Antitumor Agents. 148.§ Synthesis and Biological Evaluation of Novel 4β-Amino Derivatives of Etoposide with Better Pharmacological Profiles

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A series of novel 4β-amino derivatives of etoposide (1), which can form water-soluble salts and demonstrate excellent activity against mdr- and topo II-resistant cell lines, have been synthesized. Compared with etoposide, compounds **5–6**, **8**, and **10–16** show comparable or greater inhibition of human DNA topo II. In a cellular protein–DNA complex formation assay, compounds **5–6**, **8**, **10–14**, and **16** are more potent than **1**. A dose–response study of **8** shows that it is 20 times more active in formation of protein-linked DNA breaks than etoposide. Furthermore, both **8** and its free base **7** were found to be highly active toward etoposide-resistant KB cell lines. All compounds were also evaluated *in vitro* against a total of 56 human tumor cell lines derived from seven cancer types. Comparison of the $\log_{10}$ GI50 mean graph midpoints of **5–19** (−4.89 to −7.30) with that of **1** (−4.08) shows these new analogs to be 6–1659-fold more active than **1**.

Introduction

Etoposide (VP-16, **1**, Figure 1) is a widely-used, important antineoplastic agent. It shows good clinical effects against several types of tumors, including testicular and small cell lung cancers, lymphoma, leukemia, and Kaposi's sarcoma.[2] However, several limitations such as poor water solubility, development of drug resistance, and metabolic inactivation still exist. In order to overcome the limitations of **1** and develop compounds with better antitumor activity, our laboratory has been engaged in the design and synthesis of novel **1** analogs.[3-13] Replacement of the C-4 sugar moiety of **1** with a nonsugar substitution has proven to be significant in overcoming the drug resistance of **1**.[14,15] The C-4 nonsugar substitutions can be linked through an O-, S- or N-linkage. In general, the O-linked derivatives (ethers, esters) and the S-linked derivatives (thioethers) are inactive or show lower activity compared with the N-linked congeners.[8-10] It is also known that only compounds with β substitution at the C-4 position are active. On the other hand, both **1** and its congener teniposide (**2**, Figure 1) are poorly soluble in water and therefore require a complex formulation for drug administration.[16] Development of **1** derivatives that can form water-soluble salts will ease the difficulty of drug formulation and open the possibility of other routes of drug administration, such as oral administration. In view of the available information on structure–activity relationships, we decided to synthesize **1** derivatives with a C-4β N-linked, nonsugar, aliphatic nitrogen- or carboxyl group-containing substitution. This lead to the discovery of the title compounds which have increased antitumor activities, better water solubility, and an improved drug resistance profile.

Chemistry

The 4-alkylamino compounds were synthesized by direct nucleophilic substitution of the C-4-bromo intermediate, 4β-bromo-4′-desoxypodophyllotoxin (**4**) resulting from bromination of **3**, with appropriate alkylamines at elevated temperature (Scheme 1). The C-1α pendant aromatic ring dictates a stereoselective substitution yielding the C-4β alkylamino isomer as the major product. The alkylamino compounds were then reacted with HCl to form the hydrochloride salts. The 4-arylamino compounds were synthesized by nucleophilic substitution at room temperature (Scheme 2). The sodium salt **18** was formed by reacting the carboxylic compound **17** with NaOH.

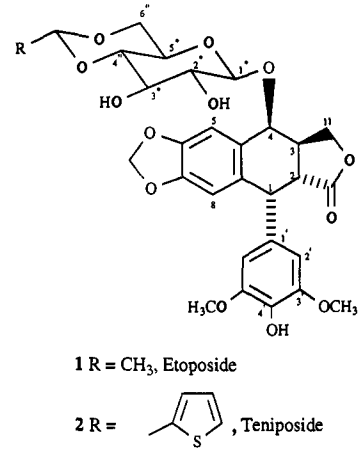

Figure 1. Structures of etoposide and teniposide.

Biological Results and Discussion

Table 1 gives the biological results for KB cytotoxicity, DNA topo II inhibition, and DNA–protein complex formation with compounds **5–19**. Compared with etoposide, compounds **5–6**, **8**, and **10–16** show comparable or greater inhibition of human DNA topo II. In the cellular protein–DNA complex formation assay, compounds **5–6**, **8**, **10–14**, and **16** are more potent than **1**. Compounds **6**, **8**, **10**, **12**, **14**, and **16** are the hydrochloride salts of **5**, **7**, **9**, **11**, **13**, and **15**, respectively. In most cases, the salts show comparable or better activity than that of their parent free base. This might be due to the increased water solubility of the compounds in a salt form. Compound **18** is the sodium salt of the carboxylic acid **17**. Compound

§ For Part 147, see ref 1.
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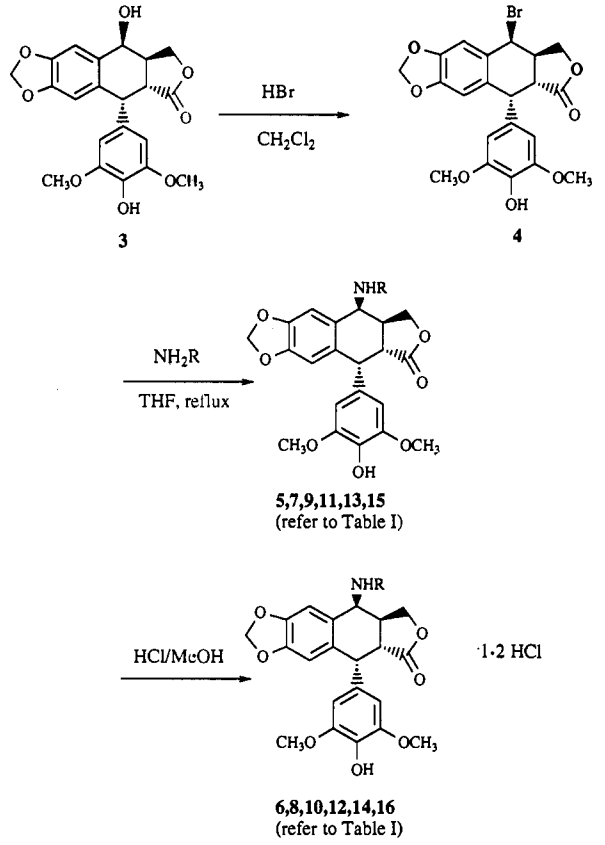
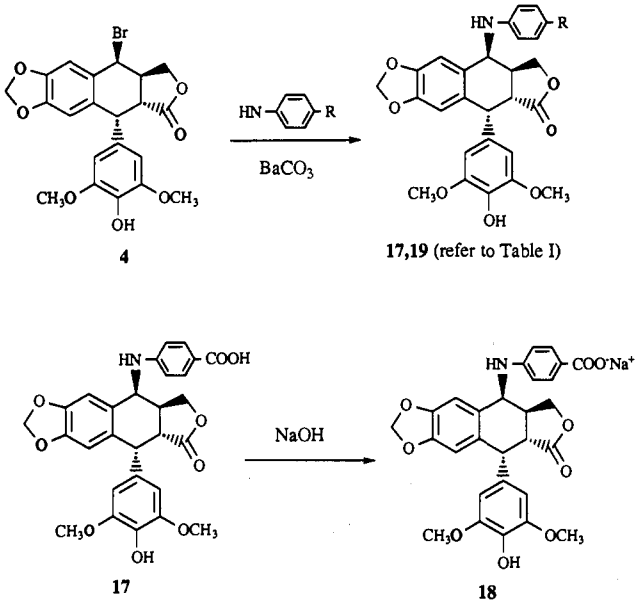
Scheme 1. Synthesis of 4β-Alkylamino Derivatives of 4'-Demethylepipodophyllotoxin



Scheme 2. Synthesis of 4β-Arylamino Derivatives of 4'-Demethylepipodophyllotoxin



18, which has a negative charge, has poor activities. In contrast, the positively charged amine salts show good activities.

The 4-amino-1-benzylpiperidine moiety of 7 and 8 caused significant cytotoxicity. A dose–response study of 8 shows that it is 20 times more active in formation of protein-linked DNA breaks than etoposide (Figure 2, 8 is as effective as etoposide at a concentration 20 times lower than etoposide), and 8 is more than 15 times more active than 1 toward KB ATCC cells (Table 2). Furthermore, both 8 and its free base 7 were found to be highly active

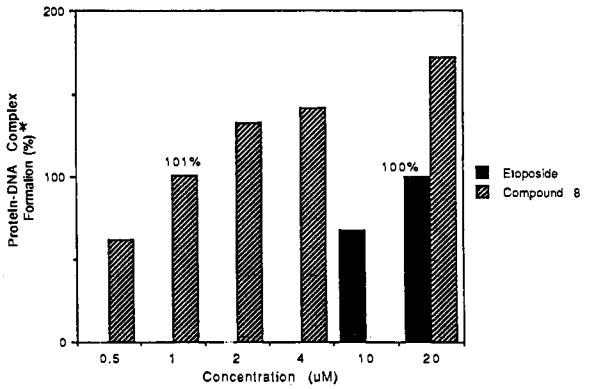


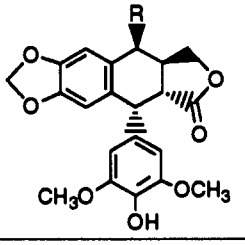
Figure 2. Dose–response of compound 8. [*Relative units, the amount of protein–DNA complex formation with etoposide at 20 μM is designated as 100%.]

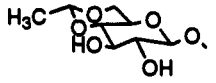
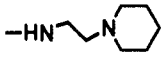
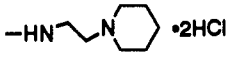
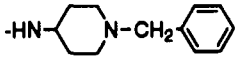
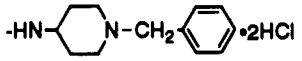
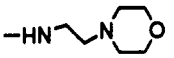
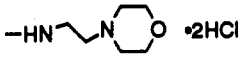
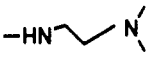
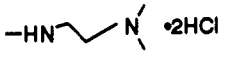
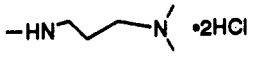
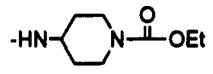
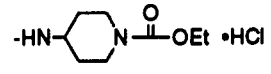
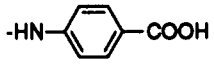
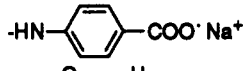
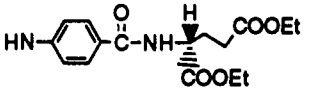
toward etoposide-resistant KB cell lines. As shown in Table 2, compounds 7 and 8 are more than 200 times and more than 400 times more active than 1, respectively, toward KB 7D cells, which have a decreased etoposide uptake and a decreased topo II level.[14-15] Both compounds are also more than 19 times more active than 1 toward the P-glycoprotein overexpressed MDR cell line KB V20c, which is cross-resistant to vincristine and etoposide. Thus, compound 8 is much more potent than etoposide in causing protein-linked DNA breaks *in vitro*, and compounds 7 and 8 can overcome both an etoposide-resistant KB variant, which has defects in cellular uptake of etoposide and decreased amounts of DNA topo II, and an etoposide/vincristine-resistant variant, which overexpresses P-glycoprotein. Compounds 11 and 13 (Table 2) also show activities toward both drug-resistant cell lines.

All compounds were also evaluated *in vitro* against a total of 56 human tumor cell lines derived from eight cancer types (leukemia, non-small cell lung cancer, small cell lung cancer, colon cancer, CNS cancer, melanoma, ovarian cancer, and renal cancer). For each compound, dose–response curves for each cell line were measured with five different drug concentrations, and the concentration causing 50% cell growth inhibition (GI50), total cell growth inhibition (TGI, 0% growth), and 50% cell death (LC50, −50% growth) compared with the control was calculated. The $\log_{10}$ GI50 of compounds **5–19** as well as of 1 are expressed in the form of mean graphs (for compound 8, refer to Figure 3). In these graphs, the mean logarithmic value of GI50 in all cell lines for each tested compound is used as the midpoint of that bar graph. Bars extending to the right represent sensitivity of the cell line to the test agent in excess of the average sensitivity of all tested cell lines. The bar scale is logarithmic; therefore, a bar 2 units to the right shows the compound achieved the GI50 for the cell line at a concentration one-hundredth the mean concentration required over all cell lines; thus, the cell line is unusually sensitive to the compound. Bars extending to the left correspondingly imply sensitivity less than the mean. The $\log_{10}$ TGI and $\log_{10}$ LC50 values for **5–19** were also measured and can be expressed with similar bar mean graphs; however, only the mean graph midpoint values of $\log_{10}$ TGI and $\log_{10}$ LC50, as well as $\log_{10}$ GI50, for **5–19** are listed in Table 3.

As demonstrated by the mean graph pattern, these compounds have similar selectivity to 1 against leukemia (e.g., CCRF-CEM, MOLT-4), non-small cell lung cancer (e.g., A549/ATCC, NCI-H460), and renal cancer (e.g., CAKI-1) cell lines. However, some compounds (e.g.,

Table 1. Biological Evaluation of 4β-Amino Derivatives of 4'-Demethylepipodophyllotoxin



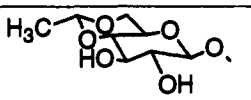
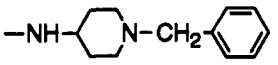
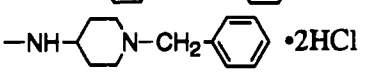
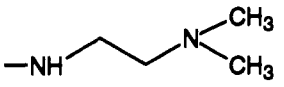
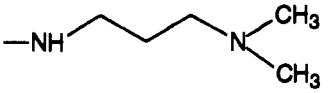
compd	R	cytotoxicity ID_{50} KB (μM)	inhibition of DNA topo II ID_{50} (μM)[b]	cellular protein–DNA complex formation (%), 20 μM
1		0.20	50	100
5		1.4	25	190
6		1.6	50	183
7		0.027	100	83
8		0.021	50	172
9		2.0	>100	77
10		4.0	50	140
11		0.4	25	203
12			25	183
13		0.18	25	186
14		1.15	25	179
15		0.74	25	17
16		1.13	25	138
17		>4.0	>100	1.9
18		>4.0	>100	6.9
19		<0.4	100	83

[a] ID_{50} was the concentration of drug which affords 50% reduction in cell number after a 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 based on the degree of inhibition at these five concentrations.

compounds **5–9**, **11–12**, and **19**) show an increased selectivity toward CNS cancer cell lines (e.g., SF-295, SF-539, SNB-78). Above average activity toward some ovarian cancer, melanoma, and colon cancer cell lines is also observed for some compounds (e.g., **5–8**, **11–14**, and **19** against ovarian cancer cell lines SK-OV-3; **8**, **11–14**, and **19** against melanoma LOXIMVI and UACC-62 cell lines; **16** against the colon cancer HCT-15 cell line). In general, all 15 compounds show greater activity than **1** toward all the cell lines. Comparison of the $\log_{10}$ GI50 mean graph midpoints of **5–19** (−4.89 to −7.30) with that of **1** (−4.08) shows these new analogs to be 6–1,659-fold more active than **1**. The mean graph midpoints of $\log_{10}$ TGI and $\log_{10}$ LC50 showed similar patterns to the $\log_{10}$ G150 mean graph midpoints.

The interaction between **1**, DNA, and topo II may occur through an intercalation-like mechanism.[17,18] The planar ABCD ring system of **1** (or its analogs) inserts between the base pairs, while the sugar moiety (or other C-4 substitutions) and the pendant E ring stretch out along the DNA minor groove and may bind to DNA and/or protein (topo II). From this model, an aliphatic nitrogen-containing C-4 side chain may interact with the phosphate residues of the DNA backbone to form a stable complex. However, if the C-4 side chain contains a carboxyl group or other negative charge-containing/generating moieties, this negative charge might repel the phosphate negative charges of the DNA backbone and prevent the formation of a stable complex. This could explain the good activities observed with the aliphatic nitrogen-containing compounds and the

Table 2. Cytotoxic Effect of Compounds 7, 8, 11, and 13 toward KB ATCC Cells and Their Resistant Variants

compd	R	ID_{50} (nM) KB ATCC	KB 7D (topo II, aMDR)	KB V20C (MDR)
etoposide		320	18000	2300 ± 990
7		27	50	26
8		24 ± 4	97.5 ± 74	21.5 ± 6.3
11		400	680	440
13		180	660	200

relatively poor activities observed with the carboxyl group-containing compounds. Furthermore, a long C-4 side chain, such as found in compounds 7 and 8, might produce stronger binding between the drug molecule and the DNA minor groove. The benzyl carbon of 7 and 8 could also provide the flexibility necessary for a good fit of the chain along the DNA minor groove curvature. Using this rationale, we synthesized 19, the glutamate diethyl ester, instead of the glutamate compound with two free carboxyl groups. Compound 19 has a long, flexible C-4 side chain, which potentially could provide good binding with the DNA minor groove. Biological evaluations of this compound were very promising; 19 is the most active compound toward the human tumor cell lines among all the 1 analogs synthesized to date. Comparison of the $\log_{10}$ GI50 mean graph midpoint value of 19 (−7.30) with that of 1 (−4.08) shows that 19 is 1659-fold more active than 1.

Because of their potent antitumor activities and their intrinsic water solubility, these novel 4-β-alkylamino derivatives of 1 show great potential for further development as useful anticancer agents.

Experimental Section

General Experimental Procedures. All melting points were taken on a Fischer-Johns melting point apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer 1320 spectrophotometer, and ^{1}H NMR spectra were obtained using a Bruker AC-300 NMR spectrometer with TMS as the internal standard. All chemical shifts are reported in ppm. Elemental analyses were performed 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 plates. Specific rotations were measured with a Rudolph Research Autopol III polarimeter. All new target compounds were characterized by melting point, ^{1}H NMR and IR spectral analyses, and elemental or MS analyses.

Synthesis of 4′-*O*-Demethyl-4β-(alkylamino)-4-desoxypodophyllotoxin Compounds (5, 7, 9, 11, 13, and 15). A solution of 4′-*O*-demethylepipodophyllotoxin (10 g, 24 mmol) (3) in 250 mL dry dichloromethane was kept at 0 °C, and dry hydrogen bromide was bubbled through the solution for about 2 h until TLC showed the total disappearance of 3. The solution was then evaporated under vacuum, and water was removed using benzene as an azeotropic mixture to remove the water formed in the reaction. The desired product (4, 4′-*O*-demethyl-4β-bromo-4-desoxypodophyllotoxin, 11.5 g) was obtained and was used in the next reaction step without further purification. Dry tetrahydrofuran (20 mL) was added to 1 g of 4 (2.16 mmol), and the suspension was heated to reflux under nitrogen. The appropriate alkylamine (2.59 mmol) was injected into the suspension, and the reaction mixture was refluxed for 6 h and then evaporated under vacuum. The desired 4′-*O*-demethyl-4β-(alkylamino)-4-desoxypodophyllotoxins were obtained by silica gel chromatography using mixed ethyl acetate, toluene, and triethylamine as eluent.

4′-*O*-Demethyl-4β-[[2″-(1‴-piperidinyl)ethyl]amino]-4-desoxypodophyllotoxin (5): yield 11.5%; crystals from acetone; mp 218–219 °C; IR (KBr) 3370, 2940, 1765, 1610, 1520, 1480, 1220, and 1110 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 6.82 (s, 1H, 5-H), 6.48 (s, 1H, 8-H), 6.30 (s, 2H, 2′,6′-H), 5.97 (s, 1H, OCH_2O), 5.96 (s, 1H, OCH_2O), 4.52 (d, J = 5 Hz, 1H, 1-H), 4.30 (m, 2H, 11-H), 3.82 (d, J = 4 Hz, 1H, 4-H), 3.77 (s, 6H, 3′,5′-OCH_3), 3.31 (dd, J = 5, 15 Hz, 1H, 2-H), 2.71 (m, 3H, 3-H, and 1″-H), 2.45 (m, 6H, 2″-H and 2‴,6‴-H), 1.58 (m, 4H, 3‴,5‴-H), 1.45 (m, 1H, 4‴-H). Anal. ($C_{28}H_{34}O_7N_2$) C, H, N.

4′-*O*-Demethyl-4β-[[4″-(1″-benzylpiperidinyl)]amino]-4-desoxypodophyllotoxin (7): yield 13.1%; crystals from acetone; mp 249–250 °C; IR (KBr) 3350, 2940, 2840, 1755, 1610, 1520, 1475, 1220, and 1110 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.32 (m, 5H, 1″-benzyl aromatic H), 6.74 (s, 1H, 5-H), 6.46 (s, 1H, 8-H), 6.28 (s, 2H, 2′,6′-H), 5.96 (s, 1H, OCH_2O), 5.94 (s, 1H, OCH_2O), 5.39 (br, 1H, 4′-OH), 4.51 (d, J = 5.1 Hz, 1H, 1-H), 4.25 (m, 2H, 11-H), 3.98 (d, J = 4 Hz, 1H, 4-H), 3.77 (s, 6H, 3′,5′-OCH_3), 3.52 (s, 2H, benzyl-CH_2), 3.28 (dd, J = 5.1, 13.8 Hz, 1H, 2-H), 2.74 (m, 1H, 3-H), 2.94–1.25 (m, 9H, piperidinyl-H). Anal. ($C_{33}H_{38}O_7N_2 \cdot {}^1/_2H_2O$) C, H, N.

4′-*O*-Demethyl-4β-[[2″-(4‴-morpholinyl)ethyl]amino]-4-desoxypodophyllotoxin (9): yield 14.8%; crystals from acetone–hexane; mp 204–205 °C; IR (KBr) 3380, 2940, 2850, 1765, 1610, 1520, 1480, 1225, and 1110 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 6.77 (a, 1H, 5-H), 6.49 (s, 1H, 8-H), 6.29 (s, 2H, 2′,6′-H), 5.95 (AB q, J = 1.6 Hz, 2H, OCH_2O), 4.53 (d, J = 5.2 Hz, 1H, 1-H), 4.29 (m, 2H,

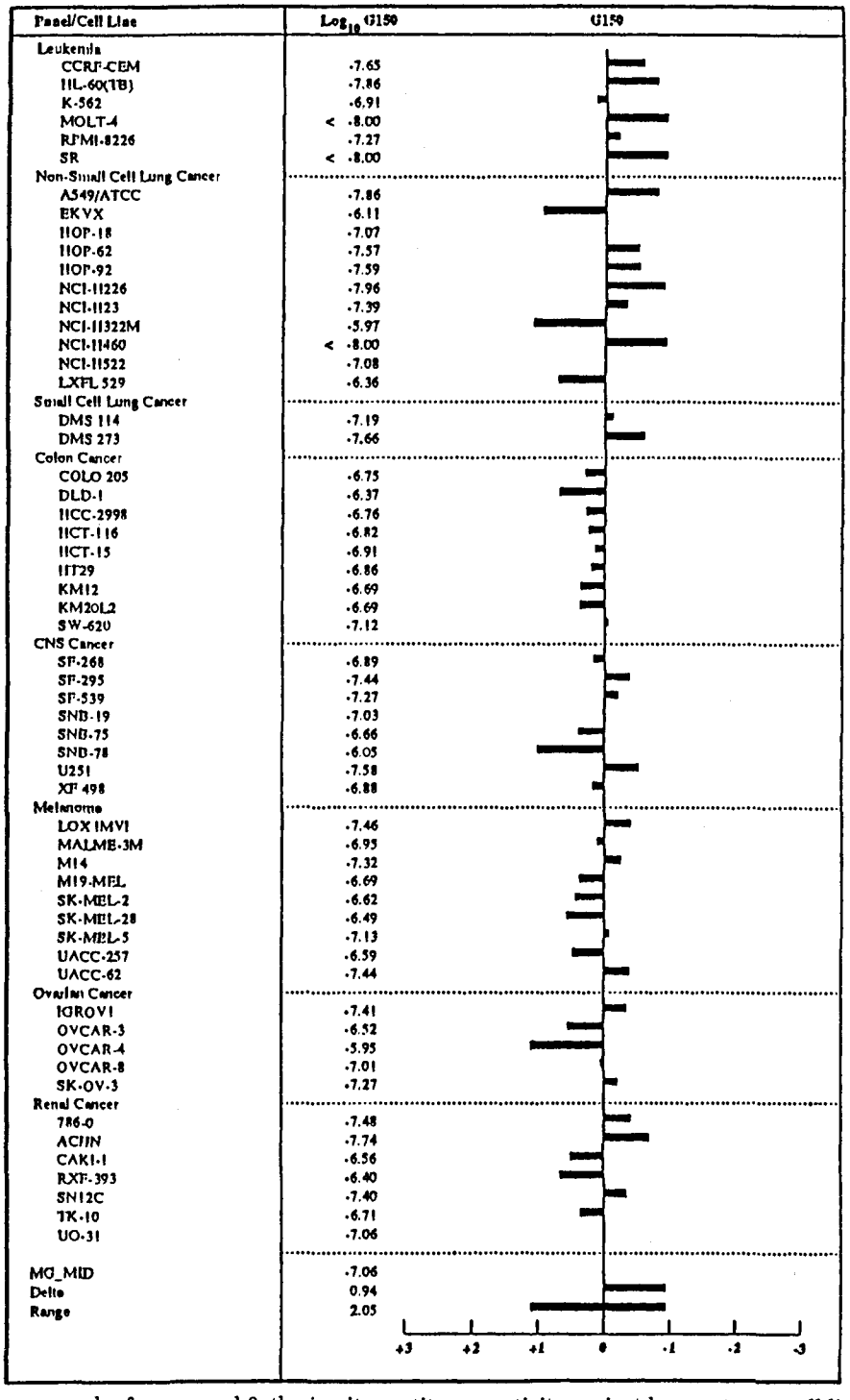


Figure 3. $\log_{10}$ GI50 mean graph of compound 8–the *in vitro* antitumor activity against human tumor cell lines. [$\log_{10}$ GI50: The logarithmic concentration required to product 50% of growth inhibition. Responses to the right of the mean $\log_{10}$ GI50 line (the midpoint of the bar graph) are more sensitive than the mean, while those on the left are more resistant (data from NCI).]

11-H), 3.82 (d, J = 4.2 Hz, 1H, 4-H), 3.77 (s, 6H, 3′,5′-OCH_3), 3.75 (m, 4H, 2‴,6‴-H), 3.31 (dd, J = 5.1, 13.9 Hz, 1H, 2-H), 2.93 (m, 1H, 1″-H), 2.83 (m, 1H, 3-H), 2.75 (m, 1H, 1″-H), 2.56 (m, 6H, 2″,3‴,5‴-H). Anal. ($C_{27}H_{32}O_8N_2 \cdot {}^1/_2H_2O$) C, H, N.

4′-*O*-Demethyl-4β-[[2″-(dimethylamino)ethyl]amino]-4-desoxypodophyllotoxin (11): yield 13.8%; crystals from acetone; mp 178–180 °C; IR (KBr) 3350, 2940, 1765, 1610, 1520, 1510, and 1475 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.81 (s, 1H, 5-H), 6.47 (s, 1H, 8-H), 6.28 (s, 2H, 2′,6′-H), 5.96 (s, 1H, OCH_2O), 5.94 (s, 1H, OCH_2O), 4.51 (d, J = 5.1 Hz, 1H, 1-H), 4.35 (m, 2H, 11-H), 3.82 (d, J = 4 Hz, 1H, 4-H), 3.77 (s, 6H, 3′,5′-OCH_3), 3.33 (dd, J = 5, 14 Hz, 1H, 2-H), 2.91 (m, 1H, 1″-H), 2.78 (m, 1H, 3-H), 2.65 (m, 1H, 1″-H), 2.45 (m, 2H, 2″-H), 2.25 (s, 6H, $N(CH_3)_2$). Anal. ($C_{25}H_{30}O_7N_2$) C, H, N.

4′-*O*-Demethyl-4β-[[3″-(dimethylamino)propyl]amino]-4-desoxypodophyllotoxin (13): yield 16.8%; crystals from MeOH–Et_2O; mp 189–190 °C; IR (KBr) 3370, 2920, 2820, 1755, 1610, 1520, 1480, 1225, and 1110 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.79 (s, 1H, 5-H), 6.48 (s, 1H, 8-H), 6.29 (s, 2H, 2′,6′-H), 5.95 (AB q, 2H, OCH_2O), 4.52 (d, J = 5.4 Hz, 1H, 1-H), 4.30 (m, 2H, 11-H), 3.80 (d, J = 3.9 Hz, 1H, 4-H), 3.77 (s, 6H, OCH_3), 3.28 (dd, J = 5.2, 13.8 Hz, 1H, 2-H), 2.95 (m, 1H, 1″-H), 2.75 (m, 1H, 3-H), 2.55 (m, 1H, 1″-H), 2.32 (m, 2H, 3″-H), 2.23 (s, 6H, $N(CH_3)_2$), 1.65 (m, 2H, 2″-H). Anal. ($C_{26}H_{32}O_7N_2$) C, H, N.

4′-*O*-Demethyl-4β-[[4″-[1″-(ethoxycarbonyl)piperidinyl]]-amino]-4-desoxypodophyllotoxin (15): yield 28.1%; crystals from CH_2Cl_2–acetone; mp 261–262 °C dec; IR (KBr) 3340, 2940, 2840, 1755, 1685, 1610, 1510, 1485, 1230, and 1110 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.74 (s, 1H, 5-H), 6.54 (s, 1H, 8-H), 6.28 (s, 2H, 2′,6′-H), 5.96 (AB q, 2H, OCH_2O), 5.40 (br, 1H, 4′-OH), 4.52 (d, J = 5.1 Hz, 1H, 1-H), 4.25 (m, 2H, 11-H), 4.14 (q, J = 7.17 Hz, 2H, CH_2-CH_3), 4.01 (d, J = 3.8 Hz, 1H, 4-H), 3.77 (s, 6H, 3′,5′-OCH_3), 3.26

Table III. $\log_{10}$ GI50, $\log_{10}$ TGI, and $\log_{10}$ LC50 Mean Graph Midpoints (MG_MID) of *in Vitro* Inhibitory Activity Tests for Compound 5–19 against Human Tumor Cell Lines[a]

compd	$\log_{10}$ GI50	$\log_{10}$ TGI	$\log_{10}$ LC50
1	-4.08	-	-
5	-6.43	-5.48	-4.51
6	-6.37	-5.43	-4.44
7	-6.76	-5.83	-4.76
8	-7.06	-6.28	-5.22
9	-5.74	-4.94	-4.35
10	-5.61	-5.00	-4.78
11	-6.81	-5.83	-4.82
12	-6.58	-5.60	-4.66
13	-6.48	-5.66	-4.90
14	-6.26	-5.55	-4.87
15	-5.87	-5.11	-4.44
16	-5.75	-5.09	-4.67
17	-4.89	-4.28	-4.02
18	-5.07	-4.80	-4.76
19	-7.30	-6.47	-5.54

[a] GI50: Drug molar concentration causing 50% cell growth inhibition. TGI: Drug concentration causing total cell growth inhibition (0% growth). LC50: drug concentration causing 50% cell death (-50% growth). MG_MID: mean graph midpoints, the average sensitivity of all cell lines toward the test agent. Data from the National Cancer Institute.

(dd, J = 5.2, 13.6 Hz, 1H, 2-H), 2.82 (m, 2H, 2″,6″-H), 2.70 (m, 1H, 3-H), 2.54 (m, 1H, 4″-H), 2.18–1.18 (m, 6H, 2″,3″,5″,6″-H), 1.27 (t, J = 6.9 Hz, 3H, CH_2CH_3). Anal. ($C_{29}H_{34}O_9N_2$) C, H, N.

Synthesis of 4′-*O*-Demethyl-4β-(alkylamino)-4-desoxypodophyllotoxin Hydrochloride Salts (6, 8, 10, 12, 14, and 16). A solution of **5**, **7**, **9**, **11**, **13**, or **15** in methanol was reacted with a stoichiometric amount of 1 M HCl in ethyl ether. The resulting solution was evaporated to dryness *in vacuo*. The residue was washed thoroughly with chloroform and ethyl ether to yield **6**, **8**, **10**, **12**, **14**, or **16**, respectively.

4′-*O*-Demethyl-4β-[2″-(1‴-piperidinyl)ethyl]amino]-4-desoxypodophyllotoxin dihydrochloride (6): yield 85%; amorphous powder from MeOH; mp 229–232 °C; IR (KBr) 3350, 2960, 2660, 1760, 1610, 1520, 1485, 1220, and 1110 cm^{-1}; ^1H NMR (d_6-DMSO–D_2O) δ 7.09 (s, 1H, 5-H), 6.55 (s, 1H, 8-H), 6.13 (s, 2H, 2′,6′-H), 6.00 (s, 1H, OCH_2O), 5.99 (s, 1H, OCH_2O), 4.53 (d, J = 5.8 Hz, 1H, 1-H), 4.39 (m, 3H, 4-H and 11-H), 3.58 (s, 6H, 3′,5′-OCH_3), 3.43 (dd, J = 5.5, 15 Hz, 1H, 2-H), 3.35–2.90 (m, 9H, 3-H, 1″-H, 2″-H, and 2‴,6‴-H), 1.73 (br, 4H, 3‴,5‴-H), 1.52 (br, 2H, 4‴-H). Anal. ($C_{28}H_{36}O_7N_2Cl_2 \cdot {}^1/_2H_2O$) C, H, N.

4′-*O*-Demethyl-4β-[[4″-(1″-benzylpiperidinyl)]amino]-4-desoxypodophyllotoxin dihydrochloride (8): yield 85.3%; amorphous powder from MeOH; mp 205–206 °C; IR (KBr) 3400, 2940, 2680, 1755, 1610, 1505, 1485, and 1235 cm^{-1}; ^1H NMR (MeOD–D_2O) δ 7.56 (m, 5H, 1″-benzyl aromatic H), 7.00 (s, 1H, 5-H), 6.61 (s, 1H, 8-H), 6.24 (s, 2H, 2′,6′-H), 6.03 (s, 1H, OCH_2O), 6.02 (s, 1H, OCH_2O), 4.07 (d, J = 5.4 Hz, 1H, 1-H), 4.54 (m, 1H, 11-H), 4.38 (m, 2H, 4-H & 11-H), 3.71 (s, 6H, 3′,5′-OCH_3), 3.65 (s, 2H, benzyl-CH_2), 3.43 (dd, J = 5.4, 14.7 Hz, 1H, 2-H), 3.30–1.80 (m, 10H, 3-H & piperidinyl-H). Anal. ($C_{33}H_{38}O_7N_2Cl_2 \cdot 1.5H_2O$) C, H, N.

4′-*O*-Demethyl-4β-[[2″-(4‴-morpholinyl)ethyl]amino]-4-desoxypodophyllotoxin dihydrochloride (10): yield 84.4%; amorphous powder from MeOH; mp 218–220 °C; IR (KBr) 3440, 2930, 2670, 1755, 1610, 1505, 1485, 1235, and 1110 cm^{-1}; ^1H NMR (D_2O) δ 6.82 (s, 1H, 5-H), 6.42 (s, 1H, 8-H), 6.15 (s, 2H, 2′,6′-H), 5.83 (s, 1H, OCH_2O), 5.80 (s, 1H, OCH_2O), 4.53 (d, J = 6.0 Hz, 1H, 4-H), 4.49 (d, J = 3.6 Hz, 1H, 1-H), 4.29 (m, 2H, 11-H), 3.78 (m, 4H, 2‴,6‴-H), 3.55 (s, 6H, 3′,5′-OCH_3), 3.50–3.00 (m, 14H, 2,3-H, 1″,2″-H, 2‴,3‴,5‴,6‴-H). Anal. ($C_{27}H_{34}O_8N_2Cl_2 \cdot H_2O$) C, H, N.

4′-*O*-Demethyl-4β-[[2″-(dimethylamino)ethyl]amino]-4-desoxypodophyllotoxin dihydrochloride (12): yield 85%; crystals from MeOH–EtOH; mp 245–248 °C dec; IR (KBr) 3350, 2940, 2690, 1755, 1610, 1520, 1485, 1220, and 1110 cm^{-1}; ^1H NMR (D_2O–MeOD) δ 7.01 (s, 1H, 5-H), 6.58 (s, 1H, 8-H), 6.30 (s, 2H, 2′,6′-H), 6.01 (s, 1H, OCH_2O), 5.98 (s, 1H, OCH_2O), 4.68 (d, J = 5.8 Hz, 1H, 1-H), 4.52 (m, 3H, 4-H and 11-H), 3.72 (s, 6H, 3′,5′-OCH_3), 3.45 (m, 5H, 2-H, 1″,2″-H), 3.25 (m, 1H, 3-H), 2.94 (s, 6H, $N(CH_3)_2$). Anal. ($C_{25}H_{32}O_7N_2Cl_2 \cdot H_2O$) C, H, N.

4′-*O*-Demethyl-4β-[[3″-(dimethylamino)propyl]amino]-4-desoxypodophyllotoxin dihydrochloride (14): yield 85%; amorphous powder from MeOH; mp 229–231 °C dec; IR (KBr) 3510, 3360, 2960, 2720, 1760, 1610, 1520, 1480, 1220, and 1110 cm^{-1}; ^1H NMR (D_2O) δ 6.79 (s, 1H, 5-H), 6.44 (s, 1H, 8-H), 6.15 (s, 2H, 2′,6′-H), 5.82 (s, 1H, OCH_2O), 5.81 (s, 1H, OCH_2O), 4.56 (m, 2H, 1-H and 4-H), 4.39 (m, 1H, 11-H), 4.27 (m, 1H, 11-H), 3.54 (s, 6H, OCH_3), 3.34 (dd, J = 5.8, 15.1 Hz, 1H, 2-H), 3.22 (m, 1H, 3-H), 3.05 (m, 4H, 1″,3″-H), 2.71 (s, 6H, $N(CH_3)_2$), 1.98 (m, 2H, 2″-H). Anal. ($C_{26}H_{34}O_7N_2Cl_2 \cdot H_2O$) C, H, N.

4′-*O*-Demethyl-4β-[[4″-[1″-(ethoxycarbonyl)piperidinyl]]-amino]-4-desoxypodophyllotoxin hydrochloride (16): yield 85%; crystals from EtOH–MeOH; mp 222–224 °C dec; IR (KBr) 3340, 2940, 1775, 1685, 1610, 1510, 1485, 1230, and 1110 cm^{-1}; ^1H NMR (MeOD) δ 6.99 (s, 1H, 5-H), 6.63 (s, 1H, 8-H), 6.25 (s, 2H, 2′,6′-H), 6.03 (s, 2H, OCH_2O), 4.72 (s, 2H, OCH_2O), 4.72 (d, J = 4.4 Hz, 1H, 1-H), 4.57 (m, 1H, 11-H), 4.50–4.00 (m, 5H, 11-H, 4-H, 4″-H, CH_2CH_3), 3.72 (s, 6H, 3′,5′-OCH_3), 3.36 (dd, J = 5.3, 14.9 Hz, 1H, 2-H), 3.23 (m, 1H, 3-H), 3.50–1.50 (m, 8H, 2″,3″,5″,6″-H), 1.27 (t, J = 7.1 Hz, 3H, CH_2CH_3). Anal. ($C_{29}H_{36}O_9N_2Cl \cdot {}^1/_2H_2O$) C, H, N.

Synthesis of 4′-*O*-Demethyl-4β-(arylamino)-4-desoxypodophyllotoxins 17 and 19. A solution containing 4′-*O*-demethyl-4β-bromo-4-desoxypodophyllotoxin (4) (0,5 g, 1.08 mmol), anhydrous barium carbonate (0.43 g, 2.16 mmol), and the appropriate arylamine (2.16 mmol) in 15 mL of dry dichloromethane under nitrogen was stirred overnight at room temperature. The reaction mixture was filtered, diluted with ethyl acetate, washed with water, dried over anhydrous magnesium sulfate, and purified via silica gel column chromatography using dichloromethane–acetone–ethyl acetate (100:5:5) as an eluent.

4′-*O*-Demethyl-4β-(4″-carboxyanilino)-4-desoxypodophyllotoxin (17): yield 23.2%; crystals from acetone; mp 205–207 °C; IR (KBr) 3380, 2905, 1765, 1700, 1605, 1520, 1480, and 1225 cm^{-1}; ^1H NMR ($CDCl_3$–acetone-d_6) δ 7.92 (d, J = 8.5 Hz, 2H, 3″,5″-H), 6.79 (s, 1H, 5-H), 6.60 (d, J = 8.7 Hz, 2H, 2″,6″-H), 6.53 (s, 1H, 8-H), 6.34 (s, 2H, 2′,6′-H), 5.97 (s, 1H, OCH_2O), 5.96 (s, 1H, OCH_2O), 5.89 (br, 1H, 4′-OH), 4.95 (d, J = 6.9 Hz, 1H, 4-H), 4.60 (d, J = 4.8 Hz, 1H, 1-H), 4.38 (m, 1H, 11-H), 3.93 (m, 1H, 11H), 3.79 (s, 6H, OCH_3), 3.19 (dd, J = 4.9, 14.1 Hz, 1H, 2-H), 3.07 (m, 1H, 3-H). Anal. ($C_{28}H_{25}O_9N$) C, H, N.

4′-*O*-Demethyl-4β-[4″-[(diethyl L-glutamat-*N*-carbonyl]-anilino]-4-desoxypodophyllotoxin (19): yield 69.4%; crystals from CH_2Cl_2–toluene; mp 137–139 °C; IR (KBr) 3380, 2990, 1775, 1735, 1610, 1510, 1485, 1230, and 1110 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.72 (d, J = 8.5 Hz, 2H, 3″,5″-H), 6.86 (d, J = 7.5 Hz, 1H, amide-H), 6.76 (s, 1H, 5-H), 6.56 (d, J = 8.6 Hz, 2H, 2″,6″-H), 6.54 (s, 1H, 8-H), 6.33 (s, 2H, 2′,6′-H), 5.98 (s, 1H, OCH_2O), 5.96 (s, 1H, OCH_2O), 5.44 (s, 1H, 4′-OH), 4.77 (m, 2H, 4-H and glutamyl-α-C·H), 4.61 (d, J = 4.3 Hz, 1H, 1-H), 4.37 (m, 1H, 11-H), 4.23 (m, 3H, 4β-NH and Et-CH_2), 4.11 (q, J = 7.1 Hz, 2H, EtCH_2), 3.92 (m, 1H, 11H), 3.79 (s, 6H, OCH_3), 3.11 (dd, J = 4.5, 14.0 Hz, 1H, 2-H), 3.05 (m, 1H, 1″-H), 2.60–2.00 (m, 4H, glutamyl-CH_2CH_2), 1.31 (t, J = 7.0 Hz, 3H, EtCH_3), 1.23 (t, J = 7.2 Hz, 3H, Et-CH_3). Anal. ($C_{37}H_{40}O_{12}N_2$) C, H, N.

4′-*O*-Demethyl-4β-(4″-carboxyanilino)-4-desoxypodophyllotoxin Sodium Salt (18). 4′-*O*-Demethyl-4β-(4″-carboxyanilino)-4-desoxypodophyllotoxin (17) (20 mg, 0.0385 mmol) was added to 3.10 mL of 0.0123 N NaOH–MeOH solution (0.0381 mmol), and the mixture was stirred at room temperature for 5 h. Water (3.1 mL) was then added to the reaction mixture. The resulting solution (50% MeOH–H_2O solution) was applied to a Sephadex LH20 column. The desired product (15 mg, yield 72%) was obtained using 50% MeOH–H_2O as eluent: amorphous powder from MeOH; mp 246–248 °C; IR (KBr) 3380, 2905, 1765, 1605, 1515, 1480, and 1225 cm^{-1}; ^1H NMR (MeOD) δ 7.81 (d, J = 8.7 Hz, 2H, 3″,5″-H), 6.76 (s, 1H, 5-H), 6.63 (d, J = 8.8 Hz, 2H, 2″,6″-H), 6.49 (s, 1H, 8-H), 6.37 (s, 2H, 2′,6′-H), 5.92 (s, 2H, OCH_2O), 4.91 (d, J = 4.1 Hz, 1H, 1-H), 4.58 (d, J = 5.0 Hz, 1H, 4-H), 4.41 (m, 1H, 11-H), 3.88 (m, 1H, 11H), 3.74 (s, 6H, OCH_3), 3.31 (m, 1H, 2-H), 3.11 (m, 1H, 3-H). Anal. ($C_{28}H_{24}O_9NNa \cdot 1.5H_2O$) 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.[7]

The *in vitro* human tumor cell line assay was performed by the National Cancer Institute.[19,20] Briefly, a total of 54 human tumor cell lines derived from eight cancer types (leukemia, non-small cell lung cancer, small cell lung cancer, colon cancer, CNS cancer, melanoma, ovarian cancer, and renal cancer) were used in this assay. Compounds were tested in different concentrations against every cell line. All lines are inoculated onto a series of standard 96-well microtitre plates on day 0, in the majority of cases at 20 000 cells/well, and then preincubated in absence of testing agent for 24 h. A testing agent was then added in five 10-fold dilutions and incubated for a further 48 h. Following this, the cells are fixed *in situ*, washed, and dried. Sulforhodamine B (SRB, protein binding dye) is added, followed by further washing and drying of the stained adherent cell mass. The bound stain is solubilized and measured spectrophotometrically on an automatic plate reader.

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References

(1) Lee, K. H.; Sun, L.; Wang, H. K. Antitumor Agents 147. Antineoplastic Alkaloids from Chinese Medicinal Plants. *Abstr. Chinese Med.*, submitted.

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

(3) Levy, R. K.; Hall, I. H.; Lee, K. H. Antitumor Agents LXII: Synthesis and Biological Evaluation of Podophyllotoxin Esters and Related Derivatives. *J. Pharm. Sci.* **1983**, *72*, 1158–1161.

(4) Thurston, L. S.; Irie, H.; Tani, S.; Han, F. S.; Liu, Z. C.; Cheng, Y. C.; Lee, K. H. Antitumor Agents. 78. Inhibition of Human DNA Topoisomerase II by Podophyllotoxin and α-Peltatin Analogues. *J. Med. Chem.* **1986**, *29*, 1547–1550.

(5) Beers, S. A.; Imakura, Y.; Dai, H. J.; Li, D. H.; Cheng, Y. C.; Lee, K. H. Antitumor Agents, 99. Synthetic Ring C Aromatized Podophyllotoxin Analogues as Potential Inhibitors of Human DNA Topoisomerase II. *J. Nat. Prod.* **1988**, *51*, 901–905.

(6) Thurston, L. S.; Imakura, Y.; Haruna, M.; Li, D. H.; Liu, Z. C.; Liu, S. Y.; Cheng, Y. C.; Lee, K. H. Antitumor Agents. 100. Inhibition of Human Topoisomerase II by Cytotoxic Ether and Ester Derivatives of Podophyllotoxin and α-Peltatin. *J. Med. Chem.* **1989**, *32*, 604–608.

(7) Lee, K. H.; Imakura, Y.; Haurna, M.; Beers, S. A.; Thurston, L. S.; Dai, H. J.; Chen, C. H. Antitumor Agent 107. New Cytotoxic 4-Alkylamino Analogues of 4′-Demethyl-epipodophyllotoxin as Inhibitors of Human DNA Topoisomerase II. *J. Nat. Prod.* **1989**, *52*, 606–613.

(8) 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.

(9) 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.

(10) Zhou, X. M.; Wang, Z. Q.; Chang, J. Y.; Chen, H. X.; Cheng, Y. C.; Lee, K. H. Antitumor Agents. 120. New 4-Substituted Benzylamine and Benzyl Ether Derivatives of 4′-*O*-Demethylepipodophyllotoxin as Potent Inhibitors of Human DNA Topoisomerase II. *J. Med. Chem.* **1991**, *34*, 3346–3350.

(11) 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.* **1992**, *35*, 866–871.

(12) Wang, Z. Q.; Hu, H.; Chen, H. X.; Cheng, Y. C.; Lee, K. H. 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. *J. Med. Chem.* **1992**, *35*, 871–877.

(13) Wang, Z. Q.; Shen, Y. C.; Chen, H. X.; Chang, J. Y.; Guo, X.; Cheng, Y. C.; Lee, K. H. Antitumor Agents 126. Novel 4β-Substituted Anilino Derivatives of 3′,4′-*O*,*O*-Didemethylpodophyllotoxin as Potent Inhibitors of Human DNA Topoisomerase II. *Pharm. Res.* **1993**, *10*, 343–350.

(14) Liu, S. Y.; Hwang, B. D.; Haruna, M.; Imakura, Y.; Lee, K. H.; Cheng, Y. C. Podophyllotoxin Analogs: Effects on DNA Topoisomerase II, Tubulin Polymerization, Human Tumor KB Cells, and Their VP-16-Resistant Variants. *Mol. Pharmacol.* **1989**, *36*, 78–82.

(15) Chang, J. Y.; Han, F. S.; Liu, S. Y.; Wang, Z. Q.; Lee, K. H.; Cheng, Y. C. Effect of 4β-Arylamino Derivatives of 4′-*O*-Demethylepipodophyllotoxin on Human DNA Topoisomerase II, Tubulin Polymerization, KB Cells, and Their Resistant Variants. *Cancer Res.* **1991**, *51*, 1755–1759.

(16) Clark, P. I.; Slevin, M. L. The Clinical Pharmacology of Etoposide and Teniposide. *Clin. Pharmacokinet.* **1987**, *12*, 223–252.

(17) Woynarowski, J. M.; Sigmund, R. D.; Beerman, T. A. DNA Minor Groove Binding Agents Interfere with Topoisomerase II Mediated Lesions Induced by Epipodophyllotoxin Derivative VM-26 and Acridine Derivative m-AMSA in Nuclei from L1210 Cells. *Biochemistry* **1989**, *28*, 3850–3855.

(18) MacDonald, T. L.; Lehnert, E. K.; Loper, J. T.; Chow, K. C.; Ross, W. E. On the Mechanism of Interaction of DNA Topoisomerase II with Chemotherapeutic Agents. In *DNA Topoisomerases in Cancer*; Potmesil, M., Kohn, K. W., Eds., Oxford University Press: New York, 1991; pp 119–214.

(19) Ferguson, P. J.; Fisher, M. H.; Stephenson, J.; Li, D. H.; Zhou, B. S.; Cheng, Y. C. Combined Modilities of Resistance in Etoposide-resistant Human KB Cell Lines. *Cancer Res.* **1988**, *8*, 5956–5964.

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