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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Design and synthesis of taxane derivatives of valproic acid as potent and selective cytotoxic agents

Himaja Malipeddi[1] · **Sunil V. Mali**[1,2] · **Moonjit Das**[1]

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Abstract Resistance to anticancer agents has important implications for cancer chemotherapy. Small changes in chemical structures of cytotoxic agents can alter their biological interactions that can be beneficial in overcoming the drug resistance problem. Valproic acid, a well-known antiepileptic drug is in advanced clinical studies for cancer treatment. In the present study, valproic acid was incorporated into the taxane moiety at various positions and the new analogs were evaluated for their in vitro cytotoxicity. The novel analog, Valprotaxel showed comparable cytotoxicity in head and neck, and colon cancer cell lines with remarkable improvement in selectivity for cancer cells compared to paclitaxel and docetaxel.

Keywords Cytotoxic agents · Valproic acid · Taxane · Valprotaxel

Introduction

Paclitaxel (**1**) and docetaxel (**2**) are widely used chemotherapeutic agents for the treatment of many types of human solid tumors such as ovarian, head and neck, bladder, lung and breast cancers, and have also been used to treat malignant glioma and brain metastates (Wani et al., 1971; Hajek et al., 1996; Lee et al., 1997; Glantz et al., 1997; Brandes et al., 2000). However, aqueous insolubility, lack of tumor cell selectivity and acquired and intrinsic tumor resistance associated with P-glycoprotein (Pgp) remain as limitations (Sparreboom et al., 1997). Drug cocktails (combinations) are currently used to overcome resistance problem in cancer chemotherapy. Small changes in chemical structure of the taxane moiety can alter interaction with Pgp. Cabazitaxel (**3**), a 7,10 methyl ether analog of docetaxel is a potent tubulin-binding taxane and a poor P-gp substrate, approved by US Food and Drug Administration in June 2010 for treatment, to prolong survival for patients with metastatic multidrug resistant (MDR) prostate cancer in the post docetaxel setting. BMS275183 (**4**), in which the C-3 phenyl ring was replaced with t-butyl group and C-4 acetate was replaced with carbonate enhanced the oral efficacy in two tumor models (Mastalerz et al., 2003) (Fig. 1). The C-10 succinate of paclitaxel was reported with reduced P-gp interactions and improved permeability across the blood–brain barrier (BBB) (Rice et al., 2003). There has been a growing interest in the concept of combining two drugs or pharmacophoric moieties in a single molecule to create a more medically effective hybrid entity.

Valproic acid (VPA, **6**), an antiepileptic drug is broad-spectrum inhibitor of histone deacetylase currently studied for cancer therapy (Göttlicher et al., 2001). The combination of VPA with other anticancer agents has been considered as a useful strategy for cancer therapy (Eyal et al., 2006; Gonzalez et al., 2008; Garcia-Manero et al., 2006; Soriano et al., 2007). Although VPA is known to induce P-gp (MDR1) expression, the derivatization of acid group of VPA results in the loss of the ability to express P-gp

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✉ Himaja Malipeddi
drmhimaja@gmail.com

[1] Pharmaceutical Chemistry Division, School of Advanced Sciences, VIT University, Vellore, Tamil Nadu, India

[2] Medicinal Chemistry Division, Piramal Healthcare Ltd., Goregaon, Mumbai, India

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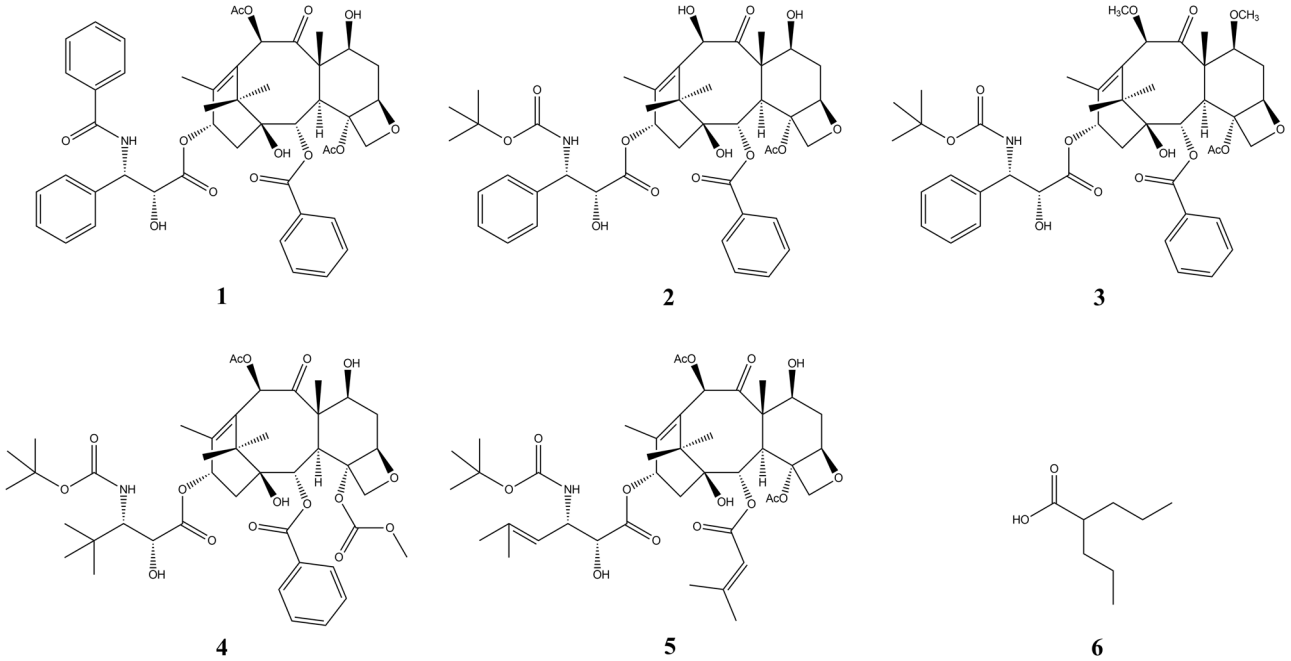


Fig. 1 Structures of taxol analogs and VPA

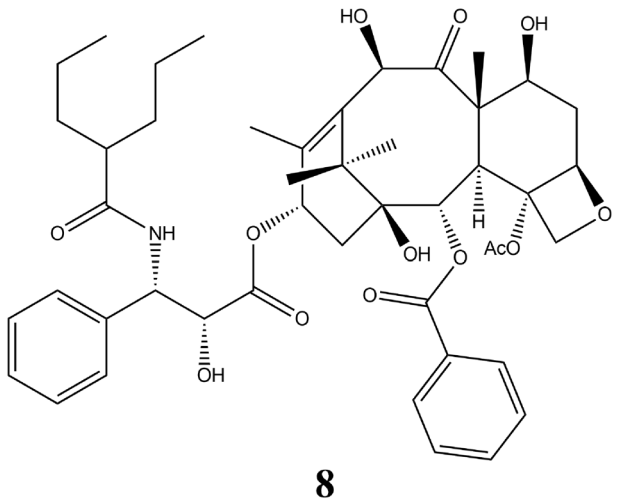


Fig. 2 Structure of valprotaxel (**8**)

(Braiteh et al., 2008). VPA, a relatively small molecule, was combined with taxane moiety covalently to generate a new chemical entity. In this paper, we present the synthesis and the in vitro cytotoxicity study of these taxol analogs. The novel compound, Valprotaxel (**8**) has shown interesting in vitro results (Fig. 2).

Materials and methods

Paclitaxel and docetaxel were purchased from Dabur Pharma Ltd, Nadia, West Bengal, India. VPA was purchased from Sigma Aldrich chemicals. All reagents and solvents were of laboratory grade and used without further purification. Nuclear magnetic resonance (NMR) spectra were obtained on Bruker AVANCE 300 Instrument. Chemical values are reported as δ values in ppm: s = singlet, d = doublet, dd = doublet of doublet, t = triplet, q = quartet, m = multiplet, bs = broad singlet. Mass spectrometry and high-resolution mass spectrometry (HRMS) data were obtained on Bruker-Micro-Q-TOF instrument.

Synthesis of (2aR,4S,4aS,6R,9S,11S,12S,12aR,12bS)-12b-acetoxy-9-(((2R,3S)-3-amino-2-hydroxy-3-phenylpropanoyl)oxy)-4,6,11-trihydroxy-4a,8,13,13-tetramethyl-5-oxo-2a,3,4,4a,5,6,9,10,11,12,12a,12b-dodecahydro-1H-7,11-methanocyclodeca-[3,4]benzo [1,2-b]oxet-12-yl benzoate (7)

Docetaxel (0.100 g, 0.123 mmol) was stirred in Tetrahydrofuran (THF) (5 ml) in an ice bath. To this was added a solution of trifluoroacetic acid (0.5 ml) in THF (2 ml). The reaction mixture was stirred in an ice bath for 2 h under nitrogen. The reaction mixture was carefully neutralized with sodium bicarbonate (8 % w/v) addition in an ice bath until pH 8 and extracted with dichloromethane (DCM) (20 ml). Organic layer was washed with brine (10 ml), dried over sodium sulfate and concentrated to get free amine compound **7**.

Yield 86 % (0.075 g); white solid; ^{1}H NMR (DMSO-d_6, 300 MHz): $\delta = 7.95$ (d, 2H, $J = 6.9$ Hz, ArH), 7.61–7.75 (m, 3H, ArH), 7.35–7.37 (m, 4H, ArH), 7.19–7.21 (m, 1H, ArH), 5.86 (br s, 1H, CH), 5.40 (d, 1H, $J = 7.2$ Hz, CH), 5.07 (br s,

1H, CH), 5.02 (d, 1H, $J = 6.9$ Hz, CH), 4.96 (s, 1H, CH), 4.88 (d, 1H, $J = 8.4$ Hz, CH), 4.54 (s, 1H, CH), 4.06 (s, 1H, CH), 3.96–4.03 (m, 3H, CH_2 & CH), 3.64 (d, 1H, $J = 7.2$ Hz, CH), 2.22–2.26 (m, 2H, CH_2), 2.11 (s, 3H, CH_3), 1.91 (s, 2H, CH_2), 1.78–1.87 (m, 2H, CH_2), 1.74 (s, 3H, CH_3), 1.64–1.68 (m, 4H, CH_2), 1.51 (s, 3H, CH_3), 1.01 (s, 3H, CH_3), 0.97 (s, 3H, CH_3); MS: (ES+) *m/z* 708.2 $[M + H]^+$.

Synthesis of (2aR,4S,4aS,6R,9S,11S,12S,12aR,12bS)-12b-acetoxy-4,6,11-trihydroxy-9-(((2R,3S)-2-hydroxy-3-phenyl-3-(2-propylpentanamido)propanoyl)oxy)-4a,8,13,13-tetramethyl-5-oxo-2a,3,4,4a,5,6,9,10,11,12,12a,12b-dodecahydro-1H-7,11-methanocyclodeca[3,4]benzo[1,2-b]oxet-12-yl benzoate (8)

Compound **7** (0.070 g, 0.099 mmol) was stirred with VPA (0.01 g, 0.099 mmol) in DCM (2 ml). DCC (0.024 g, 0.118 mmol) and dimethylaminopyridine (DMAP) (0.006 g, 0.049 mmol) were added and the reaction mixture was stirred overnight at room temperature (RT). The reaction mixture was concentrated and purified by silica gel (150–300 mesh) column chromatography using acetone: DCM gradient to yield pure compound **8**.

Yield 24 % (0.020 g); off-white solid; ^{1}H NMR ($CDCl_3$, 300 MHz): $\delta = 8.13$ (d, 2H, $J = 7.2$ Hz, ArH), 7.53–7.64 (m, 3H, ArH); 7.28–7.40 (m, 5H, ArH), 6.33 (d, $J = 9.0$ Hz, 1H, CH), 6.19 (br s, 1H, CH), 5.71 (d, 1H, $J = 6.9$ Hz, CH), 5.64 (d, 1H, $J = 6.9$ Hz, CH), 5.21 (s, 1H, CH), 4.95 (d, 1H, $J = 8.1$ Hz, CH), 4.69 (br s, 1H, CH), 4.33 (d, 1H, $J = 8.4$ Hz, CH), 4.23 (br s, 3H, CH & CH_2); 3.91 (d, 1H, $J = 7.5$ Hz, CH), 3.49 (br s, 1H, CH), 2.58–2.64 (m, 2H, CH_2); 2.38 (s, 3H, CH_3), 2.29–2.33 (m, 2H, CH_2), 2.10–2.14 (m, 1H, CH), 2.06 (s, 1H, CH), 1.84 (s, 3H, CH_3), 1.77 (s, 3H, CH_3), 1.54–1.63 (m, 4H, CH & CH_2), 1.36–1.38 (m, 3H, CH &CH_2), 1.28 (s, 3H, CH_3), 1.25 (s, 3H, CH_3), 1.11 (s, 3H, CH_3), 0.82–0.88 (m, 6H, CH_3); ^{13}C NMR ($CDCl_3$, 75.4 MHz): $\delta = 9.8$, 11.0, 14.1, 14.3, 20.7, 22.4, 22.6, 24.7, 24.9, 26.3, 31.5, 35.0, 35.1, 35.3, 35.6, 36.8, 43.1, 46.4, 47.5, 54.1, 57.7, 71.8, 72.5, 73.3, 74.5, 74.8, 78.6, 81.1, 84.2, 84.5, 126.8, 128.0, 128.7, 129.1, 129.5, 129.8, 130.1, 133.6, 136.0, 138.3, 166.9, 170.1, 173.3, 175.8, 211.3; HRMS: *m/z* ESI (+ve) for $C_{46}H_{60}NO_{13}$ Calculated; 834.4059 $[M + H]^+$. Found: 834.4026 $[M + H]^+$ (Mass accuracy: 3.99 ppm).

Synthesis of 4a,8,13,13-tetramethyl-5-oxo-2a,3,4,4a,5,6,9,10,11,12,12a,12b-dodecahydro-1H-7,11-methanocyclodeca[3,4]benzo[1,2-b]oxet-12-yl benzoate (10)

10-deacetyl baccatin–III (compound **9**) (0.200 g, 0.341 mmol) was stirred in dry DCM (5 ml) along with pyridine (5 ml) in an ice bath. To this was added a solution of allyl chloroformate (0.102 g, 0.853 mmol) in DCM (2 ml), followed by addition of DMAP (0.041 g, 0.341 mmol). The reaction mixture was stirred in an ice bath for 1 h under nitrogen, and then overnight at RT. The reaction mixture was quenched with ice water and extracted with DCM (20 ml). The organic layer was washed with brine (10 ml), dried over sodium sulfate, concentrated and purified by silica gel (150–300 mesh) column chromatography using acetone: DCM gradient to get pure compound **10**.

Yield 60 % (0.150 g); white solid; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.09 (d, 2H, $J = 7.2$ Hz, ArH), 7.60 (d, 2H, $J = 7.5$ Hz, ArH), 7.47 (t, 2H, $J = 7.5$ Hz, ArH), 6.14 (s, 1H, CH), 5.85–5.98 (m, 1H, CH), 5.60-5.64 (m, 1H, CH), 5.26-5.44 (m, 4H, CH_2), 4.95 (t, 1H, $J = 7.5$ Hz, CH), 4.87 (br s, 1H, CH), 4.69 (d, 1H, $J = 5.1$ Hz, CH), 4.59-4.64 (m, 2H, CH_2), 4.31 (t, 1H, $J = 7.2$ Hz, CH), 4.01–4.19 (m, 3H, CH2 &CH), 3.84 (d, 1H, $J = 7.2$ Hz, CH), 2.53–2.64 (m, 2H, CH_2), 2.29 (s, 3H, CH_3), 2.11 (s, 2H, CH_2), 2.07 (s, 3H, CH_3), 2.00 (s, 1H, CH), 1.84 (s, 2H, CH_2), 1.67 (s, 1H, CH), 1.06–1.13 (m, 6H, CH_3); MS: (ES+) *m/z* 735.3$[M + Na]^+$.

Synthesis of (2aR,4S,4aS,6R,9S,11S,12S,12aR,12bS)-12b-acetoxy-4,6,11-trihydroxy-4a,8,13,13-tetramethyl-5-oxo-9-((2-propylpentanoyl)oxy)-2a,3,4,4a,5,6,9,10,11,12,12a,12b-dodecahydro-1H-7,11-methanocyclodeca[3,4]benzo[1,2-b]oxet-12-yl benzoate (12)

Compound **10** (0.140 g, 0.196 mmol) was stirred with VPA (0.141 g, 0.980 mmol) in dry toluene (3 ml). DCC (0.201 g, 0.980 mmol) and DMAP (0.023 g, 0.196 mmol) were added, and the reaction mixture was heated at 80 °C for 24 h. The reaction mixture was filtered and the filtrate was concentrated to get crude **11**, which was then stirred in methanol (3 ml) along with ammonium formate and Pd-C (catalytic) for 2 h at RT. Reaction was quenched with water (2 ml), extracted with ethyl acetate (20 ml), concentrated and purified by silica gel (150–300 mesh) column chromatography using acetone: DCM gradient to get pure **12**.

Yield 40 % (0.052 g); white solid; ^{1}H NMR ($CDCl_3$, 300 MHz): $\delta = 8.06$ (d, 2H, $J = 7.5$ Hz, ArH), 7.57 (t, 1H, $J = 7.5$ Hz, ArH), 7.43 (t, 2H, $J = 7.8$ Hz, ArH), 6.28 (s, 1H, CH), 5.57 (d, 1H, $J = 7.2$ Hz, CH), 5.41-5.50 (m, 1H, CH), 5.24 (d, 1H, $J = 10.2$ Hz, CH), 4.95–4.98 (m, 1H, CH), 4.78 (t, 1H, $J = 7.2$ Hz, CH), 4.37–4.39 (m, 1H, CH), 4.26 (d, 1H, $J = 9.0$ Hz, CH), 4.15 (t, 1H, $J = 7.5$ Hz, CH), 4.03 (d, 1H, $J = 6.6$ Hz, CH), 3.84 (d, 1H, $J = 6.9$ Hz, CH), 2.45–2.53 (m, 2H, CH_2), 2.26 (s, 1H, CH), 2.19–2.25 (m, 4H, CH_2), 2.01–2.05 (m, 1H, CH), 1.97 (s, 2H, CH_2), 1.81 (s, 1H, CH), 1.62 (s, 2H, CH_2), 1.33–1.42 (m, 6H, CH_2), 1.17–1.21 (m, 2H, CH_2), 1.01–1.07 (m, 6H, CH_2), 0.84–0.92 (m, 6H, CH_3); MS: (ES+) *m/z* 671.4 $[M + H]^+$; ^{13}C NMR ($CDCl_3$, 75.4 MHz): $\delta = 9.4$, 10.8, 14.0, 14.9, 15.4, 19.4, 20.4, 20.5, 21.0, 22.5, 26.5, 27.0, 33.4, 34.2, 34.4, 34.5, 35.5, 38.6, 42.6, 45.1, 45.2, 46.1, 46.8, 56.2, 58.7, 67.9, 71.7, 72.3, 74.9, 75.7, 78.7, 79.1, 80.4, 80.8,

83.8, 84.5, 128.6, 129.3, 130.0, 132.2, 133.6, 142.6, 146.0, 167.0, 170.6, 175.0, 176.5, 203.9, 210.9. HRMS: *m/z* ESI (+ve) for $C_{37}H_{54}NO_{11}$. Calculated; 688.3691 $[M + NH_4]^+$. Found: 688.3686 $[M + NH_4]^+$ (Mass accuracy: 0.76 ppm).

Synthesis of (2aR,4S,4aS,6R,9S,11S,12S,12aR,12bS)-9-(((2R,3S)-3-benzamido-3-phenyl-2-((2-propylpentanoyl)oxy)propanoyl)oxy)-12-(benzoyloxy)-4,11-dihydroxy-4a,8,13,13-tetramethyl-5-oxo-2a,3,4,4a,5,6,9,10,11,12,12a,12b-dodecahydro-1H-7,11-methanocyclodeca[3,4]benzo[1,2-b]oxete-6,12b-diyl diacetate (13)

Paclitaxel (compound **1**) (0.050 g, 0.058 mmol) was stirred with VPA (0.008 g, 0.058 mmol) in dry DCM (2 ml) in an ice bath, followed by addition of DCC (0.014 g, 0.069 mmol), and DMAP (0.003 g, 0.029 mmol). The reaction mixture was stirred at RT for 16 h under nitrogen. The precipitated dicyclohexylurea (DCU) was filtered; the filtrate was concentrated and purified by silica gel (150–300 mesh) column chromatography using acetone: DCM gradient to get pure compound **13**.

Yield 61 % (0.035 g); white solid; ^{1}H NMR ($CDCl_3$, 300 MHz): $\delta = 8.16$ (d, 2H, $J = 7.2$ Hz, ArH), 7.75 (d, 2H, $J = 7.2$ Hz, ArH), 7.61–7.66 (m, 1H, ArH), 7.51–7.57 (m, 3H, ArH), 7.34–7.45 (m, 7H, ArH), 6.88 (d, 1H, $J = 9.0$ Hz, CH), 6.31 (s, 1H, CH), 6.27 (d, 1H, $J = 9.0$ Hz, CH), 5.98 (dd, 1H, $J = 9.0$ Hz, $J = 3.0$ Hz, CH), 5.70 (d, 1H, $J = 7.2$ Hz, CH), 5.50 (d, 1H, $J = 3.3$ Hz, CH), 5.00 (d, 1H, $J = 7.8$ Hz, CH), 4.47–4.50 (m, 1H, CH), 4.34 (d, 1H, $J = 8.4$ Hz, CH), 4.23 (d, 1H, $J = 8.4$ Hz, CH), 3.84 (d, 1H, $J = 7.2$ Hz), 3.45–3.52 (m, 1H, CH), 2.55-2.61 (m, 1H, CH), 2.51 (s, 3H, CH_3), 2.35-2.49 (m, 2H, CH_2), 2.24 (s, 3H, CH_3), 2.19 (s, 1H, CH), 2.10–2.18 (m, 1H, CH), 1.97 (s, 3H, CH_3), 1.91–1.95 (m, 2H, CH_2), 1.70 (s, 3H, CH_3), 1.49–1.56 (m, 2H, CH_2), 1.35–1.41 (m, 4H, CH_2), 1.27 (s, 3H, CH_3), 1.24 (s, 3H, CH_3), 1.15 (s, 3H, CH_3), 1.09–1.14 (m, 2H, CH_2), 0.76–0.94 (m, 6H, CH_3) ; 13C NMR ($CDCl_3$, 75.4 MHz): $\delta = 9.6$, 13.9, 14.8, 20.3, 20.4, 20.8, 22.1, 22.7, 24.9, 25.6, 26.7, 31.5, 33.9, 34.4, 34.6, 35.5, 43.1, 44.9, 45.5, 49.1, 52.8, 58.5, 71.6, 72.1, 73.8, 75.1, 75.6, 79.1, 81.0, 84.4, 126.3, 127.0, 128.3, 128.7, 129.0, 129.2, 130.2, 132.0, 132.7, 133.6, 136.9, 142.8, 167.0, 167.1, 168.0, 169.8, 171.2, 175.4, 211.0; MS: (ES+) *m/z* 978.4 $[M - H]^+$

Synthesis of (2aR,4S,4aS,6R,9S,11S,12S,12aR,12bS)-12b-acetoxy-9-(((2R,3S)-3-((tert-butoxycarbonyl)amino)-3-phenyl-2-((2-propylpentanoyl)oxy)propanoyl)oxy)-4,6,11-trihydroxy-4a,8,13,13-tetramethyl-5-oxo-2a,3,4,4a,5,6,9,10,11,12,12a,12b-dodecahydro-1H-7,11-methanocyclodeca[3,4]benzo[1,2-b]oxet-12-yl benzoate (14)

Docetaxel (compound 2) (0.050 g, 0.0619 mmol) was stirred with VPA (0.009 g, 0.0619 mmol) in DCM (2 ml) in an ice bath followed by addition of DCC (0.015 g, 0.0742 mmol) and DMAP (0.003 g, 0.030 mmol). The reaction mixture was stirred at RT for 16 h under nitrogen. The precipitated DCU was filtered; the filtrate was concentrated and purified by silica gel (150–300 mesh) column chromatography using acetone: DCM gradient to get pure compound **14**.

Yield 52 % (0.030 g); white solid; ^{1}H NMR ($CDCl_3$, 300 MHz): $\delta = 8.11$ (d, 2H, $J = 7.2$ Hz, ArH), 7.59 (t, 1H, $J = 7.2$ Hz, ArH), 7.49 (t, 2H, $J = 7.8$ Hz, ArH), 7.34–7.39 (m, 3H, ArH), 7.25–7.29 (m, 2H, ArH), 6.25 (t, 1H, $J = 8.7$ Hz CH), 5.68 (d, 1H, $J = 7.2$ Hz, ArH), 5.47 (br s, 1H, CH), 5.28 (br s, 1H, CH), 5.32 (d, 1H, $J = 9.3$ Hz, CH), 5.20 (br s, 1H, CH), 4.96 (d, 1H, $J = 7.8$ Hz, CH) 4.32 (d, 1H, $J = 8.4$ Hz, CH), 4.24–4.28 (m, 1H, CH), 4.18 (d, 1H, $J = 8.4$ Hz, CH), 3.93 (d, 1H, $J = 6.9$ Hz, CH), 2.53-2.60 (m, 1H, CH), 2.47 (s, 3H, CH_3), 2.28–2.41 (m, 2H, CH_2), 2.10–2.16 (m, 2H, CH_2), 1.95 (s, 3H, CH_3), 1.79–1.89 (m, 1H, CH), 1.74 (s, 3H, CH_3), 1.66 (s, 1H, CH), 1.51–1.57 (m, 2H, CH_2), 1.45–1.49 (m, 2H, CH_2), 1.37 (s, 9H, CH_3), 1.23 (s, 3H, CH_3), 1.14–1.19 (m, 2H, CH), 1.11 (s, 3H, CH_3), 0.92–1.03 (m, 6H, CH_3); ^{13}C NMR ($CDCl_3$, 75.4 MHz): $\delta = 10.0$, 14.0, 14.2, 20.1, 20.3, 20.9, 22.6, 26.3, 28.1, 34.3, 34.5, 35.5, 36.8, 43.0, 44.9, 46.4, 57.5, 71.6, 71.8, 74.0, 74.4, 78.9, 80.4, 80.9, 84.2, 126.0, 128.0, 128.7, 129.2, 130.2, 133.6, 135.4, 137.4, 139.2, 155.1, 167.0, 167.9, 169.7, 169.7, 175.4, 211.5; MS: (ES+) *m/z* 934.4 $[M + H]^+$

Synthesis of (2aR,4S,4aS,6R,9S,11S,12S,12aR,12bS)-9-(((2R,3S)-3-benzamido-2-hydroxy-3-phenylpropanoyl)oxy)-12-(benzoyloxy)-11-hydroxy-4a,8,13,13-tetramethyl-5-oxo-4-((2-propylpentanoyl)oxy)-2a,3,4,4a,5,6,9,10,11,12,12a,12b-dodecahydro-1H-7,11-methanocyclodeca[3,4]benzo[1,2-b]oxete-6,12b-diyl diacetate (15)

2′-alcohol of paclitaxel **1** was first protected by stirring paclitaxel (0.100 g, 0.117 mmol) with methoxyacetic acid (0.010 g, 0.117 mmol) in DCM (2 ml) in an ice bath, followed by addition of DCC (0.026 g, 0.128 mmol) and DMAP (0.007 g, 0.058 mmol). The reaction mixture was stirred at RT for 16 h under nitrogen. The precipitated DCU was filtered; filtrate was concentrated to give 2-mAc paclitaxel **1a** (0.090 g, white solid). This was stirred in DCM (2 ml) along with VPA (0.014 g, 0.097 mmol), DCC (0.024 g, 0.116 mmol), and DMAP (0.005 g, 0.048 mmol) for 24 h. The precipitated DCU was filtered and the filtrate was concentrated to get the crude compound **1b**, which was then stirred in ammoniated methanol (2 ml) at RT for 2 h. The reaction mixture was concentrated and purified by silica gel (150–300 mesh) column chromatography using acetone: DCM gradient to get pure compound **15**.

Yield 47 % (0.045 g); white solid; ^{1}H NMR ($CDCl_3$, 300 MHz): $\delta = 8.13$ (d, 2H, $J = 7.2$ Hz, ArH), 7.78 (d, 2H, $J = 7.2$ Hz, ArH), 7.64 (t, 1H, $J = 7.2$ Hz, ArH), 7.49–7.55 (m, 5H, ArH), 7.36–7.46 (m, 5H, ArH), 7.10 (d, 1H , $J = 8.7$ Hz, ArH), 6.22 (br s, 1H, CH), 6.18 (d, 1H, $J = 8.4$ Hz, ArH), 5.82 (d, 1H, $J = 6.6$ Hz, CH), 5.63–5.69 (m, 2H, CH), 4.96 (d, 1H, $J = 8.7$ Hz, CH), 4.82 (br s, 1H, CH), 4.33 (d, 1H, $J = 8.4$ Hz), 4.19–4.22 (m, 2H, CH_2), 4.15 (s, 1H, CH), 3.86–3.94 (m, 2H, CH), 3.69 (br s, 1H, CH), 3.48 (s, 2H, CH_2), 2.59–2.69 (m, 1H, CH), 2.40 (s, 3H, CH_3), 2.35 (d, 1H, $J = 9.0$ Hz, CH), 2.19 (s, 3H, CH_3), 1.92 (br s, 2H, CH_2), 1.83 (d, 1H, $J = 4.5$ Hz, CH), 1.35 (s, 1H, CH), 1.30 (s, 3H, CH_3), 1.27 (br s, 6H, CH_3), 1.22 (s, 3H, CH_3), 1.18 (s, 3H, CH_3), 0.84–0.94 (m, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 75.4 MHz): $\delta = 10.7$, 14.1, 14.6, 20.7, 22.5, 22.6, 26.5, 29.3, 29.7, 31.9, 33.4, 35.5, 43.2, 47.0, 54.8, 56.1, 59.4, 69.5, 71.7, 72.1, 73.1, 74.2, 75.3, 78.4, 81.0, 83.8, 127.0, 128.3, 128.7, 129.0, 130.1, 131.9, 132.8, 133.6, 133.8, 137.9, 140.5, 166.8, 167.0, 168.9, 169.9, 170.4, 172.4, 201.8; MS: (ES+) *m/z* 980.0 $[M + H]^+$.

Synthesis of (2aR,4S,4aS,6R,9S,11S,12S,12aR,12bS)-12b-acetoxy-9-(((2R,3S)-3-((tert-butoxycarbonyl) amino)-2-hydroxy-3-phenylpropanoyl)oxy)-6,11-dihydroxy-4a,8,13,13-tetramethyl-5-oxo-4-((2-propylpentanoyl)oxy)-2a,3,4,4a,5,6,9,10,11,12,12a,12b-dodecahydro-1H-7,11-methanocyclodeca[3,4]benzo[1,2-b]oxet-12-yl benzoate (16)

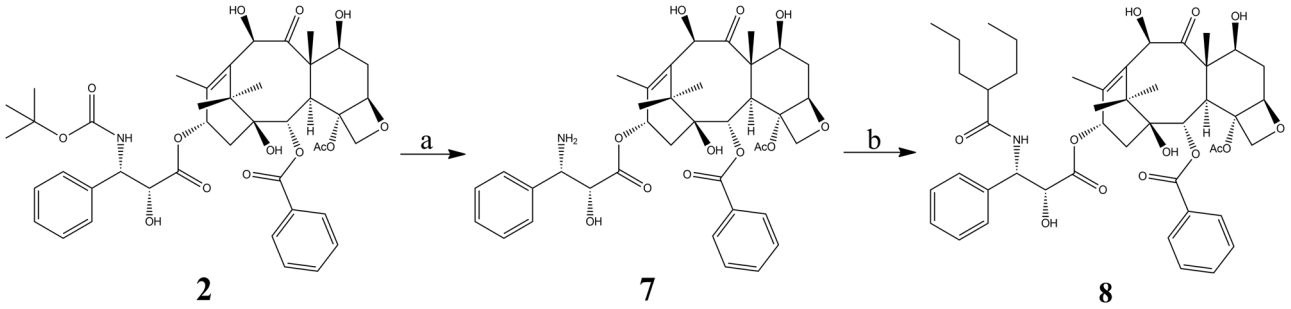
2′-alcohol and C-10 alcohol of docetaxel were first protected by stirring docetaxel **2** (0.100 g, 0.123 mmol) with methoxyacetic acid (0.038 g, 0.270 mmol) in dry DCM (4 ml), followed by addition of DCC (0.055 g, 0.270 mmol) and DMAP (0.007 g, 0.061 mmol). The reaction mixture was stirred at RT for 16 h under nitrogen. The precipitated DCU was filtered; the filtrate was concentrated to get the crude compound **2a** (white solid, 0.095 g), which was stirred in dry DCM (4 ml) along with VPA (0.014 g, 0.099 mmol), DCC (0.024 g, 0.118 mmol), and DMAP (0.006 g, 0.049 mmol) for 24 h. The precipitated DCU was filtered and the filtrate was concentrated to get the crude compound **2b**, which was further stirred in ammoniated methanol (2 ml) at RT for 2 h. The reaction mixture was concentrated and purified by silica gel (150–300 mesh) column chromatography using acetone: DCM gradient to get pure compound **16**.

Yield 48 % (0.045 g); white solid; ^{1}H NMR ($CDCl_3$, 300 MHz): $\delta = 8.12$ (d, 2H, $J = 7.8$ Hz, ArH), 7.63 (t, 1H, $J = 8.4$ Hz, ArH), 7.51 (t, 2H, $J = 7.8$ Hz, ArH), 7.34–7.40 (m, 4H, ArH), 6.18–6.26 (m, 1H, CH), 5.68 (d, 1H, $J = 6.9$ Hz, ArH), 5.46 (d, 1H, $J = 9.6$ Hz, ArH), 5.28 (br s, 1H, CH), 4.95 (d, 1H, $J = 7.8$ Hz, CH), 4.64 (br s, 1H, CH), 4.33 (t, 1H, $J = 8.4$ Hz), 4.21 (t, 1H, $J = 8.1$ Hz), 4.00-4.06 (m, 2H, CH_2), 3.47–3.55 (m, 1H, CH), 3.39 (d, 1H, $J = 5.1$ Hz, CH), 2.50–2.62 (m, 1H, CH), 2.39 (s, 2H, CH_2), 2.29–2.32 (m, 2H, CH), 1.88-1.90 (m, 2H, CH_2), 1.85 (s, 3H, CH_3), 1.69–1.75 (m, 2H, CH_2), 1.48–1.55 (m, 2H, CH_2), 1.37 (s, 6H, CH_3 &CH_2), 1.22 (s, 1H, CH), 1.12 (s, 3H, CH_3), 0.89–1.00 (m, 6H, CH_3); ^{13}C NMR ($CDCl_3$, 75.4 MHz): $\delta = 10.8$, 14.0,14.2, 20.4, 20.6, 22.4, 24.9, 25.6, 26.3, 28.2, 33.4, 33.9, 34.1, 34.4, 34.5, 35.7, 43.1, 45.2, 46.4, 49.1, 56.2, 71.5, 72.3, 73.6, 74.3, 74.5, 78.6, 80.1, 80.7, 83.7, 126.7, 128.0, 128.7, 128.8, 129.1, 130.1, 133.7, 135.5, 138.4, 138.7, 155.3, 167.0, 170.2, 172.5, 175.0, 176.6, 210.4; MS: (ES+) *m/z* 934.3 $[M + H]^+$.

Synthesis of (2aR,4S,4aS,6R,9S,11S,12S,12aR,12bS)-12b-acetoxy-9-(((2R,3S)-3-((tert-butoxycarbonyl) amino)-2-hydroxy-3-phenylpropanoyl)oxy)-4,11-dihydroxy-4a,8,13,13-tetramethyl-5-oxo-6-((2-propylpentanoyl)oxy)-2a,3,4,4a,5,6,9,10,11,12,12a,12b-dodecahydro-1H-7,11-methanocyclodeca[3,4]benzo[1,2-b]oxet-12-yl benzoate (17)

Docetaxel **2** (0.100 g, 0.123 mmol) was stirred with methoxyacetic acid (0.017 g, 0.123 mmol) in DCM (2 ml) in an ice bath followed by addition of DCC (0.030 g, 0.148 mmol) and DMAP (0.007 g, 0.061 mmol). The reaction mixture was stirred at RT for 16 h under nitrogen. The precipitated DCU was filtered; the filtrate was concentrated to get the crude 2′-mAc docetaxel **2c** (0.090 g, white solid), which was then stirred in dry DCM (2 ml) along with VPA (0.014 g, 0.102 mmol), DCC (0.025 g, 0.122 mmol) and DMAP (0.006 g, 0.051 mmol) for 24 h. The precipitated DCU was filtered; filtrate was concentrated to get the crude compound **2d**, which was stirred in ammoniated methanol (2 ml) at RT for 2 h. The reaction mixture was concentrated and purified by silica gel (150–300 mesh) column chromatography using acetone: DCM gradient to get pure compound **17**.

Yield 42 %; (0.040 g); white solid; ^{1}H NMR ($CDCl_3$, 300 MHz): $\delta = 8.12$ (d, 2H, $J = 7.8$ Hz, ArH), 7.61–7.63 (m, 1H, ArH), 7.51–7.54 (m, 2H, ArH), 7.40–7.49 (m, 5H, ArH), 6.18–6.26 (m, 1H, CH), 5.68 (d, 1H, $J = 6.9$ Hz, ArH), 5.46 (d, 1H, $J = 9.6$ Hz, ArH), 5.28 (br s, 1H, CH), 4.95 (d, 1H, $J = 8.4$ Hz, CH), 4.64 (br s, 1H, CH), 4.34 (d, 1H, $J = 8.4$ Hz), 4.21 (d, 1H, $J = 8.4$ Hz), 4.00–4.10 (m, 3H, CH), 3.44–3.55 (m, 1H, CH), 3.43 (d, 1H, $J = 5.4$ Hz, CH), 2.49–2.63 (m, 1H, CH), 2.39 (s, 3H, CH_3), 2.29–2.31 (m, 2H, CH_2), 1.91–1.96 (m, 3H, CH & CH_2), 1.88 (s, 6H, CH_3), 1.69–1.73 (m, 4H, CH_2), 1.50–1.52 (m, 2H, CH_2), 1.35–1.37 (m, 10H, CH_3 &CH_2), 1.27 (s, 3H, CH_3), 1.24 (s, 3H, CH_3), 1.17–1.19 (m, 4H, CH_2), 1.12 (s, 3H, CH_3), 0.89-0.94 (m, 6H, CH_3); ^{13}C NMR ($CDCl_3$, 75.4 MHz): $\delta = 10.9$, 14.0, 14.2, 20.4, 20.6, 22.4, 24.9, 25.6, 26.3, 28.2, 29.7, 33.4, 33.9, 34.1, 35.7, 43.0, 45.2, 46.4, 49.1, 56.2, 71.5, 72.4, 73.6, 74.3, 74.5, 75.3, 78.6, 80.1, 80.7, 83.7, 126.7, 128.7, 128.8, 129.0, 130.1, 133.7, 135.5, 138.7,



Scheme 1 Reagents and conditions: (a) TFA, THF, 45 min at 0 °C and $NaHCO_3$, 86 %; (b) VPA, DCC, DMAP, 25 °C, 16 h, 24 %

155.3, 160.0, 166.9, 170.2, 175.0, 210.4; MS: (ES+) *m/z* 956.5 $[M + Na]^+$.

Cell line maintenance

A panel of six cancer cells representing multiple cancers of clinical relevance were selected, namely, ACHN (human renal cell carcinoma, ATCC, CRL-1611), Panc-1 (human pancreatic adenocarcinoma, ATCC, CRL-1469) cultured on minimum essential medium (MEM) with 2 mM L-glutamine and 10 % fetal bovine serum (FBS), H460 (human non small cell lung carcinoma, ATCC, HTB-177), Calu-1 (human lung carcinoma, ATCC, HTB-54), cultured on RPMI, 2 mM L-glutamine and 10 % FBS, HCT-116 (human colon cancer, ATCC, CCL- 247) and FADU (Head and Neck Squmous cell carcinoma, ATCC, HTB- 43) cultured on McCoy's 5a medium and 10 % FBS and MCF10A (normal breast epithelium cells) cultured on MEM with 2 mM L-glutamine and 10 % FBS.

Cell proliferation assay

Logarithmically growing cells were plated at a density of 5×10^3 cells/well in a 96-well tissue culture grade micro-plate and allowed to recover overnight. The cells were challenged with varying concentration of different hybrids for 48 h. Control cells received standard media containing dimethyl sulfoxide (DMSO) vehicle at a concentration of 0.2 %. After 48 h of incubation, cell toxicity was determined by the cell counting kit-8 (CCK-8) reagent (Dojindo Molecular Technologies, Inc, Maryland, Japan); (WST-1 [2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)]-2H-tetrazolium, monosodium salt assay). In accordance with the manufacturer's instructions, 10 µl/well CCK-8 reagents were added and plates were incubated for 2 h. The compound induced anti-proliferation/toxicity was determined by measuring the absorbance on Tecan Sapphire multi-fluorescence micro-plate reader (Tecan, Germany, GmbH) at a wavelength of 450 nm corrected to 650 nm and normalized to controls. Each independent experiment was performed thrice in triplicate.

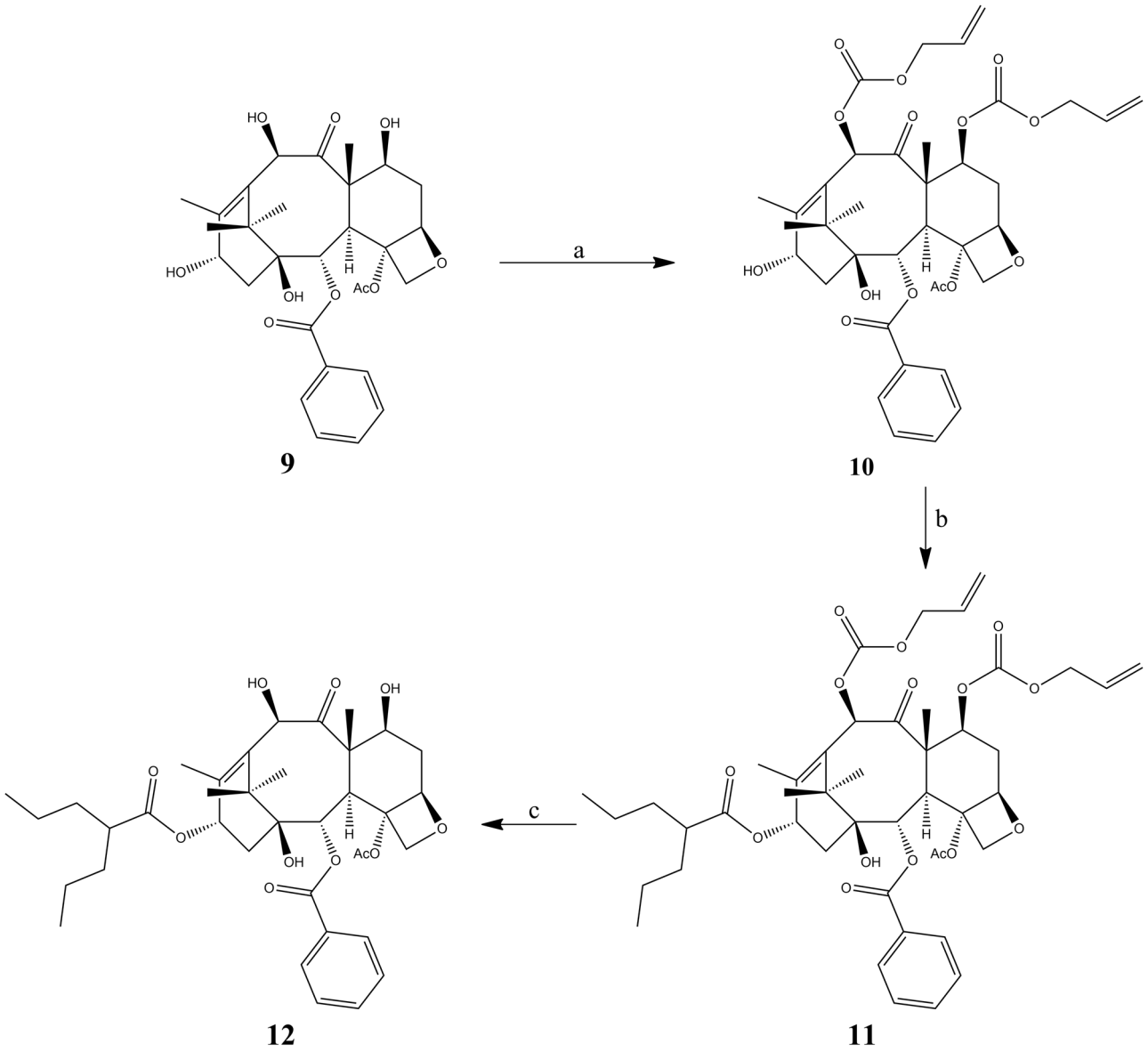
Results and discussion

VPA was incorporated into the taxane moiety at various positions viz., C-2′, C-7, C-10, C-13 using ester bond and amide at C-3′. The synthesis of C-3′ VPA amide (**8**) was accomplished by deprotecting the Boc group of docetaxel using trifluoroacetic acid (TFA), and the free amine was coupled with VPA using DCC and DMAP as shown in Scheme 1 (Querolle et al., 2003). The VPA ester at C-13 (**12**) was synthesized by protecting the C-7 and C-10 hydroxyl groups of 10-DAB-III using allyl chloroformate and pyridine in chloroform, followed by coupling with VPA using DCC/DMAP (Heo et al., 2009), and deprotection using Pd-C and ammonium formate as shown in Scheme 2.

The VPA esters of paclitaxel and docetaxel at C-2′ (**13** and **14**) were synthesized by using DCC/DMAP as the coupling agent as shown in Scheme 3. VPA esters at the C-7 position (**15** and **16**) were synthesized by protecting the 2′-hydroxy group using base labile methoxyacetate group. In case of docetaxel both C-2′ and C-10 hydroxyl group were protected by methoxyacetate. After VPA coupling, deprotection of the methoxyacetyl group was accomplished by using ammoniated methanol as shown in Scheme 3. Similarly C-10 VPA ester of docetaxel (**17**) synthesis was accomplished by protecting C-2′hydroxyl using methoxy acetate. These hybrids were evaluated for their cytotoxicity using the propidium iodide assay method and their IC_{50} values were determined.

Determination of IC_{50} for different compounds in a panel of cancer cells

PANC1, ACHN1, CALU1, H460, HCT116, FADU, and normal breast epithelium cells MCF10A were treated with different hybrids (Table 1) at an eight-point dilution set of 0.01 µM to 30 µM. After 48 h, the compound-induced

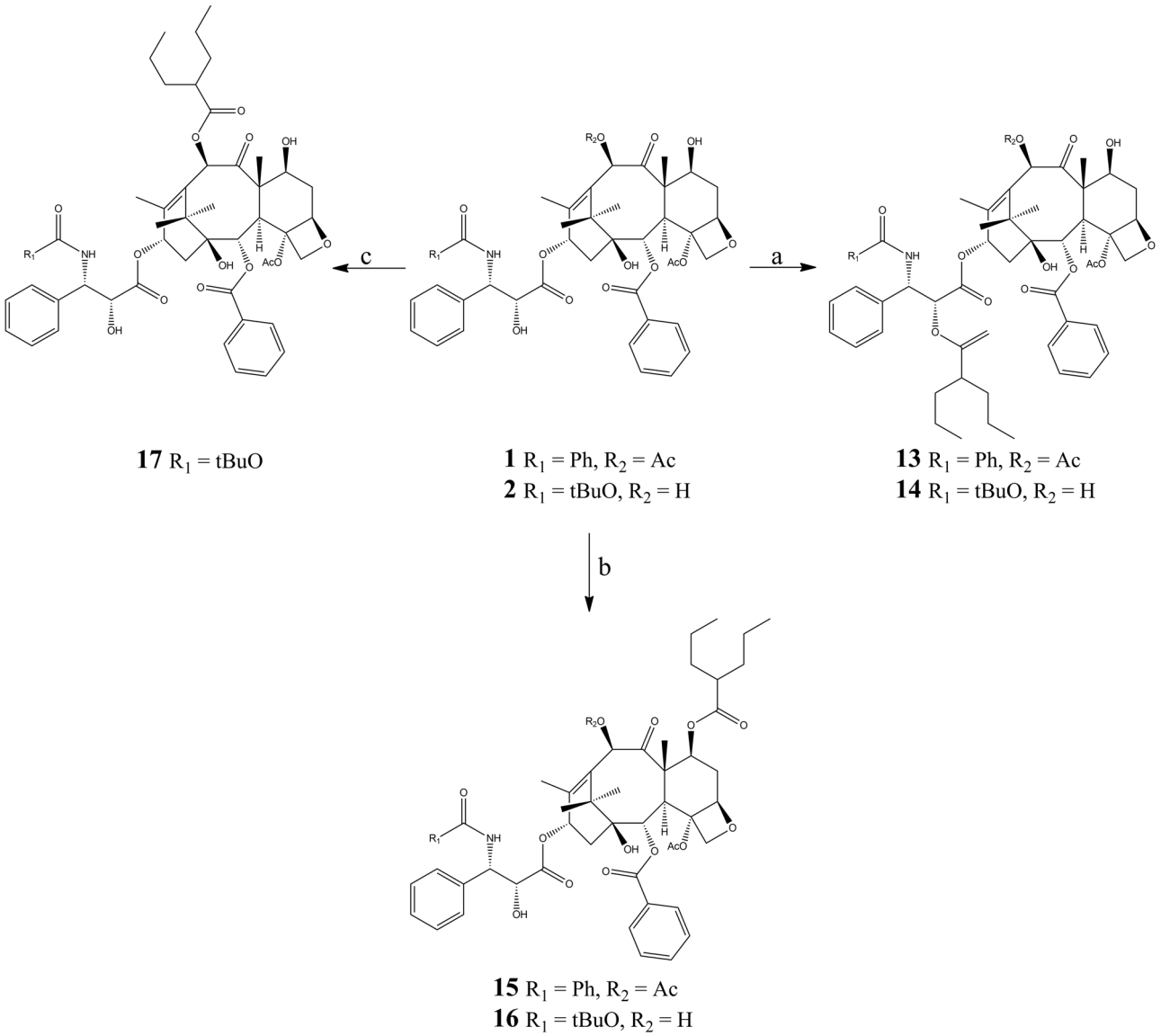


Scheme 2 Reagents and conditions: (a) allyl chloroformate, CH_2Cl_2, pyridine 0 °C and 16 h at 25 °C, 60 %; (b) VPA, DCC, DMAP, 25 °C, 16 h; (c) Pd-C, ammonium formate, methanol, 25 °C, 3 h, 40 %

toxicity was determined using a CCK-8 live cell dehydrogenase non-radioactive assay (Mosmann, 1983).

Analysis of the dose-response curve revealed that the hybrid (**8**) showed significant cytotoxicity against colon and head and neck cancer cell line with IC_{50} values of 0.01 μM and 0.08 μM respectively. This is comparable to paclitaxel, and in normal breast epithelium cells (MCF10A) it showed an IC_{50} above 10 μM, which illustrates better therapeutic index (TI = 1000) for colon cancer cell lines than paclitaxel (TI = 37.5) and docetaxel (TI = 37.5) by a big margin, whereas VPA exhibits non-selective cytotoxicity in the range of 12.1–22.1 μM against the tested cell lines. The C-13 side chain replacement with VPA (**12**) did not show any increase in cytotoxicity compared with 10-DAB-III (**9**). Hybrid **12** showed reduced cytotoxicity compared to DAB-III. The VPA esters at C-2′, C-7 and C-10 (**13**–**16**) of docetaxel and paclitaxel showed reduced cytotoxicity compared to the parent drugs. However, hybrid **17** showed better cytotoxicity in the range of 0.15–0.94 μM on the tested cancer cell lines.

The hybrids (**13**–**17**) are connected by ester bonds and are susceptible for ester hydrolysis by esterases. These hybrids may behave as prodrugs rather than hybrids as these compounds will get hydrolyzed by esterases in plasma. This study reveals that molecular hybridization of VPA and taxol works better at C-3′. Interestingly, hybrid **8**, what we called



Scheme 3 Reagents and conditions: (a) VPA, DCC, DMAP, 25 °C, 16 h, 52 %; (b) (i) 2.0 eq. methoxyacetic acid, DCC, DMAP, 25 °C, 16 h; (ii) VPA, DCC, DMAP, 25 °C, 16 h; (iii) ammoniated methanol, 25 °C, 2 h, 48 %; (c) (i) 1 eq. methoxyacetic acid, DCC, DMAP, 25 °C, 16 h; (ii) VPA, DCC, DMAP, 25 °C, 16 h; (iii) ammoniated methanol, 25 °C, 2 h, 42 %

as Valprotaxel, showed improvement in therapeutic index by a big margin compared to paclitaxel. A small change in structure at C-3′ (where instead of the N-Boc group of docetaxel we introduced the valpromide) results in improvement in therapeutic index. This selectivity and potency of Valprotaxel against various cancer cell lines makes it good candidate for further evaluation.

Conclusion

We have demonstrated the synthesis of VPA–taxane hybrids. The novel drug hybrid Valprotaxel has shown potency and selectivity towards highly proliferating cancer cells as compared to the normal cells, particularly in head, neck and colon cancer cell lines. Valprotaxel holds promise for further studies in Pgp interactions and permeability across BBB. It can also be a potential candidate for treatment of brain tumors where therapeutic benefit of paclitaxel is low and variable.

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Table 1 Cytotoxicity of VPA–taxane hybrids (IC_{50} μM)

% Cytotoxicity (IC_{50} in μM)							
Sample code	[a]ACHN	[b]Panc1	[c]Calu1	[d]H460	[e]HCT116	[f]FADU	[g]MCF1A
Paclitaxel (**1**)	0.082 ± 0.006	0.093 ± 0.012	0.03 ± 0.040	0.01 ± 0.05	0.008 ± 0.019	0.06 ± 0.012	0.3 ± 0.10
Docetaxel (**2**)	0.0075 ± 0.001	0.0095 ± 0.005	0.03 ± 0.009	0.0081 ± 0.01	0.0078 ± 0.001	0.0056 ± 0.001	0.21 ± 0.15
VPA (**6**)	16.3 ± 5.0	17.5 ± 3.2	22.1 ± 3.3	15.3 ± 2.3	16.5 ± 3.0	12.1 ± 3..3	15.1 ± 2.0
Valprotaxel (**8**)	0.12 ± 0.02	0.15 ± 0.05	1.2 ± 0.02	0.22 ± 0.04	0.010 ± 0.005	0.08 ± 0.02	10.0 ± 2.0
DAB-III (**9**)	1.8 ± 0.20	2.2 ± 0.30	10.0 ± 3.00	2.5 ± 1.2	1.9 ± 0.9	2.7 ± 0.30	3.3 ± 0.8
Hybrid **12**	5.2 ± 1.00	6.8 ± 1.6	16.5 ± 3.6	7.8 ± 1.4	7.0 ± 2.0	3.7 ± 1.6	25 ± 2.0
Hybrid **13**	0.93 ± 0.10	0.86 ± 0.15	1.06 ± 0.35	0.96 ± 0.10	1.2 ± 0.20	0.76 ± 0.10	5.7 ± 0.70
Hybrid **14**	1.2 ± 0.02	0.98 ± 0.14	0.86 ± 0.20	0.73 ± 0.15	1.1 ± 0.25	0.93 ± 0.10	5.9 ± 1.0
Hybrid **15**	1.4 ± 0.35	1.5 ± 0.35	0.98 ± 0.15	1.4 ± 0.30	1.2 ± 0.20	1.0 ± 0.35	10.0 ± 2.0
Hybrid **16**	0.76 ± 0.20	0.84 ± 0.15	1.1 ± 0.40	0.78 ± 0.10	0.59 ± 0.10	0.45 ± 0.05	3.6 ± 0.4.0
Hybrid **17**	0.32 ± 0.05	0.45 ± 0.20	0.94 ± 0.25	0.81 ± 0.20	0.20 ± 0.10	0.15 ± 0.05	7.5 ± 0.50

Data are mean of three determinations

[a] Renal cancer

[b] Pancreatic cancer

[c] Lung cancer

[d] Non-small cell lung cancer

[e] Colon cancer

[f] Head and neck

[g] Normal breast epithelium cells

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

Braiteh F, Soriano AO, Manero GG, Hong D, Johnson MM, Silva LDP, Yang H, Alexander S, Wolff J, Kurzrock J (2008) Phase I study of epigenetic modulation with 5-azacytidine and valproic acid in patients with advanced cancers. Clin Cancer Res 14:6296–6301

Brandes AA, Pasetto LM, Monfardini S (2000) New drugs in recurrent high grade gliomas. Anticancer Res 20:1913–1920

Eyal S, Lamb JG, Smith-Yockman M, Yagen B, Fibach E et al (2006) The antiepileptic and anticancer agent, valproic acid, induces P-glycoprotein in human tumour cell lines and in rat liver. Braz J Pharmacol 149:250–260

Garcia-Manero G, Kantarjian HM, Sanchez-Gonzalez B, Yang H, Rosner G et al (2006) Phase 1/2 study of the combination of 5-aza-2′-deoxycytidine with valproic acid in patients with leukemia. Blood 108(10):3271–3279

Glantz MJ, Chamberlain MC, Chang SM, Prados MD, Cole BF (1997) The role of paclitaxel in the treatment of primary and metastatic brain tumors. Sem Radiat Oncol 9:27–33

Gonzalez AD, Candelaria M, Plascencia CP, Cardenas EP, Hernandez EC, Herrera LA (2008) Valproic acid as epigenetic cancer drug: Preclinical, clinical and transcriptional effects on solid tumors. Cancer Treat Rev 34:206–222

Göttlicher M, Minucci S, Zhu P, Krämer OH, Schimpf A et al (2001) Valproic acid defines a novel class of HDAC inhibitors inducing differentiation of transformed cells. EMBO J 20:6969–6978

Hajek R, Vorlicek J, Slavik M (1996) Paclitaxel (Taxol): A review of its antitumor activity in clinical studies, mini review. Neoplasma 43:141–154

Heo JH, Park SJ, Kang JH, Lee IS, Lee JS, Park YJ, Kim KS, Lee JY (2009) Development of new efficient methods for docetaxel. Bull Korean Chem Soc 30:25–26

Lee JS, Pisters KM, Komaki R, Glisson BS, Khuri FR, Schea R, Fossella FV (1997) Paclitaxel/carboplatin chemotherapy as primary treatment of brain metastases in non-small cell lung cancer: a preliminary report. Sem Oncol 24(4 Suppl. 12): S12-S55

Mastalerz H, Ook D, Fairchild CR, Hansel S, Johnson W et al (2003) The discovery of BMS-275183: an orally efficacious novel taxane. Bioorg Med Chem 11:4315–4323

Mosmann T (1983) Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods 65:55–63

Querolle O, Dubois J, Thoret S, Dupont C, Gueritte F, Guenard D (2003) Synthesis of novel 2-O,3′-N-linked macrocyclic taxoids with variable ring size. Eur J Org Chem 2003(3): 542–550

Rice A, Michaelis ML, Georg G, Liu Y, Turunen B, Audus KL (2003) Overcoming the bloodbrain barrier to taxane delivery for neurodegenerative diseases and brain tumors. J Mol Neurosci 20:339–343

Soriano AO, Yang H, Faderl S (2007) Safety and clinical activity of the combination of 5-azacytidine, valproic acid and all trans-retinioc acid in acute myeloid leukemia and myelodysplastic syndrome. Blood 110(7):2302–2308

Sparreboom A, Van Asperen J, Mayer U, Schinkel AH, Smit JW, Meijer DKF, Borst P, Noojijen WJ, Beijnen JH, van Tellingen O (1997) Limited oral bioavailability and active epithelial excretion of paclitaxel (taxol) caused by P-glycoprotein in the intestine. J Proc Natl Acad Sci 94:2031

Wani MC, Taylor HL, Wall ME, Coggon P, McPhail AT (1971) Plant antitumor agents-VI: The isolation and structure of taxol, a novel antileukemic and antitumor agent from *Taxus brevifolia*. J Am Chem Soc 93:2325–2327