Hemisynthetic Secofriedelane Triterpenes with Inhibitory Activity against the Growth of Human Tumor Cell Lines in Vitro

Cristina Moiteiro,*,† César Manta,† Fátima Justino,† Regina Tavares,† Maria João M. Curto,† Madalena Pedro,§ Maria São José Nascimento,§ and Madalena Pinto‡

INETI, Instituto Nacional de Engenharia, Tecnologia e Inovação, Estrada do Paço do Lumiar, 1649-038 Lisboa, Portugal, Centro de Estudos de Química Orgânica, Fitoquímica e Farmacologia da Universidade do Porto, Laboratório de Microbiologia, Faculdade de Farmácia da Universidade do Porto, Rua Aníbal Cunha 164, 4050-047 Porto, Portugal, and Centro de Estudos de Química Orgânica, Fitoquímica e Farmacologia da Universidade do Porto, Laboratório de Química Orgânica, Faculdade de Farmácia da Universidade do Porto, Rua Aníbal Cunha 164, 4050-047 Porto, Portugal

Received March 20, 2004

Seco acids **7** and **9** and hydroxylated analogues **5** and **6** derived from friedelane triterpenes were synthesized stereoselectively in high yields. Compounds **5**−**9** were evaluated for their ability to inhibit in vitro the growth of three human tumor cell lines, MCF-7 (breast adenocarcinoma), NCI-H460 (non-small cell lung cancer), and SF-268 (CNS cancer). Only compounds **7** and **9** were found to possess significant growth inhibitory effects, exhibiting GI_{50} values that range from 24.6 to 32.8 μM and 10.9 to 17.6 μM, respectively.

Review articles have reported the occurrence of a large number of structurally diverse triterpenes with physiological functions associated with chemical protection of plants.[1] In particular, triterpenes that are highly oxidized at the A ring, such as seco acids, have been reported to possess a wide spectrum of biological activities.[1]

In a previous paper[2] we reported the synthesis of a secofriedelane derivative that inhibited the proliferation of human lymphocytes and the growth of several human tumor cell lines in vitro, and two hydroxylated friedelane derivatives that showed only moderate antiproliferative properties. On the basis of these previous results several new seco acids and hydroxylated derivatives prepared from friedelin (**1**) and from 3-hydroxyfriedel-3-en-2-one (**2**) were obtained in an attempt to find more effective potential antitumor agents. Compounds **1** and **2** were easily available from cork smoker wash solids, a residue obtained from the processing of cork, an important industrial activity in Portugal.[3]

Synthesis of 3,4-seco acid from controlled silylation of friedelin (**1**) via silyl enol ether (**3**) is summarized in Scheme 1. This new synthetic route, which involves stereoselective silylation of the carbonyl functionality of friedelin (**1**), achieved by reaction with trimethylsilyl iodide (TMIS) and hexamethyldisilazane (HMDS) under thermodynamic control, yielded 3-trimethylsiloxyfriedel-3-ene (**3**) in 84% yield. Cleavage of the A-ring of **3** with *m*-CPBA followed by oxidation with H_5IO_6 afforded the 3,4-seco acid **7** in high yield.[2] The nor-seco acid (**9**) was obtained by oxidation of 3-hydroxyfriedel-3-en-2-one (**2**) with potassium permanganate and phase-transfer catalyst in 90% yield, as previously reported.[4]

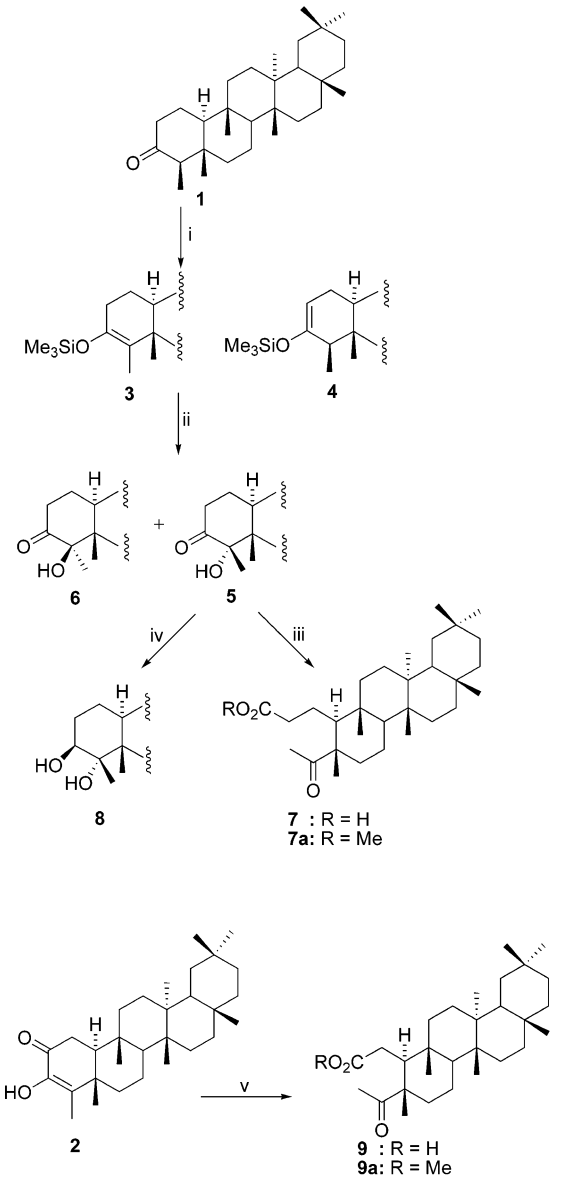
All of the seco compounds **7**, **7a**, **9**, and **9a** and hydroxylated intermediates **5** and **6** synthesized were evaluated for their capacity to inhibit in vitro growth of three human tumor cell lines, MCF-7 (breast adenocarcinoma), NCI-H460 (non-small cell lung cancer), and SF-268 (CNS cancer).

* Corresponding author. Tel: 351 210924751. Fax: 351 217168100. E-mail: cristina.moiteiro@ineti.pt.
† INETI, Instituto Nacional de Engenharia, Tecnologia e Inovação.
§ Laboratório de Microbiologia, Faculdade de Farmácia da Universidade do Porto.
‡ Laboratório de Química Orgânica, Faculdade de Farmácia da Universidade do Porto.

In our semisynthetic approach, the triterpenes friedelin (**1**) and 3-hydroxyfriedel-3-en-2-one (**2**) serve as precursors for the synthesis of highly oxygenated friedelane type seco acids (Scheme 1). Regiospecific silylation of **1** was achieved by the use of a highly electrophilic silylating agent, TMIS, in combination with HMDS[5] in nonpolar solvent at 25 °C. In this reaction we observed the consumption of the ketone **1** and the formation of a mixture of silyl enol ethers **3** and **4**, after 2 h. Increasing the reaction time to 48 h allowed conversion of the silyl enol ether **4** into the thermodynamically more stable **3**. All the spectroscopic data of **3** were identical to those described in the literature.[2] Oxidation of **3** with *m*-chloroperbenzoic acid under buffered pH conditions produced a mixture of 4α-hydroxyfriedelan-3-one (**5**) and 4*β*-hydroxyfriedelan-3-one (**6**), with predominance of **5**. Both **5** and **6** had similar IR spectra, showing the presence of hydroxyl (3448 cm^{-1}) and carbonyl (1703 cm^{-1}) groups. The EIMS of these compounds were also very similar, exhibiting molecular ions $[M]^+$ at *m*/*z* 442, in agreement with the molecular formula $C_{30}H_{50}O_2$, as well as ions at *m*/*z* 424 due to loss of one water molecule, indicating the presence of a hydroxyl group in ring A. Comparison of ^{1}H NMR spectra of **5** and **6** indicated that the main differences were in the signals for H-1, H-2, H-10, and Me-23. The configuration of the 4-hydroxy groups was derived from the shape of the signals and the coupling constants in the ^{1}H NMR spectrum. The ^{1}H NMR spectrum of **5** showed a signal at δ 2.98 assigned to the H-2ax proton that was greatly influenced by the axial 4-OH group. A proton signal at δ 2.22 (J = 13.5 Hz, J = 4.8 Hz, and J = 2.0 Hz) was in agreement with H-2eq. The signal at δ 2.11 was assigned to H-10 (J = 12.6 Hz, J = 3.3 Hz). Eight singlets corresponding to the methyl groups were observed, being Me-23 at δ 1.16. In the ^{1}H NMR spectrum of **6** the Me-23 singlet was observed at δ 1.39. A singlet at δ 3.82 (exchangeable with D_2O) was assigned to the hydroxyl group, and a two-proton multiplet at δ 2.50 was assigned to H-2. The ^{13}C NMR spectrum of **5** displayed signals at δ 213.1 and 81.0 assigned to C-3 and C-4, respectively. The HMBC and HMQC spectra of **5** and **6** allowed assignment of all the proton and carbon signals. The configuration of the C-4 OH group was confirmed by stereoselective reduc-

10.1021/np0498915 CCC: $27.50 © 2004 American Chemical Society and American Society of Pharmacognosy
Published on Web 07/09/2004

Scheme 1



tion of **6** with sodium borohydride, which gave 3β,4α-dihydroxyfriedelane (**8**).[6]

Oxidation of **5** with periodic acid at 0 °C afforded 3,4-secofriedelan-4-oxo-3-oic acid (**7**) in 84% yield, which upon methylation with diazomethane gave the corresponding methyl ester **7a**. This seco acid **7** was previously prepared by oxidative ozonolysis of friedel-3-en-3-yl benzoate,[7] which was obtained by benzoylation of friedelin. However, the benzoylation of friedelin was not regioselective, leading to a mixture of friedel-3-en-3-yl benzoate and friedel-2-en-3-yl benzoate. The IR, EIMS, and ^{13}C NMR data of **7** and **7a** were identical with data reported in the literature;[7,8] however, the ^{1}H NMR data were not described.

3-Nor-2,4-secofriedelan-4-oxo-2-oic acid (**9**) was obtained in high yield by oxidation of **2** with $KMnO_4$ under phase-transfer conditions (benzyltriethylammonium chloride),[4] which upon methylation with diazomethane afforded the corresponding methyl ester **9a** (Scheme 1). The structures of compounds **9** and **9a** were elucidated by a combination of ^{1}H, ^{13}C, DEPT, and 2D NMR (HETCOR) techniques. The

Table 1. Effect of Triterpenoids **5**−**7**, **7a**, **9**, and **9a** on the Growth of Human Tumor Cell Lines (concentration causing 50% cell growth inhibition)[a]

	GI_{50} (μM)		
compound	MCF-7 (breast)	NCI-H460 (lung)	SF-268 (CNS)
5	>110	>110	>110
6	>50	>50	>50
7	26.9 ± 1.5	32.8 ± 2.9	24.6 ± 1.4
7a	>110	>110	>110
9	10.9 ± 0.5	17.6 ± 0.2	12.9 ± 0.7
9a	101.2 ± 4.5	93.9 ± 5.4	105.7 ± 3.5

[a] Cells were exposed for 48 h to five concentrations of compounds starting from a maximum concentration of 110 μM. Compound **6** was tested at 50 μM because of its solubility. Doxorubicin, GI_{50} MCF-7= 42.8 ± 8.2 nM; GI_{50} NCI-H460 = 94.0 ± 8.7 nM; GI_{50} SF-268= 94.0 ± 7.0 nM; results are the mean ± SEM of 3−6 independent experiments performed in duplicate.

^{13}C NMR spectra of **8** and **8a** are reported for the first time (Experimental Section).

The ability of the synthetic triterpenoids **5**−**9**, **7a**, and **9a** to inhibit the in vitro growth of MCF-7, NCI-H460, and SF-268 cell lines is summarized in Table 1. As reported in our previous paper[2] the 2,3-secofriedelan-2-al-3-oic acid obtained from friedelin (**1**) was inhibitory against human lymphocyte proliferation (IC_{50} = 10.7 μM) and growth of human tumor cell lines (IC_{50} = 5.4−17.2 μM). These results demonstrated that fission of the friedelin A-ring could provide compounds having inhibitory activity against various cell lines. Although the number of derivatives tested is not sufficient to draw structure−activity relationships, some structural features important to the growth inhibitory effect can be inferred. Compound **9** was most active against the three cell lines. It was followed in potency by compound **7**. Methylation of **7** and **9** caused a drastic drop in potency. Seco acids with the same functional groups and a decrease of one methylene unit (**9** in relation to **7**) led to a 2-fold increase in inhibitory activity.

In conclusion, we present an efficient method for the preparation of secofriedelanes, some of which exhibit inhibitory activity against several human tumor cell lines in vitro.

Experimental Section

General Experimental Procedures. Melting points were determined on a Reichert Thermovar apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer 298 spectrophotometer. ^{1}H, ^{13}C, and 2D NMR (300 MHz) spectra (all in $CDCl_3$) were obtained on a Brüker-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. Elemental analyses (C, H, O) were performed on a Thermo Finnigan-CE Instruments Model Flash EA 1112 CHNS series by the CQFB FCT-UNL Analyses Laboratory. Analytical 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.

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

Extraction and Isolation. Friedelin (**1**) and 3-hydroxy-friedel-3-en-2-one (**2**) were extracted from powdered cork smoker wash solids.[3]

Silylation of 1. Compound **1** (105.9 mg, 0.249 mmol) was dissolved in pentane (4 mL) and was added, under nitrogen, to a mixture of TMIS (0.1 mL, 0.52 mmol) and HMDS (0.4 mL, 1.90 mmol). The mixture was stirred at 25 °C for 48 h,

and pentane was subsequently added. Evaporation of the solvent under reduced pressure afforded 3-trimethylsiloxy-friedel-3-ene (**3**) (104.3 mg, 0.209 mmol, 84%): white crystals; mp 195−197 °C. Physical and spectroscopic data of compound **3** are in agreement with literature.[2]

4α-Hydroxyfriedelan-3-one (5). Compound **3** (100.0 mg, 0.20 mmol) was dissolved in CH_2Cl_2 (10 mL), and then *m*-CPBA (84 mg, 0.0.37 mmol) and $NaHCO_3$ (0.5 M, 5.5 mL) were added. The mixture was stirred for 2 h at room temperature. The organic layer was separated, washed with a saturated solution of sodium sulfite, a solution of $NaHCO_3$, and with distilled H_2O, and then concentrated and dried (80 mg, 90.9%). The product was purified by column chromatography (petroleum ether−CH_2Cl_2, 2:1) to afford 4α-hydroxyfriedelan-3-one (**5**) (40 mg, 45%) and 4*β*-hydroxyfriedelan-3-one (**6**) (12 mg, 13.5%).

4α-Hydroxyfriedelan-3-one (5): white crystals (from $CHCl_3$); mp 268−271 °C (lit.[7] 269−271 °C); IR (KBr) ν_{max} 3448 (OH), 2927, 2867 (CH_2, CH_3, st), 1703 (C=O), 1458, 1384 (CH_2, CH_3), 1107, 1071 (C−OH) cm^{-1}; ^1H NMR δ ($CDCl_3$) 0.81 (3H, s, Me-25), 0.88 (3H, s, Me-24), 0.95 (3H, s, Me-30), 1.00 (6H, s, Me-26 and Me-29), 1.06 (3H, s, Me-27), 1.16 (3H, s, Me-23), 1.18 (3H, s, Me-28), 1.80 (2H, m, H-2), 2.11 (1H, dd, $J_{10ax1eq} = 3.3$ Hz, $J_{10ax1ax} = 12.6$ Hz, H-10), 2.22 (1H, ddd, $J_{2eq1eq} = 2.0$ Hz, $J_{2eq1ax} = 4.8$ Hz, $J_{gem} = 13.5$ Hz, H-2), 2.98 (1H, ddd, $J_{2ax1eq} = 7.5$ Hz, $J_{2ax1ax} = 13.5$ Hz, $J_{gem} = 13.5$ Hz, H-2); ^{13}C NMR δ ($CDCl_3$) 29.7 (C-1), 39.3 (C-2), 213.1 (C-3), 81.0 (C-4), 44.6 (C-5), 37.1 (C-6), 21.7 (C-7), 49.3 (C-8), 37.1 (C-9), 52.4 (C-10), 35.3 (C-11), 30.5 (C-12), 39.7 (C-13), 38.2 (C-14), 32.4 (C-15), 35.8 (C-16), 29.9 (C-17), 42.8 (C-18), 33.4 (C-19), 28.2 (C-20), 32.8 (C-21), 36.0 (C-22), 16.7 (C-23), 16.9 (C-24), 18.0 (C-25), 20.3 (C-26), 18.8 (C-27), 32.1 (C-28), 31.8 (C-29), 35.1 (C-30); EIMS *m/z* 442 $[M]^+$ (17), 427 (2), 412 (7), 341 (6), 289 (2), 221 (3), 218 (17), 205 (51), 123 (56), 95 (100), 55 (80); *anal.* calcd for $C_{30}H_{50}O_2$, C 81.39%, H 11.38%, found C 81.42%, H 11.63%.

4*β*-Hydroxyfriedelan-3-one (6): white crystals (from ethyl acetate); mp 218−220 °C (lit.[7] 234.5−236.0 °C); ^1H NMR δ ($CDCl_3$) 0.83 (3H, s, Me-25), 0.88 (3H, s, Me-24), 0.95 (3H, s, Me-30), 1.00 (3H, s, Me-26), 1.01 (3H, s, Me-29), 1.06 (3H, s, Me-27), 1.18 (3H, s, Me-28), 1.39 (3H, s, Me-23), 1.55 (2H, m, H-1), 1.70 (1H, m, H-10), 2.50 (2H, m, H-2), 3.82 (1H, s, OH); ^{13}C NMR δ ($CDCl_3$) 17.6 (C-1), 39.3 (C-2), 213.0 (C-3), 81.3 (C-4), 45.3 (C-5), 36.0 (C-6), 21.9 (C-7), 53.2 (C-8), 37.6 (C-9), 57.8 (C-10), 35.9 (C-11), 30.5 (C-12), 39.7 (C-13), 38.3 (C-14), 32.5 (C-15), 35.4 (C-16), 30.0 (C-17), 42.8 (C-18), 33.3 (C-19), 28.2 (C-20), 32.8 (C-21), 35.9 (C-22), 21.9 (C-23), 21.8 (C-24), 17.9 (C-25), 20.4 (C-26), 18.8 (C-27), 32.1 (C-28), 31.8 (C-29), 35.1 (C-30); EIMS *m/z* 442 $[M]^+$ (28), 427 (2), 412 (3), 341 (4), 289 (2), 221 (2), 218 (15), 205 (57), 123 (53), 95 (100), 55 (85); *anal.* calcd for $C_{30}H_{50}O_2$, C 81.39%, H 11.38%, found C 81.49%, H 11.60%.

3*β*,4α-Dihydroxyfriedelane (8). 4α-Hydroxifriedel-3-one (**5**) (16.8 mg, 0.038 mmol) was dissolved in CH_2Cl_2−MeOH (1:1, 4 mL) and cooled to −70 °C, and $NaBH_4$ (16.5 mg, 0.44 mmol) was added. The solution was stirred for 1 h at −70 °C and for 3 additional hours at room temperature. Sodium hydroxide (1 M, 10 mL) was added, and the reaction mixture was extracted with CH_2Cl_2 to give **8** (6.9 mg, 0.016 mmol, 41%), mp 240−241 °C; all the spectroscopic data were identical with the literature.[6]

3,4-Secofriedelan-4-oxo-3-oic acid (7). 4α-Hydroxyfriedelan-3-one (**5**) (54.8 mg, 0.124 mmol) was dissolved in Et_2O (11 mL) and treated with periodic acid (37 mg, 0.16 mmol) at 0 °C with stirring for 1 h. After 5 h of further stirring at room temperature, the reaction mixture was filtered and washed with Et_2O and the filtrate concentrated to give 3,4-secofriedelan-4-oxo-3-oic acid (**7**) (47.7 mg, 0.104 mmol, 84%): mp 141−144 °C (lit.[7] 205−207 °C); ^1H NMR δ 0.92 (3H, s, Me-25), 0.95 (3H, s, Me-30), 1.00 (6H, s, Me-26 and Me-29), 1.03 (3H, s, Me-27), 1.17 (3H, s, Me-28), 1.21 (3H, s, Me-24), 1.65 (1H, m, H-10), 2.15 (3H, s, Me-23), 2.17 (1H, s, COOH), 2.39 (2H, m, H-2); IR, ^{13}C NMR, and EIMS are in agreement with literature.[7,8]

Compound **7**, upon methylation with diazomethane, afforded the corresponding methyl 3,4-secofriedelan-4-oxo-3-oate (**7a**): white crystals from MeOH; mp 152−155 °C (lit.[7] 157−158 °C); ^1H NMR δ 0.91 (3H, s, Me-25); 0.95 (3H, s, Me-30); 1.00 (6H, s, Me-26 and 29); 1.03 (3H, s, Me-27); 1,17 (3H, s, Me-28); 1.21 (3H, s, Me-24); 2.15 (3H, s, Me-23), 2.34 (2H, m, H-2), 3.63 (3H, s, O-CH_3).

3-Nor-2,4-secofriedelan-4-oxo-2-oic acid (9). 3-Hydroxyfriedel-3-en-2-one (**2**) (21.6 mg, 0.049 mmol) was dissolved in CH_2Cl_2 (1 mL), then $KMnO_4$ (15.5 mg, 0.049 mmol) and benzyltriethylammonium chloride (22.5 mg, 0.049 mmol) in CH_2Cl_2 were added. The mixture was stirred 24 h at room temperature and then refluxed for 1 h. Diluted HCl (10%) was added until the manganese oxide was destroyed. Sodium sulfite (10%, 10 mL) was added, and the organic layer was separated and dried with Na_2SO_4 to give 3-nor-2,4-secofriedelan-4-oxo-2-oic acid (**9**) (20.4 mg, 0.046 mmol, 90%): as crystals (from EtOH); mp 213−214 °C (lit.[7] 215−217 °C); IR (KBr) ν_{max} 2937, 2867, 1733 (C=O ester), 1715 (C=O) cm^{-1}; ^1H NMR δ ($CDCl_3$) 0.90 (3H, s, Me-25), 0.94 (3H, s, Me-30), 0.99 (3H, s, Me-26), 1.01 (3H, s, Me-27), 1.05 (3H, s, Me-29), 1.13 (3H, s, Me-24), 1.18 (3H, s, Me-28), 1.90 (1H, dd, $J_{1eq10ax} = 4.0$ Hz, $J_{gem} = 15.6$ Hz, H-1), 2.20 (3H, s, Me-23), 2.30 (1H, dd, $J_{10ax1eq} = 4.0$ Hz, $J_{10ax1ax} = 6.6$ Hz, H-10), 2.32 (1H, s, COOH), 2.35 (1H, dd, $J_{1ax10ax} = 6.6$ Hz, $J_{gem} = 15.6$ Hz, H-1); ^{13}C NMR δ ($CDCl_3$) 32.9 (C-1), 178.2 (C-2), 233.9 (C-4), 53.4 (C-5), 37.5 (C-6), 17.5 (C-7), 52.5 (C-8), 38.2 (C-9), 49.8 (C-10), 34.4 (C-11), 29.9 (C-12), 39.6 (C-13), 38.3 (C-14), 32.3 (C-15), 35.9 (C-16), 29.9 (C-17), 42.8 (C-18), 35.3 (C-19), 28.1 (C-20), 32.8 (C-21), 39.2 (C-22), 25.3 (C-23), 17.6 (C-24), 17.8 (C-25), 20.2 (C-26), 18.7 (C-27), 32.1 (C-28), 31.8 (C-29), 34.9 (C-30); EIMS *m/z* 444 $[M]^+$ (3), 426 (2), 401 (34), 342 (2), 273 (3), 205 (43), 191 (10), 149 (14), 137 (22), 123 (35), 121 (30), 109 (47), 95 (4), 81 (6), 69 (67), 55 (53), 43 (100); *anal.* calcd for $C_{29}H_{48}O_3$, C 78.33%, H 10.88%, found C 78.18%, H 11.27%.

Compound **9**, upon methylation with diazomethane, afforded methyl 3-nor-2,4-secofriedelan-4-oxo-2 oate (**9a**): white powder from EtOH; mp 189−192 °C (lit.[7] 166−167 °C); IR (KBr) ν_{max} 3449 (OH), 2944, 2868, 1715 (C=O, ketone) cm^{-1}, 1701 (C=O carboxylic acid); ^1H NMR δ ($CDCl_3$) 0.89 (3H, s, Me-25), 0.94 (3H, s, Me-30), 0.99 (3H, s, Me-26), 1.04 (3H, s, Me-27), 1.06 (3H, s, Me-29), 1.10 (3H, s, Me-24), 1.18 (3H, s, Me-28), 1.90 (1H, dd, $J_{gem} = 16.5$, $J_{1eq10ax} = 4.0$ Hz, H-1), 2.20 (3H, s, Me-23), 2.30 (1H, dd, $J_{gem} = 2.1$, $J_{1ax10ax} = 6.0$ Hz, H-1), 2.40 (1H, dd, $J_{10ax1ax} = 6.0$; $J_{10ax1eq} = 4.0$ Hz, H-10); 3.63 (3H, s, OMe); ^{13}C NMR δ 32.7 (C-1), 174.6 (C-2), 214.4 (C-4), 53.6 (C-5), 37.4 (C-6), 17.5 (C-7), 52.6 (C-8), 38.1 (C-9), 49.8 (C-10), 34.3 (C-11), 30.1 (C-12), 39.7 (C-13), 38.3 (C-14), 32.3 (C-15), 35.9 (C-16), 29.9 (C-17), 42.8 (C-18), 35.3 (C-19), 28.2 (C-20), 32.8 (C-21), 39.2 (C-22), 25.3 (C-23), 17.6 (C-24), 17.9 (C-25), 20.2 (C-26), 18.8 (C-27), 32.1 (C-28), 31.8 (C-29), 35.0 (C-30); 51.7 (OCH_3); EIMS *m/z* 458 $[M]^+$ (4), 427 (3), 415 (44), 401 (3), 287 (3), 273 (6), 205 (56), 191 (12), 149 (22), 137 (25), 123 (40), 121 (32), 109 (51), 95 (65), 81 (43), 69 (54), 59 (100), 55 (47), 43 (70); *anal.* calcd for $C_{30}H_{50}O_3$, C 78.55%, H 10.99%, found C 78.45%, H 10.78%.

Tumor Cell Growth Assay. Compounds, prepared in DMSO, were freshly diluted with cell culture medium just prior to the assays. Final concentrations of DMSO (0.25%) did not interfere with the cell growth. The effects of compounds on the growth of tumor cell lines MCF-7 (breast adenocarcinoma), NCI-H460 (non-small cell lung cancer), and SF-268 (CNS cancer) were evaluated according to the procedure adopted by the National Cancer Institute (NCI) for the in vitro anticancer drug screening that uses the protein-binding dye sulforhodamine B (SRB) to assess growth inhibition. The methodology used was the same as originally described in the literature.[9,10] Doxorubicin was used as positive control.

Acknowledgment. This work was supported by INETI, FCT (I&D N°226/94; Ph.D. grant SFRH/BD/1456/2000), POCTI (QCA III), and FEDER. We would like to thank the National Cancer Institute, Bethesda, MD, for kindly providing the tumor cell lines. The authors are grateful to P. Puapairoj and N. Nazareth for their help with the tumor cell growth assays.

References and Notes

(1) Bass, W. J. *Phytochemistry* **1985**, *24*, 1875−1889.
(2) Moiteiro, C.; Justino, F.; Tavares, R.; Marcelo Curto, M. J.; Florêncio, M. H.; Nascimento, M. S. J.; Pedro, M.; Cerqueira, F.; Pinto, M. M. M. *J. Nat. Prod.* **2001**, *64*, 1273−1277.
(3) Moiteiro, C.; Mata, M. L.; Tavares, R.; Curto, M. M. J. Presented at the 7th European Conference on Biomass for Energy Industry and Environment Agriculture and Industry; Florence, Italy, October 5−9, P.14.112, 1992.
(4) Moiteiro, C.; Justino, F.; Feio, S.; Tavares, R.; Marcelo Curto, M. J. In *Natural Products in The New Millennium: Prospects and Industrial Application*; Rauter, A., Araújo, E., Sales, F., Justino, J., Santos, S. P., Eds.; Proceedings of the Phytochemical Society of Europe; Kluwer Academic Publishers: Dordrecht, 2002; Vol. 47, pp 421−427.
(5) Miller, R. D.; McKean, D. R. *Synthesis* **1979**, 730−732.
(6) Pradhan, B. P.; Roy, A.; Sinha, R. P. *Indian J. Chem.* **1993**, *32B*, 1070−1072.
(7) Nishihama, T.; Takahashi, T. *Bull. Chem. Soc. Jpn.* **1987**, *60*, 2117−2125.
(8) Patra, A.; Chaudhuri, S. K.; Acharyya, A. K. *Magn. Reson. Chem.* **1990**, *28*, 85−92.
(9) Skehan, P.; Storeng, R.; Scudiero, D.; Monks, A.; McMahon, J.; Vistica, D.; Warren, J. T.; Bokesch, H.; Kenney, S.; Boyd, M. R. *J. Natl. Cancer Inst.* **1990**, *82*, 1107−1112.
(10) Monks, A.; Scudiero, D.; Skehan, P.; Shoemaker, R.; Paull, K.; Vistica, D.; Hose, C.; Langley, J.; Cronise, P.; Vaigro-Wolff, A.; Gray-Goodrich, M.; Campbell, H.; Mayo, J.; Boyd, M. *J. Natl. Cancer Inst.* **1991**, *83*, 757−76.

NP0498915