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Synthesis and Antiproliferative Activity of Retroetoposide

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Abstract—Retro-4′-demethyl-4-epipodophyllotoxin **6** was synthesized in eight steps and 10% overall yield from 4′-demethyl-4-epipodophyllotoxin **12**. Subsequent coupling of **22** with 1-*O*-trimethylsilyl-4,6-*O*-ethylidene-β-D-glucoside **26** afforded retroetoposide **5** which is 10-fold less cytotoxic than etoposide against L1210 cell line.

Natural lignan podophyllotoxin **1** and etoposide **2**, a semi-synthetic analogue, have attracted considerable application[1] as a result of the potent antitumour activity of the latter compound (Fig. 1), alone or in combination, for the treatment of refractory testicular cancers and small cell lung cancers.

The mechanism of action for **2** involves topoisomerase II inhibition resulting in DNA strand scission and cell death.[2] Recently, a two-drug model for etoposide action against human topoisomerase IIα has been reported by Osheroff and co-workers with important consequences for topo II-based cancer chemotherapy.[3] The development of drug resistance, myelosuppression and poor oral bioavailability have stimulated further syntheses of analogues of **2** for better pharmacological profiles. Therefore, about 100 new analogues have been synthesized and reported,[4] and among them, etopophos **3**, a water-soluble prodrug has been introduced on the market[5] whereas GL 331 **4** in under phase II clinical trial.[6]

Besides these problems, the metabolism of etoposide results in inactivation by hydrolysis and epimerization to the corresponding 2,3-*cis*-hydroxyacid and picroetoposide which are 500- and 100-fold less active, respectively.[7] In order to avoid or minimize the C-2 epimerization and the lactone ring opening, two main strategies have been followed. The first included replacement of the γ-lactone with heterocycles.[8] The second took advantage of the preparation of C-2 substituted analogues[9] or of 2-aza-podophyllotoxin.[10] For our part, we recently proposed a third strategy based upon the synthesis of less epimerizable δ-lactone-containing analogues.[11]

Futhermore, we have focused on the acquirement of information about the structure–activity relationship (SAR) of retro-4′-demethyl-4-epipodophyllotoxin **6** having the lactone carbonyl group at C-11.

Such functionality is present in arylnaphthalene lignans[12] like justicidin E[13] **7**, retrojusticidin B **8** and retrochinensin **9**, and in aryltetraline lignans like formosolactone **10**[14] and α-conidendrin (ACON) **11**[15] (Fig. 2).

Retroarylnaphthalene lactones and retroaryltetraline lactones have been less studied.[16] Antimicrobial and anti-platelet activating factor activities have been reported in this former sub-group, and retrojusticidin B **8** has been shown to inhibit HIV-1 reverse transcriptase.[17] As for **11**, it was shown to display no relevant cytotoxic effect. However, this result may be due to the lack of some structural determinants as the methylene dioxy on the A-ring, the 4-hydroxy group on the C-ring, and the tri- or dimethoxy groups on the E-ring. In this communication, we report the synthesis and antiproliferative activity of retro-4′-demethyl-4-epipodophyllotoxin **6** and the corresponding retroetoposide **5**.

The synthesis of retrolactone **6** was achieved from 4′-demethyl-4-epipodophyllotoxin **12**[18] (Scheme 1). Bissilylation of **12**, followed by LAH reduction, led to the *trans*-diol **14**.[19] Treatment of the latter with 2.5 equivalents of pivaloyl chloride furnished a mixture of **15**, **16**, and **17** in 65, 27 and 8% yields, respectively.

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The prospect of utilizing **16** in place of **15** appeared attractive for the small-scale preparation of **6** (Scheme 2). Dess–Martin oxidation[20] of **16** and subsequent sodium chlorite oxidation gave the acid derivative **19** (74%, two steps). Following removal of the pivaloyl group with $LiEt_3BH$, cyclization of the resultant γ-hydroxy carboxylic acid **20** by treatment with DCC in the presence of 4-DMAP provided the expected γ-lactone **21**. Final deprotection of **21** was carried out in the presence of tetrabutylammonium fluoride, affording **6**.[21] Prior to the glycosidation-step, **6** was selectively protected at C-4′ as the benzyloxycarbonyl derivative **22**.

On the other hand, the sugar moiety was prepared from glucose in five steps via standard procedures (Scheme 3).

Hydroxyl protection of the 4,6-*O*-ethylidene-D-glucopyranose **23**[22] as the chloroacetyl esters furnished **24**. Selective anomeric deprotection (MeOH, pyridine) afforded **25** as a mixture (α/β = 70:30) which was then converted into the silylated glucoside donor **26** by treatment with trimethylsilyl chloride in the presence of

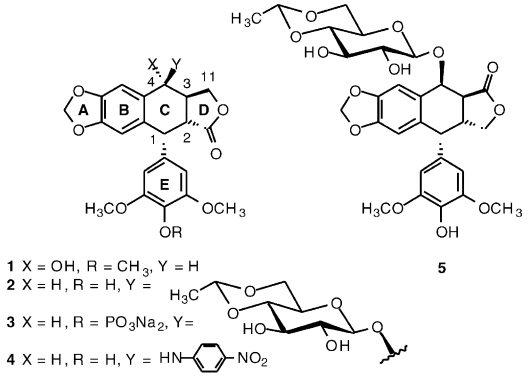


Figure 1. Structures of podophyllotoxin **1** and its derivatives **2–5**.

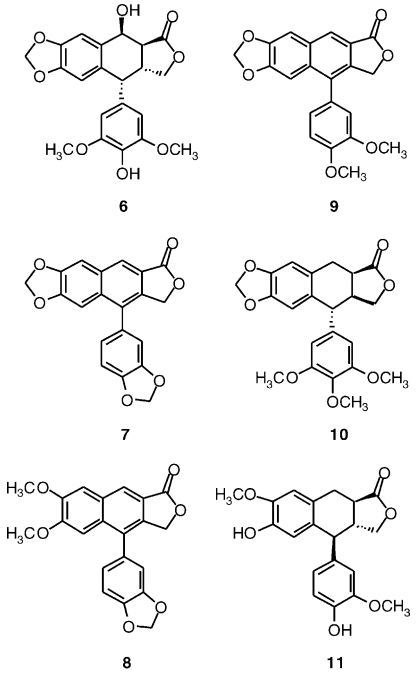
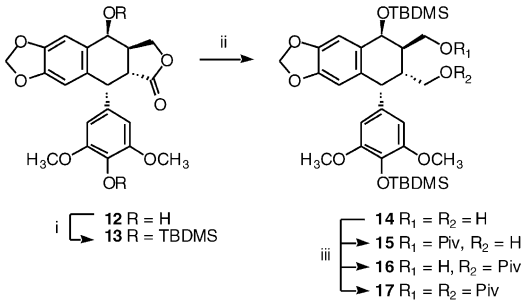
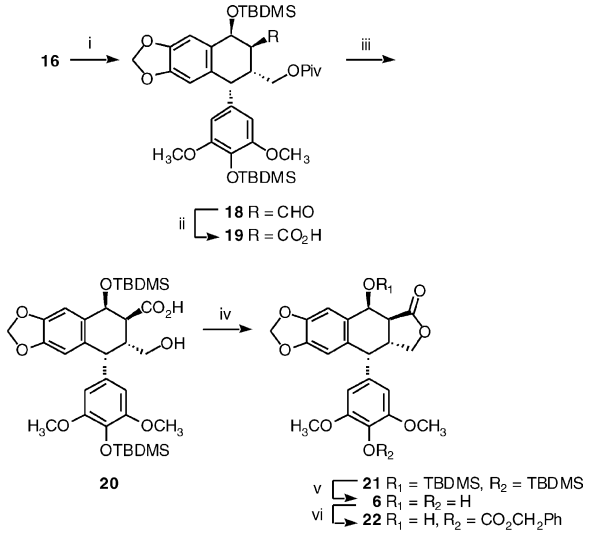


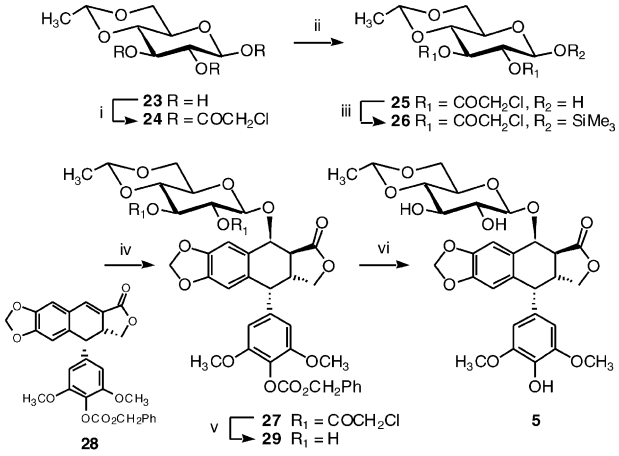
Figure 2. Retrolactones **6–11**.



Scheme 1. (i) TBDMSOTf, 2,6-lutidine, CH_2Cl_2, 0 °C, 1 h, 81%; (ii) $LiAlH_4$, THF, 0 °C to rt, 30 min, 92%; (iii) PivCl, Et_3N, 4-DMAP, CH_2Cl_2, rt, 40 min, **15** (65%), **16** (27%) and **17** (8%).



Scheme 2. (i) Periodinane, CH_2Cl_2, rt, 50 min, 90%; (ii) $NaClO_2$, NH_2SO_3H, *t*-BuOH/H_2O (2:1) rt, 1 h, 82%; (iii) $LiEt_3BH$, THF, 0 °C, 1 h, 97%; (iv) DCC, 4-DMAP, CH_2Cl_2, rt, 1 h, 83%; (v) TBAF, THF, rt, 4 h, 80%; (vi) $PhCH_2OCOCl$, Et_3N, CH_2Cl_2, 0 °C, 1 h, 85%.



Scheme 3. (i) $ClCH_2COCl$, CH_2Cl_2, pyridine, −20 to 0 °C then 3.5 h, 80%; (ii) MeOH, pyridine, 40 °C, quant yield; (iii) Me_3SiCl, Et_3N, CH_2Cl_2, 50%; (iv) **26** (0.85 equiv) **22** (1 equiv), BF_3–Et_2O, CH_2Cl_2, −60 to −30 °C for 3 h then −10 °C for 2 h, **27** (40%) and **28** (10%); (v) $Zn(OAc)_2$, MeOH, reflux for 1.15 h, 80%; (vi) H_2, 10% Pd/C, MeOH, rt, 98%.

Et_3N. The β-anomer was obtained in pure form by crystallization of the crude reaction mixture.

Kuhn-like coupling[23] (BF_3–Et_2O, −30 °C) of **22** with **26** produced a mixture of the desired glucoside **27** in 40% yield with high stereoselectivity and the unsaturated retrolactone **28** (10%), both resulting from elimination (E1 vs S_N1) due to the acidity of the α-proton, and acid-catalyzed dehydration of **22**. Finally, deprotection of the sugar by methanolysis, followed by hydrogenation, generated retroetoposide **5**.[21]

Compounds **6** and **5** were tested against L1210 cell line: retrolactone **6** was almost devoid of cytotoxic effect with an $IC_{50} = 27.4\,\mu M$. For its part, retroetoposide **5** was less active ($IC_{50} = 6.74\,\mu M$) than etoposide **2** ($IC_{50} = 0.834\,\mu M$), whereas it remains active as an inhibitor of topoisomerase II[24] and blocks the L1210 cells at the G2/M transition (66% at 50 μM). Unlike several 4-β-*O*-*N*-[2(*N*′,*N*′-dialkylamino)alkyl] carbamate derivatives[25] of **6**, retroetoposide **5** displayed poor activity. We suggest that specific intramolecular interactions between the sugar moiety and the carbonyl group at C-11 due to their proximity may affect the conformation, and thus, account for the activity of **5**. Further syntheses of retro-4′-demethyl-4-epipodophyllotoxin analogues will be reported in due course.

Acknowledgements

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21. All newly-synthesized compounds gave satisfactory analytical and spectroscopic data.
6. Mp 129–131 °C (acetone/hexane); $[\alpha]_D^{20}$ −16 (*c* 1, $CHCl_3$); IR ($CDCl_3$) 3685, 3539, 1771 cm^{-1}; ^1H NMR: (300 MHz, $CDCl_3$) δ 6.94 (s, 1H); 6.50 (s, 1H), 6.03 (s, 2H), 5.99 and 5.95 (2 d, 1H, $J = 1.2$ Hz), 5.48 (br s, 1H), 5.14 (t, $J = 3.3$ Hz), 4.47 (t, 1H, $J = 8.2$ Hz), 4.35 (d, 1H, $J = 7.2$ Hz), 3.80 (s, 6H), 3.62 (dd, 1H, $J = 11.2$, 8.2 Hz), 3.34 (m, 1H), 2.79 (dd, 1H, $J = 14$, 3.3 Hz), 2.60 (d, 1H, $J = 3.3$ Hz). MS (DCI/NH_3) *m*/*z* 418 $(M + NH_4)^+$. Anal. calcd for $C_{21}H_{20}O_8$: C, 63.00; H, 5.03. Found: C, 62.86; H, 5.05.
5. Mp 161 °C; $[\alpha]_D^{20}$ −17.5 (*c* 1, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 6.87 (s, 1H,), 6.47 (s, 1H), 5.97–5.94 (m, 4H), 5.22 (d, 1H, $J = 2.2$ Hz), 4.70–4.68 (m, 2H), 4.55 (d, 1H, $J = 7.8$ Hz), 4.36–4.33 (m, 2H), 4.19 (dd, 1H, $J = 4.6$, 10.3 Hz), 3.76 (s, 6H), 3.67–3.61 (m, 2H), 3.54–3.39 (m, 3H), 3.32–3.20 (m, 2H), 1.33 (d, 3H, $J = 5$ Hz); MS (DCI/NH_3) *m*/*z* 606 $(M + NH_4)^+$. Anal. calcd for $C_{29}H_{32}O_{13}$: C, 59.18; H, 5.43; O, 35.34. Found: C, 59.31; H, 5.40; O, 35.27.
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24. Topoisomerase II-mediated DNA relaxation assay: Supercoiled pBS DNA (0.1 μg) was incubated for 15 min at 30 °C, in a 50 mM Tris–HCl buffer, pH 7.5, containing 1 mM ATP, 120 mM KCl, 10 mM $MgCl_2$, 0.5 mM DTT, 0.1 mM EDTA and 30 μg BSA, in the presence of the drug at 50 μM (total reaction volume 10 μL). Two units of human DNA topoisomerase II were added to the duplex, preincubated as described, and incubated for 30 min at 30 °C. The DNA–topoisomerase II cleavage complexes were dissociated by addition of SDS (final concentration 0.5%) and of proteinase K (Sigma) to 500 μg/mL, followed by incubation for 30 min at 55 °C. DNA samples were then added to the electrophoresis dye mixture (5 μL) and electrophoresed (35 V/cm) in a 1% agarose gel in TBEx1, containing ethidium bromide (1 μg/mL), at room temperature for 2 h. Gels were washed and photographed under UV light.
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