Pergamon

SCIENCE DIRECT®

Bioorganic & Medicinal Chemistry 11 (2003) 5135–5142

BIOORGANIC & MEDICINAL CHEMISTRY

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

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Received 9 May 2003; accepted 18 August 2003

Abstract—The new 4β-amido analogues of podophyllotoxin or 4′-*O*-demethylepipodophyllotoxin have been prepared either by the coupling of 4β-amino podophyllotoxin or 4β-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 Et_3N. These 4β-amido and 4β-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.

Introduction

Podophyllotoxin (**1**) and desoxypodophylltoxin (**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 teniposide[5] (**4**). Etoposide is the most widely used anticancer agent for the treatment of leukemia, testicular cancer and small cell lung cancer. Its clinical efficancy is due to its ability to inhibit the enzyme DNA–topoisomerase II. These podophyllotoxin lignans block the catalytic activity of DNA–toposiomerase 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, C4β-*N*-amidopodophyllotoxin derivatives have received less attention. Further, some of these compounds have shown improved biological activity in comparison to etoposide.

Recently, C4β-amido analogue[13] 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 4β-amido aryl substituted podophylltoxin 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 C4β-amido and sulphonamido analogues of epipodophylltoxin and 4′-*O*-demethyl epipodophyllotoxin.[16]

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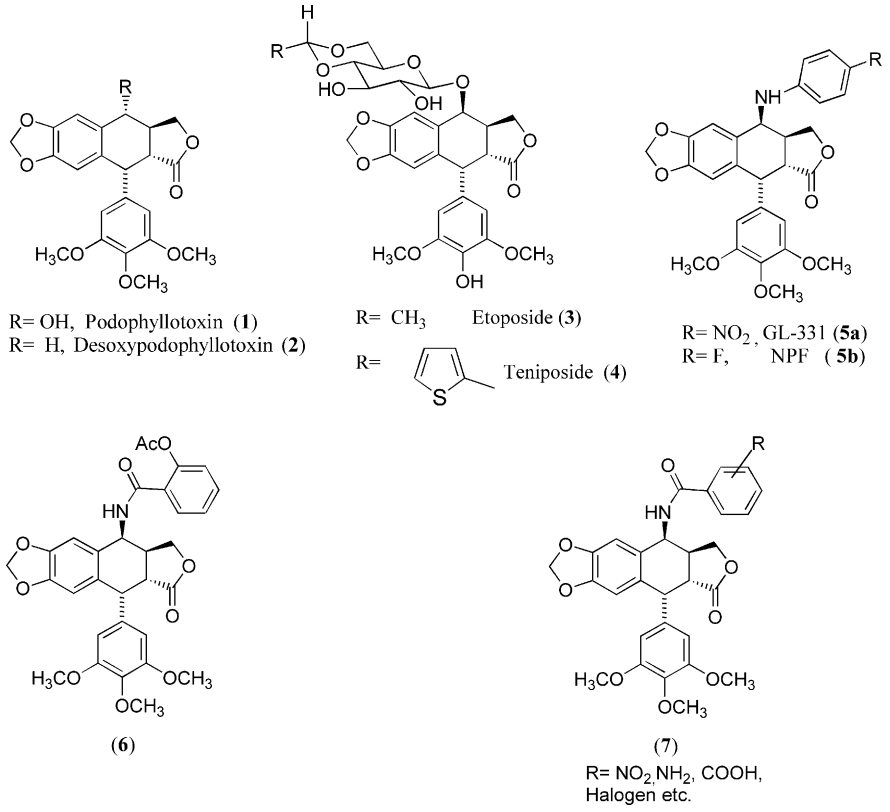
doi:10.1016/j.bmc.2003.08.019



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

Chemistry

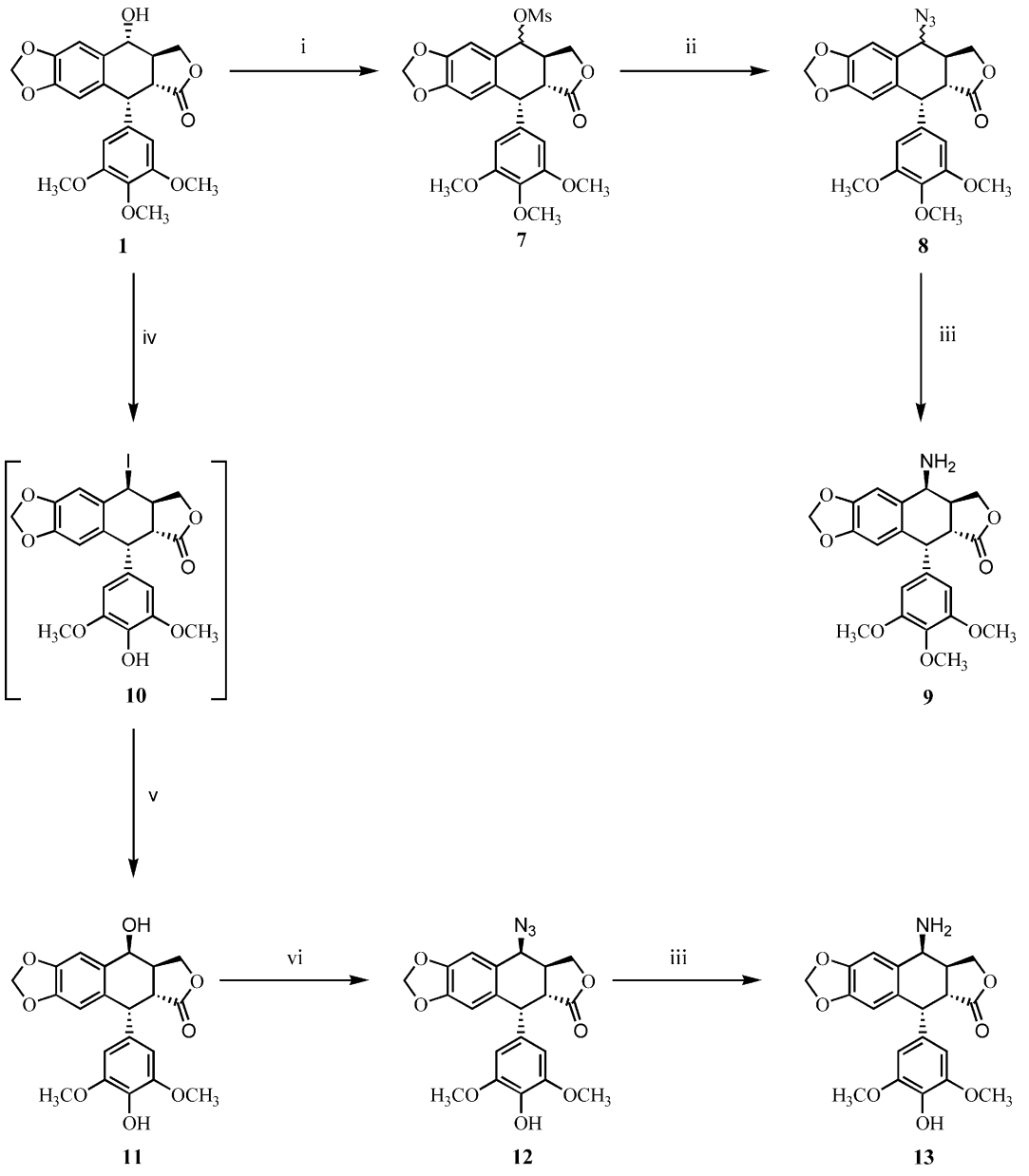
The synthesis of C4β-amido and sulphonamido analogues of podophyllotoxin have been carried out from podophyllotoxin as schemes in Schemes 1 and 2. The key intermediate for the preparation of the new analogues is C4β-aminopodophyllotoxin (**9**) C4β-amino-4′-*O*-demethylpodophyllotoxin (**13**). Podphyllotoxin is mesylated followed by azidation to give C4β-azidopodophyllotoxin (**8**). This upon reduction over Pd/C provides a 7:3 mixture of C4β-aminopodophyllotoxin and C4α-aminopodophyllotoxin. The required C4β-aminopodophyllotoxin (**9**) has been purified by column chromatography by employing chloroform–ethyl acetate (2:1) as eluent, where as C4β-amino-4′-*O*-demethylpodophyllotoxin (**13**) has been prepared from C4β-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 system[17] followed by hydrolysis in presence of barium carbonate (Scheme 1).

The intermediates, C4β-aminopodophyllotoxin (**9**) and C4β-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 C4β-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 C4β-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 C4β-amino nicotinyl derivatives of podophyllotoxin (**24–27**). Attempts to prepare these derivatives from nicotinic acid chloride did not produce the desired C4β-amidopodophyllotoxin products (Scheme 2).

Biological Evaluation

These 4β-amido and 4β-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.

4β-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 4β-sulphonamido aryl substituted podophylllotoxin derivatives (**20–23**) is highly potent

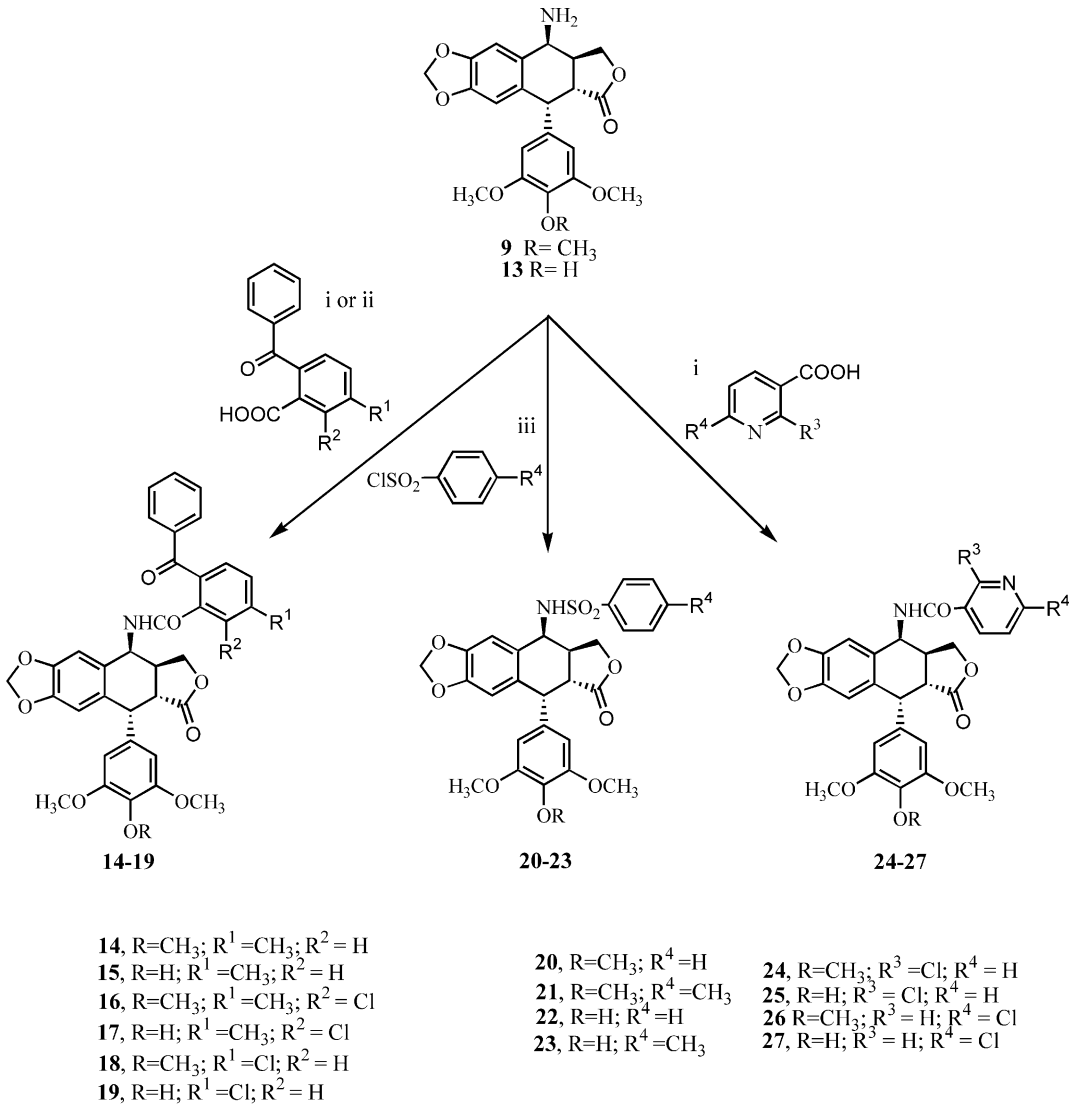


Scheme 1. Reagents: (i) $MeSO_2Cl/Et_3N$; (ii) NaN_3/DMF; (iii) $Pd/C/H_2$; (iv) $MeSO_3H/NaI$, CH_2Cl_2, rt, 5 h; (v) CH_3COCH_3/H_2O, $BaCO_3$, rt, 30 min; (vi) NaN_3/CF_3COOH.

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.



14, R=CH_3; R^1 =CH_3; R^2 = H
15, R=H; R^1 =CH_3; R^2 = H
16, R=CH_3; R^1 =CH_3; R^2 = Cl
17, R=H; R^1 =CH_3; R^2 = Cl
18, R=CH_3; R^1 =Cl; R^2 = H
19, R=H; R^1 =Cl; R^2 = H

20, R=CH_3; R^4 =H
21, R=CH_3; R^4 =CH_3
22, R=H; R^4 =H
23, R=H; R^4 =CH_3

24, R=CH_3; R^3 =Cl; R^4 = H
25, R=H; R^3 = Cl; R^4 = H
26 R=CH_3; R^3 = H; R^4 = Cl
27, R=H; R^3 = H; R^4 = Cl

Scheme 2. Reagents: (i) DCC, CH_2Cl_2, rt, 5 h; (ii) RCOCl, CH_2Cl_2, rt 5 h; (iii) CH_2Cl_2, rt, 5 h, Et_3N.

Table 1. GI_{50} 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

[a]Values 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.

4β-Aminopodophyllotoxin (9)

To a solution of 4β-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 ($CHCl_3$) 3400, 2900, 1770, 1500, 1480, 1410 cm^{-1}; ^1H NMR ($CDCl_3$) 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.

4β-Amino-4′-*O*-demthylepipodophyllotoxin (13). To a solution of 4β-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 ($CHCl_3$) 3520, 3350, 2900, 1770, 1500, 1480, 1410 cm^{-1}; ^1H NMR ($CDCl_3$) 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. 4β-Aminopodophyllotoxin or 4β-amino-4′-*O*-demthylepipodophyllotoxin (0.5 m mol) 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 $NaHCO_3$, 10% hydrochloric acid and water respectively, dried over anhydrous Na_2SO_4 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 4β-aminopodophyllotoxin or 4β-amino-4′-*O*-demthylepipodophyllotoxin (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.

4β-(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 4β-aminopodophyllotoxin (206 mg, 0.5 mmol) to give the compound **14**. Yield: 85%; mp 173–175 °C; $[\alpha]_D^{25}$ −34.5; IR ($CHCl_3$) 3360, 3290, 2900, 1745, 1690, 1590 cm^{-1}; ^1H NMR ($CDCl_3$) 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. ($C_{37}H_{33}NO_9$) calcd C: 69.91, H: 5.23, N: 2.20; found C: 69.73, H: 5.17, N: 2.11.

4β-(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 4β-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 ($CHCl_3$) 3360, 3190, 2920, 1750, 1590 cm^{-1}; ^1H NMR ($CDCl_3$) 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^{+1}), 468, 383, 346, 325. Anal. ($C_{36}H_{31}NO_9$) calcd C: 69.55, H: 5.02, N: 2.25; found C: 69.32, H: 5.05, N: 2.18.

4β-(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 4β-aminopodophyllotoxin (206 mg, 0.5 mmol) to give the product **16**. Yield 50%; mp 156–159 °C; $[\alpha]_D^{25}$ −63.46; IR ($CHCl_3$): 3300, 3260, 2900, 1725, 1670, 1580 cm^{-1}. ^1H NMR ($CDCl_3$) 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^{+1}), 578, 551, 397. Anal. ($C_{37}H_{32}ClNO_9$) calcd C: 66.31, H: 4.81, N: 2.09; found C: 66.24, H: 4.76, N: 2.06.

4β-(3″-Chloro-4″-methylbenzophenone-2″-formyl)amino-4′-*O*-demethylepipodo-phyllotoxin (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 m mol) and 4β-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 ($CHCl_3$) 3300, 3280, 2900, 1745, 1690, 1590 cm^{-1}; ^1H NMR ($CDCl_3$) 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^{+1}), 603, 577, 551, 523, 412, 397. Anal. ($C_{36}H_{30}ClNO_9$) calcd C: 65.90, H: 4.60, N: 2.13; found C: 65.75, H: 4.62, N: 2.09.

4β-(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 4β-aminopodophyllotoxin (206 mg, 0.5 mmol) to give the compound **18**. Yield 75%; mp 153–156 °C; $[\alpha]_D^{25}$ −19.25; IR ($CHCl_3$) 3300, 3250, 2890, 1750, 1680, 1590 cm^{-1}; ^1H NMR ($CDCl_3$) 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. ($C_{36}H_{30}ClNO_9$) calcd C: 65.90, H: 4.60, N: 2.13; found C: 65.73, H: 4.55, N: 2.10.

4β-(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 4β-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 ($CHCl_3$) 3380, 3250, 2900, 1735, 1690, 1580 cm^{-1}; ^1H NMR ($CDCl_3$) 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. ($C_{35}H_{28}ClNO_9$) calcd C: 65.47, H: 4.39, N: 2.18; found C: 65.35, H: 4.32, N: 2.15.

4β-(Benzene sulphonyl)aminopodophyllotoxin (20). This compound was prepared according to method B described earlier employing benzene sulphonyl chloride (0.105 mL, 0.5 m mol) and 4β-aminopodophyllotoxin (206 mg, 0.5 mmol) to give the compound **20**. Yield 80%; mp 233–235 °C; $[\alpha]_D^{25}$ −76.0; IR ($CHCl_3$) 3350, 3200, 2860, 1745, 1560 cm^{-1}; ^1H NMR ($CDCl_3$) 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. ($C_{28}H_{27}NO_9S$) calcd C: 60.75, H: 4.91, N: 2.53; found C: 60.62, H: 4.90, N: 2.47.

4β-(*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 4β-aminopodophyllotoxin (206 mg, 0.5 mmol) to give the compound **21**. Yield 85%; mp 209–212 °C; $[\alpha]_D^{25}$ −14.5; IR ($CHCl_3$) 3390, 3280, 2890, 1735, 1580 cm^{-1}; ^1H NMR ($CDCl_3$) 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. ($C_{29}H_{29}NO_9S$) calcd C: 61.36, H: 5.14, N: 2.46; found C: 61.29, H: 5.09, N: 2.40.

4β-(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 4β-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 ($CHCl_3$) 3380, 3260, 2890, 1750, 1580 cm^{-1}; ^1H NMR ($CDCl_3$) 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. ($C_{27}H_{25}NO_9S$) calcd C: 60.10, H: 4.67, N: 2.59; found C: 60.05, H: 4.64, N: 2.48.

4β-(*p*-Toluene 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 4β-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 ($CHCl_3$) 3400, 3290, 2860, 1750, 1580 cm^{-1}; ^1H NMR ($CDCl_3$) 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. ($C_{28}H_{27}NO_9S$) calcd C: 60.75, H: 4.91, N: 2.53; found C: 60.64, H: 4.88, N: 2.51.

4β-(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 4β-aminopodophyllotoxin (206 mg, 0.5 mmol) to give the compound **24**. Yield 50%; mp 166–168 °C; $[\alpha]_D^{25}$ −136.53; IR ($CHCl_3$) 3300, 3250, 2870, 1735, 1590 cm^{-1}; ^1H NMR ($CDCl_3$) 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. ($C_{28}H_{25}ClN_2O_8$) calcd C: 60.81, H: 4.55, N: 5.06; found C: 60.74, H: 4.41, N: 5.01.

4β-(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 4β-amino-4′-*O*-demethylepipodophyllotoxin (200 mg, 0.5 m mol) to give the compound **25**. Yield 45%; mp 180–182 °C; $[\alpha]_D^{25}$ −23.5; IR ($CHCl_3$) 3360, 3280, 2890, 1745, 1580 cm^{-1}; ^1H NMR ($CDCl_3$) 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. ($C_{27}H_{23}ClN_2O_8$) calcd C: 60.17, H: 4.30, N: 5.19; found C: 60.12, H: 4.21, N: 5.09.

4β-(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 4β-aminopodophyllotoxin (206 mg, 0.5 mmol) to give the compound **26**. Yield 45%; mp 173–174 °C; $[\alpha]_D^{25}$ −163.2; IR ($CHCl_3$) 3360, 3290, 2890, 1750, 1570 cm^{-1}; ^1H NMR ($CDCl_3$) 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. ($C_{28}H_{25}ClN_2O_8$) 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 ($CHCl_3$): 3360, 3280, 2910, 1750, 1590 cm^{-1}; ^1H NMR ($CDCl_3$) 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^{+2}), 538 (M^+), 503, 397, 367. Anal. ($C_{27}H_{23}ClN_2O_8$) 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% CO_2 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 solublised in Tris base (alkaline pH) and quantitated by measuring the OD at 490 nm in an ELISA reader. GI_{50} (concentration that inhibits the cell growth by 50%) is calculated according to the method of Boyd.[19]

Acknowledgements

This work was financially supported by Department of Science and Technology, India under the drugs and pharmaceutical research programme. One of the author's, B.A.K., is thankful to CSIR, New Delhi for the award of research fellowship.

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