Articles

Synthesis and Cytostatic Activity of Geiparvarin Analogues

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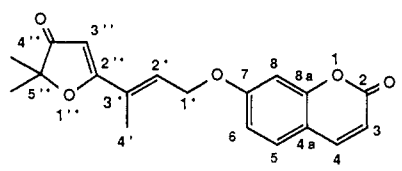
In an attempt to determine some of the structural features of geiparvarin (1) that account for its cytostatic activity in vitro, a series of geiparvarin analogues (**4a–g**) modified in the 3(2*H*)-furanone moiety have been designed and synthesized. The preparation of **4a–g** was achieved through a new approach to the 3(2*H*)-furanone ring based on the elaboration of isoxazole derivatives. Among these synthetic analogues, **4b**, the 5-methyl-5-ethyl derivative, proved as active as 1 in inhibiting the proliferation of murine and human tumor cell lines in vitro. As a rule, substitutions at the C_5 atom of the 3(2*H*)-furanone moiety of 1 slightly decreased the cytostatic activity of geiparvarin. Several geiparvarin analogues described in this study (i.e. the 5-methyl-5-ethyl derivative **4b**, 3(2*H*)-furanimine **4c**, 5-methyl derivative **4f**, and 5-ethyl derivative **4g**) showed such activity in vitro and deserve further investigation for their antitumor potentials in vivo.

Natural products isolated from extracts of plants and herbs hold much promise in providing valuable and novel antitumor agents.[1] This is the case of geiparvarin (1), a



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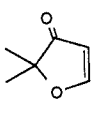
naturally occurring product that has been the subject of several synthetic investigations since its isolation from the leaves of *Geijera parviflora* Lindl and characterization in 1967.[2,3] It is important to note that the central structural feature of the molecule, namely the 3(2*H*)-furanone ring, is common to an increasing number of antitumor agents, including such diverse substances as jatrophone,[4] eremantholides A, B, and C,[5] and chilenone A.[6]

Furthermore, during studies directed to define the reactivity of the 3(2*H*)-furanone system toward potential bionucleophiles, Smith[7] has shown that simple 3(2*H*)-furanones and alkenylfuranones, such as **2** and **3**, respectively, undergo 1,4 and 1,6 conjugate addition of propanethiol under acidic and basic conditions.

Taking into account that geiparvarin itself as well as jatrophone and eremantolides reacted with propanethiol via 1,6 conjugate addition to afford Michael adducts, the same authors[7] suggested that this reactivity of the alkenyl-3(2*H*)-furanone system can be considered as the possible mode of biological action of this class of antitumor agents. For instance, geiparvarin and propenylfuranone **3** displayed activity against P-388 lymphocytic leukemia with T/C values of 130 at 400 mg/kg and 128 at 100 mg/kg, respectively.[7]



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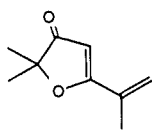

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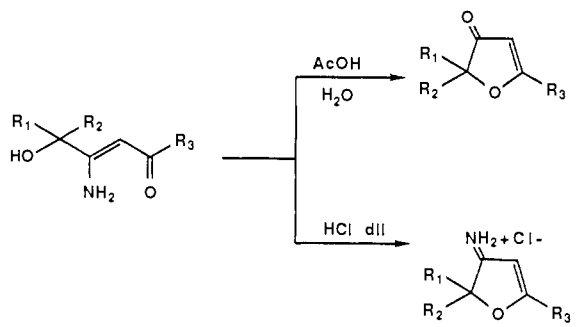
Scheme I



Our recent studies on 3(2*H*)-furanone rings functionalized at the 5-position led us to devise a simple and efficient route to 1 utilizing a new approach based on isoxazole chemistry.[8]

In order to investigate structure–activity relationships of 1, we have undertaken a program aimed at synthesizing new geiparvarin analogues modified on the 3(2*H*)-furanone ring, assuming that the coumarin moiety of the molecule plays a part only in conferring the right degree of lipophilicity. Recently, a series of geiparvarin analogues in which the coumarin ring was replaced by 4*H*-1-benzopyran-4-one and xanthone have been reported.[9]

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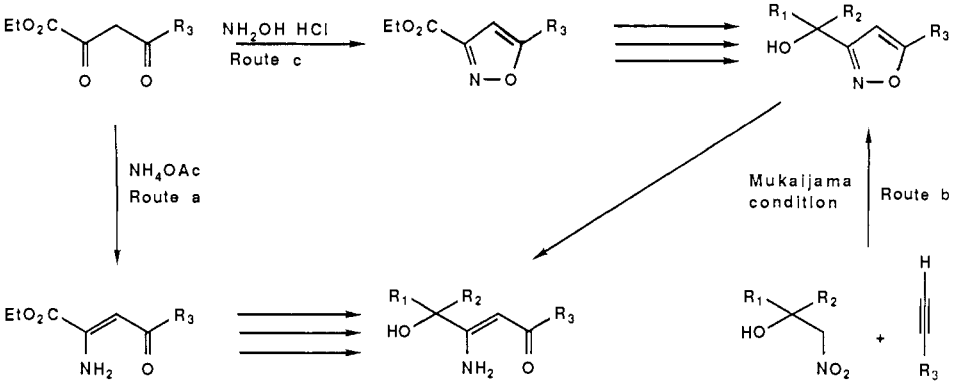
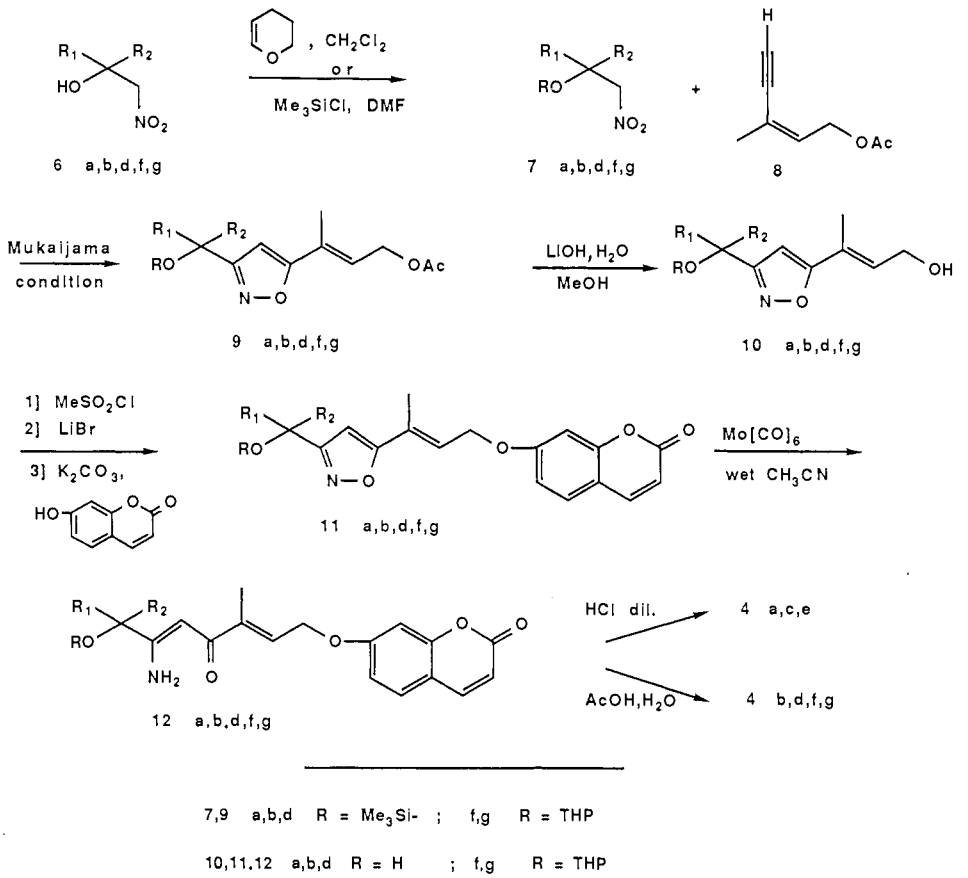
Scheme II



Scheme III



In this paper, we report the synthesis and the cytostatic activity of a series of new geiparvarin analogues (**4a–g**) as well as the cytostatic activity of a group of simple 3-(2*H*)-furanone and 3(2*H*)-furanimines (**5a–i**), some of which have previously been reported[10] and others that were newly synthesized.

Chemistry

The analogues **4a–g** as well as the newly synthesized 3(2*H*)-furanones and 3(2*H*)-furanimines (**5a–i**) were prepared according to the routes depicted in Schemes I–III.

In previous synthetic studies,[8,10,11] we have reported the controlled reaction conditions to obtain 3(2*H*)-furanones and 3(2*H*)-furanimines. This reaction is based on acid-promoted cyclocondensation of the γ-hydroxy-β-enaminone, namely treatment with acetic acid in THF/water at room temperature or with hydrochloric acid in THF at room temperature (Scheme I).

The γ-hydroxy-β-enaminone was obtained both through regiospecific reaction of ammonium acetate in the presence of acetic acid on ethyl 2,4-dioxoalkanoate followed by elaboration of the ester function and through reductive ring opening of the appropriate isoxazole. The latter in turn was obtained both through [3 + 2] cycloaddition of a nitrile oxide generated from the suitable nitro derivative under Mukaiyama conditions on an acetylenic compound and through regiospecific cyclocondensation of hydroxylamine hydrochloride on ethyl 2,4-dioxoalkanoate followed by elaboration of the ester function (Scheme II).

The choice of the synthetic route to be used in the preparation of the γ-hydroxy-β-enaminone depends on the availability of the starting material: in the case of simple 3(2*H*)-furanones and 3(2*H*)-furanimines (**5a–i**) (R_3 = alkyl,

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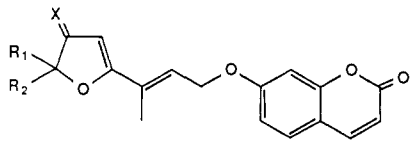
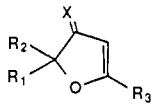
Table I. Properties of Geiparvarin Analogues (**4a–g**) and 3(2*H*)-Furanones and Iminofuranones **5a–i**



4 a - g



5 a - l

no.	R_1	R_2	R_3	X	mp, °C	salt	solvent	yield, %	formula	anal. calcd (found) C	H	N	Cl
1	Me	Me		O	ref 8								
4a	Me	Me		NH	270–275	chloride	$MeOH/Et_2O$	92	$C_{19}H_{20}NO_4Cl$	63.05 (63.09)	5.53 (5.53)	3.87 (3.88)	9.80 (9.72)
4b	Me	Et		O	118		Et_2O	94	$C_{20}H_{20}O_5$	70.57 (70.63)	5.92 (5.90)		
4c	Me	Et		NH	178–180	chloride	Me_2CO	90	$C_{20}H_{22}NO_4Cl$	63.92 (63.98)	5.86 (5.85)	3.72 (3.70)	9.44 (9.35)
4d	$-(CH_2)_5-$			O	157–160		Me_2CO	93	$C_{22}H_{22}O_5$	72.11 (72.20)	6.05 (6.09)		
4e	$-(CH_2)_5-$			NH	145–150	chloride	$MeOH/Et_2O$	88	$C_{22}H_{24}NO_4Cl$	68.13 (68.22)	6.19 (6.20)	3.61 (3.63)	9.14 (8.95)
4f	Me	H		O	165–166		MeCN	90	$C_{18}H_{16}O_5$	69.22 (69.18)	5.16 (5.20)		
4g	Et	H		O	130–131		AcOEt	92	$C_{19}H_{18}O_5$	69.92 (69.94)	5.56 (5.56)		
5a	Me	Me	*a*	O	ref 10								
5b	Ph	Ph	Me	NH	200-dec.	chloride	$CHCl_3/Et_2O$	86	$C_{17}H_{16}ClNO$	71.45 (71.44)	5.06 (5.06)	4.90 (4.90)	12.41 (12.40)
5c	H	H	*o*-MeO-Ph	O	ref 10								
5d	H	H	Ph	O	ref 10								
5e	Me	Me	*o*-MeO-Ph	O	ref 10								
5f	Me	Me	*i*-C_4H_9	NH	ref 10								
5g	Me	Me	Ph	NH	ref 10								
5h	Me	Me	*n*-C_5H_{11}	NH	ref 10								
5i	Me	Me	*i*-C_4H_9	NH	139–140	oxalate	$CHCl_3/Et_2O$	94	$C_{12}H_{19}NO_5$	56.02 (56.15)	7.44 (7.50)	5.44 (5.43)	

[a] $CH_2C(CH_3)=CHCH_2OH$.

aryl), routes a and c are the best ones, whereas in the case of complex 3(2*H*)-furanones route b is more suitable, allowing the assembly of highly functionalized partners. The synthesis of analogues **4a–g** was performed according to route b as shown in Scheme III.

The isoxazole scaffolds (**9a,b,d,f,g**) destined to become the 3(2*H*)-furanone moiety of the geiparvarin analogues (**4a–g**) could be assembled through 1,3-dipolar cycloaddition between two readily available precursors, namely the primary nitro derivatives (**7a,b,d,f,g**), easily accessible by protection of the hydroxyl function as the trimethylsilyl ether or tetrahydropyranyl ether of the Henry adducts (**6a,b,d,f,g**) between the appropriate carbonyl compound and nitromethane, and the acetate (**8**) of the commercially available enyne. Thus, the nitrile oxide generated from **7a,b,d,f,g**) under Mukaiyama conditions cycloadded regioselectively and chemoselectively to the acetyl derivative **8** to produce the 3,5-substituted isoxazoles (**9a,b,d,f,g**) in 80–90% yield. Removal of the acetyl group from the intermediates **9a,b,d** by treatment at 20 °C with lithium hydroxide solution ($MeOH/H_2O$ 9/1) followed by acid workup furnished diols **10a,b,d**. In entries f and g the tetrahydropyranyl group was maintained in order to avoid ether formation on both hydroxyl functions in the next step. Compounds **10f,g** were obtained from **9f,g** through basic hydrolysis with lithium hydroxide followed by neutral workup.

The primary hydroxyl group of **10a,b,d,f,g** reacted, selectively in the case of entries a, b, and d, with methanesulfonyl chloride in the presence of triethylamine to afford the corresponding crude mesylates, which on treatment with 7-hydroxycoumarin in the presence of lithium bromide gave rise to a 70–80% yield of the key intermediates **11a,b,d,f,g**. At this point the stage was set for the unmasking of the isoxazole nucleus to reveal the latent 1,3-diketone precursor of the 3(2*H*)-furanone moiety. In this case, reductive ring opening of **11a,b,d,f,g** cannot be performed with catalytic hydrogenation owing to the presence of a C–C double bond in the side chain.

Among the nonhydrogenolytic methods for the ring opening of the isoxazole, we choose the protocol introduced by Nitta, involving molybdenum hexacarbonyl in wet acetonitrile. This procedure generated the expected enaminones **12a,b,d,f,g** in 80–95% yield. On exposure of **12b,d,f,g** to acetic acid at 20 °C for 24 h, an easy cyclodehydration took place, affording **4b,d,f,g** in 90–95% yield. On exposure of **12a,b,d** to hydrochloric acid at room temperature for 3 h, cyclodehydration took place, affording 3(2*H*)-furanimines **4a,c,e** in 86–92% yield.

Biological Evaluation

Geiparvarin (**1**), analogues **4a–g** with modifications in the 3(2*H*)-furanone moiety, and compounds with modified 3(2*H*)-furanone (**5a**, **5c**, **8d**, **5e**) and 3(2*H*)-furanimine (**5b**, **5f**, **5g**, **5h**, **5i**) moieties were evaluated for their inhibitory effects on the proliferation of murine (L1210, FM3A) and human (Raji, Molt/4F, CEM, H9 and MT4) tumor cells (Table II).

Among the geiparvarin analogues modified in the 3-(2*H*)-furanone moiety (**4a–g**), compound **4b** proved as active as the parent compound in inhibiting the proliferation of murine and human tumor cell lines in vitro. Its 50% inhibitory dose (ID_{50}) was 2–3 μg/mL for all cell lines except for MT4. Interestingly, both **1** and **4b** proved 10-fold more inhibitory to the growth of the HTLV-I (human T-cell leukemia virus type 1) transformed T4 lymphocyte

Table II. Inhibitory Effects of the Geiparvarin Analogues **4a–g** and Their 3(2*H*)-Furanones and Iminofuranones **5a–i** on the Proliferation of Murine of Human Tumor Cells

	ID_{50},[a] μg/mL						
no.	L1210	FM3A	Raji	Molt/4F	MT4	CEM	H9
1	2.07 ± 0.66	3.20 ± 0.39	2.19 ± 0.36	2.84 ± 0.42	0.320 ± 0.077	2.00 ± 0.84	1.97 ± 0.83
4a	18.4 ± 4.6	20.7 ± 0.82	26.0 ± 2.7	25.8 ± 6.5	8.77 ± 6.52	18.8 ± 9.1	21.7 ± 9.9
4b	2.05 ± 0.49	2.96 ± 0.26	2.59 ± 0.56	3.02 ± 0.41	0.530 ± 0.132	2.49 ± 0.73	2.15 ± 0.85
4c	4.71 ± 0.68	14.1 ± 1.55	17.9 ± 4.0	3.17 ± 0.30	2.65 ± 0.23	3.57 ± 1.59	2.58 ± 0.13
4d	1.82 ± 0.58	6.61 ± 2.16	5.43 ± 1.37	12.7 ± 10.3	2.10 ± 0.91	2.72 ± 0.08	2.08 ± 0.93
4e	4.05 ± 1.05	15.8 ± 3.46	23.5 ± 3.6	20.5 ± 6.5	4.93 ± 2.15	4.95 ± 3.51	14.2 ± 12.8
4f	7.05 ± 2.66	15.6 ± 4.76	10.4 ± 2.98	3.32 ± 0.45	2.56 ± 0.68	3.56 ± 0.65	2.91 ± 0.87
4g	9.77 ± 4.71	17.2 ± 2.36	2.43 ± 0.55	2.84 ± 0.19	1.63 ± 0.49	3.27 ± 1.13	4.10 ± 3.47
5a	15.8 ± 4.01	23.3 ± 3.24	16.6 ± 5.7	15.2 ± 7.7	3.05 ± 0.61	10.3 ± 6.8	9.13 ± 4.61
5b	24.2 ± 4.02	25.4 ± 1.6	24.0 ± 5.0	25.3 ± 3.6	26.5 ± 4.5	29.3 ± 2.3	30.0 ± 7.7
5c	>100	>100	>100	>100	44.4 ± 10.6	>100	66.3 ± 31.7
5d	45.8 ± 14.0	31.4 ± 3.6	50.1 ± 2.7	31.2 ± 7.5	31.7 ± 1.1	30.2 ± 3.3	38.6 ± 4.4
5e	40.4 ± 0.26	31.7 ± 4.61	89.1 ± 18.9	53.3 ± 22.9	30.4 ± 6.3	66.1 ± 36.9	63.9 ± 41.7
5f	>100	33.0 ± 3.21	>100	>100	>100	>100	>100
5g	66.4 ± 31.3	24.7 ± 3.7	>100	>100	76.2 ± 25.6	>100	>100
5h	98.3 ± 2.89	24.2 ± 2.14	>100	>100	>100	>100	>100
5i	>100	33.2 ± 6.83	>100	>100	>100	>100	>100

[a] 50% inhibitory dose or dose required to inhibit cell proliferation by 50%.

cell line (MT4) than to the growth of the other T4 lymphocyte cell lines. As a rule, substitutions at the C-5 atom of the 3(2*H*)-furanone moiety of geiparvarin slightly decreased the cytostatic activity in vitro. Compound **4a**, the 3(2*H*)-furanimine derivative of **1**, was even 10-fold less cytostatic than geiparvarin. When the 3(2*H*)-furanone moiety of geiparvarin was linked to 4*H*-1-benzopyran-4-one or xanthone rather than to a coumarin ring, the inhibitory effects of these compounds on tumor cell proliferation was substantially decreased.[9] Compounds **5a**, **5d**, and **5e** were 5–20-fold less cytostatic, whereas **5c** was inactive against most of the tumor cell lines at 100 μg/mL. Replacement of the carbonyl group in 3(2*H*)-furanone by an imine function further decreased the cytostatic activities of these compounds. Only **5b** showed a moderate antiproliferative effect (ID_{50} = 25 μg/mL).

In conclusion, minor substitutions at C-5 of geiparvarin and deletion of the coumarin part from the furanone or furanimine moiety resulted in a modestly or substantially decreased cytostatic effect of compound **1**. However, several members of the geiparvarin analogues described in this study (i.e. **4b**, **4c**, **4f**, and **4g**) show such activity in vitro that they deserve further investigation for their antitumor potentials in vivo.

Experimental Section

Melting points were determined on a Büchi apparatus and were uncorrected. Reaction courses and product mixtures were routinely monitored by thin-layer chromatography (TLC) on silica gel precoated F_{254} Merck plates. Infrared (IR) spectra were recorded on a Perkin-Elmer 297 spectrometer. Nuclear magnetic resonance (NMR) spectra were obtained with a Brucker 200 spectrometer for solution in $CDCl_3$, and peak positions are given in parts per million downfield from tetramethylsilane as an internal standard. The various splitting patterns were designated as follow: s, singlet; br s, broad singlet; d, doublet; t, triplet; q, quartet; m, multiplet. The synthesis of **4b** and **4c** are given as examples. The other geiparvarin analogues were prepared according to those procedures from appropriate starting materials. The final compounds (**4a–g**) are listed in Table I with their analytical data. Starting materials, compounds **6a**,[12a] **6b**,[12b] **6d**,[12c] and **6f,g**,[12d] have been prepared according to known directions. Compounds **7a**, **7b**, **7d**, **7f**, and **7g** were prepared from the corresponding nitro alcohol by standard procedures.[13a,b]

Preparation of 4b and 4c. 5-[(*E*)-3-(Acetyloxy)-1-methyl-1-propenyl]-α-methyl-α-ethyl-3-isoxazolemethanol Trimethylsilyl Ether (9b, R = Me_3Si, R_1 = Me, R_2 = Et). To a solution of **7b** (R = Me_3Si, R_1 = Me, R_2 = Et) (10.7 g, 52 mmol) and 8 (21.5 g, 156 mmol) in dry benzene (50 mL) containing triethylamine (0.5 mL) was added dropwise phenyl isocyanate (14.1 mL, 130 mmol) in benzene (10 mL) at room temperature, and the mixture was allowed to stand overnight. The cooled mixture (5 °C) was filtered, and the filtrate was washed with brine (3 × 50 mL), dried, and concentrated in vacuo. The residue crude oil was flash chromatographed on silica gel (ether/light petroleum 2/8) to give **9a** as an oil (14.7 g, 87%); IR (neat) 1740, 1590 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.1 (s, 9 H), 0.87 (t, 3 H, *J* = 7 Hz), 1.6 (s, 3 H), 1.9 (q, 2 H, *J* = 7 Hz), 2.0 (s, 3 H), 4.8 (d, 2 H, *J* = 6 Hz), 6.3 (s, 1 H), 6.4 (br t, 1 H).

5-[(*E*)-3-Hydroxy-1-methyl-1-propenyl]-α-methyl-α-ethyl-3-isoxazolemethanol (10b, R = H, R_1 = Me, R_2 = Et). Lithium hydroxide monohydrate (8.4 g, 200 mmol) was added to a stirred solution of **9b** (R = Me_3Si, R_1 = Me, R_2 = Et) (27 g, 83 mmol) in methanol (200 mL) containing 30 mL of water. After 10 min at 0 °C the solution was concentrated in vacuo, and the residue was poured into 2 N hydrochloric acid (30 mL) and extracted with ethyl acetate (3 × 100 mL). The dried organic extracts were evaporated in vacuo, and the residue was flash chromatographed on silica gel (ethyl acetate/light petroleum, 1:1) to give **10a** as an oil (16.5 g, 94%): IR (neat) 3300, 1650, 1560 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.89 (t, 3 H, *J* = 7 Hz), 1.6 (s, 3 H), 1.85 (q, 2 H, *J* = 7 Hz), 1.99 (s, 3 H), 2.3 (br s, 1 H), 2.7 (br s, 1 H), 4.4 (d, 1 H, *J* = 6 Hz), 6.2 (s, 1 H), 6.4 (br t, 1 H).

7-[[(*E*)-3-[3-(1-Hydroxy-1-methylpropyl)isoxazol-5-yl]-2-butenyl]oxy]-2*H*-1-benzopyran-2-one (11b, R = H, R_1 = Me, R_2 = Et). To an ice-cooled solution of **10b** (R = H, R_1 = Me, R_2 = Et) (0.85 g, 4 mmol) in methylene chloride (10 mL) containing triethylamine (0.5 mL, 6 mmol) was added dropwise methanesulfonyl chloride (0.35 mL, 5 mmol) in methylene chloride (5 mL) with stirring. After 30 min at room temperature, the mixture was treated with 2% citric acid (3 mL) and the organic phase separated, dried, and concentrated in vacuo. The crude mesylate was immediately heated at reflux for 0.5 h in acetone solution (20 mL) containing 7-hydroxycoumarin (0.65 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 to give **11b** (1.33 g, 78%): mp 124–126 °C (AcOEt, light petroleum); IR ($CHCl_3$) 3300, 1730, 1610, 1570, 1500 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.88 (t, 3 H, *J* = 7 Hz), 1.58 (s, 3 H),

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1.85 (q, 2 H, J = 7 Hz), 2.1 (s, 3 H), 2.5 (br s, 1 H), 4.85 (d, 2 H, J = 6 Hz), 6.2 (s, 1 H), 6.25 (d, 1 H, J = 9 Hz), 6.55 (br t, 1 H), 6.8 (m, 2 H), 7.4 (d, 1 H, J = 8.5 Hz), 7.65 (d, 1 H, J = 9 Hz).

7-[[(*E*,*E*)-6-Amino-7-hydroxy-3-methyl-7-ethyl-4-oxo-2,5-octadienyl]oxy]-2*H*-1-benzopyran-2-one (12b, R = H, R_1 = Me, R_2 = Et). A solution of isoxazole **11b** (R = H, R_2 = Et) (2.13 g, 6 mmol) in acetonitrile (40 mL) containing water (50 drops) was treated with molybdenum hexacarbonyl (0.81 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 was evaporated in vacuo. The residue was flash chromatographed on silica gel (ethyl acetate/light petroleum 6/4) to give **12a** (2.0 g, 94%): mp 104–105 °C (methanol); IR ($CHCl_3$) 3450, 1730, 1615, 1550, 1500 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.94 (t, 3 H, J = 7 Hz), 1.5 (s, 3 H), 1.5–1.85 (m, 3 H), 1.96 (s, 3 H), 4.8 (d, 2 H, J = 6 Hz), 5.3 (s, 1 H), 6.25 (d, 1 H, J = 9 Hz), 6.4 (br, 2 H), 6.64 (m, 2 H), 7.76 (d, 1 H, J = 8.5 Hz), 7.7 (d, 1 H, J = 9 Hz), 10.5 (br s, 1 H).

7-[[3-(4,5-Dihydro-5-methyl-5-ethyl-4-oxo-2-furanyl)-2-butenyl]oxy]-2*H*-1-benzopyran-2-one (4b, R_1 = Me, R_2 = Et, X = O). A solution of **12b** (R = H, R_1 = Me, R_2 = Et) (0.92 g, 2.6 mmol) in tetrahydrofuran (20 mL) was stirred at room temperature for 24 h with 75% acetic acid (20 mL). The mixture was poured into brine (50 mL) and extracted with ethyl acetate (3 × 25 mL). The combined organic extracts were washed in turn with saturated aqueous sodium hydrogen carbonate (2 × 10 mL) and brine (2 × 10 mL) and dried. The solvent was removed under reduced pressure and the residue was purified by flash chromatography, eluting with methylene chloride/ethyl acetate 10/1, to give **4b** (0.83 g, 94%): mp 118 °C (ether); IR ($CHCl_3$) 1730, 1690, 1660, 1610, 1550 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.87 (t, 3 H, J = 7 Hz), 1.38 (s, 3 H), 1.8 (q, 2 H, J = 7 Hz), 2.0 (s, 3 H), 4.85 (d, 2 H, J = 6 Hz), 5.65 (s, 1 H), 6.3 (d, 1 H, J = 9 Hz), 6.9 (m, 3 H), 7.2 (d, 1 H, J = 8.5 Hz), 7.4 (d, 1 H, J = 9 Hz).

7-[[3-(4,5-Dihydro-5-methyl-5-ethyl-4-imino-2-furanyl)-2-butenyl]oxy]-2*H*-1-benzopyran-2-one Hydrochloride (4c, R_1 = Me, R_2 = Et, X = NH). A solution of enaminone **12b** (R = H, R_1 = Me, R_2 = Et) (1.2 g, 3.3 mmol) in THF (20 mL) containing 5% HCl (20 mL) was stirred at room temperature for 3 h. Evaporation of the solvent in vacuo, followed by extraction with ether (50 mL) to remove some impurities and finally with $CHCl_3$ (3 × 25 mL). The dried chloroform extracts were evaporated in vacuo, and the solid residue was crystallized from acetone to give **4c** (1.13 g, 90%): mp 178–180 °C; IR ($CHCl_3$) 3400–2500, 1720, 1610, 1520 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.85 (t, 3 H, J = 7 Hz), 1.6 (s, 3 H), 2.0 (m, 5 H), 5.0 (d, 2 H, J = 6 Hz), 6.3 (d, 1 H, J = 9 Hz), 6.4 (s, 1 H), 7.0 (m, 3 H), 7.65 (d, 1 H, J = 8.5 Hz), 8.0 (d, 1 H, J = 9 Hz), 11.5 (br s, 2 H).

Cytostatic Assays. The cytostatic effects of the compounds were assessed on the basis of the inhibition of cell proliferation; these assays were carried out with murine leukemia L1210 and mammary carcinoma FM3A cells, human B-lymphoblast Raji and T-lymphoblast Molt/4F cells, and human T4 lymphocyte MT4, CEM, and H9 cells in their exponential growth phase. This assay procedure has been described previously.[14]

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Registry No. 1, 36413-91-9; **4a**, 117310-13-1; **4b**, 117310-14-2; **4c**, 117310-15-3; **4d**, 117310-16-4; **4e**, 117310-17-5; **4f**, 117310-18-6; **4g**, 117310-19-7; **5a**, 87064-11-7; **5b**, 117310-20-0; **5c**, 82284-73-9; **5d**, 5198-63-0; **5e**, 117310-21-1; **5f**, 117310-22-2; **5g**, 117310-23-3; **5h**, 117310-24-4; **5i**, 117310-25-5; **6a**, 5447-98-3; **6b**, 22916-76-3; **6d**, 3164-73-6; **6f**, 3156-73-8; **6g**, 3156-74-9; **7a**, 55816-65-4; **7b**, 117310-26-6; **7d**, 117310-27-7; **7f**, 69386-03-4; **7g**, 117310-28-8; **8**, 35272-88-9; **9a**, 117285-95-7; **9b**, 117310-29-9; **9d**, 117310-30-2; **9f**, 117310-31-3; **9g**, 117310-32-4; **10a**, 101417-83-8; **10a** (mesylate), 101417-84-9; **10b**, 117310-33-5; **10b** (mesylate), 117310-37-9; **10d**, 117310-34-6; **10d** (mesylate), 117310-38-0; **10f**, 117310-35-7; **10f** (mesylate), 117310-39-1; **10g**, 117310-36-8; **10g** (mesylate), 117310-40-4; **11a**, 101417-85-0; **11b**, 117310-41-5; **11d**, 117310-42-6; **11f**, 117310-43-7; **11g**, 117310-44-8; **12a**, 101417-86-1; **12b**, 117310-45-9; **12d**, 117310-46-0; **12f**, 117310-47-1; **12g**, 117310-48-2; 7-hydroxycoumarin, 93-35-6.

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