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Synthesis and anti-HIV-1 activities of novel podophyllotoxin derivatives

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Abstract—In order to explore the range of biological activities of the podophyllotoxin compound class, a novel series of derivatives of podophyllotoxin, which were conjugates containing stavudine and different structural podophyllotoxin analogues, were designed, synthesized, and evaluated for their anti-HIV-1 activities in vitro. Among these compounds, **19d** and **19c** showed the highest anti-HIV-1 activities with EC_{50} values of 0.17 and 0.29 μM and TI values of 466.9 and 354.5, respectively.

In the past two decades, a worldwide search has been made for new chemotherapeutic agents targeting the human immunodeficiency virus (HIV), the causative agent of acquired immune deficiency syndrome (AIDS). Nineteen drugs, including nucleoside/nucleotide viral reverse transcriptase (RT) inhibitors (NRTIs), nonnucleoside RT inhibitors (NNRTIs), protease inhibitors (PIs), and fusion (or entry) inhibitors (FIs), are now approved for clinical use in the world.[1] However, these drugs have only limited or transient clinical benefit due to their side effects and the development of drug-resistant viral strains.[2] Therefore, current searches for new anti-HIV agents are focused on discovering compounds with novel structures and different mechanisms of action.[3]

Podophyllotoxin (**1**) as well as its congeners and derivatives exhibit pronounced biological activity mainly as strong antiviral agents and as antineoplastic drugs.[4] The podophyllotoxin derivatives etoposide (VP-16, **2**), teniposide (VM-26, **3**) and Etopophos (etoposide phosphate, **4**) are thus successfully utilized in treatment of a variety of cancers, including small-cell lung cancer, testicular carcinoma, lymphoma, and Kaposi's sarcoma (Fig. 1).[5] In addition, there are GL-331,[6] NK-611,[7] TOP53[8] and Fl 1782[9] in clinical trail for treatment kinds of tumors. As to mechanism, podophyllotoxin inhibits the assembly of tubulin into microtubules through interaction with protein at the colchicine binding site, preventing the formation of the spindle. While etoposide and congeners induce a premitotic blockade in late S stage of the cell cycle because of the inhibition of DNA topoisomerase II (Top II), an enzyme required for the unwinding of DNA during replication.[4,5] Other than their anti-cancer activity, podophyllotoxin was used as antiviral agent in treatment of herpes simplex type I and condyloma acuminatum caused by human papilloma virus and other venereal and perianal warts.[10] Lee's group recently reported their initial anti-HIV results for modified podophyllotoxin derivatives.[3,11]

Stavudine (2′,3′-didehydro-2′,3′-dideoxy-thymidine, d4T, **5**) is an anti-HIV treatment in the class of drugs called nucleoside reverse inhibitors. However, stavudine may cause some serious side effects, including peripheral neuropathy, tingling, burning, numbness, and pain in the hands or feet.[12]

To explore the range of biological activities of the podophyllotoxin compound class, we have synthesized a

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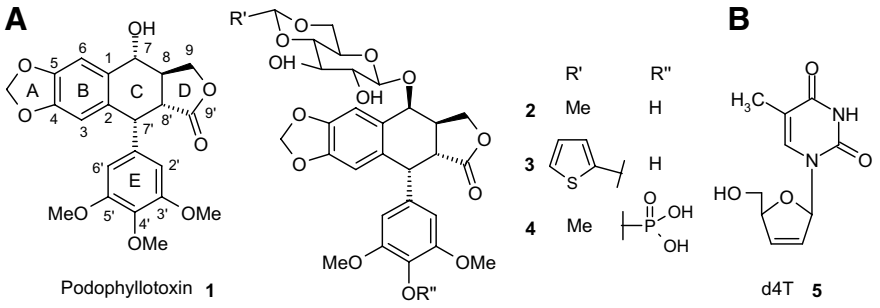


Figure 1. Podophyllotoxin, Etoposide, Teniposide, Etopophos, and Stavudine.

novel series of derivatives of podophyllotoxin, which were conjugates containing stavudine and different structural podophyllotoxin analogues, and evaluated their anti-HIV-1 activities in vitro. The preliminary results showed anti-HIV-1 activities of some synthesized compounds are more distinct compared to those of podophyllotoxin.

Compound **9** was synthesized as shown in Scheme 1, the thymidine **6** was dimesylated and treated with aqueous sodium hydroxide to afford **8** in 70% yield. Upon treatment with sodium hydrogenate (NaH) in DMF, this yielded **5** in good yield.[13] The latter was converted into its activated derivative **9** by treatment with *p*-nitrophenylchloroformate in the presence of pyridine (Scheme 1).[14]

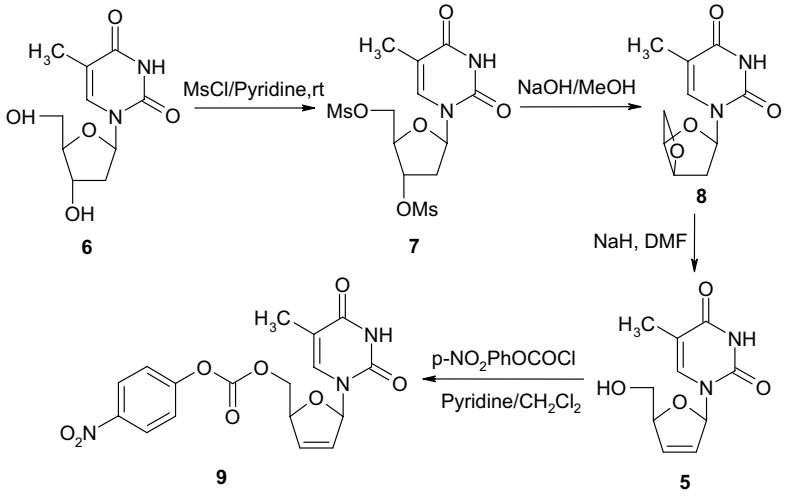
Preparation of 7-azido-compounds **11**–**14** and **17** is shown in Scheme 2. Podophyllotoxin **1** was first brominated and regioselectively 4′-demethylated by treatment with dried HBr and barium carbonate to afford **10** in 63% overall yield.[15] The intermediate compound **16** was prepared from **10** in 83% yield by use of a modified literature procedure.[16] Boron trichloride has been used in the selective cleavage of the methylenedioxy group in the presence of aromatic methoxy group, followed by mild basic hydrolysis with barium carbonate ($BaCO_3$). Methylation of **15** was prepared in 92% yield using freshly prepared CH_2N_2.

Preparation of 7-azido-compounds **11**–**14** and **17** was according to the literature.[17] In the presence of Lewis acid, such as boron trifluoride etherate ($BF_3{\cdot}Et_2O$), trifluoroacetic acid (TFA), the reaction of the SN_1 nucleophilic substitutions occurred, 7β-substituents were major products. However, the main products resulting from the reaction of 7-bromo-podophyllotoxin and its 4′-*O*-demethyl compounds with sodium azide (NaN_3) were 7α-azido derivatives.

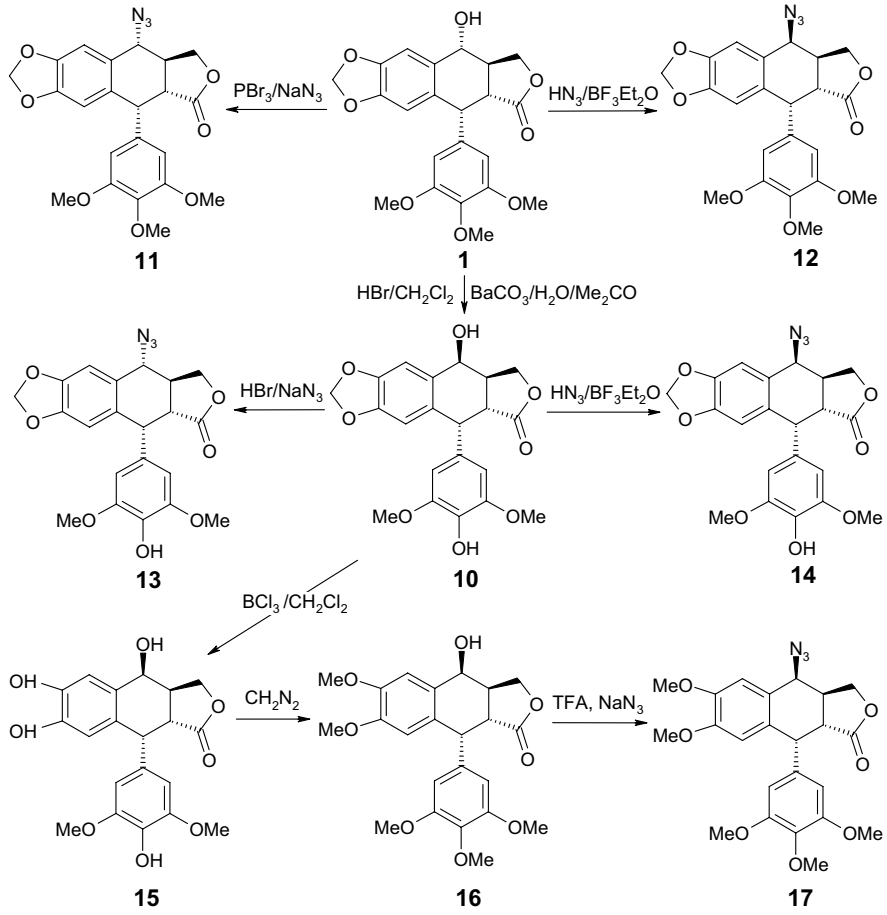
Further, the 7-amino compounds **18a**–**e** were prepared by reduction of the azides **11**–**14** and **17** using Pd as a catalyst. Finally, introduction of the carbamate chains was performed by reacting **9** with the appropriate amines **18a**–**e** in the presence of triethylamine, affording compounds **19a**–**e** (Scheme 3).[14]

In addition, 7β-cyano derivative **20** was prepared in 75% yield by reaction of **16** with trimethylsilyl cyanide, in the presence of $BF_3{\cdot}Et_2O$ at −15 °C. And 7β-carboxyl-derivative **21** was obtained in 46% yield by hydrolyzing **20** in acetic acid (Scheme 4).[18]

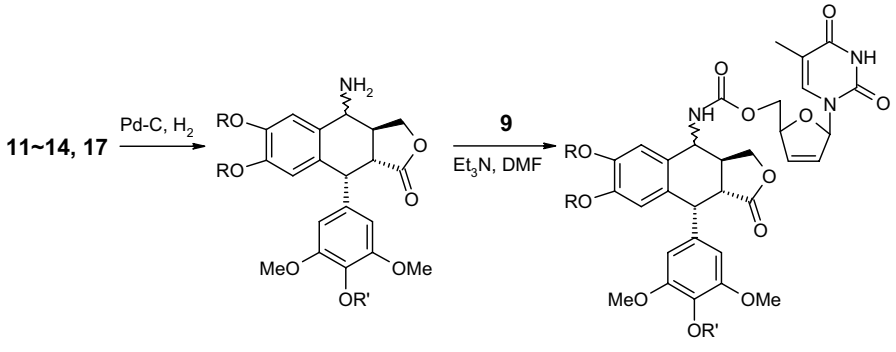
All the target compounds were corroborated by ^1H NMR, ^{13}C NMR, IR spectroscopy, and high-resolution mass spectroscopy (HRMS).[19] The assignment of the configuration at C-7 position for target compounds **19a**–**e** and **20**–**21** was based on the chemical shift for the



Scheme 1. Synthesis compound **9** from thymidine **6**.



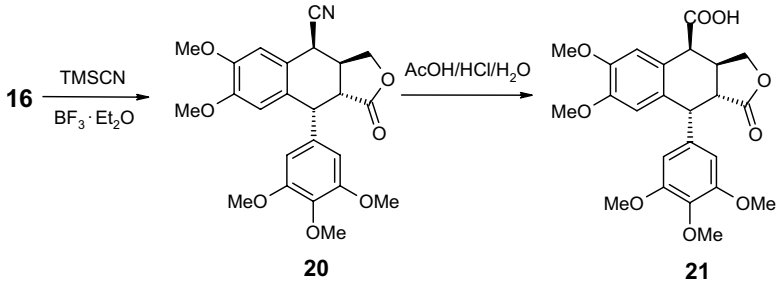
Scheme 2. Preparation of 7-azido-compounds related to podophyllotoxin **11**–**14**, **17**.



Componds			R	R'	7-configuration
11	**18a**	**19a**	R-R = -CH_2-	CH_3	7α
12	**18b**	**19b**	R-R = -CH_2-	CH_3	7β
13	**18c**	**19c**	R-R = -CH_2-	H	7α
14	**18d**	**19d**	R-R = -CH_2-	H	7β
17	**18e**	**19e**	CH_3	CH_3	7β

Scheme 3. Synthesis of compounds **18a**–**e** and **19a**–**e**.

protons at C-6 and the $J_{7,8}$ coupling constants. The protons at C-6, showing a singlet around 6.8, are always in upfield in the C-7β substituted than in the C-7α substituted compounds ($\delta_{H\text{-}6}$: 6.86 (**19a**, 7a) vs 6.78 (**19b**, 7β), 6.85 (**19c**, 7a) vs 6.77 (**19d**, 7β)). This difference is due to the anisotropic effect of the aromatic ring attached to the C-7α or C-7β substituted compounds. In addition, the C-7β substituted compounds have



Scheme 4. Synthesis of compounds **20** and **21**.

$J_{7,8} < 4.5$ Hz due to a *cis* relationship between H-7 and H-8. The C-7α substituted compounds, however, have $J_{7,8} > 8.5$ Hz because H-7 is *trans* to H-8.[20,21]

All target compounds were evaluated for inhibitory activity against HIV-1 replication in acutely infected C8166 cells following the previously described methods[22] and the results are shown in Table 1. Among these compounds, **19d** and **19c** showed the most anti-HIV-1 activity with EC_{50} values of 0.17 and 0.29 μM, and TI values of 466.9 and 354.5, respectively.

As it can be seen in Table 1, the anti-HIV-1 activities of the 4′-methoxy compounds were much less than the ones observed for the corresponding 4′-hydroxy compounds (**19a** to **19c**, **19b** to **19d**). Simultaneously, the C-7α and C-7β derivatives (**19a** to **19b**, **19c** to **19d**) did not show significant differences in their anti-HIV activities. As reported previously,[3] the A-ring modified compounds with 4,5-dimethoxy showed less cytotoxicity and higher TI value (**19e** to **19b**). These data indicate that replacing the 4,5-methylenedioxy group with 4,5-dimethoxy substitution increased their anti-HIV activities. In addition, 7β-cyano substituted 4,5-dimethoxy compound **20** showed promising anti-HIV activity (EC_{50} 1.05 μ M; TI 131.28). However, compound **21** obtained by hydrolyzing 20 was significantly less active with EC_{50} value of 46.90 μM and TI value >9.31.

Table 1. Anti-HIV-1 activities of Compounds **19a**–**e**, **20**, and **21**

Compound	IC_{50}[a] (μM)	EC_{50}[b] (μM)	TI[c]
19a	3.39	0.24	14.42
19b	68.15	5.46	12.47
19c	103.79	0.29	354.53
19d	79.14	0.17	466.91
19e	>294.55	4.39	>67.11
20	137.56	1.05	131.28
21	>436.68	46.90	>9.31
1	<0.15	0.0029	53.3
AZT	500	0.009	55,556

[a] IC_{50}, concentration of drug that causes 50% reduction in total cell number. Drugs with IC_{50} values >200 μg/mL cannot be tested at higher concentrations for a more exact IC_{50} value due to the effect of the solvent DMSO.

[b] EC_{50}, concentration of drug that reduces syncytia formation by 50%.

[c] Therapeutic Index (TI) is a ratio of the IC_{50} value/EC_{50} value. Therefore, when the IC_{50} value is >200 μg/mL (refer to footnote a, above), the TI value must also be reported as greater than.

In conclusion, modified podophyllotoxin derivatives have demonstrated significant anti-HIV-1 activity. 7β-Amide or cyano group substitution and an opened A-ring coupled with 4′-demethylation resulted in compounds with better anti-HIV-1 activity. These results are encouraging and warrant further structural modification to both decrease cytotoxicity and increase antiviral inhibitory activity. Additional biological evaluation is in progress to better define the anti- HIV-1 activity of the podophyllotoxin compounds.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2006.11.070.

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19. General procedure for synthesis of compounds **19a**–**e**:To a stirred solution of 9 (0.2 mmol, 78 mg) in DMF (10 mL) were added the appropriate amine **18a**–**e** (0.22 mmol, 1.1 equiv) and dried triethylamine (0.l mL) under argon at 50 °C and then the mixture was stirred for 5 h. The reaction mixture was evaporated in vacuo, the residue was purified by silica gel column chromatography using a mixture of CH_2C1_2/acetone 20:1 as the eluant to afford compounds **19a**–**e** in the reported yields. 7β-Amino-7-deoxy-4′-demethylepipodo-phyllotoxin d4T carbamate (**19d**) Yield: 83%; mp: 178–180 °C; $[\alpha]_{25}^{D}$ -54° (c 0.5, $CHC1_3$); IR (KBr) cm^1: 3328, 3065, 2998, 2943, 2898, 1777, 1692, 1615, 1512, 1481, 1462, 1426, 1368, 1329, 1236, 1112, 1085, 1038, 996. ^{H} NMR ($CDC1_3$, 300 M) s: 7.09(s, 1H, 6″-H), 6.92 (s, 1H, 1‴-H), 6.77 (s, 1H, 6-H), 6.47 (s, 1H, 3-H), 6.31 (d, 1H, *J* = 6.0 Hz, 3‴-H), 6.26 (s, 2H, 2′, 6′-H), 5.98 (s, 2H, OCH_2O), 5.91 (d, 1H, *J* = 5.7 Hz, 2‴-H), 5.37 (d, 1H, *J* = 6.9 Hz, NH), 5.03 (br, 1H, 4‴-H), 4.97 (dd, 1H, *J* = 7.5, 3.6 Hz, 7-H), 4.56 (d, 1H, *J* = 3.9, 7′-H), 4.36 (m, 2H, 11β-H, 5‴β-H), 4.22 (dd, 1H, *J* = 11.7, 6.0 Hz, 5‴α-H), 3.91 (t, 1H, *J* = 9.0 Hz, 9-Ha), 3.75 (s, 6H, 2OCH3), 2.88 (m, 2H, 8,8′-H), 1.74 (s, 3H, CH_3); ^{13}C NMR ($CDC1_3$, 75 M) s: 174.4, 163.5, 155.6, 150.7, 148.4, 147.6, 146.3, 135.4, 133.9, 132.9, 132.2, 130.2, 128.3, 127.1, 111.0, 109.9, 108.8, 107.6, 101.7, 90.2, 84.2, 68.5, 66.1, 56.3, 49.8, 43.3, 41.4, 37.3, 12.4. MS (ESI): 672.1 (M+Na), 667.2 ($M+NH_4$), 550.1, 413.3; HRMS (ESI): 667.2252 for $[M+NH_4]^+$ (calcd: 667.2246 for $C_{32}H_{35}O_{12}N_4$).
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