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 4β-*N*- or 4β-*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 4β-*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 4β-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 4β-arylamino analogues[9] of **2**, we report herein the strucure–activity relationships of a series of 4β-*N*- or 4β-*O*-benzyl-substituted derivatives of 4′-*O*-demethylpodophyllotoxin 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 4β-(unsubstituted benzylamino)- (**9**) and 4β-(substituted benzylamino)-4′-*O*-demethyl-4-desoxypodophyllotoxins (**10–20**) started from 4′-*O*-demethylepipodophyllotoxin (**2**). The key intermediate, 4′-*O*-demethyl-4β-azido-4-desoxypodophyllotoxin (**3**) was obtained in high yield by treating **2** with trifluoroacetic acid (TFA) and sodium azide[14] (Scheme I). In this reaction, high stereoselectivity through the formation of a benzylic carbonium ion at C-4 to yield solely the 4β-oriented secondary azide was achieved by use of TFA. Previously, the preparation of the 4β-azide was found to be complex, as it required three steps from **2** in low yield and generated considerable amounts of the 4α-isomer. The azide **3** could simply be reduced with hydrogen in the presence of 10% Pd–C to afford 4′-*O*-demethyl-4β-amino-4-desoxypodophyllotoxin (**4**) in good yield without producing the 4α-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 $SnCl_2$–H_2O gave **21** and **22**, respectively. Compounds **23** and **24** were synthesized from

† For Part 119, see ref 1.

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(1) Part 119: Wu, T. S.; Lin, Y. M.; Haruna, M.; Pan, D. J.; Shingu, T.; Chen, Y. P.; Hsu, H. Y.; Nakano, T.; Lee, K. H. *J. Nat. Prod.* **1991**, *54*, 823–829.
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(4) van Maanen, J. M. S.; Retel, J.; DeVries, Jr.; Pinedo, H. M. *J. Natl. Cancer Inst.* **1988**, *80*, 1526–1533.
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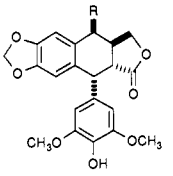
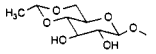
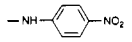
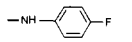
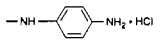
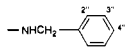
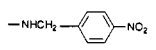
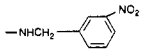
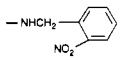
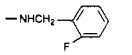
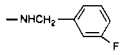
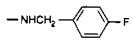
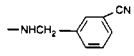
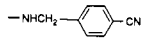
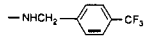
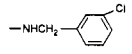
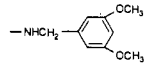
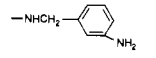
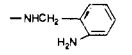
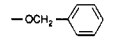
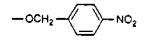
Table I. Biological Evaluation of 4β-(Benzylamino)- and 4β-Anilino-4-desoxypodophyllotoxins and 4β-*O*-Benzylepipodophyllotoxin



compd	R	cytotoxicity:[a] ID_{50}, KB, μM	inhibition of DNA topoisomerase II activity:[b] ID_{50}, μM	cellular protein–DNA complex formation, % (10 μM)
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] 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 three concentrations at 5, 10, 25, 50, or 100 μM. The ID_{50} 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 ion[15] (Scheme I) by treatment with benzyl alcohol and barium carbonate.

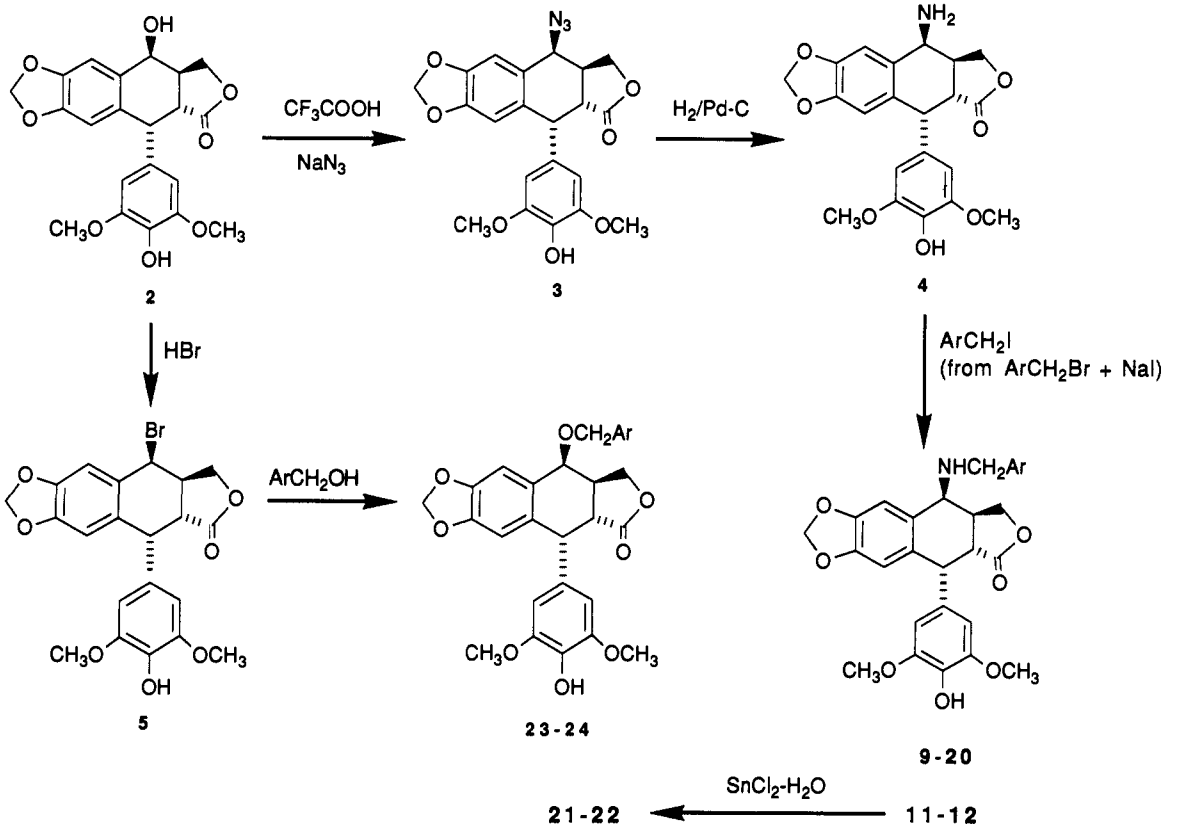
Results and Discussion

As illustrated in Table I, all 4β-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, NO_2, F, Cl, OCH_3, and NH_2 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 4β-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 4β-anilino or a 4β-*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

(15) Bellamy, F. D.; Ou, K. *Tetrahedron Lett.* **1984**, *8*, 839–841.

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-4β-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 ($CHCl_3$–CH_3COCH_3–CH_3CO_2Et = 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-H_2), 3.79 (s, 6 H, 3′,5′-OCH_3), 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-4β-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 ($CHCl_3$–CH_3CO_2Et = 2:1 and $CHCl_3$–CH_3CO_2Et–MeOH = 2:1:0.1) to give 1.18 g of 4 (70%): $[\alpha]^{25}_D$ −63° (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 ($CHCl_3$–CH_3COCH_3–CH_3CO_2Et = 100:5:5).

4′-*O*-Demethyl-4β-(benzylamino)-4-desoxypodophyllotoxin (9): yield 54%; crystals from chloroform–ethyl acetate; mp 180–181 °C; $[\alpha]^{25}_D$ −65° (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 (s, 1 H, OH), 4.54 (d, 1 H, J = 5.1, 1-H) 4.32 (m, 2-H, 11-H_2), 3.94 (d, 1 H, J = 3.8, 4-H), 3.89 (d, 2 H, J = 2.7, NCH_2), 3.78 (s, 6 H, 3′,5′-OCH_3), 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. ($C_{28}H_{27}O_7N·H_2O$) C, H, N.

4′-*O*-Demethyl-4β-[(4″-nitrobenzyl)amino]-4-desoxypodophyllotoxin (10): yield 48%; crystals from chloroform–ethyl acetate; mp 216–217 °C; $[\alpha]^{25}_D$ −61° (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-H_2), 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′-OCH_3), 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. ($C_{28}H_{26}O_9N_2·H_2O$) C, H, N.

4′-*O*-Demethyl-4β-[(3″-nitrobenzyl)amino]-4-desoxypodophyllotoxin (11): yield 45%; crystals from chloroform–ethyl acetate; mp 196–198 °C; $[\alpha]^{25}_D$ −67° (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-H_2), 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′-OCH_3), 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 (NO_2) cm^{-1} Anal. ($C_{28}H_{26}O_9N_2$) C, H, N.

4′-*O*-Demethyl-4β-[(2″-nitrobenzyl)amino]-4-desoxypodophyllotoxin (12): yield 42%; crystals from chloroform–ethyl acetate; mp 246–247 °C; $[\alpha]^{25}_D$ –46° (c = 0.25, $CHCl_3$); ^1H NMR ($CDCl_3$) 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-H_2, NCH), 4.02 (m, 2 H, 4-H, NCH), 3.78 (s, 6 H, 3′,5′-OCH_3), 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 (NO_2) cm^{-1}. Anal. ($C_{28}H_{26}O_9N_2$) C, H, N.

4′-*O*-Demethyl-4β-[(2″-fluorobenzyl)amino]-4-desoxypodophyllotoxin (13): yield 46%; crystals from chloroform–ethyl acetate; mp 174–175 °C; $[\alpha]^{25}_D$ –66° (c = 0.25, $CHCl_3$); ^1H NMR ($CDCl_3$) 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, OCH_2O), 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-H_2), 3.81 (m, 3 H, 4-H, NCH_2), 3.78 (s, 6 H, 3′,5′-OCH_3), 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. ($C_{28}H_{26}O_7NF$) C, H, N.

4′-*O*-Demethyl-4β-[(3″-fluorobenzyl)amino]-4-desoxypodophyllotoxin (14): yield 51%; crystals from ethyl acetate–hexane; mp 154–155 °C; $[\alpha]^{25}_D$ –66° (c = 0.25, $CHCl_3$); ^1H NMR ($CDCl_3$) 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-H_2), 3.85 (m, 3 H, 4-H, NCH_2), 3.78 (s, 6 H, 3′,5′-OCH_3) 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. ($C_{28}H_{26}O_7NF$) C, H, N.

4′-*O*-Demethyl-4β-[(4″-fluorobenzyl)amino]-4-desoxypodophyllotoxin (15): yield 45%; crystals from ethyl acetate–hexanes; mp 148–150 °C; $[\alpha]^{25}_D$ –65° (c = 0.25, $CHCl_3$); ^1H NMR ($CDCl_3$) 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-H_2), 3.90 (m, 2 H, 4-H, NCH), 3.78 (m, 7 H, 3′,5′-OCH_3, 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. ($C_{28}H_{26}O_7NF$) C, H, N.

4′-*O*-Demethyl-4β-[(3″-cyanobenzyl)amino]-4-desoxypodophyllotoxin (16): yield 49%; crystals from chloroform–ethyl acetate; mp 176–178 °C; $[\alpha]^{25}_D$ –66° (c = 0.25, $CHCl_3$); ^1H NMR ($CDCl_3$) 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-H_2), 3.88 (m, 3 H, 4-H), NCH_2), 3.71 (s, 6 H, 3′,5′-OCH_3), 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. ($C_{29}H_{26}O_7N_2 \cdot H_2O$) C, H, N.

4′-*O*-Demethyl-4β-[(4″-cyanobenzyl)amino]-4-desoxypodophyllotoxin (17): yield 51%; crystals from chloroform–ethyl acetate; mp 178–180 °C; $[\alpha]^{25}_D$ –64° (c = 0.25, $CHCl_3$); ^1H NMR ($CDCl_3$) 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, OCH_2O), 5.40 (s, 1 H, OH), 4.55 (d, 1 H, J = 5.2, 1-H), 4.31 (m, 2 H, 11-H_2), 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′-OCH_3), 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 (aromatic C=C) cm^{-1}. Anal. ($C_{29}H_{26}O_7N_2$) 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; $[\alpha]^{25}_D$ –61° (c = 0.25, $CHCl_3$); ^1H NMR ($CDCl_3$) 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-H_2), 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′-OCH_3), 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. ($C_{29}H_{26}O_7NF_3$) C, H, N.

4′-*O*-Demethyl-4β-[(3″-chlorobenzyl)amino]-4-desoxypodophyllotoxin (19): yield 46%; crystals from chloroform–ethyl acetate; mp 155–156 °C; $[\alpha]^{25}_D$ –65° (c = 0.25, $CHCl_3$); ^1H NMR ($CDCl_3$) 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, 2 H, J = 5.3, 1-H), 4.33 (d, 2 H, J = 9.2, 11-H_2), 3.94 (m, 2 H, 4-H, NCH), 3.80 (m, 7 H, 3′,5′-OCH_3, 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. ($C_{28}H_{26}O_7NCl \cdot H_2O$) 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; $[\alpha]^{25}_D$ –65° (c = 0.25, $CHCl_3$); ^1H NMR ($CDCl_3$) 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-H_2), 3.92 (d, 1 H, J = 4.0, 4-H), 3.84 (s, 8 H, NCH_2, 2″,6″-OCH_3), 3.77 (s, 6 H, 3′,5′-OCH_3), 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. ($C_{30}H_{31}O_9N$) 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 $MgSO_4$, and evaporated in vacuo. The crude product was purified by column chromatography (CH_2Cl_2–CH_3CO_2Et–MeOH = 100:5:5) to give **21**: yield 75%; crystals from ethyl acetate–hexane; mp 209–210 °C; $[\alpha]^{25}_D$ –66° (c = 0.25, $CHCl_3$); ^1H NMR ($CDCl_3$) 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-H_2), 3.93 (d, 1 H, J = 3.9, 4-H), 3.79 (s, 6 H, 3′,5′-OCH_3), 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. ($C_{28}H_{28}O_7N_2 \cdot H_2O$) 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; $[\alpha]^{25}_D$ –85° (c = 0.25, $CHCl_3$); ^1H NMR ($CDCl_3$) 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-H_2), 3.93 (m, 3 H, 4-H, NCH_2), 3.78 (s, 6 H, 3′,5′-OCH_3), 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. ($C_{28}H_{28}O_7N_2$) 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 $MgSO_4$, and purified by column chromatography (CH_2Cl_2–CH_3COCH_3–CH_3CO_2Et = 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; $[\alpha]^{25}_D$

−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. ($C_{28}H_{26}O_8$) C, H.

4′-*O*-Demethyl-4β-(3″-nitrobenzyl)podophyllotoxin (24). Yield 65%; crystals from ethyl acetate–chloroform; mp 209–210 °C; $[\alpha]^{25}_D$ −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. ($C_{28}H_{25}O_{10}N$) 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_8-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 dibutyryl-cyclic-GMP.[4] The development of CR 1409 and CR 1505 by Makovec and co-workers[5] and the development of the benzodiazepine MK-329 (devazepide) by Evans and co-workers[6] 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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