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What Makes Epothilones Stick?

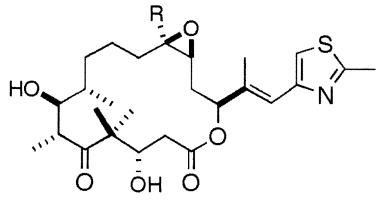
The potent epothilone tubulin polymerization promoters have been studied extensively by synthetic and SAR approaches. The paper by Buey et al. ([13], this issue of *Chemistry & Biology*) adds a new depth of mechanistic understanding by a careful analysis of the tubulin polymerization mechanism of the epothilones.

The discovery in 1979 of the ability of paclitaxel (Taxol) to promote the polymerization of tubulin heterodimers to microtubules [1] marked a turning point in the development of this compound as an anticancer agent, and it went on to become a blockbuster drug. Paclitaxel's success initiated a search for other anticancer agents that operate by the same mechanism, and several compounds that do this have been found, including discodermolide [2], eleutherobin [3], and several other natural products. Of all the "paclitaxel-like" compounds found to date, however, the epothilones have excited the most interest. Epothilones A (Figure 1[1]) and B (Figure 1[2]) were originally isolated as antifungal agents, but scientific interest in them was enormously stimulated by the discovery of their tubulin-polymerization activity in 1995 [4] and by the publication of their complete structure and stereochemistry [5]. Biological studies of the epothilones have shown that they stabilize microtubules in the same way that paclitaxel does but with somewhat higher potencies. Not only do they act as tubulin-polymerization agents in the same way that paclitaxel does, but they also compete for the same binding site on the polymer, since they act as competitive inhibitors of the binding of [^{3}H]paclitaxel to tubulin polymers [6]. In addition, epothilone B has been shown to be superior to paclitaxel in treating vinblastine-resistant CCRF-CEM tumors in the mouse [7], and desoxyepothilone B is curative against paclitaxel-resistant CCRF-CEM tumors [8]. Several total and partial syntheses of the epothilones and their analogs have been reported, and extensive SAR studies have been carried out. Their chemistry and biology have been reviewed, with two important reviews by one of the coauthors of the present paper [9, 10]. Epothilone B is the most potent antiproliferative agent among the naturally occurring tubulin polymerization promoters, with an activity from 2- to 10-fold greater than that of paclitaxel, and it is less susceptible than paclitaxel to Pgp- mediated MDR [11]. Epothilone B is in phase II trials by Novartis, and its lactam analog BMS-247550 is in phase II/III trials by Bristol-Myers Squibb. Deoxyepothilone B (Sloan-Kettering/Kosan/Roche) and C21-amino epothilone B (BMS) have also entered clinical trials [12].

Previous authors have made extensive studies of the SAR of the epothilones, as summarized in the reviews cited [9,10]. The study by Buey et al. [13] goes beyond these studies to provide an in-depth look at why certain structural modifications result in increased activity and why others give reduced activity. The authors do this by a careful analysis of the binding affinity of epothilone analogs to microtubules and by correlating this affinity with cytotoxicity. This paper is thus the first in which the microtubule binding of epothilones has been studied at a fundamental level.

The major problem with studies of the interaction of paclitaxel and paclitaxel-like compounds with tubulin is that they are too good at what they do. They bind to the polymerized form of tubulin and immediately induce further assembly so that it is not possible to study tubulin binding apart from microtubule assembly; the two reactions are inseparably linked. The present authors have previously developed a way around this difficulty by the use of microtubules stabilized by gentle crosslinking and by the use of fluorescent paclitaxel probes. These conditions allow the study of the ligand-microtubule interaction at low enough concentrations to obtain direct measurements of the binding affinity, and nonfluorescent ligands can be studied by competition with the fluorescent probes.

In the present study, the authors applied these methods to the study of the binding of epothilones A and B and various epothilone analogs to microtubules. Direct binding constants were calculated, and the reactions were carried out at various temperatures so that ΔH and ΔS values could be calculated for each ligand. The binding affinity results are presented graphically in a



1 R = H Epothilone A
2 R = Me Epothilone B

Figure 1. Epothilones A and B
Structures of Epothilone A and Epothilone B.

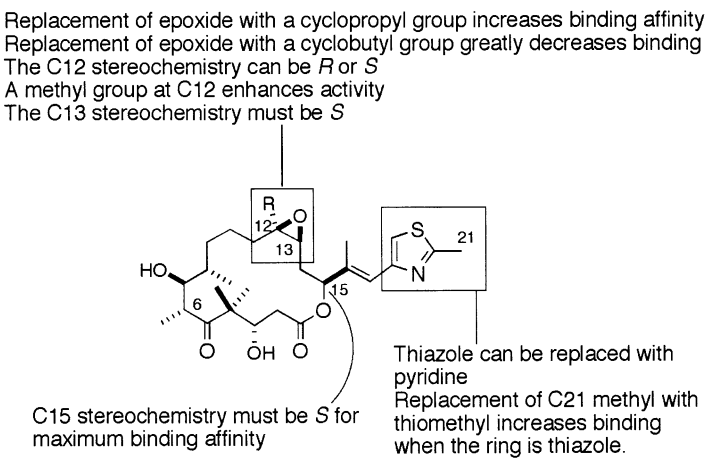


Figure 2. Design of Epothilone Analogs

Structure-activity relationships for microtubule binding of C12, cyclopropyl, and C15 side chain epothilone analogs.

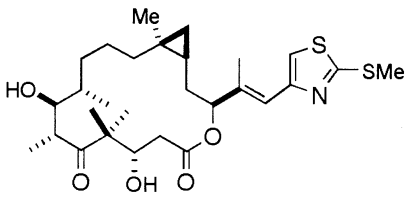
visual format, where the position of a structure in the figure is related both to its binding affinity and to its structure.

So what did these studies reveal? At a basic level the findings agree with earlier results, confirming, for example, that the *S* stereochemistries at C13 and C15 are crucial, while that at C12 can be either *R* or *S*. Replacement of the epoxide with a cyclopropane ring enhances binding affinity, as does replacement of the C21 methyl group with a thiomethyl group. These findings are summarized in Figure 2.

The analog with the greatest binding affinity was found to be compound **19** (Figure 3), with both the cyclopropane ring and thiomethyl substitutions on the epothilone B skeleton; this compound had about 25 times the binding affinity of epothilone B itself.

An analysis of the factors behind the observed SAR proved instructive. Thus the change of the C21 methyl to thiomethyl, which was favorable overall, was actually unfavorable in the enthalpy term but highly favorable in the entropy term, thus making the overall reaction an entropy-driven one. It is suggested that the sulfur atom places the methyl group in a better position for a hydrophobic interaction. In the pyridine series, however, the change of methyl to thiomethyl proved unfavorable; presumably in this case the sulfur places the methyl group in an unfavorable location. Another conclusion was that the changes were largely additive, so that the effect of making two modifications could usually be predicted from the effects of the individual modifications.

Since the normal effect of ligand binding is microtubule stabilization, a study was also done on the relationship between ligand binding and microtubule stabilization activity. It was found that these values correlated fairly well. As expected, compound **19**, with the strongest microtubule binding affinity, was also the strongest inducer of microtubule assembly, and the same was true in reverse for the analog with the weakest binding affinity.



Compound **19**

Figure 3. Compound 19

Structure of the high-affinity epothilone analog compound **19**.

All this is very interesting, but at the end of the day an analog must be able to induce cancer cell death, and so it is the cytotoxicity of the epothilones that primarily determines their value as drug candidates. A compound with excellent binding characteristics that was only weakly cytotoxic would probably not be of great interest. The authors thus compared their binding affinity data with cytotoxicity data, using the 1A9 human ovarian carcinoma cell line. They found that cytotoxicity paralleled binding affinity to a reasonable approximation, and compound **19** was indeed the most potent analog tested. Interestingly, however, compounds such as **19**, which owed their increased binding affinity in part to a favorable entropic term, were not as potent cytotoxins as their binding affinity would have predicted. Although compound **19**, for example, had a binding affinity 25 times larger than epothilone B, it was only three times more cytotoxic than the latter.

The authors point out that their method of measuring binding affinities is simpler and more sensitive to structural changes than the measurement of critical concentrations. They thus rightly claim that the measurement of binding affinities is a better design tool than measurement of microtubule stabilization. They go on to state that future improvements in cytotoxicity could be made most effectively by the preparation of compounds with increased enthalpic contributions to their binding affinity. Regrettably, the authors do not provide any insight as to how this might be done, but they have provided a useful tool for others to take advantage of.

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New Start and Finish for Complex Polyketide Biosynthesis

The polyketide vicenistatin has significant anticancer activity. In the January issue of *Chemistry & Biology*, Kakinuma and coworkers [20] reported on the vicenistatin biosynthetic gene cluster and demonstrated in vitro glycosylation of its aglycone core, raising the possibility of producing analogs with altered sugar residues.

The polyketide group of natural products includes numerous antibiotics, anticancer drugs, and immunosuppressants. Biosynthesis of these important compounds involves assembly of carbon chains from small acyl precursors. In general, chain elongation is catalyzed by a set of enzymes that together make up a polyketide synthase (PKS). A starter or growing acyl chain migrates onto the active site cysteine of a ketosynthase (KS), and an acyltransferase (AT) loads a dicarboxylic acyl extender unit onto the phosphopantetheine thiol of an acyl carrier protein (ACP). A decarboxylative Claisen condensation leaves an extended β-ketoacyl chain attached to the ACP [1]. A complete chain extension cycle can involve three more steps: conversion of the β-ketone group to an alcohol by a ketoreductase (KR), dehydration by a dehydratase (DH) to form a *trans* α-β unsaturated acyl intermediate, and finally reduction by an enoyl reductase (ER) to give a saturated chain. The condensations between acyl thioesters may be repeated many times, but PKSs rarely take β-ketone processing all the way to the third stage. As a result, ketones, hydroxyl groups, and double bonds appear at defined positions within polyketide chains.

Chemical and genetic studies have now uncovered many different types of PKS [2]. Of these, the modular PKSs are the most amenable to redesign for production of novel compounds. These systems contain a set of enzymes or modules for every cycle of chain extension, as well as a loading module for transferring the starter acyl group onto the first KS domain. This modular organization allows programmed assembly of a defined sequence of starter and extender units, together with controlled processing of each β-ketone group. The final product may be cyclized by a thioesterase (TE) to give a macrolactone.

Starter units may derive from acyl CoAs that are recruited from primary metabolism and used directly. Acetyl and propionyl starters can also be generated by decarboxylation of malonyl and methylmalonyl groups attached to loading module ACPs. Other more unusual organic acids are activated as acyl adenylates before transfer to CoA or an ACP [3]. Modular PKSs generally use malonyl and methylmalonyl-CoA as extender units. Ethylmalonyl and methoxymalonyl units are used less frequently [4]. The sequences of malonyl and methylmalonyl-specific AT domains can be distinguished by characteristic motifs. A separate region of the AT domain contains critical residues that appear to determine substrate specificity. Methylmalonate-specific AT domains use only the (*2S*) isomer of methylmalonyl CoA [5]. When this branched extender is used, condensation occurs with inversion of stereochemistry at C-2 so that a (*2R*) 2-methyl-3-ketoacyl chain is generated initially [6]. With some modules epimerization must occur to give the final alkyl stereochemistry. Exactly how this epimerization is achieved is still unclear. The epimerase activity seems to reside within the KS domain [7], but there are no obvious differences between the primary sequences of epimerizing and nonepimerizing KSs.

KR domains are responsible for determining the final stereochemistry at chiral centers derived from reduction of β-ketones to alcohols [8]. Conserved amino acid residues at a few key positions appear to be useful for predicting KR stereospecificity [9, 10]. Predictive methods suggest that the DH domains of modular PKSs normally act on (*3R*)-hydroxyacyl chains to give *trans* double bonds. Few complex polyketides have *cis* double