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Design, synthesis and cytotoxic activity of novel sulfonylurea derivatives of podophyllotoxin

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ABSTRACT

Three series of novel sulfonylurea podophyllotoxin derivatives were designed, synthesized, and evaluated for in vitro cytotoxicity against four tumor cell lines (A-549, DU-145, KB and KBvin). Compounds **14c** (IC_{50}: 1.41–1.76 μM) and **14e** (IC_{50}: 1.72–2.01 μM) showed superior cytotoxic activity compared with etoposide (IC_{50}: 2.03 to >20 μM), a clinically available anticancer drug. Significantly, most of the compounds exhibited comparable cytotoxicity against the drug-resistant tumor cell line KBvin, while etoposide lost activity completely. Preliminary structure–activity relationship (SAR) correlations indicated that the 4′-*O*-methyl functionality in podophyllotoxin analogues may be essential to maintain cytotoxic activity, while an arylsulfonylurea side chain at podophyllotoxin's 4β position can significantly improve cytotoxic activity.

1. Introduction

Podophyllotoxin (**1**) is a naturally occurring aryltetralin lignan isolated from various plant species of the *Podophyllum* family. It exerts cytotoxic activity by inhibiting microtubule assembly.[1,2] Enormous progress has been achieved concerning the structural elements required for activity. These findings led to approval of etoposide (**3**) and teniposide (**4**), two semisynthetic glucoside derivatives of -*O*-demethylepipodophyllotoxin (**2**), as well as etopophos (**5**), a water-soluble prodrug of **3**, as anticancer drugs. Unlike **1**, compounds **3**–**5** exert cytotoxic activity by inhibiting DNA topoisomerase II.[3–7]

Improved understanding of the mechanism(s) of action, along with extensive structure–activity and pharmacology studies, reenergized interest in further modification studies on the C-4 substituent of **2** to increase antitumor activity. Accordingly, various C-4 substituents were introduced into the parent molecule leading to either enhanced or comparable activity. Through C4 modification, some nonsugar substituted analogues, particularly N-linked congeners, were found to exhibit superior pharmacological properties to **3**, and several clinical trial drug candidates, including NPF (**6**),[8] GL-331 (**7**),[9] and TOP-53 (**8**),[10] emerged as alternatives to overcome the drawbacks of **3**. Overall, the excellent activity profiles of these agents, including improved water solubility, cytotoxic activity, drug resistance profiles, and antitumor spectra, suggested this compound class could be optimized through rational C-4 modification. Both a composite pharmacophore model and comparative molecular field analysis also further demonstrated that the C-4 molecular area could accommodate considerable structural diversity.[11]

Based on accumulated SAR studies and critical modeling clues, we have generated focused libraries of potent aniline, alkylamino, phenol, thiophenol, and carbohydrate derivatives functionalized at the C-4 position of **2**; among which, some compound have exhibited significant anticancer and DNA topoisomerase II inhibitory activities.[2,12] Especially, 4β-anilino substituted podophyllotoxin derivatives showed potent cytotoxic activity against some human parental and drug-resistant cancer cell lines.[13–19] From these studies, **7** proved to be more potent than **3** and underwent phase II clinical trials for the treatment of various cancers. In continuation of these efforts, we recently found that a series of aroylthiourea derivatives of 4-β-amino-4-desoxypodophyllotoxin displayed potent antitumor activity with significantly different drug-resistance profiles from those of **1**.[20] Some new compounds exhibited promising cytotoxicity against the KBvin drug resistant tumor cell line (e.g., IC_{50} 0.098 and 0.13 μM), while etoposide lost activity completely. In addition, some compounds were effective in drug-sensitive and drug-resistant xenograft models at lower doses than etoposide,

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demonstrating potential as drug candidates for anticancer chemotherapy. These encouraging results prompted us to further extend our investigation by synthesizing a novel series of sulfonylurea podophyllotoxin derivatives. The sulfonylurea group was chosen based on the facts that this group is commonly found in various drugs and introduction of a bioactive sulfonylurea group can usually potentiate the biochemical or pharmacological properties of the original molecule.[21] Therefore, in this paper, we describe our introduction of sulfonylurea groups into the podophyllotoxin skeleton via a coupling reaction and our cytotoxic activity studies on the resulting compounds (see Fig. 1).

2. Results and discussion

2.1. Chemistry

The synthetic routes to target podophyllotoxin derivatives are outlined in Schemes 1 and 2. Briefly, precursor 4-azidopodophyllotoxins **9** and **10** were prepared from **1** and **2** by employing the BF_3–Et_2O/HN_3 reagent system. Both **9** and **10** were then reduced under hydrogen atmosphere with Pd/C catalyst to yield the key intermediate 4β-amino congeners **11** and **12**, respectively, in excellent yields.[22] Next, intermediates **11** and **12** were coupled with various sulfonylcarbamates in dry toluene to afford the desired 4β-sulfonylurea podophyllotoxins **13a–l** and **14a–e**, respectively, in good yields.

As illustrated in Scheme 2, initially, silyl-protected podophyllotoxin **15** was produced in excellent yield following a classical synthetic method with *tert*-butyldimethylsilyl chloride (TBDMSCl) and imidazole in DMF. Furthermore, another key precursor anhydropodophyllotoxin (**18**) was synthesized by the following sequence, reduction of **15** with lithium aluminum hydride ($LiAlH_4$), followed by closure of the resulting diol **16** to cyclic ether **17** with a mixture of triphenylphosphine (TPP) and diethyl azodicarboxylate (DEAD), and subsequently, deprotection with tetrabutylammonium fluoride (Bu_4NF).[23] Similarly, intermediate 4β-amino-anhydropodophyllotoxin (**20**) was prepared from **18** through azidation and catalytic hydrogenation via a similar procedure to that described above for **11** and **12**. Finally, using similar methods to those for **13a–l** and **14a–e**, target compounds **21a–c** were obtained from **20** in yields ranging from 67% to 82%. All newly synthesized compounds were purified by column chromatography and their structures were confirmed by ^1H NMR, ^{13}C NMR, and ESI-MS data.

2.2. Cytotoxicity and SAR

Target compounds **13a–l**, **14a–e** and **21a–c** were evaluated for in vitro cytotoxicity against four human tumor cell lines, A549 (nonsmall cell lung cancer), DU145 (prostate cancer), KB (nasopharyngeal carcinoma), and KBvin [multi-drug resistant (MDR) KB subline selected using vincristine], using a sulforhodamine B colorimetric (SRB) assay with triplicate experiments.[24] Compound **3** was included as positive control and the results are summarized in Table 1.

Notably, compounds **14c** and **14e** showed superior activity (IC_{50} 1.41–1.76 and 1.72–2.01 μM, respectively) compared with **3** (IC_{50} 2.03–3.88 μM) against A549, DU-145, and KB tumor cell lines. Most importantly, these two compounds retained significant cytotoxicity (IC_{50} 1.76 and 2.01 μM, respectively) 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 **2** is favorable for overcoming drug-resistance.[19]

Although 4′-*O*-methylated derivatives **14c** and **14e** showed significant cytotoxicity, their corresponding 4′-hydroxyl analogues **13d** and **13i** displayed only marginal activity (IC_{50} 8.10–8.76 and

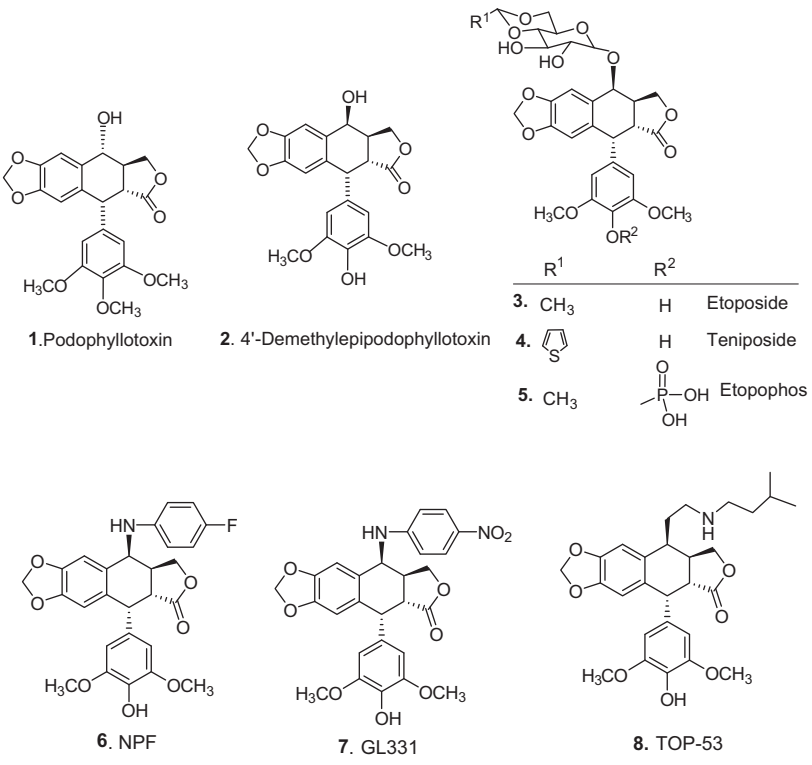
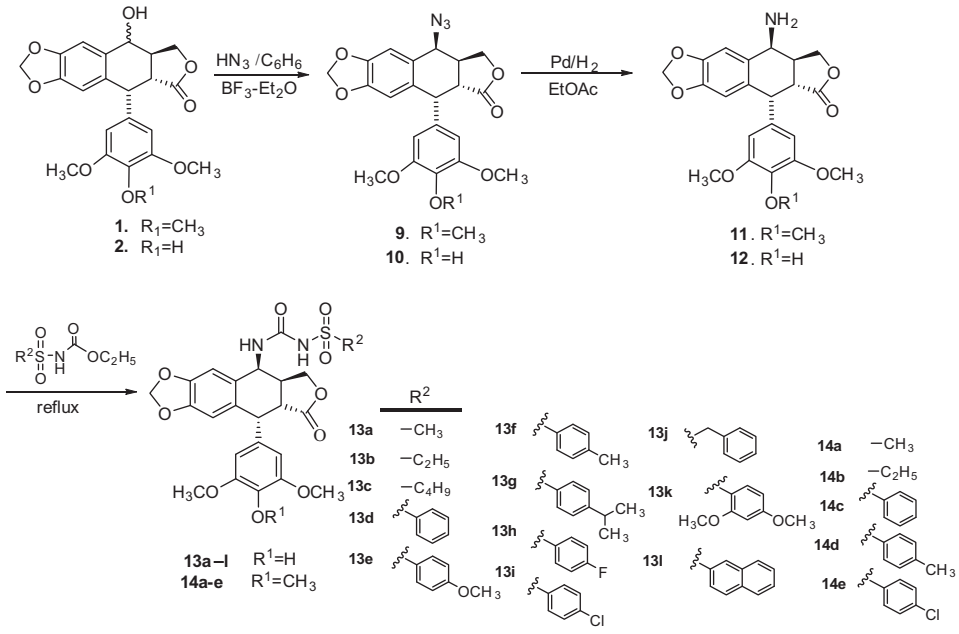
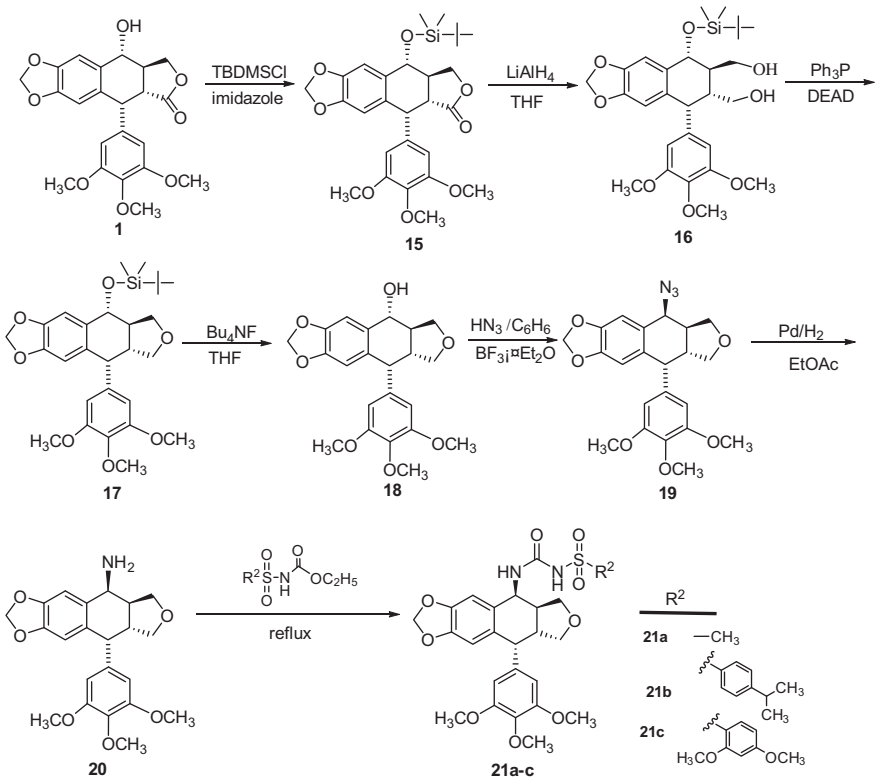


Figure 1. Structures of podophyllotoxin derivatives.



Scheme 1. Synthesis of 4β-sulfonylurea podophyllotoxin congeners **13a–l** and **14a–e**.



Scheme 2. Synthesis of 4β-sulfonylurea anhydropodophyllotoxin congeners **21a–c**.

7.95–11.52 μM, respectively). Similar results were seen in our previous study,[20] and highlight the critical role of the 4′-*O*-methyl functionality in sulfonylurea-substituted **2**-derivatives.

4′-*O*-Methylated derivatives **14a**, **14b**, and **14c** with methyl, ethyl, and 4-methylphenyl groups on the sulfonylurea side chain were obviously much less potent (IC_{50} >20 μM) than **14c** and **14e** with phenyl and 4-chlorophenyl groups in the R^2 position. These results were unexpected,[25] and further investigation is needed.

Within the 4′-*O*-demethylated series (**13a–l**), compounds **13a** and **13b** with methyl- and ethyl-sulfonylurea groups, respectively, were inactive (IC_{50} >20 μM), while **13c** with a butyl R^2 group showed marginal to weak cytotoxicity (IC_{50} 7.96–13.95 μM), indicating that the substituent's size is critical. Except for **13f**,

Table 1
In vitro cytotoxicity of **13a–l**, **14a–e** and **21a–c** against four human tumor cell lines with etoposide (**3**) as control

Compd	IC_{50} (μM)			
	A549	DU145	KB	KBvin
13a	>20	>20	>20	>20
13b	>20	>20	>20	>20
13c	7.96 ± 0.470	11.94 ± 1.042	10.87 ± 1.280	13.95 ± 0.740
13d	8.25 ± 0.842	8.10 ± 0.410	8.38 ± 0.452	8.76 ± 0.120
13e	7.67 ± 0.489	9.66 ± 0.864	9.49 ± 0.821	8.79 ± 0.585
13f	>20	>20	>20	>20
13g	7.72 ± 1.229	9.32 ± 0.337	9.51 ± 0.414	8.35 ± 0.681
13h	7.82 ± 0.424	9.61 ± 0.622	9.00 ± 0.151	7.92 ± 0.863
13i	9.03 ± 0.142	11.52 ± 0.472	10.56 ± 0.256	7.95 ± 0.500
13j	8.63 ± 0.892	11.24 ± 0.824	11.78 ± 0.291	13.99 ± 0.653
13k	8.00 ± 0.383	8.51 ± 0.816	8.08 ± 0.769	8.56 ± 0.818
13l	8.49 ± 0.291	10.53 ± 0.532	9.70 ± 0.294	8.62 ± 0.950
14a	>20	>20	>20	>20
14b	>20	>20	>20	>20
14c	1.60 ± 0.082	1.44 ± 0.080	1.41 ± 0.112	1.76 ± 0.263
14d	>20	>20	>20	>20
14e	1.89 ± 0.162	1.75 ± 0.124	1.72 ± 0.181	2.01 ± 0.323
21a	>20	>20	>20	>20
21b	11.79 ± 0.22	11.88 ± 0.152	11.02 ± 0.630	13.17 ± 0.732
21c	14.91 ± 0.583	14.29 ± 0.108	12.02 ± 0.043	>20
Etoposide	2.58 ± 0.252	2.03 ± 0.121	3.88 ± 0.199	>20

4′-O-demethylated compounds with phenylsulfonylurea groups (**13c–e** and **13g–k**) showed similar potency (IC_{50} 7.67–13.99 μM) to **13c** against all tested tumor cell lines. Cytotoxic potency was only slightly affected by the electronegativity or positions of substituents on the phenyl ring. Moreover, compound **13l** with a 2-naphthylsulfonylurea group showed comparable potency to compounds with a phenyl group.

Subsequently, to investigate whether the lactone moiety can influence cytotoxic activity, 4β-sulfonylurea anhydropodophyllotoxin compounds **21a–c** were prepared. As shown in Table 1, compound **21a** was inactive, while **21b** and **21c** were less potent than the related lactone compounds **13g** and **13k**, suggesting that the lactone moiety might be important for antitumor effects. However, further investigation is warranted including comparison with 4′-*O*-methylated lactone compounds.

3. Conclusion

In summary, three series of novel 4β-sulfonylurea podophyllotoxin derivatives were designed, synthesized, and evaluated for cytotoxicity against four tumor cell lines (A-549, DU-145, KB and KBvin) by using a sulforhodamine B colorimetric assay. Among them, compounds **14c** and **14e** were the most promising derivatives with greater potency than **3** and were selected as lead molecules for further development. The cytotoxic results revealed that the 4′-*O*-methyl functionality was essential in increasing cytotoxicity in the **1**-derived compounds, and aryl substituents on the sulfonylurea moiety were advantageous. These findings support our further optimization of **1** to develop potential anticancer drug candidates. Continuing studies to substantiate and improve activity profiles are underway in our laboratory and will be reported in due course.

4. Experimental section

4.1. Chemistry

Melting points were taken on a Kofler melting point apparatus and are uncorrected. Mass spectra were recorded on a Bruker Daltonics APEXII49e spectrometer with ESI source as ionization. ^1H and ^{13}C NMR spectra were recorded at 400 and 100 MHz on a Bruker AM-400 spectrometer using TMS as reference (Bruker Company, USA). Optical rotations were measured on an Autopol IV polarimeter (Rudolph, USA) in a 10 mm cell at 21 °C. Podophyllotoxin (**1**) was isolated from the Chinese medicinal herb *Juniperus sabina* Linnaeus, and served as the starting material for preparation of all new derivatives. The starting sulfonylcarbamates were prepared according to the procedure reported previously.[26,27] The key intermediate 4β-amino congeners **11**, **12** and **20** were synthesized by our previously reported procedures, and their structures confirmed by direct comparison with an authentic sample and previously reported spectroscopic data.[21,22]

4.2. General synthetic procedure for target compounds 13a–l, 14a–e and 21a–c

Key intermediates **11**, **12** and **20** (0.2 mmol) were added dropwise to a solution of 0.4 mmol of sulfonylcarbamate in dry toluene (20 mL). The reaction mixture was refluxed for 2–4 h and then concentrated. The residue was purified by chromatography on silica gel using $CHCl_3$/MeOH as eluant to give **13a–l**, **14a–e**, and **21a–c**, which were stable both at chemical purification stage and under assay conditions.

4.2.1. 4β-*N*-(Methylsulfonylurea)-4-deoxy-4′-demethylepipodophyllotoxin (13a)

Yield: 50%; mp: 180–182 °C; $[\alpha]_D^{21}$ − 31.4° (*c* 0.5, $CHCl_3$); ^1H NMR (400 MHz, DMSO-d_6) *δ*: 8.29 (s, 1H, –CONHSO$_2$–), 6.87 (s, 1H, 5-H), 6.53 (s, 1H, 8-H), 6.21 (s, 2H, 2′,6′-H), 6.00 and 5.98 (ABq, 2H, –OCH$_2$O–), 5.01–4.98 (m, 1H, 4-H), 4.49 (d, 1H, 1-H, *J* = 5.2 Hz), 4.32 (t, 1H, 11β-H, *J* = 8 Hz), 3.81 (t, 1H, 11α-H, *J* = 9.2 Hz), 3.61 (s, 6H, 3′,5′-OCH$_3$), 3.25 (s, 3H, 1″-H), 3.10 (dd, 1H, 2-H, *J* = 14.4, 5.2 Hz), 2.98–2.91 (m, 1H, 3-H); ^{13}C NMR (100 MHz, DMSO-d_6) *δ*: 174.7, 152.4, 147.6, 147.3, 146.8, 137.4, 134.8, 132.6, 130.2, 129.8, 128.4, 119.8, 109.5, 108.5, 101.5, 56.2, 42.9, 41.6, 40.8; ESI-MS: *m*/*z* 521.0 [M+H]$^+$.

4.2.2. 4β-*N*-(Ethylsulfonylurea)-4-deoxy-4′-demethylepipodophyllotoxin (13b)

Yield: 52%; mp: 160–162 °C; $[\alpha]_D^{21}$ − 77.6° (*c* 0.5, $CHCl_3$); ^1H NMR (400 MHz, DMSO-d_6) *δ*: 8.29 (s, 1H, –CONHSO$_2$–), 6.86 (s, 1H, 5-H), 6.53 (s, 1H, 8-H), 6.21 (s, 2H, 2′,6′-H), 6.00 and 5.98 (ABq, 2H, –OCH$_2$O–), 5.01–4.98 (m, 1H, 4-H), 4.49 (d, 1H, 1-H, *J* = 5.2 Hz), 4.32 (t, 1H, 11β-H, *J* = 8 Hz), 3.78 (t, 2H, 1″-H, *J* = 10.4 Hz), 3.61 (s, 6H, 3′,5′-OCH$_3$), 3.15 (dd, 1H, 2-H, *J* = 14.4, 5.2 Hz), 2.98–2.91 (m, 1H, 3-H), 1.25–1.16(m, 3H, 2″-H); ^{13}C NMR (100 MHz, DMSO-d_6) *δ*: 174.6, 152.3, 147.6, 147.3, 146.8, 134.8, 132.6, 130.2, 129.8, 109.7, 109.3, 108.5, 101.5, 56.2, 47.3, 42.9, 40.8, 37.0; ESI-MS: *m*/*z* 535.0 [M+H]$^+$.

4.2.3. 4β-*N*-(Butylsulfonylurea)-4-deoxy-4′-demethylepipodophyllotoxin (13c)

Yield: 49%; mp: 161–163 °C; $[\alpha]_D^{21}$ − 85.4° (*c* 0.5, $CHCl_3$); ^1H NMR (400 MHz, DMSO-d_6) *δ*: 8.28 (s, 1H, –CONHSO$_2$–), 6.84 (s, 1H, 5-H), 6.47 (s, 1H, 8-H), 6.20 (s, 2H, 2′,6′-H), 5.98 and 5.96 (ABq, 2H, –OCH$_2$O–), 5.03–5.02 (m, 1H, 4-H), 4.43 (d, 1H, 1-H, *J* = 4.8 Hz), 4.29 (t, 1H, 11β-H, *J* = 8 Hz), 4.03–4.00 (m, 1H, 11α-H), 3.60 (s, 6H, 3′,5′-OCH$_3$), 3.15-3.07 (m, 1H, 2-H), 2.97 (t, 2H, 1″-H, *J* = 7.6 Hz), 2.94–2.84 (m, 1H, 3-H), 1.58–1.56 (m, 2H, 2″-H), 1.38–1.32 (m, 2H, 3″-H), 1.16 (t, 3H, 4″-H, *J* = 6.8 Hz); ^{13}C NMR (100 MHz, DMSO-d_6) *δ*: 147.3, 136.0, 134.8, 108.5, 105.3, 56.1, 43.1, 30.9, 21.3, 13.9; ESI-MS: *m*/*z* 585.1 [M+Na]$^+$.

4.2.4. 4β-*N*-(Phenylsulfonylurea)-4-deoxy-4′-demethylepipodophyllotoxin (13d)

Yield: 59%; mp: 195–197 °C; $[\alpha]_D^{21}$ − 33.8° (*c* 0.5, $CHCl_3$); ^1H NMR (400 MHz, DMSO-d_6) *δ*: 8.27 (s, 1H, –CONHSO$_2$–), 7.92 (d,

2H, 2″,6″-H, J = 7.6 Hz), 7.69 (t, 1H, 4″-H, J = 7.6 Hz), 7.61 (t, 2H, 3″,5″-H, J = 7.6 Hz), 6.71 (s, 1H, 5-H), 6.51 (s, 1H, 8-H), 6.18 (s, 2H, 2′,6′-H), 5.99 and 5.97 (ABq, 2H, –OCH_2O–), 4.90–4.87 (m, 1H, 4-H), 4.46 (d, 1H, 1-H, J = 5.2 Hz), 4.13 (t, 1H, 11α-H, J = 8 Hz), 3.59 (s, 6H, 3′,5′-OCH_3), 3.10 (dd, 1H, 2-H, J = 14.4, 5.2 Hz), 2.88–2.78 (m, 1H, 3-H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 174.4, 167.1, 151.6, 147.4, 147.2, 146.7, 140.3, 134.7, 133.3, 132.5, 130.1, 129.6, 129.1, 127.3, 109.5, 109.1, 108.4, 101.4, 68.1, 56.0, 47.8, 42.8, 40.7, 36.7; ESI-MS: m/z 583.3 $[M+H]^+$.

4.2.5. 4β-*N*-(4″-Methoxyphenylsulfonylurea)-4-deoxy-4′-demethyl-epipodophyllotoxin (13e)

Yield: 42%; mp: 166–168 °C; $[\alpha]_D^{21}$ – 59.4° (c 0.5, $CHCl_3$); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.03 (s, 1H, –$CONHSO_2$–), 7.84 (d, 2H, 2″,6″-H, J = 8.8 Hz), 7.76 (d, 2H, 3″,5″-H, J = 8.8 Hz), 6.77 (s, 1H, 5-H), 6.51 (s, 1H, 8-H), 6.20 (s, 2H, 2′,6′-H), 6.02 and 6.01 (ABq, 2H, –OCH_2O–), 5.00–4.97 (m, 1H, 4-H), 4.50 (d, 1H, 1-H, J = 4.8 Hz), 4.46-4.41 (m, 2H, 11-H), 3.84 (s, 3H, 4″-OCH_3), 3.62 (s, 6H, 3′,5′-OCH_3), 3.16 (dd, 1H, 2-H, J = 14, 4.8 Hz), 2.96–2.82 (m, 1H, 3-H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 174.0, 162.2, 147.7, 147.3, 146.9, 134.9, 132.3, 129.9, 129.6, 114.3, 109.6, 108.6, 56.1, 42.8, 40.7, 38.0, 30.8; ESI-MS: m/z 630.5 $[M+NH_4]^+$.

4.2.6. 4β-*N*-(4″-Methylphenylsulfonylurea)-4-deoxy-4′-demethyl-epipodophyllotoxin (13f)

Yield: 56%; mp: 193–195 °C; $[\alpha]_D^{21}$ – 50.0° (c 0.5, $CHCl_3$); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.29 (br s, 1H, –$CONHSO_2$–), 7.87–7.71 (m, 2H, 2″,6″-H), 7.23–7.18 (m, 2H, 3″,5″-H), 6.78 (s, 1H, 5-H), 6.51 (s, 1H, 8-H), 6.20 (s, 2H, 2′,6′-H), 5.97 and 5.96 (ABq, 2H, –OCH_2O–), 4.93–4.92 (m, 1H, 4-H), 4.42 (d, 1H, 1-H, J = 5.2 Hz), 4.12 (br s, 2H, 11-H, J = 8 Hz), 3.59 (s, 6H, 3′,5′-OCH_3), 3.15 (m, 1H, 2-H), 2.79 (m, 1H, 3-H), 2.336 (s, 3H, 4″-CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 174.8, 172.5, 147.3, 146.7, 134.8, 132.2, 131.0, 130.4, 129.0, 126.9, 109.5, 109.2, 108.5, 101.4, 68.5, 63.0, 56.2, 48.8, 47.6, 43.0, 40.9, 37.0, 30.9, 21.4; ESI-MS: m/z 597.0 $[M+H]^+$.

4.2.7. 4β-*N*-(4″-Isopropylphenylsulfonylurea)-4-deoxy-4′-demethyl-epipodophyllotoxin (13g)

Yield: 41%; mp: 172–174 °C; $[\alpha]_D^{21}$ – 53.1° (c 0.5, $CHCl_3$); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.04 (s, 1H, –$CONHSO_2$–), 7.84 (d, 2H, 2″,6″-H, J = 8 Hz), 7.75 (d, 2H, 3″,5″-H, J = 8 Hz), 6.79 (s, 1H, 5-H), 6.51 (s, 1H, 8-H), 6.21 (s, 2H, 2′,6′-H), 5.98 and 5.97 (ABq, 2H, –OCH_2O–), 5.01–4.98 (m, 1H, 4-H), 4.51 (d, 1H, 1-H, J = 4.8 Hz), 4.44–4.41 (m, 2H, 11-H), 3.64 (s, 6H, 3′,5′-OCH_3), 3.17 (dd, 1H, 2-H, J = 14.4, 4.8 Hz), 3.01–2.91 (m, 1H, 3-H), 2.88–2.83 (m, 1H, 7″-H), 1.22-1.18 (m, 6H, 7″-CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 174.4, 172.2, 166.7, 164.8, 153.8, 147.7, 147.3, 146.7, 134.8, 132.3, 130.1, 129.9, 129.4, 127.1, 126.9, 109.6, 109.4, 108.6, 101.4, 84.7, 56.2, 50.6, 42.7, 40.7, 37.5, 30.8, 23.6; ESI-MS: m/z 624.3 $[M]^+$.

4.2.8. 4β-*N*-(4″-Fluorophenylsulfonylurea)-4-deoxy-4′-demethyl-epipodophyllotoxin (13h)

Yield: 38%; mp: 167–169 °C; $[\alpha]_D^{21}$ – 94.7° (c 0.5, $CHCl_3$); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.03 (s, 1H, –$CONHSO_2$–), 7.71 (d, 2H, 2″,6″-H, J = 8 Hz), 7.28 (d, 2H, 3″,5″-H, J = 8 Hz), 6.99 (br s, 1H, 4-NH), 6.71 (s, 1H, 5-H), 6.46 (s, 1H, 8-H), 6.18 (s, 2H, 2′,6′-H), 6.01 and 5.98 (ABq, 2H, –OCH_2O–), 5.00–4.97 (m, 1H, 4-H), 4.43 (d, 1H, 1-H, J = 7.2 Hz), 4.03–4.01 (m, 1H, 11β-H), 3.89–3.87 (m, 1H, 11α-H), 3.62 (s, 6H, 3′,5′-OCH_3), 3.15–3.13 (m, 1H, 2-H), 2.93–2.86 (m, 1H, 3-H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 174.5, 151.5, 147.7, 147.3, 146.9, 141.7, 137.3, 134.9, 132.2, 130.8, 129.9, 129.6, 116.5, 114.7, 109.6, 108.6, 101.5, 67.8, 56.2, 50.4, 44.4, 42.8, 40.7, 37.5; ESI-MS: m/z 601.0 $[M+H]^+$.

4.2.9. 4β-*N*-(4″-Chlorophenylsulfonylurea)-4-deoxy-4′-demethyl-epipodophyllotoxin (13i)

Yield 35%; mp: 162–164 °C; $[\alpha]_D^{21}$ – 50.8° (c 0.5, $CHCl_3$); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.02 (s, 1H, –$CONHSO_2$–), 8.36 (d, 2H, 2″,6″-H, J = 8.8 Hz), 7.64 (d, 2H, 3″,5″-H, J = 7.6 Hz), 6.78 (s, 1H, 5-H), 6.62 (s, 1H, 8-H), 6.18 (s, 2H, 2′,6′-H), 6.00 and 5.98 (ABq, 2H, –OCH_2O–), 4.91–4.87 (m, 1H, 4-H), 4.70 (d, 1H, 1-H, J = 5.2 Hz), 4.17 (t, 1H, 11α-H, J = 7.6 Hz), 3.60 (s, 6H, 3′,5′-OCH_3), 3.17–3.12 (m, 1H, 2-H), 2.88–2.87 (m, 1H, 3-H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 174.5, 147.3, 140.9, 129.4, 129.2, 126.9, 108.5, 106.5, 101.4, 56.1, 55.4, 30.8; ESI-MS: m/z 640.4 $[M+Na]^+$.

4.2.10. 4β-*N*-(Benzylsulfonylurea)-4-deoxy-4′-demethyl-epipodophyllotoxin (13j)

Yield: 50%; mp: 158–160 °C; $[\alpha]_D^{21}$ – 31.0° (c 0.5, $CHCl_3$); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.33 (s, 1H, –$CONHSO_2$–), 7.40 (s, 5H, Ar-H), 6.91 (br s, 1H, 4-NH), 6.85 (s, 1H, 5-H), 6.54 (s, 1H, 8-H), 6.23 (s, 2H, 2′,6′-H), 6.04 and 6.01 (ABq, 2H, –OCH_2O–), 5.09–5.08 (m, 1H, 4-H), 4.49 (d, 1H, 1-H, J = 4.8 Hz), 4.39 (t, 1H, 11β-H, J = 8 Hz), 3.79 (t, 1H, 11α-H, J = 10 Hz), 3.63 (s, 6H, 3′,5′-OCH_3), 3.17(s, 2H, –SO_2CH_2–), 3.10 (dd, 1H, 2-H, J = 14.4, 4.8 Hz), 2.98–2.95 (m, 1H, 3-H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 174.5, 152.4, 147.5, 147.3, 146.8, 134.8, 132.5, 130.9, 130.1, 129.9, 129.7, 128.7, 127.3, 109.6, 109.1, 108.5, 101.5, 68.4, 58.1, 56.1, 47.9, 42.9, 40.8, 36.8, 30.8; ESI-MS: m/z 596.5 $[M]^+$.

4.2.11. 4β-*N*-(2″,4″-Dimethoxyphenylsulfonylurea)-4-deoxy-4′-demethyl-epipodophyllotoxin (13k)

Yield: 42%; mp: 166–168 °C; $[\alpha]_D^{21}$ – 21.0° (c 0.5, $CHCl_3$); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.28 (s, 1H, –$CONHSO_2$–), 7.74 (d, 1H, 6″-H, J = 8.8 Hz), 6.86 (d, 1H, 4-NH, J = 8 Hz), 6.73 (d, 1H, 3″-H, J = 2 Hz), 6.71 (s, 1H, 5-H), 6.65 (dd, 1H, 5″-H, J = 8.8, 2 Hz), 6.53 (s, 1H, 8-H), 6.19 (s, 2H, 2′,6′-H), 6.02 and 6.01 (ABq, 2H, –OCH_2O–), 4.89–4.86 (m, 1H, 4-H), 4.51 (d, 1H, 1-H, J = 5.2 Hz), 4.11 (t, 1H, 11α-H, J = 8 Hz), 3.85 (s, 6H, 2″,4″-OCH_3), 3.60 (s, 6H, 3′,5′-OCH_3), 2.94 (dd, 1H, 2-H, J = 14.4, 5.2 Hz), 2.88–2.79 (m, 1H, 3-H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 174.3, 165.1, 158.0, 151.5, 147.5, 147.2, 146.8, 134.8, 132.5, 130.0, 129.6, 119.0, 109.6, 109.0, 108.5, 105.1, 101.5, 99.3, 68.1, 56.5, 56.1, 47.6, 42.9, 40.8, 36.7, 30.8; ESI-MS: m/z 665.1 $[M+Na]^+$.

4.2.12. 4β-*N*-(2″-Naphthylsulfonylurea)-4-deoxy-4′-demethyl-epipodophyllotoxin (13l)

Yield: 47%; mp: 176–178 °C; $[\alpha]_D^{21}$ – 16.3° (c 0.5, $CHCl_3$); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.43 (s, 1H, –$CONHSO_2$–), 8.31 (s, 1H, 3″-H), 8.23 (d, 2H, 1″,4″-H, J = 8 Hz), 8.08-8.02 (m, 4H, 5″,6″,7″,8″-H), 6.83 (s, 1H, 5-H), 6.50 (s, 1H, 8-H), 6.23 (s, 2H, 2′,6′-H), 6.00 and 5.98 (ABq, 2H, –OCH_2O–), 4.99–4.96 (m, 1H, 4-H), 4.46 (d, 1H, 1-H, J = 4.8 Hz), 4.33 (t, 1H, 11β-H, J = 8.4 Hz), 3.80 (t, 1H, 11α-H, J = 10.8 Hz), 3.62 (s, 6H, 3′,5′-OCH_3), 3.29 (dd, 1H, 2-H, J = 14.4, 5.2 Hz), 2.99–2.90 (m, 1H, 3-H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 174.7, 172.2, 170.1, 166.0, 156.4, 147.3, 146.6, 141.3, 140.3, 136.3, 134.8, 132.1, 131.8, 129.6, 129.4, 129.2, 128.5, 127.9, 127.6, 122.6, 109.5, 109.1, 108.5, 101.3, 68.2, 60.2, 56.1, 48.7, 43.0, 40.7, 36.6; ESI-MS: m/z 671.0 $[M+K]^+$.

4.2.13. 4β-*N*-(Methylsulfonylurea)-4-deoxyepipodophyllotoxin (14a)

Yield: 54%; mp: 192–194 °C; $[\alpha]_D^{21}$ – 33.3° (c 0.5, $CHCl_3$); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 6.88 (s, 1H, 5-H), 6.53 (s, 1H, 8-H), 6.26 (s, 2H, 2′,6′-H), 6.00 and 5.99 (ABq, 2H, –OCH_2O–), 5.01 (dd, 1H, 4-H, J = 7.6, 4.4 Hz), 4.54 (d, 1H, 1-H, J = 5.2 Hz), 4.34 (t, 1H,

11β-H, J = 8 Hz), 3.85–3.80 (m, 1H, 11α-H), 3.63 (s, 6H, 3′,5′-OCH_3), 3.60 (s, 3H, 4′-OCH_3), 3.22 (s, 3H, 1″-H), 3.19–3.17 (m, 1H, 2-H), 3.05–2.89 (m, 1H, 3-H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 174.6, 152.2, 147.6, 146.9, 136.5, 135.9, 132.2, 130.0, 109.6, 109.4, 108.2, 101.5, 68.6, 60.1, 55.9, 47.9, 43.2, 41.5, 40.7, 37.1, 30.9; ESI-MS: m/z 535.0 $[M+H]^+$.

4.2.14. 4β-*N*-(Ethylsulfonylurea)-4-deoxyepipodophyllotoxin (14b)

Yield: 57%; mp: 163–165 °C; $[\alpha]_D^{21}$ − 85.7° (c 0.5, $CHCl_3$); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 6.87 (s, 1H, 5-H), 6.54 (s, 1H, 8-H), 6.26 (s, 2H, 2′,6′-H), 6.00 and 5.99 (ABq, 2H, –OCH_2O–), 5.01 (dd, 1H, 4-H, J = 8, 4.8 Hz), 4.54 (d, 1H, 1-H, J = 5.2 Hz), 4.34 (t, 1H, 11β-H, J = 8 Hz), 3.79 (t, 1H, 11α-H, J = 10.4 Hz), 3.63 (s, 6H, 3′,5′-OCH_3), 3.60 (s, 3H, 4′-OCH_3), 3.20 (dd, 1H, 2-H, J = 14.4, 5.2 Hz), 2.99–2.89 (m, 1H, 3-H), 1.23 (t, 3H, 2″-H, J = 7.6 Hz); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 174.5, 152.3, 147.6, 146.9, 136.4, 135.9, 132.2, 129.8, 116.8, 109.6, 109.3, 108.2, 101.5, 97.5, 68.5, 64.1, 60.1, 55.9, 52.7, 47.2, 43.1, 40.6, 8.1; ESI-MS: m/z 549.0 $[M+H]^+$.

4.2.15. 4β-*N*-(Phenylsulfonylurea)-4-deoxyepipodophyllotoxin (14c)

Yield: 54%; mp: 158–160 °C; $[\alpha]_D^{21}$ − 34.8° (c 0.5, $CHCl_3$); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.91 (d, 2H, 2″,6″-H, J = 7.6 Hz), 7.66–7.54 (m, 3H, 3″,4″,5″-H), 6.93 (br s, 1H, 4-NH), 6.73 (s, 1H, 5-H), 6.51 (s, 1H, 8-H), 6.24 (s, 2H, 2′,6′-H), 6.01 and 5.99 (ABq, 2H, –OCH_2O–), 5.01–4.98 (m, 1H, 4-H), 4.51 (d, 1H, 1-H, J = 5.2 Hz), 4.17-4.13 (m, 1H, 11α-H), 3.65–3.60 (s, 9H, 3′,4′,5′-OCH_3), 3.21 (dd, 1H, 2-H, J = 14, 5.2 Hz), 2.96–2.87 (m, 1H, 3-H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 173.9, 166.7, 152.1, 147.7, 147.0, 140.7, 136.5, 135.8, 133.2, 133.0, 130.8, 129.6, 129.2, 127.5, 109.7, 108.2, 101.5, 60.0, 55.9, 50.6, 42.9, 40.5, 37.7; ESI-MS: m/z 596.5 $[M]^+$.

4.2.16. 4β-*N*-(4″-Methylphenylsulfonylurea)-4-deoxyepipodophyllotoxin (14d)

Yield: 49%; mp: 197–198 °C; $[\alpha]_D^{21}$ − 57.5° (c 0.5, $CHCl_3$); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.81 (d, 2H, 2″,6″-H, J = 8.4 Hz), 7.41 (d, 2H, 3″,5″-H, J = 8 Hz), 6.71 (s, 1H, 5-H), 6.52 (s, 1H, 8-H), 6.22 (s, 2H, 2′,6′-H), 5.99 (ABq, 2H, –OCH_2O–), 4.89 (dd, 1H, 4-H, J = 8, 4.8 Hz), 4.51 (d, 1H, 1-H, J = 5.6 Hz), 4.15 (t, 1H, 11β-H, J = 8 Hz), 4.01–3.95 (m, 1H, 11α-H), 3.61 (s, 6H, 3′,5′-OCH_3), 3.59 (s, 3H, 4′-OCH_3), 3.15–3.10 (m, 1H, 2-H), 2.89–2.79 (m, 1H, 3-H), 2.39 (s, 3H, 4″-CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 174.4, 152.1, 147.4, 146.8, 136.4, 135.8, 132.0, 129.3, 127.3, 109.5, 109.2, 108.2, 101.5, 68.3, 60.0, 55.9, 47.7, 43.1, 40.5, 36.9, 30.9, 21.2; ESI-MS: m/z 649.3 $[M+K]^+$.

4.2.17. 4β-*N*-(4″-Chlorophenylsulfonylurea)-4-deoxyepipodophyllotoxin (14e)

Yield: 42%; mp: 160–162 °C; $[\alpha]_D^{21}$ − 45.1° (c 0.5, $CHCl_3$); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.93 (d, 2H, 2″,6″-H, J = 8.4 Hz), 7.69 (d, 2H, 3″,5″-H, J = 8.8 Hz), 6.73 (s, 1H, 5-H), 6.52 (s, 1H, 8-H), 6.23 (s, 2H, 2′,6′-H), 5.99 and 5.98 (ABq, 2H, –OCH_2O–), 4.90 (dd, 1H, 4-H, J = 7.6, 4.4 Hz), 4.51 (d, 1H, 1-H, J = 5.2 Hz), 4.18 (t, 1H, 11α-H, J = 8.4 Hz), 3.61 (s, 6H, 3′,5′-OCH_3), 3.59 (s, 3H, 4′-OCH_3), 3.17 (dd, 1H, 2-H, J = 14.4, 5.6 Hz), 2.92–2.81 (m, 1H, 3-H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 174.4, 152.1, 147.5, 146.8, 136.5, 135.8, 132.1, 129.8, 129.4, 129.3, 108.2, 101.5, 61.6, 60.1, 55.9, 49.8, 47.8, 43.1, 36.8; ESI-MS: m/z 631.5 $[M+H]^+$.

4.2.18. 4β-*N*-(Methylsulfonylurea)-4-deoxyanhydroepipodophyllotoxin (21a)

Yield: 47%; mp: 153–155 °C; $[\alpha]_D^{21}$ − 51.1° (c 0.5, $CHCl_3$); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 6.88 (d, 1H, 4-NH, J = 8.4 Hz), 6.83 (s, 1H, 5-H), 6.45 (s, 1H, 8-H), 6.14 (s, 2H, 2′,6′-H), 5.99 and 5.97 (ABq, 2H, –OCH_2O–), 5.00 (dd, 1H, 4-H, J = 8, 3.6 Hz), 4.34 (d, 1H, 1-H, J = 5.2 Hz), 4.04–3.99 (m, 2H, 11-H), 3.92 (t, 1H, 12β-H, J = 7.6 Hz), 3.79 (t, 1H, 12α-H, J = 7.6 Hz), 3.64 (s, 6H, 3′,5′-OCH_3), 3.61 (s, 3H, 4′-OCH_3), 2.77–2.73 (m, 1H, 3-H), 2.42–2.40 (m, 1H, 2-H), 1.98 (s, 3H, 1″-CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 170.4, 152.4, 152.0, 147.2, 146.4, 136.7, 136.1, 132.5, 130.6, 109.5, 109.1, 108.9, 107.3, 107.1, 101.2, 69.0, 67.5, 67.4, 60.0, 59.9, 59.8, 55.9, 47.0, 46.9, 44.7, 44.6, 41.4, 41.3, 38.6, 20.8, 14.2; ESI-MS: m/z 543.0 $[M+Na]^+$.

4.2.19. 4β-*N*-(4″-Isopropylphenylsulfonylurea)-4-deoxyanhydroepipodophyllotoxin (21b)

yield 48.8%; mp: 197–199 °C; $[\alpha]_D^{21}$ − 76.5° (c 0.5, $CHCl_3$); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.81 (d, 2H, 2″,6″-H, J = 8 Hz), 7.48 (d, 2H, 3″,5″-H, J = 8 Hz), 6.80 (d, 1H, 4-NH, J = 8.4 Hz), 6.65 (s, 1H, 5-H), 6.43 (s, 1H, 8-H), 6.11 (s, 2H, 2′,6′-H), 5.97 and 5.96 (ABq, 2H, –OCH_2O–), 4.88–4.87 (m, 1H, 4-H), 4.31 (d, 1H, 1-H, J = 3.6 Hz), 3.88–3.87 (m, 2H, 11-H), 3.62 (s, 6H, 3′,5′-OCH_3), 3.59 (s, 3H, 4′-OCH_3), 3.00–2.98 (m, 2H, 12-H), 2.72–2.68 (m, 1H, 3-H), 2.34 (m, 2H, 2-H, 7″-H), 1.21 (d, 6H, 7″-CH_3, J = 6.8 Hz); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 154.7, 152.8, 151.6, 147.7, 146.8, 137.8, 137.1, 136.6, 132.9, 130.9, 127.8, 127.4, 109.9, 109.2, 107.7, 101.6, 69.3, 67.7, 60.4, 56.3, 47.3, 45.0, 38.9, 33.9, 23.9; ESI-MS: m/z 624.7 $[M]^+$.

4.2.20. 4β-*N*-(2″,4″-Dimethoxyphenylsulfonylurea)-4-deoxyanhydroepipodophyllotoxin (21c)

Yield: 45%; mp: 152–154 °C; $[\alpha]_D^{21}$ − 40.4° (c 0.5, $CHCl_3$); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.69 (d, 1H, 6″-H, J = 8.8 Hz), 6.74–6.62 (m, 4H, 4-NH, 5-H, 3″-H, 5″-H), 6.44 (s, 1H, 8-H), 6.11 (s, 2H, 2′,6′-H), 5.98 (ABq, 2H, –OCH_2O–), 4.84 (dd, 1H, 4-H, J = 8.4, 4 Hz), 4.33 (d, 1H, 1-H, J = 5.6 Hz), 3.91–3.88 (m, 2H, 11-H), 3.84 (s, 3H, 2″-OCH_3), 3.81 (s, 3H, 4″-OCH_3), 3.62 (s, 6H, 3′,5′-OCH_3), 3.59 (s, 3H, 4′-OCH_3), 2.98 (t, 1H, 12β-H, J = 8.4 Hz), 2.71 (t, 1H, 12α-H, J = 8.8 Hz), 2.37–2.27 (m, 2H, 3, 2-H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 172.1, 164.8, 157.8, 152.4, 152.3, 151.3, 147.2, 146.5, 136.7, 136.1, 132.3, 130.8, 119.1, 109.5, 109.4, 108.8, 107.3, 107.1, 105.0, 101.2, 101.0, 99.2, 68.9, 67.1, 59.9, 56.3, 46.7, 44.6, 40.0, 38.4, 21.1; ESI-MS: m/z 643.1 $[M+H]^+$.

4.3. Antiproliferative activity assay

Antiproliferative activity was determined by the sulforhodamine B (SRB) colorimetric assay as previously described.[26] 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.

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