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

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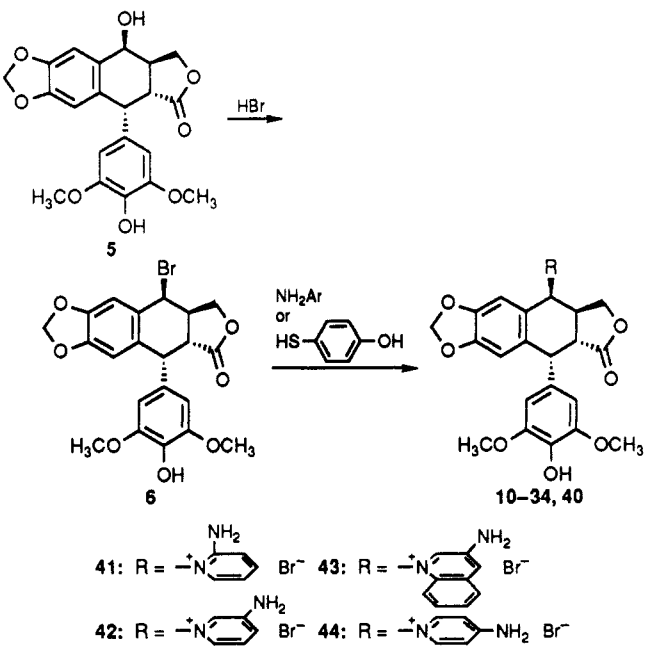
A number of 4′-O-demethylepipodophyllotoxin derivatives possessing various 4β-N-, 4β-O- or 4β-S-aromatic rings have been synthesized and evaluated for their inhibitory activity against the human DNA topoisomerase II as well as for their activity in causing cellular protein-linked DNA breakage. The results indicated, that for DNA toposoimerase II, a basic unsubstituted 4β-anilino moiety is structurally required for the enhanced activity. Substitution on this moiety with CN, $COOCH_3$, $COOC_2H_5$, OH and $COOCH_3$, $OCH_3$, $COCH_3$, $CH_2OH$, $OCH_2O$, $OCH_2CH_2O$, phenoxy, morpholino, $NO_2$, and $NH_2$ either at the para and/or the meta position yielded compounds which are as potent or more potent than etoposide. Substitution with $COOC_2H_5$ 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 4β-O-glucosidic substituent of 1 with 4β-N-hydroxyalkyl and 4β-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 compounds[12] 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]

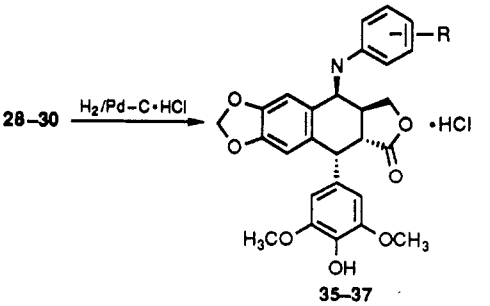
## Chemistry

The synthesis of 4-arylamino derivatives of 5, in general, followed our previous procedure.[11] As shown in Scheme I, the target compounds (10–31) of the 4-arylamino series

**Scheme I**




**Scheme II**




* To whom correspondence should be addressed.

† 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.

‡ Natural Products Laboratory, School of Pharmacy, University of North Carolina at Chapel Hill.

§ Laboratory for Molecular Modeling, School of Pharmacy, University of North Carolina at Chapel Hill.

∥ Department of Pharmacology, Yale University School of Medicine.

(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.

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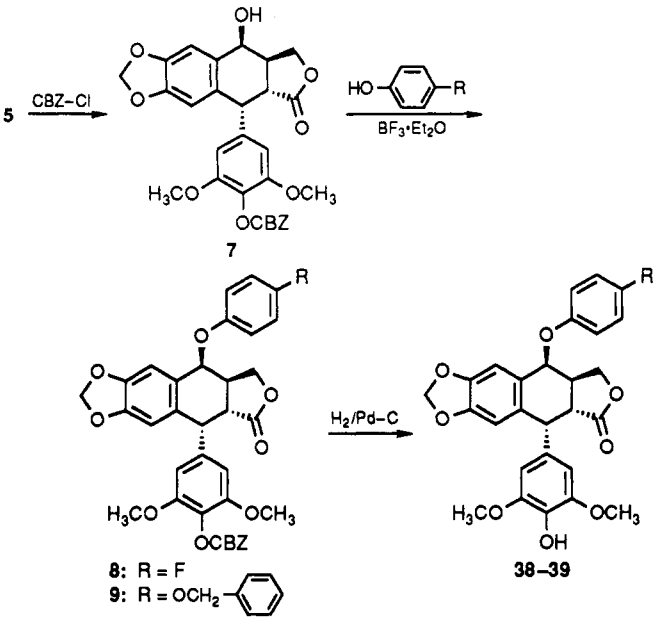
**Scheme III**




were prepared by nucleophilic substitution of appropriate arylamines with 4β-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 zwitterionic[11] (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 prepared[9] previously from **5**, was condensed with 4-fluorophenol and 4-(benzyloxy)phenol in the presence of $BF_3$–$Et_2O$[17] 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-1α pendant aromatic ring directed the substitution to be stereoselective, resulting in the formation of C-4β-oriented **10–34** and **40** as the main products.[9,11]

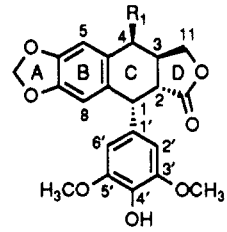
## Results and Discussion

As illustrated in Table I, the 4β-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**), $COOCH_3$ or $COOC_2H_5$ (**14**, **15**), OH and $COOCH_3$ (**17**), $OCH_3$ (**19**, **22**), $COCH_3$ (**20**), $CH_2OH$ (**21**), $OCH_2O$ (**24**), $OCH_2CH_2O$ (**25**), phenoxy (**26**), morpholino (**27**), $NO_2$ (**28**, **29**), and $NH_2$ (**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 4β-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 4β-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 μM 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

(3) Issell, B. F.; Muggia, F. M.; Carter, S. K. *Etoposide [VP-16] Current Status and New Developments*; Academic Press: New York, 1984.
(4) Saito, H.; Yoshikawa, H.; Nishimura, Y.; Kondo, S.; Takeuchi, T.; Umezawa, H. *Chem. Pharm. Bull.* **1986**, *34*, 3733.
(5) Saito, H.; Nishimura, Y.; Kondo, S.; Komuro, K.; Takeuchi, T.; *Bull. Chem. Soc. Jpn.* **1988**, *61*, 2493.
(6) 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.
(7) Beers, S. A.; Imakura, Y.; Dai, H. J.; Cheng, Y. C.; Lee, K. H. *J. Nat. Prod.* **1988**, *51*, 901.
(8) 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.
(9) 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.
(10) Liu, S. Y.; Hwang, B. D.; Haruna, M.; Imakura, Y.; Lee, K. H.; Cheng, Y. C. *Mol. Pharmacol.* **1989**, *36*, 78.
(11) 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.
(12) Compounds **15–17**, **24**, **32–34**, **39**, and **40** were designed by molecular modeling studies.
(13) 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).
(14) Klingsber, E. In *Heterocyclic Compounds, Pyridine and its Derivatives*; John Wiley and Sons: New York, 1962; Part 3, pp 1–32.
(15) 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 μM) 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 4β-(Arylamino)-4′-demethylpodophyllotoxin and Related Compounds



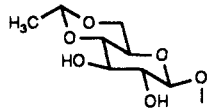
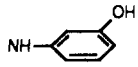
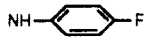
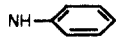
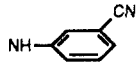
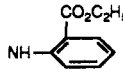
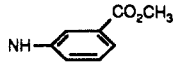
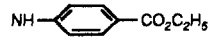
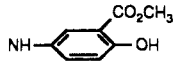
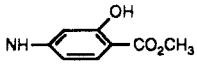
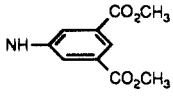
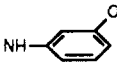
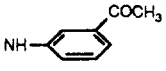
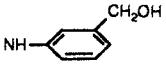
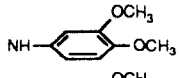
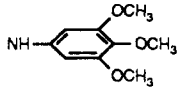
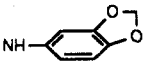
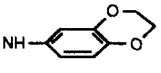
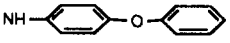
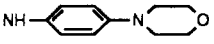
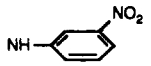
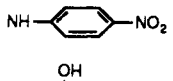
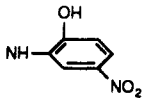
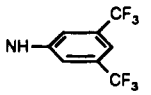
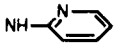
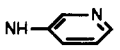
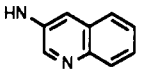
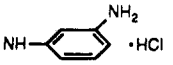
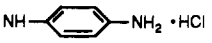
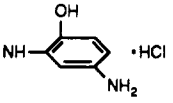
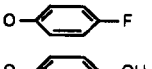
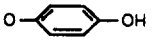
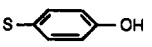
| compd | $R_1$ | cytotoxicity:[a] $ID_{50}$ KB, μM | inhibition of DNA topoisomerase II activity:[b] $ID_{50}$, μM | cellular protein–DNA complex formation, % (10 μM) |
|---|---|---|---|---|
| 1 |  | 0.20 | 50 | 100 |
| 2 | $NHCH_2CH_2OH$ | 1.60 | | 121 |
| 3 |  | 0.45 | 25 | 290 |
| 4 |  | 0.24 | 5 | 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] $ID_{50}$ 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 $ID_{50}$ 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-desoxypodophyllotoxin (6).** A solution of 4′-*O*-demethylepipodophylotoxin (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-deoxypodophyllotixin (**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-desoxypodophyllotoxin (10):** yield 57.8%; crystals from methanol; mp 172–173 °C; $[\alpha]^{25}_D$ −120° ($c$ = 1.0, $CHCl_3$); IR (KBr) 3500 (OH), 3360 (NH), 2900 (aliphatic C–H), 1755 (lactone), 1595, 1500 and 1475 (aromatic C=C) $cm^{-1}$; $^1H$ NMR ($CDCl_3$) 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, $OCH_2O$), 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′-$OCH_3$), 3.16 (dd, $J$ = 4.9, 14.0 Hz, 1 H, 2-H), 3.00 (m, 1 H, 3-H).

Anal. ($C_{27}H_{25}NO_7$) C, H, N.

**4′-*O*-Demethyl-4β-(3″-cyanoanilino)-4-desoxypodophyllotoxin (11)**: yield 64.3%; crystals from methanol; mp 191–192 °C; $[\alpha]^{25}_D$ −117° (*c* = 0.33, $CHCl_3$); 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 ($CDCl_3$) 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, $OCH_2O$), 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′-$OCH_3$), 3.14–3.00 (m, 2 H, 2-H and 3-H).

Anal. ($C_{28}H_{24}N_2O_7 \cdot {}^1/_2H_2O$) C, H, N.

**4′-*O*-Demethyl-4β-(4″-cyanoanilino)-4-desoxypodophyllotoxin (12)**: yield 45.7%; crystals from ethanol; mp 187–189 °C; $[\alpha]^{25}_D$ −145° (*c* = 1.0, $CHCl_3$); 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 ($CDCl_3$) 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, $OCH_2O$), 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′-$OCH_3$), 3.09 (dd, 1 H, 2-H), 3.05 (m, 1 H, 3-H). Anal. ($C_{28}H_{24}N_2O_7$) C, H, N.

**4′-*O*-Demethyl-4β-[2″-(ethoxycarbonyl)anilino]-4-desoxypodophyllotoxin (13)**: yield 51%; crystals from ethanol; mp 231–232 °C; $[\alpha]^{25}_D$ −102 °C (*c* = 0.5, $CHCl_3$); 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 ($CDCl_3$) 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, $OCH_2O$), 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, $CO_2CH_2CH_3$), 3.86 (t, *J* = 7.3 Hz, 1 H, 11-H), 3.81 (s, 6 H, 3′,5′-$OCH_3$), 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, $CO_2CH_2CH_3$). Anal. ($C_{30}H_{29}NO_9$) C, H, N.

**4′-*O*-Demethyl-4β-[3″-(methoxycarbonyl)anilino]-4-desoxypodophyllotoxin (14)**: yield 61%; crystals from methanol; mp 255–258 °C; $[\alpha]^{25}_D$ −98° (*c* = 0.5, $CHCl_3$); 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 ($CDCl_3$) 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, 1H, 8-H), 6.33 (s, 2 H, 2′,6′-H), 5.97 and 5.95 (AB q, *J* = 1.2 Hz, 2 H, $OCH_2O$), 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, $CO_2CH_3$), 3.81 (s, 6 H, 3′,5′-$OCH_3$), 3.14 (dd, 1 H, 2-H), 3.05 (m, 1 H, 3-H).

Anal. ($C_{29}H_{27}NO_9$) C, H, N.

**4′-*O*-Demethyl-4β-[4″-(ethoxycarbonyl)anilino]-4-desoxypodophyllotoxin (15)**: yield 48.7%; crystals from ethanol; mp 270–271 °C; $[\alpha]^{25}_D$ −145° (*c* = 0.33, $CHCl_3$), 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 ($CDCl_3$) 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, $OCH_2O$), 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, $CO_2CH_2CH_3$), 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′-$OCH_3$), 3.10 (dd, 1 H, 2-H), 3.08 (m, 1 H, 3-H), 1.38 (t, *J* = 7.1 Hz, 3 H, $CO_2CH_2CH_3$).

Anal. ($C_{30}H_{29}NO_9$) C, H, N.

**4′-*O*-Demethyl-4β-[3″-(methoxycarbonyl)-4″-hydroxyanilino]-4-desoxypodophyllotoxin (16)**: yield 49%; crystals from ethanol; mp 158–160 °C; $[\alpha]^{25}_D$ −115° (*c* = 0.5, $CHCl_3$); 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 ($CDCl_3$) 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, $OCH_2O$), 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″-$CO_2CH_3$), 3.80 (s, 6 H, 3′,5′-$OCH_3$), 3.18 (dd, 1 H, 2-H), 3.11 (m, 1 H, 3-H).

Anal. ($C_{29}H_{27}NO_{10}$).

**4′-*O*-Demethyl-4β-[3″-hydroxy-4″-(methoxycarbonyl)anilino]-4-desoxypodophyllotoxin (17)**: yield 45%; crystals from methanol; mp 177–180 °C; $[\alpha]^{25}_D$ −146° (*c* = 0.5, $CHCl_3$); 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 ($CDCl_3$) 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, $OCH_2O$), 5.97 (s, 1 H, $OCH_2O$), 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″-$CO_2CH_3$), 3.79 (s, 6 H, 3″,5″-$OCH_3$), 3.05 (m, 2 H, 2-H and 3-H).

Anal. ($C_{29}H_{27}NO_{10}$).

**4′-*O*-Demethyl-4β-[3″,5″-bis(methoxycarbonyl)anilino]-4-desoxypodophyllotoxin (18)**: yield 35%; crystals from ethanol; mp 170–173 °C dec; $[\alpha]^{25}_D$ −111° (*c* = 0.5, $CHCl_3$); 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 ($CDCl_3$) 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, $OCH_2O$), 5.97 (s, 1 H, $OCH_2O$), 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″-$CO_2CH_3$), 3.90 (t, 1 H, 11-H), 3.81 (s, 6 H, 3′,5′-$OCH_3$), 3.10 (m, 2 H, 2-H and 3-H).

Anal. ($C_{31}H_{29}NO_{11} \cdot H_2O$) C, H, N.

**4′-*O*-Demethyl-4β-(3″-methoxyanilino)-4-desoxypodophyllotoxin (19)**: yield 57%; $[\alpha]^{25}_D$ −117° (*c* = 0.5, $CHCl_3$); 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 ($CDCl_3$) 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, $OCH_2O$), 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′-$OCH_3$), 3.78 (s, 3 H, 3″-$OCH_3$), 3.13 (dd, 1 H, 2-H), 2.99 (m, 1 H, 3-H).

Anal. ($C_{28}H_{27}NO_8$) C, H, N.

**4′-*O*-Demethyl-4β-(3″-acetylanilino)-4-desoxypodophyllotoxin (20)**: yield 42%; crystals from ethyl acetate–ether; mp 259–262 °C; $[\alpha]^{25}_D$ −121° (*c* = 0.5, $CHCl_3$); IR (KBr) 3380 (NH), 2900 (aliphatic C–H), 1738 (lactone), 1660 (ester), 1590, 1575 and 1500 (aromatic C=C) $cm^{-1}$; $^1H$ NMR ($CDCl_3$) 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, $OCH_2O$), 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′-$OCH_3$), 3.12 (dd, *J* = 4.9, 14.0 Hz, 1 H, 2-H), 3.04 (m, 1 H, 3-H).

Anal. ($C_{29}H_{27}NO_8$) C, H, N.

**4′-*O*-Demethyl-4β-[3″-(hydroxymethyl)anilino]-4-desoxypodophyllotoxin (21)**: yield 43%; crystals from ether–hexane; mp 189–192 °C; $[\alpha]^{25}_D$ −110° (*c* = 0.5, $CHCl_3$); IR (KBr) 3500 (OH), 3380 (NH), 2890 (aliphatic C–H), 1745 (lactone), 1595, 1500 and 1470 (aromatic C=C) $cm^{-1}$; $^1H$ NMR ($CDCl_3$) 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, $OCH_2O$), 5.45 (s, 1 H, 4′-OH), 4.71 (d, *J* = 3.4 Hz, 1 H, 4-H), 4.65 (s, 2 H, 3″-$CH_2OH$), 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′-$OCH_3$), 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. ($C_{28}H_{27}NO_8$) C, H, N.

**4′-*O*-Demethyl-4β-(3″,4″-dimethoxyanilino)-4-desoxypodophyllotoxin (22)**: yield 39%; crystals from methanol; mp 233–234 °C dec; $[\alpha]^{25}_D$ −118° (*c* = 1, $CHCl_3$); IR (KBr) 3500 (OH), 3360 (NH), 2920 (aliphatic C–H), 1770 (lactone), 1605 and 1505 (aromatic C=C) $cm^{-1}$; $^1H$ NMR ($CDCl_3$) 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, $OCH_2O$), 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''-$OCH_3$), 3.81 (s, 3 H, 3''-$OCH_3$), 3.80 (s, 6 H, 3',5'-$OCH_3$), 3.18 (dd, $J$ = 5.0, 14.0 Hz, 1 H, 2-H), 2.96 (m, 1 H, 3-H).

Anal. ($C_{29}H_{29}NO_9$) C, H, N.

**4'-*O*-Demethyl-4β-(3'',4'',5''-trimethoxyanilino)-4-desoxypodophyllotoxin (23)**: yield 60%; crystals from ethanol–ether; mp 240–242 °C; $[\alpha]^{25}_D$ −110° ($c$ = 0.5, $CHCl_3$); IR (KBr) 3500 (OH), 3460 (NH), 2930 (aliphatic C–H), 1765 (lactone), 1600, 1500, 1476 (aromatic C=C) $cm^{-1}$; $^1H$ NMR ($CDCl_3$) 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, $OCH_2O$), 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''-$OCH_3$), 3.80 (s, 6 H, 3',5'-$OCH_3$), 3.18 (dd, 1 H, 2-H), 3.00 (m, 1 H, 3-H).

Anal. ($C_{30}H_{31}NO_{10}\cdot{}^1/_2H_2O$) C, H, N.

**4'-*O*-Demethyl-4β-[3'',4''-(methylenedioxy)anilino]-4-desoxypodophyllotoxin (24)**: yield 36%; crystals from methanol; mp 247–249 °C dec; $[\alpha]^{25}_D$ −126° ($c$ = 1, $CHCl_3$); IR (KBr) 3500 (OH), 3340 (NH), 2900 (aliphatic C–H), 1752 (lactone), 1605, 1496 and 1475 (aromatic C=C) $cm^{-1}$; $^1H$ NMR ($CDCl_3$) 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 $OCH_2O$), 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'-$OCH_3$), 3.15 (dd, 1 H, 2-H), 2.95 (m, 1 H, 3-H).

Anal. ($C_{28}H_{25}NO_9$) C, H, N.

**4'-*O*-Demethyl-4β-[3'',4''-(ethylenedioxy)anilino]-4-desoxypodophyllotoxin (25)**: yield 41%; crystals from chloroform; mp 293–296 °C dec; $[\alpha]^{25}_D$ −114° ($c$ = 0.25, $CHCl_3$); IR (KBr) 3360 (NH), 2950 (aliphatic C–H), 1730 (lactone), 1615, 1515 and 1480 (aromatic C=C) $cm^{-1}$; $^1H$ NMR ($CDCl_3$) 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, $OCH_2O$), 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''–$OCH_2CH_2O$–), 4.05 (t, 1 H, 11-H), 3.79 (s, 6 H, 3',5'-$OCH_3$), 3.15 dd, 1 H, 2-H), 2.95 (m, 1 H, 3-H).

Anal. ($C_{29}H_{27}NO_9\cdot H_2O$) C, H, N.

**4'-*O*-Demethyl-4β-(4''-phenoxyanilino)-4-desoxypodophyllotoxin (26)**: yield 51%; crystals from ethanol; mp 144–146 °C; $[\alpha]^{25}_D$ −121° ($c$ = 0.5, $CHCl_3$); IR (KBr) 3520 (OH), 3400 (NH), 2910 (aliphatic C–H), 1785 (lactone), 1615, 1510 and 1485 (aromatic C=C) $cm^{-1}$; $^1H$ NMR ($CDCl_3$) 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, $OCH_2O$), 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'-$OCH_3$), 3.18 (dd, $J$ = 4.8, 14.8 Hz, 1 H, 2-H), 3.05 (m, 1 H, 3-H).

Anal. ($C_{33}H_{29}NO_8$) C, H, N.

**4'-*O*-Demethyl-4β-(4''-morpholinoanilino)-4-desoxypodophyllotoxin (27)**: yield 46%; crystals from ethanol; mp 235–237 °C dec; $[\alpha]^{25}_D$ −129° ($c$ = 1, $CHCl_3$); IR (KBr) 3500 (OH), 3330 (NH), 2880 (aliphatic C–H), 1755 (lactone), 1620, 1510 and 1475 (aromatic C=C) $cm^{-1}$; $^1H$ NMR ($CDCl_3$) 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, $OCH_2O$), 5.95 (s, 1 H, $OCH_2O$), 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'-$OCH_3$), 3.22–2.90 (m, 2 H, 2-H, 3-H and 4 H, morpholino).

Anal. ($C_{31}H_{32}N_2O_8$) C, H, N.

**4'-*O*-Demethyl-4β-(3''-nitroanilino)-4-desoxypodophyllotoxin (28)**: yield 47%; crystals from ethanol; mp 185–187 °C; $[\alpha]^{25}_D$ −96° ($c$ = 0.5, $CHCl_3$); IR (KBr) 3390 (NH), 2900 (aliphatic C–H), 1750 (lactone), 1520 and 1345 ($NO_2$) $cm^{-1}$; $^1H$ NMR ($CDCl_3$) 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, $OCH_2O$), 5.98 (s, 1 H, $OCH_2O$), 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'-$OCH_3$), 3.11 (m, 2 H, 2-H and 3-H).

Anal. ($C_{27}H_{24}N_2O_9$) C, H, N.

**4'-*O*-Demethyl-4β-(4''-nitroanilino)-4-desoxypodophyllotoxin (29)**: yield 44%; crystals from ethyl acetate; mp 205–207 °C; $[\alpha]^{25}_D$ −170° ($c$ = 0.5, $CHCl_3$); IR (KBr) 3500 (OH), 3470 (NH), 2920 (aliphatic C–H), 1775 (lactone), 1600, 1520 and 1490 (aromatic C=C), 1330 and 1310 ($NO_2$) $cm^{-1}$; $^1H$ NMR ($CDCl_3$) 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, $OCH_2O$), 5.98 (s, 1 H, $OCH_2O$), 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'-$OCH_3$), 3.10 (m, 2 H, 2-H and 3-H).

Anal. ($C_{27}H_{24}N_2O_9$) C, H, N.

**4'-*O*-Demethyl-4β-(2''-hydroxy-5''-nitroanilino)-4-desoxypodophyllotoxin (30)**: yield 35%; crystals from ethanol; mp 192–194 °C dec; $[\alpha]^{25}_D$ −114° ($c$ = 0.5, $CHCl_3$); IR (KBr) 3540 (OH), 3420 (NH), 2925 (aliphatic C–H), 1775 (lactone), 1630 and 1600 (aromatic C=C), 1530 and 1350 ($NO_2$) $cm^{-1}$; $^1H$ NMR ($CDCl_3$) 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, $OCH_2O$), 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'-$OCH_3$), 3.17 (m, 2 H, 2-H and 3-H).

Anal. ($C_{27}H_{24}N_2O_{10}\cdot{}^1/_2H_2O$) 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; $[\alpha]^{25}_D$ −85° ($c$ = 0.5, $CHCl_3$); IR (KBr) 3500 (OH), 3360 (NH), 2930 (aliphatic C–H), 1780 (lactone), 1620, 1510 and 1485 (aromatic C=C) $cm^{-1}$; $^1H$ NMR ($CDCl_3$) 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, $OCH_2O$), 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'-$OCH_3$), 3.09 (m, 2 H, 2-H and 3-H).

Anal. ($C_{29}H_{23}F_6NO_7$) C, H, N.

**4'-*O*-Demethyl-4β-(2''-pyridylamino)-4-desoxypodophyllotoxin (32)**: yield 26.6%; crystals from ethanol; mp 215–218 °C dec; $[\alpha]^{25}_D$ −82° ($c$ = 0.33, $CHCl_3$); IR (KBr), 3500 (OH), 3360 (NH), 2950 (aliphatic C–H), 1760 (lactone), 1690, 1645, 1600 and 1460 (aromatic ring) $cm^{-1}$; $^1H$ NMR ($CDCl_3$) 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, $OCH_2O$), 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'-$OCH_3$), 3.05 (m, 2 H, 2-H and 3-H).

Anal. ($C_{26}H_{24}N_2O_7\cdot{}^1/_2H_2O$) C, H, N.

**4'-*O*-Demethyl-4β-(3''-pyridylamino)-4-desoxypodophyllotoxin (33)**: yield 10%; crystals from ethanol; mp 179–181 °C dec; $[\alpha]^{25}_D$ −99° ($c$ = 0.33, $CHCl_3$); IR (KBr) 3500 (OH), 3350 (NH), 2900 (aliphatic C–H), 1765 (lactone), 1575, 1500 and 1470 (aromatic ring) $cm^{-1}$; $^1H$ NMR ($CDCl_3$) 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, $OCH_2O$), 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'-$OCH_3$), 3.18 (dd, $J$ = 4.9, 14.1 Hz, 1 H, 2-H), 3.03 (m, 1 H, 3-H).

Anal. ($C_{26}H_{24}N_2O_7\cdot{}^1/_2H_2O$) C, H, N.

**4'-*O*-Demethyl-4β-(3''-quinolylamino)-4-desoxypodophyllotoxin (34)**: yield 49.4%; crystals from ethanol–ether; mp 243–246 °C dec; $[\alpha]^{25}_D$ −179° ($c$ = 0.5, $CHCl_3$); IR (KBr), 3460 (OH), 3380 (NH), 2900 (aliphatic C–H), 1775 (lactone), 1605, 1510 and 1480 (aromatic ring) $cm^{-1}$; $^1H$ NMR ($CDCl_3$) 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, $OCH_2O$), 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'-$OCH_3$), 3.15 (m, 2 H, 2-H and 3-H).

Anal. ($C_{30}H_{26}N_2O_7$) C, H, N.

**4'-*O*-Demethyl-4β-[(4''-hydroxyphenyl)thio]-4-desoxypodophyllotoxin (40)**: yield 73%; crystals from methanol–acetone; mp 210–212 °C; $[\alpha]^{25}_D$ −111° ($c$ = 1, $CHCl_3$); IR (KBr)

3500 (OH), 2990 (aliphatic C–H), 1800 (lactone), 1635, 1520 and 1495 (aromatic C=C); $^1$H NMR ($CDCl_3$) $\delta$ 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. ($C_{27}H_{24}O_8 \cdot {}^1/_2H_2O$) 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-4β-(3″-aminoanilino)-4-desoxypodophyllotoxin Chloride (35)**: yield 75%; mp 203–206 °C dec; $[\alpha]^{25}_D$ −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$^{-1}$; $^1$H NMR ($CD_3OD$) $\delta$ 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. ($C_{27}H_{27}N_2ClO_7$) C, H.

**4′-*O*-Demethyl-4β-(4″-aminoanilino)-4-desoxypodophyllotoxin Chloride (36)**: yield 72%; mp 187–190 °C dec; $[\alpha]^{25}_D$ −100° ($CH_3OH$); IR (KBr) 3420, 2920 and 2600 ($NH_3^+$ OH and aliphatic C–H), 1770 (lactone), 1620, 1520 and 1490 cm$^{-1}$; $^1$H NMR (DMSO-$d_6$) $\delta$ 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. ($C_{27}H_{27}N_2Cl_2O_7 \cdot H_2O$) C, H.

**4′-*O*-Demethyl-4β-(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$^{-1}$; $^1$H NMR $\delta$ ($CD_3OD$) 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. ($C_{27}H_{27}ClN_2O_8$) C, H, N.

**4′-*O*-Demethyl-4β-(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 $BF_3 \cdot OEt_2$ (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; $[\alpha]^{25}_D$ −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$^{-1}$; $^1$H NMR ($CDCl_3$) $\delta$ 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. ($C_{27}H_{23}FO_8$) C, H, N.

**4′-*O*-Demethyl-4β-(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; $[\alpha]^{25}_D$ −95° ($c$ = 0.5, acetone); IR (KBr) 3400 (OH), 2900 (aliphatic C–H), 1750 (lactone), 1600, 1500 and 1480 (aromatic C=C) cm$^{-1}$; $^1$H NMR (DMSO-$d_6$) $\delta$ 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. ($C_{27}H_{24}O_9$), 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]

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**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-$H_2NC_6H_4CN$, 2237-30-1; 4-$H_2NC_6H_4CN$, 873-74-5; 2-$H_2NC_6H_4CO_2C_2H_5$, 87-25-2; 3-$H_2NC_6H_4CO_2CH_3$, 4518-10-9; 4-$H_2NC_6H_4CO_2C_2H_5$, 94-09-7; 3-$H_2NC_6H_4OCH_3$, 536-90-3; 3-$H_2NC_6H_4COCH_3$, 99-03-6; 3-$H_2NC_6H_4CH_2OH$, 1877-77-6; 4-$H_2NC_6H_4OPh$, 139-59-3; 3-$H_2NC_6H_4NO_2$, 99-09-2; 4-$H_2NC_6H_4NO_2$, 100-01-6; 4-$FC_6H_4OH$, 371-41-5; 4-$HOC_6H_4OCH_2Ph$, 103-16-2; 4-$HSC_6H_4OH$, 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-dicarboxmethoxyaniline, 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.

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