ketonucleoside is also a weak, competitive inhibitor of AdoHcy hydrolase and does not produce time-dependent inactivation of the enzyme.

The data presented above, as well as the observation in our laboratory that AdoHcy hydrolase inactivated by NpcA or the synthetic analogue of NpcA can be reactivated by incubation with NAD^+,[6,15] strongly suggest that these inhibitors inactivate the enzyme by a cofactor depletion mechanism, which simply involves conversion of the enzyme from the NAD^+ form (catalytically active) to the NADH form (catalytically inactive), and tight-binding of the 3′-ketoNpcA to this NADH form. These data do not support the k_{cat} mechanism proposed by Wolfson et al.[7] for the NpcA-induced inactivation of AdoHcy hydrolase.

Other AdoHcy hydrolase inactivators may act through similar mechanisms to that of NpcA. For example, 4′,5′-unsaturated 5-fluoroadenosine inactivators convert enzyme-bound NAD^+ to NADH.[19] The subsequent loss of fluoride anion in this case may be due to Michael addition of water, forming a product which has a high affinity for the NADH form of AdoHcy hydrolase. Further studies on the mechanism of AdoHcy hydrolase inactivators should afford the opportunity to rationally design potent and selective inhibitors of this enzyme.

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E-Ring Desoxy Analogues of Etoposide

Sir:

Etoposide (1, VP-16, Vepesid), a clinically efficacious antineoplastic drug used for the treatment of testicular cancer and one of the most active single agents against small cell lung cancer, is the least toxic of all chemotherapeutic agents.[1] As a semisynthetic derivative of the naturally occurring lignan podophyllotoxin,[2] a potent inhibitor of microtubule assembly,[3] VP-16 apparently exerts its cytotoxic effect by DNA strand scission, retaining little of the antimitotic potency of its aglycon. In 1983, independent studies by Ross[4] and Long[5] demonstrated that DNA breakage is associated with etoposide-induced DNA/protein cross-links and correlates with both the cytotoxicity and the ability of several analogues to inhibit the catenation activity of eukaryotic topoisomerase II (topo II). During this same period, the investigations of Sinha[6] pointed to the relevance of the etoposide *o*-quinone **2** in the mechanism of action, since VP-16 gives rise to a stable phenoxy radical following in vitro peroxidative activation, and because the intermediate formed during microsomal activation irreversibly binds to both nucleic acids and proteins. Sinha's later bioactivation studies[7] have confirmed this *o*-quinone and identified the E-ring phenoxy radical by ESR spectroscopy. Liu and co-workers[8] have found that inhibition of topo II by VP-16 blocks DNA religation by stabilizing the initially formed cleavable complex, thereby leading to DNA scission. Early structure–activity relationship (SAR) data revealed that a free 4′-hydroxyl group in **1** was essential for DNA breakage[9] and antitumor activity,[10] whereas 4′-methoxy derivatives were mostly inactive. A free 4′-phenol may confer both hydrogen-bonding capability for interaction with topo II and/or DNA and also would greatly enhance the biooxidation process. Therefore, in order to probe the significance of the *o*-quinone and topo II based mechanisms, we now report the first synthesis and biological evaluation of etoposide desoxy E-ring analogues **6**, **7**, and **9**.[11]

The synthesis of the title compounds was achieved by using both total and semisynthetic techniques. Oxidation of etoposide (**1**) to *o*-quinone **2** using the method of Nemec,[12] followed by condensation with methoxylamine hydrochloride in pyridine, regioselectively delivers monoquinone oxime **3**. Hydrogenolysis of **3** gives aminophenol **4** (70% overall), which yields diazo phenoxide **5** (79%) after treatment with $NaNO_2$ in HOAc/THF. Reduction of **5** ($NaBH_4$/MeOH, 41%) provides 3′-desmethoxyetoposide (**6**, 3′-DesMeOVP). Efficient preparation of 4′-deshydroxyetoposide (**7**, 4′-DesOHVP) proceeded via

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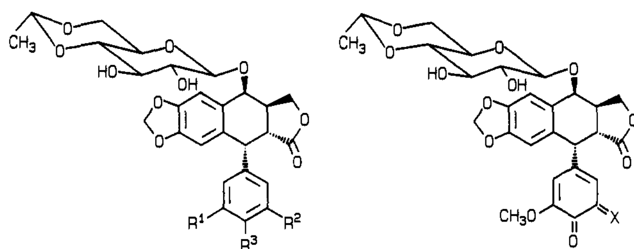

1; R^1=R^2=OCH_3, R^3=OH (VP-16)
4; R^1=OCH_3, R^2=NH_2, R^3=OH
5; R^1=OCH_3, R^2=N_2^+, R^3=O^-
6; R^1=OCH_3, R^2=H, R^3=OH (3'-DesMeOVP)
7; R^1=R^2=OCH_3, R^3=H (4'-DesOHVP)
8; R^1=R^2=OCH_3, R^3=OSO_2CF_3
9; R^1=R^2=H, R^3=OH (3',5'-DidesMeOVP)

2; X=O
3; X=$NOCH_3$

high-pressure hydrogenation (20% $Pd(OH)_2$/C, $NaHCO_3$, EtOAc/MeOH, 1200 psi H_2, 60 °C; 80%) of the corresponding 4'-triflate **8** readily derived from etoposide (Tf_2NPh, EtN(*i*-Pr)$_2$, CH_3CN, 40 °C, 192 h, 96%). Finally, we resorted to a total synthesis of the final target, 3',5'-didesmethoxyetoposide (**9**, 3',5'-DidesMeOVP), which was available from the corresponding (±)-3',5'-didesmethoxy-4'-demethyl-4'-*O*-benzylepipodophyllotoxin, itself prepared in analogous yield and fashion as we described for the parent system.[13] Glycosylation of this racemic aglycon with 4,6-*O*-ethylidene-2,3-bis-*O*-[(*β*,*β*,*β*-trichloroethoxy)carbonyl]-*β*-*d*-glucopyranose ($BF_3{\cdot}Et_2O/CH_2Cl_2$/–20 °C) gave a 60:40 diastereomeric mixture in favor of the *d*-gluco-*l*-epipodophyllotoxin (VP-16 related) versus the *d*-gluco-*d*-epipodophyllotoxin diastereomer in 85% total yield and easily separable by silica gel chromatography. Removal of the protecting groups (zinc dust, HOAc/THF, 96%; 20% $Pd(OH)_2$/C, 750 psi H_2, 30 °C, 20 h, 93%) provided pure **9**. Analogous transformation of the *d*-gluco-*d*-epipodophyllotoxin diastereomer into the fully deprotected inactive isomer followed by comparison with **9** and etoposide using CD and ^1H and ^{13}C NMR provided unambiguous structure confirmation.

A summary of the in vivo P388 leukemia data for compounds **1**, **2**, **6**, **7**, and **9** is given in Table I. Detailed descriptions of the protocol for these experiments have been described.[14] Significant activity in the P388 model is defined 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. Since VP-16 itself is often exceptionally active in this assay, it was sometimes administered on day 5 instead of day 1. Inspection of the P388 data clearly reveals that, of the 3 desoxy analogues **6**, **7**, and **9**, only **6** (3'-DesMeOVP) approached the potency and activity of VP-16. Althugh the activity of **7** (4'-DesOHVP) and **9** (3',5'-DidesMeOVP) was significant (max % T/C = 175), these analogues were both less potent than VP-16, nontoxic at the highest doses tested (which were ≥2-fold greater than the maximum tolerated dose of VP-16), and less active at the doses evaluated.

The cell panel used for our in vitro cytotoxicity study included etoposide sensitive (HCT-116), acquired-resistant (HCT/VP35), and inherently resistant (MOSER) human

Table I. Anti-P388 Leukemia Activity of Compounds **1**, **2**, **6**, **7**, and **9**

expt no.	compd	dose,[b] mg/kg per inj	T/C (LTS)[a]	max T/C (LTS)[a] for 1: dose, mg/kg per inj	ratio compd/1
1	2	120	447 (1/4)		
		80	>621 (3/4)	>595 (2/4), 60[b]	~1.0
		40	274		
2	6	60	235		
		40	245	250 (1/6), 30[b]	~1.0
		20	190		
3	7	150	175	280, 60[c]	<1.0
		30	125		
4	9	200	175	230, 100[c]	<1.0
		50	145		

[a] T/C refers to the percent of the median survival time of drug-treated mice compared to saline-treated controls. Long-term survivors (LTS) were mice alive/total on day 30 when the experiment was terminated. [b] Administered ip on days 1 and 5. [c] Administered ip on days 5 and 8.

Table II. In Vitro Cytotoxicity of Etoposide Desoxy E-Ring Analogues

compd	IC_{50},[a] μg/mL HCT-116	B16-F10	MOSER	HCT/VP35	HCT/VP35 / HCT-116
6	15.4 (4.4)	20.1 (5.1)	46.5 (52)	5.4 (4.7)*[b]	12.3 (11.0)*
7	7.3 (2.4)	25 (2.3)	45.5 (38.5)	3.4 (35.5)	0.47 (14.8)
9	62.7 (7.9)	43.4 (10.4)	42.9 (25.6)	85.1 (34.0)	1.4 (4.3)

[a] The IC_{50} is the dose that reduces by 50%, after 72 h, 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. The crystal violet assay was used except where indicated by an asterisk.[15] [b](*) The XTT assay[16] was employed and the IC_{50} values for the HCT-116 cell panel were 0.44 (0.43) for the analogue (etoposide).

colon tumor lines, as well as a VP-16 sensitive mouse melanoma line (B16-F10). A crystal violet protein stain[15] or the XTT vital stain[16] were employed to determine cell viability, and the IC_{50} ratio of HCT/VP35 to HCT-116 shown in Table II reflects the ability of an anglogue to overcome the VP-16 aquired resistance in the HCT/VP35 line. This cell line has normal levels of the 170K P-glycoprotein but diminished levels of topo II[17] so apparently the mechanism of resistance is related to the latter and does not indicate a multidrug-resistance mechanism.[18] While **9** (3',5'-Dides-MeOVP) shows significantly decreased potency relative to VP-16 in all the cell lines, and **6** (3'-DesMeOVP) is similar to the parent drug, **7** (4'-DesOHVP) shows excellent potency in both HCT cell lines and greatly overcomes the resistance in topo II deficient HCT/VP35. Perhaps related to these phenomena, we found that, using purified topo II, 4'-DesOHVP was inactive at the upper limit of the assay (100 μM) in its ability to inhibit the unknotting reaction using P4 phage DNA.[19] Both **6** and **9** showed comparable activity to VP-16 in this regard.

With use of alkaline elution techniques,[8b,10] which presumably measure the ability of a compound to elicit topo II mediated DNA strand breaks in a whole cell assay, **6** (3'-DesMeOVP) was almost as potent as VP-16, whereas **9** (3',5'-DidesMeOVP) and **7** (4'-DesOHVP) showed 40-

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and 25-fold less potency, respectively. Since it is unlikely that 7 can be biooxidized to an *o*-quinone, and because it does not inhibit purified topo II (vide supra), we postulate that its DNA breakage, cytotoxicity, and in vivo P388 antitumor activity may be due to a previously undescribed third mechanism of action.

Preliminary data from our laboratory indicate that etoposide binds to purified calf thymus DNA.[20] Subsequent to our findings, a recent report by Ross[21] also describes DNA binding by VP-16. The cytotoxicity and in vivo antitumor activity of these new analogues, and presumably etoposide as well, cannot be entirely due to the *o*-quinone mechanism. While topo II inhibition certainly is part of the in vitro mechanism of action, the comparable P388 activity of **6** (3′-DesOMeVP) and etoposide, coupled with the diminished activity of **7** (4′-DesOHVP) and **9** (3′,5′-DidesMeOVP), which are not capable of easy bioactivation to *o*-quinones, implicates the in vivo importance of the *o*-quinone mechanism. Furthermore, a free 4′-OH does not appear to be essential for DNA breakage or cytotoxicity but seems to be critical for topo II inhibition. Since **7** does not inhibit purified topo II and yet still exhibits good in vitro cytotoxic potency and ability to overcome the VP-16 aquired resistance of a topo II deficient cell line, it is likely that a third mechanism of action for **7** at least, and probably for VP-16 as well, that could relate to direct DNA binding, may be operative in the complete pharmacological profile of these agents.[22]

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(22) The DNA binding by etoposide may itself contribute to the overall cytotoxicity, perhaps via inhibition of DNA polymerase. Consideration of such possibilities should be the subject of future research, the results of which may contribute to the design of second-generation etoposide analogues endowed with better activity against resistant tumors.

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Articles

Synthesis, Cytotoxicity, and Antiviral Activity of Certain 7-[(2-Hydroxyethoxy)methyl]pyrrolo[2,3-*d*]pyrimidine Nucleosides Related to Toyocamycin and Sangivamycin

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A number of 7-[(2-hydroxyethoxy)methyl]pyrrolo[2,3-*d*]pyrimidine derivatives related to the nucleoside antibiotics toyocamycin and sangivamycin were prepared and tested for their biological activity. Treatment of the sodium salt of 4-amino-6-bromo-5-cyanopyrrolo[2,3-*d*]pyrimidine (**1**) with (2-acetoxyethoxy)methyl bromide (**2**) afforded a mixture of 4-amino-6-bromo-5-cyano-7-[(2-acetoxyethoxy)methyl]pyrrolo[2,3-*d*]pyrimidine (**3**) and the corresponding N_1 isomer. Debromination of this mixture gave the corresponding 4-amino-5-cyano-7-[(2-acetoxyethoxy)-methyl]pyrrolo[2,3-*d*]pyrimidine (**4**) and 4-amino-5-cyano-1-[(2-acetoxyethoxy)methyl]pyrrolo[2,3-*d*]pyrimidine (**5**). Deacetylation of **4** and **5** furnished 4-amino-5-cyano-7-[(2-hydroxyethoxy)methyl]pyrrolo[2,3-*d*]pyrimidine (**6**) and the corresponding N_1 isomer (**7**), respectively. The sites of attachment for the acyclic moiety for **6** and **7** were assigned on the basis of UV spectral studies as well as ^{13}C NMR spectroscopy. Conventional functional group transformation of **6** provided a number of novel 5-substituted derivatives (**8**–**10**), including the sangivamycin derivative **8**. The methyl formimidate derivative **10** was converted to the thioamide derivative **11** and the carbohydrazide derivative **12**. Compounds **6** and **8**–**12** were tested for cytotoxicity to L1210 murine leukemic cells in vitro. None of these compounds caused significant inhibition of cell growth. Evaluation of compounds **4** and **6**–**12** for activity against human cytomegalovirus (HCMV) and herpes simplex virus type 1 (HSV-1) revealed that only the thioamide (**11**) was active. It inhibited HCMV but not HSV-1 at concentrations producing only slight cytotoxicity in human foreskin fibroblasts (HFF cells) and KB cells.

The pyrrolo[2,3-*d*]pyrimidine ribonucleoside antibiotics toyocamycin and sangivamycin have exhibited an interesting range of biological effects, including antitumor[1-7] and antiviral activity.[7-12] The antineoplastic activity of toyocamycin appears to be mediated by its incorporation

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