European Journal of Medicinal Chemistry 46 (2011) 285–296

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Synthesis and biological evaluation of new 4β-anilino-4′-*O*-demethyl-4-desoxypodophyllotoxin derivatives as potential antitumor agents

Li Wang [a], Fenyan Yang [a], Xiaochun Yang [b], Xianghong Guan [a], Chunqi Hu [a], Tao Liu [a], Qiaojun He [b], Bo Yang [b], Yongzhou Hu [a,*]

[a] *ZJU-ENS Joint Laboratory of Medicinal Chemistry, College of Pharmaceutical Sciences, Zhejiang University, Hangzhou 310058, China*
[b] *Institute of Pharmacology & Toxicology, College of Pharmaceutical Sciences, Zhejiang University, Hangzhou 310058, China*

ARTICLE INFO

Article history:
Received 28 July 2010
Received in revised form
5 November 2010
Accepted 11 November 2010
Available online 19 November 2010

Keywords:
4β-Anilino-4′-*O*-demethyl-4-desoxypodophyllotoxin derivative
Cytotoxicity
Drug resistance
Tumor growth inhibition

ABSTRACT

A series of new 4β-anilino-4′-*O*-demethyl-4-desoxypodophyllotoxin derivatives were prepared and evaluated for their cytotoxicities against four human cancer cell lines including KB, KB/VCR, A549 and 95D. Most compounds showed better growth-inhibition activities against tested cell lines than that of etoposide (**VP-16**). Preliminary structure–activity relationships (SARs) were concluded and it indicated that the side chains substituted at 4β position of podophyllotoxin significantly influenced the cytotoxic activity, especially for the drug resistance profile. *In vivo* studies of compound **26c** on highly metastatic human lung cancer xenograft in nude mice showed that it can significantly inhibit tumor growth with administrating by oral route.

© 2010 Elsevier Masson SAS. All rights reserved.

1. Introduction

Etoposide (**VP-16**, **1**) (Fig. 1) and teniposide (**VM-26**, **2**), first synthesized in the 1960s, are two semi-synthetic glycoside derivatives of podophyllotoxin (**PDT**, **3**) [1]. In 1983 and 1992, **1** and **2** were officially approved respectively for clinical use against various types of cancers including breast cancer, testicular cancer, small-cell lung cancer, lymphoma, Kaposi's sarcoma and childhood leukemia [2,3]. The mechanism of **VP-16** was elucidated eleven years later after its introduction in medical practice. Being a topoisomerase II inhibitor, **VP-16** stabilizes the double-stranded DNA cleavage normally catalyzed by topoisomerase II and inhibits faithful relegation of DNA breaks. The break of double-strand DNA subsequently triggers the desired antitumor effects of the drug [4]. Recent researches reveal that the antitumor activity of **VP-16** is attributed primarily to its inhibition of topo II α, whereas the carcinogenic effect has been attributed to the β isoform [5].

Although being used extensively in clinic, **VP-16** and **VM-26** exhibited several toxic side effects such as bone-marrow depression, increased risk of secondary acute myelogenous leukemia, acquired drug-resistance and poor water-solubility, which block their further application [6]. Thus, it is valuable for medicinal chemists to overcome drug-resistance and develop more active, less toxic podophyllotoxin derivatives. After the approval of **VP-16**, NK-611 (**4**) [7], GL-331 (**5**) [8], TOP-53 (**6**) [9], etopophos (**7**) [10] and tafluposide (**8**) [11] were emerged through C_4 modification as alternatives to overcome the drawbacks of **VP-16**. The excellent activity profiles of these agents including improved water solubility, topoisomerase II inhibitory activity, drug resistance and antitumor spectra suggested the important role of C_4 substitution pattern. Molecular area-oriented chemical modification of podophyllotoxin revealed structural features critical for the topoisomerase II inhibition: (1) β-D-glucopyranose is not essential, the 4β configuration is fundamental with various substitution accommodated at C_4; (2) the free 4′-hydroxy is crucial, but 4′-ester pro-drugs are allowed; (3) the *trans*-lactone D ring with 2α, 3β configuration is very important; (4) the methylenedioxy cycle (ring A) is optimal; and (5) the free rotation of ring E is required. This study also showed that the C_4 is an optimal position tolerable to significant structural modification [12]. The later discovered comparative molecular field analysis (CoMFA) model further demonstrated that bulky substituents at C_4 might be favorable for topo-II inhibition [13].

In previous studies, we reported that 4β-anilino substituted podophyllotoxin derivatives were potent cytotoxic agents against some

* Corresponding author. Tel./fax: +86 571 88208460.
E-mail address: huyz@zju.edu.cn (Y. Hu).

0223-5234/$ – see front matter © 2010 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.ejmech.2010.11.016

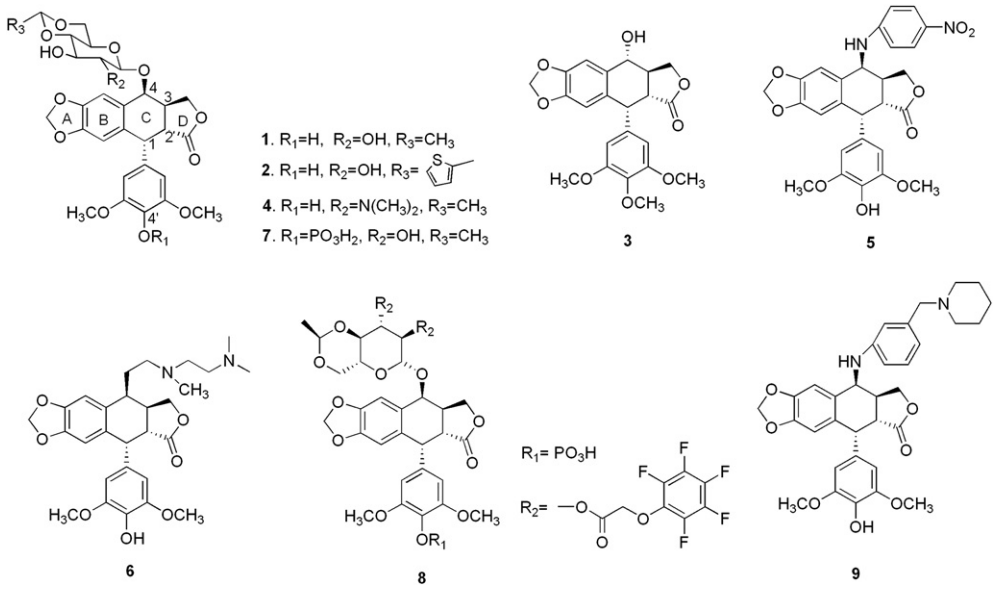


Fig. 1. Structures of etoposide (**VP-16**, **1**), teniposide (**VM-26**, **2**), podophyllotoxin (**PDT**, **3**), NK-611 (**4**), GL-331 (**5**), TOP-53 (**6**), etopophos (**7**), tafluposide (**8**) and **9**.

human cancer cell lines and drug-resistant cancer cell lines [14]. One representative compound, 4β-(*N*-[3-(piperidin-1-ylmethyl) phenyl]amino)-4′-*O*-demethyl-4-desoxypodophyllotoxin (**9**), strongly inhibited the growth of the tested drug-sensitive cancer cells with IC_{50} values ranging from 0.4 μM (Glima, U251 cell line) to 14.5 μM (lung cancer, A549 cell line), which also inhibited drug-resistant cancer cells with at least 3-fold more potent activity than that of **VP-16**. Besides, compound **9** was effective in the treatment of cancer at a lower dose than that of **VP-16** in drug-sensitive xenograft and drug-resistant xenograft models, demonstrating that it is a potential drug candidate for anticancer chemotherapy [14]. Based on the structural features of compound **9** and previously obtained biological results, we designed and synthesized three classes of podophyllotoxin derivatives. A 4β-anilino moiety was included to retain the drug-resistance profiles; a second basic group (compounds **22a**–**m**) or amide groups (compounds **26a**–**l**) were introduced to optimize activity profiles; and hydroxyl groups were introduced to modulate water solubility of target compounds (compounds **23a**–**d**). Preliminary structure–activity relationships (SARs) of synthesized podophyllotoxin derivatives were derived from the *in vitro* results. Compounds **23a**, **26c** and **26e** were chosen for *in vivo* studies.

2. Results and discussion

2.1. Chemistry

The synthetic routes of target podophyllotoxin derivatives **22a**–**m**, **23a**–**d** and **26a**–**l** are outlined in Schemes 1 and 2. Aryl aldehydes **10a**–**b** were efficiently converted into dibromoalkene derivatives **11a**–**b** by Wittig Reaction, which were then treated with secondary amines to afford intermediates **12a**–**j** [15]. The obtained aryl amides **12a**–**j** were further reduced to the corresponding amines **14a**–**m** first by borane and then by Pd/C under hydrogen atmosphere [16]. On the other hand, treatment of *p*-nitrophenol **15** with 2-chloroethanol in a potassium hydroxide solution at an elevated temperature gave 2-(4-nitrophenoxy)ethanol **17**. Reaction of 1-(bromomethyl)-4-nitrobenzene **20** with different glycols yielded the benzyloxy alcohols **20a**–**c**. Reduction of the nitro groups of **17** and **20a**–**c** using Pd/C under hydrogen atmosphere led to **18** and **21a**–**c**. Finally, the target molecules of class I **22a**–**m** and class II **23a**–**d** were prepared utilizing our recently developed synthetic protocol (Scheme 1) [17–19].

Compound **24** was prepared from **1** with the same procedure as described above. Deprotection of the amine group of **24** afforded the hydrochloride salt **25**, which was reacted with the corresponding aromatic carboxylic acids to obtain the target compounds of class III **26a**–**l** (Scheme 2).

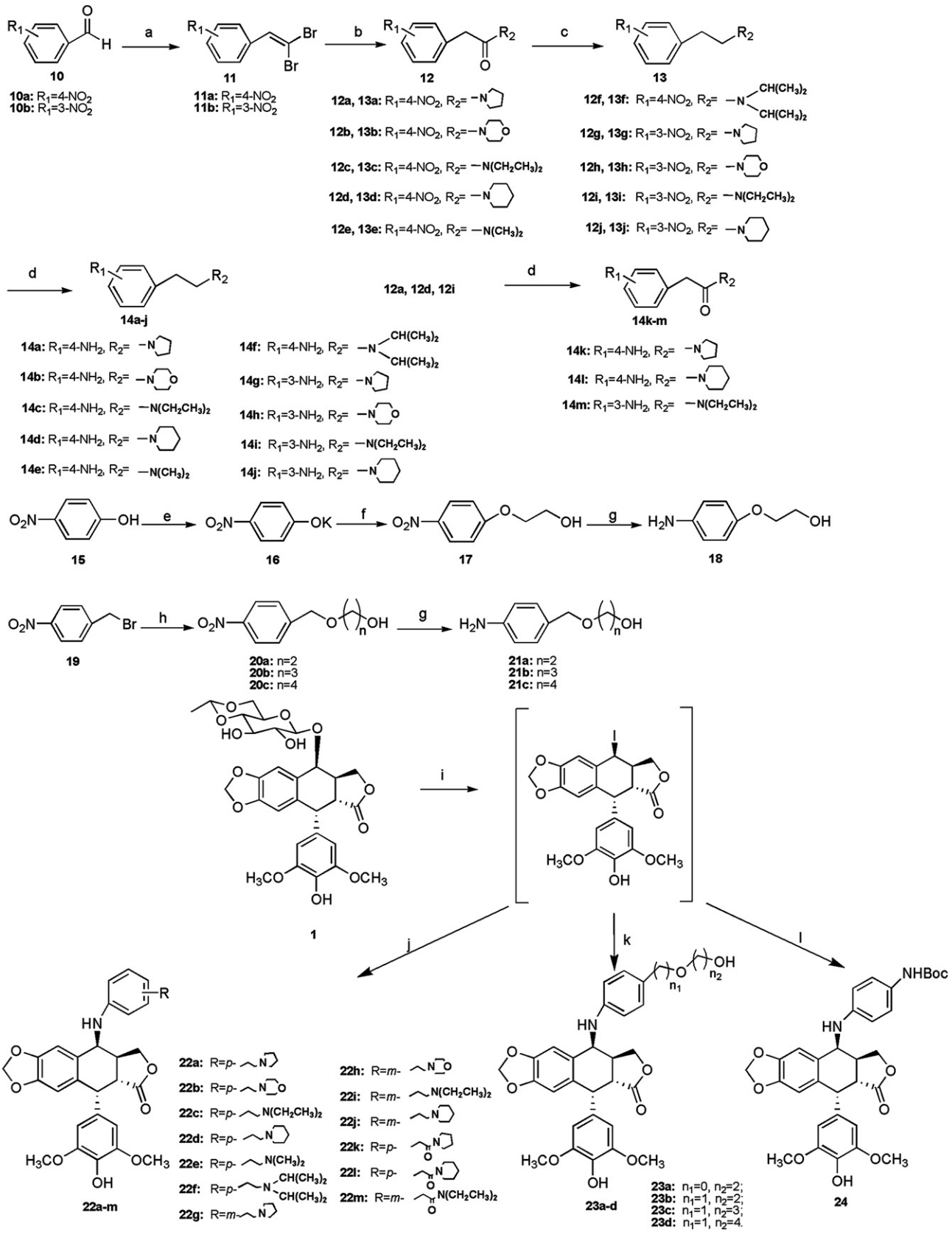
2.2. Pharmacology

2.2.1. In vitro cytotoxic activities

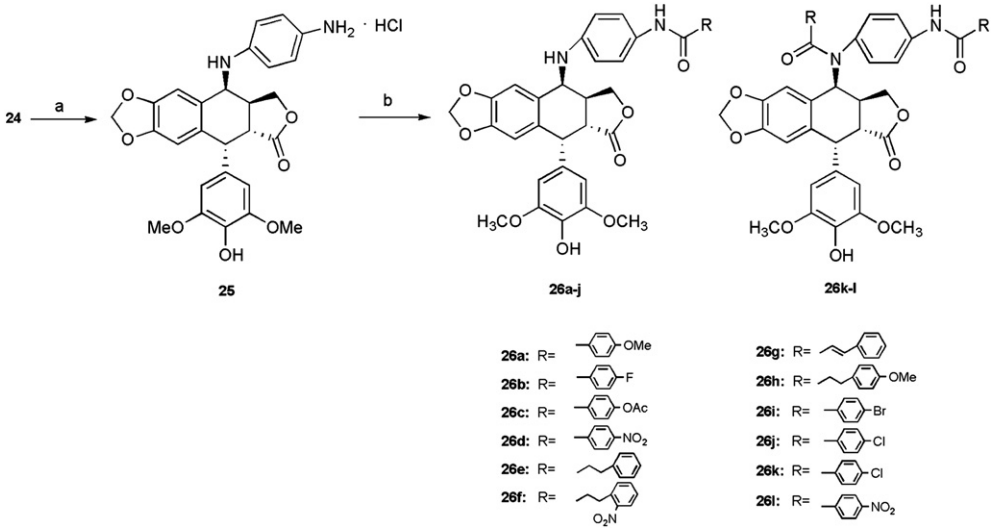
All the synthesized compounds were tested for their cytotoxic activities against four human cancer cell lines that comprised of KB, A549, 95D and drug-resistant cell line KB/VCR. **VP-16** and **9** were chosen as the positive controls. IC_{50} values were obtained using the standard MTT assay. The results are summarized in Table 1.

As shown in Table 1, most synthesized compounds exhibited potent cytotoxic activities than that of **VP-16**. The RFs of most compounds against KB cell lines were lower than that of **VP-16**. The results implied that the introduction of aniline group at C_4 position contributed to overcome drug-resistance in the tested compounds. This is in agreement with observation in other literature reports on podophyllotoxin derivatives which suggest that C_4-amino substitution is good for overcoming drug-resistance [20].

The biological results of compounds in class I indicated that the alkyl amino group on the end of 4β-side chain was important for *in vitro* cytotoxic activity. The IC_{50} values of compounds **9**, **22d** and **22j** demonstrated that the length of the alkyl linker between the benzene ring and tertiary amino influenced the cytotoxic activity obviously. In general, the compounds with a CH_2CH_2 linker (i.e. **22d** and **22j**) showed better activities than the molecule with a CH_2 linker (**9**). Compounds with pyrrolidine **22a**, morpholine **22b** or *N*,*N*-diethylamine group **22c** on the end of 4β-side chain displayed similar or more potent activities than that of compound **9**. Among them, the pyrrolidine analogue **22a** exhibited the most potent cytotoxic activity, with IC_{50} ranging from 0.036 μM to 1.57 μM against the tested cancer cell lines. Transfer of the alkyl amino group from 4-position of aniline ring on the side chain (**22a**, **22b** and **22c**) to 3-position (**22g**, **22h** and **22i**) made a slightly decreased activity. Besides, compounds **22k**–**m**, which possess amide groups on the end of 4β-side chain, maintained fairly the same cell growth inhibition potency against KB, KB/VCR and A549 cell lines compared to the activities of compounds **22a**, **22d** and **22i**, but greatly reduced the inhibition of 95D cell lines.



Scheme 1. Synthesis of **22a**–**m**, **23a**–**d** and **24**. Reagents and conditions: (a) CBr_4, PPh_3, CH_2Cl_2, 0 °C; (b) amine, H_2O, rt, 1 h (method 1); amine, THF, H_2O, KOH, rt, 12 h (method 2); (c) $BF_3 \cdot Et_2O$, $NaBH_4$, THF, reflux; (d) H_2, Pd/C, MeOH; (e) KOH, H_2O; (f) $ClCH_2CH_2OH$, H_2O; (g) H_2, Pd/C, EtOAc; (h) glycol, KOH, 80 °C, 3 h; (i) NaI, TMSCl, CH_3CN, 0 °C, 1 h; (j) $BaCO_3$, Et_3N, CH_3CN, **14a**–**m**, rt, 15–18 h; (k) $BaCO_3$, Et_3N, CH_3CN, **18** or **21a**–**c**, rt, 15–18 h; (l) $BaCO_3$, Et_3N, CH_3CN, *t*-butyl 4-aminophenylcarbamate, rt, 15–18 h.



Scheme 2. Synthesis of **26a**–**l**. Reagents and conditions: (a) concentrated HCl/EtOAc, rt, 5 h; (b) EDCI, HOBt, Et_3N, carboxylic acids, CH_2Cl_2, 0 °C-rt.

Compounds containing the hydrophilic tails in class II were designed to improve water-solubility. As illustrated in Table 1, the length of alkoxy side chain on the end of 4β-side chain was found to influence the cytotoxic activity. Compound **23a** with a short chain showed the most potent cytotoxic activity, as the number of atoms between the benzene ring and the terminal hydroxyl group increased (**23b**, **23c** and **23d**), the activities decreased except that of compound **23b** against 95D tumor cell lines.

Compounds **26a**–**l** in class III, which possess the amide moieties on *para*-position of anilino ring, displayed comparable or slightly weaker cell growth inhibition against KB and A549 cell lines in comparison with that of **VP-16**, but a cytotoxic potency superior to that of **VP-16** on KB/VCR and 95D cell lines, except the activities of compounds **26k** and **26l**. Among them, compound with fluorine atom **26b** or acetoxy group **26c** on the benzene ring of 4β-side chain showed the most potent activities. It is interesting that two di-substituted derivatives **26k** and **26l** showed decreased activities compared with those of mono-disubstituted compounds **26d** and **26j** as well as **VP-16**.

In an overall view, the activities of class II derivatives were superior to those of class III compounds, but inferior to those of class I compounds. This result revealed that the 4β-aniline substitution was beneficial for the cytotoxic activity, especially for overcoming acquired drug resistance. The activity correlated well with the alkalinity of the substituents at the tail of the 4β-aniline side chain. Compounds of class I with alkyl amino groups on the end of 4β-side chain possessed the most potent inhibition activity, compounds in class II with the hydroxyl groups ranked the second and compounds in class III bearing the substituted amide moieties showed the lowest activities among all these three classes of synthesized compounds.

2.2.2. *In vivo antitumor effects*

Based on the cytotoxic activities of the tested compounds *in vitro*, compounds **23a**, **26c** and **26e** were chosen to investigate their growth inhibitory activities against the xenograft model of a highly metastatic human lung cancer 95D in nude mice. Compound **26c** (50 mg/kg) was administered intragastrically two or three times a week for 20 days and compounds **23a** (10 mg/kg) and **26e** (10 mg/kg) were administered intramuscularly every other day for 20 days. The results of experimental therapeutic efficacies of **23a**, **26c** and **26e** are shown in Table 2 and Fig. 2.

Tumor weights of 95D in 50 mg/kg **26c** group reduced significantly ($P < 0.05$), and the inhibition rate was 57.3%. The inhibition rates of 10 mg/kg **23a** and 10 mg/kg **26e** groups were 37.5% and 9.2%, respectively.

2.2.3. *Physicochemical characteristics*

Compounds **23a**–**d** were designed to improve the water solubility, based on the structural features of compound **9**, by introducing the hydroxyl groups. In order to determine the water-lipid coefficient, calculated values of *c* Log *P* by ChemDraw 9.0 software were reported. In addition, based on the method initiated by Baker et al. [21], retention behavior of compounds **9** and **23a**–**d** was measured by RP-HPLC to indicate the hydrophobicity parameter of drugs. The retention index (RI) values of tested compounds were calculated by an equation based on the retention time of the alkan-2-ones used. The results are summarized in Table 3.

As shown in Table 3, both the calculated *c* Log *P* value and retention index value of compound **9** were higher than those of compounds **23a**–**d**, indicating that introducing the hydroxyl groups improved the water solubility.

3. Conclusions

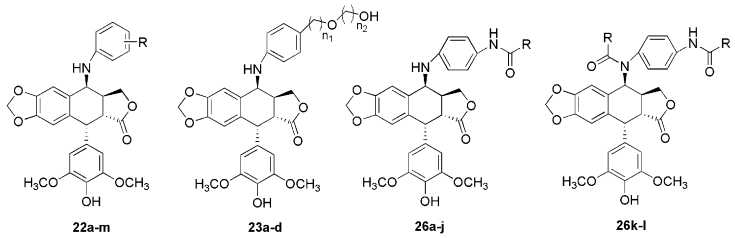
In summary, a series of new 4β-anilino-4′-*O*-demethyl-4-desoxypodophyllotoxin derivatives have synthesized and evaluated for their biological activities. Most of synthesized compounds exhibited potent cytotoxic activities against the tested cancer cell lines including MDR cancer cell lines *in vitro*. Preliminary structure–activity relationships were put forward based on the biological results. Compound **26c** was a promising agent which can be orally administered, and at 50 mg/kg it significantly inhibited high metastatic human lung cancer growth *in vivo*. Additional work is underway to perform the pre-clinical evaluation of compound **26c**.

4. Experimental protocols

4.1. *Chemistry*

Melting points were taken on a BÜCHI B-540 apparatus and are uncorrected. IR spectra were recorded on a Brüker VECTOR-22 spectrophotometer. Optical rotations were measured with a JASCO P-1010 polarimeter. NMR spectra were obtained using a Brüker Advance DMX 500-MHz spectrometer with TMS as the internal standard. All chemical shifts are reported in parts per million (ppm). Mass spectra were recorded on a Fourier Transform Ion

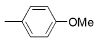
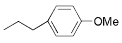
Table 1
In vitro cytotoxic activities of synthesized compounds and **VP-16**, **9** against four human cancer cell lines.



Compound	R(n_1, n_2)	IC_{50}[a] (μM)				RF[b]
		KB	KB/VCR	A549	95D	
VP-16	–	4.61	83.4	2.56	20.2	18.09
9	–	0.97	3.44	0.50	0.20	3.55
22a	p- 	0.21	1.57	0.24	0.036	7.48
22b	p- 	0.78	1.27	1.94	11.5	1.63
22c	p- 	0.45	1.53	0.40	9.84	3.4
22d	p- 	4.21	19.1	13.4	17.2	4.54
22e	p- 	2.42	3.95	19.0	17.1	1.63
22f	p- 	0.44	2.20	0.49	10.0	5
22g	m- 	0.39	1.85	3.91	1.17	4.74
22h	m- 	0.48	2.09	5.10	14.8	4.35
22i	m- 	0.77	3.87	7.49	14.6	5.03
22j	m- 	0.43	1.78	2.75	7.40	4.14
22k	p- 	1.52	2.20	15.9	>50	1.45
22l	p- 	0.40	2.33	5.20	25.8	5.83
22m	m- 	0.42	27.2	4.78	>50	64.76
23a	$n_1 = 0$, $n_2 = 2$	2.88	6.51	4.54	4.13	2.26
23b	$n_1 = 1$, $n_2 = 2$	3.12	7.39	5.11	0.55	2.37
23c	$n_1 = 1$, $n_2 = 3$	6.00	5.08	7.45	12.04	0.85
23d	$n_1 = 1$, $n_2 = 4$	6.60	9.55	8.5	20.30	1.45

(*continued on next page*)

Table 1 (*continued*)

Compound	R(n_1, n_2)	IC_{50}[a] (μM)				RF[b]
		KB	KB/VCR	A549	95D	
26a		6.76	9.28	3.41	0.44	1.37
26b		2.91	5.31	1.56	1.08	1.82
26c		1.91	8.48	2.68	0.42	4.44
26d		>50	13.7	11.0	3.41	<0.27
26e		6.88	3.70	3.31	1.15	0.54
26f		6.69	5.30	8.43	0.41	0.79
26g		6.53	2.80	>50	1.25	0.43
26h		>50	3.08	>50	1.23	<0.06
26i		8.75	5.72	18.9	1.66	0.65
26j		22.1	13.06	19.5	6.56	0.59
26k		>50	>50	>50	15.7	NT
26l		>50	14.21	>50	19.6	<0.28

[a] Cells were exposed with various concentrations of compounds and **VP-16** for 72 h.
[b] Resistance factor that was calculated as the ratio of the IC_{50} value of the MDR cells to that of the corresponding drug-sensitive parental cells.

Cyclotron Resonance Mass Spectrometer. RP-HPLC analyses were carried out on an Agilent 1200 system with DAD detector.

*4.1.1. Synthesis of key intermediates (**14a–m**, **18** and **21a–b**)*

Compounds **14a–m**, **18** and **21a–b** were synthesized according to the known methods [15–18].

*4.1.2. 4-[(4-nitrobenzyl)oxy]butan-1-ol (**20c**)*

Potassium hydroxide (145 mg, 2.6 mmol) was added to a solution of 4-nitrobenzyl bromide (512 mg, 2.4 mmol) in 1,4-butanediol (5 mL), and the resulting mixture heated at about 80 °C for 20 h until the raw product disappeared. After cooling to room temperature, the mixture was diluted with water and extracted with dichloromethane (10 mL × 3). Combined organic layers were dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by flash column chromatography on silica gel using PE/EtOAc (3:1, v/v) as eluent to afford **20c**. Yellow oil (535 mg, 83.1%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.19 (d, $J = 9.0$ Hz, 2H), 7.49 (d, $J = 9.0$ Hz, 2H), 4.61 (s, 2H), 3.66 (m, 2H), 3.56 (m, 2H), 1.73 (m, 2H), 1.68 (m, 2H).

Table 2
Inhibitory effects of **23a**, **26c** and **26e** on the tumor growth of 95D in nude mice.

Group	Average body weight (g)		Tumor volume (mm^3)		Tumor weight (g)	RTV	T/C (%)	Inhibition rate (%)
	D1	D20	D1	D20				
Control	17.5 ± 0.8	21.6 ± 0.6	153 ± 26	1370 ± 225	1.97 ± 0.31	9.3 ± 1.5	–	–
23a	17.6 ± 0.4	17.0 ± 0.4***	166 ± 18	663 ± 112*	1.23 ± 0.18	4.2 ± 1.0*	45.4	37.5
26c	18.2 ± 1.0	16.8 ± 0.5***	165 ± 37	527 ± 21*	0.84 ± 0.10*	4.3 ± 0.6*	46.3	57.3
26e	17.4 ± 0.5	20.2 ± 0.7	168 ± 27	910 ± 80	1.79 ± 0.26	5.8 ± 0.8	62.4	9.2

D1: first day treated with drug. *$P < 0.05$, ***$P < 0.001$, compared with control on the same day. Significant difference was calculated by Student's t test.

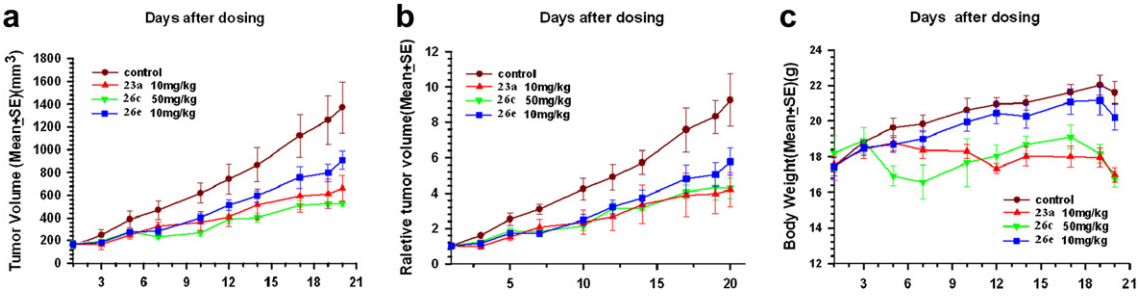


Fig. 2. (a) Effects of compounds **23a**, **26c** and **26e** on the tumor volume of transplanted tumor (95D) in nude mice ($\bar{x} \pm s$); (b) Effects of compounds **23a**, **26c** and **26e** on the relative tumor volume (RTV) of transplanted tumor (95D) in nude mice ($\bar{x} \pm s$); (c) Effects of compounds **23a**, **26c** and **26e** on the body weight of transplanted tumor (95D) in nude mice ($\bar{x} \pm s$).

*4.1.3. 4-[(4-aminobenzyl)oxy]butan-1-ol (**21c**)*

Compound **20c** (300 mg, 1.3 mmol) was dissolved in methanol (15 mL) in a pressure bottle, 5% Pd/C (30 mg) was added and the reaction mixture was stirred at room temperature under hydrogen for 12 h. When hydrogen uptake ceased, the catalyst was removed by filtration and the solvents evaporated. The resulting crude residue was purified by flash column chromatography on silica gel using PE/EtOAc (1:2, v/v) as eluent to afford **21c**. Yellow oil (162 mg, 62.3%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.11 (d, $J = 8.5$ Hz, 2H), 6.63 (d, $J = 8.5$ Hz, 2H), 4.37 (s, 2H), 3.57 (t, $J = 6.0$ Hz, 2H), 3.46 (t, $J = 6.0$ Hz, 2H), 1.67 (q, $J = 6.0$ Hz, 2H), 1.61 (q, $J = 6.0$ Hz, 2H).

*4.1.4. General procedure for the synthesis of **22**, **23** and **24***

A solution of TMSCl (0.76 mL) in dry acetonitrile (15 mL) was added dropwise to a mixture of **VP-16** (0.88 g, 1.5 mmol) and sodium iodide (0.9 g, 6.0 mmol) in dry acetonitrile at 0 °C and stirred for 1 h. Dried barium carbonate (0.9 g, 4.5 mmol) was added to the mixture. After stirring for 10 min, the pH was adjusted to 8 using anhydrous triethylamine. Then the appropriate amine (1.8 mmol) was added and the reaction mixture was stirred at room temperature for 15–18 h. The mixture was filtered; the filtrate was evaporated, washed with 20% sodium thiosulfate solution and extracted with ethyl acetate (30 mL × 3). Combined organic layers were dried over Na_2SO_4 and concentrated in vacuo. The crude product was purified by flash column chromatography on silica gel using PE/EtOAc/Et_3N (100:100–200:1, v/v/v) to give **22**, **23** and **24**.

*4.1.5. 4β-(N-[4-(2-pyrrolidin-1-ylethyl)phenyl]amino)-4′-O-demethyl-4-desoxypodophyllotoxin (**22a**)*

Yellow solid (58.3%), mp 135.2–136.7 °C; IR(KBr, cm^{-1}) 3487, 2942, 1771, 1614, 1518, 1482, 1458, 1227, 1116, 1036; $[\alpha]_D^{25}$–73.1 ($c = 0.1$, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.07 (d, $J = 8.0$ Hz, 2H, 3″,5″-H), 6.75 (s, 1H, 5-H), 6.52 (s, 1H, 8-H), 6.49 (d, $J = 8.0$ Hz, 2H, 2″,6″-H), 6.33 (s, 2H, 2′,6′-H), 5.95 and 5.97 (AB q, $J = 1.0$ Hz, 2H, $-OCH_2O-$), 4.65 (m, 1H, 1-H), 4.59 (d, $J = 4.5$ Hz, 1H, 4-H), 4.34 (t, $J = 8.0$ Hz, 1H, 11-H), 4.00 (t, $J = 8.0$ Hz, 1H, 11-H), 3.84 (br, 1H, NH), 3.79 (s, 6H, 3′,5′-OCH_3), 3.11 (m, 1H, 2-H), 3.06 (m, 4H, N–CH_2–CH_2-Ph), 2.99 (m, 1H, 3-H), 2.09 (m, 4H, pyrrolidine-H), 1.36 (t, $J = 7.5$ Hz, 4H, pyrrolidine-H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 174.82, 146.58, 146.48, 142.82, 134.15, 133.77, 131.93, 129.90, 129.68, 125.78, 112.69, 109.95, 109.10, 107.98, 101.56, 69.09, 57.24, 56.52, 53.86, 52.63, 45.84, 43.42, 41.91, 38.66, 31.29, 23.40; HR-MS (ESI+) calculated for $C_{33}H_{36}N_2O_7$ $[M + 1]^+$: 573.2556, found: $m/z = 573.2549$ $[M + 1]^+$.

*4.1.6. 4β-(N-[4-(2-morpholin-4-ylethyl)phenyl]amino)-4′-O-demethyl-4-desoxypodophyllotoxin (**22b**)*

Yellow solid (62.3%), mp 117.9–118.9 °C; IR(KBr, cm^{-1}) 3387, 2936, 1771, 1614, 1517, 1482, 1227, 1114, 1036; $[\alpha]_D^{25}$–50.4 ($c = 0.1$, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.04 (d, $J = 9.0$ Hz, 2H, 3″,5″-H), 6.76 (s, 1H, 5-H), 6.52 (s, 1H, H-8), 6.47 (d, $J = 9.0$ Hz, 2H, 2″,6″-H), 6.33 (s, 2H, 2′,6′-H), 5.94 and 5.96 (AB q, $J = 1.5$ Hz, 2H, $-OCH_2O-$), 4.64 (m, 1H, 1-H), 4.58 (d, $J = 5.0$ Hz, 1H, 4-H), 4.35 (t, $J = 8.0$ Hz, 1H, 11-H), 3.98 (t, $J = 8.0$ Hz, 1H, 11-H), 3.81 (br, 1H, 4-NH), 3.79 (s, 6H, 3′,5′-OCH_3), 3.74 (m, 4H, morphaline-H), 3.16 (m, 1H, 3-H), 3.13 (dd, $J = 5.0$, 14.0 Hz, 1H, 2-H), 3.07 (m, 1H, 3-H), 2.72 (m, 2H, N–CH_2–CH_2-Ph), 2.59 (m, 2H, N–CH_2–CH_2-Ph), 2.56 (m, 4H, morphaline-H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 175.04, 148.43, 147.77, 146.70, 146.00, 134.41, 132.10, 131.00, 130.82, 129.94, 129.03, 112.62, 110.11, 109.29, 108.27, 101.68, 69.16, 67.00, 61.24, 60.55, 56.72, 56.28, 53.82, 52.98, 43.64, 42.12, 38.92, 32.30; HR-MS (ESI+) calculated for $C_{33}H_{36}N_2O_8$ $[M + 1]^+$: 589.2505, found: $m/z = 589.2510$ $[M + 1]^+$.

*4.1.7. 4β-(N-[4-[2-(diethylamino)ethyl]phenyl]amino)-4′-O-demethyl-4-desoxypodophyllotoxin (**22c**)*

Yellow–white solid (70.8%), mp 113.1–113.9 °C; IR(KBr, cm^{-1}) 3387, 2969, 2935, 2840, 1771, 1614, 1518, 1482, 1464, 1227, 1115, 1036; $[\alpha]_D^{25}$–39.9 ($c = 0.1$, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.05 (d, $J = 8.0$ Hz, 2H, 3″,5″-H), 6.76 (s, 1H, 5-H), 6.52 (s, 1H, 8-H), 6.48 (d, $J = 8.0$ Hz, 2H, 2″,6″-H), 6.33 (s, 2H, 2′,6′-H), 5.94 and 5.96 (AB q, $J = 1.0$ Hz, 2H, $-OCH_2O-$), 4.64 (m, 1H, 1-H), 4.58 (d, $J = 5.0$ Hz, 1H, 4-H), 4.35 (t, $J = 8.0$ Hz, 1H, 11-H), 3.95 (t, $J = 8.0$ Hz, 1H, 11-H), 3.91 (br, 1H, NH), 3.79 (s, 6H, 3′,5′-OCH_3), 3.14 (dd, $J = 5.0$, 14.0 Hz, 1H, 2-H), 3.00 (m, 1H, 3-H), 2.95 (m, 6H, N–CH_2–CH_2-Ph, N–$(CH_2CH_3)_2$), 2.86 (m, 2H, N–CH_2–CH_2-Ph), 1.25 (t, $J = 7.5$ Hz, 6H, $-CH_2CH_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 175.00, 148.33, 147.66, 146.58, 146.31, 134.20, 131.98, 130.70, 130.04, 127.70, 112.65, 110.00, 109.23, 108.04, 101.63, 69.06, 56.71, 53.76, 52.75, 46.35, 43.51, 41.99, 38.79, 30.45, 9.77; HR-MS (ESI+) calculated for $C_{33}H_{38}N_2O_7$ $[M + 1]^+$: 575.2713, found: $m/z = 575.2720$ $[M + 1]^+$.

*4.1.8. 4β-(N-[4-(2-piperidin-1-ylethyl)phenyl]amino)-4′-O-demethyl-4-desoxypodophyllotoxin (**22d**)*

Yellow solid (68.2%), mp 132.3–133.3 °C; IR(KBr, cm^{-1}) 3387, 2936, 1775, 1613, 1517, 1481, 1230, 1114, 1036; $[\alpha]_D^{25}$–63.7 ($c = 0.1$, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.12 (d, $J = 9.0$ Hz, 2H, 3″,5″-H), 6.68 (d, $J = 9.0$ Hz, 2H, 2″,6″-H), 6.62 (s, 1H, 5-H), 6.57 (s, 1H, 8-H), 6.34 (s, 2H, 2′,6′-H), 5.97 (s, 2H, $-OCH_2O-$), 5.14 (m, 1H, 1-H), 4.66 (d, $J = 5.0$ Hz, 1H, 4-H), 4.28 (t, $J = 8.0$ Hz, 1H, 11-H), 4.12 (t,

Table 3
Calculated *c* Log *P* and retention index (RI) of compounds **9** and **23a–d**.

Compd.	Calculated *c* Log *P*	RI
9	3.505	590.4
23a	2.267	476.8
23b	2.116	472.4
23c	2.470	514.6
23d	2.415	572.2

J = 8.0 Hz, 1H, 11-H), 3.80 (br, 1H, NH), 3.79 (s, 6H, 3′,5′-OCH_3), 3.04 (dd, J = 5.0, 14.5 Hz, 1H, 2-H), 2.98 (m, 1H, 3-H), 2.89 (m, 4H, N−CH_2−CH_2-Ph), 2.79 (m, 4H, piperidine-H), 1.81 (m, 6H, piperidine-H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.93, 147.68, 147.35, 147.24, 146.02, 133.63, 132.08, 129.89, 129.33, 128.07, 113.96, 111.16, 109.43, 108.34, 107.38, 107.14, 101.00, 68.50, 59.71, 56.01, 55.77, 55.45, 53.28, 43.40, 43.05, 42.93, 38.32, 34.87, 23.88, 22.86; HR-MS (ESI+) calculated for $C_{34}H_{38}N_2O_7$ $[M+1]^+$: 587.2713, found: m/z = 587.2720 $[M+1]^+$.

4.1.9. 4β-(N-[4-[2-(dimethylamino)ethyl]phenyl]amino)-4′-O-demethyl-4-desoxypodophyllotoxin (**22e**)

Yellow solid (36.7%), mp 101.1−101.8 °C; IR(KBr, cm^{-1}) 3387, 2966, 2934, 1612, 1774, 1515, 1482, 1229, 1115, 1036; $[\alpha]_D^{25}$−27.3 (c = 0.1, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.11 (d, J = 8.5 Hz, 2H, 3″,5″-H), 6.66 (d, J = 8.5 Hz, 2H, 2″,6″-H), 6.64 (s, 1H, 5-H), 6.56 (s, 1H, 8-H), 6.33 (s, 2H, 2′,6′-H), 5.97 (s, 2H, −OCH_2O−), 5.13 (m, 1H, 1-H), 4.66 (d, J = 4.5 Hz, 1H, 4-H), 4.31 (t, J = 9.0 Hz, 1H, 11-H), 3.88 (t, J = 9.0 Hz, 1H, 11-H), 3.80 (s, 6H, 3′,5′-OCH_3), 3.40 (br, 1H, NH), 3.02 (dd, J = 5.0, 15.0 Hz, 1H, 2-H), 2.96 (m, 1H, 3-H), 2.93 (m, 2H, N−CH−$(CH_3)_2$), 2.92 (m, 2H, N−CH_2−CH_2-Ph), 2.87 (m, 2H, N−CH_2−CH_2-Ph), 1.28 (m, 12H, N−CH−$(CH_3)_2$); HR-MS (ESI+) calculated for $C_{31}H_{34}N_2O_7$ $[M+1]^+$: 547.2400, found: m/z = 547.2408 $[M+1]^+$.

4.1.10. 4β-(N-[4-[2-(diisopropylamino)ethyl]phenyl]amino)-4′-O-demethyl-4-desoxypodophyllotoxin (**22f**)

Light yellow solid (77.4%), mp 127.8−132.8 °C; IR(KBr, cm^{-1}) 3387, 2936, 2777, 1772, 1614, 1517, 1482, 1227, 1116, 1036; $[\alpha]_D^{25}$−57.9 (c = 0.1, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.05 (d, J = 8.0 Hz, 2H, 3″,5″-H), 6.76 (s, 1H, 5-H), 6.52 (s, 1H, 8-H), 6.49 (d, J = 8.0 Hz, 2H, 2″,6″-H), 6.33(s, 2H, 2′,6′-H), 5.95 and 5.96 (AB q, J = 1.5 Hz, 2H, −OCH_2O−), 4.64 (m, 1H, 1-H), 4.58 (d, J = 4.5 Hz, 1H, 4-H), 4.34 (t, J = 8.0 Hz, 1H, 11-H), 3.96 (t, J = 8.0 Hz, 1H, 11-H), 3.81 (br, 1H, NH), 3.79 (s, 6H, 3′,5′-OCH_3), 3.12 (dd, J = 5.0, 14.0 Hz, 1H, 2-H), 3.06 (m, 1H, 3-H), 2.84 (m, 2H, N−CH_2−CH_2-Ph), 2.79 (m, 2H, N−CH_2−CH_2-Ph), 2.52 (s, 6H, N−CH_3); HR-MS (ESI+) calculated for $C_{35}H_{42}N_2O_7$ $[M+1]^+$: 603.3026, found: m/z = 603.3032 $[M+1]^+$.

4.1.11. 4β-(N-[3-(2-pyrrolidin-1-ylethyl)phenyl]amino)-4′-O-demethyl-4-desoxypodophyllotoxin (**22g**)

Yellow solid (53.8%), mp 116.9−117.2 °C; IR(KBr, cm^{-1}) 3387, 2942, 2840, 1772, 1605, 1590, 1482, 1226, 1115, 1036; $[R]_D^{25}$−55.5 (c = 0.1, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.12 (t, J = 7.5 Hz, 8.5 Hz, 1H, 5″-H), 6.76 (s, 1H, 5-H), 6.59 (d, J = 7.0 Hz, 1H, 4″-H), 6.52 (m, 2H, 6″-H and 8-H), 6.41 (d, 7.5 Hz, 1H, 2″-H), 6.33(s, 2H, 2′,6′-H), 5.95 and 5.97 (AB q, J = 1.5 Hz, 2H, −OCH_2O−), 4.70 (m, 1H, 1-H), 4.59 (d, J = 5.0 Hz, 1H, 4-H), 4.39 (t, J = 7.5 Hz, 1H, 11-H), 3.93 (t, J = 7.5 Hz, 1H, 11-H), 3.82 (br, 1H, NH), 3.79 (s, 6H, 3′,5′-OCH_3), 3.12 (dd, J = 5.0, 14.0 Hz, 1H, 2-H), 3.11 (m, 2H, N−CH_2−CH_2-Ph), 3.03 (m, 1H, 3-H), 3.00 (m, 2H, N−CH_2−CH_2-Ph), 2.04 (m, 4H, pyrrolidine-H), 1.34 (t, J = 7.5 Hz, 4H, pyrrolidine-H); HR-MS (ESI+) calculated for $C_{33}H_{36}N_2O_7$ $[M+1]^+$: 573.2556, found: m/z = 573.2550 $[M+1]^+$.

4.1.12. 4β-(N-[3-(2-morpholin-4-ylethyl)phenyl]amino)-4′-O-demethyl-4-desoxypodophyllotoxin (**22h**)

Yellow solid (57.8%), mp 97.1−98.3 °C; IR(KBr, cm^{-1}) 3381, 2936, 1771, 1614, 1518, 1481, 1227, 1116, 1036; $[\alpha]_D^{25}$−85.5 (c = 0.1, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.12 (t, J = 7.5 Hz, 1H, 5″-H), 6.77 (s, 1H, 5-H), 6.62 (d, J = 8.5 Hz, 1H, 4″-H), 6.52 (s, 1H, 8-H), 6.43 (s, 1H, 2″-H), 6.38 (m, 1H, 6″-H), 6.34 (s, 2H, 2′,6′-H), 5.95 and 5.97 (AB q, J = 1.5 Hz, 2H, −OCH_2O−), 4.68 (m, 1H, 1-H), 4.58 (d, J = 5.0 Hz, 1H, 4-H), 4.35 (t, J = 8.0 Hz, 1H, 11-H), 3.96 (t, J = 8.0 Hz, 1H, 11-H), 3.87 (br, 1H, 4-NH), 3.81 (m, 4H, morphaline-H), 3.79(s, 6H, 3′,5′-OCH_3), 3.16 (m, 1H, 3-H), 3.12(dd, J = 5.0, 14.0 Hz, 1H, 2-H), 3.07 (m, 1H, 3-H), 2.80 (m, 2H, N−CH_2−CH_2-Ph), 2.68 (m, 2H, N−CH_2−CH_2-Ph), 2.63 (m, 4H, morphaline-H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 175.04, 148.42, 147.82, 147.76, 134.42, 132.13, 130.86, 129.89, 118.95, 113.12, 110.27, 110.10, 109.36, 108.29, 101.70, 69.15, 66.63, 60.66, 56.72, 53.62, 52.65, 46.02, 43.64, 42.12, 38.93, 33.13; HR-MS (ESI+) calculated for $C_{33}H_{36}N_2O_8$ $[M+1]^+$: 589.2505, found: m/z = 573.2515 $[M+1]^+$.

4.1.13. 4β-(N-[3-[2-(diethylamino)ethyl]phenyl]amino)-4′-O-demethyl-4-desoxypodophyllotoxin (**22i**)

Yellow solid (54.1%), mp 134.2−135.8 °C; IR(KBr, cm^{-1}) 3381, 2944, 1772, 1614, 1518, 1482, 1227, 1115, 1036; $[\alpha]_D^{25}$−105.7 (c = 0.1, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.13 (t, J = 8.5 Hz, 1H, 5″-H), 6.75 (s, 1H, 5-H), 6.58 (m, 2H, 4″-H and 6″-H), 6.52 (s, 1H, 8-H), 6.43 (s, 1H, 2″-H), 6.34 (s, 2H, 2′,6′-H), 5.95 and 5.97 (AB q, J = 1.5 Hz, 2H, −OCH_2O−), 4.72 (m, 1H, 1-H), 4.59 (d, J = 5.0 Hz, 1H, 4-H), 4.40 (t, J = 8.0 Hz, 1H, 11-H), 4.00 (br, 1H, NH), 3.93 (t, J = 8.0 Hz, 1H, 11-H), 3.80 (s, 6H, 3′,5′-OCH_3), 3.13 (dd, J = 6.5, 14.0 Hz, 1H, 2-H), 3.08 (m, 1H, 3-H), 3.00 (m, 8H, N−CH_2−CH_2-Ph, N−$(CH_2CH_3)_2$), 1.38 (t, J = 7.5 Hz, 6H, −CH_2CH_3); HR-MS (ESI+) calculated for $C_{33}H_{38}N_2O_7$ $[M+1]^+$: 575.2713, found: m/z = 575.2719 $[M+1]^+$.

4.1.14. 4β-(N-[3-(2-piperidin-1-ylethyl)phenyl]amino)-4′-O-demethyl-4-desoxypodophyllotoxin (**22j**)

Yellow solid (51.4%), mp 216.8−222 °C; IR(KBr, cm^{-1}) 3387, 2942, 2840, 1771, 1614, 1518, 1482, 1227, 1116, 1036; $[\alpha]_D^{25}$−68.9 (c = 0.1, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.11 (t, J = 7.5 Hz, 1H, 5″-H), 6.76 (s, 1H, 5-H), 6.58 (d, J = 7.0 Hz, 1H, 4″-H), 6.54 (m, 1H, 2″-H), 6.52 (s, 1H, 8-H), 6.41 (s, 1H, 6″-H), 6.34 (s, 2H, 2′,6′-H), 5.95 and 5.97 (AB q, J = 0.5 Hz, 2H, −OCH_2O−), 4.70 (m, 1H, 1-H), 4.58 (d, J = 5.0 Hz, 1H, 4-H), 4.40 (t, J = 8.0 Hz, 1H, 11-H), 3.97 (br, 1H, NH), 3.92 (t, J = 8.0 Hz, 1H, 11-H), 3.79 (s, 6H, 3′,5′-OCH_3), 3.13 (dd, J = 5.0, 14.0 Hz, 1H, 2-H), 3.05 (m, 1H, 3-H), 3.03 (m, 4H, piperidine-H), 2.97 (m, 4H, N−CH_2−CH_2-Ph), 1.34 (m, 6H, piperidine-H); HR-MS (ESI+) calculated for $C_{34}H_{38}N_2O_7$ $[M+1]^+$: 587.2713, found: m/z = 587.2705 $[M+1]^+$.

4.1.15. 4β-(N-[4-(2-oxo-2-pyrrolidin-1-ylethyl)phenyl]amino)-4′-O-demethyl-4-desoxypodophyllotoxin (**22k**)

Yellow solid (34.2%), mp 75.8−80.4 °C; IR(KBr, cm^{-1}) 3376, 2969, 2879, 1771, 1618, 1518, 1482, 1453, 1227, 1114, 1036; $[\alpha]_D^{25}$−46.0 (c = 0.1, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.12 (d, J = 10.0 Hz,, 2H, 3″,5″-H), 6.76 (s, 1H, 5-H), 6.52 (s, 1H, 8-H), 6.49 (d, J = 11.0 Hz, 2H, 2″,6″-H), 6.33 (s, 2H, 2′,6′-H), 5.95 and 5.96 (AB q, J = 1.5 Hz, 2H, −OCH_2O−), 4.65 (m, 1H, 1-H), 4.59 (d, J = 4.5 Hz, 1H, 4-H), 4.35 (t, J = 8.0 Hz, 1H, 11-H), 3.98 (t, J = 8.0 Hz, 1H, 11-H), 3.81 (br, 1H, NH), 3.79 (s, 6H, 3′,5′-OCH_3), 3.55 (s, 2H, NCO−CH_2-Ph), 3.44 (m, 4H, pyrrolidine-H), 3.13 (dd, J = 3.5, 14.0 Hz, 1H, 2-H), 2.97 (m, 1H, 3-H), 1.91 (m, 2H, pyrrolidine-H), 1.81 (m, 2H, piperidine-H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 174.47, 169.61, 147.05, 146.49, 146.03, 145.93, 133.54, 133.32, 131.34, 130.18, 129.65, 126.13, 124.07, 111.95, 108.65, 107.42, 104.77, 101.00, 70.49, 68.52, 56.01, 55.98, 46.37, 45.44, 45.08, 42.91, 42.03, 41.35, 40.64, 38.20, 25.65, 23.85; HR-MS (ESI+) calculated for $C_{33}H_{34}N_2O_8$ $[M+1]^+$: 587.2349, found: m/z = 587.2345 $[M+1]^+$.

4.1.16. 4β-(N-[4-(2-oxo-2-piperidin-1-ylethyl)phenyl]amino)-4′-O-demethyl-4-desoxypodophyllotoxin (**22l**)

Yellow solid (38.8%), mp 103.8−104.2 °C; IR(KBr, cm^{-1}) 3373, 2936, 1772, 1615, 1518, 1482, 1227, 1115, 1036; $[\alpha]_D^{25}$−72.1 (c = 0.1, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.08 (d, J = 8.5 Hz,, 2H, 3″,5″-H), 6.76 (s, 1H, 5-H), 6.50 (d, J = 8.0 Hz, 2H, 2″,6″-H), 6.52 (s, 1H, 8-H), 6.33 (s, 2H, 2′,6′-H), 5.94 and 5.96 (AB q, J = 1.0 Hz, 2H, −OCH_2O−), 4.66 (m, 1H, 1-H), 4.58 (d, J = 5.0 Hz, 1H, 4-H), 4.34 (t, J = 8.0 Hz, 1H, 11-H), 3.97 (t, J = 8.0 Hz, 1H, 11-H), 3.87 (br, 1H,

NH), 3.79 (s, 6H, 3′,5′-OCH_3), 3.62 (s, 2H, NCO–CH_2-Ph), 3.55 (m, 2H, piperidine-H), 3.40 (m, 2H, piperidine-H), 3.14 (dd, $J = 5.0$, 14.5 Hz, 1H, 2-H), 3.08 (m, 1H, 3-H), 1.58 (m, 2H, piperidine-H), 1.51 (m, 2H, piperidine-H), 1.38 (m, 2H, piperidine-H); HR-MS (ESI+) calculated for $C_{34}H_{36}N_2O_8$ $[M+1]^+$: 601.2505, found: $m/z = 601.2498$ $[M+1]^+$.

4.1.17. 4β-(N-[3-(2-oxo-2-(diethylamino)ethyl)phenyl]amino)-4′-O-demethyl-4-desoxypodophyllotoxin (**22m**)

Yellow solid (59.6%), mp 78.9–82.3 °C; IR(KBr, cm^{-1}) 3387, 2973, 2935, 1772, 1605, 1517, 1483, 1481, 1227, 1114, 1036; $[\alpha]_D^{25}$–60.3 ($c = 0.1$, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.12 (t, $J = 7.5$ Hz, 1H, 5″-H), 6.76 (s, 1H, 5-H), 6.62 (d, $J = 7.5$ Hz, 1H, 4″-H), 6.54 (m, 1H, 6″-H), 6.50 (s, 1H, 8-H), 6.42 (m, 1H, 2″-H), 6.33 (s, 1H, 2′-H), 6.29 (s, 1H, 6′-H)5.95 and 5.97 (AB q, $J = 2.0$ Hz, 2H, $-OCH_2O-$), 4.68 (m, 1H, 1-H), 4.58 (d, $J = 5.0$ Hz, 1H, 4-H), 4.33 (t, $J = 8.0$ Hz, 1H, 11-H), 3.92 (t, $J = 8.0$ Hz, 1H, 11-H), 3.85 (br, 1H, NH), 3.79 (s, 6H, 3′,5′-OCH_3), 3.62 (s, 2H, NCO–CH_2-Ph), 3.36 (m, 2H, N–$(CH_2CH_3)_2$), 3.28 (m, 2H, N–$(CH_2CH_3)_2$), 3.10 (dd, $J = 5.5$, 14.5 Hz, 1H, 2-H), 3.06 (m, 1H, 3-H), 1.36 (t, $J = 7.0$ Hz, 3H, $-CH_2CH_3$), 1.24 (t, $J = 7.0$ Hz, 3H, $-CH_2CH_3$); HR-MS (ESI+) calculated for $C_{33}H_{36}N_2O_8$ $[M+1]^+$: 589.2505, found: $m/z = 589.2512$ $[M+1]^+$.

4.1.18. 4β-(N-[4-(2-hydroxyethoxy)phenyl]amino)-4′-O-demethyl-4-desoxypodophyllotoxin (**23a**)

Yellow solid (84%), mp 126.1–127.3 °C; $[R]_D^{25}$–100.3 ($c = 0.1$, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.83 (d, $J = 7.0$ Hz, 2H, 3″,5″-H), 6.76 (s, 1H, 5-H), 6.52 (s, 1H, 8-H), 6.49 (d, $J = 7.0$ Hz, 2H, 2″,6″-H), 6.33 (s, 2H, 2′,6′-H), 5.95 and 5.96 (AB q, $J = 1.0$ Hz, 2H, $-OCH_2O-$), 4.58 (m, 2H, 4-H, 1-H), 4.34 (t, $J = 8.0$ Hz, 1H, 11-H), 4.02 (m, 3H, 11-H, $-OCH_2CH_2OH$), 3.92 (t, $J = 4.5$ Hz, 2H, $-OCH_2CH_2OH$), 3.79 (s, 6H, 3′,5′-OCH_3), 3.14 (dd, $J = 5.0$, 14.0 Hz, 1H, 2-H), 2.96 (m, 1H, 3-H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 175.01, 151.52, 148.16, 147.54, 146.48, 142.17, 134.12, 131.81, 130.96, 130.70, 116.17, 113.38, 109.92, 109.05, 108.24, 101.51, 70.13, 69.01, 61.59, 56.50, 53.37, 43.42, 41.91, 38.79; HR-MS (ESI+) calculated for $C_{29}H_{29}NO_9$ $[M+1]^+$: 536.1876, found: $m/z = 536.1875$ $[M+1]^+$.

4.1.19. 4β-(N-[4-[(2-hydroxyethoxy)methyl]phenyl]amino)-4′-O-demethyl-4-desoxypodophyllotoxin (**23b**)

Yellow solid (61%), mp 73.6–74.9 °C; $[\alpha]_D^{25}$–57.4 ($c = 0.1$, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.20 (d, $J = 8.5$ Hz, 2H, 3″,5″-H), 6.76 (s, 1H, 5-H), 6.52 (m, 3H, 2″,6″,8-H), 6.33 (s, 2H, 2′,6′-H), 5.96 (m, 2H, $-OCH_2O-$), 4.66 (m, 1H, 1-H), 4.60 (d, $J = 5.5$ Hz, 1H, 4-H), 4.47 (s, 2H, Ph-CH_2–O), 4.38 (m, 1H, 11-H), 3.97 (t, $J = 4.5$ Hz, 2H, $-OCH_2CH_2OH$), 3.80 (s, 6H, 3′,5′-OCH_3), 3.75 (t, $J = 6.0$ Hz, 2H, $-OCH_2CH_2CH_2OH$), 3.65 (t, $J = 6.0$ Hz, 2H, $-OCH_2CH_2CH_2OH$), 3.12 (dd, $J = 5.0$, 13.5 Hz, 1H, 2-H), 3.00 (m, 1H, 3-H), 1.85 (t, $J = 6.0$ Hz, 2H, $-OCH_2CH_2CH_2OH$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 175.33, 149.09, 148.55, 147.88, 147.47, 146.78, 134.40, 133.17, 132.21, 130.88, 130.17, 129.93, 128.59, 127.92, 115.31, 112.45, 110.21, 109.45, 108.37, 101.91, 74.87, 72.78, 69.37, 66.72, 62.08, 58.71, 56.75, 44.01, 42.22, 37.80; HR-MS (ESI+) calculated for $C_{30}H_{31}NO_9$ $[M+1]^+$: 550.2032, found: $m/z = 550.2025$ $[M+1]^+$.

4.1.20. 4β-(N-[4-[(3-hydroxypropoxy)methyl]phenyl]amino)-4′-O-demethyl-4-desoxypodophyllotoxin (**23c**)

Yellow solid (64%), mp 101.4–103.7 °C; $[\alpha]_D^{25}$–92.5 ($c = 0.1$, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.83 (d, $J = 7.0$ Hz, 2H, 3″,5″-H), 6.76 (s, 1H, 5-H), 6.52 (s, 1H, 8-H), 6.49 (d, $J = 7.0$ Hz, 2H, 2″,6″-H), 6.33 (s, 2H, 2′,6′-H), 5.95 and 5.96 (AB q, $J = 1.0$ Hz, 2H, $-OCH_2O-$), 4.58 (m, 2H, 4-H, 1-H), 4.34 (t, $J = 8.0$ Hz, 1H, 11-H), 4.02 (m, 3H, 11-H, $-OCH_2CH_2OH$), 3.92 (t, $J = 4.5$ Hz, 2H, $-OCH_2CH_2OH$), 3.79 (s, 6H, 3′,5′-OCH_3), 3.14 (dd, $J = 5.0$, 14.0 Hz, 1H, 2-H), 2.96 (m, 1H, 3-H); HR-MS (ESI+) calculated for $C_{31}H_{33}NO_9$ $[M+1]^+$: 564.2189, found: $m/z = 564.2195$ $[M+1]^+$.

4.1.21. 4β-(N-[4-[(4-hydroxybutoxy)methyl]phenyl]amino)-4′-O-demethyl-4-desoxypodophyllotoxin (**23d**)

Yellow solid (58.1%), mp 88.2–90 °C; $[\alpha]_D^{25}$–72.9 ($c = 0.1$, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.18 (d, $J = 8.5$ Hz, 2H, 3″,5″-H), 6.76 (s, 1H, 5-H), 6.52 (d, $J = 8.0$ Hz, 2H, 2″,6″-H), 6.53 (s, 1H, 8-H), 6.34 (s, 2H, 2′,6′-H), 5.95 and 5.97 (AB q, $J = 1.0$ Hz, 2H, $-OCH_2O-$), 4.67 (m, 1H, 1-H), 4.59 (d, $J = 5.0$ Hz, 1H, 4-H), 4.42 (s, 2H, Ph-CH_2–O), 4.35 (t, $J = 8.0$ Hz, 1H, 11-H), 3.77 (s, 6H, 3′,5′-OCH_3), 3.68 (m, 2H, $-OCH_2CH_2CH_2CH_2OH$), 3.62 (t, $J = 5.5$ Hz, 2H, $-OCH_2CH_2CH_2CH_2OH$), 3.50 (t, $J = 5.5$ Hz, 2H, $-OCH_2CH_2CH_2CH_2OH$), 3.12 (dd, $J = 5.0$, 13.5 Hz, 1H, 2-H), 2.98 (m, 1H, 3-H), 1.69 (m, 2H, $-OCH_2CH_2CH_2CH_2OH$); HR-MS (ESI+) calculated for $C_{32}H_{35}NO_9$ $[M+1]^+$: 578.2345, found: $m/z = 578.2351$ $[M+1]^+$.

4.1.22. 4β-(N-[4-(tert-butyl-(4-aminophenyl)carbamate)])-4′-O-demethyl-4-desoxypodophyllotoxin (**24**)

Yellow solid (85.7%), mp 136.1–138.6 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.20–7.21 (m, 2H), 6.74 (s, 1H), 6.51 (s, 1H), 6.48–6.50 (d, $J = 9.0$ Hz, 2H), 6.36 (ws, 1H), 6.33 (s, 2H), 5.95 and 5.97 (d, $J = 10.5$ Hz, 2H), 5.47 (ws, 1H), 4.61 (m, 1H), 4.57–4.58 (d, $J = 5.0$ Hz, 1H), 4.34–4.37 (t, $J = 8.0$ Hz, 1H), 3.96–4.00 (m, 1H), 3.79 (s, 6H), 3.13–3.16 (dd, $J = 5.0$, 14.5 Hz, 1H), 2.94–3.01 (m, 1H), 1.52 (s, 9H).

4.1.23. 4β-(N-[4-aminophenyl]amino)-4′-O-demethyl-4-desoxypodophyllotoxin hydrochloride (**25**)

Compound **24** (2.14 g, 3.63 mmol) was dissolved in saturated HCl/EtOAc (50 mL) and then stirred at room temperature for 5 h. The mixture was filtered, the filtration cake was dried overnight to obtain the desired product **25**, yellow solid (95.7%), mp 183.3–186.3 °C; ^{1}H NMR (500 MHz, DMSO) δ 9.93 (s, 1H), 7.11–7.13 (m, 2H), 6.76–6.77 (m, 3H), 6.54 (s, 1H), 6.25 (m, 2H), 6.33 (s, 2H), 5.96 and 5.99 (d, $J = 10.5$ Hz, 2H), 4.85 (m, 1H), 4.50–4.51 (d, $J = 5.0$ Hz, 1H), 4.33–4.35 (m, 2H), 3.64 (s, 6H), 3.27–3.28 (dd, $J = 5.0$, 14.5 Hz, 1H), 2.96–3.03 (m, 1H).

4.1.24. General procedure for the synthesis of **26**

To a solution of compound **25** (60 mg, 0.1 mmol), the corresponding acid (0.1 mmol) and HOBt (15 mg, 0.11 mmol) in dry dichloromethane, triethylamine and EDC·HCl (21 mg, 0.11 mmol) were added at 0 °C, this mixture was stirred at room temperature until the starting material disappeared. The reaction mixture was diluted with water and extracted with dichloromethane (10 mL × 3). Combined organic layers were dried over Na_2SO_4 and concentrated in vacuo. The crude product was purified by flash column chromatography on silica gel using PE/EtOAc (1:1, v/v) to give **26**.

4.1.25. 4β-[N-(4‴-methoxy-phenyl-1‴-carbonyl)-4″-aminoanilino]-4′-O-demethyl-4-desoxypodophyllotoxin (**26a**)

Yellow solid (54.5%), mp 143.8–145.7 °C; IR(KBr, cm^{-1}) 3384, 3379, 3060, 3036, 2960, 2932, 2875, 1771, 1728, 1647, 1607, 1515, 1482, 1464, 1253, 1228, 1115, 1036; $[\alpha]_D^{25}$–125.7 ($c = 0.1$, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.82 (d, $J = 8.5$ Hz, 2H, 2‴,6‴-H), 7.65 (s, 1H, –CONH–), 7.44 (d, $J = 8.0$ Hz, 2H, 3″,5″-H), 6.96 (d, $J = 8.5$ Hz, 3‴,5‴-H), 6.76 (s, 1H, 5-H), 6.55 (d, $J = 9.0$ Hz, 2H, 2″,6″-H), 6.52 (s, 1H, 8-H), 6.34 (s, 2H, 2′,6′-H), 5.95 and 5.97 (AB q, $J = 1.0$ Hz, 2H, $-OCH_2O-$), 4.69 (m, 1H, 1-H), 4.61 (d, $J = 5.0$ Hz, 1H, 4-H), 4.39 (t, $J = 8.0$ Hz, 1H, 11-H), 4.02 (t, $J = 8.0$ Hz, 1H, 11-H), 3.91 (s, 3H, $-OCH_3$), 3.83 (s, 6H, 3′,5′-OCH_3), 3.16 (dd, $J = 5.0$, 14.0 Hz, 1H, 2-H), 3.04 (m, 1H, 3-H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 175.04, 162.46, 148.33, 147.68, 146.56, 144.70, 134.18, 132.54, 131.99, 131.02, 130.75, 129.34, 128.95, 127.25, 122.81, 114.02, 112.64, 110.01, 109.27, 108.03, 101.65, 69.08, 68.28, 56.60, 55.58, 52.97, 43.53, 42.03, 38.83; HR-MS (ESI+) calculated for $C_{35}H_{32}N_2O_9$ $[M+1]^+$: 625.2141, found: $m/z = 625.2135$ $[M+1]^+$.

*4.1.26. 4β-[N-(4‴-fluoro-phenyl-1‴-carbonyl)-4″-aminoanilino]-4′-O-demethyl-4-desoxypodophyllotoxin (**26b**)*

Yellow solid (57%), mp 157.2–158.9 °C; IR(KBr, cm^{-1}) 3373, 2960, 2930, 1770, 1726, 1643, 1604, 1515, 1482, 1227, 1160, 1115, 1038; $[\alpha]_D^{25}$–77.5 ($c = 0.1$, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 7.86 (m, 2H, 2‴,6‴-H), 7.66 (s, 1H, –CONH–), 7.44 (d, $J = 8.5$ Hz, 2H, 3″,5″-H), 7.14 (t, $J = 8.5$ Hz, 3‴,5‴-H), 6.76 (s, 1H, 5-H), 6.55 (d, $J = 9.0$ Hz, 2H, 2″,6″-H), 6.52 (s, 1H, 8-H), 6.34 (s, 2H, 2′,6′-H), 5.95 and 5.97 (AB q, $J = 1.0$ Hz, 2H, $-OCH_2O-$), 4.66 (m, 1H, 1-H), 4.59 (d, $J = 5.5$ Hz, 1H, 4-H), 4.36 (t, $J = 8.0$ Hz, 1H, 11-H), 3.98 (t, $J = 8.0$ Hz, 1H, 11-H), 3.85 (br, 1H, –CONH), 3.79 (s, 6H, 3′,5′-OCH_3), 3.13 (dd, $J = 5.0$, 14.0 Hz, 1H, 2-H), 3.00 (m, 1H, 3-H); HR-MS (ESI+) calculated for $C_{34}H_{29}FN_2O_8$ $[M + 1]^+$: 613.1941, found: $m/z = 613.1935$ $[M + 1]^+$.

*4.1.27. 4β-[N-(4‴-acetyloxyl-phenyl-1‴-carbonyl)-4″-aminoanilino]-4′-O-demethyl-4-desoxypodophyllotoxin (**26c**)*

Yellow solid (28.1%), mp 182.2–184.5 °C; IR(KBr, cm^{-1}) 3420, 2909, 1759, 1651, 1605, 1516, 1482, 1371, 1311, 1227, 1199, 1162, 1037; $[\alpha]_D^{25}$–105.6 ($c = 0.1$, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 7.87 (d, $J = 8.5$ Hz, 2H, 2‴,6‴-H), 7.71 (s, 1H, –CONH–), 7.44 (d, $J = 8.5$ Hz, 2H, 3″,5″-H), 7.20 (d, $J = 8.5$ Hz, 3‴,5‴-H), 6.76 (s, 1H, 5-H), 6.55 (d, $J = 9.0$ Hz, 2H, 2″,6″-H), 6.53 (s, 1H, 8-H), 6.34 (s, 2H, 2′,6′-H), 5.95 and 5.97 (AB q, $J = 1.0$ Hz, 2H, $-OCH_2O-$), 4.65 (m, 1H, 1-H), 4.58 (d, $J = 4.5$ Hz, 1H, 4-H), 4.36 (t, $J = 8.0$ Hz, 1H, 11-H), 3.98 (t, $J = 8.0$ Hz, 1H, 11-H), 3.85 (br, 1H, –CONH), 3.79 (s, 6H, 3′,5′-OCH_3), 3.12 (dd, $J = 5.0$, 14.0 Hz, 1H, 2-H), 2.98 (m, 1H, 3-H). 2.33 (s, 3H, $-COCH_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 174.9, 169.1, 164.9, 153.2, 148.3, 147.6, 146.5, 144.8, 134.2, 132.6, 131.9, 130.7, 130.6, 128.9, 128.5, 122.8, 121.9, 112.5, 109.9, 109.2, 108.0, 101.5, 68.9, 56.4, 52.8, 41.9, 38.7, 21.1; HR-MS (ESI+) calculated for $C_{36}H_{32}N_2O_{10}$ $[M + 1]^+$: 653.2091, found: $m/z = 653.2085$ $[M + 1]^+$.

*4.1.28. 4β-[N-(4‴-nitro-phenyl-1‴-carbonyl)-4″-aminoanilino]-4′-O-demethyl-4-desoxypodophyllotoxin (**26d**)*

Orange solid (43.6%), mp 230.1–231.7 °C; IR(KBr, cm^{-1}) 3413, 2964, 2848, 1772, 1642, 1602, 1515, 1481, 1229, 1107, 1038; $[\alpha]_D^{25}$–97.1 ($c = 0.1$, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 8.34 (d, $J = 8.5$ Hz, 2H, 2‴,6‴-H), 8.03 (d, $J = 8.5$ Hz, 2H, 3‴,5‴-H), 7.70 (s, 1H, –CONH–), 7.47 (d, $J = 8.0$ Hz, 2H, 3″,5″-H), 6.77 (s, 1H, 5-H), 6.58 (d, $J = 9.0$ Hz, 2H, 2″,6″-H), 6.54 (s, 1H, 8-H), 6.34 (s, 2H, 2′,6′-H), 5.97 and 5.98 (AB q, $J = 1.0$ Hz, 2H, $-OCH_2O-$), 4.68 (m, 1H, 1-H), 4.61 (d, $J = 5.0$ Hz, 1H, 4-H), 4.37 (t, $J = 8.0$ Hz, 1H, 11-H), 3.99 (t, $J = 8.0$ Hz, 11-H), 3.87 (br, 1H, –CONH–), 3.79 (s, 6H, 3′,5′-OCH_3), 3.13 (dd, $J = 5.0$, 14.0 Hz, 1H, 2-H), 3.00 (m, 1H, 3-H); HR-MS (ESI+) calculated for $C_{34}H_{29}N_3O_{10}$ $[M + 1]^+$: 640.1886, found: $m/z = 640.1892$ $[M + 1]^+$.

*4.1.29. 4β-[N-(phenyl-1‴-ethylcarbonyl)-4″-aminoanilino]-4′-O-demethyl-4-desoxypodophyllotoxin (**26e**)*

Yellow solid (60.6%), mp 156.8–158.5 °C; IR(KBr, cm^{-1}) 3473, 2907, 1771, 1642, 1603, 1515, 1482, 1227, 1114, 1037; $[R]_D^{25}$–84.27 ($c = 0.1$, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 8.15 (d, $J = 9.0$ Hz, 2H, 3‴,6‴-H), 7.41 (d, $J = 9.0$ Hz, 2H, 4‴,5‴-H), 6.91 (s, 1H, –CONH–), 6.73 (s, 1H, 5-H), 6.53 (s, 1H, 8-H), 6.48 (d, $J = 8.5$ Hz, 2H, 2″,6″-H), 6.33 (s, 2H, 2′,6′-H), 5.95 and 5.97 (AB q, $J = 1.0$ Hz, 2H, $-OCH_2O-$), 4.62 (m, 1H, 1-H), 4.59 (d, $J = 5.0$ Hz, 1H, 4-H), 4.34 (t, $J = 8.0$ Hz, 1H, 11-H), 3.95 (t, $J = 8.0$ Hz, 11-H), 3.79 (s, 6H, 3′,5′-OCH_3), 3.15 (t, $J = 7.5$ Hz, 2H, $-NHCOCH_2CH_2$-Ph), 3.11 (dd, $J = 5.0$, 14.0 Hz, 1H, 2-H), 2.99 (m, 1H, 3-H), 2.66 (t, $J = 7.5$ Hz, 2H, $-NHCOCH_2CH_2$-Ph); ^{13}C NMR (125 MHz, $CDCl_3$) δ 174.96, 170.37, 148.22, 147.56, 146.48, 144.52, 140.80, 134.11, 131.86, 130.65, 128.94, 128.61, 128.43, 126.33, 122.49, 122.17, 115.38, 112.41, 109.90, 109.13, 107.98, 101.53, 68.96, 56.50, 52.82, 43.42, 41.89, 39.20, 38.69, 31.72; HR-MS (ESI+) calculated for $C_{36}H_{34}N_2O_8$ $[M + 1]^+$: 623.2349, found: $m/z = 623.2355$ $[M + 1]^+$.

*4.1.30. 4β-[N-(2‴-nitro-phenyl-1‴-ethylcarbonyl)-4″-aminoanilino]-4′-O-demethyl-4-desoxypodophyllotoxin (**26f**)*

Brown solid (31.9%), mp 158.3–159.2 °C; $[R]_D^{25}$–133.3 ($c = 0.1$, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 8.15 (d, $J = 9.0$ Hz, 2H, 3‴,6‴-H), 7.41 (d, $J = 9.0$ Hz, 2H, 4‴,5‴-H), 6.91 (s, 1H, –CONH–), 6.73 (s, 1H, 5-H), 6.53 (s, 1H, 8-H), 6.48 (d, $J = 8.5$ Hz, 2H, 2″,6″-H), 6.33 (s, 2H, 2′,6′-H), 5.95 and 5.97 (AB q, $J = 1.0$ Hz, 2H, $-OCH_2O-$), 4.62 (m, 1H, 1-H), 4.59 (d, $J = 5.0$ Hz, 1H, 4-H), 4.34 (t, $J = 8.0$ Hz, 1H, 11-H), 3.95 (t, $J = 8.0$ Hz, 11-H), 3.79 (s, 6H, 3′,5′-OCH_3), 3.15 (t, $J = 7.5$ Hz, 2H, $-NHCOCH_2CH_2$-Ph), 3.11 (dd, $J = 5.0$, 14.0 Hz, 1H, 2-H), 2.99 (m, 1H, 3-H), 2.66 (t, $J = 7.5$ Hz, 2H, $-NHCOCH_2CH_2$-Ph); HR-MS (ESI+) calculated for $C_{36}H_{33}N_3O_{10}$ $[M + 1]^+$: 668.2199, found: $m/z = 668.2190$ $[M + 1]^+$.

*4.1.31. 4β-[N-(phenyl-1‴-vinylcarbonyl)-4″-aminoanilino]-4′-O-demethyl-4-desoxypodophyllotoxin (**26g**)*

Yellow solid (29.8%), mp 153.7–155.2 °C; $[\alpha]_D^{25}$–144.0 ($c = 0.1$, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 7.73 (m, 1H, –CH=CH), 7.54 (d, $J = 5.0$ Hz, 2H, 2‴,6‴-H), 7.47 (d, $J = 5.5$ Hz, 2H, 3″,5″-H), 7.39 (m, 3H, 3‴,4‴,5‴-H), 7.15(s, 1H, –CH=CH), 6.77(s, 1H, 5-H), 6.57 (s, 1H, 8-H), 6.54 (m, $J = 4.0$ Hz, 2H, 2″,6″-H), 6.35 (s, 2H, 2′,6′-H), 5.97 and 5.98 (AB q, $J = 1.0$ Hz, 2H, $-OCH_2O-$), 4.66 (m, 1H, 1-H), 4.60 (d, $J = 4.5$ Hz, 1H, 4-H), 4.37 (t, $J = 8.0$ Hz, 1H, 11-H), 4.00 (t, $J = 8.0$ Hz, 11-H), 3.80 (s, 6H, 3′,5′-OCH_3), 3.13 (dd, $J = 5.0$, 14.0 Hz, 1H, 2-H), 3.01 (m, 1H, 3-H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 175.19, 164.11, 148.46, 147.80, 146.69, 144.79, 141.98, 134.99, 134.30, 131.18, 130.88, 129.10, 128.14, 122.48, 121.20, 112.73, 109.39, 108.16, 101.77, 72.06, 69.19, 65.84, 56.73, 53.03, 43.66, 42.13, 38.92; HR-MS (ESI+) calculated for $C_{36}H_{32}N_2O_8$ $[M + 1]^+$: 621.2192, found: $m/z = 621.2187$ $[M + 1]^+$.

*4.1.32. 4β-[N-(4‴-methoxy-phenyl-1‴-ethylcarbonyl)-4″-aminoanilino]-4′-O-demethyl-4-desoxypodophyllotoxin (**26h**)*

Grey–white solid (71.8%), mp 149.4–151.2 °C; IR(KBr, cm^{-1}) 3469, 2935, 2838, 1771, 1646, 1611, 1514, 1482, 1228, 1114, 1036; $[\alpha]_D^{25}$–95.5 ($c = 0.1$, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 7.24 (m, 2H, 2‴,6‴-H), 7.16 (d, $J = 8.5$ Hz, 2H, 3″,5″-H), 6.84 (m, 3H, 3″,4″,5″-H), 6.74 (s, 1H, 5-H), 6.52 (s, 1H, 8-H), 6.48 (d, $J = 8.0$ Hz, 2H, 2″,6″-H), 6.33 (s, 2H, 2′,6′-H), 5.95 and 5.97 (AB q, $J = 1.0$ Hz, 2H, $-OCH_2O-$), 4.62 (m, 1H, 1-H), 4.59 (d, $J = 5.0$ Hz, 1H, 4-H), 4.34 (t, $J = 8.0$ Hz, 1H, 11-H), 3.96 (t, $J = 8.0$ Hz, 11-H), 3.79 (m, 9H, 3′,5′-OCH_3, $-OCH_3$), 3.11 (dd, $J = 5.0$, 14.0 Hz, 1H, 2-H), 3.00 (m, 1H, 3-H), 2.98 (t, $J = 7.5$ Hz, 2H, –NHCO–CH_2CH_2-Ph), 2.59 (t, $J = 7.5$ Hz, 2H, –NHCO–CH_2CH_2-Ph); HR-MS (ESI+) calculated for $C_{37}H_{36}N_2O_9$ $[M + 1]^+$: 653.2454, found: $m/z = 653.2460$ $[M + 1]^+$.

*4.1.33. 4β-[N-(4‴-bromo-phenyl-1‴-carbonyl)-4″-aminoanilino]-4′-O-demethyl-4-desoxypodophyllotoxin (**26i**)*

Purple solid (46.8%), mp 160.9–162.6 °C; $[\alpha]_D^{25}$–46.9 ($c = 0.1$, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 7.74 (d, $J = 8.5$ Hz, 2H, 2‴,6‴-H), 7.62 (d, $J = 8.5$ Hz, 2H, 3‴,5‴-H), 7.45 (d, $J = 9.0$ Hz, 2H, 3″,5″-H), 6.77 (s, 2H, –CONH–), 6.56 (d, $J = 8.5$ Hz, 2H, 2″,6″-H), 6.34 (s, 2H, 2′,6′-H), 5.97 and 5.99 (AB q, $J = 1.0$ Hz, 2H, $-OCH_2O-$), 4.67 (m, 1H, 1-H), 4.61 (m, 1H, 4-H), 4.40 (m, 1H, 11-Ha), 3.96 (m, 1H, 11-Hb), 3.80 (s, 6H, 3′,5′-OCH_3), 3.14 (m, 1H, 2-H), 3.00 (m, 1H, 3-H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 175.23, 165.07, 148.53, 147.86, 146.76, 145.13, 144.00, 134.37, 134.15, 132.18, 131.24, 130.89, 129.13, 128.95, 126.59, 123.68, 122.72, 115.73, 112.76, 108.22, 105.46, 101.84, 69.23, 58.74, 56.78, 53.09, 43.71, 40.34, 38.98; HR-MS (ESI+) calculated for $C_{34}H_{29}BrN_2O_8$ $[M + 1]^+$: 674.1087, found: $m/z = 674.1082$ $[M + 1]^+$.

*4.1.34. 4β-[N-(4‴-chlorophenyl-2‴-carbonyl)-4″-aminoanilino]-4′-O-demethyl-4-desoxypodophyllotoxin (**26j**)*

Purple solid (14.3%), mp 165.6–167.2 °C; $[\alpha]_D^{25}$–63.1 ($c = 0.1$, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 7.82 (d, $J = 8.5$ Hz, 2H, 2‴,6‴-H), 7.64 (d, $J = 8.5$ Hz, 2H, 3‴,5‴-H), 7.44 (d, $J = 9.0$ Hz, 2H, 3″,5″-H), 6.76

(m, 1H, 5-H, –CONH–), 6.55 (d, $J = 8.5$ Hz, 2H, 2″,6″-H), 6.34 (s, 2H, 2′,6′-H), 5.95 and 5.96 (AB q, $J = 1.0$ Hz, 2H, $-OCH_2O-$), 4.67 (m, 1H, 1-H), 4.60 (m, 1H, 4-H), 4.39 (m, 1H, 11-Ha), 3.99 (m, 1H, 11-Hb), 3.80 (s, 6H, 3′,5′-OCH_3), 3.14 (m, 1H, 2-H), 3.00 (m, 1H, 3-H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 175.25, 164.98, 148.57, 147.90, 146.80, 145.17, 144.03, 138.19, 134.42, 133.67, 132.21, 130.86, 129.31, 128.81, 123.11, 122.75, 115.77, 112.80, 110.22, 109.46, 108.26, 101.86, 69.26, 60.75, 58.76, 56.81, 53.12, 43.74, 42.23, 39.00; HR-MS (ESI+) calculated for $C_{34}H_{29}ClN_2O_8$ $[M+1]^+$: 629.1646, found: $m/z = 629.1650$ $[M+1]^+$.

4.1.35. 4β-[N-(4‴-nitro-phenyl-1‴-carbonyl)-4″-aminoanilino]-[N-(4⁗-nitro-phenyl-1⁗-carbonyl)]-4′-O-demethyl-4-desoxypodophyllotoxin (**26k**)

According to the procedure used to prepare **26a**, reaction of **25** (60 mg, 0.1 mmol) with 4-nitrobenzoic acid (33 mg, 0.2 mmol) provided **26k**, red solid (37.7%), mp 217–220 °C; IR(KBr, cm^{-1}) 3471, 3110, 2940, 2907, 1774, 1749, 1643, 1601, 1515, 1482, 1348, 1265, 1129, 1037; $[\alpha]_D^{25}$–72.7 ($c = 0.1$, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.32 (m, 8H, 2‴,3‴,5‴,6‴,2⁗,3⁗,5⁗,6⁗-H), 8.03 (d, $J = 8.5$ Hz, 2H, 3″,5″-H), 7.78(s, 1H, –CONH–), 7.48 (d, $J = 8.0$ Hz, 2H, 2″,6″-H), 6.78 (s, 1H, 5-H), 6.58 (d, $J = 8.5$ Hz, 2H, 2′,6′-H), 6.57 (s, 1H, 8-H), 5.98 and 5.99 (AB q, $J = 1.0$ Hz, 2H, $-OCH_2O-$), 4.69 (m, 1H, 1-H), 4.67 (d, $J = 5.0$ Hz, 1H, 4-H), 4.41 (t, $J = 8.0$ Hz, 1H, 11-H), 4.00 (t, $J = 8.0$ Hz, 11-H), 3.71 (s, 6H, 3′,5′-OCH_3), 3.19 (dd, $J = 5.0$, 14.0 Hz, 1H, 2-H), 3.00 (m, 1H, 3-H); HR-MS (ESI+) calculated for $C_{41}H_{32}N_4O_{18}$ $[M+1]^+$: 789.1999, found: $m/z = 789.1995$ $[M+1]^+$.

4.1.36. 4β-[N-(4‴-chloro-phenyl-1‴-carbonyl)-4″-aminoanilino]-[N-(4⁗-chloro-phenyl-1⁗-carbonyl)]-4′-O-demethyl-4-desoxypodophyllotoxin (**26l**)

According to the procedure used to prepare **26a**, reaction of **25** (60 mg, 0.1 mmol) with 4-chlorobenzoic acid (31 mg, 0.2 mmol) provided **26l**, brown solid (17.4%), mp 143.1–145 °C; $[\alpha]_D^{25}$–62.8 ($c = 0.1$, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.13 (d, $J = 9.0$ Hz, 2H, 2‴,6‴-H), 7.81 (m, 2H, 2⁗,6⁗-H), 7.44 (m, 8H, 2″, 3″, 5″, 6″, 3‴, 5‴, 3⁗, 5⁗-H), 6.78 (s, 1H, 5-H), 6.57 (m, 3H, 2′,6′, 8-H), 5.98 and 6.00 (AB q, $J = 1.0$ Hz, 2H, $-OCH_2O-$), 4.68 (m, 2H, 4-H, 1-H), 4.43 (t, $J = 8.0$ Hz, 1H, 11-Ha), 4.00 (t, $J = 8.0$ Hz, 11-Hb), 3.70 (s, 6H, 3′,5′-OCH_3), 3.19 (dd, $J = 5.0$, 14.0 Hz, 1H, 2-H), 3.02 (m, 1H, 3-H); HR-MS (ESI+) calculated for $C_{41}H_{32}ClN_2O_9$ $[M+1]^+$: 768.1455, found: $m/z = 768.1452$ $[M+1]^+$.

4.2. Pharmacology

4.2.1. Cytotoxicity assay

The cytotoxic activity *in vitro* against several cancer cell lines was measured by MTT assay. The cancer cell lines included oral squamous cell carcinoma (KB), vincristine (VCR)-selected MDR-positive KB/VCR, human non-small cell lung cancer (A549) and highly metastatic human lung cancer (95D). MTT solution (2.5 mg/mL in RPIM-1640, Sigma, St. Louis, MO) was added (20.0 μL/well), and the plates were incubated for an additional 4 h at 37 °C. The purple formazan crystals were dissolved in 100.0 μL DMSO. After 5 min, the plates were read on a Multiskan Spectrum (Thermo Electron Corporation, Marietta, Ohio) at 570 nm. Assays were performed in triplicate on three independent experiments. The concentration of drug inhibit for 50% of cells (IC_{50}) was calculated using the software of Dose-Effect Analysis with Microcomputers.

4.2.2. Antitumor activity in 95D bearing mice

95D highly metastatic human lung cancer model was established by injection of a highly metastatic human lung cancer 95D cells (1×10^7 cells per animal, subcutaneously into the armpit of the nude mice) to 4- to 5-week-old BALB/C female nude mice (National Rodent Laboratory Animal Resource, Shanghai Branch, China). Treatments were initiated when tumors reached a mean group size of about 100–200 mm^3. Nude mices were administered intragastrically with **26c** (50 mg/kg, 5 mg/mL in 0.9% sterile sodium chloride solution) two or three times a week for 20 days. Compounds **23a** (10 mg/kg, 2 mg/mL in 0.9% sterile sodium chloride solution) and **26e** (10 mg/kg, 2 mg/mL in 0.9% sterile sodium chloride solution) were administered intramuscularly once every day for 20 days. Mice weight and tumor volume were recorded every other day until animals were sacrificed.

All experiments were approved by an internal ethical committee and in accordance with the institutional guidelines. Tumor volume (mm^3) was measured with calipers and calculated as $(W^2 \times L)/2$, where W is the width and L is the length. The T/C% value was determined by calculating relative tumor volume (RTV) as T/C% = 100 × (mean RTV of treated group)/(mean RTV of control group). The tumor growth inhibition rate was calculated by using the formula IR (%) $= (1 - TW_t/TW_c) \times 100$, where TW_t and TW_c were the mean tumor weight of treatment and control groups.

4.3. Retention index

4.3.1. Chromatographic conditions

The RP-HPLC analysis was performed on an Agilent 1200 Series instrument fitted with an Eclipse Plus C_{18} (5 μm, 4.6 mm × 250 mm). The optimum separation in HPLC was carried out with a mobile phase composed of 1% HOAc: acetonitrile (45:55, v/v) at a flow-rate of 1.0 mL/min. The volume of sample injected was 20 μL, the column temperature was set at 25 °C, and the elution was detected at 254 nm, according to the UV absorption of the sample.

4.3.2. RI evaluation

Each of the compounds **9** and **23a**–**d** was dissolved in acetonitrile (0.1 mg/mL) and then injected into the HPLC apparatus. The retention time of these five compounds was recorded. The capacity factors (k'_x) and RI values of the samples were calculated using the equations as follows:

$$k'_x = (t_x - t_0)/t_0$$

$$\mathrm{RI} = 100\mathrm{N} + 100(\lg k'_x - \lg k'_n)/(\lg k'_{n+1} - \lg k'_n)$$

where x = compounds **9** or **23a**–**d** measured, n = alkan-2-one eluting immediately before x, $n+1$ = alkan-2-one eluting immediately after x and N = carbon number of alkan-2-one. The k' values were calculated using the retention time of $NaNO_3$ as a dead time.

Acknowledgements

This study was financially supported by the National Key Tech Project for Major Creation of New drugs (No. 2009ZX09501-003) and the Fundamental Research Funds for the Central Universities (No. KYJD038).

References

[1] D. Guianvarc'h, M. Duca, C. Boukarim, L. Kraus-Berthier, S. Leonce, A. Pierre, B. Pfeiffer, P. Renard, P.B. Arimondo, C. Monneret, D. Dauzonne, J. Med. Chem. 47 (2004) 2365–2374.
[2] E.L. Baldwin, N. Osheroff, Curr. Med. Chem.: Anti-Cancer Agents 5 (2005) 363–372.
[3] A. Kamal, B.A. Kumar, M. Arifuddin, Tetrahedron Lett. 44 (2003) 8457–8459.
[4] J.L. Nitiss, Nat. Rev. Cancer 9 (2009) 338–350.
[5] J. Yang, A. Bogni, E.G. Schuetz, M. Ratain, M.E. Dolan, H. McLeod, L. Gong, C. Thorn, M.V. Relling, T.E. Klein, R.B. Altman, Pharmacogenet. Genom. 19 (2009) 552–553.
[6] Y.J. You, Curr. Pharm. Des. 11 (2005) 1695–1717.

[7] I. Rassmann, T. Schilling, H. Schrodel, A. KaeserFrohlich, K. Burk, J. Rastetter, A.R. Hanauske, Eur. J. Cancer 31A (1995) 111.
[8] T.S. Huang, C.H. Shu, W.K. Yang, J. WhangPeng, Cancer Res. 57 (1997) 2974–2978.
[9] T. Utsugi, J. Shibata, Y. Sugimoto, K. Aoyagi, K. Wierzba, T. Kobunai, T. Terada, T. OhHara, T. Tsuruo, Y. Yamada, Cancer Res. 56 (1996) 2809–2814.
[10] S.Z. Fields, L.N. Igwemezie, S. Kaul, L.P. Schacter, R.J. Schilder, P.P. Litam, B.S. Himpler, C. Mcaleer, J. Wright, R.H. Barbhaiya, C.J. Langer, P. Odwyer, Clin. Cancer Res. 1 (1995) 105–111.
[11] C. Etievant, A. Kruczynski, J.M. Barret, D. Perrin, B.T. Hill, Biochem. Pharmacol. 65 (2003) 755–763.
[12] R.M. Moreas, F.E. Dayan, C. Canel, Stud. Nat. Prod. Chem. 26 (2002) 149–182.
[13] S.J. Cho, A. Tropsha, M. Suffness, Y.C. Cheng, K.H. Lee, J. Med. Chem. 39 (1996) 1383–1395.
[14] C.Q. Hu, D.Q. Xu, W.T. Du, S.J. Qian, L. Wang, J.S. Lou, Q.J. He, B. Yang, Y.Z. Hu, Mol. Biosyst. 6 (2010) 410–420.
[15] D.H. Huh, J.S. Jeong, H.B. Lee, H. Ryu, Y.G. Kim, Tetrahedron 58 (2002) 9925–9932.
[16] R.J. Gentile, R.J. Murray, J.E. MacDonald, W.C. Shakespeare, WO 9505363 A1.
[17] Q.Y. Liu, H.T. Xi, Y. Jiang, X.Q. Sun, Huaxue Gongye Yu Gongcheng 21 (2004) 425–428.
[18] M.R. Pitts, J.R. Harrison, C.J. Moody, J. Chem. Soc., Perkin Trans. 1 (2001) 955–977.
[19] Y.Z. Hu, W.T. Du, Q.J. He, B. Yang, X.C. Yang, CN 101074233.
[20] Z.Y. Xiao, K.F. Bastow, J.R. Vance, R.S. Sidwell, H.K. Wang, M.S. Chen, Q. Shi, K.H. Lee, J. Med. Chem. 47 (2004) 5140–5148.
[21] J.K. Baker, R.E. Skelton, T.N. Riley, J.R. Bagley, J. Chromatogr. Sci. (1980) 153–158.