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SYNTHESIS AND ANTILEUKEMIC ACTIVITY OF ETOPOSIDE A-RING ANALOGS

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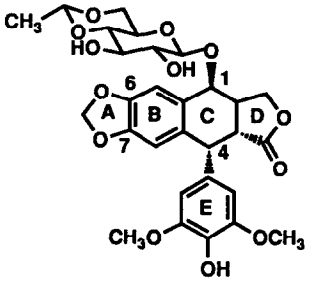
ABSTRACT: The A ring of the clinical antitumor agent, etoposide, was opened to a protected 6,7-diphenol which was derivatized to form a number of new analogs. These compounds displayed activity inferior to etoposide when evaluated against several tumor cell lines *in vitro* and against murine P388 leukemia *in vivo*.

Etoposide (VP-16, Vepesid) (**1**), is an antitumor agent currently in clinical use for the treatment of testicular and small cell lung cancer.[1] Etoposide is prepared from the naturally occurring cytotoxic lignan podophyllotoxin[2] (**2**) which is a potent inhibitor of microtubule assembly.[3] Sikkimotoxin (**3**), also a naturally occurring lignan [4], contains methoxy substituents at the 6 and 7 position rather than the methylenedioxy moiety present in **2**.



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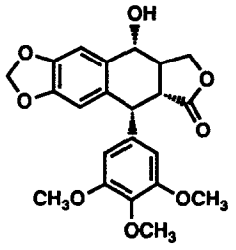

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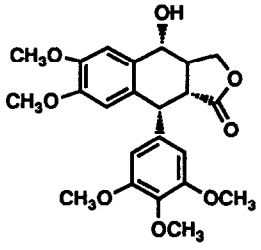

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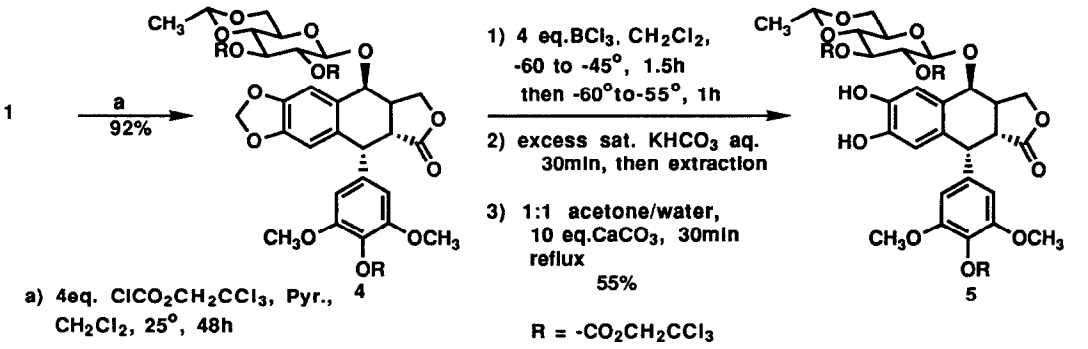
Sikkimotoxin has been shown to be less cytotoxic than podophyllotoxin and neither is useful for the treatment of human cancers.[5] In contrast, etoposide displays little antimitotic activity and apparently its cytotoxic effects are due to DNA strand scission.[6] Several mechanisms for this result have been suggested including inhibition of the enzyme topoisomerase II,[7,8] oxidation of the E ring to a reactive quinone,[9] and more recently, a direct, noncovalent interaction of etoposide with DNA has been proposed.[10] One possible explanation for such an interaction with DNA is that the A and B rings allow etoposide to be a weak intercalator. Since the biological targets of etoposide are significantly different than those of podophyllotoxin, we decided to determine how substituent changes in the A ring region of the molecule would affect antitumor activity.

Etoposide was protected as the tri-carbonate (**4**) in high yield (Scheme 1). The methylenedioxy group of **4** was detached with BCl_3 by modifying conditions used by Schreier in his sikkimotoxin

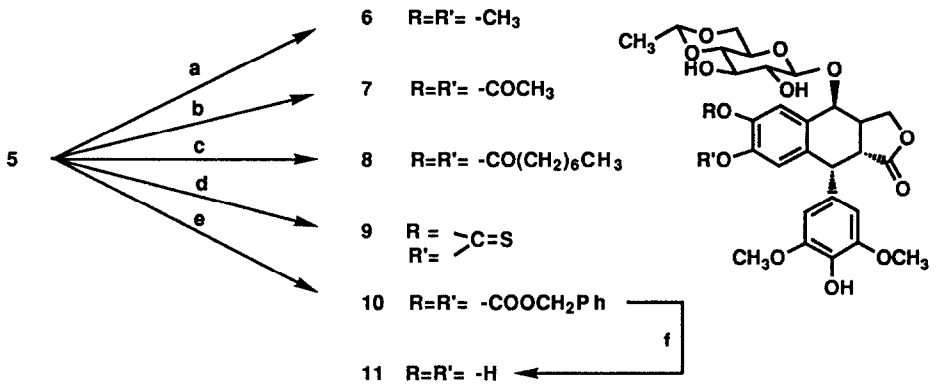
synthesis.[11] The correct temperature range during the BCl_3 cleavage reaction and adherence to the workup described in Scheme 1 are critical. The yield of **5** obtained from a reaction utilizing 20g of **4** was 55% after purification by flash chromatography over silica gel.

Scheme 1



Reaction of bisphenol **5** with excess diazomethane, acetic anhydride, octanoyl chloride, thiophosgene, or benzyl chloroformate followed by subsequent reductive deprotection (Zinc/Acetic acid) provided the corresponding dimethylether (**6**), diacetate (**7**), dioctanoate (**8**), cyclic

Scheme 2



a] i) excess CH_2N_2 in Et_2O, 1:1 THF: MeOH, 1eq. phenol, 25°, 48h; ii) reductive deprotection*; 43% overall. **b]** i) 3.5eq. Ac_2O, $EtN(iPr)_2$, CH_3CN, 25°, 4.5h; ii) reductive deprotection*; 24% overall. **c]** i) 2.2eq $CH_3(CH_2)_6COCl$, $EtN(iPr)_2$, CH_3CN, 25°, 25min; ii) reductive deprotection*; 73% overall. **d]** i) 1.2 eq. $Cl_2C{=}S$, $EtN(iPr)_2$, CH_3CN, 2°, 15min; ii) reductive deprotection*; 44% overall. **e]** i) 3.3eq $PhCH_2OCOCl$, $EtN(iPr)_2$, CH_3CN, 25°, 40min; ii) reductive deprotection*; 32% overall. **f]** i) 10% Pd/C, 30 PSI H_2, EtOH, 45min (82%).

*Reductive deprotection describes the following sequence: i) 1.4:1 dioxane/ AcOH, Zinc dust, 25°, 3-6h; ii) $CHCl_3$/ water; iii) SiO_2 flash chromatography.

thiocarbonate (**9**) and dibenzyl carbonate (**10**) respectively after flash chromatographic purification (Scheme 2).[12]

Attempted reductive deprotection of **5** provided only impure dihydroxy etoposide (**11**) which resisted further purification. Fortunately, hydrogenation of dibenzyl carbonate **10** provided an 82% yield of pure **11** after flash chromatography (Scheme 2).

The cell panel used for the *in vitro* cytotoxicity study of **5-11** included etoposide sensitive (HCT-116) and acquired-resistant (HCT-VP35) human colon carcinoma cell lines (Table 1). An XTT vital stain[13] was used to determine cell viability. The HCT-VP35 cell line was determined to have normal levels of 170K P-glycoprotein but diminished levels of topoisomerase II, thus suggesting that a multidrug resistance mechanism may not be operative.[14]. All of the analogs tested were found to be less potent than the parent compound. Interestingly, some of the analogs were able to partially, or completely, overcome resistance in the HCT-VP35 cell line, as evidenced by comparison of the ratio of (IC_{50} HCT-VP35) / (IC_{50} HCT-116) for the analogs compared to the same ratio for **1**. The analogs **9** and **11** had not reached an IC_{50} at the highest doses tested so no conclusions about their ability to overcome resistance can be drawn from this data. The decreased potency observed *in vitro* for a more polar etoposide analog such as **11** may be due to poor transport into the cell, as we have observed similar results with other, relatively polar, etoposide analogs.

Table I. In Vitro Cytotoxicity of Etoposide A Ring Analogs
IC_{50}, ug/mL[a]

Compound	HCT-116	HCT - VP35	Ratio[b]
(**1**)	0.34	1.78	5.2
(**5**)	10.22	8.95	0.87
(**6**)	1.61	10.33	6.4
(**7**)	19.57	27.07	1.4
(**8**)	8.03	21.67	2.7
(**9**)	>15.6	>15.6	----
(**10**)	5.80	11.8	2.0
(**11**)	>15.6	>15.6	----

[a] The IC_{50} is the dose that reduces by 50%, after 72h, cell growth *in vitro* as compared to controls. IC_{50} values of etoposide in the same assay are shown in parentheses. All values indicated are the average of at least two runs. [b] Ratio of (IC_{50} HCT-VP35)/ (IC_{50}HCT-116).

The *in vivo* P388 leukemia data for compounds **5-9** and **11** are summarized in Table 2. Descriptions of the protocol for these experiments have previously been described in detail.[15] Significant activity in the P388 model is described as a T/C of > 125% and refers to the percent of the median survival time of drug treated mice compared to saline treated controls.

Although several of the analogs retained significant activity, all of the analogs prepared were less potent than **1**, and less active at the doses tested. The doses of the analogs were always

Table II. Anti-P388 Leukemia[a] Activity of Compounds 5-9 and 11.

Compound	dose,[b] mg/kg per inj	T/C [c]	Data for etoposide (1) T/C (LTS)[d] day, mg/kg/day
5	300 150 75	105 100 100	>480 (5/6), day 48,150
6	280 140 70	228 189 189	>567 (4/6), day 51,150
7	200 100 50	194 167 144	>567 (4/6), day 51,150
8	300 150 75	135 125 120	>480 (5/6), day 48,150
9	300 150 75	170 155 130	>260 (6/6), day 26,120
11	300 150 75	165 130 125	>320 (2/6), day 53,120

[a] P388 leukemia, 10^6 cells, were implanted ip on day-0 into CDF_1 mice. [b] Administered ip on days 5 and 8. [c] T/C refers to the percent of the median survival time of drug treated mice compared to saline treated controls. [d] Long -term survivors (LTS) were (mice alive/total) on the day indicated in the next column. .

higher (in most cases 2 fold) than the maximum tolerated dose of etoposide. None of the analogs tested had long term survivors on the last day the various experiments were evaluated. In contrast, **1** always had at least two long term survivors and frequently the majority of the mice were surviving at the optimum dose.

The 6,7-dimethoxy, and diacetoxy analogs (**6,7**) retained the most activity while the 6,7 dihydroxy tricarbonate (**5**) was completely inactive. Protection of the two sugar hydroxy groups and the E ring phenol as lipophilic carbonates significantly changes the polarity of the drug, which may account for the reduced potency *in vitro* and the total loss of *in vivo activity* observed for compound **5**. This analog is actually slightly more cytotoxic to the etoposide resistant cell line than to the etoposide sensitive line in the *in vitro* assay. The bis octyl carbonate **8** is also more lipophilic than the parent **1** and also exhibited a significant loss of *in vivo* activity. Analog **8** however, displayed decreased potency against the resistant cell line relative to the sensitive line. It is interesting to note that when two lipophilic carbonate substituents are attached to the 6,7-positions of the A-ring, as in **8** and **10**, the *in vitro* profile still resembles etoposide but when the three lipophilic substituents are attached to the pendant E-ring phenol and the two sugar hydroxy groups, as in compound **5**, there is little difference in potency between the etoposide resistant and sensitive cell lines.

In conclusion, the methylenedioxy A ring of etoposide is not essential for antitumor activity but is important for optimal activity. Use of these intermediates to synthesize analogs which have the potential to be improved DNA intercalators is in progress.

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8. The structural elements which are important for the proposed simultaneous interaction of etoposide and the enzyme topoisomerase II with DNA are undefined at the molecular level. An *in vitro* study examining the interaction of these and other etoposide analogs with purified topoisomerase II is essentially complete and will be submitted for publication in the near future by B.H. Long.
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12. All new compounds in this communication gave satisfactory analytical and spectroscopic data in full accord with their structures. Select 300 MHz NMR data for some compounds is included below. Tricarbonate **5**: ^{1}H NMR ($CDCl_3$) d 6.91 (s, 1H), 6.61 (s, 1H), 6.30 (s, 2H), 6.16 (bs, 2H), 5.20 (t, J=9.0Hz, 1H), 5.02-4.60 (m, 11H), 4.45 (t, J=9.7Hz, 1H), 4.19 (m, 2H), 3.72 (s, 6H), 3.63 (t, 11.7Hz, 1H), 3.47 (m, 1H), 3.25 (dd,13.8, 4.8Hz, 1H), 2.90 (m,1H), 1.39 (d, 4.2Hz, 3H). Dimethyl ether **6**: ^{1}H NMR (dmso-d6) d 8.24 (s, 1H,-OH), 6.97 (s,1H), 6.55 (s,1H), 6.15 (s, 2H), 5.25 (dd, 11.8,5.0 Hz, 4.7Hz, 1H), 4.95 (m,1H), 4.71 (m,1H), 4.49 (m, 2H), 4.27 (m, 2H), 4.07 (m, 1H), 3.78 (s, 3H), 3.61 (s, 3H), 3.58 (s, 6H), 3.52 (t, J=9.9Hz,

1H), 3.32 (bs, 2H, -OH), 3.32-3.10 (m,3H), 3.06 (m, 1H), 2.87 (m, 1H), 1.22 (d, J=4.8Hz, 3H). Diacetate **7**: ^{1}H NMR ($CDCl_3$) d 8.23 (s,1H, -OH), 7.32 (s, 1H), 6.08 (s, 2H), 5.22 (m, 2H,-OH) 4.98 (d, J=3.2Hz, 1H), 4.66 (m, 1H), 4.58 (d, 5.12Hz, 1H), 4.44 (d, J=7.3Hz, 1H), 4.23 (d, J=9.1Hz, 2H), 4.01 (dd, J=10.1, 4.4Hz, 1H), 3.53 (s, 6H), 3.52-3.07 (m, 4H), 3.27 (bs, 2H, -OH), 3.02 (m, 1H), 2.87 (m, 1H), 2.24 (s, 3H), 1.18 (d, J=5.0Hz, 3H).

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