

Articles

Antitumor Agents. 120.[†] New 4-Substituted Benzylamine and Benzyl Ether Derivatives of 4'-O-Demethylepipodophyllotoxin as Potent Inhibitors of Human DNA Topoisomerase II

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A number of new 4'-O-demethylepipodophyllotoxin derivatives possessing various 4 β -N- or 4 β -O-benzyl groups have been synthesized and evaluated for their inhibitory activity against the human DNA topoisomerase II as well as for their activity in causing cellular protein-linked DNA breakage. The 4 β -N-benzyl derivatives 9–22 are, in general, as active or more active than etoposide (1). The most active compounds are 14, 16, and 17, which are more than 2-fold more potent than 1. The results indicated that a basic unsubstituted 4 β -benzylamino moiety is structurally required for the enhanced activity. Replacement of the benzyl nitrogen with oxygen gave compounds (23 and 24) which are inactive. The ability of these compounds to inhibit human DNA topoisomerase II and to cause protein-linked DNA breakage appears to have no direct correlation with cytotoxicity in KB cells.

Etoposide (VP-16, 1), a podophyllotoxin-derived glucoside, is an important drug used in the treatment of small-cell lung cancer, testicular carcinoma, leukemia, lymphoma, and Kaposi's sarcoma.^{2,3} Although 1 has been widely used in the clinic, the development of drug resistance, myelosuppression, and poor oral bioavailability^{4,5} has encouraged further synthesis of analogues related to 1 for better pharmacological profiles. Previous studies in these laboratories^{6–9} aimed at the development of amino analogues of 1 to overcome the aforementioned problems have yielded numerous compounds which can be easily converted to water-soluble products. In addition, many of these compounds, such as 6–8, are not only more potent than 1 in inhibiting the human DNA topoisomerase II and in causing the protein-linked DNA breakage but are also active against 1-resistant KB cells. As an extension to our studies among these amino analogues, such as the 4 β -arylamino analogues⁹ of 2, we report herein the structure-activity relationships of a series of 4 β -N- or 4 β -O-benzyl-substituted derivatives of 4'-O-demethylepipodophyllotoxin as inhibitors of human DNA topoisomerase II. The human DNA topoisomerase II has been shown to be a target enzyme of 1.^{10–13}

Chemistry

The preparation of 4 β -(unsubstituted benzylamino)- (9) and 4 β -(substituted benzylamino)-4'-O-demethyl-4-desoxypodophyllotoxins (10–20) started from 4'-O-demethylepipodophyllotoxin (2). The key intermediate, 4'-O-demethyl-4 β -azido-4-desoxypodophyllotoxin (3) was obtained in high yield by treating 2 with trifluoroacetic acid (TFA) and sodium azide¹⁴ (Scheme I). In this reaction, high stereoselectivity through the formation of a benzylic carbonium ion at C-4 to yield solely the 4 β -oriented secondary azide was achieved by use of TFA. Previously, the preparation of the 4 β -azide was found to

be complex, as it required three steps from 2 in low yield and generated considerable amounts of the 4 α -isomer. The azide 3 could simply be reduced with hydrogen in the presence of 10% Pd-C to afford 4'-O-demethyl-4 β -amino-4-desoxypodophyllotoxin (4) in good yield without producing the 4 α -isomer.

As shown in Scheme I, the preparation of 9–20 was achieved by treatment of 4 with benzyl iodide, prepared by treating benzyl bromide with NaI, to overcome the sluggish problem of reacting 4 with benzyl bromide as encountered before.⁹

Reduction of 11 and 12 with SnCl₂–H₂O gave 21 and 22, respectively. Compounds 23 and 24 were synthesized from

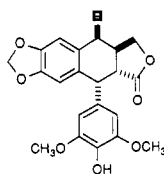
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[†] For Part 119, see ref 1.

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Table I. Biological Evaluation of 4 β -(Benzylamino)- and 4 β -Anilino-4-desoxypodophyllotoxins and 4 β -O-Benzylepipodophyllotoxin

compd	R	cytotoxicity: ^a ID ₅₀ , KB, μ M	inhibition of DNA topoisomerase II activity: ^b ID ₅₀ , μ M	cellular protein-DNA complex formation, % (10 μ M)
1		0.20	50	100
6		0.49	10	323
7		0.24	5	213
8		0.80	5	330
9		2.20	25	181
10		0.40	50	216
11		<0.40	25	130
12		1.80	50	144
13		1.90	100	126
14		3.00	25	216
15		>4.00	50	169
16		<0.40	25	225
17		<0.40	25	284
18		2.30	100	99
19		1.20	50	159
20		1.70	100	144
21		<0.40	25	191
22		<0.40	25	184
23		2.40	>100	<25
24		1.90	>100	<25

^a ID₅₀ was the concentration of drug which affords 50% reduction in cell number after a 3-day incubation. ^b Each compound was examined with three concentrations at 5, 10, 25, 50, or 100 μ M. The ID₅₀ value was established on the basis of the degree of inhibition at these three concentrations.

5, obtained by treatment of 2 with HBr as reported previously, via a S_N1 mechanism due to the formation of a C-4 benzylic carbonium ion¹⁵ (Scheme I) by treatment with benzyl alcohol and barium carbonate.

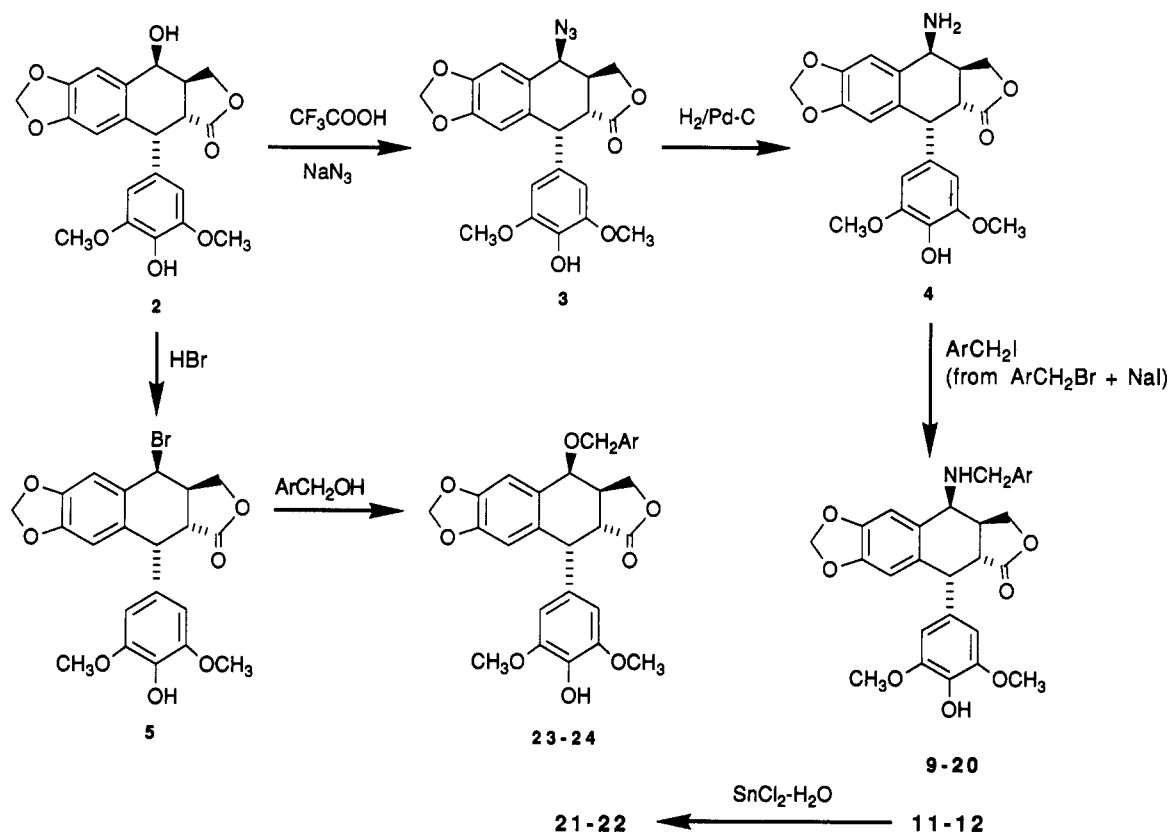
Results and Discussion

As illustrated in Table I, all 4 β -benzylamino-substituted compounds (9–22) showed comparable or superior activity to 1 in inhibiting the human DNA topoisomerase II and causing cellular protein–DNA strand breakage. All of these compounds possess substituents, except for 9, such as CN,

NO₂, F, Cl, OCH₃, and NH₂ at 2'', 3'', or 4''. The most active compounds are 14, 16, and 17, which are more than 2-fold more potent than 1 in inhibiting the human DNA topoisomerase II and causing cellular protein–DNA strand breakage. Replacement of the benzyl nitrogen with an oxygen gave rise to inactive compounds 23 and 24.

Since the basic unsubstituted 4 β -benzylamino compound 9 is almost 2-fold more potent than 1, coupled with results obtained from our previous studies, we would conclude that either a 4 β -anilino or a 4 β -N-benzyl ring system is required for inhibiting the human DNA topoisomerase II activity and for causing cellular protein-linked DNA breakage ability, and that the latter two kinds of activity

Scheme I



appear to have no correlation with the *in vitro* cytotoxicity of these compounds.

Experimental Section

General Experimental Procedures. All melting points were taken on a Fischer-Johns melting point apparatus and were uncorrected. IR spectra were recorded on a Perkin-Elmer 1320 spectrophotometer, and ^1H NMR spectra were obtained by using a Bruker AC-300 NMR spectrometer; all chemical shifts were reported in ppm from TMS. Elemental analyses were performed by Atlantic MicroLab, Inc., Norcross, GA. Optical rotations were measured with a Rudolph Research autopol III polarimeter. Analytical thin-layer chromatography (TLC) was carried out on Merck precoated silica gel 60 F-254. EM Kieselgel 60 (230-400 mesh ASTM) was used for column chromatography. All new target compounds were characterized by melting point, optical rotation, ^1H NMR, and IR spectral analyses as well as elemental analyses.

4'-O-Demethyl-4 β -azido-4-desoxypodophyllotoxin (3). To 1.60 g (4.00 mmol) of 4'-O-demethylepipodophyllotoxin (2) and 1.32 g (20.00 mmol) of sodium azide in 8 mL of CHCl_3 was added 4 mL of trifluoroacetic acid (5.19 mmol) dropwise. The reaction mixture was stirred for 15 min. Saturated aqueous sodium bicarbonate solution was added. The organic layer was washed with water and dried over MgSO_4 . After the solvent was removed, the crude product was purified by column chromatography ($\text{CHCl}_3\text{-CH}_3\text{COCH}_3\text{-CH}_3\text{CO}_2\text{Et} = 100:5:5$) to give 1.5 g of 3 (94%): mp 215-217 °C (crystals from chloroform and ethyl acetate); ^1H NMR (CDCl_3) δ 6.82 (s, 1 H, 5-H), 6.60 (s, 1 H, 8-H), 6.28 (s, 2 H, 2',6'-H), 6.04 (s, 1 H, OCHO), 6.02 (s, 1 H, OCHO), 5.43 (s, 1 H, OH), 4.78 (d, 1 H, $J = 3.7$, 1-H), 4.64 (d, 1 H, $J = 5.2$, 4-H), 4.32 (d, 2 H, $J = 9.2$, 11-H₂), 3.79 (s, 6 H, 3',5'-OCH₃), 3.18 (dd, 1 H, $J = 5.1$, $J = 13.9$, 2-H), and 2.95 (m, 1 H, 3-H); IR (KBr) 3400 (OH), 2920 (aliphatic C-H), 2100 (azide), 1720 (lactone C=O), 1602, and 1460 (aromatic C=C) cm^{-1} .

4'-O-Demethyl-4 β -amino-4-desoxypodophyllotoxin (4). To a solution of 3 (1.5 g, 3.53 mmol) in 80 mL of ethyl acetate was added 300 mg of 10% palladium on activated carbon. The mixture was stirred overnight under hydrogen. The reaction mixture was filtered, and the filtrate was evaporated. The crude product was purified by column chromatography ($\text{CHCl}_3\text{-CH}_3\text{CO}_2\text{Et} = 2:1$ and

$\text{CHCl}_3\text{-CH}_3\text{CO}_2\text{Et-MeOH} = 2:1:0.1$) to give 1.18 g of 4 (70%): $[\alpha]_D^{25} -63^\circ$ ($c = 0.25$, CHCl_3). The spectral data and melting point of 4 are consistent with those reported in ref 6.

General Procedure for the Synthesis of Compounds 9-20. To a solution of substituted benzyl bromide (0.79 mmol) in acetone (3 mL) was added sodium iodide (128 mg, 0.85 mmol). The reaction mixture was stirred for 20 min and then filtered. The filtrate was evaporated to give the corresponding benzyl iodide. To 4 (0.66 mmol) in 1,2-dichloroethane (4 mL) were added the substituted benzyl iodide (0.79 mmol) and the anhydrous barium carbonate (1.2 equiv) under nitrogen. After stirring for 40 h at 75-80 °C, the mixture was filtered. The filtrate was evaporated to give a crude product which was purified by column chromatography ($\text{CHCl}_3\text{-CH}_3\text{COCH}_3\text{-CH}_3\text{CO}_2\text{Et} = 100:5:5$).

4'-O-Demethyl-4 β -(benzylamino)-4-desoxypodophyllotoxin (9): yield 54%; crystals from chloroform-ethyl acetate; mp 180-181 °C; $[\alpha]_D^{25} -65^\circ$ ($c = 0.25$, CHCl_3); ^1H NMR (CDCl_3) δ 7.37 (m, 5 H, 2'',3'',4'',5'',6''-H), 6.54 (s, 1 H, 5-H), 6.48 (s, 1 H, 8-H), 6.29 (s, 2 H, 2',6'-H), 5.96 (s, 1 H, OCHO), 5.94 (s, 1 H, OCHO), 5.40 (d, 1 H, OH), 4.54 (d, 1 H, $J = 5.1$, 1-H), 4.32 (m, 2-H, 11-H₂), 3.94 (d, 1 H, $J = 3.8$, 4-H), 3.89 (d, 2 H, $J = 2.7$, NCH₂), 3.78 (s, 6 H, 3',5'-OCH₃), 3.34 (dd, 1 H, $J = 5.1$, 14.0, 2-H), and 2.82 (m, 1 H, 3-H); IR (KBr) 3300 (OH, NH), 2850 (aliphatic C-H), 1760 (lactone), 1590, and 1490 (aromatic C=C) cm^{-1} . Anal. ($\text{C}_{28}\text{H}_{27}\text{O}_7\text{N-H}_2\text{O}$) C, H, N.

4'-O-Demethyl-4 β -[(4''-nitrobenzyl)amino]-4-desoxypodophyllotoxin (10): yield 48%; crystals from chloroform-ethyl acetate; mp 216-217 °C; $[\alpha]_D^{25} -61^\circ$ ($c = 0.25$, CHCl_3); ^1H NMR (CDCl_3) δ 8.23 (d, 2 H, $J = 8.5$, 3'',5''-H), 7.55 (d, 2 H, $J = 8.5$, 2'',6''-H), 6.67 (s, 1 H, 5-H), 6.52 (s, 1 H, 8-H), 6.30 (s, 2 H, 2',6'-H), 5.99 (s, 1 H, OCHO), 5.95 (s, 1 H, OCHO), 5.41 (s, 1 H, OH), 4.57 (d, 1 H, $J = 5.2$, 1-H), 4.29 (m, 2 H, 11-H₂), 4.16 (d, 1 H, $J = 14.3$, NCH), 3.96 (d, 1 H, $J = 3.9$, 4-H), 3.91 (d, 1 H, $J = 14.3$, NCH), 3.75 (s, 6 H, 3',5'-OCH₃), 3.32 (dd, 1 H, $J = 5.2$, 14.0, 2-H), and 2.86 (m, 1 H, 3-H); IR (KBr) 3380 (OH, NH), 2890 (aliphatic C-H), 1750 (lactone), 1600, 1515, 1470 (aromatic C=C), 1470, and 1330 (NO_2) cm^{-1} . Anal. ($\text{C}_{28}\text{H}_{26}\text{O}_9\text{N}_2\text{-H}_2\text{O}$) C, H, N.

4'-O-Demethyl-4 β -[(3''-nitrobenzyl)amino]-4-desoxypodophyllotoxin (11): yield 45%; crystals from chloroform-ethyl acetate; mp 196-198 °C; $[\alpha]_D^{25} -67^\circ$ ($c = 0.25$, CHCl_3); ^1H NMR (CDCl_3) δ 8.19 (s, 1 H, 2''-H), 8.14 (d, 1 H, $J = 8.0$, 4''-H), 7.70

(d, 1 H, $J = 7.6$, 6''-H), 7.53 (t, 1 H, $J = 8.0$, 5''-H), 6.63 (s, 1 H, 5-H), 6.48 (s, 1 H, 8-H), 6.26 (s, 2 H, 2',6'-H), 5.95 (s, 1 H, OCHO), 5.99 (s, 1 H, OCHO), 5.38 (s, 1 H, OH), 4.54 (d, 1 H, $J = 5.3$, 1-H), 4.30 (m, 2 H, 11-H₂), 4.12 (d, 1 H, $J = 13.7$, NCH), 3.94 (d, 1 H, $J = 3.9$, 4-H), 3.88 (d, 1 H, $J = 13.7$, NCH), 3.75 (s, 6 H, 3',5'-OCH₃), 3.28 (dd, 1 H, $J = 5.3$, 14.0, 2-H), and 2.84 (m, 1 H, 3-H); IR (KBr) 3360 (OH, NH), 2900 (aliphatic C—H), 1760 (lactone), 1610, 1470 (aromatic C=C), 1520, and 1330 (NO₂) cm⁻¹. Anal. (C₂₈H₂₆O₉N₂) C, H, N.

4'-O-Demethyl-4β-[(2''-nitrobenzyl)amino]-4-desoxypodophyllotoxin (12): yield 42%; crystals from chloroform-ethyl acetate; mp 246–247 °C; $[\alpha]_D^{25} -46^\circ$ ($c = 0.25$, CHCl₃); ¹H NMR (CDCl₃) δ 8.02 (d, 1 H, $J = 8.0$, 3''-H), 7.61 (m, 2 H, 4'',6''-H), 7.50 (t, 1 H, $J = 8.2$, 5''-H), 6.64 (s, 1 H, 5-H), 6.49 (s, 1 H, 8-H), 6.29 (s, 2 H, 2',6'-H), 5.96 (s, 1 H, OCHO), 5.93 (s, 1 H, OCHO), 5.41 (s, 1 H, OH), 4.55 (d, 1 H, $J = 5.2$, 1-H), 4.42–4.27 (m, 3 H, 11-H₂, NCH), 4.02 (m, 2 H, 4-H, NCH), 3.78 (s, 6 H, 3',5'-OCH₃), 3.31 (dd, 1 H, $J = 5.2$, 14.0, 2-H), and 2.88 (m, 1 H, 3-H); IR (KBr) 3380 (OH, NH), 2880 (aliphatic C—H), 1740 (lactone), 1600, 1470 (aromatic C=C), 1500, and 1330 (NO₂) cm⁻¹. Anal. (C₂₈H₂₆O₉N₂) C, H, N.

4'-O-Demethyl-4β-[(2''-fluorobenzyl)amino]-4-desoxypodophyllotoxin (13): yield 46%; crystals from chloroform-ethyl acetate; mp 174–175 °C; $[\alpha]_D^{25} -66^\circ$ ($c = 0.25$, CHCl₃); ¹H NMR (CDCl₃) δ 7.36 (m, 2 H, 4'',5''-H), 7.18 (m, 2 H, 3'',5''-H), 6.47 (s, 1 H, 5-H), 6.44 (s, 1 H, 8-H), 6.28 (s, 2 H, 2',6'-H), 5.94 (s, 2 H, OCH₂O), 5.40 (s, 1 H, OH), 4.53 (d, 1 H, $J = 5.2$, 1-H), 4.35 (d, 2 H, $J = 9.2$, 11-H₂), 3.81 (m, 3 H, 4-H, NCH₂), 3.78 (s, 6 H, 3',5'-OCH₃), 3.34 (dd, 1 H, $J = 5.2$, 14.0, 2-H), and 2.83 (m, 1 H, 3-H); IR (KBr) 3350 (OH, NH), 2900 (aliphatic C—H), 1755 (lactone), 1600, 1500, and 1475 (aromatic C=C) cm⁻¹. Anal. (C₂₈H₂₆O₇NF) C, H, N.

4'-O-Demethyl-4β-[(3''-fluorobenzyl)amino]-4-desoxypodophyllotoxin (14): yield 51%; crystals from ethyl acetate-hexane; mp 154–155 °C; $[\alpha]_D^{25} -66^\circ$ ($c = 0.25$, CHCl₃); ¹H NMR (CDCl₃) δ 7.31 (m, 1 H, 5''-H), 7.13–6.85 (m, 3 H, 2'',4'',6''-H), 6.59 (s, 1 H, 5-H), 6.49 (s, 1 H, 8-H), 6.24 (s, 2 H, 2',6'-H), 5.99 (s, 1 H, OCHO), 5.95 (s, 1 H, OCHO), 5.40 (s, 1 H, OH), 4.55 (d, 1 H, $J = 5.2$, 1-H), 4.34 (m, 2 H, 11-H₂), 3.85 (m, 3 H, 4-H, NCH₂), 3.78 (s, 6 H, 3',5'-OCH₃), 3.34 (dd, 1 H, $J = 5.2$, 14.0, 2-H) and 2.83 (m, 1 H, 3-H); IR (KBr) 3390 (OH, NH), 2905 (aliphatic C—H), 1760 (lactone), 1610, 1520, and 1480 (aromatic C=C) cm⁻¹. Anal. (C₂₈H₂₆O₇NF) C, H, N.

4'-O-Demethyl-4β-[(4''-fluorobenzyl)amino]-4-desoxypodophyllotoxin (15): yield 45%; crystals from ethyl acetate-hexanes; mp 148–150 °C; $[\alpha]_D^{25} -65^\circ$ ($c = 0.25$, CHCl₃); ¹H NMR (CDCl₃) δ 7.33 (m, 2 H, 2'',6''-H), 7.09 (m, 2 H, 3'',5''-H), 6.59 (s, 1 H, 5-H), 6.49 (s, 1 H, 8-H), 6.29 (s, 2 H, 2',6'-H), 5.98 (s, 1 H, OCHO), 3.94 (s, 1 H, OCHO), 5.40 (s, 1 H, OH), 4.54 (d, 1 H, $J = 5.3$, 1-H), 4.34 (m, 2 H, 11-H₂), 3.90 (m, 2 H, 4-H, NCH), 3.78 (m, 7 H, 3',5'-OCH₃, NCH), 3.32 (dd, 1 H, $J = 5.3$, 14.0, 2-H), 2.85 (m, 1 H, 3-H); IR (KBr) 3330 (OH, NH), 2880 (aliphatic C=C), 1750 (lactone), 1630, 1500, and 1480 (aromatic C=C) cm⁻¹. Anal. (C₂₈H₂₆O₇NF) C, H, N.

4'-O-Demethyl-4β-[(3''-cyanobenzyl)amino]-4-desoxypodophyllotoxin (16): yield 49%; crystals from chloroform-ethyl acetate; mp 176–178 °C; $[\alpha]_D^{25} -66^\circ$ ($c = 0.25$, CHCl₃); ¹H NMR (CDCl₃) δ 7.61 (m, 3 H, 2'',4'',6''-H), 7.48 (t, 1 H, 5''-H), 6.62 (s, 1 H, 5-H), 6.56 (s, 1 H, 8-H), 6.28 (s, 2 H, 2',6'-H), 5.97 (s, 1 H, OCHO), 5.93 (s, 1 H, OCHO), 5.41 (s, 1 H, OH), 4.55 (d, 1 H, $J = 5.2$, 1-H), 4.26 (m, 2 H, 11-H₂), 3.88 (m, 3 H, 4-H, NCH₂), 3.71 (s, 6 H, 3',5'-OCH₃), 3.30 (dd, $J = 5.2$, 14.0, 2-H), and 2.84 (m, 1 H, 3-H); IR (KBr) 3360 (OH, NH), 2900 (aliphatic C—H), 2220 (C=N), 1755 (lactone), 1600, 1500, and 1480 (aromatic C=C) cm⁻¹. Anal. (C₂₉H₂₆O₇N₂H₂O) C, H, N.

4'-O-Demethyl-4β-[(4''-cyanobenzyl)amino]-4-desoxypodophyllotoxin (17): yield 51%; crystals from chloroform-ethyl acetate; mp 178–180 °C; $[\alpha]_D^{25} -64^\circ$ ($c = 0.25$, CHCl₃); ¹H NMR (CDCl₃) δ 7.65 (d, 2 H, $J = 8.1$, 3'',5''-H), 7.48 (d, 2 H, $J = 8.1$, 2'',6''-H), 7.26 (s, 2 H, 2',6'-H), 6.64 (s, 1 H, 5-H), 6.50 (s, 1 H, 8-H), 5.95 (AB q, 2 H, $J = 1.2$, OCH₂O), 5.40 (s, 1 H, OH), 4.55 (d, 1 H, $J = 5.2$, 1-H), 4.31 (m, 2 H, 11-H₂), 4.08 (d, 1 H, $J = 14.0$, NCH), 3.93 (d, 1 H, $J = 3.9$, 4-H), 3.86 (d, 1 H, $J = 14.0$, NCH), 3.75 (s, 6 H, 3',5'-OCH₃), 3.30 (dd, 1 H, $J = 5.2$, 14.0, 2-H) and 2.84 (m, 1 H, 3-H); IR (KBr) 3360 (OH, NH), 2900 (aliphatic C—H), 2220 (C=N), 1750 (lactone), 1600, 1500, and 1450 (aro-

matic C=C) cm⁻¹. Anal. (C₂₉H₂₆O₇N₂) C, H, N.

4'-O-Demethyl-4β-[[4''-(trifluoromethyl)benzyl]amino]-4-desoxypodophyllotoxin (18): yield 49%; crystals from ethyl acetate-hexane; mp 207–209 °C; $[\alpha]_D^{25} -61^\circ$ ($c = 0.25$, CHCl₃); ¹H NMR (CDCl₃) δ 7.64 (d, 2 H, $J = 8.0$, 3'',5''-H), 7.48 (d, 2 H, $J = 8.0$, 2'',6''-H), 6.64 (s, 1 H, 5-H), 6.50 (s, 1 H, 8-H), 6.36 (s, 2 H, 2',6'-H), 5.98 (s, 1 H, OCHO), 5.94 (s, 1 H, OCHO), 5.40 (s, 1 H, OH), 4.56 (d, 1 H, $J = 5.2$, 1-H), 4.34 (d, 2 H, $J = 9.0$, 11-H₂), 4.05 (d, 1 H, $J = 13.6$, NCH), 3.94 (d, 1 H, $J = 3.6$, 4-H), 3.88 (d, 1 H, $J = 13.6$, NCH), 3.78 (s, 6 H, 3',5'-OCH₃), 3.33 (dd, 1 H, $J = 5.2$, 14.0, 2-H), and 2.86 (m, 1 H, 3-H); IR (KBr) 3350 (OH, NH), 2940 (aliphatic C—H), 1620, 1510, and 1480 (aromatic C=C) cm⁻¹. Anal. (C₂₉H₂₆O₇NF₃) C, H, N.

4'-O-Demethyl-4β-[(3''-chlorobenzyl)amino]-4-desoxypodophyllotoxin (19): yield 46%; crystals from chloroform-ethyl acetate; mp 155–156 °C; $[\alpha]_D^{25} -65^\circ$ ($c = 0.25$, CHCl₃); ¹H NMR (CDCl₃) δ 7.36 (s, 1 H, 2''-H), 7.28 (m, 3 H, 4'',5'',6''-H), 6.60 (s, 1 H, 5-H), 6.58 (s, 1 H, 8-H), 6.29 (s, 2 H, 2',6'-H), 5.99 (s, 1 H, OCHO), 5.95 (s, 1 H, OCHO), 5.40 (s, 1 H, OH), 4.55 (d, 1 H, $J = 5.3$, 1-H), 4.33 (d, 2 H, $J = 9.2$, 11-H₂), 3.94 (m, 2 H, 4-H, NCH), 3.80 (m, 7 H, 3',5'-OCH₃, NCH), 3.32 (dd, 1 H, $J = 5.3$, 14.0, 2-H), and 2.82 (m, 1 H, 3-H); IR (KBr) 3350 (OH, NH), 2890 (aliphatic C—H), 1750 (lactone), 1600, 1500, and 1470 (aromatic C=C) cm⁻¹. Anal. (C₂₈H₂₆O₇NClH₂O) C, H, N.

4'-O-Demethyl-4β-[(3'',5''-dimethoxybenzyl)amino]-4-desoxypodophyllotoxin (20): yield 57%; crystals from chloroform-ethyl acetate; mp 186–187 °C; $[\alpha]_D^{25} -65^\circ$ ($c = 0.25$, CHCl₃); ¹H NMR (CDCl₃) δ 6.59 (s, 1 H, 5-H), 6.51 (d, 2 H, $J = 2.2$, 2'',6''-H), 6.47 (s, 1 H, 8-H), 6.41 (t, 1 H, $J = 2.2$, 4''-H), 6.28 (s, 2 H, 2',6'-H), 5.96 (s, 1 H, OCHO), 5.92 (s, 1 H, OCHO), 5.40 (s, 1 H, OH), 4.54 (d, 1 H, $J = 5.2$, 1-H), 4.34 (m, 2 H, 11-H₂), 3.92 (d, 1 H, $J = 4.0$, 4-H), 3.84 (s, 8 H, NCH₂, 2'',6''-OCH₃), 3.77 (s, 6 H, 3',5'-OCH₃), 3.32 (dd, 1 H, $J = 5.2$, 14.0, 2-H), and 2.81 (m, 1 H, 3-H); IR (KBr) 3360 (OH, NH), 2920 (aliphatic C—H), 1750 (lactone), 1600, 1510, and 1470 (aromatic C=C) cm⁻¹. Anal. (C₃₀H₃₀O₉N₂) C, H, N.

4'-O-Demethyl-4β-[(3''-aminobenzyl)amino]-4-desoxypodophyllotoxin (21): Tin(II) chloride dihydrate (110 mg, 0.5 mmol) was added to 11 (50 mg, 0.1 mmol) in ethyl acetate (2 mL). After refluxing under nitrogen for 1 h, the mixture was filtered, diluted with ethyl acetate, washed with water, dried over MgSO₄, and evaporated in vacuo. The crude product was purified by column chromatography (CH₂Cl₂-CH₃CO₂Et-MeOH = 100:5:5) to give 21: yield 75%; crystals from ethyl acetate-hexane; mp 209–210 °C; $[\alpha]_D^{25} -66^\circ$ ($c = 0.25$, CHCl₃); ¹H NMR (CDCl₃) δ 7.17 (t, 1 H, $J = 7.7$, 5''-H), 6.73 (d, 1 H, $J = 7.7$, 6''-H), 6.68 (s, 1 H, 2''-H), 6.64 (d, 1 H, $J = 7.7$, 4''-H), 6.64 (d, 1 H, 4''-H), 6.56 (s, 1 H, 5-H), 6.47 (s, 1 H, 8-H), 6.29 (s, 2 H, 2',6'-H), 5.96 (s, 1 H, OCHO), 5.92 (s, 1 H, OCHO), 5.40 (s, 1 H, OH), 4.53 (d, 1 H, $J = 5.2$, 1-H), 4.33 (m, 2 H, 11-H₂), 3.93 (d, 1 H, $J = 3.9$, 4-H), 3.79 (s, 6 H, 3',5'-OCH₃), 3.34 (dd, 1 H, $J = 5.2$, 12.0, 2-H), and 2.80 (m, 1 H, 3-H); IR (KBr) 3440 (NH), 3360 (OH, NH), 2900 (aliphatic C—H), 1760 (lactone), 1660, 1500, and 1480 (aromatic C=C) cm⁻¹. Anal. (C₂₈H₂₆O₇N₂H₂O) C, H, N.

4'-O-Demethyl-4β-(2''-aminobenzyl)-4-desoxypodophyllotoxin (22): Compound 22 was prepared from 12 in a manner analogous to that described above for the preparation of 21 and 11: yield 60%; crystals from ethyl acetate-hexane; mp 138–140 °C; $[\alpha]_D^{25} -85^\circ$ ($c = 0.25$, CHCl₃); ¹H NMR (CDCl₃) δ 7.15 (m, 2 H, 3'',5''-H), 6.75 (m, 3 H, 4'',6''-H, 5-H), 6.49 (s, 1 H, 8-H), 6.27 (s, 2 H, 2',6'-H), 5.98 (s, 1 H, OCHO), 5.94 (s, 1 H, OCHO), 5.40 (s, 1 H, OH), 4.55 (d, 1 H, $J = 5.2$, 1-H), 4.16 (m, 2 H, 11-H₂), 3.93 (m, 3 H, 4-H, NCH₂), 3.78 (s, 6 H, 3',5'-OCH₃), 3.20 (dd, 1 H, $J = 5.2$, 14.0, 2-H), and 2.84 (m, 1 H, 3-H); IR (KBr) 3400 (NH), 3344 (OH, NH), 2900 (aliphatic C—H), 1750 (lactone), 1600, 1500, and 1470 (aromatic C=C) cm⁻¹. Anal. (C₂₈H₂₆O₇N₂) C, H, N.

Synthesis of Compounds 23 and 24. A mixture of 4'-O-demethyl-4β-bromo-4-desoxypodophyllotoxin (5), anhydrous barium carbonate (1.2 equiv) and the appropriate benzyl alcohol (1.2 equiv) in dry dichloromethane was refluxed under nitrogen for 1 day. The reaction mixture was filtered, diluted with ethyl acetate, washed with water, dried over anhydrous MgSO₄, and purified by column chromatography (CH₂Cl₂-CH₃COCH₃-CH₃CO₂Et = 100:5:5) to yield 23 and 24.

4'-O-Demethyl-4β-benzylpodophyllotoxin (23). Yield 66%; crystals from chloroform-ethyl acetate; mp 214–215 °C; $[\alpha]_D^{25}$

-67° ($c = 0.25$, CHCl_3); ^1H NMR (CDCl_3) δ 7.37 (m, 5 H, 2'',3'',4'',5'',6''-H), 6.71 (s, 1 H, 5-H), 6.56 (s, 1 H, 8-H), 6.26 (s, 2 H, 2',6'-H), 6.99 (s, 1 H, OCHO), 6.97 (s, 1 H, OCHO), 5.40 (s, 1 H, OH), 4.60 (m, 4 H, 4-H, 1-H, OCH_2), 4.45 (m, 2 H, 11-H), 3.76 (s, 6 H, 3',5'- OCH_3), 3.46 (dd, 1 H, $J = 5.2$, 14.0, 2-H), and 2.92 (m, 1 H, 3-H); IR (KBr) 3440 (OH), 2900 (aliphatic C—H), 1750 (lactone), 1600, 1500, and 1480 (aromatic C=C) cm^{-1} . Anal. ($\text{C}_{28}\text{H}_{28}\text{O}_8$) C, H.

4'-O-Demethyl-4 β -(3''-nitrobenzyl)podophyllotoxin (24). Yield 65%; crystals from ethyl acetate-chloroform; mp 209–210 °C; $[\alpha]_D^{25}$ -66° ($c = 0.25$, CHCl_3); ^1H NMR (CDCl_3) δ 8.18 (m, 2 H, 2'',4''-H), 7.65 (d, 1 H, 6''-H), 7.56 (t, 1 H, 5''-H), 6.77 (s, 1 H, 5-H), 6.60 (s, 1 H, 8-H), 6.27 (s, 2 H, 2',6'-H), 6.04 (s, 1 H, OCHO), 6.00 (s, 1 H, OCHO), 5.41 (s, 1 H, OH), 4.71 (m, 4 H, 4-H, 11-H, CH_2O), 4.41 (m, 2 H, 1-H, 11-H), 3.78 (s, 6 H, 3',5'- OCH_3), 3.50 (dd, 1 H, $J = 5.4$, 14.0, 2-H), and 2.97 (m, 1 H, 3-H) IR (KBr) 3380 (OH), 2900 (aliphatic C—H), 1755 (lactone), 1600, 1500, and 1480 (aromatic C=C), 1520, and 1350 (NO_2) cm^{-1} . Anal. ($\text{C}_{28}\text{H}_{25}\text{O}_{10}\text{N}$) C, H, N.

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Cholecystokinin Antagonists: (*R*)-Tryptophan-Based Hybrid Antagonists of High Affinity and Selectivity for CCK-A Receptors

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The intriguing structural similarities of glutamic acid based cholecystokinin (CCK) antagonists (A-64718 and A-65186) and the benzodiazepine CCK antagonist MK-329 (L-364,718) have been reported. Efforts to include the weak CCK antagonist benzotript into this construct utilizing a similar approach have resulted in a novel series of benzotript-based hybrid antagonists N^α -(3'-quinolylcarbonyl)-(*R*)-tryptophan di-*n*-pentylamide (9, A-67396), N^α -(4',8'-dihydroxy-2'-quinolylcarbonyl)-(*R*)-tryptophan di-*n*-pentylamide (23, A-70276), and N^α -(3'-quinolylcarbonyl)-(*R*)-5'-hydroxytryptophan di-*n*-pentylamide (36, A-71134) which possess respectively binding affinities of 23, 21, and 11 nM for the pancreatic CCK-A receptor and which inhibit CCK $_B$ -induced amylase secretion. Compound 9 possesses a selectivity of >500-fold for the pancreatic CCK-A receptor over the CCK-B receptor.¹

Introduction

Within the past few years considerable research has been directed toward the development of cholecystokinin (CCK) antagonists as potential therapeutic agents for appetitive disorders, anxiety, potentiation of opiate analgesia, and treatment of gastrointestinal, pancreatic, and possibly psychiatric disorders.² As a result of these studies a number of potent and selective CCK antagonists have been reported and are currently being investigated.³ Prior to these advances only a few, weak nonselective CCK antagonists were available including proglumide (Milid), benzotript, and dibutyl-cyclic-GMP.⁴ The development of CR 1409 and CR 1505 by Makovec and co-workers⁵ and the development of the benzodiazepine MK-329 (devazepide) by Evans and co-workers⁶ were significant in that they produced the first potent, non-peptide-based CCK antagonists (Figure 1) exhibiting high affinity and selectivity for the CCK-A receptors, which are found predominantly in the periphery and typically characterized in the pancreas, gallbladder, and ileum. The selectivity of such agents for CCK-A receptors over CCK-B receptors that predominant in brain has provided pharmacological tools both for central nervous system (CNS) receptor mapping studies and for the delineation of the functional roles for CCK-A receptors.⁷ Thus, investigations with MK-329

have confirmed the existence of CCK-A receptors, albeit in low abundance, in several brain regions including the

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