Antitumor Agents. 111.† New 4-Hydroxylated and 4-Halogenated Anilino Derivatives of 4′-Demethylepipodophyllotoxin as Potent Inhibitors of Human DNA Topoisomerase II

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A series of C-4 hydroxylated and halogenated anilino derivatives of epipodophyllotoxin or 4′-demethylepipodophyllotoxin 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 **11–17** and **22** are more potent than etoposide in causing DNA breakage, while compounds **11–13**, **15**, **16**, and **20** are as active or more active than etoposide in their inhibition of the human DNA topoisomerase II. The cytotoxicity in KB cells appears to have no direct correlation with their ability to inhibit DNA topoisomerase II and to cause protein-linked DNA breaks in cells.

Etoposide (**1**) reached clinical study in the early 1970s. Its clinical efficacy has stimulated a renewed interest in the medicinal chemistry of podophyllotoxin-derived antitumor agents.[2-8] It has recently been shown that **1** and related compounds are potent inhibitors of 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.[9-11] Structure-activity relationship studies have demonstrated a direct correlation between cytotoxicity, DNA breakage, and mice-derived topoisomerase II inhibition activities among the podophyllotoxin analogues.[12] In our previous study[13] we found that substitution of the glycosidic moiety of **1** by an 4-alkylamino side chain, as in 4′-demethyl-4β-[(2″-hydroxyethyl)amino]-4-desoxypodophyllotoxin, showed potent inhibitory activity on human DNA topoisomerase II as well as stronger activity in causing cellular protein-linked DNA breakage. The enzyme-inhibitory activity correlates reasonably well with its activity in causing protein-linked DNA breakage in KB cells. The in vitro cytotoxicity (KB) appears to have no correlation with the inhibitory activity of the human DNA topoisomerase II. As part of our continuing efforts along this line, we describe herein the preparation and structure–activity relationships of a series of 4-arylamino compounds derived from 4′-demethylepipodophyllotoxin or podophyllotoxin.

Chemistry

The 4-arylamino derivatives of epipodophyllotoxin (**8–10**) were synthesized from podophyllotoxin (**2**), while the 4-arylamino derivatives of 4′-demethylepipodophyllotoxin (**11–23**) were prepared from 4′-demethylepipodophyllotoxin (**3**) (Scheme I). These syntheses employed a modification of Kuhn's method.[14] The key intermediates, 4β-bromo-4-desoxypodophyllotoxin (**4**) and 4′-demethyl-4β-bromo-4-desoxypodophyllotoxin (**6**), were obtained via bromination of **2** and **3**, respectively. They were accompanied by 4β-methoxy-4-desoxypodophyllotoxin (**5**) and 4′-demethyl-4β-methoxy-4-desoxypodophyllotoxin (**7**). The formation of **5** and **7** were due to the replacement of the bromo atom in **4** and **6** with trace amounts of methanol present in dichloromethane. As the bromo intermediates (**4** and **6**) were highly reactive and susceptible toward nucleophilic attack even by moisture, and **5** and **7** were relatively stable toward the next step of the reaction, the crude bromination products (**4** and **6**) were subjected to the next step of the reaction to yield **8–23** without further purification.

Thus, the compounds **8–23** were synthesized by nucleophilic substitution from the bromination products (**4** and **6**) with appropriate arylamines. Presumably, this substitution underwent via a S_N1 mechanism, which occurred on the C-4 benzylic carbonium ion. The bulky C-1α pentant aromatic ring directed the substitution to be stereoselective, resulting in the formation of C-4β-oriented **8–23** as the main products, accompanied by C-4α isomers of **8–23**. The ratio for the β- and α-isomers varied from a range of 6:1 to 12:1.[15]

The assignment of the configuration at C-4 for **8–23** was based not only on the difference between the $J_{3,4}$ coupling

† For part 110, see ref 1.

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(1) Part 110: Yamagishi, T.; Haruna, M.; Yan, X. Z.; Chang, J. J.; Lee, K. H. *J. Nat. Prod.* **1989**, *52*, 1071.
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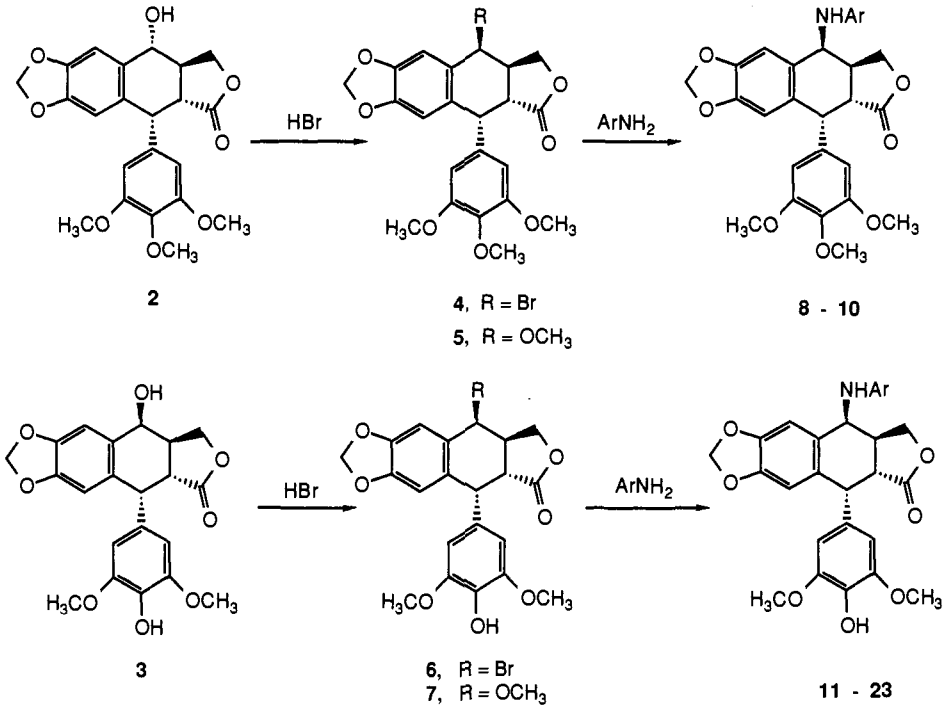
(13) Lee, K. H.; Imakura, Y.; Haruna, M.; Beers, S. A.; Thurston, L. S.; Dai, H. J.; Chen, C. H.; Liu, S. Y.; Cheng, Y. C. *J. Nat. Prod.* **1989**, *52*, 606.
(14) Kuhn, M.; Keller-Juslen, C.; Von Wartburg, A. *Helv. Chim. Acta* **1969**, *52*, 944.
(15) When the reaction mixtures were fractionated on a silica gel column for purification, the C-4β-substituted compounds were always collected before C-4α-substitution.

0022-2623/90/1833-1364$02.50/0

Scheme I



constants[13] but also on the different chemical shift for the proton at C-5. The C-4β-substituted products showed H-5 as a singlet at δ 6.70–6.81, whereas, in the case of C-4α-substituted isomers, it appeared as a singlet at δ 7.00–7.20. This difference was clearly due to the anisotropic effect of the aromatic ring attached at either the β or the α C-4 amino group on H-5.

Results and Discussion

Previous studies on the structure–activity relationships among podophyllotoxin and related compounds have suggested the following structural requirements for antitumor activity:[2,3] (a) the presence of a 4′-phenolic hydroxyl group, (b) the presence of a glucoside moiety on the C-4 hydroxyl group and a 4″,6″-*O*-cyclic acetal moiety on the hexapyranose ring, (c) the β-configuration at C-4 position, and (d) the trans-fused lactone ring.

As illustrated in Table I, compounds **8–10** lacking a free OH group at C-4′ showed no DNA scission activity and no inhibition on DNA topoisomerase II. On the contrary, the 4′-demethyl derivatives (**11–13**) demonstrated potent activity in both models. This coincides very well with the above-mentioned structural requirement (a). The cytotoxicity of **8–10** could be due to their potential effects on tubulin polymerization as seen in podophyllotoxin. The lack of the correlation between cytotoxicity and induced protein-linked DNA breaks in cells by these compounds is not surprising. It was previously shown that etoposide-induced DNA break is insufficient to cause cell death.[16] The possible existence of other biochemical determinants of those compounds' action, such as their potential effects on tubulin polymerization in cells, could also contribute to the lack of correlation between these parameters. Furthermore, the cytotoxic action of the compound will also depend on the uptake of the compound into cells.

The compounds (**11–17**, **20**, and **22**) possessing no glucoside moiety at the C-4 hydroxy group showed comparable or superior activity to **1**. It clearly indicated that the replacement of the 4-*O*-glycosyl moiety by a simpler arylamino group still retained the activity of **1**. The glucosidic ethylidene cyclic acetal moiety was not so essential for the biological activity as mentioned above in (b).

As described in our previous studies dealing with 4′-demethyl-4β-(alkylamino)-4-desoxypodophyllotoxins,[13] those compounds having a β-hydroxyethylene chain (e.g. 4′-demethyl-4β-[(2″-hydroxyethyl)amino]-4-desoxypodophyllotoxin) showed potent inhibition on enzyme as well as on DNA strand breakage. Prolongation of the ethylene side chain, as in the hydroxypropyl analogue, led to reduction in potency. A similar situation was seen in compounds **11–13**. The most active substance among them in the three assay systems (Table I) was **12**. Compound **12** possessed an oxygen atom meta to the nitrogen atom. Although there are three carbon atoms separating the oxygen atom from the nitrogen atom, the bond distance in a benzene ring is shorter than that between sp^3 hybridized carbon atoms. In addition, the bond distance between oxygen and sp^2 carbons is less than that between sp^3 hybridized carbon atoms. The same applies for the bond distance between nitrogen and sp^2 vs nitrogen and sp^3 carbon atoms. The result is that the distance between oxygen and nitrogen in **12** is not as far as it was in 4′-demethyl-4β-[(2″-hydroxyethyl)amino]-4-desoxypodophyllotoxin.

Table I also illustrated two factors necessary for optimum activity in three bioassay systems of the halogenated compounds (**14–23**) which are different from those of the hydroxyl compounds (**11–13**). First, the distance between the halogen and the nitrogen is important. The compound showed the greatest activity when the halogen (F, Cl, Br, I) is in the para position. The activity then decreased as the halogen was moved into the meta position with the least activity occurring when the halogen is in the ortho position. The second factor involves the size of the substituted halogen. Although the distance-dependent activity relationship is still seen when fluorine (similar in size to

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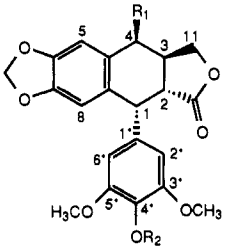
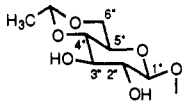
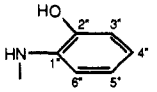
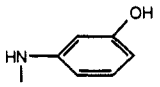
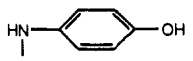
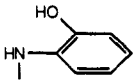
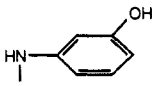
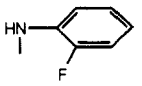
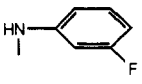
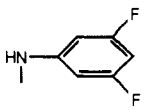
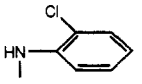
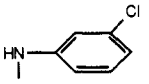
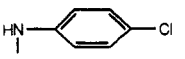
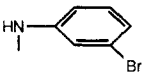
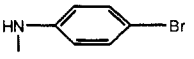
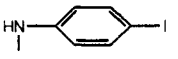
Table I. Biological Evaluation of 4-(Arylamino)epipodophyllotoxins



no.	R_1	R_2	cytotoxicity,[a] ID_{50} KB, μM	inhibn of DNA topoisomerase II activity		cellular protein–DNA complex formation, % (10 μM)
				100 μM[b]	ID_{50},[c] μM	
1		H	0.20	+++	50	100
8		CH_3	4.11	+		6
9		CH_3	0.31	+		37
10		CH_3	0.59	+		21
11		H	4.54	++++	25	151
12		H	0.45	++++	5	290
13		H	2.26	+++	25	211
14		H	0.25	++		121
15		H	0.23	+++	50	158
16		H	0.24	++++	10	213
17		H	1.08	++	50	115
18		H	2.34	+		32
19		H	2.29	++		51
20		H	0.22	+++	50	99
21		H	2.36	+		62
22		H	0.22	++	100	179
23		H	0.34	+		64

[a] ID_{50} was the concentration of drug that affords 50% reduction in cell number after a 3-day incubation. [b] +, ++, +++, ++++, and – denote 25%, 50%, 75%, >75%, and 0% inhibition. [c] Each compound was examined with three concentrations at 25, 50, and 100 μM. The ID_{50} value was established on the basis of the degree of inhibition at these three concentrations.

hydrogen) is used as the halogen, all four fluorine compounds endow with comparable activity with 1 in three bioassay systems.

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 ^{1}H NMR spectra were obtained by using either a JEOL FX-60 or a Bruker 250 NMR spectrometer; all chemical shifts were reported in parts per million from TMS. Elemental analyses were performed by Galbraith Laboratories, Inc., Knoxville, TN, or by Atlantic MicroLab Inc., Norcross, GA. Mass spectral analyses were determined on a V.G. Micromass 70-70 instrument at 70 eV with a direct inlet system. 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 CC. Preparative TLC was performed on Analtech precoated silica gel GF (500 μm, 20 × 20 cm). All new target compounds were characterized by melting point and ^{1}H NMR and IR spectral analyses as well as elemental analyses.

Synthesis of Compounds 8–10. Podophyllotoxin (500 mg, 1.2 mmol) was dissolved in dry dichloromethane (10 mL) and kept at 0 °C. Hydrogen bromide gas was introduced into the solution for 45 min, after which time the solvent was evaporated in vacuo, and anhydrous tetrahydrofuran (15 mL), anhydrous barium carbonate (474 mg, 2.4 mmol), and the appropriate hydroxyanilines (142 mg, 1.3 mmol) were added. The mixture stood at room temperature overnight and then was filtered and concentrated. The crude product was purified by column chromatography (silica gel (45 g) with dichloromethane–acetone–ethyl acetate 100:5:5 as an eluant).

4β-(2″-Hydroxyanilino)-4-desoxypodophyllotoxin (8): amorphous crystals from ether, mp 145–148 °C; IR (KBr) 3480 (OH), 3410 (NH), 2900 (aliphatic CH), 1760 (lactone), 1580, 1475 (aromatic C=C) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 6.90 (t, J = 6.6 Hz, 1 H, 4″-H), 6.78 (s, 1 H, 5-H), 6.65 (m, 2 H, 3″,6″-H), 6.53 (m, 2 H, 8-H, 5″-H), 6.34 (s, 2 H, 2′,6′-H), 5.96 (AB q, J ≈ 1.0, 3.5 Hz, 2 H, OCH_2O), 5.02 (s, 1 H, exchangeable, 2″-OH), 4.68 (m, 1 H, 4-H), 4.62 (d, J = 4.9 Hz, 1 H, 1-H), 4.38 (t, J = 8.6 Hz, 1 H, 11-H), 4.33 (m, 1 H, exchangeable, NH), 4.00 (t, J ≈ 8.6 Hz, 1 H, 11-H), 3.82 (s, 3 H, 4′-OCH_3), 3.76 (s, 6 H, 3′,5′-OCH_3), 3.25 (dd, J = 5.1, 14.0 Hz, 1 H, 2-H), 3.05 (m, 1 H, 3-H); MS m/z = 505 (M^+). Anal. ($C_{28}H_{27}NO_8 \cdot {}^3/_2H_2O$) C, H.

4β-(3-Hydroxyanilino)-4-desoxypodophyllotoxin (9): amorphous powder from ether, mp 148–150 °C; IR (KBr) 3370 (OH, NH), 2900 (aliphatic CH), 1760 (lactone), 1585, 1475 (aromatic C=C) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.05 (t, J = 8.0 Hz, 1 H, 5″-H), 6.77 (s, 1 H, 5-H), 6.52 (s, 1 H, 8-H), 6.32 (s, 2 H, 2′,6′-H), 6.25 (dd, J = 2.2, 8.0 Hz, 1 H, 4″-H), 6.14 (dd, J = 2.2, 8.0 Hz, 1 H, 6″-H), 6.05 (t, J = 2.2 Hz, 1 H, 2″-H), 5.96 (AB q, J = 1.3, 3.8 Hz, 2 H, OCH_2O), 4.64 (d, J = 3.9 Hz, 1 H, 4-H), 4.49 (d, J = 5.0 Hz, 1 H, 1-H), 4.4 (t, J = 8.7 Hz, 1 H, 11-H), 4.03 (t, J = 8.7 Hz, 1 H, 11-H), 3.81 (s, 3 H, 4′-OCH_3), 3.76 (s, 6 H, 3′,5′-OCH_3), 3.18 (dd, J = 5.0 Hz, 14.0 Hz, 1 H, 2-H), 3.02 (m, 1 H, 3-H); MS m/z = 505 (M^+). Anal ($C_{28}H_{27}NO_8 \cdot H_2O$) C, H.

4β-(4″-Hydroxyanilino)-4-desoxypodophyllotoxin (10): crystals from chloroform, mp 145–150 °C; IR (KBr) 3310 (OH, NH), 3010 (aromatic CH), 2900 (aliphatic CH), 1730 (lactone), 1575, 1475 (aromatic C–H) cm^{-1}; ^{1}H NMR ($CDCl_3$, D_2O exchange) δ 6.75 (d, J = 8.3 Hz, 3 H, 5-H, 3″,5″-H), 6.53 (s, 1 H, 8-H), 6.45 (d, J = 8.3 Hz, 2 H, 2″,6″-H), 6.23 (s, 2 H, 2′,6′-H), 5.95 (AB q, J = 1.0, 4.0 Hz, 2 H, OCH_2O), 4.60 (d, J = 4.2 Hz, 1 H, 4-H), 4.57 (d, J = 4.6 Hz, 1 H, 1-H), 4.38 (t, J = 6.0 Hz, 1 H, 11-H), 4.05 (t, J = 6.0 Hz, 1 H, 11-H), 3.83 (s, 3 H, 4′-OCH_3), 3.75 (s, 6 H, 3′,5′-OCH_3), 3.18 (dd, J = 4.6 Hz, 14.0 Hz, 1 H, 2-H), 3.0 (m, 1 H, 3-H). Anal ($C_{28}H_{27}NO_8 \cdot {}^1/_2H_2O$) C, H.

4′-Demethyl-4β-bromo-4-desoxypodophyllotoxin (6). A solution of 4′-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 to dryness, and the desired product (11.5 g) was obtained, which was used for the next reaction without further purification .

Synthesis of Compounds 11–23. A solution containing 4′-demethyl-4β-bromo-4-deoxypodophyllotoxin (**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, and purified via column chromatography (30 g of silica gel with dichloromethane–acetone–ethyl acetate 100:5:5 or toluene–ethyl acetate 3:1 as eluant).

4′-Demethyl-4β-(2″-hydroxyanilino)-4-desoxypodophyllotoxin (11): yield 28%; crystals from ether, mp 175 °C; IR (KBr) 3360 (OH, NH), 2900 (aliphatic C–H), 1750 (lactone), 1600, 1475 (aromatic C=C) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 6.88 (t, J = 7.4 Hz, 1 H, 4″-H), 6.78 (s, 1 H, 5-H), 6.65 (m, 2 H, 3″,6″-H), 6.50 (m, 2 H, 8-H, 5″-H), 6.35 (s, 2 H, 2′,6′-H), 5.96 (AB q, J = 1.2, 3.5 Hz, 2 H, OCH_2O), 5.44 (s, 1 H, exchangeable, 4′-OH), 5.10 (s, 1 H, exchangeable, 2″-OH), 4.67 (d, J = 4.0 Hz, 1 H, 4-H), 4.61 (d, J = 4.8 Hz, 1 H, 1-H), 4.38 (t, J = 8.5 Hz, 1 H, 11-H), 3.98 (t, J = 8.5 Hz, 1 H, 11-H), 3.79 (s, 6 H, 3′,5′-OCH_3), 3.24 (dd, J = 4.8, 14 Hz, 1 H, 2-H), 3.02 (m, 1 H, 3-H); MS m/z = 491 (M^+). Anal. ($C_{27}H_{25}NO_8$) C, H.

4′-Demethyl-4β-(3″-hydroxyanilino)-4-desoxypodophyllotoxin (12): yield 32%; amorphous powder from ether, mp 163–166 °C; IR (KBr) 3480 (OH), 3380 (NH), 2900 (aliphatic CH), 1750 (lactone), 1590, 1474 (aromatic C=C) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.05 (t, J = 8.0 Hz, 1 H, 5″-H), 6.78 (s, 1 H, 5-H), 6.52 (s, 1 H, 8-H), 6.33 (s, 2 H, 2′,6′-H), 6.24 (dd, J = 2.2, 8.0 Hz, 1 H, 4″-H), 6.15 (dd, J = 1.7, 8.0 Hz, 1 H, 6″-H), 6.07 (t, J = 2.2 Hz, 1 H, 2″-H), 5.97 (d, J = 4.4 Hz, 2 H, OCH_2O), 5.43 (s, 1 H, exchangeable, 4′-OH), 4.82 (s, 1 H, exchangeable, 3″-OH), 4.65 (d, J = 3.9 Hz, 1 H, 4-H), 4.58 (d, J = 4.8 Hz, 1 H, 1-H), 4.37 (t, J = 8.7 Hz, 1 H, 11-H), 4.0 (t, J = 8.7 Hz, 1 H, 11-H), 3.79 (s, 6 H, 3′,5′-OCH_3), 3.1 (dd, J = 4.8, 14.1 Hz, 1 H, 2-H), 2.98 (m, 1 H, 3-H); MS m/z = 491 (M^+). Anal. ($C_{27}H_{25}NO_8 \cdot H_2O$) C, H.

4′-Demethyl-4β-(4″-hydroxyanilino)-4-desoxypodophyllotoxin (13): yield 33%; amorphous powder from ether, mp 162–165 °C; IR (KBr) 3525 (OH), 3345 (NH), 3010 (aromatic CH), 2900 (aliphatic CH), 1745 (lactone), 1600, 1475 (aromatic C=C) cm^{-1}; ^{1}H NMR (DMSO-d_6, D_2O exchange) δ 6.69 (s, 1 H, 5-H), 6.55 (s, 4 H, 2″,3″,5″,6″-H), 6.48 (s, 1 H, 8-H), 6.23 (s, 2 H, 2′,6′-H), 5.94 (d, J = 9.7 Hz, 2 H, OCH_2O), 4.68 (d, J = 4.3 Hz, 1 H, 4-H), 4.46 (d, J = 5.4 Hz, 1 H, 1-H), 4.29 (t, J = 7.6 Hz, 1 H, 11-H), 3.76 (J = 7.6 Hz, 1 H, 11-H), 3.61 (s, 6 H, 3′,5′-OCH_3), 3.28 (dd, J = 5.4, 15.8 Hz, 1 H, 2-H), 2.95 (m, 1 H, 3-H). Anal. ($C_{27}H_{25}NO_8 \cdot H_2O$) C, H.

4′-Demethyl-4β-(2″-fluoroanilino)-4-desoxypodophyllotoxin (14): yield 43%; crystals from methanol, mp 197–198 °C; $[\alpha]^{25}_D$ −128° (c = 0.25, $CHCl_3$); IR (KBr) 3500 (OH), 4480 (NH), 2890 (aliphatic C–H), 1755 (lactone), 1610, 1505, and 1475 (aromatic C=C) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.04 (m, 2 H, 3″,6″-H), 6.76 (s, 1 H, 5-H), 6.72 (m, 1 H, 5″-H), 6.60 (t, J = 7.2 Hz, 1 H, 4″-H), 6.54 (s, 1 H, 8-H), 6.34 (s, 2 H, 2′,6′-H), 5.97 (d, J = 7.3 Hz, 2 H, OCH_2O), 5.46 (s, 1 H, exchangeable, 4′-OH), 4.69 (d, J = 4.2 Hz, 1 H, 4-H), 4.62 (d, J = 4.9 Hz, 1 H, 1-H), 4.38 (t, J = 8.2 Hz, 1 H, 11-H), 4.10 (t, 1 H, exchangeable, NH), 3.82 (t, J = 8.2 Hz, 1 H, 11-H), 3.79 (s, 6 H, 3′,5′-OCH_3), 3.15 (dd, J = 5.0, 14.0 Hz, 1 H, 2-H), 3.00 (m, 1 H, 3-H). Anal. ($C_{27}H_{24}FNO_7$) C, H, N.

4′-Demethyl-4β-(3″-fluoroanilino)-4-desoxypodophyllotoxin (15): yield 60%; crystals from methanol, mp 201–203 °C dec; $[\alpha]^{25}_D$ −132° (c = 1, $CHCl_3$); IR (KB) 3500 (OH), 3360 (NH), 2900 (aliphatic C–H), 1750 (lactone), 1605, 1500, and 1475 (aromatic C=C) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.15 (t, J = 7.4 Hz, 1 H, 5″-H), 6.76 (s, 1 H, 5-H), 6.53 (s, 1 H, 8-H), 6.49 (dd, J = 1.2, 7.4 Hz, 1 H, 4″-H), 6.40 (s, 2 H, 2′,6′-H), 6.32 (d, J = 1.2 Hz, 1 H, 2″-H), 6.24 (dd, J = 1.2, 7.4 Hz, 1 H, 6″-H), 5.97 (AB q, J = 1.2, 7.9 Hz, 2 H, OCH_2O), 5.44 (s, 1 H, exchangeable, 4′-OH), 4.67 (s, 1 H, exchangeable, NH), 4.63 (d, J = 4.0 Hz, 1 H, 4-H), 4.59 (d, J = 5.0 Hz, 1 H, 1-H), 4.39 (t, J = 8.5 Hz, 1 H, 11-H), 3.98 (t, J = 8.5 Hz, 1 H, 11-H), 3.79 (s, 6 H, 3′,5′-OCH_3), 3.11 (dd, J = 5.0, 14.0 Hz, 1 H, 2-H), 3.00 (m, 1 H, 3-H). Anal. ($C_{27}H_{24}FNO_7$) C, H, N.

4′-Demethyl-4β-(4″-fluoroanilino)-4-desoxypodophyllotoxin (16): yield 45%; crystals from ethanol, mp 176–177 °C; $[\alpha]^{25}_D$ −100° (c = 0.8, $CHCl_3$); IR (KBr) 3540 (OH), 3420 (NH), 2900 (aliphatic C–H), 1740 (latone), 1610, 1500, and 1480 (aromatic C=C) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 6.94 (t, J = 6.7, 2 H, 3″,5″-H), 6.75 (s, 1 H, 5-H), 6.53 (s, 1 H, 8-H), 6.49 (q, J = 2.2, 6.2 Hz, 2

H, 2″,6″-H), 6.33 (s, 2 H, 2′,6′-H), 5.96 (AB q, J = 1.2, 7.5 Hz, 2 H, OCH_2O), 5.43 (s, 1 H, exchangeable, 4′-OH), 4.60 (d, 2 H, 4-H and 1-H), 4.37 (t, J = 7.5 Hz, 1 H, 11-H), 3.99 (t, J = 7.5 Hz, 1 H, 11-H), 3.79 (s, 6 H, 3′,5′-OCH_3), 3.73 (br, 1 H, exchangeable, NH), 3.13 (t, J = 5.0, 14.0 Hz, 1 H, 2-H), 3.00 (m, 1 H, 3-H). Anal. ($C_{27}H_{24}FNO_7$) C, H, N.

4′-Demethyl-4β-(3″,5″-difluoroanilino)-4-desoxypodophyllotoxin (17): yield 50%; crystals from ethanol, mp 180–183 °C; $[\alpha]^{25}_D$ −132° (c = 0.33, $CHCl_3$); IR (KBr) 3500 (OH), 3370 (NH), 2890 (aliphatic C–H), 1750 (lactone), 1620, 1590, 1500, and 1475 (aromatic C=C) cm^{-1}; ^1H NMR ($CDCl_3$) 6.75 (s, 1 H, 5-H), 6.54 (s, 1 H, 8-H), 6.32 (s, 2 H, 2′,6′-H), 6.23 (m, 1 H, 4″-H), 6.07 (m, 2 H, 2″,6″-H), 5.98 (AB q, J = 1.3, 9.0 Hz, 2 H, OCH_2O), 5.45 (s, 1 H, exchangeable, 4′-OH), 4.61 (m, 2 H, 4-H and 1-H), 4.39 (t, J = 8.5 Hz, 1 H, 11-H), 4.10 (d, J = 6.1 Hz, 1 H, exchangeable, NH), 3.85 (t, J = 8.5 Hz, 1 H, 11-H), 3.81 (s, 6 H, 3′,5′-OCH_3), 3.08 (dd, J = 4.8, 14.1 Hz, 1 H, 2-H), 3.02 (m, 1 H, 3-H). Anal. ($C_{27}H_{23}NF_2O_9$) C, H, N.

4′-Demethyl-4β-(2″-chloroanilino)-4-desoxypodophyllotoxin (18): yield 38%; crystals from ethyl acetate–ether, mp 253–255 °C; $[\alpha]^{25}_D$ −90° (c = 1.0, $CHCl_3$); IR (KBr) 3500 (OH), 3450 (NH), 2895 (aliphatic CH), 1751 (lactone), 1590, 1500, and 1472 (aromatic C=C) cm^{-1}; ^1H NMR ($CDCl_3$) 7.31 (dd, J = 1.4, 7.9 Hz, 1 H, 3″-H), 7.18 (t, J = 8.8 Hz, 1 H, 5″-H), 6.76 (s, 1 H, 5-H), 6.73 (t, J = 9.0 Hz, 4″-H), 6.58 (d, J = 8.2 Hz, 1 H, 6″-H), 6.54 (s, 1 H, 8-H), 6.35 (s, 2 H, 2′,6′-H), 5.98 (AB q, J = 1.2, 4.2 Hz, 2 H, $OCOH_2O$), 5.44 (s, 1 H, exchangeable, 4′-OH), 4.73 (t, J = 4.9 Hz, 1 H, 4-H), 4.64 (d, J = 4.9 Hz, 1 H, 1-H), 4.49 (d, J = 6.0 Hz, 1 H, exchangeable, NH), 4.36 (t, J = 8.3 Hz, 1 H, 11-H), 3.91 (t, J = 8.3 Hz, 1 H, 11-H), 3.80 (s, 6 H, 3′,5′-OCH_3), 3.17 (dd, J = 4.8, 14.0 Hz, 1 H, 2-H), 3.04 (m, 1 H, 3-H). Anal. ($C_{27}H_{24}ClNO_7$) C, H, N.

4′-Demethyl-4β-(3″-chloroanilino)-4-desoxypodophyllotoxin (19): yield 48%; crystals from ethyl acetate–ether, mp 174–176 °C; $[\alpha]^{25}_D$ −112° (c = 1.0, $CHCl_3$); IR (KBr) 3500 (OH), 3360 (NH), 2920 (aliphatic CH), 1752 (lactone), 1580 and 1450 (aromatic C=C) cm^{-1}; ^1H NMR ($CDCl_3$) 7.12 (t, J = 8.1 Hz, 1 H, 5″-H), 6.76 (s, 1 H, 5-H), 6.74 (dd, J = 1.0, 8.1 Hz, 1 H, 4″-H), 6.53 (br, 2 H, 8-H and 2″-H), 6.42 (dd, J = 1.6, 6.5 Hz, 1 H, 6″-H), 6.33 (s, 2 H, 2′,6′-H), 5.97 (AB q, J = 1.0, 8.7 Hz, 2 H, OCH_2O), 5.43 (s, 1 H, exchangeable, 4′-OH), 4.66 (br, 1 H, 4-H), 4.59 (d, J = 4.8 Hz, 1 H, 1-H), 4.39 (t, J = 7.7 Hz, 1 H, 11-H), 3.99 (t, J = 7.7 Hz, 1 H, 11-H), 3.96 (br, 1 H, exchangeable, NH), 3.79 (s, 6 H, 3′,5′-OCH_3), 3.11 (dd, J = 5.8, 14.0 Hz, H, 2-H), 3.01 (m, 1 H, 3-H). Anal. ($C_{27}H_{24}ClNO_7$) C, H, N.

4′-Demethyl-4β-(4″-chloroanilino)-4-desoxypodophyllotoxin (20): yield 52%; crystals from ethyl acetate–ether, mp 253–255 °C; $[\alpha]^{25}_D$ −125° (c = 0.75, $CHCl_3$); IR (KB) 3500 (OH), 3360 (NH), 2900 (aliphatic CH), 1758 (lactone), 1605, 1590, and 1475 (aromatic C=C) cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.17 (d, J = 8.7 Hz, 2 H, 3″,5″-H), 6.74 (s, 1 H, 5-H), 6.53 (s, 1 H, 8-H), 6.48 (d, J = 8.7 Hz, 2 H, 2″,6″-H), 6.32 (s, 2 H, 2′,6′-H), 5.96 (AB q, J = 1.0, 6.8 Hz, 2 H, OCH_2O), 5.43 (s, 1 H, exchangeable, 4′-OH), 4.63 (d, J = 4.2 Hz, 1 H, 4-H), 4.59 (d, J = 4.9 Hz, 1 H, 1-H), 4.38 (t, J = 8.0 Hz, 1 H, 11-H), 3.96 (J = 8.0 Hz, 1 H, 11-H), 3.79 (s, 6 H, 3′,5′-OCH_3), 3.12 (dd, J = 4.9, 14.1 Hz, 1 H, 2-H), 2.99 (m, 1 H, 3-H). Anal. ($C_{27}H_{24}ClNO_7$) C, H, N.

4′-Demethyl-4β-(3″-bromoanilino)-4-desoxypodophyllotoxin (21): yield 55%; crystals from methanol–ether, mp 177–179 °C; $[\alpha]^{25}_D$ −105° (c = 1, $CHCl_3$); IR (KBr) 3450 (OH), 3340 (NH), 2900 (aliphatic CH), 1740 (lactone), 1590, 1500, and 1475 (aromatic C=C) cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.07 (t, J = 8.0 Hz, 1 H, 5″-H), 6.90 (dd, J = 0.9, 7.9 Hz, 1 H, 4″-H), 6.75 (s, 1 H, 5-H), 6.70 (br, 1 H, 2″-H), 6.53 (s, 1 H, 8-H), 6.47 (dd, J = 1.7, 8.3 Hz, 1 H, 6″-H), 6.33 (s, 2 H, 2′,6′-H), 5.97 (dd, J = 1.2, 9.3 Hz, 2 H, OCH_2O), 5.43 (s, 1 H, exchangeable, 4′-OH), 4.65 (d, J = 4.2 Hz, 1 H, 4-H), 4.60 (d, J = 4.8 Hz, 1 H, 1-H), 4.39 (t, J = 7.3 Hz, 1 H, 11-H), 3.96 (t, J = 7.3 Hz, 1 H, 11-H), 3.90 (d, J = 6.2 Hz, 1 H, exchangeable, NH), 3.80 (s, 6 H, 3′,5′-OCH_3), 3.10 (dd, J = 4.9, 14.0 Hz, 1 H, 2-H), 3.02 (m, 1 H, 3-H). Anal. ($C_{27}H_{24}BrNO_7$) C, H, N.

4′-Demethyl-4β-(4″-bromoanilino)-4-desoxypodophyllotoxin (22): yield 57%; crystals from ethyl acetate–ethanol, mp 227–230 °C; $[\alpha]^{25}_D$ −110° (c = 0.5, $CHCl_3$); IR (KBr) 3500 (OH), 3330 (NH), 2900 (aliphatic CH), 1755 (lactone), 1605, 1590, and 1475 (aromatic C=C) cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.30 (d, J = 8.9 Hz, 2 H, 3″,5″-H), 6.75 (s, 1, 5-H), 6.53 (s, 1 H, 8-H), 6.44 (d, J = 8.9 Hz, 2 H, 2″,6″-H), 6.32 (s, 2 H, 2′,6′-H), 5.98 (AB q, J = 1.3, 8.3 Hz, 2 H, OCH_2O), 5.42 (s, 1 H, exchangeable, 4′-OH), 4.62 (m, 2 H, 4-H and 1-H), 4.36 (t, J = 8.5 Hz, 1 H, 11-H), 3.95 (t, 8.5 Hz, 11-H), 3.86 (d, J = 7.8, 1 H, exchangeable, NH), 3.79 (s, 6 H, 3′,5′-OCH_3), 3.11 (dd, J = 4.8, 14.1 Hz, 1 H, 2-H), 3.00 (m, 1 H, 3-H). Anal. ($C_{27}H_{24}BrNO_7$) C, H, N.

4′-Demethyl-4β-(4″-iodoanilino)-4-desoxypodophyllotoxin (23): yield 41%; crystals from ethanol, mp 198–200 °C dec; $[\alpha]^{25}_D$ −111° (c = 0.5, $CHCl_3$); IR (KBr) 3540 (OH), 3420 (NH), 2900 (aliphatic CH), 1770 (lactone), 1610, 1585, and 1480 (aromatic C=C) cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.46 (d, J = 8.8 Hz, 2 H, 3′,5′-H), 6.74 (s, 1 H, 5-H), 6.65 (s, 1 H, 8-H), 6.35 (d, J = 8.8 Hz, 2 H, 2″,6″-H), 6.32 (s, 2 H, 2′,6′-H), 5.59 (AB q, J = 1.1, 8.9 Hz, 2 H, OCH_2O), 5.44 (s, 1 H, exchangeable, 4′-OH), 4.62 (m, 1 H, 4-H), 4.58 (d, J = 4.9 Hz, 1 H, 1-H), 4.34 (t, J = 8.5 Hz, 1 H, 11-H), 3.94 (m, 2 H, 11-H, and NH), 3.78 (s, 6 H, 3′,5′-OCH_3), 3.09 (dd, J = 4.9, 14.1 Hz, 1 H, 2-H), 2.99 (m, 1 H, 3-H). Anal. ($C_{27}H_{24}INO_7$) 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.[9]

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