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Original article

Synthesis and evaluation of aroylthiourea derivatives of 4-β-amino-4'-O-demethyl-4-desoxypodophyllotoxin as novel topoisomerase II inhibitors

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ABSTRACT

A novel series of aroylthiourea derivatives of 4-β-amino-4′-*O*-demethyl-4-desoxy- podophyllotoxin were synthesized. Their cytotoxicities against three cancer cell lines were investigated by MTT assay. The kDNA decatenation assay indicated that compounds **5a**, **5f**, **5h** and **5l** inhibited topoisomerase II-mediated kDNA decatenation. DNA flow cytometric analysis revealed that compound **5a** induced cell cycle arrest at G2/M phase in HCT-116 cell line.

1. Introduction

DNA topoisomerases solve the topological problems associated with DNA replication, transcription, recombination, and other nuclear processes by introducing temporary single or double strand breaks in the DNA [1]. DNA topoisomerase II controls DNA topology by transient cleavage of the DNA double helix. This enzyme has been established as important molecular target of anticancer drug [2].

Podophyllotoxin (Fig. 1) is a well known naturally occurring antitumor lignan lactone isolated from the genus *Podophyllum* [3]. Semisynthetic podophyllotoxin derivatives etoposide (**VP-16**) and teniposide are in clinical use as antineoplastic agents due to their ability to inhibit the enzyme DNA topoisomerase II [4,5]. However, their clinical use has encountered certain limitations such as drug resistance and poor water solubility [6]. In order to obtain better therapeutic agents, a great number of etoposide analogs have been synthesized. The numerous synthesized analogs have allowed the improvement of the knowledge of structure–activity relationships. One of the major breakthroughs in this field underlining that the sugar moiety of etoposide is not essential for topoisomerase II inhibition [7,8].

Recently, new synthetic N-linked congeners, as the known antitumor agents GL-331 and NPF, exhibit improved cytotoxicity and DNA topoisomerase II inhibition activity [9–13]. Moreover, literature reported thiourea derivatives [14] and carbamate derivatives [13,15] possess potent anti-HIV and antitumor activities. To our best knowledge, there is no such effort addressed to synthesize aroylthiourea derivatives of 4-β-amino-4′-*O*-demethyl-4-desoxypodophyllotoxin. Structure–activity relationships (SAR) studies suggested that the essential structural features for topoisomerase II inhibitory activity are 4′-hydroxyl group, 4-β-stereochemistry and 4-*N*-linkage.

We previously discovered a series of aroylthiourea derivatives of 4-β-amino-4- desoxypodophyllotoxin as anticancer agents [16]. Representative compound **HY-1** (Fig. 1) possessed potent DNA topoisomerase II inhibitory activity and cytotoxicity. These results prompted us to further synthesize a library of aroylthiourea derivatives of 4-β-amino-4′-*O*-demethyl-4-desoxypodophyllotoxin as novel DNA topoisomerase II inhibitors.

Herein, we report the synthesis and antitumor activity of these aroylthiourea derivatives of 4-β-amino-4′-*O*-demethyl-4-desoxypodophyllotoxin (**5a**–**5l**, Scheme 1). It is found that compound **5a** possesses promising DNA topoisomerase II inhibitory activity.

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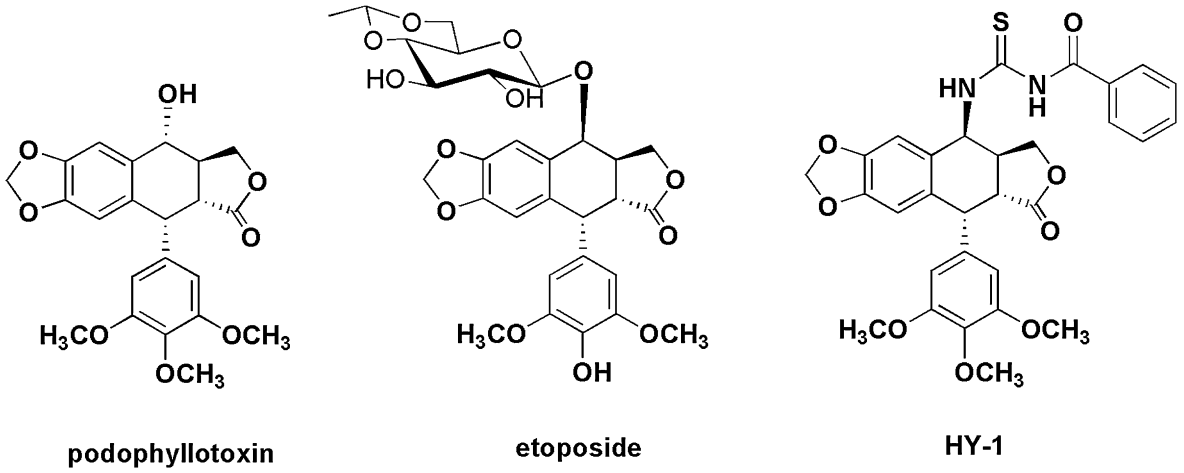


Fig. 1. Podophyllotoxin, etoposide and HY-1.

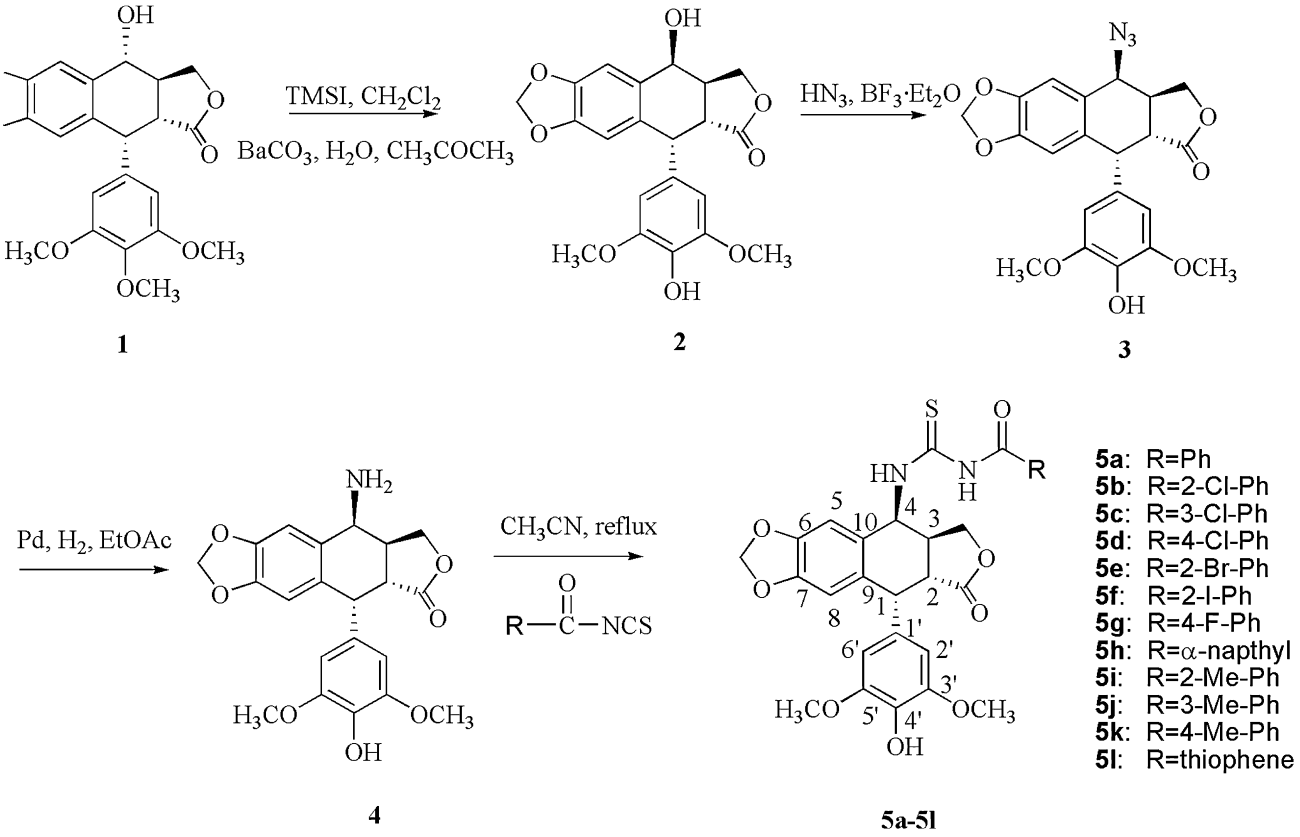
2. Results and discussion

2.1. Synthesis of aroylthiourea derivatives of 4-β-amino-4′-O-demethyl-4-desoxypodophyllotoxin

The synthetic route used to synthesize title compounds is outlined in Scheme 1. 4′-demethylepipodophyllotoxin (**2**) was prepared in 79% overall yield by treatment of podophyllotoxin (**1**) with TMSI in methylene chloride at 0 °C followed by weak basic hydrolysis with barium carbonate [17]. In the presence of boron trifluoride etherate, the reaction of hydrazoic acid with 4′-demethylepipodophyllotoxin (**2**) gave 4-β-azido derivatives (**3**) [13]. Nextly, 4-β-amino-4′-*O*-demethyl-4-desoxypodophyllotoxin (**4**) was prepared by reduction of **3** using Pd/C as a catalyst according to the procedure reported previously [18]. Coupled corresponding aroylisothiocyanates [19] with **4** in dry acetonitrile over a 2 h reflux period gave a series of aroylthioureas **5a**–**5l** in the yields of 53%–70%.

2.2. Cytotoxicities of compounds *5a*–*5l* against three cancer cell lines

The title compounds were evaluated for their cytotoxic activities against three cancer cell lines including HepG2, A549 and HCT-116 using a MTT growth inhibition assay. IC_{50} values were summarized in Table 1 and represented the concentration inducing a 50% decrease of cell growth after 3 days incubation.



Scheme 1. Synthesis of aroylthiourea derivatives of 4-β-amino-4′-*O*-demethyl-4-desoxypodophyllotoxin **5a**–**5l**.

Table 1
Cytotoxicities against three cancer cell lines of **5a**–**5l**.

Compd	IC_{50} (μM)		
	HepG2	HCT-116	A549
5a	6.1	6.3	9.8
5b	7.7	7.7	10.3
5c	7.6	6.8	17.9
5d	7.6	7.2	11.5
5e	5.1	6.2	11.4
5f	6.7	6.7	15.1
5g	8.3	6.8	10.8
5h	18.7	2.2	6.0
5i	5.2	4.7	12.2
5j	4.6	5.5	13. 3
5k	3.0	6.8	11.3
5l	4.4	5.0	9.5
4	1.5	2.1	5.2
Etoposide	4.9	13.5	21.4

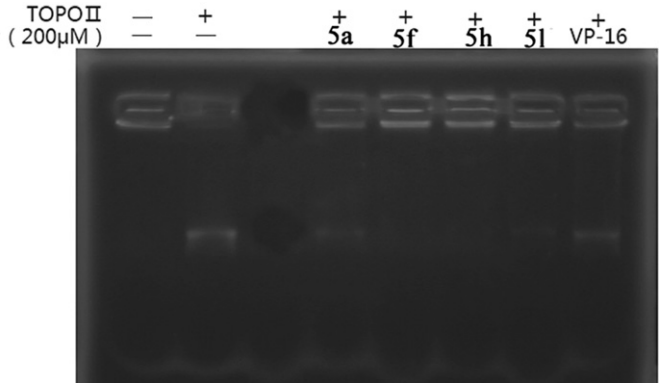


Fig. 2. Effects of 200 μM compound **5a**, **5f**, **5h**, **5l** and etoposide on human topoisomerase II-mediated decatenation activity of kDNA.

From the IC_{50} values, it is clear that most of these derivatives displayed potent cytotoxic activities against three cancer cell lines. However, these derivatives showed comparatively lesser activity against A549 cell line than other two cell lines. Compound **5a** and **5l**, without halogen moiety possessed the highest cytotoxicity on three cancer cell lines. Moreover, **5a** and **5l** possessed better cytotoxicity than etoposide. These results indicate that the halogen-substitution did not contribute to the cytotoxic potency of these compounds. Compound **5h**, bearing α-naphthyl group, was found a potent effect on A549 and HCT-116 cell lines, but less potent on HepG2 cell line, which indicated a different mode of action involved.

In summary, we have prepared a series of aroylthiourea derivatives of 4-β-amino-4′-*O*-demethyl-4-desoxypodophyllotoxin. The synthetic compounds were cytotoxic against three cancer cell lines at the micromolar range.

*2.3. Inhibition of human topoisomerase II kDNA decatenation by compounds **5a**, **5f**, **5h** and **5l***

To further understand the action mechanisms of antitumor activity of these compounds, the effect on the catalytic activity of human topoisomerase II was evaluated using kDNA decatenation assay. As shown in Fig. 2, compounds **5a**, **5f**, **5h** and **5l** completely inhibited the catalytic activity of topoisomerase II at 200 μM, which was much better than the effect of etoposide at same concentration. The results indicated that these compounds exerted their antitumor activities through inhibition of human topoisomerase II catalytic activity.

*2.4. Induction of cell cycle arrest in HCT-116 cells by compound **5a***

On the basis of the above results, further biological evaluations have been focused on compound **5a**. To gain further insight into the mode of action of compound **5a**, we examined the effects of compound **5a** on cell cycle by flow cytometry in HCT-116 cells. Interestingly, a concentration dependent change was observed in the cell cycle pattern (Fig. 3). Our results demonstrated that treatment of HCT-116 cells for 24 h with 0, 1.25, 2.5, 5 and 10 μM **5a** increased the percentage of cells at G2/M phase from 35.67% to 96.45%. The flow cytometric data clearly indicated that **5a** caused HCT-116 cell cycle arrest at G2/M phase follow the same pattern of etoposide.

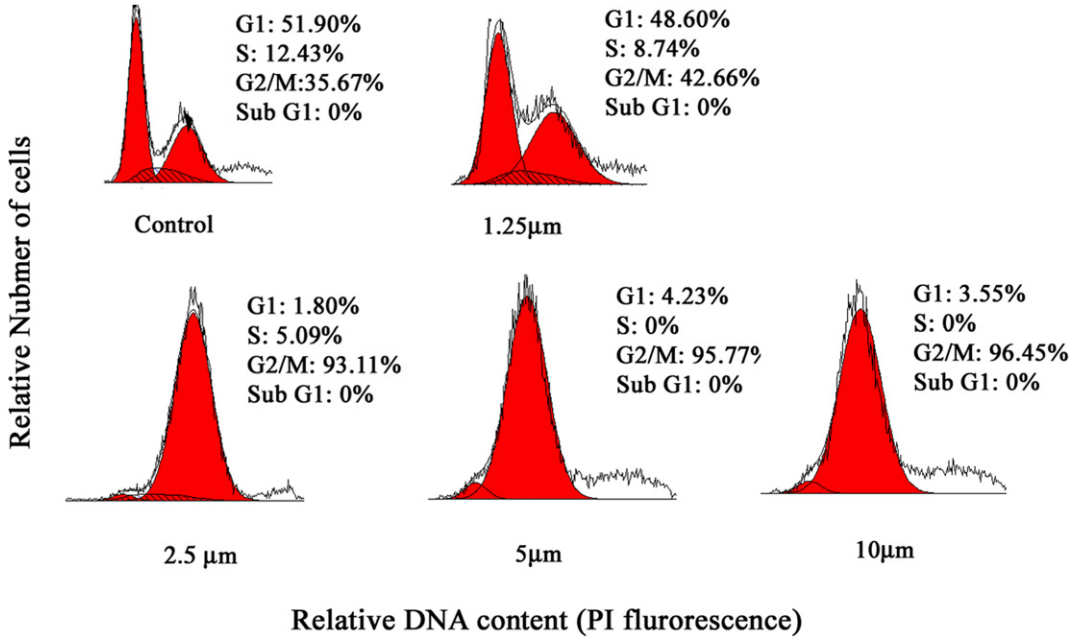


Fig. 3. Effects of compound **5a** on cell cycle of HCT-116 cells. Cells were incubated with 0, 1.25 μM, 2.5 μM, 5 μM, 10 μM **5a** for 24 h and stained with propidium iodide (PI). Their DNA content was analyzed by fluorescence flow cytometry.

3. Conclusion

The present work lead to the development of aroylthiourea derivatives of 4-β-amino-4′-*O*-demethyl-4-desoxypodophyllotoxin as novel DNA topoisomerase II inhibitors. All of these results confirm that the replacement of the glycoside moiety of etoposide with 4-β-*N*-aroylthiourea residue is favorable to topoisomerase II inhibitory activity. Two of the novel agents **5a** and **5l** have shown higher growth inhibitory effect on HepG2, A549 and HCT-116 cancer cell lines than all the other tested compounds. Their mode of action could be interpreted as inhibitory effect on the catalytic activity of DNA topoisomerase II, which caused HCT-116 cell cycle arrest at G2/M phase.

4. Experimental

4.1. Syntheses

Solvents were purified in the usual way. TLC was performed on precoated Merck silica Gel 60 F_{254} plates. Flash chromatography was performed on silica gel (100–200 mesh, Qingdao, China). ^{1}H NMR and ^{13}C NMR spectra were taken on a Bruker 300 MHz spectrometer with tetramethylsilane (TMS) as an internal standard, and chemical shifts were recorded in ppm values. The high resolution spectra were obtained on a Q-TOF Global Mass (ESIMS).

General procedure for the synthesis of compounds **5a**–**5l**: 1 mmol of 4-β-amino-4′-*O*-demethyl-4-desoxypodophyllotoxin (**4**) was added to a solution of 1.2 mmol of corresponding aroylisothiocyanate [19] in 10 mL of dry acetonitrile. The mixture was refluxed on a water bath for approximately 2 h (monitored by TLC). After the reaction completed, the result solution was evaporated under reduced pressure. The residue was purified by flash chromatography on silica gel (1:3, EtOAc-petroleum ether) to afford a solid.

4.1.1. 4β-(3-Benzoylthioureido)-4′-O-demethyl-4-desoxypodophyllotoxin (**5a**)

White solid, yield 54%; $[\alpha]_D^{25}$ -79 (c 0.3, CH_2Cl_2); ^{1}H NMR ($CDCl_3$): δ 2.14 (s, 1 H, 1- NH), 2.92 (dd, J = 4.8, 14.4 Hz, 1 H, 2-H), 3.07 (m, 1 H, 3-H), 3.77 (s, 6 H, 3′, 5′-OCH_3), 3.99 (d, J = 5.4, 1 H, 4-H), 4.32 (m, 2 H, 11-H), 4.62 (d, J = 4.8 Hz, 1 H, 1-H), 5.86 (brs, 1 H, 2-NH), 5.94 (s, 1 H, OCH_2O), 5.96 (s, 1 H, OCH_2O), 6.30 (s, 2 H, ArH), 6.52 (s, 1 H, ArH), 6.83 (s, 1 H, ArH), 7.48 (d, J = 7.5 Hz, 1H, ArH), 7.51 (d, J = 5.7 Hz, 1H, ArH), 7.62 (t, J = 5.7 Hz, 1 H, ArH), 7.80 (s, 1 H, ArH), 7.83 (s, 1 H, ArH); ^{13}C NMR ($CDCl_3$): δ 37.5, 42.3, 43.6, 54.1, 56.5, 68.7, 76.6, 77.0, 77.4, 101.6, 108.6, 109.1, 110.2, 127.5, 127.8, 129.2, 130.1, 132.5, 133.9, 146.5, 147.7, 148.8, 167.1, 173.9, 180.6; HRMS calcd for $C_{29}H_{26}N_2O_8NaS$ 585.1308, found 585.1299; mp 156–158 °C from ethanol.

4.1.2. 4β-[3-(2-Chlorobenzoyl)thioureido]-4′-O-demethyl-4-desoxypodophyllotoxin (**5b**)

Yellow solid, yield 55%; $[\alpha]_D^{25}$ -84 (c 0.3, CH_2Cl_2); ^{1}H NMR ($CDCl_3$): δ 2.13 (s, 1 H, 1-NH), 2.93 (dd, J = 4.8, 14.4 Hz, 1 H, 2-H), 3.07 (m, 1 H, 3-H), 3.76 (s, 6 H, 3′, 5′-OCH_3), 3.80 (d, J = 5.1, 1H, 4-H), 4.32 (m, 2 H, 11-H), 4.62 (d, J = 4.8 Hz, 1H, 1-H), 5.86 (brs, 1 H, 2-NH), 5.94 (s, 1 H, OCH_2O), 5.96 (s, 1 H, OCH_2O), 6.28 (s, 2 H, ArH), 6.52 (s, 1 H, ArH), 6.84 (s, 1 H, ArH), 7.38 (m, 1 H, ArH), 7.42 (dd, J = 1.8, 7.2 Hz, 1 H, ArH), 7.56 (dd, J = 2.1, 7.2 Hz, 1 H, ArH), 7.63 (d, J = 6.9 Hz, 1 H, ArH); ^{13}C NMR ($CDCl_3$): δ 37.5, 42.3, 43.6, 54.1, 56.5, 68.7, 76.6, 77.0, 77.4, 101.6, 108.0, 109.1, 110.2, 127.6, 130.1, 130.6, 132.6, 133.4, 146.6, 147.7, 148.8, 166.2, 173.9, 180.1; HRMS calcd for $C_{29}H_{25}N_2O_8NaSCl$ 619.0918, found 619.0931; mp 161–163 °C from ethanol.

4.1.3. 4β-[3-(3-Chlorobenzoyl)thioureido]-4′-O-demethyl-4-desoxypodophyllotoxin (**5c**)

Yellow solid, yield 55%; $[\alpha]_D^{25}$ -75 (c 0.3, CH_2Cl_2); ^{1}H NMR ($CDCl_3$): δ 2.13 (s, 1 H, 1-NH), 2.93 (dd, J = 4.8, 14.4 Hz, 1 H, 2-H), 3.07 (m, 1 H, 3-H), 3.76 (s, 6 H, 3′, 5′-OCH_3), 3.80 (d, J = 4.5, 1 H, 4-H), 4.32 (m, 2 H, 11-H), 4.62 (d, J = 4.8 Hz, 1 H, 1-H), 5.86 (brs, 1 H, 2-NH), 5.94 (d, J = 1.2, 1 H, OCH_2O), 5.96(d, J = 1.2, 1 H, OCH_2O), 6.29 (s, 2 H, ArH), 6.50 (s, 1 H, ArH), 6.81 (s, 1 H, ArH), 7.43 (d, J = 1.8 Hz, ArH), 7.56 (d, J = 1.8 Hz, ArH), 7.69 (d, J = 2.1 Hz, 1 H, ArH), 7.82 (s, 1 H, ArH); ^{13}C NMR ($CDCl_3$): δ 37.5, 42.3, 43.6, 54.1, 56.5, 68.7, 76.6, 77.0, 77.5, 101.7, 107.9, 109.1, 110.2, 125.6, 127.6, 128.1, 130.1, 130.4, 132.5, 133.1, 133.8, 134.2, 135.4, 146.5, 147.7, 148.8, 166.1, 173.9, 180.5; HRMS calcd for $C_{29}H_{25}N_2O_8NaSCl$ 619.0918, found 619.0916; mp 161–163 °C from ethanol.

4.1.4. 4β-[3-(4-Chlorobenzoyl)thioureido]-4′-O-demethyl-4-desoxypodophyllotoxin (**5d**)

Yellow solid, yield 54%; $[\alpha]_D^{25}$ -76 (c 0.3, CH_2Cl_2); ^{1}H NMR ($CDCl_3$): δ 2.13 (s, 1 H, 1-NH), 2.81 (dd, J = 5.1 Hz, 14.4 Hz, 1 H, 2-H), 2.97 (m, 1 H, 3-H), 3.65 (s, 6 H, 3′, 5′-OCH_3), 3.71 (d, J = 4.5, 1 H, 4-H), 3.88 (m, 2 H, 11-H), 4.37 (d, J = 4.8 Hz, 1H, 1-H), 5.86 (brs, 1 H, 2-NH), 5.94 (s, 1 H, OCH_2O), 5.96(s, 1 H, OCH_2O), 6.18 (s, 2 H, ArH), 6.40 (s, 1 H, ArH), 6.71(s, 1 H, ArH), 7.32(s, 1 H, ArH), 7.35 (s, 1 H, ArH), 7.75 (s, 1 H, ArH), 7.78 (s, 1 H, ArH); ^{13}C NMR ($CDCl_3$): δ 37.4, 42.2, 43.5, 53.7, 56.4, 68.7, 76.8, 77.2, 77.6, 101.5, 108.0, 109.0, 110.0, 127.8, 129.0, 129.6, 129.9, 130.0, 132.4, 134.4, 139.9, 146.7, 147.6, 148.6, 167.1, 173.9, 181.1; HRMS calcd for $C_{29}H_{25}N_2O_8NaSCl$ 619.0918, found 619.0923; mp 157–158 °C from ethanol.

4.1.5. 4β-[3-(2-Bromobenzoyl)thioureido]-4′-O-demethyl-4-desoxypodophyllotoxin (**5e**)

Yellow solid, yield 57%; $[\alpha]_D^{25}$ -76 (c 0.3, CH_2Cl_2); ^{1}H NMR ($CDCl_3$): δ 2.13 (s, 1 H, 1-NH), 2.93 (dd, J = 4.8, 14.4 Hz, 1 H, 2-H), 3.07 (m, 1 H, 3-H), 3.76 (s, 6 H, 3′, 5′-OCH_3), 3.80 (d, J = 4.8, 1 H, 4-H), 4.32 (m, 2 H, 11-H), 4.62 (d, J = 4.8 Hz, 1 H, 1-H), 5.86 (brs, 1 H, 2-NH), 5.94 (s, 1 H, OCH_2O), 5.96 (s, 1 H, OCH_2O), 6.29 (s, 2 H, ArH), 6.51 (s, 1 H, ArH), 6.84 (s, 1 H, ArH), 7.38 (m, 1 H, ArH), 7.42 (m, 1 H, ArH), 7.61 (dd, J = 2.1, 7.2 Hz, 1 H, ArH), 7.64 (dd, J = 1.8, 7.2 Hz, 1H, ArH); ^{13}C NMR ($CDCl_3$): δ 37.5, 42.3, 43.6, 54.1, 56.5, 68.7, 76.6, 77.0, 77.4, 101.6, 108.0, 109.1, 110.2, 119.5, 127.6, 127.9, 130.1, 130.1, 132.6, 133.2, 134.1, 134.2, 146.6, 147.7, 148.8, 167.2, 173.9, 180.0; HRMS calcd for $C_{29}H_{25}N_2O_8NaSBr$ 663.0413, found 663.0406; mp 162–163 °C from ethanol.

4.1.6. 4β-[3-(2-Iodobenzoyl)thioureido]-4′-O-demethyl-4-desoxypodophyllotoxin (**5f**)

Yellow solid, yield 55%; $[\alpha]_D^{25}$ -81 (c 0.3, CH_2Cl_2); ^{1}H NMR ($CDCl_3$): δ 2.13 (s, 1 H, 1-NH), 2.93 (dd, J = 4.8, 14.4 Hz, 1 H, 2-H), 3.07 (m, 1 H, 3-H), 3.76 (s, 6 H, 3′, 5′-OCH_3), 3.80 (d, J = 4.8, 1H, 4-H), 4.32 (m, 2 H, 11-H), 4.62 (d, J = 4.8 Hz, 1 H, 1-H), 5.86 (brs, 1 H, 2-NH), 5.94 (s, 1 H, OCH_2O), 5.97(s, 1 H, OCH_2O), 6.29 (s, 2 H, ArH), 6.51 (s, 1 H, ArH), 6.84 (s, 1 H, ArH), 7.20 (m, 1 H, ArH), 7.22 (m, 1 H, ArH), 7.56 (d, J = 2.1 Hz, 1 H, ArH), 7.63 (d, J = 1.8 Hz, 1 H, ArH); ^{13}C NMR ($CDCl_3$): δ 37.5, 42.3, 43.6, 54.1, 56.5, 68.7, 76.6, 77.0, 77.4, 92.1, 101.6, 108.0, 109.1, 110.2, 127.6, 128.5, 128.8, 130.1, 132.6, 132.9, 138.5, 140.2, 146.6, 147.7, 148.8, 168.9, 173.9, 180.2; HRMS calcd for $C_{29}H_{25}N_2O_8NaSI$ 711.0274, found 711.0267; mp 165–167 °C from ethanol.

4.1.7. 4β-[3-(4-Fluorbenzoyl)thioureido]-4′-O-demethyl-4-desoxypodophyllotoxin (**5g**)

Yellow solid, yield 53%; $[\alpha]_D^{25}$ -82 (c 0.3, CH_2Cl_2); ^{1}H NMR ($CDCl_3$): δ 2.12 (s, 1 H, 1-NH), 2.90 (dd, J = 4.8, 14.4 Hz, 1 H, 2-H), 3.07 (m, 1 H, 3-H), 3.76 (s, 6 H, 3′, 5′-OCH_3), 3.80 (d, J = 4.8, 1 H, 4-H), 4.32 (m, 2 H, 11-H), 4.62 (d, J = 4.8 Hz, 1 H, 1-H), 5.86 (brs, 1 H, 2-NH), 5.94 (s, 1 H, OCH_2O), 5.96(s, 1 H, OCH_2O), 6.28 (s, 2 H, ArH), 6.51 (s, 1 H, ArH), 6.82 (s, 1 H, ArH), 7.18 (d, J = 2.1 Hz, 1 H, ArH), 7.21(d, J = 2.1 Hz, 1 H, ArH), 7.82 (d, J = 1.8 Hz, 1 H, ArH), 7.84 (d, J = 1.8 Hz, 1 H, ArH); ^{13}C NMR ($CDCl_3$): δ 37.5, 42.3, 43.6, 54.1, 56.5, 68.7, 76.6, 77.0, 77.4, 101.6, 107.9, 109.1, 110.2, 116.3, 116.6, 127.7, 130.1, 130.3, 130.4, 132.5, 134.2, 146.5,

147.7, 148.8, 166.1, 173.9, 180.5; HRMS calcd for $C_{29}H_{25}N_2O_8NaSF$ 603.1213, found 603.1219; mp 157–159 °C from ethanol.

4.1.8. 4β-(3-α-Naphthoylthioureido)-4′-O-demethyl-4-desoxypodophyllotoxin (**5h**)

Yellow solid, yield 65%; $[\alpha]_D^{25}$ -80 (c 0.3, CH_2Cl_2); ^{1}H NMR ($CDCl_3$): δ 2.16 (s, 1 H, 1- NH), 3.00 (dd, J = 4.8, 14.4 Hz, 1 H, 2-H), 3.11 (m, 1 H, 3-H), 3.79 (s, 6 H, 3′, 5′-OCH_3), 3.80 (d, J = 5.1, 1 H, 4-H), 4.32 (m, 2 H, 11-H), 4.67 (d, J = 4.8 Hz, 1 H, 1-H), 5.91 (brs, 1 H, 2-NH), 5.98 (s, 1 H, OCH_2O), 6.00(s, 1 H, OCH_2O), 6.33 (s, 2 H, 2′, 6′-ArH), 6.55 (s, 1 H, 8-ArH), 6.92 (s, 1 H, 5-ArH), 7.60 (m, 3 H, 3″, 6″, 7″-H), 7.80 (d, J = 6.9 Hz, 1 H, 2″-H), 7.92 (d, J = 7.5 Hz, 1 H, 5″-H), 8.05 (d, J = 8.4 Hz, 1 H, 4″-H); 8.30 (d, J = 8.4 Hz, 1 H, 8″-H); ^{13}C NMR ($CDCl_3$): δ 37.5, 42.3, 43.7, 54.2, 56.53 68.8, 76.6, 77.5, 101.7, 107.9, 109.2, 110.2, 124.5, 126.7, 127.1, 127.7, 128.8, 129.8, 130.2, 132.6, 133.4, 133.8, 134.2, 146.5, 147.8, 148.8, 169.3, 174.1, 180.7; HRMS calcd for $C_{33}H_{28}N_2O_8S$ 613.1660, found 613.1665; mp 148–150 °C from ethanol.

4.1.9. 4β-[3-(2-Methylbenzoyl)thioureido]-4′-O-demethyl-4-desoxypodophyllotoxin (**5i**)

Yellow solid, yield 62%; $[\alpha]_D^{25}$ -90 (c 0.3, CH_2Cl_2); ^{1}H NMR ($CDCl_3$): δ 2.15 (s, 1 H, 1-NH), 2.47 (s, 3 H, CH_3), 2.93 (dd, J = 4.8, 14.4 Hz, 1 H, 2-H), 3.08 (m, 1 H, 3-H), 3.78 (s, 6 H, 3′, 5′-OCH_3), 3.80 (d, J = 5.1, 1 H, 4-H), 4.29 (m, 2 H, 11-H), 4.63 (d, J = 4.8 Hz, 1 H, 1-H), 5.86 (brs, 1 H, 2-NH), 5.96 (s, 1 H, OCH_2O), 5.98 (s, 1 H, OCH_2O), 6.31 (s, 2 H, 2′, 6′-ArH), 6.53 (s, 1 H, 8-ArH), 6.86 (s, 1 H, 5-ArH), 7.43 (m, 4 H, 3″, 4″, 5″, 6″-ArH); ^{13}C NMR ($CDCl_3$): δ 20.2, 29.7, 37.5, 42.3, 43.7, 54.1, 56.5, 68.8, 76.6, 77.0, 77.5, 101.7, 108.0, 109.2, 110.3, 126.3, 127.2, 127.8, 130.2, 132.0, 132.3, 132.6, 134.3, 137.9, 146.6, 147.8, 148.8, 169.5, 174.0, 180.7; HRMS calcd for $C_{30}H_{29}N_2O_8S$ 577.1644, found 577.1645; mp 144–146 °C from ethanol.

4.1.10. 4β-[3-(3-Methylbenzoyl)thioureido]-4′-O-demethyl-4-desoxypodophyllotoxin (**5j**)

Yellow solid, yield 69%; $[\alpha]_D^{25}$ -76 (c 0.3, CH_2Cl_2); ^{1}H NMR ($CDCl_3$): δ 2.03 (s, 1 H, 1-NH), 2.42 (s, 3 H, CH_3), 2.92 (dd, J = 4.8, 14.4 Hz, 1 H, 2-H), 3.08 (m, 1 H, 3-H), 3.76(s, 6 H, 3′, 5′-OCH_3), 3.96 (d, J = 5.1, 1 H, 4-H), 4.50 (m, 2 H, 11-H), 4.62 (d, J = 4.8 Hz, 1 H, 1-H), 5.85 (brs, 1 H, 2-NH), 5.94 (s, 1 H, OCH_2O), 5.97 (s, 1 H, OCH_2O), 6.31 (s, 2 H, 2′, 6′-ArH), 6.52 (s, 1 H, 8-ArH), 6.83 (s, 1 H, 5-ArH), 7.55(m, 4 H, 2″, 4″, 5″, 6″-ArH), ^{13}C NMR ($CDCl_3$): δ 21.7, 31.7, 37.5, 42.3, 43.7, 54.1, 56.5, 61.9, 71.1, 76.6, 77.1, 77.4, 77.5, 101.7, 108.0, 109.2, 110.2, 127.6, 127.8, 128.3, 130.0, 130.2, 132.5, 134.2, 145.1, 146.5, 147.7, 148.8, 167.1, 174.0, 180.6; HRMS calcd for $C_{30}H_{29}N_2O_8S$, 577.1642, found 577.1645; mp 123–125 °C from ethanol.

4.1.11. 4β-[3-(4-Methylbenzoyl)thioureido]-4′-O-demethyl-4-desoxypodophyllotoxin(**5k**)

Yellow solid, yield 69%; $[\alpha]_D^{25}$ -89 (c 0.3, CH_2Cl_2); ^{1}H NMR ($CDCl_3$): δ 2.14 (s, 1 H, 1- NH), 2.41 (s, 3 H, CH_3), 2.90 (dd, J = 4.8, 14.4 Hz, 1 H, 2-H), 3.05 (m, 1 H, 3-H), 3.75 (s, 6 H, 3′, 5′-OCH_3), 3.77 (d, J = 5.1, 1 H, 4-H), 4.40 (m, 2 H, 11-H), 4.63 (d, J = 4.8 Hz, 1 H, 1-H), 5.87 (brs, 1 H, 2-NH), 5.94 (s, 1 H, OCH_2O), 5.97 (s, 1H, OCH_2O), 6.31 (s, 2 H, 2′, 6′-ArH), 6.52 (s, 1 H, 8-ArH), 6.84 (s, 1 H, 5-ArH), 7.50 (m, 4 H, 2″, 3″, 5″, 6″-ArH), ^{13}C NMR ($CDCl_3$): δ 21.4, 37.5, 42.3, 43.7, 54.1, 56.5, 68.7, 76.6, 77.1, 77.5, 101.7, 108.0, 109.2, 110.2, 124.6, 127.8, 128.1, 129.2, 130.2, 131.2, 132.5, 134.2, 134.8, 139.4, 146.5, 147.8, 148.8, 167.4, 174.0, 180.6; HRMS calcd for $C_{30}H_{29}N_2O_8S$, 577.1643 found 577.1645; mp 134–136 °C from ethanol.

4.1.12. 4β-(3-Thienylthioureido)-4′-O-demethyl-4-desoxypodophyllotoxin (**5l**)

Yellow solid, yield 70%; $[\alpha]_D^{25}$ -81 (c 0.3, CH_2Cl_2); ^{1}H NMR ($CDCl_3$): δ 2.02 (s, 1 H, 1-NH), 2.90 (dd, J = 4.8, 14.4 Hz, 1 H, 2-H), 3.08 (m, 1 H, 3-H), 3.74 (s, 6 H, 3′, 5′-OCH_3), 3.77 (d, J = 5.1, 1 H, 4-H), 4.40 (m, 2 H, 11-H), 4.61 (d, J = 4.8 Hz, 1 H, 1-H), 5.83 (brs, 1 H, 2-NH), 5.93 (s, 1 H, OCH_2O), 5.97 (s, 1 H, OCH_2O), 6.30 (s, 2 H, 2′, 6′-ArH), 6.51 (s, 1 H, 8-ArH), 6.81(s, 1 H, 5-ArH), 7.50 (m, 3 H, 2″, 3″, 5″-ArH), ^{13}C NMR ($CDCl_3$): δ 37.5, 42.3, 43.7, 54.2, 56.5, 68.7, 76.6, 77.0, 77.5, 101.7, 108.0, 109.1, 110.2, 127.7, 128.6, 130.2, 131.0, 132.5, 134.3, 134.7, 135.7, 146.6, 147.8, 148.8, 167.4, 173.9, 180.2; HRMS calcd for $C_{27}H_{25}N_2O_8S_2$, 569.1059 found 569.1073; mp 134–136 °C from ethanol.

4.2. Cell culture

Three human cell lines, HepG2 (Hepatocellular carcinoma), A549 (lung carcinoma), HCT-116 (colon carcinoma) were cultured on RPMI-1640 medium supplemented with fetal bovine serum (10%), penicillin (100 U/mL) and streptomycin (100 μg/mL) in 25 cm^2 culture flasks at 37 °C in a humidified atmosphere with 5% CO_2.

4.3. Cell viability

Cell viability was assessed by the MTT assay. Cells were harvested from the culture during the exponential growth phase, and seeded into multiwell culture plates at 5×10^4–1×10^5 cells/mL in fresh medium. After overnight growth, cells were treated with compounds (predissolved in DMSO) at selected concentrations for a period of 3 days. The medium was then discarded and replaced with MTT dye. Plates were incubated at 37 °C for 4 h. The resulting formazan crystals were solubilized in lysis buffer (sodium dodecyl sulfate (SDS) 10 g, *N*, *N*-dimethylformamide (DMF) 25 mL, H_2O 25 mL, Acetic acid 1 mL, pH 4.7), and the optical density was read at 570 nm with a microplate reader (Biotek synergy 2).

4.4. Topoisomerase II-mediated kDNA decatenation assay

A gel assay was carried out as previously described [20] to determine if compound **5a** inhibited the catalytic decatenation activity of topoisomerase II. kDNA consists of highly catenated networks of circular DNA, which can be decatenated by topoisomerase II in an ATP-dependent reaction.

4.5. Cell cycle analysis

We used the human colon carcinoma HCT-116 cell line for cell cycle analysis. Briefly, cells were grown in RPMI-1640 supplemented with 10% (v/v) heat-inactivated fetal bovine serum, 2 mM L-glutamine, 100 units/mL penicillin, and 100 μg/mL streptomycin at 37 °C in a humidified atmosphere with 5% CO_2. Untreated and drug treated cells were centrifuged and fixed overnight in 70% ethanol at 4 °C. Washed three times with PBS, incubated for 1 h with 1 mg/mL RNase A and 20 μg/mL propidium iodide at room temperature, and analyzed with a FACSCalibur flow cytometer (BD).

4.6. Statistical analysis

Data were presented as means ± SE and analyzed by SPSS software. Pictures were processed with Photoshop software. Mean values were obtained from at least three independent experiments.

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