Synthesis and biological evaluation of carbon-substituted C-4 derivatives of podophyllotoxin

Yvonne Lear and Tony Durst

Abstract: Several C-4 carbon-substituted analogues of podophyllotoxin, **1**, were prepared by treatment of **1** with allyltrimethylsilane or trimethylsilylcyanide in the presence of boron trifluoride etherate. Alternatively, carbon substituents were introduced via additions to the carbobenzyloxy-protected C-4′-dimethylated podophyllotoxone. These 4′-dimethylated derivatives showed promising in vitro antitumour activity and were equally active against human colon cell line HT116 and two multidrug resistant cell lines. The alcohol **6** was evaluated in vivo but was found to be inactive.

Key words: podophyllotoxin analogues, podophyllotox-4-one, C4 carbon substituted podophyllotoxins.

Résumé : On a préparé plusieurs analogues de la podophyllotoxine **1** substitués sur le carbone en position 4, en traitant le composé **1** avec l'allyltriméthylsilane ou le cyanure de triméthylsilane en présence de l'éthérate de trifluorure de bore. Alternativement, on a introduit les substituants sur le carbone via des additions sur la podophyllotoxone déméthylée en C-4′ et protégée par le groupe carbobenzyloxy. Ces dérivés déméthylés en C-4′ exhibent in vitro une activité anti tumorale prometteuse et sont également actifs contre la ligne cellulaire du colon humain HT116 et contre deux lignes cellulaires résistantes a de nombreux médicaments. On a évalué l'alcool **6** in vivo mais elle est inactive.

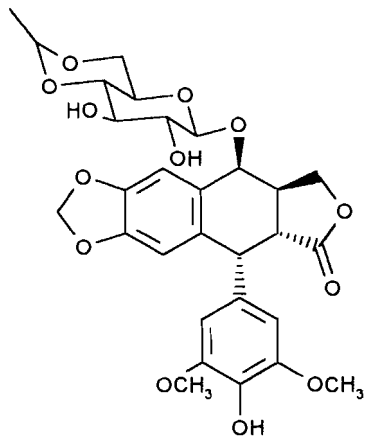
Mots clés : analogues de la podophyllotoxine, podophyllotox-4-one, podophyllotoxines substituées en C4.

[Traduit par la rédaction]

Introduction

The clinical use of Etoposide **1** as an antitumour agent (1–3) has spurred interest in the modification of its aglycone podophyllotoxin **2** in order to furnish derivatives possessing superior antitumour activity. These efforts have included a number of modifications at the C-4 position of the molecule. New derivatives have included ethers, esters (4), amines (5, 6), sulfides, sulfoxides, and sulfones (7). Except for an isolated example described in 1960 (8), there appear to have been no reports describing the synthesis of derivatives of podophyllotoxin bearing carbon substituents at C-4. Such derivatives should have greater chemical stability than the typical podophyllotoxin derivatives since the C-4 heteroatom bond is susceptible to heterolytic cleavage, which generates a benzylic carbocation intermediate and hence either new substitution or elimination products.

This report describes the synthesis of several derivatives of 4′-demethylpodophyllotoxin in which the OH bond at C-4 has been replaced by a carbon–carbon bond, and the evaluation of their biological activity. These new analogues retain the crucial structural features of the Etoposide aglycone, such as the *trans*-fused lactone (3) and the C-4′ hydroxyl group on the pendant aromatic ring (9). In addition, these derivatives have



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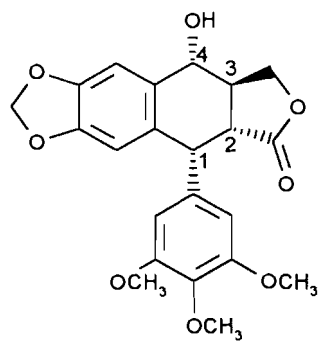

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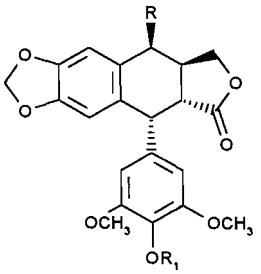
the preferred β stereochemistry at C-4 for optimal activity (10).

Results and discussion

The desired carbon substituents at C-4 were introduced using either podophyllotoxin or 4′-*O*-carbobenzyloxypodophyllotoxin as starting materials. Thus treatment of podophyllotoxin with allyltrimethylsilane in dichloromethane at 0°C in the presence of boron trifluoride etherate (11, 12), afforded 4-β-allyl-4-deoxypodophyllotoxin, **3**, mp 131°C, in 95% yield. The 4-β-stereochemistry was expected since trapping of the intermediate benzylic cation should occur preferentially from the sterically less hindered β side. This was confirmed by the 5.8 Hz coupling constant between H3 and H4, which is consistent with that observed for similar compounds (13). The coupling constants for the remaining C-ring protons ($J_{1,2}$ = 5.2 Hz and $J_{2,3}$ = 14.3 Hz) show that the podophyllotoxin stereochemistry has been retained.

The intermediate **3** was readily converted to the alcohol **4** in 90% yield by reaction with borane – methyl sulfide followed by $NaOH–H_2O_2$ work-up. Oxidation of **4** with Jones reagent gave the carboxylic acid **5**. Both **4** and **5** were demethylated at C-4′ using anhydrous HBr in dichloromethane to afford **6** and **7**, respectively. The demethylation of **5** to give **7** was highly problematic, giving low yields of the product **7**, which was impossible, due to decomposition, to purify completely either by silica gel chromatography or recrystallization. The spectroscopic properties of these compounds were in agreement with the assigned structures and are reported in the experimental section.



3	R = $CH_2CH{=}CH_2$	$R_1 = CH_3$
4	R = $CH_2CH_2CH_2OH$	$R_1 = CH_3$
5	R = CH_2CH_2COOH	$R_1 = CH_3$
6	R = $CH_2CH_2CH_2OH$	R_1 = H
7	R = CH_2CH_2COOH	R_1 = H
8	R = CN	$R_1 = CH_3$
9	R = $COOCH_2CH_3$	$R_1 = CH_3$
10	R = CN	R_1 = H
11	R = $COOCH_2CH_3$	R_1 = H
12	R = OH	R_1 = Cbz

The preparation of the 4-cyano and 4-carboethoxy derivatives **10** and **11**, respectively, has thus far been unsuccessful. The required 4-cyano-4-deoxypodophyllotoxin, **8**, was obtained in 88% yield from **2** upon treatment with trimethylsilylcyanide and boron trifluoride etherate. Unfortunately, the demethylation of the 4′-methoxy group with HBr under the usual conditions afforded only a small amount of a highly insoluble product, tentatively assigned as **10** on the basis of its MS and ^{1}H NMR data. Hydrolysis of **8** in refluxing ethanolic HCl gave **9** on the basis of MS and ^{1}H NMR data; unfortunately 4′-*O*-demethylation of **9** to provide target compound **11** using HBr or BBr_3 has not been successful.

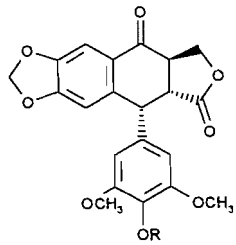
4′-*O*-Carbobenzyloxypodophyllotoxone, **13**, available by pyridinium dichromate oxidation of the corresponding protected 4′-*O*-carbobenzoxyepipodophyllotoxin **12** (10), was converted into a compound presumed to be **15**, in 80% yield, upon reaction with lithium phenylacetylide. This intermediate was not characterized but was hydrogenated over palladium to remove the Cbz protecting group and reduce the alkyne at C-4 to give **17**, mp 163–164°C, whose structure was verified by NMR and mass spectral data. In a model study, reaction of podophyllotoxone (**8**) with *n*-BuLi gave a mixture of **16** and its C-4 epimer as the major products. A single crystal X-ray diffraction determined that the minor isomer had the α geometry at C-4. Based on this result, it was concluded that the larger phenyl acetylide anion would also give mainly the β geometry, hence products **15** and **17**.

Biological evaluation

The in vitro cytotoxicity of three of the above compounds was evaluated against the human colon cell line HCT 116 and two drug-resistant cell lines HCT116/VM46 and HCT116/VP35 (Table 1). The former exhibits multidrug resistance to lipophilic anticancer drugs such as taxol, Etoposide (VP-16), Teniposide (VM-26), doxorubicin, and vinblastine by overexpressing *p*-glycoprotein, which acts as a cell surface drug efflux pump to limit the accumulation of the above-mentioned drugs in cancer cells. The latter line has reduced levels of topoisomerase II and is resistant to topoisomerase-active drugs such as Etoposide and Teniposide. The results of the tests and comparison to Etoposide and Teniposide are shown in Table 1.

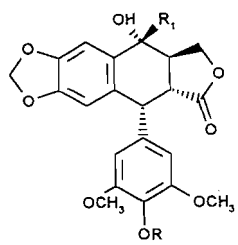
In these in vitro tests, the 4-hydroxypropyl derivative **6** was most active amongst the new structures against all three cell lines. While the alcohol **6** was approximately 4 times less active than Teniposide against the nonresistant strain, it was somewhat more active than Teniposide against the resistant strains. Compound **6** has comparable activity, with an IC_{50} of approximately 0.7 μM, against each of the three cell lines. The in vitro activity of the tertiary alcohol **17** is reduced by a factor of about 3 and that of the acid **7** by more than 10 relative to **6**. Interestingly for **17**, as for **6**, the response of the multidrug-resistant cell lines is similar to that of the nonresistant line HCT 116. This indicates that **6** and **17** are not substrates for the *p*-glycoprotein efflux pump or they inhibit the pump.

Compound **6** was subsequently evaluated in vivo against a distally implanted M109 tumour model in CDF1 mice by administering it interperitoneally as a solution in H_2O – carbomethoxy cellulose at dosages ranging from 50 to 120 mg/kg. Survival times at 50 and 80 mg/kg were identical to that of the control group (test/control, T/C = 100–105). At the highest



13 R = Cbz

14 R = CH_3



15 R = Cbz R_1 = C≡CPh

16 R = CH_3 R_1 = Bu

17 R = H R_1 = CH_2CH_2Ph

Table 1. In vitro toxicity of podophyllotoxin derivatives in human colon carcinoma cell lines.

Compound tested	IC_{50} (μM)		
	HCT116	HCT116/VM46	HCT116/VP35
6	0.76	0.76	0.66
7	9.5	13	11
17	2.2	2.4	2.2
Etoposide	1.5	8.6	7.4
Teniposide	0.18	0.92	0.76

dosage employed the T/C fell to 87. The other C-4 substituted derivatives were not tested in vivo. Biological testing was carried out by Bristol-Myers Squibb following established protocol.

Experimental

NMR spectra were recorded on either A Varian XL 300 or Bruker AMX 500 spectrometer. Melting points were recorded on a Thomas Scientific melting point apparatus. THF was freshly distilled from sodium and benzophenone prior to use. Chromatography solvents were distilled routinely prior to use. Podophyllotoxin was donated by Bristol-Myers Squibb, Wallingford, Connecticut.

4-β-Allyl-4-deoxypodophyllotoxin 3

Podophyllotoxin, **2** (1 g, 2.4 mmol, 1 equiv.) was stirred with boron trifluoride etherate (750 μL, 2.5 equiv.) and allyltrimethylsilane (80 μL, 2 equiv.) in dry CH_2Cl_2 (1 mL) over 4 Å molecular sieves at 25°C for 4 h. Water (3 mL) was added and the product was extracted with CH_2Cl_2, washed with H_2O, dried ($MgSO_4$), and evaporated to give a white solid. Chromatography on silica gel (1:1 hexane:ethyl acetate) and recrystallization from hexane – ethyl acetate afforded the pure compound (1.05 g, 95% yield); mp 137–138°C. IR: 1775 cm^{-1} (lactone). ^1H NMR ($CDCl_3$): 6.73 (s, 1H), 6.47 (s, 1H), 6.29 (s, 2H), 5.95 (s, 1H), 5.94 (s, 1H), 5.81 (m, 1H), 5.11–5.15 (m, 2H), 4.56 (d, $J_{1,2}$ = 5.2 Hz, 1H), 4.23–4.30 (m, 2H), 3.77 (s, 3H), 3.71 (s, 6H), 3.27–3.40 (m, 1H), 3.05–3.09 (m, 1H), 2.96–3.01 (m, $J_{2,3}$ = 14.3 Hz, $J_{3,4}$ = 5.8 Hz, 1H), 2.57–2.60 (m, 1H), 2.43–2.45 (m, 1H). ^{13}C NMR ($CDCl_3$): 175.1, 152.4, 147.0, 146.9, 137.0, 136.6, 136.1, 133.0, 130.8, 116.9, 110.1, 108.7, 108.4, 101.2, 69.0, 60.7, 56.2, 44.0, 42.2, 38.5, 37.7, 36.1. HRMS calcd. for $C_{25}H_{26}O_7$: 438.1679; found: 438.1681.

4β-(3-Hydroxypropyl)-4-deoxypodophyllotoxin 4

Borane methyl sulfide complex (100 μL 10 M solution) was added dropwise to a solution of **3** (925 mg, 2.1 mmol) in dry CH_2Cl_2 (6 mL) at 0°C. The solution was stirred at 25°C for 4 h, then diluted with 10 mL EtOH. The pH of the solution was maintained at 8 using 3 N aqueous NaOH while H_2O_2 (0.3 mL, 30% in H_2O) was added at 0°C. Water (10 mL) was added and the product extracted with CH_2Cl_2, dried ($MgSO_4$), and the solvent evaporated, leaving a white solid that was recrystallized from hexane – ethyl acetate to give the product (915 mg, 95% yield); mp 118–119°C. IR: 3612, 1775 cm^{-1}. ^1H NMR ($CDCl_3$): 6.69 (s, 1H), 6.43 (s, 1H), 6.25 (s, 2H), 5.92 (s, 1H), 5.90 (s, 1H), 4.53 (d, J = 4.8 Hz, 1H), 4.36 (dd, J = 8.5 Hz, 7.1 Hz, 1H), 4.08–4.13 (m, 1H), 3.77 (s, 3H), 3.72 (s, 6H), 2.95–3.00 (m, 1H), 2.85–2.95 (m, 2H). ^{13}C NMR ($CDCl_3$): 175.1, 152.4, 147.0, 146.8, 137.1, 136.2, 133.8, 130.5, 110.2, 108.8, 108.5, 101.2, 69.0, 62.5, 60.7, 56.2, 44.1, 42.2, 39.3, 36.2, 32.1, 29.7. HRMS calcd. for $C_{25}H_{28}O_8$: 456.1775; found: 456.1773.

4β-(3-Propanoic acid)-4-deoxypodophyllotoxin 5

Jones reagent (2 mL, 0.4 M) was added dropwise to a solution of 160 mg **4** dissolved in acetone until an orange colour persisted. Stirring was continued for 15 min. The reaction mixture was brought to pH 7 using saturated $NaHCO_3$ solution. The mixture was extracted using CH_2Cl_2. The organic layer was washed with H_2O, dried ($MgSO_4$), and the solvent evaporated. The product was isolated by column chromatography on silica gel using 5% MeOH, 63% ethyl acetate, and 32% hexanes as eluent and was recrystallized from hexane – ethyl acetate. Yield: 105 mg (66%); mp 197°C. IR (CH_2Cl_2): 2600–3300 cm^{-1}. ^1H NMR: 6.77 (s, 1H), 6.44 (s, 1H), 6.24 (s, 2H), 5.93 (s, 1H), 5.91 (s, 1H), 4.54 (br, 1H), 4.36 (m, 1H), 4.12 (m, 1H), 3.77 (s, 3H), 3.71 (s, 6H), 3.11 (br, 1H), 2.98 (br, 1H), 2.41–2.49 (m, 2H), 2.02–2.07 (m, 1H), 1.86–1.90 (m, 1H). ^{13}C NMR: 177.2, 174.7, 152.5, 147.1, 137.2, 136.1, 132.7, 130.3, 110.4, 108.8, 108.5, 101.3, 68.6, 60.8, 56.2, 44.1, 41.9, 38.5, 36.1, 32.7, 27.7. HRMS calcd. for $C_{25}H_{26}O_9$: 470.1577; found: 470.1590.

4β-(3-Hydroxypropyl)-4-deoxy-4′-demethylpodophyllotoxin 6

A solution of 10 mL dichloroethane and 3 mL diethyl ether saturated with anhydrous HBr at 0°C was added to 750 mg **4**. The solution was stirred at 0°C for 30 min and at 25°C for 90 min. The solution was neutralized by adding solid K_2CO_3. The organic layer was diluted with CH_2Cl_2 (10 mL) and washed with H_2O (×3), with brine, dried ($MgSO_4$), and the solvent evaporated. The compound was isolated by radial chromatography on silica with ethyl acetate as eluent and was recrystallized from CH_2Cl_2–hexane. Yield: 440 mg (60%); mp 129–130°C. IR: 3000–3500 (br) cm^{-1}. ^1H NMR ($CDCl_3$): 6.69 (s, 1H), 6.43 (s, 1H), 6.27 (s, 2H), 5.92 (s, 1H), 5.90 (s, 1H), 4.52 (d, $J_{1,2}$ = 4.7 Hz, 1H), 4.30–4.33 (m, 1H), 4.11–4.16 (m, 1H), 3.75 (s, 6H), 3.06 (m, 1H), 2.96 (m, 1H), 1.83 (m, 1H), 1.70 (m, 1H), 1.54–1.69 (m, 4H). ^{13}C NMR ($CDCl_3$): 146.3, 131.7,

110.2, 108.8, 108.1, 101.2, 68.9, 62.6, 56.4, 44.0, 42.3, 39.3, 36.1, 21.1, 29.7, 14.6. HRMS calcd. for $C_{24}H_{26}O_8$: 442.1614; found: 442.1630.

4β-(3-Propanoic acid)-4′-demethyl-4-deoxypodophyllotoxin 7

A solvent mixture of 25% ether in dichloroethane was saturated with HBr(g) at 0°C. The solvent mixture (10 mL) was added to 95 mg **5** and stirred at 0°C for 30 min and at 25°C for 2 h. The solution was neutralized with saturated $NaHCO_3$, extracted with CH_2Cl_2, dried ($MgSO_4$), and evaporated. Chromatography on silica gel (5% MeOH, 65% ethyl acetate, 30% hexanes as eluent) by preparative TLC and recrystallization from hexane – ethyl acetate yielded 20 mg (20%) of the impure demethylated compound **7**, which during each attempted purification decomposed considerably; mp 198–200°C, ^{1}H NMR: 6.69 (s, 1H), 6.44 (s, 1H), 6.27 (s, 2H), 5.92 (d, 1H), 5.90 (d, 1H), 4.53 (d, 1H), 4.31–4.33 (m, 1H), 4.12–4.18 (m, 1H), 3.75 (s, 6H), 3.05–3.08 (m, 1H), 2.97 (d, 1H), 2.94 (s, 1H). ^{13}C NMR: 191.0, 175.3, 146.3, 134.2, 133.0, 131.0, 110.2, 108.8, 108.1, 101.2, 68.9, 62.6, 56.4, 44.0, 42.3, 39.3, 36.1, 32.1, 29.7. HRMS calcd. for $C_{24}H_{24}O_9$: 456.1420; found: 456.1440.

4β-Cyano-4-deoxypodophyllotoxin 8

Boron trifluoride etherate (120 μL, 2 equiv.) was added to a solution of podophyllotoxin (200 mg, 1 equiv.) and trimethylsilyl cyanide (70 μL, 1.1 equiv.) in dry CH_2Cl_2 (3 mL) at 0°C. The solution was stirred for 20 min at 0°C and at 25°C for 1.5 h, when TLC showed that no starting material remained. Water was added (10 mL) and extraction carried out with CH_2Cl_2. The organic extracts were dried ($MgSO_4$) and the solvent evaporated to leave a yellow solid that was pure by NMR (180 mg, 88%); mp 158–159°C. IR: 2308, 1784 cm^{-1}. ^{1}H NMR ($CDCl_3$): 6.83 (s, 1H), 6.56 (s, 1H), 6.27 (s, 1H), 6.01 (s, 1H), 5.99 (s, 1H), 4.67 (d, $J_{1,2}$ = 4.6 Hz, 1H), 4.45–4.50 (m, 1H), 4.35 (d, J = 9.4 Hz, 1H), 4.21 (d, $J_{3,4}$ = 5.1 Hz, 1H), 3.80 (s, 3H), 3.74 (s, 6H), 3.05–3.15 (m, 1H), 2.90–3.05 (m, 1H). ^{13}C NMR ($CDCl_3$): 172.6, 152.4, 148.6, 147.7, 137.2, 134.4, 131.1, 122.1, 117.4, 110.7, 108.2, 107.9, 101.7, 68.5, 60.6, 56.0, 43.3, 43.1, 33.5, 33.0. HRMS calcd. for $C_{23}H_{21}O_7N$: 423.1301; found: 423.1314.

4β-Carboethoxy-4-deoxypodophyllotoxin 9

8 150 mg was refluxed in a solution of 3 mL HCl in 6 mL ethanol for 1 h. The solution was brought to pH 7 with saturated aqueous $NaHCO_3$ solution, extracted with CH_2Cl_2, dried ($MgSO_4$), and the solvent was evaporated to yield 95 mg white solid (57% yield); mp 138–139°C. IR: 1776, 1732 cm^{-1}. ^{1}H NMR: 7.11 (s, 1H), 6.47 (s, 1H), 6.07 (s, 1H), 5.94 (s, 1H), 5.92 (s, 1H), 4.44 (d, $J_{1,2}$ = 5.4 Hz, 2H), 4.1–4.2 (m, 1H), 3.83 (d, J = 9.2 Hz, 1H), 3.78 (m, 1H), 3.77 (s, 3H), 3.72 (s, 6H), 3.15–3.20 (m, 1H), 3.00 (dd, $J_{1,2}$ = 5.4 Hz, $J_{2,3}$ = 13.0 Hz, 1H), 1.25 (t, 3H). ^{13}C NMR: 175.6, 171.5, 153.0, 147.7, 147.2, 135.6, 130.5, 121.7. 109.4, 108.9, 106.4, 101.3, 70.2, 61.0, 60.8, 56.2, 46.8, 46.4, 42.2, 32.1, 29.7, 25.7, 14.2. HRMS calcd. for $C_{25}H_{26}O_9$: 470.1577; found: 470.1579.

4′-*O*-Carbobenzyloxypodophyllotoxone 13

A solution of 100 mg 4′-*O*-carbobenzyloxyepipodophyllotoxin **12** (10) and 300 mg pyridinium dichromate in 2 mL dry CH_2Cl_2 was stirred for 26 h at 25°C. Separation by silica gel column chromatography using hexane:ethyl acetate (1:1) and recrystallization from CH_2Cl_2–hexane afforded 82 mg desired product (82% yield); mp 99°C. IR (CH_2Cl_2): 1773, 1733 cm^{-1}. ^{1}H NMR ($CDCl_3$): 7.56 (s, 1H), 7.34–7.44 (m, 5H), 6.70 (s, 1H), 6.42 (s, 2H), 6.11 (s, 1H), 6.09 (s, 1H), 5.27 (s, 2H), 4.87 (d, $J_{1,2}$ = 4.3 Hz, 1H), 4.56–4.57 (m, 1H), 4.34–4.38 (m, 1H), 3.71 (s, 1H), 3.45–3.51 (m, 1H), 3.29 (dd, $J_{1,2}$ = 4.3 Hz, $J_{2,3}$ = 15.6 Hz, 1H). ^{13}C NMR ($CDCl_3$): 191.9, 172.6, 152.8, 151.7, 147.8, 140.7, 134.9, 134.6, 128.1, 127.9, 109.4, 106.7, 105.8, 102.1, 70.1, 66.6, 55.9, 46.3, 44.3, 43.0. FABMS (M + 1) calcd. 533.1369; found: 533.1397.

4′-*O*-Carbobenzoxy-4-(phenylethynyl)-epipodophyllotoxin 15

*n*BuLi (60 μL, 2 N in hexanes, 1 equiv.) was added to a solution of penylacetylene (20 μL, 1 equiv.) in dry THF (2 mL) at −78°C. A solution of 13 (90 mg, 1 equiv.) in dry THF (2 mL) was added and stirring continued at −78°C for 30 min. The reaction was quenched at −78°C with saturated aqueous NH_4Cl. The mixture was allowed to warm to room temperature and was extracted with CH_2Cl_2. The organic layer was washed with H_2O, dried ($MgSO_4$), and the solvent evaporated, leaving 82 mg white solid. The mixture was used for the next step without further purification.

4-Butyl-4-deoxypodophyllotoxin 16

To a solution of podophyllotoxone (6) (250 mg, 0.6 mmol, 1 equiv.) in 3 mL dry THF at −78°C was added 250 μL *n*-BuLi (2.4 M in hexanes, 0.6 mmol, 1 equiv.). The solution was stirred at −78°C for 15 min, then quenched with saturated NH_4Cl. Chromatography on silica gel using hexane:ethyl acetate (1:1) afforded 40 mg of the α-substituted isomer and 70 mg of the β-substituted isomer.

α Isomer: mp 105–106°C. IR: 3425br, 1777 cm^{-1}. ^{1}H NMR: 6.98 (s, 1H), 6.52 (s, 1H), 6.31 (s, 2H), 6.00 (s, 1H), 5.96 (s, 1H), 4.58 (d, $J_{1,2}$ = 4.69 Hz, 1H), 4.27–4.38 (m, 2H), 3.79 (s, 1H), 3.71 (s, 6H), 3.22 (dd, $J_{1,2}$ = 4.9 Hz, $J_{2,3}$ = 14.1 Hz, 1H), 2.88–2.97 (m, 1H), 1.77–1.84 (m, 2H), 1.05–1.4 (m, 4H), 0.89 (t, 3H). ^{13}C NMR: 175.4, 152.5, 147.9, 136.5, 135.1, 134.3, 132.5, 110.5, 107.8, 104.9, 101.5, 72.9, 67.1, 60.7, 55.9, 44.6, 41.7, 39.4, 39.3, 27.8, 23.1, 13.9.

β Isomer: mp 110°C. IR: 3593 br, 1774 cm^{-1}. ^{1}H NMR: 7.05 (s, 1H), 6.45 (s, 1H), 6.29 (s, 2H), 5.96 (s, 2H), 4.56 (d, $J_{1,2}$ = 4.9 Hz, 1H), 4.45 (dd, 1H), 4.27 (s, 1H), 3.78 (s, 3H), 3.73 (s, 6H), 3.08 (dd, $J_{1,2}$ = 4.9 Hz, $J_{2,3}$ = 14.9 Hz, 1H), 2.98 (m, 1H), 1.82–1.88 (m, 2H), 1.2–1.4 (m, 4H), 0.88 (t, 3H). ^{13}C NMR: 174.5, 152.5, 147.7, 147.4, 137.2, 136.3, 135.4, 131.3, 109.6, 108.4, 106.0, 101.4, 74.6, 67.7, 60.7, 56.3, 44.6, 44.5, 44.4, 39.4, 27.2, 23.25, 13.9. HRMS calcd. for $C_{26}H_{30}O_8$: 470.1954; found: 470.1967.

4-(2-Phenylethyl)-4′-demethylepipodophyllotoxin 17

A mixture of **15** (70 mg) and 10% Pd/C (25 mg) in ethyl acetate (5 mL) was stirred under hydrogen atmosphere for 20 min, when TLC showed that no starting material remained. Recrystallization from $CHCl_3$ gave the desired product (25 mg, 45% yield); mp 163–164°C. IR: 3529, 1778 cm^{-1}. ^{1}H NMR (acetone-d_6): 8.00 (s, 1H), 7.28 (s, 1H), 7.18–7.25 (m, 2H), 7.12–7.15 (m, 1H), 6.47 (s, 1H), 6.42 (s, 2H), 5.97 (s, 2H), 4.66 (a,

1H), 4.57 (d, 1H, $J_{1,2}$ = 5.6 Hz), 4.42 (d, 1H, J = 1.5 Hz), 4.40 (s, 1H), 3.70 (s, 6H), 3.36 (dd, 1H, $J_{1,2}$ = 14.9 Hz, $J_{2,3}$ = 5.7 Hz), 3.14–3.21 (m, 1H), 2.75–2.78 (m, 1H), 2.17–2.21 (m, 1H), ^{13}C NMR (acetone-d_6): 175.0, 147.9, 147.6, 147.5, 143.2, 138.1, 135.8, 132.6, 132.2, 128.9, 126.3, 110.0, 107.2, 101.9, 79.0, 74.2, 68.0, 56.5, 45.4, 45.0, 44.4, 41.4, 31.5. HRMS calcd. for $C_{29}H_{28}O_8$: 504.1780; found: 504.1764.

Biological testing

Biological testing was carried out at Bristol-Myers Squibb Laboratories. To determine in vitro cytotoxicity, the cell lines used were human colon carcinoma cell line HCT 116, the VM-26 resistant cell line HCT116/VM46, and the VP-16 resistant cell line HCT116/VP35. Cells were plated and 24 h later drugs were added and serially diluted. After 72 h of continuous drug exposure, the tetrazolium dye XTT was added. Since dehydrogenase enzyme in live cells reduces the XTT to a form that absorbs light at 450 nm, spectrophotometric methods can be used to determine to percentage of live cells. The results in Table 1 reflect drug concentration required to inhibit cell proliferation to 50% of that of untreated control cells.

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