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Synthesis and anticancer activity of new derivatives of podophyllotoxin

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A series of analogues of etoposide (VP-16,1), the C-4 alkylamino-substituted 4'-dimethyl-epipodophyllotoxins (4a-g), have been synthesized and studied for their activity to inhibit L1210 and KB cells *in vitro*. Compounds 4a, 4b, 4c and 4f are as potent or more potent than VP-16 in their inhibition of both L1210 and KB cells.

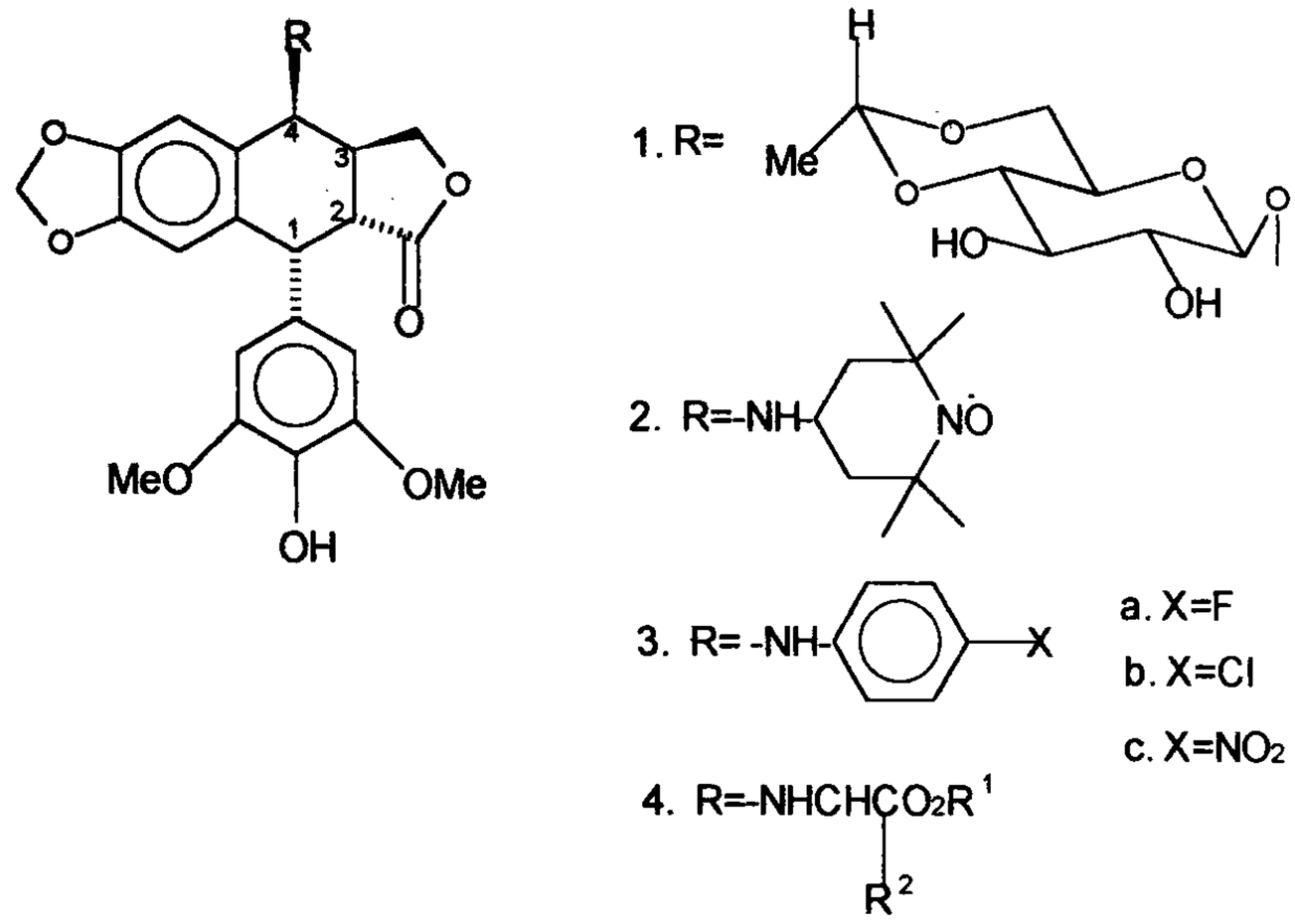
THE clinical efficacy and intriguing mechanism of podophyllotoxin-derived glucoside, etoposide (VP-16, 1), has greatly stimulated interest in the synthesis of new active analogues of podophyllotoxin[1–6]. The approach to modify 1 based on replacement of the glucose moiety with an amino sugar has led to some highly active analogues[3], suggesting that β-anomeric configuration was indispensable for the antitumour activity and that the amino substituent of the sugar moiety was important for increasing the activity. Changes in the 4β-glycosyl group are also of interest for simplified structure which might retain the activity of 1 and its amino glucoside analogues, and be accessible to practical industrialization. In the previous papers[4,7,8], we reported the synthesis of an amino nitroxyl spin-labeled analogue of 1, GP-7(2), which exhibited superior pharmacological properties to 1. A series of 4β-alkylamino and arylamino derivatives of 4'-demethylepipodophyllotoxin, such as 3a–c, have also demonstrated by the strong antitumour activity that considerable simplification in the sugar structure might be permitted so long as the amino group was retained[5,6,9]. These findings prompted us to change the C-4 glucose moiety in VP-16 to a configurationally similar amino-acid ester, and to synthesize seven new derivatives of podophyllotoxin (4a–g).

4β alkylamino derivatives of 4'-demethylepipodophyllotoxin (4a–g) were synthesized by direct nucleophilic substitution (SN1) of appropriate L-amino-acid ester (7a–g) with 4β-bromo-4'-demethyl-4-deoxypodophyllotoxin (6) resulting from podophyllotoxin (5). The bulky C-1α pentant aromatic ring dictates the substitution to be stereoselective in yielding the C-4β alkylamino isomers as the major products. In some cases, the C-4α isomers were also observed. The assignment of the configuration at C-4 for compounds 4a–g was based on the difference of $J_{3,4}$ coupling constants. The C-4β-substituted compounds 4a–g have a $J_{3,4} \approx 4.0$ Hz as seen in 1 and 3 (refs. 5, 6), due to a *cis* relationship between H-3 and H-4. The C-4α substituted derivatives, however, have a $J_{3,4} \geq 10.0$ Hz as H-3 is *trans* to H-4 (ref. 6).

We have tested the inhibitory activities of compounds 4a–g against leukaemia L1210 and KB cells *in vitro*. ID_{50} values of compounds 1 and 4a–g are 0.40, 0.28, 0.42, 0.38, 0.70, 1.60, 0.42 and 1.25 μM for L1210 cells, and 0.22, 0.20, 0.10, 0.18, 0.56, 0.84, 0.28 and 1.00 μM for KB cells, respectively. Therefore, compounds 4a, 4b, 4c and 4f are as potent or more potent than VP-16 in their inhibition of both L1210 and KB cells. These results demonstrate the possibility of considerable simplification in the sugar structure of 1 and suggest further elaboration of the 4β-amino substituent to optimize the structure of this class of anticancer compounds. Further study for anticancer activity of synthesized compounds is in progress.

All melting points were taken on Yanaco melting point apparatus and uncorrected. IR spectra were obtained on a nicolet-5DX spectrophotometer, and ^{1}HNMR spectra were obtained by using either a Bruker AM-400 or JMS-FX-90Q NMR spectrometer. All chemical shifts are reported in ppm from TMS. Elemental analysis were taken on a YANACO-CHN-CODER MT-3 instrument. MS analysis were determined on a VG-7070E-HF instrument at 70 eV.

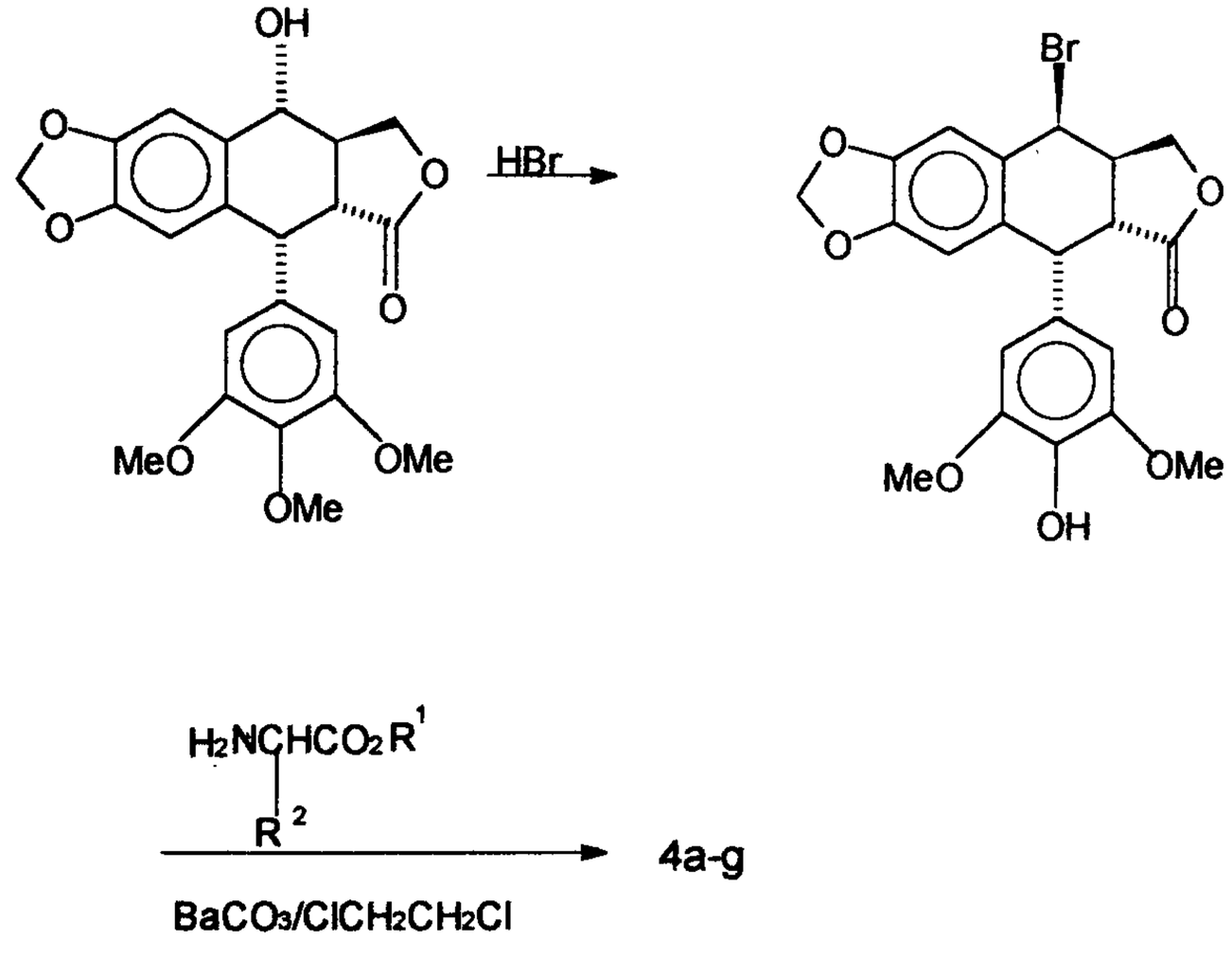
A solution containing 4β-bromo-4'-demethyl-4-deoxypodophyllotoxin (6) (ref. 1) (1.5 mmol), anhydrous barium carbonate (2.0 mmol), and the appropriate L-amino-acid ester (7a-g) (2.0 mmol) in 20 ml of dry 1,2-dichloroethane under nitrogen was stirred overnight at room temperature. The reaction mixture was filtered, diluted with ethyl acetate, washed with water, dried, and purified via column chromatography (50 g of silica gel with dichloromethane-acetone 8:1 as eluant). Yields ranged from 18 to 47%.



a.R^1=Et,R^2=H;b.R^1=Me,R^2=H;.c.R^1=R^2 =Me; d.R^1 =Me,R^2 = -$CHMe_2$

e.R^1=Me,R^2=-CH_2Ph;f.R^1=Me,R^2=-CH_2Ph-OH(p);g.R^1=Et,R^2=-CH_2CHMe_2

Scheme 1.



Scheme 2.

Compound 4a: m.p. 230–231°C; MS m/z $[M]^+$ 485; IR (KBr) 3500 (OH), 3427 (NH), 1762 (lactone), 1734 (ester), 1614, 1515 and 1483 (aromatic C=C) cm^{-1}; ^{1}HNMR ($CDCl_3$), δ 7.04 (s, 1H, H-5) 6.34 (s, 1H, H-8), 6.15 (s, 2H, H-2', 6'), 5.87 (s, 2H, OCH_2O), 4.70 (s, 1H, OH), 4.40 (d, J = 5.0 Hz, 1H, H-1), 4.22–3.96 (m, 4H, H-11 and CO_2CH_2), 3.82 (d, J = 4.0 Hz, 1H, H-4), 3.64 (s, 6H, 3', 5'-OCH_3), 3.30 (s, 2H, $NHCH_2$), 3.24 (dd, J = 5.0, 14.0 Hz, 1H, H-2), 2.75 (m, 1H, H-3), 1.92 (s, 1H, NH), 1.24 (t, J = 7.2 Hz, 3H, CH_2CH_3). Anal. calculated for $C_{25}H_{27}NO_9$, C 61.85, H 5.61, N 2.89; found C 61.83, H 5.65, N 2.88.

Compound 4b: m.p. 227–228°C; MS m/z $[M]^+$ 471; IR (KBr) 3500 (OH), 3420 (NH), 1760 (lactone), 1734

(ester), 1610, 1511 and 1485 (aromatic C–C) cm^{-1}; ^{1}HNMR ($CDCl_3$), δ 7.00 (s, 1H, H-5) 6.39 (s, 1H, H-8), 6.21 (s, 2H, H-2', 6'), 5.91 (m, 2H, OCH_2O), 5,10 (s, 1H, 4'-OH), 4.49 (d, J = 5.0 Hz, 1H, H-1), 4.28 (m, 2H, H-11), 3.89 (d, J = 4.0 Hz, 1H, H-4), 3.72 (s, 3H, CO_2CH_3), 3.65 (s, 6H, 3', 5'-OCH_3), 3.37 (s, 2H, NHC$\underline{H}_2$), 3.29 (dd, J = 5.0 H, 14.0 Hz, 1H, H-2), 2.99 (m, 1H, H-3), 2.20 (s, 1H, NH). Anal. calculated for $C_{24}H_{25}NO_9$, C 61.14, H 5.34, N 2.97; found C 61.17, H 5.31, N 3.01.

Compound 4c: m.p. 214–215°C; MS m/z $[M]^+$ 485; IR (KBr) 3435 (OH, NH), 1772 (lactone), 1742 (ester), 1612, 1515 and 1483 (aromatic C=C) cm^{-1}; ^{1}HNMR ($CDCl_3$), δ 7.02 (s, 1H, H-5) 6.38 (s, 1H, H-8), 6.22 (s, 2H, H-2', 6'), 5.85 (s, 2H, OCH_2O), 5.12 (s, 1H, 4'-OH), 4.42 (d, J = 4.8 Hz, 1H, H-1), 4.20–4.00 (m, 2H, H-11), 3.92 (d, J = 4.0 Hz, 1H, H-4), 3.78 (s, 3H, CO_2CH_3), 3.70 (s, 6H, 3', 5'-OCH_3), 3.65 (q, J = 6.8 Hz, 1H, NHC$\underline{H}$), 3.13 (m, 1H, H-2), 2.80 (m, 1H, H-3), 2.06 (s, 1H, NH), 1.15 (d, J = 6.8 Hz, 3H, CHC$\underline{H}_3$), Anal. calculated for $C_{25}H_{27}NO_9$, C 61.85, H 5.61, N 2.89; found C 61.90, H 5.62, N 2.84.

Compound 4d: m.p. 178–179°C; MS m/z $[M]^+$ 513; IR (KBr) 3470 (OH, NH), 1770 (lactone), 1736 (ester), 1610, 1508 and 1482 (aromatic C=C) cm^{-1}; ^{1}HNMR ($CDCl_3$), δ 7.00 (s, 1H, H-5) 6.39 (s, 1H, H-8), 6.23 (s, 2H, H-2', 6'), 5.83 (AB_q, J = 1.0, 4.5 Hz, 2H, OCH_2O), 5.00 (s, 1H, OH), 4.38–4.22 (m, 3H, H-1 and H-11), 3.90 (d, J = 4.0 Hz, 1H, H-4), 3.82 (s, 3H, CO_2CH_3), 3.77 (s, 6H, 3', 5'-OCH_3), 3.69 (d, J = 7.4 Hz, 1H, NHC$\underline{H}$), 3.16 (dd, J = 5.0, 12.0 Hz, 1H, H-2), 2.80 (m, 1H, H-3), 2.30 (m, 1H, C$\underline{H}$Me$_2$), 2.21 (s, 1H, NH), 1.10 (d, J = 7.4 Hz, 6H, CH(C$\underline{H}_3$)$_2$). Anal. calculated for $C_{27}H_{31}NO_9$, C 63.15, H 6.08, N 2.73; found C 63.10, H 6.05, N 2.72.

Compound 4e: m.p. 170–172°C; MS m/z $[M]^+$ 561; IR (KBr) 3500 (OH), 3380 (NH), 1776 (lactone), 1726 (ester), 1612, 1516 and 1485 (aromatic C=C) cm^{-1}; ^{1}HNMR (DMSO-d_6), δ 7.35 (m, 5H, C_6H_5), 6.89 (s, 1H, H-5), 6.55 (s, H, H-8), 6.34 (s, 2H, H-2', 6'), 5.90 (s, 2H, OCH_2O), 5.41 (s, 1H, OH), 4.44 (d, J = 8 Hz, 1H, H-1), 4.38–4.09 (m, 3H, H-11, NH), 3.90 (d, J = 4.2 Hz, 1H, H-4), 3.70 (s, 3H, CO_2CH_3), 3.68 (s, 6H, 3', 5'-OCH_3), 3.64 (t, J = 7.0 Hz, 1H, NHC$\underline{H}$), 3.32 (d, J =7.0 Hz, 2H, C$\underline{H}_2$Ph), 3.10 (m, 2H, H-2, 3). Anal. calculated for $C_{31}H_{31}NO_9$, C 66.30, H 5.56, N 2.49; found C 66.38, H 5.58, N 2.47.

Compound 4f: m.p. 197–198°C; MS m/z $[M]^+$ 577; IR (KBr) 3450 (OH, NH), 1775 (lactone), 1734 (ester), 1610, 1505 and 1480 (aromatic C=C) cm^{-1}; ^{1}HNMR (DMSO-d_6, D_2O exchange), δ 6.90 and 6.70 (d, J = 8.3 Hz, 4H, C_6H_4), 6.82 (s, 1H, H-5), 6.51 (s, 1H, H-8), 6.26 (s, 2H, H-2', 6'), 5.95 (s, 2H, OCH_2O), 4.48 (d, J = 5.0 Hz, 1H, H-1), 4.37 (m, 2H, H-11), 3.95 (s, 3H, CO_2CH_3), 3.83 (d, J = 4.1 Hz, 1H, H-4), 3.75 (s, 6H, 3', 5'-OCH_3), 3.67 (t, J = 7.0 Hz, 1H, NHC$\underline{H}$), 3.30 (d, J = 7.0 Hz, 2H, CH_2Ph), 3.18–3.11 (m, 2H, H-2, 3), Anal. calculated for $C_{31}H_{31}NO_{10}$, C 64.46, H 5.41, N 2.43; found C 64.31, H 5.39, N 2.44.

Compound 4g: m.p. 130–131°C; MS m/z $[M]^+$ 541; IR (KBr) 3443 (OH, NH), 1772 (lactone), 1733 (ester), 1605, 1503 and 1485 (aromatic C=C) cm^{-1}; ^{1}HNMR ($CDCl_3$), δ 6.94 (s, 1H, H-5), 6.46 (s, 1H, H-8), 6.19 (s, 2H, H-2', 6'), 5.91 (m, 2H, OCH_2O), 5.32 (s, 1H, OH), 4.47 (d, J = 5.0 Hz, 1H, H-1), 4.28–4.00 (m, 4H, H-11, CO_2CH_2), 3.80 (d, J = 4.2 Hz, 1H, H-4), 3.74 (s, 6H, 3', 5'-OCH_3), 3.66 (t, J = 7.0 Hz, 1H, NHC$\underline{H}$), 3.30 (m, 1H, H-2), 2.94 (m, 1H, H-3), 2.18 (s, 1H, NH), 1.85 (m, 3H, C$\underline{H}_2$C$\underline{H}$Me$_2$), 1.28 (t, J = 7.2 Hz, 3H, CH$_2$C$\underline{H}_3$), 0.97 (d, J = 6.6 Hz, 6H, CH(C$\underline{H}_3$)$_2$). Anal. calculated for $C_{29}H_{35}NO_9$, C 64.31, H 6.51, N 2.59; found C 64.21, H 6.50, N 2.62.

Drugs were dissolved in Me_2SO at a concentration of 20 mM as the stock solution and diluted before use with H_2O to the desired concentration of each drug. The cytotoxicity was determined *in vitro* using L1210 and KB cells grown in RPMI No. 1640 medium supplemented with fetal calf serum according to the published procedure[9,10].

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