Original Article

Synthesis and Cytotoxicities of Novel Podophyllotoxin Xyloside Derivatives

Natural Product Communications
July 2019: 1–6

DOI: 10.1177/1934578X19860668
journals.sagepub.com/home/npx

Cheng-Ting Zi[1,2]*, Liu Yang[2]*, Bang-Lei Zhang[1], Yan Li[2], Zhong-Tao Ding[3], Zi-Hua Jiang[4], Jiang-Miao Hu[2], and Jun Zhou[2]

Abstract

Novel podophyllotoxin xyloside derivatives **8** to **11** were synthesized and evaluated for their cytotoxicities against a panel of 5 human cancer cell lines (HL-60, SMMC-7721, A-549, MCF-7, SW480) using [3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyltetrazolium bromide] assays. These derivatives showed good to moderate activities, with compound **9** having an IC_{50} value of 4.42 μM against the A-549 cell line. Overall, compound **9** might be a promising candidate for further development.

Keywords

podophyllotoxin, butyrylated xyloside, cytotoxicity, synthesis

Received: March 3rd, 2019; Accepted: April 12th, 2019.

Podophyllotoxin (**1**, Figure 1) is a lignan isolated from the roots and rhizomes of *Podophyllum hexandrum* and *P. peltatum*[1] and shows strong cytotoxic activity against various cancer cell lines through inhibiting microtubule assembly.[2] The poor water solubility and unacceptable toxic side effects of podophyllotoxin have limited its application as a drug in cancer chemotherapy.[3] Many derivatives of podophyllotoxin have been developed for clinical use as antineoplastic agents. For example, the 2 semi-synthetic glucosidic cyclic acetals of podophyllotoxin, etoposide (**2**) and teniposide (**3**), are in clinical use for the treatment of a variety of malignancies, including lung cancer, lymphomas, and Kaposi's sarcoma.[4] However, their therapeutic uses are often hindered by problems such as poor bioavailability and drug resistance.

More recently, the glycoconjugates of small molecule anticancer drugs have become an attractive strategy in order to improve drug efficacy and pharmacokinetics and reduce side effects.[5-7] In our earlier study, we reported the synthesis and cytotoxicities of a group of per-butyrylated glycoside derivatives of podophyllotoxin (eg, **4**, Figure 1).[8] We found that derivatives with per-butyrylated glycosides (D-glucose/D-galactose/D-mannose/D-arabinose/L-rhamnose/maltose/lactose) generally displayed higher activities, but the per-butyrylated xyloside derivatives of podophyllotoxin are still not known. Mani et al reported that the xyloside derivatives reduce tumor growth both in vitro and in vivo.[9-11] In addition, butyrate is a well-known histone deacetylase (HDAC) inhibitor and its anticancer effect shows promising therapeutic potential.[12] Reported here are the chemical synthesis of xyloside derivatives of podophyllotoxin and their in vitro cytotoxicities against the 5 human cancer cell lines: HL-60 (leukemia), SMMC-7721 (hepatoma), A-549 (lung cancer), MCF-7 (breast cancer), and SW480 (colon cancer).

The novel xyloside derivatives of podophyllotoxin **8** to **11** were synthesized according to the synthetic route shown in Scheme 1. 2,3,4-Tri-*O*-butyryl-α-D-xylopyranose (**6**) was prepared with 60% yield by the treatment of D-xylose (**5**) with iodine and butyric anhydride followed by treatment with ammonia solution (25%) in acetonitrile.[13,14] The preparation of 4'-demethylepipodophyllotoxin (**7**) involving a similar method has been reported in the literature.[15] Then, compound **6** was allowed to reacted with compounds **1** and **7** in the

[1] Key Laboratory of Pu-er Tea Science, Ministry of Education, College of Science, Yunnan Agricultural University, Kunming, China
[2] State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, China
[3] Key Laboratory of Medicinal Chemistry for Nature Resource, Ministry of Education, School of Chemical Science and Technology, Yunnan University, Kunming, China
[4] Department of Chemistry, Lakehead University, Thunder Bay, Canada

*The authors Cheng-Ting Zi and Liu Yang contributed equally to the work.

Corresponding Authors:
Zi-Hua Jiang, Department of Chemistry, Lakehead University, 955 Oliver Road, Thunder Bay, ON, Canada P7B 5E1.
Email: zict@ynau.edu.cn
Jiang-Miao Hu, State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, China.
Email: zict@ynau.edu.cn

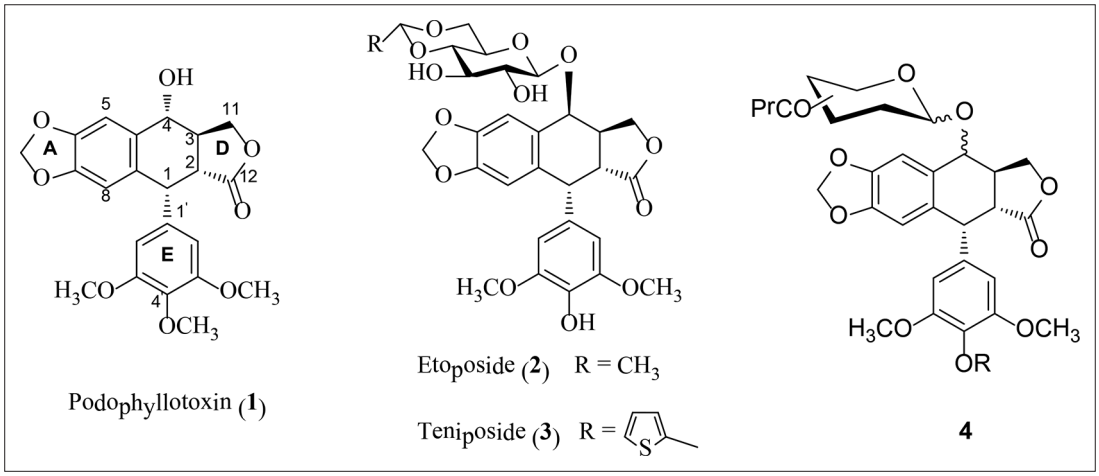
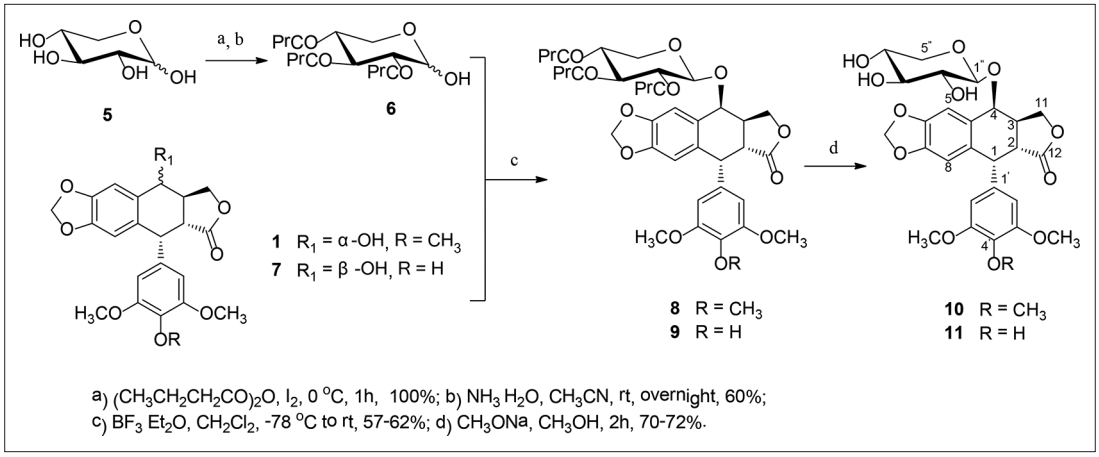


Figure 1. Structures of podophyllotoxin (**1**), etoposide (**2**), teniposide (**3**), and per-butyrylated glycoside derivatives of podophyllotoxin (**4**).

presence of boron trifluoride•etherate (BF_3•Et_2O) at -78°C to give the per-butyrylated xyloside derivatives **8** and **9** of podophyllotoxin with 57% to 62% yields.[8] These products were treated with sodium methylate in methanol at room temperature for 2 hours to yield podophyllotoxin xyloconjugates **10** and **11** in good yields (70%-72%). The chemical structures of the synthesized compounds were characterized by ^{1}H-NMR and ^{13}C-NMR, electrospray ionization mass spectrometry (ESI-MS), and high-resolution mass spectrometry (HRESIMS).

To evaluate the cytotoxicity we used the [3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyltetrazolium bromide] (MTT) method.[16] The podophyllotoxin derivatives **8** to **11** were tested for their cytotoxicity against 5 cancer cell lines. Etoposide and cisplatin were taken as positive control drugs. Their activities were expressed by the IC_{50} value (concentration of drug inhibiting 50% cell growth) and the cytotoxicity data are presented in Table 1. As can be seen from Table 1, compounds having a free xylose residue (compounds **10** and **11** having IC_{50} > 40 μM) show weak activity, while compounds **8** and **9** show good cytotoxicities against all cancer cell lines tested, and were observed to be less toxic to the human bronchial epithelial (BEAS-2B) cell line. This indicates that compounds **8** and **9** are more sensitive to certain tested cancer cells than normal cells and possess good selectivity.

Compound **9** was found to show the highest cytotoxicity against the HL-60 cell line with an IC_{50} value of 2.9 μM. Compound **9** also showed higher potency against the A-549 cell line, with an IC_{50} value of 4.42 μM, which is significantly more potent than the control drug etoposide (having IC_{50} of 11.92 μM against the A-549 cell line). Furthermore, compound **9** with a hydroxy group at the C-4' position in the E ring is more active than compound **8** which has a methoxyl group at the C-4' position. Previously, we had reported the cytotoxic activity of 4β-triazole-podophyllotoxin xylosides (all having IC_{50} >40 μM against 5 cancer cell lines).[17] However, we have found in this study that per-butyrylation of the xylose residue leads to increased cytotoxic activity.



Scheme 1. Synthesis of compounds **8** to **11**.

Table 1. In Vitro Anticancer Activity (IC_{50}, μM) of Xyloside of Podophyllotoxin Derivatives **8** to **11**.

Compounds	IC_{50}(μM)					
	HL-60	SMMC-7721	A-549	MCF-7	SW480	BEAS-2B
8	8.41	18.16	18.51	26.07	28.02	29.70
9	2.90	10.39	4.42	16.36	21.65	25.56
10	>40	>40	>40	>40	>40	NT
11	>40	>40	>40	>40	>40	NT
Etoposide (**2**)	0.31	8.12	11.92	32.82	17.11	NT
Cisplatin	1.17	6.43	9.24	15.86	13.42	NT

NT, not tested.

As compounds **10** and **11** do not show in vitro efficacy, we have calculated the ClogP and polar surface area (PSA) values of compounds **8** to **11** by Sybyl,[18] and the data are shown in Table 2. According to known procedures,[18-20] for the most potent topoisomerase (I/II) inhibitors, the PSA value should be less than 100 $Å^2$ and the logP value less than 5. We found that 2 compounds **10** and **11** have PSA values of 211.3 and 236.5 $Å^2$, respectively, which are expected to have less cell permeability than compounds **8** and **9** (having PSA values of 155.0 and 183.4 $Å^2$, respectively).

Experimental

General

The melting points were measured by an X-4 melting point apparatus and were uncorrected. Optical rotations were obtained with a Jasco P-1020 Automatic Digital Polariscope. MS data were obtained in the ESI mode on API Qstar Pulsar instrument; HRMS data were obtained in the ESI mode on LCMS-IT-TOF (Shimadzu, Kyoto, Japan); ^{1}H-NMR and ^{13}C-NMR spectra were recorded on Bruker AV-400 or DRX-500 (Bruker BioSpin GmbH, Rheinstetten, Germany) instruments, using tetramethylsilane as an internal standard. Column chromatography (CC): silica gel (200-300 mesh; Qingdao Makall Group Co., Ltd, Qingdao, China). D-xylose and *n*-butyric anhydride were purchased from Aladdin Chemical Co., Ltd (Guangzhou, China); MTT was purchased from Sigma-Aldrich (St Louis, MO, USA). Dichloromethane and acetonitrile were distilled over calcium hydride. All reagents were commercially available and used without further purification unless indicated otherwise. 4'-Demethylepipodophyllotoxin (**7**) was synthesized according to the described methods.[15] All reactions were monitored using thin-layer chromatography (TLC) on silica gel plates.

*Synthesis of 2,3,4-Tri-O-Butyryl-a-D-Xylopyranose (**6**)*

D-xylose (750 mg, 5 mmol) was suspended in *n*-butyric anhydride (3.5 mL, 20 mmol) and stirred at 0°C. Iodine (50 mg) was added and stirring was continued for 1 hour. The reaction mixture was diluted with CH_2Cl_2 (30 mL) and washed successively with aqueous saturated sodium thiosulfate and aqueous saturated sodium bicarbonate solutions. The organic layer was then dried over sodium sulfate and concentrated in vacuo to give the per-butyrylated crude product. The crude product was dissolved in acetonitrile (10 mL), and 25% ammonia solution (0.2 mL, 10 mmol) was added dropwise slowly. The mixture was stirred at room temperature for 6 hours until no starting material was detected by TLC analysis. The solvent was evaporated and the residue was purified by CC (silica gel, petroleum ether 60°C-90°C: ethyl acetate = 4:1) to afford the product **6** (1.1 g, 60%).

a/β = 6:1.

^{1}H-NMR ($CDCl_3$, 400 MHz) δ 5.52 (d, 1H, J = 9.7 Hz, C^3-H), 5.35 (d, 1H, J = 3.5 Hz, $C^{4''}$-H), 4.96 (m, 1H, $C^{2''}$-H), 4.81 (d, 1H, J = 4.0 Hz, $C^{1''}$-H), 3.82 to 3.75 (m, 2H, $C^{5''}$-CH_2), 2.28 to 2.18 (m, 6H, 3 × $COCH_2$), 1.59 to 1.53 (m, 6H, 3 × CH_2CH_3), 0.90 to 0.84 (m, 9H, 3 × CH_2CH_3).

^{13}C-NMR ($CDCl_3$, 100 MHz) δ 173.0 (C=O), 172.7 (C=O), 172.6 (C=O), 95.9 (C-1''), 71.1, 69.1, 68.9, 58.3 (C-5''), 36.0 ($COCH_2$), 35.9 ($COCH_2$), 35.8 ($COCH_2$), 18.3 (CH_2CH_3), 18.3 (CH_2CH_3), 18.2 (CH_2CH_3), 13.5 (CH_2CH_3), 13.5 (CH_2CH_3), 13.5 (CH_2CH_3).

ESIMS: m/z 383 $[M+Na]^+$.

Table 2. The ClogP Values and PSA of Xyloside of Podophyllotoxin Derivatives **8** to **11**.

Compounds	Molecular formula	MP (°C)	Yield (%)	ClogP	PSA ($Å^2$)
8	$C_{39}H_{48}O_{15}$	92-93	57	5.53	155.0
9	$C_{38}H_{46}O_{15}$	102-104	62	5.20	183.4
10	$C_{27}H_{30}O_{12}$	153-155	70	0.46	211.3
11	$C_{26}H_{28}O_{12}$	162-165	72	0.13	236.5
Etoposide (**2**)	$C_{29}H_{32}O_{13}$	-	-	0.03	198.6

PSA, polar surface area.

*Synthesis of Per-Butyrylated Xyloside Podophyllotoxin Derivatives **8, 9** (General Method)*

To a mixture of 72 mg (0.2 mmol) of 2,3,4-tri-*O*-butyryl-α-D-xylopyranose (**5**) and 83 mg (0.2 mmol) of podophyllotoxin (**1**) or 80.0 mg (0.2 mmol) of 4'-demethylepipodophyllotoxin (**5**) in CH_2Cl_2 (2 mL) was added dropwise a solution of boron trifluoride•etherate (25 μL, 0.02 mmol) in CH_2Cl_2 (1 mL) at -78°C. After another 1 hour of stirring at room temperature, triethylamine (0.1 mL) was added to the mixture, and acetic acid (0.1 mL) was added. The solvent was evaporated and the residue was purified by CC (silica gel, petroleum ether 60°C-90°C:ethyl acetate = 9:1→4:1) to afford per-butyrylated xyloside podophyllotoxin derivatives.

4β-(1"-*O*-(2",3",4"-Tri-*O*-Butyryl-β-D-Xylopyranose))Podophyllotoxin (8)

White powder.
Yield: 57%.
MP: 92°C to 93°C.
$[a]_D^{21.8}$: -50.4 (*c* 0.20, $CHCl_3$).
^{1}H-NMR ($CDCl_3$, 400 MHz) *δ* 6.78 (s, 1H, C^5-H), 6.54 (s, 1H, C^8-H), 6.22 (s, 2H, $C^{2'}$, $C^{6'}$-H), 6.00 to 5.97 (m, 2H, OCH_2O), 5.23 (t, 1H, *J* = 8.0 Hz, $C^{3"}$-H), 5.00 to 4.90 (m, 2H, $C^{4"}$-H, $C^{2"}$-H), 4.87 (d, 1H, *J* = 4.0 Hz, C^4-H), 4.73 (d, 1H, *J* = 7.2 Hz, $C^{1"}$-H), 4.57 (d, 1H, *J* = 5.2 Hz, C^1-H), 4.45 to 4.40 (m, 1H, C^{11}-CHα), 4.29 to 4.25 (m, 1H, $C^{5"}$-CH_a), 4.13 (dd, 1H, *J* = 4.0, 12.0 Hz, C^{11}-CHβ), 3.78 (s, 3H, $C^{4'}$-OCH_3), 3.72 (s, 6H, $C^{3'}$, $C^{5'}$-OCH_3), 3.39 to 3.34 (m, 1H, $C^{5"}$-CH_b), 3.20 (dd, 1H, *J* = 4.0, 12.0 Hz, C^2-H), 3.92 to 3.83 (m, 1H, C^3-H), 2.28 to 1.97 (m, 6H, 3 × $COCH_2$), 1.67 to 1.37 (m, 6H, 3 × CH_2CH_3), 0.92 to 0.78 (m, 9H, 3 × CH_2CH_3).
^{13}C-NMR ($CDCl_3$, 100 MHz) *δ* 174.6 (C-12), 172.5 (C=O), 172.4 (C=O), 171.7 (C=O), 152.5 (C-3', C-5'), 148.7 (C-6), 146.9 (C-7), 137.3 (C-4'), 135.0 (C- C-1'), 132.9 (C-9), 127.8 (C-10), 110.9 (C-8), 109.1 (C-5), 108.3 (C-2', C-6'), 101.6 (OCH_2O), 99.2 (C-1"), 72.9 (C-4), 71.0, 70.6, 68.6, 67.6 (C-11), 62.4 (4'-OCH_3), 60.7 (C-5"), 56.2 (3', 5'-OCH_3), 43.8 (C-2), 40.9 (C-1), 37.5 (C-3), 35.9 ($COCH_2$), 35.8 ($COCH_2$), 35.7 ($COCH_2$), 18.3 (CH_2CH_3), 18.2 (CH_2CH_3), 18.1 (CH_2CH_3), 13.5 (CH_2CH_3), 13.5 (CH_2CH_3), 13.4 (CH_2CH_3).
ESIMS: *m/z* 779 $[M+Na]^+$.
HRESIMS: calcd for $C_{39}H_{48}O_{15}Na$ $[M+Na]^+$ 779.2885, found 779.2786.

4β-(1"-*O*-(2",3",4"-Tri-*O*-Butyryl-β-D-Xylopyranose))-4'-Demethylepipodophyllotoxin (9)

White powder.
Yield: 62%.
MP: 102°C to 104°C.
$[a]_D^{21.8}$: -67.0 (*c* 0.11, $CHCl_3$).
^{1}H-NMR ($CDCl_3$, 500 MHz) *δ* 6.78 (s, 1H, C^5-H), 6.54 (s, 1H, C^8-H), 6.23 (s, 2H, $C^{2'}$, $C^{6'}$-H), 5.99 to 5.96 (m, 2H, OCH_2O), 5.41 (brs, 1H, $C^{4'}$-OH), 5.22 (t, 1H, *J* = 10.0 Hz, $C^{3"}$-H), 5.00 to 4.90 (m, 2H, $C^{2"}$-H, $C^{4"}$-H), 4.86 (d, 1H, *J* = 3.0 Hz, C^4-H), 4.72 (d, 1H, *J* = 7.5 Hz, $C^{1"}$-H), 4.56 (d, 1H, *J* = 5.0 Hz, C^1-H), 4.43 to 4.39 (m, 1H, C^{11}-CHα), 4.25 (t, 1H, *J* = 5.0 Hz, $C^{5"}$-CH_a), 4.13 (dd, 1H, *J* = 5.0, 10.0 Hz, C^{11}-CHβ), 3.75 (s, 6H, $C^{3'}$, $C^{5'}$-OCH_3), 3.38 to 3.34 (m, 1H, $C^{5"}$-CH_b), 3.18 (dd, 1H, *J* = 5.0, 15.0 Hz, C^2-H), 2.90 to 2.85 (m, 1H, C^3-H), 2.27 to 2.00 (m, 6H, 3 × $COCH_2$), 1.63 to 1.38 (m, 6H, 3 × CH_2CH_3), 0.91 to 0.78 (m, 9H, 3 × CH_2CH_3).
^{13}C-NMR ($CDCl_3$, 125 MHz) *δ* 174.6 (C-12), 172.5 (C=O), 172.4 (C=O), 171.7 (C=O), 148.7 (C-6), 146.8 (C-7), 146.4 (C-3', C-5'), 134.1 (C-4'), 133.0 (C-1'), 130.5 (C-9), 127.8 (C-10), 110.9 (C-8), 110.4 (C-5), 107.9 (C-2', C-6'), 101.5 (OCH_2O), 99.2 (C-1"), 73.0 (C-4), 71.0, 70.6, 68.6, 67.6 (C-11), 62.4 (C-5"), 56.4 (3', 5'-OCH_3), 43.7 (C-2), 41.0 (C-1), 37.5 (C-3), 36.0 ($COCH_2$), 35.9 ($COCH_2$), 35.8 ($COCH_2$), 18.3 (CH_2CH_3), 18.2 (CH_2CH_3), 18.1 (CH_2CH_3), 13.5 (CH_2CH_3), 13.5 (CH_2CH_3), 13.4 (CH_2CH_3).
ESIMS: *m/z* 765 $[M+ Na]^+$.
HRESIMS: calcd for $C_{38}H_{46}O_{15}Na$ $[M+ Na]^+$ 765.2729, found 765.2656.

*Synthesis of Xyloside Derivatives of Podophyllotoxin **10, 11** (General Method)*

To a solution of per-butyrylated xyloside podophyllotoxin derivatives **8** and **9** (0.01 mmol) in methanol (1.0 mL) was added sodium methoxide (0.03 mmol). The resulting mixture was stirred for 2 hours and then the pH of the mixture was adjusted to 7.0 by addition of anhydrous Amberlite ion-exchange resin IRA-400 with the aid of pH indicator paper. The resin was removed by filtration, the filtrate concentrated and the residue was purified by CC (chloroform/methanol 9:1) to afford the podophyllotoxin xyloconjugates.

4β-(1"-*O*-(β-D-Xylopyranose))Podophyllotoxin (10)

White powder.
Yield: 70%.
MP: 153°C155°C.
$[a]_D^{25.0}$: -42.6 (*c* 0.10, CH_3OH).
^{1}H-NMR (CD_3OD, 500 MHz) *δ* 6.53 (s, 2H, $C^{2'}$, $C^{6'}$-H), 6.51 (s, 1H, C^5-H), 6.46 (s, 1H, C^8-H), 5.94 to 5.92 (m, 2H, OCH_2O), 4.79 (d, 1H, *J* = 7.2 Hz, $C^{1"}$-H), 4.41 to 4.39 (m, 3H), 4.11 (t, 1H, *J* = 7.2 Hz), 3.84 to 3.82 (m, 2H), 3.78 (s, 6H, $C^{3'}$, $C^{5'}$-OCH_3), 3.76 (s, 3H, $C^{4'}$-OCH_3), 3.64 to 3.62 (m, 2H), 3.52 to 3.51 (m, 2H), 3.21 to 3.20 (m, 1H, C^3-H), 3.12 to 3.10 (m, 1H, C^2-H).
^{13}C-NMR ($CDCl_3$, 125 MHz) *δ* 176.6 (C-12), 154.7 (C-3', C-5'), 149.1 (C-7), 148.1 (C-6), 139.2 (C-4'), 138.0 (C-1'), 133.3 (C-9), 130.2 (C-10), 110.2 (C-1"), 108.7 (C-8), 106.5 (C-2', C-6'), 103.4 (C-5), 102.5 (OCH_2O), 78.0 (C-4), 76.1, 74.9, 73.8, 71.1, 66.9

(C-11), 61.1 (4'-OCH_3), 56.6 (3', 5'-OCH_3), 46.4 (C-2), 45.0 (C-1), 40.7 (C-3).
ESIMS: m/z 569 $[M+Na]^+$.
HRESIMS: calcd for $C_{27}H_{30}O_{12}Na$ $[M+Na]^+$ 569.1629, found 569.1618.

4β-(1''-*O*-(β-D-Xylopyranose))-4'-Demethylepipodophyllotoxin (11)

White powder.
Yield: 72%.
MP: 162°C to 165°C.
$[a]_D^{25.0}$: -60.7 (c 0.12, CH_3OH).
^{1}H-NMR (CD_3OD, 500 MHz) δ 6.50 (s, 1H, C^5-H), 6.49 (s, 2H, $C^{2'}$, $C^{6'}$-H), 6.47 (s, 1H, C^8-H), 5.93 to 5.92 (m, 2H, OCH_2O), 4.77 (d, 1H, J = 7.2 Hz, $C^{1''}$-H), 4.38 to 4.36 (m, 3H), 4.12 to 4.10 (m, 1H), 3.80 to 3.79 (m, 2H), 3.78 (s, 6H, $C^{3'}$, $C^{5'}$-OCH_3), 3.70 to 3.62 (m, 3H), 3.22 to 3.20 (m, 1H, C^3-H), 3.19 to 3.10 (m, 1H, C^2-H).
^{13}C-NMR ($CDCl_3$, 125 MHz) δ 176.7 (C-12), 149.4 (C-3', C-5'), 149.0 (C-7), 148.1 (C-6), 135.5 (C-1'), 133.6 (C-4'), 133.5 (C-9), 130.2 (C-10), 110.3 (C-1''), 109.3 (C-8), 106.5 (C-2', C-6'), 103.7 (C-5), 102.5 (OCH_2O), 78.0 (C-4), 76.1, 75.0, 71.2, 70.0, 66.9 (C-11), 56.8 (3', 5'-OCH_3), 46.3 (C-2), 45.2 (C-1), 40.6 (C-3).
ESIMS: m/z 555 $[M+Na]^+$.
HRESIMS: calcd for $C_{26}H_{28}O_{12}Na$ $[M+Na]^+$ 555.1473, found 555.14696.

Cytotoxicity Assay

Five human cancer lines, human myeloid leukemia (HL-60), hepatocellular carcinoma (SMMC-7721), lung cancer (A-549), breast cancer (MCF-7), and colon cancer (SW480), were used in the cytotoxicity assay. All the cells were cultured in RMPI-1640 or DMEM medium (Hyclone, Logan, UT, USA), supplemented with 10% fetal bovine serum (Hyclone, USA) in 5% CO_2 at 37°C. The cytotoxicity assay was performed according to the MTT method in 96-well microplates.[16] Briefly, adherent cells (100 μL) were seeded into each well of a 96-well cell culture plate and allowed to adhere for 12 hours before drug addition, while suspended cells were seeded just before drug addition, both with an initial density of 1×10^5 cells/mL in 100 μL of medium. Each tumor cell line was exposed to the test compound at various concentrations in triplicate for 48 hours. After the incubation, MTT (100 μg) was added to each well, and the incubation continued for 4 hours at 37°C. The cells were lysed with SDS (200 μL) after removal of 100 μL of medium. The optical density of the lysate was measured at 595 nm in a 96-well microtiter plate reader (Bio-Rad 680). IC_{50} values were calculated by Reed and Muench's method.[21,22]

Calculated Molecular Descriptors

Compounds **8** to **11** were built and energy minimized under the Tripos force field with 0.05 kcal/(mol Å). The Gasteiger-Huckel method was used to calculate charges, and energy minimization was performed by the Powell method with 2000 iterations. The global molecular descriptor (ClogP) and PSA were calculated using the Molecular Spreadsheet application in Sybyl 2.0.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was financial supported by National Nature Science Foundation of China (No. 21602196); the Yunnan Provincial Science and Technology Department (Nos. 2017ZF003-04, 2017FD084, and 2017FG001-046); and Yunnan Agricultural Universit Natural Science Foundation for Young Scientists (No. 2015ZR08).

References

1. Canel C, Moraes RM, Dayan FE, Ferreira D. Podophyllotoxin. *Phytochemistry*. 2000;54(2):115-120.
2. Umesha B, Basavaraju YB, Mahendra C. Synthesis and biological screening of pyrazole moiety containing analogs of podophyllotoxin. *Med Chem Res*. 2015;24(1):142-151.
3. Budman DR. Early studies of etoposide phosphate, a water-soluble prodrug. *Semin Oncol*. 1996;23(6 Suppl 13):8-14.
4. Kamal A, Ali Hussaini SM, Rahim A, Riyaz S. Podophyllotoxin derivatives: a patent review (2012 - 2014). *Expert Opin Ther Pat*. 2015;25(9):1025-1034.
5. Calvaresi EC, Hergenrother PJ. Glucose conjugation for the specific targeting and treatment of cancer. *Chem Sci*. 2013;4(6):2319-2333.
6. Lin Y-S, Tungpradit R, Sinchaikul S, et al. Targeting the delivery of glycan-based paclitaxel prodrugs to cancer cells via glucose transporters. *J Med Chem*. 2008;51(23):7428-7441.
7. Arafa HMM. Possible contribution of beta-glycosidases and caspases in the cytotoxicity of novel glycoconjugates in colon cancer cells. *Invest New Drugs*. 2010;28(3):306-317.
8. Zi C-T, Yang D, Dong F-W, et al. Synthesis and antitumor activity of novel per-butyrylated glycosides of podophyllotoxin and its derivatives. *Bioorg Med Chem*. 2015;23(7):1437-1446.
9. Mani K, Belting M, Ellervik U, et al. Tumor attenuation by 2(6-hydroxynaphthyl)-beta-D-xylopyranoside requires priming of heparan sulfate and nuclear targeting of the products. *Glycobiology*. 2004;14(5):387-397.
10. Mani K, Havsmark B, Persson S, et al. Heparan/chondroitin/dermatan sulfate primer 2-(6-hydroxynaphthyl)-O-beta-D-xylopyranoside preferentially inhibits growth of transformed cells. *Cancer Res*. 1998;58(6):1099-1104.
11. Nilsson U, Johnsson R, Fransson Lars-Åke, Ellervik U, Mani K. Attenuation of tumor growth by formation of antiproliferative glycosaminoglycans correlates with low acetylation of histone H3. *Can Res*. 2010;70(9):3771-3779.

12. Miller SJ. Cellular and physiological effects of short-chain fatty acids. *Mini Rev Med Chem*. 2004;4(8):839-845.
13. Kartha KPR, Field RA. Iodine: a versatile reagent in carbohydrate chemistry IV. Per-O-acetylation, regioselective acylation and acetolysis. *Tetrahedron*. 1997;53(34):11753-11766.
14. Fiandor J, García-López MT, De Las Heras FG, Méndez-Castrillón PP. A Facile Regioselective 1- *O* -Deacylation of Peracylated Glycopyranoses. *Synthesis*. 1985;1985(12):1121-1123.
15. Hansen HF, Jensen RB, Willumsen AM, et al. New compounds related to podophyllotoxin and congeners: synthesis, structure elucidation and biological testing. *Acta Chem Scand*. 1993;47(12):1190-1200.
16. Malich G, Markovic B, Winder C. The sensitivity and specificity of the MTS tetrazolium assay for detecting the in vitro cytotoxicity of 20 chemicals using human cell lines. *Toxicology*. 1997;124(3):179-192.
17. Zi CT, Li GT, Li Y, et al. Synthesis and anticancer activity of 4β-triazole-podophyllotoxin glycosides. *Nat Prod Bioprospect*. 2015;5:83-90.
18. Zi CT, Yang L, Dong FW, et al. Synthesis and antitumor activity of camptothecin-4β-triazolopodophyllotoxin conjugates. *Nat Prod Res*. 2019;12:1-9.
19. Onawole AT, Sulaiman KO, Adegoke RO, Kolapo TU. Identification of potential inhibitors against the Zika virus using consensus scoring. *J Mol Graph Model*. 2017;73:54-61.
20. Pajouhesh H, Lenz GR. Medicinal chemical properties of successful central nervous system drugs. *NeuroRx*. 2005;2(4):541-553.
21. Zamoiskii EA. Evaluation of Reed-Muench method in determination of activity of biological preparations. *Zh Mikrobiol Epidemiol Immunobiol*. 1956;27(1):77-83.
22. Stanic M. A simplification of the estimation of the 50 percent endpoints according to the reed and Muench method. *Pathol Microbiol*. 1963;26:298-302.