Novel molecules that interact with microtubules and have functional activity similar to Taxol™

Lifeng He, George A. Orr and Susan Band Horwitz

Taxol™ is an antitumor drug approved by the FDA for the treatment of ovarian, breast and non-small-cell lung carcinomas. Originally isolated from the bark of the Pacific yew, *Taxus brevifolia*, it was the first natural product described that stabilized microtubules. In the past five years, a group of novel natural products, including the epothilones, discodermolide, eleutherobin, sarcodictyins and the laulimalides, all of which have biological activities similar to those of Taxol, has been discovered. In this review, we discuss each of these novel microtubule-stabilizing agents and the search for a common pharmacophore among them, taking into consideration recent advances in our understanding of the taxanes and tubulin.

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▼ Taxol (paclitaxel) is a natural product originally isolated from the bark of the slow growing Pacific yew, *Taxus brevifolia*. In 1971, Wall and Wani and their collaborators published the isolation and structural determination of Taxol[1]. The Taxol molecule, named by Wall, is a complex diterpene with a taxane ring structure fused with a four-membered oxetane ring, an ester side-chain at position C-13 and a benzoyl side-chain at the C-2 position (Fig. 1). The drug is cytotoxic to tumor cells growing in tissue culture and in several rodent tumor model systems. Taxotere™ (docetaxel) is synthesized from 10-deacetylbaccatin III, a precursor isolated from the needles of the European yew, *Taxus baccata*, and has a *tert*-butoxycarbonyl group at the C-3′ and a hydroxyl group on the C-10 position (Fig. 1). Although not identical, its biological and clinical activities resemble those of Taxol[2].

Concerted and persistent efforts made by medicinal chemists, pharmacologists and oncologists in the ensuing years moved Taxol into the clinic and continually improved its response rates. The drug has been approved by the FDA for the treatment of ovarian, breast and non-small-cell lung carcinomas as well as Kaposi's sarcoma[3]. Several new Taxol derivatives and oral preparations of the drug are in clinical trials both as single agents and in combination with other drugs.

Taxol has a unique mechanism of action: it was the first compound known to interact exclusively with a polymer form of tubulin. It was originally reported by our laboratory in 1979 that Taxol promoted the *in vitro* assembly of tubulin in the absence of GTP, which is normally required for microtubule assembly, and that the microtubules formed were stable against conditions favoring depolymerization, such as cold temperature, Ca^{2+} and dilution[4]. Taxol binds to β-tubulin in the microtubule specifically and reversibly with a stoichiometry of almost one (relative to the α,β-tubulin dimer)[5,6]. Another unique activity of Taxol is its ability to induce the formation of characteristic microtubule bundles in cells[7]. By contrast, other antimitotic agents, such as colchicine and the *vinca* alkaloids, inhibit microtubule assembly through their interaction with the tubulin dimer. However, at nanomolar drug concentrations, Taxol exhibited effects similar to those of nanomolar concentrations of colchicine and the *vinca* alkaloids, in that it suppressed microtubule dynamics[8,9].

Taxol binds to a hydrophobic pocket in β-tubulin

Taxol analogs containing both photoreactive moieties and tritium tags have played an important role in the identification of the binding sites for Taxol in β-tubulin. In an early study performed in our laboratory, [^{3}H]Taxol

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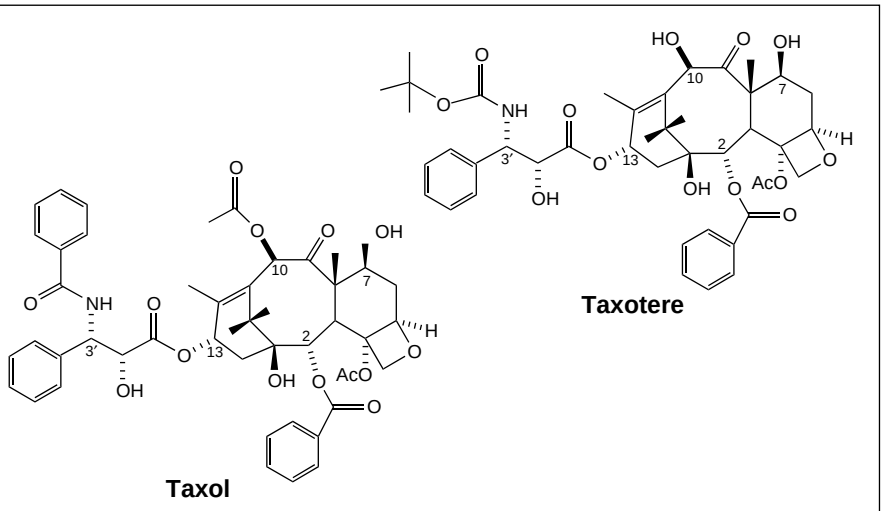


Figure 1. Chemical structures of Taxol™ and Taxotere™.

was used to photolabel tubulin and it was found that the radiolabeled Taxol preferentially bound to β-tubulin[10]. There was insufficient photoincorporation of [^{3}H]Taxol to determine the exact site of interaction, and so it became necessary to employ photoaffinity radiolabeled Taxol analogs for these studies. Therefore, [^{3}H]3′-(*p*-azidobenzamido)-Taxol and [^{3}H]2-(*m*-azidobenzoyl)-Taxol were used to label tubulin (Fig. 2). The C-3′ *p*-benzamido and the C-2 *m*-azido-benzoyl groups of the Taxol analogs crosslinked to amino acid residues 1–31 and 217–233 in β-tubulin, respectively[11,12]. Recently, Arg282 in β-tubulin was identified as the amino acid residue crosslinked by [^{3}H]7-(benzoyldihydrocinnamoyl)-Taxol (Fig. 2)[13].

During the course of these photoaffinity studies, Nogales and Downing and their coworkers made a major breakthrough in solving the atomic structure of the α,β-tubulin dimer[14,15]. A 3.7 Å density map for α,β-tubulin was obtained by electron crystallography of zinc-induced tubulin sheets. This not only provided information on the structural and functional composition of the α,β-tubulin dimer, but enabled scientists to envision the binding of Taxol in the context of the tubulin structure.

Combining the results obtained from photoaffinity labeling studies and the atomic structure of α,β-tubulin, it has been proposed that Taxol binds to a hydrophobic pocket in α,β-tubulin in a T-shaped conformation[16]. The C7-OH of Taxol is close to Thr274, the C-2 benzoyl group fits into a pocket formed by His227 and Asp224, both of which are part of the H7 helix, and the C3′ benzamido moiety is close to Val23 (Ref. 13), in β-tubulin (Fig. 3). This Taxol binding pocket is close to the M-loop, at the lateral interface of adjacent protofilaments and in contact with the H3 helix in the neighboring β-tubulin subunit (Fig. 4). Therefore, Taxol might stabilize microtubules by binding to this pocket in β-tubulin and strengthening the lateral interactions between protofilaments[14–17].

The binding of Taxol could also impact the structure of the microtubule in other ways. The C2-benzoyl group of Taxol binds to the H7 helix, a structure in β-tubulin that is also in contact with the exchangeable nucleotide (GTP/GDP) binding site (Fig. 4). The H7 helix could have a controlling effect on the conformation of the whole tubulin molecule as has been observed in FtsZ, the bacterial homolog of tubulin[17–19]. The hydrolysis of GTP to GDP in β-tubulin is a major regulatory mechanism that influences microtubule stability[20,21]. By interacting with the H7 helix, Taxol could induce a conformation of β-tubulin that mimics the GTP-bound β-tubulin. Therefore, in this hypothesis GTP and Taxol, each of which interacts with the H7 helix in a different way, are able to promote microtubule assembly (Fig. 4). Microtubules formed with either Taxol or non-hydrolyzable GTP analogs are stable in the presence of Ca^{2+} or at cold temperatures, which normally destabilize microtubules[22,23].

Microtubules formed with Taxol or other microtubule-stabilizing agents

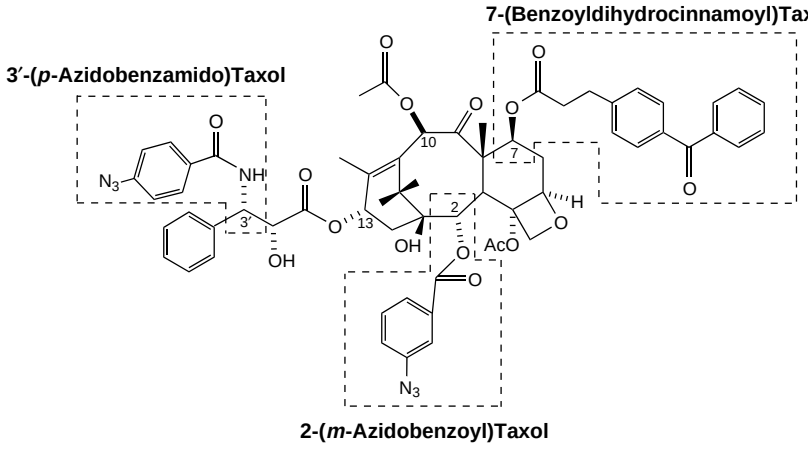


Figure 2. Photoaffinity labeling analogs of Taxol™.

are composed of fewer numbers of protofilaments than those formed with GTP or GMPCPP (a non-hydrolyzable GTP analog), indicating altered conformation upon the binding of these compounds[6,24]. Because the M-loop is a flexible component at the lateral interface it is likely that the binding of Taxol and other microtubule-stabilizing agents alter the conformation of the M-loop.

It is interesting to note that the region in α-tubulin that is equivalent to the Taxol binding pocket in β-tubulin is occupied by an eight amino-acid peptide (A362–A369), which forms part of the S9–S10 loop (Fig. 4)[14,15]. It was proposed that this loop acts as an endogenous microtubule-stabilizing factor[14–17]. This naturally led to the hypothesis that Taxol and other microtubule-stabilizing molecules exert their activity by mimicking the function of this endogenous regulatory factor. It would be of interest to know why this stabilizing loop is present only in α-tubulin. Furthermore, what is the advantage of having this difference in the structure and function of the α- and β-tubulin subunits? Another difference between α- and β-tubulin relates to GTP that is non-exchangeable in α-tubulin, but is exchangeable and hydrolyzed to GDP in β-tubulin during microtubule polymerization and depolymerization. Finding out why these differences exist will help us to better understand the regulation of microtubule stability.

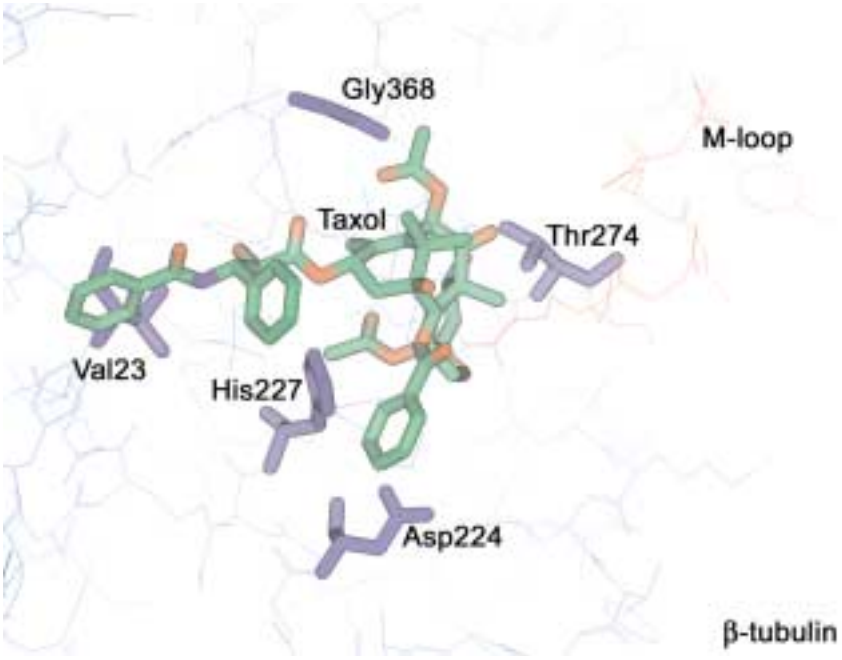


Figure 3. Taxol™ binds at a hydrophobic pocket in β-tubulin. Taxol binds into a hydrophobic pocket in β-tubulin on the luminal surface of the microtubule. The C-7 hydroxyl group of the drug is close to Thr274, the C-2 benzoyl ring fits into a pocket formed by His227 and Asp224 and the C3′-moiety is close to Val23.

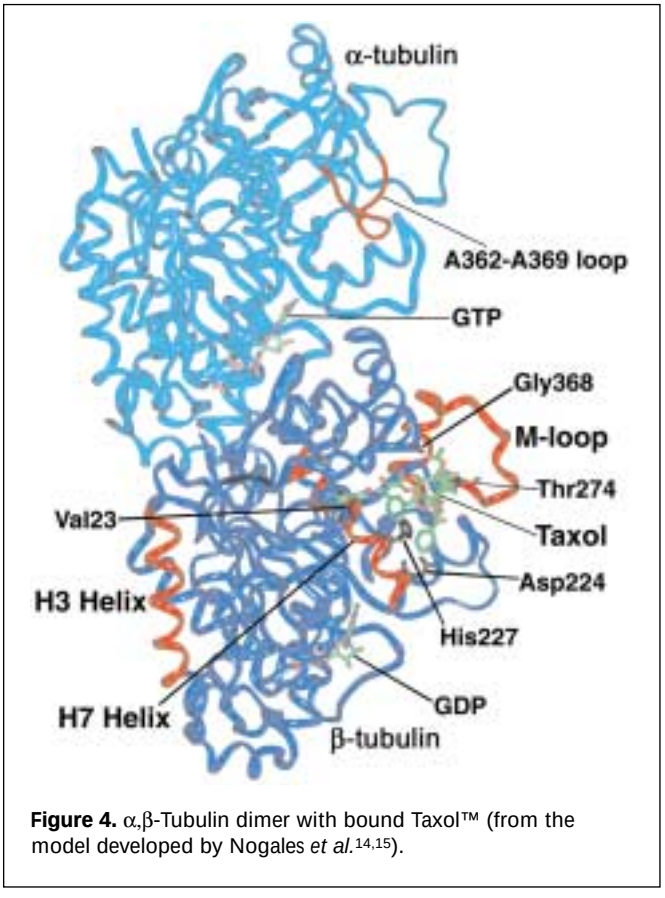


Figure 4. α,β-Tubulin dimer with bound Taxol™ (from the model developed by Nogales *et al.*[14,15]).

Problems in drug formulation and drug resistance

Taxol is dissolved in Cremophor EL for intravenous administration to cancer patients because of its extreme hydrophobicity. Although not fully documented, it is thought that Cremophor EL could be responsible for the hypersensitivity reactions seen in some patients[3]. The search for better ways to administer Taxol is a continuing project.

Cancers treated with Taxol or other hydrophobic antitumor drugs can become resistant to the drugs by acquiring the multidrug resistance (MDR) phenotype[25,26]. The expression of P-glycoprotein (P-gp), a transmembrane transporter that significantly reduces the intracellular concentration of these drugs, is associated with this phenotype. Tumors expressing P-gp are not only resistant to Taxol, but also to other hydrophobic antitumor agents to which they

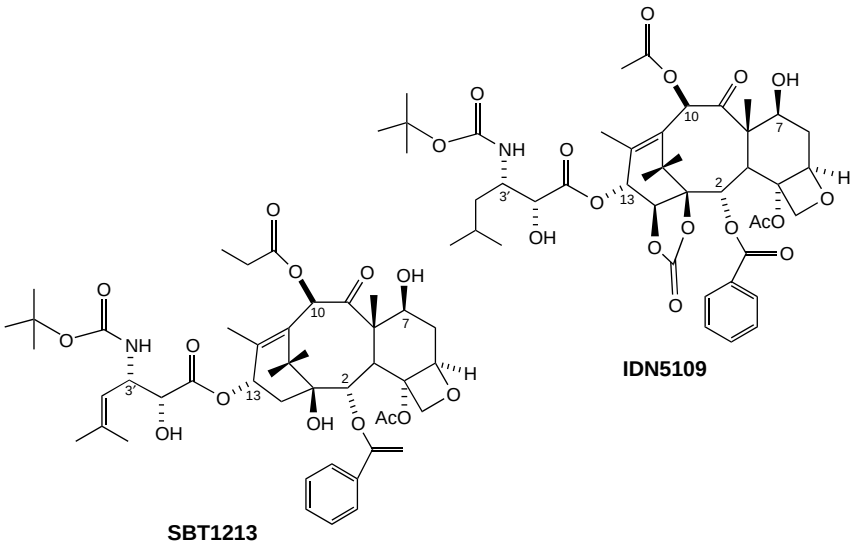


Figure 5. Chemical structures of SBT1213 and IDN5109.

demonstrate cross resistance[27]. These problems, in addition to the clinical success of Taxol, have triggered an interest in the design of improved taxanes and a search for novel microtubule-stabilizing agents that act in a manner similar to Taxol.

Ojima and collaborators are designing new taxanes that can circumvent P-gp-mediated drug resistance and/or can be administered orally. They have designed and synthesized a class of 3′-(2-methyl-1-propenyl) and 3′-(2-methylpropyl) taxoids that are not only more cytotoxic than Taxol in drug-sensitive MCF-7 breast cancer cells, but also maintain activity in MCF-7 Taxol-resistant cells that express P-gp (Refs 28–30). Further structure–activity relationship (SAR) studies have indicated that the ability of these taxoids to overcome the MDR phenotype is dependent on the proper modification at the C-10 position of the taxane ring. It is not yet clear how the C-10 modifications (e.g. SBT1213) impact the activity of taxoids in MDR cancer cells (Fig. 5). However, this research provides a new approach to the design of taxanes that might be able to circumvent P-gp-mediated drug resistance in human tumors.

One taxane, 13-(*N-boc*-β-isobutyl-isoserinyl)-14-hydroxybaccatin-1,14-carbonate (IDN5109; Fig. 5), was originally selected because it was not a substrate for P-gp (Ref. 31). Its oral bioavailability was tested in mice, and 120 mg kg^{-1} of IDN5109 administered orally was as active as intravenous administration of Taxol (40 mg kg^{-1}) at causing tumor regression of two human ovarian-cancer xenografts[32]. In a separate study, oral administration of IDN5109 (90 mg kg^{-1}) exhibited comparable efficacy to intravenous administration of Taxol (54 mg kg^{-1}) against MX-1, a human breast carcinoma model in mice[33]. These results hinted at a possible relationship between lack of transport by P-gp and increased oral bioavailability of taxanes[34].

Another taxane derivative of interest is DHA–paclitaxel, in which docosahexaenoic acid (DHA), a natural fatty acid, is covalently linked by an ester bond to the C-2′ hydroxyl group of Taxol (Fig. 6). This drug was designed in an effort to introduce tumor-targeting capacity to the Taxol molecule. Compared with paclitaxel, it has been shown to exhibit superior therapeutic effects in some animal tumor model studies[35,36]. Pharmacokinetic studies revealed that its area-under-curve (AUC) value, calculated from the intratumor drug concentration–time curve, is significantly greater than that of Taxol and its concentration is sustained above the therapeutic concentration for a much longer time than Taxol. It has been hypothesized that DHA–paclitaxel remains in the bloodstream as an inactive drug until it reaches the tumor where it is metabolized to an active taxane.

The value of any of these new taxane molecules in the clinic remains to be demonstrated.

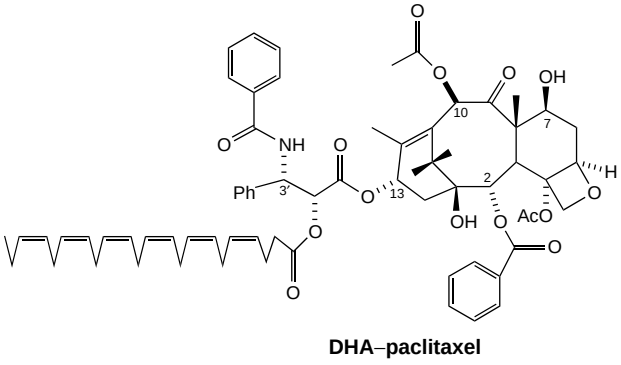
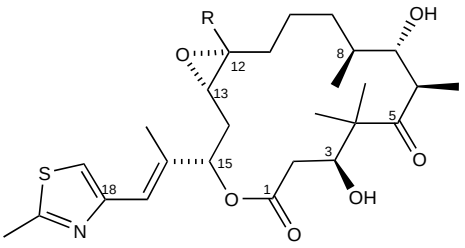


Figure 6. Chemical structure of DHA–paclitaxel.

Novel natural products with similar mechanisms of action to Taxol

The epothilones

Discovery and activities Epothilones A and B were first isolated from myxobacterium *Sorangium cellulosum* strain 90, by Höfle and Reichenbach and their coworkers, as an



Epothilone A (R = H)
Epothilone B (R = Me)

Figure 7. Chemical structures of the epothilones.

antifungal and cytotoxic compound (Fig. 7)[37]. In 1995, Bollag *et al.*[38] reported that the epothilones mimic the microtubule-stabilizing effect of Taxol. Like Taxol, epothilones A and B were able to promote GTP-independent tubulin polymerization that resulted in stable microtubules. Cells treated with the epothilones exhibited microtubule bundles, a characteristic morphological change of Taxol treated cells, and such bundles of microtubules were stable at 4°C. The epothilones inhibited the growth of cultured cancer cells at low drug concentrations and induced mitotic arrest and subsequent apoptosis in cells[38].

Drug-binding competition studies revealed that the epothilones competitively inhibited the binding of [^{3}H]Taxol to microtubules, suggesting that both drugs bind to the same, or overlapping, sites in β-tubulin. A crucially important characteristic of the epothilones is that they retain their potency in cells that express P-gp[38], thereby suggesting that these drugs might have an advantage over Taxol in the treatment of drug-resistant tumors[39].

Total synthesis Since the discovery of the epothilones as potent microtubule-stabilizing agents, the total synthesis of these compounds has been of intense interest to many organic chemists. The total synthesis was first accomplished between late 1996 and early 1997 by the laboratories of Danishefsky[40] and Nicolaou[41].

To ensure an adequate supply of the epothilones for clinical studies, efforts are being made to produce large quantities of these compounds. This problem is being approached by cloning the gene cluster responsible for epothilone biosynthesis and expressing the genes concomitantly in the actinomycete, *Streptomyces coelicolor*[42], a bacterium that grows approximately tenfold as rapidly as the natural producer, *S. cellulosum*. In a separate project, a cluster of 22 open reading frames in the *S. cellulosum* genome were sequenced and related to the expression of different modules involved in the production of the epothilones in myxobacteria[43]. The identification of these genes and their functions will facilitate the production and modification of the epothilones.

SAR studies The SAR of the epothilone molecule has been characterized in several laboratories[41,44]. In summary, the C-1 to C-8 part of the macrolide ring is extremely intolerant of any modifications and maintaining the size of the macrolide ring is essential for biological activity. The epoxide at the C-12, C-13 positions can be replaced by a double bond, a phenyl ring or a cyclopropane ring. It has been reported that Z-12,13-desoxyepothilone B is superior to epothilone B in reducing tumor size in a mouse model carrying an MDR-tumor xenograft[45]. The addition of small substituents, such as ethyl or vinyl, at the C-12 position can be tolerated. The linker region (from C-15 to C-18) is sensitive to modifications and the thiazole ring on the side chain can be replaced by other aromatic rings, like phenyl or oxazolyl, with a partial loss in activity.

Epothilone clinical trials Currently, several independent clinical trials are being carried out in parallel with epothilone B, desoxyepothilone B and new derivatives of the epothilones[46–50]. Each drug has its unique features and it is not clear at this time which, if any, of these compounds will become useful antitumor drugs for the treatment of human tumors.

Epothilone resistance in cultured cell lines The fact that the epothilones are neither substrates for P-gp nor inducers of P-gp, make them ideal candidates for the study of drug-resistance mechanisms that do not operate via P-gp. In the presence of P-gp, it has been difficult to determine if microtubule-stabilizing agents induce other mechanisms of drug resistance that are normally masked by the expression of P-gp.

To date, two reports of epothilone-resistant cell lines have been published and point mutations in β-tubulin have been identified in epothilone-resistant cell lines. In the work done by Giannakakou *et al.*[51], epothilone-resistance in a human ovarian carcinoma cell line A2780 (1A9) has been characterized. Two clones, 1A9/A8 and 1A9/B10, both of which are 25–57-fold resistant to epothilone A and B, were isolated in a single-step selection with epothilone A or B, respectively, and shown not to express the MDR gene. Sequencing of the predominant β-tubulin isotype, Class I β-tubulin, identified one point mutation in each clone: β274Thr to Ile in 1A9/A8 and β282Arg to Gln in 1A9/B10 (Ref. 51). Through molecular modeling, using the 3.7 Å structural model of the tubulin dimer developed by Nogales *et al.*[14,15], the authors proposed that the β274Thr to Ile mutation might disrupt the hydrogen bond between

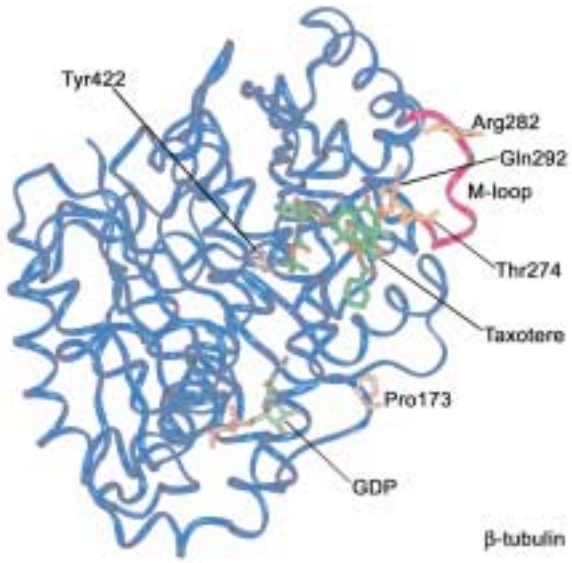


Figure 8. β-Tubulin mutations in epothilone-resistant cells. Point mutations have been identified in epothilone-resistant cell lines. In 1A9/A8 and 1A9/B10 clones, the mutations are at β274 (Thr to Ile) and β282 (Arg to Gln), respectively[51]. These two residues are close to the hypothetical binding site for the epothilones and the M-loop. In A549.EpoB40, HeLa.EpoA9 and HeLa.EpoB1.8 cells, the mutations are at β292 (Gln to Glu), β173 (Pro to Ala) and β422 (Tyr to Tyr/Cys), respectively[52]. Gln292 is close to the epothilone-binding pocket and the M-loop, Pro173 is on a loop-forming part of the GTP/GDP binding pocket and Tyr422 is on the H12 helix at the C-terminus of β-tubulin.

this residue and the C7-OH group of the epothilones and that the β282Arg to Gln mutation could impact both the binding of the epothilones to β-tubulin and the lateral contacts between adjacent protofilaments (Fig. 8).

Our group has selected a series of epothilone B-resistant non-small-cell lung tumour cell lines (e.g. A549.EpoB40), one epothilone A-resistant cell line (Hela.EpoA9) and one epothilone B-resistant (HeLa.EpoB1.8) cell line using stepwise increases in the concentration of epothilone[52]. Cytotoxicity studies of these epothilone-resistant cell lines revealed that they are cross resistant to other microtubule-stabilizing agents, such as Taxol and Taxotere. No MDR1 expression was detected in these resistant cell lines and sequence analysis of Class I β-tubulin revealed that there was a point mutation in each of the resistant cell lines: β292Gln to Glu in A549.EpoB40 (maintained in 40 nM epothilone B), β173Pro to Ala in HeLa.EpoA9 (maintained in 9 nM epothilone A) and β422Tyr to Tyr/Cys in HeLa.EpoB1.8 (maintained in 1.8 nM epothilone B). β292Gln is located near the M-loop and Thr274. Therefore, a mutation to Glu might impact both the binding of the epothilone molecule and the lateral interactions between neighboring protofilaments (Fig. 8).

β173Pro and β422Tyr are located at a loop forming part of the nucleotide (GTP) binding pocket and on the H12 helix at the C-terminus of β-tubulin, respectively (Fig. 8). By disrupting the binding and hydrolysis of GTP and the binding of microtubule-associated proteins (MAPs) to the C-terminal external surface of β-tubulin, mutations at these residues could decrease the endogenous microtubule-stabilizing activities of GTP and MAPs to compensate for the microtubule-stabilizing activity of the epothilones. Consistent with this hypothesis, the resistant cell lines are hypersensitive to microtubule-destabilizing drugs, such as vinblastine and colchicine. Our studies indicate that in addition to mutations that directly influence drug binding and lateral contacts within microtubules, mutations could lead to diminished endogenous microtubule-stabilizing activity resulting in drug resistance.

There are many other issues that must be explored in an attempt to understand mechanisms of epothilone resistance. For example, the stability of cellular microtubules is regulated by many factors, such as MAPs (e.g. MAP4)[53] and microtubule-destabilizing proteins (e.g. stathmin)[54–56]. Alterations in the expression or activities of these proteins could change the sensitivity of cells to microtubule-stabilizing agents.

In both studies of epothilone-resistant cells that have been reported[51,52], it was observed that the doubling time for the resistant cell lines was significantly longer than that of the parental cell lines, indicating a slower growth rate. Is this the result of an impaired microtubule network because of the expression of mutated β-tubulin or a distinct mechanism of drug resistance? Reduction in the rate of cell division could make cells less vulnerable to microtubule-stabilizing agents. It is interesting to note that only one point mutation in Class I β-tubulin was detected in each of the epothilone-resistant cell lines. Is this just a coincidence or a pattern? Are the residues mutated specifically for drug resistance or are they just random mutations at crucial regions that are involved in the regulation of microtubule stability?

Discodermolide

Discovery and unique activities Discodermolide (Fig. 9) was originally isolated from a marine sponge, *Discodermia dissoluta*, and found to have immunosuppressive activity[57–59]. In 1996, it was reported that discodermolide was a microtubule-stabilizing molecule with similarities to Taxol[60,61]. Discodermolide promotes the assembly of microtubules in the absence of GTP or MAPs, induces microtubule bundle formation in cells and competitively inhibits the binding of [^{3}H]Taxol to microtubules[60,62].

Despite the resemblance of its activities to those of Taxol, discodermolide exhibited several unique functions.

For example, when the drug was added to a tubulin solution *in vitro*, assembly occurred instantaneously indicating a major effect on the initiation and nucleation processes of microtubule assembly[62,63]. Examination by electron microscopy revealed that microtubules formed with discodermolide were significantly shorter than those formed in the presence of Taxol. Microtubule bundles present in cells treated with discodermolide were also distinguishable from those formed in Taxol-treated cells. The bundles consisted of short microtubules located at the peripheral region of the cells[63].

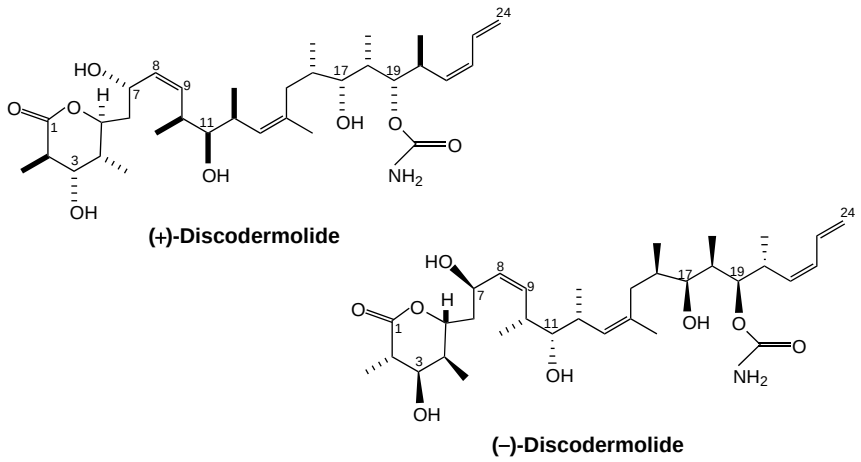


Figure 9. Chemical structures of (+)- and (–)-discodermolide.

Of particular interest is the observation that discodermolide, unlike the epothilones and eleutherobin, could not substitute for Taxol in a Taxol-resistant cell line that requires low concentrations of Taxol for normal growth[39]. Furthermore, it was found that the combination of Taxol and discodermolide, but not of Taxol and epothilone B, exhibited synergistic drug effects in cultured cell lines. It is not known at this time if this synergistic combination of Taxol and discodermolide can be replicated in human tumors. No apparent cross-resistance to discodermolide was observed in epothilone-resistant cell lines that carry β-tubulin mutations[52]. These findings have suggested that discodermolide possesses distinct activities in addition to those overlapping with the activities of Taxol and the epothilones. The unique activities of discodermolide could be related to its dramatic effects on tubulin initiation and nucleation or might indicate that the drug has intracellular targets other than the microtubule.

SAR and total synthesis The SAR profile of discodermolide is complicated, yet interesting. It was found that although both the (+)- and (–)- enantiomers were active as inhibitors of cell proliferation, their effects appeared to be distinct. The natural product, (+)-discodermolide, arrested cells at the G_2/M phase of the cell cycle, whereas synthetic (–)-discodermolide has been reported to block cells in S-phase[64].

SAR studies with acetylated (+)-discodermolide analogs indicated that the addition of acetate groups at either the C-3, C-7 and C-11 positions resulted in either no significant change in activity or an increase in cytotoxicity[65,66]. However, acetylation at the C-17 position resulted in a dramatic decrease in cytotoxicity. The removal of either the C-3 hydroxyl, the C-14 methyl or both the C-3 and the C-7 hydroxyl groups of (+)-discodermolide resulted in partial loss of activity in both the *in vitro* tubulin-assembly assay and cytotoxicity studies. The C(8,9) *E* analog of (+)-discodermolide was ~50% as potent as (+)-discodermolide in the *in vitro* tubulin-assembly assay, but was significantly less cytotoxic than (+)-discodermolide[63]. It is not known if the (–)-discodermolide stabilizes microtubules.

The completion of a gram-scale synthesis of (+)-discodermolide by Smith and his associates[67], and the total synthesis reported by other groups, has ensured a supply of this drug for biological and clinical studies.

Eleutherobin, sarcodictyin, the laulimalides and other new molecules Eleutherobin and sarcodictyins A and B (Fig. 10) are a group of natural products isolated from different marine sources. Eleutherobin was isolated from an *Eleutherobia* species of soft coral found in the Indian Ocean near

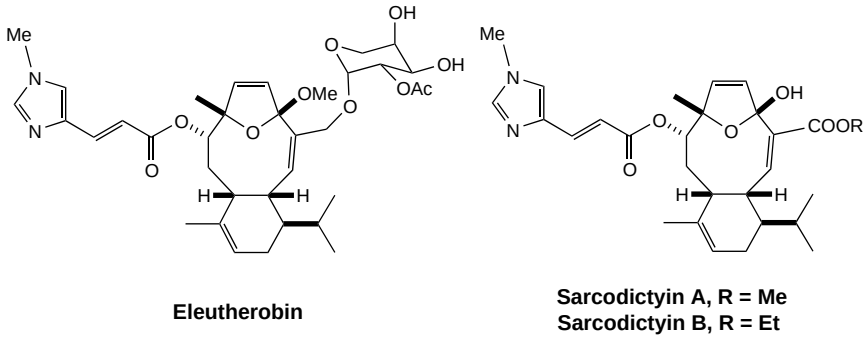


Figure 10. Chemical structures of eleutherobin and the sarcodictyins.

Bennett's Shoal in Western Australia[68,69] and the sarcodictyins were isolated from the Mediterranean coral *Sarcodictyon roseum*[70]. Despite the distant geographical locations of the marine soft corals from which these compounds were isolated, the core structure of eleutherobin and the sarcodictyins are almost identical. Their activities resemble the microtubule-stabilizing functions of Taxol with slightly reduced potencies. However, interest in these compounds has been diminished because they express cross-resistance in cells that are P-gp-positive[71]. It is possible, however, that these compounds might possess advantages that have not yet been realized.

The laulimalides, laulimalide and isolaulimalide (Fig. 11), are another group of active microtubule-stabilizing natural products isolated from a lipophilic extract of the marine sponge *Cacospongia mycofijiensis*, found in the Marshall Islands territory of the Pacific Ocean[72]. Others had previously isolated laulimalide from sponges collected in Indonesia, Vanuatu and Okinawa (Japan). The laulimalides possess activities similar to that of Taxol but, like epothilone and discodermolide, they maintain their potency in cells that express P-gp (Ref. 72).

Common pharmacophore studies

The similarity in the biological activities of Taxol, the epothilones, discodermolide and eleutherobin has triggered a search for a common pharmacophore for these molecules. The goal has been to identify common structural properties among these compounds and to use the information to design antitumor drugs with superior therapeutic activity. Several models have been published over the past five years.

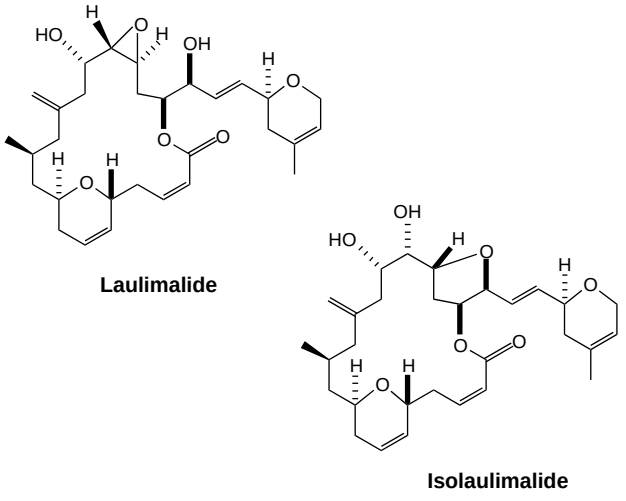


Figure 11. Chemical structures of the laulimalides.

Early models

The first common pharmacophore models that were proposed were based on a comparison of the chemical structures of Taxol and the epothilones. In 1996, Winkler and Axelsen proposed that the macrolide ring of the epothilones matches with a hypothetical side-chain complex formed by the C-2 and C-13 side-chains of Taxol and that the thiazole side-chain in the epothilones mimics the acetyl group at the C-10 position in the taxane ring[73]. In 1999, the laboratories of Ojima and of Snyder proposed two additional models. In the Ojima model, the macrolide ring of the epothilone molecule was also matched with the putative side-chain complex formed by the C-2 and C-13 side-chains of Taxol[74]. However, the thiazole side-chain was superimposed with the C-13 side-chain of Taxol. The model proposed by Snyder and his collaborators in 1999 has similarities to those reported by Winkler and by Ojima, that is, the macrolide ring and the thiazole side-chain of the epothilones match with the side-chain complex and the C-13 side-chain of Taxol, respectively[75]. None of these models took into account the structure of the tubulin dimer.

Closer examination of the crystal structure of α,β-tubulin and the sites of interaction between Taxol and β-tubulin, made it obvious that the search for a common pharmacophore should be performed in the context of the structure of the α,β-tubulin dimer. For example, the side-chain complex formed by the C-2 and C-13 side-chains of Taxol might not really exist when Taxol binds to β-tubulin, because the C-2 and C-13 side-chains are located on the opposite sides of His227 in β-tubulin (Fig. 3). The formation of a complex between the C-2 and C-13 side-chains might not allow their interaction with their binding sites in β-tubulin.

Recent models

The development of a 3.7 Å structural model of α,β-tubulin by Nogales and Downings[14,15] has made it possible to model microtubule-stabilizing agents in the context of its binding target. In 2000, Fojo and his collaborators[51] proposed two possible common pharmacophore models using the β-tubulin mutations identified in epothilone-resistant cells as an indirect indication of the binding sites of the epothilones in β-tubulin. Because the mutations found in the epothilone-resistant cells, β274Thr to Ile and β282Arg to Gln, are located near the taxane binding site in β-tubulin, they proposed that the macrolide ring of the epothilones mimics the taxane ring[51]. Because of the lack of accurate information on the binding of the epothilone molecule in β-tubulin, they were unable to identify the orientation of the thiazole side-chain and, therefore, proposed two

alternative models: in Model I, the thiazole side-chain was matched with the C-2 side-chain of Taxol, and in Model II, the thiazole side-chain was superimposed with the C-13 side-chain of Taxol. Our group also published a common pharmacophore model in 2000, based on the study of a biologically active baccatin III analog and the model is essentially equivalent to the Model I described by Fojo. We hypothesized that the thiazole side-chain of epothilone corresponds to the C-2 side-chain of 2-*m*-azido baccatin III and that both side chains bind to a pocket formed by His227 and Asp224 in β-tubulin (Fig. 12)[76]. The macrolide ring system overlaps with the taxane ring system and binds to a pocket composed of Thr274, Gly368, His227 and Asp224.

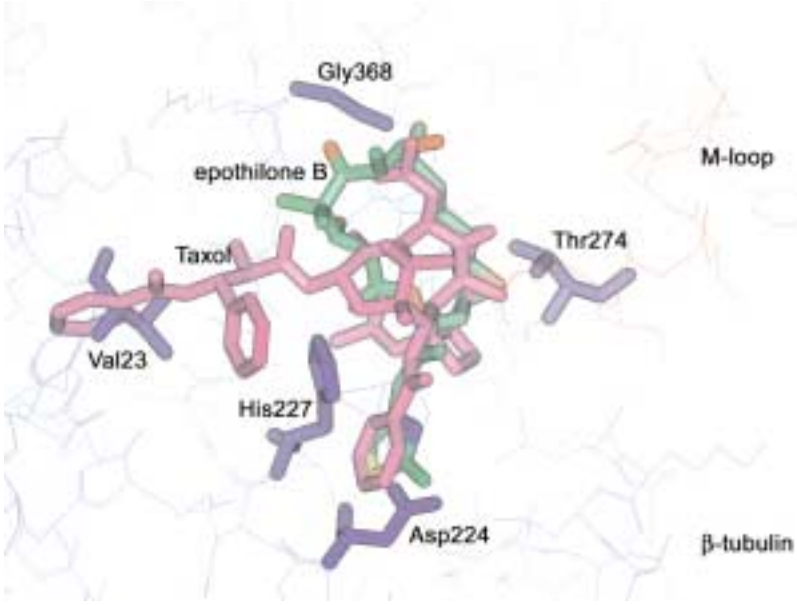


Figure 12. Common pharmacophore model for Taxol™ and epothilone B. The macrolide ring and the thiazole side-chain of epothilone B match with the taxane ring and the C-2 benzoyl ring of Taxol, respectively.

Common features of microtubule-stabilizing compounds

Taxol, the epothilones, eleutherobin/sarcodictyins and laulimalide have distinct chemical structures. However, some common features emerge as we examine the composition of these molecules; they all contain a core ring system and one or two side chains. The core ring systems for Taxol, the epothilones, eleutherobin/sarcodictyin and the laulimalides are composed of 14, 16, 14 and 20 members, respectively. The side chains of these compounds all contain a 2–4 carbon long linker connected with an aromatic ring. These common features suggest that the core ring systems and the side chains of these compounds might bind to common binding sites in β-tubulin, resulting in a similarity in their biological functions.

Although the discodermolide molecule does not possess a closed core ring system, studies of its conformation in solution revealed that its backbone folds into a ring-like structure[77,78]. It is possible that it binds to the same, or overlapping, binding sites in β-tubulin as other microtubule-stabilizing agents with the folded main chain matching the core ring systems and the C-19 side-chain or the lactone ring mimicking the side chains.

The key to the precise modeling of these compounds is their correct docking in β-tubulin. At this time, because of a lack of information describing direct interactions between these molecules and their binding sites, the models are mostly hypothetical. Results obtained from electron crystallography and photoaffinity labeling studies that examine direct interactions between ligands and their binding sites will enable the accurate positioning of these molecules in β-tubulin.

Future directions

Although we praise the discovery of novel microtubule-stabilizing molecules, we also realize that many issues must be addressed before these molecules can be used as antitumor drugs in humans.

Toxicity and pharmacokinetics

The success of an antitumor drug represents a balance between its therapeutic effect and toxicity. The ideal antitumor drug is one that is safe to the host, but kills malignant cells. In the studies of the newly discovered microtubule-stabilizing agents discussed here, emphasis has been placed on their superior potency compared with Taxol and their activity in MDR cells that express P-gp. However, the delivery and metabolism of drugs in the human body is far more complicated than that which can be observed in the laboratory. For example, the endogenous expression of P-gp in the gastrointestinal tract might be an essential detoxification mechanism for the human body[79–81]. Cytotoxic agents that are not substrates for P-gp, such as the epothilones and discodermolide, might be superior to Taxol in treating MDR-cancers and might not induce the MDR phenotype in tumors. However, they might have more severe toxic

side-effects to the host. Therefore, the toxicity of these molecules in humans must be examined carefully .

Pharmacokinetics also has an important role in the treatment of cancer because the antitumor drugs must remain in the body at reasonable concentrations for a sufficient period of time to exert their therapeutic effects. It is not clear whether these novel agents can be formulated and administered to achieve these criteria because their water-solubility, metabolism and molecular weights are different from those of Taxol.

Drug resistance

By nature, cancer cells are vulnerable to mutations and tend to grow without proper control. Although some of these novel natural products could circumvent P-gp-mediated drug resistance, cancer cells might develop other mechanisms to enhance their survival in the presence of these drugs. As previously discussed, cells treated with the epothilones acquired mutations in β-tubulin that were either related to decreased drug-binding or the presence of less-stable microtubules that could counteract the microtubule-stabilizing activity of the epothilones. Such cells carrying β-tubulin mutations are cross-resistant to the taxanes. This indicates that mutations in β-tubulin might be a potential mechanism of drug resistance in cancers treated with these novel microtubule-stabilizing agents. Cancer patients that were treated previously with Taxol or Taxotere and, therefore, selected for mutations in β-tubulin, might have a poor response to these new agents[82]. Alterations in the expression or activity of endogenous factors that regulate the stability of microtubules, such as the MAP family or stathmin, could also impact on the sensitivity of cancer cells to microtubule-stabilizing drugs.

Targeting of cancers

One limitation of microtubule-stabilizing drugs is that they are toxic to almost all of the cells in the body because of the ubiquitous presence of microtubules. The compounds themselves lack the ability to either target a specific cancer or to distinguish cancer cells from normal cells. However, with new drug delivery technologies and increased knowledge of the chemistry of these molecules, it might be possible to introduce cancer-targeting capabilities to these microtubule-stabilizing drugs by linking them with cancer-targeting vehicles, such as antibodies specific for cancer cells[83,84], or by combining them with regimens that might sensitize cancer cells to microtubule-stabilizing drugs. The discovery of these novel microtubule-stabilizing agents presents us with new alternative drugs and promises the development of antitumor drugs that act like Taxol but could possess superior therapeutic activity.

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