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Synthesis, Modeling, and Anti-Tubulin Activity of a D-Seco Paclitaxel Analogue

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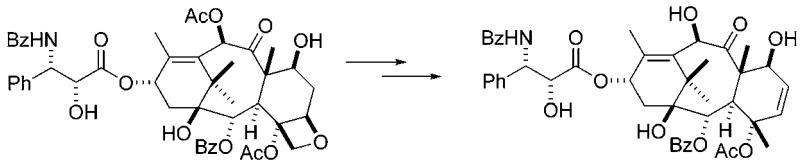
ABSTRACT



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We have previously described a model of paclitaxel–microtubule binding that led to the prediction that analogues of paclitaxel lacking any D ring could stabilize microtubules as well as paclitaxel if the substituent present at C4 did not have unfavorable steric interactions with the binding pocket. We report the synthesis of a 4-methyl paclitaxel analogue, compound 1, which bears this prediction out. Compound 1 is as potent as paclitaxel at microtubule stabilization in vitro; however, it has only about one-four-hundredth the cytotoxicity of paclitaxel.

As part of our research into the oft-discussed role of the oxetane ring on the biological activity of the anticancer agent paclitaxel,[1] we recently reported the synthesis of a series of D-seco analogues, with an acetoxy group at C20 (see compound **2**).[2] None of the compounds showed activity in cytotoxicity and tubulin assembly assays. Following the receptor models developed by one of us,[3,4] we ascribed the lack of activity to an unfavorable steric effect between the protein and the C20 acetoxy group.[2] Consistent with predictions[1] based on this viewpoint, Dubois and co-workers demonstrated that a docetaxel analogue with only a carbocyclic ring (cyclopropyl) at this position (**3**), stabilizes microtubules, with bioactivity in the microtubule assay being reduced by only half relative to the parent compound.[5] The oxygen in the oxetane ring is therefore not a requirement for bioactivity in these compounds. With the aim of testing whether any ring is necessary at this position, we designed and synthesized compound **1**, in which C20 consists only of a methyl group. The minireceptor model predicted that this compound would stabilize microtubules (MTs), and indeed it does: it is equipotent with paclitaxel. However, it has very low activity in cytotoxicity assays, which is also the case

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(1) Wang, M.; Cornett, B.; Nettles, J.; Liotta, D. C.; Snyder, J. P. *J. Org. Chem.* **2000**, *65*, 1059−1068.

(2) Barboni, L.; Datta, A.; Dutta, D.; Georg, G. I.; Vander Velde, D. G.; Himes, R. H.; Wang, M.; Snyder, J. P. *J. Org. Chem.* **2001**, *66*, 3321−3329.

(3) Wang, M.; Xia, X.; Kim, Y.; Hwang, D.; Jansen, J. M.; Botta, M.; Liotta, D. C.; Snyder, J. P. *Org. Lett.* **1999**, *1*, 43−46.

(4) Snyder, J. P.; Nettles, J. H.; Cornett, B.; Downing, K. N.; Nogales, E. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 5312−5316.

(5) Dubois, J.; Thoret, S.; Guéritte, F.; Guénard, D. *Tetrahedron Lett.* **2000**, *41*, 3331−3334.

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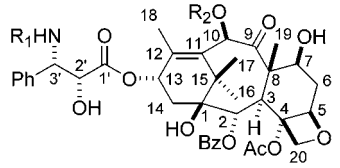
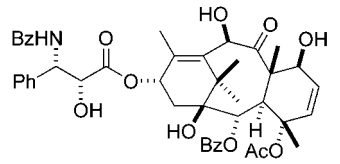
for cyclopropyl **3**.[6] The closely related compound that lacks the 20-methyl group has dramatically reduced microtubule-stabilizing activity as well as low cytotoxicity.[7]



Paclitaxel: R_1 = Bz; R_2 = Ac

Docetaxel: R_1 = Boc; R_2 = H



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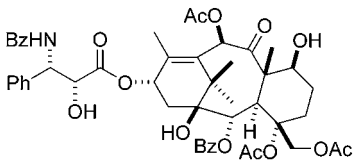

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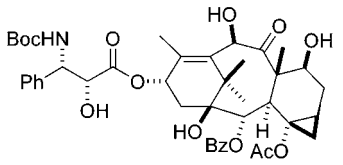

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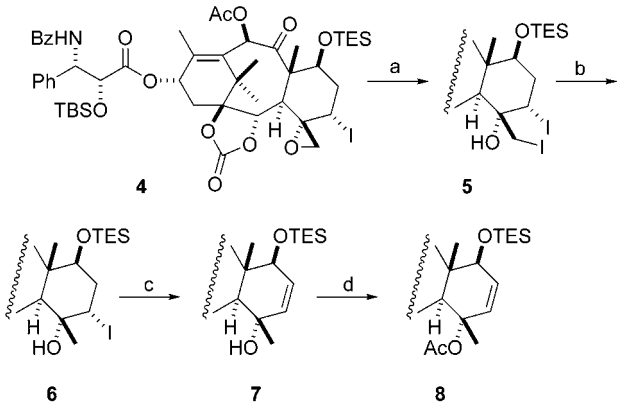
Our strategy for the synthesis of **1** involves the introduction of an easily removable group at C20 by nucleophilic ring opening of the C4−C20 oxirane intermediate **4**, which was prepared from paclitaxel using a reported protocol.[8] For this purpose, the introduction of a halide at C20 as reported by Dubois and co-workers[5] was selected as the most direct procedure, since halogen atoms are easily removed by hydrogenolysis.[9] Accordingly, compound **4** was treated with $(Bu^t)_4NI$ in the presence of a Lewis acid ($MgBr_2 \cdot OEt_2$) to form the 4-hydroxy-20-iodo analogue **5**[5] (87%, Scheme 1).

Hydrogenolysis of the C20 and C5 iodo groups was first attempted using a Pd/C catalyst,[9] but only starting material was recovered. After some experimentation, it was found that treatment of **5** with H_2 (balloon pressure) and Adams catalyst (PtO_2) gave compound **6** (79%), in which only the primary halogen was removed. This unusual pattern of reactivity may be due to steric hindrance at C5 by the substituents at C4. Since attempts to remove both iodines

Scheme 1[a]

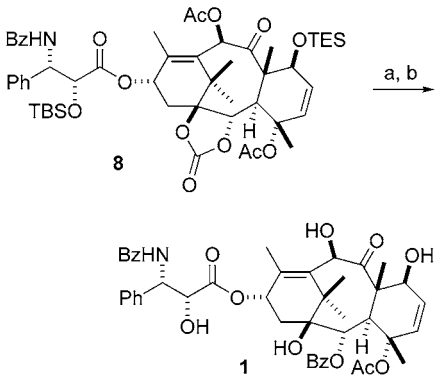


[a] Reagents and conditions: (a) $(Bu^t)_4NI$, $MgBr_2 \cdot OEt_2$, CH_2Cl_2, rt, 8 h (87%); (b) H_2, PtO_2, EtOAc, rt, 48 h (84%); (c) $NaBH_3CN$, DMPU, 70 °C, 15 h (73%); (d) Ac_2O, DMAP, toluene, 80 °C, 48 h (60%).

using higher H_2 pressure were unsuccessful, we treated the 5α iodine analogue **6** with $NaBH_3CN$ in DMPU[10] and obtained the dehydrohalogenation product **7** (73%).

Acetylation of **7** gave **8** (60%), which was treated with an excess of phenyllithium. After workup, the reaction mixture, consisting of multiple products that were not further characterized, was treated with HF-pyridine, affording compound **1** in 3% yield (Scheme 2).

Scheme 2[a]

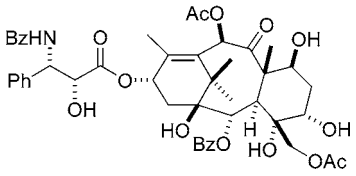


[a] Reagents and conditions: (a) PhLi, THF, −78 °C, 3 h; (b) HF/py, THF, 0 °C, 4 h (3%).

Our previous work on D-seco compounds led to the conclusion that the "conformational lock" on paclitaxel, originally ascribed to the oxetane ring, was more likely due to the presence of the 4-acetyl group.[2] Removal of the 4-acetyl leads to changes in the shape of the diterpene, as reflected in some key *J*-couplings, and also to loss of microtubule-stabilizing activity. It is instructive to compare

(6) Dubois, J.; Guéritte, F. Private communucation.
(7) Deka, V.; Dubois, J.; Thoret, S.; Guéritte, F.; Guénard, D. *Org Lett.* **2003**, ASAP.
(8) Gunatilaka, A. A. L.; Ramdayal, F. D.; Sarragiotto, M. H.; Kingston, D. G. I.; Sackett, D. L.; Hamel, E. *J. Org. Chem.* **1999**, *64*, 2694−2703.
(9) Pinder, A. R. *Synthesis* **1980**, 425−452.
(10) Hutchins, R. O.; Kandasamy, D.; Maryanoff, C. A.; Masilamani, D.; Maryanoff, B. E. *J. Org. Chem.* **1977**, *42*, 82−91.

1 to paclitaxel and to another compound we have previously analyzed, 20-acetoxy-4-deacetyl-5-epi-20*O*-secopaclitaxel (**9**), which lacks both the oxetane ring and the 4-acetate.[11]



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Two important descriptors of the overall shape of the diterpene are the coupling constant between H2 and H3, which serves as a reporter of the dihedral angle along the bottom portion of the B ring, and the coupling between H13 and H14α and 14β, which serves a similar function for the A ring. In paclitaxel, J_{2-3} is 7.2 Hz; in **1**, it is 7.4 Hz (identical within experimental error). In paclitaxel and in **1**, H13 has nearly equal couplings to both protons at C14, and the signal is a broad triplet. In **9**, J_{2-3} is 5.3 Hz and H13 is a doublet of doublets with *J*-couplings of 4.4 and 10.3 Hz. Thus, in paclitaxel, the A ring sustains a boat conformation, while in **9**, it adopts an envelope conformation. The main point of difference between **1** and paclitaxel is the 5−6 double bond, which leads to a flattening of the normal chair conformation of the C ring and may also provide some conformational rigidity. In light of the overall conformational similarity to paclitaxel, this is evidently a local perturbation.

We have also previously reported on solvent-dependent NOE and *J*-couplings for paclitaxel, which were interpreted in terms of a fast conformational equilibrium between multiple families of conformations, two "collapsed" and one extended.[12] The solution NMR data indicate that the same is true for compound **1**. In chloroform, the $J_{2'-3'}$ coupling constant is 2.4 Hz, very similar to that of paclitaxel (2.6 Hz). In DMSO, $J_{2'-3'}$ increases to 6.3 Hz (for paclitaxel, 7.0 Hz), indicating an increase in the population of a "polar collapsed" conformation.

The D-seco compound **1** is equipotent with paclitaxel in promoting the polymerization of tubulin to MTs in our in vitro assay.[13] Electron microscopy was used to verify that the products of the assembly reaction were microtubules. However, in a cytotoxicity assay employing the MCF-7 cell line, it had about 0.25% of the activity of paclitaxel. (The ED_{50} for paclitaxel in these actively dividing cells is 4 nM; for **1**, the ED_{50} was 1.6 μM.) The docetaxel cyclopropyl analogue **3**, which similarly lacks an oxygen in the D ring, also loses much more cytotoxicity than microtubule binding. In a KB cell line, **3** was found to be about 1% as cytotoxic as paclitaxel.[6]

(11) Boge, T. C.; Hepperle, M.; Vander Velde, D. G.; Gunn, C. W.; Grunewald, G. L.; Georg, G. I. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 3041−3046.

(12) (a) Snyder, J. P.; Nevins, N.; Cicero, D. O,; Jansen, J. *J. Am. Chem. Soc.* **2000**, *122*, 724−725. (b) Vander Velde, D. G.; Georg, G. I.; Grunewald, G. L.; Gunn, C. W.; Mitscher, L. A. *J. Am. Chem. Soc.* **1993**, *113*, 11650−11651.

(13) Liu, Y.; Ali, S. Y.; Boge, T. C.; Georg, G. I.; Victory, S.; Zygmunt, J.; Marquez, R. T.; Himes, R. H. *Combi. Chem. High Thr. Scr.* **2002**, *5*, 39−48.

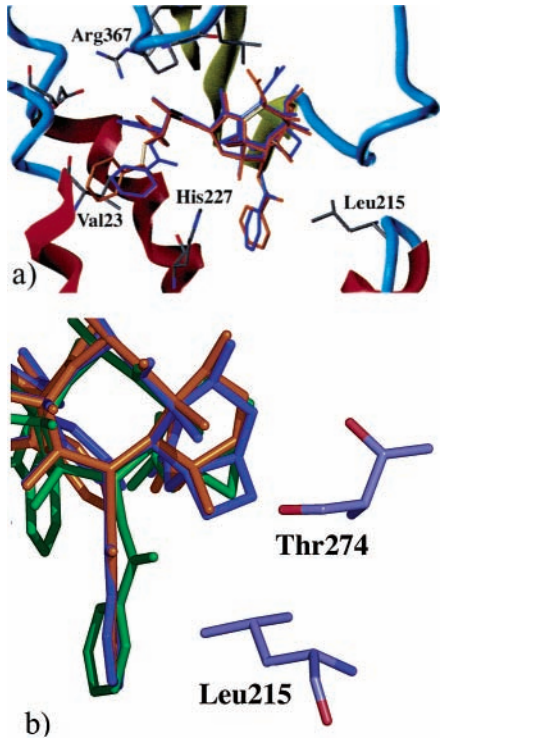

Figure 1. (a) Superposition of paclitaxel (blue) and D-seco **1** (orange) in the electron crystallographic paclitaxel/tublin model[4] resulting from flexible docking of **1** into the binding site. The C20 carbons of the two analogues are juxtaposed and in close contact with Leu215. (b) Overlap of paclitaxel, **1**, and **3** (green) in the subsite around ring-D.

Two computational approaches have led to the prediction that the D-seco analogue **1** is expected to be highly active in the microtubule stabilization assay. First, we performed a flexible docking experiment with **1** in the paclitaxel−tubulin model derived from electron crystallography (EC).[3] The DOCK procedure[14] delivered a binding mode nearly identical to that of paclitaxel as pictured in Figure 1. Bound to the protein, the molecule adopts the T-conformation in common with paclitaxel and analogues.[3] Not surprisingly, the rigid tricyclic A−C ring core facilitates the alignment of the C20 methyl group in **1** and the corresponding methylene carbon in the oxetane D-ring. As previously noted,[1] the hydrogens on both C20 carbons appear to experience close approach with the methyls of Leu215 (r(H- - -H) = 2.6−3.1 Å). While the binding forms of **1** and paclitaxel are not those observed as major contributors to the solution conformation, the geometric similarity in solution as documented in the previous section is matched by the similarities in the model binding sites. In an earlier study, we observed the T-conformation as a low-population (<10%) torsional isomer in $CDCl_3$.[12a] Given the overall resemblance of paclitaxel and **1** in chloroform, it can be safely projected that D-seco **1** enjoys low-population residence in the T-shape in condensed media as well.

(14) Ewing, T. J. A.; Kuntz, E. E. *J. Comput. Chem.* **1997**, *18*, 1175−1189; cf. http://www.cmpharm.ucsf.edu/kuntz/kuntz.html.

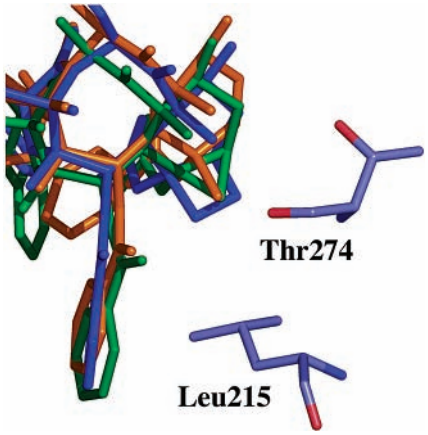

Figure 2. Superposition of paclitaxel (blue), D-seco **1** (orange), and cyclopropyl **3** (green) in the third-generation minireceptor; the B, C and D rings of the taxanes are illustrated along with two minireceptor residues in the vicinity of ring-D.

The second assessment of the binding capacity of D-seco **1** took advantage of the 3D-QSAR minireceptor approach. Prior to our work devoted to determination of the EC-derived conformation of paclitaxel bound to tubulin,[3] we developed a second-generation minireceptor model based on the "non-polar-collapsed" conformation of the ligand and a compatible conformation of epothilone A.[15] As a result of unsuccessful attempts to accommodate the activity of various C20 oxygenated D-seco taxanes with this model,[2] it became clear that a minireceptor more faithful to the protein structure was required. Consequently, a third-generation model based on T-paclitaxel and the evolving D-seco SAR was subsequently developed[16] within the context of the PrGen package[17] and employed here and in the previous work[2] to examine various D-ring-ruptured paclitaxel analogues. Compound **1** surfaced as a structure that was both synthetically accessible and endowed with highly favorable predicted tubulin-polymerization capacity. The D-ring region of our latest minireceptor is portrayed in Figure 2 with the superimposed structures of paclitaxel, **1**, and **3** in their optimized locations.

With paclitaxel estimated binding affinity as a yardstick (8.9×10^{-6}), the corresponding affinities of **1** and **3** were calculated to be 2.1×10^{-7} and 4.5×10^{-7}, respectively. Both are predicted to be somewhat more effective as microtubule-stabilizing agents by comparison to paclitaxel. The second-generation minireceptor model yielded the same result for **3**[1] prior to its reported synthesis and testing,[6] the latter demonstrating the compound to be very similar to paclitaxel in its microtubule binding. The origin of the overestimation of the binding capacity of **1** is illustrated by comparing Figures 1b and 2. Optimization of the structure in the protein-truncated minireceptor causes the C20 methyl in **1** to shift approximately 1 Å away from Leu215, thereby reducing the steric compression implied by the full protein model (Figure 1).

Contrary to long-held dogma, the results for cyclopropane **3** demonstrated for the first time that neither the intact oxetane ring nor an oxygen on C-5 are necessary for taxane efficacy with tubulin.[1,5] The microtubule-stabilizing prediction for D-seco **1**, its synthesis, and subsequent demonstration as a potent stabilizer of microtubles constitute another important exception to the "oxetane rule." Obviously, the D ring is not necessary for maintaining the conformational properties of the taxane diterpene so long as its rupture or removal is appropriately compensated elsewhere in the molecule. The situation is reminiscent of the unusual bioactivity of C2-*meta*-azido baccatin, a molecule lacking the otherwise "essential" C13 taxane side chain.[18] Unlike the latter case, however, the full paclitaxel microtubule assembly activity is retained by D-ring-free **1**.

The unexpectedly low cytotoxicity of **1** deserves comment. Taxane cytotoxicity reflects other factors besides microtubule binding, including water solubility, partitioning into cells, and active efflux, among others. We have preliminary data to suggest that **1** does indeed partition into cells about as efficiently as paclitaxel, and in a future publication, we will elaborate on its biological effects and its potential utility.

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Supporting Information Available: Experimental procedures and spectral data for compounds **1** and **5**−**8**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(15) Wang, M.; Xia, X.; Kim, Y.; Hwang, D.; Jansen, J. M.; Botta, M.; Liotta, D. C.; Snyder, J. P. *Org. Lett.* **1999**, *1*, 43−46.
(16) Wang, M.; Lakdawala, A.; Snyder, J. P. Unpublished results.
(17) (a) Vedani, A.; Zbinden, P.; Snyder, J. P.; Greenidge, P. A. *J. Am. Chem. Soc.* **1995**, *117*, 4987−4994. (b) Zbinden, P.; Dobler, M.; Folkers, G.; Vedani, A. *Quant. Struct.-Act. Relat.* **1998**, *17*, 122−130.
(18) He, L.; Jagtap, P. G.; Kingston, D. G.; Shen, H. J.; Orr, G. A.; Horwitz, S. B. *Biochemistry* **2000**, *39*, 3972−3978.