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Synthesis and Biological Activity of A-*nor*-Paclitaxel Analogues

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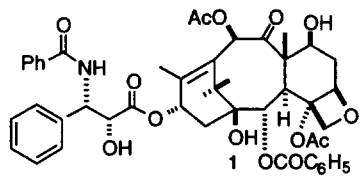
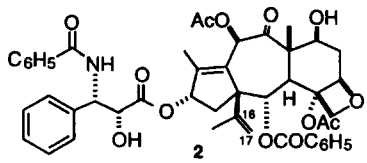
Abstract—A number of paclitaxel analogues with a 5-membered A-ring (A-*nor*-paclitaxels, or (15→1)-*abeo*-paclitaxels) have been prepared in order to determine whether analogues of this class might have improved bioactivity as compared with paclitaxel. Most of the compounds synthesized were less active than paclitaxel, but one analogue was equivalent to paclitaxel in a tubulin-assembly assay, and another analogue was more cytotoxic than paclitaxel in two different cell lines of the NCI screen. © 1997 Elsevier Science Ltd.

Introduction

The novel diterpenoid paclitaxel (Taxol®, **1**) continues to provide significant benefits in the clinical management of breast and ovarian cancers.[1] Because of its structural complexity and its important biological activity, many different analogues of paclitaxel have been prepared with the ultimate aim of discovering an analogue with improved bioactivity.[2] In our own research program, we have primarily been concerned with examining the effects of modifications to the functional groups around the taxene nucleus of paclitaxel,[3–5] and both we[6] and others[7,8] have found that certain paclitaxel analogues modified at the 2-position have improved potencies as compared with paclitaxel.

In addition to studies of the effect of functional group modifications on tubulin-assembly activity and cytotoxicity, we have also investigated the more fundamental question of whether the nature of the taxene ring system can be modified without losing potency. The first experiment along these lines was the synthesis of the A-*nor*-paclitaxel **2** from paclitaxel;[9] the resulting material was found to be about one third as potent as paclitaxel in a tubulin disassembly assay, but to be significantly less cytotoxic than paclitaxel.[9]



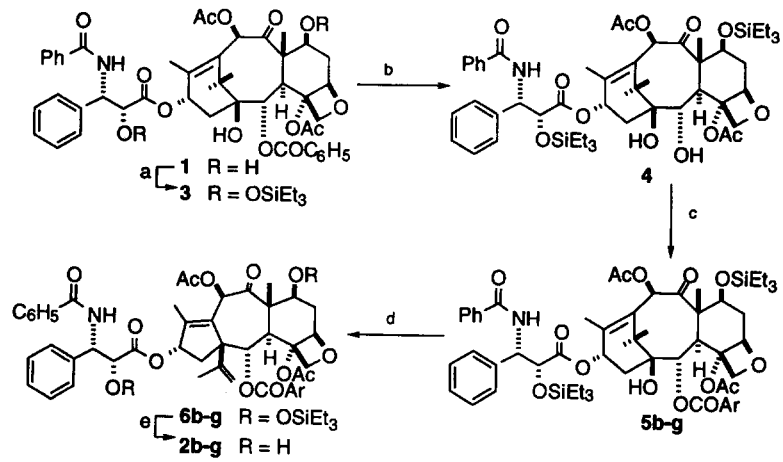


As noted above, selected substitutions on the 2-benzoyl group of paclitaxel have produced analogues with improved tubulin-assembly activity and cytotoxicity.[6–8] Given this fact, it was natural to investigate whether the same modifications could enhance the activity of A-*nor*-paclitaxel. This paper describes the syntheses of the A-*nor*-paclitaxel analogues **2b–2g** and of the additional analogues **7–10**, and studies of their cytotoxicities, tubulin-assembly activities, and (in two cases) in vivo activities.

Results and Discussion

Synthesis of C-2 substituted benzoyl analogues of A-*nor*-paclitaxel

The synthesis of the A-*nor*-paclitaxel analogues **2b–2g** is shown in Scheme 1. Protection of paclitaxel as its 2′,7-*O*-triethylsilyl derivative **3** was followed by selective hydrolysis of the 2-benzoate with sodium hydroxide under phase-transfer conditions.[6] Reacylation of the 2-debenzoyl derivative **4** with selected substituted benzoic acids then gave the protected 2-*O*-debenzoyl-2-*O*-aroyl paclitaxels **5b–g**. Rearrangement to the A-*nor* analogues was effected by treatment with thionyl chloride in pyridine; this reaction proceeded cleanly and in good yield to give the analogues **6b–g** without any concomitant alcohol products, which are observed when ring contraction is effected by acid treatment.[10] Deprotection with methanolic HCl afforded the A-*nor* analogues

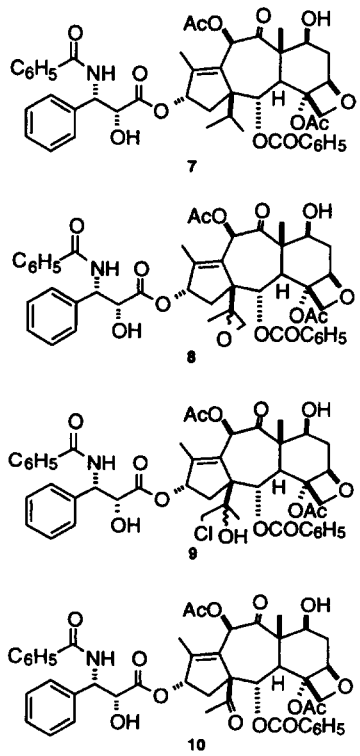


Scheme 1. (a) Et_3SiCl, imidazole, DMF, rt, 1 h, **3**, 95%. (b) Bu_4NHSO_4, 2 N NaOH, C_6H_6, CH_2Cl_2, rt, 1 h, **4**, 65%. (c) ArCOOH, DCC, 4-PP, $C_6H_5CH_3$, **5b–g**, 65–75%. (d) $SOCl_2$, py, CH_2Cl_2, rt, **6b–g**, 40–65%. (e) MeOH/5%/HCl, rt, **2b–g**, 90–95%.

2b–g. The structures of the analogues were established by NMR and MS, and specifically by comparison with the ^1H NMR spectrum of the parent compound.[9]

Synthesis of A-*nor*-paclitaxel analogues modified at C-16,17

In addition to the analogues **2b–g**, it was of interest to investigate the effect of modifications of the C-1 isopropenyl group on activity. The dihydro derivative **7** had been prepared previously,[9] and the derivatives **8–10** were prepared by standard methods. Thus treatment of the A-*nor*-paclitaxel **2** with MCPBA yielded the epoxide **8**, while treatment with methanolic HCl gave the chlorohydrin **9**. Both **8** and **9** were obtained as a mixture of diastereomers, and no attempt was made to separate the mixture. Ozonolysis of **2** yielded the ketone **10**.



Biological evaluation

The A-*nor*-paclitaxel analogues **2**, **2b–g**, and **7–10** were subjected to two different tubulin-assembly assays. All the compounds were assayed at room temperature with purified tubulin under a modification of the conditions previously described,[11] and some of them were also assayed with microtubular protein (tubulin unresolved from microtubule-associated proteins) at 37 °C at Bristol–Myers Squibb. Cytotoxicity assays were obtained against Burkitt lymphoma CA46 cells and both normal and paclitaxel-resistant HCT 116 cells. In addition, the analogues **2**, **2b,c** and **d** were assayed in the NCI 60-cell line panel.[12] These data are summarized in Table 1.

In contrast to earlier results, which indicated that the A-*nor*-paclitaxel **2** was about one third as active as paclitaxel in a simple tubulin-assembly assay,[9] it was found to be at least 10-fold less active than paclitaxel (**1**) under the somewhat different conditions of the current assay. In agreement with earlier results,[9] it was also significantly less cytotoxic than paclitaxel to CA46 cells and also to four selected cell lines from the NCI tumor panel.

The aroyl analogues **2b–g** were uniformly less active than paclitaxel in the tubulin-assembly assay and less cytotoxic to CA46 cells, although some analogues (**2d** and **e**) were only slightly less potent than paclitaxel in the Bristol–Myers Squibb assay. Interestingly, however, the *m*-azido analogue **2c** was more cytotoxic than paclitaxel to the NSCLC H226 and the breast MDA-MB-435 cell lines, and was only slightly less cytotoxic than paclitaxel to the CNS SF539 cell line. These findings parallel those observed for paclitaxel itself, where the *m*-azido analogue was more cytotoxic than other analogues,[6] and suggests that other analogues with this substituent are worth investigating.

Table 1. Activity data for compounds **1–10**

Compd	Ar	EC_{50}[a]	Tubulin Polymerization ratio[b]	IC_{50}[c]	IC_{50}[d]	IC_{50}[e]	Log_{10} TGI[f] NSCLC H226	Colon COLO205	CNS SF539	Breast MDA-MB-435
1	N.A.	4.1 ± 0.6	1.0	9	2.3	327	−7.04(1)	−7.04(1)	−7.51(1)	−8.23(1)
2	N.A.	>40		>100	N.D.[g]	N.D.	>−5.00	−5.81(1)	−5.80(1)	−6.51(1)
2b		>40		>100	N.D.	N.D.	−5.18(2)	−6.10(2)	−6.33(2)	−7.19(2)
2c		>40		>100	N.D.	N.D.	<−8.00	−6.58(2)	−7.27(2)	<−8.00
2d		>40	1.2	>100	39	419	>−5.00	−5.81(2)	−6.67(2)	−7.30(2)
2e		>40	1.6	>100	33.5	364	N.D.	N.D.	N.D.	N.D.
2f		>40	17	>100	283	2063	N.D.	N.D.	N.D.	N.D.
2g		>40		>100	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
7	N.A.	>40	12	>100	75.5	903	N.D.	N.D.	N.D.	N.D.
8	N.A.	3.8 ± 0.9	2.2	20	15.3	523	N.D.	N.D.	N.D.	N.D.
9	N.A.	17	9.3	50	33.8	916	N.D.	N.D.	N.D.	N.D.
10	N.A.	>40	2.9	>100	314	>9000	N.D.	N.D.	N.D.	N.D.

[a] EC_{50} (μM) for polymerization in 1.0 M glutamate at rt in the absence of GTP (data from EH laboratory).
[b] EC_{50} (μM) for tubulin polymerization relative to paclitaxel (data from Bristol–Myers Squibb).
[c] IC_{50} (nM) for cytotoxicity to Burkitt lymphoma CA46 cells.
[d] IC_{50} (nM) for cytotoxicity to HCT116 cells.
[e] IC_{50} (nM) for cytotoxicity to HCT116/VM46 cells.
[f] TGI is the molar concentration, expressed as a logarithm, required to completely inhibit the growth of the indicated cell line. The number of determinations averaged for each sample is given in parentheses.
[g] N.D. = not determined.

The A-*nor*-paclitaxels with modified isopropenyl functions also proved interesting. The most potent analogue was the epoxide **8**, which was comparable to paclitaxel in both tubulin-assembly assays and half as cytotoxic against CA46 cells. The chlorohydrin **9** also showed moderate tubulin-assembly activity and cytotoxicity: it is not known whether this is due to its partial conversion to the epoxide **8** under the conditions of the biochemical experiment. The ketone **10** appeared to be inactive with purified tubulin but had significant tubulin-assembly activity in the microtubule-protein assay. However, it had no significant cytotoxicity. Overall, these data suggest that the reduced tubulin-assembly activity of most of these compounds derives from the C(1)- substituent rather than from the contracted A-ring.

The apparent discrepancies between the two assembly assays with compounds **2d,e** and **10**, led us to examine these agents in further detail. In the assay with purified tubulin, assembly with paclitaxel was measured by centrifugal recovery of polymer formed at room temperature in 1.0 M glutamate in the absence of GTP. In the assay with microtubule protein, assembly was measured by turbidimetry at 37 °C in the absence of GTP, with the reaction dependent on the microtubule-associated proteins that copurify with tubulin in the initial preparation of the protein. In both assays assembly without paclitaxel requires the presence of GTP, but the drug eliminates the absolute requirement for the nucleotide in tubulin assembly.

In previous studies with a series of paclitaxel analogues modified at position C-7,[13] we observed that these agents were all unable to induce assembly in 1.0 M glutamate at 15 °C but induced assembly at 37 °C, resulting in polymers that subsequently were resistant to cold-induced disassembly. Paclitaxel, in contrast, induced vigorous polymerization reactions in 1.0 M glutamate at reaction temperatures as low as 10 °C.[11,13] We therefore examined the ability of compounds **2d**, **e** and **10** to induce the polymerization of purified tubulin in 1.0 M glutamate at 37 °C, both by turbidimetry and by collecting polymer by centrifugation. We obtained different results with each of the agents. Compound **2e** appeared to precipitate as soon as it was added to 1.0 M glutamate, causing a marked rise in turbidity, but we found no evidence of a 37 °C assembly reaction with this agent. Compound **2d** did prove to be active at 37 °C in both assays, while compound **10** remained inactive (data not shown).

Two of the analogues, the *m*-methoxy analogue **2e** and the dihydro analogue **7**, were selected for in vivo testing against the mouse M109 tumor. In this assay, which responds to paclitaxel at doses of 40 mg/kg, no significant response was seen with either **2e** or **7** at doses up to 200 mg/kg. It thus appears that the limited in vitro activity seen for compounds of this type does not translate directly into in vivo activity.

In conclusion, the conversion of paclitaxel to its A-*nor* analogues usually produces compounds with decreased tubulin-assembly activity and cytotoxicity, but certain selected analogues can have improved in vitro activity as compared with paclitaxel. This enhanced in vitro activity has however not yet been transferred to an in vivo system.

Experimental

General experimental procedures. All chemicals were procured from Aldrich Chemical Company and were used without further purification. All anhydrous reactions were performed under argon. Dichloromethane was dried over calcium hydride. All reactions were monitored by TLC (silica gel, GF) and analyzed with UV light and developed with vanillin spray. ^{1}H NMR and ^{13}C NMR spectra were obtained in $CDCl_3$ at 270 and 400 MHz for proton spectra and assigned primarily by comparison of chemical shifts and coupling constants with those of related compounds. Chemical shifts (δ) are expressed in ppm, and coupling constants (J) are given in Hertz. ^{13}C NMR spectra were assigned with the aid of HETCOR and DEPT spectra. Some ^{1}H NMR spectra showed the presence of traces of ethyl acetate; paclitaxel and its derivative retain ethyl acetate very tightly, and it is difficult to remove completely even on prolonged treatment in vacuo at 38 °C. Exact mass measurements were performed at the Midwest Center for Mass Spectrometry.

2′,7-Bis(*O*-triethylsilyl)paclitaxel (3). To a solution of paclitaxel (**1**) (170.6 mg, 0.2 mmol) in dry DMF (1.0 mL) was added imidazole (136.0 mg, 2.0 mmol) followed by triethylsilyl chloride (0.30 mL, 2.0 mmol) and the reaction mixture was stirred at room temperature for 2 h. The reaction mixture was diluted with EtOAc (20 mL), washed successively with H_2O (2 × 20 mL) and brine. The organic layer was separated, dried over Na_2SO_4, and evaporated under reduced pressure to yield a transparent syrup. The crude material was chromatographed over silica gel using hexanes:EtOAc (3:1) as eluent to obtain compound **3** as a white solid (204 mg, 94%). Mp 122–124 °C; 1H NMR δ 0.45 (6H, q, J = 7.8, $SiCH_2$), 0.58 (6H, q, J = 7.8, $SiCH_2$), 0.81 (9H, t, J = 7.8, $SiCH_2CH_3$), 0.90 (9H, t, J = 7.8, $SiCH_2CH_3$), 1.20 (3H, s, 16-CH_3), 1.25 (3H, s, 17-CH_3), 1.70 (3H, s, 19-CH_3), 2.05 (3H, br s, 18-CH_3), 2.16 (3H, s, 10-OAc), 2.32 (1H, m, 14-H), 2.50 (1H, m, 6-H), 2.54 (3H, s, 4-OAc), 3.83 (1H, d, J = 7.0, 3-H), 4.22 (1H, d, J = 8.0, 20-H), 4.32 (1H, d, J = 8.0, 20-H), 4.48 (1H, dd, J = 10.6, 6.6, 7-H), 4.70 (1H, d, J = 2.1, 2′-H), 4.94 (1H, br d, J = 8.8, 5-H), 5.70 (2H, m, 2-H and 3′-H), 6.24 (1H, br t, 13-H), 6.45 (1H, s, 10-H), 7.10 (1H, d, J = 8.9, 3′N-H), 7.30–7.60 (11H, m, Ar-H), 7.74 (2H, dd, J = 8.5, 1.1, 3′-NBz orthoH), 8.13 (2H, dd, J = 8.5, 1.4, 2-OBz orthoH); FABMS m/z $[M+Na]^+$ 1104 (100).

Reaction of 2′,7-bis(*O*-triethylsilyl)paclitaxel (3) with sodium hydroxide under phase-transfer conditions. 2′,7-Bis-(*O*-triethylsilyl)paclitaxel **3** (216.0 mg, 0.20 mmol) was dissolved in C_6H_6:CH_2Cl_2 (8:2, 20 mL). To this solution were added tetrabutyl ammonium hydrogen sulfate (800 mg, 2.35 mmol), followed by 2 N NaOH solution (20 mL). The reaction mixture was stirred at room temperature and monitored by TLC. After 1 h, TLC showed the presence of a new spot with low R_f (0.3) along with starting compound (R_f 0.75). The reaction mixture was then diluted with C_6H_6 (25 mL) and washed with H_2O (2 × 25 mL) and brine. The organic layer was separated, dried over Na_2SO_4 and evaporated to give crude product. Purification of crude product by preparative TLC (silica gel; 1000 μm, hexanes:EtOAc, 1:1) gave 2-*O*-debenzoyl-2′,7-bis(*O*-triethylsilyl) paclitaxel (**4**) (70.0 mg, 73% based on unrecovered starting material), 7-*O*-triethylsilyl baccatin (15.0 mg, 20% based on unrecovered starting material) and 2′,7-bis-(*O*-triethylsilyl)paclitaxel **3** (110 mg). Compound **4**: ^{1}H NMR δ 0.48 (6H, q, J = 7.8, $SiCH_2$), 0.58 (6H, q, J = 7.8, $SiCH_2$), 0.81 (9H, t, J = 7.8, $SiCH_2CH_3$), 0.90 (9H, t, J = 7.8, $SiCH_2CH_3$), 1.05 (3H, s, 16-CH_3), 1.10 (3H, s, 17-CH_3), 1.51 (3H, s, 19-CH_3), 1.95 (3H, br s, 18-CH_3), 2.15 (3H, s, 10-OAc), 2.40 (3H, s, 4-OAc), 2.50 (1H, m, 6-H), 3.25 (1H, br s, 2-OH), 3.49 (1H, d, J = 6.8, 3-H), 3.92 (1H, t, J = 5.8, 2-H), 4.42 (1H, dd, J = 10.6, 6.7, 7-H), 4.62 (3H, br s, 20-H and 2′-H), 4.98 (1H, br d, J = 8.8, 5-H), 5.63 (1H, br d, J = 9.5, 3′-H), 6.20 (1H, br t, 13-H), 6.37 (1H, s, 10-H), 7.08 (1H, d, J = 9.5, 3′N-H), 7.15–7.50 (8H, m, Ar-H), 7.78 (2H, dd, J = 8.5, 1.3, 3′-NBz orthoH). FABMS m/z $[M+Na+H]^+$ 1001.

Reaction of 2-*O*-debenzoyl-2′,7-bis(*O*-triethylsilyl)-paclitaxel (4) with substituted benzoic acids and DCC. A substituted benzoic acid (0.1 mmol) was dissolved in dry toluene (0.2 mL). To this solution DCC (20.6 mg, 0.1 mmol) and 4-pyrrolidinopyridine (1.0 mg, 0.006 mmol) were added. The mixture was stirred at room temperature for 5 min and then 2-*O*-debenzoyl-2′,7-bis(*O*-triethylsilyl)paclitaxel (**4**, 10.0 mg, 0.01 mmol) was added. The reaction mixture was stirred at room temperature or heated in an oil bath at 55 °C until the starting compound was consumed (TLC analysis). The reaction mixture was diluted with EtOAc (10 mL) and filtered through a pad of silica gel and celite; the pad was then washed with EtOAc (5 mL). The filtrate was concentrated on a rotary evaporator to give crude product. Further purification of crude product by column chromatography over silica gel using hexanes:EtOAc, 3:1, as eluent gave a 2-*O*-aroyl-2′,7-bis(*O*-triethylsilyl)paclitaxel analogue **5** in 65–75% yield.

Synthesis of 2-*O*-aroyl-2′,7-bis(*O*-triethylsilyl)-A-*nor*-paclitaxels 6. To a solution of a 2-*O*-aroyl-2′,7-bis(*O*-triethylsilyl)paclitaxel **5** (0.01 mmol) in CH_2Cl_2 (1 mL) was added pyridine (0.02 mL) by syringe followed by the addition of thionyl chloride (0.005 mL). The mixture was stirred at room temperature for 10 min. TLC analysis (hexanes:EtOAc, 3:1) showed that starting material was consumed, and a new spot with a higher R_f was formed. The mixture was worked up by diluting with CH_2Cl_2 and washing with dil $NaHCO_3$ (10%), dil HCl (1%), water, and brine, drying over Na_2SO_4, and evaporation under reduced pressure to yield crude product as a thick syrup. The crude product was purified by preparative TLC (silica gel; 500 μm, hexanes:EtOAc, 3:1) to obtain 2-*O*-aroyl-2′,7-bis(*O*-triethylsilyl)-A-*nor*-paclitaxel **6** in 85–95% yield. Representative NMR of compound **6f**: ^{1}H NMR δ 0.43 (6H, q, J = 7.8, $SiCH_2$), 0.57 (6H, q, J = 7.8, $SiCH_2$), 0.76 (9H, t, J = 7.8, $SiCH_2CH_3$), 0.90 (9H, t, J = 7.8, $SiCH_2CH_3$), 1.66 (6H, s, 16-CH_3 -H), 4.71 (1H, br s, olefinic H), 4.79 (1H, s, olefinic H), 5.06 (1H, br d, J = 8.8, 5-H), 5.59 (1H, d, J = 7.0, 2-H), 5.66 (1H, br d, J = 8.9, 3′ H), 5.84 (1H, br t, 13-H), 6.39 (1H, s, 10-H), 7.09 (1H, d, J = 8.9, 3′N-H), 7.20–7.81 (11H, m, ArH), 7.96 (1H, d, J = 7.5, 2 OBz 4-H), 8.30 (1H, d, J = 7.6 2-OBz 6-H), 8.40 (1H, br s, 2 OBz 2-H).

Reaction of 2-*O*-aroyl-2′,7-bis(*O*-triethylsilyl)-A-*nor*-paclitaxel with MeOH/HCl. A 2-*O*-aroyl-2′,7-bis(*O*-triethylsilyl)-A-*nor*-paclitaxel **6** (0.005 mmol) was dissolved in freshly prepared methanolic HCl (5% v/v). The mixture was stirred at room temperature for 40–45 min, diluted with EtOAc (10 mL) and washed with dil $NaHCO_3$ solution, water and brine. The organic layer was dried over Na_2SO_4 and concentrated to give crude product. Further purification was carried out using preparative TLC (silica gel; 500 μm, hexanes:EtOAc; 1:1) to yield a 2-*O*-aroyl-A-*nor*-paclitaxel **2** (70–90%).

Compound **2**: ^{1}H NMR δ 1.61 (3H, s, 19-CH_3), 1.66 (3H, s, 16-CH_3) 1.72 (3H, s, 18-CH_3), 1.93 (1H, m, 6-H), 2.05 (1H, dd, J = 8.1, 13.3, 14-H), 2.19 (3H, s, 10-OAc), 2.38 (3H, s, 4-OAc), 2.42 (1H, dd, J = 6.4, 13.3, 14-H), 2.60 (1H, d, J = 3.5, 7-OH), 2.62 (1H, m, 6-H), 3.39 (1H, d, J = 4.5, 2′-OH), 3.51 (1H, d, J = 8.1, 3-H), 4.20 (1H, d, J = 8.1, 20-H), 4.33 (1H, d, J = 8.1, 20-H), 4.65 (1H, m, 7-H), 4.70 (2H, m, 2′-H and olefinic H), 4.79 (1H, s, olefinic H), 5.06 (1H, br d, J = 8.2, 5-H), 5.51 (1H, d, J = 8.1, 2-H), 5.73 (2H, m, 3′-H and 13-H), 6.34 (1H, s, 10-H), 6.94 (1H, d, J = 9.2, 3′N-H), 7.30–7.58 (1H, m, ArH), 7.69 (2H, dd, J = 1.4, 7.1, 3′N Bz 2 and 6 H), 8.12 (2H, dd, J = 1.5, 7.1, 2-OBz 2 6 H). ^{13}C NMR δ 8.24, 11.64, 20.40, 20.62, 21.86, 35.80, 39.11, 44.35, 54.77, 56.61, 63.78, 70.75, 71.68, 72.54, 73.29, 74.47, 78.88, 78.96, 84.85, 113.46, 126.77, 126.97, 128.09, 128.54, 128.72, 128.88, 129.04, 129.99, 131.77, 133.53 133.81, 135.35, 138.22, 144.75, 145.20, 165.23, 166.74, 170.44, 171.26, 172.52, 203.22. HR-FABMS calcd for $C_{47}H_{49}O_{13}NNa$ ([M+Na]$^+$): 858.3101; observed 858.3113.

Compound **2b**: ^{1}H NMR δ 1.62 (3H, s, 19-CH_3), 1.70 (3H, s, 16-CH_3) 1.71 (3H, s, 18-CH_3), 1.94 (1H, m, 6-H), 2.08 (1H, dd, J = 7.6, 14.0, 14-H), 2.20 (3H, s, 10-OAc), 2.40 (3H, s, 4-OAc), 2.43 (1H, m, 14-H), 2.64 (1H, m, 6-H), 3.28 (1H, br s, 2′-OH), 3.54 (1H, d, J = 8.4, 3-H), 4.14 (1H, d, J = 8.1, 20-H), 4.27 (1H, d, J = 8.1, 20-H), 4.63 (1H, m, 7-H), 4.73 (2H, m, 2′-H and olefinic H), 4.82 (1H, s, olefinic H), 5.07 (1H, br d, J = 8.4, 5-H), 5.53 (1H, d, J = 8.4, 2-H), 5.72 (2H, m, 3′-H and 13-H), 6.34 (1H, s, 10-H), 6.82 (1H, d, J = 9.4, 3′N-H), 7.20–7.58 (1H, m, ArH), 7.75 (1H, d, J = 7.8, 2 Bz 4-H), 8.41 (1H, d, J = 7.9, 2-OBz 6-H), 8.45 (1H, br s, 2-OBz 2-H). HR-FABMS calcd for $C_{48}H_{49}O_{13}N_2$ ([M+H]$^+$): 861.3235; observed 861.3240.

Compound **2c**: ^{1}H NMR δ 1.62 (3H, s, 19-CH_3), 1.67 (3H, s, 16-CH_3) 1.71 (3H, s, 18-CH_3), 1.93 (1H, m, 6-H), 2.06 (1H, dd, J = 7.5, 14.1, 14-H), 2.20 (3H, s, 10-OAc), 2.36 (3H, s, 4-OAc), 2.44 (1H, dd, 14-H), 2.54 (1H, d, J = 3.6, 7-OH), 2.62 (1H, m, 6-H), 3.38 (1H, d, J = 4.5, 2′OH), 3.51 (1H, d, J = 8.2, 3-H), 4.22 (1H, d, J = 8.0, 20-H), 4.29 (1H, d, J = 8.0, 20-H), 4.65 (1H, m, 7-H), 4.68 (1H, br s, 2′-H), 4.72 (1H, br s, olefinic H), 4.81 (1H, s, olefinic H), 5.06 (1H, br d, J = 8.1, 5-H), 5.54 (1H, d, J = 8.2, 2-H), 5.70 (2H, m, 3′-H and 13-H), 6.33 (1H, s, 10-H), 6.84 (1H, d, J = 9.2, 3′N-H), 7.30–7.46 (10H, m, ArH), 7.62 (2H, d, J = 7.0, 3′NBz 2 and 6-H), 7.78 (1H, br s, 2-OBz 6 H), 7.92 (1H, d, J = 1.9, 2 OBz 2 H). HR-FABMS calcd for $C_{47}H_{49}O_{13}N_4$ ([M+H]$^+$): 877.3296; obsd 877.3304.

Compound **2d**: ^{1}H NMR δ 1.62 (3H, s, 19-CH_3), 1.67 (3H, s, 16-CH_3) 1.70 (3H, s, 18-CH_3), 1.93 (1H, m, 6-H), 2.02 (1H, dd, J = 7.5, 14.0, 14-H), 2.19 (3H, s, 10-OAc), 2.37 (3H, s, 4-OAc), 2.41 (1H, dd, 14-H), 2.61 (1H, m, 6-H), 3.30 (1H, br s, 2′-OH), 3.50 (1H, d, J = 8.3, 3-H), 4.23 (2H, ABd, J = 8.0, 20-H2), 4.63 (1H, m, 7-H), 4.70 (1H, br s, 2′-H), 4.75 (1H, br s, olefinic H), 4.81 (1H, s, olefinic H), 5.08 (1H, br d, J = 8.3, 5-H), 5.53 (1H, d, J = 8.3, 2-H), 5.70 (2H, m, 3′-H and 13-H), 6.32 (1H, s, 10-H), 6.78 (1H, d, J = 9.2, 3′N-H), 6.95 (1H, m, 2 Ar 4-H), 7.30–7.42 (8H, m, ArH), 7.65 (4H, m, 3′-NBz ortho H and 2-OBz ortho H). HR-FABMS calcd for $C_{47}H_{48}O_{13}NF_2$ ([M+H]$^+$) 872.3094; obsd 872.3064.

Compound **2e**: ^{1}H NMR δ 1.62 (3H, s, 19-CH_3), 1.64 (3H, s, 16-CH_3) 1.71 (3H, s, 18-CH_3), 1.92 (1H, m, 6-H), 2.04 (1H, dd, J = 7.8, 13.5, 14-H), 2.19 (3H, s, 10-OAc), 2.34 (3H, s, 4-OAc), 2.42 (1H, dd, J = 6.5, 13.4, 14-H), 2.55 (1H, d, J = 3.8 , 7-OH), 2.63 (1H, m, 6-H), 3.43 (1H, d, J = 4.7, 2′-OH), 3.48 (1H, d, J = 8.1 3-H), 3.81 (3H, s, OCH_3), 4.28 (2H, ABd, J = 8.0, 20-H2), 4.66 (2H, m, 7-H, 2′ H), 4.73 (1H, br s, olefinic H), 4.81 (1H, s, olefinic H), 5.06 (1H, br d, J = 8.0, 5-H), 5.55 (1H, d, J = 8.2, 2-H), 5.70 (2H, m, 3′-H and 13-H), 6.32 (1H, s, 10-H), 6.94 (1H, d, J = 9.1, 3′N-H), 7.09 (1H, dd, J = 1.4, 7.5, 2-OBz 4-H), 7.30–7.50 (9H, m, ArH), 7.60 (1H, d, J = 1.4, 2.5, 2-OBz 2′-H), 7.72 (3H, m, 2-OBz 6-H and 3′N-OBz 2, 6-H). HR-FABMS calcd for $C_{48}H_{51}O_{14}NNa$ ([M+Na]$^+$): 888.3207; obsd 888.3194.

Compound **2f**: ^{1}H NMR δ 1.62 (3H, s, 19-CH_3), 1.67 (3H, s, 16-CH_3) 1.72 (3H, s, 18-CH_3), 1.93 (1H, m, 6-H), 2.07 (1H, dd, J = 7.6, 13.2, 14-H), 2.20 (3H, s, 10-OAc), 2.38 (3H, s, 4-OAc), 2.46 (1H, dd, J = 6.6, 13.2, 14-H), 2.58 (1H, d, J = 3.5, 7-OH), 2.65 (1H, m, 6-H), 3.36 (1H, br s, 2′-OH), 3.54 (1H, d, J = 8.3, 3-H), 4.17 (1H, d, J = 7.9, 20-H), 4.28 (1H, d, J = 7.9, 20-H), 4.66 (1H, m, 7-H), 4.71 (1H, br s, 2′-H), 4.74 (1H, br s, olefinic H), 4.81 (1H, s, olefinic H), 5.07 (1H, br d, J = 8.0, 5-H), 5.53 (1H, d, J = 8.3, 2-H), 5.72 (2H, m, 3′-H and 13-H), 6.34 (1H, s, 10-H), 6.88 (1H, d, J = 9.2, 3′N-H), 7.28–7.62 (11H, m, ArH), 7.79 (1H, d, J = 7.8, 2 Bz 4 H), 8.37 (2H, br s, 2-OBz ortho H). HR-FABMS: calcd for $C_{48}H_{49}O_{13}NF_3$ ([M+H]$^+$): 904.3156; obsd 904.3112.

Compound **2g**: ^{1}H NMR δ 1.61 (3H, s, 19-CH_3), 1.70 (3H, s, 16-CH_3) 1.71 (3H, s, 18-CH_3), 1.95 (1H, m, 6-H), 2.07 (1H, dd, J = 7.6, 13.2, 14-H), 2.20 (3H, s, 10-OAc), 2.40 (3H, s, 4-OAc), 2.42 (1H, dd, J = 6.6, 13.2, 14-H), 2.59 (1H, d, J = 3.9, 7-OH), 2.63 (1H, m, 6-H), 3.30 (1H, br s, 2′-OH), 3.52 (1H, d, J = 8.3, 3-H), 4.17 (1H, d, J = 8.1, 20-H), 4.28 (1H, d, J = 8.1, 20-H), 4.66 (1H, m, 7-H), 4.73 (2H, m, 2′-H -H and 13-H), 6.34 (1H, s, 10-H), 6.83 (1H, d, J = 9.2, 3′N-H), 7.28–7.60 (9H, m, ArH), 7.62 (2H, dd, J = 1.4,7.1, 3′NBz ortho H), 7.79 (1H, dd, J = 1.9, 7.9, 2 OBz 4-H), 8.18 (1H, d, J = 1.9, 2 OBz 2H). HR-FABMS calcd for $C_{47}H_{48}O_{13}NCl_2$ ([M+H]$^+$): 904.2503; obsd 904.2497.

15,16-Dihydro A-*nor*-paclitaxel (7). A mixture of A-*nor*-paclitaxel (16.7 mg, 0.02 mmol) and Pd/C (100 mg, 10%) in ethyl acetate (0.5 mL) was stirred under H_2 (1 atm) at room temperature. The reaction was stopped after 2 h, when hydrogen uptake had ceased. The reaction mixture was filtered, the residue washed with ethyl acetate, and the combined ethyl acetate fractions concentrated to yield homogeneous 15,16-dihydro-A-*nor*-paclitaxel **7**. ^{1}H NMR δ 0.72 (3H, d, J = 6.8, 16-CH_3), 0.77 (3H, d, J = 6.7, 17-CH_3) 1.52 (3H, s, 19-CH_3), 1.76 (3H, s, 18-CH_3), 1.94 (1H, m, 6-H), 2.05 (1H, dd, J = 7.3, 12.4, 14-H), 2.17 (3H, s, 10-OAc), 2.22 (1H, m, 14-H), 2.34 (3H, s, 4-OAc), 2.58 (1H, m, 6-H), 3.46 (1H, d, J = 4.7, 2′-OH), 3.55 (1H, d, J = 8.1, 3-H), 4.15 (1H, d, J = 8.1, 20-H), 4.35 (1H, d, J = 8.1, 20-H), 4.57 (1H, m, 7-H), 4.72 (1H, m, 2′-H), 5.02 (1H, br d, J = 8.0, 5-H), 5.39 (1H, d, J = 8.1, 2-H), 5.78 (2H, m, 3′-H and 13-H), 6.27 (1H, s, 10-H), 7.03 (1H, d, J = 9.0, 3′N-H), 7.30–7.58 (11H, m, ArH), 7.73 (2H, dd, J = 1.5, 7.0, 3′NBz ortho H), 8.08 (2H, dd, J = 1.5,7.0, 2 OBz ortho H); ^{13}C NMR 8.37, 11.35, 17.87, 18.86, 20.39, 22.02, 34.99, 35.36, 36.20, 44.66, 54.90, 56.68, 63.85, 71.38, 72.60, 73.41, 74.56, 76.68, 79.07, 80.16, 84.90 126.81, 127.02, 128.17, 128.62, 128.73, 128.93, 129.25, 129.96, 131.85, 133.55 133.83, 136.38, 138.12, 144.31, 165.44, 166.79, 170.34, 171.02, 172.63, 203.72. HR-FABMS calcd for $C_{47}H_{51}O_{13}NNa$ ([M+Na]$^+$): 860.2894; obsd 860.3169.

15,16-Epoxy-A-*nor*-paclitaxel (8). To a solution of a A-*nor*- paclitaxel (16.7 mg, 0.02 mmol) in dry CH_2Cl_2 (1.0 mL) was added *m*-chloroperbenzoic acid (50%) (34.4 mg, excess). The mixture was stirred at room temperature for 4 h, diluted with CH_2Cl_2 (10 mL) and the resulting solution washed with dil $NaHCO_3$, H_2O, and brine. The organic layer was separated, dried over Na_2SO_4, and evaporated to give crude product. Further purification on preparative TLC (silica gel, 500 μm) using hexanes:EtOAc (1:1) as eluent gave epoxide **8** (16.0 mg, 93%). ^{1}H NMR δ 1.14 (3H, s, 16-CH_3), 1.64 (3H, s, 19-CH_3) 1.76 (3H, s, 18-CH_3), 2.04 (1H, m, 6-H), 2.14 (1H, m, 14-H), 2.21 (3H, s, 10-OAc), 2.36 (1H, d, J = 3.5, 17-H), 2.42 (3H, s, 4-OAc), 2.58 (2H, m, 6-H and 14-H), 2.77 (1H, d, J = 3.5, 17-H), 3.53 (1H, d, J = 8.1, 3-H), 4.14 (1H, d, J = 8.1, 20-H), 4.38 (1H, d, J = 8.1, 20-H), 4.68 (1H, m, 7-H), 4.75 (1H, br d, J = 2.2, 2′-H), 5.06 (1H, br d, J = 8.6, 5-H), 5.46 (1H, d, J = 8.1, 2-H), 5.79 (1H, d, J = 9.3, 3′-H), 6.00 (1H, br t, 13-H), 6.31 (1H, s, 10-H), 7.00 (1H, d, J = 9.3, 3′N-H), 7.32–7.73 (11H, m, ArH), 7.72 (2H, d, J = 7.1, 3′NBz ortho H), 8.15 (2H, d, J = 7.1, 2 OBz ortho H). HR-FABMS calcd for $C_{47}H_{49}O_{14}NNa$ ([M+Na]$^+$): 874.3050; obsd 874.3058.

Ozonolysis of A-*nor*-paclitaxel. Dry ozone gas was passed through a solution of A-*nor*-paclitaxel **2a** (16.7 mg, 0.02 mmol) in dichloromethane (2.0 mL) at −78 °C for 30 min. Nitrogen gas was flushed through a reaction mixture for 10 min. Dimethyl sulfide (0.2 mL) was added to the reaction mixture, which was allowed to warm to room temperature. The reaction mixture was evaporated on a rotary evaporator under reduced pressure until it became a thick syrup. The crude product was purified on preparative TLC (silica gel, 500 μm) using hexanes:EtOAc (1:1) as an eluent to yield the 1-keto compound **10** (15.4 mg, 92%). ^{1}H NMR δ 1.68 (6H, s, 18-CH_3 and 19-CH_3), 1.97 (2H, m, 14 and 6H), 2.09 (3H, s, 10-OAc), 2.16 (3H, s, 16-CH_3), 2.17 (3H, s, 4-OAc), 2.43 (1H, dd, J = 7.6, 14.7, 14-H), 2.60 (1H, m, 6-H), 3.36 (1H, d, J = 9.2, 3-H), 3.50 (1H, br s, 2′-OH), 4.41 (2H, ABd, J = 8.2, 20-H_2), 4.58 (2H, m, 7-H and 2′-H), 5.02 (1H, br d, J = 8.1, 5-H), 5.50 (1H, br d, J = 8.8, 3′-H), 5.85 (1H, t, J = 7.6, 13-H), 6.02 (1H, d, J = 9.2, 2-H), 6.27 (1H, s, 10-H), 6.83 (1H, d, J = 8.8, 3′N-H), 7.26–7.60 (13-H, m, ArH), 8.08 (2H, d, J = 6.9, 2-OBz orthoH); ^{13}C NMR 8.38, 12.50, 20.38, 21.70, 25.56, 35.43, 36.50, 44.10, 55.13, 56.07, 69.42, 71.17, 71.78, 71.91, 73.24, 74.62, 79.17, 79.81, 84.75 126.91, 126.93, 128.21, 128.59, 128.75, 128.88, 130.04, 131.83, 132.01, 133.53, 133.70, 137.92, 147.39, 165.08, 166.77, 170.45, 171.14, 172.46, 202.62, 204.36. HR-FABMS calcd for $C_{46}H_{47}O_{14}NNa$ ([M+Na]$^+$) 860.2894; obsd 860.2889.

Reaction of epoxide 8 with MeOH/HCl. A solution of epoxide **8** (8.5 mg, 0.01 mmol) in methanolic HCl (5% v/v, 0.2 mL) was stirred at room temperature for 30 min. The reaction mixture was diluted with EtOAc (10.0 mL) and washed with dilute $NaHCO_3$, H_2O, and brine. The organic layer was dried over Na_2SO_4 and evaporated to yield crude product. This was purified on preparative TLC (silica gel, 500 μm) using hexanes:EtOAc (1:1) as an eluent to yield chloro alcohol **9** (7.8 mg, 88%). ^{1}H NMR δ 1.18 (3H, s, 16-CH_3), 1.64 (3H, s, 19-CH_3) 1.77 (3H, s, 18-CH_3), 1.92 (1H, dd, J = 9.1, 14.7, 6-H), 2.16 (1H, m, 14-H), 2.19 (3H, s, 10-OAc), 2.38 (3H, s, 4-

OAc), 2.56 (2H, m, 6-H and 14-H), 3.33 (1H, d, J = 11.0, 17-H), 3.45 (2H, m, C17-H and OH), 3.63 (1H, d, J = 7.8, 3-H), 4.11 (1H, d, J = 8.1, 20-H), 4.37 (1H, d, J = 8.1, 20-H), 4.42 (1H, m, 7-H), 4.78 (1H, dd, J = 2.5, 4.8, 2(-H), 5.00 (1H, br d, J = 8.4, 5-H), 5.75 (1H, d, J = 7.8, 2-H), 5.81 (1H, d, J = 9.2, 3′-H), 5.93 (1H, br t, 13-H), 6.29 (1H, s, 10-H), 7.02 (1H, d, J = 9.2, 3′N-H), 7.34–7.58 (11H, m, ArH), 7.75 (2H, d, J = 7.6, 3′NBz ortho H), 8.13 (2H, d, J = 7.1, 2-OBz orthoH). FABMS ($[M+H]^+$) 867.6.

Determination of EC_{50} values for paclitaxel analogues in 1.0 M glutamate. As described previously, the EC_{50} value obtained for paclitaxel-induced assembly in glutamate at room temperature varies inversely with the glutamate concentration.[11] In the earlier study this ranged from >40 μM at 0.1 M glutamate, through 23 μM at 0.4 M glutamate, 11 μM at 0.5 M glutamate, 3.5 μM at 0.7 M glutamate, to 2.6 μM at 1.0 M glutamate. After establishing that the analogues presented here were less active than paclitaxel, we chose the 1.0 M glutamate condition for detailed comparative studies, as presented in Table 1. Reaction mixtures contained 1.0 mg/mL (10 μM) tubulin, 1.0 M monosodium glutamate (pH 6.6 with HCl in 2 M stock solution), 4% (v/v) dimethyl sulfoxide, and varying drug concentrations. Incubation was for 15 min at room temperature, followed by a 10 min centrifugation at room temperature at 14,000 rpm in an Eppendorf model 5415C microcentrifuge. The EC_{50} was defined as the drug concentration that resulted in a 50% reduction of protein in the supernatant as compared with control reaction mixtures without drug.

Growth of human Burkitt lymphoma CA46 cells. Cells were grown in 5.0 mL suspension cultures under 5% CO_2 for 24 h at 37 °C, as described previously.[4] The IC_{50} value was defined as the drug concentration that reduced the increase in cell number (cell growth) by 50%.

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