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Anti-AIDS agents. Part 61: Anti-HIV activity of new podophyllotoxin derivatives ☆

Xiao-Kang Zhu,[a] Jian Guan,[a] Zhiyan Xiao,[c] L. Mark Cosentino[b] and Kuo-Hsiung Lee[a,*]

[a]*Natural Products Laboratory, School of Pharmacy, University of North Carolina, Chapel Hill, NC 27599-7360, USA*
[b]*Biotech Research Laboratories, 3 Taft Court, Rockville, MD 20850, USA*
[c]*Department of Synthetic Medicinal Chemistry, Chinese Academy of Medicinal Sciences and Peking Union Medical College, Beijing 100050, China*

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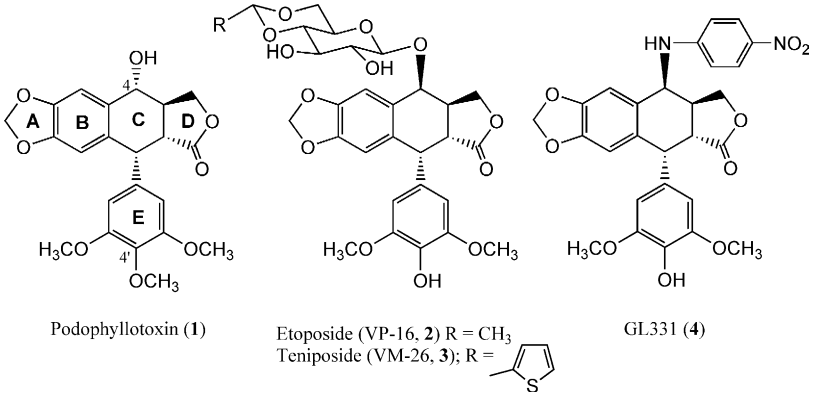
Abstract—A series of novel podophyllotoxin derivatives containing structural modifications at C-4 (**7**–**14**), C-4′ (**16**–**17**), and the methylenedioxy A-ring (**23**–**28**) was synthesized and tested for inhibition of HIV replication. Four of these compounds (**25**–**28**) were previously reported to show EC_{50} values of <0.001 μg/mL and therapeutic index (TI) values >120. Three of the newly tested compounds (**8**, **12**, and **20**) showed good activity with EC_{50} values of 0.012, <0.001, and 0.389 μg/mL and TI values of 19.1, >16, and 19.4, respectively. A comparison of the anti-HIV activity of these derivatives suggested that an opened A-ring with 6,7-dimethoxy substitution and a 4′-demethylated E ring enhanced anti-HIV activity.

1. Introduction

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),[2,3] non-nucleoside RT inhibitors (NNRTIs),[4] protease inhibitors (PIs),[4] and fusion (or entry) inhibitors (FIs),[5] are now approved for clinical use in the US. However, these drugs have only limited or transient clinical benefit due to their adverse side effects and the development of drug-resistant viral strains.[6–9] Therefore, current searches for new anti-HIV agents are focused on discovering compounds with novel structures and different mechanisms of action.

Podophyllotoxin (**1**) is a lignan isolated from the roots of the North American *Podophyllum peltatum* Linnaeus,



Podophyllotoxin (**1**)

Etoposide (VP-16, **2**) R = CH_3
Teniposide (VM-26, **3**); R =

GL331 (**4**)

☆ For Part 60, see Ref. 1.

* Corresponding author. Tel.: +1-919-962-0066; fax: +1-919-966-3893; e-mail: khlee@unc.edu

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the Tibetan *P. emodi* Wall, or the Taiwanese species *Podophyllum pleinthum*. Hence[10] extensive structural modification and anti-tumor studies of **1** have resulted in clinically useful anti-cancer drugs, such as etoposide (**2**) and teniposide (**3**). In our continuing studies on the synthesis and biological evaluation of podophyllotoxin- and etoposide-related derivatives, we have prepared several series of C-4 modified podophyllotoxins bearing various substituents including alkyl- and aryl-amino groups [e.g., **4** (GL331)], oxy and thio ethers, and ketones.[11–16] Additional structural changes have been made in the methylenedioxy A-ring, C-ring, and the lactone D-ring.[15,17,18] To explore the range of biological activities of the podophyllotoxin compound class, we recently reported our initial anti-HIV results[19] for modified podophyllotoxin derivatives. Based on these promising results, we have extended our studies and herein report results of analogues containing structural modifications at C-4 (**7**–**14**), C-4′ (**16**–**17**), and the methylenedioxy A-ring (**23**–**24**).

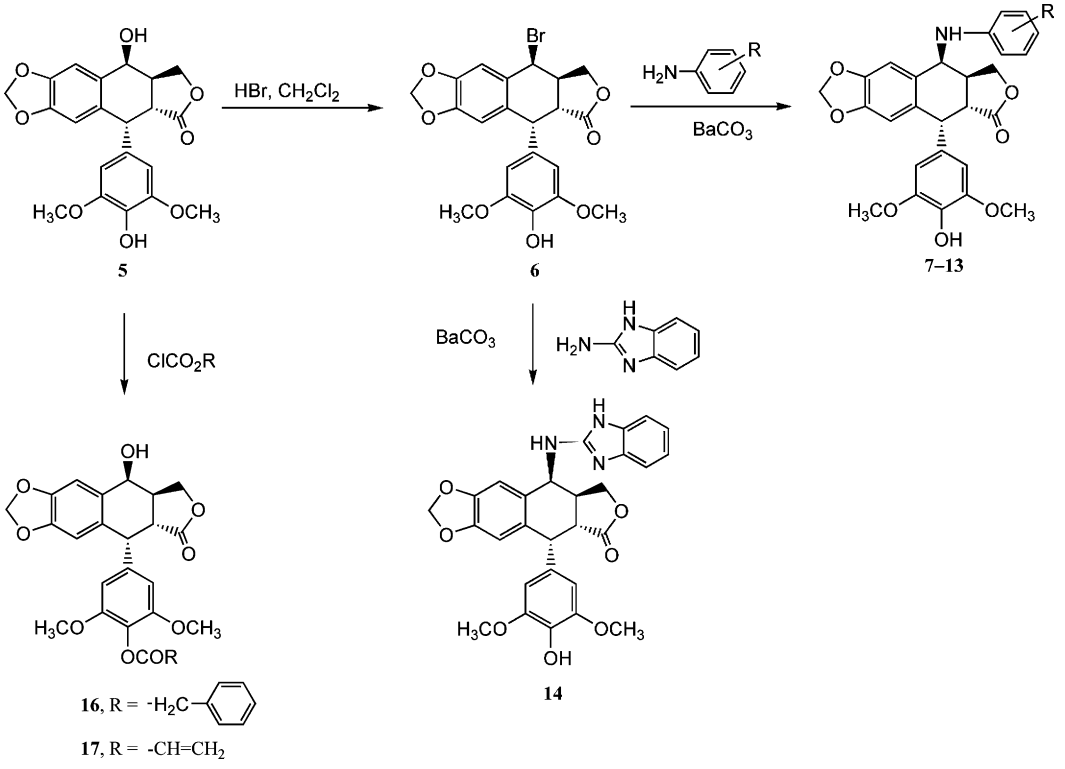
2. Chemistry

4′-Demethyl-epipodophyllotoxin (**5**) was prepared from podophyllotoxin according to a previously published method[12] and intermediate **6** was synthesized from **5** via a modified Kuhn's method.[16] As shown in Scheme 1, compounds **7**–**14** were prepared by treating **6** with appropriately substituted arylamines in the presence of barium carbonate. Although this reaction occurs via an S_N1 mechanism,[15] the bulky C-1α pendent aromatic ring directs a stereoselective substitution, resulting in preferential formation of C-4β-oriented products. The 4′-hydroxy group of **5** was selectively reacted with benzyl or vinyl chloroformate to give the carbonate derivatives **16** and **17**.

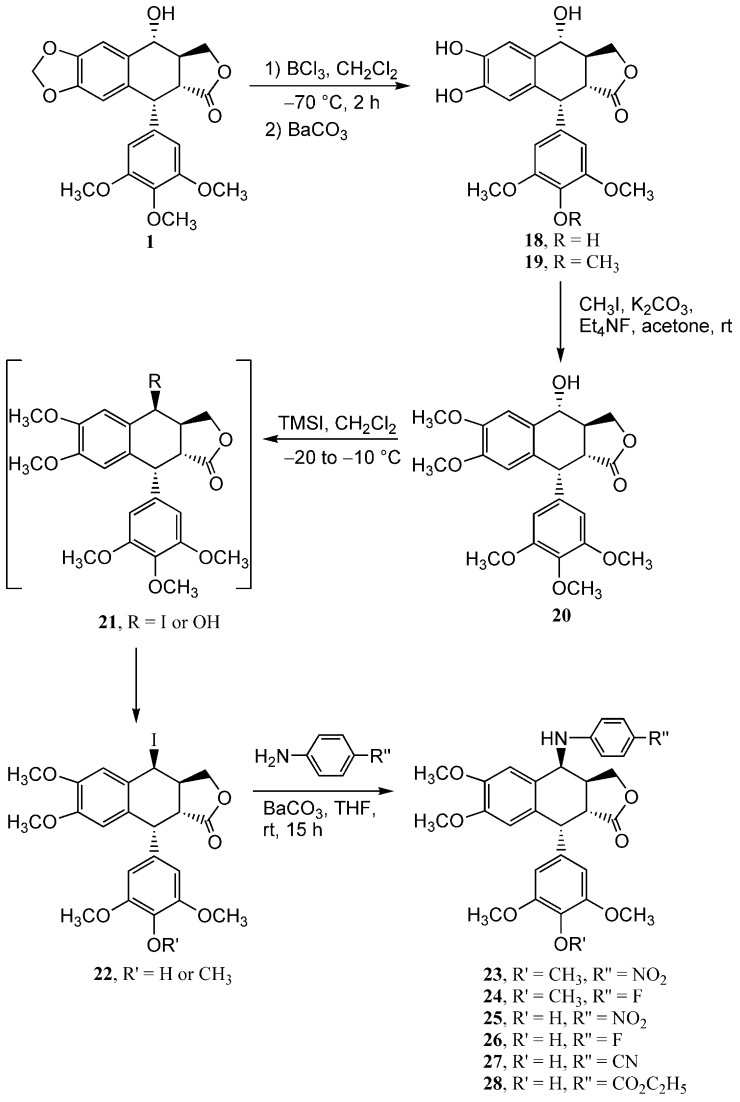
6,7-*O*-Demethylene-4′-*O*-demethyl-podophyllotoxin (**18**) and 6,7-*O*-demethylene-podophyllotoxin (**19**) were prepared by treating podophyllotoxin (**1**) with BCl_3 followed by weak basic hydrolysis with $BaCO_3$.[15] Methylation of both **18** and **19** was achieved using methyl iodide with K_2CO_3 and Et_4NF as catalysts. 4′-Demethyl-6,7-*O*-demethylene-6,7-*O*-dimethyl-4β-substituted-amino-4-desoxypodophyllotoxin derivatives (**25**–**28**) were prepared by an efficient four-step synthetic sequence starting from podophyllotoxin (Scheme 2). In a one-pot synthesis, intermediate **20** underwent 4-halogenation and -epimerization coupled with 4′-demethylation using trimethylsilyl iodide (TMSI) to give **22**. Then without purification, amination of the 4-position of **22** with the appropriate *p*-substituted aniline yielded the target compounds **25**–**28** as the major products. This protocol proceeded effectively to afford **25**–**28** without any detectable 6,7-demethylation (Scheme 2). Compounds with an intact 4′-methoxy group (e.g., **23** and **24**) were minor products.

3. Biological results and discussion

All three compound types were evaluated for inhibitory activity against HIV-1 replication in acutely infected H9 cells. Table 1 shows the data for Type-1 compounds, which have different substituents at the 4-position of 4′-demethyl-desoxypodophyllotoxin (**7**–**15**, **2**, and **4**).



Scheme 1.



Scheme 2.

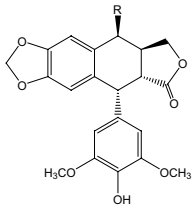
Among these compounds, **8** and **12** showed the highest anti-HIV activity with EC_{50} values of 0.012 and <0.001 μg/mL and TI values of 19.1 and >16, respectively.

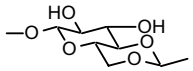
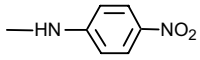
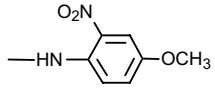
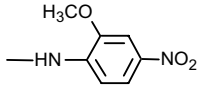
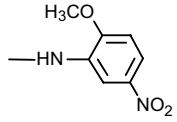
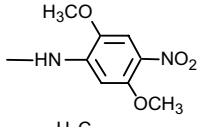
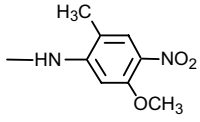
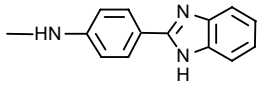
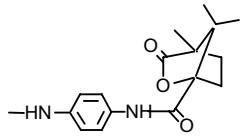
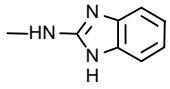
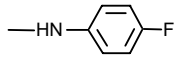
As shown in Table 1, addition of an *o*-methoxy group to **4** increased anti-HIV activity and therapeutic index (see compound **8**). Among the three disubstituted analogues, the 2-methoxy-4-nitroanilino analogue (**8**) showed the highest anti-HIV potency, the 2-methoxy-5-nitroanilino derivative (**9**) was less active, and the 2-nitro-4-methoxyanilino compound (**7**) was inactive. Both trisubstituted compounds (**10** and **11**) were inactive. Replacing the *p*-nitro group of **4** with bulky substituents (benzamidizole, **12**, or camphanoylamido, **13**) led to significantly increased anti-HIV activity, as well as cytotoxicity. However, placing a benzimidazole directly at the 4-position of podophyllotoxin resulted in total loss of anti-HIV activity (**14**).

The Type-2 compounds (podophyllotoxin and epipodophyllotoxins) in Table 2 (**1**, **5**, **16**, and **17**) were less active than the Type-1 compounds. The most active compounds were the phenol **5** and its corresponding vinyl carbamate **17** with EC_{50} values of 0.057 and 0.034 μg/mL, respectively. The benzyl carbamate (**16**) was devoid of anti-HIV activity.

Data for the Type-3 compounds are shown in Table 3. As reported previously,[19] the 4β-substituted anilino-6,7-dimethoxy compounds **25**–**28** showed promising activity (EC_{50} < 0.001 μg/mL; TI > 120 ~> 166). However, compounds **4** and **15** (both with an intact methylenedioxy ring A) were significantly less active with EC_{50} values of 0.055 and 0.1 μg/mL and TI values of 2.43 and 10, respectively (Table 1). A similar order of activity was found with podophyllotoxin and two derivatives (**19** and **20**) with a 4α-OH and various 6,7-substitution patterns. Compound **20** (6,7-dimethoxy) was active (EC_{50} 0.389 μg/mL, TI 19.4), **19** (6,7-dihydroxy) was less active (EC_{50} 4.30 μg/mL, TI 2.23), and **1** (6,7-methylenedioxy) was inactive (Table 2). These data indicate that replacing the 6,7-methylenedioxy group with 6,7-dimethoxy substitution increased anti-HIV activity. In a final comparison, the 4′-methoxy compounds **23** and **24** were

Table 1. Anti-HIV activity of podophyllotoxin derivatives-Type 1



Compd	R	EC_{50} (μg/mL)[a]	IC_{50} (μg/mL)[b]	TI[c]
2		0.030	1.27	42.7
4		0.055	0.132	2.43
7		Not active	1.69	—
8		0.012	0.23	19.1
9		0.29	0.229	7.92
10		Not active	1.76	—
11		Not active	38.7	—
12		<0.001	0.016	>16
13		0.0023	0.018	7.82
14		Not active	0.206	—
15[d]		0.1	1.0	10

[a] EC_{50} refers to the concentration of drug that causes 50% reduction in total virus replication.

[b] IC_{50} refers to the concentration of drug that causes 50% reduction in total cell number. Drugs that have IC_{50} values >100 μg/mL cannot be tested at higher concentrations for a more exact IC_{50} value due to the effect of the solvent, DMSO.

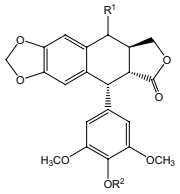
[c] Therapeutic Index is a ratio of the IC_{50} value/EC_{50} value. Therefore, when the IC_{50} value is >100 μg/mL (refer to footnote a, above), the TI value must also be reported as greater than.

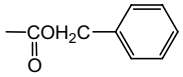
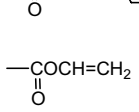
[d] From Ref. 19.

much less active than the corresponding 4′-hydroxy compounds **25** and **26**.

In summary, modified podophyllotoxin derivatives have demonstrated significant anti-HIV activity. Bulky, *para*-substitution on the 4β-aniline (**12** and **13**) and an opened A-ring (**25**–**28**) coupled with 4′-demethylation resulted in compounds with better anti-HIV activity. These results are encouraging and warrant further structural modification to both decrease cytotoxicity and increase antiviral inhibitory activity. Although the precise biological target is not known, studies by Hara et al.[20] on the anti-HIV activity of numerous aryltetralin lignan analogues provide some insight and suggestions. Their work showed

Table 2. Anti-HIV activity of podophyllotoxin derivatives-Type 2



Compd	R^1	R^2	EC_{50} (μg/mL)[a]	IC_{50} (μg/mL)[a]	TI[a]
1		CH_3	Not active	0.0025	—
5		H	0.057	0.171	3.01
16			Not active	0.201	—
17			0.034	0.136	4.02

[a] See Table 1.

that lignan analogues bind to reverse transcriptase at the same site as many other RT inhibitors. Additional biological evaluation is in progress to better define the anti-HIV activity of the podophyllotoxin compounds.

4. Experimental

All melting points were taken on Fisher–Johns and Mel-Temp II melting point instruments and are uncorrected. IR spectra were recorded on a Perkin–Elmer 1320 spectrophotometer. ^{1}H NMR spectra were obtained using Bruker AC-300 and WM 250 NMR spectrometers with TMS as the internal standard. All chemical shifts are reported in ppm. FABMS and HRFABMS spectral analyses were determined on a JOEL HX-110 instrument. Analytical thin-layer chromatography (TLC) was carried out on Merck precoated aluminum silica gel sheets (Kieselgel 60 F-254). Optical rotations were measured with a JASCO DIP-1000 polarimeter. All target compounds were characterized by ^{1}H and IR spectral analyses and MS analyses.

4.1. Synthesis of 4β-poly-substituted-anilino-4′-demethyl-desoxypodophyllotoxins (7–14)

A solution containing 4′-*O*-demethyl-4β-bromo-4-desoxypodophyllotoxin (**6**) (695 g, 1.5 mmol), anhydrous $BaCO_3$ (590 g, 3.0 mmol), and the appropriate arylamine (1.65 mmol) in 15 mL of dry dichloroethane under nitrogen was stirred overnight at room temperature. The reaction mixture was filtered, diluted with EtOAc, washed with water, dried over anhydrous $MgSO_4$, and purified via silica gel column chromatography using CH_2Cl_2–acetone–EtOAc (100:5:5) as eluent. The physical and spectral data of all compounds agreed with those reported by us previously.[21]

4.2. Synthesis of 4′-*O*-demethyl-4′-acyl-epipodophyllotoxins (16 and 17)

To a solution of 4′-*O*-demethyl-epipodophyllotoxin (**5**) (2 mmol) in 10 mL of anhydrous dichloroethane and 1 mL of pyridine was added the appropriate chloroformate in 2 mL of dichloroethane dropwise under nitrogen at room temperature. The reaction mixture was then stirred for 1–3 days, concentrated in vacuo to remove solvent. The residue was diluted with CH_2Cl_2, washed with water, dried over anhydrous magnesium sulfate, and purified via silica gel column chromatography using CH_2Cl_2–acetone–EtOAc (100:5:5) as eluent.

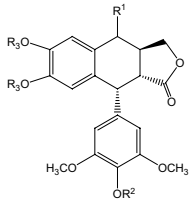
4.3. 4′-*O*-Demethyl-4′-carbobenzoxy-epipodophyllotoxin (16)

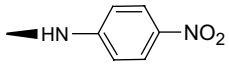
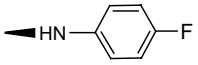
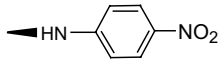
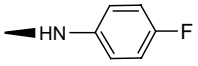
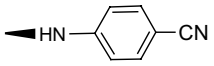
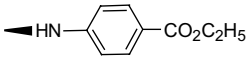
Yield 64%; crystals from MeOH; mp 116–118 °C; $[\alpha]_D^{21.4}$ −29.4 (*c* 0.17, $CHCl_3$); IR (KBr) 3495 (OH), 2900 (aliphatic C–H), 1755 (lactone), 1595, 1500, 1475, 1450, 1420 (aromatic C=C) cm^{-1}; MS *m*/*e*: 534 $[M]^+$; ^{1}H NMR (DMSO, D_2O exchange): δ 6.95 (s, 1 H, 5-H), 6.54 (s, 1H, 8-H), 6.33 (s, 2H, 2′, 6′-H), 6.01 (s, 2H, 12-H), 5.48 (d, $J = 5.8$ Hz, 1H, exchangeable, 4-OH), 4.74 (m, 1H, 4-H), 4.60 (d, $J = 5.8$ Hz, 1H, 1-H), 4.36 (t, $J = 7.9$ Hz, 1H, 11-H), 4.19 (t, $J = 7.9$ Hz, 1H, 11-H), 3.62 (s, 6H, 3′, 5′-OCH_3), 3.32 (m, 1H, 2-H), 2.82 (m, 1H, 3-H); Anal. ($C_{29}H_{26}O_{10}$) C, H.

4.4. 4′-*O*-Demethyl-4′-vinyloxy-carbonyl-epipodophyllotoxin (17)

Yield 54.3%; crystals from acetone; mp 246–249 °C; $[\alpha]_D^{23.5}$ −59.1 (*c* 0.18, $CHCl_3$); IR (KBr) 3530 (OH), 2900 (aliphatic C–H), 1765 (lactone), 1580, 1475, 1450 (aromatic C=C) cm^{-1}; MS *m*/*e*: 470 $[M]^+$; ^{1}H NMR ($CDCl_3$, D_2O exchange): δ 7.13 (dd, $J = 6.2$ Hz, 13.8 Hz,

Table 3. Anti-HIV activity of podophyllotoxin derivatives-Type 3



Compd	R^1	R^2	R^3	EC_{50} (g/mL)[a]	IC_{50} (μg/mL)[a]	TI[a]
19	OH	CH_3	H	4.30	9.57	2.23
20	OH	CH_3	CH_3	0.389	7.53	19.4
23		CH_3	CH_3	2.45	5.29	2.16
24		CH_3	CH_3	0.151	1.43	9.50
25[a]		H	CH_3	<0.001	0.158	>158
26[a]		H	CH_3	<0.001	0.166	>166
27[a]		H	CH_3	<0.001	0.141	>141
28[a]		H	CH_3	<0.001	0.120	>120
AZT				0.0239	500	22,650

[a] See Table 1.

1H, 1″-H), 6.90 (s, 1H, 5-H), 6.57 (s, 1H, 8-H), 6.34 (s, 2H, 2′, 6′-H), 6.00 (d, $J = 7.0$ Hz, 2 H, 12-H), 5.03 (dd, $J = 2.1$, 13.9 Hz, 1H, 4-H), 4.89 (t, $J = 3.7$ Hz, 1H, 1-H), 4.66 (m, 2H, 2″-H), 4.41 (m, 2H, 11-H), 3.73 (s, 6H, 3′, 5′-OCH_3), 3.32 (dd, $J = 5.1$, 14.1 Hz, 1H, 2-H), 2.84 (m, 1H, 3-H); Anal. ($C_{24}H_{22}O_{10}$) C, H.

4.5. Synthesis of 4′-*O*-demethyl-6,7-*O*-demethylene-6,7-dimethyl-4-substituted-aniline-4-desoxypodophyllotoxins (25–28) and 6,7-*O*-demethylene-6,7-dimethyl-4-substituted-aniline-4-desoxypodophyllotoxin (23, 24)

To a solution of 6,7-*O*-demethylene-6,7-*O*-dimethylpodophyllotoxin **20** (86 g, 0.2 mmol), prepared as previously described in Ref. 15, in dry 1,2-dichloroethane (10 mL) was added a solution of TMSI (140 mg, 0.1 mL, 0.7 mmol) at −20 °C. The reaction mixture was stirred for 6 h at −20 to −10 °C then concentrated in vacuo at room temperature to give a brown residue. This crude product was dissolved in dry 1,2-dichloroethane (5 mL), and then $BaCO_3$ (80 mg, 0.4 mmol) and the appropriate substituted aniline (0.22 mmol) were added successively. The mixture was stirred for 12 h at room temperature, filtered, and concentrated under reduced pressure. The crude product was purified on a silica gel preparative TLC plate with hexane–MeOH–acetone (90:5:5) as solvent system to give the 4′-*O*-demethylated compound (**25**–**28**) as major product (52% yield for **25**) and the undemethylated compound as minor product (6% yield for **23**). Spectral and physical data of all compounds agreed with those reported in our previous literature.[15]

4.6. Anti-HIV assay

The T cell line, H9, was maintained in continued 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/milliliter. The appropriate amount of virus for a multiplicity of infection (m.o.i.) 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 performing cell counts by a Coulter Counter in 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 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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References and notes

1. For Part 60, see: Yu, D.; Chen, C. H.; Brossi, A.; Lee, K. H. *J. Med. Chem.*, submitted for publication.
2. Fischl, M. A.; Richman, D. D.; Grieco, M. H.; Gottlieb, M. S.; Volberding, P. A.; Laskin, O. L.; Leedom, J. M.; Groopman, J. E.; Mildvan, D.; Schoolley, R. T., et al. *New Engl. J. Med.* **1987**, *317*, 185.
3. De Clercq, E. *AIDS Res. Human Retroviruses* **1992**, *8*, 119.
4. Office of Special Health Issues, Food & Drug Administration, December 22, 1998.
5. Moyle, G. *J. Antimicrob. Chemother.* **2003**, *51*, 213.
6. Schinazi, R. F.; Mead, J. R.; Feorino, P. M. *AIDS Res. Human Retroviruses* **1992**, *8*, 963.
7. Shirahata, T.; Yarchoan, R.; O'Brien, M. C.; Husson, R. N.; Anderson, B. D.; Kojima, E.; Shimoda, T.; Broder, S.; Mitsuya, H. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *91*, 562.
8. Johnson, M. I.; Horth, D. F. *Sciences* **1993**, *260*, 1286.
9. Ho, D. D.; Moudgil, T.; Alam, M. *New Engl. J. Med.* **1989**, *321*, 1622.
10. Shibata, S.; Murata, T.; Fujita, M. *Yakugaku Zasshi* **1962**, *82*, 777.
11. Beer, S. A.; Imakura, Y.; Dai, H. J.; Li, D. H.; Cheng, Y. C.; Lee, K. H. *J. Nat. Prod.* **1988**, *51*, 901.
12. Thurston, L. S.; Imakura, Y.; Haruna, M.; Li, D. H.; Liu, Z. C.; Liu, S. Y.; Cheng, Y. C.; Lee, K. H. *J. Med. Chem.* **1989**, *32*, 604.
13. Wang, Z. Q.; Kuo, Y. H.; Schnur, D.; Bowen, J. P.; Liu, S. Y.; Han, F. S.; Cheng, J. Y.; Cheng, Y. C.; Lee, K. H. *J. Med. Chem.* **1990**, *33*, 2660.
14. Zhou, X. M.; Wang, Z. Q.; Chen, H. X.; Cheng, Y. C.; Lee, K. H. *Pharma. Res.* **1993**, *10*, 214.
15. Wang, Z. Q.; Hu, H.; Chen, H. X.; Cheng, Y. C.; Lee, K. H. *J. Med. Chem.* **1992**, *35*, 871.
16. Zhang, Y. L.; Guo, X.; Cheng, Y. C.; Lee, K. H. *J. Med. Chem.* **1994**, *37*, 446.
17. Zhou, X. M.; Lee, K. J. H.; Cheng, J.; Wu, S. S.; Chen, H. X.; Guo, X.; Cheng, Y. C.; Lee, K. H. *J. Med. Chem.* **1994**, *37*, 287.
18. Wang, Z. Q.; She, Y. C.; Chen, H. X.; Chang, J. Y.; Guo, X.; Cheng, Y. C.; Lee, K. H. *Pharm. Res.* **1993**, *10*, 343.
19. Lee, C. T. L.; Lin, V. C. K.; Zhang, S. X.; Zhu, X. K.; Van Vliet, D.; Hu, H.; Beer, S. A.; Wang, Z. Q.; Cosentino, L. M.; Morris-Natschke, S. L.; Lee, K. H. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 2897.
20. Hara, H.; Fujihashi, T.; Sakata, T.; Kaji, A.; Kaji, H. *AIDS Res. Human Retroviruses* **1997**, *13*, 695.
21. Zhu, K. K.; Guan, J.; Tachibana, Y.; Bastow, K. F.; Cheng, H. H.; Cheng, Y. C.; Lee, K. H. *J. Med. Chem.* **1999**, *42*, 2441.