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ANTI-AIDS AGENTS. 29.[1] ANTI-HIV ACTIVITY OF MODIFIED PODOPHYLLOTOXIN DERIVATIVES

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Abstract: Podophyllotoxin derivatives containing structural modifications at C-4 and in the methylenedioxy A-ring, lactone D-ring, and phenyl E-ring have been tested for inhibition of HIV replication. The four most promising compounds (**6**, **7**, **8**, and **19**), with the methylenedioxy A-ring opened and methylated and the 4′-position demethylated, had EC_{50}'s less than 0.001 μM and therapeutic indices greater than 120.

Podophyllum peltatum, commonly known as the American mandrake or Mayapple, and the related Indian species *Podopyllum emodi* have been used medicinally for centuries. Podophyllotoxin (**2**) is a bioactive lignan isolated from these plant sources, and this compound has been the focus of extensive chemical modification leading to clinically useful anticancer drugs, for example, etoposide (**1**). This latter compound varies both in structure and mechanism of action from podophyllotoxin. Structurally, etoposide has an OH instead of an OMe at C-4′ and is glycosylated with the opposite stereochemistry at C-4. Mechanistically, etoposide inhibits the enzyme DNA topoisomerase II and, subsequently, increases DNA cleavage, while the potent antimitotic agent podophyllotoxin inhibits microtubule assembly by binding reversibly to tubulin, an essential protein in cell division, and preventing tubulin polymerization. Furthermore, with etoposide, bio-oxidation to an E-ring ortho-quinone can be linked to covalent binding to proteins, and metal- and photo-induced cleavage of DNA can be linked to hydroxy radical formation by metal-etoposide complexes.[2]

In our continuing studies on the synthesis and biological evaluation of podophyllotoxin- and etoposide-related derivatives, we have made several series of C-4 modified podophyllotoxins bearing various substituents

including alkyl- and aryl-amino groups, oxy and thio ethers, and ketones.[3–8] Other structural changes have been made in the methylenedioxy group of the A-ring, the C-ring and the lactone D-ring.[9–11] To investigate the range of biological activities of the podophyllotoxin/etoposide compound class, we report herein the initial anti-HIV results for these and related compounds.

The compounds tested are shown in Figure 1, and the biological data are shown in Table 1.[12] The parent compound **2** was cytotoxic at all concentrations tested. Podophyllotoxin derivatives **3**, **4**, and **9**, which all contain a C-4 hydroxy group, did not suppress HIV replication. Other structural variants that either did not show any antiviral inhibition or were active only at cytotoxic levels included the diamino instead of the dimethoxy substitution in the E-ring (**25**), a ketone (**16**, **17**), diethylamine hydrochloride (**10**), diethylaminoethylamine (**11**),

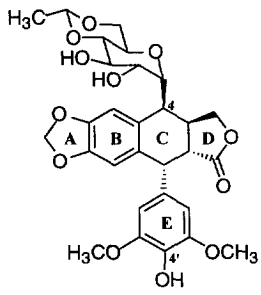
Table 1. Anti-HIV Activity of Podophyllotoxin Derivatives.

Compound	Anti-HIV Activity EC_{50} (μM)	Cytotoxicity IC_{50} (μM)	Therapeutic Index (TI = IC_{50}/EC_{50})
1	0.03	1.27	42.7
2	----	Toxic at all concentrations	----
3	No suppression	1.85	----
4	No suppression	45	-----
5	0.16	0.32	2
6	<0.001	0.141	>141
7	<0.001	0.120	>120
8	<0.001	0.158	>158
9	No suppression	0.07	----
10	No suppression	>100	----
11	10	8	0.8
12	No suppression	1.9	----
13	0.8	1.7	2.1
14	0.45	0.8	1.8
15	<0.00128	0.07	55.8
16	0.8	1.7	2.1
17	3	9	3
18	2.2	9.7	4.4
19	<0.001	0.166	>166
20	7	29	4.1
21	0.1	1.0	10
22	0.45	1.5	3.3
23	0.45	1.8	4
24	0.12	1.5	12.5
25	1.7	6.6	3.9
AZT	0.009	500	55,556

ethylthio (**5**, **12**), *p*-hydroxyphenylthio (**13**), or *p*-fluorophenyoxy (**14**) group instead of a hydroxy group at C-4.

Seven compounds (**18**–**24**) contain a C-4 *p*-fluoroanilino group along with variations in either the phenyl E-ring (**22**, trimethoxy), the lactone ring (**21**, lactone vs. **23**, lactol vs. **24**, cyclic ether), or the methylenedioxy A-ring (**18**, dimethoxy vs. **22**, methylenedioxy; **19**, dimethoxy vs. **20**, dihydroxy). Except for this *p*-fluoroanilino group, compound **22** was identical in structure to podophyllotoxin. Although **22** was active in the HIV assay, it had a TI

Figure 1. Structures of Compounds **1**–**25**.



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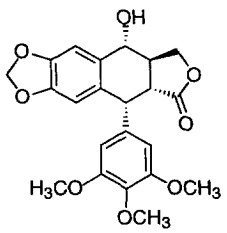

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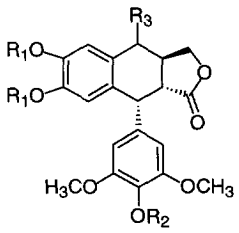
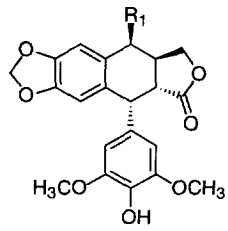
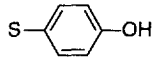
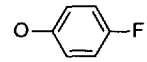
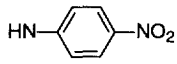
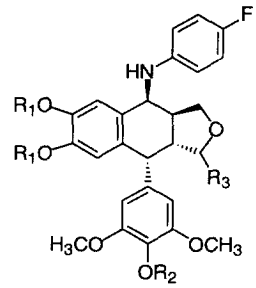


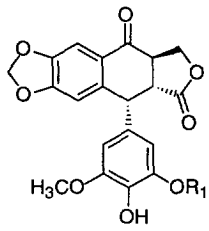
Compound	R_1	R_2	R_3
3	H	H	OH
4	H	H	OH
5	-CH_2-	CH_3	SEt
6	CH_3	H	HN—⟨⟩—CN
7	CH_3	H	HN—⟨⟩—CO_2Et
8	CH_3	H	HN—⟨⟩—NO_2



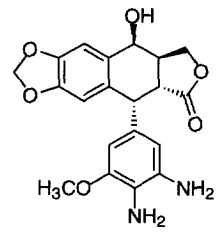
Compound	R_1
9	OH
10	$NEt_2 \cdot HCl$
11	$NH(CH_2)_2NEt_2$
12	SEt
13	
14	
15	



Compound	R_1	R_2	R_3
18	CH_3	CH_3	=O
19	CH_3	H	=O
20	H	H	=O
21	- CH_2 -	H	=O
22	- CH_2 -	CH_3	=O
23	- CH_2 -	H	OH
24	- CH_2 -	H	H



Compound	R_1
16	CH_3
17	H



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value of only 3.3. Converting the A-ring methylenedioxy to a dimethoxy substitution (**18**) decreased both activity and cytotoxicity. Compound **21**, the C-4′ demethylated analog of **22**, had a threefold higher TI value. With this 4′-hydroxy, 3′,5′-dimethoxy substituent pattern, the activity and cytotoxicity did not vary significantly when the lactone ketone (**21**) was either reduced to a hydroxyl (**23**) or replaced by a methylene (**24**). While compound **20**, which contains dihydroxy substitution in the A-ring, showed the lowest activity in the *p*-fluoroanilino series, the corresponding derivative **19**, which has two methoxy groups at these positions, was quite active in the HIV

inhibition assay (EC_{50} < 0.001 μM) and also had a good therapeutic index (>166). Several additional arylamino substituted compounds were prepared and tested including *p*-cyano- (**6**), *p*-ethylcarbonate- (**7**), and nitro- (**8**) anilino compounds. All of these compounds had a similar activity to **19** (EC_{50} values <0.001 μM) with therapeutic indices all greater than 120.

In summary, modified podophyllotoxin derivatives have demonstrated an anti-HIV activity. The data for compounds **6**, **7**, **8**, and **19**, with the A-ring opened, methylated and the 4′-positon demethylated, are encouraging and warrant further structural modification to both decrease cytotoxicity and increase antiviral inhibitory activity. Further biological evaluation is in progress to better define the anti-HIV activity of these compounds.

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12. **Anti-HIV Assay.** The T cell line, H9, was maintained in continuous culture with complete medium (RPMI 1640 with 10% fetal calf serum supplemented with L-glutamine at 5% CO_2 and 37 °C). Aliquots of this cell line were only used in experiments when in log-phase growth. Test samples were first dissolved in dimethyl sulfoxide. The following final drug concentrations were routinely used for screening: 100, 20, 4 and 0.8 μg/mL.

For active agents, additional dilutions were prepared for subsequent testing so that an accurate EC_{50} value (defined below) could be achieved. As the test samples were being prepared, an aliquot of the H9 cell line was infected with HIV-1 (IIIB isolate) while another aliquot was mock-infected with complete medium. The stock virus used for these studies typically had a $TCID_{50}$ value of 10^4 Infectious Units/mL. The appropriate amount of virus for a multiplicity of infection (moi) between 0.1 and 0.01 Infectious Units/cell was added to the first aliquot of H9 cells. The other aliquot only received culture medium, and these mock-infected cells were used for toxicity determinations (IC_{50}, defined below). After a 4 h incubation at 37 °C and 5% CO_2, both cell populations were washed three times with fresh medium and then added to the appropriate wells of a 24 well-plate containing the various concentrations of the test drug or culture medium (positive infected control/negative drug control). In addition, AZT was also assayed during each experiment as a positive drug control. The plates were incubated at 37 °C and 5% CO_2 for 4 days. Cell-free supernatants were collected on Day 4 for use in our in-house p24 antigen ELISA assay. P24 antigen is a core protein of HIV and therefore is an indirect measure of virus present in the supernatants. Toxicity was determined by performing cell counts by a Coulter Counter on the mock-infected H9 cells which had either received culture medium (no toxicity), test sample, or AZT. If a test sample had suppressive capability and was not toxic, its effects were reported in the following terms: IC_{50}, the concentration of test sample which was toxic to 50% of the mock-infected H9 cells; EC_{50}, the concentration of the test sample which was able to suppress HIV replication by 50%; and Therapeutic Index (TI), the ratio of IC_{50} to EC_{50}.

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