Acknowledgment. The authors thank Mike Fisher of the Cancer Research Center, UNC-Chapel Hill for KB cell culture assay. This work was supported by grants from the American Cancer Society CH-370 (K.H.L.) and the National Cancer Institute CA 44358 (Y.C.C.)

Registry No. 1, 33419-42-0; 2, 138261-30-0; 3, 6559-91-7; 4, 138261-31-1; 5, 138261-32-2; 6, 138261-33-3; 7, 138261-34-4; 8, 138261-35-5; 9, 138261-36-6; 10, 138261-37-7; 11, 138261-38-8; 12, 138261-39-9; 13, 138261-40-2; DNA topoisomerase II, 80449-01-0; 4-nitroaniline, 100-01-6; 3-nitroaniline, 99-09-2; 3-aminophenol, 591-27-5; 2,3-dihydro-1,4-benzodioxin-6-amine, 22013-33-8; 1,3-benzodioxol-5-amine, 14268-66-7; 4-fluoroaniline, 371-40-4; 4-chloroaniline, 106-47-8; 4-bromoaniline, 106-40-1.

Antitumor Agents. 124.† New 4β-Substituted Aniline Derivatives of 6,7-*O*,*O*-Demethylene-4′-*O*-demethylpodophyllotoxin and Related Compounds as Potent Inhibitors of Human DNA Topoisomerase II

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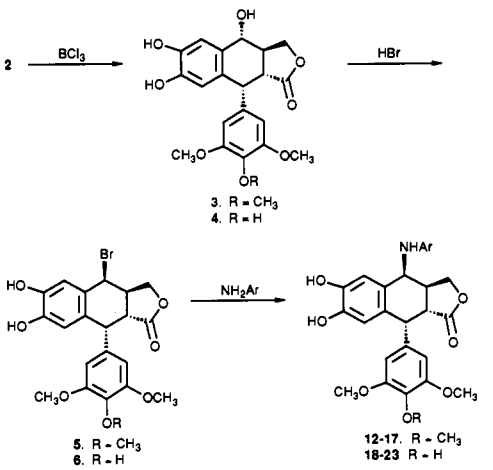
A series of 6,7-*O*,*O*-demethylene-4′-*O*-demethyl-4β-(substituted anilino)-4-desoxypodophyllotoxins (18–23), 6,7-*O*,*O*-demethylene-6,7-*O*,*O*-dimethyl-4′-*O*-demethyl-4β-(substituted anilino)-4-desoxypodophyllotoxins (28–31), and their corresponding 4′-*O*-methyl analogues (12–17 and 24–27) have 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. Compounds 18–23 are 2-fold more potent than etoposide and compounds 12, 16, 17, 30, and 31 are as active as etoposide in their inhibition of the human DNA topoisomerase II. Compounds 19 and 20 and 29–31 are as active or more active than etoposide in causing protein-linked DNA breakage. These results indicate that a free C-4′ hydroxy group is essential for the DNA breakage activity, and that the hydroxyl groups at C-6 and -7 positions may be involved in an interaction which is responsible for the inhibitory activity of DNA topoisomerase II. The maintenance of an intact methylene dioxy-type ring-A system would contribute to enhanced activity. In addition, the sterically less hindered substitution at C-6 and C-7 positions may be important for optimal interactions with DNA topoisomerase II. There is no correlation between the ability of these compounds to inhibit DNA topoisomerase II and their ability to cause protein-linked DNA breaks in cells. This may relate to the difference in uptake of these compounds. The better correlation was observed between the protein-linked DNA breaks and the cytotoxicity in KB cells of these compounds.

Etoposide (1) is an important anticancer drug used in the clinic for the treatment of small-cell lung cancer, testicular cancer, lymphoma, and leukemia.[2,3] It has recently been shown that 1 and related compounds are potent inhibitors of DNA topoisomerase II. These compounds inhibit the catalytic activity of the target enzyme by stabilizing a cleavable enzyme–DNA complex in which the DNA is cleaved and covalently linked to the enzyme.[4-6]

To date a number of structural modifications on 1 have been reported. These include (1) the replacement of the glucose moiety with an amino sugar,[7] (2) changes of the glucose moiety with a simpler group, such as a 4β-substituted anilino group and a 4β-*O*-aminoethyl group,[8-10] (3) the conversion of the lactone ring D to the hydrazide, hydroxy acid, and diol or cyclic ether,[2,3] and (4) the modification of ring E, by halogenation to the 2′-halo compounds, oxidation to the 3′,4′-orthoquinones and the ring-E desoxy analogues.[11-13]

However, very little work has been done on the modification of ring A.[14,15] The 6,7-*O*,*O*-demethyleneetoposide has not yet been reported. In order to investigate the effect of ring A on the biological activity of the molecule, we have synthesized a series of 6,7-*O*,*O*-demethylene-4′-*O*-demethyl-4β-(substituted anilino)-4-desoxypodophyllotoxins (**18–23**), 6,7-*O*,*O*-demethylene-6,7-*O*,*O*-dimethyl-4′-*O*-demethyl-4β-(substituted anilino)-4-desoxypodophyllotoxins (**28–31**), and their corresponding 4′-*O*-methyl analogues (**12–17** and **24–27**) for evaluating their inhibitory activity

Scheme I



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† For part 123, see ref 1.

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(1) Part 123: Hu, H.; Wang, Z. Q.; Liu, S. Y.; Cheng, Y. C.; Lee, K. H. Antitumor Agents 123. Synthesis and Human DNA Topoisomerase II Inhibitory Activity of 2′-Chloro Derivatives of Etoposide and 4β-(Arylamino)-4′-*O*-demethylpodophyllotoxins. *J. Med. Chem.*, previous article in this issue.

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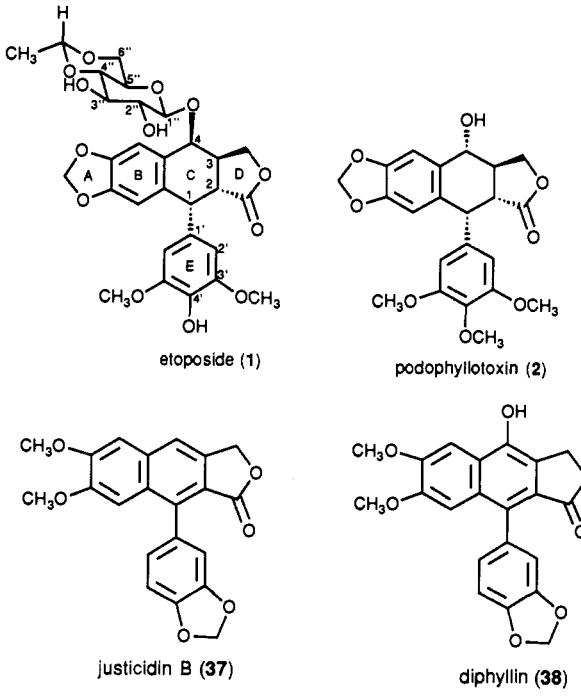
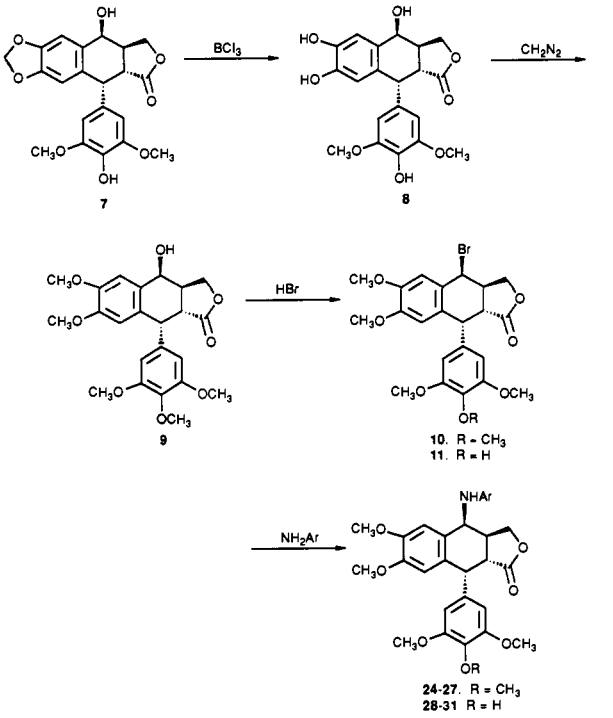


Figure 1.

against the human DNA topoisomerase II as well as their activity in causing cellular protein-linked DNA breakage.

Scheme II



The ring-A-opened compounds have been found in the naturally occurring podophyllotoxin (2) congeners, such as justicidin B (37) and diphyllin (38)[16] as shown in Figure 1. Compounds 37 and 38 were reported to be much more effective against Sindbis virus than 2.[17]

Chemistry

The preparation of compounds 12–23 is shown in Scheme I. The intermediates, 6,7-*O*,*O*-demethylenepodophyllotoxin (3) and its 4′-*O*-demethyl compound (4), were synthesized from podophyllotoxin (2) by use of a slightly modified literature procedure.[14] Boron trichloride has been used in the selective cleavage of the methylenedioxy group in the presence of aromatic methoxy groups.[14,18,19] The cleavage of the methoxy group usually requires either higher temperature or longer reaction time. However, in our experiments the resulting 3 was further demethylated at C-4′ to afford 4 in a yield of 3–10% even at very low temperature (−70 to −65 °C) within 3 h.

The bromination of 3 and 4 was carried out according to our previous method to furnish the bromides 5 and 6, respectively, which were used directly for the next step of reaction due to their instability.[8,9]

Normally, the nucleophilic substitution of a bromide derived from 2 or its related compounds by an aniline requires 16–20 h as discussed in our previous work.[9] However, bromides 5 and 6, which do not contain the

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methylenedioxy bridge between C-6 and C-7, required a shorter reaction time of 2–3 h.

The preparation of target compounds **24–31** is shown in Scheme II. Compound **8** was synthesized from 4′-*O*-demethylepipodophyllotoxin (**7**) in an analogous method as described for the preparation of **3** and **4** with a high yield of 85% after recrystallization.[20]

Although compounds **24–27** were easy to prepare from intermediate **9**, the preparation of partially methylated **28–31** met with some difficulty. Attempted selective methylation only on the 6,7-hydroxy group by controlling the amount of diazomethane, the reaction temperature or the reaction time, as well as the selective blockage of the 4′-hydroxy group by using several types of protecting groups were unsuccessful. Finally, a full O-methylation of **8** was applied to afford **9**. Bromination of **9** with HBr at room temperature for 14 h yielded a mixture of the bromides **10** and **11**, which upon treatment with substituted anilines gave rise to **24–27** and **28–31**.

Results and Discussion

As shown in Table I, all target compounds (**12–31**) were assayed for their cytotoxicity in KB cells and their ability to inhibit human DNA topoisomerase II and to cause the protein-linked DNA breakage in comparison with etoposide (**1**). These results provided the following conclusions: (1) Compounds in group II (**19** and **20**) and IV (**29–31**), which possess the free hydroxy group at 4′-position, showed comparable or superior activity to **1** in causing protein-linked DNA strand breakage. Methylation of the OH group at C-4′ yielded compounds, such as **12–17** in group I and **24–27** in group III, which were all inactive. Thus, a free 4′-hydroxy group is essential for the DNA breakage activity. This data is in agreement with the previous reports from us and others.[8,21–23] (2) The ring-A-opened C-4′ OH-bearing 6,7-dihydroxy compounds (**18–23**) exhibited significant activity in inhibiting DNA topoisomerase II. They were approximately 2-fold more potent than **1**. Even among their corresponding C-4′ OH methylated compounds (**12–17**), compounds **12**, **16**, and **17** were as potent as **1** in inhibiting the target enzyme. This suggested that hydroxy groups at C-6 and -7 positions play as important a role as the 4′-hydroxy group in an interaction that is involved in the enzyme inhibitory activity. This was further supported by the data from compounds in group III (**24–27**), in which three hydroxy groups at 6-, 7-, and 4′-positions have all been blocked by methyl group. These compounds showed no activity in inhibiting the DNA topoisomerase II as well as in causing the DNA breakage. Thus, the data discussed above seem to imply that a sterically less hindered substitution at the C-6 and C-7 positions is important for optimal interactions with DNA topoisomerase II. (3) There was no significant correlation between the ability of tested compounds in causing protein-linked DNA breakage and in inhibiting DNA topoisomerase II. This could be due to the difference in uptake of these compounds. However, the better correlation between protein-linked DNA breaks and cytotoxicity in KB cells was observed in these compounds. (4) The maintenance of an intact methylenedioxy-type ring-A system, in general, appears to be more important than a ring-A-opened system in contributing to the enhanced ability in inhibiting DNA topoisomerase II and in causing protein-linked DNA breakage. Thus, the methylenedioxy-bearing **32–35**[9] in group V are much more potent than their corresponding C-6 and C-7 free OH-bearing **19–22**.

Experimental Section

General Experimental Procedures. All melting points were taken on a Fischer-Johns melting point apparatus and were uncorrected. The IR spectra were recorded on a Perkin-Elmer 1320 spectrophotometer, and ^{1}H 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. Silica gel 60 (32–63 μm) from Universal Scientific Inc. was used for column chromatography. Preparative TLC was performed on Analtech precoated silica gel GF (1000 μm, 20 × 20 cm). All new target compounds were characterized by melting point, optical rotation, and ^{1}H NMR and IR spectroscopy as well as elemental analyses.

6,7-*O*,*O*-Demethylenepodophyllotoxin (3). To a solution of boron trichloride in dichloromethane (1 M, 120 mL) precooled at −70 to −65 °C was added dropwise podophyllotoxin (**2**) (12.4 g, 30 mmol) in dichloromethane (100 mL) over 2 h. After the mixture was stirred at the same temperature for an additional 1 h, the mixture was poured into 500 mL of ice–water, extracted with ethyl acetate three times. The combined organic layers were washed with brine until the pH was 6–7, dried over anhydrous sodium sulfate, and filtered. The filtrate was evaporated in vacuo to give a white solid (12.7 g). The solid was put into a mixture of acetone–water–calcium carbonate (120 mL, 120 mL, 8 g) and refluxed for 3.5 h. The white suspension was filtered off, and the filtrate was neutralized by 1 N hydrochloric acid until pH was 2–3 and extracted by ethyl acetate five times. The combined organic layers were washed with brine until the pH was 6, dried over anhydrous sodium sulfate, and evaporated in vacuo to afford 10.1 g of a mixture of **3** and **4**. Purification of this mixture by flash column chromatography on silica gel (200 g) with a mixture of chloroform–acetone–methanol (100:10:5) as the eluting solvent gave 8.2 g of **3** as a white solid (68% yield): mp 226–228 °C; crystals from ethyl acetate; $[\alpha]^{25}_D$ −120° (c = 0.5, ethyl alcohol); IR (KBr) 3518, 3400, 3000, 1760, 1578, and 1500 cm^{-1}; ^{1}H NMR (acetone-d_6) δ 7.90 (br s, 2 H, OH-6,7), 7.20 (s, 1 H, H-5), 6.47 (s, 1 H, H-8), 6.46 (s 2 H, H-2′,6′), 4.75 (d, J = 9.7 Hz, 1 H, H-4), 4.50 (t, 2 H, H-1,11), 4.12 (t, J = 10.3 Hz, 1 H, H-11), 3.68 (s, 9 H, OCH_3-3′,4′,5′), 3.04 (dd, J = 14.3, 4.8 Hz, 1 H, H-2), and 2.83 (m, 1 H, H-3). Anal. ($C_{21}H_{22}O_8$) C, H.

6,7-*O*,*O*-Demethylene-4′-*O*-demethylpodophyllotoxin (4). This compound was obtained from the aforementioned flash column by further elution as white solids (0.81 g): mp 208–211 °C dec, crystals from ethyl acetate; $[\alpha]^{25}_D$ −101° (c = 0.25, acetone); IR (KBr) 3430, 3160, 1772, 1638, and 1540 cm^{-1}; ^{1}H NMR (acetone-d_6) δ 7.88 and 7.87 (s and s, 2 H, OH-6,7), 7.19 (s, 1 H, H-5), 7.07 (s, 1 H, OH-4′), 6.47 (s, 1 H, H-8), 6.45 (s, 2 H, H-2′,6′), 4.74 (d, J = 9.5 Hz, 1 H, H-4), 4.71 (s, 1 H, OH-4), 4.49 (m, 2 H, H-1,11), 4.11 (t, J = 10.2 Hz, 1 H, H-11), 3.69 (s, 6 H, OCH_3-3′,5′), 2.96 (dd, J = 14.2, 4.9 Hz, 1 H, H-2), and 2.81 (m, 1 H, H-3). Anal. ($C_{20}H_{20}O_8$) C, H.

6,7-*O*,*O*-Demethylene-4β-bromo-4-desoxypodophyllotoxin (5). To a suspension of **3** (5.5 g, 13.6 mmol) in 150 mL of dry dichloromethane precooled at 0–5 °C was bubbled with dry hydrogen bromide gas for 1 h. The solution was then evaporated in vacuo to dryness. The crude product (6.6 g) obtained was used for the preparation of compounds **12–17**.

6,7-*O*,*O*-Demethylene-4β-bromo-4′-*O*-demethyl-4-desoxypodophyllotoxin (6). By use of the same method outlined for

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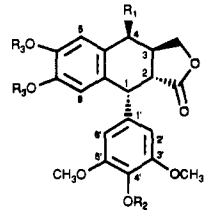
Table I. Biological Evaluation of 4β-(Arylamino)-6,7-*O*,*O*-demethylene-4-desoxypodophyllotoxins



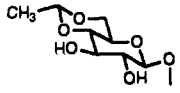
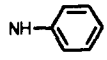
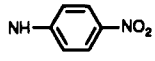
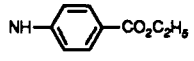
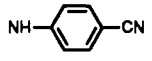
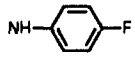
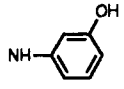
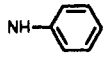
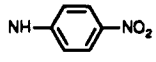
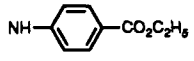
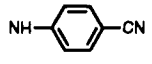
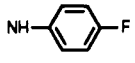
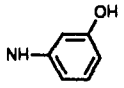
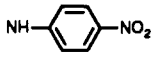
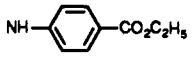
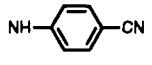
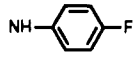
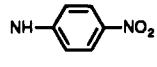
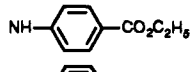
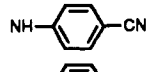
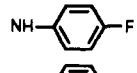
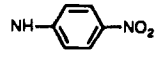
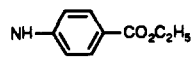
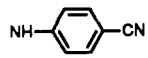
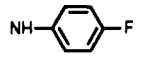
group	compd	R_1	R_2	R_3	cytotoxicity:[a] IC_{50} KB, μM	inhibition of DNA topoisomerase II:[b] ID_{50}, μM	cellular protein–DNA complex formation (%), 10 μM
I	1		H	CH_2[c]	0.20	50	100
	12		CH_3	H	>2.10	50	9
	13		CH_3	H	>1.90	>100	23
	14		CH_3	H	>1.80	100	22
	15		CH_3	H	>2.00	>100	33
	16		CH_3	H	>2.00	50	11
	17		CH_3	H	>2.00	50	4
II	18		H	H	>2.20	25	84
	19		H	H	1.50	20	99
	20		H	H	1.46	20	138
	21		H	H	2.00	20	62
	22		H	H	>2.10	25	52
	23		H	H	>2.10	20	18
III	24		CH_3	CH_3	7.30	>100	8
	25		CH_3	CH_3	5.50	>100	9
	26		CH_3	CH_3	5.80	>100	12
	27		CH_3	CH_3	7.60	>100	8
IV	28		H	CH_3	1.30	100	75
	29		H	CH_3	<0.70	100	127
	30		H	CH_3	<0.77	50	125
	31		H	CH_3	0.78	50	108
V	32		H	CH_2[c]	0.49	10	323

Table I (Continued)

group	compd	R_1	R_2	R_3	cytotoxicity:[a] IC_{50} KB, μM	inhibition of DNA topoisomerase II:[b] ID_{50}, μM	cellular protein–DNA complex formation (%), 10 μM
	33		H	CH_2[c]	0.84	5	207
	34		H	CH_2[c]	0.64	10	211
	35		H	CH_2[c]	0.24	5	213

[a] IC_{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 10, 20, 25, 50, and 100 μM. The ID_{50} value was established on the basis of the degree of inhibition at these concentrations. [c] This corresponds to a C-6 and -7 methylenedioxy group.

the synthesis of **5**, compound **6** was prepared from **4** and used for the preparation of target compounds **18–23** without purification.

Synthesis of Compounds 12–17. A solution containing **5** (300 mg, 0.62 mmol), anhydrous barium carbonate (250 mg, 1.27 mmol), and the appropriate substituted anilines (0.65 mmol) in 5 mL of dry THF under nitrogen was stirred for 2–3 h at room temperature. The barium salts were filtered, and the filtrate was evaporated to dryness. The solid obtained was purified via a flash column chromatography [10 g of silica gel, TLC standard grade with toluene–ethyl acetate (25:40) as an eluant].

6,7-*O*,*O*-Demethylene-4β-anilino-4-desoxypodophyllotoxin (12): yield 53%; mp 216–218 °C; crystals from dichloromethane–acetone; $[\alpha]^{25}_D$ −124° (*c* = 0.2, acetone); IR (KBr) 3400, 2900, 1755, 1595, and 1500 cm^{-1}; ^1H NMR (acetone-d_6) δ 8.00 (br s, 2 H, OH-6,7), 7.13 (t, *J* = 7.6 Hz, 2 H, H-3′,5′), 6.77 (s, 1 H, H-5), 6.73 (d, *J* = 8.0 Hz, 1 H, H-4″), 6.63 (t, *J* = 7.6 Hz, 2 H, H-2″,6″), 6.38 (s, 2 H, H-2′,6′), 5.19 (d, 1 H, NH), 4.81 (d, *J* = 3.9 Hz, 1 H, H-4), 4.46 (d, *J* = 5.0 Hz, 1 H, H-1), 4.38 (t, 1 H, H-11), 3.88 (q, 1 H, H-11), 3.69 (s, 6 H, OCH_3-3′,5′), 3.65 (s, 3 H, OCH_3-4′), 3.25 (dd, *J* = 14.1, 5.0 Hz, 1 H, H-2), and 3.12 (m, 1 H, H-3). Anal. ($C_{27}H_{27}NO_7$) C, H, N.

6,7-*O*,*O*-Demethylene-4β-(4″-nitroanilino)-4-desoxypodophyllotoxin (13): yield 72%; mp 163–166 °C, crystals from acetone–dichloromethane; $[\alpha]^{25}_D$ −136° (*c* = 0.25, acetone); IR (KBr) 3370, 2950, 1665, 1600, 1505, and 1320 cm^{-1}; ^1H NMR (acetone-d_6 and $CDCl_3$) δ 8.07 (d, *J* = 8.9 Hz, 2 H, H-3″,5″), 6.85 (d, *J* = 8.9 Hz, 2 H, H-2″,6″), 6.82 (s, 1 H, H-5), 6.68 and 6.65 (s and s, 2 H, OH-6,7), 6.52 (s, 1 H, H-8), 6.39 (s, 2 H, H-2′,6′), 5.18 (dd, 1 H, H-4), 4.55 (d, *J* = 4.5 Hz, 1 H, H-1), 4.43 (t, *J* = 7.0 Hz, 1 H, H-11), 3.85 (t, *J* = 7.0 Hz, 1 H, H-11), 3.68 (s, 6 H, OCH_3-3′,5′), 3.66 (s, 3 H, OCH_3-4′), and 3.24 (m, 2 H, H-2,3). Anal. ($C_{27}H_{26}N_2O_9 \cdot {}^1/_2H_2O$) C, H; N: calcd, 5.26; found, 4.76.

6,7-*O*,*O*-Demethylene-4β-[4″-(ethoxycarbonyl)anilino]-4-desoxypodophyllotoxin (14): yield 68%; mp 158–160 °C, crystals from ethyl acetate; $[\alpha]^{25}_D$ − 99° (*c* = 0.5, acetone); IR (KBr) 3350, 2920, 1760, 1680, and 1595 cm^{-1}; ^1H NMR (acetone-d_6) δ 8.03 (br s, 2 H, OH-6,7), 7.82 (d, *J* = 6.8 Hz, 2 H, H-3″,5″), 6.81 (d, *J* = 6.8 Hz, 2 H, H-2″,6″), 6.80 (s, 1 H, H-5), 6.51 (s, 1 H, H-8), 6.41 (s, 2 H, H-2′,6′), 6.03 (d, *J* = 7.8 Hz, 1 H, NH), 5.03 (dd, *J* = 7.8, 3.8 Hz, 1 H, H-4), 4.53 (d, *J* = 4.7 Hz, 1 H, H-1), 4.42 (dd, *J* = 7.9, 6.8 Hz, 1 H, H-11), 3.85 (t, *J* = 7.9 Hz, 1 H, H-11), 3.69 (s, 6 H, OCH_3-3′,5′), 3.68 (s, 3 H, OCH_3-4′), 3.28 (dd, *J* = 14.1, 4.7 Hz, 1 H, H-2), and 3.21 (m, 1 H, H-3). Anal. ($C_{30}H_{31}NO_9$) C, H, N.

6,7-*O*,*O*-Demethylene-4β-(4″-cyanoanilino)-4-desoxypodophyllotoxin (15): yield 55%; mp 158–151 °C, crystals from toluene–ethyl acetate; $[\alpha]^{25}_D$ −117° (*c* = 0.5, acetone); IR (KBr) 3350, 2930, 2202, 1758, 1600, and 1325 cm^{-1}; ^1H NMR (acetone-d_6) δ 8.09 and 8.02 (s and s, 2 H, OH-6,7), 7.52 (d, *J* = 8.6 Hz, 2 H, H-3″,5″), 6.90 (d, *J* = 8.6 Hz, 2 H, H-2″,6″), 6.83 (s, 1 H, H-5), 6.55 (s, 1 H, H-8), 6.41 (s, 2 H, H-2′,6′), 6.23 (d, *J* = 8.6 Hz, 1 H, NH), 5.05 (br s, 1 H, H-4), 4.55 (d, *J* = 4.8 Hz, 1 H, H-1), 4.42 (t, 1 H, H-11), 3.86 (t, 1 H, H-11), 3.68 (s, 6 H, OCH_3-3′,5′), 3.67 (s, 3 H, OCH_3-4′), 3.25 (dd, 1 H, H-2), and 3.20 (m, 1 H, H-3). Anal. ($C_{28}H_{26}N_2O_7 \cdot {}^1/_2H_2O$) C, H; N: calcd, 5.42; found, 4.96.

6,7-*O*,*O*-Demethylene-4β-(4″-fluoroanilino)-4-desoxypodophyllotoxin (16): yield 59%; mp 193–195 °C, crystals from ethyl acetate; $[\alpha]^{25}_D$ − 107° (*c* = 0.5, acetone); IR (KBr) 3400, 2950, 1760, 1590, and 1510 cm^{-1}; ^1H NMR (acetone-d_6) δ 7.98 and 7.97 (s and s, 2 H, OH-6,7), 6.93 (t, 2 H, H-3″,5″), 6.76 (m, 3 H, H-5 and H-2″,6″), 6.51 (s, 1 H, H-8), 6.39 (s, 2 H, H-2′,6′), 5.19 (br s, 1 H, NH), 4.80 (d, *J* = 3.9 Hz, 1 H, H-4), 4.51 (d, *J* = 4.9 Hz, 1 H, H-1), 4.41 (t, *J* = 7.9 Hz, 1 H, H-11), 3.91 (t, *J* = 7.9 Hz, 1 H, H-11), 3.69 (s, 6 H, OCH_3-3′,5′), 3.67 (s, 3 H, OCH_3-4′), 3.25 (dd, *J* = 13.1, 4.9 Hz, 1 H, H-2), and 3.16 (m, 1 H, H-3). Anal. ($C_{27}H_{26}NFO_7$) C, H, N.

6,7-*O*,*O*-Demethylene-4β-(3″-hydroxyanilino)-4-desoxypodophyllotoxin (17): yield 49%; mp 148–150 °C, crystals from methanol–dichloromethane; $[\alpha]^{25}_D$ −98° (*c* = 0.5, acetone); IR (KBr) 3400, 2950, 1755, 1600, and 1520 cm^{-1}; ^1H NMR (acetone-d_6) δ 8.03 (s, 1 H, OH-3″), 8.00 and 7.95 (s and s, 2 H, OH-6,7), 6.95 (t, *J* = 8.3 Hz, 1 H, H-5″), 6.80 (s, 1 H, H-5), 6.49 (s, 1 H, H-8), 6.47 (d, *J* = 4.4 Hz, 1 H, NH), 6.41 (s, 2 H, H-2′,6′), 6.24 (br s, 2 H, H-2″,4″), 6.16 (dd, *J* = 8.3, 1.8 Hz, 1 H, H-6″), 4.77 (d, *J* = 4.1 Hz, 1 H, H-4), 4.49 (d, *J* = 5.0 Hz, 1 H, H-1), 4.37 (t, *J* = 7.9 Hz, 1 H, H-11), 3.91 (t, *J* = 7.9 Hz, 1 H, H-11), 3.68 (s, 9 H, OCH_3-3′,4′,5′), 3.25 (dd, *J* = 14.0, 5.0 Hz, 1 H, H-2), and 3.10 (m, 1 H, H-3). Anal. ($C_{27}H_{27}NO_8$) C, H, N.

Synthesis of Compounds 18–23. Compounds **18–23** were prepared from **6** (100 mg, 0.21 mmol), anhydrous barium carbonate (80 mg, 0.41 mmol), and the appropriate substituted anilines (0.22 mmol) by the similar procedure used for the preparation of **12–17** described above.

6,7-*O*,*O*-Demethylene-4β-anilino-4′-*O*-demethyl-4-desoxypodophyllotoxin (18): yield 73%; mp 150–153 °C, crystals from ethyl acetate; $[\alpha]^{25}_D$ −112° (*c* = 0.2, acetone); IR (KBr) 3400, 3180, 1760, 1605, and 1515 cm^{-1}; ^1H NMR (acetone-d_6) δ 8.02 (br s, 2 H, OH-6,7), 7.13 (t, 3 H, OH-4′ and H-3″,5″), 6.78 (s, 1 H, H-5), 6.72 (d, *J* = 8.0 Hz, 1 H, H-4″), 6.62 (t, *J* = 7.7 Hz, 2 H, H-2″,6″), 6.38 (s, 1 H, OCH_3-2′,6′), 5.19 (d, 1 H, NH), 4.82 (d, *J* = 3.9 Hz, 1 H, H-4), 4.46 (d, *J* = 4.9 Hz, 1 H, H-1), 4.40 (t, 1 H, H-11), 3.89 (t, 1 H, H-11), 3.68 (s, 6 H, OCH_3-3″,5″), 3.25 (dd, *J* = 14.0, 4.9 Hz, 1 H, H-2), and 3.12 (m, 1 H, H-3). Anal. ($C_{26}H_{25}NO_7 \cdot {}^1/_2H_2O$) C, H; N: calcd, 2.93; found, 2.45.

6,7-*O*,*O*-Demethylene-4β-(4″-nitroanilino)-4′-*O*-demethyl-4-desoxypodophyllotoxin (19): yield 79%; mp 185–188 °C dec, crystals from ethyl acetate–toluene; $[\alpha]^{25}_D$ − 130° (*c* = 0.25, acetone); IR (KBr) 3370, 2940, 1765, 1600, 1518, and 1320 cm^{-1}; ^1H NMR (acetone-d_6 and $CDCl_3$) δ 8.04 (d, *J* = 8.9 Hz, 2 H, H-3″,5″), 6.89 (d, *J* = 8.9 Hz, 2 H, H-2″,6″), 6.82 (s, 1 H, H-5), 6.68 and 6.65 (s and s, 2 H, OH-6,7), 6.52 (s, 1 H, H-8), 6.37 (s, 2 H, H-2′,6′), 5.13 (br s, 1 H, H-4), 4.51 (d, 1 H, H-1), 4.43 (t, 1 H, H-11), 3.85 (t, 1 H, H-11), 3.68 (s, 6 H, OCH_3-3′,5′), and 3.22 (m, 2 H, H-2,3). Anal. ($C_{26}H_{24}N_2O_9 \cdot {}^1/_2H_2O$) C, H; N: calcd, 5.41; found, 4.64.

6,7-*O*,*O*-Demethylene-4β-[4″-(ethoxycarbonyl)anilino]-4′-*O*-demethyl-4-desoxypodophyllotoxin (20): yield 75%; mp 149–152 °C, crystals from dichloromethane–ethyl acetate; $[\alpha]^{25}_D$ −104° (*c* = 0.5, acetone); IR (KBr) 3360, 2950, 1750, 1675, 1595, and 1510 cm^{-1}; ^1H NMR (acetone-d_6) δ 7.99 and 7.96 (s and s, 2 H, OH-6,7), 7.83 (d, *J* = 8.8 Hz, 2 H, H-3″,5″), 7.09 (s, 1 H, OH-4′), 6.81 (d, *J* = 8.8 Hz, 2 H, H-2″,6″), 6.79 (s, 1 H, H-5), 6.52 (s, 1 H, H-8), 6.40 (s, 2 H, H-2′,6′), 6.05 (d, 1 H, NH), 5.02 (dd, *J* = 6.9, 2.7 Hz, 1 H, H-4), 4.50 (d, *J* = 4.3 Hz, 1 H, H-1), 4.41 (t, *J* = 6.7 Hz, 1 H, H-11), 3.85 (t, *J* = 6.7 Hz, 1 H, H-11), 3.69 (s, 6 H, OCH_3-3′,5′), and 3.28–3.10 (m, 2 H, H-2,3). Anal. ($C_{29}H_{29}NO_9$) C, H, N.

6,7-*O*,*O*-Demethylene-4β-(4″-cyanoanilino)-4′-*O*-demethyl-4-desoxypodophyllotoxin (21): yield 62%; mp 153–156

°C dec, crystals from ethyl acetate–toluene; $[\alpha]^{25}_D$ −108° (c = 0.5, acetone); IR (KBr) 3360, 2920, 2200, 1755, 1596, and 1510 cm^{-1}; ^1H NMR (acetone-d_6) δ 8.08 and 8.00 (s and s, 2 H, OH-6,7), 7.50 (d, J = 8.7 Hz, 2 H, H-3″,5″), 7.13 (s, 1 H, OH-4′), 6.89 (d, J = 8.7 Hz, 2 H, H-2″,6″), 6.81 (s, 1 H, H-5), 6.53 (s, 1 H, H-8), 6.38 (s, 2 H, H-2′,6′), 6.21 (d, J = 8.6 Hz, 1 H, NH), 5.03 (dd, 1 H, H-4), 4.51 (d, J = 4.2 Hz, 1 H, H-1), 4.42 (t, 1 H, H-11), 3.84 (dd, 1 H, H-11), 3.68 (s, 6 H, OCH_3-3′,5′), and 3.20 (m, 2 H, H-2,3). Anal. ($C_{27}H_{24}N_2O_7 \cdot {}^1/_2H_2O$) C, H; N: calcd, 5.62; found, 4.84.

6,7-*O*,*O*-Demethylene-4β-(4″-fluoroanilino)-4′-*O*-demethyl-4-desoxypodophyllotoxin (22): yield 42%; mp 151–153 °C, crystals from ethyl acetate–toluene; $[\alpha]^{25}_D$ −80° (c = 0.5, acetone); IR (KBr) 3400, 2950, 1755, 1615, and 1510 cm^{-1}; ^1H NMR (acetone-d_6) δ 7.97 and 7.96 (s and s, 2 H, OH-6,7), 7.04 (s, 1 H, OH-4′), 6.94 (t, 2 H, H-3″,5″), 6.86 (m, 3 H, H-2″,6″ and H-5), 6.51 (s, 1 H, H-8), 6.40 (s, 2 H, H-2′,6′), 5.18 (s, 1 H, NH), 4.80 (d, J = 4.1 Hz, 1 H, H-4), 4.51 (d, J = 4.9 Hz, 1 H, H-1), 4.41 (t, 1 H, H-11), 3.91 (t, 1 H, H-11), 3.69 (s, 6 H, OCH_3-3′,5′), 3.26 (dd, J = 13.1, 4.9 Hz, 1 H, H-2), and 3.09 (m, 1 H, H-3). Anal. ($C_{26}H_{24}NFO_7 \cdot {}^1/_2H_2O$) C, H; N: calcd, 2.55; found, 2.09.

6,7-*O*,*O*-Demethylene-4β-(3″-hydroxyanilino)-4′-*O*-demethyl-4-desoxypodophyllotoxin (23): yield 42%; mp 180–183 °C, crystals from ethyl acetate–toluene; $[\alpha]^{25}_D$ −109° (c = 0.5, acetone); IR (KBr) 3400, 2950, 1710, 1600, and 1510 cm^{-1}; ^1H NMR (acetone-d_6) δ 8.05 (s, 1 H, OH-3″), 8.00 and 7.96 (s and s, 2 H, OH-6,7), 7.09 (s, 1 H, OH-4′), 6.95 (t, J = 8.2 Hz, 1 H, H-5″), 6.78 (s, 1 H, H-5), 6.50 (s, 1 H, H-8), 6.23 (br s, 2 H, H-2″,4″), 6.15 (dd, J = 8.2, 1.8 Hz, 1 H, H-6″), 5.10 (d, J = 6.7 Hz, 1 H, NH), 4.78 (d, J = 3.8 Hz, 1 H, H-4), 4.47 (d, J = 4.8 Hz, 1 H, H-1), 4.33 (t, J = 8.2 Hz, 1 H, H-11), 3.93 (dd, J = 14.0, 4.8 Hz, 1 H, H-2), and 3.16 (m, 1 H, H-3). Anal. ($C_{26}H_{25}NO_8 \cdot {}^1/_2H_2O$) C, H; N: calcd, 2.85; found, 2.38.

6,7-*O*,*O*-Demethylene-4′-*O*-demethylepipodophyllotoxin (8). Compound 8 (9.3 g, 80%) was prepared from 4′-*O*-demethylepipodophyllotoxin (7) (12.0 g, 30 mmol) by an analogous method used for the synthesis of 3 and 4 from 2. Compound 8: mp 227–229 °C; crystals from ethanol–acetone; $[\alpha]^{25}_D$ −97° (c = 0.37, acetone); IR (KBr) 3500, 3400, 1770, 1600, 1500, and 1460 cm^{-1}; ^1H NMR (DMSO-d_6) δ 8.90 (s, 2 H, OH-6,7), 8.23 (s, 1 H, OH-4′), 6.78 (s, 1 H, H-5), 6.33 (s, 1 H, H-8), 6.19 (s, 2 H, H-2′,6′), 5.25 (s, 1 H, OH-4), 4.63 (s, 1 H, H-4), 4.35 (d, J = 5.0 Hz, 1 H, H-1), 4.30 (t, J = 9.3 Hz, 1 H, H-11), 4.14 (t, J = 9.3 Hz, 1 H, H-11), 3.60 (s, 6, H, OCH_3-3′,5′), 3.21 (dd, J = 15.4, 5.0 Hz, 1 H, H-2), and 2.74 (m, 1 H, H-3). Anal. ($C_{20}H_{20}O_8$) C, H.

6,7-*O*,*O*-Demethylene-6,7-*O*,*O*-dimethyl-4′-*O*-demethylepipodophyllotoxin (9). To a solution of 8 (1.5 g, 3.8 mmol) in ether–methanol (5:3, 160 mL) was added freshly prepared diazomethane (according to the Technical Information Bulletin #AL-113, Aldrich). The mixture was stirred for 3 h at room temperature. The crystals precipitated in the reaction mixture and were collected and recrystallized from ether–methanol to afford 9 (1.6 g, 94%): mp 108–110 °C; $[\alpha]^{25}_D$ −62.4° (c = 0.25, acetone); IR (KBr) 3420, 2960, 1770, 1580, 1520, and 1460 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.92 (s, 1 H, H-5), 6.56 (s, 1 H, H-8), 6.28 (s, 2 H, H-2′,6′), 4.91 (s, 1 H, H-4), 4.66 (d, J = 4.6 Hz, 1 H, H-1), 4.40 (m, 2 H, H-11), 3.95 (s, 3 H, OCH_3-4′), 3.80 (s, 6 H, OCH_3-6′,7′), 3.72 (s, 6 H, OCH_3-3′,5′), 3.28 (dd, J = 15.1, 4.6 Hz, 1 H, H-2), and 2.82 (m, 1 H, H-3). Anal. ($C_{23}H_{25}O_8 \cdot {}^1/_2CH_3OH$) C, H.

6,7-*O*,*O*-Demethylene-6,7-*O*,*O*-dimethyl-4β-bromo-4-desoxypodophyllotoxin (10) and 6,7-*O*,*O*-Demethylene-6,7-*O*,*O*-dimethyl-4′-*O*-demethyl-4β-bromo-4-desoxypodophyllotoxin (11). To a solution of 9 (310 mg, 0.72 mmol) in anhydrous dichloromethane (8 mL) cooled at 0 °C was bubbled dry hydrogen bromide for 45 min. The reaction mixture was sealed and allowed to stand at room temperature for 14 h. The solution was then evaporated in vacuo, followed by using benzene as an azeotropic mixture to remove the water. The resulting mixture of 10 and 11 (498 mg) was used directly for the preparation of 24 and 28.

Synthesis of 6,7-*O*,*O*-Demethylene-6,7-*O*,*O*-dimethyl-4β-(4″-nitroanilino)-4-desoxypodophyllotoxin (24) and 6,7-*O*,*O*-Demethylene-6,7-*O*,*O*-dimethyl-4′-*O*-demethyl-4β-(4″-nitroanilino)-4-desoxypodophyllotoxin (28). A solution containing a mixture of 10 and 11 (498 mg), anhydrous barium carbonate (500 mg, 7.53 mmol), and 4-nitroaniline (99.5 mg, 0.72 mmol) in dry 1,2-dichloroethane (8 mL) was stirred under nitrogen overnight at room temperature. The reaction mixture was filtered. The filtrate was diluted with ethyl acetate, washed with water, and dried (Na_2SO_4). The resulting residue was purified by column chromatography [silica gel with toluene–ethyl acetate (4:1.5) as an eluant] to yield 24 (220 mg) and 28 (71 mg). **Compound 24**: mp 124–126 °C, crystals from ethyl acetate–chloroform; $[\alpha]^{25}_D$ −155° (c = 0.26, acetone); IR (KBr) 3360, 2920, 1770, 1580, 1500, 1470, 1330, and 1310 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.17 (d, J = 8.9 Hz, 2 H, H-3″,5″), 6.75 (s, 1 H, H-5), 6.60 (d, J = 8.9 Hz, 2 H, H-2″,6″), 6.58 (s, 1 H, H-8), 6.32 (s, 2 H, H-2′,6′), 4.89 (t, J = 5.0 Hz, 1 H, H-11), 4.70 (d, J = 3.0 Hz, 1 H, H-4), 4.67 (d, J = 6.7 Hz, 1 H, H-1), 4.44 (t, J = 5.0 Hz, 1 H, H-11), 3.88 (s, 3 H, OCH_3-6), 3.83 (s, 3 H, OCH_3-4′), 3.82 (s, 3 H, OCH_3-7), 3.76 (s, 6 H, OCH_3-3′,5′), and 3.11 (br s, 2 H, H-2,3). Anal. ($C_{29}H_{30}N_2O_9$) C, H; N: calcd, 5.09; found, 4.60. **Compound 28**: mp 177–178 °C, crystals from ethanol; $[\alpha]^{25}_D$ −136° (c = 0.26, acetone); IR (KBr) 3340, 2960, 2920, 1770, 1580, 1510, 1460, 1320, and 1300 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.18 (d, J = 9.1 Hz, 2 H, H-3″,5″), 6.73 (s, 1 H, H-5), 6.60 (d, J = 9.1 Hz, 2 H, H-2″,6″), 6.58 (s, 1 H, H-8), 6.33 (s, 2 H, H-2′,6′), 5.45 (s, 1 H, OH-4′), 4.89 (q, 1 H, H-11), 4.69 (d, J = 1.5 Hz, 1 H, H-4), 4.63 (d, J = 6.6 Hz, 1 H, H-1), 4.43 (q, 1 H, H-11), 3.88 (s, 3 H, OCH_3-6), 3.82 (s, 3 H, OCH_3-7), 3.79 (s, 6 H, OCH_3-3′,5′), and 3.10 (br s, 2 H, H-2,3). Anal. ($C_{28}H_{28}N_2O_9 \cdot {}^1/_2C_6H_5CH_3$) C, H, N.

Compounds **25–27** and **29–31** were prepared from a mixture of 10 and 11 (312 mg) by an identical procedure for the synthesis of 24 and 28.

6,7-*O*,*O*-Demethylene-6,7-*O*,*O*-dimethyl-4β-[4″-(ethoxycarbonyl)anilino]-4-desoxypodophyllotoxin (25): 198 mg; mp 127–130 °C, crystals from ether; $[\alpha]^{25}_D$ − 98° (c = 0.26, acetone); IR (KBr) 3360, 2940, 1770, 1680, 1600, 1500, and 1460 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.95 (d, J = 8.4 Hz, 2 H, H-3″,5″), 6.76 (s, 1 H, H-5), 6.58 (s, J = 8.4 Hz, 2 H, H-2″,6″), 6.56 (s, 1 H, H-8), 6.33 (s, 2 H, H-2′,6′), 4.84 (d, J = 3.3 Hz, 1 H, H-4), 4.68 (s, J = 4.5 Hz, 1 H, H-1), 4.41 (t, J = 8.3 Hz, 1 H, H-11), 4.35 (q, J = 7.2 Hz, 2 H, $CO_2CH_2CH_3$), 3.95 (t, J = 8.3 Hz, 1 H, H-11), 3.87 (s, 3 H, OCH_3-6), 3.83 (s, 3 H, OCH_3-4′), 3.82 (s, 3 H, OCH_3-7), 3.76 (s, 6 H, OCH_3-3′,5′), 3.14 (dd, J = 13.3, 4.5 Hz, 1 H, H-2), 3.05 (m, 1 H, H-3), and 1.38 (t, J = 7.2 Hz, 3 H, $CO_2CH_2CH_3$). Anal. ($C_{32}H_{35}NO_9 \cdot {}^1/_4C_6H_5CH_3$) C, H, N.

6,7-*O*,*O*-Demethylene-6,7-*O*,*O*-dimethyl-4′-*O*-demethyl-4β-[4″-(ethoxycarbonyl)anilino]-4-desoxypodophyllotoxin (29): 45 mg; mp 125–127 °C; $[\alpha]^{25}_D$ −119° (c = 0.25, acetone); IR (KBr) 3360, 2940, 1770, 1680, 1600, 1510, and 1460 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.95 (d, J = 8.7 Hz, 2 H, H-3″,5″), 6.76 (s, 1 H, H-5), 6.58 (d, J = 8.7 Hz, 2 H, H-2″,6″), 6.56 (s, 1 H, H-8), 6.33 (s, 2 H, H-2′,6′), 5.44 (s, 1 H, OH-4′), 4.82 (t, 1 H, H-4), 4.67 (d, J = 4.5 Hz, 1 H, H-1), 4.36 (t, J = 7.3 Hz, 1 H, H-11), 4.33 (q, J = 7.0 Hz, 2 H, $CO_2CH_2CH_3$), 3.94 (t, 1 H, H-11′), 3.87 (s, 3 H, OCH_3-6), 3.82 (s, 3 H, OCH_3-7), 3.79 (s, 6 H, OCH_3-3′,5′), 3.14 (dd, J = 13.3, 4.5 Hz, 1 H, H-2), 3.05 (m, 1 H, H-3), and 1.38 (t, J = 7.0 Hz, 3 H, $CO_2CH_2CH_3$). Anal. ($C_{31}H_{33}NO_9$) C, H, N.

6,7-*O*,*O*-Demethylene-6,7-*O*,*O*-dimethyl-4β-(4″-cyanoanilino)-4-desoxypodophyllotoxin (26): 129 mg, mp 127–128 °C, crystals from ethanol; $[\alpha]^{25}_D$ −140° (c = 0.26, acetone); IR (KBr) 3340, 2960, 2920, 2200, 1770, 1600, 1505, and 1460 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.52 (d, J = 8.4 Hz, 2 H, H-3″,5″), 6.73 (s, 1 H, H-5), 6.61 (d, J = 8.4 Hz, 2 H, H-2″,6″), 6.56 (s, 1 H, H-8), 6.31 (s, 2 H, H-2′,6′), 4.82 (t, 1 H, H-4), 4.68 (d, J = 3.9 Hz, 1 H, H-1), 4.42 (m, 2 H, H-11), 3.87 (s, 3 H, OCH_3-6), 3.83 (s, 3 H, OCH_3-4′), 3.82 (s, 3 H, OCH_3-7), 3.75 (s, 6 H, OCH_3-3′,5′), and 3.10 (m, 2 H, H-2,3). Anal. ($C_{30}H_{30}N_2O_7$) C, H, N.

6,7-*O*,*O*-Demethylene-6,7-*O*,*O*-dimethyl-4′-*O*-demethyl-4β-(4″-cyanoanilino)-4-desoxypodophyllotoxin (30): 38 mg; mp 158–161 °C; $[\alpha]^{25}_D$ −130° (c = 0.27, acetone); IR (KBr) 3360, 2920, 2210, 1770, 1600, 1520, and 1460 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.52 (d, J = 8.0 Hz, 2 H, H-3″,5″), 6.73 (s, 1 H, H-5), 6.60 (d, J = 8.0 Hz, 2 H, H-2″,6″), 6.57 (s, 1 H, H-8), 6.32 (s, 2 H, H-2′,6′), 5.44 (s, 1 H, OH-4′), 4.81 (t, 1 H, H-4), 4.68 (d, J = 3.6 Hz, 1 H, H-1), 4.40 (br s, 2 H, H-11), 3.88 (s, 3 H, OCH_3-6), 3.81 (s, 3 H, OCH_3-7), 3.79 (s, 6 H, OCH_3-3′,5′), and 3.10 (m, 2 H, H-2,3). Anal. ($C_{29}H_{28}N_2O_7 \cdot {}^1/_2C_6H_5CH_3$) C, H, N.

6,7-*O*,*O*-Demethylene-6,7-*O*,*O*-dimethyl-4β-(4″-fluoroanilino)-4-desoxypodophyllotoxin (27): 178 mg; mp 240–243 °C, crystals from ethanol–acetone; $[\alpha]^{25}_D$ −95° (*c* = 0.30, acetone); IR (KBr) 3360, 2940, 1770, 1600, 1570, 1510, and 1460 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.97 (t, 2 H, H-3″,5″), 6.75 (s, 1 H, H-5), 6.55 (s, 1 H, H-8), 6.51 (t, 2 H, H-2″,6″), 6.33 (s, 2 H, H-2′,6′), 4.66 (m, 2 H, H-1,4), 4.40 (t, 1 H, H-11), 4.01 (t, 1 H, H-11), 3.86 (s, 3 H, OCH_3-6), 3.83 (s, 3 H, OCH_3-4′), 3.82 (s, 3 H, OCH_3-7), 3.75 (s, 6 H, OCH_3-3′,5′), 3.20 (dd, *J* = 13.7, 5.0 Hz, 1 H, H-2), and 3.02 (m, 1 H, H-3). Anal. ($C_{29}H_{30}FNO_7 \cdot {}^1/_2H_2O$) C, H, N.

6,7-*O*,*O*-Demethylene-6,7-*O*,*O*-dimethyl-4′-*O*-demethyl-4β-(4″-fluoroanilino)-4-desoxypodophyllotoxin (31): 45 mg; mp 221–224 °C; $[\alpha]^{25}_D$ −93° (*c* = 0.25, acetone); IR (KBr) 3380, 2940, 1760, 1600, 1510, and 1460 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.96 (t, 2 H, H-3″,5″), 6.74 (s, 1 H, H-5), 6.55 (s, 1 H, H-8), 6.50 (t, 2 H, H-2″,6″), 6.34 (s, 2 H, H-2′,6′), 5.43 (s, 1 H, OH-4′), 4.65 (m, 2 H, H-1,4), 4.39 (t, 1 H, H-11), 4.00 (t, 1 H, H-11), 3.86 (s, 3 H, OCH_3-6), 3.82 (s, 3 H, OCH_3-7), 3.75 (s, 6 H, OCH_3-3′,5′), 3.18 (dd, *J* = 14.7, 4.9 Hz, 1 H, H-2), and 3.01 (m, 1 H, H-3). Anal. ($C_{28}H_{28}FNO_7 \cdot {}^1/_4H_2O$) C, H, N.

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.[24]

Acknowledgment. The authors thank Mike Fisher of the Cancer Research Center, UNC-Chapel Hill for KB cell culture assay. This work was supported by grants from the American Cancer Society CH-370 (K. H. L.) and the National Cancer Institute CA 44358 (Y. C. C.).

Registry No. **2**, 518-28-5; **3**, 1174-97-6; **4**, 138456-90-3; **5**, 138355-75-6; **6**, 138355-76-7; **7**, 6559-91-7; **8**, 138456-91-4; **9**, 1178-09-2; **10**, 138456-92-5; **11**, 138355-77-8; **12**, 138355-78-9; **13**, 138355-79-0; **14**, 138355-80-3; **15**, 138355-81-4; **16**, 138355-82-5; **17**, 138355-83-6; **18**, 138355-84-7; **19**, 138355-85-8; **20**, 138355-86-9; **21**, 138355-87-0; **22**, 138355-88-1; **23**, 138355-89-2; **24**, 138355-90-5; **25**, 138355-91-6; **26**, 138355-92-7; **27**, 138355-93-8; **28**, 138355-94-9; **29**, 138355-95-0; **30**, 138355-96-1; **31**, 138355-97-2; **32**, 127882-73-9; **33**, 127882-59-1; **34**, 127882-56-8; **35**, 125830-36-6; NH_2Ph, 62-53-3; *p*-$NH_2C_6H_4NO_2$, 100-01-6; *p*-$NH_2C_6H_4CO_2C_2H_5$, 94-09-7; *p*-$NH_2C_6H_4CN$, 873-74-5; *p*-$NH_2C_6H_4F$, 371-40-4; *m*-$NH_2C_6H_4OH$, 591-27-5; DNA topoisomerase II, 80449-01-0.

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New Nonpeptide Angiotensin II Receptor Antagonists. 1. Synthesis, Biological Properties, and Structure–Activity Relationships of 2-Alkyl Benzimidazole Derivatives

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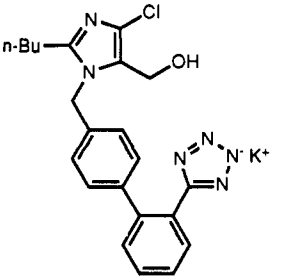
On the basis of an extension of the literature lead 1, a series of benzimidazoles have been synthesized and shown to be angiotensin II (AII) receptor antagonists. The structure–activity relationships of these new antagonists have been explored and the key binding interactions defined. Molecular mechanics calculations were carried out on analogues of imidazole AII antagonists and conformationally restricted analogues were synthesized. The benzimidazole antagonists displaced AII in binding studies in vitro with IC_{50} values in the range 10^{-5}–10^{-7} M and antagonized the hypertensive effects of AII in vivo (rats) following intravenous administration with ED_{50} values in the range of 5–20 mg/kg.

Angiotensin II (AII), a powerful endogenous vasconstrictor produced by the renin–angiotensin system (RAS), is a major regulator of blood pressure in mammals.[1] Blockade of the RAS, through inhibition of angiotensin converting enzyme (ACE), has provided an effective means of lowering blood pressure in the majority of hypertensive patients.[2] However, ACE not only cleaves angiotensin I to produce AII, but also hydrolyzes a variety of other biologically significant peptides.[3] Thus, alternative and potentially more selective approaches to blockade of the RAS have been sought, such as inhibitors of the more specific enzyme renin.[4] Another obvious target has been receptor antagonists of AII itself. Until recently all potent AII receptor antagonists reported have been peptide analogues of AII[5,6] and have suffered from the problems normally associated with peptides such as poor oral absorption, short plasma half lives and rapid clearance.[7] In addition many exhibit partial agonism.[7] Therefore the recent discovery by the Du Pont group of a series of imidazole derivatives which are potent, nonpeptide AII antagonists has provided an important advance in the area.[8]

The most studied compound of this new class of AII antagonists, DuP 753, is currently undergoing clinical eval-



DUP 753

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