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Single-site chemical modification at C10 of the baccatin III core of paclitaxel and Taxol C reduces P-glycoprotein interactions in bovine brain microvessel endothelial cells

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Abstract—A single-site modification of paclitaxel analogs at the C10 position on the baccatin III core that reduces interaction with P-glycoprotein in bovine brain microvessel endothelial cells is described. Modification and derivatization of the C10 po\sition were carried out using a substrate controlled hydride addition to a key C9 and C10 diketone intermediate. The analogs were tested for tubulin assembly and cytotoxicity, and were shown to retain potency similar to paclitaxel. P-glycoprotein interaction was examined using a rhodamine assay and it was found that simple hydrolysis or epimerization of the C10 acetate of paclitaxel and Taxol C can reduce interaction with the P-glycoprotein transporter that may allow for increased permeation of taxanes into the brain.
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Chemotherapy of CNS localized tumors has been limited by the inability of drugs to bypass the blood–brain barrier (BBB) and little progress has been made since the advent of the nitrosoureas.[1] While primary CNS tumors are relatively uncommon, metastatic tumors of the brain outnumber primary lesions at least 10–1.[2] Therefore, the development of agents able to permeate the BBB would provide clinicians with additional options for the treatment of brain localized tumors, especially those with multiple lesions.

Paclitaxel (**1**, Fig. 1) is a microtubule-binding chemotherapeutic agent that causes cell cycle arrest at the G_2/M phase leading to cellular apoptosis.[3] Paclitaxel is approved for the treatment of breast, ovarian, and non-small cell lung cancers, Kaposi's sarcoma, and is active against a wide variety of cancer cell types.[4]

Paclitaxel does not cross the blood–brain barrier and is not effective against cancers localized in the cranial cavity.[5] The primary mechanism of decreased paclitaxel BBB penetration is active efflux by P-glycoprotein (Pgp), which is a membrane bound protein produced by the multi-drug resistant gene cassette (MDR1). Pgp knockout mice show nearly a fourfold increase in the

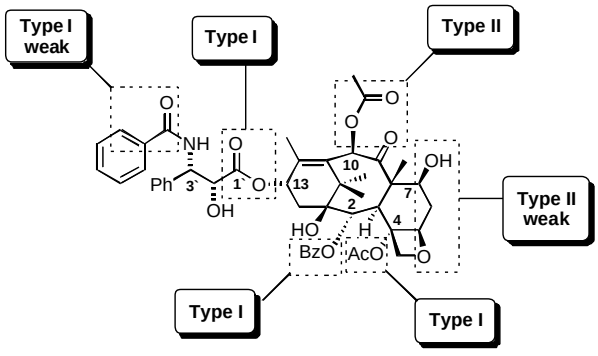

Figure 1. Paclitaxel (**1**) recognition elements for Pgp.

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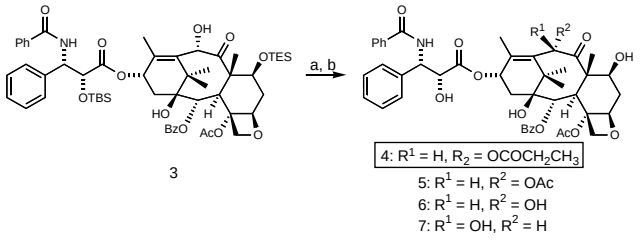
concentration of brain paclitaxel levels as compared to normal mice.[6] Paclitaxel has a relatively low toxicity to primary cortical neurons and has been shown to protect them from β-amyloid protein-induced toxicity, which has been implicated in Alzheimer's disease.[7] Thus, if paclitaxel could be modified to evade Pgp, it would provide a potential means for the treatment of brain-related cancers and other CNS diseases.

A series of recognition elements required for Pgp interaction have been described recently by us.[8,9] This analysis identified clusters common to Pgp substrates that consist of hydrogen bond acceptors organized in a defined spatial arrangement, and their relative frequency was correlated with the strength of interaction with Pgp. Two motifs, termed type I and type II, were identified that possess different relative recognition affinities for Pgp. We analyzed paclitaxel (Fig. 1) and identified a strongly and a weakly interacting type II unit, three standard type I units, and one weakly interacting type I unit that consists of an electron-donating heteroatom and a weak π-system in close proximity to one another. The type II unit is considered to have the highest Pgp affinity.

Ojima et al. have demonstrated, using photoaffinity-labeling experiments, that Pgp contains a binding site specific for taxanes.[10] They have also shown that the NCI/ADR-RES (formerly named MCF-7ADR)[11] cell line (a multi-drug resistant breast cancer phenotype known to overexpress Pgp) is very sensitive to substitution of the C10 moiety in 3′-dephenyl-3′-isobutylpaclitaxel analogs,[12] although this effect was not observed when the same modifications were made on paclitaxel.[13] Since the C9 carbonyl-C10 ester motif is a type II unit, we surmised that it might be largely responsible for Pgp interaction. All Pgp inducers carry at least one type II unit and it has been suggested by us that this unit interacts more strongly with the efflux protein.[8] Thus, we reasoned that the chemical modification of this motif through changes of the C10 oxidation state and/or absolute configuration might decrease the affinity of certain taxanes for Pgp. This hypothesis is supported by the recent report that TXD-258 (7-*O*-methyl-10-*O*-methyldocetaxel) is able to cross the BBB.[14] This analog has a C10 –OMe group in place of the acetoxy group usually found at this position in paclitaxel analogs (this alters the C10 type II ester functionality). We have also found that deletion of the C10 ester unit in certain paclitaxel analogs decreases interaction with Pgp in a concentration-dependent manner.[15] In the work presented here, we sought to transform the type II interaction to a weaker type I element via C10 ester hydrolysis or by altering the spatial distance between the oxygen heteroatoms through C10 epimerization in paclitaxel and Taxol C.[16]



Scheme 1. Synthesis and chemical structures of paclitaxel analogs. Reagents and conditions: (a) $(CH_3CH_2CO)_2O$, DMAP, 0 °C; 82%; (b) HF/pyridine, pyridine, 0 °C; 75%.

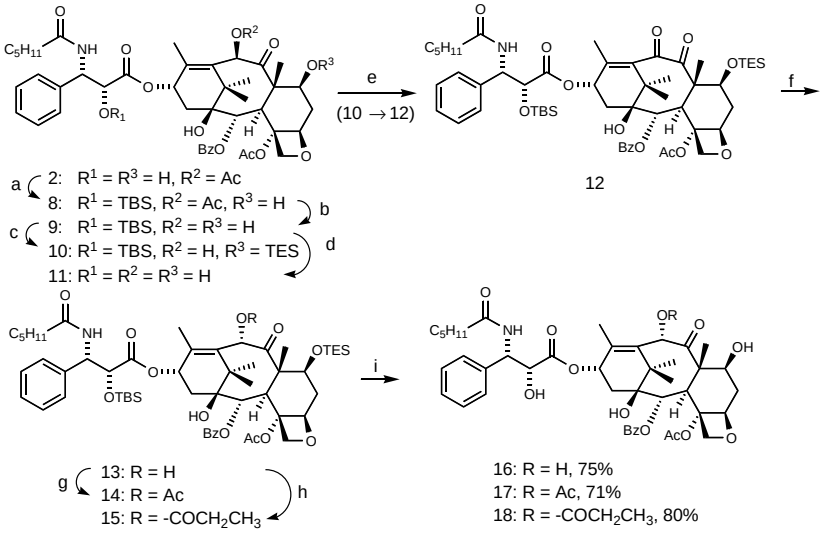
Our group has developed an efficient route to C10 modified taxanes that utilizes a stereoselective hydride addition from the β-face of the baccatin III core to afford the α-C10 epimeric products 10-*epi*-paclitaxel (**5**) and 10-*epi*-10-deacetyl-paclitaxel **6** (shown in Scheme 1).[17] Propionyl analog **4** was prepared similarly by reacting **3** with propionic anhydride, followed by HF-mediated deprotection (Scheme 1).

Selective protection of the C2′ hydroxyl group of Taxol C (**2**) as the silyl ether furnished **8** in good yield (Scheme 2). Hydrazine-mediated cleavage[18] of the C10 acetate provided the C10 hydroxyl analog **9**, which was regioselectively protected at the C7 hydroxyl group with triethylsilyl chloride to afford **10** in excellent yield. Deprotection of the β-hydroxy taxane **9** provided the 10β-deacetyl-Taxol C analog **11** in 84% yield. Dess–Martin periodinane oxidation of the C10 hydroxy group in **10** occurred smoothly resulting in the C9–C10 diketone **12**.

Reduction of **12** using sodium borohydride complexed in diglyme was required to provide sufficient amounts of the α-C10 compound **13**, as solid sodium borohydride gave mostly the C9–C10 diol resulting from reduction of the C9 carbonyl group. Acetylation and propionylation occurred as expected in good yields affording **14** and **15** in 75% and 85%, respectively, using the corresponding anhydrides. Hydrogen fluoride deprotection furnished target compounds **16**, **17**, and **18** in 75%, 71%, and 80% yield, respectively. All compounds displayed spectroscopic properties in agreement with their structures.

Table 1 displays the effective dose ratios [ED_{50}/$ED_{50(paclitaxel)}$] for each compound in a tubulin assembly assay and cytotoxicity studies in the MCF-7 (parental cell line) and NCI/ADR-RES (multi-drug resistant phenotype) cell lines. Our original hypothesis was that the NCI/ADR-RES line would serve as an initial indicator of the Pgp evading potential as this line has been shown to express high levels of MDR-1 and Pgp.[19] Modification of the C10 center did not significantly affect cytotoxicity or tubulin assembly across the range of taxanes. In addition, none of the compounds in Table 1 showed a large increase in cytotoxicity for the multi-drug resistant phenotype. This was unexpected, as we had anticipated significant potency of these compounds in this cell line.

Each analog was evaluated for potential interactions with Pgp relative to the negative (no addition) and positive controls [cyclosporine A (CsA) and paclitaxel, both of which are known Pgp substrates and cause a net increase in cellular rhodamine concentrations] in primary cultures of bovine brain microvessel endothelial cells (BMECs),[20] which we have previously demonstrated to



Scheme 2. Synthesis of Taxol C analogs. Reagents and conditions: (a) TBSCl, imidazole, CH_2Cl_2; 87%; (b) $N_2H_4{\cdot}H_2O$, EtOH; 89%; (c) TESCl, DMAP, CH_2Cl_2; 85%; (d) HF/pyridine, pyridine, 0 °C; 84%; (e) Dess–Martin periodinane, CH_2Cl_2; 99%; (f) $NaBH_4$ (solution in diglyme); 50%; (g) Ac_2O, DMAP, pyridine, 0 °C; 75%; (h) $(CH_3CH_2CO)_2O$, DMAP, pyridine, 0 °C; 86%; (i) HF/pyridine, pyridine, 0 °C.

Table 1. ED_{50} ratios (compound/paclitaxel) for in vitro tubulin assembly and cytotoxicity for compounds **1**–**2**, **4**–**7**, **11**, and **16**–**18**[13]

Compounds	Tubulin assembly	MCF-7	NCI/ADR-RES
1	1	1	1
2	2.0	2.2	0.48
4	4.1	2.6	1.1
5	0.8	0.7	1.3
6	3.2	1.4	0.7
7	2.1	7.5	6.6
11	2.2	4.8	3.8
16	1.6	9.2	3.1
17	0.21	3.2	1.6
18	2.1	3.8	2.9

Paclitaxel has a mean ED_{50} of 3.23 nM ± 1.84 and 1.53 μM ± 1.26 in the MCF-7 and NCI/ADR-RES lines, respectively.

express Pgp.[21] A compound that does not interact, or interacts weakly, with Pgp should not significantly change the rhodamine level relative to the negative control.

The results with paclitaxel (**1**) and Taxol C (**2**) (Fig. 2) demonstrate that compounds containing the β-C10 acetate group strongly interact with Pgp. As is evident, there is a marked loss of interaction when the β-C10 acetate is hydrolyzed (Fig. 2, compounds **7** and **11**) for both series. This is likely due to the conversion of the strongly interacting type II unit to a weaker type I motif. The epimerization of the β-OH to the α configuration does not cause a significant further change in interaction with Pgp in this assay (Fig. 2, compounds **6** and **16**). This decreased interaction with the efflux protein is mostly retained upon acetylation of the α-hydroxyl moiety causing just a slight increase for compound **5** and a decrease for compound **17** in rhodamine accumulation. This is significant as for both series the β-acetate compounds interact appreciably with the Pgp transporter relative to rhodamine. C10 α-substituents reside within the concave 'cup' of the baccatin portion of the taxanes. Thus, it is conceivable that the acetate unit is no longer able to interact with the complementary amino acid residue in the Pgp binding site. Additionally, it is feasible that the epimerization of this unit changes the spatial distance of the outer two electron donors, disrupting or weakening its hydrogen bonding ability. Interestingly, as the size of the α-substituent increases (Fig. 2, compounds **4** and **18**), Pgp interaction is observed at levels near that of the β-acetate compounds or positive controls.

We previously reported that C10-deacetoxypaclitaxel and C10-deacetoxy-C7-deoxypaclitaxel interacted appreciably with Pgp in the rhodamine assay at 10 μM but not at 5 μM.[15] However, C7-deoxypaclitaxel inter-

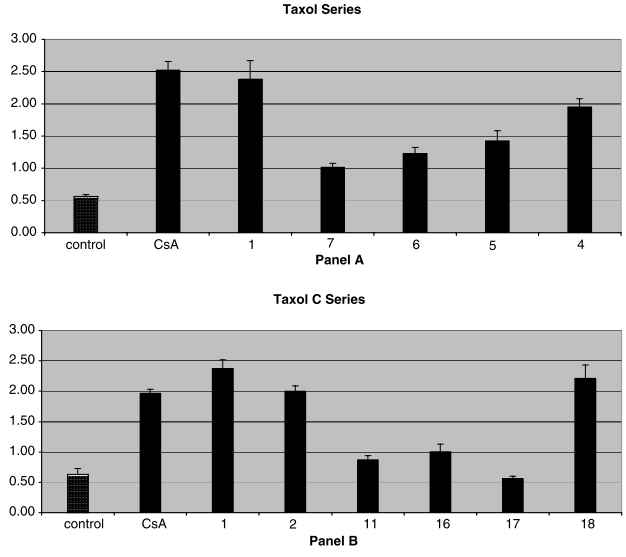


Figure 2. Rhodamine uptake results for compounds **4**–**7** (A, paclitaxel series) and **2**, **11**, and **16**–**18** (B, Taxol C series), in BMECs. Paclitaxel and the derivatives were present at a concentration of 10 μM. The concentrations of cyclosporin A (CsA) and rhodamine were 5 μM.

acted with Pgp at both concentrations. Thus, it seems that the C10 acetate plays a pivotal role in Pgp recognition. It is interesting to note that the compounds presented in this study (**5**–**7**, **11**, **15**, and **17**) seem to do little to rhodamine accumulation even at the 10 μM concentration.

Although the rhodamine assay is an indirect measurement of Pgp interaction, we have previously established it as a viable indicator of relative BBB permeation by comparison to bidirectional permeation data obtained from BMEC monolayers of related compounds.[20] Further studies regarding these compounds in the BMEC permeation assay are underway.

Although paclitaxel has six purported recognition elements, the simple modification of only one of these groups is sufficient to decrease interaction with Pgp. Although we do not yet know the relative contributions of each recognition element in binding to Pgp, it is clear that the element involving C9 and C10 plays a significant role. This is underscored by observations that certain modifications at C10 resulted in compounds with higher activity against Pgp-overexpressing cancer cell lines[12] or with ability to cross the BBB.[14] We did not find a correlation between our compounds' activity against the NCI/ADR-RES cell line and rhodamine uptake in BMECs. The discrepancy suggests the possibility that other efflux systems may be present in the MDR cell line that recognizes these compounds.[22]

In summary, we have described a novel single point chemical modification of the baccatin portion of taxanes that significantly decreases affinity for the P-glycoprotein transporter system. Additionally, we have begun to elucidate a preliminary structure–activity relationship for Pgp and taxanes with respect to the recognition elements postulated. The concept and data presented illustrate that it might be possible to deliver chemically modified taxanes across the blood–brain barrier. This is in contrast to current strategies for brain delivery of non-CNS permeable drugs, including Pgp inhibition,[5a] and BBB disruption.[1] Long-term application of this research has the potential to lead to effective agents for CNS cancer treatment.

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