Antitumor Agents. 125.[1] New 4β-Benzoylamino Derivatives of 4′-*O*-Demethyl-4-desoxypodophyllotoxin and 4β-Benzoyl Derivatives of 4′-*O*-Demethylpodophyllotoxin as Potent Inhibitors of Human DNA Topoisomerase II

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A series of 4β-benzoylamino (**5–17**) derivatives of 4′-*O*-demethyl-4-desoxypodophyllotoxin and 4β-benzoyl (**18–20**) derivatives of 4′-*O*-demethyl podophyllotoxin 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 **5–13** and **15–17** are more potent than etoposide in causing DNA breakage, while compounds **9**, **10**, **13**, **14**, **16**, and **20** are more active than etoposide in their inhibition of the human DNA topoisomerase II. The order for the enzyme inhibitory activity of the derivatives of 4′-*O*-demethyl-4-desoxypodophyllotoxin is 4β-arylamino > 4β-benzylamino > 4β-benzoylamino.

KEY WORDS: etoposide; DNA topoisomerase II; amino analogues of etoposide; KB cells; cytotoxicity.

INTRODUCTION

Etoposide (VP-16; **1**) (Fig. 1) is useful in the treatment of a variety of cancers, including small cell lung cancer, testicular cancer, lymphoma, and leukemia. In combination chemotherapy, it has been used successfully with cisplatin and several other antitumor drugs including cyclophosphamide, doxorubicin, and vincristine (2–4). Although it is widely used in the clinic, the use of **1** as a single agent has not proven curative in patients with germinal neoplasms. Prior treatment seems to be an important factor in determining the therapeutic outcome of VP-16-containing chemotherapy regimens (5,6). VP-16 is poorly soluble in water and has limited stability in physiological solution. It also becomes important to overcome the development of myelosuppression and resistance to **1** (4,6,7). All of these considerations have encouraged further synthesis of analogues of **1** with better pharmacological profiles.

It was reported that some amide derivatives of 4′-*O*-demethyl-4-desoxypodophyllotoxin (**5–15**; where *R* = P-NH-4,6-*O*-ethylideneglucopyranosyl) demonstrated a comparable antileukemic activity against L-1210 lymphocytic leukemia *in vivo* and less toxicity compared to **1** (8). Previously, we have demonstrated that some of the 4β-amino analogues, such as W-68 and NPF, are 40-fold more cytotoxic than **1** against P-388/adriamycin resistant cells (P-388/adr), which overexpressed MDR1 and decreased the content of topoisomerase II, whereas they exhibited almost the same activity against P-388 cells. In addition, NPF was found to be at least seven times less toxic *in vivo* compared to **1** (9,10). Since these 4β amino analogues possess potent activity in inhibiting DNA topoisomerase II and in causing cellular protein-linked DNA breakage (11–13), we have synthesized and evaluated a series of 4β-benzoylamino derivatives of 4′-*O*-demethyl-4-desoxypodophyllotoxin and 4β-benzoyl derivatives of 4′-*O*-demethylpodophyllotoxin as inhibitors of human DNA topoisomerase II and as part of our ongoing program aimed at elucidating the structure–activity relationships among these 4β-amino analogues of **1**. The enzyme inhibitory activity of the amide derivatives of 4′-*O*-demethyl-4-desoxypodophyllotoxin mentioned above has not yet been reported. The DNA topoisomerase II has been proposed as a target enzyme of **1**, as **1** inhibits the catalytic activity of DNA topoisomerase II by stabilizing a cleavable enzyme–DNA complex, in which DNA is cleaved and covalently linked to the enzyme (14–17).

MATERIALS AND METHODS

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 using a Bruker AC-300 NMR spectrometer. All chemical shifts are reported as parts per million 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 60F-25 EM Kieselgel 60 (230- to 400-mesh ASTM). All new compounds were characterized by melting point, optical rotation, ^{1}H NMR, and IR spectra as well as elemental analyses.

Synthesis of Compounds 5–15

To a solution of an appropriate substituted benzoic acid (0.25 mmol) in THF (3 mL) was added DCC (57 mg, 0.28 mmol). After 10 min, compound **2** (100 mg, 0.25 mmol) was added to the mixture, and the mixture was stirred at room temperature overnight. The mixture was filtered and the filtrate was evaporated to give a crude product of **5–15**. This product was purified by preparative TLC (chloroform:ethyl acetate:acetone:methanol = 100:5:5:5).

4′-O-Demethyl-4β-(benzoylamino)-4-desoxypodophyllotoxin (5). Yield, 73%; m.p., 213–214°C; crystals from chloroform–ethyl acetate; $[\alpha]^{25}_D$-68° (*c* = 0.25, acetone). IR (KBr) ν_{max}: 3500, 3300, 2910, 1750, 1720, 1610, 1500, and 1470 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 7.78 (2H, d, *J* = 7.4 Hz, H-2″,6″), 7.57–7.45 (3H, m, H-3″,4″,5″), 6.83 (1H, s, H-5), 6.57 (1H, s, H-8), 6.33 (2H, s, H-2′,6′), 6.27 (1H, d, *J* = 6.8

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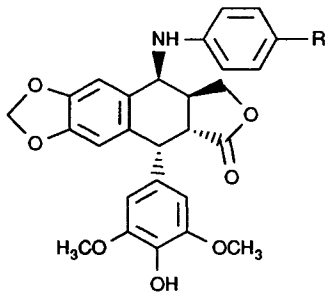


W-68, R = NO_2

NPF, R = F

Fig. 1. Etoposide (VP-16; 1).

Hz, H-4), 6.01 and 5.99 (2H, s and s, OCH_2O), 5.45 (2H, brs, NH and OH-4'), 4.64 (1H, d, J = 4.3 Hz, H-1), 4.51 (1H, t, J = 9.2 Hz, H-11), 3.92 (1H, t, J = 9.2 Hz, H-11), 3.80 (6H, s, OCH_3-3',5'), 3.06 (1H, m, H-3), and 2.93 (1H, dd, J = 14.2, 4.8 Hz, H-2). *Anal.* Calcd for $C_{28}H_{25}NO_8 \cdot \frac{1}{2}H_2O$: C, 65.63; H, 5.08; N, 2.73. Found: C, 65.65; H, 5.23; N, 2.71.

4'-O-Demethyl-4β-[(2"-hydroxybenzoyl)amino]-4-desoxypodophyllotoxin (6). Yield, 61%; m.p. 172–174°C; crystals from chloroform; $[\alpha]^{25}_D$-69° (c = 0.25, acetone). IR (KBr) ν_{max}: 3490, 3350, 3120, 2905, 1760, 1630, 1590, 1550, and 1470 cm^{-1}. ^1H NMR ($CDCl_3$) δ 7.45 (1H, t, J = 7.5 Hz, H-4"), 7.35 (1H, d, J = 7.5 Hz, H-6"), 7.04 (1H, d, J = 7.5 Hz, H-3"), 6.88 (1H, t, J = 7.5 Hz, H-5"), 6.82 (1H, s, H-5), 6.58 (1H, s, H-8), 6.47 (1H, d, J = 6.7 Hz, H-4), 6.40 (2H, s, H-2',6'), 6.01 and 6.00 (2H, s and s, OCH_2O), 5.44 (2H, brs, NH and OH-4'), 4.64 (1H, d, J = 4.9 Hz, H-1), 4.49 (1H, t, H-11), 3.87 (1H, t, H-11), 3.76 (6H, s, OCH_3-3',5'), 3.05 (1H, m, H-3), and 2.96 (1H, dd, J = 14.3, 4.9 Hz, H-2). *Anal.* Calcd for $C_{28}H_{25}NO_9 \cdot 1\frac{1}{2}H_2O$: C, 61.53; H, 5.13; N, 2.56. Found: C, 61.76; H, 5.28; N, 2.56.

4'-O-Demethyl-4β-[(2"-fluorobenzoyl)amino]-4-desoxypodophyllotoxin (7). Yield, 71%; m.p., 163–164°C; crystals from ethyl acetate–hexane; $[\alpha]^{25}_D$-58° (c = 0.25, acetone). IR (KBr) ν_{max}: 3310, 3120, 2910, 1760, 1640, 1600, 1500, and 1470 cm^{-1}. ^1H NMR ($CDCl_3$) δ 8.10 (1H, m, H-4"), 7.43 (1H, m, H-6"), 7.33 (1H, m, H-3"), 7.14 (1H, m, H-5"), 6.85 (2H, m, H-4,5), 6.57 (1H, s, H-8), 6.34 (2H, s, H-2',6'), 6.01 and 5.99 (2H, s and s, OCH_2O), 5.45 (2H, brs, NH and OH), 4.65 (1H, d, J = 4.7 Hz, H-1), 4.50 (1H, t, H-11), 3.92 (1H, t, H-11), 3.81 (6H, s, OCH_3-3',5'), 3.00 (1H, m, H-3), and 2.95 (1H, dd, J = 14.1, 4.9 Hz, H-2). *Anal.* Calcd for $C_{28}H_{24}NO_9$: C, 64.49; H, 4.61; N, 2.69. Found: C, 64.10; H, 4.99; N, 3.00.

4'-O-Demethyl-4β-[(3"-fluorobenzoyl)amino]-4-desoxypodophyllotoxin (8). Yield, 68%; m.p., 186–188°C; crystals from chloroform–ethyl acetate; $[\alpha]^{25}_D$ −62° (c = 0.25, acetone). IR (KBr) ν_{max}: 3490, 3300, 2900, 1750, 1630, 1570, 1500, and 1460 cm^{-1}. ^1H NMR ($CDCl_3$) δ 7.46 (3H, m, H-2",4",6"), 7.27 (1H, m, H-5"), 6.82 (1H, s, H-5), 6.57 (1H, s, H-8), 6.33 (3H, m, H-2',6' and H-4), 6.01 and 5.99 (2H, s and s, OCH_2O), 5.50 (2H, brs, NH and OH-4'), 4.63 (1H, d, J = 5.7 Hz, H-1), 4.51 (1H, t, H-11), 3.89 (1H, m, H-11), 3.80 (6H, s, OCH_3-3',5'), 3.05 (1H, m, H-3), and 2.93 (1H, dd, J = 14.3, 4.8 Hz, H-2). *Anal.* Calcd for $C_{28}H_{24}NFO_8 \cdot \frac{1}{2}H_2O$: C, 63.39; H, 4.71; N, 2.64. Found: C, 63.01; H, 4.72; N, 2.92.

4'-O-Demethyl-4β-[(4"-fluorobenzoyl)amino]-4-desoxypodophyllotoxin (9). Yield, 69%; m.p., 242–244°C; crystals from chloroform–ethyl acetate; $[\alpha]^{25}_D$-78° (c = 0.25, acetone). IR (KBr) ν_{max}: 3410, 3120, 2910, 1760, 1630, 1590, 1510, and 1480 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.80 (2H, m, H-2",6"), 7.16 (2H, m, H-3",5"), 6.82 (1H, s, H-5), 6.57 (1H, s, H-8), 6.33 (2H, s, H-2',6'), 6.25 (1H, d, J = 6.8 Hz, H-4), 6.01 and 6.00 (2H, s and s, OCH_2O), 5.43 (2H, brs, NH and OH-4'), 4.63 (1H, d, J = 4.7 Hz, H-1), 4.50 (1H, t, H-11), 3.87 (1H, m, H-11), 3.80 (6H, s, OCH_3-3',5'), 3.04 (1H, m, H-3), and 2.93 (1H, dd, J = 14.2, 4.7 Hz, H-2). *Anal.* Calcd for $C_{28}H_{24}NFO_8$: C, 64.49; H, 4.61; N, 2.69. Found: C, 64.31; H, 5.09.

4'-O-Demethyl-4β-[(4"-acetoxybenzoyl)amino]-4-desoxypodophyllotoxin (10). Yield, 51%; m.p., 175–176°C; crystals from ethyl acetate–hexane; $[\alpha]^{25}_D$-61° (c = 0.25, acetone). IR (KBr) ν_{max}: 3350, 3100, 2980, 1760, 1740, 1620, 1590, 1505, and 1470 cm^{-1}. ^1H NMR ($CDCl_3$) δ 8.12 (2H, d, J = 8.7 Hz, H-2",6"), 7.19 (2H, d, J = 8.7 Hz, H-3",5"), 6.82 (1H, s, H-5), 6.57 (1H, s, H-8), 6.33 (2H, s, H-2',6'), 6.25 (1H, d, J = 6.8 Hz, H-4), 6.01 and 6.00 (2H, s and s, OCH_2O), 5.43 (2H, s, NH and OH-4'), 4.63 (1H, d, J = 4.7 Hz, H-1), 4.50 (1H, q, H-11), 3.86 (1H, m, H-11), 3.76 (6H, s, OCH_3-3',5'), 3.04 (1H, m, H-3), 2.96 (1H, dd, J = 14.3, 4.7 Hz, H-2), and 2.33 (3H, s, CH_3CO). *Anal.* Calcd for $C_{30}H_{27}NO_{10}$: C, 64.17; H, 4.81; N, 2.50. Found: C, 64.01; H, 4.99; N, 2.44.

4'-O-Demethyl-4β-[(4"-acetylbenzoyl)amino]-4-desoxypodophyllotoxin (11). Yield, 70%; m.p., 178–180°C (dec); crystals from ethyl acetate–hexane; $[\alpha]^{25}_D$-68° (c = 0.25, acetone). IR (KBr) ν_{max}: 3500, 3350, 2920, 1770, 1680, 1640, 1600, 1520, and 1480 cm^{-1}. ^1H NMR ($CDCl_3$) δ 8.05 (2H, d, J = 8.2 Hz, H-3",5"), 7.87 (2H, d, J = 8.2 Hz, H-2",6"), 6.83 (1H, s, H-5), 6.58 (1H, s, H-8), 6.36 (3H, m, H-2',6' and H-4), 6.02 and 6.00 (2H, s and s, OCH_2O), 5.45 (2H, s, NH and OH-4'), 4.64 (1H, d, J = 4.8 Hz, H-1), 4.51 (1H, t, H-11), 3.89 (1H, m, H-11), 3.73 (6H, s, OCH_3-3',5'), 3.06 (1H, m, H-3), 2.94 (1H, dd, J = 14.2, 4.8 Hz, H-2), and 2.66 (3H, s, CH_3CO). *Anal.* Calcd for $C_{30}H_{27}NO_9 \cdot \frac{1}{2}H_2O$: C, 64.98; H, 5.05; N, 2.53. Found: C, 64.95; H, 5.09; N, 2.75.

4'-O-Demethyl-4β-[(3"-cyanobenzoyl)amino]-4-desoxypodophyllotoxin (12). Yield, 68%; m.p., 190–192°C; crystals from ethyl acetate–hexane; $[\alpha]^{25}_D$-62° (c = 0.25, acetone). IR (KBr) ν_{max}: 3300, 3100, 2910, 2200, 1760, 1640, 1590, 1500, and 1470 cm^{-1}. ^1H NMR ($CDCl_3$) δ 8.06 (2H, m, H-2",6"), 7.84 (1H, d, J = 7.5 Hz, H-4"), 7.62 (1H, t, J = 7.5 Hz, H-5"), 6.82 (1H, s, H-5), 6.58 (1H, s, H-8), 6.38 (1H, d, J = 6.7 Hz, H-4), 6.33 (2H, s, H-2',6'), 6.02 and 6.00 (2H, s and s, OCH_2O), 5.45 (2H, brs, NH and OH-4'), 4.64 (1H, d, J = 4.8 Hz, H-1), 4.50 (1H, t, H-11), 3.87 (1H, t, H-11), 3.80 (6H, s, OCH_3-3',5'), 3.07 (1H, m, H-3), and 2.94 (1H, dd, J = 14.3, 4.9 Hz, H-2). *Anal.* Calcd for $C_{29}H_{24}N_2O_8 \cdot \frac{1}{2}H_2O$: C, 64.81; H, 4.66; N, 5.21. Found: C, 64.89; H, 4.72; N, 5.21.

4'-O-Demethyl-4β-[(4"-cyanobenzoyl)amino]-4-desoxypodophyllotoxin (13). Yield, 73%; m.p., 198–202°C; crystals from ethyl acetate–chloroform; $[\alpha]^{25}_D$-64° (c = 0.25, acetone). IR (KBr) ν_{max}: 3320, 3100, 2980, 2200, 1760, 1640, 1600, 1500, and 1470 cm^{-1}. ^1H NMR ($CDCl_3$) δ 7.89 (2H, d, J = 8.5 Hz, H-3",5"), 7.77 (2H, d, J = 8.5 Hz, H-2",6"), 6.81

(1H, s, H-5), 6.58 (1H, s, H-8), 6.33 (3H, m, H-2′,6′ and H-4), 6.02 and 6.00 (2H, s and s, OCH_2O), 5.44 (2H, s, NH and OH-4′), 4.64 (1H, d, J = 5.0 Hz, H-1), 4.50 (1H, q, H-11), 3.83 (1H, q, H-11), 3.80 (6H, s, OCH_3-3′,5′), 3.06 (1H, m, H-3), and 2.91 (1H, dd, J = 14.3, 5.0 Hz, H-2). *Anal.* Calcd for $C_{29}H_{24}N_2O_8 \cdot \frac{1}{2}H_2O$: C, 64.81; H, 4.66; N, 5.21. Found: C, 64.77; H, 4.71; N, 5.21.

4′-O-Demethyl-4β-[(3″-nitrobenzoyl)amino]-4-desoxypodophyllotoxin (14). Yield, 80%; m.p., 194–195°C; crystals from ethyl acetate–chloroform; $[\alpha]^{25}_D$-46° (c = 0.25, acetone). IR (KBr) ν_{max}: 3350, 3120, 1750, 1640, 1600, 1510, and 1470 cm^{-1}. ^1H NMR ($CDCl_3$) δ 8.58 (1H, s, H-2″), 8.38 (1H, d, J = 7.4 Hz, H-4″), 8.21 (1H, d, J = 7.5 Hz, H-6″), 7.26 (1H, t, H-5″), 6.82 (1H, s, H-5), 6.54 (2H, m, H-4,8), 6.32 (2H, s, H-2′,6′), 6.00 (2H, s, OCH_2O), 5.45 (2H, brs, NH and OH-4′), 4.62 (1H, d, J = 4.7 Hz, H-1), 4.49 (1H, t, H-11), 3.87 (1H, t, H-11), 3.80 (6H, s, OCH_3-3′,5′), 3.08 (1H, m, H-3), and 2.96 (1H, dd, J = 14.4, 4.7 Hz, H-2). *Anal.* Calcd for $C_{28}H_{24}N_2O_{10} \cdot \frac{1}{2}H_2O$: C, 60.32; H, 4.76; N, 5.03. Found: C, 60.71; H, 4.51; N, 4.96.

4′-O-Demethyl-4β-[(4″-nitrobenzoyl)amino]-4-desoxypodophyllotoxin (15). Yield, 69%; m.p., 204–206°C; crystals from ethyl acetate–chloroform; $[\alpha]^{25}_D$-52° (c = 0.25, acetone). IR (KBr) ν_{max}: 3450, 3150, 2980, 1760, 1640, 1600, 1540, and 1470 cm^{-1}. ^1H NMR ($CDCl_3$) δ 8.31 (2H, d, J = 8.7 Hz, H-3″,5″), 7.97 (2H, d, J = 8.7 Hz, H-2″,6″), 6.83 (1H, s, H-5), 6.58 (1H, s, H-8), 6.56 (1H, d, J = 6.5 Hz, H-4), 6.32 (2H, s, H-2′,6′), 6.02 and 5.99 (1H, s and s, OCH_2O), 5.45 (2H, brs, NH and OH-4′), 4.63 (1H, d, J = 4.8 Hz, H-1), 4.51 (1H, t, H-11), 3.88 (1H, t, H-11), 3.80 (6H, s, OCH_3-3′,5′), 3.05 (1H, m, H-3), and 2.95 (1H, dd, J = 14.3, 5.0 Hz, H-2). *Anal.* Calcd for $C_{28}H_{24}N_2O_{10} \cdot \frac{1}{2}H_2O$: C, 60.32; H, 4.76; N, 5.03. Found: C, 60.33; H, 4.78; N, 4.76.

Synthesis of Compounds 16 and 17

To a solution of **14** or **15** (25 mg, 0.05 mmol) in ethyl acetate (3.0 mL) was added 10% palladium on activated carbon (3.0 mg). The reaction mixture was stirred under hydrogen for 2 hr at room temperature under atmospheric pressure. The catalyst was filtered off and the filtrate was evaporated to give a crude product. The crude product was purified by preparative TLC (chloroform:acetone:ethyl acetate:methanol = 100:5:5:3) to yield **16** or **17**, respectively.

4′-O-Demethyl-4β-[(3′-aminobenzoyl)amino]-4-desoxypodophyllotoxin (16). Yield, 95%; m.p., 180–182°C; crystals from ethyl acetate–chloroform; $[\alpha]^{25}_D$-53° (c = 0.25, acetone). IR (KBr) ν_{max}: 3360, 3120, 2920, 1760, 1640, 1600, 1570, and 1460 cm^{-1}. ^1H NMR ($CDCl_3$) δ 7.20 (1H, t, J = 7.7 Hz, H-5″), 7.11 (1H, s, H-2″), 7.02 (1H, d, J = 7.7 Hz, H-6″), 6.82 (2H, m, H-4″ and H-5), 6.55 (1H, s, H-8), 6.32 (2H, s, H-2′,6′), 5.98 (1H, d, J = 5.1 Hz, H-4), 5.99 and 5.97 (2H, s and s, OCH_2O), 5.40 (2H, brs, NH and OH-4′), 4.61 (1H, d, J = 4.6 Hz, H-1), 4.48 (1H, t, H-11), 3.85 (1H, m, H-11), 3.79 (6H, s, OCH_3-3′,5′), 3.01 (1H, m, H-3), and 2.90 (1H, dd, J = 14.3, 4.6 Hz, H-2). *Anal.* Calcd for $C_{28}H_{26}N_2O_8$: C, 64.86; H, 5.02; N, 5.41. Found: C, 64.73; H, 5.24; N, 5.25.

4′-O-Demethyl-4β-[(4″-aminobenzoyl)amino]-4-desoxypodophyllotoxin (17). Yield, 69%; m.p., 204–206°C; crystals from ethyl acetate–chloroform; $[\alpha]^{25}_D$-56° (c = 0.25, acetone). IR (KBr) ν_{max}: 3450, 3350, 3150, 2960, 1760, 1630, 1600, 1500, and 1470 cm^{-1}. ^1H NMR ($CDCl_3$) δ 7.60 (2H, d, J = 8.5 Hz, H-2″,6″), 6.82 (1H, s, H-5), 6.67 (2H, d, J = 8.5 Hz, H-3″,5″), 6.33 (2H, s, H-2′,6′), 6.10 (1H, d, J = 6.8 Hz, H-4), 6.00 and 5.98 (2H, s and s, OCH_2O), 5.43 (2H, brs, NH and OH-4′), 4.63 (1H, d, J = 4.6 Hz, H-1), 4.49 (1H, t, H-11), 3.91 (1H, t, H-11), 3.80 (6H, s, OCH_3-3′,5′), 3.03 (1H, m, H-3), and 2.93 (1H, dd, J = 14.2, 4.6 Hz, H-2). *Anal.* Calcd for $C_{28}H_{26}N_2O_8 \cdot \frac{1}{2}H_2O$: C, 63.76; H, 5.12; N, 5.31. Found: C, 64.02; H, 5.30; N, 5.12.

Synthesis of Compounds 18–20

To a solution of appropriate benzoic acids (0.34 mmol) in pyridine was added phenyl sulfonyl chloride (72 μL, 0.56 mmol) at 0°C. After 10 min the cooling bath was removed and the temperature was raised to 20–25°C. 4′-*O*-Demethyl-4-*O*-carbobenzoxyepipodophyllotoxin (**3**) (150 mg, 0.28 mmol) was added to the reaction mixture. After the mixture was stirred overnight, it was poured into ice water, extracted with dichloromethane, dried (Na_2SO_4), and evaporated. The resulting product was purified by preparative TLC (chloroform:acetone:ethyl acetate = 100:5:5) to give 4′-*O*-demethyl-4′-*O*-CBZ protected **18–20**, respectively. These compounds were then subjected to catalytic hydrogenation in 4 mL of ethyl acetate with 30 mg of palladium on activated carbon for 2 hr at room temperature to yield **18–20**, respectively.

4′-O-Demethyl-4β-benzoylpodophyllotoxin (18). Yield, 90%; m.p., 213–214°C; crystals from ethyl acetate–chloroform; $[\alpha]^{25}_D$-79° (c = 0.25, acetone). IR (KBr) ν_{max}: 3120, 1760, 1690, 1600, 1500, and 1470 cm^{-1}. ^1H NMR ($CDCl_3$) δ 8.03 (2H, d, J = 7.2 Hz, H-2″,6″), 7.60 (1H, t, J = 7.2 Hz, H-4″), 7.47 (2H, t, J = 7.2 Hz, H-3″,5″), 6.96 (1H, s, H-5), 6.60 (1H, s, H-8), 6.41 (1H, d, J = 3.5 Hz, H-4), 6.27 (2H, s, H-2′,6′), 6.01 and 5.97 (2H, s and s, OCH_2O), 5.43 (1H, s, OH-4′), 4.73 (1H, d, J = 4.9 Hz, H-1), 4.40 (1H, t, H-11), 3.98 (1H, t, H-11), 3.81 (6H, s, OCH_3-3′,5′), 3.36 (1H, dd, J = 14.1, 4.9 Hz, H-2), and 3.10 (1H, m, H-3). *Anal.* Calcd for $C_{28}H_{24}O_9$: C, 66.67; H, 4.76. Found: C, 66.67; H, 4.76.

4′-O-Demethyl-4β-(3″-aminobenzoyl)podophyllotoxin (19). Yield, 89%; m.p., 228–229°C; crystals from ethyl acetate–chloroform; $[\alpha]^{25}_D$-78° (c = 0.25, acetone). IR (KBr) ν_{max}: 3460, 3360, 2950, 1760, 1695, 1600, 1495, and 1470 cm^{-1}. ^1H NMR ($CDCl_3$) δ 7.40 (1H, d, J = 8.0 Hz, H-6″), 7.31 (1H, s, H-2″), 7.23 (1H, t, J = 8.0 Hz, H-5″), 6.95 (1H, s, H-5), 6.90 (1H, d, J = 8.0 Hz, H-4″), 6.59 (1H, s, H-8), 6.37 (1H, d, J = 3.6 Hz, H-4), 6.34 (2H, s, H-2′,6′), 6.01 and 5.97 (2H, s and s, OCH_2O), 5.45 (1H, s, OH-4′), 4.72 (1H, d, J = 5.0 Hz, H-1), 4.39 (1H, t, H-11), 3.97 (1H, t, H-11), 3.81 (6H, s, OCH_3-3′,5′), 3.34 (1H, dd, J = 14.1, 5.0 Hz, H-2), and 3.08 (1H, m, H-3). *Anal.* Calcd for $C_{28}H_{25}NO_9$: C, 64.74; H, 4.82; N, 2.70. Found: C, 64.58; H, 4.95; N, 2.65.

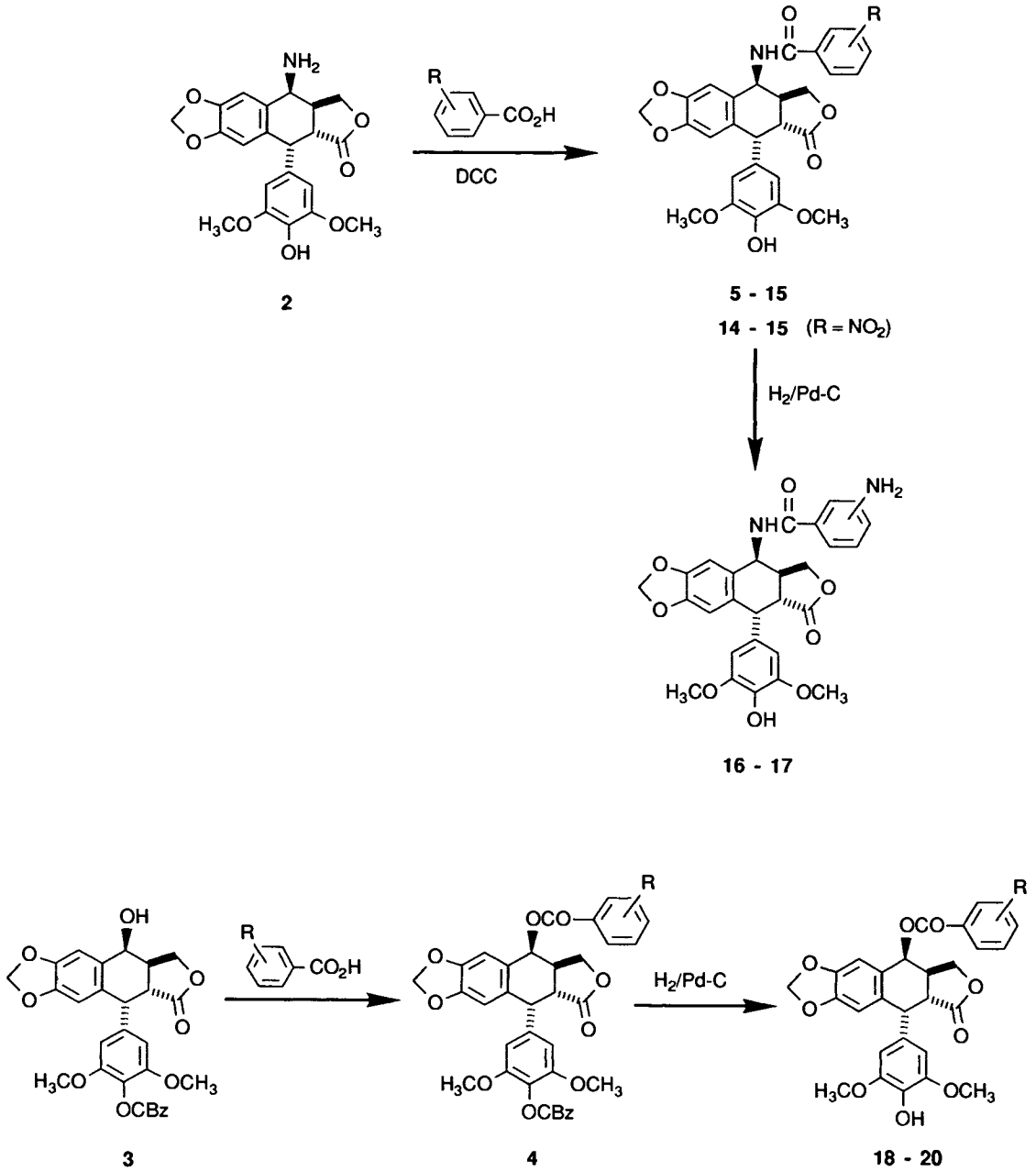
4′-O-Demethyl-4β-(4″-aminobenzoyl)podophyllotoxin (20). Yield, 84%; m.p., 228–229°C; crystals from ethyl acetate–chloroform; $[\alpha]^{25}_D$-62° (c = 0.25, acetone). IR (KBr) ν_{max}: 3460, 3120, 2940, 1760, 1660, 1590, 1500, and 1470 cm^{-1}. ^1H NMR ($CDCl_3$) δ 7.83 (2H, d, J = 8.5 Hz, H-2″,6″), 6.95 (1H, s, H-5), 6.64 (2H, d, J = 8.5 Hz, H-3″,5″), 6.58 (1H, s, H-8), 6.34 (3H, brs, H-2′,6′ and H-4), 6.00 and 5.97

(2H, s and s, OCH_2O), 5.44 (1H, s, OH-4'), 4.71 (1H, d, J = 4.9 Hz, H-1), 4.38 (1H, t, H-11), 3.97 (1H, t, H-11), 3.80 (6H, s, OCH_3-3',5'), 3.34 (1H, dd, J = 14.1, 4.9 Hz, H-2), and 3.06 (1H, m, H-3). *Anal.* Calcd for $C_{28}H_{25}NO_9 \cdot \frac{1}{3}H_2O$: C, 64.00; H, 4.89; N, 2.67. Found: C, 64.16; H, 5.01; N, 2.64.

RESULTS AND DISCUSSION

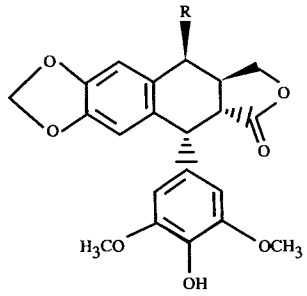
As shown in Scheme I, 4β-(substituted benzoylamino)-4'-*O*-demethyl-4-desoxypodophyllotoxins (**5–15**) were synthesized by amidation of the appropriate substituted benzoic acids with 4'-*O*-demethyl-4β-amino-4-desoxypodophyllotoxin (**2**), which was obtained from podophyllotoxin as described previously (13) (Table I). The yields in this synthesis are in the range of 61–73% calculated from **2**. In the presence of DCC, the acid selectively reacts with the amino group to form the amide rather than with a hydroxyl group to form the ester (18). Compounds **16** and **17** were prepared from their corresponding **14** and **15** by catalytic hydrogenation with Pd-C. Treatment of **3**, prepared previously from 4'-*O*-demethylepipodophyllotoxin (19), with a substituted benzoic acid in the presence of benzenesulfonyl chloride (20) afforded the ester (**4**). Removal of the phenolic protecting group of **4** by catalytic hydrogenation furnished **18**, **19**, and **20**. The yields in these syntheses are in the range of 84–90%, calculated from **3**.

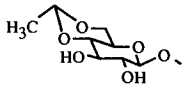
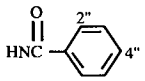
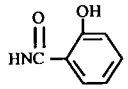
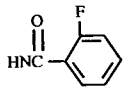
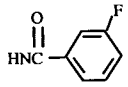
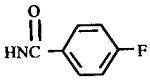
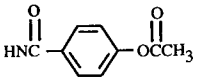
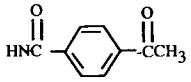
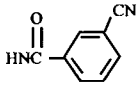
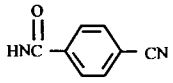
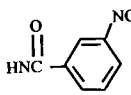
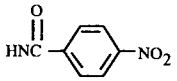
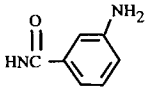
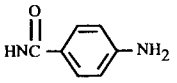
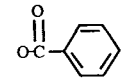
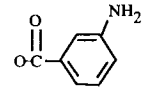
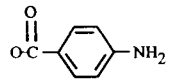
The selection of F, OH, $OCOCH_3$, $COCH_3$,CN,NO_2, and NH_2 as substituents of the amide or ester phenyl ring is



Scheme I

Table I. Biological Evaluation of 4β-(Substituted Benzoyl)-Amino-4′-*O*-demethyl-4-desoxypodophyllotoxins and 4β-(Substituted Benzoyl)-4′-*O*-Demethylpodophyllotoxins



Compound	*R*	Cytotoxicity, ID_{50} KB (μM)[a]	Inhibition of DNA toposomerase II activity, ID_{50} (μM)[b]	Cellular protein–DNA complex formation (%) 10 μM
1		0.20	50	100
5		1.27	>50	177
6		<1.92	50	160
7		2.50	100	128
8		1.92	>50	116
9		0.65	25	117
10		1.09	25	137
11		1.83	50	124
12		1.89	50	149
13		0.19	25	159
14		0.60	10	86
15		0.73	50	160
16		0.81	25	149
17		1.16	50	120
18		<0.20	50	94
19		1.56	50	100
20		1.19	25	94

[a] ID_{50} is the concentration of drug that affords 50% reduction in cell number after a 3-day incubation.

[b] Each compound was examined with five concentrations, 10, 20, 25, 50, and 100 μM. The ID_{50} value was established on the basis of the degree of inhibition at these five concentrations.

based on the fact that these phenyl substituents contribute to the enhanced inhibitory activity against DNA topoisomerase II and ability to cause protein-linked DNA breakage as observed in our previous studies on 4β-amino analogues (9–13). As illustrated in Table I, all 4β-substituted benzoyl amides and benzoyl esters (**5–20**) showed comparable or superior activity to **1** in inhibiting the human DNA topoisomerase II and in causing cellular protein–DNA strand breakage, except for **7**, in which its inhibitory activity against the enzyme is less than that of **1**. The most active compounds are **12**, **13**, **15**, and **16**. However, the enzyme inhibitory activity for these amides is less than that for their corresponding 4β-arylamino (12) and 4β-benzylamino compounds (13) which possess the same substituents. A comparison of the amides with the esters showed that the amides are more potent than their corresponding esters. This result is in agreement with our previous observation that the 4β-nitrogen substituted aryl compounds are, in general, more potent than their corresponding 4β-etheral derivatives in inhibiting the human DNA topoisomerase II and in causing cellular protein-linked DNA breakage (11–13). There is a lack of correlation among the ability of compounds in causing protein linked DNA breaks and in producing cytotoxicity (KB).

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