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A Unified and Quantitative Receptor Model for the Microtubule Binding of Paclitaxel and Epothilone

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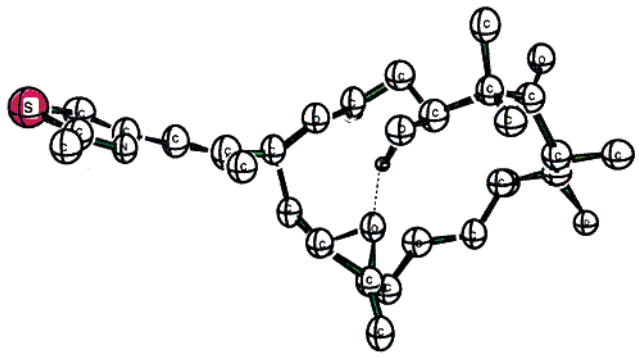
ABSTRACT



Paclitaxel and epothilone represent the two major classes of antimicrotubule agents that promote tubulin polymerization and, presumably, mitotic arrest during cell division. A common minireceptor binding site model at *β*-tubulin has been constructed for these structurally divergent compounds. Utilizing 20 amino acids identified in photoaffinity labeling experiments, the 3-D model correlates measured and predicted K_i's with $r = 0.99$ and rms($\Delta G_{calc} - \Delta G_{exp}$) = 0.2 kcal/mol. In addition, the model predicts the affinity of compounds not used in the training set and explains much of the SAR for the paclitaxel and epothilone families.

The capability of paclitaxel (paclitaxel) to treat a number of drug refractory cancers has been related to its unique mechanism of action. Upon binding to microtubules, the drug is believed to promote their assembly and stability, and thereby interfere with cell division.[1] Epothilone A and B mimic the biological effects of paclitaxel both in vitro and in cultured tumor cell lines,[2−4] while evidencing both apoptosis[2b] and a considerably enhanced toxicity against P-glycoprotein expressing multiple drug resistant cells.[4] The recent X-ray crystal structure[2a] of epothilone B quickly stimulated the total synthesis of natural compounds and a stunning number of potent analogues.[5]

The biosimilarity of paclitaxel and the epothilones is underscored by the observation that the 16-membered macrolides are competitive inhibitors of [^{3}H]paclitaxel binding. The suggestion that the compounds bind to the same

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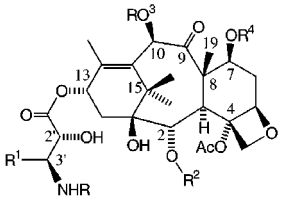
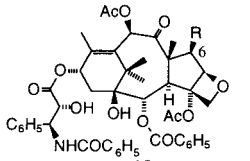


1, R = R^2 = COPh, R^1 = Ph, R^3 = COMe, R^4 = H, taxol
2, R = CO_2tBu, R^1 = Ph, R^2 = COPh, R^3 = R^4 = H, taxotere
3(*2′S,3′R*), R = R^2 = COPh, R^1 = Ph, R^3 = R^4 = H
4, R = COPh, R^1 = C_6H_{11}, R^2 = COPh, R^3 = COMe, R^4 = H
5, R = R^2 = COPh, R^1 = Ph, R^4 = H, C-10 H
6, R = R^2 = COPh, R^1 = Ph, R^3 = COMe, R^4 = H, C-9 OH
7, R = R^2 = COPh, R^1 = Ph, R^3 = COMe, R^4 = H, C-4 OH
8, R = COPh, R^1 = Ph, R^4 =H, R^3 = COMe, C-2 H
9, R = R^2 = COPh, R^1 = Ph, R^3 = COMe, R^4 = H, 2-epi-OBz
10, R = R^2 = COPh, R^1 = Ph, R^3 = R^4 = $COCH_2NH_2$
11, R = COPh, R^1 = Ph, R^2 = CH_2(4-CF_3)Ph, R^3 = COMe, R^4 = H
12, taxol with C-13 side chain: $OCOCH_2CH_3$

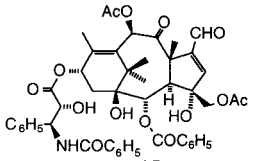
microtubule binding site[4] raises the question as to how the widely divergent structural classes interact with the tubulin protein at the molecular level. We have addressed this issue by constructing a receptor model of the paclitaxel−tubulin binding site, docking suitable epothilone conformers into it, and developing a semiquantitative correlation of ligand binding.

Initiation of the study required knowledge of a highly probable bioactive conformation for paclitaxel and its analogues. Results from NMR investigations coupled to conformational analysis has led to two extreme models for paclitaxel−tubulin binding. One is derived from studies in $CDCl_3$ and locates the C-2 benzoyl phenyl in a hydrophobic cluster with either *tert*-butyl (taxotere) or phenyl (paclitaxel) at the terminus the C-3′ NHR moiety.[6] The X-ray crystal structure of taxotere[7] (docetaxel) illustrates a related motif. The second model follows from C-2 Bz/C-3′ Ph phenyl clustering in D_2O/[D_6]DMSO or CH_3OD/D_2O;[8] a conformational profile is found likewise in the X-ray structure of paclitaxel.[9] Adopting the hydrophobic solvent hypothesis ($CDCl_3$),[10,11] we used a superposition of three highly active paclitaxel analogues ((2′*R*,3′*S*)-paclitaxel, (2′*S*,3′*R*)-paclitaxel,[12] and taxotere) energetically minimized in Macromodel[13] (AMBER*/H_2O/GBSA) to construct a first-round paclitaxel−tubulin minireceptor consisting of 20 amino acids.[14,15] Both baccatin core and side chains in paclitaxel contribute to the model. The receptor residues selected[16] were taken from two patches of β-tubulin in contact with the ligand as determined by photoaffinity labeling.

For epothilone B (**17**), a 10 000 step Monte Carlo conformational search was performed with AMBER*/H_2O/GBSA leading to a global minimum found seven times. Within a 5 kcal mol^{-1} energy window, 568 unique conformations were obtained. The X-ray crystal structure[2a] was located at 7.4 kcal mol^{-1} above the global minimum. Despite the modest agreement between measured ([D_6]DMSO) and calculated coupling constants (X-ray structure) for epothilone B,[2a] it is most likely that the solution structure is a weighted average of an undetermined number of low-energy conformations.



13, R = OH
14, R= OAc



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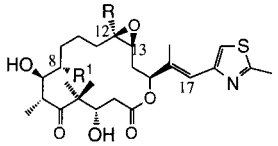
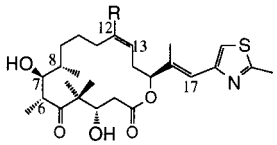


16, R = H, R^1 = Me, Epothilone A
17, R = R^1 = Me, Epothilone B
18, R = R^1 = Me, C-17 oxazole
19, R = Me, R^1 = Me, trans-$\Delta^{12,13}$
20(*12S,13R*), R = H, R^1 = Me
21, R = R^1 = H



22, R = Me, C-17 pyridine
23(*6S,7R*), R = H
24, R = H, Desoxyepothilone A
25, R = Me, Desoxyepothilone B
26, R = Me, trans-$\Delta^{12,13}$

Eleven additional taxoids and epothilones with a range of tubulin polymerization tendencies were selected for the training set (Table 1, Supporting Information). Each was

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optimized with AMBER*/H_2O/GBSA, SEAL-fitted[17] onto the template paclitaxel conformation and then transferred to the minireceptor. The resulting complexes were optimized in PrGen with correlation coupling to provide the refined minireceptor and scored with a three-term free-energy function.[15,18] The most favorable orientation of paclitaxel and the corresponding super-position of the highest ranked epothilone B conformation (**17**) are depicted in parts a and b of Figure 1, respectively. Very different from the epothilone

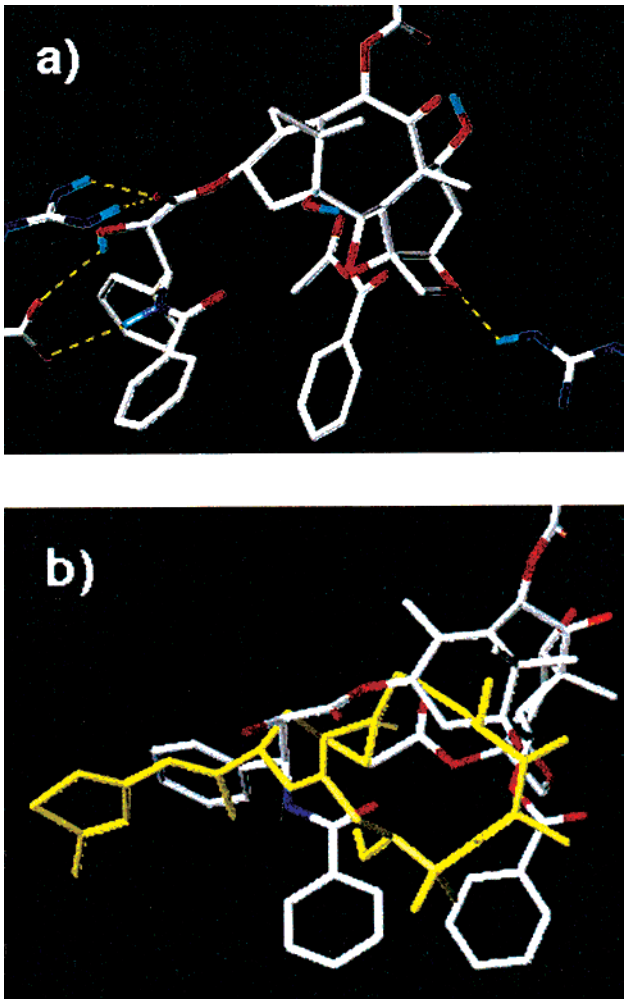

Figure 1. (a) A subsite of the microtubule minireceptor model showing the predicted binding conformations and hydrogen bonding pattern for paclitaxel, and (b) superposition of paclitaxel and epothilone-B in the minireceptor.

B and 26-substituted epothilone B[19] X-ray crystal structure forms, **17** is curled within the region of the binding site occupied by the C-2 and C-13 side chains of paclitaxel and the atoms of the rigid tetracyclic core connecting them. The epothilone thiazole ring matches the location of the paclitaxel phenyl ring emanating from C-3′ (Figure 1b). It is noteworthy that the epoxide ring forms an intramolecular cross-ring hydrogen bond. In the solid state, the epoxy macrolide amphidinolide shows a similar constitution.[20] In contrast to the X-ray structure, our candidate for the bioactive conformation of epothilone was found only 2.6 kcal mol^{-1} above the global minimum.

Biological activities were assembled from the literature (ID_{50} or EC_{50})[5,12] under the assumption that all substances are acting through the paclitaxel−tubulin binding site (Table 1[21]). These values in turn were expressed as rough K_i's and free energies of binding (ΔG) for the purpose of the 3D SAR correlation. On the basis of the training set of structures (Table 1), the current model shows a high correlation ($r = 0.99$) and a low rms($\Delta G_{calc} - \Delta G_{exp}$) = 0.2 kcal mol^{-1} (Figure 2).

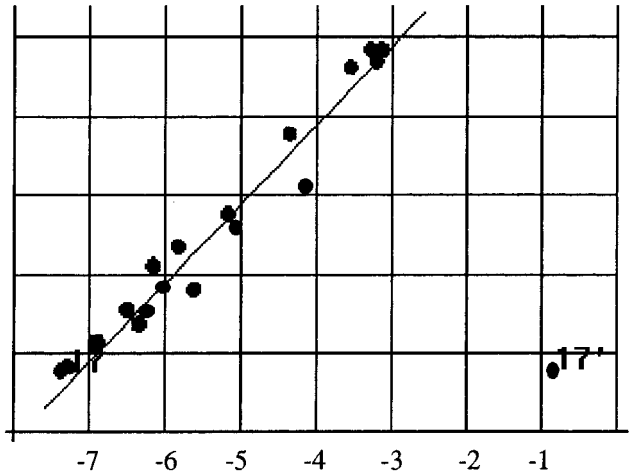

Figure 2. Free energy correlation predicts the binding of taxoids and epothilone B to microtubules (training set, white; test set, gray); $r = 0.99$ and rms($\Delta G_{calc} - \Delta G_{exp}$) = 0.2 kcal mol^{-1} for the training set. Horizontal axis is ΔG_{calc} (kcal/mol); vertical, ΔG_{exp} (kcal/mol). See Table 1 for numerical values.

A paclitaxel−epothilone pharmacophore[22,23] with the thiazole ring of the macrolide coincident with the C-10 acetate

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(18) Zbinden P.; Dobler, M.; Folkers, G.; Vedani, A. *Quant. Struct.-Act. Relat.* **1998**, *17*, 122−130.

of paclitaxel has been proposed recently.[24] An orientation of this type (**17′**) is readily docked into the paclitaxel−tubulin receptor model. However, the predicted binding free energy is 5−6 kcal mol^{-1} lower than that of paclitaxel and epothilone B (Table 1, Figure 2). We take this to imply that the thiazole ring of epothilone prefers the same locus as one of the essential paclitaxel phenyl rings as shown in Figure 1b. Accordingly, our model posits that the heterocyclic ring or its equivalent is critical for epothilone biology. It concurs with the SAR work of the Danishefsky and Nicolaou groups showing a loss of activity for a truncated, shortened, or kinked epothilone thiazole side chain.[5e,f]

Desoxyepothilones A (**24**) and B (**25**) have been shown to bind to microtubules under paclitaxel-like conditions.[5d] In addition, for certain cell lines they promote levels of cell killing equivalent to or better than paclitaxel itself. In the model of Figure 1, the docked olefins are predicted to bind with almost the same affinity as paclitaxel (Table 1). The outcome can be rationalized by noting first that the epoxy analogues do not form hydrogen bonds with the minireceptor. Thus, no productive receptor ligand interactions are lost in the absence of epoxide. Second, outside the binding pocket the desoxy derivatives are less well solvated. The relative affinities of epothilones and their desoxy analogues would appear to be controlled to a large extent by the energetics of desolvation as the ligands migrate from aqueous solution to the hydrophobic tubulin binding site.

The taxoids included in both training and test sets (Table 1) account for many of the SAR observations in recent publications, for example: (1) Modification of paclitaxel by placing an H at C-10 (**5**) or an OH at C-9 (**6**) does not alter the drug's ability to polymerize tubulin. (By contrast, the suppression of activity by OH at C-4 (**7**) or H at C-2 (**8**) underscores the critical nature of the lost side chains.) (2) 3′-Cyclohexyl analogues (**4**) exhibit higher activity than paclitaxel. (3) *Para* substituents on the C-2 benzoyl group (e.g., **11**) reduce activity in the tubulin assay. (4) Contraction of ring C (**13** and **14**) compromises activity, while both contraction and rupture of rings C and D, respectively, eliminates it altogether (**15**). (5) Conversion of the C-13 side chain to a propionic acid ester (**12**) likewise severely diminishes ligand-induced tubulin aggregation.

The present model can accommodate much of the SAR for epothilones as well. (1) Replacement of the thiazole termini on the side chain with oxazole (**18**) or pyridine (**19**) causes only a slight reduction in the degree of microtubule assembly. (2) As shown by comparison of epothilone B **17** with its desmethyl analogue (**23**), an (S)-methyl group at C-8 is important for ligand−tubulin binding. In the mini-receptor complex this methyl overlaps the region occupied by the C-15 dimethyl unit of paclitaxel. (3) Altering the *cis* to the *trans* configuration about the epoxide (**17** vs **20**) or the C═C bond (**25** vs **26**) results in a drop of 5−20 in tubulin polymerization efficiency. (4) A methyl at C-12 persistently enhances target polymerization (**24** vs **26**). The group occupies the same hydrophobic region as the essential C-4 acetate methyl in paclitaxel. (5) That the model is also sensitive to a change in stereochemistry at C-6 and C-7 is shown by the low K_i predicted for **22** in agreement with experiment.

In conclusion, the epothilones are conformationally mobile 16-membered macrolide rings that include the X-ray conformation as a low population contributor when evaluated by the widely used AMBER*/H_2O/GBSA force field. The semiquantitative paclitaxel−tubulin minireceptor model predicts a novel binding mode for an internally H-bonded epothilone conformer and accounts nicely for the tubulin polymerization properties of a range of epothilones including the desoxyepothilones. We anticipate the paclitaxel−tubulin receptor surrogate will prove useful as a construct for identifying novel paclitaxel mimics. Preliminary efforts have been made to incorporate the eleuthrobins[25] and sarcodictyns[26] into the model and will be the subject of a future communication.

Supporting Information Available: Table 1 containing data for training and test set molecules; Cartestian coordinates for the 20 amino acid minireceptor and the bound conformations of paclitaxel and epothilone. This material is available free of charge via the Internet at http://pubs.acs.org.

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