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(8*R*)-3β,8-Dihydroxypolypoda-13*E*,17*E*,21-triene Induces Cell Cycle Arrest and Apoptosis in Treatment-Resistant Prostate Cancer Cells

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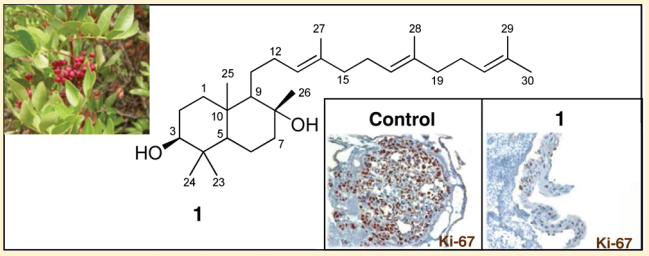
Supporting Information

ABSTRACT: Mastic, a resinous exudate from *Pistacia lentiscus*, has been reported to exhibit selective cytotoxicity against different cancer cell lines. There are, however, no data published correlating distinct mastic-derived compounds with the postulated cytotoxic activity. A polypodane-type bicyclic triterpenoid, (8*R*)-3β,8-dihydroxypolypoda-13*E*,17*E*,21-triene (**1**), was isolated from *P. lentiscus* oleogum resin. In androgen-independent PC-3 prostate cancer cells, **1** potently inhibited the expression of cyclins D1 and E, but had no effect on the expression of the cyclin kinase inhibitor $p21^{Waf1/Cip1}$. Inhibition of the expression of cell cycle-regulating cyclins resulted in cell cycle arrest in the G_o/G_1 phase, reduction in the number of cells in the S phase, and the triggering of apoptosis, as detected by increased expression of phosphatidylserine on the cell surface and by formation of DNA laddering. In addition, **1** suppressed the formation of prostate cancer colonies in soft agar and inhibited proliferation, angiogenesis, and the growth of prostate tumors xenografted onto chick chorioallantoic membranes without overt systemic toxicity. Taken together, these data show that **1** triggers apoptosis in chemoresistant, androgen-independent human prostate cancer cells in vitro and in vivo. Thus, **1** may serve as a lead compound for targeting so far incurable androgen-insensitive prostate cancers.



Secondary metabolites of plants have been used traditionally for the treatment of cancer. Even today, plant-derived natural products and their derivatives and synthetic mimics still make up a considerable portion of the currently available anticancer drugs.[1]

Prostate cancer is a very commonly diagnosed cancer in men.[2] Radical prostatectomy, androgen-deprivation, and radiation therapy are common treatment options for patients with localized disease. However, approximately 35% of such patients will experience disease recurrence within a 10-year period. For patients who no longer respond to androgen-deprivation, a docetaxel-based therapy is currently the only possibility of improving survival time. However, the typical extension of the median survival time with docetaxel chemotherapy of only two or three months may be regarded as very modest.[3] Therefore, novel compounds preferably targeting tumor-specific signaling pathways in relation to prostate cancer are urgently required.

Mastic is a resinous exudate from the stems and main leaves of *Pistacia lentiscus* L. (Anacardiaceae). *P. lentiscus* oleogum has been used for the treatment of peptic ulcers and was later shown to possess antibacterial efficacy against *Helicobacter pylori*.[4,5] In addition, *P. lentiscus* oleogum extracts exhibited selective cytotoxic activity against various cancer cell lines, especially leukemia and colon cancer,[6,7] and also demonstrated antineoplastic activity by inhibiting the growth of human colorectal tumor xenografts in immunodeficient mice.[8] In hormone-dependent prostate cancer, *P. lentiscus* oleogum extract inhibited the expression and function of androgen receptors.[9] However, there are, so far, no data to correlate the purified constituents of *P. lentiscus* oleogum resin with its postulated cytotoxic or antitumor activity.

The composition of *P. lentiscus* oleogum resin has not been investigated extensively. However, *P. lentiscus* was the first plant shown to produce bi-, tri-, tetra-, and pentacyclic isoprenoid skeletons from epoxysqualene.[10] Hence, its oleogum resin contains mainly triterpenoids.[11] The bicyclic 2,6-naphthalenediol, (8*R*)-3β,8-dihydroxypolypoda-13*E*,17*E*,21-triene (**1**), was isolated initially from *P. lentiscus* oleogum resin.[10,12] Later, **1** was also isolated from Guggulu, the gum resin from *Commiphora mukul*.[13,14] Guggulu is used widely in traditional Ayurvedic medicine to treat inflammation, obesity, and lipid disorders. Therefore, its constituents have been analyzed for anti-inflammatory properties. Compared to other terpenoids, **1** possesses rather weak inhibitory activities for the cyclooxygenase enzymes COX-1 and COX-2 and on lipid peroxidation.[14] Interestingly,

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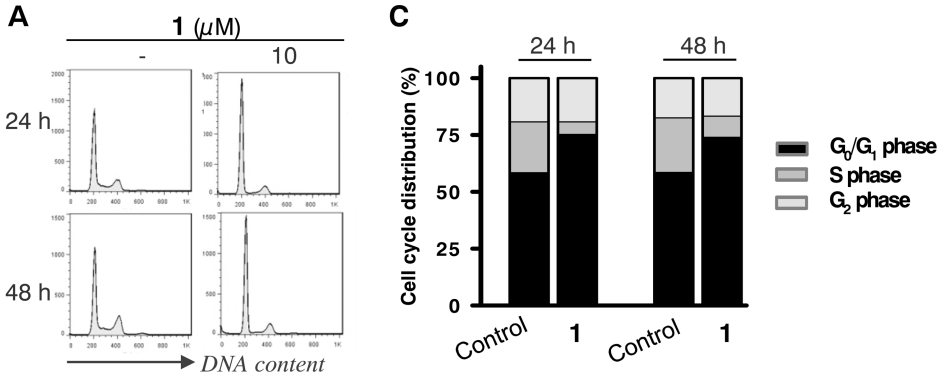
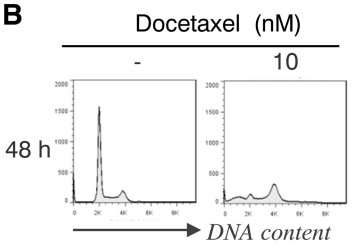




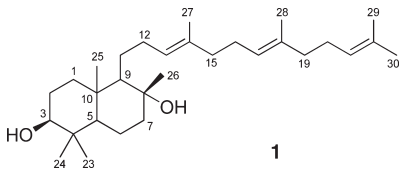
D

	Control		1	
	24 h	48 h	24 h	48 h
G_0/G_1	58.7 ± 1.4	58.8 ± 2.2	75.4 ± 0.8**	74.2 ± 1.2**
S	22.4 ± 1.3	23.9 ± 1.1	5.6 ± 0.3**	9.3 ± 0.9**
G_2/M	18.9 ± 0.3	17.3 ± 1.2	18.9 ± 0.3	17.3 ± 1.2

Figure 1. Treatment of prostate cancer cells with **1** induces G_0/G_1 cell cycle arrest. PC-3 cells were treated with 10 μM **1** (A) or docetaxel as a positive control (B), stained with propidium iodide, and analyzed by flow cytometry. Representative histograms are shown. The distribution of cells in the different phases of the cell cycle was analyzed using ModFit software and is presented as a graph (C) and table (D). Data are means ± SEM from three experiments, Newman-Keuls test, $^{**}p < 0.01$.

the structurally related myhrranol A exhibited anti-inflammatory properties and was shown to inhibit NO production in a LPS-stimulated murine macrophage cell line.[15] It also reduced various parameters of inflammation in adjuvant-induced murine air-pouch granulomas such as the exudative pouch fluid, angiogenesis, and granuloma weight.[16] The putative cytotoxic and antineoplastic properties of **1** have not been addressed so far.

The purpose of this study was to demonstrate the potential antitumor activity of the bicyclic triterpenoid **1** isolated from *P. lentiscus* oleogum resin on the treatment-resistant PC-3 prostate cancer cell line.[17] This study shows that **1** inhibits proliferation and induces apoptosis of chemoresistant androgen-independent prostate cancer cells in vitro, as well as in vivo in tumor xenografts grown on chicken chorioallantoic membranes (CAM).



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RESULTS AND DISCUSSION

It has been reported previously that *P. lentiscus* oleogum resin dissolved in DMSO inhibits the expression and functionality of the androgen receptor in the LNCaP prostate cancer cell line, which requires androgen for proliferation.[9] The growth of hormone-dependent tumors, such as LNCaP cells, can be inhibited by androgen ablation. However, advanced tumors may not express androgen receptors growing hormone-independently. Importantly, they exhibit resistance to radiation and chemotherapy,[2] which is inevitably linked to an unfavorable prognosis. For the current study, the PC-3 human prostate adenocarcinoma cell line was used, since this exhibits hormone- and anchorage-independent growth,[18] does not express androgen receptors, and displays chemoresistance.[17]

The bicyclic triterpenoid **1** was isolated in this study from the oleogum resin of *P. lentiscus*. Although **1** has been isolated previously[10,12] and its ^{1}H NMR spectrum at 400 MHz was reported,[12] not all protons have been assigned. Previous authors used the conversion of **1** to a known compound, (+)-ambreinolide, to establish the absolute configuration of **1**.[12] The complete assignments of ^{1}H and ^{13}C NMR spectroscopic data of **1** are reported here for the first time (Experimental Section).

PC-3 prostate cancer cells were treated with **1** for 24 and 48 h, and the amounts of cells in different phases of the cell cycle were analyzed. Compound **1** induced accumulation of the cells in the G_0/G_1 phase, whereas the amount of cells in the S phase was significantly reduced (Figure 1A,C,D). In contrast to **1**, the reference substance docetaxel (Taxotere), an antimitotic agent used to treat hormone-refractory prostate cancer, induced cell accumulation in the G_2/M phase (Figure 1B).

The mammalian cell cycle is controlled by cyclin-dependent kinases, which are regulated by activators (cyclins) and inhibitors (cyclin kinase inhibitors). The D-type cyclins are important during the G_1 phase of the cell cycle, in which cells initiate DNA synthesis. Activation of cyclin D/cyclin-dependent kinase complexes leads to expression of E-type cyclins essential to drive the G_1/S transition.[19] The activity of cell-cycle cyclin-dependent kinases is deregulated in cancer cells. Cyclin D1 was shown to be overexpressed in prostate adenocarcinoma samples and metastatic lymph node specimens from patients with prostate cancer.[20,21] This increases cell proliferation, migration, and invasion.[22] Accordingly, overexpression of cyclin D1 is associated with metastatic prostate cancer to the bones.[23] PC-3 cells express

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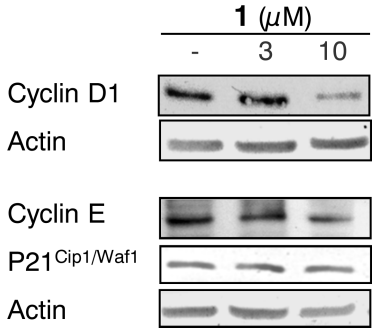

Figure 2. Compound **1** induces downregulation of proteins involved in cell proliferation. Expressions of cyclin D1 and cyclin E and cyclin kinase inhibitor $p21^{Waf1/Cip1}$ were analyzed in PC-3 cells treated with **1** for 24 (cyclin D1) and 48 h (cyclin E and $p21^{Waf1/Cip1}$). Actin, loading control; results are representative out of three.

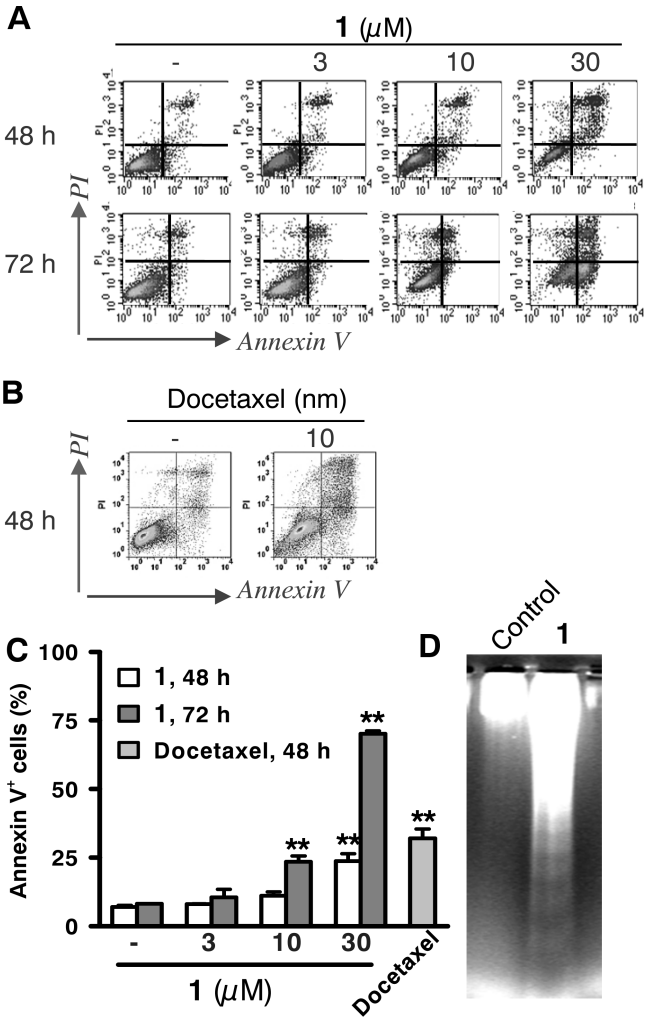

Figure 3. Compound **1** induces apoptosis in prostate cancer cells. PC-3 cells were treated with **1** (A) or docetaxel (B), stained with annexin V/propidium iodide, and analyzed by flow cytometry. Representative dot plots are shown. (C) PC-3 cells were treated with either **1** or docetaxel (10 nM), and the amounts of apoptotic annexin V^+ cells were analyzed using CellQuest software and are presented as means ± SEM of three experiments; Newman-Keuls test, $^{**}p < 0.01$. (D) Characteristic DNA fragmentation in PC-3 cells treated with **1** (10 μM) for 72 h.

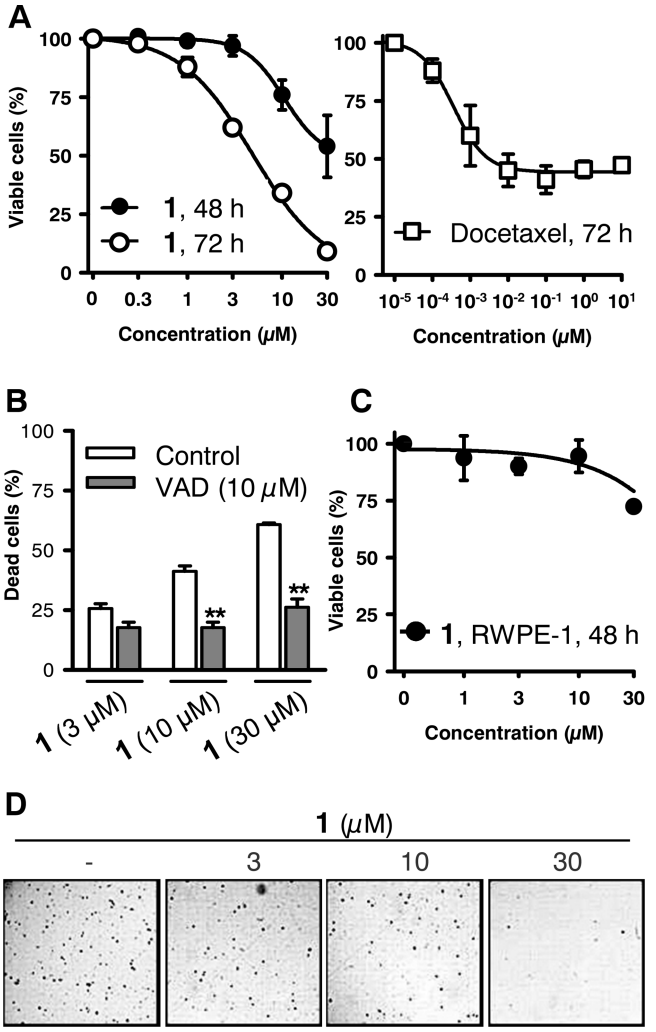

Figure 4. Compound **1** inhibits proliferation of human prostate cancer cells in vitro. (A) PC-3 cells were treated with **1** or docetaxel, and cell viability was analyzed using the XTT assay. (B) The pan-caspase inhibitor Z-VAD-FMK inhibits death of PC-3 cells induced by **1**. Cell death was analyzed by a LDH release assay. Data are means ± SEM of three experiments; Newman-Keuls test, $^{**}p < 0.01$. (C) Similarly, normal prostate epithelial cells, RWPE-1, were treated with **1**, and cell viability was analyzed using the XTT assay. The data are means ± SEM of 3−5 experiments, each performed in triplicate. (D) Compound **1** inhibits 3D anchorage-independent growth of prostate cancer cells. Cells were seeded in soft agar containing **1** at a density of 1000 cells/cm^2 for 21 days. The colonies were visualized with crystal violet, with **1** shown to inhibit proliferation.

high amounts of cyclin D1 and cyclin E, as shown by analysis of Western immunoblots (Figure 2). Treatment with **1** induced a decrease in cyclin D1 expression after 24 h and in cyclin E after 48 h (Figure 2). Down-regulation of cyclin D1 expression might account for the cell-cycle arrest observed in cells treated with **1**. Indeed, down-regulation of cyclin D1 was sufficient to inhibit proliferation of a prostate cancer cell line in vitro.[24]

The Cip and Kip family of cyclin kinase inhibitors are able to block proliferation in different cells.[19] Among these, p21 is known to be a mediator of p53-induced growth arrest. PC-3 cells do not express p53, so, accordingly, the p21 levels were not changed in those cells treated with **1** (Figure 2), and, in contrast

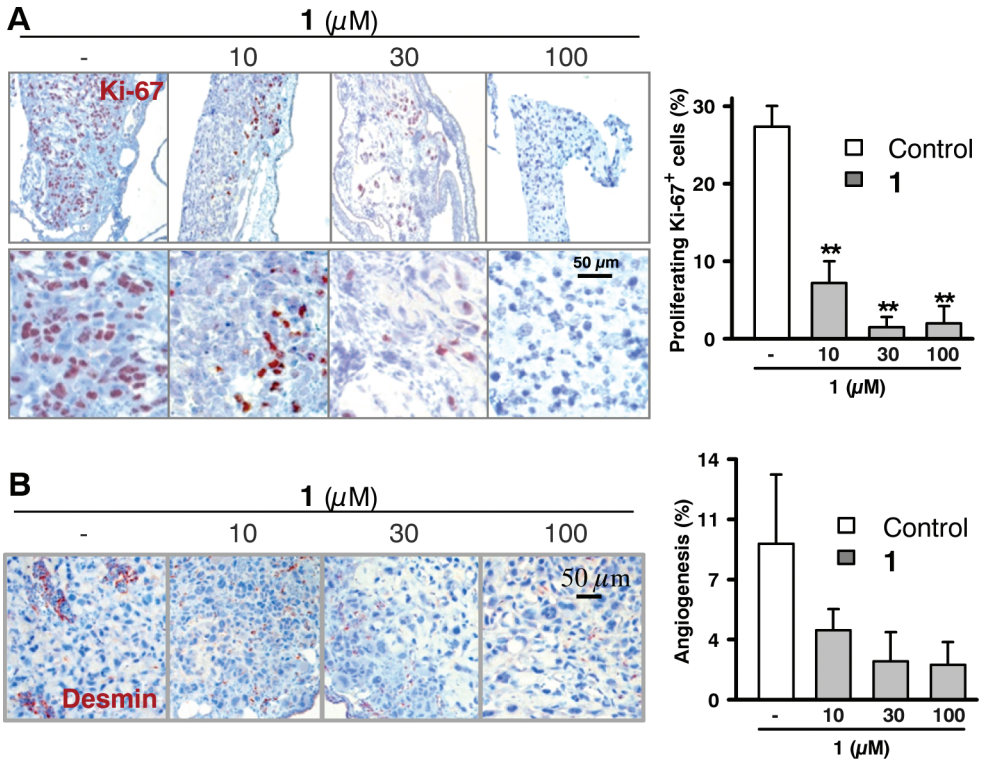

Figure 5. Compound **1** inhibits proliferation (A) and angiogenesis (B) of cancer cells xenografted on chicken chorioallantoic membranes (CAM). Xenografts were treated topically with **1** for 4 days, and the tumors were analyzed by immunohistochemistry. K_i-67, proliferation antigen (brown), desmin, angiogenesis marker (red), nuclei counterstained with hematoxylin (blue), $n = 3-6$, original magnifications 100× and 200×, $^{**}p < 0.01$.

to cyclin D1, this could not account for the accumulation of the cells in G_0/G_1 phase.

It was further determined whether compound **1** could induce apoptosis in PC-3 cells. Treatment of the cells with **1** triggered a time- and concentration-dependent expression of phosphatidylserine on the outer cell membrane leaflet similar to that observed in docetaxel-treated cells, as determined by flow cytometry, using fluorescein isothiocyanate-labeled annexin V and PI (Figure 3A−C). The DNA of terminally apoptotic cells shows a characteristic laddering pattern of oligonucleosomal fragments. This is a result of internucleosomal chromatin cleavage by endogenous endonucleases in multiples of 180 base pairs.[25] This fragmentation is regarded as the ultimate hallmark of apoptosis. The present data revealed that **1** induces a characteristic DNA fragmentation in PC-3 cells after 72 h of treatment (Figure 3D).

To determine the cytotoxic activity of **1** in the PC-3 cells, cell proliferation was analyzed using the XTT assay. Compound **1** induced a time- and concentration-dependent cytotoxic effect on prostate cancer cells with IC_{50} values of about 30 and 4 μM at 48 and 72 h, respectively (Figure 4A). In comparison to **1**, docetaxel was more potently cytotoxic to PC-3 cells (Figure 4A). However, a significant proportion of the cells remained resistant to docetaxel, even when the concentration of this anticancer agent was increased by 3 orders of magnitude up to 10 μM, whereas there were no cells resistant to **1** when used at concentrations ≥ 30 μM (Figure 4A). The pan-caspase inhibitor Z-VAD-FMK inhibited significantly the death of prostate cancer cells induced by **1**, indicating that this substance induces a caspase-dependent apoptosis (Figure 4B). In contrast, **1** did not show any significant cytotoxic activity in normal prostate epithelial RWPE-1 cells, suggesting selectivity of the compound for prostate cancer cells (Figure 4C). These data show that **1** exhibits a selective, concentration- and time-dependent cytotoxicity on androgen-independent prostate cancer cells in vitro.

In addition, the effects of **1** were determined on colony formation by prostate cancer cells during nonanchored cell growth in soft agar. This assay has been shown previously to correlate well with clinical responses and has proven useful for the analysis of resistance to chemotherapeutic agents.[26] When compound **1** was added to the culture medium, it reduced the formation of three-dimensional PC-3 colonies concentration-dependently (Figure 4D).

To verify the antitumor activity of **1** in vivo, PC-3 cells were xenotransplanted on the CAM of fertilized chicken eggs. Immunohistochemical analysis of tumor sections for the proliferation antigen K_i-67 revealed a concentration-dependent inhibitory effect for **1** on tumor size and cell proliferation, as evidenced by the reduced amount of K_i-67 positive cells (Figure 5A). A critical step in tumor development is angiogenesis, the formation of new blood vessels. The effect of **1** on tumor angiogenesis was analyzed by immunohistochemistry using desmin as a marker of the early stages of capillary sprouting.[27] The antitumor effect of **1** was accompanied by a concentration-dependent reduced formation of new blood vessels in the PC-3 tumor xenografts grown on the CAM (Figure 5B).

Taken together, the present data show that (8*R*)-3β,8-dihydroxypolypoda-13*E*,17*E*,21-triene (**1**) triggers apoptosis in androgen-independent and chemoresistant human PC-3 prostate cancer cells in vitro and in vivo. To the best of our knowledge, this is the first report on an antitumor efficacy of a polypodane-type bicyclic triterpenoid. Therefore, **1** may serve as a novel lead for treatment of so far incurable androgen-insensitive prostate cancers.

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EXPERIMENTAL SECTION

General Experimental Methods. The optical rotation was measured on a JASCO 1030 polarimeter (JASCO, Gross-Umstadt, Germany). The ^{1}H and ^{13}C NMR spectra, DEPT 135, and two-dimensional homo- and heterocorrelation NMR spectra ($^{1}H,^{1}H$-COSY, HSQC, and HMBC) were recorded on a Bruker AV500 NMR spectrometer operating at resonance frequencies of 500.13 MHz for ^{1}H and 125.75 MHz for ^{13}C. The sample was measured in $CDCl_3$ at 297 K using a 5 mm TCI Cryoprobe. Mass spectrometric analysis was performed using a Finnigan MAT SSQ-7000 instrument in the chemical ionization mode. Low-pressure chromatography was performed using a Sykam chromatography system equipped with a Rheodyne 7010 injection valve, a Shimadzu SPD 6A UV detector, and a Lichrospher RP18 column (250 × 25 mm, 5 μm; Merck, Germany). The preparative HPLC system consisted of a Shimadzu LC-9A pump equipped with a Rheodyne 7010 injection valve, a Dionex UVD 340 S photodiode array detector, and a Reprosil PUR ODS-3 column (250 × 8 mm, 5 μm; Maisch, Germany). The analytical HPLC system consisted of a Shimadzu LC-9A pump equipped with a Rheodyne 7010 injection valve, a Dionex UVD 340 S photodiode array detector, and a Reprosil PUR ODS-3 column (250 × 3 mm, 5 μm; Maisch). TLC analysis was performed on precoated silica gel plates (Kieselgel 60 F_{254}, Merck) and precoated reversed-phase plates (HPTLC silica gel 60 RP-18 $WF_{254}S$, Merck). Spots were detected under UV light at λ_{254} and λ_{366} nm with sulfuric acid as spray reagent. Docetaxel was obtained from Santa Cruz Biotechnology (Santa Cruz, CA).

Plant Material. The oleogum resin of *P. lentiscus* was purchased from Friedrich Nature Discovery, Euskirchen, Germany, lot number 10895. A voucher specimen was deposited in the Herbarium of Ulm University (Ulm, Germany), number ULM-20881. A HPLC trace of *P. lentiscus* oleogum resin dissolved in DMSO is shown in the Supporting Information.

Extraction and Isolation. *(8R)-3β,8-Dihydroxypolypoda-13E,17E,21-triene (CAS 89362-84-5, **1**) ($C_{30}H_{52}O_2$):* **1** was purified to chemical homogeneity (>99.9% purity) by reversed-phase high-performance liquid chromatography.[10,12] Thus, *P. lentiscus* oleogum (1.2 g) was dissolved in DMSO, applied onto a Lichrospher RP18 column (250 × 25 mm, 5 μm particle size), and eluted with acetonitrile−water (75:25, v/v) containing 0.2% acetic acid as a mobile phase (13 mL/min). The fractions containing **1** (33 mg) were further purified using a Reprosil PUR ODS-3 column (250 × 8 mm, 5 μm particle size) and methanol−water (90:10) containing 0.2% acetic acid as mobile phase (4.5 mL/min) and detected at 215 nm. The purity of the isolated compound was checked by an analytical HPLC run on a Reprosil PUR ODS-3 column (250 × 3 mm, 5 μm particle size) with a linear gradient, mobile phase A (80:19.8:0.2 methanol−water−acetic acid) to mobile phase B (95:4.8:0.2 methanol−water−acetic acid) (0.56 mL/min), and by TLC analyses (C_{18} and SiO_2). The purity of the isolated compound **1** (24 mg) was >99.9%.

*(8R)-3β,8-Dihydroxypolypoda-13E,17E,21-triene (**1**):* $[\alpha]^{20}_{D}$ +6 (*c* 0.0045, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 5.16 (1H, t, *J* = 7.3 Hz, H-13), 5.12 (1H, t, *J* = 7.3 Hz, H-17), 5.11 (1H, t, *J* = 7.3 Hz, H-21), 3.24 (1H, dd, *J* = 11.6, 4.6 Hz, H-3), 2.08 (2H, m, H-12), 2.05 (2H, m, H-16), 2.05 (2H, m, H-20), 2.00 (2H, m, H-15), 2.00 (2H, m, H-19), 1.89 (1H, m, H-7a), 1.73 (1H, m, H-1a), 1.69 (3H, s, H-30), 1.67 (1H, m, H-2a), 1.65 (1H, m, H-6a), 1.62 (3H, s, H-27), 1.61 (3H, s, H-28), 1.61 (3H, s, H-29), 1.59 (1H, m, H-2b), 1.46 (1H, m, H-11a), 1.38 (1H, m, H-7b), 1.31 (1H, m, H-11b), 1.15 (3H, s, H_3-26), 1.14 (1H, m, H-1b), 1.13 (1H, m, H-6b), 1.03 (1H, m, H-9), 1.00 (3H, s, H-24), 0.91 (1H, m, H-5), 0.81 (3H, s, H-25), 0.77 (3H, s, H-23); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 135.5 (C, C-14), 135.1 (C, C-18), 131.4 (C, C-22), 125.4 (CH, C-13), 124.5 (CH, C-21), 124.3 (CH, C-17), 78.9 (CH, C-3), 74.0 (C, C-8), 61.3 (CH, C-9), 55.1 (CH, C-5), 44.5 (CH_2, C-7), 39.9 (CH_2, C-15), 39.9 (CH_2, C-19), 39.0 (C, C-4), 38.9 (C, C-10), 38.0 (CH_2, C-1), 31.4 (CH_2, C-12), 28.2 (CH_3, C-24), 27.3 (CH_2, C-2), 26.8 (CH_2, C-16), 26.8 (CH_2, C-20), 25.8 (CH_3, C-30), 25.7 (CH_2, C-11), 23.9 (CH_3, C-26), 20.3 (CH_2, C-6), 17.8 (CH_3, C-29), 16.3 (CH_3, C-27), 16.1 (CH_3, C-28), 15.6 (CH_3, C-25), 15.5 (CH_3, C-23); its positive-ion CIMS showed the molecular formula $C_{30}H_{52}O_2$, through the presence of a peak at *m/z* 444 $[M]^+$.

Cell Culture and Xenografts. Androgen-independent PC-3 prostate cancer cells obtained from ATCC (CRL-1435) were cultured in Ham's F12-K supplemented with 10% fetal calf serum. Normal prostate epithelial cells derived from the peripheral zone of a histologically normal adult human prostate transfected with a single copy of the human papilloma virus 18 to establish the RWPE-1 (ATCC, CRL-11609) cell line were grown in keratinocyte serum-free medium (Invitrogen) and were analyzed at 48 h. For the in vivo experiments, 0.7×10^6 PC-3 cells in the log growth phase were transplanted in 20 μL of medium/matrigel (1:1, v/v) onto the chicken chorioallantoic membrane. Starting from day 1 after seeding, the cells were treated topically once daily with either 20 μL of the compound **1** or solvent control for four consecutive days. The xenografts were collected, fixed, paraffin imbedded, and analyzed immunohistochmically, as previously described.[28] Quantification of immunohistochemical staining was performed using ImageJ software (NIH). For the colony formation assay, the cells were seeded at a density 1000 cells/cm^2 in 0.35% agar in the presence of 10 μM **1** or solvent. The colonies were allowed to grow for 21 days, fixed, and stained with crystal violet.

Cell Extracts and Western Immunoblots. Aliquots of whole cell lysates containing equal amounts of protein were separated by SDS-PAGE, transferred, probed with specific antibodies, and detected as described.[29]

Detection of Cytotoxicity. Cytotoxicity of the compounds was analyzed by monitoring the mitochondrial reduction of XTT or release of LDH (Roche Diagnostics, Mannheim, Germany). Where applicable, control samples were treated with solvent. Briefly, 3−5 × 10^3 cells in 200 μL of culture medium/well were seeded in 96-well plates and treated for various time periods. Before the end of each experiment, 50 μL of XTT labeling mixture (final concentrations: 125 μM sodium XTT and 25 μM phenazine methosulfate) per well was added, and the plates were incubated for another 4 h. The absorbance of the sample was measured at 450 nm using a microtiter plate reader. In some experiments the pan-caspase inhibitor Z-VAD-FMK (10 μM, Calbiochem) was added together with **1**. Forty-eight hours later, the supernatants were analyzed for LDH activity according to the manufacturer's instructions.

Quantification of Apoptosis by Annexin V and Propidium Iodide Double Staining. Apoptosis was quantified by annexin V/propidium iodide staining followed by flow cytometry. Briefly, PC-3 cells were exposed to **1** or docetaxel for 48 and 72 h. Adherent cells were harvested by citric saline (1.35 M KCl, 0.15 M sodium citrate). The cells were stained with FITC-labeled annexin V and propidium iodide (BD Biosciences) and analyzed by flow cytometry.

Cell Cycle Analysis. Progression of cells through the cell cycle was examined using flow cytometry. Cells were treated with **1** or docetaxel, harvested by trypsinization, and fixed in 70% ethanol. DNA was stained with propidium iodide in the presence of 40 μg/mL DNase-free RNase A. The DNA content was analyzed using a FACScan, and cell-cycle analysis was performed using the ModFit software (BD Biosciences).

DNA Fragmentation Assay. PC-3 cells were cultured with test compounds or the solvent for 72 h. Floating cells were collected by centrifugation, washed, lysed with genomic DNA extraction buffer (100 mM Tris HCl, pH 8.0, 2 mM EDTA, 0.8% (w/v) SDS, 5 mg/mL RNase A, and 6.25 mg/mL proteinase K), and analyzed by electrophoresis in 1.5% agarose gel.

Statistical Analysis. Statistical analysis was performed using Newman−Keuls test, $^{*}p < 0.05$, $^{**}p < 0.01$.

■ ASSOCIATED CONTENT

Supporting Information. HPLC trace of *P. lentiscus* oleogum resin and mass spectrum of **1**. This information is available free of charge via the Internet at http://pubs.acs.org.

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