

Antitumor Agents. 113.[†] New 4 β -Arylamino Derivatives of 4'-O-Demethylepipodophyllotoxin and Related Compounds as Potent Inhibitors of Human DNA Topoisomerase II

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A number of 4'-O-demethylepipodophyllotoxin derivatives possessing various 4 β -N-, 4 β -O- or 4 β -S-aromatic rings have been synthesized and evaluated for their inhibitory activity against the human DNA topoisomerase II as well as for their activity in causing cellular protein-linked DNA breakage. The results indicated, that for DNA topoisomerase II, a basic unsubstituted 4 β -anilino moiety is structurally required for the enhanced activity. Substitution on this moiety with CN, COOCH₃, COOC₂H₅, OH and COOCH₃, OCH₃, COCH₃, CH₂OH, OCH₂O, OCH₂CH₂O, phenoxy, morpholino, NO₂, and NH₂ either at the para and/or the meta position yielded compounds which are as potent or more potent than etoposide. Substitution with COOC₂H₅ and OH at the ortho position afforded inactive compounds. Replacement of the aryl nitrogen with oxygen or sulfur gave compounds which are much less active or inactive. However, replacement of the phenyl ring with a pyridine nucleus furnished compounds which are as active or slightly more active than etoposide. There is a lack of correlation between the ability of these compounds in inhibiting DNA topoisomerase II and in causing protein-linked DNA breaks.

The clinical efficacy and intriguing mechanism of the podophyllotoxin-derived glucoside, etoposide (VP-16, 1), has greatly stimulated interest in further studies on the modification of the C-4 substituent of 1 for better antitumor activity.¹⁻⁵ Since one of the principal mechanisms of action of 1 is the inhibition of catalytic activity of DNA topoisomerase II and concurrent enzyme-mediated production of lethal DNA strand breaks, a systematic study on the synthesis and evaluation of simpler analogues related to 1 with enhanced activity and/or activity against 1-resistant tumor cell lines, using DNA topoisomerase II as a target enzyme, has been undertaken in our laboratories.⁶⁻¹¹ In our previous studies, replacement of the 4 β -O-glucosidic substituent of 1 with 4 β -N-hydroxyalkyl and 4 β -hydroxylated and halogenated anilino moieties have yielded a number of compounds which are as potent or more potent than 1 in inhibiting the human DNA topoisomerase II and causing cellular protein-linked DNA breakage (e.g. 2-4).⁹⁻¹¹ In addition, the 1 resistant KB cells developed by us were found to be less resistant to at least one of such compounds, such as 2. These significant findings prompted us to further synthesize and evaluate various new 4-arylamino derivatives and related compounds originated from 4'-O-demethylepipodophyllotoxin (5) as potent inhibitors of DNA topoisomerase II and as useful antitumor agents. It should be noted that extensive computer-assisted molecular modeling studies were employed; and that some of the compounds¹² described herein were predicted, prior to their laboratory preparation, to exhibit biological activity on the basis of our modeling hypothesis. Moreover, all of the active compounds fit the computational models and will be described in a subsequent publication.¹³

Chemistry

The synthesis of 4-arylamino derivatives of 5, in general,

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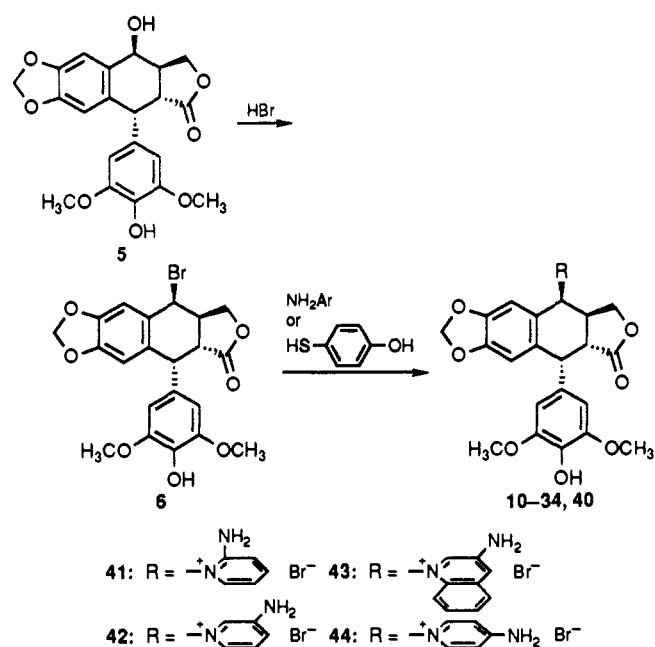
[†] For part 112, see: Kuo, Y. H.; Chen, C. H.; King, M. L.; Yang Kuo, L. M.; Wu, T. S.; Haruna, M.; Lee, K. H. *J. Nat. Prod.* 1990, 53, 422.

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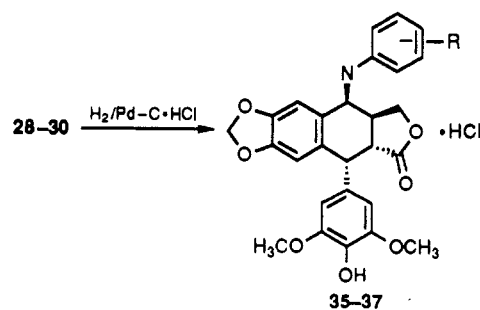
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Scheme I



Scheme II

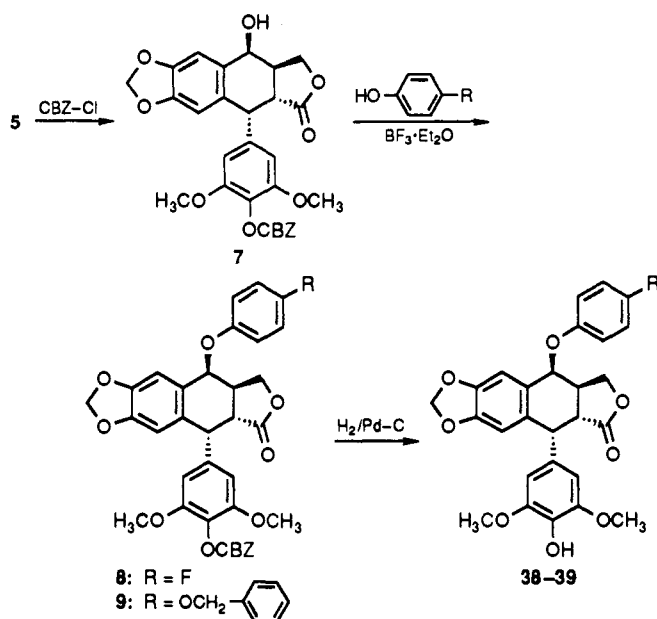


followed our previous procedure.¹¹ As shown in Scheme I, the target compounds (10-31) of the 4-arylamino series

(1) Jardine, I. In *Anticancer Agents Based on Natural Product Models*; Cassady, J. M., Douros, J., Eds.; Academic Press: New York, 1980; p 319 and references therein.

(2) Levy, R. K.; Hall, I. H.; Lee, K. H. *J. Pharm. Sci.* 1983, 72, 1158.

Scheme III



were prepared by nucleophilic substitution of appropriate arylamines with 4 β -bromo-4'-desoxypodophyllotoxin (6), which was obtained by bromination of 5 as described previously.¹¹ The yields in this synthesis were in a range of 35–65%, calculated from 5. It is noteworthy, however, when this same method was used for the synthesis of 32–34, using 2-amino- and 3-aminopyridines and 3-aminoquinoline, respectively, as starting materials, quaternization of the pyridine nitrogen occurred. The pyridinium salts (41–43) were isolated as the predominant reaction products.¹⁴ When 4-aminopyridine was used as a starting material for the same reaction, compound 44 was obtained quantitatively, as the quaternization took place only on the ring nitrogen due to the formation of a partly zwitterionic¹¹ (bond-delocalized) formula as reported previously by Walker et al.^{15,16}

Scheme II showed the preparation of diamino compounds (35–37) from their corresponding nitro substances (28–30) by catalytic hydrogenation. In this transformation, an increasing amount of Pd–C in an acidic solvent was used to offset the inactivating effect of the amino group on Pd–C. Compounds 35–37 were obtained as stable hydrochloric acid salts.

The synthesis of 38 and 39 was based on the procedure of Saito et al.⁵ and shown in Scheme III. Compound 7, which was prepared⁹ previously from 5, was condensed with 4-fluorophenol and 4-(benzyloxy)phenol in the presence of BF₃–Et₂O¹⁷ to afford 8 and 9, respectively. Removal of the phenolic protecting group by catalytic hydrogenation furnished 38 and 39. The preparation of 40 was achieved by treatment of 6 with 4-mercaptophenol as shown in Scheme I.

As discussed previously,¹¹ compounds 10–34 and 40 were synthesized by nucleophilic substitution from the bromo compound (6) with appropriate arylamines or mercaptophenol via a S_N1 mechanism which occurred on the C-4 benzylic carbonium ion. The bulky C-1 α pendant aromatic ring directed the substitution to be stereoselective, resulting in the formation of C-4 β -oriented 10–34 and 40 as the main products.^{9,11}

Results and Discussion

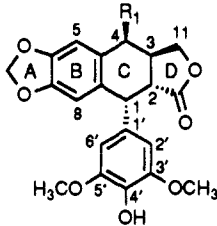
As illustrated in Table I, the 4 β -arylamino compounds 10–12, 14, 15, 17, 19–22, 24–29, 32–35, and 36 showed comparable or superior activity to 1 in inhibiting the human DNA topoisomerase II and causing cellular protein–DNA strand breakage. All of these compounds, in general, possess substituents, such as CN (11, 12), COOCH₃ or COOC₂H₅ (14, 15), OH and COOCH₃ (17), OCH₃ (19, 22), COCH₃ (20), CH₂OH (21), OCH₂O (24), OCH₂CH₂O (25), phenoxy (26), morpholino (27), NO₂ (28, 29), and NH₂ (35, 36) at either 3''- and/or 4''-position. In terms of their activity against DNA topoisomerase II the most active compounds are 4, 15, and 36, which are 10-fold more potent than 1. Those which are at least 2-fold more active than 1 include 3, 10, 11, 18, 19, 21, 22, 25, 26, 27, 29, and 35. Substitution occurred at the 2''-position of 10 which led to inactive compounds (13, 30, and 37). Replacing the aryl nitrogen with either an oxygen (38 and 39) or a sulfur (40) gave rise to much less active or inactive compounds, respectively. Further replacement of the phenyl ring with a pyridine nucleus furnished compounds (32–34) which are as potent or slightly more potent than 1.

That the basic unsubstituted 4 β -anilino compound (10) is at least twice more potent than 1 would suggest the structural requirement of this activity-enhancing moiety, in which the basic nitrogen and the aromatic ring might be involved in binding to the target enzyme or cause a bond delocalization to generate a charged 4 β -N atom. This would facilitate the formation of the C-4 benzylic carbonium ion, thereby allowing the alkylation of the target enzyme to occur. When the potential of their activity is causing protein linked DNA breakage, as in compound 1, cells treated with those compounds at 10 μ M were examined. All these showed anti-DNA topoisomerase II activity could also cause protein linked DNA breaks in cells as shown in Table I. There was a lack of correlation between the ability of compounds in causing protein linked DNA

- Issell, B. F.; Muggia, F. M.; Carter, S. K. *Etoposide [VP-16] Current Status and New Developments*; Academic Press: New York, 1984.
- Saito, H.; Yoshikawa, H.; Nishimura, Y.; Kondo, S.; Takeuchi, T.; Umezawa, H. *Chem. Pharm. Bull.* 1986, 34, 3733.
- Saito, H.; Nishimura, Y.; Kondo, S.; Komuro, K.; Takeuchi, T.; *Bull. Chem. Soc. Jpn.* 1988, 61, 2493.
- Thurston, L. S.; Irie, H.; Tani, S.; Han, F. S.; Liu, Z. C.; Cheng, Y. C.; Lee, K. H. *J. Med. Chem.* 1986, 29, 1547.
- Beers, S. A.; Imakura, Y.; Dai, H. J.; Cheng, Y. C.; Lee, K. H. *J. Nat. Prod.* 1988, 51, 901.
- Thurston, L. S.; Imakura, Y.; Haruna, M.; Li, D. H.; Liu, Z. C.; Liu, S. Y.; Cheng, Y. C.; Lee, K. H. *J. Med. Chem.* 1989, 32, 604.
- Lee, K. H.; Imakura, Y.; Haruna, M.; Beers, S. A.; Thurston, L. S.; Dai, H. J.; Chen, C. H.; Liu, S. Y.; Cheng, Y. C. *J. Nat. Prod.* 1989, 52, 606.
- Liu, S. Y.; Hwang, B. D.; Haruna, M.; Imakura, Y.; Lee, K. H.; Cheng, Y. C. *Mol. Pharmacol.* 1989, 36, 78.
- Lee, K. H.; Beers, S. A.; Mori, M.; Wang, Z. Q.; Kuo, Y. H.; Li, L.; Liu, S. Y.; Cheng, Y. C. *J. Med. Chem.* 1990, 33, 1364.
- Compounds 15–17, 24, 32–34, 39, and 40 were designed by molecular modeling studies.
- Part of the molecular modeling studies were presented at the 198th Meeting of the American Chemical Society, Miami Beach, FL, 1989 (American Chemical Society: Washington, DC, 1989).
- Klingsber, E. In *Heterocyclic Compounds, Pyridine and its Derivatives*; John Wiley and Sons: New York, 1962; Part 3, pp 1–32.
- Walker, G. N.; Moore, M. A.; Weaver, B. N. *J. Org. Chem.* 1962, 26, 2740.

(16) Compounds 41–44 were characterized by spectral analyses (NMR, MS, and IR). Compound 43 showed only moderate activity (<50% inhibition at 100 μ M) in inhibiting DNA topoisomerase II, while 41, 42, and 44 were almost inactive.

(17) Keller-Juslen, C.; Kuhn, M.; Von Wartburg, A. *J. Med. Chem.* 1971, 14, 936.

Table I. Biological Evaluation of 4 β -(Arylamino)-4'-demethylpodophyllotoxin and Related Compounds


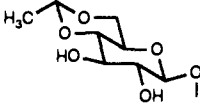
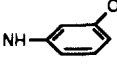
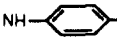
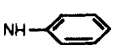
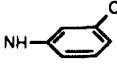
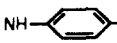
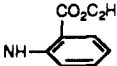
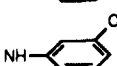
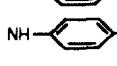
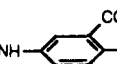
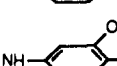
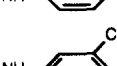
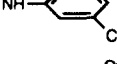
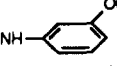
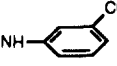
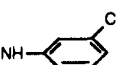
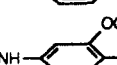
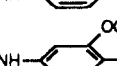
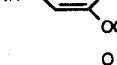
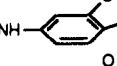
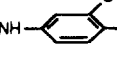
compd	R ₁	cytotoxicity: ^a ID ₅₀ KB, μ M	inhibition of DNA topoisomerase II activity: ^b ID ₅₀ , μ M	cellular protein- DNA complex formation, % (10 μ M)
1		0.20	50	100
2	NHCH ₂ CH ₂ OH	1.60		121
3		0.45	25	290
4		0.24	5	213
10		0.71	25	243
11		0.69	25	137
12		0.64	10	211
13		1.0	>100	4
14		2.7	50	249
15		0.84	5	207
16		1.0	50	83
17		5.8	50	129
18		<1.0	25	50
19		3.8	25	104
20		1.7	50	150
21		<1.0	25	235
22		<1.0	25	180
23		0.63	>100	47
24		<1.0	50	164
25		0.68	10	279
26		1.0	25	97
27		0.66	10	140

Table I (Continued)

compd	R ₁	cytotoxicity: ^a ID ₅₀ KB, μM	inhibition of DNA topoisomerase II activity: ^b ID ₅₀ , μM	cellular protein- DNA complex formation, % (10 μM)
28		1.0	50	230
29		0.49	10	323
30		1.0	>100	15
31		3.4	>100	21
32		0.71	50	97
33		0.24	50	148
34		<1.0	50	123
35		4.0	25	140
36		0.8	5	330
37		3.3	>100	11
38		0.5	>100	57
39		<1.0	50	34
40		<1.0	>100	10

^a ID₅₀ was the concentration of drug which affords 50% reduction in cell number after 3-day incubation. ^b Each compound was examined with five concentrations at 5, 10, 25, 50, and 100 μM. The ID₅₀ value was established on the basis of the degree of inhibition at these three concentrations.

breaks and inhibition of DNA topoisomerase II. This could be due to the difference of uptake or of the nature of the interaction of topoisomerase II and DNA stabilized by those compounds. The most interesting feature is the lack of correlation between protein linked DNA breaks and cytotoxicity. This could suggest the rate limit of each step for cytotoxicity of these class of compounds is not only this action on DNA topoisomerase II. Those issues are currently under investigation. In addition, further synthesis and evaluation of this class of compounds as potent DNA topoisomerase II inhibitors and antitumor agents are in progress.

Experimental Section

General Experimental Procedures. All melting points were taken on a Fischer-Johns melting point apparatus and were uncorrected. IR spectra were recorded on a Perkin-Elmer 1320 spectrophotometer, and ¹H NMR spectra were obtained by using a Bruker AC-300 NMR spectrometer; all chemical shifts were reported in ppm from TMS. Elemental analyses were performed by Atlantic Microlab, Inc., Norcross, GA. Optical rotations were measured with a Rudolph Research autopol III polarimeter. Analytical thin-layer chromatography (TLC) was carried out on Merck precoated silica gel 60 F-254. EM Kieselgel 60 (230–400 mesh ASTM) was used for column chromatography. All new target compounds were characterized by melting point, optical rotation, and ¹H NMR and IR spectral analyses, as well as, elemental analyses.

4'-O-Demethyl-4β-bromo-4-desoxytopodophyllotoxin (6). A solution of 4'-O-demethylepipodophyllotoxin (10 g, 24 mmol) in

250 mL of dry dichloromethane was kept at 0 °C, and dry hydrogen bromide gas was bubbled through the solution. After 45 min, nitrogen was also bubbled through the solution to drive off excess hydrogen bromide. The solution was then evaporated in vacuo, followed by using benzene as an azeotropic mixture to remove the water thoroughly formed in the reaction. The desired product (11.5 g) was obtained, which was used for the next reaction step without further purification.

Synthesis of Compounds 10–34 and 40. A solution containing 4'-O-demethyl-4β-bromo-4-desoxytopodophyllotoxin (6) (300 mg, 0.65 mmol), anhydrous barium carbonate (153 mg, 0.78 mmol), and the appropriate arylamine (0.78 mmol) in 7 mL of dry 1,2-dichloroethane under nitrogen was stirred overnight at room temperature. The reaction mixture was filtered, diluted with ethyl acetate, washed with water, dried over anhydrous magnesium sulfate, and purified via column chromatography (30 g silica gel with dichloromethane–acetone–ethyl acetate 100:5:5 or toluene–ethyl acetate 3:1 as an eluant).

4'-O-Demethyl-4β-anilino-4-desoxytopodophyllotoxin (10): yield 57.8%; crystals from methanol; mp 172–173 °C; [α]_D²⁵ –120° (c = 1.0, CHCl₃); IR (KBr) 3500 (OH), 3360 (NH), 2900 (aliphatic C–H), 1755 (lactone), 1595, 1500 and 1475 (aromatic C=C) cm⁻¹; ¹H NMR (CDCl₃) δ 7.22 (t, J = 7.5 Hz, 2 H, 3'',5''-H), 6.80 (m, 2 H, 4''-H and 5-H), 6.50 (m, 3 H, 2''-H, 6''-H, and 8-H), 6.33 (s, 2 H, 2',6'-H), 5.96 and 5.98 (AB q, J = 1.3 Hz, 2 H, OCH₂O), 5.42 (s, 1 H, exchangeable, 4'-OH), 4.68 (br, 1 H, 4-H), 4.60 (d, J = 4.9 Hz, 1-H), 4.38 (t, J = 8.4 Hz, 1 H, 11-H), 4.01 (t, J = 8.4 Hz, 1 H, 11-H), 3.85 (br, 1 H, exchangeable, NH), 3.79 (s, 6 H, 3',5'-OCH₃), 3.16 (dd, J = 4.9, 14.0 Hz, 1 H, 2-H), 3.00 (m, 1 H, 3-H).

Anal. (C₂₇H₂₅NO₇) C, H, N.

4'-O-Demethyl-4 β -(3''-cyanoanilino)-4-desoxypodophyllotoxin (11): yield 64.3%; crystals from methanol; mp 191–192 °C; $[\alpha]_D^{25}$ -117° ($c = 0.33$, CHCl₃); IR (KBr) 3450 (OH), 3360 (NH), 2900 (aliphatic C-H), 2225 (CN), 1750 (lactone), 1595, 1500 and 1450 (aromatic C=C) cm⁻¹; ¹H NMR (CDCl₃) δ 7.31 (t, $J = 7.6$ Hz, 5''-H), 7.07 (d, $J = 7.6$ Hz, 4''-H), 6.80 (d, 2 H, 2''-H, 6''-H), 6.74 (s, 1 H, 5-H), 6.55 (s, 1 H, 8-H), 6.33 (s, 2 H, 2',6'-H), 6.01 and 5.99 (AB q, $J = 1.3$ Hz, 2 H, OCH₂O), 5.48 (s, 1 H, exchangeable, 4'-OH), 4.69 (d, $J = 3.8$ Hz, 1 H, 4-H), 4.62 (d, $J = 4.5$ Hz, 1 H, 1-H), 4.41 (t, $J = 8.5$ Hz, 1 H, 11-H), 3.92 (t, $J = 8.5$ Hz, 1 H, 11-H), 3.81 (s, 6 H, 3',5'-OCH₃), 3.14–3.00 (m, 2 H, 2-H and 3-H).

Anal. (C₂₈H₂₄N₂O₇·¹/₂H₂O) C, H, N.

4'-O-Demethyl-4 β -(4''-cyanoanilino)-4-desoxypodophyllotoxin (12): yield 45.7%; crystals from ethanol; mp 187–189 °C; $[\alpha]_D^{25}$ -145° ($c = 1.0$, CHCl₃); IR (KBr) 3500 (OH), 3360 (NH), 2890 (aliphatic C-H), 2210 (CN), 1765 (lactone), 1600, 1510 and 1475 (aromatic C=C) cm⁻¹; ¹H NMR (CDCl₃) δ 7.50 (d, $J = 8.7$ Hz, 2 H, 3',5''-H), 6.74 (s, 1 H, 5-H), 6.57 (d, $J = 8.7$ Hz, 2 H, 2'',6''-H), 6.55 (s, 1 H, 8-H), 6.32 (s, 2 H, 2',6'-H), 5.99 and 5.97 (AB q, $J = 1.2$ Hz, 2 H, OCH₂O), 5.44 (s, 1 H, exchangeable, 4'-OH), 4.78 (m, 1 H, exchangeable, NH), 4.63 (d, $J = 4.2$ Hz, 1 H, 4-H), 4.36 (m, 2 H, 11-H), 3.85 (m, 1 H, 1-H), 3.79 (s, 6 H, 3',5'-OCH₃), 3.09 (dd, 1 H, 2-H), 3.05 (m, 1 H, 3-H). Anal. (C₂₈H₂₄N₂O₇) C, H, N.

4'-O-Demethyl-4 β -[2''-(ethoxycarbonyl)anilino]-4-desoxypodophyllotoxin (13): yield 51%; crystals from ethanol; mp 231–232 °C; $[\alpha]_D^{25}$ -102° ($c = 0.5$, CHCl₃); IR (KBr) 3500 (OH), 3330 (NH), 2900 (aliphatic C-H), 1770 (lactone), 1670 (ester), 1600, 1580, 1500 and 1480 (aromatic C=C) cm⁻¹; ¹H NMR (CDCl₃) δ 8.00 (dd, $J = 1.6$, 8.0 Hz, 1 H, 3''-H), 7.43 (t, $J = 8.0$ Hz, 1 H, 4''-H), 6.77 (s, 1 H, 5-H), 6.71 (t, 1 H, $J = 8.0$ Hz, 5''-H), 6.64 (d, 1 H, $J = 8.0$ Hz, 6''-H), 6.53 (s, 1 H, 8-H), 6.36 (s, 2 H, 2',6'-H), 5.97 and 5.95 (AB q, $J = 1.3$ Hz, 2 H, OCH₂O), 5.46 (s, 1 H, exchangeable, 4'-OH), 4.86 (d, $J = 4.2$ Hz, 1 H, 4-H), 4.63 (d, $J = 4.9$ Hz, 1 H, 1-H), 4.35 (t, $J = 7.3$ Hz, 1 H, 11-H), 4.28 (q, 2 H, $J = 7.2$ Hz, CO₂CH₂CH₃), 3.86 (t, $J = 7.3$ Hz, 1 H, 11-H), 3.81 (s, 6 H, 3',5'-OCH₃), 3.16 (dd, $J = 4.9$, 14.1 Hz, 1 H, 2-H), 3.05 (m, 1 H, 3-H), 1.38 (t, $J = 7.2$ Hz, 3 H, CO₂CH₂CH₃). Anal. (C₃₀H₂₆NO₉) C, H, N.

4'-O-Demethyl-4 β -[3''-(methoxycarbonyl)anilino]-4-desoxypodophyllotoxin (14): yield 61%; crystals from methanol; mp 255–258 °C; $[\alpha]_D^{25}$ -98° ($c = 0.5$, CHCl₃); IR (KBr) 3500 (OH), 3370 (NH), 2900 (aliphatic C-H), 1740 (lactone), 1700 (ester), 1600, 1500 and 1475 (aromatic C=C) cm⁻¹; ¹H NMR (CDCl₃) δ 7.45 (d, $J = 7.8$ Hz, 1 H, 6''-H), 7.35 (t, $J = 7.8$ Hz, 1 H, 5''-H), 7.22 (br, 1 H, 2''-H), 6.75 (br, 2 H, 5-H and 4''-H), 6.54 (s, 1 H, 8-H), 6.33 (s, 2 H, 2',6'-H), 5.97 and 5.95 (AB q, $J = 1.2$ Hz, 2 H, OCH₂O), 5.45 (br, 1 H, exchangeable, 4'-OH), 4.76 (d, $J = 4.6$ Hz, 1 H, 4-H), 4.51 (d, $J = 4.6$ Hz, 1 H, 1-H), 4.40 (t, 1 H, 11-H), 3.96 (t, 1 H, 11-H), 3.91 (s, 3 H, CO₂CH₃), 3.81 (s, 6 H, 3',5'-OCH₃), 3.14 (dd, 1 H, 2-H), 3.05 (m, 1 H, 3-H).

Anal. (C₂₉H₂₇NO₉) C, H, N.

4'-O-Demethyl-4 β -[4''-(ethoxycarbonyl)anilino]-4-desoxypodophyllotoxin (15): yield 48.7%; crystals from ethanol; mp 270–271 °C; $[\alpha]_D^{25}$ -145° ($c = 0.33$, CHCl₃); IR (KBr) 3500 (OH), 3370 (NH), 2940 (aliphatic C-H), 1762 (lactone), 1695 (ester), 1610, 1520 and 1480 (aromatic C=C) cm⁻¹; ¹H NMR (CDCl₃) δ 7.92 (d, $J = 8.8$ Hz, 2 H, 3'',5''-H), 6.77 (s, 1 H, 5-H), 6.55 (d, $J = 8.8$ Hz, 2 H, 2'',6''-H), 6.54 (s, 1 H, 8-H), 6.33 (s, 2 H, 2',6'-H), 5.99 and 5.97 (AB q, $J = 1.1$ Hz, 2 H, OCH₂O), 5.44 (s, 1 H, exchangeable, 4'-OH), 4.78 (d, $J = 3.3$ Hz, 1 H, 4-H), 4.62 (d, $J = 4.5$ Hz, 1 H, 1-H), 4.40 (t, $J = 7.5$ Hz, 1 H, 11-H), 4.37 (q, $J = 7.1$ Hz, 2 H, CO₂CH₂CH₃), 4.32 (m, 1 H, exchangeable, NH), 3.92 (t, $J = 7.5$ Hz, 1 H, 11-H), 3.80 (s, 6 H, 3',5'-OCH₃), 3.10 (dd, 1 H, 2-H), 3.08 (m, 1 H, 3-H), 1.38 (t, $J = 7.1$ Hz, 3 H, CO₂CH₂CH₃).

Anal. (C₃₀H₂₆NO₉) C, H, N.

4'-O-Demethyl-4 β -[3''-(methoxycarbonyl)-4''-hydroxyanilino]-4-desoxypodophyllotoxin (16): yield 49%; crystals from ethanol; mp 158–160 °C; $[\alpha]_D^{25}$ -115° ($c = 0.5$, CHCl₃); IR (KBr) 3500 (OH), 3370 (NH), 2980 (aliphatic C-H), 1760 (lactone), 1660 (ester), 1600, 1500 and 1470 (aromatic C=C) cm⁻¹; ¹H NMR (CDCl₃) δ 7.25 (br, 1 H, 2''-H), 6.94 (d, $J = 8.0$ Hz, 1 H, 5''-H), 6.91 (s, 1 H, 4''-OH), 6.89 (s, 1 H, 5-H), 6.74 (dd, $J = 1.1$, 8.0 Hz, 6''-H), 6.72 (s, 1 H, 8-H), 6.53 (s, 2 H, 2',6'-H), 5.98 and 5.96 (AB

q, $J = 1.1$ Hz, 2 H, OCH₂O), 5.43 (s, 1 H, 4'-H), 4.61 (m, 2 H, 4-H and 1-H), 4.39 (t, $J = 7.3$ Hz, 1 H, 11-H), 4.10 (t, $J = 7.3$ Hz, 1 H, 11-H), 3.95 (s, 3 H, 3''-CO₂CH₃), 3.80 (s, 6 H, 3',5'-OCH₃), 3.18 (dd, 1 H, 2-H), 3.11 (m, 1 H, 3-H).

Anal. (C₂₉H₂₇NO₁₀).

4'-O-Demethyl-4 β -[3''-hydroxy-4''-(methoxycarbonyl)anilino]-4-desoxypodophyllotoxin (17): yield 45%; crystals from methanol; mp 177–180 °C; $[\alpha]_D^{25}$ -146° ($c = 0.5$, CHCl₃); IR (KBr) 3500 (OH), 3360 (NH), 2900 (aliphatic C-H), 1750 (lactone), 1650 (ester), 1620, 1520 and 1480 (aromatic C=C) cm⁻¹; ¹H NMR (CDCl₃) δ 7.65 (d, $J = 8.5$ Hz, 1 H, 5''-H), 6.76 (s, 1 H, 5-H), 6.54 (s, 1 H, 8-H), 6.32 (s, 2 H, 2',6'-H), 6.06 (dd, $J = 1.2$, 8.5 Hz, 1 H, 6''-H), 6.04 (d, $J = 1.2$ Hz, 1 H, 2''-H), 5.99 (s, 1 H, OCH₂O), 5.97 (s, 1 H, OCH₂O), 5.43 (s, 1 H, 4'-OH), 4.72 (m, 1 H, NH), 4.61 (d, $J = 4.3$ Hz, 1 H, 4-H), 4.41 (dd, 1 H, 11-H), 4.30 (d, $J = 4.0$ Hz, 1 H, 1-H), 3.92 (t, 1 H, 11-H), 3.90 (s, 3 H, 4''-CO₂CH₃), 3.79 (s, 6 H, 3',5''-OCH₃), 3.05 (m, 2 H, 2-H and 3-H).

Anal. (C₂₉H₂₇NO₁₀).

4'-O-Demethyl-4 β -[3'',5''-bis(methoxycarbonyl)anilino]-4-desoxypodophyllotoxin (18): yield 35%; crystals from ethanol; mp 170–173 °C dec; $[\alpha]_D^{25}$ -111° ($c = 0.5$, CHCl₃); IR (KBr) 3500 (OH), 3380 (NH), 2900 (aliphatic C-H), 1780 (lactone), 1720 (ester), 1605, 1510 and 1485 (aromatic C=C) cm⁻¹; ¹H NMR (CDCl₃) δ 8.08 (br, 1 H, 4''-H), 7.42 (br, 2 H, 2'',6''-H), 6.72 (s, 1 H, 5-H), 6.55 (s, 1 H, 8-H), 6.34 (s, 2 H, 2',6'-H), 5.99 (s, 1 H, OCH₂O), 5.97 (s, 1 H, OCH₂O), 5.45 (s, 1 H, 4'-OH), 4.80 (m, 1 H, NH), 4.63 (d, $J = 4.4$ Hz, 1 H, 4-H), 4.45 (t, 1 H, 11-H), 4.17 (d, $J = 5.5$ Hz, 1 H, 1-H), 3.94 (s, 6 H, 3'',5''-CO₂CH₃), 3.90 (t, 1 H, 11-H), 3.81 (s, 6 H, 3',5'-OCH₃), 3.10 (m, 2 H, 2-H and 3-H).

Anal. (C₃₁H₂₉NO₁₁·H₂O) C, H, N.

4'-O-Demethyl-4 β -[3''-methoxyanilino]-4-desoxypodophyllotoxin (19): yield 57%; $[\alpha]_D^{25}$ -117° ($c = 0.5$, CHCl₃); crystals from ethyl acetate; mp 277–279 °C; IR (KBr) 3360 (NH), 2900 (aliphatic C-H), 1740 (lactone), 1600, 1500 and 1470 (aromatic C=C) cm⁻¹; ¹H NMR (CDCl₃) δ 7.11 (t, $J = 8.2$ Hz, 1 H, 5''-H), 6.78 (s, 1 H, 5-H), 6.53 (s, 1 H, 8-H), 6.35 (dd, $J = 1.7$, 8.2 Hz, 1 H, 4''-H), 6.33 (s, 2 H, 2',6'-H), 6.10 (dd, $J = 1.7$, 8.2 Hz, 1 H, 6''-H), 6.10 (t, $J = 1.7$ Hz, 1 H, 2''-H), 5.97 and 5.95 (AB q, $J = 0.7$ Hz, 2 H, OCH₂O), 5.43 (s, 1 H, 4'-OH), 4.68 (d, 1 H, 4-H), 4.59 (d, $J = 4.8$ Hz, 1 H, 1-H), 4.30 (t, 1 H, 11-H), 4.01 (t, 1 H, 11-H), 3.84 (m, 1 H, NH), 3.79 (s, 6 H, 3',5'-OCH₃), 3.78 (s, 3 H, 3''-OCH₃), 3.13 (dd, 1 H, 2-H), 2.99 (m, 1 H, 3-H).

Anal. (C₂₈H₂₇NO₉) C, H, N.

4'-O-Demethyl-4 β -[3''-acetylanilino]-4-desoxypodophyllotoxin (20): yield 42%; crystals from ethyl acetate-ether; mp 259–262 °C; $[\alpha]_D^{25}$ -121° ($c = 0.5$, CHCl₃); IR (KBr) 3380 (NH), 2900 (aliphatic C-H), 1738 (lactone), 1660 (ester), 1590, 1575 and 1500 (aromatic C=C) cm⁻¹; ¹H NMR (CDCl₃) δ 7.34 (m, 2 H, 5''-H and 6''-H), 7.16 (br, 1 H, 2''-H), 6.75 (dd, $J = 1.8$, 8.0 Hz, 1 H, 4''-H), 6.75 (s, 1 H, 5-H), 6.54 (s, 1 H, 8-H), 6.33 (s, 2 H, 2',6'-H), 5.98 and 5.96 (AB q, $J = 1.2$, 2 H, OCH₂O), 5.45 (s, 1 H, 4'-OH), 4.79 (m, 1 H, NH), 4.61 (d, $J = 4.9$ Hz, 1 H, 4-H), 4.42 (t, 1 H, 11-H), 4.02 (d, $J = 4.9$ Hz, 1 H, 1-H), 3.92 (t, 1 H, 11-H), 3.81 (s, 6 H, 3',5'-OCH₃), 3.12 (dd, $J = 4.9$, 14.0 Hz, 1 H, 2-H), 3.04 (m, 1 H, 3-H).

Anal. (C₂₉H₂₇NO₉) C, H, N.

4'-O-Demethyl-4 β -[3''-(hydroxymethyl)anilino]-4-desoxypodophyllotoxin (21): yield 43%; crystals from ether-hexane; mp 189–192 °C; $[\alpha]_D^{25}$ -110° ($c = 0.5$, CHCl₃); IR (KBr) 3500 (OH), 3380 (NH), 2890 (aliphatic C-H), 1745 (lactone), 1595, 1500 and 1470 (aromatic C=C) cm⁻¹; ¹H NMR (CDCl₃) δ 7.20 (t, $J = 8.1$ Hz, 1 H, 5''-H), 6.78 (d, 2 H, 5-H and 4''-H), 6.62 (br, 1 H, 2''-H), 6.53 (s, 1 H, 8-H), 6.48 (dd, $J = 1.8$, 8.1 Hz, 1 H, 6''-H), 6.34 (s, 2 H, 2',6'-H), 5.97 and 5.95 (AB q, $J = 0.9$, 2 H, OCH₂O), 5.45 (s, 1 H, 4'-OH), 4.71 (d, $J = 3.4$ Hz, 1 H, 4-H), 4.65 (s, 2 H, 3''-CH₂OH), 4.60 (d, $J = 4.9$ Hz, 1 H, 1-H), (t, 1 H, 11-H), 3.99 (t, 1 H, 11-H), 3.80 (s, 6 H, 3',5'-OCH₃), 3.77 (m, 1 H, NH), 3.15 (dd, $J = 4.9$, 14.0 Hz, 1 H, 2-H), 3.00 (m, 1 H, 3-H).

Anal. (C₂₈H₂₇NO₉) C, H, N.

4'-O-Demethyl-4 β -[3'',4''-dimethoxyanilino]-4-desoxypodophyllotoxin (22): yield 39%; crystals from methanol; mp 233–234 °C dec; $[\alpha]_D^{25}$ -118° ($c = 1$, CHCl₃); IR (KBr) 3500 (OH), 3360 (NH), 2920 (aliphatic C-H), 1770 (lactone), 1605 and 1505 (aromatic C=C) cm⁻¹; ¹H NMR (CDCl₃) δ 6.78 (s, 1 H, 5-H), 6.75 (d, $J = 8.5$ Hz, 1 H, 5''-H), 6.53 (s, 1 H, 8-H), 6.34 (s, 2 H, 2',6'-H), 6.17 (s, 1 H, 2''-H), 6.05 (d, $J = 8.5$ Hz, 1 H, 6''-H), 5.96 (AB q,

$J = 1.2$ Hz, 2 H, OCH₂O), 5.43 (s, 1 H, 4'-OH), 4.60 (d, 2 H, 4-H and 1-H), 4.38 (t, $J = 8.3$ Hz, 1 H, 11-H), 4.05 (t, $J = 8.3$ Hz, 1 H, 11-H), 3.83 (s, 3 H, 4'-OCH₃), 3.81 (s, 3 H, 3''-OCH₃), 3.80 (s, 6 H, 3',5'-OCH₃), 3.18 (dd, $J = 5.0, 14.0$ Hz, 1 H, 2-H), 2.96 (m, 1 H, 3-H).

Anal. (C₂₉H₂₉NO₉) C, H, N.

4'-O-Demethyl-4β-(3'',4'',5''-trimethoxyanilino)-4-desoxypodophyllotoxin (23): yield 60%; crystals from ethanol-ether; mp 240–242 °C; $[\alpha]_D^{25} -110^\circ$ ($c = 0.5$, CHCl₃); IR (KBr) 3500 (OH), 3460 (NH), 2930 (aliphatic C-H), 1765 (lactone), 1600, 1500, 1476 (aromatic C=C) cm⁻¹; ¹H NMR (CDCl₃) δ 6.80 (s, 1 H, 5-H), 6.53 (s, 1 H, 8-H), 6.34 (s, 2 H, 2',6''-H), 5.98 and 5.96 (AB q, $J = 1.2$ Hz, 2 H, OCH₂O), 5.77 (s, 2 H, 2'',6''-H), 5.45 (br, 1 H, 4'-OH), 4.62 (m, 2 H, 4-H and 1-H), 4.35 (t, 1 H, 11-H), 4.08 (t, 1 H, 11-H), 3.81 (s, 9 H, 3',4'',5''-OCH₃), 3.80 (s, 6 H, 3',5'-OCH₃), 3.18 (dd, 1 H, 2-H), 3.00 (m, 1 H, 3-H).

Anal. (C₃₀H₃₁NO₁₀·¹/₂H₂O) C, H, N.

4'-O-Demethyl-4β-[3'',4''-(methylenedioxy)anilino]-4-desoxypodophyllotoxin (24): yield 36%; crystals from methanol; mp 247–249 °C dec; $[\alpha]_D^{25} -126^\circ$ ($c = 1$, CHCl₃); IR (KBr) 3500 (OH), 3340 (NH), 2900 (aliphatic C-H), 1752 (lactone), 1605, 1496 and 1475 (aromatic C=C) cm⁻¹; ¹H NMR (CDCl₃) δ 6.76 (s, 1 H, 5-H), 6.68 (d, $J = 8.1$ Hz, 1 H, 5''-H), 6.52 (s, 1 H, 8-H), 6.33 (s, 2 H, 2',6''-H), 6.17 (d, $J = 1.2$, 1 H, 2''-H), 5.96 (q, 3 H, 6''-H and OCH₂O), 5.90 (s, 2 H, 7''-H), 5.43 (s, 1 H, exchangeable, 4'-OH), 4.59 (d, $J = 4.9$ Hz, 1 H, 4-H), 4.56 (d, $J = 3.9$ Hz, 1 H, 1-H), 4.37 (t, 1 H, 11-H), 4.05 (t, 1 H, 11-H), 3.79 (s, 6 H, 3',5'-OCH₃), 3.15 (dd, 1 H, 2-H), 2.95 (m, 1 H, 3-H).

Anal. (C₂₈H₂₆NO₉) C, H, N.

4'-O-Demethyl-4β-[3'',4''-(ethylenedioxy)anilino]-4-desoxypodophyllotoxin (25): yield 41%; crystals from chloroform; mp 293–296 °C dec; $[\alpha]_D^{25} -114^\circ$ ($c = 0.25$, CHCl₃); IR (KBr) 3360 (NH), 2950 (aliphatic C-H), 1730 (lactone), 1615, 1515 and 1480 (aromatic C=C) cm⁻¹; ¹H NMR (CDCl₃) δ 6.76 (s, 1 H, 5-H), 6.73 (d, $J = 9.1$ Hz, 1 H, 5''-H), 6.51 (s, 1 H, 8-H), 6.33 (s, 2 H, 2',6''-H), 6.09 (br, 2 H, 2'',6''-H), 5.96 and 5.94 (AB q, $J = 1.2$ Hz, 2 H, OCH₂O), 5.45 (br 1 H, 4'-OH), 4.58 (d, 1 H, 4-H), 4.55 (d, 1 H, 1-H), 4.37 (t, 1 H, 11-H), 4.25–4.21 (m, 4 H, 3',4''-OCH₂CH₂O-), 4.05 (t, 1 H, 11-H), 3.79 (s, 6 H, 3',5'-OCH₃), 3.15 dd, 1 H, 2-H), 2.95 (m, 1 H, 3-H).

Anal. (C₂₉H₂₇NO₉·H₂O) C, H, N.

4'-O-Demethyl-4β-(4''-phenoxyanilino)-4-desoxypodophyllotoxin (26): yield 51%; crystals from ethanol; mp 144–146 °C; $[\alpha]_D^{25} -121^\circ$ ($c = 0.5$, CHCl₃); IR (KBr) 3520 (OH), 3400 (NH), 2910 (aliphatic C-H), 1785 (lactone), 1615, 1510 and 1485 (aromatic C=C) cm⁻¹; ¹H NMR (CDCl₃) δ 7.32 (m, 2 H, 3'',5''-H (phenoxy)), 7.00 (m, 5 H, 3',5''-H and 1'',5''-H (phenoxy)), 6.80 (s, 1 H, 5-H), 6.55 (t, 3 H, 8-H and 2'',6''-H), 6.34 (s, 2 H, 2',6''-H), 5.97 and 5.95 (AB q, $J = 1.3$ Hz, 2 H, OCH₂O), 5.46 (br, 1 H, 4'-OH), 4.62 (m, 2 H, 4-H and 1-H), 4.38 (t, $J = 7.7$ Hz, 1 H, 11-H), 4.04 (t, $J = 7.7$ Hz, 1 H, 11-H), 3.79 (s, 6 H, 3',5'-OCH₃), 3.18 (dd, $J = 4.8, 14.8$ Hz, 1 H, 2-H), 3.05 (m, 1 H, 3-H).

Anal. (C₃₃H₂₉NO₉) C, H, N.

4'-O-Demethyl-4β-(4''-morpholinoanilino)-4-desoxypodophyllotoxin (27): yield 46%; crystals from ethanol; mp 235–237 °C dec; $[\alpha]_D^{25} -129^\circ$ ($c = 1$, CHCl₃); IR (KBr) 3500 (OH), 3330 (NH), 2880 (aliphatic C-H), 1755 (lactone), 1620, 1510 and 1475 (aromatic C=C) cm⁻¹; ¹H NMR (CDCl₃) δ 6.86 (d, $J = 9.5$ Hz, 2 H, 3'',5''-H), 6.76 (s, 1 H, 5-H), 6.52 (br, 3 H, 8-H and 2'',6''-H), 6.35 (s, 2 H, 2',6''-H), 5.97 (s, 1 H, OCH₂O), 5.95 (s, 1 H, OCH₂O), 5.44 (s, 1 H, exchangeable, 4'-OH), 4.61 (m, 2 H, 4-H and 1-H), 4.37 (t, $J = 7.0$ Hz, 1 H, 11-H), 4.08 (t, $J = 7.0$ Hz, 1 H, 11-H), 3.82 (br, 4 H, morpholino), 3.80 (s, 6 H, 3',5'-OCH₃), 3.22–2.90 (m, 2 H, 2-H, 3-H and 4 H, morpholino).

Anal. (C₃₁H₃₂N₂O₈) C, H, N.

4'-O-Demethyl-4β-(3''-nitroanilino)-4-desoxypodophyllotoxin (28): yield 47%; crystals from ethanol; mp 185–187 °C; $[\alpha]_D^{25} -96^\circ$ ($c = 0.5$, CHCl₃); IR (KBr) 3390 (NH), 2900 (aliphatic C-H), 1750 (lactone), 1520 and 1345 (NO₂) cm⁻¹; ¹H NMR (CDCl₃) δ 7.63 (α, $J = 8.8$ Hz, 1 H, 4''-H), 7.38 (m, 2 H, 2'',5''-H), 6.85 (d, $J = 8.8$ Hz, 1 H, 6''-H), 6.75 (s, 1 H, 5-H), 6.56 (s, 1 H, 8-H), 6.33 (s, 2 H, 2',6''-H), 6.00 (s, 1 H, OCH₂O), 5.98 (s, 1 H, OCH₂O), 5.45 (s, 1 H, 4'-OH), 4.78 (m, 1 H, NH), 4.62 (d, $J = 3.2$ Hz, 4-H), 4.55 (t, 1 H, 11-H), 4.25 (d, 1 H, 1-H), 3.93 (t, 1 H, 11-H), 3.81 (s, 6 H, 3',5'-OCH₃), 3.11 (m, 2 H, 2-H and 3-H).

Anal. (C₂₇H₂₄N₂O₉) C, H, N.

4'-O-Demethyl-4β-(4''-nitroanilino)-4-desoxypodophyllotoxin (29): yield 44%; crystals from ethyl acetate; mp 205–207 °C; $[\alpha]_D^{25} -170^\circ$ ($c = 0.5$, CHCl₃); IR (KBr) 3500 (OH), 3470 (NH), 2920 (aliphatic C-H), 1775 (lactone), 1600, 1520 and 1490 (aromatic C=C), 1330 and 1310 (NO₂) cm⁻¹; ¹H NMR (CDCl₃) δ 8.15 (d, $J = 9.1$ Hz, 2 H, 3'',5''-H), 6.76 (s, 1 H, 5-H), 6.60 (d, 3 H, 8-H and 2'',6''-H), 6.32 (s, 2 H, 2',6''-H), 6.00 (s, 1 H, OCH₂O), 5.98 (s, 1 H, OCH₂O), 5.46 (s, 1 H, 4'-OH), 4.83 (m, 1 H, NH), 4.62 (m, 2 H, 4-H and 1-H), 4.41 (t, 1 H, 11-H), 3.90 (t, 1 H, 11-H), 3.80 (s, 6 H, 3',5'-OCH₃), 3.10 (m, 2 H, 2-H and 3-H).

Anal. (C₂₇H₂₄N₂O₉) C, H, N.

4'-O-Demethyl-4β-(2''-hydroxy-5''-nitroanilino)-4-desoxypodophyllotoxin (30): yield 35%; crystals from ethanol; mp 192–194 °C dec; $[\alpha]_D^{25} -114^\circ$ ($c = 0.5$, CHCl₃); IR (KBr) 3540 (OH), 3420 (NH), 2925 (aliphatic C-H), 1775 (lactone), 1630 and 1600 (aromatic C=C), 1530 and 1350 (NO₂) cm⁻¹; ¹H NMR (CDCl₃) δ 7.62 (dd, $J = 2.6, 8.6$ Hz, 1 H, 4''-H), 7.38 (d, $J = 2.6$ Hz, 1 H, 6''-H), 6.80 (d, $J = 8.6$ Hz, 1 H, 3''-H), 6.34 (s, 1 H, 5-H), 6.29 (s, 1 H, 8-H), 6.19 (s, 2 H, 2',6''-H), 6.00 and 5.98 (AB q, $J = 1.2$ Hz, 2 H, OCH₂O), 5.50 (s, 1 H, 4'-OH), 4.78 (m, 1 H, NH), 4.63 (d, $J = 4.6$ Hz, 1 H, 4-H), 4.58 (d, $J = 6.1$ Hz, 1 H, 1-H), 4.23 (t, 1 H, 11-H), 3.92 (t, 1 H, 11-H), 3.81 (s, 6 H, 3',5'-OCH₃), 3.17 (m, 2 H, 2-H and 3-H).

Anal. (C₂₇H₂₄N₂O₁₀·¹/₂H₂O) C, H, N.

4'-O-Demethyl-4β-[3'',5''-bis(trifluoromethyl)anilino]-4-desoxypodophyllotoxin (31): yield 59%; crystals from ether-hexane; mp 165–168 °C; $[\alpha]_D^{25} -85^\circ$ ($c = 0.5$, CHCl₃); IR (KBr) 3500 (OH), 3360 (NH), 2930 (aliphatic C-H), 1780 (lactone), 1620, 1510 and 1485 (aromatic C=C) cm⁻¹; ¹H NMR (CDCl₃) δ 7.02 (br, 1 H, 4''-H), 6.93 (br, 2 H, 2'',6''-H), 6.78 (s, 1 H, 5-H), 6.57 (s, 1 H, 8-H), 6.33 (s, 2 H, 2',6''-H), 6.01 and 5.99 (AB q, $J = 1.1$ Hz, 2 H, OCH₂O), 5.45 (s, 1 H, 4'-OH), 4.76 (m, 1 H, NH), 4.63 (d, $J = 3.5$ Hz, 1 H, 4-H), 4.39 (m, 1 H, 11-H), 4.33 (d, $J = 5.8$ Hz, 1 H, 1-H), 3.88 (m, 1 H, 11-H), 3.80 (s, 6 H, 3',5'-OCH₃), 3.09 (m, 2 H, 2-H and 3-H).

Anal. (C₂₉H₂₃F₆NO₇) C, H, N.

4'-O-Demethyl-4β-(2''-pyridylamino)-4-desoxypodophyllotoxin (32): yield 26.6%; crystals from ethanol; mp 215–218 °C dec; $[\alpha]_D^{25} -82^\circ$ ($c = 0.33$, CHCl₃); IR (KBr), 3500 (OH), 3360 (NH), 2950 (aliphatic C-H), 1760 (lactone), 1690, 1645, 1600 and 1460 (aromatic ring) cm⁻¹; ¹H NMR (CDCl₃) δ 8.11 (d, 1 H, 6''-H), 7.45 (m, 1 H, 4''-H), 6.81 (s, 1 H, 5-H), 6.67 (m, 1 H, 5''-H), 6.55 (s, 1 H, 8-H), 6.45 (d, 1 H, 3''-H), 6.34 (s, 2 H, 2',6''-H), 5.98 and 5.96 (AB q, $J = 1.3$ Hz, 2 H, OCH₂O), 5.34 (br, 1 H, exchangeable, 4'-OH), 5.35 (m, 1 H, exchangeable, NH), 4.60 (d, $J = 4.2$ Hz, 1 H, 4-H), 4.24 (m, 2 H, 1-H and 11-H), 3.85 (m, 1 H, 11-H), 3.78 (s, 6 H, 3',5'-OCH₃), 3.05 (m, 2 H, 2-H and 3-H).

Anal. (C₂₆H₂₄N₂O₇·¹/₂H₂O) C, H, N.

4'-O-Demethyl-4β-(3''-pyridylamino)-4-desoxypodophyllotoxin (33): yield 10%; crystals from ethanol; mp 179–181 °C dec; $[\alpha]_D^{25} -99^\circ$ ($c = 0.33$, CHCl₃); IR (KBr) 3500 (OH), 3350 (NH), 2900 (aliphatic C-H), 1765 (lactone), 1575, 1500 and 1470 (aromatic ring) cm⁻¹; ¹H NMR (CDCl₃) δ 8.08 (d, $J = 5.5$ Hz, 1 H, 6''-H), 8.02 (br, 1 H, 2''-H), 7.16 (m, 1 H, 5''-H), 6.85 (dd, 1 H, 4''-H), 6.75 (s, 1 H, 5-H), 6.55 (s, 1 H, 8-H), 6.32 (s, 2 H, 2',6''-H), 5.99 and 5.97 (AB q, $J = 1.3$ Hz, 2 H, OCH₂O), 4.65 (d, $J = 4.9$ Hz, 1 H, 4-H), 4.60 (m, 1 H, 1-H), 4.20 (t, $J = 8.2$ Hz, 1 H, 11-H), 3.90 (m, 2 H, 11-H and NH), 3.80 (s, 6 H, 3',5'-OCH₃), 3.18 (dd, $J = 4.9, 14.1$ Hz, 1 H, 2-H), 3.03 (m, 1 H, 3-H).

Anal. (C₂₆H₂₄N₂O₇·¹/₂H₂O) C, H, N.

4'-O-Demethyl-4β-(3''-quinolylamino)-4-desoxypodophyllotoxin (34): yield 49.4%; crystals from ethanol-ether; mp 243–246 °C dec; $[\alpha]_D^{25} -179^\circ$ ($c = 0.5$, CHCl₃); IR (KBr), 3460 (OH), 3380 (NH), 2900 (aliphatic C-H), 1775 (lactone), 1605, 1510 and 1480 (aromatic ring) cm⁻¹; ¹H NMR (CDCl₃) δ 8.46 (d, $J = 2.9$ Hz, 2''-H), 7.97 (m, 1 H, 4''-H), 7.65 (m, 1 H, 7''-H), 7.48 (m, 2 H, 5'',6''-H), 6.99 (d, $J = 2.9$ Hz, 8''-H), 6.76 (s, 1 H, 5-H), 6.57 (s, 1 H, 8-H), 6.35 (s, 2 H, 2',6''-H), 6.00 and 5.98 (AB q, $J = 1.1$ Hz, 2 H, OCH₂O), 5.48 (s, 1 H, exchangeable, 4'-OH), 4.78 (d, $J = 3.5$ Hz, 1 H, 4-H), 4.64 (d, $J = 4.8$ Hz, 1 H, 1-H), 4.45 (t, 1 H, 11-H), 4.23 (d, 1 H, exchangeable, NH), 3.99 (t, 1 H, 11-H), 3.81 (s, 6 H, 3',5'-OCH₃), 3.15 (m, 2 H, 2-H and 3-H).

Anal. (C₃₀H₂₆N₂O₇) C, H, N.

4'-O-Demethyl-4β-[(4''-hydroxyphenyl)thio]-4-desoxypodophyllotoxin (40): yield 73%; crystals from methanol-acetone; mp 210–212 °C; $[\alpha]_D^{25} -111^\circ$ ($c = 1$, CHCl₃); IR (KBr)

3500 (OH), 2990 (aliphatic C-H), 1800 (lactone), 1635, 1520 and 1495 (aromatic C=C); $^1\text{H NMR}$ (CDCl_3) δ 7.30 (dd, $J = 2.1, 8.7$ Hz, 3',5'-H), 6.93 (s, 1 H, 5-H), 6.81 (dd, $J = 2.1, 8.7$ Hz, 2'',6''-H), 6.45 (s, 1 H, 8-H), 6.28 (s, 2 H, 2',6'-H), 5.98 and 5.96 (AB q, $J = 1.3$ Hz, 2 H, OCH_2O), 5.42 (s, 1 H, 4'-OH), 5.27 (s, 1 H, 4''-OH), 4.61 (d, $J = 4.4$ Hz, 1 H, 4-H), 4.54 (d, $J = 5.2$ Hz, 1 H, 1-H), 4.45 (t, 1 H, 11-H), 4.15 (t, 1 H, 11-H), 3.77 (s, 6 H, 3',5'- OCH_3), 3.35 (dd, $J = 5.2, 12.9$ Hz, 1 H, 2-H), 3.15 (m, 1 H, 3-H).

Anal. ($\text{C}_{27}\text{H}_{24}\text{O}_8 \cdot \frac{1}{2}\text{H}_2\text{O}$) C, H, N.

Synthesis of Compounds 35-37. A solution of 28-30 (140 mg) in ethyl acetate (10 mL) was adjusted with 1 N hydrogen chloride solution in methanol to pH = 1-2. After 10% palladium on activated carbon (70 mg) was added, the solution was stirred under hydrogen for 3 h. The catalyst was filtered off and washed with methanol. The filtrate and washings were combined and evaporated to give a solid. The solid was washed with ether five times to drive the free hydrogen chloride away, followed by crystallization from methanol-ether to afford 35-37.

4'-O-Demethyl-4 β -(3''-aminoanilino)-4-desoxypodophyllotoxin Chloride (35): yield 75%; mp 203-206 °C dec; $[\alpha]_D^{25}$ -108° ($c = 0.5$, CH_3OH); IR (KBr) 3450, 3360 and 3000 (NH_3^+ and OH), 2900 (aliphatic C-H), 1765 (lactone), 1605, 1510, 1500 and 1475 cm^{-1} ; $^1\text{H NMR}$ (CD_3OD) δ 6.89 (t, $J = 7.8$ Hz, 1 H, 5''-H), 6.76 (s, 1 H, 5-H), 6.48 (s, 1 H, 8-H), 6.35 (s, 2 H, 2',6'-H), 6.08 (m, 3 H, 2'',4'', and 6''-H), 5.92 (s, 2 H, OCH_2O), 4.76 (d, $J = 4.2$ Hz, 4-H), 4.56 (d, $J = 5.0$ Hz, 1-H), 4.39 (t, $J = 7.9$ Hz, 1 H, 11-H), 3.95 (t, $J = 7.9$ Hz, 1 H, 11-H), 3.73 (s, 6 H, 3',5'- OCH_3).

Anal. ($\text{C}_{27}\text{H}_{27}\text{N}_2\text{ClO}_7$) C, H.

4'-O-Demethyl-4 β -(4''-aminoanilino)-4-desoxypodophyllotoxin Chloride (36): yield 72%; mp 187-190 °C dec; $[\alpha]_D^{25}$ -100° (CH_3OH); IR (KBr) 3420, 2920 and 2600 (NH_3^+ OH and aliphatic C-H), 1770 (lactone), 1620, 1520 and 1490 cm^{-1} ; $^1\text{H NMR}$ ($\text{DMSO}-d_6$) δ 9.90 (br, 4''- NH_3^+ , exchangeable), 7.12 (d, $J = 8.6$ Hz, 2 H, 2'',6''-H), 6.55 (t, 3 H, 3'',5''-H and 5-H), 6.38 (br, 1''-NH, exchangeable), 6.23 (s, 2 H, 2',6'-H), 6.01 (s, 1 H, OCH_2O), 5.95 (s, 1 H, OCH_2O), 4.85 (br, 1 H, 4-H), 4.50 (d, $J = 4.9$ Hz, 1 H, 1-H), 4.35 (t, $J = 7.6$ Hz, 1 H, 11-H), 3.65 (br, 7 H, 11-H and 3',5'- OCH_3), 3.28 (dd, $J = 4.9, 14.1$ Hz, 1 H, 2-H), 3.00 (br, 1 H, 3-H).

Anal. ($\text{C}_{27}\text{H}_{27}\text{N}_2\text{ClO}_7 \cdot \text{H}_2\text{O}$) C, H.

4'-O-Demethyl-4 β -(2''-hydroxy-5''-aminoanilino)-4-desoxypodophyllotoxin Chloride (37): yield 82%; mp > 300 °C; IR (KBr) 3350 (NH_3^+ and OH), 1760 (lactone), 1600 and 1500 cm^{-1} ; $^1\text{H NMR}$ (CD_3OD) 6.76 (d, $J = 8.2$ Hz, 1 H, 3''-H), 6.72 (s, 1 H, 5-H), 6.58 (d, $J = 1.9$ Hz, 1 H, 6''-H), 6.00 (s, 1 H, 8-H), 6.49 (dd, $J = 1.9, 8.2$ Hz, 1 H, 4''-H), 6.26 (s, 2 H, 2',6'-H), 6.00 (s, 1 H, OCH_2O), 5.97 (s, 1 H, OCH_2O), 5.10 (d, 1 H, 4-H), 4.75 (br, 1 H, 1-H), 4.45 (m, 2 H, 11-H), 3.65 (s, 6 H, 3',5'- OCH_3), 3.45 (dd, 1 H, 2-H), 3.00 (m, 1 H, 3-H).

Anal. ($\text{C}_{27}\text{H}_{27}\text{ClN}_2\text{O}_8$) C, H, N.

4'-O-Demethyl-4 β -(4''-fluorophenyl)podophyllotoxin (38). Compound 7 was prepared by using the literature method.^{5,18} To a solution of a mixture of 7 (300 mg, 0.55 mmol) and 4-fluorophenol (75 mg, 0.66 mmol) in dichloroethane (6 mL) was added $\text{BF}_3 \cdot \text{OEt}_2$ (137 μL), and the solution was kept at -15 °C under nitrogen. The mixture was stirred for 1 h. After being quenched with pyridine (150 μL), the mixture was washed with brine and dried over anhydrous MgSO_4 . Evaporation of the solvent gave crude 8 (360 mg). 8 was dissolved in ethyl acetate (30 mL), and the solution was stirred under an atmosphere of hydrogen in the presence of 10% Pd/C (100 mg) at room temperature for 2 h. After removal of catalyst, the solvent was evaporated to afford

white solid which was purified by column chromatography (silica gel, 30 g) with benzene-ethyl acetate (3:1) as an eluant to give 38 152 mg (55%) calculated from 7. 38 was crystallized from methanol: mp 191-194 °C dec; $[\alpha]_D^{25}$ -140° ($c = 1$, CHCl_3); IR (KBr) 3500 (OH), 3360 (NH), 2900 (aliphatic C-H), 1755 (lactone), 1600, 1500 and 1480 (aromatic C=C) cm^{-1} ; $^1\text{H NMR}$ (CDCl_3) δ 7.03 (m, 2 H, 2'',6''-H), 6.84 (m, 2 H, 3'',5''-H), 6.61 (s, 1 H, 5-H), 6.59 (s, 1 H, 8-H), 6.32 (s, 2 H, 2',6'-H), 5.97 and 5.95 (AB q, $J = 1.2$ Hz, 2 H, OCH_2O), 5.44 (s, 1 H, 4'-OH), 5.34 (d, $J = 3.5$ Hz, 1 H, 4-H), 4.70 (d, $J = 5.0$ Hz, 1 H, 1-H), 4.38 (t, $J = 7.6$ Hz, 1 H, 11-H), 4.21 (t, $J = 7.6$ Hz, 1 H, 11-H), 3.79 (s, 6 H, 3',5'- OCH_3), 3.49 (dd, $J = 5.0, 14.0$ Hz, 1 H, 2-H), 3.06 (m, 1 H, 3-H).

Anal. ($\text{C}_{27}\text{H}_{23}\text{FO}_8$) C, H, N.

4'-O-Demethyl-4 β -(4''-hydroxyphenyl)podophyllotoxin (39). 39 was obtained from 7 (300 mg, 0.56 mmol) and 4-(benzyloxy)phenol (126 mg, 0.62 mmol) via the intermediate 9 by the similar procedure for the preparation of 38 from 7: yield 65% calculated from 7; crystals from toluene; mp 163-166 °C; $[\alpha]_D^{25}$ -95° ($c = 0.5$, acetone); IR (KBr) 3400 (OH), 2900 (aliphatic C-H), 1750 (lactone), 1600, 1500 and 1480 (aromatic C=C) cm^{-1} ; $^1\text{H NMR}$ ($\text{DMSO}-d_6$) δ 6.89 (d, $J = 8.8$ Hz, 2 H, 3'',5''-H), 6.71 (d, $J = 8.8$ Hz, 2 H, 2'',6''-H), 6.58 (s, 1 H, 5-H), 6.31 (s, 1 H, 8-H), 6.22 (s, 2 H, 2',6'-H), 6.01 (s, 1 H, OCH_2O), 5.09 (s, 1 H, OCH_2O), 5.45 (br, 1 H, 4'-OH), 4.59 (d, $J = 5.0$ Hz, 1 H, 4-H), 4.50 (br, 1 H, 1-H), 4.40 (t, $J = 7.6$ Hz, 1 H, 11-H), 3.97 (t, $J = 7.6$ Hz, 1 H, 11-H), 3.64 (s, 6 H, 3',5'- OCH_3), 3.05 (br, 1 H, 2-H), 2.70 (br, 1 H, 3-H).

Anal. ($\text{C}_{27}\text{H}_{24}\text{O}_9$) C, H.

Biological Assay. Assays for the inhibition of human DNA topoisomerase II and the cellular protein-linked DNA breaks as well as the cytotoxicity in KB cells were carried out according to the procedures described previously.⁹

Acknowledgment. The authors thank Mike Fisher of the Pharmacology Department, UNC-Chapel Hill for KB cell culture assay. This work was supported by grants from the American Cancer Society CH-370 A/B (K. H. Lee) and NIH No. CA 44358 (Y. C. Cheng). One of us (J.B.P.) would like to thank the American Cancer Society (Institutional Grant No. IN-15-30) for their support.

Registry No. 5, 6559-91-7; 6, 16477-16-0; 7, 23363-33-9; 8, 127882-52-4; 9, 127882-53-5; 10, 127882-54-6; 11, 127882-55-7; 12, 127882-56-8; 13, 127882-57-9; 14, 127882-58-0; 15, 127882-59-1; 16, 127882-60-4; 17, 127882-61-5; 18, 127882-62-6; 19, 127882-63-7; 20, 127882-64-8; 21, 127882-65-9; 22, 127882-66-0; 23, 127882-67-1; 24, 127882-68-2; 25, 127882-69-3; 26, 127882-70-6; 27, 127882-71-7; 28, 127882-72-8; 29, 127882-73-9; 30, 127882-74-0; 31, 127913-87-5; 32, 127882-75-1; 33, 127882-76-1; 34, 127882-77-3; 35, 127882-78-4; 36, 127882-79-5; 37, 127882-80-8; 38, 127882-81-9; 39, 127882-82-0; 40, 127882-83-1; PhNH_2 , 62-53-3; 3- $\text{H}_2\text{NC}_6\text{H}_4\text{CN}$, 2237-30-1; 4- $\text{H}_2\text{NC}_6\text{H}_4\text{CN}$, 873-74-5; 2- $\text{H}_2\text{NC}_6\text{H}_4\text{CO}_2\text{C}_2\text{H}_5$, 87-25-2; 3- $\text{H}_2\text{NC}_6\text{H}_4\text{CO}_2\text{CH}_3$, 4518-10-9; 4- $\text{H}_2\text{NC}_6\text{H}_4\text{CO}_2\text{C}_2\text{H}_5$, 94-09-7; 3- $\text{H}_2\text{NC}_6\text{H}_4\text{OCH}_3$, 536-90-3; 3- $\text{H}_2\text{NC}_6\text{H}_4\text{COCH}_3$, 99-03-6; 3- $\text{H}_2\text{NC}_6\text{H}_4\text{CH}_2\text{OH}$, 1877-77-6; 4- $\text{H}_2\text{NC}_6\text{H}_4\text{OPh}$, 139-59-3; 3- $\text{H}_2\text{NC}_6\text{H}_4\text{NO}_2$, 99-09-2; 4- $\text{H}_2\text{NC}_6\text{H}_4\text{NO}_2$, 100-01-6; 4- $\text{FC}_6\text{H}_4\text{OH}$, 371-41-5; 4- $\text{HOC}_6\text{H}_4\text{OCH}_2\text{Ph}$, 103-16-2; 4- $\text{HSC}_6\text{H}_4\text{OH}$, 637-89-8; DNA topoisomerase II, 80449-01-0; methyl 5-amino-2-hydroxybenzoate, 42753-75-3; methyl 2-hydroxy-4-aminobenzoate, 4136-97-4; 3,5-dicarboxymethoxyaniline, 99-27-4; 3,4-dimethoxyaniline, 6315-89-5; 3,4,5-trimethoxyaniline, 24313-88-0; 3,4-(methylenedioxy)aniline, 14268-66-7; 3,4-(ethylenedioxy)aniline, 22013-33-8; 4-(4-morpholinyl)aniline, 2524-67-6; 2-amino-4-nitrophenol, 99-57-0; 3,5-bis(trifluoromethyl)aniline, 328-74-5; 2-pyridinamine, 504-29-0; 3-pyridinamine, 462-08-8; 3-quinolinamine, 580-17-6.

(18) Kuhn, M.; Von Wartburg, A. *Helv. Chim. Acta* 1969, 52, 948.