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Original article

In vitro and *in vivo* anticancer activity of 2-deacetoxytaxinine J and synthesis of novel taxoids and their *in vitro* anticancer activity☆

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ABSTRACT

The taxane diterpneoid 2-deacetoxytaxinine J (2-DAT-J) **1** has been isolated from the bark of Himalayan yew, *Taxus baccata* L. spp. *wallichiana* in a reasonably good yield (0.1%) and its anticancer activity against breast cancer cell lines (MCF-7 and MDA-MB-231) and normal human kidney epithelial cell line (HEK-293) has been studied. 2-DAT-J (**1**) showed significant *in vitro* activity against breast cancer cell line at a concentration of 20 μM and 10 μM in MCF-7 and MDA-MB-231 respectively. Few novel taxoids were derived (**7**, **8** and **10**–**13**) from the naturally occurring 2-DAT-J (**1**) and screened for their anticancer activity. The structure–activity relationship studies indicated that the cinnamoyl group on C-5 and acetyl group on C-10 are essential for the anticancer activity. 2-DAT-J (**1**) was also tested for its *in vivo* activity on DMBA-induced mammary tumors in virgin female Sprague Dawley rats at a dose of 10 mg/kg body weight orally for 30 days and showed significant regression in mammary tumors as compared to vehicle treated group ($p < 0.05$).

1. Introduction

Taxanes are an integral part of the treatment of multiple cancer types, including breast and ovarian cancer. Taxol® (**3**) which belongs to taxane class of compound, is a diterpenoid pseudo alkaloid and was first isolated from the bark of the western yew, *Taxus brevifolia*, by Wall and Wani's group in collaboration with National Cancer Institute (NCI) of USA, and is a particularly significant new lead for the treatment of cancer [1]. Thus far, it has been approved by the FDA of US for the treatment of advanced ovarian and metastatic breast cancer and is currently in phase II and III clinical trials for lung and other cancers [2]. The outstanding cytotoxic activity of taxol is believed to arise from its unique propensity to hinder cell replication by preventing microtubules from depolymerization [3]. The only source of taxol was from the needles (0.033%) and bark (0.01) of the pacific yew tree *T. brevifolia*, which cannot be considered as a renewable source because of its slow growth and, therefore, the supply of taxol was quite limited [4]. Ample approaches towards the semi-synthetic routes to taxol have been developed.

Apart from this, six total syntheses of taxol have been published to date [5]. On the other hand, the supply of the key terpenoid fragment baccatin III (**2**) (0.2%) and 10-deacetylbaccatin III (**4**) (0.1%) is obtained from the leaves, a rapidly renewed resource of *Taxus baccata*. The side chain attached to the main ring of baccatin III is *N*-bezoyl-(2*R*,3*S*)-phenylisoserine (**5**) in the synthesis of taxol (Fig. 1) [6].

The shortest route was reported by Wender et al., starting from (1*R*)-(+)-verbenone which involves 37 steps [5d] in an overall yield of about 0.37%. The scarcity of taxol from the natural source and economically not viable synthetic methods have led to search for alternative compound isolable in useful quantities.

In continuation of our drug discovery program on anticancer agents we isolated 2-DAT-J (**1**) in reasonably good yield (0.1%) from the Indian *T. baccata* (spp. *wallichiana*). Chattopadhyay and co-workers [7] isolated **1** from the same species. 2-DAT-J (**1**) was also reported from several other species such as *Taxus canadensis* [8], *Taxus mairei* [9], *Taxus chinensis* [10], *T. brevifolia* [11], *Taxus cuspidate* [12] and *Taxus yunnanensis* [13]. It exhibits cytotoxicity against L1210 murine leukemia cells and KB human epidermoid carcinoma cells and has effects on the Ca^{2+} induced microtubule depolymerization [14]. Taxoid **1** devoid of cytotoxicity and tubulin affinity is

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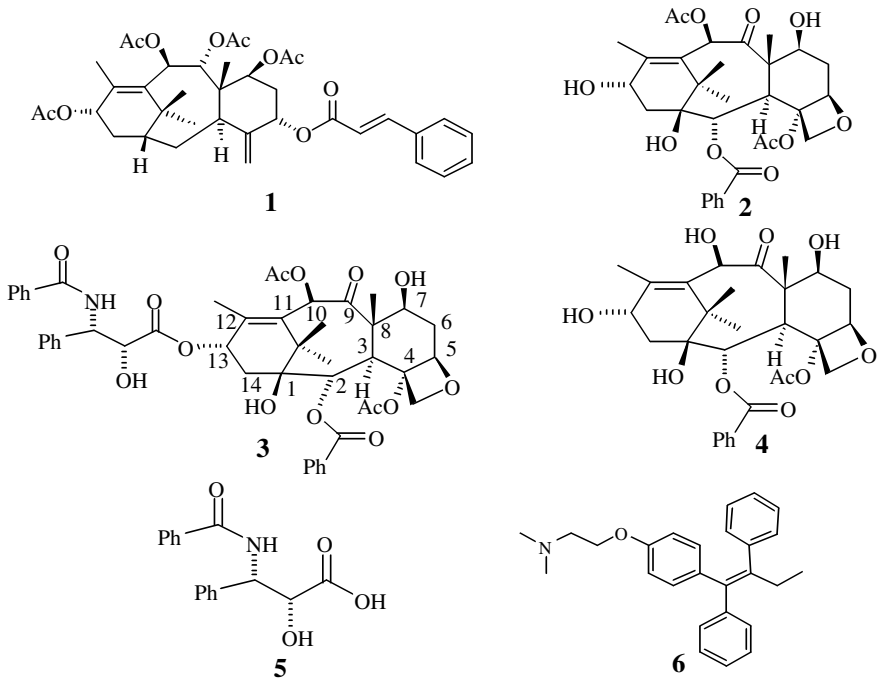


Fig. 1. 2-Deacetoxytaxinine J (**1**); baccatin III (**2**); paclitaxel (**3** Taxol®: BMS-181339); 10-deacetylbaccatin III (**4**); phenylisoserine (**5** taxol C-13 side chain); Tamoxifen (**6**).

a powerful inhibitor of P-gp activity, acting as efficient reversing agent in multi-drug resistant (MDR) cancer cells [15]. It increases vincristine accumulation (266%) in MDR 2780 AD cells as compared to verapamil (254%). Recently Botta and co-workers prepared 2-DAT-J (**1**) in one step from commercially available 2-deacetoxyaustropicatine (2-DAS) and synthesized a small library of 2-DAT-J (**1**) analogues to develop potential MDR-reversing agents against MDR-resistant tumor cell lines [16]. Several groups reported the *in vitro* anticancer activity of 2-DAT-J (**1**), to our knowledge *in vivo* anticancer activity in animal models has not yet been reported.

The present study was focused to develop alternative compound for taxol. Towards this end, we here report the *in vivo* anticancer activity of 2-DAT-J (**1**) for the first time and synthesis of few novel taxoids and their *in vitro* anticancer activity.

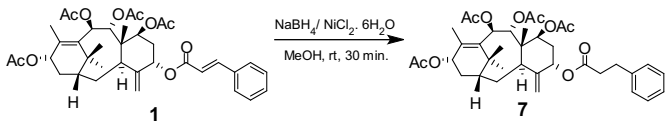
2. Results and discussion

2.1. Chemistry

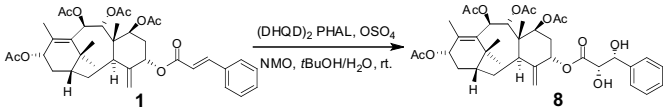
Chemical transformation of the active compound was carried out to study the structure–activity relationship. Initially the selective hydrogenation of olefinic bond [17] of cinnamoyl group was carried out on 2-DAT-J (**1**) using $NaBH_4$, $NiCl_2 \cdot 6H_2O$ to obtain the dihydro derivative **7** (Scheme 1).

To increase the hydrophilic character of the compound Sharpless asymmetric dihydroxylation [18] was carried out on **1** using OsO_4, which provided dihydroxylated derivative **8** (Scheme 2).

To find out the role of the cinnamoyl moiety in 2-DAT-J (**1**) we attempted to remove the cinnamoyl group from 2-DAT-J (**1**).



Scheme 1. Regioselective hydrogenation of 2-DAT-J (**1**) by using $NaBH_4/NiCl_2 \cdot 6H_2O$.

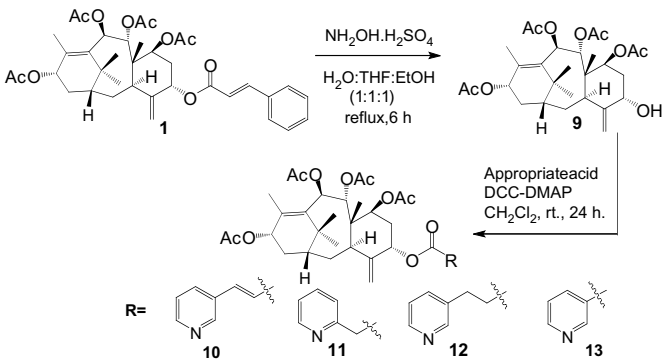


Scheme 2. Dihydroxylation of 2-DAT-J (**1**).

Hydrolysis of 2-DAT-J (**1**) under basic conditions such as NaOH or KOH led to mixture of several compounds. Therefore $NH_2OH \cdot H_2SO_4$ was used to regioselectively decinnamoylate **1** without disturbing other acetyl groups [19]. Further we used this decinnamoylated derivative **9** to substitute the cinnamoyl group with heterocyclic acids to furnish **10**–**13** (Scheme 3).

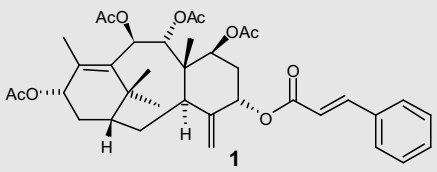
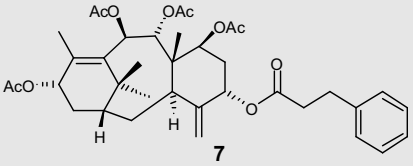
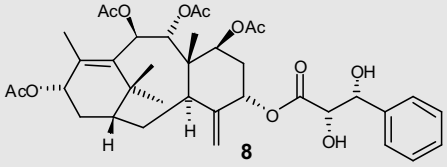
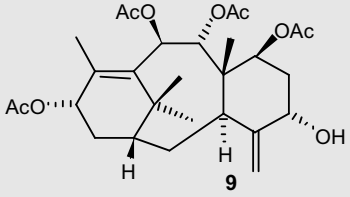
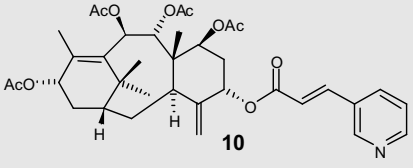
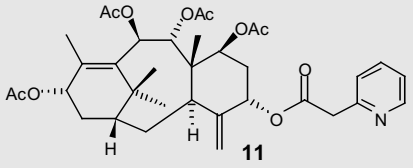
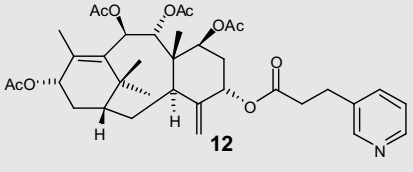
2.2. Biology

2-DAT-J (**1**), its derivatives **7**, **8** and its analogues **9**–**15** were screened for their *in vitro* anticancer activity against two breast cancer cell lines, MCF-7 (ER-positive) and MDA-MB-231 (ER-positive) and a normal human kidney epithelial cell line



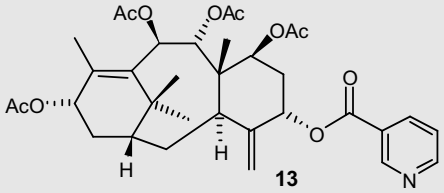
Scheme 3. Synthesis of ester analogues of 2-DAT-J (**1**).

Table 1
Anticancer activity of 2-deacetoxytaxinine J (2-DAT-J) **1** and its derivatives.

Entry	Compound	IC_{50} (μM)[c]		
		MCF-7	MDA-MB-231	HEK-293
1	 **1**	20	10	>50
2	 **7**	15	20	10
3[a]	 **8**	>50	13	>50
4	 **9**	>50	>50	>50
5	 **10**	34	>50	>50
6	 **11**	28	>50	>50
7	 **12**	39	>50	>50

(*continued on next page*)

Table 1 (*continued*)

Entry	Compound	IC_{50} (μM)[c]		
		MCF-7	MDA-MB-231	HEK-293
8	 **13**	>50	>50	>50
9	Tamoxifen (**6**)	12	24	[b]
10	Taxol (**3**)	[b]	[b]	43

[a] Stereochemistry was not determined.
[b] Not done.
[c] Values are mean of three independent experiments.

(HEK-293) and the results are presented in Table 1 [20,21]. The natural compound 2-DAT-J (**1**) showed significant activity against breast cancer cell line at a concentration of 20 μM and 10 μM in MCF-7 and MDA-MB-231 respectively. The marketed drugs such as Tamoxifen showed an IC_{50} of 12 μM (MCF-7) and 24 μM (MDA-MB-231). The dihydro derivative **7** of 2-DAT-J is more potent than the parent compound (**1**) and also other analogues against MCF-7 cell line, however, **7** showed significant cytotoxicity towards normal cell line (HEK-293). The compound **7** has an IC_{50} of 15 μM, 20 μM and 10 μM against MCF-7, MDA-MB-231 and HEK-293 respectively. The more hydrophilic dihydroxylated compound **8** exhibited significant activity against only MDA-MB-231 cell line. Removal of cinnamoyl group from **1** to provide **9** completely diminished the activity in all the cell lines tested, which indicates the importance of cinnamoyl group in the biological activity. Only three analogues, in which the cinnamoyl group was replaced with heterocyclic aromatic acids such as **10** (IC_{50} of 34 μM), **11** (IC_{50} of 28 μM), **12** (IC_{50} of 39 μM) exhibited moderate activity against MCF-7 cell lines. Nicotinic acid derivative **13** did not show significant activity in all the tested cell lines.

2-DAT-J (**1**) was also tested for its *in vivo* activity on DMBA-induced mammary tumors in virgin female Sprague Dawley rats at a dose of 10 mg/kg body weight orally for 30 days and showed significant regression in mammary tumors as compared to vehicle treated group ($p < 0.05$) (Fig. 2) [22].

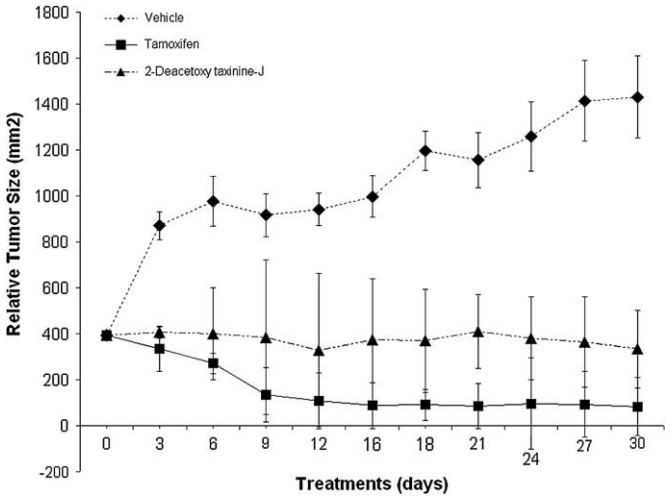


Fig. 2. Effect of 2-DAT-J (**1**) on mammary tumors of female Sprague Dawley rats.

3. Conclusion

In conclusion, we have isolated a taxane diterpenoid 2-DAT-J (**1**) from the bark of *T. baccata* (spp. *wallichiana*) in reasonably good yield and prepared few novel taxoid derivatives and studied their anticancer activity. The natural compound 2-DAT-J (**1**) has comparable *in vitro* activity with marketed drug, Tamoxifen. 2-DAT-J (**1**) also induced significant tumor regression in DMBA-induced mammary tumors in Sprague Dawley rats at a dose of 10 mg/kg body weight by oral route. The structure–activity relationship indicated that the cinnamoyl group on C-5 is essential for the anticancer activity (entries 1 and 4 in Table 1). Replacing the cinnamoyl group with other heterocyclic acids as in **10**–**13** did not improve the activity.

4. Experimental section

4.1. General methods

Melting points were recorded on Buchi-530 capillary melting point apparatus and are uncorrected. IR spectra were recorded on Perkin–Elmer AC-1 spectrometer. ^1H NMR spectra were run on Bruker Advance DPX 300 MHz and 200 MHz in $CDCl_3$. ^{13}C NMR spectra were recorded at 75 MHz and 50 MHz in $CDCl_3$. Chemical shifts are reported as values in ppm relative to $CHCl_3$ (7.26) in $CDCl_3$ and TMS was used as internal standard. ESI mass spectra were recorded on JEOL SX 102/DA-6000. Chromatography was executed with silica gel (60–120 mesh) using mixtures of ethyl acetate and hexane as eluants. Ethyl acetate and hexane were dried and purified by distillation prior to use.

4.2. Isolation of 2-deacetoxytaxinine J (*1*)

The plant material (10 kg of stem bark) of *T. baccata* (spp. *wallichiana*) belongs to the family of Taxaceae. It was collected in the month of June, 2002 from Dhakudi, Bhageshwar district, Uttaranchal state, India by the Botany Division of CDRI, Lucknow and a voucher specimen (No. KRA 23932) was deposited in the departmental herbarium of CDRI. The bark of the plant was extracted with 4 L of ethyl alcohol four times in a percolator. The resultant alcoholic extract was (16 L) combined and concentrated under reduced pressure to give alcoholic extract. This was fractionated with chloroform and butanol successively. The resultant chloroform fraction was subjected to conventional silica gel column chromatography using hexane and ethyl acetate solvent system to

give 2-deacetoxytaxinine J (**1**). Compound **1** was characterized by using ^{1}H NMR, ^{13}C NMR, IR and mass spectral data and comparing with literature data.

Mp: 146–147 °C; IR (KBr) 2991, 2947, 2878, 1742, 1632, 1438, 1375, 1243, 1158, 1021, 770 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.77 (d, $J = 16.1$ Hz, 1H), 7.49 (m, 2H), 7.40 (m, 3H), 6.57 (d, $J = 16.1$ Hz, 1H), 6.30 (d, $J = 11.1$ Hz, 1H), 5.94 (d, $J = 11.1$ Hz, 1H), 5.81 (t, $J = 8.4$ Hz, 1H), 5.69 (dd, $J = 11.3$, 5.8 Hz, 1H), 5.57 (s, 1H), 5.38 (s, 1H), 5.02 (s, 1H), 3.30 (d, $J = 4.2$ Hz, 1H), 2.72 (m, 1H), 2.33 (s, 3H), 2.07 (s, 3H), 2.04 (s, 3H), 1.99 (s, 3H), 1.94–1.79 (m, 6H), 1.71 (s, 3H), 1.63 (s, 3H), 1.10 (s, 3H), 0.88 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 170.9, 170.5, 170.1, 169.5, 166.4, 146.6, 146.0, 137.5, 135.3, 134.4, 130.8, 129.2 (2C), 128.3 (2C), 118.7, 116.2, 77.0, 75.1, 71.9, 70.8, 70.3, 46.6, 40.4, 39.6, 37.7, 34.8, 32.1, 31.4, 27.5, 21.7 (2C), 21.2 (2C), 21.1, 15.5, 13.4; MS (ESI) m/z 673.1 $(M + 23)^+$.

4.3. 3-Phenyl-propionic acid 7,9,10,13-tetraacetoxy-8,12,15,15-tetramethyl-4-methylene-tricyclo[9.3.1.03,8]pentadec-11-en-5-yl ester (**7**)

To a magnetically stirred solution of 2-deacetoxytaxinine J (**1**) (250 mg, 0.38 mmol) in methanol (25 mL) was added gradually $NiCl_2 \cdot 6H_2O$ (46 mg, 0.19 mmol) at rt. When the clear solution acquired a greenish color, the whole reaction mixture was brought to 5 °C and $NaBH_4$ (21 mg, 0.57 mmol) was added portionwise. After addition of $NaBH_4$, the whole solution was stirred for 30 min at 5 °C to rt. Methanol was removed by vacuum, and then the reaction mixture was dissolved in ethyl acetate and neutralized with 10% HCl solution, the organic layer was washed with water, dried over anhyd Na_2SO_4 and evaporated under reduced pressure. Then the crude product was chromatographed on silica gel to afford the desired compound **7** (168 mg, 67%): IR (KBr) 2929, 1737, 1596, 1438, 1243, 1163, 1022, 767 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.29 (m, 2H), 7.20 (m, 3H), 6.26 (d, $J = 11.0$ Hz, 1H) 5.91 (d, $J = 11.0$ Hz, 2H), 5.55 (dd, $J = 11.3$, 5.3 Hz, 1H), 5.42 (t, $J = 3.3$ Hz, 1H), 5.29 (s, 1H), 4.96 (s, 1H), 2.99 (t, $J = 7.8$ Hz, 2H), 2.89 (d, $J = 5.6$ Hz, 1H), 2.85–2.56 (m, 3H), 2.18 (s, 3H), 2.08 (s, 3H), 2.04 (s, 3H), 1.99 (s, 3H), 1.93–1.72 (m, 6H), 1.67 (s, 3H), 1.64 (s, 3H), 1.14 (s, 3H), 0.86 (s, 3H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 172.4, 171.0, 170.7, 170.2, 169.7, 147.1, 140.5, 137.8, 134.9, 129.1 (2C), 128.7 (2C), 126.8, 116.1, 77.2, 75.2, 72.0, 70.9, 70.5, 46.5, 39.8, 40.8, 38.1, 36.9, 34.4, 31.7, 31.5, 31.4, 27.9, 27.8, 21.8, 21.4, 21.3, 21.3, 15.5, 13.7; MS (FAB) m/z 593 $(M - OAc\ (59))^+$.

4.4. 2,3-Dihydroxy-3-phenyl-propionic acid 7,9,10,13-tetraacetoxy-8,12,15,15-tetramethyl-4-methylene-tricyclo[9.3.1.03,8]pentadec-11-en-5-yl ester (**8**)

To a magnetically stirred solution of 2-deacetoxytaxinine J (**1**) (250 mg, 0.38 mmol) and $(DHDQ)_2$ PHAL (5 mol%) in *t*BuOH/H_2O mixture (1:1, 10 mL) were added gradually NMO (50 wt% in water, 1.1 mmol) and OsO_4 (9 mg, 0.038 mmol). The pH was adjusted to 5 by addition of 2 N H_2SO_4, and the reaction mixture was stirred for 48 h at room temperature. Sodium sulfite (100 mg) was added, and the reaction mixture was stirred for an additional hour. It was then extracted with ethyl acetate (3 × 25 mL), the organic layer was washed with water, dried over anhyd Na_2SO_4 and evaporated under reduced pressure. Then the crude product was chromatographed on silica gel to afford the desired compound **8** (110 mg, 42%). IR (KBr) 2943, 2858, 1739, 1654, 1375, 1238, 1115, 1025, 770 cm^{-1}; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.37 (m, 5H), 6.25 (d, $J = 11.0$ Hz, 1H), 5.90 (d, $J = 11.0$ Hz, 2H), 5.46 (d, $J = 11.0$ Hz, 2H), 5.03 (s, 2H), 4.53 (s, 1H), 4.30 (m, 1H), 2.80 (s, 1H), 2.58 (m, 1H), 2.21 (s, 3H), 2.08 (s, 3H), 2.03 (s, 3H), 1.98 (s, 3H), 1.94–1.79 (m, 6H), 1.76 (s, 3H), 1.62 (s, 3H), 1.11 (s, 3H), 0.91 (s, 3H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 172.5, 171.3, 170.6, 170.3, 169.7, 146.1, 140.2, 137.5, 135.2, 129.0, 128.8 (2C), 127.0 (2C), 117.4, 76.8, 76.4, 75.7, 74.6, 71.9, 70.9, 70.5, 46.6, 40.7, 39.8, 37.9, 34.4, 31.8, 31.5, 27.9, 27.6, 21.8, 21.4, 21.2, 21.1, 15.8, 13.7; MS (ESI) m/z 708.4 $(M + 23)^+$.

4.5. Acetic acid 7,10,13-triacetoxy-5-hydroxy-8,12,15,15-tetramethyl-4-methylene-tricyclo[9.3.1.03,8]pentadec-11-en-9-yl ester (**9**)

To a magnetically stirred solution of 2-deacetoxytaxinine J (**1**) (500 mg, 0.7 mmol) in a mixture of solvents (H_2O:THF:EtOH (1:1:1), 10 mL) was added hydroxylamine·sulphuricacid salt (250 mg, 3,5 mmol) and triethylamine (4.9 mmol) at rt. The whole solution was stirred for 6 h under reflux. Solvents were removed by vacuum, then the reaction mixture was dissolved in water (100 mL), extracted with ethyl acetate (3 × 100 mL). The combined organic layer was dried over anhyd Na_2SO_4 and evaporated under reduced pressure. Then the crude product was chromatographed on silica gel to afford the desired compound **9** (363 mg, 89%); Mp: 188–190 °C; IR (KBr) 3548, 2984, 2942, 1743, 1654, 1439, 1374, 1249, 1136, 1024, 758 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.25 (d, $J = 11.0$ Hz, 1H), 5.81 (d, $J = 11.0$ Hz, 1H), 5.70 (m, 2H), 5.17 (s, 1H), 5.83 (s, 1H), 4.29 (s, 1H), 3.20 (d, $J = 4.8$ Hz, 1H), 2.76 (m, 1H), 2.19 (s, 3H), 2.05 (s, 6H), 2.07 (s, 3H), 1.97 (s, 3H), 1.99–1.67 (m, 6H), 1.56 (s, 3H), 0.98 (s, 3H), 0.80 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 170.5, 170.2, 169.8, 169.4, 151.6, 137.9, 136.1, 112.7, 73.5, 72.3, 70.2, 69.9, 46.9, 39.8, 39.1, 36.2, 35.6, 32.5, 32.3, 29.8, 27.1, 26.4, 21.6, 21.2, 21.1, 21.0, 16.1, 12.7; MS (ESI) m/z 543.0 $(M + 23)^+$.

4.6. 3-Pyridin-3-yl-acrylic acid 7,9,10,13-tetraacetoxy-8,12,15,15-tetramethyl-4-methylene-tricyclo[9.3.1.03,8]-pentadec-11-en-5-yl ester1(**10**)

To a magnetically stirred solution of compound **9** (250 mg, 0.5 mmol), DCC (5 mmol) and DMAP (5 mmol) in dry CH_2Cl_2 (10 mL) was added gradually (*E*)-3-(pyridin-3-yl)acrylic acid (1 mmol) at rt. The whole solution was stirred for 24 h at rt. The reaction mixture was cooled, filtered and washed with dry and cold CH_2Cl_2, the filtrate was evaporated under reduced pressure. Then the crude product was chromatographed on silica gel to afford the desired compound **10** (72%): IR (KBr) 3021, 2928, 2855, 1743, 1654, 1523, 1424, 1372, 1217, 1046, 757 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.76 (d, $J = 1.1$ Hz, 1H), 8.64 (dd, $J = 4.6$, 1.3 Hz, 1H), 7.83 (m, 1H), 7.76 (d, $J = 16.1$ Hz, 1H), 7.37 (m, 1H), 6.65 (d, $J = 16.1$ Hz, 1H), 6.29 (d, $J = 11.3$ Hz, 1H), 5.95 (d, $J = 11.3$ Hz, 1H), 5.81 (t, $J = 8.7$ Hz, 1H), 5.66 (dd, $J = 10.9$, 5.2 Hz, 1H), 5.59 (s, 1H), 5.40 (s, 1H), 5.04 (s, 1H), 3.03 (d, $J = 4.5$ Hz, 1H), 2.76 (m, 1H), 2.32 (s, 3H), 2.08 (s, 3H), 2.04 (s, 3H), 1.99 (s, 3H), 1.95–1.79 (m, 6H), 1.73 (s, 3H), 1.64 (s, 3H), 1.10 (s, 3H), 0.89 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 171.0, 170.7, 170.3, 169.7, 165.9, 151.8, 151.8, 150.2, 146.5, 142.4, 137.4, 135.6, 134.6, 130.3, 124.2, 120.9, 116.6, 77.4, 75.6, 71.2, 70.4, 46.7, 40.5, 37.9, 34.9, 32.3, 31.6, 31.4, 27.6, 26.6, 25.8, 21.9, 21.4, 21.3, 15.7, 13.6; MS (ESI) m/z 652.2 $(M + H)^+$, 674.2 $(M + 23)^+$.

4.6.1. Pyridin-2-yl-acetic acid 7,9,10,13-tetraacetoxy-8,12,15,15-tetramethyl-4-methylene-tricyclo[9.3.1.03,8]pentadec-11-en-5-yl ester (**11**)

Mp: 84–86 °C; IR (KBr) 3329, 2931, 2853, 1741, 1628, 1576, 1438, 1374, 1243, 1157, 1023, 754 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.56 (d, $J = 4.3$ Hz, 1H), 7.69 (dt, $J = 7.7$, 1.7 Hz, 1H), 7.32 (d, $J = 7.7$ Hz, 1H), 7.21 (m, 1H), 6.27 (d, $J = 11.1$ Hz, 1H), 5.97 (d, $J = 8.6$ Hz, 1H), 5.93 (d, $J = 11.1$ Hz, 1H), 5.99 (dd, $J = 11.5$, 5.14 Hz, 1H), 5.48 (s, 1H), 5.31 (s, 1H), 4.99 (s, 1H), 4.00 (s, 1H), 2.98 (d, $J = 5.4$ Hz, 1H), 2.68 (m, 1H), 2.19 (s, 3H), 2.08 (s, 3H), 2.06 (s, 3H), 2.04 (s, 3H), 1.99 (s, 3H), 1.96–1.68 (m, 7H), 1.65 (s, 3H), 1.15 (s, 3H), 0.87 (s, 3H); ^{13}C NMR ($CDCl_3$,

75 MHz) δ 170.8, 170.4, 170.2, 169.9, 169.4, 154.2, 149.8, 146.8, 137.7, 137.0, 134.7, 123.9, 122.5, 116.1, 76.8, 75.5, 71.9, 70.9, 70.3, 46.4, 43.9, 40.7, 39.7, 37.9, 34.2, 31.6, 31.4, 27.8, 27.7, 21.7, 21.6, 21.2, 21.0, 15.2, 13.6; MS (ESI) m/z 640.1 $(M+H)^+$.

*4.6.2. 3-Pyridin-3-yl-propionic acid 7,9,10,13-tetraacetoxy-8,12,15,15-tetramethyl-4-methylene-tricyclo[9.3.1.03,8]pentadec-11-en-5-yl ester (**12**)*

Mp: 90–92 °C; IR (KBr) 3021, 2929, 2853, 1734, 1623, 1579, 1432, 1373, 1217, 1024, 757 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 8.49 (d, $J=3.5$ Hz, 2H), 7.56 (d, $J=7.8$, Hz, 1H), 7.25 (m, 1H), 6.24 (d, $J=11.2$ Hz, 1H), 5.92 (m, 2H), 5.22 (dd, $J=11.3$, 5.3 Hz, 1H), 5.42 (t, $J=3.2$ Hz, 1H), 5.29 (s, 1H), 4.97 (s, 1H), 3.00 (m, 3H), 2.88 (d, $J=5.3$ Hz, 1H), 2.78 (m, 2H), 2.17 (s, 3H), 2.09 (s, 3H), 2.05 (s, 3H), 1.99 (s, 3H), 1.93–1.80 (m, 6H), 1.77 (s, 3H), 1.64 (s, 3H), 1.14 (s, 3H), 0.88 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 171.6, 170.5, 170.4, 170.0, 169.4, 150.0, 148.2, 146.7, 137.4, 136.0, 135.7, 134.9, 123.9, 116.0, 76.9, 75.3, 72.7, 70.9, 70.2, 46.3, 40.7, 39.7, 38.0, 36.8, 34.3, 32.9, 31.5, 31.3, 27.7, 27.6, 21.6, 21.3, 21.2, 21.0, 15.2, 13.5; MS (ESI) m/z 654.2 $(M+H)^+$, 676.2 $(M+23)^+$.

*4.6.3. Nicotinic acid 7,9,10,13-tetraacetoxy-8,12,15,15-tetramethyl-4-methylene-tricyclo[9.3.1.03,8]pentadec-11-en-5-yl ester (**13**)*

Mp: 166–168 °C; IR (KBr) 2952, 1743, 1627, 1590, 1437, 1375, 1238, 1115, 1025, 745 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 9.34 (d, $J=1.7$ Hz, 1H), 8.81 (dd, $J=4.8$, 1.5 Hz, 1H), 8.38 (dt, $J=7.9$, 1.9 Hz, 1H), 7.49 (dd, $J=7.9$, 4.9 Hz, 1H), 6.65 (d, $J=16.1$ Hz, 1H), 6.27(d, $J=11.3$ Hz, 1H), 5.96 (d, $J=11.3$ Hz, 1H), 5.76 (m, 1H), 5.65 (t, $J=2.8$ Hz, 1H), 5.55 (s, 1H), 5.18 (s, 1H), 2.88 (d, $J=4.7$ Hz, 1H), 2.64 (m, 1H), 2.20 (s, 3H), 2.11 (s, 3H), 2.06 (s, 3H), 2.02 (s, 3H), 1.99 (s, 3H), 1.95–1.78 (m, 3H), 1.63 (s, 3H), 1.09 (s, 3H), 1.08 (s, 3H), 0.91 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 170.6, 170.5, 170.2, 169.5, 165.1, 153.9, 151.4, 146.0, 137.7, 137.4, 135.1, 126.6, 123.8, 118.1, 77.0, 76.9, 71.8, 70.5, 70.2, 46.5, 40.4, 39.6, 38.2, 34.7, 32.0, 31.3, 27.6, 27.5, 21.7, 21.7, 21.0, 19.9, 15.4, 13.8; MS (ESI) m/z 626.2 $(M+H)^+$, 648.1 $(M+23)^+$.

4.7. Cell lines

MCF-7 is an estrogen receptor positive breast cancer cell line derived from pleural effusion and most commonly used cell line for screening of anticancer breast agents [20], whereas MDA-MB-231 is an estrogen receptor negative model of aggressive breast cancer. HEK-293 is a transformed normal epithelial cell line originally derived from human fetal kidney epithelium.

4.8. In vitro assay for anticancer activity

The cytotoxic activity of the compounds was determined using MTT assay [16]. 1×10^4 cells/well were seeded in 200 μl DMEM, supplemented with 10% FBS in each well of 96-well microculture plates and incubated for 24 h at 37 °C in a CO_2 incubator. Compounds, diluted to the desired concentrations in culture medium, were added to the wells with respective vehicle control. After 18 h of incubation, 10 μl MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) (5 mg/mL) was added to each well and the plates were further incubated for 4 h. Then the supernatant from each well was carefully removed, formazon crystals were dissolved in 100 μl of DMSO and absorbance at 540 nm wavelength was recorded.

4.9. In vivo anticancer activity

Animal studies were conducted with prior approval of Institutional Animal Ethics Committee, CDRI, Lucknow. Mammary tumors were induced in virgin female Sprague Dawley rats with gastric instillation of 10 mg/100 g body weight, of 7,12-dimethylbenzanthracene (DMBA) under prescribed anesthesia. Animals were palpated biweekly for tumor development, tumor size measured and were grouped into Group A ($n=3$): orally with 10 mg/kg body weight of 2-DAT-J (**1**) in 0.5 mL of vehicle daily for 30 days; Group B ($n=3$): orally with 10 mg/kg body weight of Tamoxifen citrate in 0.5 mL of vehicle daily for 30 days; and Group C ($n=3$): orally 0.5 mL vehicle only for 30 days. Tumor sizes were measured with Vernier calipers on two days interval.

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Appendix. Supplementary data

Spectra (^1H NMR, ^{13}C NMR and Mass) of all the compounds associated with this article are available as supplementary data. Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.ejmech.2009.04.022.

References

[1] (a) J. Crown, M. O'Leary, Lancet 335 (2000) 1176–1178;
(b) M.C. Wani, H.L. Taylor, M.E. Wall, P. Coggon, A.T. McPhail, J. Am. Chem. Soc. 93 (1971) 2325–2327.
[2] (a) E.K. Rowinsky, L.A. Cazenave, R.C. Donehower, J. Natl. Cancer Inst. 82 (1990) 1247–1259;
(b) W.J. Slichenmyer, D.D. Von Hoff, J. Clin. Pharmacol. 30 (1990) 770–788;
(c) W.J. Slichenmyer, D.D. Von Hoff, Anticancer Drugs 2 (1991) 519–530;
(d) M. Suffness, Ann. Rep. Med. Chem. 28 (1993) 305.
[3] (a) P.B. Schiff, J. Fant, S.B. Horwitz, Nature 277 (1979) 665–667;
(b) S.B. Horwitz, Trends Pharmacol. Sci. 13 (1992) 134–136;
(c) J.M. Andreu, J.F. Diaz, J. Garcia de Ancos, R. Gil, F.J. Mendrano, E. Nogales, E. Pantos, E. Towns-Andrews, J. Mol. Biol. 226 (1992) 169–184;
(d) J.F. Diaz, J.M. Andreu, Biochemistry 32 (1993) 2747–2755;
(e) M.A. Jordan, R.J. Toso, D. Thrower, L. Wilson, Proc. Natl. Acad. Sci. U.S.A. 90 (1993) 9552–9556;
(f) I. Arnal, R.H. Wade, Curr. Biol. 5 (1995) 900–908;
(g) E. ter Har, R.J. Kowalski, E. Hamel, C.M. Lin, R.E. Longley, S.P. Gunasekara, H.S. Rosenkranz, B.W. Day, Biochemistry 35 (1996) 243–250;
(h) D.T. Hung, J. Chen, S.L. Schreiber, Chem. Biol. 3 (1996) 287–293.
[4] N.C. Wheeler, K. Jech, S. Masters, S.W. Brobst, A.B. Alvarado, A.J. Hoover, K.M. Snader, J. Nat. Prod. 55 (1992) 432–440.
[5] (a) R.A. Holton, H.B. Kim, C. Somoza, F. Liang, R.J. Biediger, P.D. Boatman, M. Shindo, C.C. Smith, S. Kim, H. Nadizadeh, Y. Suzuki, C. Tao, P. Vu, S. Tang, P. Zhang, K.K. Murthi, L.N. Gentile, J.H. Liu, J. Am. Chem. Soc. 116 (1994) 1599–1600;
(b) K.C. Nicolaou, H. Ueno, J.J. Liu, P.G. Nantermet, Z. Yang, J. Renaud, K. Paulvannan, R. Chadha, J. Am. Chem. Soc. 117 (1995) 653–656;
(c) S.J. Danishefsky, J.J. Masters, W.B. Young, J.T. Link, L.B. Snyder, T.V. Magee, D.K. Jung, R.C.A. Isaacs, W.G. Bornmann, C.A. Alaimo, C.A. Coburn, M.J. Di Grandi, J. Am. Chem. Soc. 118 (1996) 2843–2859;
(d) P.A. Wender, N.F. Badham, S.P. Gonway, P.E. Floreancing, T.E. Glass, J.B. Houze, N.E. Krauss, D. Lee, D.G. Marquess, P.L. McGrane, W. Meng, M.G. Natchus, A.J. Shuker, J.C. Sutton, R.E. Taylor, J. Am. Chem. Soc. 119 (1997) 2757–2758;
(e) T. Mukaiyama, I. Shiina, H. Iwadare, M. Saitoh, T. Nishimura, N. Ohkawa, H. Sakoh, K. Nishimura, Y. Tani, M. Hasegawa, K. Yamada, K. Saitoh, Chem.—Eur. J. 5 (1999) 121–161;
(f) H. Kusama, R. Hara, S. Kawahara, T. Nishimori, H. Kashima, N. Nakamura, K. Morihara, I. Kuwajima, J. Am. Chem. Soc. 122 (2000) 3811–3820.
[6] (a) J.N. Denis, A.E. Greene, D. Guenard, F. Gueritte-Voegelein, L. Mangatal, P. Potier, J. Am. Chem. Soc. 110 (1988) 5917–5919;
(b) L. Mangatal, M.T. Adeline, A.E. Greene, D. Guenard, F. Gueritte-Voegelein, P. Potier, Tetrahedron 45 (1989) 4177–4190;
(c) H.-J. Ha, G.-S. Park, Y.-G. Ahn, G.S. Lee, Bioorg. Med. Chem. Lett. 8 (1998) 1619–1622 and references therein.
[7] (a) L. Jigyu, M. Zhida, M. Niwa, Acta Chim. Sin. 46 (1988) 1053;
(b) S.K. Chattopadhyay, R.P. Sharma, Phytochemistry 39 (1995) 935–936.
[8] L.O. Zamir, J. Zhang, J.H. Wu, F. Sauriol, O. Mamer, Tetrahedron 55 (1999) 14323–14340.
[9] S.J. Yang, J.M. Fang, Y.S. Cheng, Phytochemistry 43 (1996) 839–842.

[10] J.Y. Liang, K.S. Huang, A.A.L. Gunatilaka, Planta Med. 64 (1998) 187–188.
[11] K.V. Rao, J. Juchum, Phytochemistry 47 (1998) 1315–1324.
[12] K. Juńichi, S. Hideyuki, Heterocycles 47 (1998) 1111–1134.
[13] N.T. Nguyen, A.H. Banskota, Y. Tezuka, T. Nobukawa, S. Kadota, Phytochemistry 64 (2003) 1141–1147.
[14] J. Kobayashi, A. Ogiwara, H. Hosoyama, H. Shigemori, N. Yoshida, T. Sasaki, Y.L.S. Iwasaki, M. Naito, T. Tsuruo, Tetrahedron 50 (1994) 7401–7416.
[15] J. Kobayashi, H. Hosoyama, X.X. Wang, H. Shigemori, Y. Koiso, S. Iwasaki, T. Sasaki, M. Naito, T. Tsuruo, Bioorg. Med. Chem. Lett. 7 (1997) 393–398.
[16] M. Botta, S. Armaroli, D. Castagnolo, G. Fontana, P. Perad, E. Bombardelli, Bioorg. Med. Chem. Lett. 17 (2007) 1579–1583.
[17] J.M. Khurana, P. Sharma, Bull. Chem. Soc. Jpn. 77 (2004) 549–552.
[18] M.A. Andersson, R. Epple, V.V. Fokin, K.B. Sharpless, Angew. Chem., Int. Ed. 41 (2002) 472–475.
[19] (a) I. Fenoglio, G.M. Nano, D.G. Vander Velde, G. Appendino, Tetrahedron Lett. 37 (1996) 3203–3206;
(b) D.A. Sun, A. Nikolakakis, F. Sauriol, O. Mamer, L. Zamir, Bioorg. Med. Chem. 9 (2001) 1985–1992.
[20] H.D. Soule, J. Vazguez, A. Long, S. Albert, M. Brennan, J. Natl. Cancer Inst. 51 (1973) 1409–1416.
[21] K.V. Sashidhara, J.N. Rosaiah, A. Kumar, H.K. Bid, R. Konwar, N. Chattopadhyay, Phytother. Res. 21 (2007) 1105–1108.
[22] C. Huggins, L.C. Grand, P.F. Brillantes, Nature 189 (1961) 204–207.