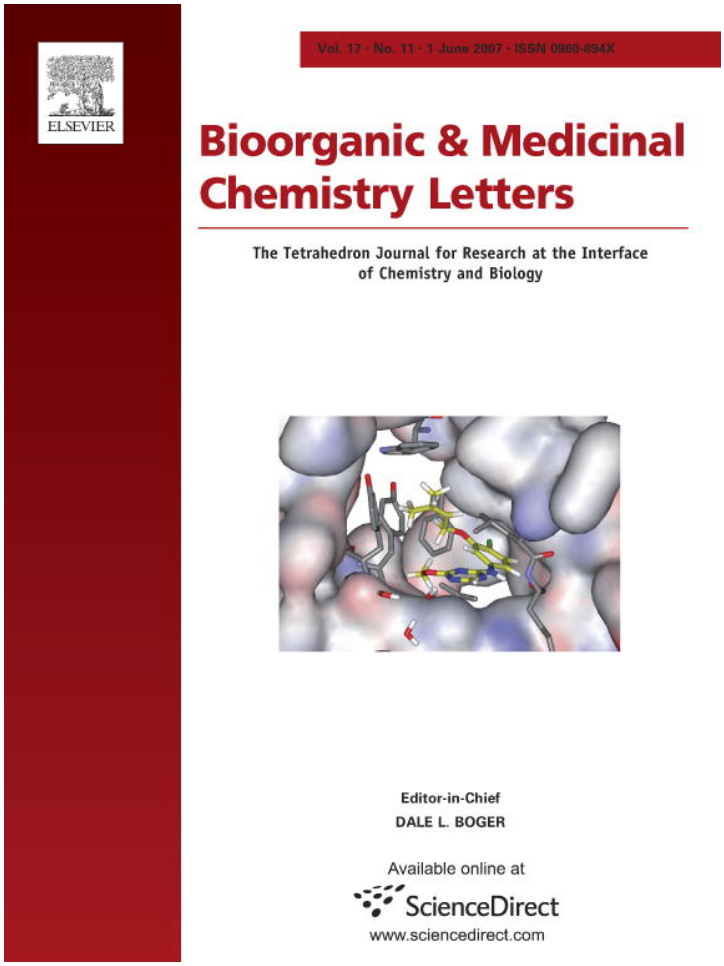

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Synthesis, biological evaluations, and tubulin binding poses of C-2α sulfur linked taxol analogues

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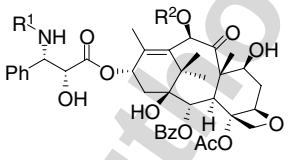
Abstract—In combination with chemical modifications, bioassays, and computational simulation techniques, C-2 benzoylthio, and benzylthio taxoids were synthesized, biologically evaluated, and their binding conformations rationalized, in order to probe the interaction of taxane molecule with β-tubulin.

Chemical and biological evaluation of the antitumor agent paclitaxel (PTX, Taxol®, **1**) has been a mainstream focus for natural product research since the mid 1990s. The subject remains an active field in the attempt to overcome problems of resistance[1a,b] and toxicity.[1c] Both PTX[2] and its analogue docetaxel **2** (DTX)[3a,b] are currently used clinically for the treatment of ovarian, breast, and non-small lung cancers, especially at the recurrent or metastatic stages.



1: R^1 = Bz, R^2 = Ac **2:** R^1 = Boc, R^2 = H

Many other antitubulin agents possessing a similar mechanism of action are also found, including the most widely studied classes such as epothilones, sarcodictyins, eleutherobin, discodermolide, and laulimalide.[4] Compounds that prevent the formation of microtubules but likewise perturb the tubulin-microtubule equilibrium (e.g., colchicine congeners, vinca alkaloids,[5a] hemiasterlins[5b], and noscapine analogues[5c]) have also contributed to the rather large family of tubulin-based antineoplastic agents in recent years.

With respect to PTX, extensive structure-activity relationship (SAR) studies over the past two decades have led to several generalizations. Perhaps the most crucial one concerns three key side chains on the southern hemisphere of the molecule: the C-13 *N*-benzoyl phenylisoserne, the C-2 benzoate, and the C-4 acetate moieties. Both in terms of atomic constitution and molecular conformation, all are critical to taxane tubulin binding and cytotoxicity.[6a,b] At the conformational level, although there are upwards of a dozen different forms of PTX conformers in solution,[7] three extremes have at times been proposed as that bound to β-tubulin: the polar, nonpolar, and T-Taxol conformations.[8] Only the latter has led to the design and synthesis of tethered taxanes that show potency superior to PTX in a combination of microtubule stabilization and cytotoxicity assays.[9]

SAR studies at the C-2 position have shown that both the nature and stereochemistry of the 2-benzoyl group

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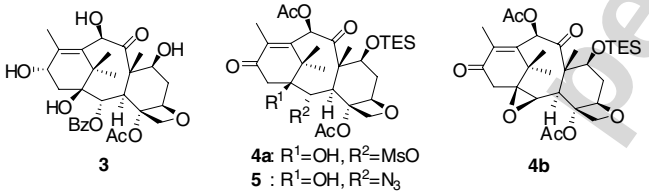
in PTX derivatives are determinants of activity.[10,11] On the other hand, many paclitaxel analogues with aroyl[12] or alkyl[13] ester groups at the C-2 position have been reported with modest to highly potent cytotoxicity.

We also undertook the preparation of a series of C2 heteroatom-substituted taxoids to probe the PTX C-2 structural requirements. Based on the methodology reported in our first communication,[14a] we were able to develop a cytotoxicity SAR for the 2-amido analogues,[14b] its C-10 modified analogues,[14c] and 2-debenzoyloxy-2α-phenylthio 10-acetyl docetaxel[14d] whose inactivity can be attributed to its shorter chain length at C-2 compared with benzoate.

Although a photoaffinity labeling experiment indicated that C-2 substituents are close to residues 217–231 of β-tubulin[15] and X-ray crystallographic data gave the location of β-tubulin and its taxane ligand,[18] the binding conformation as well as the residues in β-tubulin and functional groups in taxane involved in binding process cannot be determined with precision.

Here we report preparation of the first PTX analogues bearing 2α-benzoylthio and benzylthio moieties designed on the basis of isostere replacement principle, and their initial SARs as well. Their conformations correlated with their binding affinity to β-tubulin are also explored.



Following a known procedure,[14a] 10-DAB **3** was converted into the key epoxide intermediate **4b**. All reactions proceeded in satisfactory yields from 88–97%. Epoxide **4b** exists as an inseparable mixture containing 2-mesylate **4a** (ca. 2:1). Treatment of the **4a** and **4b** mixture with NaN_3 afforded the double S_N2 product 2-azido baccatin **5** in ca. 85% yield, implying that **4a** was converted to **4b** during the course of the reaction.

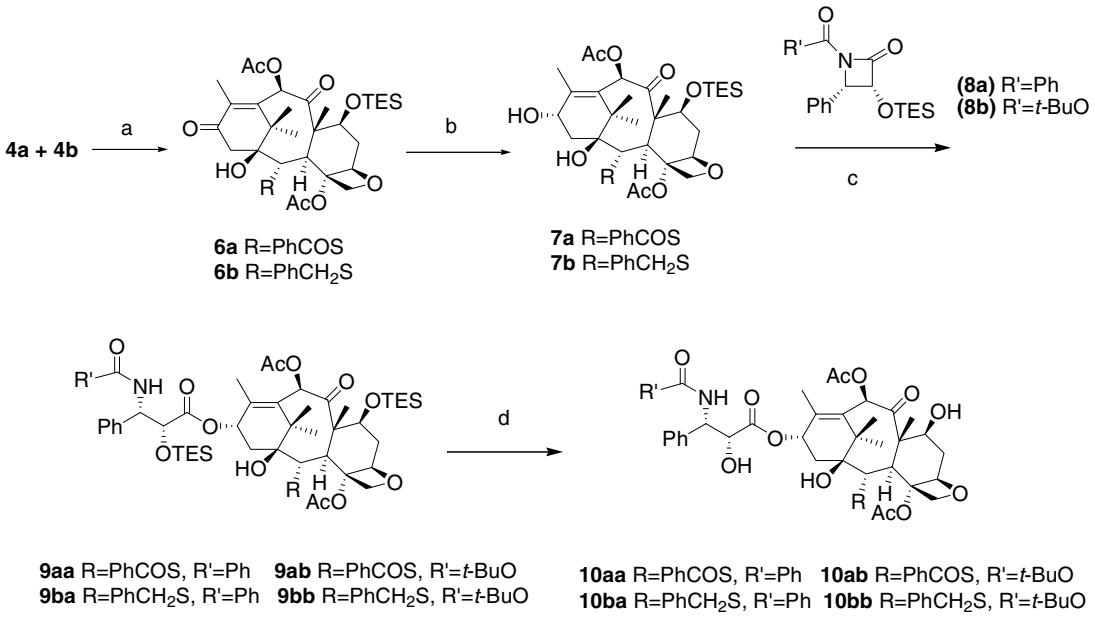
Following the same strategy, the mixture of **4a** and **4b** was treated with sodium thiobenzoate to furnish **6a** in 56% yield. The ^1H shifts for H-2 in **6a** are almost identical to the 2-benzoate counterpart, while H-3 is shifted slightly downfield by 0.2 ppm. The latter reflects a modified environment around H-3 arising from changes in the C-2 thioester geometry (bond lengths and angles) by comparison with the corresponding ester. Subsequent reduction of **6a**–**7a** (80%) and incorporation of the C-13 side chains with enantiopureβ-lactams **8a**–**b** afforded products **9aa** and **9ab**, which were then deprotected at C-2′ to the desired 2-PhCOS analogues **10aa** (PTX side chain) and **10ab** (DTX side chain) in 42–44% yields over two steps (Scheme 1). It is interesting to note that the H-13 chemical shifts in **10aa** and **10ab** appear at δ4.9, an upfield shift of 1.2 ppm by comparison with the C-2 benzoates in **1** and **2**. In addition, the quaternary carbons at 198 ppm in the ^{13}C NMR of **10aa** and **10ab** were attributed to C-1′ after careful assignment with 1D and 2D NMR techniques. These observations fall outside expectations since taxoid H-13 and C-1′ resonances (C-13 esters or amides[16]) to date have been observed at 5.5–6.2 ppm and 165–175 ppm, respectively. This should be due to conformational distortion after the introduction of C-2 thioester.

C-2 sulfide baccatin **6b** can also be obtained by treatment of the mixture of **4a** and **4b** with sodium benzylsulfide at room temperature in 83% yield. The most significant changes in the ^1H NMR for **6b** are found at H-2 and H-3. That the protons are shifted upfield by 0.3 and 1.5 ppm, respectively, can be attributed to the loss of the C-2 carbonyl group. Following reduction, coupling of side chains, and deprotection reactions, the final products **10ba** and**10bb** were afforded (Scheme 1).

Next, we determined the free energy changes of binding for all four S-linked taxoids **10aa**–**10bb** at 35 °C as −28 to −30 KJ/mol. This 28–35% reduction in ΔG for binding corresponds to 2000–10000 times loss of binding affinity. The same compounds were subjected to MTT-determined cytotoxicities and found to be much less active in both sensitive and MDR tumor cell lines. In agreement with the potency difference between PTX (**1**) and DTX (**2**), the taxoids **10aa** and **10ba** with a PTX side chain are 3–5 fold less active than **10ab** and **10bb** with the DTX side chain. But there appeared to be no obvious difference in the activity between 2-PhCOS and 2-$PhCH_2S$ series (Table 1).

In order to explain the low activity of those taxanes, the ligand-tubulin interaction was analyzed with molecular dynamics based on T-conformation.

The diminutive binding affinities and cytotoxicities of **10** can be attributed to the alteration of local geometry around the sulfur atoms by comparison with the classic C-2 benzoyl ester. In the latter case, the C-2 phenyl ring of PTX encapsulated by tubulin in the electron crystallographic structure[17] is bounded on three sides in a rather narrow pocket. The opposite faces of the ring are within van der Waals contact of His227 and Leu215, while the *para*-position is in equally short contact with the protein backbone at Asp224. Extension of the C-2 substituent either perpendicular to the faces of the ring or further from the baccatin core can be predicted to create one or more steric clashes. To test the idea that the latter distance increment could cause a steric problem for series **10**, thioester **10aa** was constructed by an O to S modification of T-Taxol, optimized with the MMFF/GBSA force field[18], and docked into β-tubulin by superposing the taxane on the location of the original PTX ligand. To relax the protein-ligand complex, 20 °K molecular dynamics was performed for the thio-PTX ligand and nearby side chains in a 10 Å sphere around the binding site. Once unfavorable steric



Scheme 1. Synthesis of C2 S-linked taxoids **10aa–bb**. Reagents and conditions: (a) RNa, DMF, r.t; (b) $NaBH_4$, MeOH–THF, −15 °C; (c) LHMDS, THF, −50 → −40 °C; (d) HF–Py, 0 °C → r.t.

Table 1. Cytotoxicity and binding data for taxoids **10**

Compound	R	R′	IC_{50} (μM)[a,b]		ΔG (KJ/mol)[c]
			A2780	A2780/AD	
10ab	PhCOS	Boc	2.4 ± 1.0	6.96 ± 0.67 (2.9)	−30.1 ± 0.6
10aa	PhCOS	Bz	11.7 ± 0.7	27 ± 7 (2.3)	−28.4 ± 1.1
10bb	$PhCH_2S$	Boc	3.5 ± 1.4	8.3 ± 2.7 (2.3)	−29.9 ± 0.7
10ba	$PhCH_2S$	Bz	9.9 ± 1.0	9.4 ± 1.7 (0.94)	−28.6 ± 0.1
PTX(**1**)	PhCOO	Bz	0.0016 ± 0.0002	0.92 ± 0.13 (575)	−42.1 ± 0.2

[a] IC_{50} values in μM were determined after two days exposure to drugs using the MTT cell proliferation assay. Data are means ± SE of four independent experiments.

[b] The numbers in parentheses are the calculated relative resistance obtained by dividing the IC_{50} value of the resistant line by the IC_{50} value of the parental line.

[c] Free energy changes derived from equilibrium binding constants of the ligands to microtubules at 35 °C ($-\Delta G_{0app} = RT\ln K_{(binding)}$).

contacts were removed, the temperature was slowly increased to 300 °K and the system allowed to reach a stable state. At this point, the entire taxane molecule had risen out of the binding site by 1–2 Å, and the terminal C-2 phenyl ring had slipped from the tight His/Leu/Asp sub-pocket to be repositioned in the first water shell around the protein.

A similar set of calculations for thioether **10ba** causes a comparable extrusion of the molecule from the binding pocket. In this case, however, the saturated C-2 side chain deviates from planarity and causes a significant rearrangement of the surrounding residues. Of particular note, the terminal phenyl ring is now perpendicular to His227 and located in a pocket opened by the movement of the Leu217 and Leu215 residues. Figure 1 depicts the final conformation of the C2–S–CH_2–Ph moiety and the displacement of the baccatin core by comparison with PTX.

In conclusion, C2 S-linked paclitaxel analogues are found to be much less active in both tubulin binding ability and cytotoxicity, although for different reasons as revealed by molecular simulations. It is noteworthy

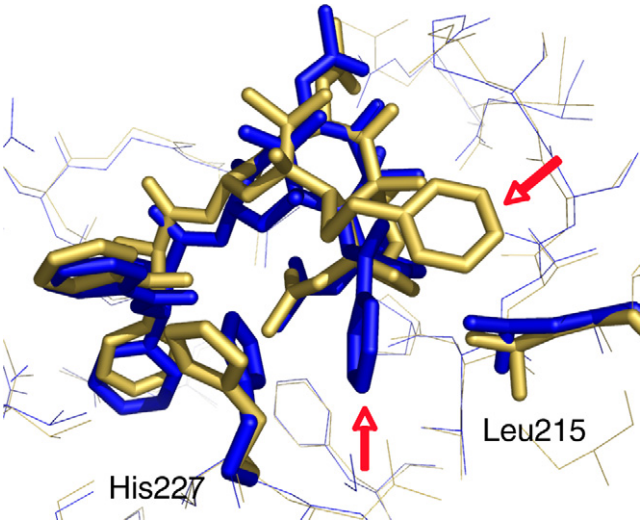


Figure 1. A superposition of PTX (blue) and thioether **10ba** (light gold) in the β-tubulin taxane binding site. For PTX, the C-2 phenyl is sandwiched between His227 and Leu215 (red arrow). Compound **10ba**'s C-2 SCH_2Ph group is predicted to adopt a staggered conformation that relocates the terminal phenyl upward (red arrow) while causing His227 to shift to the left (See text and supplement for computational details).

that T-Taxol conformation has been proven[9,19,20] to be a good predictor for the tubulin binding/bioactivity of taxanes, and here it gives another example for its powerfulness.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2007.03.026.

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