

Synthesis and Biological Evaluation of New Podophyllic Aldehyde Derivatives with Cytotoxic and Apoptosis-Inducing Activities

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Several series of nonlactonic podophyllic aldehyde analogues were prepared and evaluated against several human tumor cell lines. They had different combinations of aldehyde, imine, amine, ester, and amide functions at C-9 and C-9' of the cyclolignan skeleton. All the compounds synthesized showed cytotoxicity levels in the μM range and below. Within the new series tested, compounds having an aldehyde or imine at C-9 and an ester at C-9' were the most potent, with GI_{50} values in the nM range, some of them being several times more potent against HT-29 and A-549 carcinoma than against MB-231 melanoma cells. Cell cycle studies and analysis of the microtubule-disrupting capacity have demonstrated the existence of two different mechanisms of cell death induction for compounds with closely related structures.

Introduction

Podophyllotoxin, **1**, is a naturally occurring cyclolignan which is the main component of Podophyllum resin, whose pharmacological properties have been well-recognized for centuries.¹ A number of biological activities such as cytotoxicity, antiviral, cathartic, antirheumatic, insecticidal, etc., have been reported for podophyllotoxin and several related compounds and derivatives.^{2–5} Among these, the antitumor activity has been the most attractive, making this cyclolignan a lead compound for anticancer drug design and development. Several semisynthetic derivatives are in clinical use for the treatment of a variety of malignancies⁶ including lung and testicular carcinoma, lymphoma, nonlymphocytic leukemia, etc. Such is the case for etoposide, teniposide, and etopophos, a more soluble prodrug of etoposide (Figure 1). Most interestingly, these semisynthetic derivatives and the parent compound podophyllotoxin differ substantially in their mechanisms of action. While podophyllotoxin inhibits the assembly of tubulin into microtubules, preventing the formation of the achromatic spindle and arresting cell division in metaphase, etoposide and analogues inhibit DNA topoisomerase II, preventing religation of the double-stranded breaks.^{1,2}

In a previous paper,⁷ we made a proposal regarding the interaction between cyclolignans and biomolecules that would imply the existence of another, hitherto unknown, mechanism for antineoplastic cyclolignans on the basis of ring fusion strengthening and chemical reactivity of *trans*-lactonic podolignans. This unknown mechanism would explain why some lignan derivatives had been found to be as cytotoxic as podophyllotoxin and etoposide, without appreciable

inhibition of tubulin polymerization, while being only very weak inhibitors of Topo II in vitro.⁸ Additionally, it has been reported that antitubulin lignans (podophyllotoxin,⁹ deoxy-podophyllotoxin,¹⁰ LL-15,^{11,12} and other podophyllotoxin analogues¹³), as well as related Topo II inhibitors (etoposide,¹⁴ GL331¹⁵), induce apoptotic cell death through independent mechanisms, and such an effect would also enhance their cytotoxicity.

Over the years, looking for more potent, less toxic, and more selective analogues, our group has been engaged in the design and synthesis of better podophyllotoxin related drugs and has prepared a large number of cyclolignans through chemical modification of most rings and positions of the lignan skeleton.^{12,16,17} In the course of such studies, we prepared a potent and selective cytotoxic cyclolignan, the podophyllic aldehyde **2** (Figure 1) that lacked the *trans*- γ -lactone ring, which was previously considered as one of the most important features needed for the bioactivity of podophyllotoxin analogues. Consequently, the podophyllic aldehyde became our lead compound, to be improved by means of further structure modification. The formation of imines from **2** was particularly noteworthy because they not only maintained the cytotoxicity level of the parent aldehyde but also improved considerably the selectivity against adriamycin-resistant HT-29 colon carcinoma.¹² Additional assays demonstrated that both podophyllic aldehyde **2** and its imine derivatives inhibited tubulin polymerization,¹¹ and notably, that these molecules were able to induce delayed cellular apoptosis after 48 h of treatment.¹² Those studies confirmed that the γ -lactone ring is not an essential feature for cytotoxicity, whereas the presence of an electrophilic function at C-7/C-9 seemed important for the antineoplastic potency observed for this type of cyclolignan.

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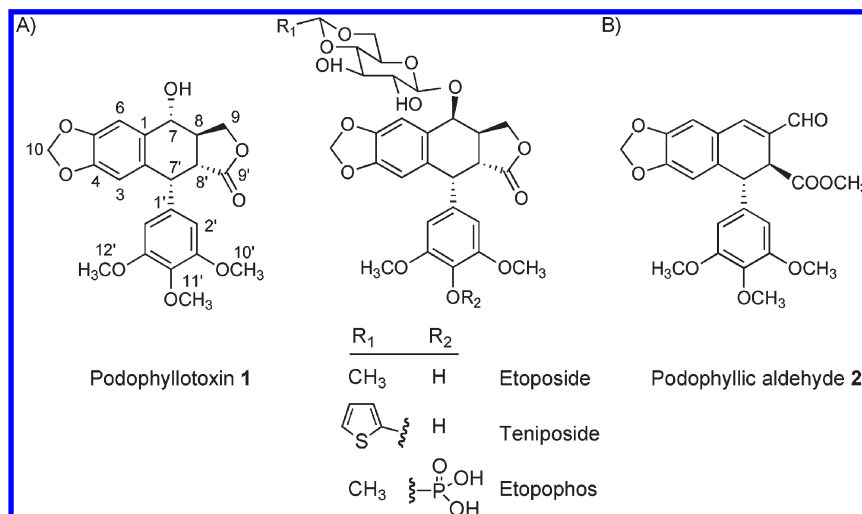


Figure 1. Structures of podophyllotoxin and related compounds. (A) Cyclolignans in clinical use. (B) Selective cytotoxic aldehyde.

All the derivatives of **2** prepared previously kept the β -axially oriented carbomethoxy group at C-9', so we have planned to investigate the influence of the nature and size of this molecular fragment on the cytotoxicity and selectivity. Hence, here we report the preparation and evaluation of new podophyllic aldehyde derivatives, having longer and structurally varied ester chains at C-9', and also some bioisosteric amide analogues. There are several bibliographic citations¹⁸ dealing with esters and amides at positions C-7 and C-4', and even with substitution of the γ -lactone by a γ -lactam,¹⁹ that report good cytotoxicity results. However, only a few derivatives²⁰ with such groupings at position C-9' have so far been described and no report has been found on compounds containing the aldehyde function at C-9 simultaneously. Furthermore, although the imines previously prepared by us¹² were more potent than the parent aldehyde, it could be assumed that they may hydrolyze during the cytotoxicity assay, thus releasing the aldehyde and the corresponding amine. Consequently, we have now transformed several imines into the corresponding amines to analyze the influence of such stabilization on the activity and selectivity.

In addition to the results of cytotoxicity evaluation, we also present here those of cell-cycle arrest studies, which have been performed with the aim of confirming our previous mechanistic findings for podophyllic aldehyde **2**. These results now give experimental biological support to our previous proposal that there is a third cytotoxicity mechanism for podophyllotoxin related lignans.

Results and Discussion

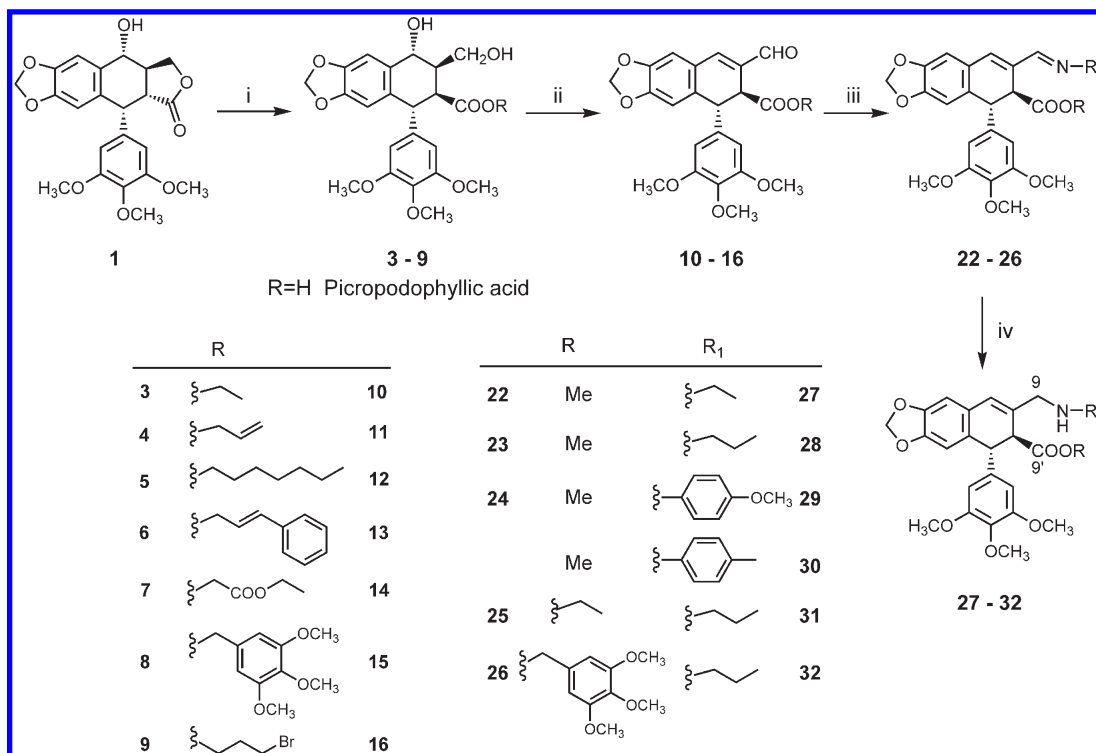
Chemistry. The natural cyclolignan podophyllotoxin **1**, isolated from *Podophyllum* resin,²¹ was the starting material for all the products presented here. The numbering of the compounds used in this work (Figure 1) is in accordance with IUPAC rules for lignans.²²

The aldehyde **2** and the imines **22–24** were obtained as previously described by us.¹² Aldehydes **10–16** were prepared by Swern oxidation of the corresponding dihydroxyesters **3–9**. The latter were prepared, in moderate to good yields, through esterification of picropodophyllic acid obtained by opening of the lactone ring under basic conditions, followed by treatment with the corresponding alkyl bromide (Scheme 1). The ester formation was carried out using

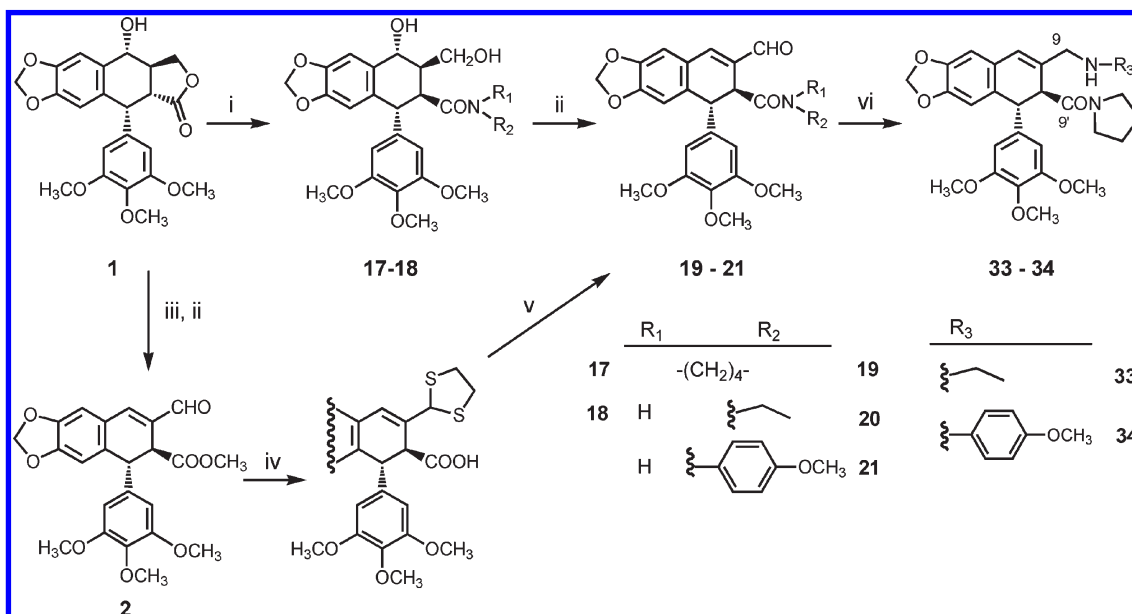
potassium carbonate or sodium hydride as bases in the minimum volume of dry DMF. This aspect was critical because dilution leads to a relactonization with the formation of the 8'-epimeric lactone, picropodophyllotoxin.

Another series of derivatives involves those compounds having an amide function at C-9'. It is known that the conversion of carboxylic esters to amides is often a useful procedure to obtain amides;²³ in our case, aldehyde **2**, bearing a methyl ester, could be considered a good starting substrate to obtain amides at C-9', however, simple esters like methyl or ethyl esters are not very reactive and require strongly basic catalysis.²³ Looking for an alternative procedure to get the amides, and because we knew, from our previous studies, that the γ -lactone of **1** is very reactive toward nucleophiles,^{7,24} we decided to explore the direct aminolysis²⁵ of **1** with primary and secondary amines. Thus, when pyrrolidine and ethylamine were used, as reactants and solvents, under reflux, the dihydroxyamides **17** and **18** were obtained, respectively, in good yields. Further Swern oxidation of **17** and **18** afforded the aldehyde-amides **19** and **20**, respectively (Scheme 2).

When aromatic amines, such as aniline, *p*-anisidine, or *p*-toluidine, were used as reagents, the reaction failed and unchanged **1** or mixtures of **1** and picropodophyllotoxin were recovered, even if the reaction was attempted under microwave irradiation or in the presence of potassium *tert*-butoxide. In view of the difficulties encountered in getting *N*-aryl amides from the lactone, and despite its low reactivity, we reconsidered the possibility of obtaining them by transformation of the carbomethoxy group present in aldehyde **2**.²³ To avoid the direct reaction between the aldehyde function of **2** and aniline, the aldehyde group was first protected as its dithioacetal by treatment with 1,2-ethanedithiol. The dithiolane-ester was then tested as reaction substrate, under reflux or microwave irradiation, without catalyst or in presence of potassium *tert*-butoxide or trimethylaluminum, but the reaction did not progress appreciably, giving only recovered starting material. After such disappointing results, we opted for the saponification of the methyl ester with potassium hydroxide in methanol to get the free carboxylic acid, which was then successfully treated with *p*-anisidine in the presence of DCC and OHBTZ.²⁶ Final removal of the aldehyde protection with SeO₂ yielded the desired aldehyde-amide **21**.

Scheme 1. Preparation of Cyclolignans with Ester Functions at C-9^a

^a (i) (1) KOH/MeOH, (2) HCl/H₂O, pH = 4, (3) base/DMF, R-Br; (ii) Swern; (iii) R₁-NH₂, (iv) NaBH₄, MeOH/0 °C.

Scheme 2. Preparation of Cyclolignans with Amide Functions at C-9^a

^a (i) R₁-NH-R₂; (ii) Swern oxidation; (iii) (1) KOH/MeOH, H⁺/H₂O, pH = 4 (2) CH₂N₂/Et₂O; (iv) (1) (CH₂SH)₂, TMSCl/CH₂Cl₂, (2) KOH/MeOH, (3) HCl/H₂O; (v) (1) *p*-anisidine/THF, DCC/HOBtZ, (2) SeO₂/AcOH; (vi) (1) R₃-NH₂, (2) NaBH₄, MeOH/0 °C.

The aldehydes **2**, **10**, **15**, and **19** were transformed into the corresponding imines by condensation with aliphatic and aromatic primary amines in excess, applying either a classical method, in solution and with a drying agent, or a solvent-free method, under microwave irradiation using montmorillonite K-10 clay as the solid support. In the second case, reaction times were considerably reduced, but the extraction from the solid support was not too efficient and a small amount of the parent aldehyde was always present, as deduced from ¹H

NMR spectra of the extracted crude reaction products. The condensation could not be monitored by TLC because hydrolysis occurred during elution, so progress was followed through the evolution of the aldehyde proton signal in the ¹H NMR spectra until its complete disappearance. Such sensitivity to hydrolysis also made the purification of the imines difficult and crude reaction products were used in the next reaction step, without further purification. However, when the amines used as reagents and solvent were volatile enough

Table 1. Antineoplastic Cytotoxicity of Podophyllic Aldehyde Derivatives (GI₅₀ μ M)

	MB-231	A-549	HT-29
1	- ^a	0.012	0.024
2	0.20	0.061	0.038
3	0.09	0.07	0.09
4	0.11	0.12	0.08
5	0.18	0.19	0.16
6	0.18	0.20	0.15
7	0.16	0.17	0.18
9	0.034	> 17	0.022
10	0.54	0.18	0.20
11	0.15	0.03	0.02
12	2.5	1.0	1.0
13	0.13	0.032	0.036
14	0.11	0.013	0.028
15	0.042	0.034	0.020
16	0.17	0.13	0.14
17	7.8	6.8	7.8
18	10	9.8	10
19	8.2	6.6	7.3
20	7.1	4.5	8.0
21	4.6	1.2	0.80
22	-	0.022	0.0022
23	1.3	0.041	0.041
24	-	0.094	0.0094
25	1.2	0.23	0.39
26	0.13	0.0052	0.0043
27	2.6	0.7	0.4
28	5.5	1.5	1.0
29	2.4	1.0	0.7
30	1.5	0.9	0.8
31	0.37	0.23	0.27
32	6.4	1.9	2.0
33	> 20	> 20	> 20
34	> 17	15	15

^a - = cytotoxicity not tested.

to be removed under vacuum, the resulting crude imines were technically pure by NMR and suitable for full characterization and evaluation. This was the case for compounds **25** and **26**. The imines **22–24** were previously characterized by us.¹²

The reduction of the crude products of imine formation was done with sodium borohydride in methanol at 0 °C. Column chromatography of the reduction products afforded the amines **27–34** in acceptable yields from the corresponding aldehydes (Schemes 1 and 2). In the case of amino-esters **27–32**, it is necessary to keep in mind that migration of the Δ^7 double bond to the $\Delta^{8(9)}$ position and/or γ -lactam ring closure could take place²⁷ if temperatures over 30 °C are attained during reaction workup and solvent removal.

Biological Results. Cytotoxicity. Most cyclolignans reported here were evaluated in vitro to determine their cytotoxicities²⁸ against the human tumor cell lines: MB-231 (breast carcinoma), A-549 (lung carcinoma), and HT-29 (colon carcinoma). The results found are shown in Table 1, and several considerations related to the influence of the functionalities at positions C-9 and C-9' can be analyzed.

All the compounds tested, considered as analogues of the podophyllic aldehyde **2**, were cytotoxic and some of them resulted several times more potent against HT-29 and A-459 carcinoma than against MB-231 melanoma cell lines.

The dihydroxyesters **3–9** showed GI₅₀ values in the μ M range or below, with no significant differences between them, and compared to **2**, no significant differences between the three lines tested were observed. Only compound **9** showed a

notable selectivity, being exceptionally noncytotoxic against A-549 cells at the maximum concentration tested (10 μ g/mL, 17 μ M) while displaying GI₅₀ values in the same range as podophyllotoxin and podophyllic aldehyde against the other lines tested.

In general, the aldehyde-ester derivatives **10–16** maintained the cytotoxicity of aldehyde **2**, showing GI₅₀ values below the μ M range and being a few times more potent against HT-29 and A-549 than against MB-231 cells. Compared to dihydroxyesters **3–9**, those aldehydes with an alkyl-chain ester were less potent (**10**, **11**, **12**, **16** vs **3**, **4**, **5**, **9**, respectively), whereas those having an aromatic ring or an additional ester function in the chain, turned out to be more potent than their hydroxylic precursors (**13**, **14** vs **6**, **7**).

As happened with previous imines reported by us,¹² the derivatives **22–26** were the most potent analogues, with GI₅₀ values against HT-29 and A-549 cells in the nM range, while the GI₅₀ values found on MB-231 were several times higher. Thus, the imino-esters **23** and **26** were around 30 times more potent against HT-29 and A-549 than against MB-231, with a significant improvement in the potency of the precursor aldehydes.

Regarding the C-9' side-chain ester function, it can be noted that, in a similar way as for the above-mentioned dihydroxyesters and aldehyde-esters, and particularly for A-549 and HT-29 cell lines, those imines containing a benzene ring or an additional ester function were also more potent than those having an aliphatic alkyl, alkenyl, or haloalkyl fragment in the ester chain (**13**, **14**, **15** vs **10–12**, **16**). This comparison can be extended to the imine **25** (ethyl ester), which turned out to be 1 order of magnitude less potent than the imine **26** (trimethoxybenzyl ester) against MB-231 cells and around two orders less potent against A-549 and HT-29 cells. However, it is interesting to note that the opposite occurs in the case of amino-esters when compounds **31** (ethyl ester) and **32** (trimethoxybenzyl ester) are compared, the latter being around one order less cytotoxic against the three cell lines tested.

The reduction of the imines to the corresponding amines **27–32** led to a fair decrease of cytotoxicity though retained a certain degree of selectivity toward HT-29 cells. Finally, the substitution of the ester moiety by an amide group led to the less potent compounds, mostly independent of the type of the amide *N*-substitution and the function (hydroxyl, aldehyde, or amine) located at C-9. Only the anisyl amide **21** showed a GI₅₀ value in the μ M range.

Cell Cycle Effects. To gain further insight into the mode of action of these compounds, we examined the effects of compounds **11–16**, **20**, and **21** on cell cycle by flow cytometry in HT-29 cells. The results are shown in Table 2 and Figure 2. The concentrations of the above compounds required to elicit these effects on cell cycle are consistent with the corresponding GI₅₀ values for cell proliferation assays because the concentration of cells used for flow cytometry analysis exceeded by about 10–20 times that used in cell proliferation tests. The G₂/M arrest was observed for 24 and 48 h, and then this cell cycle arrest led eventually to cell death, with over 30% apoptosis after 72 h treatment with 1 μ M of the above-mentioned compounds (Table 2).

As could be expected due to the close structural similarity with podophyllotoxin **1** and podophyllic aldehyde **2**, which have been reported to disrupt the microtubule network and to compete with colchicine in its binding to tubulin,^{11,12} compounds **11**, **13**, **14**, and **15** induced a nearly total arrest

Table 2. Effect of Compounds **11**–**16**, **20**, and **21** on Cell Cycle and Apoptosis in HT-29 Cells^a

compd	conc, μ M	% cells											
		24 h treatment				48 h treatment				72 h treatment			
		sub-G ₁	G ₀ /G ₁	S	G ₂ /M	sub-G ₁	G ₀ /G ₁	S	G ₂ /M	sub-G ₁	G ₀ /G ₁	S	G ₂ /M
control		0.5	65.0	8.7	25.8	1.0	63.8	8.0	27.2	1.8	64.3	9.1	24.8
11	1	0.3	3.9	4.5	91.3	1.8	10.9	3.9	83.4	41.6	33.6	16.8	8.0
12	1	19.6	14.6	42.6	23.2	29.2	25.3	29.6	15.9	82.3	16.7	0.4	0.6
13	1	0.4	1.4	1.8	96.4	5.2	5.3	4.2	85.3	33.7	21.1	22.0	23.2
14	1	0.5	1.0	0.9	97.6	6.3	4.7	3.4	85.6	30.6	16.4	19.2	33.8
15	1	0.5	0.9	1.1	97.5	8.6	6.8	5.1	79.5	32.3	18.3	12.9	36.5
16	1	29.2	46.9	9.7	14.2	34.6	38.9	8.4	18.1	67.7	31.6	0.3	0.4
20	1	25.0	51.7	9.8	13.5	61.7	33.0	3.0	2.3	93.8	5.3	0.5	0.4
21	1	13.6	48.0	13.2	25.2	45.2	50.4	1.8	2.6	52.6	36.4	8.4	2.6

^a HT-29 cells were incubated with the above compounds at 1 μ M for the indicated times, and the proportion of cells in each phase of the cell cycle was quantitated by flow cytometry. Cells in the sub-G₁ region represent apoptotic cells. Untreated control cells were run in parallel. Data shown are representative of three independent experiments performed.

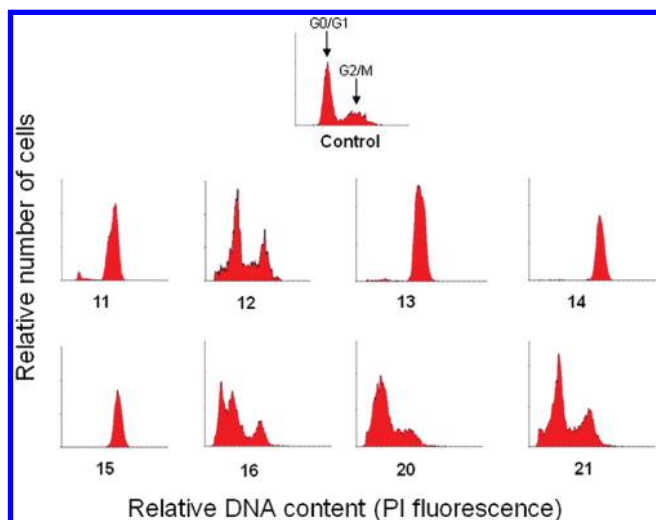


Figure 2. Effects of compounds **11**–**16**, **20**, and **21** on cell cycle in HT-29 cells. Cells were incubated with 1 μ M of the indicated compounds for 24 h and stained with propidium iodide (PI). Their DNA content was analyzed by fluorescence flow cytometry. The positions of the G₀/G₁ and G₂/M peaks are indicated by arrows. The experiment shown is representative of three performed.

of cells (higher than 90%) at the G₂/M phase after 24 h of treatment. This was also in agreement with their high cytotoxicity and the very low GI₅₀ values, ranging between 20 and 36 nM, observed for these compounds in the cell proliferation assays (Table 1). The G₂/M arrest was similarly observed for HT-29 cells treated with these compounds at 0.2 μ M (data not shown).

Most interestingly, the aldehyde-esters **12** and **16** and the aldehyde-amides **20** and **21** were shown to directly induce apoptosis, when used at 1 μ M for 24–72 h, without any previous G₂/M arrest (Table 2 and Figure 2), suggesting a different mode of action for these compounds.

By analyzing the effect of the above compounds on the microtubule network, we found that compounds **11**, **13**, **14**, and **15** disrupted microtubules (Figure 3, and data not shown) after 24 h incubation at 1 μ M, whereas compounds **12**, **16**, **20**, and **21** had a much lower effect, in some cases negligible, if any, on microtubules (Figure 3, and data not shown). Thus, these results on microtubule network square with those of cell cycle. Compounds **11**, **13**, **14**, and **15** inhibited microtubule polymerization and promoted cell cycle arrest in G₂/M phase, whereas compounds **12**, **16**, **20**, and **21** seemed to act through a distinct mechanism.

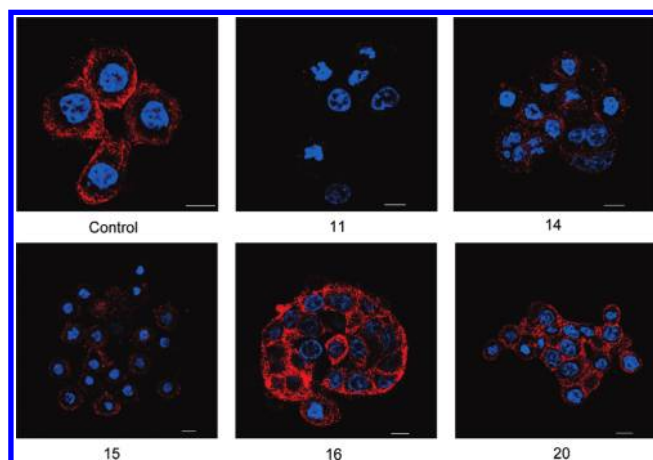


Figure 3. - Effects of compounds **11**, **14**, **15**, **16**, and **20** on the microtubule network of HT-29 cells. Cells were incubated in the absence (control) or in the presence of 1 μ M of compounds **11**, **14**, **15**, **16**, and **20** for 24 h and then fixed and processed for immunofluorescence of microtubules (red fluorescence) and nuclei (blue fluorescence) as described in Experimental Section. Bar, 10 μ m. The photomicrographs shown are representative of at least three independent experiments performed.

On these grounds, the strong G₂/M arrest following treatment with **11**, **13**, **14**, and **15** appears to be mediated by microtubule disruption. Interference with the microtubule assembly/disassembly processes leads to apoptosis through mechanisms that are not completely understood.²⁹ Because those lignans included in this research would have not any potential anti-Topo II activity, the mechanistic difference observed between compounds **11**, **13**, **14**, and **15**, which lead to total G₂/M arrest before inducing apoptosis, and their closely related partners **12**, **16**, **20**, and **21**, which promote cell death without any significant cell cycle arrest, serves as an experimental proof of the existence of a previously unknown mechanism of action for podophyllotoxin lignans. Nevertheless, to get further insight into this mechanism, which results in cell death by eventually affecting microtubule structure or dynamics, an extended chemical and biological research program must be planned and executed.

In summary, new nonlactonic cyclolignans derived from podophyllic aldehyde **2** and having different structural arrangements at C-9 and C-9' were prepared and evaluated against several human tumor cell lines. All the compounds showed GI₅₀ cytotoxicity levels in the μ M–nM range.

In general, those compounds containing an ester function at C-9' were more potent than the corresponding amides and the combination of an aldehyde or an imine function at C-9 with an ester at C-9' was found to be the best for potency and selectivity against HT-29 colon carcinoma. On the other hand, it was observed that functions conferring chemical stability have a negative influence on cytotoxicity and that the presence of a highly electrophilic function at C-9 (aldehyde or imine) is the most important feature for cytotoxicity and potency. These findings also support our previous proposal about the importance of this position, which seems to constitute a site for interaction with the biological targets.^{7,24} Cell cycle studies have revealed the existence of two different modes of action for these compounds, apparently involving tubulin polymerization, microtubule assembly, or dynamics. The mechanistic difference displayed by compounds with such similar structures is evidence for a third mechanism of action for cyclolignans and will provide the basis for further biological and chemical studies focused on investigating this subject.

Experimental Section

Chemistry. NMR spectra were recorded on a Bruker AC 200 at 200 MHz for ¹H and 50.3 MHz for ¹³C in deuteriochloroform with TMS as internal standard. Chemical shift values are expressed in ppm followed by multiplicity and coupling constants (*J*) in Hz. The complete NMR signals are given for the first compound of each series described here, and only characteristic signals are indicated for the remainder. Optical rotations were recorded on a Perkin-Elmer 241 polarimeter in chloroform solution and UV spectra on a Hitachi 100-60 spectrophotometer in ethanol solution. IR spectra were obtained on a Nicolet Impact 410 spectrophotometer, and wave numbers are given in cm⁻¹. HRMS were run in a VG-TS-250 spectrometer working at 70 eV. Elemental analyses (C, H, N) were obtained with a LECO CHNS-932 and were within ±0.4% of the theoretical values. Solvents and reagents were purified by standard procedures as necessary, and the chlorinated solvents, including deuteriochloroform, were filtered through sodium bicarbonate prior its use in order to eliminate acid traces.

General Methods for the Synthesis of Diols 3–9, 17, and 18.
Method A. A mixture of picropodophylllic acid (obtained from podophyllotoxin¹²) and K₂CO₃ was dissolved in dry DMF and stirred at room temperature for 30 min. Then the alkylating agent was added and the mixture stirred at room temperature from 45 min to 1 h. The crude product was diluted with EtOAc and filtered, and the solutions were washed with satd aq NaCl, dried over Na₂SO₄, and filtered. The solvent was removed under vacuum to give the corresponding picropodophyllate derivatives.

Method B. A solution of NaH in dry DMF was cooled to 0 °C and kept under an argon atmosphere. Picropodophylllic acid dissolved in DMF was added and the mixture stirred during 15 min. Then the alkylating agent was added and the mixture left to attain the room temperature and kept stirring for 45 min more. The reaction was quenched with MeOH, which was then removed under vacuum and the crude diluted with EtOAc. The organic phase was washed with satd aq NaCl, dried over Na₂SO₄, filtered, and the solvent removed to get the corresponding picropodophyllate derivatives.

Ethyl Picropodophyllate (3). From picropodophylllic acid (200 mg, 0.460 mmol), K₂CO₃ (192 mg, 1.39 mmol), and ethyl bromide (1 mL, 13 mmol) in dry DMF (1.5 mL) using method A during 1 h to yield compound **3** (185 mg, 85%). ¹H NMR (CDCl₃): δ 1.13 (t, 3H, *J* = 7.1 Hz, C9'-OCH₂CH₃), 2.45 (m, 1H, H8), 3.33 (dd, 1H, *J* = 8.2, 3.6 Hz, H8'), 3.65 (m, 2H, H9), 3.76 (s, 6H, H10', H12'), 3.80 (s, 3H, H11'), 4.05 (c, 2H, *J* = 7.1 Hz,

C9'-OCH₂CH₃), 4.25 (d, 1H, *J* = 8.2 Hz, H7'), 4.85 (m, 1H, H7), 5.88 (s, 2H, H10), 6.34 (s, 3H, H3, H2', H6'), 6.83 (s, 1H, H6). ¹³C NMR (CDCl₃): δ 14.1 (C9'-OCH₂CH₃), 43.7 (C7'), 45.6 (C8), 47.1 (C8'), 56.2 (C10', C12'), 60.9 (C11', C9'-OCH₂CH₃), 62.6 (C9), 69.8 (C7), 101.1 (C10), 106.4 (C2', C6'), 108.4 (C6), 109.4 (C3), 130.1 (C1), 130.9 (C2), 136.7 (C4'), 140.0 (C1'), 146.8 (C4), 147.7 (C5), 153.1 (C3', C5'), 174.4 (C9'). IR (film) cm⁻¹: 3403 (OH), 1725 (COOR), 1125 and 1035 (OH). HRMS: calcd for C₂₄H₂₈O₉Na, 483.1622; found, 483.1611. [α]_D²⁰ -81.6° (*c* 0.9%).

Allyl Picropodophyllate (4). From picropodophylllic acid (80 mg, 0.190 mmol), K₂CO₃ (105 mg, 0.760 mmol), and allyl bromide (1 mL, 11 mmol) in dry DMF (2 mL) using method A during 50 min. The reaction product was chromatographed on silica gel, eluting with 30% CH₂Cl₂/EtOAc to give compound **4** (17 mg, 38%). ¹H NMR (CDCl₃): δ 4.51 (d, 2H, *J* = 5.5 Hz, C9'-OCH₂CH=CH₂), 5.16 (d, 1H, *J* = 10.6 Hz, C9'-OCH₂CH=CH₂), 5.14 (d, 1H, *J* = 16.1 Hz, C9'-OCH₂CH=CH₂), 5.80 (m, 1H, C9'-OCH₂CH=CH₂). ¹³C NMR (CDCl₃): δ 65.5 (C9'-OCH₂CH=CH₂), 118.4 (C9'-OCH₂CH=CH₂), 131.8 (C9'-OCH₂CH=CH₂), 173.9 (C9'). IR (film) cm⁻¹: 3442, 1126, 1038 (OH), 1731 (COOR). HRMS: calcd for C₂₅H₂₈O₉Na, 495.1625; found, 495.1632. [α]_D²⁰ -77.6° (*c* 0.8%).

Heptyl Picropodophyllate (5). From picropodophylllic acid (140 mg, 0.324 mmol), K₂CO₃ (179 mg, 1.30 mmol), and 1-heptyl bromide (1.0 mL, 6.3 mmol) in dry DMF (1.5 mL) using method A during 45 min to obtain diol-ester **5** (95 mg, 55%). ¹H NMR (CDCl₃): δ 0.85 (t, 3H, *J* = 6.9 Hz, C9'-O-(CH₂)₆-CH₃), 1.15 (m, 8H, C9'-O-CH₂CH₂-(CH₂)₄-CH₃), 1.49 (m, 2H, C9'-O-CH₂CH₂-(CH₂)₄-CH₃), 3.99 (t, 2H, *J* = 6.6, C9'-O-CH₂CH₂-(CH₂)₄-CH₃). ¹³C NMR (CDCl₃): δ 14.1 (C9'-O-(CH₂)₆-CH₃), 22.6, 25.7, 28.5, 28.8 (C9'-O-CH₂CH₂-(CH₂)₄-CH₃), 31.7 (C9'-O-CH₂CH₂-(CH₂)₄-CH₃), 65.1 (C9'-O-CH₂CH₂-(CH₂)₄-CH₃), 174.4 (C9'). IR (film) cm⁻¹: 3439, 1127, 1039 (OH), 1728 (COOR). HRMS: calcd for C₂₉H₃₈O₉Na, 553.2908; found, 553.2389.

Cinnamyl Picropodophyllate (6). From picropodophylllic acid (150 mg, 0.350 mmol), NaH (16 mg, 0.52 mmol), and cinnamyl bromide (137 mg, 0.690 mmol) in dry DMF (5 mL) using method B to yield diol-ester **6** (170 mg, 89%). ¹H NMR (CDCl₃): δ 4.68 (d, 2H, *J* = 6.2 Hz, C9'-OCH₂CH=CH-C₆H₅), 6.16 (dt, 1H, *J* = 16.5, 6.2 Hz, C9'-OCH₂CH=CH-C₆H₅), 6.54 (d, 1H, *J* = 16.5 Hz, C9'-OCH₂CH=CH-C₆H₅), 7.30 (m, 5H, C9'-OCH₂CH=CH-C₆H₅). ¹³C NMR (CDCl₃): δ 65.5 (C9'-OCH₂CH=CH-C₆H₅), 122.7 (C9'-OCH₂CH=CH-C₆H₅), 126.0 (C), 126.6 (2CH), 128.2 (CH), 128.7 (2CH, C9'-OCH₂CH=CH-C₆H₅), 134.4 (C9'-OCH₂CH=CH-C₆H₅), 173.9 (C9'). IR (film) cm⁻¹: 3400, 1125, 1036 (OH), 1727 (COOR). HRMS: calcd for C₃₁H₃₂O₉Na, 571.1938; found, 571.1973. [α]_D²⁰ -22.5° (*c* 0.8%). UV (EtOH) λ_{max} 205 (lg ε 4.1), 251 (lg ε 4.2), 359 (lg ε 4.3).

Ethoxycarbonylmethyl Picropodophyllate (7). From picropodophylllic acid (80 mg, 0.18 mmol), NaH (8.3 mg, 0.28 mmol), and ethyl bromoacetate (41.0 mL, 0.370 mmol) in dry DMF (2.5 mL) using method B to obtain compound **7** (53 mg, 55%). ¹H NMR (CDCl₃): δ 1.22 (t, 3H, *J* = 7.1 Hz, C9'-OCH₂-COOCH₂CH₃), 4.16 (c, 2H, *J* = 7.1 Hz, C9'-OCH₂-COOCH₂CH₃), 4.37 and 4.75 (AB system, *J* = 16.0 Hz, C9'-OCH₂-COOCH₂CH₃). ¹³C NMR (CDCl₃): δ 14.1 (C9'-OCH₂-COOCH₂CH₃), 61.8 (C9'-OCH₂-COOCH₂CH₃), 62.2 (C9'-OCH₂-COOCH₂CH₃), 168.3 (C9'-OCH₂-COOCH₂CH₃), 173.2 (C9'). IR (film) cm⁻¹: 3430, 1125, 1036 (OH), 1741 (COOR). HRMS: calcd for C₂₆H₃₀O₁₁Na, 541.1680; found, 541.1661.

3,4,5-Trimethoxybenzyl Picropodophyllate (8). From picropodophylllic acid (160 mg, 0.370 mmol), K₂CO₃ (77 mg, 0.55 mmol), and 3,4,5-trimethoxybenzyl bromide (145 mg, 0.55 mmol) in dry DMF (2.5 mL) using method A during 1 h to yield compound **8** (158 mg, 70%). ¹H NMR (CDCl₃): δ 3.80 (s, 9H, C9'-OCH₂-C₆H₂(OCH₃)₃), 4.91 and 5.01 (AB system,

$J = 10$ Hz, $C9'-OCH_2-C_6H_2(OCH_3)_3$, 6.33 (s, 2H, $C9'-OCH_2-C_6H_2(OCH_3)_3$). IR (film) cm^{-1} : 3490, 1125, 1036 (OH), 1728 (COOR).

3-Bromopropyl Picropodophyllate (9). From picropodophyllic acid (250 mg, 0.58 mmol), K_2CO_3 (240 mg, 1.74 mmol), and 1,3-dibromopropane (0.177 mL, 1.74 mmol) in dry DMF (1.8 mL) using method A during 1 h to obtain compound **9** (263 mg, 82%). 1H NMR ($CDCl_3$): δ 2.03 (q, 2H, $J = 6.2$ Hz, $C9'-CH_2CH_2-CH_2Br$), 3.20 (t, 2H, $J = 6.2$ Hz, $C9'-CH_2CH_2CH_2Br$), 4.12 (m, 2H, $C9'-OCH_2CH_2CH_2Br$). ^{13}C NMR ($CDCl_3$): δ 29.1 ($C9'-OCH_2CH_2CH_2Br$), 31.4 ($C9'-OCH_2CH_2CH_2Br$), 62.5 ($C9'-OCH_2CH_2CH_2Br$), 174.3 ($C9'$). IR (film) cm^{-1} : 3440, 1125, 1036 (OH), 1728 (COOR). HRMS: calcd for $C_{25}H_{29}O_9NaBr$, 575.0887; found, 575.0950. $[\alpha]^{20}_D -64.9^\circ$ (c 0.9%).

Picropodophyllic Acid Pyrrolidinyl Amide (17). Pyrrolidine (0.8 mL, 9.6 mmol) was added to a solution of **1** (200 mg, 0.48 mmol) in dry benzene (20 mL), and the mixture was stirred at $50^\circ C$ under argon atmosphere during 46 h. Solvent and residual amine were evaporated in vacuum to give the corresponding dihydroxyamide **17** in quantitative yield. 1H NMR ($CDCl_3$): δ 1.50–1.90 (m, 4H, $C9'-N-(CH_2)_4-$), 3.20–3.50 (m, 4H, $C9'-N-(CH_2)_4-$). ^{13}C NMR ($CDCl_3$): δ 24.2, 25.9, 46.0, 46.8 ($C9'-N-(CH_2)_4-$), 173.9 ($C9'$). IR (film) cm^{-1} : 3380, 1126, 1038 (OH), 1611 (CON- $(CH_2)_4-$). HRMS: calcd for $C_{26}H_{31}NO_8 + H$, 486.2122; found, 486.2127.

N-Ethyl Picropodophyllamide (18). A mixture of **1** (100 mg, 0.241 mmol) and ethylamine (70% in water, 10 mL, 18 mmol) was stirred and heated under reflux during 1.5 h. The excess of amine was removed under vacuum to give **18** in quantitative yield. 1H NMR ($CDCl_3$): δ 0.85 (t, 3H, $J = 7.2$ Hz, $C9'-NHCH_2CH_3$), 2.98 (m, 1H, $C9'-NHCH_2CH_3$), 3.12 (m, 1H, $C9'-NHCH_2CH_3$), 5.75 (m, 1H, NH). ^{13}C NMR ($CDCl_3$): δ 14.3 ($C9'-NHCH_2CH_3$), 34.4 ($C9'-NHCH_2CH_3$), 174.9 ($C9'$). IR (film) cm^{-1} : 3360, 1126, 1038 (NH, OH), 1645 (CONH). HRMS: calcd for $C_{24}H_{29}NO_8 + H$, 460.1966; found, 460.1976. $[\alpha]^{20}_D -46.5^\circ$ (c 0.9%).

Swern Oxidation for the Synthesis of α,β -Unsaturated Aldehydes. To a precooled ($-55^\circ C$) and stirred solution of oxalyl chloride (3 equiv) in dry CH_2Cl_2 (5 mL) was added dropwise a solution of DMSO (6 equiv) in dry CH_2Cl_2 (2 mL). After 5 min at $-55^\circ C$, a solution of the corresponding diol (1 equiv) in dry CH_2Cl_2 was slowly added. The mixture was stirred at the same temperature for 30 min, and then triethylamine (10 equiv) was added dropwise. The mixture was warmed to $0^\circ C$ over 1 h, quenched with water, and extracted with CH_2Cl_2 or EtOAc. The organic phase was washed with 2 N HCl and satd aq solutions of $NaHCO_3$ and NaCl and the solvent was evaporated off. Column chromatography of the reaction products with mixtures of EtOAc/ CH_2Cl_2 gave the corresponding aldehydes.

Ethyl 9-Deoxy-9-oxo- α -apicropodophyllate (10). From ethyl picropodophyllate **3** (90 mg, 0.20 mmol). The Swern reaction product was chromatographed on silica gel, eluting with 2% EtOAc/ CH_2Cl_2 to give compound **10** (55 mg, 64%). 1H NMR ($CDCl_3$): δ 1.10 (t, 3H, $J = 6.9$ Hz, $C9'-OCH_2CH_3$), 3.74 (s, 6H, $H10'$, $H12'$), 3.78 (s, 3H, $H11'$), 3.97 (d, 1H, $J = 4.0$ Hz, $H8'$), 4.05 (m, 2H, $C9'-OCH_2CH_3$), 4.59 (d, 1H, $J = 3.8$ Hz, $H7'$), 5.99 (d, 1H, $J = 1.8$ Hz, $H10a$), 6.01 (d, 1H, $J = 1.8$ Hz, $H10b$), 6.21 (s, 2H, $H2'$, $H6'$), 6.65 (s, 1H, $H3$), 6.88 (s, 1H, $H6$), 7.34 (s, 1H, $H7$), 9.60 (s, 1H, $H9$). ^{13}C NMR ($CDCl_3$): δ 14.1 ($C9'-OCH_2CH_3$), 44.8 ($C7'$), 46.4 ($C7'$), 56.1 ($C10'$, $C12'$), 60.8 ($C11'$), 61.4 ($C9'-OCH_2CH_3$), 101.8 ($C10$), 104.9 ($C2'$, $C6'$), 108.9 ($C6$), 110.1 ($C3$), 125.2 ($C1$), 133.4 ($C8$), 133.8 ($C2$), 137.1 ($C4'$), 137.3 ($C1'$), 145.5 ($C7$), 147.4 ($C5$), 150.5 ($C4$), 153.2 ($C3'$, $C5'$), 171.7 ($C9'$), 191.3 ($C9$). IR (film) cm^{-1} : 1726 (COOR), 1671 (CHO). HRMS: calcd for $C_{24}H_{24}O_8Na$, 463.1363; found, 463.1348.

Allyl 9-Deoxy-9-oxo- α -apicropodophyllate (11). From allyl picropodophyllate **4** (85 mg, 0.18 mmol). The Swern reaction product was chromatographed on silica gel, eluting with 2% EtOAc/ CH_2Cl_2 to give compound **11** (41 mg, 50%). 1H NMR ($CDCl_3$): δ 4.52 (m, 2H, $C9'-OCH_2CH=CH_2$), 5.14 (d, 1H,

$J = 11.7$ Hz, $C9'-OCH_2CH=CH_2$), 5.16 (d, 1H, $J = 16.1$ Hz, $C9'-OCH_2CH=CH_2$), 5.80 (m, 1H, $C9'-OCH_2CH=CH_2$), 7.34 (s, 1H, $H7$), 9.60 (s, 1H, $H9$). ^{13}C NMR ($CDCl_3$): δ 65.8 ($C9'-OCH_2CH=CH_2$), 117.9 ($C9'-OCH_2CH=CH_2$), 131.7 ($C9'-OCH_2CH=CH_2$), 133.1 ($C8$), 145.7 ($C7$), 171.4 ($C9'$), 191.3 ($C9$). IR (film) cm^{-1} : 1732 (COOR), 1670 (CHO). Anal. ($C_{25}H_{24}O_8$) C, H, N. HRMS: calcd for $C_{25}H_{24}O_8Na$, 475.1363; found, 475.1381.

Heptyl 9-Deoxy-9-oxo- α -apicropodophyllate (12). From heptyl picropodophyllate **5** (95 mg, 0.18 mmol). The Swern reaction product was chromatographed on silica gel, eluting with 5% EtOAc/ CH_2Cl_2 to give compound **12** (41 mg, 44%). 1H NMR ($CDCl_3$): δ 0.86 (t, 3H, $J = 6.6$ Hz, $C9'-O-(CH_2)_6-CH_3$), 1.2 (m, 8H, $C9'-O-CH_2CH_2-(CH_2)_4-CH_3$), 1.5 (m, 2H, $C9'-O-CH_2CH_2-(CH_2)_4-CH_3$), 4.01 (t, 2H, $J = 6.6$ Hz, $C9'-O-CH_2CH_2-(CH_2)_4-CH_3$), 7.33 (s, 1H, $H7$), 9.60 (s, 1H, $H9$). ^{13}C NMR ($CDCl_3$): δ 14.1 ($C9'-O-(CH_2)_6-CH_3$), 22.6, 25.7, 28.4, 28.8 ($C9'-O-CH_2CH_2-(CH_2)_4-CH_3$), 31.7 ($C9'-O-CH_2CH_2-(CH_2)_4-CH_3$), 65.5 ($C9'-O-CH_2CH_2-(CH_2)_4-CH_3$), 133.4 ($C8$), 145.4 ($C7$), 191.3 ($C9$). IR (film) cm^{-1} : 1728 (COOR), 1672 (CHO). Anal. ($C_{29}H_{34}O_8$) C, H, N. HRMS: calcd for $C_{29}H_{34}O_8Na$, 533.2146; found 533.2162. $[\alpha]^{20}_D -135^\circ$ (c 1.1%).

Cinnamyl 9-Deoxy-9-oxo- α -apicropodophyllate (13). From cinnamyl picropodophyllate **6** (150 mg, 0.274 mmol). The Swern reaction product was chromatographed on silica gel, eluting with 5% EtOAc/ CH_2Cl_2 to give aldehyde **13** (50 mg, 53%). 1H NMR ($CDCl_3$): δ 4.68 (d, 2H, $J = 5.8$ Hz, $C9'-OCH_2CH=CH-C_6H_5$), 6.15 (dt, 1H, $J = 16.0$, 5.8 Hz, $C9'-OCH_2CH=CH-C_6H_5$), 6.49 (d, 1H, $J = 16.0$ Hz, $C9'-OCH_2CH=CH-C_6H_5$), 7.25 (m, 5H, $C9'-OCH_2CH=CH-C_6H_5$), 7.36 (s, 1H, $H7$), 9.62 (s, 1H, $H9$). ^{13}C NMR ($CDCl_3$): δ 65.8 ($C9'-OCH_2CH=CH-C_6H_5$), 122.8 ($C9'-OCH_2CH=CH-C_6H_5$), 125.5, 126.6 (2CH), 128.1, 128.6 (2CH, $C9'-OCH_2CH=CH-C_6H_5$), 133.2 ($C8$), 133.9 ($C9'-OCH_2CH=CH-C_6H_5$), 145.6 ($C7$), 191.1 ($C9$). IR (film) cm^{-1} : 1730 (COOR), 1669 (CHO). Anal. ($C_{31}H_{28}O_8$) C, H, N. HRMS: calcd for $C_{31}H_{28}O_8Na$, 551.1676; found 551.1682. $[\alpha]^{20}_D -131^\circ$ (c 0.9%).

Ethoxycarbonylmethyl 9-Deoxy-9-oxo- α -apicropodophyllate (14). From ethoxycarbonylmethyl picropodophyllate **7** (50 mg, 0.10 mmol). The Swern reaction product was chromatographed on silica gel, eluting with 10% EtOAc/ CH_2Cl_2 to give compound **14** (20 mg, 46%). 1H NMR ($CDCl_3$): δ 1.20 (t, 3H, $J = 7.3$ Hz, $C9'-OCH_2COOCH_2CH_3$), 4.15 (c, 2H, $J = 7.3$ Hz, $C9'-OCH_2COOCH_2CH_3$), 4.53 and 4.59 (AB system, $J = 15.9$ Hz, $C9'-OCH_2COOCH_2CH_3$), 7.39 (s, 1H, $H7$), 9.61 (s, 1H, $H9$). ^{13}C NMR ($CDCl_3$): δ 14.1 ($C9'-OCH_2COOCH_2CH_3$), 61.3 ($C9'-O-CH_2COOCH_2CH_3$), 61.3 ($C9'-OCH_2COOCH_2CH_3$), 132.4 ($C8$), 145.9 ($C7$), 167.3 ($C9'-OCH_2COOCH_2CH_3$), 170.9 ($C9'$), 191.1 ($C9$). IR (film) cm^{-1} : 2720 (CHO), 1743 (COOR), 1670 (CHO). Anal. ($C_{26}H_{26}O_{10}$) C, H, N. HRMS: calcd for $C_{26}H_{26}O_{10}Na$, 521.1418; found 521.1410. $[\alpha]^{20}_D -110^\circ$ (c 0.7%).

3,4,5-Trimethoxybenzyl 9-Deoxy-9-oxo- α -apicropodophyllate (15). From 3,4,5-trimethoxybenzyl picropodophyllate **8** (220 mg, 0.36 mmol). The reaction product was chromatographed on silica gel, eluting with 20% EtOAc/ CH_2Cl_2 to give compound **15** (68 mg, 78%). 1H NMR ($CDCl_3$): δ 3.82 (s, 9H, $C9'-OCH_2-C_6H_2(OCH_3)_3$), 4.99 (s, 2H, $C9'-OCH_2-C_6H_2(OCH_3)_3$), 6.18 (s, 2H, $C9'-OCH_2-C_6H_2(OCH_3)_3$), 7.35 (s, 1H, $H7$), 9.61 (s, 1H, $H9$). ^{13}C NMR ($CDCl_3$): δ 56.1 (2C, $C9'-OCH_2-C_6H_2(OCH_3)_3$), 60.8 (1C, $C9'-OCH_2-C_6H_2(OCH_3)_3$), 67.1 (1C, $C9'-OCH_2-C_6H_2(OCH_3)_3$), 104.8 (2CH, $C9'-OCH_2-C_6H_2(OCH_3)_3$), 131.3 (1C, $C9'-OCH_2-C_6H_2(OCH_3)_3$), 133.1 ($C8$), 137.7 (1C, $C9'-OCH_2-C_6H_2(OCH_3)_3$), 145.7 ($C7$), 153.2 (2C, $C9'-OCH_2-C_6H_2(OCH_3)_3$), 191.2 ($C9$). IR (film) cm^{-1} : 1730 (COOR), 1669 (CHO). Anal. ($C_{32}H_{32}O_{11}$) C, H, N. HRMS: calcd for $C_{32}H_{32}O_{11}Na$, 615.1837; found 615.1844. $[\alpha]^{20}_D -108^\circ$ (c 1%).

3-Bromopropyl 9-Deoxy-9-oxo- α -apicropodophyllate (16). From 3-bromopropyl picropodophyllate **9** (630 mg, 1.14 mmol)

to yield aldehyde **16** (520 mg, 86%). ^1H NMR (CDCl_3): δ 2.05 (m, 2H, $\text{C9}'\text{-CH}_2\text{CH}_2\text{CH}_2\text{Br}$), 3.26 (t, 2H, $J = 6.6$ Hz, $\text{C9}'\text{-OCH}_2\text{CH}_2\text{CH}_2\text{Br}$), 4.16 (m, 2H, $\text{C9}'\text{-OCH}_2\text{CH}_2\text{CH}_2\text{Br}$), 7.34 (s, 1H, H7), 9.60 (s, 1H, H9). ^{13}C NMR (CDCl_3): δ 29.2 ($\text{C9}'\text{-OCH}_2\text{CH}_2\text{CH}_2\text{Br}$), 31.5 ($\text{C9}'\text{-OCH}_2\text{CH}_2\text{CH}_2\text{Br}$), 62.9 ($\text{C9}'\text{-OCH}_2\text{CH}_2\text{CH}_2\text{Br}$), 145.7 (C7), 191.2 (C9). IR (film) cm^{-1} : 1731 (COOR), 1669 (CHO). Anal. ($\text{C}_{25}\text{H}_{25}\text{BrO}_8\text{Na}$) C, H, N. HRMS: calcd for $\text{C}_{25}\text{H}_{25}\text{BrO}_8\text{Na}$, 555.0625; found 555.0635.

9-Deoxy-9-oxo- α -apopicropodophylllic Acid Pyrrolidinyl Amide (19). From the diol-amide **17** (167 mg, 0.344 mmol). The reaction product was chromatographed on silica gel, eluting with 50% acetone/ CH_2Cl_2 to give compound **19** (144 mg, 90%). ^1H NMR (CDCl_3): δ 1.65–1.98 (m, 4H, $\text{C9}'\text{-N}(\text{CH}_2)_4$), 3.05 (m, 1H, $\text{C9}'\text{-N}(\text{CH}_2)_4$), 3.28 (m, 1H, $\text{C9}'\text{-N}(\text{CH}_2)_4$), 3.43 (m, 1H, $\text{C9}'\text{-N}(\text{CH}_2)_4$), 3.70 (m, 1H, $\text{C9}'\text{-N}(\text{CH}_2)_4$), 7.33 (s, 1H, H7), 9.53 (s, 1H, H9). ^{13}C NMR (CDCl_3): δ 24.3, 26.0, 46.0, 47.0 ($\text{C9}'\text{-N}(\text{CH}_2)_4$), 134.4 (C8), 146.8 (C7), 191.9 (C9). IR (film) cm^{-1} : 1668 (CHO), 1636 (CON). Anal. ($\text{C}_{26}\text{H}_{27}\text{NO}_7$) C, H, N. HRMS: calcd for $\text{C}_{26}\text{H}_{27}\text{NO}_7 + \text{H}$, 466.1860; found 466.1862. $[\alpha]_{\text{D}}^{20} = -200^\circ$ (c 0.85%).

N-Ethyl 9-Deoxy-9-oxo- α -apopicropodophyllamide (20). From the diol-amide **18** (80 mg, 0.17 mmol). The reaction product was chromatographed on silica gel, eluting with 50% EtOAc/ CH_2Cl_2 to give compound **20** (26 mg, 35%). ^1H NMR (CDCl_3): δ 1.04 (t, 3H, $J = 7.2$ Hz, $\text{C9}'\text{-NHCH}_2\text{CH}_3$), 3.17 (m, 2H, $\text{C9}'\text{-NHCH}_2\text{CH}_3$), 7.35 (s, 1H, H7), 9.57 (s, 1H, H9). ^{13}C NMR (CDCl_3): δ 14.7 ($\text{C9}'\text{-NHCH}_2\text{CH}_3$), 34.5 ($\text{C9}'\text{-NHCH}_2\text{CH}_3$), 132.9 (C8), 148.1 (C7), 193.5 (C9). IR (film) cm^{-1} : 3367 (NH), 1667 (CHO), 1640 (CONH). Anal. ($\text{C}_{24}\text{H}_{25}\text{NO}_7$) C, H, N. HRMS: calcd for $\text{C}_{24}\text{H}_{25}\text{NO}_7 + \text{H}$, 440.1704; found 440.1713. $[\alpha]_{\text{D}}^{20} = -190^\circ$ (c 0.31%).

N-(4-Methoxyphenyl) 9-Deoxy-9-oxo- α -apopicropodophyllamide (21). A mixture of aldehyde **2** (100 mg, 0.235 mmol), 1,2-ethanedithiol (0.040 mL, 0.47 mmol), and chlorotrimethylsilane (7 μL , 0.05 mmol) in dry CH_2Cl_2 (10 mL) was stirred at room temperature for 24 h. The crude reaction product was diluted with CH_2Cl_2 , washed with 4% NaOH and brine, dried over Na_2SO_4 , filtered, and concentrated in vacuum to yield the protected aldehyde-ester (100 mg, 84%) that was dissolved in MeOH (10 mL), treated with 5% KOH/MeOH (20 mL), and stirred for 22 h. Then 0.2 M NaH_2PO_4 (200 mL) was added to the reaction mixture and MeOH removed in vacuum and then acidified to pH = 4 by adding 2N HCl and extracted with EtOAc. The organic phase was washed with brine, dried, filtered, and evaporated off to give the free acid.

A solution of the dithiolane-acid (69 mg) in dry THF (4 mL) was treated with *p*-anisidine (280 mg, 2.28 mmol), dicyclohexylcarbodiimide (29 mg, 0.14 mmol), and 1-hydroxybenzotriazol (19 mg, 0.14 mmol) at room temperature for 21 h. The reaction mixture was diluted with EtOAc, washed with 2 N HCl, satd aq Na_2CO_3 , and brine. The organic phase was dried, filtered, and concentrated to yield the protected amide. A solution of this amide (70 mg, 0.12 mmol) in acetic acid (4.0 mL) was treated with SeO_2 (65 mg, 0.59 mmol), and the mixture was stirred at room temperature for 24 h. The crude was extracted with EtOAc, and the organic phase was washed with satd aq NaHCO_3 and NaCl, dried over Na_2SO_4 , and evaporated off. The reaction product was chromatographed on silica gel, eluting with 10% EtOAc/ CH_2Cl_2 to give compound **21** (24 mg, 20% from aldehyde **2**). ^1H NMR (CDCl_3): δ 3.76 (s, 3H, $\text{C9}'\text{-NH-C}_6\text{H}_4\text{-OCH}_3$), 6.78 and 7.39 (AB system, $J = 9.0$ Hz, $\text{C9}'\text{-NH-C}_6\text{H}_4\text{-OCH}_3$), 7.40 (s, 1H, H7), 8.27 (s, 1H, NH), 9.60 (s, 1H, H9). ^{13}C NMR (CDCl_3): δ 55.5 ($\text{C9}'\text{-NH-C}_6\text{H}_4\text{-OCH}_3$), 114.1 (2CH, $\text{C9}'\text{-NH-C}_6\text{H}_4\text{-OCH}_3$), 121.1 (2CH, $\text{C9}'\text{-NH-C}_6\text{H}_4\text{-OCH}_3$), 131.2 (C, $\text{C9}'\text{-NH-C}_6\text{H}_4\text{-OCH}_3$), 131.9 (C8), 148.6 (C7), 156.3 (C, $\text{C9}'\text{-NH-C}_6\text{H}_4\text{-OCH}_3$), 168.4 (C9), 193.9 (C9). IR (film) cm^{-1} : 3328 (NH), 1668 (CHO), 1640 (CONH). Anal. ($\text{C}_{29}\text{H}_{27}\text{NO}_8$) C, H, N. HRMS: calcd for $\text{C}_{29}\text{H}_{27}\text{NO}_8 + \text{H}$, 518.1809; found 518.1827.

General Methods for the Synthesis of Imines. A mixture of aldehyde (1 equiv), anhydrous MgSO_4 (4 equiv), and the corresponding amine (2 equiv) was dissolved in CH_2Cl_2 (3 mL) and stirred at room temperature during 3–13 d. The reaction mixture was diluted with CH_2Cl_2 , filtered, and concentrated under vacuum to give the imines in quantitative yields. Imines were obtained pure enough to carry on complete characterization without being necessary any purification step. Imines **22–24** have been described previously.¹²

Ethyl 9-Deoxy-9-propylimino- α -apopicropodophyllate (25). From aldehyde **10** (150 mg, 0.340 mmol) and propyl amine (56 μL , 0.82 mmol) during 2 d to yield imine **25** (135 mg, 83%). ^1H NMR (CDCl_3): δ 0.82 (t, 3H, $J = 7.3$, $\text{NCH}_2\text{CH}_2\text{CH}_3$), 1.11 (t, 3H, $J = 7.2$, $\text{C9}'\text{-OCH}_2\text{CH}_3$), 1.58 (m, 2H, $\text{NCH}_2\text{CH}_2\text{CH}_3$), 3.40 (m, 2H, $\text{NCH}_2\text{CH}_2\text{CH}_3$), 4.05 (m, 2H, $\text{C9}'\text{-OCH}_2\text{CH}_3$), 6.78 (s, 1H, H7), 7.94 (s, 1H, H9). ^{13}C NMR (CDCl_3): δ 11.7 ($\text{NCH}_2\text{CH}_2\text{CH}_3$), 14.1 ($\text{C9}'\text{-OCH}_2\text{CH}_3$), 24.1 ($\text{NCH}_2\text{CH}_2\text{CH}_3$), 60.9 ($\text{C9}'\text{-OCH}_2\text{CH}_3$), 63.1 ($\text{NCH}_2\text{CH}_2\text{CH}_3$), 131.7 (C8), 135.1 (C7), 161.2 (C9), 172.6 (C9'). IR (film) cm^{-1} : 1726 (COOCH_3). HRMS: calcd for $\text{C}_{27}\text{H}_{21}\text{NO}_7 + \text{H}$, 482.2173; found 482.2170.

3,4,5-Trimethoxybenzyl 9-Deoxy-9-propylimino- α -apopicropodophyllate (26). From aldehyde **15** (90 mg, 0.15 mmol) and propylamine (25 μL , 0.30 mmol) during 3 d. ^1H NMR (CDCl_3): δ 0.76 (t, 3H, $J = 7.2$, $\text{NCH}_2\text{CH}_2\text{CH}_3$), 1.50 (m, 2H, $\text{NCH}_2\text{CH}_2\text{CH}_3$), 3.40 (m, 2H, $\text{NCH}_2\text{CH}_2\text{CH}_3$), 3.78 (s, 6H, $\text{C9}'\text{-OCH}_2\text{-C}_6\text{H}_2(\text{OCH}_3)_3$), 3.81 (s, 3H, $\text{C9}'\text{-OCH}_2\text{-C}_6\text{H}_2(\text{OCH}_3)_3$), 4.96 (s, 2H, $\text{C9}'\text{-OCH}_2\text{-C}_6\text{H}_2(\text{OCH}_3)_3$), 6.41 (s, 2H, $\text{C9}'\text{-OCH}_2\text{-C}_6\text{H}_2(\text{OCH}_3)_3$), 6.80 (s, 1H, H7), 7.94 (s, 1H, H9). ^{13}C NMR (CDCl_3): δ 11.7 ($\text{NCH}_2\text{CH}_2\text{CH}_3$), 24.1 ($\text{NCH}_2\text{CH}_2\text{CH}_3$), 56.1 (2C, $\text{C9}'\text{-OCH}_2\text{-C}_6\text{H}_2(\text{OCH}_3)_3$), 60.9 (1C, $\text{C9}'\text{-OCH}_2\text{-C}_6\text{H}_2(\text{OCH}_3)_3$), 63.1 ($\text{NCH}_2\text{CH}_2\text{CH}_3$), 66.9 ($\text{C9}'\text{-OCH}_2\text{-C}_6\text{H}_2(\text{OCH}_3)_3$), 105.1 (2CH, $\text{C9}'\text{-OCH}_2\text{-C}_6\text{H}_2(\text{OCH}_3)_3$), 131.5 (1C, $\text{C9}'\text{-OCH}_2\text{-C}_6\text{H}_2(\text{OCH}_3)_3$), 131.6 (C8), 135.2 (C7), 137.7 (1C, $\text{C9}'\text{-OCH}_2\text{-C}_6\text{H}_2(\text{OCH}_3)_3$), 153.2 (2C, $\text{C9}'\text{-OCH}_2\text{-C}_6\text{H}_2(\text{OCH}_3)_3$), 161.1 (C9), 172.4 (C9'). IR (film) cm^{-1} : 1726 (COOR). HRMS: calcd for $\text{C}_{35}\text{H}_{40}\text{NO}_{10} + \text{H}$, 634.2647; found, 634.2658.

General Methods for the Synthesis of Amines. Method C. The solution of the imine, obtained as described above, in MeOH (20 mL) was treated with excess of NaBH_4 at 0 $^\circ\text{C}$ for 1 h. Water was added to the reaction mixture, and MeOH was partially removed in vacuum to give an aqueous solution that was adjusted with satd Na_2CO_3 to pH > 7 and then extracted with EtOAc. The organic phase was washed with brine, dried over Na_2SO_4 , and finally filtered and solvent removed to give the corresponding amine.

Method D. The aldehyde and the corresponding amine were dissolved in CH_2Cl_2 , and montmorillonite K-10 was added and well mixed. The removal of the solvent gave a solid mixture, which was subjected to microwave irradiations during 1–2 min. The mixture was extracted with EtOAc and the organic phase was dried over Na_2SO_4 , filtered, and evaporated off to get a reaction product that was reduced with NaBH_4 , as described above for method C.

Methyl 9-Ethylamino-9-deoxy- α -apopicropodophyllate (27). From imine **22** (92 mg, 0.21 mmol) and NaBH_4 (92 mg, 2.4 mmol) using method C. The reaction product was chromatographed on silica gel, eluting with 50% EtOAc/MeOH to give compound **27** (42 mg, 40%). ^1H NMR (CDCl_3): δ 0.93 (t, 3H, $J = 6.9$, NHCH_2CH_3), 2.30 (m, 2H, NHCH_2CH_3), 3.30 and 3.48 (AB system, $J = 14.4$, H9), 3.61 (m, 1H, H8'), 3.62 (s, 3H, $\text{C9}'\text{-OCH}_3$), 3.74 (s, 6H, H10', H12'), 3.77 (s, 3H, H11'), 4.48 (d, 1H, $J = 3.7$, H7'), 5.90 (d, 1H, $J = 1.6$, H10a), 5.92 (d, 1H, $J = 1.6$, H10b), 6.27 (s, 2H, H2', H6'), 6.44 (s, 1H, H7), 6.56 (s, 1H, H3), 6.65 (s, 1H, H6). ^{13}C NMR (CDCl_3): δ 14.5 (NHCH_2CH_3), 42.6 (NHCH_2CH_3), 48.6 (C7'), 49.7 (C8'), 52.1 ($\text{C9}'\text{-OCH}_3$), 53.4 (C9), 56.2 (C10', C12'), 60.7 (C11'), 101.0 (C10), 105.4 (C2', C6'), 106.9 (C6), 109.5 (C3), 126.4 (C7), 127.2 (C1), 129.1 (C2), 131.0 (C8), 137.1 (C4'), 138.3 (C1'), 146.9 (C5), 147.2 (C4), 153.1 (C3', C5'), 172.9 (C9'). IR (film) cm^{-1} : 3400

(NH), 1732 (COOR). HRMS: calcd for $C_{25}H_{29}NO_7 + H$, 456.2017; found, 456.2003.

Methyl 9-Propylamino-9-deoxy- α -apopicropodophyllate (28). From imine **23** (155 mg, 0.33 mmol) and $NaBH_4$ (155 mg, 4.09 mmol) using method C. The reaction product was chromatographed on silica gel, eluting with 5% EtOH/ CH_2Cl_2 to give compound **28** (100 mg, 64%). 1H NMR ($CDCl_3$): δ 0.77 (t, 3H, $J = 7.5$, $NHCH_2CH_2CH_3$), 1.30 (m, 2H, $NHCH_2CH_2CH_3$), 2.25 (m, 2H, $NHCH_2CH_2CH_3$), 3.29 and 3.48 (AB system, $J = 14.4$, H9), 6.45 (s, 1H, H7). ^{13}C NMR ($CDCl_3$): δ 11.6 ($NHCH_2CH_2CH_3$), 22.5 ($NHCH_2CH_2CH_3$), 50.0 ($NHCH_2CH_2CH_3$), 53.6 (C9), 126.5 (C7), 131.1 (C8). IR (film) cm^{-1} : 3396 (NH), 1729 (COOCH₃). HRMS: calcd for $C_{26}H_{31}NO_7 + H$, 470.2173; found, 479.2183.

Methyl 9-(4-Methoxyphenyl)amino-9-deoxy- α -apopicropodophyllate (29). From aldehyde **2** (100 mg, 0.230 mmol) and *p*-anisidine (296 mg, 2.35 mmol) on K-10 montmorillonite (1.2 g) using method D during 2 min. The reaction product was chromatographed on silica gel, eluting with 10% EtOAc/ CH_2Cl_2 to give compound **29** (50 mg, 40%). 1H NMR ($CDCl_3$): δ 3.72 (s, 3H, $NH-(C_6H_4)-OCH_3$), 3.75 (m, 2H, H9a, H9b), 6.50 (s, 1H, H7), 6.40 and 6.69 (AB system $J = 9.0$, $NH-(C_6H_4)-OCH_3$). ^{13}C NMR ($CDCl_3$): δ 49.7 (C9), 55.6 ($NH-(C_6H_4)-OCH_3$), 113.8 (2CH, $NH-(C_6H_4)-OCH_3$), 114.7 (2CH, $NH-(C_6H_4)-OCH_3$), 124.8 (C7), 131.2 (C8), 141.9 (C, $NH-(C_6H_4)-OCH_3$), 152.0 (C, $NH-(C_6H_4)-OCH_3$). IR (film) cm^{-1} : 3400 (NH), 1728 (COOCH₃). HRMS: calcd for $C_{30}H_{31}NO_8 + H$, 534.2122; found, 534.2137.

Methyl 9-(4-Methylphenyl)amino-9-deoxy- α -apopicropodophyllate (30). From aldehyde **2** (100 mg, 0.235 mmol) and *p*-toluidine (251 mg, 2.35 mmol) using method D during 1 min. The reaction product was chromatographed on silica gel, eluting with 5% EtOAc/ CH_2Cl_2 to give compound **30** (80 mg, 71%). 1H NMR ($CDCl_3$): δ 2.20 (s, 3H, $NH-(C_6H_4)-CH_3$), 3.75 (m, 2H, H9), 6.50 (s, 1H, H7), 6.35 and 6.89 (AB system, $J = 8.1$, $NH-(C_6H_4)-CH_3$). ^{13}C NMR ($CDCl_3$): δ 20.3 ($NH-(C_6H_4)-CH_3$), 49.1 (C9), 112.8 (2CH, $NH-(C_6H_4)-CH_3$), 124.8 (C7), 126.6 (C, $NH-(C_6H_4)-CH_3$), 129.6 (2CH, $NH-(C_6H_4)-CH_3$), 131.2 (C8), 145.6 (C, $NH-(C_6H_4)-CH_3$). IR (film) cm^{-1} : 3407 (NH), 1729 (COOCH₃). HRMS: calcd for $C_{30}H_{31}NO_7 + H$, 518.2173; found, 518.2200.

Ethyl 9-Propylamino-9-deoxy- α -apopicropodophyllate (31). From imine **25** (135 mg, 0.28 mmol) and $NaBH_4$ (135 mg, 3.57 mmol) using method C to yield amine **31** (100 mg, 74%). 1H NMR ($CDCl_3$): δ 0.76 (t, 3H, $J = 7.3$, $NHCH_2CH_2CH_3$), 1.30 (m, 2H, $NHCH_2CH_2CH_3$), 2.20 (m, 2H, $NHCH_2CH_2CH_3$), 3.25 (d, 1H, $J = 14.3$, H9a), 3.47 (d, 1H, $J = 14.3$, H9b), 6.40 (s, 1H, H7). ^{13}C NMR ($CDCl_3$): δ 11.7 ($NHCH_2CH_2CH_3$), 23.1 ($NHCH_2CH_2CH_3$), 50.7 ($NHCH_2CH_2CH_3$), 53.9 (C9), 125.3 (C7), 132.6 (C8). IR (film) cm^{-1} : 3350 (NH), 1725 (COOR). Anal. ($C_{27}H_{33}NO_7$) C, H, N. HRMS: calcd for $C_{27}H_{33}NO_7 + H$, 484.2330; found, 484.2338.

3,4,5-Trimethoxybenzyl 9-Propylamino-9-deoxy- α -apopicropodophyllate (32). From imine **26** (90 mg, 0.14 mmol) and $NaBH_4$ (135 mg, 3.57 mmol) using method C. The reaction product was chromatographed on silica gel, eluting with 7% EtOH/ CH_2Cl_2 to give compound **32** (44 mg, 45%). 1H NMR ($CDCl_3$): δ 0.74 (t, 3H, $J = 7.3$, $NHCH_2CH_2CH_3$), 1.25 (m, 2H, $NHCH_2CH_2CH_3$), 2.17 (m, 2H, $NHCH_2CH_2CH_3$), 3.26 and 3.45 (AB system, $J = 14.6$, H9), 6.44 (s, 1H, H7). ^{13}C NMR ($CDCl_3$): δ 11.6 ($NHCH_2CH_2CH_3$), 23.0 ($NHCH_2CH_2CH_3$), 50.6 ($NHCH_2CH_2CH_3$), 54.0 (C9), 125.7 (C7), 132.0 (C8). IR (film) cm^{-1} : 3345 (NH), 1726 (COOR). HRMS: calcd for $C_{35}H_{41}NO_{10} + H$, 636.2803; found, 636.2831.

9-Ethylamino-9-deoxy- α -apopicropodophyllilic Acid Pyrrolidinyl Amide (33). Following the general method for the preparation of imines, aldehyde **19** (80 mg, 0.17 mmol) reacted with ethyl amine (70% in water, 0.390 mL, 6.88 mmol) during 12 d to yield the corresponding imine, which was directly reduced to the amine **33** using method C (50 mg, 68%). 1H NMR ($CDCl_3$): δ

1.11 (t, 3H, $J = 7.1$, $NHCH_2CH_3$), 2.65 (m, 2H, $NHCH_2CH_3$), 3.33 and 3.44 (AB system, $J = 11.0$, H9), 6.48 (s, 1H, H7). ^{13}C NMR ($CDCl_3$): δ 14.2 ($NHCH_2CH_3$), 43.0 ($NHCH_2CH_3$), 52.7 (C9), 128.1 (C7), 131.8 (C8). IR (film) cm^{-1} : 3300 (NH), 1629 (CON). HRMS: calcd for $C_{28}H_{34}N_2O_6 + H$, 495.2490; found, 495.2508.

9-(4-Methoxyphenyl)amino-9-deoxy- α -apopicropodophyllilic Acid Pyrrolidinyl Amide (34). The corresponding imine, obtained from aldehyde **19** (80 mg, 0.17 mmol) and *p*-anisidine (847 mg, 6.88 mmol) during 12 d, was directly reduced using method C. The reaction product was chromatographed on silica gel, eluting with 50% acetone/ CH_2Cl_2 to give compound **34** (70 mg, 71%). 1H NMR ($CDCl_3$): δ 3.72 (s, 3H, $NH-(C_6H_4)-OCH_3$), 3.73 and 3.95 (AB system, $J = 15.5$, H9), 6.53 (s, 1H, H7), 6.54 (d, 2H, $J = 9.1$, $NH-(C_6H_4)-OCH_3$), 6.73 (d, 2H, $J = 9.1$, $NH-(C_6H_4)-OCH_3$). ^{13}C NMR ($CDCl_3$): δ 49.1 (C9), 55.8 ($NH-(C_6H_4)-OCH_3$), 114.0 (2CH, $NH-(C_6H_4)-OCH_3$), 114.8 (2CH, $NH-(C_6H_4)-OCH_3$), 125.8 (C7), 134.3 (C8), 142.6 (C, $NH-(C_6H_4)-OCH_3$), 152.3 (C, $NH-(C_6H_4)-OCH_3$). IR (film) cm^{-1} : 3341 (NH), 1625 (CON). HRMS: calcd for $C_{33}H_{36}N_2O_7 + H$, 573.2595; found, 573.2600.

Cell Growth Inhibition Assays. A colorimetric assay using sulforhodamine B (SRB) was adapted for a quantitative measurement of cell growth and viability, following a previously described method.²⁸ Cells were seeded in 96-well microtiter plates, at 5×10^3 cells per well in aliquots of 195 μ L of RPMI medium, and they were allowed to attach to the plate surface by growing in a drug-free medium for 18 h. Afterward, samples were added in aliquots of 5 μ L (dissolved in DMSO/ H_2O , 3:7). After 72 h exposure, the antitumor effect was measured by the SRB methodology: cells were fixed by adding 50 μ L of cold 50% (wt/vol) trichloroacetic acid (TCA) and incubating for 60 min at 4 °C. Plates were washed with deionized water and dried; 100 μ L of SBR solution (0.4 wt %/vol in 1% acetic acid) was added to each microtiter well and incubated for 10 min at room temperature. Unbound SRB was removed by washing with 1% acetic acid. Plates were air-dried, and bound stain was solubilized with Tris buffer. Optical densities were read on an automated spectrophotometer plate reader at single wavelength of 490 nm. Data analyses were generated automatically by the LIMS implementation. Using control OD values (C), test OD values (T) and time zero OD values (T_0), the drug concentration that caused a 50% growth inhibition (GI₅₀ value) was calculated from the equation: $100[(T - T_0)/(C - T_0)] = 50$. Each value represents the mean from triplicate determinations.

Cell Cycle Analysis. For cell cycle analysis, we used the human colon adenocarcinoma HT-29 cell line grown in RPMI supplemented with 10% (v/v) heat-inactivated fetal calf serum, 2 mM L-glutamine, 100 units/mL penicillin, and 100 μ g/mL streptomycin at 37 °C in a humidified atmosphere of 5% CO_2 and 95% air. Cells were periodically tested for *Mycoplasma* infection and found to be negative. Untreated and drug-treated cells ($(3-5) \times 10^5$) were centrifuged and fixed overnight in 70% ethanol at 4 °C. Then cells were washed three times with PBS, incubated for 1 h with 1 mg/mL RNase A and 20 μ g/mL propidium iodide at room temperature, and analyzed with a Becton Dickinson FACSCalibur flow cytometer (San Jose, CA) as described previously.^{30,31} Quantification of apoptotic cells was calculated as the percentage of cells in the sub-G1 peak in cell cycle analysis.

Confocal Microscopy. HT-29 cells were grown on poly-L-lysine coated coverslips, and after drug treatment coverslips were washed three times with HPEM (25 mM HEPES, 60 mM PIPES, 10 mM EGTA, 3 mM $MgCl_2$, pH6.6), fixed with 4% paraformaldehyde in HPEM buffer for 15 min, and permeabilized with 0.5% Triton X-100 as previously described.³² Coverslips were incubated with a specific Ab-1 antialpha-tubulin mouse monoclonal antibody (diluted 1:150 in PBS) (Calbiochem) for 1 h, washed four times with PBS, and then incubated with CY3-conjugated sheep antimouse IgG

(Jackson ImmunoResearch) for 1 h at 4 °C. After washing four times with PBS, cell nuclei were stained with DAPI (Sigma) for 5–10 min, washed with PBS, and then samples were analyzed by confocal microscopy using a ZeissLSM310 laser scan confocal microscope. A drop of SlowFade light antifading reagent (Molecular Probes) was added to preserve fluorescence. Negative controls, lacking the primary antibody or using an irrelevant antibody, showed no staining.

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Supporting Information Available: Complete ^1H and ^{13}C NMR data for all synthesized analogues. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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