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An efficient one-pot synthesis of benzothiazolo-4β-anilino-podophyllotoxin congeners: DNA topoisomerase-II inhibition and anticancer activity

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ABSTRACT

An efficient one-pot iodination methodology for the synthesis of benzothiazolo-4β-anilino-podophyllotoxin (**5a**–**h**) and benzothiazolo-4β-anilino-4-*O*-demethylepipodophyllotoxin (**6a**–**h**) congeners has been successfully developed by using zirconium tetrachloride/sodium iodide. Interestingly, this protocol demonstrates enhancement of stereoselectivity apart from the improvement in the yields in comparison to previous methods reported for such related podophyllotoxin derivatives. These compounds have been designed and synthesized using association strategy by coupling of 4β-podophyllotoxin and 4β-demethylepipodophyllotoxin with a variety of substituted aminoaryl benzothiazoles. Some of the representative compounds have been evaluated for their cytotoxicity against selected human cancer cell lines and DNA topoisomerase-II inhibition activity.

A variety of reagent systems have been reported in the literature for the iodination of alcohols.[1–6] However, most of the reaction procedures suffer from several drawbacks such as long reaction times, harsh reaction conditions, non-commercially available materials and tedious work-up procedures. In this article, we wish to report the synthesis of 4β-arylaminopodophyllotoxin derivatives employing zirconium tetrachloride in combination with sodium iodide ($ZrCl_4$/NaI) as an efficient and selective reagent. This reagent system has not been explored much in the literature except for the iodination of simple benzyl as well as allyl alcohols.[7] Moreover, zirconium salts have been biologically screened that exhibit lower toxicities and, for example,[8] the LD_{50} of $ZrCl_4$ given orally to rats is 1688 mg kg^{-1}. In this context we decided to explore this protocol for the one-pot conversion of structurally diverse and sterically hindered 4β-podophyllotoxin to their iodides. These iodo intermediates have been utilised for the preparation of a new class of substituted benzothiazolo-4β-anilino-podophyllotoxins.

Podophyllotoxin and its derivatives are important members of the lignane class of natural products derived from plants of the genus *Podophyllum* (**1**, Fig. 1).[9,10] This antimitotic cyclolignan, was extensively investigated as an antitumour agent, but failed in clinical studies due to severe gastrointestinal side effects.[11] However, some of the semisynthetic derivatives led to the development of new anticancer drugs like etoposide (**2**) and teniposide (**3**),[12–15] that are currently being used in clinic for the treatment of a variety of malignancies (lung and testicular cancers, lymphoma, nonlymphocytic leukaemia, and glioblastoma multiforme).[16] Etoposide is known to block the catalytic activity of DNA topoisomerase II by stabilizing a cleavage enzyme-DNA complex in which the DNA is cleaved and covalently linked to the enzyme.[17] Inspite of its widely usage in clinic there are several limitations for these semisynthetic podophyllotoxin derivatives, which has inspired to further search for new effective anticancer agents based on this scaffold.[18]

Similarly, the structurally simple and synthetically accessible, 2-(4-aminophenyl)benzothiazole class of compounds have shown potent and selective antitumour properties. The original member of this series, 2-(4-aminophenyl)benzothiazole **4a** (CJM 126) is found to exhibit potent and selective in vitro activity against certain cancer cell lines.[19] This compound subsequently superseded as a lead compound with the discovery that certain 3′-substituents (CH_3, Cl, Br, I) conferred low nanomolar growth inhibitory activity in an extended set of human tumour cell lines, from which 2-(4-amino-3-methylphenyl)benzothiazole **4b** (DF 203) has been selected as a potent and selective antitumour agent (Fig. 1).[20] In a research programme, we have been involved in the design and synthesis of new podophyllotoxin derivatives as potential DNA topoisomerase-II inhibiting anticancer agents.[21–30] In continuation of these efforts, we have undertaken the synthesis of some new analogues of podophyllotoxin by linking it to a benzothiazole moiety through an arylamino spacer at C-4 position of the podophyllotoxin scaffold with a view to combine the pharmacological characteristics of both these chromophores.

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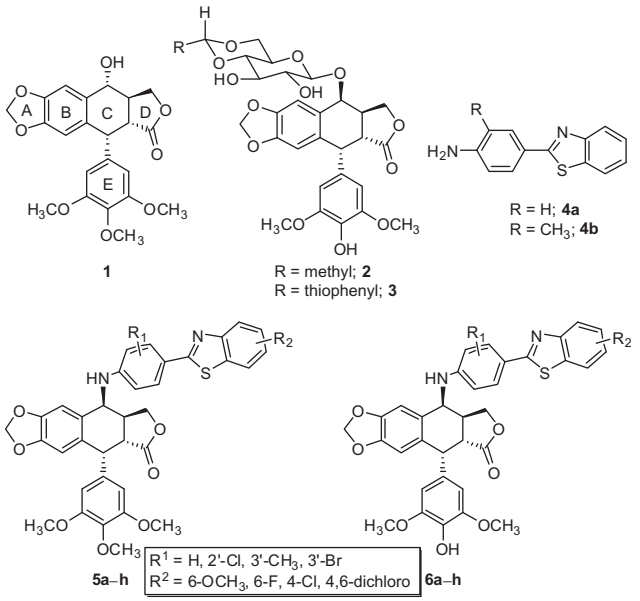
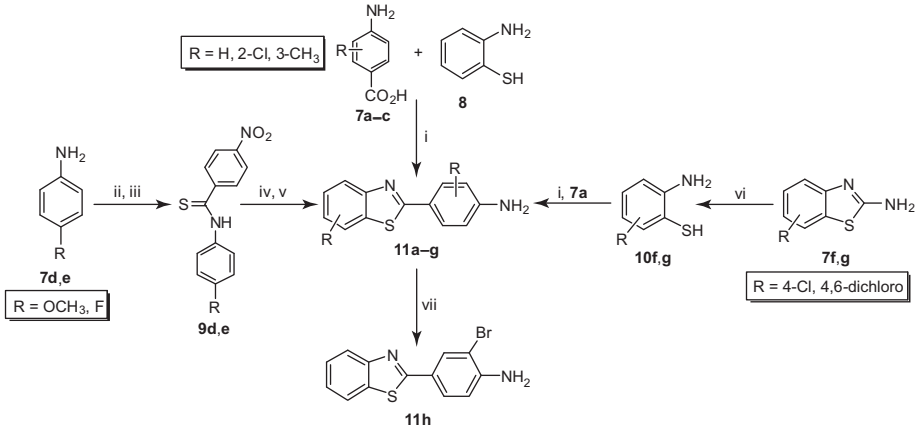


Figure 1. Naturally occurring podophyllotoxin (**1**), clinically using etoposide (**2**), teniposide (**3**), CJM-126 (**4a**), DF-203 (**4b**) and the new substituted benzothiazolo-4β-anilino-podophyllotoxin (**5a**–**h**) and 4β-*N*-demethylepipodophyllotoxin congeners (**6a**–**h**).

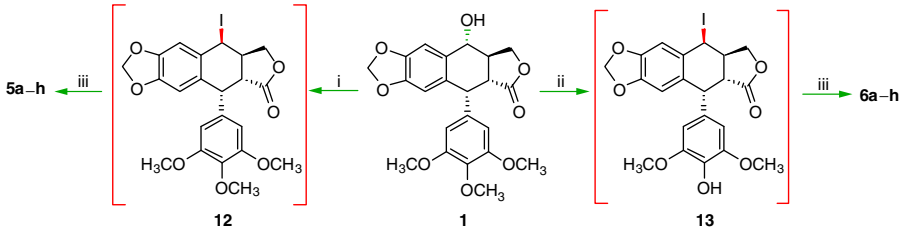
A new class of substituted benzothiazolo-4β-anilino-podophyllotoxin (**5a**–**h**) and 4β-*N*-demethylepipodophyllotoxin congeners (**6a**–**h**) were synthesized by the coupling of various substituted and unsubstituted 2-(4-aminophenyl)benzothiazoles (**11a**–**h**) with podophyllotoxin (**1**). Initially, we synthesized a number of aryl substituted-benzothiazoles with an amino functionality. All the substituted-benzothiazoles were prepared by employing the literature methods. Compounds **11a**–**c** were accomplished in a single step by the mixing of substituted aminobenzoic acid (**7a**–**c**) and 2-aminothiophenol (**8**) in polyphosphoric acid (PPA) at higher temperatures.[31] Then, the 2-(4-aminophenyl)-6-methoxybenzothiazole (**11d**) and 2-(4-aminophenyl)-6-fluorobenzothiazole (**11e**) were obtained from different approaches.[32] Reaction of substituted anilines (**7d**,**e**) with *p*-nitrobenzoylchloride yields the appropriate benzanilides (**9d**,**e**). These were converted to their thiobenzanilides by using Lawesson's reagent. The thiobenzanilides were then converted to their 2-(4-nitrophenyl)-6-benzothiazoles by the Jacobson's cyclization[21] employing $K_3[Fe(CN)_6]$. Reduction of these nitro compounds with stannous chloride affords the benzothiazoles (**11d**,**e**) as shown in Scheme 1. Later, 2-(4-aminophenyl)-4-chlorobenzothiazole (**11f**) and 2-(4-aminophenyl)-4,6-dichlorobenzothiazole (**11g**) were prepared from substituted 2-aminobenzothiazoles (**7f**,**g**). These benzothiazoles converted to their appropriate 2-aminothiophenols by using 50% aqueous KOH and ethylene glycol (**10f**,**g**). The thiophenol was heated with 4-aminobenzoic acid (**7a**) in polyphosphoric acid to afford the appropriate benzothiazoles (**11f**,**g**) as shown in Scheme 1. Finally, the 2-(4-amino-3-bromophenyl)benzothiazole (**11h**) was synthesized from 2-(4′-aminophenyl)benzothiazole (**11a**) in the presence of bromine.

2-(4′-Aminophenyl)benzothiazole linked podophyllotoxin congeners (**5a**–**h** and **6a**–**h**) were synthesized by employing $ZrCl_4$/NaI reagent system. The reaction proceeds via the formation of 4β-iodoepipodophyllotoxin (**12**) or 4β-iodo-4-*O*-demethylpodophyllotoxin (**13**) as intermediates. Moreover, as the iodo intermediates are highly reactive and susceptible to nucleophilic attack in the presence of slightest moisture, these have been subjected in the crude form for the next step of the reaction to yield the final products. The synthetic strategy developed for the preparation of these 2-(4-aminophenyl)benzothiazole substituted popodophyllotoxin congeners is shown in Scheme 2. It is interesting to observe that by employing CH_3CN as the solvent produced 2-(4-aminophenyl)benzothiazole substituted epipodophyllotoxin analogues (**5a**–**h**)[33] without 4-*O*-demethylation in 70–85% yield, whereas as the use of CH_2Cl_2 as the solvent produces 2-(4′-aminophenyl)benzothiazole substituted 4′-*O*-demethylepipodophyllotoxin (**6a**–**h**)[33] in 60–75% yield. The substitution via S_N1 mechanism occurs on the C-4 benzylic carbonium ion, wherein the bulky pendent aromatic ring-E and $ZrCl_4$/NaI directs the substitution stereoselectively resulting in high yield of C-4β isomer as the main product. The present method employing $ZrCl_4$/NaI provides the desired products in good yields with the required stereoselectivity. The ratio for the formation of C-4β and C-4α isomers is about 9:1.

A series of newly synthesized compounds (**5a**–**h** and **6a**–**h**) have been screened for their cytotoxicity on selected human cancer cell lines (Zr-75-1, MCF7, KB, Gurav, DWD, A2780, Colon, A549, PC3, oral, lung, and SiHa). However, few compounds **5a**,**b**,**d** and **6a**,**b**,**f** are showing significant in vitro cytotoxic activity against the selected cancer cell lines that comprise of colon (Colo 205), lung (Hop-62), and oral (HT1080, DWD). The screening procedure is based on the routine method adopted by the Developmental Therapeutic Programme of National Cancer Institute (NCI)[34] and the results are described in Table 1. All the new compounds were compared with the standard drug tested, that is, adriamycin (ADR) and podopyllo-



Scheme 1. Reagents and conditions: (i) PPA, 220 °C, 3 h, 50−60%; (ii) *p*-nitrobenzoylchloride, pyridine, reflux, 2 h, 90%; (iii) Lawesson's reagent, chlorobenzene, reflux, 3 h, 73%; (iv) $K_3[Fe(CN)_6]$, 30% aq NaOH, reflux, 30 min, 25%; (v) $SnCl_2{\cdot}2H_2O$, ethanol, ref lux, 4 h, 90%; (vi) 50% KOH, ethylene glycol, reflux, 48 h, 60%; (vii) **11a**, Br_2, CH_2Cl_2, −5 °C, 2 min, 75%.



Scheme 2. Reagents and conditions: (i) $ZrCl_4$/NaI, CH_3CN rt, 15 min; (ii) $ZrCl_4$/NaI, CH_2Cl_2, rt, 5 h; (iii) **11a**–**h**, THF, $BaCO_3$, rt, 8 h, 70–85%.

Table 1
In vitro (IC_{50} μM) cytotoxicity data for some representative compounds

Compounds	Colo 205 (colon)	Hop-62 (lung)	Oral	
			HT1080	DWD
5a	8.1	>80	8.2	22
6a	2.7	30.1	9.7	2.3
5d	7.0	15.2	9.5	5.2
6b	8.3	8.6	>80	6.5
5f	7.1	60.3	9.1	7.3
6f	8.3	10.1	>80	9.1
ADR	5.1	5.4	4.7	2.8
Podophyllotoxin	5.0	13.1	8.1	6.1

ADR = Adriamycin, podophyllotoxin are the control drug.

toxin, with the concentration of the compound produces to 50% inhibition of cell growth (IC_{50}).

It is interesting to note that one of the most active compounds being the 4′-*O*-demethyl-4β-[4-(1,3-benzothiazole-2-yl)anilino]-4-desoxypodophyllotoxin derivative **6a** with an IC_{50} 2.7 μM (colon 205) and 2.3 μM (DWD) as more potent than the standard adriamycin and podophyllotoxin (5.1 and 5.0 μM). These new podophyllotoxin analogues exhibit significant anticancer activity against Colo 205, HT1080, and DWD cell lines, and moderate activity against Hop-62 cell lines as illustrated in Table 1. It is observed from the data that by linking benzothiazole moiety thorough an arylamino spacer at C-4 position of the podophyllotoxin scaffold has shown moderate improvement in the activity for certain cell lines. However, by combining these two chromophores the activity has not altered significantly in comparison to the activity of the individual pharmacophores.

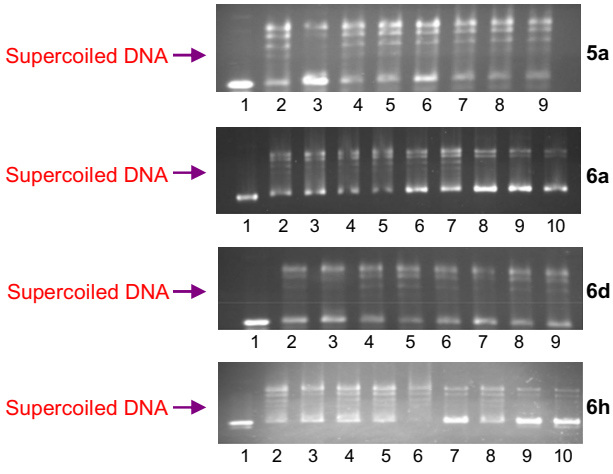


Figure 2. Lane number 1 contain supercoiled PBR322 DNA in the absence of topisomerase II, where the upper band is nicked open circular (OC) DNA and the lower brighter band is supercoiled covalently closed circular (CCC) DNA. Lane 2 shows CCC DNA related by topoisomerase II. There are various topoisomers and the upper thick band of relaxed CCC DNA co-migrates with nicked DNA, which is also relaxed. Lane 3 shows the inhibitory effect of *m*-AMSA. Lane 4–10 shows the inhibitory effects of **5a**, **6a**, **6d** and **6h** in increasing concentrations, that is, 10, 50, 100, 200, 300, 500 μM, and 1 mM, respectively.

DNA topoisomerase-II inhibition is the pharmacological target of clinical relevance for most of the podophyllotoxin semisynthetic derivatives. Therefore, some of the representative compounds (**5a** and **6a**,**d**,**h**) have been evaluated for its inhibition by employing previous procedure.[35] The compounds **6a** and **6h** from this series exhibited interesting DNA topoisomerase-II inhibition activity, suggesting that these new molecules also exhibit biological activity based on this mechanism similar to the etoposide prototypes. All these compounds exhibited comparable in vitro inhibition of topo-II catalytic activity to *m*-AMSA and the results are illustrated in Figure 2. Moreover, these results agree with the hypothesis that the bulky substitution at C-4 position can enhance the activity profile for such compounds.

In conclusion, we have designed and synthesized a series of benzothiazolo-4β-anilino-podophyllotoxin derivatives by employing zirconium tetrachloride and sodium iodide reagent system, almost in a stereoselective manner with enhanced yields. These compounds have also shown potent in vitro cytotoxic activity with better topoisomerase-II inhibition. Moreover, efforts are in progress to improve the bioavailability of such compounds.

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33. *4β-[4-(1,3-Benzothiazole-2-yl)anilino]-4-desoxypodophyllotoxin* (**5a**). To a solution of podophyllotoxin (**1**) (414 mg, 1 mmol) in dry CH_3CN (10 mL), sodium iodide (298 mg, 2 mmol) was added and stirred for 5 min. To this stirred suspension $ZrCl_4$ (458 mg, 2 mmol) was added drop wise at room temperature and the stirring was continued for another 15 min at same temperature. This solution was then evaporated in vacuo and used for the next reaction without further purification. To the crude product, anhydrous $BaCO_3$ (395 mg, 2 mmol) and 2-(4′-aminophenyl)benzothiazole (**11a**) (271 mg, 1.2 mmol) in 10 mL of dry THF under nitrogen was added and stirred for 8 h at room temperature. The reaction mixture was filtered, diluted with EtOAc and washed with water, 10% aqueous sodium thiosulphate solution, dried over anhydrous Na_2SO_4 and purified via column chromatography using ethyl acetate/hexane (1:1) as eluent to afford **5a** in 80% yield. Mp: 172–175 °C, $[\alpha]_D^{25} = -120.0$ ($c = 1.0$ in $CHCl_3$), ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.69–3.04 (m, 2H), 3.77 (s, 6H), 3.8 (s, 3H), 3.97 (t, 1H, $J = 9.6$ Hz), 4.29–4.40 (m, 2H), 4.55 (d, 1H, $J = 4.4$ Hz), 4.63–4.76 (m, 1H), 5.96 (d, 2H, $J = 4.7$ Hz), 6.27 (s, 2H), 6.52 (s, 1H), 6.63 (d, 2H, $J = 8.1$ Hz), 6.8 (s, 1H), 7.32 (t, 1H, $J = 7.4$ Hz), 7.44 (t, 1H, $J = 7.4$ Hz), 7.84 (d, 1H, $J = 8.1$ Hz), 7.96 (m, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 38.6, 41.7, 43.5, 52.1, 56.3, 60.6, 68.6, 101.5, 108.6, 109.2, 109.8, 112.1, 121.4, 123.7, 124.5, 126.1, 129.4, 129.8, 131.8, 134.5, 135.0, 147.5, 148.2, 149.8, 152.7, 154.2, 168.0, 174.3. IR (KBr): υ 3362, 2925, 1772, 1604, 1505, 1483 cm^{-1}. MS (FAB): *m/z* 623 [M$^+$+H]. *4β-[4-(1,3-Benzothiazole-2-yl)-3-chloroanilino]-4-desoxypodophyllotoxin* (**5b**). Yield (443 mg, 85%), Mp: 156–160 °C, $[\alpha]_D^{25} = -122$ ($c = 1.0$ in $CHCl_3$), ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.93–3.07 (m, 2H), 3.73 (s, 6H), 3.76 (s, 3H), 3.94 (t, 1H, $J = 9.0$ Hz), 4.38 (t, 2H, $J = 7.5$ Hz), 4.45–4.60 (m, 1H), 4.72–4.89 (m, 1H), 5.93 (s, 2H), 6.23 (s, 2H), 6.46 (s, 1H), 6.6 (d, 1H, $J = 8.3$ Hz), 6.70 (s, 1H), 6.75 (s, 1H), 7.34 (t, 1H, $J = 8.3$ Hz), 7.45 (t, 1H, $J = 8.3$ Hz), 7.87 (d, 1H, $J = 7.5$ Hz), 7.98 (d, 1H, $J = 8.3$ Hz), 8.21 (d, 1H, $J = 8.3$ Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 38.3, 41.6, 43.4, 51.9, 56.1, 56.2, 68.5, 101.6, 108.1, 109.2, 109.7, 110.9, 113.0, 121.1, 121.5, 122.5, 124.7, 126.0, 129.1, 131.7, 132.9, 134.1, 134.8, 135.4, 137.1, 147.4, 148.3, 149.5, 152.3, 152.6, 164.3, 174.2. IR (KBr): υ 3398, 2924, 1771, 1615, 1508, 1481 cm^{-1}. MS (FAB): *m/z* 659 [M$^+$+2]. *4β-[4-(1,3-Benzothiazole-2-yl)-2-methylanilino]-4-desoxypodophyllotoxin* (**5c**). Yield (426 mg, 84%), Mp: 152–155 °C, $[\alpha]_D^{25} = -139.0$ ($c = 1.0$ in $CHCl_3$), ^{1}H NMR (200 MHz, $CDCl_3$): δ 2.16 (s, 3H), 2.79–3.08 (m, 1H), 3.15–3.39 (dd, 1H, $J = 14.6$, 4.4 Hz), 3.78 (s, 6H), 3.82 (s, 3H), 3.90 (t, 1H, $J = 9.2$ Hz), 4.42 (t, 1H, $J = 7.7$ Hz), 4.63 (d, 1H, $J = 4.2$ Hz), 4.85–5.09 (m, 2H), 5.98 (s, 2H), 6.32 (s, 2H), 6.56 (s, 1H), 6.8 (s, 1H), 7.10–7.58 (m, 4H), 7.82–8.04 (m, 3H). IR (KBr): υ 3411, 2923, 1773, 1607, 1515, 1483 cm^{-1}. MS (FAB): *m/z* 637 [M$^+$+H]. *4β-[4-(6-Methoxy-1,3-benzothiazole-2-yl)anilino]-4-desoxypodophyllotoxin* (**5d**). Yield (415 mg, 80%), Mp: 102–105 °C, $[\alpha]_D^{25} = -85.0$ ($c = 1.0$ in $CHCl_3$), ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.69–3.01 (m, 2H), 3.75 (s, 6H), 3.78 (s, 3H), 3.87 (s, 3H), 4.39–4.45 (m, 2H), 4.51 (d, 1H, $J = 3.7$ Hz), 4.73–5.03 (m, 1H), 5.92 and 5.95 (ABq, 2H, $J = 1.5$ Hz), 6.25 (s, 2H), 6.48 (s, 1H), 6.60 (d, 2H, $J = 8.3$ Hz), 6.77 (s, 1H), 7.0 (dd, 1H, $J = 9.0$, 2.2 Hz), 7.28 (d, 1H, $J = 2.2$ Hz), 7.80–7.88 (m, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 38.5, 41.7, 43.5, 52.0, 55.7, 56.2, 60.7, 68.6, 101.5, 104.2, 108.1, 109.1, 109.8, 112.1, 115.1, 122.8, 123.8, 129.0, 129.7, 131.7, 131.9, 134.9, 135.6, 137.1, 147.5, 148.2, 148.4, 149.2, 152.5, 157.3, 165.7, 174.4. IR (KBr): υ 3366, 2924, 2853, 1772, 1606, 1506, 1481 cm^{-1}. MS (FAB): *m/z* 675 [M$^+$+Na]. *4β-[4-(6-Fluoro-1,3-benzothiazole-2-yl)anilino]-4-desoxypodophyllotoxin* (**5e**). Yield (410 mg, 81%), Mp: 168–172 °C, $[\alpha]_D^{25} = -130.0$ ($c = 1.0$ in $CHCl_3$), ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.79–3.01 (m, 2H), 3.75 (s, 6H), 3.78 (s, 3H), 3.83–3.99 (m, 1H), 4.34–4.54 (m, 3H), 4.75–5.18 (m, 1H), 5.93 (s, 1H), 5.96 (s, 1H), 6.25 (s, 2H), 6.49 (s, 1H), 6.61 (d, 2H, $J = 8.6$ Hz), 6.77 (s, 1H), 7.15 (m, 1H), 7.5 (dd, 1H, $J = 8.6$, 2.6 Hz), 7.84–7.91 (m, 3H). IR (KBr): υ 3334, 2912, 2839, 1774, 1604, 1506, 1481 cm^{-1}. MS (FAB): *m/z* 641 [M$^+$+H]. *4β-[4-(4-Chloro-1,3-benzothiazole-2-yl)anilino]-4-desoxypodophyllotoxin* (**5f**). Yield (420 mg, 83%), Mp: 136–140 °C, $[\alpha]_D^{25} = -117.0$ ($c = 1.0$ in $CHCl_3$), ^{1}H NMR (200 MHz, $CDCl_3$): δ 2.79–3.01 (m, 2H), 3.76 (s, 6H), 3.80 (s, 3H), 4.37–4.49 (m, 2H), 4.53–4.67 (m, 1H), 4.75–5.45 (m, 1H), 5.93 and 5.97 (ABq, 2H, $J = 1.5$ Hz), 6.26 (s, 2H), 6.5 (s, 1H), 6.59 (d, 2H, $J = 9.0$ Hz), 6.80 (s, 1H), 7.21–7.39 (m, 1H), 7.44 (d, 1H, $J = 8.3$ Hz), 7.72 (d, 1H, $J = 8.3$ Hz), 7.96 (d, 2H, $J = 8.3$ Hz). IR (KBr): υ 3392, 2923, 1773, 1610, 1506, 1481 cm^{-1}. MS (FAB): *m/z* 658 [M$^+$+H]. *4β-[4-(4,6-Dichloro-1,3-benzothiazole-2-yl)anilino]-4-desoxy podophyllotoxin* (**5g**). Yield (460 mg, 84%), Mp: 160–163 °C, $[\alpha]_D^{25} = -102.0$ ($c = 0.5$ in $CHCl_3$), ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.69–3.02 (m, 2H), 3.76 (s, 6H), 3.8 (s, 3H), 4.38–4.49 (m, 2H), 4.55–4.64 (m, 1H), 4.75–4.99 (m, 1H), 5.95 (s, 1H), 5.98 (s, 2H), 6.28 (s, 2H), 6.5 (s, 1H), 6.61 (d, 2H, $J = 8.3$ Hz), 6.79 (s, 1H), 7.48 (s, 1H), 7.71 (s, 1H), 8.95 (d, 2H, $J = 8.3$ Hz). IR (KBr): υ 3390, 2908, 1773, 1605, 1508, 1481 cm^{-1}. MS (FAB): *m/z* 691 [M$^+$+H]. *4β-[4-(1,3-Benzothiazole-2-yl)-2-bromoanilino]-4-desoxypodophyllotoxin* (**5h**). Yield (452 mg, 81%), Mp: 125–128 °C, $[\alpha]_D^{25} = -90.0$ ($c = 1.0$ in $CHCl_3$), ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.89–3.10 (m, 1H), 3.18 (dd, 1H, $J = 14.6$, 4.5 Hz), 3.78 (s, 6H), 3.82 (s, 3H), 4.42 (t, 1H, $J = 7.5$ Hz), 4.68 (d, 1H, $J = 3.8$ Hz), 4.88–5.46 (m, 2H), 6.01 (s, 2H), 6.35 (s, 2H), 6.58 (s, 1H), 6.63 (d, 1H, $J = 8.0$ Hz), 6.79 (s, 1H), 7.32–7.55 (m, 2H), 7.85–8.1 (m, 3H), 8.29 (d, 1H, $J = 2.1$ Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 38.4, 41.7, 43.5, 52.3, 56.2, 60.7, 68.3, 101.6, 107.6, 108.3, 109.0, 110.0, 121.4, 122.6, 124.8, 126.3, 128.2, 129.2, 131.8, 132.0, 134.5, 134.3, 137.7, 145.9, 147.8, 148.5, 152.6, 154.0, 166.2, 174.0. IR (KBr): υ 3394, 2924, 2854, 1776, 1594, 1510, 1484 cm^{-1}. MS (FAB): *m/z* 740 [M$^+$+K]. *4′-O-Demethyl-4β-[4-(1,3-benzothiazole-2-yl)anilino]-4-desoxypodophyllotoxin* (**6a**). Yield (310 mg, 62%), Mp: 143–147 °C, $[\alpha]_D^{25} = -116.0$ ($c = 1.0$ in $CHCl_3$), ^{1}H NMR (200 MHz, DMSO-d_6+$CDCl_3$): δ 2.69–3.04 (m, 1H), 3.28 (dd, 1H, $J = 14.3$, 4.4 Hz), 3.76 (s, 6H), 3.88–4.16 (m, 2H), 4.34 (t, 1H, $J = 8.9$ Hz), 4.53 (d, 1H, $J = 4.5$ Hz), 4.85 (br s, 1H), 5.95 (d, 2H, $J = 4.6$ Hz), 6.28 (s, 2H), 6.50 (s, 1H), 6.72 (d, 2H, $J = 8.9$ Hz), 6.8 (s, 1H), 7.28 (t, 1H, $J = 7.1$ Hz), 7.4 (t, 1H, $J = 7.1$ Hz), 7.84 (m, 4H). IR (KBr): υ 3392, 2914, 1773, 1610, 1510, 1483 cm^{-1}. MS (FAB): *m/z* 626 [M$^+$+NH$_4$]. *4-O-Demethyl-4β-[4-(1,3-benzothiazole-2-yl)-3-chloroanilino]-4-desoxypodophyllotoxin* (**6b**). Yield (340 mg, 65%), Mp: 157–160 °C, $[\alpha]_D^{25} = -124.0$ ($c = 1.0$ in $CHCl_3$), ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.79–3.02 (m, 2H), 3.8 (s, 6H), 4.25–4.40 (m, 2H), 4.55–4.65 (m, 1H), 4.75–4.99 (m, 1H,), 5.40 (br s, 1H), 5.93 and 5.98 (ABq, 2H, $J = 1.5$ Hz), 6.29 (s, 2H), 6.51 (s, 1H), 6.6 (dd, 1H, $J = 9.0$, 2.2 Hz), 6.69 (d, 1H, $J = 2.2$ Hz), 6.77 (s, 1H), 7.37 (t, 1H, $J = 8.3$ Hz), 7.48 (t, 1H, $J = 8.3$ Hz), 7.9 (d, 1H, $J = 7.5$ Hz), 8.03 (d, 1H, $J = 7.5$ Hz), 8.22 (d, 1H, $J = 9.0$ Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 38.3, 41.8, 43.3, 52.0, 56.4, 68.4, 101.5, 107.2, 107.8, 109.1, 109.8, 111.1, 112.9, 121.1, 122.7, 124.8, 126.2, 127.4, 129.3, 130.3, 132.0, 133.0, 134.1, 135.5, 136.6, 146.4, 147.0, 147.5, 148.3, 149.3, 174.3. IR (KBr): υ 3395, 2920, 1772, 1607, 1520, 1482 cm^{-1}. MS (FAB): *m/z* 646 [M$^+$+2]. *4-O-Demethyl-4β-[4-(1,3-benzothiazole-2-yl)-2-methylanilino]-4-desoxypodophyllotoxin* (**6c**). Yield (381 mg, 75%), Mp: 133–135 °C, $[\alpha]_D^{25} = -130.0$ ($c = 1.0$ in $CHCl_3$), ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.21 (s, 3H), 2.89–3.10 (m, 1H), 3.18 (dd, 1H, $J = 14.5$, 4.5 Hz), 3.8 (s, 6H), 3.90 (t, 1H, $J = 9.7$ Hz), 4.41 (t, 1H, $J = 7.6$ Hz), 4.64 (d, 1H, $J = 4.2$ Hz), 4.88–5.67 (m, 1H), 6.01 (d, 2H, $J = 4.1$ Hz), 6.35 (s, 2H), 6.58 (s, 1H), 6.69 (s, 1H), 7.14–7.50 (m, 4H), 7.82–8.05 (m, 3H). IR (KBr): υ 3397, 2907, 1773, 1607, 1508, 1484 cm^{-1}. MS (FAB): *m/z* 623 [M$^+$+H]. *4-O-Demethyl-4β-[4-(6-methoxy-1,3-benzothiazole-2-yl)anilino]-4-desoxypodophyllotoxin* (**6d**). Yield (329 mg, 63%), Mp: 142–146 °C, $[\alpha]_D^{25} = -109.0$ ($c = 1.0$ in $CHCl_3$), ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.89–3.06 (m, 2H), 3.80 (s, 6H), 3.89 (s, 3H), 4.35–4.56 (m, 2H), 4.60 (d, 1H, $J = 4.0$ Hz), 4.77–4.90 (m, 1H), 5.40 (br s, 1H), 5.95 (s, 1H), 5.98 (s, 1H), 6.30 (s, 2H), 6.52 (s, 1H), 6.61 (d, 2H, $J = 8.8$ Hz), 6.79 (s, 1H), 7.02 (dd, 1H, $J = 9.2$, 2.4 Hz), 7.31 (d, 1H, $J = 2.4$ Hz), 7.82–7.91 (m, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 38.4, 41.7, 43.3, 52.0, 55.7, 56.4, 68.6, 101.5, 104.2, 107.8, 109.1, 109.8, 112.0, 115.1, 122.8, 123.7, 128.9, 129.7, 130.4, 131.9, 134.0, 146.4, 147.4, 148.2, 148.6, 149.2, 157.2, 174.5. IR (KBr): υ 3367, 2922, 1771, 1605, 1509, 1484 cm^{-1}. MS (FAB): *m/z* 677 [M$^+$+K]. *4-O-Demethyl-4β-[4-(6-fluoro-1,3-benzothiazole-2-yl)anilino]-4-desoxypodophyllotoxin* (**6e**). Yield (378 mg, 74%), Mp: 200–205 °C, $[\alpha]_D^{25} = -128.0$ ($c = 1.0$ in $CHCl_3$), ^{1}H NMR (300 MHz, DMSO-d_6+$CDCl_3$): δ 2.89–3.00 (m, 1H), 3.24 (dd, 1H, $J = 14.1$, 5.2 Hz), 3.7 (s, 6H), 3.80 (t, 1H, $J = 10.4$ Hz), 4.29 (t, 1H, $J = 7.4$ Hz), 4.48 (d, 1H, $J = 5.2$ Hz), 4.82–5.13 (m, 1H), 5.90 (s, 2H), 6.23 (s, 2H), 6.45 (s, 1H), 6.67 (d, 2H, $J = 8.9$ Hz), 6.75 (s, 1H), 7.10–7.39 (m, 1H), 7.52 (dd, 1H, $J = 8.1$, 2.9 Hz), 7.76–7.89 (m, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 38.5, 41.8, 43.3, 52.1, 56.4, 68.6, 101.5, 107.5, 107.8, 109.1, 109.9, 112.1, 114.4, 114.7, 123.1, 123.3, 123.5, 129.2, 129.6, 130.3, 132.0, 143.1, 146.4, 147.6, 148.3, 149.5, 150.7, 158.5, 161.7, 167.7, 174.5. IR (KBr): υ 3356, 2903, 2834, 1773, 1605, 1509, 1483 cm^{-1}. MS (FAB): *m/z* 627 [M$^+$+H]. *4-O-Demethyl-4β-[4-(4-chloro-1,3-benzothiazole-2-yl)anilino]-4-desoxypodophyllotoxin* (**6f**). Yield (346 mg, 66%), Mp: 155–158 °C, $[\alpha]_D^{25} = -124.0$ ($c = 1.0$ in $CHCl_3$), ^{1}H NMR (200 MHz, $CDCl_3$): δ 2.80–3.08 (m, 2H), 3.8 (s, 6H), 4.35–4.55 (m, 2H), 4.60 (d, 1H, $J = 3.5$ Hz), 4.80–5.18 (m, 1H), 5.42 (br s, 1H), 5.94 and 5.97 (ABq, 2H, $J = 1.4$ Hz), 6.32 (s, 2H), 6.53 (s, 1H), 6.62 (d, 2H, $J = 8.5$ Hz), 6.79 (s, 1H), 7.24–7.39 (m, 1H), 7.47 (dd, 1H, $J = 8.2$, 1.4 Hz), 7.74 (dd, 1H, $J = 8.2$, 1.4 Hz), 7.78 (d, 2H, $J = 8.5$ Hz). IR (KBr): υ 3364, 2925, 1773, 1610, 1508, 1485 cm^{-1}. MS (FAB): *m/z* 645 [M$^+$+2]. *4-O-Demethyl-4β-[4-(4,6-dichloro-1,3-benzothiazole-2-yl)anilino]-4-desoxypodophyllotoxin* (**6g**). Yield (330 mg, 60%), Mp: 155–158 °C, $[\alpha]_D^{25} = -124.0$ ($c = 0.5$ in $CHCl_3$), ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.01–3.77 (m, 2H), 3.79 (s, 6H), 3.88–4.23 (m, 1H), 4.34–4.47 (m, 1H), 4.54–4.69 (m, 1H), 4.86–5.14 (m, 1H), 5.38 (br s, 1H), 5.98 (s, 2H), 6.32 (s, 2H), 6.53 (s, 1H), 6.73 (d, 2H, $J = 8.5$ Hz), 6.83 (s, 1H), 7.46 (s, 1H), 7.78 (s, 1H), 7.89 (d, 2H, $J = 8.5$ Hz). IR (KBr): υ 3362, 2925, 1774, 1597, 1507, 1482 cm^{-1}. MS (FAB): *m/z* 695 [M$^+$+NH$_4$]. *4-O-Demethyl-4β-[4-(1,3-benzothiazole-2-yl)-2-bromoanilino]-4-desoxypodophyllotoxin* (**6h**). Yield (339 mg, 60%), Mp: 177–180 °C, $[\alpha]_D^{25} = -91.0$ ($c = 1.0$ in $CHCl_3$), ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.69–3.13 (m, 2H), 3.81 (s, 6H), 4.4 (t, 1H, $J = 7.5$ Hz), 4.68 (d, 1H, $J = 3.8$ Hz), 4.85–5.17 (m, 2H), 5.45 (s, 1H), 6.01 (s, 2H), 6.35 (s, 2H), 6.59 (s, 1H), 6.64 (d, 1H, $J = 8.0$ Hz), 6.79 (s, 1H), 7.32–7.56 (m, 2H), 7.85–8.06 (m, 3H), 8.29 (d, 1H, $J = 2.2$ Hz). IR (KBr): υ 3393, 2923, 2852, 1774, 1598, 1520, 1487 cm^{-1}. MS (FAB): *m/z* 689 [M$^+$+2].
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