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Synthesis of 4 β -amido and 4 β -sulphonamido Analogues of Podophyllotoxin as Potential Antitumour Agents

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Abstract—The new 4 β -amido analogues of podophyllotoxin or 4'-*O*-demethylepipodophyllotoxin have been prepared either by the coupling of 4 β -amino podophyllotoxin or 4 β -amino-4'-*O*-demethyl epipodophyllotoxin with the corresponding acids in presence of DCC in dichloromethane or by treating the appropriate acid chloride or sulphonyl chloride in presence of Et₃N. These 4 β -amido and 4 β -sulphonamido derivatives of podophyllotoxin have been evaluated for their cytotoxicity against six human cancer cell lines. Some of these analogues have shown promising anticancer activity.

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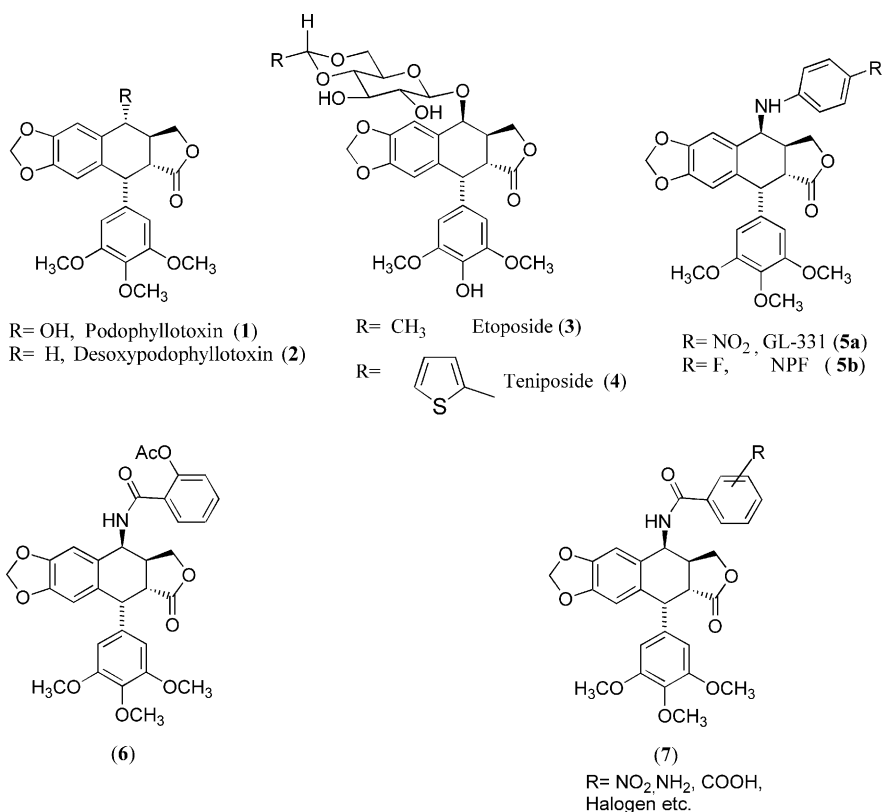
Introduction

Podophyllotoxin (**1**) and desoxypodophyllotoxin (**2**) are two well known naturally occurring aryltetralin lignans and the former is the major constituent of a number of plant species of the *podophyllum* family.^{1,2} Both these compounds are cytotoxic and their derivatives are in use as antitumour agents, for example etoposide^{3,4} (**3**) and teniposide⁵ (**4**). Etoposide is the most widely used anticancer agent for the treatment of leukemia, testicular cancer and small cell lung cancer. Its clinical efficacy is due to its ability to inhibit the enzyme DNA-topoisomerase II. These podophyllotoxin lignans block the catalytic activity of DNA-topoisomerase II by stabilizing a cleavable enzyme DNA-complex in which the DNA is cleaved and covalently linked to enzyme.⁶ This lead has stimulated a renewed interest in the chemical and biochemical studies of podophyllotoxin derived antitumour agents.⁷ The replacement of the C-4 sugar moiety of etoposide (VP-16) with a nonsugar substitution has proven to be significant in overcoming the drug resistance of etoposide.⁸ The C-4 non-sugar substitutions can be linked through *O*-, *S*- or *N*-linkage. In general, the *O*-linked derivatives (ethers, esters) and the *S*-linked derivatives (thioethers) are inactive or show lower activity in comparison to the *N*-linked

congeners,^{9–11} for example NPF (**5a**) and GL-331 (**5b**). Presently, GL-331 is undergoing phase II clinical trials against gastric carcinoma, colon cancer, non-small cell carcinoma, and etoposide-resistant malignancies.¹² It has also been indicated in the literature that bulky substitution at the C4 position usually enhances the cytotoxicity and DNA topoisomerase II inhibition activity.^{11b,c} It is observed that, amongst the C4-*N*-substituted congeners of podophyllotoxin that have been synthesized and developed, C4 β -*N*-amidopodophyllotoxin derivatives have received less attention. Further, some of these compounds have shown improved biological activity in comparison to etoposide.

Recently, C4 β -amido analogue¹³ has been synthesized and evaluated for its biological activity. This compound (**6**) exhibits superior anticancer activity compared to etoposide against some of the human cancer cell lines. In earlier studies a series of 4 β -amido aryl substituted podophyllotoxin derivatives (**7**)¹⁴ have been prepared and evaluated for their biological activity. It is observed from the results that some of these compounds exhibit enhanced DNA-topoisomerase II inhibition in comparison to etoposide. In view of the available information on structure-activity relationship studies and in continuation of our ongoing project on the design and development of structurally modified podophyllotoxin congeners,¹⁵ it was considered of interest to prepare C4 β -amido and sulphonamido analogues of epipodophyllotoxin and 4'-*O*-demethyl epipodophyllotoxin.¹⁶

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Hence, their preparation and biological evaluation, particularly anticancer activity is reported herein.

Chemistry

The synthesis of C4 β -amido and sulphonamido analogues of podophyllotoxin have been carried out from podophyllotoxin as schemes in Schemes 1 and 2. The key intermediate for the preparation of the new analogues is C4 β -aminopodophyllotoxin (**9**) C4 β -amino-4'-*O*-demethylpodophyllotoxin (**13**). Podophyllotoxin is mesylated followed by azidation to give C4 β -azidopodophyllotoxin (**8**). This upon reduction over Pd/C provides a 7:3 mixture of C4 β -aminopodophyllotoxin and C4 α -aminopodophyllotoxin. The required C4 β -aminopodophyllotoxin (**9**) has been purified by column chromatography by employing chloroform–ethyl acetate (2:1) as eluent, where as C4 β -amino-4'-*O*-demethylpodophyllotoxin (**13**) has been prepared from C4 β -azido-4'-*O*-demethylpodophyllotoxin. (**12**) The azido compound (**12**) has been obtained from the azidation of 4'-*O*-demethylepipodophyllotoxin (**11**) which in turn has been obtained from the sequential in situ iodination in presence of methane sulphonic acid/NaI reagent system¹⁷ followed by hydrolysis in presence of barium carbonate (Scheme 1).

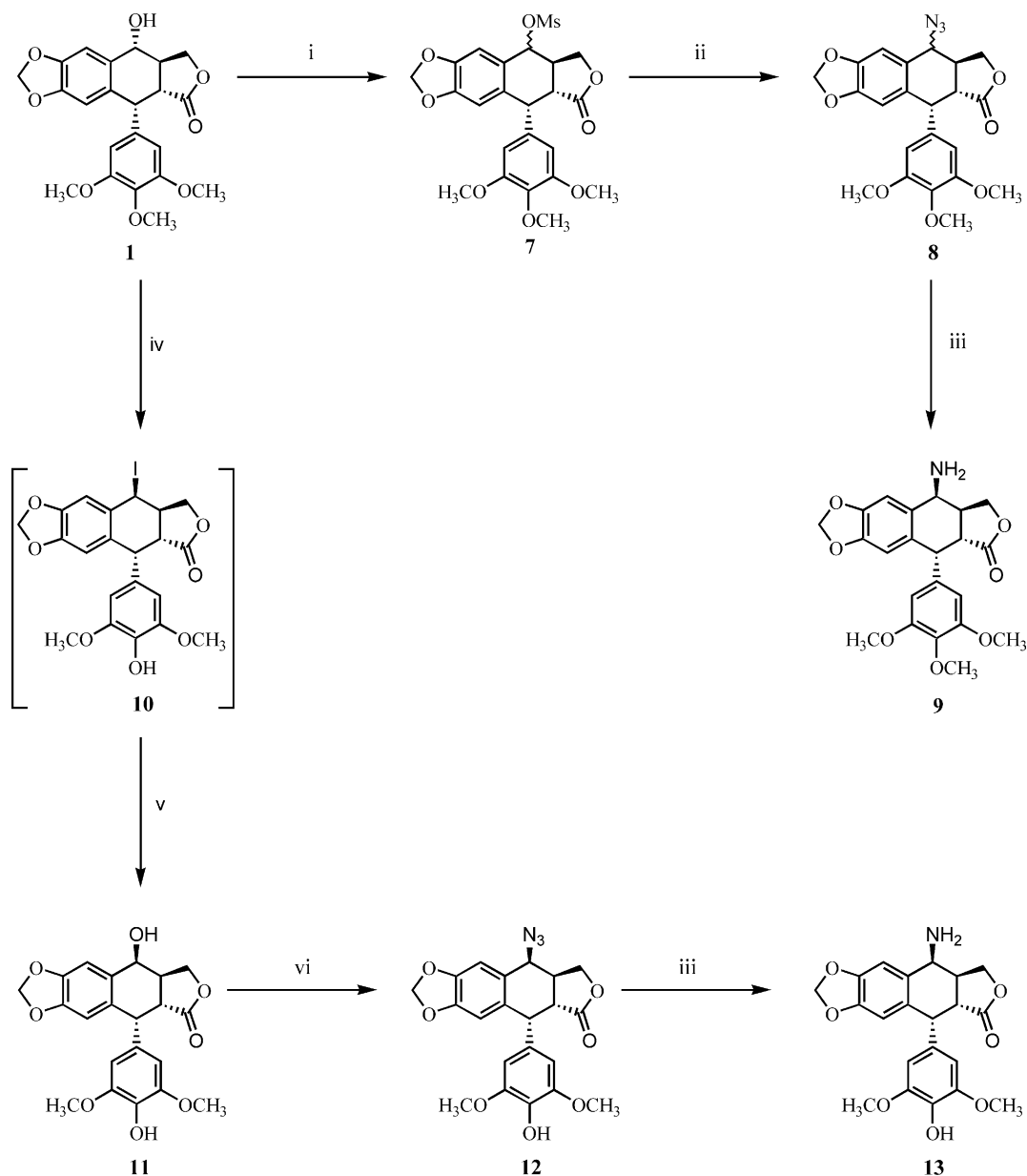
The intermediates, C4 β -aminopodophyllotoxin (**9**) and C4 β -amino-4'-*O*-demethyl podophyllotoxin (**13**) have been coupled with 2-benzoyl substituted benzoic acids in presence of *N,N'*-dicyclohexylcarbodiimide (DCC) to afford the corresponding C4 β -amido aryl substituted podophyllotoxin and their 4'-*O*-demethyl derivatives

(**14–19**). This reaction has also been performed by employing 2-benzoyl substituted benzoic acid chlorides to produce the corresponding amido analogues. It has been observed that the work up is cleaner and simpler and yields are improved when these coupling reactions are carried out by acid chloride method. Similarly, aryl substituted sulphonyl chlorides have been coupled to the intermediates (**9**) and (**13**) to afford the corresponding C4 β -sulphonamido-podophyllotoxin analogues (**20–23**). Furthermore, substituted nicotinic acid has been coupled to the intermediates (**9**) and (**13**) in presence of DCC to give the corresponding C4 β -amino nicotinyl derivatives of podophyllotoxin (**24–27**). Attempts to prepare these derivatives from nicotinic acid chloride did not produce the desired C4 β -amidopodophyllotoxin products (Scheme 2).

Biological Evaluation

These 4 β -amido and 4 β -sulphonamido analogues of podophyllotoxin have been tested for their cytotoxic activities against six human cancer cell lines that comprise of DU145, HT29, MCF7, MCF7ADR, NCIH460 and U251. The screening procedure is based on the routine method adopted by the Developmental Therapeutic Programme of National Cancer Institute (NCI), Bethesda, MD, USA.

4 β -Amido-2-substituted benzophenone analogues of podophyllotoxin (**14–19**) show moderate anticancer activity. It is interesting to observe that one of the compounds (**20**) in 4 β -sulphonamido aryl substituted podophyllotoxin derivatives (**20–23**) is highly potent

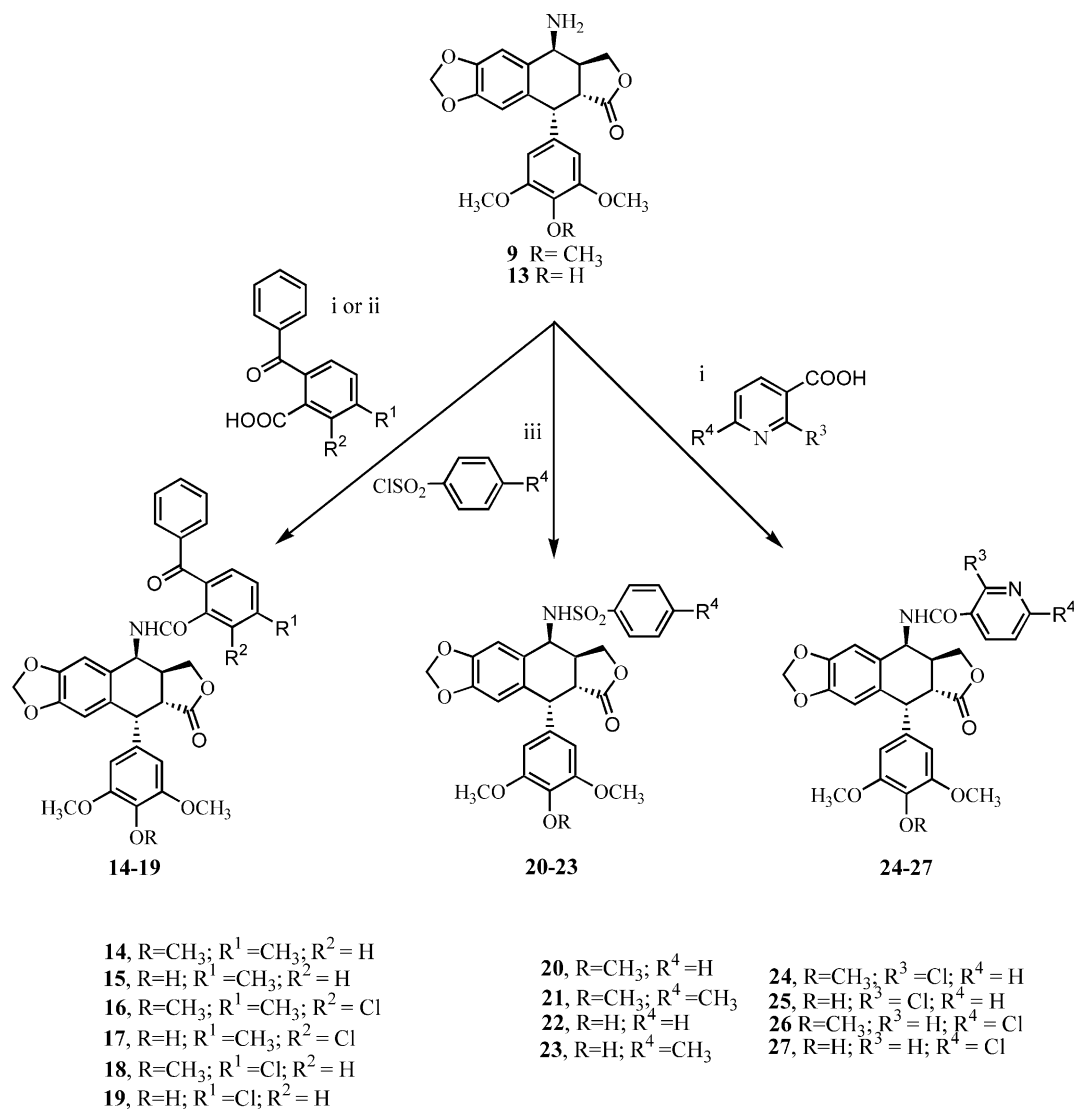


Scheme 1. Reagents: (i) MeSO₂Cl/Et₃N; (ii) NaN₃/DMF; (iii) Pd/C/H₂; (iv) MeSO₃H/NaI, CH₂Cl₂, rt, 5 h; (v) CH₃COCH₃/H₂O, BaCO₃, rt, 30 min; (vi) NaN₃/CF₃COOH.

against all the six cancer cell lines. In the same series a methyl substituent in the aryl ring at the 4''-position slightly reduces the activity. However, the 4'-*O*-demethyl analogue of compound (**20**) drastically reduces the activity in almost all the cancer cell lines. The analogues of 4β-nicotinylamido substitution (**24–27**) exhibit promising cytotoxic activity. A number of these new analogues possess comparable or superior in vitro anticancer activity. In the literature mostly 4'-*O*-demethyl derivatives have been investigated for their anticancer activity. Another interesting aspect observed in this investigation is that a large number of analogues exhibit improved cytotoxicity particularly in the presence of 4'-*O*-methyl functionality (Table 1).

Conclusion

In conclusion, we can assert that the promising results obtained for the new 4β-amido and 4β-sulphanamido analogues of podophyllotoxin described in this study make them potential candidates to take up further synthesis and evaluation of such new analogues. This investigation has also highlighted the importance of 4'-*O*-methyl functionality in such analogues for anticancer activity. This will allow researcher in this area to prepare podophyllotoxin related derivatives incorporating such functionality. Some of the potential candidates are undergoing detailed investigation particularly biochemical studies.



Scheme 2. Reagents: (i) DCC, CH₂Cl₂, rt, 5 h; (ii) RCOCl, CH₂Cl₂, rt, 5 h; (iii) CH₂Cl₂, rt, 5 h, Et₃N.

Table 1. GI₅₀ values of some of the 4β-amido and 4β-sulphonamido derivatives of podophyllotoxin

Compd	GI ₅₀ (μM)					
	DU145 (Prostate)	HT29 (Colon)	MCF7 (Breast)	MCF7ADR (Adr. res. breast)	NCIH460 (Lung)	U251 (CNS)
Etoposide ^a	0.8	59	4.3	116	1.1	6.4
14	1.9	1.1	5.4	4.0	3.0	2.6
16	8.8	3.5	4.3	1.3	3.8	4.7
18	3.4	3.2	8.2	2.0	5.2	2.8
19	3.3	9.5	10.0	15.5	0.3	3.9
20	0.02	<0.004	0.03	0.01	0.02	0.01
21	2.3	1.7	5.0	1.3	4.3	2.7
22	27.7	28.9	46.2	11.2	30.8	25.0
24	0.16	0.03	0.05	0.59	0.08	0.02
25	2.7	1.8	3.5	1.9	3.7	3.3
26	0.05	<0.019	0.12	0.95	0.03	0.04

^aValues from NCI database.

Experimental

NMR spectra are recorded on Varian Gemini 200 MHz spectrometer, using TMS as an internal reference. IR spectra are recorded on Perkin-Elmer model 683 or 1310 spectrometers with sodium chloride optics. Mass

spectra are recorded on CEC-21-100B, Finnigan Mat 1210 or Micromass 7070 spectrometers operating at 70 eV using a direct inlet system. Optical rotations are measured on Jasco Dip 360 digital polarimeter. Melting points are determined on an electro thermal melting point apparatus and are uncorrected. TLC is performed

with E. Merck precoated silica gel plates (60F-254) with iodine as a developing agent. Acme, India silica gel, 100–200 mesh for column chromatography is used.

4 β -Aminopodophyllotoxin (9)

To a solution of 4 β -azidopodophyllotoxin (1.54 g, 3.53 mmol) in 80 mL of ethyl acetate was added 300 mg, of 10% palladium on activated carbon. The mixture was stirred overnight under hydrogen, the reaction mixture was filtered, and the filtrate was evaporated. The crude product was purified by column chromatography by employing chloroform/ethyl acetate (2:1) as an eluent to give compound **9**. Yield 75%; mp 120–124 °C; $[\alpha]_D^{25}$ –62.0; IR (CHCl₃) 3400, 2900, 1770, 1500, 1480, 1410 cm⁻¹; ¹H NMR (CDCl₃) δ 2.8 (m, 1H), 3.2 (m, 1H), 3.8 (s, 9H), 4.3 (m, 2H), 4.6 (d, 1H, J =2.63 Hz), 4.8 (d, 1H, J =7.8 Hz), 6.00 (d, 2H, J =5.2 Hz), 6.2 (s, 2H), 6.5 (s, 1H), 6.8 (s, 1H); MS 413 (M⁺), 396, 229, 185, 168.

4 β -Amino-4'-O-demethylepipodophyllotoxin (13). To a solution of 4 β -azido-4'-O-demethyl-epipodophyllotoxin (1.5 g, 3.53 mmol) in 80 mL of ethyl acetate was added 300 mg, of 10% palladium on activated carbon. The mixture was stirred overnight under hydrogen. The reaction mixture was filtered and the filtrate was evaporated. The crude product was purified by column chromatography by employing chloroform/ethyl acetate (2:1) as an eluent to give compound **13**. Yield 70%; mp 134–138 °C; $[\alpha]_D^{25}$ –63.0; IR (CHCl₃) 3520, 3350, 2900, 1770, 1500, 1480, 1410 cm⁻¹; ¹H NMR (CDCl₃) δ 2.8 (m, 1H), 3.2 (m, 1H), 3.8 (s, 6H), 4.3 (m, 2H), 4.6 (d, 1H, J =8.0 Hz), 4.8 (d, 1H, J =6.0 Hz), 5.3 (s, 1H), 6.00 (d, 2H, J =6.1 Hz), 6.2 (s, 2H), 6.5 (s, 1H), 6.8 (s, 1H); MS 400 (M⁺), 387, 229, 185, 168.

General procedure for compounds 14–27

Method A. 4 β -Aminopodophyllotoxin or 4 β -amino-4'-O-demethylepipodophyllotoxin (0.5 mmol) was dissolved in 20 mL of dried dichloromethane, followed by addition of appropriate carboxylic acid (0.5 mmol) and DCC (0.5 mmol). The reaction mixture was stirred at room temperature for 5 h. Two drops of acetic acid was added. The reaction mixture was filtered. The filtrate was washed with saturated solution of NaHCO₃, 10% hydrochloric acid and water respectively, dried over anhydrous Na₂SO₄ and chromatographed through silica gel using ethyl acetate/hexane (3:7) as an eluent to obtain the pure products.

Method B. To a solution containing 4 β -aminopodophyllotoxin or 4 β -amino-4'-O-demethylepipodophyllotoxin (0.5 mmol) triethylamine (1 mL) in 20 mL of dichloromethane, appropriate acid chloride or sulphonyl chloride (0.05 mmol) in 10 mL of dichloromethane was added under nitrogen and stirred at room temperature for about 5 h till the completion of the reaction as monitored by TLC. The reaction mixture was washed with water, extracted with ethyl acetate, dried over anhydrous sodium sulphate and subjected to column chromatography using ethyl acetate/hexane (3:7) as an eluent to afford pure products.

4 β -(4''-Methylbenzophenone-2''-formyl)aminopodophyllotoxin (14). This compound was prepared according to the methods described earlier employing 4-methylbenzophenone-2-carboxylic acid (120 mg, 0.5 mmol), DCC (103 mg, 0.5 mmol) and 4 β -aminopodophyllotoxin (206 mg, 0.5 mmol) to give the compound **14**. Yield: 85%; mp 173–175 °C; $[\alpha]_D^{25}$ –34.5; IR (CHCl₃) 3360, 3290, 2900, 1745, 1690, 1590 cm⁻¹; ¹H NMR (CDCl₃) δ 2.9 (s, 3H), 2.8 (m, 2H), 3.8 (s, 3H), 3.9 (s, 6H), 4.1–4.4 (m, 2H), 4.8 (m, 2H), 5.9 (s, 2H), 6.0 (s, 1H), 6.4 (s, 1H), 6.5 (s, 2H), 6.7 (d, 1H, J =6.2 Hz), 7.1 (m, 2H), 7.3 (m, 2H), 7.4 (m, 1H), 7.5 (m, 2H), 7.7 (d, 2H), 7.8 (d, 1H, J =3.8 Hz); MS 658 (M⁺ sodium salt), 635 (M⁺), 604, 552, 524, 496, 468, 439, 397, 369, 339. Anal. (C₃₇H₃₃NO₉) calcd C: 69.91, H: 5.23, N: 2.20; found C: 69.73, H: 5.17, N: 2.11.

4 β -(4''-Methylbenzophenone-2''-formyl)amino-4'-O-demethylepipodophyllotoxin (15). This compound was prepared according to the methods described earlier employing 4-methylbenzophenone-2-carboxylic acid (120 mg, 0.5 mmol), DCC (103 mg, 0.5 mmol) and 4 β -amino-4'-O-demethylepipodophyllotoxin (200 mg, 0.5 mmol) to give the compound **15**. Yield 85%; mp 184–186 °C; $[\alpha]_D^{25}$ –165.0; IR (CHCl₃) 3360, 3190, 2920, 1750, 1590 cm⁻¹; ¹H NMR (CDCl₃) δ 2.4 (s, 3H), 3.2 (m, 2H), 3.8 (m, 2H), 3.9 (s, 6H), 4.1 (m, 2H), 4.4 (m, 2H), 6.0 (d, 2H, J =3.5 Hz), 6.1 (s, 1H), 6.4 (s, 1H), 6.5 (s, 1H), 6.7 (m, 2H), 7.2 (m, 4H), 7.4 (m, 3H), 7.6 (m, 1H), 7.8 (d, 1H, J =5.1 Hz); MS 622 (M⁺), 468, 383, 346, 325. Anal. (C₃₆H₃₁NO₉) calcd C: 69.55, H: 5.02, N: 2.25; found C: 69.32, H: 5.05, N: 2.18.

4 β -(3''-Chloro-4''-methylbenzophenone-2''-formyl)aminopodophyllotoxin (16). This compound was prepared according to the methods described earlier employing 3-chloro-4-methylbenzophenone-2-carboxylic acid (137 mg, 0.5 mmol), DCC (103 mg, 0.5 mmol) and 4 β -aminopodophyllotoxin (206 mg, 0.5 mmol) to give the product **16**. Yield 50%; mp 156–159 °C; $[\alpha]_D^{25}$ –63.46; IR (CHCl₃): 3300, 3260, 2900, 1725, 1670, 1580 cm⁻¹. ¹H NMR (CDCl₃) δ 2.4 (s, 3H), 2.8 (m, 2H), 3.8 (s, 9H), 3.9–4.2 (m, 2H), 4.5–4.6 (m, 2H), 5.8 (d, 1H, J =5.4 Hz), 5.9 (s, 2H), 6.2 (s, 2H), 6.5 (s, 1H), 6.7 (s, 1H), 7.0 (m, 1H), 7.3 (m, 1H), 7.5 (m, 1H), 7.7 (s, 1H), 7.8 (s, 1H); MS 671 (M⁺), 578, 551, 397. Anal. (C₃₇H₃₂ClNO₉) calcd C: 66.31, H: 4.81, N: 2.09; found C: 66.24, H: 4.76, N: 2.06.

4 β -(3''-Chloro-4''-methylbenzophenone-2''-formyl)amino-4'-O-demethylepipodophyllotoxin (17). This compound was prepared according to the methods described earlier employing 3-chloro-4-methylbenzophenone-2-carboxylic acid (137 mg, 0.5 mmol), DCC (103 mg, 0.5 mmol) and 4 β -amino-4'-O-demethylepipodophyllotoxin (200 mg, 0.5 mmol) to give the compound **17**. Yield in 80%; mp 177–179 °C; $[\alpha]_D^{25}$ –26.25; IR (CHCl₃) 3300, 3280, 2900, 1745, 1690, 1590 cm⁻¹; ¹H NMR (CDCl₃) δ 2.4 (s, 3H), 2.8 (m, 2H), 3.8 (s, 6H), 4.2 (m, 2H), 4.5 (m, 1H), 5.3 (br, 1H), 5.9 (d, 2H, J =2.5 Hz), 6.2 (s, 2H), 6.5 (s, 1H), 6.7 (s, 1H), 7.1 (d, 1H, J =5.6 Hz), 7.4 (m, 2H), 7.5 (m, 3H), 7.8 (m, 2H), 8.2 (d, 1H, J =4.2 Hz); MS 656 (M⁺), 603, 577, 551, 523, 412, 397. Anal. (C₃₆H₃₀ClNO₉) calcd C: 65.90, H: 4.60, N: 2.13; found C: 65.75, H: 4.62, N: 2.09.

4 β -(4''-Chlorobenzophenone-2''-formyl)aminopodophyllotoxin (18). This compound was prepared according to the methods described earlier employing 4-chlorobenzophenone-2-carboxylic acid (130 mg, 0.5 mmol), DCC (103 mg, 0.5 mmol) and 4 β -aminopodophyllotoxin (206 mg, 0.5 mmol) to give the compound **18**. Yield 75%; mp 153–156 °C; $[\alpha]_D^{25}$ –19.25; IR (CHCl₃) 3300, 3250, 2890, 1750, 1680, 1590 cm⁻¹; ¹H NMR (CDCl₃) δ 3.3 (m, 3H), 3.5 (m, 1H), 3.7 (s, 3H), 3.8 (s, 6H), 4.1 (m, 2H), 4.5 (m, 1H), 5.6 (d, 1H), 5.9 (d, 2H, J = 3.7 Hz), 6.5 (s, 1H), 6.7 (s, 2H), 7.3 (t, 3H), 7.4 (m, 1H), 7.5 (m, 3H), 7.7 (m, 1H), 7.8 (d, 1H, J = 5.2 Hz); MS 656, 603, 577, 551, 523, 412, 397. Anal. (C₃₆H₃₀ClNO₉) calcd C: 65.90, H: 4.60, N: 2.13; found C: 65.73, H: 4.55, N: 2.10.

4 β -(4''-Chlorobenzophenone-2''-formyl)amino-4'-O-demethylepipodophyllotoxin (19). This compound was prepared according to the methods described earlier employing 4-chlorobenzophenone-2-carboxylic acid (130 mg, 0.5 mmol), DCC (103 mg, 0.5 mmol) and 4 β -amino-4'-O-demethylepipodophyllotoxin (200 mg, 0.5 mmol) to give the compound **19**. Yield 85%; mp 182–184 °C; $[\alpha]_D^{25}$ –72.0; IR (CHCl₃) 3380, 3250, 2900, 1735, 1690, 1580 cm⁻¹; ¹H NMR (CDCl₃) δ 2.8 (m, 1H), 3.4 (m, 1H), 3.8 (s, 6H), 4.2 (m, 1H), 4.6 (m, 1H), 5.2 (m, 1H), 5.3 (m, 1H), 5.8 (m, 1H), 6.0 (d, 2H, J = 2 Hz), 6.3 (s, 2H), 6.5 (s, 1H), 6.7 (s, 1H), 7.1 (m, 1H), 7.3 (m, 1H), 7.4 (m, 2H), 7.5 (m, 1H), 7.6 (m, 2H), 7.7 (m, 1H), 7.8 (d, 1H, J = 5.2 Hz); MS 642, 484, 396, 382, 369, 337. Anal. (C₃₅H₂₈ClNO₉) calcd C: 65.47, H: 4.39, N: 2.18; found C: 65.35, H: 4.32, N: 2.15.

4 β -(Benzene sulphonyl)aminopodophyllotoxin (20). This compound was prepared according to method B described earlier employing benzene sulphonyl chloride (0.105 mL, 0.5 mmol) and 4 β -aminopodophyllotoxin (206 mg, 0.5 mmol) to give the compound **20**. Yield 80%; mp 233–235 °C; $[\alpha]_D^{25}$ –76.0; IR (CHCl₃) 3350, 3200, 2860, 1745, 1560 cm⁻¹; ¹H NMR (CDCl₃) δ 2.9 (m, 2H), 3.8 (s, 9H), 4.3–4.4 (m, 1H), 4.5 (m, 2H), 5.6 (s, 1H), 5.9 (d, 2H, J = 2.5 Hz), 6.1 (s, 2H), 6.2 (s, 2H), 6.1 (s, 1H), 7.7 (m, 3H), 8.0 (d, 2H, J = 10.0 Hz); MS 553, 397. Anal. (C₂₈H₂₇NO₉S) calcd C: 60.75, H: 4.91, N: 2.53; found C: 60.62, H: 4.90, N: 2.47.

4 β -(*p*-Toulene sulphonyl)aminopodophyllotoxin (21). This compound was prepared according to method B described earlier employing *p*-toulene sulphonyl chloride (95 mg, 0.5 mmol) and 4 β -aminopodophyllotoxin (206 mg, 0.5 mmol) to give the compound **21**. Yield 85%; mp 209–212 °C; $[\alpha]_D^{25}$ –14.5; IR (CHCl₃) 3390, 3280, 2890, 1735, 1580 cm⁻¹; ¹H NMR (CDCl₃) δ 2.5 (s, 3H), 2.8 (m, 1H), 3.0 (m, 1H), 3.7 (s, 6H), 3.8 (s, 3H), 4.3 (m, 2H), 4.4 (m, 1H), 4.5 (m, 1H), 4.9 (d, 1H, J = 5.7 Hz), 5.7 (s, 1H), 5.9 (s, 2H), 6.2 (s, 2H), 6.4 (s, 1H), 7.4 (d, 2H, J = 4.5 Hz), 7.8 (d, 2H, J = 4.0 Hz); MS 567(M⁺), 397, 229. Anal. (C₂₉H₂₉NO₉S) calcd C: 61.36, H: 5.14, N: 2.46; found C: 61.29, H: 5.09, N: 2.40.

4 β -(Benzene sulphonyl)amino-4'-O-demethylepipodophyllotoxin (22). This compound was prepared according to method B described earlier employing benzene sulphonyl chloride (0.105 mL, 0.5 mmol) and 4 β -amino-4'-O-

demethylepipodophyllotoxin (200 mg, 0.5 mmol) to give the compound **22**. Yield 75%; mp 1763–165 °C; $[\alpha]_D^{25}$ –207.5. IR (CHCl₃) 3380, 3260, 2890, 1750, 1580 cm⁻¹; ¹H NMR (CDCl₃) δ 2.8 (m, 1H), 3.2 (m, 1H), 3.7 (s, 6H), 4.0 (m, 2H), 4.4 (m, 1H), 4.7 (m, 1H), 5.9 (d, 2H, J = 5.2 Hz), 6.0 (s, 1H), 6.2 (s, 2H), 6.3 (s, 1H), 6.4 (s, 1H), 7.6 (m, 3H), 7.9 (d, 2H, J = 4.3 Hz), 8.1 (s, 1H); MS 539, 397. Anal. (C₂₇H₂₅NO₉S) calcd C: 60.10, H: 4.67, N: 2.59; found C: 60.05, H: 4.64, N: 2.48.

4 β -(*p*-Toulene sulphonyl)amino-4'-O-demethylepipodophyllotoxin (23). This compound was prepared according to the method B described earlier employing *p*-toulene sulphonyl chloride (95 mg, 0.5 mmol) and 4 β -amino-4'-O-demethylepipodophyllotoxin (200 mg, 0.5 mmol) to give the compound **23**. Yield 80%; mp 154–156 °C; $[\alpha]_D^{25}$ –55.0; IR (CHCl₃) 3400, 3290, 2860, 1750, 1580 cm⁻¹; ¹H NMR (CDCl₃) δ 2.5 (s, 3H), 2.8–3.0 (m, 2H), 3.8 (s, 6H), 4.3 (m, 2H), 4.4 (m, 2H), 5.6 (s, 1H), 5.9 (d, 2H, J = 4.4 Hz), 6.2 (s, 2H), 6.4 (s, 1H), 7.3 (s, 1H), 7.4 (m, 2H), 7.8 (m, 3H), 8.1 (d, 1H, J = 3.8 Hz). MS 553, 397. Anal. (C₂₈H₂₇NO₉S) calcd C: 60.75, H: 4.91, N: 2.53; found C: 60.64, H: 4.88, N: 2.51.

4 β -(2''-Chloropyridine-3''-formyl)aminopodophyllotoxin (24). This compound was prepared according to method A described earlier employing 2-chloronicotinic acid (78 mg, 0.5 mmol), DCC (103 mg, 0.5 mmol) and 4 β -aminopodophyllotoxin (206 mg, 0.5 mmol) to give the compound **24**. Yield 50%; mp 166–168 °C; $[\alpha]_D^{25}$ –136.53; IR (CHCl₃) 3300, 3250, 2870, 1735, 1590 cm⁻¹; ¹H NMR (CDCl₃) δ 2.8–3.0 (m, 2H), 3.7 (s, 9H), 4.0–4.2 (t, 1H), 4.4–4.6 (m, 2H), 5.9 (d, 2H, J = 5.5 Hz), 6.3 (s, 2H), 6.5 (s, 1H), 6.9 (s, 1H), 7.3 (m, 1H), 8.2 (m, 1H), 8.2 (d, 1H, J = 11.1 Hz), 8.4 (m, 1H); MS 552, 523, 496, 467, 397. Anal. (C₂₈H₂₅ClN₂O₈) calcd C: 60.81, H: 4.55, N: 5.06; found C: 60.74, H: 4.41, N: 5.01.

4 β -(2''-Chloropyridine-3''-formyl)amino-4'-O-demethylepipodophyllotoxin (25). This compound was prepared according to method A described earlier employing 2-chloronicotinic acid (78 mg, 0.5 mmol), DCC (103 mg, 0.5 mmol) and 4 β -amino-4'-O-demethylepipodophyllotoxin (200 mg, 0.5 mmol) to give the compound **25**. Yield 45%; mp 180–182 °C; $[\alpha]_D^{25}$ –23.5; IR (CHCl₃) 3360, 3280, 2890, 1745, 1580 cm⁻¹; ¹H NMR (CDCl₃) δ 2.9 (m, 1H), 3.1 (m, 1H), 3.8 (s, 6H), 4.0 (m, 1H), 4.5–4.6 (m, 2H), 5.2 (br, 1H), 6.0 (d, 2H, J = 7.1 Hz), 6.3 (s, 2H), 6.6 (s, 1H), 6.8 (s, 1H), 7.0 (m, 1H), 7.1 (m, 1H), 8.2 (m, 1H), 8.5 (d, 1H, J = 4.7 Hz); MS 538, 503, 397, 367. Anal. (C₂₇H₂₃ClN₂O₈) calcd C: 60.17, H: 4.30, N: 5.19; found C: 60.12, H: 4.21, N: 5.09.

4 β -(6''-Chloropyridine-3''-formyl)aminopodophyllotoxin (26). This compound was prepared according to the methods described earlier employing 2-chloronicotinic acid (78 mg, 0.5 mmol), DCC (103 mg, 0.5 mmol) and 4 β -aminopodophyllotoxin (206 mg, 0.5 mmol) to give the compound **26**. Yield 45%; mp 173–174 °C; $[\alpha]_D^{25}$ –163.2; IR (CHCl₃) 3360, 3290, 2890, 1750, 1570 cm⁻¹; ¹H NMR (CDCl₃) δ 2.8–3.2 (m, 1H), 3.8 (s, 9H), 4.0–4.2 (m, 2H), 4.4–4.4 (m, 2H), 5.9 (d, 2H, J = 5.2 Hz), 6.3 (s, 1H), 6.5 (s, 2H), 6.9 (s, 1H), 7.0 (m, 1H), 7.4 (m, 1H),

8.3 (m, 1H), 8.8 (m, 1H), 8.9 (m, 1H); MS 552, 523, 496, 467, 397. Anal. (C₂₈H₂₅ClN₂O₈) calcd C: 60.81, H: 4.55, N: 5.06; found C: 60.69, H: 4.53, N: 5.01.

4β-(6''-Chloropyridine-3''-formyl)amino-4'-O-demethylepipodophyllotoxin (27). This compound was prepared according to the method A described earlier, employing 6-chloronicotinic acid (78 mg, 0.5 mmol), DCC (103 mg, 0.5 mmol) and 4β-amino-4'-O-demethylepipodophyllotoxin (200 mg, 0.5 mmol) to give the compound **27**. Yield: 40%; mp 185–187 °C; $[\alpha]_D^{25}$: –25.25; IR (CHCl₃): 3360, 3280, 2910, 1750, 1590 cm⁻¹; ¹H NMR (CDCl₃) δ 2.9–3.1 (m, 1H), 3.2–3.4 (m, 1H), 3.8 (s, 6H), 3.9–4.0 (m, 2H), 4.3–4.6 (m, 2H), 5.4 (br, 1H), 5.9 (d, 2H, *J* = 5.4 Hz), 6.2 (d, 2H, *J* = 2.7 Hz), 6.5 (s, 1H), 6.8 (s, 1H), 7.1 (m, 1H), 7.4 (d, 1H, *J* = 10.8 Hz), 8.2 (d, 1H, *J* = 16.2 Hz), 8.8 (d, 1H, *J* = 3.2 Hz); MS 540 (M⁺), 538 (M⁺), 503, 397, 367. Anal. (C₂₇H₂₃ClN₂O₈) calcd C: 60.17, H: 4.30, N: 5.19; found C: 60.14, H: 4.23, N: 5.13.

Biological evaluation

In vitro evaluation of cytotoxic activity. In routine screening, each agent is tested over a broad concentration range (10-fold dilutions starting from >100 μM to ~10 nM) against six human cancer cell lines comprised of different tumour types. Standard compound doxorubicin is tested in each assay as a positive control. The cells are maintained in growing condition in RPMI 1640 medium containing 10% fetal calf serum and incubated at 37 °C under 5% CO₂ atmosphere. All cell lines are inoculated onto a series of standard 96-well microtitre plate on day zero, followed by 24-h incubation in the absence of test compound. The inoculation densities used in this screen are as per the procedure of Monks et al.¹⁸ All NCEs are dissolved in DMSO and diluted further in culture medium. An aliquot of each dilution is added to the growing cells in 96 well plates and incubated for 48 h. After incubation the assay is terminated by adding 50 μL of trichloro acetic acid (TCA) and incubating at 4 °C for 30 min. The precipitated cells are washed and stained with sulphorhodamine B dye for 30 min and the excess dye is washed off with acetic acid. Adsorbed dye is solubilised in Tris base (alkaline pH) and quantitated by measuring the OD at 490 nm in an ELISA reader. GI₅₀ (concentration that inhibits the cell growth by 50%) is calculated according to the method of Boyd.¹⁹

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