acetate Salt. The foregoing *tert*-butyl ester **23** (1 g, 2.48 mmol) was dissolved in CF_3COOH (10 mL). After the reaction mixture was stirred under N_2 at ambient temperature and in the dark for 10 min, the solution was concentrated under reduced pressure. The yellow oily residue was triturated with EtOAc (50 mL), and the precipitate was filtered off and washed well with petrol to give a pale yellow powder: 1.11 g (97%); mp >300 °C; NMR (Me_2SO-d_6) δ 2.41 (s, 3 H, C^2-CH_3), 3.24 (t, J = 1.9 Hz, 1 H, C≡CH), 4.36 (d, J = 1.9 Hz, 2 H, CH_2C≡C), 4.82 (s, 2 H, CH_2N), 6.83 (d, J = 9.0 Hz, 2 H, benzene 3',5'-H), 7.59 (d, J = 8.4 Hz, 1 H, quinazoline 8-H), 7.75 (d, J = 9.0 Hz, 3 H, benzene 2',6'-H, quinazoline 7-H), 7.99 (d, J = 1.7 Hz, 1 H, quinazoline 5-H); MS m/z 348 (M^+ + 1). Anal. ($C_{19}H_{15}N_3O_3 \cdot CF_3CO_2H$) C, H, N.

Preparation of Antifolate Polyglutamate *tert*-Butyl Esters. Tetra-*tert*-butyl *N*-[*N*-[*N*-[5-[*N*-[(3,4-Dihydro-2-methyl-4-oxoquinazolin-6-yl)methyl]-*N*-methylamino]-2-thenoyl]-L-γ-glutamyl]-L-γ-glutamyl]-L-glutamate (43). Thenoic acid **40** (0.329 g, 1 mmol) and tetra-*tert*-butyl L-γ-glutamyl-L-γ-glutamyl-L-glutamate (**19**) (0.944 g, 1.5 mmol) were dissolved in dry DMF (15 mL) at room temperature, and to this solution was added diethyl cyanophosphoridate (0.359 g, 2.2 mmol) and then Et_3N (0.222 g, 2.2 mmol). The mixture was stirred under nitrogen and in the dark for 2 h and then diluted with EtOAc (100 mL) and H_2O (100 mL). The water layer was separated and extracted with EtOAc (2 × 100 mL). The combined EtOAc extracts were washed with 10% aqueous citric acid (2 × 50 mL), saturated $NaHCO_3$ (100 mL), and dilute NaCl (100 mL), then dried (Na_2SO_4), filtered, and concentrated in vacuo. The residue was purified by chromatography on a silica gel column (Merck 15111) using EtOAc and then 1% MeOH in EtOAc as the eluent. The crude product **43** was crystallized from CH_2Cl_2/petrol, giving a white powder: 0.555 g (59%); mp 123-124 °C; NMR (Me_2SO-d_6) δ 1.38, 1.40 (2 × s, 36 H, $C(CH_3)_3$), 1.72, 1.87, 1.95 (3 × m, 6 H, CH_2^β), 2.16, 2.24 (2 × t, 6 H, CH_2^γ), 2.34 (s, 3 H, 2-CH_3), 3.04 (s, 3 H, N-CH_3), 4.07, 4.20 (2 × m, 3 H, CH^α), 4.66 (s, 2 H, CH_2N), 5.99 (d, J = 4.2 Hz, 1 H, thiophene 4'-H), 7.57 (m, 2 H, thiophene 3'-H + quinazoline 8-H), 7.66 (dd, J = 8.4, 2.0 Hz, 1 H, quinazoline 7-H), 7.94 (d, J = 1.7 Hz, 1 H, quinazoline 5-H), 8.14 (m, 3 H, CONH), 12.25 (s, 1 H, lactam NH); MS m/z 940 (M^+). Anal. ($C_{47}H_{68}N_6O_{12}S \cdot 0.5H_2O$) C, H, N, S.

The procedure was repeated with the appropriate primary amines **18-22** and appropriate thenoic or benzoic acids **26, 35, 40, 52,** and **53** to give the coupled antifolate *tert*-butyl esters **27-30, 38, 39, 42, 44-46, 54,** and **55**. Yields and mass spectral and analytical data of these products are given in Table II. The ^1H NMR spectra of these compounds were consistent with the assigned structures.

Preparation of Antifolate Polyglutamates. *N*-[*N*-[*N*-[5-[*N*-[(3,4-Dihydro-2-methyl-4-oxoquinazolin-6-yl)-methyl]-*N*-methylamino]-2-thenoyl]-L-γ-glutamyl]-L-γ-glutamyl]-L-glutamic Acid Trifluoroacetate Salt (48). A solution of **43** (0.150 g, 0.16 mmol) in TFA (10 mL) was stirred at room temperature for 1 h in the dark and under a nitrogen atmosphere. The solution was then concentrated in vacuo and the residue triturated with anhydrous Et_2O (30 mL). The solid was isolated by filtration, washed with Et_2O (4 × 10 mL), and dried in vacuo over P_2O_5, giving a pale yellow powder: 0.131 g, (92%); mp 150-152 °C; NMR (Me_2SO-d_6) δ 1.75-2.00 (3 × m, 6 H, CH_2^β), 2.18, 2.25 (2 × t, 2 H, 4 H, CH_2^γ), 2.38 (s, 3 H, 2-CH_3), 3.04 (s, 3 H, N-CH_3), 4.16, 4.28 (2 × m, 2 H, 1 H, CH^α), 4.67 (s, 2 H, CH_2N), 5.99 (d, J = 4.2 Hz, 1 H, thiophene 4'-H), 7.58 (2 × d, 2 H, thiophene 3'-H + quinazoline 8-H), 7.70 (dd, J = 8.4 Hz, 1 H, quinazoline 7-H), 7.96 (d, 1 H, quinazoline 5-H), 8.15 (m, 3 H, CONH), 12.46 (bd, COOH); MS m/z 717 (M^+ + 1). Anal. ($C_{31}H_{36}N_6O_{12}S \cdot 0.9CF_3COOH \cdot Et_2O$) C, H, N, S.

The procedure was repeated with the appropriate *tert*-butyl-protected polyglutamates to yield the antifolate poly-γ-glutamates **10, 12, 31-34, 36, 37, 47** and **49-51**, all of which had ^1H NMR spectra consistent with the assigned structures. Yields and analytical data are gathered in Table III.

Acknowledgment. This work was supported by grants from the Cancer Research Campaign and Medical Research Council. We thank S. Howell and M. E. Harrison at the School of Pharmacy, University of London for determining all FAB mass spectra, and M. H. Baker for the electron impact mass spectra. Carboxypeptidase G_2 was a generous gift from Drs. R. G. Melton and R. F. Sherwood at Porton Down. The authors wish to thank Ms. L. Nevill for assistance in typing the manuscript.

Supplementary Material Available: ^1H NMR spectral data of "desglutamyl" compounds **26, 35, 52, 53**, *tert*-butyl-protected polyglutamate esters **27-30, 38, 39, 42-46, 54, 55**, and polyglutamate trifluoroacetate salts **10, 12, 31-34, 36, 37, 47-51** (3 pages). Ordering information is given on any current masthead page.

Antitumor Agents. 123.† Synthesis and Human DNA Topoisomerase II Inhibitory Activity of 2'-Chloro Derivatives of Etoposide and 4β-(Arylamino)-4'-*O*-demethylpodophyllotoxins

Hong Hu,‡ Zhe-Qing Wang,‡ Su-Ying Liu,§ Yung-Chi Cheng,§ and Kuo-Hsiung Lee*,‡

Natural Products Laboratory, Division of Medicinal Chemistry and Natural Products, School of Pharmacy, University of North Carolina, Chapel Hill, North Carolina 27599, and Department of Pharmacology, Yale University School of Medicine, New Haven, Connecticut 06510. *Received July 22, 1991*

The 2'-chloro derivatives of etoposide and 4β-(arylamino)-4'-*O*-demethylpodophyllotoxins 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 showed that none of the compounds are active as a result of the C-2' chloro substitution on ring E. This would suggest that the free rotation of ring E is essential for the aforementioned enzyme inhibitory activity. In addition, these 2'-chloro derivatives showed no significant cytotoxicity (KB).

Etoposide (VP-16, **1**) shows significant clinical activity against small-cell lung cancer, testicular cancer, lymphoma, and leukemia.[2] It has been proposed that **1** and related compounds exert their lethal effects by the inhibition of

* To whom correspondence should be addressed.

† For part 122, see ref 1.

‡ Natural Products Laboratory, Division of Medicinal Chemistry and Natural Products, School of Pharmacy.

§ Department of Pharmacology, Yale University School of Medicine.

(1) Part 122; Li, L. P.; Wang, H. K.; Fujioka, T.; Chang, J. J.; Kozuka, M.; Konoshima, T.; Estes, J. R.; McPhail, D. R.; McPhail, A. T.; Lee, K. H. Structure and Stereochemistry of Amorphispironone, a Novel Cytotoxic Spironone Type Rotenoid from *Amorpha fruticosa*. *J. Chem. Soc. Chem. Commun.*, submitted.

0022-2623/92/1835-0866$03.00/0

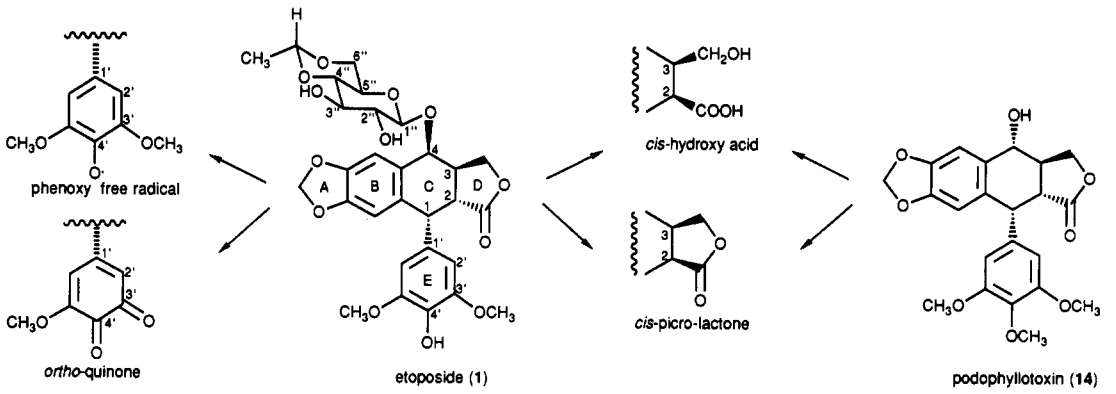
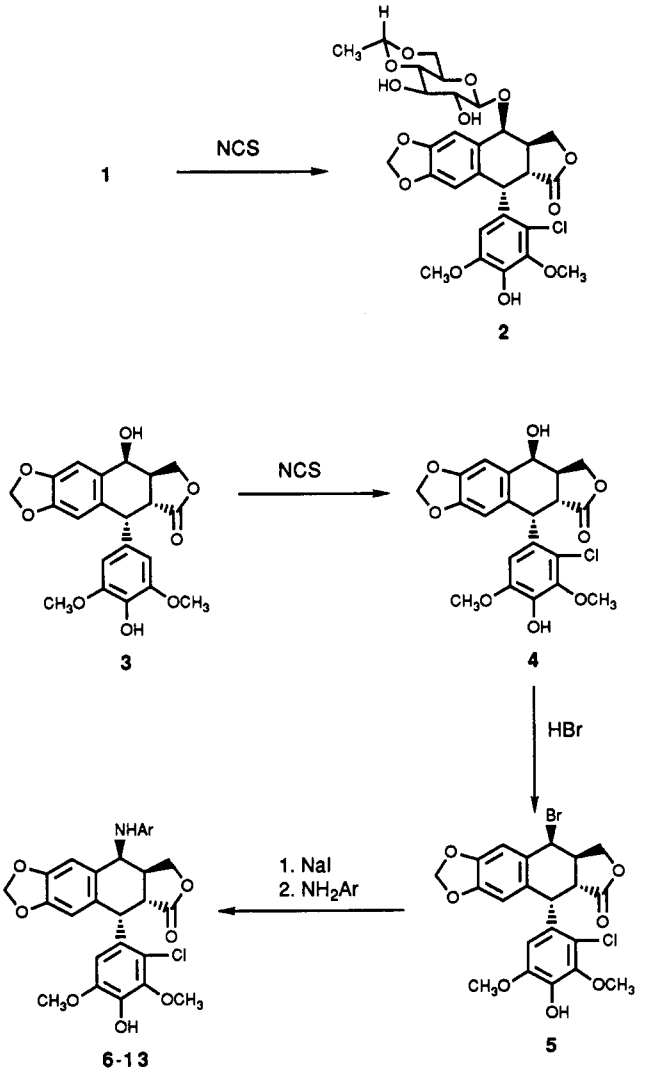


Figure 1. Structures of etoposide, podophyllotoxin, and their metabolites.

DNA topoisomerase II. These drugs block the catalytic activity of DNA topoisomerase II by stabilizing a cleavable enzyme–DNA complex in which the DNA is cleaved and covalently linked to the enzyme upon treatment with protein denaturants.[3-5] The cytotoxic effects of etoposide and related compounds might also be associated with the phenoxy free radical and its resulting *o*-quinone species (Figure 1) formed by biological oxidation of the drugs.[6,7] The phenoxy free radical and *o*-quinone derivatives of 1 were believed to be produced by metabolic activation of 1, which could bind to critically important cellular macromolecules as alkylating species causing dysfunction and, subsequently, cell death.[8,9]

Structure–activity relationship studies have shown that the trans C/D ring juncture of both 1 and podophyllotoxin (14) is essential for the antitumor activity, since the corresponding cis-hydroxy acid and cis-picro-lactone of 1 and 14 (Figure 1), which were found in vitro and in vivo,[10,11]

Scheme I



exhibited no biological activity in vitro.[12,13] The cis-picro-lactone isomer produced under physiological conditions is also readily formed by the treatment of 1 or 14

(2) O'Dwyer, P.; Leyland-Jones, B.; Alonso, M. T.; Marsoni, S.; Wittes, R. E. Etoposide (VP-16-213): Current Status of an Active Anticancer Drug. *N. Engl. J. Med.* **1985**, *312*, 692–700.

(3) Chen, G. L.; Yang, L.; Rowe, T. C.; Halligan, B. D.; Tewey, K.; Liu, L. Nonintercalative Antitumor Drugs Interfere with the Breakage-Reunion Reaction of Mammalian DNA Topoisomerase II. *J. Biol. Chem.* **1984**, *259* (21), 13560–13566.

(4) Ross, W.; Rowe, T.; Glisson, B.; Yalowich, J.; Liu, L. Role of Topoisomerase II in Mediating Epipodophyllotoxin-induced DNA Cleavage. *Cancer Res.* **1984**, *44*, 5857–5860.

(5) Rowe, T.; Kuppfer, G.; Ross, W. Inhibition of Epipodophyllotoxin Cytotoxicity by Interference with Topoisomerase-mediated DNA Cleavage. *Biochem. Pharmacol.* **1985**, *34* (14), 2483–2494.

(6) van Maanen, J. M. S.; De Ruiter, C.; Kootstra, P. R.; De Vries, J.; Pinedo, H. M. Free Radical Formation of VP16-213. *Proc. Am. Assoc. Cancer Res.* **1984**, *25*, 384.

(7) van Maanen, J. M. S.; De Ruiter, C.; Kootstra, P. R.; Broersen, J.; De Vries, J.; Laffeur, M. V. M.; Retel, J.; Kriek, E.; Pinedo, H. M. Metabolic Activation of the Antitumor Agent VP16-213. *Proc. Am. Assoc. Cancer Res.* **1986**, *27*, 308.

(8) Haim, N.; Roman, J.; Nemec, J.; Sinha, B. K. Peroxidative Free Radical Formation and *O*-demethylation of Etoposide (VP-16) and Teniposide (VM-26). *Biochem. Biophys. Res. Commun.* **1986**, *135* (1), 215–220.

(9) van Maanen, J. M. S.; De Ruiter, C.; De Vries, J.; Kootstra, P. R.; Gobars, G.; Pinedo, H. M. The Role of Metabolic Activation by Cytochrome P-450 in Covalent Binding of VP16-213 to Rat Liver and HeLa Cell Microsomal Proteins. *Eur. J. Cancer Clin. Oncol.* **1985**, *21* (9), 1099–1106.

(10) Creaven, P. J. The Clinical Pharmacology of VM-26 and VP16-213. *Cancer Chemother. Pharmacol.* **1982**, *7*, 133–140.

(11) Evans, W. E.; Sinkule, J. A.; Crom, W. R.; Dow, L.; Look, A. T.; Rivera, G. Pharmacokinetics of Teniposide (VM-26) and Etoposide (VP16-213) in Children with Cancer. *Cancer Chemother. Pharmacol.* **1982**, *7*, 147–150.

(12) Dow, L. W.; Sinkule, J. A.; Look, A. T.; Horvath, A.; Evans, W. E. 4′-Demethylepipodophyllotoxin-9-(4,6-*O*-2-ethylidene-β-D-glucopyranoside) and 4′-Demethylepipodophyllotoxin-9-(4,6-*O*-2-thenylidene-β-D-glucopyranoside) and Their Metabolites on Human Leukemic Lymphoblasts. *Cancer Res.* **1983**, *43*, 5699–5706.

(13) Jardine, I. Podophyllotoxins. In *Anticancer Agents Based on Natural Product Models*, Cassady, J. M., Douros, J. D., Eds.; Academic: New York, 1980; p 323.

with a base, such as ammonia, sodium carbonate, and sodium hydroxide. The mechanism of converting trans-fused lactone to the more stable, but less active or inactive, cis-fused form has been interpreted via the formation of an enol at C-2 by proton abstraction, followed by an inversion of configuration at C-2.[14]

The introduction of a chloro atom into the C-2′ position of **14** was found to increase the stability to epimerization at C-2 by bases. Thus, the physiologically active trans-lactone configuration of the 2′-chloro derivative of **14** was retained, even in the presence of alkoxide ions.[14] On the basis of this finding, we have synthesized and evaluated the 2′-chloro derivatives of 1 and 4β-(arylamino)-4′-*O*-demethylpodophyllotoxins. Our previous studies have shown that a number of 4β-(arylamino)-4′-*O*-demethylpodophyllotoxins (e.g. **15–18**), obtained by substituting the 4β-*O*-glucosidic moiety in 1 with 4β-arylamino moieties, are more potent than 1 in inhibiting the human DNA topoisomerase II and in causing the cellular protein-linked DNA breakage.[15,16]

Chemistry

As shown in Scheme I, the 2′-chloroetoposide (**2**) was synthesized from etoposide (**1**), while the 2′-chloro derivatives of 4β-(arylamino)-4′-*O*-demethylepipodophyllotoxins (**6–13**) were prepared from 4′-*O*-demethylepipodophyllotoxin (**3**). The 2′-chloro derivatives (**2** and **4**) were obtained via *N*-chlorosuccinimide (NCS) chlorination of 1 and **3**, respectively.[17] The key intermediate in the synthesis of **6–13**, 4β-bromo-2′-chloro-4′-*O*-demethyl-4-desoxypodophyllotoxin (**5**), was obtained by bromination of **4** as described previously.[15] Since **5** was highly reactive and susceptible to nucleophilic attack even by moisture, the crude **5** was subjected to the next step of the reaction to yield **6–13** without further purification.

However, compound **5** was found to have a decrease in the rate of nucleophilic reaction at C-4 as a result of the 2′-chloro substitution. It was, therefore, transformed into the iodo compound before the introduction of appropriate arylamines. The 2′-chloro substitution also led to a change in the chemical shift of proton at C-6′ and methoxy groups at C-3′ and -5′. The proton at C-6′ was found to have a constant upfield shift to δ 6.01–6.28 compared to that of the corresponding 2′-unsubstituted derivatives in which it was seen at δ 6.32–6.33.[14,15] The methoxy groups at C-3′ and C-5′ were no longer equivalent chemically and magnetically. The protons of the methoxy group at C-3′ showed a constant downfield shift to δ 3.74–3.96, while that at C-5′ showed a constant upfield shift to δ 3.54–3.73. Assignment of the methoxy groups was according to the ^{1}H NOE spectra. The proton at C-6′ showed an 18% increase in NOE when the methoxy group residing at the higher field was irradiated. By comparison, the methoxy groups at C-3′ and -5′ in 2′-unsubstituted derivatives appeared as a singlet within δ 3.76–3.80.[15,16] These data are in agreement with those previously reported for 4β,2′-dichlorodesoxypodophyllotoxin[14] except for the reactivity at C-4. It was demonstrated that the 2′-chloro substitution led to an increase in the rate of hydrolysis of the 4β-chloro group.[14]

As discussed previously,[15] compounds **6–13** were synthesized by nucleophilic substitution from the iodo compound, derived from **5**, with appropriate arylamines 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 **6–13** as the predominant products. The yields in the synthesis of **6–13** were in a range of 31–71%, calculated from **4**.

Results and Discussion

As illustrated in Table I, 2′-chloroetoposide (**2**) as well as 2′-chloro-4β-(arylamino)-4′-*O*-demethylpodophyllotoxins (**6–13**) showed not only no significant cytotoxicity against KB cells but also much less activity in inhibiting the human DNA topoisomerase II and essentially no activity in causing the cellular protein–DNA strand breakage. Compared with 4β-(arylamino)-4′-*O*-demethylpodophyllotoxins (**15–18**)[15,16] which are at least 2-fold more active than 1, the corresponding 2′-chloro-substituted compounds (**6**, **8**, **9**, and **11**) are inactive. This would suggest that the free rotation of ring E is required for biological activity.

The rotation of ring E is restricted as a result of the introduction of chloro group into the C-2′ position, since there is a steric hindrance between the lactone carbonyl and the 2′-chloro atom when ring E is rotated around the C_1—$C_{1'}$ bond. The more favorable ring E rotamer is most likely to be the one that ring E is closely perpendicular to ring C with H-6′ lying within the shielding area of ring B and Cl-2′ toward the outside of the paper plane. This conformation is supported by the NMR data. The proton at C-6′ and the methoxy group at C-5′ in all 2′-chloro derivatives described herein showed a constant upfield shift. In addition, the introduction of a 2′-chloro group led to a decrease in the rate of the *o*-quinone production. On oxidation of 4′-*O*-demethylepipodophyllotoxin (**3**) with sodium periodide in acetic acid, its *o*-quinone was formed in 20 min at room temperature. However, under the same condition, no quinonoid product was formed from its 2′-chloro derivative (**4**). The *o*-quinone formation from **4** required prelonged time and elevated temperature (e.g. 24 h at 80 °C). The oxidation of **2** was also found to be more difficult than that of **1**. The complete oxidation of **1** with sodium periodide in ethyl acetate containing catalytic amount of acetic acid was accomplished in 2 h at room temperature. While only a trace amount of the *o*-quinone of **2** was produced under the same condition, the complete oxidation of **2** required 7 days. The difficulty of *o*-quinone formation from **2** or **4** is more likely to be responsible for the lack of cytotoxicity and enzyme inhibitory activity of 2′-chloro derivatives. Since the *o*-quinone species formed enzymatically or chemically from 1 has been shown to bind proteins[18] and to inactivate single- and double-strand DNA[7] which might be responsible for the subsequent cellular damage and death, investigation on whether the

(14) Ayres, D. C.; Lim, C. K. Modification of the Pendant Ring of Podophyllotoxin. *Cancer Chemother. Pharmacol.* **1982**, *7*, 99–101.

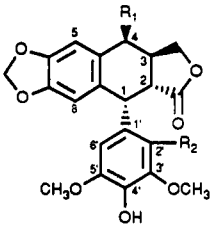
(15) Lee, K. H.; Beers, S. A.; Mori, M.; Wang, Z. Q.; Kuo, Y. H.; Li, L.; Liu, S. Y.; Chang, J. Y.; Han, F. S.; Cheng, Y. C. Antitumor Agents. 111. New 4-Hydroxylated and 4-Halogenated Anilino Derivatives of 4′-Demethylepipodophyllotoxin as Potent Inhibitors of Human DNA Topoisomerase II. *J. Med. Chem.* **1990**, *33*, 1364–1368.

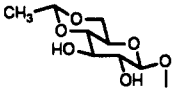
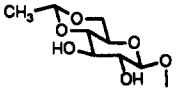
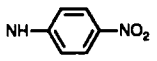
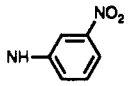
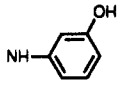
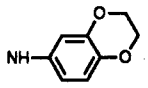
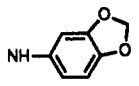
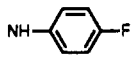
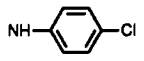
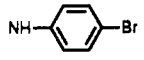
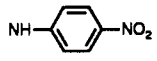
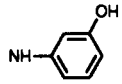
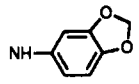
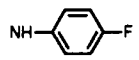
(16) Wang, Z. Q.; Kuo, Y. H.; Schnur, D.; Bowen, J. P.; Liu, S. Y.; Han, F. S.; Chang, J. Y.; Cheng, Y. C.; Lee, K. H. Antitumor Agents. 113. New 4β-Arylamino Derivatives of 4′-*O*-Demethylepipodophyllotoxin and Related Compounds as Potent Inhibitors of Human DNA Topoisomerase II. *J. Med. Chem.* **1990**, *33*, 2660–2666.

(17) Mitchell, R. H.; Lai, Y. H.; Williams, R. V. N-Bromosuccinimide-Dimethylformide: A Mild, Selective Nuclear Monobromination Reagent for Reactive Aromatic Compound. *J. Org. Chem.* **1979**, *44* (25), 4733–4735.

(18) Haim, N.; Nemec, J.; Roman, J.; Sinha, B. K. *In Vitro* Metabolism of Etoposide (VP-16-213) by Liver Microsomes and Irreversible Binding of Reactive Intermediates to Microsomal Proteins. *Biochem. Pharmacol.* **1987**, *36*, 527–536.

Table I. Biological Evaluation of 4β-(Arylamino)-2′-chloro-4′-*O*-demethylpodophyllotoxin and Related Compounds



compd	R_1	R_2	cytotoxicity:[a] IC_{50} KB, μM	inhibition of DNA topoisomerase II:[b] ID_{50}, μM	cellular protein–DNA complex (%), 10 μM
1		H	0.2	50	100
2		Cl	>6.4	>50	6.1
4	OH	Cl	>22.3	>50	15.6
6		Cl	>7.2	>100	0
7		Cl	>7.2	>100	0
8		Cl	5.7	>100	0
9		Cl	>7.0	>100	0
10		Cl	>7.2	>100	0
11		Cl	>7.5	>100	0
12		Cl	>7.3	>100	0
13		Cl	>6.8	>100	0
15		H	0.49	10	323
16		H	0.45	25	290
17		H	0.68	10	279
18		H	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 5, 10, 25, 50, and 100 μM. The ID_{50} value was established on the basis of the degree of inhibition at these concentrations.

formation of an *o*-quinone from 2′-chloro derivatives is a prerequisite for the enzyme inhibitory activity is 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. Silica gel 60 (32–63 microns) 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, ^1H NMR, and IR spectral analyses as well as elemental analysis.

2′-Chloroetoposide (2). A solution of *N*-chlorosuccinimide (NCS) (14.4 mg, 0.1 mmol) in 1 mL of DMF was added to a solution of etoposide (53 mg, 0.09 mmol) in 1 mL of DMF. The resulting mixture was stirred at room temperature for 3.5 h. The

reaction mixture was diluted with ethyl acetate, washed with water, dried over anhydrous magnesium sulfate, and purified by preparative TLC with ethyl acetate–acetone–hexane (2.5:0.5:1) as an eluent: yield 36%; crystals from ethanol; mp 155–157 °C; $[\alpha]^{25}_D$ –83° (c = 0.26, acetone); IR (KBr) 3420, 1770, 1500, 1470, and 1400 cm^{-1}; ^{1}H NMR (acetone-d_6) δ 7.86 (s, 1 H, 4′-OH), 6.95 (s, 1 H, 5-H), 6.37 (s, 1 H, 8-H), 6.15 (s, 1 H, 6′-H), 6.00 and 5.98 (s and s, 2 H, OCH_2O), 5.13–5.11 (m, 2 H, 1-H and 4-H), 4.75 (q, J = 9.9 Hz, 1 H, 7″-H), 4.65 (d, J = 7.5 Hz, 1 H, 1″-H), 4.50 (d, 2 H, 2″, 3″-OH), 4.36–4.31 (m, 2 H, 11, 11′-H), 4.18 (dd, J = 10.4, 4.5 Hz, 1 H, 2-H), 3.84 (s, 3 H, 3′-OCH_3), 3.58 (s, 3 H, 5′-OCH_3), 3.57–3.25 (m, 7 H, 3, 2″, 3″, 4″, 5″, 6″-H), 2.83 (d, J = 9.9 Hz, 3 H, 7″-CH_3). Anal. ($C_{29}H_{31}ClO_{13}$) C, H.

2′-Chloro-4′-*O*-demethylepipodophyllotoxin (4). The same method described for the preparation of **2** was used to prepare **4**. Purification of **4** was performed by column chromatography with chloroform–acetone–ethyl acetate (10:0.5:1) as an eluant: yield 75%; crystals from ethanol; mp 141–142 °C; $[\alpha]^{25}_D$ –31.4° (c = 0.5, acetone); IR (KBr) 3420, 2930, 2900, 1770, and 1480 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 9.08 (s, 1 H, 4′-OH), 6.92 (s, 1 H, 5-H), 6.25 (s, 1 H, 8-H), 6.00 (s, 1 H, 6′-H), 5.97 (s, 2 H, OCH_2O), 5.46 (d, J = 5.6 Hz, 1 H, 4-OH), 4.94 (d, J = 6.7 Hz, 1 H, 1-H), 4.77 (t, 1 H, 4-H), 4.38 (t, 1 H, 11-H), 4.15 (t, 1 H, 11′-H), 3.74 (s, 3 H, 3′-OCH_3), 3.54 (s, 3 H, 5′-OCH_3), 3.40 (dd, J = 14.8, 6.7 Hz, 1 H, 2-H), and 3.01 (m, 1 H, 3-H). Anal. ($C_{21}H_{19}ClO_8$) C, H, Cl.

4β-Bromo-2′-chloro-4′-*O*-demethyl-4-desoxypodophyllotoxin (5). A solution of 2′-chloro-4′-*O*-demethylepipodophyllotoxin (**4**) (352 mg, 0.81 mmol) in 20 mL of dry dichloromethane was kept at 0 °C, and dry hydrogen bromide gas was bubbled through the solution. After 1 h, the solution was then evaporated in vacuo, followed by using benzene as an azeotropic mixture to remove the water formed in the reaction. The desired product (400 mg) was obtained, which was used for the next step reaction without further purification. Purification of **5** can be achieved by recrystallization from benzene–ethyl ether: mp 151–153 °C; $[\alpha]^{25}_D$ –32° (c = 0.29, acetone); IR (KBr) 3460, 1760, 1440, 1400, and 1220 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 6.83 (s, 1 H, 5-H), 6.34 (s, 1 H, 8-H), 5.89 (s, 3 H, 6′-H and OCH_2O), 5.60 (d, J = 3.0 Hz, 1 H, 4-H), 5.60 (s, 1 H, 4′-H), 5.29 (s, br s, 1 H, 1-H), 4.45 (t, 1 H, 11-H), 4.33 (t, 1 H, 11′-H), 3.92 (s, 3 H, 3′-OCH_3), 3.73 (s, 3 H, 5′-OCH_3), 3.48 (dd, J = 9.6, 7.2 Hz, 1 H, 2-H), and 3.02 (s, br s, 1 H, 3-H).

Synthesis of Compounds 6–13. A solution containing 4′-*O*-demethyl-2′-chloro-4β-bromo-4-desoxypodophyllotoxin (**5**) (50 mg, 0.1 mmol) and sodium iodide (15 mg, 0.1 mmol) in 2 mL of dry acetone was stirred for 30 min at room temperature. The reaction mixture was filtered, evaporated in vacuo, and then 2 mL of dichloroethane, anhydrous barium carbonate (50 mg, 0.26 mmol), and the appropriate arylamine (0.12 mmol) were added. After the resulting mixture was stirred overnight at room temperature under a nitrogen atmosphere, it was filtered, diluted with ethyl acetate, washed with water, dried over anhydrous magnesium sulfate, and purified by preparative TLC with dichloromethane–acetone–ethyl acetate 100:5:5 or toluene–ethyl acetate 3:1 as an eluant.

4′-*O*-Demethyl-2′-chloro-4β-(4″-nitroanilino)-4-desoxypodophyllotoxin (6): yield 71%; crystals from ethanol; mp 254–256 °C; $[\alpha]^{25}_D$ –145° (c = 0.16, acetone); IR (KBr) 3300, 1770, 1600, 1500, 1480, and 1320 cm^{-1}; ^{1}H NMR (CD_3OD) δ 8.03 (d, J = 9 Hz, 2 H, 3″,5″-H), 6.70 (d, J = 9 Hz, 2 H, 2″,6″-H), 6.67 (s, 1 H, 5-H), 6.25 (s, 1 H, 8-H), 6.09 (s, 1 H, 6′-H), 5.84 (s, 2 H, OCH_2O), 5.12 (d, J = 4.8 Hz, 1 H, 1-H), 5.01 (s, br s, 1 H, 4-H), 4.55 (m, 2 H, 11,11′-H), 3.79 (s, 3 H, 3′-OCH_3), 3.59 (s, 3 H, 5′-OCH_3), and 3.29 (m, 2 H, 2, 3-H). Anal. ($C_{27}H_{23}ClN_2O_9 \cdot {}^1/_2H_2O$) C, H, N.

4′-*O*-Demethyl-2′-chloro-4β-(3″-nitroanilino)-4-desoxypodophyllotoxin (7): yield 36%; crystals from ethanol–acetone; mp 177–179 °C; $[\alpha]^{25}_D$ –81° (c = 0.29, acetone); IR (KBr) 3360, 1760, 1520, 1480, and 1350 cm^{-1}; ^{1}H NMR (acetone-d_6) δ 7.89 (s, 1 H, 4′-OH), 7.56 (dd, J = 3.0, 2.1 Hz, 1 H, 2″-H), 7.49 (dd, J = 7.8, 2.1 Hz, 1 H, 4″-H), 7.42 (t, J = 7.8 Hz, 1 H, 5″-H), 7.19 (dd, J = 7.8, 3.0 Hz, 1 H, 6″-H), 6.87 (s, 1 H, 5-H), 6.35 (s, 1 H, 8-H), 6.28 (s, 1 H, 6′-H), 6.08 (d, J = 7.5 Hz, 1 H, NH), 5.97 and 5.95 (s and s, 2 H, OCH_2O), 5.15 (m, 2 H, 1-H and 4-H), 4.52 (dd, J = 8.1, 7.8 Hz, 1 H, 11-H), 3.90 (dd, J = 8.1, 7.8 Hz, 1 H, 11′-H), 3.86 (s, 3 H, 3′-OCH_3), 3.64 (s, 3 H, 5′-OCH_3), 3.58 (m, 1 H, 3-H), and 3.43 (dd, J = 14.7, 6 Hz, 1 H, 2-H). Anal. calcd: C, 58.39; H, 4.18; N, 5.05; found: C, 58.89; H, 4.68; N, 4.64.

4′-*O*-Demethyl-2′-chloro-4β-(3″-hydroxyanilino)-4-desoxypodophyllotoxin (8): yield 50%; crystals from ethanol–acetone; mp 167–169 °C; $[\alpha]^{25}_D$ –158° (c = 0.1, acetone); IR (KBr) 3400, 3340, 1770, 1510, 1490, and 1460 cm^{-1}; ^{1}H NMR (acetone-d_6) δ 7.85 (s, 1 H, 4′-OH), 7.56 (s, 1 H, 3″-H), 6.74–6.62 (m, 5 H, 5,2″,4″,5″,6″-H), 6.31 (s, 1 H, 8-H), 6.27 (s, 1 H, 6′-H), 5.95 and 5.93 (s and s, 2 H, OCH_2O), 5.10 (d, J = 5.1 Hz, 1 H, 1-H), 4.81 (s, br s, 1 H, 4-H), 4.45 (m, 1 H, 11-H), 3.97 (m, 1 H, 11′-H), 3.85 (s, 3 H, 3′-OCH_3), 3.63 (s, 3 H, 5′-OCH_3), and 3.43 (s, br s, 2 H, 2,3-H). Anal. ($C_{27}H_{24}ClNO_8 \cdot {}^1/_2H_2O$) C, H, N.

4′-*O*-Demethyl-2′-chloro-4β-[3″,4″-(ethylenedioxy)-anilino]-4-desoxypodophyllotoxin (9): yield 62%; crystals from ethanol–acetone; mp 245–247 °C; $[\alpha]^{25}_D$ –118° (c = 0.27, acetone); IR (KBr) 3460, 3400, 2980, 2930, 1760, 1620, 1590, 1500, and 1470 cm^{-1}; ^{1}H NMR (acetone-d_6) δ 7.87 (s, 1 H, 4′-OH), 6.76 (s, 1 H, 5-H), 6.65 (d, J = 9.3 Hz, 1 H, 5″-H), 6.31 (s, 1 H, 8-H), 6.27 (m, 3 H, 6′,2″,6″-H), 5.95 and 5.94 (s and s, 2 H, OCH_2O), 5.10 (d, J = 5.5 Hz, 1-H), 4.82 (s, br s, 1 H, 4-H), 4.44 (t, 1 H, 11-H), 4.23–4.16 (m, 2 H, OCH_2CH_2O), 3.95 (t, 1 H, 11′-H), 3.85 (s, 3 H, 3′-OCH_3), 3.63 (s, 3 H, 5′-OCH_3), and 3.40 (m, 2 H, 2,3-H). Anal. ($C_{29}H_{26}ClNO_9$) C, H, N.

4′-*O*-Demethyl-2′-chloro-4β-[3″,4″-(methylenedioxy)-anilino]-4-desoxypodophyllotoxin (10): yield 31%; crystals from ethanol; mp 155–158 °C; $[\alpha]^{25}_D$ –136° (c = 0.26, acetone); IR (KBr) 3400, 3100, 1770, 1500, and 1480 cm^{-1}; ^{1}H NMR (acetone-d_6) δ 7.87 (s, 1 H, 4′-OH), 6.77 (s, 1 H, 5-H), 6.67 (d, J = 8.2 Hz, 1 H, 5″-H), 6.43 (d, J = 2.1 Hz, 1 H, 2″-H), 6.31 (s, 1 H, 8-H), 6.25 (s, 1 H, 6′-H), 6.19 (dd, J = 8.2, 2.1 Hz, 1 H, 6″-H), 5.96 and 5.94 (s and s, 2 H, 6,7-OCH_2O), 5.87 (s, 2 H, 3″,4″-OCH_2O), 5.09 (d, J = 5.2 Hz, 1 H, 1-H), 4.86 (s, br s, 1 H, 4-H), 4.45 (t, 1 H, 11-H), 3.96 (t, 1 H, 11′-H), 3.85 (s, 3 H, 3′-OCH_3), 3.63 (s, 3 H, 5′-OCH_3), and 3.40 (m, 2 H, 2,3-H). Anal. ($C_{28}H_{24}ClNO_9 \cdot H_2O$) C, H, N.

4′-*O*-Demethyl-2′-chloro-4β-(4″-fluoroanilino)-4-desoxypodophyllotoxin (11): yield 51%; crystals from ethanol–acetone; mp 157–158 °C; $[\alpha]^{25}_D$ –105° (c = 0.26, acetone); IR (KBr) 3300, 3150, 1770, 1500, and 1480 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 6.96 (t, J = 8.5 Hz, 2 H, 3″,5″-H), 6.68 (m, 3 H, NH and 2″,6″-H), 6.40 (s, 1 H, 5-H), 6.00 (s, 1 H, 6′-H), 5.96 (s, 2 H, OCH_2O), 5.58 (s, 1 H, 8-H), 5.21 (s, br s, 1 H, 4′-OH), 4.64 (d, J = 3.0 Hz, 1 H, 1-H), 4.45 (t, 1 H, 11-H), 3.96 (s, 3 H, 3′-OCH_3), 3.70 (s, 3 H, 5′-OCH_3), and 3.34–3.20 (m, 2 H, 2,3-H). Anal. ($C_{27}H_{23}ClFNO_7 \cdot {}^1/_2EtOH$) C, H, N.

4′-*O*-Demethyl-2′-chloro-4β-(4″-chloroanilino)-4-desoxypodophyllotoxin (12): yield 42%; crystals from ethanol; mp 179–180 °C; $[\alpha]^{25}_D$ –94° (c = 0.27, acetone); IR (KBr) 3320, 1770, 1600, 1500, and 1460 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.20 (d, J = 8.7 Hz, 2 H, 3″,5″-H), 6.69 (s, 1 H, 5-H), 6.52 (d, J = 8.7 Hz, 2 H, 2″,6″-H), 6.40 (s, 1 H, 8-H), 6.01 (s, 1 H, 6′-H), 5.94 (s, 2 H, OCH_2O), 5.59 (s, 1 H, 4′-OH), 5.20 (s, br s, 1 H, 4-H), 4.67 (d, J = 3.0 Hz, 1 H, 1-H), 4.45 (q, 1 H, 11-H), 3.97 (q, 1 H, 11′-H), 3.96 (s, 3 H, 3′-OCH_3), 3.71 (s, 3 H, 5′-OCH_3), and 3.26 (m, 2 H, 2,3-H). Anal. ($C_{27}H_{23}Cl_2NO_7 \cdot {}^1/_2H_2O$) C, H, N.

4′-*O*-Demethyl-2′-chloro-4β-(4″-bromoanilino)-4-desoxypodophyllotoxin (13): yield 50%; crystals from ethanol; mp 207–208 °C; $[\alpha]^{25}_D$ –115° (c = 0.24, acetone); IR (KBr) 3340, 3150, 2980, 2900, 1770, 1600, and 1480 cm^{-1}; ^{1}H NMR (acetone-d_6) δ 7.28 (dd, J = 8.7, 2.0 Hz, 2 H, 3″,5″-H), 6.79 (s, 1 H, 5-H), 6.76 (d, J = 8.7, 2.0 Hz, 2 H, 2″,6″-H), 6.27 (s, 1 H, 6′-H), 5.96 and 5.95 (s and s, 2 H, OCH_2O), 5.11 (d, J = 6.1 Hz, 1 H, 1-H), 4.97 (s, br s, 1 H, 4-H), 4.47 (q, 1 H, 11-H), 3.89 (q, 1 H, 11′-H), 3.85 (s, 3 H, 3′-OCH_3), 3.63 (s, 3 H, 5′-OCH_3), 3.47 (m, 1 H, 3-H), and 3.40 (dd, J = 14.1, 6.1 Hz, 1 H, 2-H). Anal. ($C_{27}H_{23}BrClNO_7 \cdot {}^1/_2EtOH$) 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.[19]

(19) 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. Antitumor Agents. 107. New Cytotoxic 4-Alkylamino Analogues of 4′-Demethylepipodophyllotoxin As Inhibitors of Human DNA Topoisomerase II. *J. Nat. Prod.* **1989**, *52* (3), 606–613.

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

Zhe-Qing Wang,‡ Hong Hu,‡ Hong-Xin Chen,§ Yung-Chi Cheng,§ and Kuo-Hsiung Lee*,‡

Natural Products Laboratory, Division of Medicinal Chemistry and Natural Products, School of Pharmacy, University of North Carolina, Chapel Hill, North Carolina 27599, and Department of Pharmacology, Yale University School of Medicine, New Haven, Connecticut 06510. *Received July 29, 1991*

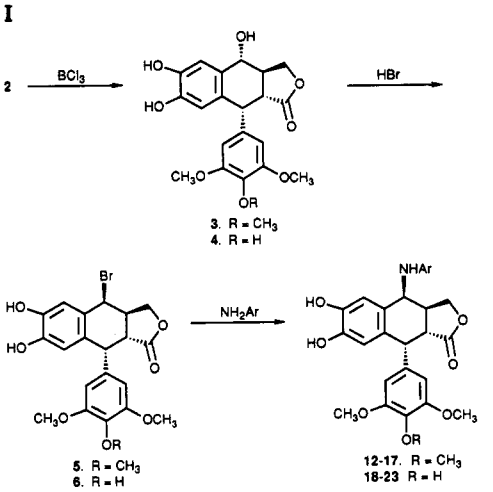
A series of 6,7-*O*,*O*-demethylene-4′-*O*-demethyl-4β-(substituted anilino)-4-desoxypodophyllotoxins (**18–23**), 6,7-*O*,*O*-demethylene-6,7-*O*,*O*-dimethyl-4′-*O*-demethyl-4β-(substituted anilino)-4-desoxypodophyllotoxins (**28–31**), and their corresponding 4′-*O*-methyl analogues (**12–17** and **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



* To whom correspondence should be addressed.

† For part 123, see ref 1.

‡ Natural Products Laboratory, Division of Medicinal Chemistry and Natural Products, School of Pharmacy.

§ Department of Pharmacology, Yale University School of Medicine.

(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.

(2) Jardine, I. Podophyllotoxins. In *Anticancer Agents Based on Natural Product Models*; Cassady, J. M., Douros, J., Eds.; Academic Press: New York, **1980**; p 319–351 and references therein.

(3) Issell, B. F.; Muggia, F. M.; Carter, S. K. The Chemistry of Etoposide. In *Etoposide (VP-16) Current Status and New Developments*; Academic Press: Orlando, FL, **1984**; p 15 and references therein.

0022-2623/92/1835-0871$03.00/0