Bioorganic & Medicinal Chemistry 21 (2013) 2363–2369

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Design, synthesis and potent cytotoxic activity of novel podophyllotoxin derivatives

Wen-Qun Li [a,†], Xu-Li Wang [a,†], Keduo Qian [b], Ying-Qian Liu [a,*], Chih-Ya Wang [b], Liu Yang [d], Jin Tian [a], Susan L. Morris-Natschke [b], Xing-Wen Zhou [a], Kuo-Hsiung Lee [b,c,*]

[a] School of Pharmacy, Lanzhou University, Lanzhou 730000, PR China
[b] Natural Products Research Laboratories, UNC Eshelman School of Pharmacy, University of North Carolina, Chapel Hill, NC 27599, United States
[c] Chinese Medicine Research and Development Center, China Medical University and Hospital, Taichung, Taiwan
[d] Environmental and Municipal Engineering School, Lanzhou Jiaotong University, Lanzhou 730000, PR China

ARTICLE INFO

Article history:
Received 8 November 2012
Revised 18 January 2013
Accepted 28 January 2013
Available online 17 February 2013

Keywords:
Podophyllotoxin
Acyl thiourea
Synthesis
Cytotoxic activity

ABSTRACT

Twenty new acyl thiourea derivatives of podophyllotoxin and 4′-demethylepipodophyllotoxin were prepared and screened for their cytotoxicity against four human tumor cell lines, A-549, DU-145, KB, and KBvin. With IC_{50} values of 0.098–1.13 μM, compounds **13b**, **13c**, and **13o** displayed much better cytotoxic activity than the control etoposide. Most importantly, **13b** and **13o** exhibited promising cytotoxicity against the drug resistant tumor cell line KBvin with IC_{50} values of 0.098 and 0.13 μM, respectively, while etoposide lost activity completely. Structure–activity relationship (SAR) correlations of the new derivatives have been established. Compounds **13b** and **13o** merit further development as a new generation of epipodophyllotoxin-derived antitumor clinical trial candidates.

1. Introduction

Topoisomerases are enzymes that control the changes in DNA structure by catalyzing the breaking and rejoining of the phosphodiester backbone of DNA strands during the normal cell cycle. Topoisomerases are classified into two categories. Monomeric type I enzymes catalyze the formation of DNA single-strand breaks, while multimeric type II enzymes engender DNA double-strand breaks. Topoisomerase-II is highly involved in DNA replication, transcription, chromosome condensation, and de-condensation, and therefore, represents an important cellular target for clinical anticancer drugs, such as the epipodophyllotoxins (etoposide and teniposide), the anthracyclines (daunorubicin and doxorubicin), and the aminoacridines.

Podophyllotoxin (**1**) is a naturally occurring aryltetralin lignan isolated from various plant species within the *Podophyllum* family. Its powerful cytotoxic properties have been attributed to its ability to bind to tubulin during mitosis and thus inhibit microtubule assembly.[1,2] During early chemical modification studies, 4′-demethylepipodophyllotoxin (**2**) was obtained by 4′-O-demethylation and stereo transformation of α to β at the C-4 position. Investigation of semisynthetic glucoconjugates based on the epipodophyllotoxin system led to two clinical anticancer drugs, etoposide (**3**) and teniposide (**4**), as well as a water-soluble prodrug of **3**, etopophos (**5**) (Fig. 1).[3–5] The structural modification also led to a change in the mechanism of action of these ligands, while **1** acts as antimicrotubule agent, **3** and **4** act as topoisomerase-II inhibitors.[6,7] Their clinical success and intriguing mechanism of action have stimulated great interest in further exploration of epipodophyllotoxin derivatives with better antitumor activity.

Some nonsugar substituted analogues, particularly N-linked congeners, such as NPF (**6**)[8] and GL-331(**7**),[9] and C-linked congeners, such as TOP-53 (**8**) (Fig. 1),[10] were then found to exhibit superior pharmacological properties compared to **3**. Among them, compound **7** was brought into phase II clinical trials.[9] Overall, these variants displayed improved water solubility and cytotoxic activity, as well as drug resistant and antitumor profiles, suggesting the possibility of further optimizing epipodophyllotoxins through rational C-4 modifications. Both the composite pharmacophore model and comparative molecular field analysis also confirmed that the C-4 molecular area could accommodate considerable structural diversity.[11]

Based on critical modeling clues, we have successfully prepared several potent epipodophyllotoxin derivatives during the past few years.[2,12–17] In our continuing work in this research area, we discovered that thiourea was a good ligand for metal ions, which play important roles in tumorigenesis. Moreover, thiourea derivatives possessed good inhibitory activity against protein tyrosine kinases

* Corresponding authors. Tel.: +1 919 962 0066; fax: +1 919 966 3893 (K.-H.L.).
E-mail addresses: khlee@email.unc.edu (K.-H. Lee), yqliu@lzu.edu.cn (Y.-Q. Liu).
† These authors contributed equally to this work.

0968-0896/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.bmc.2013.01.069

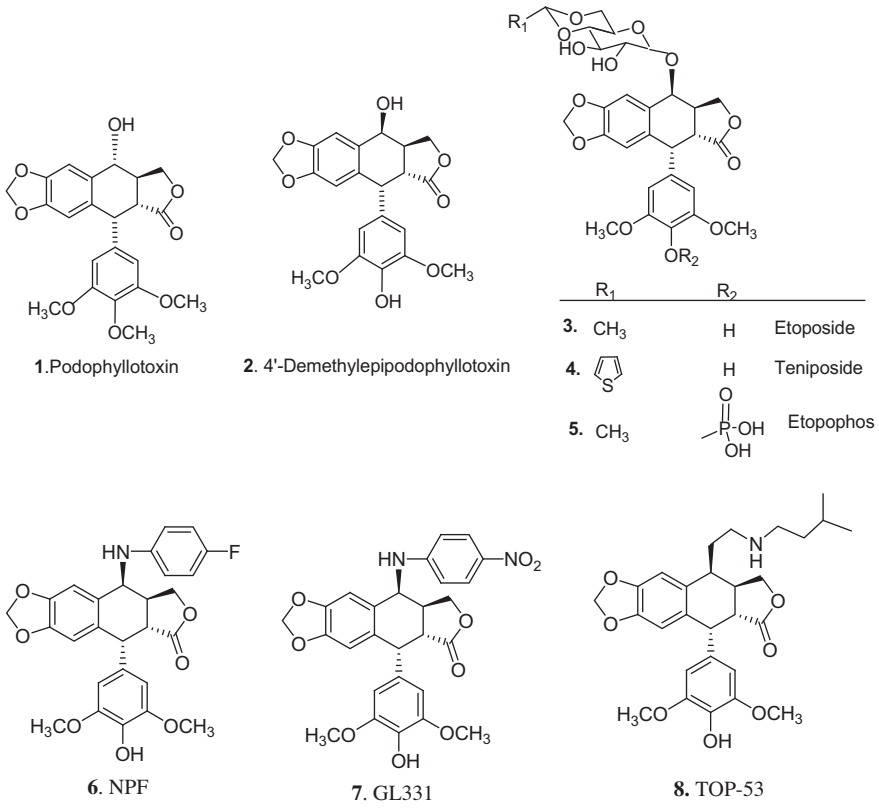


Figure 1. Structures of podophyllotoxin derivatives.

(PTKs), which also are critical in many aspects of tumorigenesis.[18,19] Thus, incorporation of a thiourea group could possibly potentiate biochemical or pharmacological properties of a bioactive original molecule.[20,21] Therefore, it was intriguing to introduce thiourea groups into podophyllotoxins (**1** and **1a**) via coupling reactions, in order to investigate the impact on the derivatives' cytotoxicity against human tumor cell lines. Herein, this paper reports the design, synthesis, and cytotoxic activity of the newly synthesized acyl thiourea epipodophyllotoxin derivatives **13a–o** and **14a–e**.

2. Results and discussion

2.1. Chemistry

The synthetic route to target compounds **13a–o** and **14a–e** is depicted in Scheme 1. The starting materials, **1** and **1a**, were first converted to 4β-chloroamido podophyllotoxins (**9** and **10**) in excellent yields by reaction with $ClCH_2CN$ in the presence of 60% w/w methanesulfonate/aluminium oxide ($MsOH/Al_2O_3$). Subsequently, the chloroacetyl group was cleaved with thiourea in the presence of acetic acid (HOAc) to furnish the key intermediates 4β-aminopodophyllotoxins (**11** and **12**).[22] Intermediates **11** and **12** were then coupled with the appropriate acylisothiocyanates in dry acetonitrile to provide a series of acyl thiourea derivatives **13a–o** and **14a–e** in 56–95% yields.[23] All newly synthesized compounds were purified by column chromatography and their structures were characterized by ^1H NMR, ^{13}C NMR and HRMS analyses.

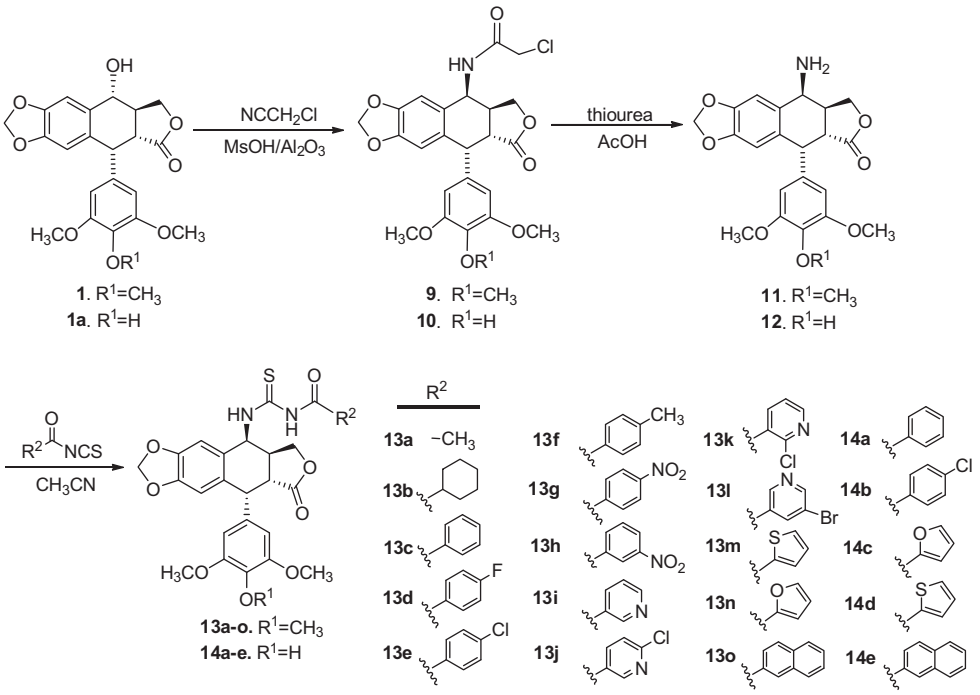
2.2. Cytotoxicity and SAR

Target compounds **13a–o** and **14a–e** were evaluated for their in vitro cytotoxicity against a panel of human tumor cell lines, including A549 (non-small cell lung cancer), DU145 (prostate cancer cell line), KB (nasopharyngeal carcinoma), and KBvin [multidrug resistant (MDR) KB subline selected using vincristine], using a sulforhodamine B colorimetric (SRB) assay with triplicate experiments.[24] Compound **3** was included as control and the results are summarized in Table 1.

Except for compounds **13j–l**, **13n**, and **14b**, most of the new compounds showed moderate to potent cytotoxic activity against the four human tumor cell lines. Among them, compounds **13b–e** and **13o** showed superior activity with IC_{50} values of 0.099–1.44 μM compared to that of **3** (IC_{50} 2.03–3.88 μM) against A549, DU-145, and KB tumor cell lines. Most importantly, these compounds retained their significant cytotoxicity (IC_{50} 0.098–2.01 μM) against the drug resistant KBvin tumor cell line, while **3** lost its activity completely (IC_{50} >20 μM). This result is in agreement with our prior observation that C4-amino substitution of epipodophyllotoxin is good for overcoming drug-resistance.[25]

The 4′-O-methylated derivatives (**13c**, **13e**, and **13m–13o**) were more potent than their corresponding 4′-hydroxyl analogues (**14a–14e**). While **13c** and **13o** showed significant cytotoxicity with IC_{50} values of 0.81–1.13 and 0.099–0.13 μM, respectively, their corresponding **14a** and **14e** displayed only marginal activity with IC_{50} values of 8.96–12.00 and 6.55–11.75 μM, respectively. The 4′-O-demethylation of **13e** (IC_{50} 1.16–2.01 μM) led to a complete loss of cytotoxicity in the corresponding **14b**. These results highlight the critical role of the 4′-*O*-methyl functionality in acyl thiourea substituted epipodophyllotoxin derivatives.

Within the 4′-O-methylated series (**13a–o**), cyclohexyl (**13b**), phenyl (**13c**), and naphthyl (**13o**) R^2 groups within the acyl thiourea side chains led to significantly enhanced cytotoxicity, as these derivatives displayed IC_{50} values of 0.098–0.32, 0.81–1.13, and 0.099–0.13 μM, respectively. Notably, annular aliphatic substituted **13b,** with a cyclohexyl R^2 group, exhibited better activity



Scheme 1. Synthesis of 4β-amino-4-desoxypodophyllotoxin (**13a**–**o**) and 4β-amino 4′-demethyl-desoxypodophyllotoxin (**14a**–**e**).

Table 1
In vitro cytotoxicity of **13a**–**o** and **14a**–**e** against four human tumor cell lines with etoposide (3) as control

Compd	IC_{50} (μM)			
	A549	DU-145	KB	KBvin
13a	13.12 ± 0.246	12.30 ± 0.176	13.43 ± 0.121	13.63 ± 0.664
13b	0.19 ± 0.010	0.17 ± 0.007	0.32 ± 0.014	0.098 ± 0.005
13c	1.13 ± 0.050	0.97 ± 0.089	0.90 ± 0.059	0.81 ± 0.101
13d	1.37 ± 0.050	1.15 ± 0.059	1.44 ± 0.049	1.38 ± 0.051
13e	1.62 ± 0.039	1.16 ± 0.047	1.20 ± 0.040	2.01 ± 0.045
13f	11.31 ± 0.291	10.52 ± 0.561	10.48 ± 0.389	12.25 ± 0.461
13g	2.06 ± 0.095	1.57 ± 0.094	1.33 ± 0.075	1.65 ± 0.071
13h	10.31 ± 0.431	9.82 ± 0.091	9.27 ± 0.395	9.86 ± 0.270
13i	15.47 ± 2.261	11.40 ± 0.146	14.20 ± 0.503	16.84 ± 0.811
13j	>20	>20	>20	>20
13k	>20	>20	>20	>20
13l	>20	>20	>20	>20
13m	12.29 ± 0.333	11.19 ± 0.173	11.56 ± 0.306	12.51 ± 0.338
13n	>20	>20	>20	>20
13o	0.11 ± 0.002	0.099 ± 0.004	0.12 ± 0.000	0.13 ± 0.007
14a	12.00 ± 0.214	9.88 ± 1.070	8.96 ± 0.490	9.93 ± 0.966
14b	>20	>20	>20	>20
14c	13.74 ± 1.000	8.49 ± 0.160	8.74 ± 0.508	9.46 ± 0.207
14d	9.82 ± 0.624	7.34 ± 0.303	8.40 ± 0.502	9.24 ± 0.501
14e	11.75 ± 1.317	6.55 ± 0.073	7.23 ± 0.312	10.29 ± 1.267
3	2.58 ± 0.252	2.03 ± 0.121	3.88 ± 0.199	>20

than compound **13a**, with a methyl R^2 group, which showed only marginal IC_{50} values of 12.30–13.63 μM. This observation indicates that the size of the acyl substituent is critical. Compounds with electron-withdrawing substituents on the phenyl ring (**13d–e**, **13g**) showed similar or slightly reduced activity (IC_{50} 1.15–2.06 μM) compared with non-substituted **13c**. An electron-donating group was detrimental to cytotoxicity, as compound **13f** (*para*-methylphenyl) had drastically reduced IC_{50} values of 10.48–12.25 μM. The transfer of a electron-withdrawing nitro group from the *para*-position (**13g**) to the *meta*-position (**13h**) also decreased the cytotoxicity remarkably (IC_{50} 9.27–10.31 μM), suggesting that the electron distribution and substituents on benzoyl thiourea side chains play an important role in the derivatives' cytotoxic activity.

The introduction of heterocyclic groups into the acyl thiourea side chains of **13i–n** resulted in dramatically decreased cytotoxicity. For instance, compounds with furan (**13n**), thiophene (**13m**), and pyridine-containing groups (**13i–13l**), all exhibited marginal or no activity against the four human tumor cell lines. This finding further confirmed that the electron density and distribution of the substituents within the acyl thiourea side chain is important towards the compounds' activity.

3. Conclusion

In summary, new acyl thiourea derivatives of epipodophyllotoxin were designed and synthesized successfully, and their cytotoxic activity was evaluated against four human tumor cell lines. Results showed that the C4-acyl thiourea compound retained cytotoxicity against the drug resistant KBvin tumor cell line, while etoposide (**3**) lost its activity completely. The best compounds **13b**, **13c**, and **13o** exhibited significant cytotoxicity against all four human tumor cell lines with IC_{50} values of 0.098–1.13 μM, 20-fold better than that of the control **3**. The SAR study found that the size, electron density, and distribution of the substituents within the acyl thiourea side chain are critical to the derivatives' activity. With a concise synthesis and potent cytotoxic profiles, the new acyl thiourea epipodophyllotoxins, especially **13b** and **13o**, are promising new candidates for further development as anticancer agents. Studies on the enzymatic interaction of these new acyl thiourea epipodophyllotoxins with the topoisomerase-II target are currently ongoing.

4. Experimental section

4.1. Chemistry

Melting points were taken on a Kofler melting point apparatus and are uncorrected. High resolution mass spectroscopic analyses

were performed on ZAB-HS and BrukerDaltonics APEXII49e instruments. ^{1}H and ^{13}C NMR spectra were recorded at 400 MHz and 100 MHz on a Bruker AM-400 spectrometer using TMS as reference (Bruker Company, USA). Podophyllotoxin (**1**) was isolated from the Chinese medicinal herb *Juniperus sabina* Linnaeus, and served as the starting material for the preparation of all new derivatives.

4.1.1. General synthetic procedure for the key intermediates 4β-amino-4-desoxypodophyllotoxin (11) and 4β-amino-4′-demethyl-4-desoxypodophyllotoxin (12)

To a stirred mixture of podophyllotoxin (4 mmol) and $ClCH_2CN$ (10 mL), a homogeneous mixture of $MsOH/Al_2O_3$ (60 mass %, 1 g) was added, and the mixture was irradiated by an ultrasonic generator in a water bath at 60 °C for 30 min. The solvent was evaporated under reduced pressure and the residue was poured onto ice water and extracted with EtOAc (3 × 50 mL). Combined organic extracts were dried over anhydrous Na_2SO_4, the mixture was filtered, and the filtrate was evaporated under reduced pressure to give the crude product, which was purified by flash column chromatography ($CHCl_3$/MeOH, 9.5:0.5) to give the pure intermediate **9** in 90% yield. Subsequently, a solution of amide **9** (5 mmol) and thiourea (0.46 g, 6 mmol) in HOAc (50 mL) was sonicated at 80 °C for 4 h, and then the solution was neutralized with aq 20% $NaHCO_3$ and extracted with $CHCl_3$ (3 × 30 mL). The combined organice extracts were washed with brine (30 mL) and dried ($MgSO_4$), filtered, and concentrated in vacuo. The residue was purified by column chromatography to give compound **11** as a white solid in 82% yield. Analogously, amide **10** and subsequently amine **12** were prepared starting from the podophyllotoxin derivative **1a** in 93% and 85% yields, respectively.

4.1.1.1. 4β-Amino-4-desoxypodophyllotoxin (11). MS (FAB) *m/z* 414 (M+1); ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.84 (s, 1H, H-5), 6.47 (s, 1H, H-8), 6.36 (s, 2H, H-2′,6′), 5.97 (s, 2H, OCH_2O), 4.58 (d, *J* = 5.1 Hz, lH, H-I), 4.32 (m, 2H, H-11), 4.25 (d, *J* = 4.0 Hz, 1H, H-4), 3.80 (s, 3H, 4′-OCH_3), 3.72 (s, 6H, 3′,5′-OCH_3), 3.32 (q, 1H, H-2), 2.92–2.63 (m, 1H, H-3), 1.84 (d, 2H, 4-NH_2); ^{13}C NMR ($CDC1_3$, 100 MHz): δ 37.9, 40.2, 43.7, 48.9, 56.4, 68.1, 101.3, 107.9, 108.6, 110.2, 131.1, 131.2, 133.9, 134.1, 146.3, 147.2, 147.6, 175.4. HRMS *m/z* calcd for $C_{22}H_{23}NO_7$: 414.1366 $[M+H]^+$, found: 414.1365 $[M+H]^+$.

4.1.1.2. 4β-Amino-4′-demethyl-4-desoxypodophyllotoxin (12). MS (EI) *m/z* 400 (M+1); ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.81 (s, 1H, H-5), 6.50 (s, lH, H-8), 6.30 (s, 2H, H-2′,6′), 5.98 and 5.95 (2s, 2H, OCH_2O), 4.56 (d, *J* = 5.2 Hz, 1H, H-l), 4.30 (d, *J* = 10 Hz, 2H, H-11), 4.18 (d, *J* = 4.0 Hz, 1H, H-4), 3.78 (s, 6H, 3′,5′-OMe), 3.15 (dd, *J* = 5.2, 14 Hz, 1H, H-2), 2.80 (m, 1H, H-3); ^{13}C NMR ($CDC1_3$, 100 MHz): δ 38.2, 40.2, 43.7, 48.9, 56.4, 68.1, 101.3, 107.9, 108.6, 110.2, 131.1, 131.2, 133.9, 134.1, 146.3, 147.3, 147.6, 175.4. HRMS *m/z* calcd for $C_{21}H_{21}NO_7$: 400.1822 $[M+H]^+$, found: 400.1825 $[M+H]^+$.

4.1.2. General synthetic procedure for compounds 13a–o and 14a–e

The key intermediates **11** and **12** (1 mmol) were added to a solution of 1.5 mmol of an acyl isothiocyanate in dry acetonitrile (10 mL). The reaction mixture was stirred for 2 h at rt and then concentrated. The residue was purified by chromatography on silica gel using EtOAc–petroleum ether to give **13a–o** and **14a–e**.

4.1.2.1. 4β-(3-Acetyl-thioureido)-4-deoxypodophyllotoxin (13a). Yield: 75%; mp: 168–172 °C; ^{1}H NMR (40 0 MHz, $CDCl_3$): δ 2.17 (s, 3H, $COCH_3$), 2.28 (dd, *J* = 14.4, 5.2 Hz, 1H, 1H-H), 3.04–3.12 (m, 1H, 3-H), 3.76 (s, 6H, 3′,5′-OCH_3), 3.81 (s, 3H, 4′-OCH_3), 3.93–3.99 (m, 1H, 11α-H), 4.50–4.54 (m, 1H, 11β-H), 4.64 (d, 1H, 1-H), 5.82 (dd, *J* = 8.0, 4.8 Hz, 1H, 4-H), 5.97 and 6.00 (ABq, 2H, –OCH_2O–), 6.30 (s, 2H, 2′,6′-H), 6.54 (s, 1H, 8-H), 6.80 (s, 1H, 5-H), 8.96 (s, 1H, –CSNHCO–), 10.72 (d, 1H, *J* = 8.0 Hz, 4-NH); ^{13}C NMR (100 MHz, $CDCl_3$): 24.4, 37.4, 42.1, 43.7, 53.9, 56.2, 60.7, 68.6, 101.7, 108.1, 109.0, 110.2, 127.6, 132.3, 134.6, 137.2, 147.7, 148.7, 152.6, 171.2, 173.9, 180.2. HRMS *m/z* calcd for $C_{25}H_{26}N_2O_8S$: 515.1483 $[M+H]^+$, found: 515.1487 $[M+H]^+$.

4.1.2.2. 4β-(3-Cyclohexanecarbonyl-thioureido)-4-deoxypodophyllotoxin (13b). Yield: 78%; mp: 153–157 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.23–1.93 (m, 10H, 2″,3″,4″,5″,6″-H), 2.17–2.21 (m, 1H, 1″-H), 2.90 (dd, *J* = 14.4, 5.2 Hz, 1H, 2-H), 3.06–3.08 (m, 1H, 3-H), 3.76 (s, 6H, 3′,5′-OCH_3), 3.81 (s, 3H, 4′-OCH_3), 3.94–3.99 (m, 1H, 11α-H), 4.50–4.54 (m, 1H, 11β-H), 4.63 (d, 1H, 1-H), 5.82 (dd, *J* = 8.0, 4.8 Hz, 1H, 4-H), 5.98 and 6.00 (ABq, 2H, –OCH_2O–), 6.30 (s, 2H, 2′,6′-H), 6.53 (s, 1H, 8-H), 6.82 (s, 1H, 5-H), 8.66 (s, 1H, –CSNHCO–), 10.80 (d, 1H, *J* = 8.0 Hz, 4-NH). ^{13}C NMR (100 MHz, $CDCl_3$): 25.2, 25.3, 26.9, 28.8, 28.9, 37.5, 42.1, 43.7, 45.8, 53.9, 56.2, 60.7, 68.7, 101.7, 108.1, 109.1, 110.1, 127.6, 132.3, 134.6, 137.2, 147.7, 148.7, 152.6, 173.9, 177.1, 180.6. HRMS *m/z* calcd for $C_{30}H_{34}N_2O_8S$: 583.2109 $[M+H]^+$, found: 583.2120 $[M+H]^+$.

4.1.2.3. 4β-(3-Benzoyl-thioureido)-4-deoxypodophyllotoxin (13c). Yield: 83%; mp: 162–165 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.96 (dd, *J* = 14.4, 5.2 Hz, 1H, 2-H), 3.09–3.14 (m, 1H, 3-H), 3.77 (s, 6H, 3′,5′-OCH_3), 3.82 (s, 3H, 4′-OCH_3), 4.00–4.06 (t, 1H, 11α-H), 4.53–4.57 (m, 1H, 11β-H), 4.67 (d, 1H, 1-H), 5.89 (dd, *J* = 7.6, 4.8 Hz, 1H, 4-H), 5.97 and 6.00 (ABq, 2H, –OCH_2O–), 6.33 (s, 2H, 2′,6′-H), 6.55 (s, 1H, 8-H), 6.87 (s, 1H, 5-H), 7.54 (t, 2H, 3″,5″-H), 7.66 (t, 1H, 4″-H), 7.83 (d, 2H, 2″,6″-H), 9.11 (s, 1H, –CSNHCO–), 10.98 (d, 1H, *J* = 8.0 Hz, 4-NH). ^{13}C NMR (100 MHz, $CDCl_3$): 37.6, 42.2, 43.8, 54.1, 56.2, 56.3, 60.4, 60.7, 60.8, 68.7, 101.7, 108.2, 109.2, 110.2, 127.5, 127.7, 129.3, 131.2, 132.3, 134.1, 134.6, 137.3, 147.8, 148.8, 152.7, 167.0, 171.1, 173.9, 180.5. HRMS *m/z* calcd for $C_{30}H_{28}N_2O_8S$: 599.1459 $[M+Na]^+$, found: 599.1474 $[M+Na]^+$.

4.1.2.4. 4β-[3-(4-Fluorobenzoyl)-thioureido]-4-deoxypodophyllotoxin (13d). Yield: 86%; mp: 161–164 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.07–3.16 (m, 1H, 3-H), 3.21 (dd, *J* = 14.8, 5.2 Hz, 1H, 2-H), 3.61 (s, 3H, 4′-OCH_3), 3.66 (s, 6H, 3′,5′-OCH_3), 3.99–4.04 (m, 1H, 11α-H), 4.41–4.45 (m, 1H, 11β-H), 4.62 (d, 1H, 1-H), 5.86 (dd, *J* = 8.0, 4.4 Hz, 1H, 4-H), 6.02 and 6.03 (ABq, 2H, –OCH_2O–), 6.31 (s, 2H, 2′,6′-H), 6.58 (s, 1H, 8-H), 6.87 (s, 1H, 5-H), 7.33 (t, 2H, 3″,5″-H), 7.98–8.01 (m, 2H, 2″,6″-H), 10.92 (d, 1H, *J* = 8.0 Hz, 4-NH), 11.54 (s, 1H, –CSNHCO–); ^{13}C NMR (100 MHz, DMSO-d_6): 36.7, 40.6, 42.5, 52.6, 55.3, 59.4, 67.8, 101.0, 107.7, 108.4, 109.2, 114.8, 115.0, 127.9, 128.2, 128.3, 131.1, 131.2, 131.9, 135.0, 135.9, 146.3, 147.3, 151.5, 163.1, 165.6, 166.3, 173.5, 180.2. HRMS *m/z* calcd for $C_{30}H_{27}FN_2O_8S$: 617.1364 $[M+Na]^+$, found: 617.1374 $[M+Na]^+$.

4.1.2.5. 4β-[3-(4-Chlorobenzoyl)-thioureido]-4-deoxypodophyllotoxin (13e). Yield: 85%; mp: 170–173 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.96 (dd, *J* = 14.4, 5.2 Hz, 1H, 2-H), 3.10–3.15 (m, 1H, 3-H), 3.77 (s, 6H, 3′,5′-OCH_3), 3.82 (s, 3H, 4′-OCH_3), 4.02 (t, 1H, 11α-H), 4.53–4.57 (m, 1H, 11β-H), 4.67 (d, 1H, 1-H), 5.88 (dd, *J* = 8.0, 4.8 Hz, 1H, 4-H), 5.99 and 6.01 (ABq, 2H, –OCH_2O–), 6.32 (s, 2H, 2′,6′-H), 6.56 (s, 1H, 8-H), 6.86 (s, 1H, 5-H), 7.52 (d, *J* = 8.8 Hz, 2H, 3″,5″-H), 7.78 (d, *J* = 8.8 Hz, 2H, 2″,6″-H), 9.07 (s, 1H, –CSNHCO–), 10.91 (d, 1H, *J* = 7.6 Hz, 4-NH); ^{13}C NMR (100 MHz, $CDCl_3$): 37.5, 42.2, 43.8, 54.2, 56.2, 60.8, 68.7, 101.7, 108.2, 109.1, 110.2, 127.6, 128.9, 129.6, 132.3, 134.6, 137.3, 140.6, 147.8, 148.8, 152.7, 166.0, 173.8, 180.3. HRMS *m/z* calcd

for $C_{30}H_{27}ClN_2O_8S$: 628.1515 $[M+NH_4]^+$, found: 628.1510 $[M+NH_4]^+$.

4.1.2.6. 4β-[3-(4-Methylbenzoyl)-thioureido]-4-deoxypodophyllotoxin (13f). Yield: 78%; mp: 162–165 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.45 (s, 3H, 4″-CH_3), 2.96 (dd, J = 14.4, 5.2 Hz, 1H, 2-H), 3.10–3.14 (m, 1H, 3-H), 3.77 (s, 6H, 3′,5′-OCH_3), 3.82 (s, 3H, 4′-OCH_3), 4.01–4.06 (m, 1H, 11α-H), 4.53–4.57 (m, 1H, 11β-H), 4.66 (d, 1H, 1-H), 5.89 (dd, J = 8.0, 4.4 Hz, 1H, 4-H), 5.97 and 6.00 (ABq, 2H, –OCH_2O–), 6.32 (s, 2H, 2′,6′-H), 6.55 (s, 1H, 8-H), 6.87 (s, 1H, 5-H), 7.33 (d, J = 8.0 Hz, 2H, 3″,5″-H), 7.72 (d, J = 8.4 Hz, 2H, 2″,6″-H), 9.07 (s, 1H, –CSNHCO–), 11.03 (d, 1H, J = 8.0 Hz, 4-NH); ^{13}C NMR (100 MHz, $CDCl_3$): 21.7, 37.6, 42.2, 43.8, 54.1, 56.2, 60.7, 68.7, 101.7, 108.2, 109.2, 110.2, 127.5, 127.8, 128.3, 130.0, 132.3, 134.6, 137.3, 145.1, 147.8, 148.8, 152.7, 167.0, 173.9, 180.6. HRMS m/z calcd for $C_{31}H_{30}N_2O_8S$: 613.1615 $[M+Na]^+$, found: 613.1622 $[M+Na]^+$.

4.1.2.7. 4β-[3-(4-Nitrobenzoyl)-thioureido]-4-deoxypodophyllotoxin (13g). Yield: 56%; mp: 171–174 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.95 (dd, J = 14.4, 5.2 Hz, 1H, 2-H), 3.10–3.19 (m, 1H, 3-H), 3.77 (s, 6H, 3′,5′-OCH_3), 3.82 (s, 3H, 4′-OCH_3), 3.99–4.04 (m, 1H, 11α-H), 4.53–4.57 (m, 1H, 11β-H), 4.68 (d, 1H, 1-H), 5.87 (dd, J = 8.0, 4.8 Hz, 1H, 4-H), 5.99 and 6.01 (ABq, 2H, –OCH_2O–), 6.31 (s, 2H, 2′,6′-H), 6.57 (s, 1H, 8-H), 6.85 (s, 1H, 5-H), 8.03 (d, J = 8.4 Hz, 2H, 2″,6″-H), 8.38 (d, J = 8.8 Hz, 2H, 3″,5″-H), 9.25 (s, 1H, –CSNHCO–), 10.79 (d, 1H, J = 8.0 Hz, 4-NH). ^{13}C NMR (100 MHz, $CDCl_3$): 37.5, 42.2, 43.8, 54.3, 56.3, 60.8, 68.6, 101.7, 108.2, 109.0, 110.3, 124.3, 127.4, 128.8, 132.4, 134.5, 136.7, 137.3, 147.8, 148.9, 150.8, 152.7, 165.1, 173.8, 179.9. HRMS m/z calcd for $C_{30}H_{27}N_3O_{10}S$: 644.1309 $[M+Na]^+$, found: 644.1326 $[M+Na]^+$.

4.1.2.8. 4β-[3-3(3-Nitrobenzoyl)-thioureido]-4-deoxypodophyllotoxin (13h). Yield: 63%; mp: 168–172 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.96 (dd, J = 14.4, 5.2 Hz, 1H, 2-H), 3.10–3.18 (m, 1H, 3-H), 3.77 (s, 6H, 3′,5′-OCH_3), 3.82 (s, 3H, 4′-OCH_3), 4.00–4.05 (m, 1H, 11α-H), 4.54–4.68 (m, 1H, 11β-H), 4.69 (d, 1H, 1-H), 5.88 (dd, J = 8.0, 4.8 Hz, 1H, 4-H), 5.99 and 6.01 (ABq, 2H, –OCH_2O–), 6.32 (s, 2H, 2′,6′-H), 6.57 (s, 1H, 8-H), 6.86 (s, 1H, 5-H), 7.78 (t, J = 8.0 Hz, 1H, 5″-H), 8.18 (d, J = 8.0 Hz, 1H, 6″-H), 8.51 (d, J = 8.0 Hz, 1H, 4″-H), 8.72 (s, 1H, 2″-H), 9.28 (s, 1H, –CSNHCO–), 10.82 (d, 1H, J = 8.0 Hz, 4-NH). ^{13}C NMR (100 MHz, $CDCl_3$): 37.5, 42.2, 43.8, 54.3, 56.2, 60.8, 68.6, 101.7, 108.2, 109.0, 110.3, 122.7, 127.4, 128.2, 130.6, 132.4, 133.0, 133.1, 134.5, 137.3, 147.8, 148.5, 148.9, 152.7, 164.8, 173.8, 179.9. HRMS m/z calcd for $C_{30}H_{27}N_3O_{10}S$: 644.1309 $[M+Na]^+$, found: 644.1317 $[M+Na]^+$.

4.1.2.9. 4β-(3-Nicotinoyl-thioureido)-4-deoxypodophyllotoxin (13i). Yield: 80%; mp: 173–176 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.95 (dd, J = 14.4, 4.8 Hz, 1H, 2-H), 3.07–3.16 (m, 1H, 3-H), 3.75 (s, 6H, 3′,5′-OCH_3), 3.80 (s, 3H, 4′-OCH_3), 3.98–4.03 (m, 1H, 11α-H), 4.50–4.52 (m, 1H, 11β-H), 4.66 (d, 1H, 1-H), 5.86 (dd, J = 8.0, 4.8 Hz, 1H, 4-H), 5.97 and 5.99 (ABq, 2H, –OCH_2O–), 6.30 (s, 2H, 2′,6′-H), 6.54 (s, 1H, 8-H), 6.84 (s, 1H, 5-H), 7.46 (dd, J = 8.0, 4.8 Hz, 1H, 6″-H), 8.13–8.15 (m, 1H, 5″-H), 8.83 (dd, J = 4.8, 1.2 Hz, 1H, 4″-H), 9.09 (d, 1H, J = 2.0 Hz, 2″-H), 9.58 (s, 1H, –CSNHCO–), 10.87 (d, 1H, J = 8.0 Hz, 4-NH). ^{13}C NMR (100 MHz, $CDCl_3$): 37.5, 42.1, 43.7, 54.1, 56.2, 60.3, 60.7, 68.6, 101.7, 108.1, 109.0, 110.2, 123.7, 127.4, 127.5, 132.3, 134.5, 135.3, 137.2, 147.7, 148.7, 148.8, 152.6, 154.2, 165.8, 173.8, 180.2. HRMS m/z calcd for $C_{29}H_{27}N_3O_8S$: 578.1592 $[M+H]^+$, found: 578.1606 $[M+H]^+$.

4.1.2.10. 4β-[3-(4-Chloronicotinoyl)-thioureido]-4-deoxypodophyllotoxin(13j). Yield: 84%; mp: 172–175 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.94 (dd, J = 14.4, 4.8 Hz, 1H, 2-H), 3.12–3.15 (m, 1H, 3-H), 3.77 (s, 6H, 3′,5′-OCH_3), 3.82 (s, 3H, 4′-OCH_3), 3.99–4.04 (m, 1H, 11α-H), 4.52–4.57 (m, 1H, 11β-H), 4.63 (d, 1H, 1-H), 5.87 (dd, J = 8.0, 4.8 Hz, 1H, 4-H), 5.98 and 6.01 (ABq, 2H, –OCH_2O–), 6.32 (s, 2H, 2′,6′-H), 6.57 (s, 1H, 8-H), 6.85 (s, 1H, 5-H), 7.51 (d, J = 8.4 Hz, 1H, 5″-H), 8.10 (dd, J = 8.0, 2.4 Hz, 1H, 6″-H), 8.88 (d, 1H, J = 2.4 Hz, 2″-H), 9.20 (s, 1H, –CSNHCO–), 10.77 (d, 1H, J = 8.0 Hz, 4-NH); ^{13}C NMR (100 MHz, $CDCl_3$): 37.5, 42.2, 43.8, 54.3, 56.3, 60.8, 68.6, 101.8, 108.3, 109.1, 110.3, 124.9, 126.3, 127.5, 132.4, 134.5, 137.4, 137.7, 147.8, 148.9, 152.7, 156.7, 164.6, 173.8, 179.9. HRMS m/z calcd for $C_{29}H_{26}ClN_3O_8S$: 634.1021 $[M+Na]^+$, found: 634.1027 $[M+Na]^+$.

4.1.2.11. 4β-[3-(2-Chloronicotinoyl)-thioureido]-4-deoxypodophyllotoxin (13k). Yield: 88%; mp: 183–186 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.94 (dd, J = 14.4, 4.8 Hz, 1H, 2-H), 3.09–3.12 (m, 1H, 3-H), 3.76 (s, 6H, 3′,5′-OCH_3), 3.80 (s, 3H, 4′-OCH_3), 3.98–4.03 (m, 1H, 11α-H), 4.50–4.55 (m, 1H, 11β-H), 4.66 (d, 1H, 1-H), 5.85 (dd, J = 7.6, 4.8 Hz, 1H, 4-H), 5.99 and 6.01 (ABq, 2H, –OCH_2O–), 6.30 (s, 2H, 2′,6′-H), 6.55 (s, 1H, 8-H), 6.87 (s, 1H, 5-H), 7.41 (dd, J = 7.6, 4.8 Hz, 1H, 5″-H), 8.07 (dd, J = 7.6, 2.0 Hz, 1H, 6″-H), 8.56 (dd, 1H, J = 4.8, 2.0 Hz, 4″-H), 9.76 (s, 1H, –CSNHCO–), 10.64 (d, 1H, J = 8.0 Hz, 4-NH); ^{13}C NMR (100 MHz, $CDCl_3$): 37.5, 42.2, 43.7, 54.1, 56.2, 60.7, 68.6, 101.7, 108.1, 109.1, 110.2, 122.9, 127.4, 128.4, 132.4, 134.5, 137.2, 139.8, 147.2, 147.8, 148.8, 152.6, 152.7, 164.7, 173.8, 179.7. HRMS m/z calcd for $C_{29}H_{26}ClN_3O_8S$: 634.1021 $[M+Na]^+$, found: 634.1041 $[M+Na]^+$.

4.1.2.12. 4β-{3-(5-Bromonicotinoyl)-thioureido]-4-deoxypodophyllotoxin (13l). Yield: 68%; mp: 173–176 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.94 (dd, J = 14.4, 4.8 Hz, 1H, 2-H), 3.12–3.15 (m, 1H, 3-H), 3.77 (s, 6H, 3′,5′-OCH_3), 3.82 (s, 3H, 4′-OCH_3), 3.99–4.04 (m, 1H, 11α-H), 4.53–4.57 (m, 1H, 11β-H), 4.68 (d, 1H, 1-H), 5.86 (dd, J = 7.6, 4.8 Hz, 1H, 4-H), 5.99 and 6.01 (ABq, 2H, –OCH_2O–), 6.31 (s, 2H, 2′,6′-H), 6.57 (s, 1H, 8-H), 6.85 (s, 1H, 5-H), 8.29 (s, 1H, 4″-H), 8.92 (s, 1H, 6″-H), 8.99 (s, 1H, 2″-H), 9.27 (s, 1H, –CSNHCO–), 10.74 (d, 1H, J = 8.0 Hz, 4-NH); ^{13}C NMR (100 MHz, $CDCl_3$): 37.5, 42.2, 43.7, 54.3, 56.3, 60.8, 68.6, 101.8, 108.2, 109.0, 110.3, 127.4, 132.4, 134.5, 137.3, 137.8, 146.4, 147.8, 148.9, 152.7, 155.4, 164.2, 173.8, 179.8. HRMS m/z calcd for $C_{29}H_{26}BrN_3O_8S$: 678.0516 $[M+Na]^+$, found: 678.0529 $[M+Na]^+$.

4.1.2.13. 4β-[3-(2-Thiophenecarbonyl)-thioureido]-4-deoxypodophyllotoxin (13m). Yield: 92%; mp: 170–173 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.94 (dd, J = 14.4, 4.8 Hz, 1H, 2-H), 3.08–3.12 (m, 1H, 3-H), 3.77 (s, 6H, 3′,5′-OCH_3), 3.82 (s, 3H, 4′-OCH_3), 3.99–4.04 (m, 1H, 11α-H), 4.52–4.56 (m, 1H, 11β-H), 4.65 (d, 1H, 1-H), 5.87 (dd, J = 7.6, 4.8 Hz, 1H, 4-H), 5.97 and 6.00 (ABq, 2H, –OCH_2O–), 6.32 (s, 2H, 2′,6′-H), 6.54 (s, 1H, 8-H), 6.84 (s, 1H, 5-H), 7.19 (dd, J = 5.2, 4.0 Hz, 1H, 4″-H), 7.71–7.74 (m, 2H, 3″,5″-H), 8.98 (s, 1H, –CSNHCO–), 10.81 (d, 1H, J = 8.0 Hz, 4-NH); ^{13}C NMR (100 MHz, $CDCl_3$): 37.6, 42.2, 43.8, 54.1, 56.3, 60.7, 68.7, 101.7, 108.3, 109.1, 110.1, 127.6, 128.6, 130.8, 132.3, 134.6, 134.7, 135.6, 137.3, 147.8, 148.8, 152.7, 161.3, 173.8, 180.1. HRMS m/z calcd for $C_{28}H_{26}N_2O_8S_2$: 600.1469$[M+NH_4]^+$, found: 600.1480 $[M+NH_4]^+$.

4.1.2.14. 4β-[3-(2-Furoyl)-thioureido]-4-deoxypodophyllotoxin (13n). Yield: 89%; mp: 158–161 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.93 (dd, J = 14.4, 4.8 Hz, 1H, 2-H), 3.09–3.12 (m, 1H, 3-H), 3.77 (s, 6H, 3′,5′-OCH_3), 3.82 (s, 3H, 4′-OCH_3), 3.99–4.04 (m, 1H, 11α-H), 4.53–4.57 (m, 1H, 11β-H), 4.65 (d, 1H, 1-H), 5.87 (dd, J = 7.6, 4.8 Hz, 1H, 4-H), 5.97 and 6.00 (ABq, 2H, –OCH_2O–), 6.31 (s, 2H, 2′,6′-H), 6.54 (s, 1H, 8-H), 6.64 (dd, J = 3.6, 1.2 Hz, 1H, 4″-H), 6.85 (s, 1H, 5-H), 7.32 (d, J = 3.6 Hz, 1H, 5″-H), 7.63 (d, J = 1.2 Hz, 1H, 3″-H), 9.19 (s, 1H, –CSNHCO–), 10.73 (d, 1H, J = 8.0 Hz, 4-NH); ^{13}C NMR (100 MHz, $CDCl_3$): 37.5, 42.2, 43.7,

54.0, 56.2, 60.7, 68.7, 101.7, 108.1, 109.1, 110.1, 113.5, 119.3, 127.7, 132.3, 134.6, 137.2, 144.6, 146.6, 147.7, 148.8, 152.6, 156.8, 173.9, 180.0. HRMS *m/z* calcd for $C_{28}H_{26}N_2O_9S$: 567.1432 $[M+H]^+$, found: 567.1448 $[M+H]^+$.

4.1.2.15. 4β-[3-(2-Naphthoyl)-thioureido]-4-deoxypodophyllotoxin (13o). Yield: 77%; mp: 156–159 °C; ^1H NMR (400 MHz, $CDCl_3$): δ 3.00 (dd, J = 14.4, 5.2 Hz, 1H, 2-H), 3.10–3.20 (m, 1H, 3-H), 3.78 (s, 6H, 3′,5′-OCH_3), 3.83 (s, 3H, 4′-OCH_3), 4.05–4.10 (m, 1H, 11α-H), 4.56–4.60 (m, 1H, 11β-H), 4.69 (d, 1H, 1-H), 5.93 (dd, J = 8.0, 4.4 Hz, 1H, 4-H), 5.98 and 6.01 (ABq, 2H, –OCH_2O–), 6.34 (s, 2H, 2′,6′-H), 6.57 (s, 1H, 8-H), 6.90 (s, 1H, 5-H), 7.63–7.68 (m, 2H, 5″,6″-H), 7.84 (dd, J = 8.4, 1.2 Hz, 1H, 3″-H), 7.93 (d, J = 8.0 Hz, 1H, 2″-H), 7.99 (d, J = 8.4 Hz, 2H, 4″,7″-H), 8.39 (s, 1H, 8″-H), 9.27 (s, 1H, –CSNHCO–), 11.07 (d, 1H, J = 8.0 Hz, 4-NH). ^{13}C NMR (100 MHz, $CDCl_3$): 37.6, 42.2, 43.8, 54.2, 56.3, 60.8, 68.7, 101.7, 108.3, 109.2, 110.2, 122.8, 127.6, 127.8, 127.9, 128.3, 129.0, 129.2, 129.3, 129.5, 132.3, 132.4, 134.6, 135.7, 137.4, 147.8, 148.8, 152.7, 167.2, 173.9. HRMS *m/z* calcd for $C_{34}H_{30}N_2O_8S$: 649.1615 $[M+Na]^+$, found: 649.1629 $[M+Na]^+$.

4.1.2.16. 4β-(3-Benzoyl-thioureido)-4′-demethyl-4-deoxypodophyllotoxin (14a). Yield: 84%; mp: 177–180 °C; ^1H NMR (400 MHz, $CDCl_3$): δ 2.95 (dd, J = 14.4, 5.2 Hz, 1H, 2-H), 3.09–3.14 (m, 1H, 3-H), 3.80 (s, 6H, 3′,5′-OCH_3), 4.00–4.05 (m, 1H, 11α-H), 4.51–4.55 (m, 1H, 11β-H), 4.66 (d, 1H, 1-H), 5.49 (s, 1H, 4′-OH), 5.90 (dd, J = 8.0, 4.8 Hz, 1H, 4-H), 5.97 and 6.00 (ABq, 2H, –OCH_2O–), 6.34 (s, 2H, 2′,6′-H), 6.55 (s, 1H, 8-H), 6.87 (s, 1H, 5-H), 7.54 (t, 2H, 3″,5″-H), 7.66 (t, 1H, 4″-H), 7.84 (d, 2H, 2″,6″-H), 9.11 (s, 1H, –CSNHCO–), 10.99 (d, 1H, J = 8.0 Hz, 4-NH). ^{13}C NMR (100 MHz, $CDCl_3$): 37.5, 42.3, 43.6, 54.1, 56.4, 56.5, 68.7, 101.7, 107.9, 109.1, 110.2, 127.5, 127.7, 129.3, 130.1, 131.2, 132.5, 134.0, 134.2, 146.5, 147.7, 148.8, 167.0, 173.9, 180.5. HRMS *m/z* calcd for $C_{29}H_{26}N_2O_8S$: 585.1302 $[M+Na]^+$, found: 585.1323 $[M+Na]^+$.

4.1.2.17. 4β-[3-(4-Chlorobenzoyl)-thioureido]-4′-demethyl-4-deoxypodophyllotoxin (14b). Yield: 92%; mp:180–183 °C; ^1H NMR (400 MHz, $CDCl_3$): δ 2.94 (dd, J = 14.4, 4.8 Hz, 1H, 2-H), 3.07–3.15 (m, 1H, 3-H), 3.80 (s, 6H, 3′,5′-OCH_3), 3.99–4.04 (m, 1H, 11α-H), 4.51–4.55 (m, 1H, 11β-H), 4.66 (d, 1H, 1-H), 5.49 (s, 1H, 4′-OH), 5.89 (dd, J = 8.0, 4.8 Hz, 1H, 4-H), 5.97 and 6.00 (ABq, 2H, –OCH_2O–), 6.33 (s, 2H, 2′,6′-H), 6.56 (s, 1H, 8-H), 6.85 (s, 1H, 5-H), 7.52 (d, J = 8.4 Hz, 2H, 3″,5″-H), 7.78 (d, J = 8.4 Hz, 2H, 2″,6″-H), 9.07 (s, 1H, –CSNHCO–), 10.90 (d, 1H, J = 8.0 Hz, 4-NH). ^{13}C NMR (100 MHz, $CDCl_3$): 37.5, 42.3, 43.6, 54.2, 56.4, 56.5, 60.4, 68.6, 101.7, 107.9, 109.1, 110.2, 127.6, 128.9, 129.6, 129.7, 130.1, 132.5, 134.2, 140.6, 146.5, 147.7, 148.8, 166.0, 171.1, 173.9, 180.3. HRMS *m/z* calcd for $C_{29}H_{25}ClN_2O_8S$: 619.0912 $[M+Na]^+$, found: 619.0944 $[M+Na]^+$.

4.1.2.18. 4β-[3-(2-Furoyl)-thioureido]-4′-demethyl-4-deoxypodophyllotoxin (14c). Yield: 86%; mp: 180–184 °C;^1H NMR (400 MHz, $CDCl_3$): δ 2.92 (dd, J = 14.4, 4.8 Hz, 1H, 2-H), 3.07–3.12 (m, 1H, 3-H), 3.80 (s, 6H, 3′,5′-OCH_3), 3.98–4.03 (m, 1H, 11α-H), 4.50–4.54 (m, 1H, 11β-H), 4.64 (d, 1H, 1-H), 5.47 (s, 1H, 4′-OH), 5.87 (dd, J = 8.8, 4.8 Hz, 1H, 4-H), 5.96 and 5.99 (ABq, 2H, –OCH_2O–), 6.33 (s, 2H, 2′,6′-H), 6.54 (s, 1H, 8-H), 6.64 (dd, J = 3.6, 1.2 Hz, 1H, 4″-H), 6.84 (s, 1H, 5-H), 7.32 (d, J = 3.6 Hz, 1H, 5″-H), 7.63 (d, J = 1.2 Hz, 1H, 3″-H), 9.19 (s, 1H, –CSNHCO–), 10.72 (d, 1H, J = 8.0 Hz, 4-NH). ^{13}C NMR (100 MHz, $CDCl_3$): 37.5, 42.3, 43.6, 54.0, 56.4, 56.5, 60.4, 68.7, 101.6, 107.9, 109.1, 110.1, 113.5, 119.2, 127.7, 130.1, 132.5, 134.2, 144.6, 146.5, 146.6, 147.7, 148.8, 156.8, 171.1, 173.9, 180.0. HRMS *m/z* calcd for $C_{27}H_{24}N_2O_9S$: 575.1095 $[M+Na]^+$, found: 575.1114 $[M+Na]^+$.

4.1.2.19. 4β-[3-(Thiophene-2-carbonyl)-thioureido]-4′-demethyl-4-deoxypodophyllotoxin (14d). Yield: 93%; mp: 194–197 °C; ^1H NMR (400 MHz, $CDCl_3$): δ 2.93 (dd, J = 14.4, 4.8 Hz, 1H, 2-H), 3.09–3.10 (m, 1H, 3-H), 3.80 (s, 6H, 3′,5′-OCH_3), 3.99–4.04 (m, 1H, 11α-H), 4.50–4.54 (m, 1H, 11β-H), 4.64 (d, 1H, 1-H), 5.49 (s, 1H, 4′-OH), 5.87 (dd, J = 8.0, 4.8 Hz, 1H, 4-H), 5.97 and 6.00 (ABq, 2H, –OCH_2O–), 6.33 (s, 2H, 2′,6′-H), 6.54 (s, 1H, 8-H), 6.85 (s, 1H, 5-H), 7.19 (dd, J = 4.8, 4.0 Hz, 1H, 4″-H), 7.71–7.74 (m, 2H, 3″,5″-H), 8.97 (s, 1H, –CSNHCO–), 10.81 (d, 1H, J = 8.0 Hz, 4-NH); ^{13}C NMR (100 MHz, $CDCl_3$): 37.5, 42.3, 43.6, 54.1, 56.4, 56.5, 60.4, 68.7, 101.7, 107.9, 109.1, 110.2, 127.6, 128.6, 130.1, 130.9, 132.5, 134.2, 134.7, 135.6, 146.5, 147.7, 148.8, 171.1, 173.9, 180.1. HRMS *m/z* calcd for $C_{27}H_{24}N_2O_8S_2$: 591.0866 $[M+Na]^+$, found: 591.0876 $[M+Na]^+$.

4.1.2.20. 4β-[3-(2-Naphthoyl)-thioureido]-4′-demethyl-4-deoxypodophyllotoxin (14e). Yield: 87%; mp: 178–181 °C; ^1H NMR (400 MHz, $CDCl_3$): δ 2.98 (dd, J = 14.4, 4.8 Hz, 1H, 2-H), 3.09–3.18 (m, 1H, 3-H), 3.80 (s, 6H, 3′,5′-OCH_3), 3.04–4.10 (m, 1H, 11α-H), 4.53–4.57 (m, 1H, 11β-H), 4.67 (d, 1H, 1-H), 5.49 (s, 1H, 4′-OH), 5.92 (dd, J = 8.0, 4.4 Hz, 1H, 4-H), 5.98 and 6.00 (ABq, 2H, –OCH_2O–), 6.35 (s, 2H, 2′,6′-H), 6.56 (s, 1H, 8-H), 6.89 (s, 1H, 5-H), 7.62–7.67 (m, 2H, 5″, 6″-H), 7.84 (dd, J = 8.4, 1.6 Hz, 1H, 3″-H), 7.92 (d, J = 7.6 Hz, 1H, 2″-H), 7.98 (d, J = 8.4 Hz, 2H, 4″,7″-H), 8.38 (s, 1H, 8″-H), 9.28 (s, 1H, –CSNHCO–), 11.07 (d, 1H, J = 8.0 Hz, 4-NH); ^{13}C NMR (100 MHz, $CDCl_3$): 37.5, 42.3, 43.6, 54.2, 56.4, 56.5, 60.4, 68.7, 101.7, 107.9, 109.1, 110.2, 122.8, 127.6, 127.7, 127.9, 128.3, 129.0, 129.2, 129.3, 129.4, 130.1, 132.3, 132.5, 134.2, 135.7, 146.5, 147.7, 148.8, 167.2, 171.1, 173.9, 180.5. HRMS *m/z* calcd for $C_{33}H_{28}N_2O_8S$: 635.1459[M+Na]$^+$, found: 635.1477 $[M+Na]^+$.

4.2. Antiproliferative activity assay

Antiproliferative activity was determined by the sulforhodamine B (SRB) colorimetric assay as previously described.[21] In brief, the cells (3–5 × 10^3 cells/well) were seeded in 96-well plates filled with RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS) containing various concentrations of samples, and incubated for 72 h. At the end of the exposure period, the attached cells were fixed with cold 50% trichloroacetic acid for 30 min followed by staining with 0.04% SRB (Sigma Chemical Co.) for 30 min. The bound SRB was solubilized in 10 mM Tris-base and the absorbance was measured at 515 nm on a Microplate Reader ELx800 (Bio-Tek Instruments, Winooski, VT) with a Gen5 software. All results were representative of three or more experiments.

Acknowledgments

This work was supported financially by the National Natural Science Foundation of China (30800720); the Post-Doctor Research Foundation (20090450142); the Fundamental Research Funds for the Central Universities (860120); and the Young Scholars Science Foundation of Lanzhou Jiaotong University (2011011). Thanks are also due to the support of Taiwan Department of Health Cancer Research Center of Excellence (DOH-100-TD-C-111–005).

References and notes

1. Lee, K. H. *J. Nat. Prod.* **2004**, *67*, 273.
2. Liu, Y. Q.; Yang, L.; Tian, X. *Curr. Bioact. Compds.* **2007**, *3*, 37.
3. De Jong, R. S.; Slijfer, E. A. M.; Uges, B. R. A.; Mulder, N. H.; de Vries, E. G. E. *Br. J. Cancer* **1997**, *76*, 1480.
4. Lee, K. H.; Beers, S. A.; Mori, M.; Wang, Z.; Kuo, Y. H.; Li, L.; Liu, S. Y.; Chang, J. Y.; Han, F. S.; Chen, Y. C. *J. Med. Chem.* **1990**, *33*, 1364.
5. Silverberg, L. J.; Kelly, S.; Vemishetti, P.; Vipond, D. H.; Gibson, F. S.; Harrison, B.; Spector, R.; Dillon, J. L. *Org. Lett.* **2000**, *2*, 3281.

6. Macdonald, T. L.; Lehnert, E. K.; Loper, J. T.; Chow, K. C.; Ross, W. E. In *DNA Topoisomerases in Cancer*; Potmesil, M., Kohn, K. W., Eds.; Oxford University: New York, 1991; pp 199–214.
7. You, Y. J. *Curr. Pharm. Des.* **2005**, *11*, 1695.
8. Zhang, Y. L.; Tropscha, A.; McPhail, A. T.; Lee, K. H. *J. Med. Chem.* **1994**, *37*, 1460.
9. Huang, T. S.; Lee, C. C.; Chao, Y.; Shu, C.-H.; Chen, L. T.; Chen, L. L.; Chen, M.-H.; Yuan, C. C.; Peng, W. *J. Pharm. Res.* **1999**, *16*, 997.
10. Byl, J. A. W.; Cline, S. D.; Utsugi, T.; Kobunai, T.; Yamada, Y.; Osheroff, N. *Biochemistry* **2001**, *40*, 712.
11. Bromberg, K. D.; Burgin, A. B.; Osheroff, N. *J. Biol. Chem.* **2003**, *278*, 7406.
12. Tian, X.; Zhang, F.-M.; Li, W.-G. *Life Sci.* **2002**, *70*, 2433.
13. Liu, Y. Q.; Tian, X. *Synth. Commun.* **2005**, *35*, 2749.
14. Thurston, L. S.; Irie, H.; Tani, S.; Han, F. S.; Liu, Z. C.; Cheng, Y. C.; Lee, K. H. *J. Med. Chem.* **1986**, *29*, 1547.
15. Hu, H.; Liu, S. Y.; Cheng, Y. C.; Lee, K. H.; Wang, Z. Q. *J. Med. Chem.* **1992**, *35*, 866.
16. Liu, Y. Q.; Li, L. H.; Li, W. Q.; Feng, G.; Yang, L. *Chem. Pap.* **2011**, *65*, 739.
17. Liu, Y. Q.; Wei, D. F.; Zhao, Y. L.; Cheng, W. d.; Lu, Y.; Ma, Y. q.; Li, X.; Han, C.; Wei, Y. X.; Cao, H. M.; Zhao, C. Y. *Bioorg. Med. Chem.* **2012**, *20*, 6285.
18. Li, H. Q.; Yan, T.; Yang, Y.; Shi, L.; Zhou, C. F.; Zhu, H. L. *Bioorg. Med. Chem.* **2010**, *18*, 305.
19. Jiang, J. D.; Roboz, J.; Weisz, I.; Deng, L.; Ma, L.; Holland, J. F.; Bekesi, J. G. *Anticancer Drug Des.* **1998**, *13*, 735.
20. Thanh, N. D.; Thi, N.; Mai, T. *Carbohydr. Res.* **2009**, *344*, 2399.
21. Moeker, J.; Teruya, K.; Rossit, S.; Wilkinson, B. L.; Lopez, M.; Bornaghi, L. F.; Innocenti, A.; Supuran, C. T.; Poulsen, S. A. *Bioorg. Med. Chem.* **2012**, *20*, 2392.
22. Liu, Y. Q.; Yang, L.; Li, L. H.; Li, H. Y. *Chem. Pap.* **2010**, *64*, 533.
23. Furuta, T.; Sakai, T.; Senga, T.; Osawa, T.; Kubo, K.; Shimizu, T.; Suzuki, R.; Yoshino, T.; Endo, M.; Miwa, A. *J. Med. Chem.* **2006**, *49*, 2186.
24. Skehan, P.; Storeng, R.; Scudiero, D.; Monks, A.; McMahon, J.; Vistica, D.; Warren, J. T.; Bokesch, H.; Kenney, S.; Boyd, M. R. *J. Natl. Cancer Inst.* **1990**, *82*, 1107.
25. Xiao, Z. Y.; Bastow, K. F.; Vance, J. R.; Sidwell, R. S.; Wang, H. K.; Chen, M. S.; Shi, Q.; Lee, K. H. *J. Med. Chem.* **2004**, *47*, 5140.