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Synthesis and biological evaluation of new taxoids derived from 2-deacetoxytaxinine J

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Dedicated to Professor Fulvio Gualtieri on the occasion of his 70th birthday

Abstract—A small library of 2-deacetoxytaxinine J (DAT-J) **1** derivatives was synthesised and tested in vitro for their reversal activity in human mammary carcinoma MDR cell line MCF7-R. One of the new taxoids showed to be active at 0.1 μM when tested in combination with paclitaxel.

Paclitaxel (Taxol®), a complex natural diterpene extracted from *Taxus brevifolia*,[1] and its derivative docetaxel are two of the most important anticancer agents for the treatment of ovarian and breast cancer.[2] Clinical use of these anticancer drugs has disclosed that paclitaxel and docetaxel have a number of undesired side effects as well as multidrug resistance (MDR). MDR induced by taxoid treatment in tumour cells is a significant obstacle to the success of chemotherapy in many cancers. In particular, MDR is a phenomenon whereby tumour cells that have been exposed to one cytotoxic agent develop cross resistance to a range of structurally and functionally unrelated compounds. The drug resistance developed in cancer cells may be mediated by a number of mechanisms; in particular it has been related to the overexpression in cancer cells of particular proteins such as P-glycoprotein (P-gp) which extrude hydrophobic anticancer compounds and maintain their intracellular concentration below a cytotoxic level.[3] Several natural and semisynthetic taxoids, devoid of cytotoxicity and tubulin affinity, are powerful inhibitors of P-gp activity, acting as efficient reversing agents and allowing accumulation of paclitaxel in MDR cancer cells.[4] New taxanes exhibited >99% MDR reversal activity against breast cancer cell lines, at 1–3 μM level.[3a] Among the natural taxoids, 2-deacetoxytaxinine J (DAT-J) **1** emerged as the most active P-gp inhibitor with a potency higher than that of verapamil;[4a] compound **1** showed an increase of vincristine accumulation (266%) in MDR 2780 AD cells as compared to verapamil (254%). DAT-J **1** is extracted in low concentration from several yew species, but it can be synthesised from the natural alkaloid 2′-deacetoxyaustropicatine (DAS) **2** available in multigram amounts from *Taxus* x media Rehd. Cv. HicKsii.[5] Moreover, unlike other cinnamates related to taxine, **1** does not show cardiac toxicity,[6] and might thus serve as an important starting material for the synthesis of new reversal agents. For this reason, the development of a procedure, using 2-deacetoxytaxinine J **1** as starting material for the preparation of new bioactive taxinine analogues, would be significative (see Figs. 1 and 2).

Herein the preparation of a small library of DAT-J **1** analogues is reported; these derivatives have been subjected to biological screening as new potential MDR-reversing agents against multidrug-resistant breast tumour cell lines.

Keywords: Taxoids; 2-Deacetoxytaxinine J; Multidrug resistance (MDR).
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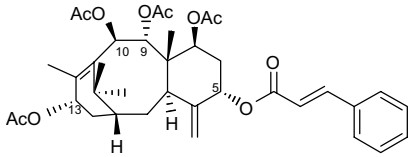

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Figure 1. The structure of 2-deacetoxytaxinine J **1**.

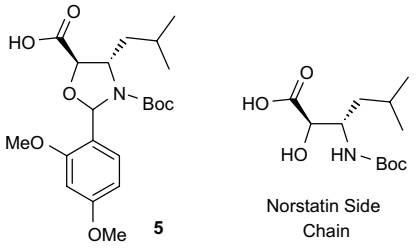
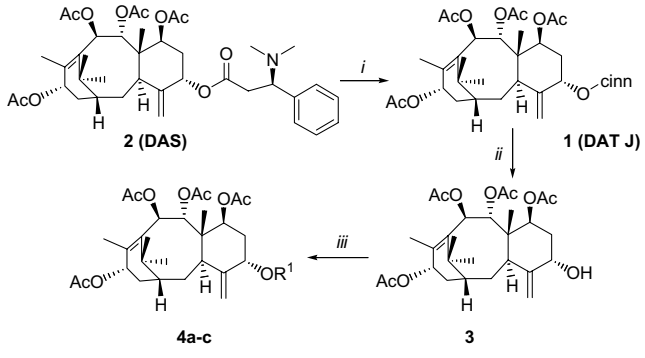


Figure 2. Free acids of protected norstatin side chain **5** and norstatin side chain of ortataxel.

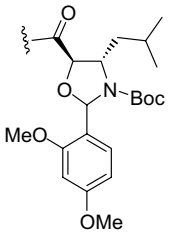
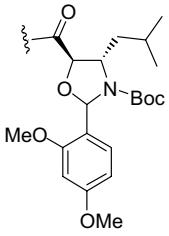
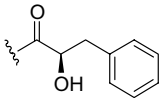
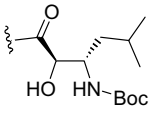
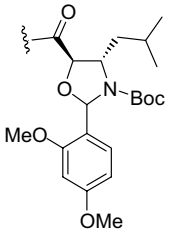
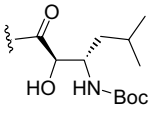
2-Deacetoxytaxinine J (DAT-J) **1** was prepared in one step from commercially available 2-deacetoxyaustropicatine (DAS) **2** by elimination with *m*-CPBA[5] (Scheme 1). DAT-J **1** was converted into **3** by removal of the cinnamoyl group with NH_2OH.[5b,7] Compound **3**, which presents a free hydroxyl group at C-5 position, was further elaborated into a first series of derivatives **4a–c** presenting different side chains.[4a,8] The choice of the side chains was investigated on the basis of the literature data and interesting and easily available scaffolds. Compound **4a** was previously synthesised and evaluated in vitro for its cytotoxicity against human cancer cell lines (TFK-1) by Horiguchi et al.[9]

Esterification of **3** with the protected norstatin side chain of ortataxel **5**[10,11] (5 equiv) and benzoylchloride (6 equiv) affords, respectively, derivatives **4b** and **4c** in high yields (see Scheme 1 and Table 1).

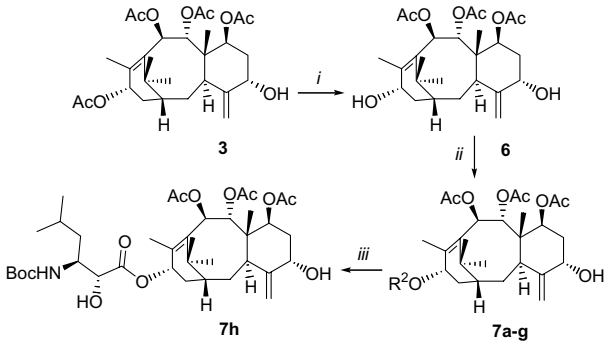


Scheme 1. Reagents and conditions: (i) 1.2 equiv *m*-CPBA, THF, rt; (ii) 9.3 equiv $NH_2OH{\cdot}HCl$, NaOAc, $EtOH/H_2O$ 1:1, reflux; (iii) 3 equiv TESCl, imidazole, DMF (for **4a**, R^1 = TES, 77%); 5 equiv **5**, EDC, toluene, DMAP (for **4b**, R^1 = protected norstatin side chain, 75%); 6 equiv BzCl, Et_3N, toluene (for **4c**, R^1 = Bz, 70%).

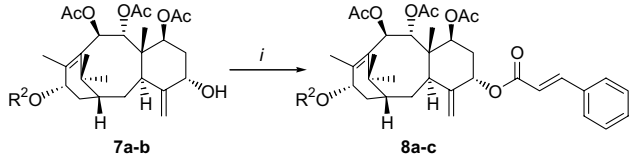
Table 1. Derivatives of DAT-J **1**

Compound	R^1
4a	TES
4b	
4c	Bz
	R^2
7a	
7b	Cinnamoyl
7c	3-Nicotinoyl
7d	Bz
7e	Bn
7f	Phenylcarbamoyl
7g	
7h	
8a	
8b	Cinnamoyl
8c	

Compound **3** was then converted into diol **6** by selective hydrolysis with LiOH at 4 °C[5b,7] (Scheme 2). Diol **6** presents two secondary hydroxyls at C-5 and at C-13 positions; on the basis of the steric hindrance at C-5 position, a series of derivatives **7a–h** was synthesised in high yield, introducing different side chains selectively at C-13 position of **6** (Scheme 2, Table 1). This latter compound was elaborated first into **7a** and **7b**,



Scheme 2. Reagents and conditions: (i) 7 equiv $LiOH{\cdot}H_2O$, MeOH/H_2O 1:1, 4 °C; (ii) 2 equiv acid, EDC, toluene, DMAP (for **7a**, R^2 = protected norstatin side chain, 85%; for **7b**, R^2 = cinnamoyl, 79%; for **7c**, R^2 = 3-nicotinoyl, 75%); 2 equiv BzCl, Et_3N, CH_2Cl_2 (for **7d**, R^2 = Bz, 82%); 2 equiv BnBr, NaH, THF (for **7e**, R^2 = Bn, 88%); 2 equiv PhNCO, Et_3N, CH_2Cl_2, reflux (for **7f**, R^2 = PhNCO, 91%); 2 equiv TBDMS-phenyllactic acid, EDC, toluene, DMAP and then TBAF, THF (for **7g**, R^2 = phenyllactoyl, 65% for 2 steps); (iii) HCl 0.1 N, MeOH (91%).

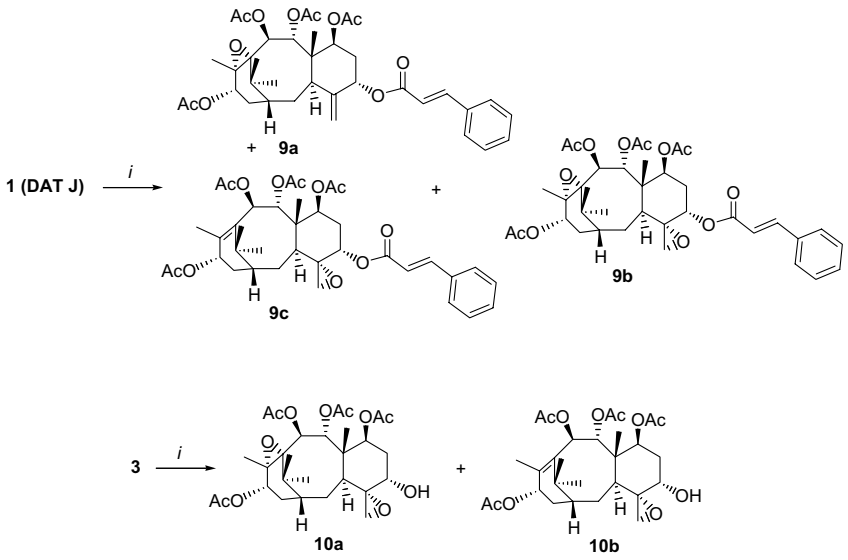


Scheme 3. Reagents and conditions: (i) 5 equiv cinnamic acid, EDC, toluene, DMAP (for **8a**, R^2 = protected norstatin side chain, 62%; for **8b**, R^2 = cinnamoyl, 60%;); 5 equiv cinnamic acid, EDC, toluene, DMAP and then HCl 0.1 N, MeOH (for **8c**, R^2 = norstatin side chain, 54% for 2 steps).

respectively, by esterification with the protected norstatin side-chain acid **5** and with cinnamic acid. Reaction of **6** with nicotinic acid (2 equiv) in presence of EDC and DMAP affords **7c**. Esterification of compound **6** with benzoyl chloride (2 equiv) affords taxinine **7d** while compound **7e**, which represents a further simplification of the side chain of **7d**, was synthesised treating starting material **6** with benzyl bromide (2 equiv) in presence of NaH. Reaction of **6** with phenylisocyanate (2 equiv) in CH_2Cl_2 under reflux affords the carbamate **7f**. Finally, compound **7g** was obtained in two steps, by esterification of **6** with TBDMS-(*R*)-phenyllactic acid (2 equiv) followed by removal of silyl group with TBAF. Compound **7a**, presenting at C-13 position the protected norstatin side chain of ortataxel, was further elaborated into **7h** by hydrolysis with 0.1 N HCl.[12]

Further elaboration of compounds **7a** and **7b** into derivatives **8a** and **8b** was accomplished by esterification at position C-5 with cinnamic acid. Hydrolysis of protected norstatin side chain of **8a** afforded also derivative **8c** (see Scheme 3 and Table 1).

The synthesis of new oxidised derivatives of **1** was then examined; in particular five compounds derived from epoxidation of DAT-J **1** and the taxinine **3** were synthesised.[5a] Horiguchi et al. first reported the epoxidation of 2-deacetoxytaxinine-J **1** and its derivatives with *m*-CPBA, and evaluated the biological activity of new compounds as potential cytotoxic agents against human cancer cell lines (TFK-1).[13] Kobayashi et al. also reported the epoxidation of the 4(20)-exomethylene in taxinine and taxinine A, and evaluated their activity as MDR-reversing agents.[14] We decided to oxidise **1** with *m*-CPBA in the presence of NaOAc following the reported procedures; the reaction afforded a mixture of the three compounds **9a** (12% yield), **9b** (25% yield) and **9c** (52% yield) which were separated by chromatography. Moreover epoxidation of taxane **3** under the same conditions afforded only the two derivatives **10a** (28% yield) and **10b** (57% yield) which were separated by chromatography. The stereochemistry of **9a–c** and **10a**,**b** was unequivocally established by NOE experiments and through a comparison with data reported in the literature.[13,14] (Scheme 4).



Scheme 4. Reagents and condition: (i) 1.5 equiv *m*-CPBA, NaOAc, CH_2Cl_2, rt.

Biological evaluation. The DAT-J **1** analogues synthesised in this work were tested in vitro for their MDR revertant activity in human mammary carcinoma cell line MCF7-R, which was resistant to paclitaxel. The compounds were tested at non-cytotoxic concentration (i.e., 1.0 and 0.1 μM) in combination with paclitaxel. The activity was expressed as IC_{50} (nM) that is the concentration that caused 50% inhibition of cancer cell growth. The results are presented in Table 2.

Introduction of different side chains or ester groups at C-5 position results in loss of activity; in fact, compounds **4b**,**c** and **8a–c** were found to be totally inactive. The derivative **4a** which presents a silyl group at C-5 showed potent activity in increasing the cytotoxicity of paclitaxel against MCF7-R cell line at 1.0 μM. The lowest concentration was inactive. At the opposite, the introduction of a side chain or an ester function at C-13 position seems to be crucial for the MDR activity; in fact, compound **6** which presents free hydroxyl groups at C-5 and C-13 positions showed low revertant activity at the higher concentration tested (1.0 μM). Compound **7h** with deprotected norstatin side chain of ortataxel at C-13 showed potent paclitaxel-MDR revertant activity at 1.0 μM but became inactive at 0.1 μM. Compounds **7b** and **7g** were active at both concentrations tested; in particular **7d**[15] showed to be very active at 0.1 μM. The epoxidised compounds **9a–c** showed a good activity at 1.0 μM concentration; among these, the derivative **9a** with an epoxide at C-11 and C-12 showed also a weak activity at lower concentration.

In conclusion, a small library of 2-deacetoxytaxinine J (DAT-J) **1** derivatives was synthesised and tested in vitro for their reversal activity in human mammary carcinoma MDR cell line MCF7-R. Among the new compounds, **7d** showed to be very active at very low concentration; **7d** presented a 67% paclitaxel IC_{50} reduction when tested in combination with paclitaxel at 0.1 μM.

Table 2. Effects of MDR-reversing agents on the cytotoxicity of paclitaxel against MCF7-R cell line

Compound	Concentration tested in combination with paclitaxel (μM)	Paclitaxel IC_{50} (nM) MCF7-R	% paclitaxel IC_{50} reduction
4a	1.0	47 ± 8.7	92
	0.1	662 ± 67	0
6	1.0	325 ± 46	47
	0.1	641 ± 54	0
7b	1.0	45 ± 4.0	92
	0.1	386 ± 78	35
7d	1.0	31 ± 8.0	95
	0.1	204 ± 52	67
7g	1.0	66 ± 8.7	89
	0.1	463 ± 59	24
7h	1.0	44 ± 13	93
	0.1	597 ± 79	0
9a	1.0	46 ± 9.8	92
	0.1	463 ± 56	22
9b	1.0	178 ± 24	70
	0.1	583 ± 94	2
9c	1.0	92 ± 14	85
	0.1	613 ± 82	0
Paclitaxel	None	593 ± 41	

Acknowledgements

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15. *Experimental procedure for the synthesis of compound **7d**.* Taxinine **6** (115 mg, 0.24 mmol) was dissolved in dry CH_2Cl_2 (10 mL) and the resulting solution was cooled at 0 °C; Et_3N (43 μL, 0.31 mmol), BzCl (34 μL, 0.29 mmol) and a catalytic amount of DMAP were added and the mixture was stirred at room temperature for 2 h. The solution was diluted with CH_2Cl_2 and washed with $NaHCO_3$ and brine. The organic layers were dried (Na_2SO_4), filtered and evaporated to give the crude **7d**. The crude product was purified by flash chromatography on silica gel, using AcOEt/petroleum ether 1:2 as eluant. Yield: 82%. ^1H NMR ($CDCl_3$): δ 8.08–8.02 (2H, m, Ph), 7.52–7.41 (3H, m, Ph), 6.31 (1H, d, J = 10.9 Hz, H-10), 5.85 (1H, d, J = 10.9 Hz, H-9), 5.87–5.85 (1H, m, H-13), 5.75 (1H, dd, J = 5.4, 11.5 Hz, H-7), 5.05 (1H, s, H-20), 4.75 (1H, s, H-20), 4.25 (1H, m, H-5), 3.30 (1H, m, H-3), 2.90 (1H, br s, O*H*), 3.03–2.84 (1H, m, H-14a), 2.36 (3H, s, CH_3-18), 2.05 (3H, s, CH_3-10), 2.01 (3H, s, CH_3-7), 1.96 (3H, s, CH_3-9), 1.91–1.66 (5H, m, H-1, H-2a,b, H-6a,b), 1.59 (3H, s, CH_3-17), 1.23–1.10 (1H, m, H-14b), 1.06 (3H, s, CH_3-16), 0.80 (3H, s, CH_3-19). MS : 583 (M + 1), 605 (M + Na). Anal. Calcd for $C_{33}H_{42}O_9$: C, 68.02; H, 7.27. Found: C, 68.23; H, 7.42.