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SYNTHESIS OF PODOPHYLLOTOXIN DERIVATIVES AS POTENTIAL ANTITUMOR AGENTS

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*A series of novel podophyllotoxin derivatives was synthesized by coupling 4β-amino-4′-demethyl-4-desoxypodophyllotoxin (**3a**) or 4β-amino-4-desoxypodophyllotoxin (**3b**) with* N*-substituted 5-formylindole (**2a–h**). Their structures were identied by spectroscopic techniques. These novel derivatives were evaluated for cytotoxicity* in vitro *against HepG2 and HeLa cell lines. Compared with etoposide, most of the compounds showed more potent cytotoxicities against two tumor cell lines. Judging from the IC_{50} values, compound **5n** is a promising agent, which is about 15 and 5 times more toxic than etoposide against HepG2 and HeLa cell lines, respectively.*

Keywords: podophyllotoxin, indibulin, cytotoxicity.

Podophyllotoxin is a lignan with potent antiviral and cytotoxic properties [1]. These remarkable properties make podophyllotoxin an important lead compound for the development of new therapeutic agents. Extensive structural modifications of podophyllotoxin have been carried out, which culminated in the clinical introduction of two semisynthetic glucoconjugate analogues of etoposide and teniposide. The chemical modifications lead to the change in the mechanism of action of these lignans, wherein podophyllotoxin acts as antimicrotubule agent, whereas its aforementioned derivatives act as topoisomerase-II inhibitors [2]. However, their high toxicity, low water solubility, acquired drug resistance, and gastrointestinal disturbances have limited the applications of etoposide and teniposide in cancer chemotherapy [3]. In order to obtain better therapeutic agents, more diverse analogues like NK611, GL-311, NPF, etc. have been synthesized [4–6]. The numerous synthesized analogs have allowed improvement in the knowledge of structure–activity relationships. One of the major breakthroughs is the knowledge that the sugar moiety of etoposide is not essential for topoisomerase II inhibition. Synthetic studies on podophyllotoxin have showed that the *O*-linked (ethers, esters) and *S*-linked (thioethers) compounds are less active in comparison to the *N*-linked congeners [7].

Indibulin is a novel, synthetic, small molecule with antitumor activity based upon destabilization of microtubules [8]. This clinical candidate is active against a variety of tumors *in vitro* and *in vivo* [9]. Furthermore, indibulin lacks the neurotoxicity typically associated with other tubulin-binding drugs such as the taxanes and vinca alkaloids, which might be related to its unknown tubulin-binding mode [9]. Both podophyllotoxin and indibulin are potent microtubulin inhibitors, but the indibulin-binding sites of the two compounds are different [10]. This prompted us to design hybrids of indibulin and podophyllotoxin with the aim of discovering novel tubulin inhibitors that can lower podophyllotoxin′s toxicity by binding to a novel tubulin-binding domain.

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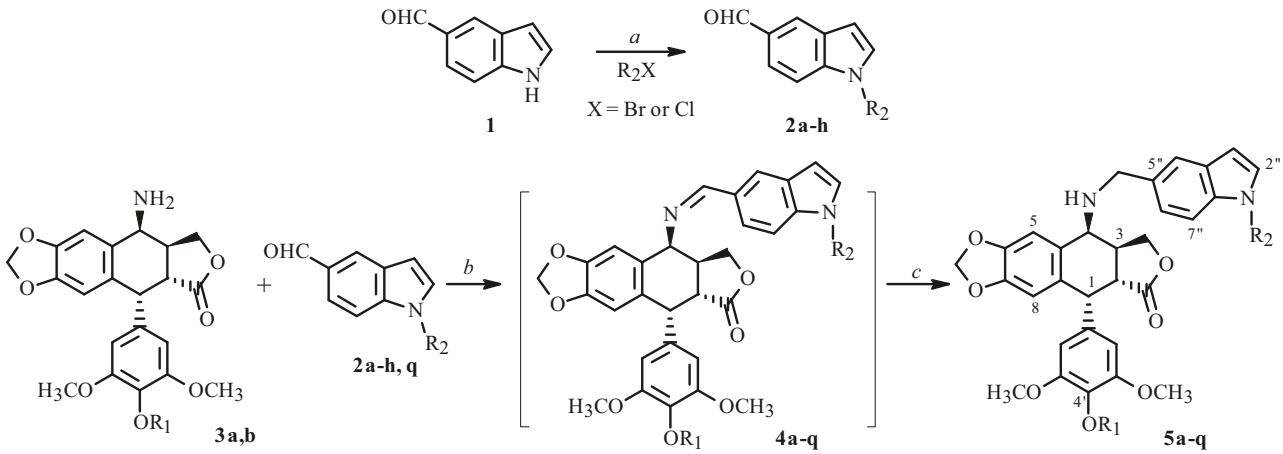
TABLE 1. Cytotoxic Activities of Compounds **5a–q** against HepG2 and HeLa Cell Lines (IC_{50}, μM)*

Compound	HepG2	HeLa	Compound	HepG2	HeLa
5a	9.35 ± 0.86	0.97 ± 0.09	**5j**	25.61 ± 2.69	> 10
5b	1.72 ± 0.15	3.50 ± 0.23	**5k**	5.24 ± 0.49	4.61 ± 0.38
5c	–	77.61 ± 6.75	**5l**	1.74 ± 0.11	0.56 ± 0.38
5d	–	> 10	**5m**	3.04 ± 0.20	35.70 ± 3.26
5e	5.41 ± 0.52	3.97 ± 0.42	**5n**	0.34 ± 0.02	0.60 ± 0.04
5f	10.97 ± 0.83	6.91 ± 0.58	**5o**	3.52 ± 0.34	0.53 ± 0.04
5g	21.4 ± 0.19	7.52 ± 0.64	**5p**	1.78 ± 0.15	2.52 ± 0.02
5h	3.04 ± 0.03	0.76 ± 0.06	**5q**	2.85 ± 0.27	2.93 ± 0.19
5i	21.2 ± 2.05	0.064 ± 0.005	Etoposide[a]	5.17 ± 0.36	3.16 ± 0.38

*IC_{50} is defined as the concentration of drug required to inhibit cell growth by 50% compared with untreated control; it is expressed as mean ±SD of at least three determinations.

[a]Reference control (> 98%).



3a: R_1 = H, **3b:** R_1 = CH_3; **5a:** R_1 = R_2 = H; **5b:** R_1 = CH_3, R_2 = H; **5c:** R_1 = H, R_2 = 3-Cl-$C_6H_4CH_2$
5d: R_1 = CH_3, R_2 = 3-Cl-$C_6H_4CH_2$; **5e:** R_1 = H, R_2 = 2-F-$C_6H_4CH_2$; **5f:** R_1 = CH_3, R_2 = 2-F-$C_6H_4CH_2$
5g: R_1 = H, R_2 = $C_6H_5CH_2$; **5h:** R_1 = CH_3, R_2 = $C_6H_5CH_2$; **5i:** R_1 = H, R_2 = *p*-$CH_3OC_6H_4CH_2$
5j: R_1 = CH_3, R_2 = *p*-$CH_3OC_6H_4CH_2$; **5k:** R_1 = H, R_2 = 4-*tert*-C_4H_9-$C_6H_4CH_2$
5l: R_1 = CH_3, R_2 = 4-*tert*-C_4H_9-$C_6H_4CH_2$; **5m:** R_1 = H, R_2 = 4-CH_3-$C_6H_4CH_2$
5n: R_1 = CH_3, R_2 = 4-CH_3-$C_6H_4CH_2$; **5o:** R_1 = H, R_2 = C_2H_5; **5p:** R_1 = CH_3, R_2 = C_2H_5
5q: R_1 = CH_3, R_2 = 2-Cl-$C_6H_4CH_2$

a. NaH, DMF, 0°C – r.t., 8 – 12 h; *b*. acetic acid, anhydrous ethanol, r.t., 3–6 h; *c*. $NaBH_4$, anhydrous ethanol, 0°C, 3–6 h

Scheme 1

Scheme 1 outlines the synthetic routes of podophyllotoxin derivatives. The *N*-substituted 5-formylindoles **2a–h** were readily synthesized by the reaction of 5-formylindole (**1**) with an aralkyl chloride or bromoethane in the presence of NaH. *N*-substituted 5-formylindoles **2a–h** were coupled with 4*β*-amino-4′-demethyl-4-desoxypodophyllotoxin **3a** or 4*β*-amino-4-desoxypodophyllotoxin **3b** in the presence of acetic acid and anhydrous ethanol and then reduced by $NaBH_4$ to generate the title compounds **5a–q**. Compounds **3a** and **3b** were prepared from 4′-demethylpodophyllotoxin and podophyllotoxin according to known procedures [10].

The cytotoxic activities of the synthesized compounds were tested against HepG2 and HeLa cell lines. IC_{50} values were obtained by the standard MTT assay. As shown in Table 1, some synthesized compounds exhibited more potent cytotoxic activities than etoposide. The cytotoxic activities of compounds **5b**, **5h**, and **5l–q** against HepG2 cell line were higher than etoposide. Compounds **5a**, **5h**, **5i**, **5l**, and **5n–q** showed comparative or even superior cytotoxic activities against HeLa cell line than etoposide. On the basis of IC_{50} values, compound **5n** showed more potent cytotoxic activity than compound **5b**, which implied that the introduction of 4-methylbenzyl at N1 of 5-formylindole enhanced the activity of podophyllotoxin derivatives.

In summary, a series of novel podophyllotoxin derivatives has been synthesized and evaluated for their cytotoxic activities. Most of the synthesized compounds exhibited more potent cytotoxic activities than etoposide. Compound **5n** exhibited promising cytotoxicity against HepG2 and HeLa cell lines (IC_{50} values 0.34 and 0.60 μM, respectively). Additional work is under way to study the antitumor effects of compound **5n** *in vivo*.

EXPERIMENTAL

General Experimental. All materials and reagents were obtained from commercial sources and used without further purification unless stated. Podophyllotoxin (98% purity) and 4′-demethylpodophyllotoxin (98% purity) were purchased from Qingze Corporation of Nanjing in China. CH_2Cl_2 was redistilled over P_2O_5. Melting points were determined on an electric X-4 digital visual melting point apparatus and were uncorrected. PMR spectra were obtained using a Bruker ARX-300 instrument (300 MHz) with tetramethylsilane (TMS) as the internal standard. Mass spectral data were obtained on Agilent 6210 TOP-MS and reported as *m/z*. Optical rotations were obtained by using a Jasco DIP-370 digital polarimeter.

N-Substituted 5-formylindoles (**2a–h**) were synthesized by the literature method [10].

General Procedure for the Synthesis of 5a–q. *N*-Substituted 5-formylindoles **2a–h**, **q** (2.0 mmol) and 4*β*-amino-4′-demethyl-4-desoxypodophyllotoxin (**3a**) or 4*β*-amino-4-desoxypodophyllotoxin (**3b**) (2.2 mmol) were dissolved in 5 mL anhydrous ethanol. To this mixture, 3–5 drops of acetic acid were then added slowly with stirring. After 1–2 h stirring at room temperature, $NaBH_4$ (15.0 mmol) was added to the mixture at one time. After an additional 2–3 h stirring at 0°C, the resulting liquid was neutralized by 1 N HCl until pH 7, concentrated under reduced pressure, and then extracted with CH_2Cl_2 (40 mL × 3) after addition of 30 mL water. The organic phase was washed with water, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was chromatographed on silica gel (CH_2Cl_2–acetone, 100:1–100:2) to give **5a–q**.

4*β*-(((1*H*-Indol-5-yl)-methyl)amino)-4′-demethyl-4-desoxypodophyllotoxin (5a) was obtained by silica gel column chromatography (eluent CH_2Cl_2–acetone, 100:2). Yield 71%, white solid, mp 184–186°C, $[\alpha]_D^{25}$ –67.4° (*c* 0.23, $CHCl_3$). HR-positive ESI-MS *m/z* 529.1970 (calcd for $C_{30}H_{29}N_2O_7$, 529.1975), $C_{30}H_{28}N_2O_7$.

PMR spectrum (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 2.77 (1H, m, H-3), 3.35 (1H, dd, J = 5.1, 13.8, H-2), 3.74 (6H, s, 3′, 5′-OCH_3), 3.94 (1H, m, H-10″), 3.95 (1H, d, J = 4.2, H-4), 3.95 (1H, m, H-11*β*), 4.31 (1H, t, J = 7.5, H-10″), 4.41 (1H, dd, J = 8.1, 10.8, H-11*α*), 4.51 (1H, d, J = 5.1, H-1), 5.89 (1H, d, J = 1.2, OCH_2O), 5.90 (1H, d, J = 1.2, OCH_2O), 6.25 (2H, s, H-2′, 6′), 6.43 (1H, s, H-8), 6.49 (1H, s, H-5), 6.52 (1H, t, J = 3.0, H-3″), 7.16 (1H, dd, J = 1.5, 8.1, H-6″), 7.23 (1H, t, J = 3.0, H-2″), 7.39 (1H, d, J = 8.1, H-7″), 7.57 (1H, br.s, H-4″).

4*β*-(((1*H*-Indol-5-yl)-methyl)amino)-4-desoxypodophyllotoxin (5b) was obtained by silica gel column chromatography (eluent CH_2Cl_2–acetone, 100:1). Yield 73%, white solid, mp 185–187°C, $[\alpha]_D^{25}$ –70.2° (*c* 0.29, $CHCl_3$), HR-positive ESI-MS *m/z* 543.2124 (calcd for $C_{31}H_{31}N_2O_7$, 543.2131), $C_{31}H_{30}N_2O_7$.

PMR spectrum (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 2.79 (1H, m, H-3), 3.38 (1H, dd, J = 5.1, 13.8, H-2), 3.72 (6H, s, 3′, 5′-OCH_3), 3.78 (3H, s, 4′-OCH_3), 3.96 (1H, m, H-10″), 3.98 (1H, d, J = 3.0, H-4), 3.98 (1H, m, H-11*β*), 4.32 (1H, t, J = 7.8, H-10″), 4.43 (1H, dd, J = 9.0, 9.6, H-11*α*), 4.52 (1H, d, J = 5.1, H-1), 6.24 (2H, s, H-2′, 6′), 6.43 (1H, s, H-8), 6.49 (1H, s, H-5), 6.54 (1H, d, J = 2.7, H-3″), 5.91 (2H, s, OCH_2O), 7.18 (1H, br.d, J = 8.4, H-6″), 7.22 (1H, d, J = 2.7, H-2″), 7.40 (1H, d, J = 8.4, H-7″), 7.58 (1H, br.s, H-4″).

4*β*-(((1-(3-Chlorobenzyl)-indol-5-yl)-methyl)amino)-4′-demethyl-4-desoxypodophyllotoxin (5c) was obtained by silica gel column chromatography (eluent CH_2Cl_2–acetone, 100:3). Yield 72%, white solid, mp 179–181°C, $[\alpha]_D^{25}$–50.5° (*c* 0.16, $CHCl_3$). HR-positive ESI-MS *m/z* 653.2050 (calcd for $C_{37}H_{34}ClN_2O_7$, 653.2055), $C_{37}H_{33}ClN_2O_7$.

PMR spectrum (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 2.78 (1H, m, H-3), 3.34 (1H, dd, J = 5.1, 13.8, H-2), 3.75 (6H, s, 3′, 5′-OCH_3), 3.93 (1H, m, H-11*β*), 3.93 (1H, m, H-10″), 3.96 (1H, br.s, H-4), 4.30 (1H, t, J = 6.9, H-10″), 4.40 (1H, dd, J = 8.4, 9.9, H-11*α*), 4.50 (1H, d, J = 5.1, H-1), 5.29 (2H, s, CH_2), 5.90 (1H, br.s, OCH_2O), 5.92 (1H, br.s, OCH_2O), 6.25 (2H, s, H-2′, 6′), 6.43 (1H, s, H-8), 6.50 (1H, s, H-5), 6.54 (1H, d, J = 3.3, H-3″), 6.93 (1H, m, H-6‴), 7.09 (1H, br.s, H-2‴), 7.14 (1H, d, J = 3.3, H-2″), 7.15 (1H, br.d, J = 8.4, H-6″), 7.20 (1H, d, J = 8.4, H-7″), 7.21–7.25 (2H, m, H-4‴, 5‴), 7.59 (1H, br.s, H-4″).

4*β*-(((1-(3-Chlorobenzyl)-indol-5-yl)-methyl)amino)-4-desoxypodophyllotoxin (5d) was obtained by silica gel column chromatography (eluent CH_2Cl_2–acetone, 100:2). Yield 71%, white solid, mp 178–180°C, $[\alpha]_D^{25}$ –48.3° (*c* 0.23, $CHCl_3$). HR-positive ESI-MS *m/z* 667.2209 (calcd for $C_{38}H_{36}ClN_2O_7$, 667.2211), $C_{38}H_{35}ClN_2O_7$.

PMR spectrum (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 2.79 (1H, m, H-3), 3.36 (1H, dd, J = 5.1, 13.8, H-2), 3.72 (6H, s, 3′, 5′-OCH_3), 3.78 (3H, s, 4′-OCH_3), 3.94 (1H, m, H-11*β*), 3.94 (1H, m, H-10″), 3.96 (1H, d, J = 4.2, H-4), 4.31 (1H, t, J = 7.5,

H-10″), 4.41 (1H, dd, J = 8.1, 9.6, H-11α), 4.51 (1H, d, J = 5.1, H-1), 5.28 (2H, s, CH_2), 5.91 (1H, br.s, OCH_2O), 5.92 (1H, br.s, OCH_2O), 6.24 (2H, s, H-2′, 6′), 6.44 (1H, s, H-8), 6.50 (1H, s, H-5), 6.54 (1H, d, J = 3.3, H-3″), 6.93 (1H, m, H-6‴), 7.09 (1H, br.s, H-2‴), 7.14 (1H, d, J = 3.3, H-2″), 7.15 (1H, d, J = 8.7, H-6″), 7.21 (1H, d, J = 8.7, H-7″), 7.21–7.24 (2H, m, H-4‴, 5‴), 7.59 (1H, br.s, H-4″).

4β-(((1-(2-Fluorobenzyl)-indol-5-yl)-methyl)amino)-4′-demethyl-4-desoxypodophyllotoxin (5e) was obtained by silica gel column chromatography (eluent CH_2Cl_2–acetone, 100:2). Yield 71%, white solid, mp 178–180°C, $[\alpha]_D^{25}$ –44.1° (*c* 0.40, $CHCl_3$). HR-positive ESI-MS *m/z* 637.3242 (calcd for $C_{37}H_{34}FN_2O_7$, 637.3250), $C_{37}H_{33}FN_2O_7$.

PMR spectrum (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 2.77 (1H, m, H-3), 3.34 (1H, dd, J = 5.1, 13.8, H-2), 3.75 (6H, s, 3′, 5′-OCH_3), 3.94 (1H, m, H-10″), 3.95 (1H, d, J = 3.9, H-4), 3.95 (1H, m, H-11β), 4.28 (1H, t, J = 7.8, H-10″), 4.40 (1H, dd, J = 8.1, 10.8, H-11α), 4.50 (1H, d, J = 5.1, H-1), 5.37 (2H, s, CH_2), 5.89 (1H, d, J = 1.2, OCH_2O), 5.91 (1H, d, J = 1.2, OCH_2O), 6.25 (2H, s, H-2′, 6′), 6.43 (1H, s, H-8), 6.50 (1H, s, H-5), 6.54 (1H, d, J = 3.3, H-3″), 6.82 (1H, td, J = 7.5, 1.8, H-3‴), 6.98 (1H, td, J = 7.5, 1.2, H-5‴), 7.07 (1H, td, J = 8.4, 1.2, H-6‴), 7.15 (1H, br.d, J = 8.4, H-6″), 7.18 (1H, d, J = 3.3, H-2″), 7.23 (1H, m, H-4‴), 7.34 (1H, d, J = 8.4, H-7″), 7.58 (1H, br.s, H-4″).

4β-(((1-(2-Fluorobenzyl)-indol-5-yl)-methyl)amino)-4-desoxypodophyllotoxin (5f) was obtained by silica gel column chromatography (eluent CH_2Cl_2–acetone, 100:1). Yield 69%, white solid, mp 176–178°C, $[\alpha]_D^{25}$ –43.4° (*c* 0.18, $CHCl_3$). HR-positive ESI-MS *m/z* 651.2504 (calcd for $C_{38}H_{36}FN_2O_7$, 651.2507), $C_{38}H_{35}FN_2O_7$.

PMR spectrum (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 2.78 (1H, m, H-3), 3.39 (1H, dd, J = 5.4, 13.8, H-2), 3.71 (6H, s, 3′, 5′-OCH_3), 3.78 (3H, s, 4′-OCH_3), 3.93 (1H, m, H-11β), 3.93 (1H, m, H-10″), 3.97 (1H, d, J = 3.9, H-4), 4.29 (1H, t, J = 7.8, H-10″), 4.41 (1H, dd, J = 8.4, 10.8, H-11α), 4.51 (1H, d, J = 5.4, H-1), 5.37 (2H, s, CH_2), 5.90 (1H, s, OCH_2O), 5.91 (1H, s, OCH_2O), 6.24 (2H, s, H-2′, 6′), 6.43 (1H, s, H-8), 6.51 (1H, s, H-5), 6.53 (1H, d, J = 3.0, H-3″), 6.82 (1H, td, J = 7.5, 1.5, H-3‴), 6.97 (1H, td, J = 7.5, 1.2, H-5‴), 7.06 (1H, td, J = 8.4, 1.2, H-6‴), 7.16 (1H, br.d, J = 8.4, H-6″), 7.18 (1H, d, J = 3.0, H-2″), 7.23 (1H, m, H-4‴), 7.34 (1H, d, J = 8.4, H-7″), 7.58 (1H, br.s, H-4″), .

4β-(((1-Benzyl-indol-5-yl)-methyl)amino)-4′-demethyl-4-desoxypodophyllotoxin (5g) was obtained by silica gel column chromatography (eluent CH_2Cl_2–acetone, 100:2). Yield 74%, white solid, mp 173–175°C, $[\alpha]_D^{25}$ –92.0° (*c* 0.22, $CHCl_3$). HR-positive ESI-MS *m/z* 619.2438 (calcd for $C_{37}H_{35}N_2O_7$, 619.2444), $C_{37}H_{34}N_2O_7$.

PMR spectrum (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 2.78 (1H, m, H-3), 3.34 (1H, dd, J = 5.1, 13.8, H-2), 3.74 (6H, s, 3′, 5′-OCH_3), 3.95 (1H, m, H-11β), 3.95 (1H, m, H-10″), 3.96 (1H, d, J = 3.9, H-4), 4.28 (1H, t, J = 7.8, H-10″), 4.40 (1H, dd, J = 8.1, 10.8, H-11α), 4.50 (1H, d, J = 5.1, H-1), 5.28 (2H, s, CH_2), 5.89 (1H, d, J = 1.2, OCH_2O), 5.91 (1H, d, J = 1.2, OCH_2O), 6.25 (2H, s, H-2′, 6′), 6.43 (1H, s, H-8), 6.49 (1H, s, H-5), 6.52 (1H, d, J = 3.0, H-3″), 7.07 (2H, dd, J = 1.8, 7.8, H-2‴, 6‴), 7.13 (1H, br.d, J = 8.4, H-6″), 7.15 (1H, d, J = 3.0, H-2″), 7.25–7.31 (3H, m, H-3‴, 4‴, 5‴), 7.27 (1H, d, J = 8.4, H-7″), 7.59 (1H, br.s, H-4″).

4β-(((1-Benzyl-indol-5-yl)-methyl)amino)-4-desoxypodophyllotoxin (5h) was obtained by silica gel column chromatography (eluent CH_2Cl_2–acetone, 100:1). Yield 72%, white solid, mp 176–178°C, $[\alpha]_D^{25}$ –79.2° (*c* 0.28, $CHCl_3$). HR-positive ESI-MS *m/z* 633.2595 (calcd for $C_{38}H_{37}N_2O_7$, 633.2601), $C_{38}H_{36}N_2O_7$.

PMR spectrum (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 2.79 (1H, m, H-3), 3.35 (1H, dd, J = 5.1, 13.8, H-2), 3.71 (6H, s, 3′, 5′-OCH_3), 3.78 (3H, s, 4′-OCH_3), 3.94 (1H, m, H-11β), 3.94 (1H, m, H-10″), 3.95 (1H, d, J = 3.9, H-4), 4.28 (1H, t, J = 7.8, H-10″), 4.40 (1H, dd, J = 8.1, 10.8, H-11α), 4.50 (1H, d, J = 5.1, H-1), 5.32 (2H, s, CH_2), 5.93 (2H, s, OCH_2O), 6.23 (2H, s, H-2′, 6′), 6.44 (1H, s, H-8), 6.50 (1H, s, H-5), 6.54 (1H, d, J = 3.0, H-3″), 7.08 (2H, dd, J = 1.8, 7.8, H-2‴, 6‴), 7.15 (1H, d, J = 3.0, H-2″), 7.25 (1H, m, H-6″), 7.25–7.31 (3H, m, H-3‴, 4‴, 5‴), 7.27 (1H, d, J = 8.4, H-7″), 7.60 (1H, br.s, H-4″).

4β-(((1-(4-Methoxybenzyl)-indol-5-yl)-methyl)amino)-4′-demethyl-4-desoxypodophyllotoxin (5i) was obtained by silica gel column chromatography (eluent CH_2Cl_2–acetone, 100:2). Yield 76%, white solid, mp 172–174°C, $[\alpha]_D^{25}$ –48.4° (*c* 0.20, $CHCl_3$). HR-positive ESI-MS *m/z* 649.2544 (calcd for $C_{38}H_{37}N_2O_8$, 649.2550), $C_{38}H_{36}N_2O_8$.

PMR spectrum (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 2.77 (1H, m, H-3), 3.35 (1H, dd, J = 5.1, 13.2, H-2), 3.75 (6H, s, 3′, 5′-OCH_3), 3.76 (3H, s, 4‴-OCH_3), 3.94 (1H, m, H-11β), 3.94 (1H, m, H-10″), 3.96 (1H, d, J = 3.9, H-4), 4.29 (1H, t, J = 7.8, H-10″), 4.40 (1H, dd, J = 8.4, 10.5, H-11α), 4.51 (1H, d, J = 5.1, H-1), 5.26 (2H, s, CH_2), 5.90 (1H, d, J = 1.2, OCH_2O), 5.92 (1H, d, J = 1.2, OCH_2O), 6.25 (2H, s, H-2′, 6′), 6.43 (1H, s, H-8), 6.49 (1H, s, H-5), 6.50 (1H, d, J = 3.0, H-3″), 6.80 (2H, d, J = 8.7, H-3‴, 5‴), 7.05 (2H, d, J = 8.7, H-2‴, 6‴), 7.12 (1H, d, J = 3.0, H-2″), 7.15 (1H, br.d, J = 8.4, H-6″), 7.30 (1H, d, J = 8.4, H-7″), 7.57 (1H, br.s, H-4″).

4β-(((1-(4-Methoxybenzyl)-indol-5-yl)-methyl)amino)-4-desoxypodophyllotoxin (5j) was obtained by silica gel column chromatography (eluent CH_2Cl_2–acetone, 100:1). Yield 74%, white solid, mp 174–176°C, $[\alpha]_D^{25}$–39.3° (*c* 0.17, $CHCl_3$). HR-positive ESI-MS *m/z* 663.2700 (calcd for $C_{39}H_{39}N_2O_8$, 663.2706), $C_{39}H_{38}N_2O_8$.

PMR spectrum (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 2.79 (1H, m, H-3), 3.37 (1H, dd, J = 5.4, 13.8, H-2), 3.71 (6H, s, 3′, 5′-OCH_3), 3.75 (3H, s, 4‴-OCH_3), 3.78 (3H, s, 4′-OCH_3), 3.96 (1H, m, H-11β), 3.96 (1H, m, H-10″), 3.98 (1H, d, J = 3.9, H-4), 4.31 (1H, t, J = 7.8, H-10″), 4.41 (1H, dd, J = 8.1, 10.8, H-11α), 4.52 (1H, d, J = 5.4, H-1), 5.28 (2H, s, CH_2), 5.89 (1H, d, J = 1.2, OCH_2O), 5.91 (1H, d, J = 1.2, OCH_2O), 6.24 (2H, s, H-2′, 6′), 6.43 (1H, s, H-8), 6.47 (1H, s, H-5), 6.50 (1H, d, J = 3.0, H-3″), 6.80 (2H, d, J = 8.7, H-3‴, 5‴), 7.05 (2H, d, J = 8.7, H-2‴, 6‴), 7.13 (1H, d, J = 3.0, H-2″), 7.15 (1H, dd, J = 8.4, 1.5, H-6″), 7.29 (1H, d, J = 8.4, H-7″), 7.57 (1H, br.s, H-4″).

4β-(((1-(4-*tert*-Butylbenzyl)-indol-5-yl)-methyl)amino)-4′-demethyl-4-desoxypodophyllotoxin (5k) was obtained by silica gel column chromatography (eluent CH_2Cl_2–acetone, 100:2). Yield 73%, white solid, mp 178–180°C, $[\alpha]_D^{25}$ –38.5° (*c* 0.27, $CHCl_3$). HR-positive ESI-MS *m*/*z* 675.3066 (calcd for $C_{41}H_{43}N_2O_8$, 675.3070), $C_{41}H_{42}N_2O_8$.

PMR spectrum (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 1.28 (9H, s, 4‴-C-CH_3), 2.78 (1H, m, H-3), 3.37 (1H, dd, J = 5.1, 13.8, H-2), 3.75 (6H, s, 3′, 5′-OCH_3), 3.92 (1H, m, H-11β), 3.92 (1H, m, H-10″), 3.98 (1H, d, J = 4.2, H-4), 4.30 (1H, t, J = 7.5, H-10″), 4.44 (1H, dd, J = 8.4, 9.9, H-11α), 4.51 (1H, d, J = 5.1, H-1), 5.29 (2H, s, CH_2), 5.92 (2H, s, OCH_2O), 6.25 (2H, s, H-2′, 6′), 6.43 (1H, s, H-8), 6.50 (1H, s, H-5), 6.52 (1H, d, J = 3.0, H-3″), 7.04 (2H, d, J = 8.7, H-2‴, 6‴), 7.14 (1H, d, J = 3.0, H-2″), 7.17 (1H, d, J = 8.4, H-6″), 7.30 (2H, d, J = 8.7, H-3‴, 5‴), 7.31 (1H, d, J = 8.4, H-7″), 7.58 (1H, br.s, H-4″).

4β-(((1-(4-*tert*-Butylbenzyl)-indol-5-yl)-methyl)amino)-4-desoxypodophyllotoxin (5l) was obtained by silica gel column chromatography (eluent CH_2Cl_2–acetone, 100:1). Yield 72%, white solid, mp 181–182°C, $[\alpha]_D^{25}$ –41.6° (*c* 0.18, $CHCl_3$). HR-positive ESI-MS *m*/*z* 689.3225 (calcd for $C_{42}H_{45}N_2O_7$, 689.3227), $C_{42}H_{44}N_2O_7$.

PMR spectrum (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 1.27 (9H, s, 4‴-C-CH_3), 2.79 (1H, m, H-3), 3.38 (1H, dd, J = 5.1, 13.8, H-2), 3.72 (6H, s, 3′, 5′-OCH_3), 3.78 (3H, s, 4′-OCH_3), 3.92 (1H, m, H-11β), 3.92 (1H, m, H-10″), 3.98 (1H, d, J = 3.6, H-4), 4.32 (1H, t, J = 7.5, H-10″), 4.43 (1H, dd, J = 9.0, 10.5, H-11α), 4.51 (1H, d, J = 5.1, H-1), 5.29 (2H, s, CH_2), 5.90 (1H, s, OCH_2O), 5.91 (1H, s, OCH_2O), 6.24 (2H, s, H-2′, 6′), 6.43 (1H, s, H-8), 6.50 (1H, s, H-5), 6.52 (1H, d, J = 3.0, H-3″), 7.14 (1H, d, J = 3.0, H-2″), 7.16 (1H, J = 8.4, H-6″), 7.02 (2H, d, J = 8.1, H-2‴, 6‴), 7.28 (2H, d, J = 8.1, H-3‴, 5‴), 7.34 (1H, d, J = 8.4, H-7″), 7.58 (1H, br.s, H-4″).

4β-(((1-(4-Methylbenzyl)-indol-5-yl)-methyl)amino)-4′-demethyl-4-desoxypodophyllotoxin (5m) was obtained by silica gel column chromatography (eluent CH_2Cl_2–acetone, 100:2). Yield 70%, white solid, mp 183–185°C, $[\alpha]_D^{25}$ –46.1° (*c* 0.19, $CHCl_3$). HR-positive ESI-MS *m*/*z* 633.2588 (calcd for $C_{38}H_{37}N_2O_7$, 633.2601), $C_{38}H_{36}N_2O_7$.

PMR spectrum (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 2.33 (3H, s, 4‴-CH_3), 2.78 (1H, m, H-3), 3.38 (1H, dd, J = 4.8, 14.0, H-2), 3.78 (6H, s, 3′, 5′-OCH_3), 3.93 (1H, m, H-11β), 3.93 (1H, m, H-10″), 3.98 (1H, d, J = 3.3, H-4), 4.31 (1H, t, J = 7.6, H-10″), 4.44 (1H, dd, J = 8.8, 10.0, H-11α), 4.53 (1H, d, J = 4.8, H-1), 5.31 (2H, s, CH_2), 5.95 (2H, s, OCH_2O), 6.29 (2H, s, H-2′, 6′), 6.47 (1H, s, H-8), 6.55 (1H, s, H-5), 6.56 (1H, d, J = 2.4, H-3″), 7.03 (2H, d, J = 8.0, H-2‴, 6‴), 7.12 (2H, d, J = 8.0, H-3‴, 5‴), 7.16 (1H, d, J = 2.4, H-2″), 7.19 (1H, br.d, J = 8.4, H-6″), 7.32 (1H, d, J = 8.4, H-7″), 7.62 (1H, br.s, H-4″).

4β-(((1-(4-Methylbenzyl)-indol-5-yl)-methyl)amino)-4-desoxypodophyllotoxin (5n) was obtained by silica gel column chromatography (eluent CH_2Cl_2–acetone, 100:1). Yield 71%, white solid, mp 181–183°C, $[\alpha]_D^{25}$ –47.8° (*c* 0.10, $CHCl_3$). HR-positive ESI-MS *m*/*z* 647.2751 (calcd for $C_{39}H_{39}N_2O_7$, 647.2757), $C_{39}H_{38}N_2O_7$.

PMR spectrum (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 2.32 (3H, s, 4‴-CH_3), 2.80 (1H, m, H-3), 3.38 (1H, dd, J = 4.8, 14.0, H-2), 3.74 (6H, s, 3′, 5′-OCH_3), 3.81 (3H, s, 4′-OCH_3), 3.95 (1H, m, H-11β), 3.95 (1H, m, H-10″), 3.98 (1H, d, J = 3.2, H-4), 4.33 (1H, t, J = 7.6, H-10″), 4.44 (1H, dd, J = 8.8, 10.0, H-11α), 4.53 (1H, d, J = 4.8, H-1), 5.31 (2H, s, CH_2), 5.93 (1H, s, OCH_2O), 5.94 (1H, s, OCH_2O), 6.28 (2H, s, H-2′, 6′), 6.46 (1H, s, H-8), 6.54 (1H, s, H-5), 6.54 (1H, br.s, H-3″), 7.03 (2H, d, J = 7.6, H-2‴, 6‴), 7.12 (2H, d, J = 7.6, H-3‴, 5‴), 7.16 (1H, br.s, H-2″), 7.17 (1H, br.d, J = 8.4, H-6″), 7.32 (1H, d, J = 8.4, H-7″), 7.61 (1H, br.s, H-4″), .

4β-(((1-Ethyl-indol-5-yl)-methyl)amino)-4′-demethyl-4-desoxypodophyllotoxin (5o) was obtained by silica gel column chromatography (eluent CH_2Cl_2–acetone, 100:3). Yield 68%, white solid, mp 169–171°C, $[\alpha]_D^{25}$ –62.4° (*c* 0.19, $CHCl_3$). HR-positive ESI-MS *m*/*z* 557.2283 (calcd for $C_{32}H_{33}N_2O_7$, 557.2288), $C_{32}H_{32}N_2O_7$.

PMR spectrum (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 1.47 (3H, t, J = 7.2, H_3-2″), 2.77 (1H, m, H_3-3), 3.38 (1H, dd, J = 5.1, 13.8, H-2), 3.74 (6H, s, 3′, 5′-OCH_3), 3.92 (1H, m, H-11β), 3.92 (1H, m, H-10″), 3.97 (1H, d, J = 3.3, H-4), 4.18 (2H, q, J = 7.2, H_2-1‴), 4.31 (1H, t, J = 7.5, H-10″), 4.43 (1H, dd, J = 8.4, 10.5, H-11α), 4.51 (1H, d, J = 5.1, H-1), 5.90 (2H, s, OCH_2O), 6.25 (2H, s, H-2′, 6′), 6.43 (1H, s, H-8), 6.47 (1H, d, J = 3.3, H-3″), 6.51 (1H, s, H-5), 7.14 (1H, d, J = 3.3, H-2″), 7.18 (1H, dd, J = 1.2, 8.4, H-6″), 7.35 (1H, d, J = 8.4, H-7″), 7.56 (1H, br.s, H-4″).

4β-(((1-Ethyl-indol-5-yl)-methyl)amino)-4-desoxypodophyllotoxin (5p) was obtained by silica gel column chromatography (eluent CH_2Cl_2–acetone, 100:2). Yield 71%, white solid, mp 172–173°C, $[\alpha]_D^{25}$ –61.8° (*c* 0.18, $CHCl_3$). HR-positive ESI-MS *m*/*z* 571.2439 (calcd for $C_{33}H_{35}N_2O_7$, 571.2444), $C_{33}H_{34}N_2O_7$.

PMR spectrum (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 1.49 (3H, t, J = 7.2, H_3-2‴), 2.79 (1H, m, H-3), 3.39 (1H, dd, J = 5.6, 14.0, H-2), 3.72 (6H, s, 3′, 5′-OCH_3), 3.80 (3H, s, 4′-OCH_3), 3.95 (1H, m, H-11β), 3.95 (1H, m, H-10″), 4.00 (1H, d, J = 4.0, H-4), 4.21 (2H, q, J = 7.2, H_2-1‴), 4.34 (1H, t, J = 7.6, H-10″), 4.45 (1H, dd, J = 8.4, 10.8, H-11α), 4.54 (1H, d, J = 5.6, H-1), 5.93 (2H, s, OCH_2O), 6.28 (2H, s, H-2′, 6′), 6.46 (1H, s, H-8), 6.50 (1H, d, J = 2.8, H-3″), 6.55 (1H, s, H-5), 7.16 (1H, d, J = 2.8, H-2″), 7.21 (1H, br.d, J = 8.4, H-6″), 7.39 (1H, d, J = 8.4, H-7″), 7.59 (1H, br.s, H-4″).

4β-(((1-(2-Chlorobenzyl)-indol-5-yl)-methyl)amino)-4-desoxypodophyllotoxin (5q) was obtained by silica gel column chromatography (eluent CH_2Cl_2–acetone, 100:1). Yield 73%, white solid, mp 180–182°C, $[\alpha]_D^{25}$ –47.5° (*c* 0.21, $CHCl_3$). HR-positive ESI-MS *m*/*z* 667.2196 (calcd for $C_{38}H_{36}ClN_2O_7$, 667.2211), $C_{38}H_{35}ClN_2O_7$.

PMR spectrum (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 2.79 (1H, m, H-3), 3.37 (1H, dd, J = 5.1, 13.8, H-2), 3.72 (6H, s, 3′, 5′-OCH_3), 3.78 (3H, s, 4′-OCH_3), 3.94 (1H, m, H-11β), 3.96 (1H, m, H-10″), 3.97 (1H, d, J = 3.9, H-4), 4.31 (1H, t, J = 7.5, H-10″), 4.41 (1H, dd, J = 8.1, 9.6, H-11α), 4.52 (1H, d, J = 5.1, H-1), 5.29 (2H, s, CH_2), 5.91 (1H, s, OCH_2O), 5.92 (1H, s, OCH_2O), 6.24 (2H, s, H-2′, 6′), 6.44 (1H, s, H-8), 6.50 (1H, s, H-5), 6.56 (1H, d, J = 2.7, H-3″), 7.06 (1H, td, J = 7.5, 1.2, H-6‴), 7.16 (1H, d, J = 2.7, H-2″), 7.20 (1H, td, J = 7.5, 1.2, H-3‴), 7.24 (2H, m, H-4‴, 5‴), 7.27 (1H, d, J = 7.8, H-6″), 7.41 (1H, d, J = 7.8, 1.0, H-7″), 7.61 (1H, br.s, H-4″).

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