HCl$) C, H, N.

***n*-Propyl 5-(*n*-Propoxycarbonyl)-1*H*-benzimidazole-2-carbamate (21).** Compound **21** was prepared by using a procedure similar to that used in the preparation of **19**. In this case, compound **6a** was refluxed for 4 h in the presence of *n*-propanol. Compound **21** was prepared, in a yield of 87%: mp 225 °C; IR (KBr) ν_{max} 3480, 2965, 2880, 2820–2700, 1720–1710, 770 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.00 (t, 6 H, 2 × CH_3), 4.45–6.40 (bs, 3 H, NH and OH, exchangeable with D_2O), 7.67 (dd, 2 H, Ar-H), 8.14 (s, 1 H, Ar-H). Anal. ($C_{15}H_{19}N_3O_4 \cdot 0.25H_2O$) C, H, N.

Isopropyl 5-(Isopropoxycarbonyl)-1*H*-benzimidazole-2-carbamate (22). Method A. Compound **22** was prepared by using a procedure similar to that used in the preparation of **19**. In this case, compound **6a** was refluxed for 4 h in the presence of 2-propanol. Compound **22** was isolated in a yield of 84%: mp 236 °C; IR (KBr) ν_{max} 3400, 2980, 1725–1720, 775–765 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.45 (t, 12 H, 2 × $(CH_3)_2$), 5.30 (5, 2 H, 2 × OCH), 7.23–8.63 (m, 3 H, Ar-H), 10.90 (bs, 1 H, NHCO, exchangeable with D_2O), 13.70 (bs, 1 H, NH, exchangeable with D_2O). Anal. ($C_{15}H_{19}N_3O_4 \cdot 0.25H_2O$) C, H, N.

Method B. Methyl 5-(isopropoxycarbonyl)-1*H*-benzimidazole-2-carbamate (**9**) (2.5 g, 0.009 mmol) was added to a premixed slurry of sodium borohydride (4.0 g, 0.1053 mmol) in 2-propanol (125 mL) and stirred on a steam bath for 12 h. The solvent was evaporated under reduced pressure and the resulting residue was diluted with water (30 mL) and extracted with ethyl acetate (3 × 150 mL). The organic layers were combined and dried over sodium sulfate. Removal of the drying agent and concentration of the solvent, under reduced pressure, afforded product **22** as a colorless solid, which was recrystallized from diethyl ether, yield 1.76 g (64%): The IR, ^1H NMR, and TLC data of this product were identical with those described above.

Methyl 5-[(Cyclopropylmethoxy)carbonyl]-1*H*-benzimidazole-2-carbamate (10) and Cyclopropylmethyl 5-[(Cyclopropylmethoxy)carbonyl]-1*H*-benzimidazole-2-carbamate (23). Thionyl chloride (7.0 mL) was added to an ice-cold stirred suspension of **5c** (3.2 g, 0.014 mmol) in dry benzene (45 mL). The resulting reaction mixture was stirred at reflux temperature for 6–7 h. The solvent was evaporated under vacuum and the resulting residue was triturated with dry benzene (10 mL). The solid compound was collected by filtration and air-dried to yield 3.88 g (97%) of **6a**; mp >305 °C.

A mixture of **6a** and cyclopropylmethyl alcohol (20 mL) was stirred at room temperature for 8–10 h, then at 100 °C for 2 h. The excess alcohol was removed by evaporation under reduced pressure. The resulting residue was chromatographed over silica gel 60 F_{254} (43 g, 70–230 mesh, column size 2.5 × 30 cm) eluting with $CHCl_3$. The fractions containing pure **10**, as determined by TLC, were pooled and evaporated to afford the desired ester **10** in 12% yield: mp 292–296 °C; IR (KBr) ν_{max} 3400, 2960, 1720, 763 cm^{-1}; ^1H NMR (DMSO-d_6) δ 0.27–0.74 (m, 4 H, CH_2CH_2), 1.24 (m, 1 H, OCH), 3.84 (s, 3 H, OCH_3), 4.14 (d, 2 H, OCH_2),

7.30–8.32 (M, 3 H, Ar-H), 12.00 (bs, 1 H, NH, exchangeable with D_2O). Anal. ($C_{14}H_{15}N_3O_4$) C, H, N.

To obtain **23** the fractions which contained both products **10** and **23**, as determined by TLC, were combined and evaporated under reduced pressure. This material was once again submitted to chromatography (silica gel, 75 g, 70–230 mesh, column size 5 × 60 cm) using chloroform/methanol (97:3, v/v). Although this procedure was not totally successful in separating **10** and **23**, the two fractions that contained the pure lower R_f product **23** were combined and evaporated to afford 4% of the transesterified product **23**: mp 252–253 °C; IR (KBr) ν_{max} 3400, 2960–2700, 1710, 768 cm^{-1}; ^1H NMR (DMSO-d_6) δ 0.1–0.83 (m, 8 H, 2 × CH_2CH_2), 0.90–1.52 (m, 2 H, 2 × CH), 4.12 (d, 4 H, 2 × (OCH_2), 7.37–8.40 (m, 3 H, Ar-H), 12.07 (bs, 1 H, NH, exchangeable with D_2O). Anal. ($C_{17}H_{19}N_3O_4$) C, H, N.

The unresolved fractions containing both compounds **10** and **23** were treated with cyclopropylmethyl alcohol (15 mL) and ethereal HCl (saturated at 0 °C, 3 drops) and stirred at 125 °C for 12 h. Evaporation of the reaction mixture provided a 70% yield of pure **23**: The IR, ^1H NMR, and TLC data of this product were identical with those described above.

General Procedure for Methyl 5-Carbamoyl-1*H*-benzimidazole-2-carbamates (24–34). Thionyl chloride (5.0 mL) was added to an ice-cold stirred suspension of **5a** (1.25 g, 0.0053 mmol) in dry benzene (30 mL). The resulting reaction mixture was stirred at reflux temperature for 6 h and then cooled to room temperature. The excess of solvent was evaporated under reduced pressure and the resulting residue was resuspended in dry benzene (15 mL) and the benzene was evaporated under vacuum. This procedure was repeated three times to remove traces of thionyl chloride. To a stirred suspension of this residue in dry tetrahydrofuran (40 mL) was added a solution of the appropriate amine (0.015 mmol) in dry tetrahydrofuran (20 mL). Stirring was continued overnight at room temperature. The solvent was removed under reduced pressure and the resulting solid was suspended in dry diethyl ether (60 mL). The solid product was collected by filtration and purified by either column chromatography over silica gel 60 F_{254} (60–200 or 70–230 mesh, column size 2.5 × 30 cm) using $CHCl_3$/MeOH (9:1, v/v) as an eluant or by recrystallization from methanol or a methanol/diethyl ether mixture.

Methyl 5-(Dimethylcarbamoyl)-1*H*-benzimidazole-2-carbamate (24). With the general method, this compound was prepared in a 79% yield: mp 241–242 °C; crystallized from a methanol/diethyl ether mixture; IR (KBr) ν_{max} 3360, 1732, 1660, 770–760 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.97 (s, 6 H, $N(CH_3)_2$), 3.48 (bs, 1 H, NHCO), 3.80 (s, 3 H, OCH_3), 6.94–7.60 (m, 3 H, ArH). Anal. ($C_{12}H_{14}N_4O_3$) C, H, N.

Methyl 5-(Ethylcarbamoyl)-1*H*-benzimidazole-2-carbamate (25). With the general method this compound was prepared in a 94% yield: mp >300 °C; crystallized from methanol; IR (KBr) ν_{max}, 3340, 2760, 1740–1730, 1660, 770, 775 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.2 (t, 3 H, CCH_3), 2.83–3.77 (m, 4 H, NHCO and CH_2), 3.80 (s, 3 H, OCH_3), 7.04–8.56 (m, 4 H, ArH and –CONH–). Anal. ($C_{12}H_{14}N_4O_3$) C, H, N.

Methyl 5-(Isopropylcarbamoyl)-1*H*-benzimidazole-2-carbamate (26). With the general method this compound was prepared in 93% yield: mp >300 °C; crystallized from methanol; IR (NBr) ν_{max} 3300, 2970, 1725–1710, 1660–1630, 765–750 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.2 (t, 3 H, $CCH_3)_2$), 3.40 (bs, 1 H, NHCO), 3.83 (s, 3 H, OCH_3), 4.17 (m, 1 H, CHC), 7.30–8.63 (m, 4 H, ArH and CONH), 12.00 (bs, 2 H, NH and H_2O). Anal. ($C_{13}H_{16}N_4O_3$) C, H, N.

Methyl 5-(Neopentylcarbamoyl)-1*H*-benzimidazole-2-carbamate (27). With the general method this compound was prepared in 90% yield: mp 265 °C; crystallized from methanol; IR (KBr) ν_{max} 3400, 2957, 1725, 1660, 760 cm^{-1}; ^1H NMR (DMSO-d_6) δ 0.84 (s, 9 H, $C(CH_3)_2$), 2.83–3.67 (m, 3 H, NCH_2 and NHCO), 3.83 (s, 3 H, OCH_3), 7.14–8.55 (m, 4 H, Ar-H and CONH). Anal. ($C_{15}H_{20}N_4O_3$) C, H, N.

Methyl 5-[(*p*-Fluorophenyl)carbamoyl]-1*H*-benzimidazole-2-carbamate (28). With the general method this compound was prepared in 81% yield: mp >300 °C; IR (KBr) ν_{max} 3390 and 3320, 1730–1715, 1670–1640, 822, 765 cm^{-1}; ^1H NMR (DMSO-d_6) δ 3.43 (bs, 1 H, NHCO), 3.84 (s, 3 H, OCH_3), 6.90–8.53 (m, 7 H, ArH), 10.30 (bs, 1 H, CONH), 12.0 (bs, 1 H, NH). Anal. ($C_{18}H_{13}N_4FO_3$) C, H, N.

Methyl 5-[[*p*-(Trifluoromethyl)phenyl]carbamoyl]-1*H*-benzimidazole-2-carbamate (29). With the general method, compound **29** was prepared in 73% yield: mp >300 °C; IR (KBr) ν_{max} 1760, 1640, 790, 750 cm^{-1}; ^1H NMR (DMSO-d_6) δ 3.20 (s, 1 H, NHCO(, 3.87 (s, 3 H, OCH_3), 5.80 (bs, CONH and H_2O), 7.50–9.07 (m, 7 H, ArH). Anal. ($C_{17}H_{13}N_4F_3O_3 \cdot HCl \cdot H_2O$) C, H, N.

Methyl 5-(Piperazinocarbonyl)-1*H*-benzimidazole-2-carbamate (30). To a cold stirred suspension of acid chloride derivative **6a** (2.157 g, 0.0085 mmol) in dry tetrahydrofuran (30 mL) was added a solution of anhydrous piperazine (0.800 g, 0.0102 mmol) in dry tetrahydrofuran (30–40 mL). The reaction mixture was stirred for 14 h at room temperature. TLC on silica gel ($CHCl_3$/MeOH 9:1, v/v) showed the reaction to be incomplete, hence an additional amount of anhydrous piperazine (1.10 g) was added and the mixture was stirred at reflux temperature for an additional 45 min and then cooled. The separated material was collected by filtration and washed thoroughly with tetrahydrofuran. The solid was suspended in methanol (200 mL) and the mixture was boiled on a steam bath for 15 min and then cooled to room temperature. The solid was collected by filtration and air-dried. This material (1.63 g) was the acid **5a** as determined by spectroscopic and analytical data. The desired product **30** crystallized from the methanol filtrate as a colorless solid, 0.486 g: mp 282–287 °C; IR (KBr) ν_{max} 3360, 2930, 1735, 1645, 765–70 cm^{-1}; ^1H NMR ($CDCl_3$ + DMSO-d_6) δ 2.78 (m, 4 H, $(CH_2)_2NC$), 3.50–4.40 (bs, 7 H, OCH_3, $N(CH_2)_2$), 7.0–8.05 (m, 3 H, Ar-H), 8.33–10.00 (bm, 3 × NH, exchangeable with D_2O). Anal. ($C_{14}H_{17}N_5O_3 \cdot 0.5H_2O$) C, H, N.

Methyl 5-[(4-Methylpiperazinyl)carbonyl]-1*H*-benzimidazole-2-carbamate (31). With the above general method, compound **31** was prepared in 72% yield: mp 243–250 °C; IR (KBr) ν_{max} 3350, 2942, 2790, 1738, 1648, 787, 772 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.10–3.0 (m, 7 H, $(CH_2)_2NCH_3$), 3.43–4.27 (m, 7 H, CON $(CH_2)_2$, OCH_3, 7.03–7.92 (m, 3 H, Ar-H). Anal. ($C_{19}H_{26}N_6O_4$) C, H, N.

Methyl 5-[[4-(*N*,*N*-Dimethylcarbamoyl)piperazinyl]carbonyl]-1*H*-benzimidazole-2-carbamate (32). With the above general method compound **32** was prepared in 84% yield: mp 239–240 °C; IR (KBr) ν_{max} 3350, 2970, 2860, 1728, 1645–1638, 770 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.93 (s, 6 H, N $(CH_3)_2$), 3.37 (t, 4 H, N $(CH_2)_2$), 3.78 (4 H, $N(CH_2)_2$), 4.05 (s, 3 H, OCH_3), 7.18–7.87 (m, 3 H, Ar-H), 11.50 (bs, 1 H, NH, exchangeable with D_2O). Anal. ($C_{19}H_{26}N_6O_4$) C, H, N.

Methyl 5-[[4-(*N*,*N*-Diethylcarbamoyl)piperazinyl]carbonyl]-1*H*-benzimidazole-2-carbamate (33). With the general method compound **33** was prepared in 76% yield: mp 232 °C; IR (KBr) ν_{max} 3350, 2940–2860, 1728, 1698, 770 cm^{-1}; ^1H NMR ($CHCl_3$) δ 1.22 (t, 6 H, 2 × CH_3), 3.03–3.98 (m, 12 H, 2 × OCH_2), $N(CH_2)_4$), 4.07 (s, 3 H, OCH_3), 7.13–7.87 (m, 3 H, Ar-H), 11.20 (bs, 1 H, NH, exchangeable with D_2O). Anal. ($C_{19}H_{26}N_6O_4$) C, H, N.

Methyl 5-(Morpholinocarbonyl)-1*H*-benzimidazole-2-carbamate (34). With the above general method compound **34** was prepared in 98% yield: mp 236 °C; IR (KBr) ν_{max} 3300, 2960, 1748, 1655, 770; ^1H NMR ($CDCl_3$ + DMSO-d_6) δ 3.95 (bs, 8 H, $O(CH_2)_2N)_2$), 4.24 (s, 3 H, OCH_3), 7.40–8.33 (m, 3 H, ArH), 11.90 (bs, 1 H, NH, exchangeable with D_2O). Anal. ($C_{14}H_{16}N_4O_4$) C, H, N.

Methyl 5-Amino-1*H*-benzimidazole-2-carbamate (35). A suspension of compound **5c** (2.50 g) in methanol (100 mL) was catalytically hydrogenated in a Parr apparatus at 80 °C and 50 psi hydrogen in the presence of 5% Pd–C (1.50 g) for 20 h. The catalyst was removed by filtration through Celite. The filtrate on concentration afforded a white colorless solid, which was collected by filtration and air-dried to yield 1.65 g (68%) of **35**: mp >300 °C; IR (KBr) ν_{max} 3350, 1718, 795–785 cm^{-1}; ^1H NMR (DMSO-d_6) δ 3.70 (s, 3 H, CH_3), 6.23–7.37 (m, 3 H, Ar-H), 11.33 (bs, 1 H, NH, exchangeable with D_2O). Anal. ($C_9H_{10}N_4O_2 \cdot 0.5H_2O$) C, H, N.

Methyl 5-[(*m*-Fluorobenzoyl)amino]-1*H*-benzimidazole-2-carbamate (36). *m*-Fluorobenzoyl chloride (0.5 mL, 0.0041 mmol) was added to a stirred suspension of **35** (0.700 g, 0.0034 mmol) and anhydrous potassium carbonate (0.300 g, 0.0022 mmol) in dry *N*,*N*-dimethylformamide (5 mL) at 0 °C. The resulting mixture turned into a clear solution after 15–30 min. Stirring

was continued at room temperature for 24 h and then the reaction mixture was poured into water. The separated solid was collected by filtration and air-dried, yield 0.75 g (67%): mp >310 °C; IR (KBr) ν_{max} 3420–3300, 1730, 1660–1648, 815–789, 750 cm^{-1}; ^1H NMR (DMSO-d_6) δ 3.84 (s, 3 H, OCH_3), 6.86–8.27 (m, 7 H, Ar-H), 10.60 (s, 1 H, NH, exchangeable with D_2O), 11.87 (bs, 1 H, NH, exchangeable with D_2O). Anal. ($C_{16}H_{13}N_4FO_3$) C, H, N.

Methyl 5-[(*p*-Fluorobenzoyl)amino]-1*H*-benzimidazole-2-carbamate (37). Compound **37** was prepared by a method similar to that employed for the synthesis of compound **36**: yield 80%; mp >300 °C; IR (KBr) ν_{max} 3405, 1725, 1682–1645, 750 cm^{-1}; ^1H NMR (DMSO-d_6) δ 3.82 (s, 3 H, OCH_3), 7.13–7.74 (m, 4 H, Ar-H), 7.80–8.44 (m, 3 H, Ar-H), 10.20 (s, 1 H, NHCO, exchangeable with D_2O), 11.83 (s, 1 H, NH). Anal. ($C_{16}H_{13}N_4FO_3$) C, H, N.

Methyl 5-[(*o*-Fluorobenzoyl)amino]-1*H*-benzimidazole-2-carbamate (38). Compound **38** was prepared by a method similar to that employed for the synthesis of compound **36**: yield 79%; mp 310–311 °C; IR (KBr) ν_{max} 3405, 1725, 1682–1645, 1608–1600, 750 cm^{-1}; ^1H NMR (DMSO-d_6) δ 3.82 (s, 3 H, OCH_3); 6.93–8.48 (m, 7 H, Ar-H), 10.30 (s, 1 H, NH exchangeable with D_2O), 11.95 (bs, 1 H, NH, exchangeable with D_2O). Anal. ($C_{16}H_{13}N_4FO_3$) C, H, N.

Antitumor Studies. Cell Culture. The in vivo cytotoxicity against L1210 cells was evaluated as described previously.[15] L1210 cells were grown in static suspension culture with Fischer's medium for leukemic cells of mice, and the growth rate over a 3-day period was determined in the presence of the indicated concentrations of the test compound, by counting the cells twice each day. Growth rate was defined as the slope of the plot of the log of the cell number against time for a treated culture, as a percentage of the slope for the control culture. Experimentally, this parameter was determined by calculating the ratio of the population doubling time of control cells (average, 12 h) to the population doubling time of treated cells. The IC_{50} was defined as the concentration required to reduce the growth rate to 50% of the control.

The in vitro cytotoxicity against H. Ep. 2 human epidermoid carcinoma cells (ATCC CCL23) was evaluated in monolayer cultures. H. Ep. 2 cells were maintained in exponential growth in Basal Medium of Eagle with Hanks' salts (BME) (GIBCO Laboratories, Grand Island, NY) and 0.5 g $NaHCO_3$/L, supplemented with 15% heat inactivated (56 °C, 30 min) bovine calf serum (Hyclone Laboratories, Logan, UT). Cells were harvested from the surface of culture flasks for subculturing or for counting with 0.005% trypsin (2 × crystalline, 3080 NF units/mg, GIBCO Laboratories, Grand Island, NY) and 0.1% EDTA, disodium salt, in BME without serum. For growth rate determinations, 2×10^4 cells were placed in replicate 25 cm^2 flasks, in control medium. After one day of incubation the medium was changed to compound-containing medium. Then growth was monitored by harvesting and counting the cells in two flasks from each treatment group, on days 1, 2, and 4 after adding the compounds. The data was plotted and analyzed as described previously[15] and above for L1210 cells. The average control population doubling time was 19 h.

Viability Determinations. The ability of L1210 cells to form colonies after the indicated treatments was evaluated in soft agar medium as described previously.[15] The fraction of treated cells forming colonies was expressed as the percent of the colony formation by control, untreated cells (average control value, 53% + 4 (SEM), n = 17).

Mitotic Index Determinations. L1210 cells (5 mL, 10^5/mL) were incubated for the indicated times with 10 μM test compound. Then the cells were separated from the medium by centrifugation and resuspended in 0.5 mL of water. Ten milliliters of Carnoy's fixative (ethanol/glacial acetic acid, 3:1) was added and the cell suspension allowed to stand for 10 min at room temperature. The cells were sedimented by centrifugation, resuspended in 0.5 mL Carnoy's fixative, and dispersed by trituration with a Pasteur pipet. Drops of this suspension of fixed cells were placed on slides, air-dried overnight, and stained with Giemsa stain in phosphate buffer (0.067 M KH_2PO_4, 0.067 M Na_2HPO_4; pH 6.5), destaining with water. The fraction of the cells containing metaphase or later mitotic figures (mitotic index) was evaluated by microscopic observation of three slides per data point, 1000 cells per slide for controls and 500 cells per slide for treated cells.

Antifilarial Studies. All compounds were evaluated for antifilarial activity against the adult worms of *B. pahangi*, *A. viteae*, and/or *L. carinii* in jirds (*Meriones unguiculatus*, males), usually using dual infections of *B. pahangi* and either *A. viteae* or *L. carinii*. The jirds were given either 24 four-day-old larvae of *L. carinii* by subcutaneous inoculation 76–133 days prior to treatment[16] or 10 (5 male and 5 female) adult *A. viteae* by surgical implantation in subcutaneous tissue 21–28 days prior to treatment. The jirds were also given either 50 infective larvae of *B. pahangi* by intraperitoneal inoculation 60–100 days prior to drug treatment or 20 (10 male and 10 female) adult *B. pahangi* surgically implanted[17] into the peritoneal cavity 4–60 days pretreatment. The drugs were administered as solutions or suspensions in aqueous 1% (hydroxyethyl)cellulose and 0.1% Tween 80 (HEC Tween 80) once daily for 5 days to three to five implanted jirds. Fifty-five to 70 days after the first drug dose, surviving animals were sacrificed and examined for adult worms by searching the pleural cavity (*L. carinii*), peritoneal cavity (*B. pahangi*), and the subcutaneous tissue and fascial plane of muscle (*A. viteae*). The number of surviving worms at necropsy was scored as a percentage relative to controls. Compounds were considered to be active when the number of adult worms was less than 40% of the controls.

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Registry No. **5a**, 65003-40-9; **5b**, 135696-70-7; **5c**, 40483-97-4; **6a**, 135696-94-5; **7**, 121649-64-7; **7**·HCl, 135696-71-8; **8**, 121649-65-8; **8**·HCl, 135696-72-9; **9**, 121649-66-9; **10**, 135696-73-0; **11**, 135696-74-1; **12**, 135696-75-2; **13**, 135696-76-3; **14**, 135696-77-4; **15**, 135891-47-3; **15**·HCl, 135720-68-2; **16**, 135696-78-5; **17**, 135891-48-4; **17**·HCl, 135696-79-6; **18**, 135696-80-9; **19**, 121649-63-6; **19**·HCl, 135696-81-0; **20**, 135891-49-5; **20**·$^1/_2$HCl, 135696-82-1; **21**, 135696-83-2; **22**, 135720-69-3; **23**, 135696-84-3; **24**, 67476-41-9; **25**, 67476-48-6; **26**, 135696-85-4; **27**, 135696-86-5; **28**, 89791-14-0; **29**, 135696-87-6; **29**·HCl, 135696-87-6; **30**, 135696-88-7; **31**, 65003-31-8; **32**, 135696-89-8; **33**, 135696-90-1; **34**, 65003-30-7; **35**, 57438-18-3; **36**, 135696-91-2; **37**, 135696-92-3; **38**, 135696-93-4; $CH_2OHCH_2CH(CH_3)_2$, 626-89-1; $CH_2OHC(CH_3)_3$, 75-84-3; $CH_2OHCH{=}CH_2$, 107-18-6; $CH_2OHC{\equiv}CH$, 107-19-7; $CH_2OHCH_2CHCH_3CH_2C(CH_3)_3$, 1573-33-7; CH_2OH-*p*-C_6H_4F, 459-56-3; $NH_2CH(CH_3)_2$, 75-31-0; $NH_2CH_2C(CH_3)_3$, 5813-64-9; *p*-$NH_2C_6H_4F$, 371-40-4; *p*-$NH_2C_6H_4CF_3$, 455-14-1; $NH(CH_3)_2$, 124-40-3; $ClCOC_6H_4$-*m*-F, 1711-07-5; $ClCOC_6H_4$-*p*-F, 403-43-0; $ClCOC_6H_4$-*o*-F, 393-52-2; 2-methylthiopseudourea sulfate, 867-44-7; 3,4-diaminobenzoic acid, 619-05-6; (hydroxymethyl)cyclopropane, 2516-33-8; *N*-(2-hydroxyethyl)pyrrolidine, 2955-88-6; 2-thiophenemethanol, 636-72-6; piperazine, 110-85-0; (*N*,*N*-dimethylcarbamoyl)piperazine, 41340-78-7; (*N*,*N*-diethylcarbamoyl)piperazine, 119-54-0; morpholine, 110-91-8; *N*-methylpiperazine, 109-01-3.

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