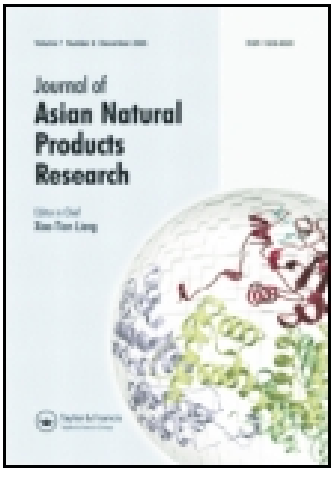

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Synthesis and biological evaluation of novel podophyllotoxin analogs as antitumor agents

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Synthesis and biological evaluation of novel podophyllotoxin analogs as antitumor agents

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A series of 4β *N*-indole-substituted podophyllotoxin derivatives were synthesized. Nine target compounds were evaluated against human cancer cell lines (HeLa, K562, and K562/A02) using MTT assay including three imine derivatives **8**, **9**, and **10** *in vitro*. The result showed that the three compounds had higher antitumor activity than their reduced forms. Among them, compounds **8**, **9**, **11**, and **16** were superior to the positive control VP-16.

Keywords: podophyllotoxin; indole; antitumor; thiophene

1. Introduction

Podophyllotoxin as an antitumor aryltetralin lignin is isolated from the roots of the North American *Podophyllum peltatum* Linnaeus and Tibetan *Podophyllum emodi* Wall [1,2]. Due to its antineoplasctic and antiviral properties, podophyllotoxin is concerned by more and more researchers [3]. But due to severe cytotoxicity of podophyllotoxin (**1**) (Figure 1), it has not been used as an anticancer drug [4]. Some derivatives of C-4 position of podophyllotoxin such as etoposide [5], etoposide (VP-16) and teniposide used in clinic have lower side effects than podophyllotoxin as antitumor agent [6]. Chemical modifications lead to the change in the mechanism of action of these ligands, wherein podophyllotoxin acts as an antimicrotubule agent, whereas its aforementioned derivatives act as topoisomerase-II inhibitors [7–9]. However, its current therapeutic use is hindered by side effects associated with myelosuppression, neutropenia, nausea, drug resistance, and poor water solubility [10,11]. To overcome the disadvantages and obtain more prodrugs, diverse analogs such as GL-331 (2), NPF(3), TOP-53(4), NK-611(5), and so on (Figure 1) which are under clinical trial have been developed.

Due to the fact that the chemical and biological studies of heterocyclic compounds have played an important role for a long time in medicine, more and more antitumor drugs are expected to be found. However, compounds with indole group in which the benzene and pyrrole rings are fused through the 2- and 3-positions of the pyrrole nucleus can also be found in various natural products, such as indole alkaloids.

In addition, indole derivatives are found to possess several biological activities including antibiotic, anti-inflammatory, analgesic, anticonvulsant, antimalarial,

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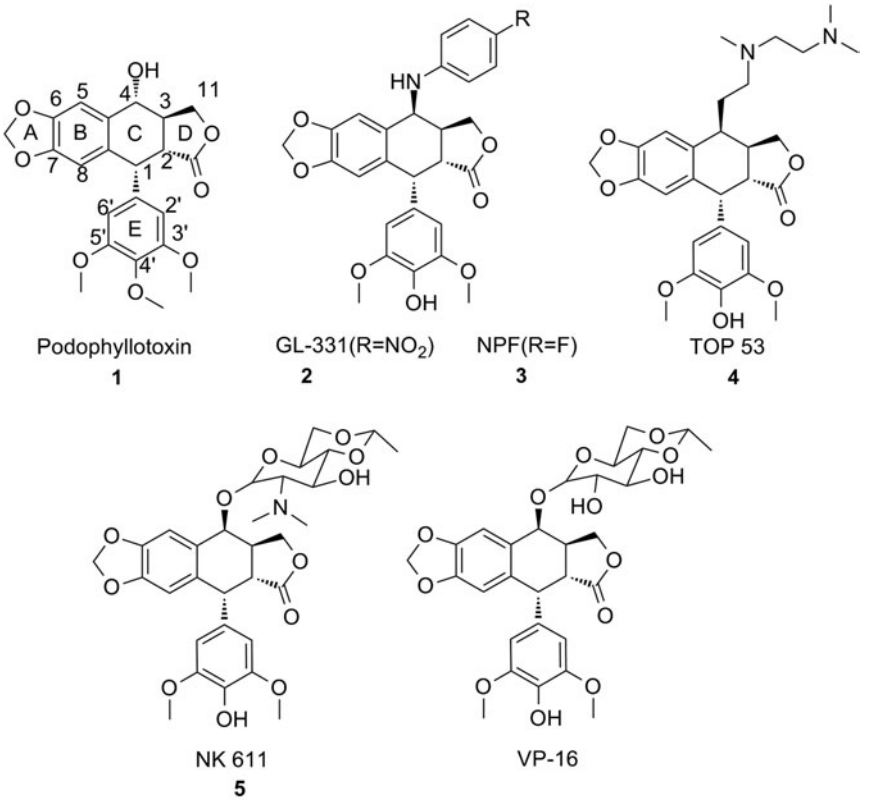


Figure 1. Structures of podophyllotoxin, NPF, GL331, TOP 53, NK61, and VP-16.

anticancer, antiulcer, and so on [12,13]. Besides, thiophene has a similar construct like furan, which is an important part of ribose-5-phosphate [14,15]. For example, thienyl derivatives of Meldrum's acid possess neurotropic activity comprising both depriming and activating components [16]. Therefore, it may be an important construct for antitumor activity.

In recent years, our group has been engaged in structural modification of podophyllotoxin in order to get high-efficiency and low-toxicity antitumor agents, and multiple compounds were found by active screening, e.g., indole-3-gloryl podophyllotoxin designed according to the association strategy. And the antitumor activity of imine derivatives was first screened as intermediate. Finally, we got nine podophyllotoxin derivatives and we compared the different antitumor activities between imine and amine products.

2. Results and discussion

In this project, nine novel podophyllotoxin derivatives were designed and synthesized. Then, the cytotoxicity of compounds was tested *in vitro* by the standard MTT assay using HeLa cells for the first time.

The synthesis of these compounds consisted of two parts. The first part was the synthesis of *N*-substituted indole. In the presence of NaH, indole should be put into dimethylformamide (DMF) with environmental temperature below 0°C [17]. In particular, attention needs to be paid that the raw material should be added separately in order to reduce secondary reaction. From the result of the reaction

Table 1. *In vitro* activity of synthesized compounds (**8**–**16**) and positive controls (VP-16) against HeLa, K562, and K562/A02 cell lines.

	IC_{50}[a] (10^{-6} mol l^{-1})		
No.	HeLa	K562	K562/A02
8	0.79	>100	>100
9	16.46	4.77	4.23
10	27.20	3.64	2.43
11	>100	60.94	1.45
12	>100	76.56	14.04
13	>100	76.56	>100
14	>100	86.88	90.54
15	25.26	80.30	93.32
16	1.15	>100	>100
VP-16	21.44	12.45	8.02

[a] IC_{50}: concentration that causes a 50% reduction of cell growth.

rate and absorptivity at different temperatures, we found that we could get higher reaction absorptivity and the product could be used in the next reaction without further purification when the temperature was kept at 25–30°C.

The second part was the synthesis of *N*-substituted indole-4-imine-deoxypodophyllotoxins; much glacial acetic acid was needed in this reaction, and the compounds must be sealed and stored in the dark as they could decompose easily.

The cytotoxicity of all compounds was tested *in vitro* against HeLa, K562, and K562/A02 cells using the standard MTT assay. The result is shown in Table 1, and all the imine derivatives showed stronger cytotoxicity than their reduced analogs against HeLa cells. The possible reason could be that the imines with many electrons showed superior antitumor cytotoxicity. Compounds **8** and **9** showed stronger activity with IC_{50} values of 0.79 and 16.46 μmol l^{-1}, respectively, than **10** (IC_{50} 27.20 μmol l^{-1}) against HeLa cells. It was suggested that the substituted phenyl group might enhance activity. However, compound **11** showed superior activity with IC_{50} value of 1.45 μmol l^{-1} against K562/A02.

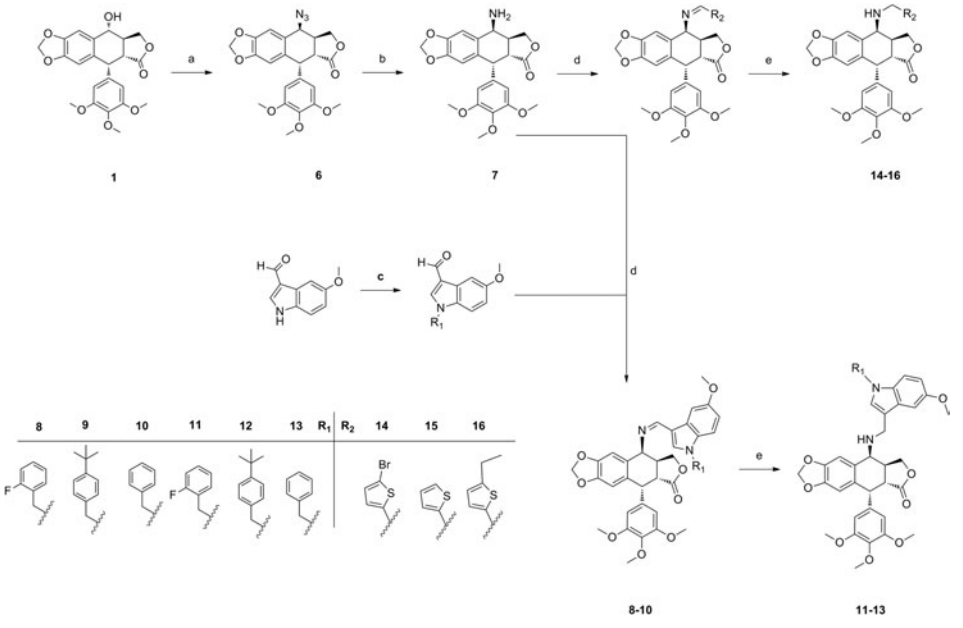
Compared with compounds **8**–**13**, compounds **14**–**16** were supposed that the C-4 position had many electrons, which led to stronger activity. Except for compound **14**, other new compounds showed moderate cytotoxic activity against human tumor cell lines. Among them, compound **16** showed superior activity with an IC_{50} value of 1.15 μmol l^{-1}, compared with VP-16 with an IC_{50} value of 14.50 μmol l^{-1} against HeLa cells. Compound **16** was more potent than compounds **15** and **14**. This observation indicated the critical role of the substituted group. An electron-withdrawing group was detrimental to cytotoxicity, as the activity of compound **14** drastically reduced with IC_{50} value of more than 100 μmol l^{-1}. However, the transfer of an electron-donating group was benefitful to antitumor activity. This suggested that the electron distribution and substituents on thiophene played an important role in cytotoxic activity of the derivatives.

We can see that the series of deoxypodophyllotoxin derivatives have potential to be new drugs, and more work is ongoing to confirm this.

3. Experimental

3.1 General experimental procedures

^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Advance 2B/400 (Bruker Technologies, Bremen, Germany) spectrometer (400 MHz) or Varian Mercury Vx300 MHz (Varian, Palo Alto, CA, USA) in $CDCl_3$, with TMS as an internal standard. Chemical shifts were recorded in (ppm) values relative to TMS, and *J* values are expressed in hertz. MS were recorded using an Agilent-6210 LC/TOF-MS spectrometer (Agilent Technologies, Waldbronn, Germany). Podophyllotoxin was purchased from Nanjing Qingze Pharmaceutical Technology Development



Scheme 1. The synthesis route of target compounds; conditions and reagents: (a) NaN_3, CF_3COOH, CH_2Cl_2; (b) $HCOONH_4$, 10% Pd/C, $CH_3COOC_2H_5$; (c) NaH, DMF; (d) HAc, CH_3OH; (e) $NaBH_4$, CH_3OH.

Co., Ltd (Nanjing, China). Other chemical reagents were purchased from Alfa-reagent (Tianjin, China).

3.2 General reaction

Podophyllotoxin (4.00 g, 10 mmol) dissolved in anhydrous CH_2Cl_2 was allowed to stir around the ice bath. Then, NaN_3 (2.06 g, 40 mmol) was added carefully. CF_3COOH (10 ml) must be dribbled in 30 min at 0°C. After stirring for 1 h, the mixture should be refluxed for 4 h at room temperature. To make sure the pH value of the mixture was adjusted to 7, saturated aqueous $NaHCO_3$ was needed. Then, the organic extracts were dried over anhydrous Na_2SO_4, concentrated and crystallized from CH_2Cl_2–$CH_3COOC_2H_5$ (1:1) to afford a white product (3.99 g, 91% yield). And 10% Pd/C (1.00 g) and $HCOONH_4$ (2.25 g, 40 mmol) were added to the solution of 4β-N_3 podophyllotoxin dissolved in 50 ml $CH_3COOC_2H_5$. The mixture was stirred under heat for 5 h and filtered. The filtrate was washed with saturated brine, then dried over anhydrous Na_2SO_4, and rotary evaporated to get a white compound **7** (3.63 g, 90% yield).

NaH (0.34 g, 10 mmol) was dissolved in DMF (10 ml) in a 50-ml three-neck bottle at 0°C. Then indole (5 mmol) was added to the mixture gradually. Upon completion of the addition, the flask was removed from the ice bath and the solution was allowed to stir at room temperature. After 5 h, R_1X (6 mmol) was added at 0°C. Then, the reaction was kept at room temperature and monitored by TLC until the starting material was gone. With stirring, the mixture was poured into cold water until precipitation was observed. The precipitate was collected on a Buchner funnel, washed twice with distilled water, and dried in air under reduced pressure. Then the powder was used for reaction.

R_2-CHO or (*N*)-R_1-5-methoxyindole-3-carboxaldehyde and acetic acid with catalytic dosage were added to a stirred solution of compound **7** in dry CH_3OH at

room temperature. After 4 h, appropriate $NaBH_4$ was added to the solution at 0°C, and after an additional 4 h, 5% HCl was added to the mixture to adjust pH value to 7. The mixture was poured into a large amount of water, and the precipitate was filtered to get the product (Scheme1).

3.2.1 *Compound **8**: N′-4β-imino-[1-(2-fluoro)-benzyl-5-methoxyl-1H-indol-3-methylene]-4-deoxypodophyllotoxin*

White powder; $[\alpha]_D^{20}$ − 26.1 ($c = 0.52$, CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.01–3.20 (1H, m, H-3), 3.77 (6H, s, 3′, 5′-OCH_3), 3.81 (6H, s, 4′, 5″-OCH_3), 3.83 (1H, d, $J = 4.8$ Hz, H-2), 4.23–4.29 (2H, m, H-11), 4.56 (1H, d, $J = 4.2$ Hz, H-4), 4.70 (1H, d, $J = 4.8$ Hz, H-1), 5.32 (2H, s, CH_2-1‴), 5.87 (2H, s, H-13), 6.37 (2H, s, H-2′, 6′), 6.53 (1H, s, H-5), 6.65 (1H, s, H-8), 6.80–6.90 (2H, m, H-3‴, 6‴), 6.93–7.05 (2H, m, H-4‴,5‴), 7.06–7.28 (2H, m, H-6″, 7″), 7.74 (1H, d, $J = 2.4$ Hz, H-2″), 8.53 (1H, s, H-10″); ^{13}C NMR (300 MHz, $CDCl_3$): δ 174.6 (C-12), 160.6 (C-2‴), 158.2 (C═N), 153.4 (C-5″), 151.5 (C-3′, 5′), 146.7 (C-7), 146.1 (C-6), 135.1 (C-1’), 135.1 (C-4′), 132.4 (C-10), 132.3 (C-8″), 131.2 (C-9″), 131.3 (C-2″), 130.0 (C-5‴), 128.1 (C-9), 125.6 (C-6‴), 123.6(C-4″), 123.5 (C-1‴), 122.5 (C-4‴), 122.4 (C-6″), 114.5 (C-3‴), 113.3 (C-3″), 112.3 (C-7″), 111.2 (C-8), 109.5 (C-5), 107.5 (C-2′, 6′), 100.5 (C-13), 67.5 (C-CH_2), 67. 4 (C-11), 59.7 (C-4), 55.3 (C-3′, 5′-OCH_3), 54.7 (C-5″-OCH_3), 54.5 (C-4′-OCH_3), 43.2 (C-1), 43.3 (C-2), 37.0 (C-3); HR-ESI-MS: *m/z* 679.2468 $[M + H]^+$ (calcd for $C_{39}H_{36}FN_2O_8$, 679.2456).

3.2.2 *Compound **9**: N’-4β-imino[1-(4-t-butyl)-benzyl-5-methoxyl-1H-indol-3-methylene]-4-deoxypodophyllotoxin*

White powder; $[\alpha]_D^{20}$ − 65.8 ($c = 0.49$, CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.29 (9H, s, 4‴-$CH(CH_3)_3$), 3.09–3.12 (2H, m, H-2, 3), 3.77 (6H, s, 3′, 5′-OCH_3), 3.81 (6H, br s, 4′, 5″-OCH_3), 4.21–4.32 (2H, m, H-11), 4.56 (1H, d, $J = 4.2$ Hz, H-4), 4.70 (1H, d, $J = 5.1$ Hz, H-1), 5.24 (2H, s, CH_2-1‴), 5.87 (1H, s, H-13), 5.93 (1H, s, H-13), 6.37 (2H, s, H-2′, 6′), 6.53 (1H, s, H-5), 6.65 (1H, s, H-8), 6.88 (1H, dd, $J = 2.4$, 8.9 Hz, H-6″), 7.06 (2H, $J = 8.2$ Hz, H-3‴, 5‴), 7.19 (1H, d, $J = 8.9$ Hz, H-7″), 7.32 (2H, d, $J = 8.2$ Hz, H-2‴,6‴), 7.40 (1H, s, H-2″), 7.74 (1H, d, $J = 2.4$ Hz, H-4″), 8.52 (1H, s, H-10″); ^{13}C NMR (300 MHz, $CDCl_3$): δ 175.6 (C-12), 152.5 (C-3′, 5′), 147.6 (C-7), 147.1 (C-6), 136.1 (C-1’), 133.3 (C-4′), 131.8 (C-10), 133.2 (C-9), 104.3 (C-5), 110.8 (C-8), 108.5 (C-2′, 6′), 104.3 (C-13), 68.4 (C-11), 60.7 (C-4), 55.7 (C-4′-OCH_3), 56.3 (C-3′, 5′-OCH_3), 50.3 (C-indol-OCH_3), 44.2 (C-1), 41.7 (C-2), 38.0 (C-3), 131.6 (C-2″), 114.0 (C-3″), 125.8 (C-4″), 154.5 (C-5″), 126.8 (C-7″), 110.8 (C-6″), 131.6 (C-8″), 130.3 (C-9″), 55.7 (1″-CH_2), 155.5 (C═N), 126.8 (C-2‴,6‴), 125.8 (C-3‴,5‴), 113.2 (C-4‴), 133.4 (C-1‴), 34.5 (4‴-CH), 31.2 (C-$(CH_3)_3$); HR-ESI-MS: *m/z* 717.3196 $[M + H]^+$ (calcd for $C_{43}H_{45}N_2O_8$, 717.3176).

3.2.3 *Compound **10**: N’-4β-imino(1-benzyl-5-methoxyl-1H-indol-3-methylene)-4-deoxypodophyllotoxin*

White powder; $[\alpha]_D^{20}$ − 35.9 ($c = 0.56$, CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.09–3.12 (2H, m, H-2, 3), 3.77 (6H, s, H-3′, 5′-OCH_3), 3.81 (6H, s, H-4′, 5″-OCH_3), 4.22–4.32 (2H, m, H-11), 4.55 (1H, d, $J = 3.9$ Hz, H-4), 4.69 (1H, d, $J = 4.8$ Hz, H-1), 5.41 (2H, s, CH_2-1‴), 6.01 (1H, s, C-13), 5.94 (1H, s, C-13), 6.37 (2H, s, H-2′, 6′), 6.65 (1H, s, H-5), 6.53 (1H, s, H-8), 6.87 (1H, d, $J = 2.1$ Hz, H-6″), 7.06 (2H, $J = 6.6$ Hz, H-3‴, 5‴), 7.74 (1H, d, $J = 2.1$ Hz, H-7″), 7.31 (2H, d, $J = 6.6$ Hz, H-2‴, 6‴), 7.39 (1H, s, H-4″), 7.16 (1H, s, H-2″), 8.53 (1H, s, H-10″); ^{13}C NMR (300 MHz, $CDCl_3$): δ 175.6 (C-12), 152.6 (C-3′, 5′), 147.7 (C-7), 136.1 (C-1′), 136.3 (C-4′), 128.9 (C-9), 132.5 (C-

10), 108.9 (C-5), 110.4 (C-8), 108.9 (C-2′, 6′), 101.3 (C-13), 68.5 (C-11), 60.8 (C-4), 55.7 (C-4′-OCH_3), 56.3 (C-3′, 5′-OCH_3), 50.7 (C-indol-OCH_3), 44.2 (C-2), 41.7 (C-1), 38.0 (C-3), 128.9 (C-3‴, 5‴), 127.0 (C-2‴, 6‴), 126.7 (C-4‴), 155.6 (C═N), 137.2 (C-2″), 113.3 (C-3″), 128.0 (C-4″), 154.5 (C-5″); HR-ESI-MS: *m/z* 661.2627 $[M + H]^+$ (calcd for $C_{39}H_{37}N_2O_8$, 661.2570).

*3.2.4 Compound **11**: N′-4β-[1-(2-fluoro)-benzyl-5-methoxyl-1H-indol-3-methylene]-amino-4-deoxypodophyllotoxin*

White powder; $[\alpha]_D^{20}$ − 53.3 ($c = 0.39$, CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.70 (1H, d, $J = 4.8$ Hz, H-1), 3.83 (1H, d, $J = 4.8$ Hz, H-2), 3.01–3.20 (1H, m, H-3), 4.56 (1H, d, $J = 4.2$ Hz, H-4), 6.65 (1H, s, H-5), 6.53 (1H, s, H-8), 4.23–4.29 (2H, m, H-11), 6.37 (2H, s, H-2′, 6′), 5.93 (2H, s, H-13), 6.80–6.90 (2H, m, H-3‴, 6‴), 6.93–7.05 (2H, m, H-4‴, 5‴), 7.06–7.28 (3H, m, H-6″, 7″, 4″), 7.74 (1H, s, H-2″), 3.80 (2H, s, CH_2-N); ^{13}C NMR (300 MHz, $CDCl_3$): δ 177.3 (C-12), 161.6 (C-2‴), 154.4 (C-5″), 153.4 (C-3′,5′), 147.4 (C-7), 146.7 (C-6), 136.4 (C-1′), 135.1 (C-4′), 132.0 (C-9), 129.3 (C-8″), 127.9 (C-9″), 132.3 (C-2″), 117.6 (C-5‴), 124.5 (C-10), 127.7 (C-6‴), 115.6 (C-4″), 121.6 (C-1‴), 120.3 (C-4‴), 110.2 (C-6″), 109.7 (C-3‴), 113.9 (C-3″), 107.3 (C-7″), 106.2 (C-8), 105.9 (C-5), 104.9 (C-2′,6′), 100.9 (C-13), 67.5 (C-CH_2), 67.4 (C-11), 60.9 (C-4), 55.8 (C-N), 56.3 (C-3′, 5′-OCH_3), 56.3 (C-indol-OCH_3), 55.9 (C-4′-OCH_3), 43.8 (C-1), 41.5 (C-2), 38.8 (C-3); HR-ESI-MS: *m/z* 681.2661 $[M + H]^+$ (calcd for $C_{39}H_{38}FN_2O_8$, 681.2612).

*3.2.5 Compound **12**: N′-4β-[1-(4-t-butyl)-benzy-5-methoxyl-1H-indol-3-methylene]-amino-4-deoxypodophyllotoxin*

White powder; $[\alpha]_D^{20}$ − 41.7 ($c = 0.46$, CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.29 (9H, s, 4‴-$CH(CH_3)_3$), 2.64–3.00 (2H, m, H-3, 2), 3.44 (2H, s, CH_2-N), 3.72 (6H, s, H-3′, 5′-OCH_3), 3.77 (6H, s, H-4′, 5‴-OCH_3), 3.90–4.12 (2H, m, H-11), 4.31 (1H, d, $J = 1.5$ Hz, H-4), 4.50 (1H, d, $J = 4.9$ Hz, H-1), 5.25 (2H, s, CH_2-1‴), 5.97 (2H, s, H-13), 6.24 (2H, s, H-2′, 6′), 6.33 (1H, s, H-8), 6.45 (1H, s, H-5), 6.87 (1H, dd, $J = 2.2$, 8.9 Hz, H-6″), 7.01–7.12 (2H, m, H-3‴, 5‴), 7.21 (1H, d, $J = 2.2$ Hz, H-7″), 7.50–7.54 (2H, m, H-2‴, 6‴), 7.70 (1H, d, $J = 8.9$ Hz, H-4″); ^{13}C NMR (300 MHz, $CDCl_3$): δ 174.6 (C-12), 151.6 (C-3′, 5′), 146.6 (C-7), 146.1 (C-6), 136.1 (C-1′), 136.3 (C-4′), 135.1 (C-9), 132.3 (C-10), 109.4 (C-5), 111.3 (C-8), 107.9 (C-2′, 6′), 103.3 (C-13), 67.4 (C-11), 59.7 (C-4), 54.7 (C-4′-OCH_3), 55.3 (C-3′, 5′-OCH_3), 49.3 (C-indol-OCH_3), 42.0 (C-1), 43.2 (C-2), 40.7 (C-3), 33.5 (4‴-CH), 30.2 (C-$(CH_3)_3$), 126.3 (C-2‴, 6‴), 124.8 (C-3‴, 5‴), 113.1 (C-4‴), 125.8 (C-1‴), 135.7 (C-2″), 112.2 (C-3″), 125.5 (C-4″), 153.5 (C-5″), 66.8 (N-CH_2), 67.4 (1″-CH_2Ar); HR-ESI-MS: *m/z* 719.3383 $[M + H]^+$ (calcd for $C_{43}H_{47}N_2O_8$, 719.3332).

*3.2.6 Compound **13**: N′-4β-(1-benzyl-5-methoxyl-1H-indol-3-methylene)-amino-4-deoxypodophyllotoxin*

White powder; $[\alpha]_D^{20}$ − 31.6 ($c = 0.49$, CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.66–2.90 (2H, m, H-2, 3), 3.39 (2H, s, N-CH_2), 3.73 (6H, s, 3′, 5′-OCH_3), 3.79 (3H, s, 4′-OCH_3), 3.85 (3H, s, 5″-OCH_3), 4.05–4.10 (2H, m, H-11), 4.35 (1H, t, $J = 7.7$ Hz, H-4), 4.53 (1H, d, $J = 5.2$ Hz, H-1), 5.27 (2H, s, CH_2-1‴), 5.93 (2H, s, C-13), 6.25 (2H, s, H-2′, 6′), 6.45 (1H, s, H-8), 6.58 (1H, s, H-5), 6.85 (1H, dd, $J = 8.9$, 2.3 Hz, H-6″), 7.17 (1H, d, $J = 2.3$ Hz, H-7″), 7.27 (1H, d $J = 8.9$ Hz, H-4″), 7.00–7.15 (3H, m, H-3‴, 4‴, 5‴), 7.25 (2H, br s, H-6‴, 2‴), 7.69 (1H, s, H-2″); ^{13}C NMR (300 MHz, $CDCl_3$): δ 174.6 (C-12), 154.6 (C-3′, 5′), 146.7 (C-7), 146.1 (C-6), 132.4 (C-1′),

135.3 (C-4′), 127.8 (C-9), 127.1 (C-10), 107.9 (C-5), 110.4 (C-8), 107.8 (C-2′, 6′), 103.8 (C-13), 67.5 (C-11), 59.7 (C-4), 55.3 (C-4′-OCH_3), 54.8 (C-3′, 5′-OCH_3), 49.6 (C-N), 49.2 (C-indol-OCH_3), 43.2 (C-2), 40.7 (C-1), 37.0 (C-3), 132.3 (C-1‴), 127.9 (C-2‴, 6‴), 126.0 (C-3‴, 5‴), 125.7 (C-4‴), 135.2 (C-2″), 112.3 (C-3″), 128.0 (C-4″), 154.0 (C-5″), 112.2 (C-6″), 109.4 (C-7″), 128.7 (C-8″); HR-ESI-MS: *m/z* 663.2747 $[M + H]^+$ (calcd for $C_{39}H_{39}N_2O_8$, 663.2706).

3.2.7 Compound **14**: N′-4β-(5-bromo-thiophene-2-methylene)-amino-4-deoxypodophyllotoxin

White powder; $[\alpha]_D^{20}$ − 61.4 ($c = 0.55$, CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.75–2.90 (1H, m, H-3), 3.20–3.30 (1H, m, H-2), 3.73 (6H, s, H-3′, 5′-OCH_3), 3.79 (3H, s, H-4′-OCH_3), 3.95 (2H, s, CH_2-N), 4.09 (1H, d, $J = 14.2$ Hz, H-4), 4.34 (2H, d, $J = 8.2$ Hz, H-11), 4.54 (1H, d, $J = 5.1$ Hz, H-1), 5.96 (2H, s, H-13), 6.24 (1H, s, H-2′, 6′), 6.48 (1H, s, H-8), 6.62 (1H, s, H-5), 6.74 (1H, s, H-4″), 6.92 (1H, s, H-3″); ^{13}C NMR (300 MHz, $CDCl_3$): δ 173.4 (C-12), 151.1 (C-3′, 5′), 146.6 (C-7), 145.8 (C-6), 143.8 (C-5″), 134.2 (C-1′), 135.9 (C-4′), 130.4 (C-9), 128.4 (C-10), 124.3 (C-3″, 4″), 110.5 (C-8), 109.4 (C-5), 107.3 (C-2′, 6′), 100.5 (C-13), 67.7 (C-11), 60.1 (C-4), 55.7 (C-3′, 5′-OCH_3), 54.6 (C-4′-OCH_3), 48.4 (C-N), 43.3 (C-1), 40.9 (C-2), 38.1 (C-3); HR-ESI-MS: *m/z* 588.0733 $[M + H]^+$ (calcd for $C_{27}H_{27}BrNO_7S$, 588.0692).

3.2.8 Compound **15**: N′-4β-(thiophen-2-methylene)-amino-4-deoxypodophyllotoxin

White powder; $[\alpha]_D^{20}$ − 46.2 ($c = 0.51$, CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.70–2.83 (1H, m, H-3), 3.31 (1H, dd, $J = 5.0$, 13.6 Hz, H-2), 3.73 (6H, s, 3′, 5′-OCH_3), 3.79 (3H, s, 4′-OCH_3), 4.00–4.10 (2H, m, H-11), 4.09 (1H, d, $J = 3.1$ Hz, H-4), 4.30–4.42 (2H, m, N-CH_2), 4.54 (1H, d, $J = 5.0$ Hz, H-1), 5.93 (2H, d, $J = 5.1$ Hz, H-13), 6.24 (2H, s, H-2′, 6′), 6.46 (1H, s, H-8), 6.52 (1H, s, H-5), 6.98 (2H, br s, H-3″, 4″), 7.25 (1H, br s, H-2″); ^{13}C NMR (300 MHz, $CDCl_3$): δ 174.9 (C-12), 152.3 (C-3′, 5′), 147.6 (C-7), 147.1 (C-6), 125.5 (C-5″), 137.0 (C-1′), 135.6 (C-4′), 132.2 (C-9), 131.5 (C-10), 126.9 (C-2″), 125.4 (C-3″), 124.9 (C-4″), 110.5 (C-8), 109.9 (C-5), 108.5 (C-2′, 6′), 101.6 (C-13), 69.1 (C-11), 61.4 (C-4), 56.9 (C-3′, 5′-OCH_3), 55.7 (C-4′-OCH_3), 49.4 (C-N), 44.6 (C-1), 42.2 (C-2), 39.5 (C-3); HR-ESI-MS: *m/z* 510.1624 $[M + H]^+$ (calcd for $C_{27}H_{28}NO_7S$, 510.1586).

3.2.9 Compound **16**: N′-4β-(5-ethyl-thiophene-2-methylene)-amino-4-deoxypodophyllotoxin

White powder; $[\alpha]_D^{20}$ − 57.5 ($c = 0.46$, CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.31 (3H, t, $J = 7.4$ Hz, 2″-CH_2CH_3), 2.65–2.90 (3H, m, H-3, 2″-CH_2), 3.30 (1H, dd, $J = 4.8$, 13.7 Hz, H-2), 3.73 (6H, s, H-3′, 5′-OCH_3), 3.79 (3H, s, 4′-OCH_3), 4.00 (3H, br s, H-4, 11), 3.43–4.37 (2H, m, N-CH_2), 4.53 (1H, d, $J = 4.8$ Hz, H-1), 5.93 (2H, d, $J = 3.8$ Hz, C-13), 6.25 (2H, s, H-2′, 6′), 6.45 (1H, s, H-8), 6.54 (1H, s, H-5), 6.65 (1H, s, H-3″), 6.76 (1H, s, H-4″); ^{13}C NMR (300 MHz, $CDCl_3$): δ 174.3 (C-12), 151.6 (C-3′, 5′), 147.5 (C-7), 146.3 (C-6), 139.6 (C-5″), 135.0 (C-1′), 136.4 (C-4′), 131.8 (C-9), 130.9 (C-10), 124.6 (C-3″), 122.4 (C-4″), 109.8 (C-8), 107.9 (C-2′, 6′), 107.5 (C-5), 101.0 (C-13), 68.5 (C-11), 60.8 (C-4), 56.3 (C-3′, 5′-OCH_3), 55.0 (C-4′-OCH_3), 49.1 (C-N), 43.9 (C-1), 41.6 (C-2), 38.9 (C-3), 24.0 (C-2″-CH_2), 16.5 (C-2″-CH_2CH_3); HR-ESI-MS: *m/z* 538.1941 $[M + H]^+$ (calcd for $C_{29}H_{32}NO_7S$, 538.1899).

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