Synthetic Secofriedelane and Friedelane Derivatives as Inhibitors of Human Lymphocyte Proliferation and Growth of Human Cancer Cell Lines in Vitro

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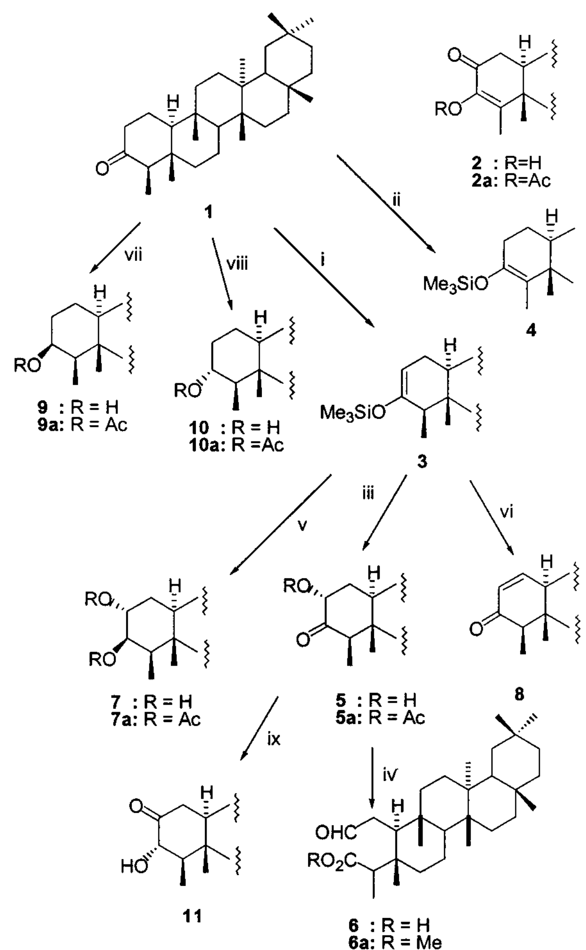
Controlled silylation of friedelin (**1**) from cork smoker wash solids, a byproduct generated during processing of corkboard by steam baking, gave 3-trimethylsiloxyfriedel-2-ene (**3**) in high yields. Oxidation of **3** with OsO_4/NMMO produced 2α-hydroxyfriedelan-3-one (cerin) (**5**), from which the new 2,3-secofriedelan-2-al-3-oic acid (**6**) was obtained quantitatively by periodic acid oxidation. Oxidation of **3** with DDQ afforded friedel-1-en-3-one (**8**). Reductive ozonolysis of **3** gave 2α,3β-dihydroxyfriedelane, pachysandiol A (**7**). Compound **6** proved to be a potent inhibitor of human lymphocyte proliferation (IC_{50} = 10.7 μM) and of the growth of a human cancer cell line (GI_{50} = 5.4−17.2 μM). ^{13}C NMR data for compounds (**3**, **4**, **5**, **6a**, **7**, and **8**) are described for the first time.

Processing of cork of *Quercus suber* L. (Fagaceae) is an important industrial activity in Portugal, generating large amounts of cork powder and cork smoker wash solids, a black wax obtained in the process of making corkboard from ground cork under pressure by treatment with superheated steam.

Cork smoker wash solids are a convenient source of friedelin (**1**) and 3-hydroxyfriedel-3-en-2-one (**2**).[1−3] Many triterpenoid compounds are reported to have antiinflammatory, antiviral, antibacterial, anticancer, or suppression of tumor promotion activities.[4,5] Friedelin (**1**) has reported diuretic activity.[6] There are no systematic studies of structure−activity relationships based on chemical modification of friedelane triterpenoids. Compound **1** offers the possibility of various structural modifications in ring-A; thus we have focused on the preparation of derivatives including seco acids and dihydroxylated and α,β-unsaturated systems (Scheme 1). We report here the synthesis and biological activity of several fridelane derivatives.

Scheme 1



Results and Discussion

Extraction of cork smoker wash solids with petroleum ether gave friedelin (**1**) (8.0%) and 3-hydroxyfriedel-3-en-2-one (**2**) (5.4%). Small amounts of β-sitosterol (1%), campesterol (0.3%), β-amyrin, and sitost-4-en-3-one (0.3%) were also identified, the latter having not been previously reported in this material.[7] Compounds **1** and **2** and its derivative, the 3-acetoxyfriedel-3-en-2-one (**2a**), have ^1H and ^{13}C NMR spectra identical to those described in the literature.[8−11]

It has been reported that, unlike the 4,4-dimethyl-3-keto triterpenoids, attempts to carry out the transformation of **1** into the respective seco acids with *m*-chloroperbenzoic acid[12,13] as with other peracids,[14] in the presence of a strong acid such as *p*-toluenesulfonic acid (*p*-TsOH), gave only the ε-lactone instead of the desired seco acids. For example, the oxidation of **1** with hydrogen peroxide in the presence of selenium dioxide gave a seco acid in only 2.1% yield, besides the ε-lactone (48%).[15] Although α-acetoxylation of **1** has also been achieved by oxidation with lead tetraac-

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Table 1. ^{1}H NMR Data (δ) for Compounds **3**−**7** in $CDCl_3$ (J in Hz)

position	3	4	5[a]	6a	7[b]
1α	1.93 (m)	2.30 (m)	1.97 (m)	2.29 (ddd, J_{gem} = 18.6, $J_{1,10}$ = 3.9, $J_{1,2}$ = 1.5)	1.58 (m)
1β				2.45 (ddd, J_{gem} = 18.6, $J_{1,10}$ = 5.4, $J_{1,2}$ = 1.5)	1.83 (td, $J_{gem} = J_{1\beta,10\alpha}$ = 13.0, $J_{1\beta,2\beta}$ = 2.7)
2α	4.79 (dd, J = 1.5, 1.5)	1.80 (m)			
2β		2.04 (m)	4.08 (ddd, J = 3.3, 3.3, 3.3)		3.99 (q, $J_{2\beta,1\beta} = J_{2\beta,1\alpha} = J_{2\beta,3\alpha}$ = 2.7)
4α	1.95 (m)		2.82 (q, J = 6.6)	2.30 (q, J = 6.9)	1.58 (m)
10α	1.45 (m)	1.45 (m)	1.75 (dd, $J_{10\alpha,1\alpha}$ = 3.0, $J_{10\alpha,1\beta}$ = 10.8)	2.02 (dd, J = 3.9, 5.4)	1.78 (dd, $J_{10\alpha,1\alpha}$ = 3.3, $J_{10\alpha,1\beta}$ = 13.0)
23	0.84 (d, J = 7.2)	1.47 (s)	0.89 (d, J = 6.6)	1.09 (d, J = 6.9)	0.94 (d, J = 7.2)
24	0.90 (s)	0.83 (s)	0.72 (s)	0.91 (s)	0.94 (s)
25	0.75 (s)	0.95 (s)	0.89 (s)	0.89 (s)	0.85 (s)
26	1.00 (s)	1.00 (s)	1.05 (s)	1.03 (s)	1.01 (s)
27	1.00 (s)	1.00 (s)	1.00 (s)	0.98 (s)	0.99 (s)
28	1.17 (s)	1.17 (s)	1.00 (s)	1.17 (s)	1.17 (s)
29	1.00 (s)	1.00 (s)	1.18 (s)	0.98 (s)	0.99 (s)
30	0.94 (s)	0.96 (s)	0.95 (s)	0.94 (s)	0.94 (s)
$OSi(Me)_3$	0.17 (s)	0.16 (s)			
CO_2Me				3.62 (s)	
C*H*O				9.76 (br s)	

[a] δ 2.18 (d, J = 3.3, OH). [b] δ 3.54 (br t, H-3α).

etate in the presence of boron trifluoride etherate, the synthesis is not regioselective and the yields range from 22 to 28%.[16]

The regiochemistry of silyl enol ether formation via kinetically and thermodynamically enolate derivatives, the ease with which these silyl enol ethers may be prepared in nearly quantitative yields, and the enhanced reactivity of silyloxyalkenes toward electrophilic species make such substances attractive intermediates for α-hydroxylation methodology.[17,18] A route leading to seco friedelanes was devised via friedelin enol silyl ethers. Conversion of ketones to silyl enol ethers is a well-known process; however, the formation of mixtures in the application of such methods is often observed.[19−21] Friedelin (**1**) reacts with trimethylsilyl chloride in the presence of LDA to give no more than 52% of 3-trimethylsiloxyfriedel-2-ene (**3**), while with Et_3N and trimethylsilyl trifluoromethanesulfonate a 1:1 mixture of the isomers **3** and **4** in 84% yield is obtained. Nevertheless, regiospecific silylation of **1** was achieved by reaction with N,O-bis(trimethylsilyl)acetamide in the presence of sodium and HMPA under kinetic control to give 98% of 3-trimethylsiloxyfriedel-2-ene (**3**) alone. In contrast, heating **1** in the presence of the same reagents at 40−50 °C for 3 h gave the silyl enol ether of thermodynamic enolate **4** as the major product (62%).

The ^{1}H NMR spectrum of **3** clearly shows one vinylic proton as a triplet at δ 4.79 (J = 1.5 Hz) assigned to H-2 and a singlet signal of nine protons at δ 0.17. Although, the mass spectra of compounds **3** and **4** are similar, these compounds can be distinguished by comparison of their ^{1}H NMR spectra; thus, in compound **4**, the absence of the olefinic proton was noted and the Me-23 was revealed as a singlet downfield (δ 1.47) in relation to Me-23 in **3** (δ 0.84, d), due to the double bond position. The ^{1}H NMR and ^{13}C NMR data of both **3** and **4** are shown in Tables 1 and 2, respectively.

Oxidation of **3** with a catalytic amount of osmium tetroxide together with *N*-methylmorpholine *N*-oxide ($NMMO{\cdot}H_2O$) afforded 2α-hydroxyfriedelan-3-one (cerin) (**5**) in 59% yield, a compound previously reported as a constituent of cork and not occurring in cork smoker wash solids.[22] The IR spectrum of **5** showed the presence of hydroxyl and carbonyl groups. The EIMS of **5** exhibited a molecular ion at *m*/*z* 442. The configuration of the 2-hydroxy group was derived from the shape of the 2β-H signal

Table 2. ^{13}C NMR Data (δ) for Compounds **3**−**8** in $CDCl_3$

position	3	4	5	6a	7	8
1	20.8	31.8	32.4	29.7	23.8	130.3
2	102.5	39.7	76.9	201.6	71.3	148.7
3	151.3	142.0	212.0	175.9	76.4	201.4
4	49.3	128.5	52.7	48.3	43.6	57.9
5	36.7	37.8	42.7	47.7	37.7	43.7
6	41.8	18.3	40.8	41.3	41.3	40.0
7	18.4	18.3	17.5	18.4	18.1	18.6
8	53.0	53.0	52.7	51.0	53.2	52.5
9	36.8	37.1	38.0	38.6	36.5	36.8
10	55.9	57.1	52.7	52.7	52.2	62.4
11	35.4	35.4	35.6	36.1	35.4	35.5
12	30.4	30.7	30.2	30.2	30.6	30.3
13	39.7	39.9	39.4	39.8	39.6	40.1
14	38.2	38.5	38.9	38.4	38.3	38.4
15	32.9	33.0	32.7	32.4	32.2	32.3
16	36.1	36.2	36.5	36.2	36.0	36.1
17	30.1	30.1	29.8	30.2	29.9	30.1
18	42.9	43.1	42.4	43.1	42.7	43.1
19	35.4	35.4	35.0	35.4	35.2	35.0
20	28.2	28.2	27.8	28.2	28.1	28.2
21	32.5	32.4	32.0	33.1	32.7	33.0
22	39.3	39.3	40.8	39.3	39.3	39.3
23	9.9	9.7	6.3	12.2	10.8	6.6
24	14.0	20.7	13.8	18.2	15.8	13.8
25	17.5	17.8	17.9	17.8	17.4	17.9
26	18.6	18.6	18.4	18.7	18.7	19.8
27	20.4	20.1	19.9	20.2	20.1	18.6
28	32.1	32.2	32.1	32.2	32.0	32.2
29	31.9	31.9	31.7	31.9	31.7	31.8
30	35.0	35.0	34.7	34.9	35.0	34.9
$OSiMe_3$	0.3	0.7				
OMe				51.0		

in the ^{1}H NMR spectrum of **5**. The oxymethine proton at δ 4.08 (ddd, J = 3.3, 3.3, 3.3 Hz) indicated an equatorial orientation. Irradiation of H-1 at δ 1.97 collapsed the ddd of the oxymethine proton into a doublet, due to coupling with the OH group (δ 2.18, d, J = 3.3 Hz). Cerin (**5**) was also obtained from the reaction of compound **3** with *m*-chloroperbenzoic acid. This alternative route is advantageous in terms of yield (85%) and lower toxicity of the oxidizing reagent. Cerin acetate (**5a**) was obtained by acetylation of compound **5**.[23]

Oxidation of **5** with periodic acid at 0 °C yielded 2,3-secofriedelan-2-al-3-oic acid (**6**), characterized as its methyl ester **6a**.[24] The molecular ion at *m*/*z* 472 together with the isotopic peaks at *m*/*z* 473 and 474 in the EIMS were in

Table 3. Effect of Compounds on the Growth of Human Cancer Cell Lines (concentration causing 50% cell growth inhibition)

compounds	GI_{50} μM				
	MCF-7 (breast)	TK-10 (renal)	UACC-62 (melanoma)	NCI−H460 (lung)	SF-268 (CNS)
1, **2**, **6a**, **8**−**11**	>100	>100	>100	>100	>100
5	59.5 ± 12.0	90.5 ± 13.3	86.0 ± 22.6	67.8 ± 3.2	95.8 ± 9.5
6	7.0 ± 0.4	10.1 ± 1.1	9.9 ± 1.1	5.4 ± 0.7	17.2± 0.9
7	66.4 ± 5.6	95.7 ± 16.9	83.3 ± 15.5	79.4 ± 2.7	15.5 ± 2.1

[a] Doxorubicin, GI_{50} MCF-7 = 5.5×10^{-2} μM; GI_{50} TK-10 = 57.0×10^{-2} μM; GI_{50} UACC-62 = 9.4×10^{-2} μM; GI_{50} NCI-H460 = 0.81×10^{-2} μM; GI_{50} SF-268 = 9.3×10^{-2} μM.

agreement with the molecular formula $C_{31}H_{52}O_3$, and elimination of the aldehyde carbonyl and ester moieties was indicated by a fragment ion at *m/z* 385. The ^{1}H NMR spectrum of **6a** (Table 1) showed signals for seven tertiary methyl groups in addition to the secondary Me-23 group at δ 1.09 as a doublet (J = 6.9 Hz). The double doublet at δ 2.02 was assigned to H-10, coupled to the H-1 protons ($J_{10,1}$ = 5.4 and 3.9 Hz). One of the H-1 protons appeared at δ 2.45, ddd, coupled to the H-10, gem-proton H-1, and aldehyde proton (J = 5.4, 18.6, and 1.5 Hz), respectively. The methyl ester group and the aldehyde proton appear as a singlet (δ 3.62) and a broad singlet at δ 9.76, respectively. The ^{13}C NMR spectrum of **6a** showed carbonyl signals at δ 201.6 and 175.9 assignable to the aldehyde and the ester groups, respectively, and with C-1 downfield at δ 29.7 due to the proximity of the aldehyde group. The IR absorption bands at 2735 and 1732 cm^{-1} further supported the presence of aldehydic and ester carbonyl functionalities in the molecule.

Ozonolysis of reactive enol silyl ethers to produce carboxylic acids is also a well-established procedure for regiospecific cleavage.[25] Attempts to cleave ring-A of **3** by reductive ozonolysis did not give the expected 2,3-seco-friedelane derivative, giving instead 2α,3β-dihydroxyfriedelane (**7**), pachysandiol-A, previously isolated from *Pachysandra terminalis* Sieb et Zucc (Buxaceae)[26] and from leaves of *Lithocarpus* sp. (Fagaceae).[27] An anomalous oxidation had already been observed in the ozonization of a hindered enol silyl ether.[28] Compound **7** was characterized by ^{1}H and ^{13}C NMR data (Tables 1 and 2) and by comparison with an authentic sample obtained by reduction of cerin (**5**) with sodium borohydride. Acetylation of **7** gave 2α,3β-diacetoxyfriedelane (**7a**), with spectral data in accordance with those published for pachysandiol-A diacetate **7a**.[26]

α,β-Unsaturation of ring-A of **1** was achieved by treatment of **3** with DDQ to afford friedel-1-en-3-one (**8**) in 76% yield. This pathway leading to **8** is an improvement over methods previously reported, such as reaction of **1** with DDQ in dioxane (9%),[14] H_2O_2, and SeO_2 (2.5%)[15] and of 2α-bromofriedel-3-one with LiBr and Li_2CO_3 (20%).[14] Compound **8** was identified by comparing the physical and spectral data with literature values.[14,15] 3β-Hydroxyfriedelane (**9**) and 3α-hydroxyfriedelane (**10**) were obtained by stereoselective reduction of friedeline (**1**) in quantitative yields.

The reactive silyl enol ether **3** is a convenient intermediate for the synthesis of a variety of friedelane derivatives. In particular, 2α,3β-dihydroxyfriedelane (**7**) is made available as a starting material for the production of known secofriedelane derivatives.[5,27]

The inhibitory activity of the 11 compounds against the in vitro proliferation of human lymphocytes was examined. Phytohemaglutinin was used as a T-mitogen. Cyclosporin A, tested in the same system, served as the internal control (IC_{50} = 0.34 μM). Only compounds **5**, **6**, and **7** showed a dose-dependent inhibitory effect against lymphocyte proliferation. No inhibitory effect was observed with triterpenes **1**, **2**, **6a**, **8**, **9**, **10**, **10a**, and **11** when tested at 100 μM.

Compounds **5** and **7** exhibited a moderate suppression of T-cell proliferation (IC_{50} = 82.3 ± 4.0 and 79.1 ± 2.2 μM, respectively), while **6** was a potent inhibitor (IC_{50} = 10.7 ± 1.6 μM) of the mitogenic response of human lymphocytes to PHA. No lymphocytotoxicity was observed when these compounds were tested at their IC_{50} concentrations (cell viability of 92.7%, 113.0%, and 89.0%, respectively), which leads to the conclusion that the inhibitory activity of these compounds was associated with cell proliferation rather than to a toxic effect.

All the compounds were tested on the growth of five human cancer cell lines, MCF-7 (breast), TK-10 (renal), UACC-62 (melanoma), NCI-H460 (lung), and SF-268 (CNS), and the results are summarized in Table 3. Only compounds **5**, **6**, and **7** inhibited the growth of the cancer cell lines tested in a dose-dependent manner. Compounds **1**, **2**, **8**, **9**, **10**, **10a**, **11**, and **6a**, the corresponding methyl ester of **6**, were inactive on all the cell lines even when tested at 100 μM, and **5** and **7** showed slight inhibition (GI_{50} > 50 μM). The seco compound **6** was a potent inhibitor of MCF-7 (breast), TK-10 (renal), UACC-62 (melanoma), and NCI-H460 (lung) cell lines, with GI_{50} values ranging from 5.4 to 10.1 μM (Table 3). Curiously, diol **7** was more inhibitory against SF-268 (GI_{50} = 15.5 ± 2.1 μM) than against the other four cell lines tested.

Experimental Section

General Experimental Procedures. Melting point determinations were performed on a Reichert Thermovar apparatus and are uncorrected. UV and IR spectra were recorded on a Hitachi 100-60 or 150-20 and Perkin-Elmer 298 spectrophotometers, respectively. ^{1}H, ^{13}C NMR, DEPT, and 2D NMR (300 MHz) spectra (all in $CDCl_3$) were obtained on a GE-300 spectrometer, and chemical shifts are given in parts per million (δ) using Me_4Si as internal standard. EIMS were measured on a Kratos MS-25 RF spectrometer at 70 eV attached to a Mach 3 system. HRMS were performed on a JEOL JMS-SX/SX 102A mass spectrometer. TLC was carried out on precoated silica gel 60 F_{254} sheets (Merck), and detection was achieved by spraying with 20% ethanolic H_2SO_4 followed by heating, or 10% ethanolic 2,4-DNP; silica gel (Merck, 230−400 mesh) was used for column chromatography. All solvents were dried and distilled before use. Ozone was produced from O_2 (20 L/h) in a Wallace & Tiernan ozonizer BA.023 at 2000 V. LDA was freshly prepared from diisopropylamine and *n*-BuLi in THF.

Fetal bovine serum and RPMI-1640 medium were obtained from Gibco BRL. Cyclosporin A, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), *N,N*-dimethylformamide (DMF), dimethyl sulfoxide (DMSO), gentamicin, L-glutamine, Histopaque-1077, phytohemagglutinin (PHA), doxorubicin, sodium lauryl sulfate (SDS), and sulforhodamine B (SRB) were purchased from Sigma

Chemical Co. Stock solutions of compounds were prepared in DMSO and stored at −20 °C, providing uniform samples for retests. These frozen concentrates were then diluted to the desired final concentrations prior to the assays. Final concentrations of DMSO showed no interference with the biological activities tested.

Material. Cork smoker wash solids were a gift from Corticeira Amorim Algarve, Portugal.

Extraction and Isolation. Powdered cork smoker wash solids (1.2 kg) were extracted using a Soxhlet apparatus with petroleum ether for 2 h, the extract was evaporated to dryness in vacuo to give a residue (230 g, 23%). The crude extract was applied to a silica gel column eluted with petroleum ether and petroleum ether−CH_2Cl_2 mixtures of increasing polarity to give the following: waxes (0.6 g, 4.3%), friedelin (**1**) (19.0 g, 8.0%), 3-hydroxyfriedel-3-en-2-one (**2**) (12.5 g, 5.4%), β-amirin, β-sitosterol (1%), campesterol (0.3%), and sitost-4-en-3-one (0.3%). These compounds were isolated and identified by comparison with literature data and/or authentic samples.

Silylation of (1). Compound **1** (300 mg, 0.7 mmol) was added, under nitrogen, to a mixture of *N,O*-bis(trimethylsilyl)acetamide (1.2 mL, 4.9 mmol), HMPA (0.6 mL, 3.5 mmol), and an excess of sodium. The mixture was stirred at room temperature for 1 h, and pentane and iced water were subsequently added. The organic layer was separated and dried over Na_2SO_4. Evaporation of the solvent under reduced pressure afforded 3-trimethylsiloxyfriedel-2-ene (**3**) (344 mg, 0.69 mmol, 98.1%): white crystals (from petroleum ether−CH_2Cl_2); mp 188−190 °C; IR (KBr) ν_{max} 2920, 2860 (C−H), 1670 (C=C), 1050 (Si−O−C), 900, 840 (Si−C) cm^{-1}; ^1H and ^{13}C NMR data in Tables 1 and 2; EIMS *m/z* 498 $[M]^+$ (30), 483 (72), 273 (9), 205 (24), 195 (22), 123 (50), 109 (62), 95 (95), 75 (96), 73 (100); HREIMS *m/z* 498.4270 (calcd for $C_{33}H_{58}OSi$, 498.4257).

Similar reaction at 40−50 °C for 3 h gave a mixture of **3** (19.6%) and 3-trimethylsiloxyfriedel-3-ene (**4**) (62.0%): mp 195−197 °C; ^1H and ^{13}C data in Tables 1 and 2; EIMS *m/z* 498 $[M]^+$ (4), 483 (45), 207 (41), 205 (8), 209 (16), 135 (12), 123 (18), 119 (21), 95 (42), 75 (100), 73 (45); HREIMS *m/z* 498.4228 (calcd for $C_{33}H_{58}OSi$, 498.4257).

Oxidation of compound **1** with fresh LDA and chlorotrimethylsilane in THF at −78 °C gave **3** (52%) and unreacted starting material. Compound **1** in CCl_4 with Et_3N and trimethylsilyl trifluoromethanesulfonate in CH_2Cl_2 at room temperature gave 84% of a 1:1 mixture of **3** and **4**.

2α-Hydroxyfriedelan-3-one (Cerin) (5). Compound **3** (37.5 mg, 0.08 mmol) was dissolved in acetone (30 mL) and cooled to −5 °C, then OsO_4 (4.0 mg, 0.016 mmol) in *tert*-butyl alcohol (0.3 mL) and NMMO (11.4 mg, 0.097 mmol) in H_2O (0.16 mL) and acetone (0.32 mL) were added dropwise. The mixture was stirred for 3 h at 0 °C and then overnight at room temperature. Sodium bisulfite (14.0 mg) and Florisil (54.0 mg) were added with stirring for 30 min, the mixture was filtered, and the solvent was evaporated under reduced pressure. The mixture was acidified to pH 2 with 1 M H_2SO_4, saturated with salt, and extracted with AcOEt (3 × 4 mL). The organic layer separated, dried, and concentrated. The white residue (33 mg) was purified by silica gel column chromatography (CH_2Cl_2−MeOH, 1%) to yield 2α-hydroxyfriedelan-3-one (**5**) (21.0 mg, 0.047 mmol, 62.7%): white powder; mp 230 °C (lit.[23] 246−250 °C); ^1H and ^{13}C NMR data in Tables 1 and 2; IR and EIMS are in agreement with literature.[23]

2,3-Secofriedelan-2-al-3-oic acid (6). 2α-Hydroxyfriedelan-3-one (**5**) (7.0 mg, 0.016 mmol) was dissolved in Et_2O (1.5 mL) and treated with periodic acid (4.5 mg, 0.019 mmol) at 0 °C with stirring for 1 h. After 3 h at room temperature, the reaction mixture was filtered and washed with Et_2O, and the filtrate concentrated to give 2,3-secofriedelan-2-al-3-oic acid (**6**) (7.3 mg, 0.015 mmol, 94%), which upon methylation with diazomethane afforded the corresponding methyl ester **6a**: mp 156−161 °C; IR (KBr) ν_{max} 2735 (C−H aldehyde), 1732 (C=O ester and aldehyde) cm^{-1}; ^1H and ^{13}C NMR data in Tables 1 and 2; EIMS *m/z* 473 (9), 472 $[M]^+$ (20), 457 (15), 441 (5), 385 (14), 273 (40), 218 (48), 205 (68), 123 (66), 95 (99), 69 (100), 88 (39); HREIMS *m/z* 472.3937 (calcd for $C_{31}H_{52}O_3$, 472.3916).

2α,3β-Dihydroxyfriedelane (7). 3-Trimethylsiloxyfriedel-2-ene (**3**) (32.5 mg, 0.065 mmol) was dissolved in CH_2Cl_2−MeOH (4:1, 10 mL) and cooled to −70 °C. It was then treated with ozone (30 L/min) for 1 h, followed by addition of sodium borohydride (5.3 mg). The solution was stirred overnight at room temperature. Excess hydride was destroyed with diluted HCl (2%, 2 mL) and the reaction mixture extracted with CH_2Cl_2 to give a crude solid (25.8 mg, 79.4%) and purified by silica gel column chromatography (CH_2Cl_2−MeOH, 1%) to give **7** (16.0 mg, 0.036 mmol, 55.4%): mp 270−273 °C (lit.[26] 291−292 °C); IR (KBr) ν_{max}3440, 2940 cm^{-1}; ^1H and ^{13}C NMR data in Tables 1 and 2; EIMS *m/z* 444 $[M]^+$ (5), 429 (10), 410 (3), 291 (12), 149 (65), 123 (32), 109 (38), 95 (48), 69 (100).

Acetylation of 7. Compound **7** (1.5 mg) was dissolved in pyridine (1 mL) and treated with Ac_2O (1 mL) overnight at room temperature. Evaporation yielded 2α,3β-diacetoxyfriedelane (**7a**) (1 mg, 56%) as a white solid. Physical and spectroscopic data are in agreement with the literature.[26]

Friedel-1-en-3-one (8). 3-Trimethylsiloxyfriedel-2-ene **3** (17.0 mg, 0.034 mmol) was dissolved in C_6H_6 (1 mL) and added dropwise to a solution of DDQ (9.0 mg, 0.04 mmol) in C_6H_6 (3 mL). After stirring overnight at room temperature, saturated aqueous $NaHCO_3$ was added (3 mL). The organic layer was separated, and the aqueous layer was washed with Et_2O (2 × 25 mL). The organic layers were evaporated under reduced pressure, and the product was purified by preparative chromatography (petroleum ether−CH_2Cl_2, 2:1) to afford **3** (1.6 mg, 9.4%) and friedel-1-en-3-one (**8**) (11.0 mg, 0.026 mmol, 76.0%): white needles (from CH_2Cl_2−Me_2CO); ^{13}C NMR (see Table 2). Physical and spectroscopic data are in agreement with the literature.[14]

3β-Hydroxyfriedelane (9). Compound **1** (24.9 mg, 0.058 mmol) was dissolved in CH_2Cl_2−MeOH (1:1 v/v, 4 mL) cooled to −78 °C and treated with sodium borohydride (25 mg, 0.66 mmol). The solution was stirred for 1 h at −78 °C and overnight at room temperature, sodium hydroxide (1 M, 15 mL) was added, and the reaction mixture was extracted with dichloromethane to give **9** (25.0 mg, 0.058 mmol, 100%) as white crystals (from CH_2Cl_2): mp 283−285 °C (lit.[1] 287−288 °C); $[\alpha]^{25}_D$ +17.26 (*c* 3.65, $CHCl_3$). Physical and spectroscopic data were compared with an authentic sample.[1,9]

3α-Hydroxyfriedelane (10). Compound **1** (100.0 mg, 0.234 mmol) was dissolved in butanol (25 mL) and treated with an excess of sodium and HMPT. The mixture was refluxed for 1.5 h, and H_2O was subsequently added. The organic layer was separated, and the aqueous layer was washed with butanol (50 mL). The organic layers were dried and concentrated to afforded **10** (100.1 mg, 0.234 mmol, 100%): mp 296−300 °C (lit.[1] 302−304 °C).

Lymphocyte Assays. The effects of compounds on the mitogenic response of human lymphocytes to PHA (10 μg/mL) were evaluated using a colorimetric MTT assay as previously described.[29] Mononuclear cells were isolated from heparinized peripheral venous blood of healthy vol-

unteers by Histopaque-1077 density centrifugation. Cyclosporin A was used as positive control. The toxicity of the compounds against the human lymphocytes was determined by an assay based on the ability of metabolically active cells to reduce the colorless tetrazolium salt MTT to a colored formazan product.[30] Briefly, in flat-bottom 96-well plates, (2−3) × 10^6 cells/mL in RPMI-1640 medium containing 10% heat-inactivated fetal bovine serum, 2 mM glutamine, and 50 μg/mL of gentamicin were exposed for 24 h to the various concentrations of each sample. Following this incubation period, the MTT solution (1 mg/mL) was added. After incubation for 4 h the MTT formazan products were solubilized with SDS/DMF solution (20% SDS in a 50% solution of DMF, pH 4.7) overnight at 37 °C. Absorbance (OD 550 nm) of the colored solution was measured with a plate reader. Lymphocytoxicity, defined in terms of the percentage of viable cells, was present when the viability of the exposed cells was less than 70% of the nonexposed control cells.

Cell Growth Inhibitory Assay. Human tumor cell lines MCF-7 (breast cancer), TK-10 (renal cancer), UACC-62 (melanoma), NCI-H460 (lung), and SF-268 (CNS) were kindly provided by the National Cancer Institute (NCI, Bethesda, MD). Cells were routinely maintained as adherent cell cultures in RPMI-1640 medium containing 5% heat-inactivated fetal bovine serum, 2 mM glutamine, and 50 μg/mL gentamicin at 37 °C in a humidified air incubator containing 5% CO_2. They were subcultured weekly, and the culture medium was changed twice a week. According to their growth profiles, the optimal plating densities of each cell line were determined to ensure exponential growth throughout the experimental period. Cells were tested for lack of mycoplasma contamination every 3−5 months. The effects of the compounds on the growth of human cancer cell lines were evaluated according to the procedure adopted in the NCI's in vitro anticancer drug screening that uses the SRB assay to assess growth inhibition.[31] Growth inhibition of 50% (GI_{50}) was calculated as described elsewhere.[31] IC50 and GI_{50} values, expressed as means ± SEM were obtained from 3−6 independent experiments performed in duplicate.

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