Supporting Information

Synthesis, Modeling and Anti-Tubulin Activity of a D-Seco Paclitaxel Analog

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Experimental:

General Methods. NMR spectra were taken on Bruker Avance 400 or Varian VXR 300 spectrometers. Cytotoxicity and tubulin binding were carried out according to established protocols.¹ Commercially available reagents and solvents were used without further purification. All anhydrous reactions were performed under a positive pressure of nitrogen. CH₂Cl₂ was dried by distillation from CaH₂, and THF by distillation from Na-benzophenone. Solvent evaporation was done under reduced pressure. Column chromatography was carried out with silica gel as the stationary phase.

Preparation of compound 5: To a stirred solution of compound 4 (655 mg, 0.611 mmol) in anhydrous CH₂Cl₂ at ambient temperature, Bu^t₄Ni (1.830 mmol, 675 mg) and MgBr₂·Et₂O (0.775 mmol, 200 mg) were added. The reaction mixture was stirred under N₂ at rt for 5 h (monitored by TLC, hexane/EtOAc 4:1) then further Bu^t₄Ni (0.542 mmol, 200 mg) and MgBr₂·Et₂O (0.388 mmol, 100 mg) were added. After 3 h the reaction mixture was diluted with CH₂Cl₂ and washed successively with water, 5% NaHCO₃ and brine. The organic phase was dried over anhydrous Na₂SO₄ and evaporated to dryness. The crude product was purified by silica gel chromatography (hexane/EtOAc 85:15) to give compound 5 (638 mg, 87%) as an amorphous solid. ¹H NMR (CDCl₃, 400 MHz): δ -0.33 (s, 3H), -0.13 (s, 3H), 0.59 (q, *J* = 7.9 Hz, 6H), 0.80 (s, 9H), 0.94 (t, *J* = 7.9 Hz, 9H), 1.13 (s, 3H), 1.22 (s, 3H), 1.37 (s, 3H), 2.11 (m, 1H), 2.19 (s, 3H), 2.33 (m, 1H), 2.48 (dd, *J* = 15.7, 9.9 Hz, 1H), 2.50 (bs, 3H), 3.01 (dd, *J* = 15.7, 5.6 Hz, 1H), 3.85 (d, *J* = 10.8 Hz, 1H), 3.98 (d, *J* = 5.0 Hz, 1H), 4.18 (d, *J* = 10.8 Hz, 1H), 4.29 (s, 1H), 4.34 (d, *J* = 5.0 Hz, 1H), 4.57 (dd, *J* = 10.5, 3.8 Hz, 1H), 4.61 (d, *J* = 2.0 Hz, 1H), 5.15 (bs, 1H), 6.03 (m, 1H), 6.05 (bd, *J* = 8.6 Hz, 1H), 6.60 (s, 1H), 7.14 (d, *J* = 8.5 Hz, 1H), 7.26 (m, 2H), 7.34 (bt, *J* = 7.4 Hz, 2H), 7.47 (m, 3H), 7.52 (m, 1H), 7.80 (m, 2H); ¹³C NMR (100 MHz, CDCl₃): δ -5.95, -5.58, 5.16, 6.76, 13.77, 14.38, 17.82, 18.22, 19.77, 20.77, 25.53, 26.37, 32.81, 39.05, 41.07, 43.88, 45.38, 55.62, 62.15, 71.02 (2C), 71.30, 75.17, 75.24, 82.08,

89.89, 127.06, 127.22, 127.58, 128.31, 128.77, 131.86, 133.16, 133.81, 138.84, 143.38, 151.97, 167.15, 169.02, 171.18, 200.76; MS (FAB+) m/z 1200.3 ($M + H$)⁺; $[\alpha]^{20}_D +17.8$ (EtOAc, c 2.06).

Preparation of compound 6: To a solution of compound **5** (617 mg, 0.515 mmol) in EtOAc (30 ml, HPLC grade) at rt, PtO₂ (Adams catalyst, 300 mg) was added. The suspension was stirred under H₂ atmosphere (balloon pressure) for 3 h; then additional PtO₂ (100 mg) was added. After 17 h about 50% of starting material was present (TLC monitoring: hexane/EtOAc 4:1) and, since the catalyst was black and flaky, a further 150 mg of catalyst was added. After 24 h the mixture was filtered, evaporated to dryness and chromatographed (silica gel; hexane/EtOAc 85:15) to give starting material (137 mg, 22%) and **6** as an amorphous solid (328 mg, 57%, 79% conversion). ¹H NMR (CDCl₃, 300 MHz): δ -0.34 (s, 3H), -0.15 (s, 3H), 0.58 (q, J = 7.9 Hz, 6H), 0.77 (s, 9H), 0.93 (t, J = 7.9 Hz, 9H), 1.11 (s, 3H), 1.17 (s, 3H), 1.36 (s, 3H), 1.73 (s, 3H), 2.16 (s, 3H), 2.19 (m, 1H), 2.34 (m, 2H), 2.45 (bs, 3H), 2.94 (dd, J = 15.9, 5.5 Hz, 1H), 3.57 (d, J = 5.5 Hz, 1H), 4.06 (bs, 1H), 4.40 (d, J = 5.5, 1H), 4.56 (dd, J = 10.4, 3.9, 1H), 4.62 (d, J = 1.8, 1H), 4.63 (m, 1H), 6.00 (dd, J = 8.8, 1.8 Hz, 1H), 6.08 (m, 1H), 6.57 (s, 1H), 7.13 (d, J = 8.8 Hz, 1H), 7.24 (m, 2H), 7.31 (m, 2H), 7.46 (m, 2H), 7.53 (m, 2H), 7.77 (m, 2H); ¹³C NMR (100 MHz, CDCl₃): δ -6.02, -5.69, 5.10, 6.67, 12.61, 17.61, 18.12, 19.53, 20.72, 25.43, 25.58, 26.39, 36.46, 39.15, 40.73, 46.07, 47.46, 55.03, 62.08, 70.54, 71.07, 71.58, 75.24, 75.28, 82.38, 90.10, 126.99, 127.23, 127.45, 128.17, 128.71, 131.77, 132.94, 133.80, 138.78, 142.97, 152.34, 166.56, 168.99, 171.30, 201.46; MS (FAB+) m/z 1074.3 ($M + H$)⁺; $[\alpha]^{20}_D +9.1$ (CHCl₃, c 1.13).

Preparation of compound 7: To a stirred solution of compound **6** (328 mg, 0.306 mmol) in DMPU (20 ml) under N₂ atmosphere, NaBH₃CN (77 mg, 1.223 mmol) was added. The reaction mixture was stirred overnight at 70 °C under N₂ atmosphere, then the reaction mixture was quenched with 2N HCl and extracted with EtOAc. The organic solution was washed consecutively with 5% NaHCO₃, H₂O and brine, dried over Na₂SO₄ and evaporated to dryness. The crude product was purified by silica gel chromatography (hexane/EtOAc 85:15) to yield compound **7** (210 mg, 73%) as an amorphous solid. ¹H NMR (CDCl₃, 400 MHz): δ -0.29 (s, 3H), -0.11 (s, 3H), 0.64 (q, J = 8.0 Hz, 6H), 0.84 (s, 9H), 0.95 (t, J = 8.0 Hz, 9H), 1.18 (s, 3H), 1.22 (s, 3H), 1.25 (s, 3H), 1.64 (s, 3H), 2.14 (bs, 3H), 2.20 (s, 3H), 2.52 (dd, J = 15.8, 10.0 Hz, 1H), 2.94 (dd, J = 15.8, 4.2 Hz, 1H), 2.98 (d, J = 6.2 Hz, 1H), 3.84 (s, 1H), 4.31 (d, J = 6.2 Hz, 1H), 4.45 (d, J = 1.5 Hz, 1H), 4.78 (bs, 1H), 5.52 (dd, J = 10.3, 1.5 Hz, 1H), 5.69 (dd, J = 10.3, 1.9 Hz, 1H), 5.89 (bd, J = 10.0 Hz, 1H), 5.99 (bd, J = 8.5 Hz, 1H), 6.58 (s, 1H), 7.26 (m, 2H), 7.35 (m, 4H), 7.47 (m, 2H), 7.53 (m, 1H), 7.78 (m, 2H); ¹³C NMR (100 MHz, CDCl₃): δ -5.99, -5.69, 5.19, 6.66, 12.75, 16.77, 18.14, 18.90, 20.79, 25.44, 27.06, 27.25, 32.70, 40.55, 51.06, 54.95, 61.51, 70.97, 71.81, 72.15, 75.34, 75.69, 81.80, 89.84, 126.56, 127.00, 127.62, 128.33, 125.66, 128.70, 131.80, 132.43, 133.82, 138.18, 139.63, 140.60, 152.71, 166.93, 169.20, 170.41, 203.16; MS (FAB+) m/z 946.7 ($M + H$)⁺; $[\alpha]^{20}_D -19.5$ (CHCl₃, c 0.57).

Preparation of compound 8: To a stirred solution of compound **7** (263 mg, 0.278 mmol) in toluene (30 ml) under N₂ atmosphere, DMAP (1360 mg, 11.130 mmol) and acetic anhydride (Ac₂O) (1136 mg, 11.130 mmol, 1.05 ml) were added. The reaction mixture was stirred overnight at 80°C under N₂ atmosphere and further Ac₂O (1 ml) and DMAP (1350 mg) were added. After 24 h further Ac₂O (0.5 ml) and DMAP (700 mg) were added. After 5 h the reaction mixture was concentrated under vacuum, diluted with

EtOAc and extracted consecutively with 2N HCl, 5% NaHCO₃, H₂O and brine, dried over Na₂SO₄ and evaporated to dryness. The crude product was purified by silica gel chromatography (hexane/EtOAc 85:15) to give starting material (80 mg) and compound **8** (80 mg, 30%) as an amorphous solid. ¹H NMR (CDCl₃, 300 MHz): δ -0.26 (s, 3H), -0.05 (s, 3H), 0.58 (q, *J* = 7.9 Hz, 6H), 0.80 (s, 9H), 0.88 (t, *J* = 7.9 Hz, 9H), 1.22 (s, 3H), 1.24 (s, 3H), 1.27 (s, 3H), 1.83 (s, 3H), 1.98 (bs, 3H), 2.15 (s, 3H), 2.16 (s, 3H), 2.58 (dd, *J* = 15.6, 9.8 Hz, 1H), 2.98 (dd, *J* = 15.6, 6.7 Hz, 1H), 3.01 (d, *J* = 6.7 Hz, 1H), 4.36 (d, *J* = 6.7 Hz, 1H), 4.62 (d, *J* = 1.8 Hz, 1H), 4.63 (bs, 1H), 5.57 (d, *J* = 9.1 Hz, 1H), 5.67 (dd, *J* = 9.1, 1.2 Hz, 1H), 6.13 (bt, *J* = 8.5 Hz, 1H), 6.40 (dd, *J* = 9.2, 1.8 Hz, 1H), 6.47 (s, 1H), 7.10 (d, *J* = 9.2, 1H), 7.31 (m, 5H), 7.49 (m, 3H), 7.79 (m, 2H); ¹³C NMR (75 MHz, CDCl₃): δ -5.57, -5.11, 5.26, 6.68, 13.46, 15.96, 18.09, 20.05, 20.79, 23.12, 25.57, 25.65, 26.17, 32.29, 41.17, 48.89, 55.54, 61.23, 70.51, 72.08, 75.16, 75.43, 81.25, 83.30, 89.86, 126.45, 127.01, 127.94, 128.64, 128.78, 129.13, 130.78, 131.83, 132.23, 134.02, 138.07, 142.24, 152.66, 166.65, 169.16, 169.33, 171.57, 202.51; MS (ES+) *m/z* 1010.7 (M + Na)⁺; [α]_D²⁰ -33.6 (CHCl₃, *c* 1.12).

Preparation of compound 1: To a stirred solution of compound **8** (80 mg, 0.081 mmol) in anhydrous THF (3 ml) at -78 °C under N₂ atmosphere, phenyllithium (PhLi, 0.405 mmol, 225 μ l of 1.8M solution in cyclohexane-ether) was added. After stirring at -78 °C for 1 h, TLC (hexane/EtOAc 4:1) showed the initial formation of a compound with a lower *R_f* than that of the starting material and further 100 μ l of PhLi were added. After 1.5 h 100 μ l of PhLi were added and the reaction mixture was stirred at -78 °C for further 30 minutes (the TLC showed the formation of many spots). The mixture was then quenched with EtOAc and the organic phase was washed with 5% NaHCO₃, water and brine. The EtOAc layer was then dried over Na₂SO₄ and evaporated to dryness. The mixture was dissolved in anhydrous THF (1 ml) in a teflon vial, the solution was stirred at 0 °C and a cold solution (-20 °C) of hydrogen fluoride-pyridine (HF/Py, 100 μ l) was added. The reaction mixture was stirred at room temperature for 3 h and then further 50 μ l of HF/Py were added. After 1 h, the mixture was diluted with EtOAc (5 ml) and neutralised with a saturated NaHCO₃ solution. The solution was then extracted successively with 2N HCl, 5% NaHCO₃, water and brine. The EtOAc layer was then dried over Na₂SO₄ and evaporated to dryness. The crude product was purified by preparative TLC (silica gel, hexane/EtOAc 2:3) to give compound **1** (2 mg, 3%). ¹H NMR (CDCl₃, 300 MHz): δ 1.13 (s, 3H, H-16), 1.15 (s, 3H, H-17), 1.34 (s, 3H, H-19), 1.44 (s, 3H, H-20), 1.65 (bs, 3H, H-18), 2.21 (s, 3H, 4-OAc), 2.28 (dd, *J* = 15.5, 7.3 Hz, 1H, H-14), 2.38 (dd, *J* = 15.5, 9.4 Hz, 1H, H-14), 3.91 (d, *J* = 7.4 Hz, 1H, H-3), 4.02 (d, *J* = 1.8 Hz, 1H, 10-OH), 4.41 (m, 1H, H-7), 4.43 (d, *J* = 3.2 Hz, 1H, 2'-OH), 4.77 (dd, *J* = 3.2, 2.4 Hz, 1H, H-2'), 5.31 (d, *J* = 1.8 Hz, 1H, H-10), 5.53 (dd, *J* = 10.4, 1.2 Hz, 1H, H-6), 5.55 (d, *J* = 7.4 Hz, 1H, H-2), 5.70 (dd, *J* = 10.4, 2.4 Hz, 1H, H-5), 5.83 (dd, *J* = 9.2, 2.4 Hz, 1H, H-3'), 6.07 (bt, *J* = 8.2 Hz, 1H, H-13), 7.25-7.62 (m, 12H, NH + 11 aromatic protons), 7.82 (bd, *J* = 7.9 Hz, 2H, *o*-OBz), 8.07 (bd, *J* = 7.7 Hz, 2H, *o*-NHBz). ¹³C NMR (75 MHz, CDCl₃): δ 12.46 (q, C-19), 15.00 (q, C-18), 19.16 (q, C-16), 22.88 (q, 4-OAc), 26.70 (q, C-17), 28.34 (q, C-20), 36.19 (t, C-14), 42.52 (s, C-15), 49.30 (d, C-3), 54.61 (d, C-3'), 60.02 (s, C-8), 71.46 (d, C-13), 72.80 (d, C-7), 73.99 (d, C-2'), 74.34 (d, C-10), 74.81 (d, C-2), 78.83 (s, C-1), 83.32 (s, C-4), 126.04 (d, C-6), 127.02 (d, *m*-NBz), 128.05 (d, *p*-Ph), 127.21, 128.62, 128.68 (2 signals), 129.65, 129.77 (*o*-OBz, *o*-NHBz, *o*-Ph, *m*-Ph, C1-OBz, C1-Ph), 130.02 (d, *m*-OBz), 131.85 (d, *p*-NBz), 133.56 (d, *p*-OBz), 133.95 (d, C-5), 134.82 (s, C-

11), 137.14 (s, C-12), 138.27 (s, C1-NHBz), 166.40 (s, NHBz), 167.34 (s, OBz), 170.74 (s, 4-OAc), 171.24 (s, C-1'), 210.73 (s, C-9); $[\alpha]_{\text{D}}^{20} -55.8$ (CHCl_3 , c 0.09). High resolution mass spectrum: ES+ in methanol, 5 mM NH_4OAc : 813.3612, calc. for $\text{M}+\text{NH}_4$, $\text{C}_{45}\text{H}_{53}\text{N}_2\text{O}_2=813.3599$ (1.3 mmu)

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- ¹ Liu, Y.; Ali, S. Y.; Boge, T. C.; Georg, G. I.; Victory, S.; Zygmunt, J.; Marquez, R. T.; Himes, R. H. A Systematic SAR Study of C10 Modified Paclitaxel Analogues Using a Combinatorial Approach. *Combi. Chem. & High Thr. Scr.* **2002**, 5, 39-48.