Geiparvarin Analogues. 2.[1] Synthesis and Cytostatic Activity of 5-(4-Arylbutadienyl)-3(2*H*)-furanones and of *N*-Substituted 3-(4-Oxo-2-furanyl)-2-buten-2-yl Carbamates

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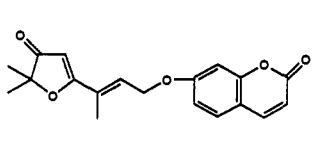
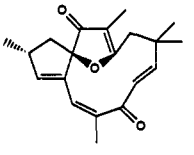
In an attempt to determine some of the structural features of geiparvarin (1) that account for its cytostatic activity in vitro, a series of geiparvarin analogues (**10a–i**, **1**, **12**, and **14–16**) which contain novel modifications in the region of the olefinic double bond and of the coumarin moiety have been designed and synthesized. Among the derivatives containing a carbamate moiety, only the analogues containing a carbamate group linked to an alkyl moiety **10b–i** were endowed with potent cytostatic activity, whereas the corresponding benzene derivative **10a** was devoid of any antiproliferative activity. 6-Methoxygeiparvarin **10l** proved equally effective as geiparvin (1), while compounds containing an additional double bond at the side chain (**12** and **14–16**) were invariably 5–100-fold less effective than geiparvarin. Diene derivative **15**, bearing a coumarin moiety, was essentially inactive against murine (L1210, FM3A) tumor cells but exhibited good activity against human (Molt/4F, MT-4) tumor cells.

Introduction

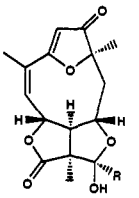
Geiparvarin (1),[2] jatrophone (2),[3] and eremantholides A, B, and C (3)[4] constitute a group of compounds of considerable current interest. The stimulus for this interest arises from observations that these compounds display significant inhibitory activity against a variety of cell lines including sarcoma 180, Lewis lung carcinoma, P-388 lymphocytic leukemia, and Walker 256 carcinosarcoma.[5-7] The common central structural feature of these molecules is the 3(2*H*)-furanone ring which seems to be responsible for the biological action of this class of compounds. Smith and co-workers[5] postulated that 1,6 conjugate addition (Michael addition) of bionucleophiles to the 3(2*H*)-furanone ring system could be the basis for the possible mode of action of this class of compounds. To obtain insight in the structure–activity relationship of geiparvarin (1), various new geiparvarin analogues were synthesized and examined for biological activity. In these studies a simple and efficient synthetic route to 1 was utilized based on a new approach toward isoxazole chemistry;[8,9] moreover, modifications of the basic 3(2*H*)-furanone ring system were carried out.[10]



1
Geiparvarin



2
Jatropbone



3

Eremantbolide
A: R = iPr
B: R = sec-Bu
C: R = CMe=CH$_2$

Recently, a series of geiparvarin analogues in which the coumarin portion was replaced by other aromatic rings have been reported.[11,12] Here we describe the synthesis and cytostatic activity of a series of new geiparvarin analogues which present novel modifications in the region of the olefinic double bond and of the coumarin moiety. Compounds **10a–i** were synthesized with the objective to obtain geiparvarin analogues with increased hydrophilicity. These compounds contain a carbamate moiety, a structural feature (bioisoster of an allylic fragment) present in other antitumor agents. The synthesis of methoxygeiparvarin **10l** was inspired by the observation that 6-methoxy-7-hydroxycoumarin (scopoletin)[13] has antitumor activity. We

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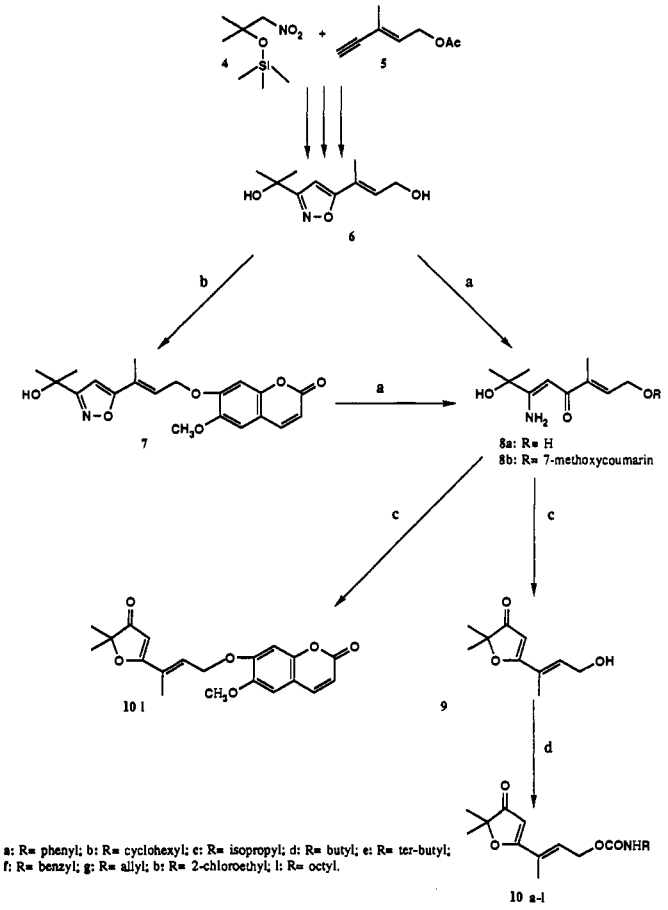
Scheme I[a]



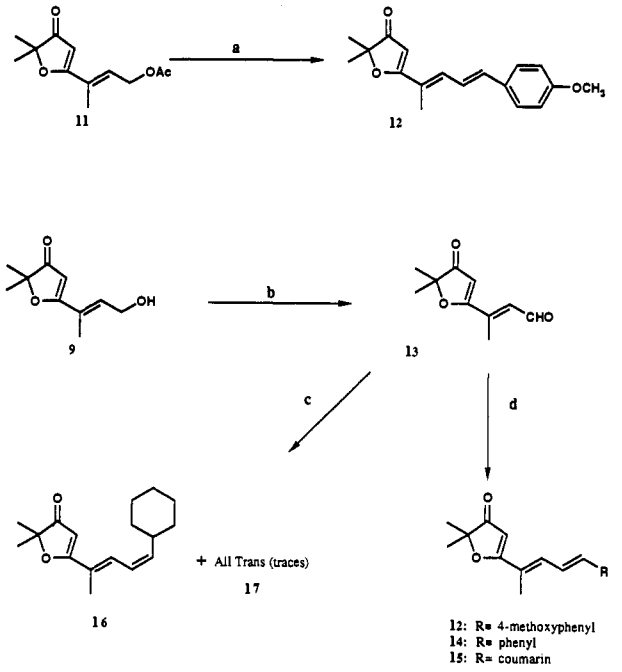
[a] (a) $Mo(CO)_6$, CH_3CN, H_2O, reflux; (b) CH_3SO_2Cl, TEA, K_2CO_3, LiBr, 7-methoxycoumarin; (c) AcOH, H_2O, THF; (d) RNCO, toluene, reflux.

felt that combination of the two units, namely the 3-(2*H*)-furanone ring system and scopoletin, would eventually lead to novel geiparvarin analogues with higher cytostatic activity. Finally, compounds **12** and **14–16** with an additional double bond, which extends the conjugation of the alkenyl-3(2*H*)-furanone system, could increase the ability to afford Michael-type adducts.

Chemistry

The strategy used for the synthesis of the 3(2*H*)-furanone ring system of compounds **10a–l**, depicted in Scheme I, is the same as that recently described for the total synthesis of geiparvarin.[8,9] Isoxazole **6** was obtained through a [3 + 2] cycloaddition of the nitrile oxide generated from nitro derived **4** on enyne **5** (Mukaijama conditions). The primary hydroxyl group of **6** reacted selectively with methansulfonyl chloride in the presence of triethylamine to afford the crude mesylate, which on treatment with 6-methoxy-7-hydroxycoumarin, in the presence of lithium bromide, gave rise to a 57% yield of intermediate **7**. On exposure of isoxazoles **7** and **6** to molybdenum hexacarbonyl in wet acetonitrile, a clean transformation of the isoxazole ring to β-enaminones **8a,b** was obtained. The acid-catalyzed cyclodehydration of **8a,b** with 75% acetic acid produced the desired 3(2*H*)-furanones **10l** and **9** in a 45 and 53% yield, respectively. Simple reaction of alcohol **9** with the appropriate isocyanate in toluene solution at reflux afforded carbamates **10a–i** in a 35–80% yield. The 5-(4-substituted-butadienyl)-3(2*H*)-furanones (**12**, **14–16**) were obtained through a Wittig reaction between the appropriate phosphonium salt and aldehyde **13**. The latter was prepared in 60% yield by

Scheme II[a]



[a] (a) NaOMe, tributylphosphine, $Pd(PPh_3)_4$, 4-methoxybenzaldehyde; (b) CrO_3, pyridine, CH_2Cl_2; (c) *n*-BuLi, $X^-{}^+Ph_3CH_2C_6H_{11}$; (d) *t*-BuOK, $X^-{}^+Ph_3CH_2$-R.

oxidation of alcohol **9** with chromium(VI) oxide–pyridine complex. In the case of compounds **12**, **14**, and **15**, generation of the Wittig reagent was achieved with potassium *tert*-butoxide in anhydrous dimethyl sulfoxide, while for the derivative **16** *n*-butyllithium in tetrahydrofuran/hexane solution at −50 °C has been used. Compound **12** was obtained in a very low yield (5%) and all attempts to improve the preparation were unsatisfactory. In order to obtain a larger amount of compound **12** we turned our attention to an alternative strategy. Recently it has been described that allylic nitro derivatives and allylic acetates react directly with tributylphosphine through the mediation of palladium(0) complex to afford allylic phosphorus ylides which are subsequently utilized for olefination of aldehydes with appreciable *E* stereoselectivity.[14,15] When applied, this protocol gave transformation of **11** to diene **12** in a 42% yield (Scheme II). The *E* configuration of the newly generated double bond of compounds **12** and **14** has been unambiguously determined by the presence of a doublet at δ 6.8–7.0 with coupling constant of 15 Hz.[7] Moreover, the structure of **14** was confirmed by X-ray analysis. Compound **15** showed a doublet, in the presence of $Eu(fod)_3$, centered at δ 7.14 with J = 15.3 Hz.

The stereochemistry of (1*E*,3*Z*)-diene **16** was proven by evaluation of its ^1H NMR coupling constants. Traces of **17**, obtained during the preparation of **16**, confirmed the attributed structure. The vinylic protons of **16** appeared at δ 5.70 (dd, J = 11.5 and 8.3 Hz) for H-4′, at δ 6.20 (dd, J = 11.5 and 10.7 Hz) for H-3′, and at δ 7.27 (d, J = 10.7 Hz) for H-2′. For compound **17** the vinylic protons appeared at δ 6.12 (dd, J = 15.2 and 7.05 Hz) for H-4′, at δ

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Table I. Inhibitory Effects of Geiparvarin Analogues on the Proliferation of Murine Leukemia L1210, Murine Mammary Carcinoma FM3A, Human T-Lymphoblast Molt/4F, and Human T-Lymphocyte MT-4 Cells

compd	IC_{50},[a] μg/mL			
	L1210	FM3A	Molt/4F	MT-4
10a	≥200	>200	>200	103 ± 1.00
10b	3.7 ± 0.10	13 ± 1.20	3.4 ± 0.30	2.4 ± 0.10
10c	4.9 ± 0.66	7.2 ± 0.64	6.6 ± 0.92	2.3 ± 0.90
10d	2.6 ± 0.65	7.7 ± 0.03	5.3 ± 2.13	1.2 ± 0.20
10e	4.1 ± 0.31	7.1 ± 0.23	6.1 ± 1.45	2.1 ± 0.98
10f	4.7 ± 1.14	8.1 ± 0.07	6.0 ± 2.25	2.0 ± 0.75
10g	6.2 ± 1.08	7.6 ± 0.56	7.7 ± 0.11	2.8 ± 0.82
10h	5.1 ± 3.44	20.8 ± 1.90	10.7 ± 0.50	6.4 ± 1.95
10i	2.0 ± 0.22	7.1 ± 0.60	1.7 ± 0.09	1.3 ± 0.22
10l	2.0 ± 0.20	3.9 ± 0.10	2.9 ± 0.30	0.6 ± 0.10
12	18 ± 0.80	25 ± 2.80	10 ± 1.40	5.7 ± 2.40
14	14 ± 0.10	15 ± 0.50	14 ± 0.80	6.5 ± 1.80
15	63 ± 11.70	>100	13 ± 1.95	2.9 ± 1.12
16	23 ± 13.00	10.8 ± 2.05	7.1 ± 4.83	8.4 ± 2.73
geiparvarin[b]	2.0 ± 0.70	3.2 ± 0.40	2.8 ± 0.40	0.3 ± 0.10

[a] 50% inhibitory concentration, or compound concentration required to inhibit tumor cell proliferation by 50%. [b] Data taken from ref 10.

6.36 (dd, J = 15.2 and 11.2 Hz) for H-3′, and at δ 7.03 (d, J = 11.2 Hz) for H-2′.

Biological Evaluation

Geiparvarin (1), methoxygeiparvarin (**10l**), carbamates **10a–i**, and dienes **12** and **14–16** were evaluated for their inhibitory effects on the proliferation of murine (L1210, FM3A) and human (Molt/4F and MT-4) tumor cells (Table I). Among the geiparvarin analogues containing a carbamate group linked to a benzene (**10a**) or an alkyl (**10b–i**) moiety, only alkyl derivatives were endowed with potent cytostatic activity "in vitro". The antiproliferating effects of the test compounds seem to be mainly correlated with the presence of a carbamate moiety linked to an alkyl chain: surprisingly, the benzene-containing geiparvarin derivative **10a** was devoid of any antiproliferative activity at a concentration as high as 200 μg/mL. The reason for this marked difference in cytostatic activity is unclear. Compound **10l**, which is the methoxy derivative of geiparvarin, proved equally effective in inhibiting tumor cell proliferation in vitro as the parent compound. Compounds **10b** and **10l** have also been evaluated for their antitumor properties in DBA/2 mice inoculated intraperitoneally with 10^6 L1210 cells. None of the compounds had marked antitumor activities at subtoxic doses (data not shown). Compounds **12** and **14–16**, which contain an additional double bond between the alkenyl-3(2*H*)-furanone system and the coumarin, aryl, or cyclohexyl moieties, were invariably 5–100-fold less effective than geiparvarin against both murine and human tumor cell lines. Interestingly compound **15**, bearing the coumarin moiety, was less inhibitory than **12**, **14**, and **16** to murine tumor cell proliferation but retained an appreciable cytostatic activity against the human tumor cell lines. The introduction of a *p*-methoxy group at the benzene moiety did not affect the cytostatic properties. Compound **16** was prepared in view of the pronounced activity of **10b**, but no improvement in the cytostatic activity was observed with respect to the corresponding phenyl (**14**) and coumarin (**15**) analogues or geiparvarin itself.

In conclusion, our results can be summarized as follows. Modifications in the region of the olefinic double bond (compounds **12** and **14–16**) results in an almost complete loss of activity when compared to geiparvarin. The biological data suggest that the oxymethylene function is essential for cytostatic activity, and probably rigid analogues are unable to accommodate particular conformational requirements. The introduction of an olefinic double bond, which increases the ability of the substrate to afford Michael-type adducts, usually give compounds which are less active than geiparvarin. Moreover, the pronounced activity of compound **15**, carrying a coumarin moiety, toward human tumor cells points to an important role of this type of heterocycle in the modulation of the cytostatic activity against human tumor cells. Finally the potent activity of compounds **10a–i** remain unexplained so far, but may be related to the presence of the carbamoyl group substituted with an alkyl chain.

Experimental Section

Melting points were obtained in open capillary tubes and are uncorrected. Reaction courses and product mixture were routinely monitored by thin-layer chromatography (TLC) on silica gel precoated F254 Merck plates. Infrared spectra (IR) were measured on a Perkin-Elmer 257 instrument. Nuclear magnetic resonance (^{1}H NMR) spectra were determined for solution in $CDCl_3$ with a Bruker AC-200 spectrometer and peak positions are given in parts per million downfield from tetramethylsilane as internal standard. Petroleum ether refers to the fractions boiling in the range 40–60 °C. Column chromatographies were performed with Merck 60–200 mesh silica gel. All drying operations were performed over anhydrous magnesium sulfate. Column chromatography (medium pressure) was carried out by using the "flash" technique.[16] Microanalysis were in agreement with calculated values within ±0.4%.

Starting Materials. Compounds **6** and **11** were prepared according to the procedure that has been previously described.[8,9] Scopoletin is commercially available.

6-Methoxy-7-[[(*E*)-3-[3-(1-hydroxy-1-methylethyl)isoxazol-5-yl]-2-butenyl]oxy]-2*H*-1-benzopyran-2-one (7). To an ice-cooled solution of **6** (0.78 g, 4 mmol) in methylene chloride (10 mL) containing triethylamine (0.8 mL, 6 mmol) was added methansulfonyl chloride (0.38 mL, 5 mmol) in methylene chloride (5 mL) dropwise with stirring. After 30 min at room temperature the mixture was treated with 2% citric acid (2 × 20 mL) and the organic phase was separated, dried, and concentrated in vacuo. The crude mesylate was immediately heated at reflux for 0.5 h in acetone solution (20 mL) containing 6-methoxy-7-hydroxycoumarin (0.77 g, 4 mmol), potassium carbonate (0.55 g, 4 mmol), and lithium bromide (0.1 g). After removal of the solvent in vacuo, the residue was poured into water (50 mL) and extracted with ethyl acetate (2 × 50 mL). The combined extracts were washed with 5% aqueous ammonia (3 × 20 mL) and brine (30 mL) and dried. The solvent was removed in vacuo and the residue was chromatographed on silica gel (ethyl acetate–petroleum ether) to give **7** as solid material (0.85 g, 57%): mp 140–142 °C (ethyl acetate); IR (KBr) 3400, 1720, 1610, 1600 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.61 (s, 6 H, $(CH_3)_2$), 2.12 (s, 3 H, CH_3C=), 2.85 (br, 1 H, OH),

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3.94 (s, 3 H, CH_3O), 4.88 (d, 2 H, J = 6.8 Hz, CH_2O), 6.27 (d, 1 H, J = 9.3 Hz, coumarin H-3), 6.31 (s, 1 H, isoxazole), 6.55 (br t, 1 H, CH=), 6.80 (s, 1 H, coumarin), 6.89 (s, 1 H, coumarin), 7.64 (d, 1 H, J = 9.3 Hz, coumarin *H*-4).

(*E*,*E*)-6-Amino-3,7-dimethyl-1,7-dihydroxy-4-oxo-2,5-octadiene (8a). Compound **8a** was obtained in the same manner as that described for **8b** (75%): oil; IR ($CHCl_3$) 3400, 1690, 1620, 1550 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.42 (s, 6 H, $(CH_3)_2$), 1.81 (s, 3 H, CH_3C=), 2.17 (s, 1 H), OH), 3.85 (br, 1 H, OH), 4.29 (m, 2 H, CH_2O), 5.42 (s, 1 H, H-2), 6.39 (br t, 1 H, H-5), 6.65 (br, 1 H, NH), 10.35 (br, 1 H, NH). Anal. ($C_{10}H_{17}NO_3$) C, H, N.

6-Methoxy-7-[[(*E*,*E*)-6-amino-7-hydroxy-3,7-dimethyl-4-oxo-2,5-octadienyl]oxy]-2*H*-1-benzopyran-2-one (8b). A solution of isoxazole **7** (2.2 g, 6 mmol) in acetonitrile (40 mL) containing water (50 drops) was treated with molybdenum hexacarbonyl (0.79 g, 3 mmol) and heated at reflux for 1.5 h with stirring. Celite (5 g) was added to the cooled solution and the resulting mixture evaporated in vacuo. The residue was flash chromatographed on silica gel (ethyl acetate–petroleum ether) to give **8b** as a solid (1.5 g, 67%): mp 160–163 °C (ethyl acetate–petroleum ether); IR (KBr) 3480, 1730, 1620, 1560, 1520 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.67 (s, 6 H, $(CH_3)_2C$), 1.92 (s, 3 H, CH_3C=), 2.80 (br, 1 H, OH), 3.95 (s, 3 H, CH_3O), 4.75 (d, 2 H, J = 6 Hz, CH_2O), 5.45 (s, 1 H, H-5′), 6.21 (d, 1 H, J = 9.5 Hz, coumarin H-3), 6.66 (br t, 1 H, H-2′), 6.92 (s, 1 H, coumarin), 6.98 (s, 1 H, coumarin), 7.64 (d, 1 H, J = 9.5 Hz, coumarin H-4), 9.55 (br, 2 H, NH_2). Anal. ($C_{20}H_{23}NO_6$) C, H, N.

2,2-Dimethyl-5-[(*E*)-1-methyl-3-hydroxy-1-propenyl]-3-(2*H*)-furanone (9). This compound was prepared in the same manner as that described for **10l**: yield 53%; melting point and IR and ^1H NMR spectroscopic data of **9** are identical with those reported in the literature.[17]

General Procedure for Preparation of N-Substituted (*E*)-3-(4,5-Dihydro-5,5-dimethyl-4-oxo-2-furanyl)-2-butenyl Carbamates (10a–i). To a solution of the alcohol **9** (0.5 g, 2.7 mmol) in dry toluene (15 mL) was added the opportune isocyanate (4 mmol) and the solution was heated at reflux for 3 h. The solution was concentrated in vacuo; the residue was solubilized in diethyl acetate, washed with 5% aqueous ammonia (2 × 25 mL) and brine (3 × 50 mL), and dried. After removal of the solvent, compounds **10a–i** were obtained as solids: **10a**, 80%, mp 115–116 °C (ethyl acetate); **10b**, 45%, mp 95–96 °C (diethyl ether); **10c**, 35%, mp 115–118 °C (diethyl ether); **10d**, 66%, mp 43–45 °C (diethyl ether–petroleum ether); **10e**, 57%, mp 116–118 °C (ethyl acetate–petroleum ether); **10f**, 65%, mp 103–105 °C (dichloromethane–petroleum ether); **10g**, 50%, mp 75–76 °C (ethyl acetate–petroleum ether); **10h**, 62%, mp 69–70 °C (diethyl ether–petroleum ether); **10i**, 56%, mp 86–87 °C (diethyl ether).

IR and ^1H NMR spectroscopic data of *N*-phenylcarbamate **10a** are given as an example: IR (KBr) 3300, 1720–1690, 1650, 1550 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.40 (s, 6 H, $(CH_3)_2$), 1.98 (s, 3 H, CH_3C=), 4.91 (d, 2 H, J = 6.5 Hz, CH_2O), 5.59 (s, 1 H, furan), 6.64 (br t, 1 H, CH=), 6.9 (br, 1 H, NH), 7.1 (m, 1 H, Ar), 7.39 (m, 4 H, Ar). Anal. ($C_{17}H_{19}NO_4$) C, H, N.

6-Methoxy-7-[[(*E*)-3-(4,5-dihydro-5,5-dimethyl-4-oxo-2-furanyl)-2-butenyl]oxy]-2*H*-1-benzopyran-2-one (10l). A solution of **8b** (0.93 g, 2.5 mmol) in tetrahydrofuran (20 mL) was stirred at room temperature for 24 h with 75% acetic acid (20 mL). The mixture was concentrated in vacuo and the residue poured into water and extracted with ethyl acetate (3 × 20 mL). The combined extracts were dried and evaporated in vacuo. The residue was chromatographed on silica gel (ethyl acetate–petroleum ether) to give **10l** as a solid (0.4 g, 45%): mp 190 °C dec (ethyl acetate); IR (KBr) 1720, 1680, 1650, 1610, 1550 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.43 (s, 6 H, $(CH_3)_2$), 2.02 (s, 3 H, CH_3C=), 3.94 (s, 3 H, CH_3O), 4.91 (d, 2 H, J = 6.8 Hz, CH_2O), 5.62 (s, 1 H, furan), 6.31 (d, 1 H, J = 9.3 Hz, coumarin H-3), 6.79 (br t, 1 H, H-2′), 6.84 (s, 1 H, coumarin), 6.91 (s, 1 H, coumarin), 7.66 (d, 1 H, J = 9.3 Hz, coumarin H-4). Anal. ($C_{20}H_{20}O_6$) C, H.

2,2-Dimethyl-5-[(*E*,*E*)-1-methyl-4-(*p*-methoxyphenyl)butadienyl]-3(2*H*)-furanone (12). A mixture of tributylphosphine (0.303 g, 1.5 mmol), tetrakis(triphenylphosphine)palladium (0.058 g, 0.05 mmol), and **11** (0.330 g, 1.5 mmol) in a mixed solvent of tetrahydrofuran (4 mL) and methanol (4 mL) was stirred at 65 °C for 24 h. To the mixture was added sodium methoxide (0.08 g, 1.5 mmol) at 25 °C and the reaction was stirred at 25 °C for 2 h. After this time the *p*-methoxybenzaldehyde (0.12 mL, 1.0 mmol) was added, and after being stirred at 25 °C for 24 h and at 65 °C for 1 h, the reaction mixture was diluted with ether (50 mL), washed with brine (3 × 25 mL), dried over $MgSO_4$, and concentrated in vacuo. The crude product was chromatographed on silica gel (diethyl ether–petroleum ether) to give **12** as a solid: (0.170 g, 42%): mp 120–122 °C (cyclohexane); IR ($CHCl_3$) 1670, 1580 cm^{-1} ^1H NMR ($CDCl_3$) δ 1.44 (s, 6 H, $(CH_3)_2$), 2.1 (s, 3 H, CH_3C=), 3.85 (s, 3 H, CH_3O), 5.60 (s, 1 H, furan), 6.95 (d, 1 H, J = 15 Hz, H-4′), 7.25–7.4 (m, 6 H, Ar, H-3′ and H-2′). Anal. ($C_{18}H_{20}O_3$) C, H.

2,2-Dimethyl-5-[(*E*)-3-formyl-2-propen-2-yl]-3(2*H*)-furanone (13). To a solution of pyridine (3.5 mL) and methylene chloride (25 mL) was added chromium(VI) oxide (0.18 g, 1.9 mmol) portionwise at 0 °C with stirring. The resulting mixture was well-stirred for 1 h at room temperature. After this time, compound **9** (0.5 g, 2.7 mmol) solubilized in methylene chloride (5 mL) was added dropwise at 0 °C and the reaction was stirred at room temperature for other 2 h. The mixture was filtered over Celite and the organic phase was concentrated in vacuo. The residue was chromatographed on silica gel (diethyl ether–petroleum ether) to give **13** as solid (0.289 g, 60%): mp 98–100 °C (diethyl ether–petroleum ether); IR ($CHCl_3$) 1730, 1710 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.4 (s, 6 H, $(CH_3)_2$), 2.42 (s, 3 H, CH_3C=), 5.9 (s, 1 H, furan), 6.7 (d, 1 H, J = 7.5 Hz, CH=), 10.25 (d, 1 H, J = 7.5 Hz, CHO). Anal. ($C_{10}H_{12}O_3$) C, H.

2,2-Dimethyl-5-[(*E*,*E*)-1-methyl-4-phenylbutadienyl]-3-(2*H*)-furanone (14). The benzyltriphenylphosphonium chloride salt (3.89 g, 10 mmol) was added, at room temperature, to a stirred solution of potassium *tert*-butoxide (1.2 g, 10 mmol) in anhydrous dimethyl sulfoxide (10 mL). The mixture was stirred for 1 h until dissolution was complete. Aldehyde **13** (1.3 g, 7.3 mmol) in dimethyl sulfoxide (5 mL) was then added dropwise to the yellow solution. The mixture was stirred at 25 °C for 1 h and then quenched with saturated aqueous sodium chloride (200 mL) and extracted with diethyl ether (2 × 100 mL). The combined ether extracts were washed with saturated aqueous sodium chloride, dried, and evaporated under reduced pressure. The solid residue was chromatographed on silica gel (eluent: diethyl ether–petroleum ether) to give **14** as a solid (0.9 g, 48%): mp 77–80 °C (hexane); IR (KBr) 1670, 1580 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.42 (s, 6 H, $(CH_3)_2$), 2.08 (s, 3 H, CH_3C=), 5.58 (s, 1 H, furan), 6.9 (d, 1 H, J = 15 Hz, H-4′), 7.25–7.4 (m, 7 H, Ar, H′3′ and H-2′). Anal. ($C_{17}H_{18}O_2$) C, H.

7-[(*E*,*E*)-4-Methyl-4-(4,5-dihydro-5,5-dimethyl-4-oxo-2-furanyl)butadienyl]-2*H*-1-benzopyran-2-one (15). Compound **15** was obtained in the same manner as that described for **14** (49%): mp 208–210 °C (ethyl acetate–petroleum ether); IR (KBr) 1715, 1670, 1600, 1540 cm^{-1}; ^1H NMR ($CDCl_3$ + 20% $(Eu(fod)_3)$ δ 1.42 (s, 6 H, $(CH_3)_2$), 2.23 (s, 3 H, CH_3C=), 5.81 (s, 1 H, furan), 6.42 (d, 1 H, J = 9.5 Hz, coumarin H-3), 7.14 (d, 1 H, J = 15.3 Hz, H-1′), 7.30 (d, 1 H, J = 11.3 Hz, H-3′), 7.57–7.71 (m, 4 H, coumarin and H-2′), 7.98 (d, 1 H, J = 9.5 Hz, coumarin H-4). Anal. ($C_{20}H_{18}O_4$) C, H.

2,2-Dimethyl-5-[(1*E*,3*Z*)-1-methyl-4-cyclohexylbutadienyl]-3(2*H*)-furanone (16). To a stirred suspension of cyclohexyltriphenylphosphonium bromide salt (1.3 g, 3.1 mmol) in 5 mL of dry tetrahydrofuran was added dropwise at −50 °C *n*-butyllithium (1.9 mL, 1.55 M, 3.1 mmol) in hexane. The resulting red solution was stirred for additional 20 min at room temperature and then 0.28 g (1.5 mmol) of aldehyde **13** was added at 0 °C. The mixture was allowed to stir at this temperature for 10 min. The dark-green suspension was diluted with diethyl ether (30 mL) and saturated ammonium chloride (15 mL). The organic layer was separated, washed with brine, and dried (Na_2SO_4). The crude product was chromatographed on silica gel (diethyl ether–petroleum ether) (0.2 g, 50%): mp 105–106 °C (hexane); IR (KBr) 1670, 1590, 1530 cm^{-1}, ^1H NMR ($CDCl_3$) δ 1.11–1.84 (m, 10 H, cyclohexane), 1.42 (s, 6 H, $(CH_3)_2$), 1.99 (s, 3 H, CH_3C=), 2.15 (m, 1 H, CHC=), 5.57 (s, 1 H, furan), 5.70 (dd, 1 H J = 11.5 and 8.3 Hz, H-4′), 6.20 (dd, 1 H, J = 11.5 and 10.7 Hz, H-3′), 7.27

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(d, 1 H, J = 10.7 Hz, H-2'). Anal. ($C_{17}H_{24}O_2$) C, H.

The 1*E*,3*E*-isomer **17** was obtained in traces as a solid: IR (KBr) 1670, 1600, 1550 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.11-1.84 (m, 10 H, cyclohexane), 1.42 (s, 6 H, $(CH_3)_2$), 1.99 (s, 3 H, CH_3C=), 2.15 (m, 1 H, CHC=), 5.53 (s, 1 H, furan), 6.12 (dd, 1 H, J = 15.2 and 7.05 Hz, H-4'), 6.36 (dd, 1 H, J = 15.2 and 11.2 Hz, H-3'), 7.03 (d, 1 H, J = 11.2 Hz, H-2').

Inhibition of L1210, FM3A, Molt/4F, and MT-4 Cell Proliferation. All assays were performed in flat-bottomed Microtests III plates (96 wells) (Falcon) as previously described.[18,19] Briefly, the cells were suspended in growth medium and added to the microplate wells at a density of 5×10^4 L1210, FM3A, or Molt/4F cells/well (200 mL) or 6.25×10^4 MT-4 cells/well in the presence of varying concentrations of the test compounds. The cells were then allowed to proliferate for 48 h (L1210 cells) or 72 h (other cell lines) at 37 °C in a humidified, CO_2-controlled atmosphere. At the end of the incubation period, the cells were counted in a Coulter counter (Coulter Electronics Ltd., Harpenden, Herts, U.K.). The IC_{50} was defined as the concentration of compound that reduced the number of viable cells by 50%.

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Antimitotic Agents. Alterations at the 2,3-Positions of Ethyl (5-Amino-1,2-dihydropyrido[3,4-*b*]pyrazin-7-yl)carbamates

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The reaction of ethyl (6-amino-4-chloro-5-nitropyridin-2-yl)carbamate (**2**) with α-amino ketone oximes gave 4-[(2-oxoethyl)amino]pyridine oximes **3**, which were reductively cyclized to give a series of ethyl (1,2-dihydropyrido[3,4-*b*]pyrazin-7-yl)carbamates (**6**). In another approach, α-nitro ketones, α-oximino ketones, and α-nitro alcohols were reduced to give α-amino alcohols, which were reacted with **2** to give 4-[(2-hydroxyethyl)amino]pyridines (**5**). Oxidation of these alcohols with the chromium trioxide–pyridine reagent gave the corresponding ketones (**4**), which were also reductively cyclized to give **6**. Structure–activity relationship studies indicated that alterations at the 2- and 3-positions of the pyrazine ring of **6** had a significant effect on cytotoxicity and the inhibition of mitosis in cultured lymphoid leukemia L1210 cells. Compounds that exhibited in vitro cytotoxicities at less than 1 nM showed the same level of in vivo activity, whereas the less potent compounds showed wide variations in their in vivo activity.

A number of the 1,2-dihydropyrido[3,4-*b*]pyrazines inhibit the proliferation of cultured L1210 cells at nanomolar concentrations and exhibit activity in mice against lymphocytic leukemia P388.[1-3] Previous work has shown that these types of compounds interact with tubulin and compete with colchicine for binding to tubulin.[4] The correlation of cytotoxicity with antimitotic activity indicated that the compounds prepared in this study also compete with colchicine for binding to tubulin. This mode of action is thought to cause the accumulation of cells at mitosis with both cultured cells and ascites cells in vivo. Previous work indicated that oxidation of the 1,2-dihydro moiety of the pyrazine ring to give the corresponding heteroaromatic ring system and increasing the basicity at the 1-position by the preparation of 1-aminoimidazo[4,5-*c*]pyridines (3-deaza-9-aminopurines) and 2-aminopyrido[3,4-*b*]pyrazines either decreased or destroyed activity.[5,6] In contrast, a methyl group at the 2-position of the pyrazine ring and the presence of electron-donating substituents in the 3-phenyl group either increased or maintained activity.[1] Because

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