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Synthesis and biological activity of macrocyclic taxane analogues

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Abstract—A series of paclitaxel analogues possessing a macrocyclic structure between the 7 and 10 positions has been prepared. These compounds possess in vitro activity against a paclitaxel resistant cell line and have in vivo activity comparable to paclitaxel.

First approved for clinical use in 1992, the taxane anticancer drug TAXOL® (paclitaxel **1**, Fig. 1) has become one of the most exciting advances in cancer chemotherapy ever discovered. Because TAXOL® has demonstrated activity against ovarian, breast, lung, Kaposi's sarcoma, bladder, prostate, esophageal, head and neck, cervical, and endometrial cancers, considerable efforts are still being expended on clinical trials to fully elucidate the usefulness of this drug.[1] Recent efforts toward the discovery of novel taxanes have introduced clinical candidates endowed with in vivo efficacy superior to paclitaxel in both paclitaxel resistant and sensitive human tumor xenograft models.[2]

Significant progress has also been made toward the discovery of orally active taxanes.[3] As the clinical potential of TAXOL® continues to grow, the development of novel taxane analogues with greater efficacy, reduced toxicity, and an expanded spectrum of activity may result in significant improvements in patient response. TAXOTERE® (docetaxel **2**, Fig. 1), a semisynthetic taxane has also been approved for clinical use.

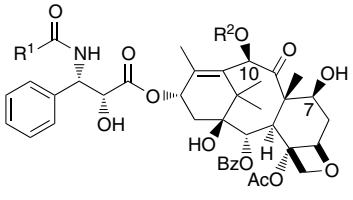

Figure 1. Paclitaxel **1** ($R^1 = Ph$, $R^2 = Ac$) docetaxel **2** ($R^1 = O$-*tert*-Bu, $R^2 = H$).

In the context of discovering analogues that overcome paclitaxel resistance mediated through the P-glycoprotein efflux mechanism, modifications at the northern hemisphere of the molecule, specifically, the 7 and 10 positions, have been implicated and pursued. SAR studies performed by a number of different groups have demonstrated that these positions are quite tolerant to chemical manipulation; probably due to the fact that these residues do not interact directly with tubulin.[4] C7 paclitaxel ethers have shown in vitro potency against both paclitaxel sensitive and resistant cell lines.[5] Analogues modified at the C10 position exhibit improved in vitro potency against resistant cell lines.[6] It has been hypothesized that modifications of this region of the molecule may affect paclitaxel's susceptibility to P- glycoprotein mediated efflux. With this information in hand we contemplated the synthesis of novel analogues **7** and **13** derived by linking the C7 and C10 positions of the taxane skeleton in a macrocyclic array.[7] Such analogues with the 7 and 10 positions simultaneously blocked might be endowed with in vivo activity against tumors expressing the MDR phenotype. P-glycoprotein mediated efflux is thought to be responsible for development of the MDR (multidrug resistant) phenotype. Alteration of the binding motif between paclitaxel analogues and P-glycoprotein could result in activity against tumors that are unresponsive to treatment or that have become resistant as a result of taxane treatment. It has been proposed that the ability of C10 taxane analogues

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to overcome MDR in vitro is the result of reduced binding affinity for P-glycoprotein.[8] The proximity of C7 to the P-glycoprotein binding site of paclitaxel has been demonstrated by photoaffinity studies.[9]

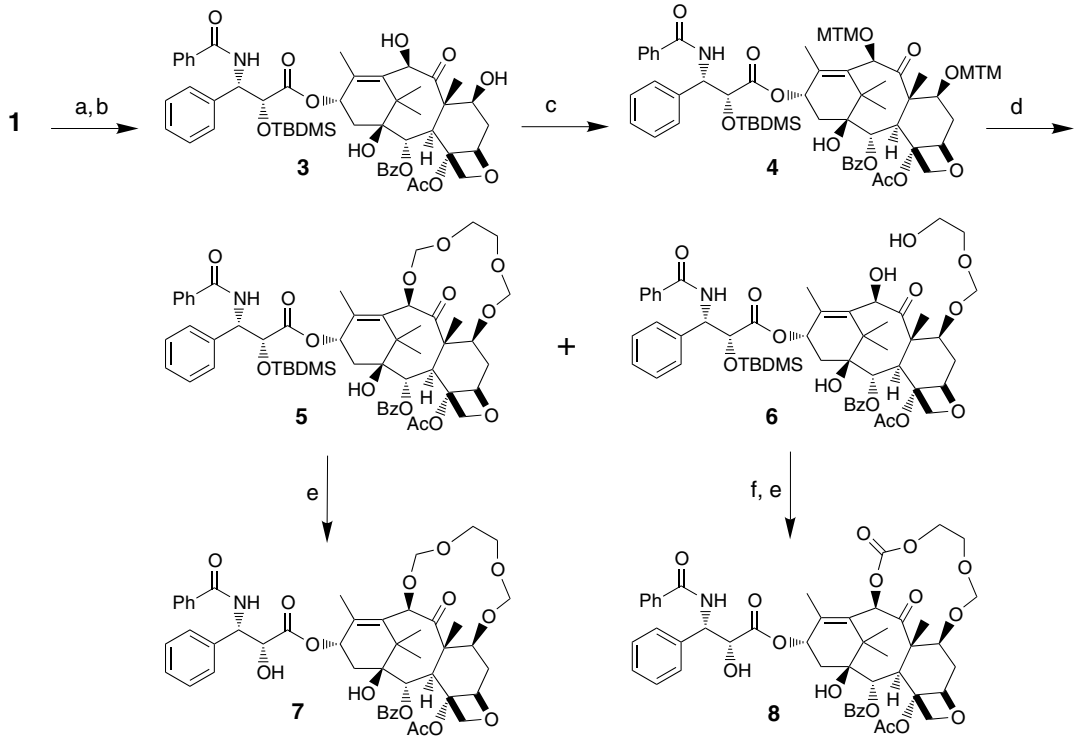
Previously, researchers in our group had developed the use of MTM ethers to introduce the MOM and other ethers into the 7-position of paclitaxel.[10] We believed this technology would prove useful for the introduction of macrocyclic elements into paclitaxel.

Our synthesis began with the preparation of 2′-*tert*-butyldimethylsilyl-paclitaxel, which was the converted to 10-deacetylpaclitaxel (**3**) upon treatment with hydrazine hydrate in ethanol.[11] This compound was then converted to the 7,10-bismethyl-thiomethoxy analogue **4**. Exposure of this compound to 10 equiv of a diol, for example, ethylene glycol, in the presence of *N*-iodosuccinimide, silver trifluoromethanesulfonate and 4 Å molecular sieves[12] provided a 1:1 mixture of the 7, 10-macrocyclic analogue **5** along with the 7-substituted 10-deacetyl analogue **6**, which were readily separable by chromatography. The structures were confirmed by spectroscopic methods. The superior reactivity of the 7-position when compared to the 10-position parallels the reactivity observed for acetylation and silylation.[13] Desilylation was effected by treatment with TBAF at −10 °C to provide compound **7**. Cyclization of compound **6** could be accomplished by treatment with a slight excess of triphosgene to afford the cyclic carbonate **8** (Scheme 1). Table 1 lists the novel macrocyclic taxanes prepared using this series of reactions.

Variation of the C13 side chain was accomplished utilizing the standard sequence of reactions. Reductive de-esterification of 7,10-bismethyl-thiomethoxypaclitaxel (**4**) with tetrabutylammonium borohydride,[14] provided the analogous baccatin compound in good yield. The 13-hydroxy group was protected as its trimethylsilyl derivative to give compound **10**, which was treated with ethylene glycol as outlined above. In this case, the acyclic by-product was not isolated. Desilylation of **11** was effected by treatment with TBAF and the resulting baccatin analogue was acylated with β-lactam **12** using a variation of the Ojima–Holton methodology.[15] Desilylation of the intermediate with TBAF afforded macrocyclic analogue **13** (Scheme 2).

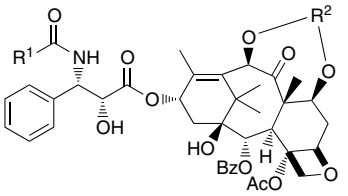
The macrocyclic taxane analogues were evaluated for inhibition of tubulin polymerization,[16] and for in vitro cytotoxicity against the paclitaxel sensitive human colon carcinoma cell line HCT-116 and against the human breast carcinoma cell line A2780. These analogues were also evaluated against the paclitaxel resistant cell lines HCT116/mdr[17] and A2780/tub.[18] Table 1 summarizes this data.

The macrocyclic analogues were significantly more potent against the paclitaxel resistant cell line, HCT116/mdr. This cell line is known to express elevated levels of P-glycoprotein and is about 100-fold resistant to paclitaxel. This suggests that these analogues are less susceptible to efflux by P-glycoprotein. The precise mechanism of this reduced susceptibility remains to be elucidated. Macrocyclic analogues **8** and **13** were slightly



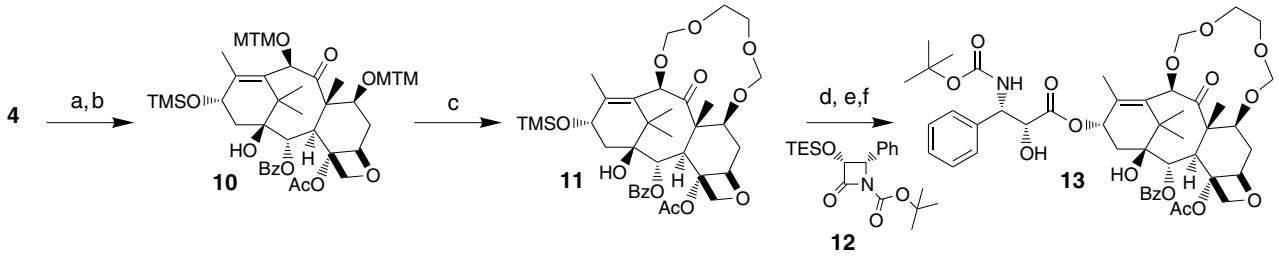
Scheme 1. Reagents and conditions: (a) TBSCl, imidazole, DMF (94%); (b) hydrazine hydrate, EtOH (95%); (c) dimethyl sulfide, benzoyl peroxide, CH_3CN (80%); (d) diol, NIS, AgOTf, 4 Å sieves, THF (25–55%); (e) TBAF, THF, −10 °C (76–95%); (f) triphosgene, pyridine, DCM, 0 °C–rt (13%).

Table 1. In vitro activity of macrocyclic taxane analogues[20]



Compound	R^1	R^2	Tubulin[a]	HCT116 IC_{50} (nM)[b]	HCT/mdr R/S ratio[c]	A2780 IC_{50} (nM)[d]	A2780/tub R/S ratio[e]
1	Ph	10-Ac, 7-H	1.0	2.2[f]	126[f]	2.4[f]	31[f]
7	Ph	$CH_2OCH_2CH_2OCH_2$	1.49	3.0	10	2.6	15
8	Ph	$(CO)OCH_2CH_2OCH_2$	0.73	1.9	31	2.1	32
13	*t*-BuO	$CH_2OCH_2CH_2OCH_2$	0.67	0.8	12	18.5	6
14	Ph	$CH_2OCH_2C(CH_3)_2CH_2OCH_2$	2.87	17.2	11	18.0	23

[a] Concentration of test compound to give a change of 0.01 OD/h expressed as a ratio to the concentration of paclitaxel.
[b] IC_{50} values determined by MTS assay after 72 h drug exposure.
[c] HCT ratio Ana/PT = HCT116 IC_{50} value of analogue/HCT116 IC_{50} value of paclitaxel in the same experiment (or A2780 cell line).
[d] HCT/mdr R/S ratio = HCT116/mdr IC_{50} value/HCT116 IC_{50} value.
[e] A2780/tub R/S ratio = A2780/tub IC_{50} value/A2780 IC_{50} value.
[f] Mean values of five tests.



Scheme 2. Reagents and conditions: (a) tetrabutylammonium borohydride, DCM, 0 °C–rt (50%); (b) TMSOTf, DIPEA, DMF, 0 °C–rt (68%); (c) ethylene glycol, NIS, AgOTf, 4 Å sieves, THF (53%); (d) TBAF, THF, −10 °C (55%); (e) LHMDS, **12**, THF, −55 °C (98%); (f) TBAF, THF, −10 °C (95%).

more potent than paclitaxel in inhibiting tubulin polymerization, while analogues **7** and **14** were slightly less potent than paclitaxel, indicating that the macrocycle is not interfering with tubulin binding or altering the mechanism of action. The lack of improved efficacy against the paclitaxel resistant cell line A2780/tub is consistent with this hypothesis.

Furthermore, compounds **7** and **13** were shown to possess activity in vivo against the ip/ip M109 Madison murine lung carcinoma screen (Table 2).[19] The in vivo activity of these analogues was equivalent to that observed for a reference dose of paclitaxel. Compound **14**, the *gem*-dimethyl macrocyclic analogue, which displayed reduced in vitro potency, was inactive in the in vivo screen. These results suggest that the presence of an unsubstituted macrocycle can be tolerated at the tubulin binding site. Analogues **7** and **13** have the potential to demonstrate greater in vivo efficacy against tumors exhibiting an MDR phenotype and are worthy of further evaluation.

In conclusion, a series of novel C7/C10 macrocyclic taxane analogues has been prepared. Several compounds in this series possess in vitro potency superior to paclitaxel, and exhibit reduced cross-resistance to a paclitaxel resistant cell line. Analogues **7** and **13** also displayed in vivo activity comparable to paclitaxel. The data are consistent with the hypothesis that these analogues are more potent than paclitaxel versus MDR resistant cell lines because of their recalcitrance to P-glycoprotein mediated efflux. This study serves to further define the SAR of the northern hemisphere of paclitaxel.

Table 2. In vivo activity of macrocyclic taxane analogues **7**, **13**, and **14** evaluated concomitantly with **1**

Compound	T/C % (dose in mg/kg/injection)	
	Analogue	Paclitaxel (**1**)
7	174 (200)	174 (60)
13	190 (100)	197 (60)
14	118 (120)	165 (60)

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17. HCT116/mdr (also known as HCT116/VM46) is a cell line, which overexpresses the drug efflux pump P-glycoprotein and is about 100-fold resistant to paclitaxel. This cell line was established by exposing HCT-116 to VM-26 (a topo II active agent) in order to generate a cell line resistant to these drugs.
18. A2780/tub (also known as A2780/Tax) is a cell line, which contains a tubulin mutation and is about 35-fold resistant to paclitaxel.
19. This is our primary SAR determining in vivo screen. Balb/c $\times$DBA/2 F_1 hybrid mice were implanted intraperitoneally, as described by William Rose in *Cancer Treatment Reports* **1981**, *65*, 299–312, with 0.5 mL of a 2% (w/v) brei of M109 lung carcinoma. Mice were treated with compounds under study by receiving intraperitoneal injections of various doses days 5 and 8 post-implant. Mice were followed daily for survival until approximately 75–90 days post-tumor implant. One group of mice per experiment remained untreated and served as the control group. Median survival times of compound-treated (T) mice were compared to the median survival time of the control (C) mice. The ratio of the two values for each compound-treated group of mice was multiplied by 100 and expressed as a percentage (i.e., % T/C). A %T/C value greater than 125 is considered active.
20. *Spectral characteristics for* **7**: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.04 (d, $J = 7.09$ Hz, 2H), 7.60 (d, $J = 7.07$ Hz, 2H), 7.55–7.28 (bm, 11H), 7.04 (d, $J = 8.93$ Hz, 1H), 6.17 (m, 1H), 5.93 (s, 1H), 5.72 (dd, $J = 2.43$ Hz, $J = 8.94$ Hz, 1H), 5.59 (d, $J = 6.88$ Hz, 1H), 4.87 (d, $J = 7.92$ Hz, 1H), 4.77 (d, $J = 6.77$ Hz, 1H), 4.71–4.65 (m, 3H), 4.42 (d, $J = 7.72$ Hz, 1H), 4.24–3.95 (bm, 6H), 3.77 (d, $J = 6.84$ Hz, 1H), 3.65 (bs, 1H), 3.54 (d, $J = 10.88$ Hz, 1H), 3.05 (d, $J = 7.71$ Hz, 1H), 2.47 (m, 1H), 2.30 (s, 3H), 2.23 (dd, $J = 4.39$ Hz, $J = 8.93$ Hz, 1H), 1.96 (s, 1H), 1.92 (s, 3H), 1.80 (m, 1H), 1.69 (s, 3H), 1.11 (s, 3H), 1.08 (s, 3H); LRMS (ESI): 896 [$(M-1)^-$, 100%]; HRMS (ESI) calcd for $C_{49}H_{55}NO_{15}$ Na: 920.3469, found: 920.3497. *Spectral characteristics for* **13**: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.10 (d, $J = 7.2$ Hz, 2H), 7.60 (m, 1H), 7.48 (m, 2H), 7.42–7.25 (bm, 6H), 6.25 (m, 1H), 6.03 (s, 1H), 5.66 (d, $J = 6.9$ Hz, 1H), 5.35 (d, $J = 9.4$ Hz, 1H), 5.29 (m, 1H), 4.95 (d, $J = 7.9$ Hz, 1H), 4.86 (d, $J = 6.7$ Hz, 1H), 4.79 (d, $J = 6.7$ Hz, 1H), 4.75 (d, $J = 7.7$ Hz, 1H), 4.62 (bs, 1H), 4.50 (d, $J = 7.7$ Hz, 1H), 4.30 (d, $J = 8.4$ Hz, 1H), 4.24 (dd, $J = 7.2$ Hz, $J = 10.5$ Hz, 1H), 4.17 (d, $J = 8.3$ Hz, 1H), 4.10 (m, 2H), 3.85 (d, $J = 6.7$ Hz, 1H), 3.64 (d, $J = 11.0$ Hz, 1H), 3.32 (d, $J = 4.9$ Hz, 1H), 3.14 (d, $J = 8.0$ Hz, 1H), 2.56 (m, 1H), 2.36 (s, 3H), 2.28 (d, $J = 8.9$ Hz, 2H), 2.04 (s, 3H), 1.86 (m, 1H), 1.76 (s, 3H), 1.34 (s, 9H), 1.21 (s, 3H), 1.19 (s, 3H); LRMS (ESI^+): 911.5 [$(M+NH_4)^+$, 100%], 894.5 [$(M+1)^+$, 95%]; LRMS (ESI^-): 892.6 [$(M-1)^-$, 100%]. Spectral data for analogues **8** and **14** is available upon request.