

# Microtubule-Binding Natural Products for Cancer Therapy

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## Abstract

Natural products, especially microtubule-binding natural products, play important roles in the war against cancer. From the clinical use of vinblastine in 1961, paclitaxel in 1992, to ixabepilone in 2007, microtubule-binding natural products have continually contributed to the development of cancer therapy. The present review summarizes the development of representative microtubule-

binding natural products including agents binding to the colchicine-binding site, the *Vinca* alkaloid-binding site, the taxane-binding site and other binding sites. Future directions for the development of new anticancer microtubule-binding natural products are discussed. Finding new formulations, new targets and new sources of microtubule-binding natural products may enable more members of this kind of agent to be introduced into the clinic for cancer therapy.

## Introduction

Natural products are excellent sources for drug discovery and development. For cancer therapy, many anticancer agents used in the clinic are either natural products or derivatives from natural sources including plants, animals and microorganisms (also of marine origin). For example, vincristine, irinotecan, etoposide and paclitaxel are plant-derived compounds; actinomycin D, mitomycin C, bleomycin, doxorubicin and L-asparaginase are drugs coming from microbial sources, and citarabine is the first drug with a marine origin [1,2]. In the past 50 years of war against cancer, these natural products have saved or prolonged the lives of millions of cancer patients. However, most of these natural products are cytotoxic agents targeting nonspecific targets expressed by both cancer cells and, to a lesser extent, by normal proliferating cells. Therefore, the use of these agents has been limited by their lack of selectivity between cancer cells and normal cells. Furthermore, with the development of targeted cancer therapies using monoclonal antibodies and synthetic protein kinase inhibitors since the late 1990s, cytotoxic natural products have gradually fallen out of fashion. As a result, no novel category of natural products for the treatment of cancer was approved by the FDA from 1997 to 2006. Nevertheless, the advance-

ment of targeted therapies has been slow and is still not able to meet the large and urgent need for novel cancer therapy agents, especially for solid tumors. Therefore, recently, natural products have become attractive again as one of the most important sources of innovative drugs. In 2007, two natural products, ixabepilone and temsirolimus, were approved by the FDA for cancer therapy. These facts suggest a possible new wave of natural products in oncology [3].

In the present review, we will focus on microtubule-binding natural products used for cancer therapy. Microtubules have been and will still be one of the important targets for future cancer drugs because of their distinct roles in cell division and universal presence in all eukaryotic cells. Microtubule-binding natural products, including *Vinca* alkaloids and taxanes, have been the most commonly prescribed anticancer therapies for decades. Many of these drugs remain among the most widely prescribed today for the treatment of cancer. Furthermore, one of the newly approved anticancer natural products in 2007, ixabepilone, is also a microtubule-binding agent. A number of microtubule-targeting natural products are still in clinical trials. In this review, the microtubule and the general anticancer mechanism of microtubule-binding agents are introduced. Then, representative microtubule-binding natural products with anticancer effects are re-

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viewed. These natural products are classified according to their binding sites on the microtubule, i.e., the colchicine-binding site, the *Vinca* alkaloid-binding site, the taxane-binding site or other binding sites. For each class of natural products, information provided to show its development includes the natural source of isolation, chemical structure characteristics, potent synthesized analogues, mechanism of microtubule-binding and anticancer effects, and others. By analyzing the development of microtubule-binding natural products, it might be possible to clarify directions for future development of this kind of anticancer agent. Published papers related to the topic of present review were searched in Pubmed using key words such as “microtubule and cancer” and more specific ones like “taxanes”. References were included or excluded based on the type of papers, time of publication and citation rates. Generally, reviews and recently published research papers with high citation rates were used for the present review.

## Microtubules

Microtubules, actin microfilaments and intermediate filaments are the main components of the cytoskeleton. Microtubules are hollow rods with an outer diameter of about 25 nm and an inner diameter of about 14 nm. Microtubules are composed of molecules of tubulin, a heterodimer consisting of two tightly linked polypeptides called  $\alpha$ -tubulin and  $\beta$ -tubulin [4]. Up to now, six isotopes of  $\alpha$ -tubulin and seven isotopes of  $\beta$ -tubulin have been identified, showing tissue-, cell- and tumor-specific patterns of expression as well as differences in drug binding. The heterodimers of  $\alpha$ -tubulin and  $\beta$ -tubulin assemble head to tail into linear protofilaments. Then, thirteen protofilaments further organize into a hollow cylinder, the backbone of the microtubule. The final structure of the microtubule is organized in a polar manner with  $\alpha$ -tubulin exposed at one end (the minus end) and  $\beta$ -tubulin exposed at the other end (the plus end) [5]. Notably microtubules are highly dynamic structures, which means that they are in continuous assembly/disassembly. The functions of microtubules include controlling the position of organelles, directing intracellular trafficking of vesicles, organelles and proteins, and pulling the chromosomes apart at mitosis [6]. The dynamic structures are perfectly equipped to transmit signals throughout the cell. In particular, when cells enter mitosis, the microtubule network is reorganized from an intracellular lattice-like structure into the mitotic spindle. The proper orientation and segregation of chromosomes require highly coordinated microtubule dynamics. Therefore, microtubules are intimately involved with the replication of cells [7]. In all, the correct arrangement of microtubules is essential for intracellular trafficking, cellular motility and mitotic chromosome segregation.

## Microtubule-Binding Reagents and their Mechanisms in Cancer Therapy

### Microtubule-binding reagents

Since microtubule dynamics play an indispensable role in cell division, cell motility, cellular transport, cell polarity and cell signaling, the microtubule appears as a highly attractive target for anticancer drug design. An increasing number of antitumor drugs target microtubules and exhibit their anticancer activities by altering the polymerization dynamics of microtubules, disrupting

mitosis, and thus inhibit cell proliferation and induce apoptosis. In fact, in view of the success of this class of drugs, it has been argued that microtubules represent the single best cancer target identified to date and microtubule-targeted drugs might continue to be an important chemotherapeutic class of drugs [8,9]. Generally, on the basis of their effects on microtubule assembly at high concentrations, microtubule-binding agents are usually divided into two distinct categories: microtubule-stabilizing and microtubule-destabilizing agents. Microtubule-stabilizing agents stabilize tubulin polymers by initiating tubulin polymerization as well as hyper-stabilizing existing microtubules under normally destabilizing conditions. Microtubule-destabilizing agents destabilize microtubules by inhibiting the assembly of tubulin heterodimers into microtubule polymers or depolymerizing existing ones [10,11]. And, according to the difference in binding site, microtubule-binding agents can be divided into (1) agents binding to the colchicine-binding site; (2) agents binding to the *Vinca* alkaloid-binding site; (3) agents binding to the taxane-binding site; or (4) agents binding to other sites. The colchicine site is located at the interface between  $\alpha$  and  $\beta$  subunits of the tubulin dimer, adjacent to the GTP-binding site of  $\alpha$ -tubulin. The *Vinca* alkaloid-binding site is close to the exchangeable GTP site on  $\beta$ -tubulin. The taxane-binding site is the NH<sub>2</sub> terminal 31 amino acids of  $\beta$ -tubulin, a deep hydrophobic pocket.

### Targets of microtubule-binding reagents in cancer therapy

The targets of microtubule-binding agents in cancer therapy include both cancer cells and vascular endothelial cells. Microtubule-binding agents, either microtubule-stabilizing agents or microtubule-destabilizing agents, could cause disruption of microtubule dynamics and subsequent mitotic arrest and cell death in cancer cells. The mechanism of their cytotoxic effects on tumor cells has been well studied [12,13]. The clinical success of the presently available microtubule-binding agents in cancer therapy was based mostly on their direct cytotoxic effects on tumor cells. Meanwhile, recent studies have suggested that vascular endothelial cells could be another important target of microtubule-binding agents in cancer therapy. Endothelial cells are highly dependent on the tubulin cytoskeleton for their normal functions, including motility, invasion, attachment, alignment and proliferation. Furthermore, compared with normal endothelial cells, tumor-related endothelial cells are much more sensitive to the activity of microtubule-binding agents [14]. Microtubule-binding agents could exhibit both antiangiogenic and vascular-disrupting actions and their multiple actions on endothelial cells cause a much greater reduction in the blood flow of tumors than that of normal tissues [15]. Both preclinical and clinical studies have suggested that microtubule-binding agents might be a particularly useful class of drugs for vascular-targeted therapy [16,17]. Efforts have been made to isolate or rescreen microtubule-binding agents whose actions more selectively target the tumor vasculature relative to their direct cytotoxic effects on cancer cells. Combretastatins are the first microtubule-binding agents identified to have tumor vascular disrupting activity at well-tolerated doses [18]. Combretastatin A-4 phosphate exhibits antiangiogenic activity both *in vitro* and *in vivo* [19] and shows promising results in clinical trials [20]. Moreover, other vascular-targeted agents such as CA-1-P (Oxi4503), AVE8062, ABT-751, TZT-1027, CYT997, Dolastatin 10, MPC-6827 (Azixa), NPI-2358, EPC2407, and MN-029, have also progressed to clinical trials for cancer [21].

### Drug resistance of microtubule-binding agents

Like other antimitotic agents, the usefulness of microtubule-binding agents is often limited by the development of drug resistance. Drug resistance to microtubule-binding agents could be mediated by multiple mechanisms. Among the causes of drug resistance, multidrug resistance (MDR) mediated by ATP-binding cassette (ABC) transporters or alteration of tubulin, the target of microtubule-binding agents, might be the most important [22–24]. MDR is defined as the acquired resistance of cancer cells to many chemically diverse anticancer drugs with different mechanisms of action [25]. The predominant cause of MDR is the overexpression and increase in drug transport activity of ABC transporters. In addition, P-glycoprotein (P-gp), also known as ABCB1 or MDR1, is arguably the most important member of the ABC family [26]. The ABC transport systems contain two nucleotide-binding domains and two membrane domains. ATP is bound and hydrolyzed at the nucleotide-binding domains and the vectorial transport of substrates across the cell membrane is mediated by the membrane domains. Recently, the crystal structures of the bacterial P-gp homologues had been reported and the results shed light on the possible conformational states adopted by ABC transporters during transport [27]. MDR mediated by ABC transporters might affect the efficacy of microtubule-binding agents that could be recognized and transported by ABC transporters such as P-gp. Besides ABC transporters, alterations in tubulin such as aberrant expression of class III  $\beta$ -tubulin as well as changes in microtubule regulation were also associated with MDR to microtubule-binding agents [22]. The key difference between class I and class III  $\beta$ -tubulin is an amino acid substitution (Arg277 in class III  $\beta$ -tubulin instead of Ser277 in class I  $\beta$ -tubulin) leading to a different three-dimensional conformation. The changed structure of class III  $\beta$ -tubulin might prevent stable binding of microtubule-binding agents such as taxanes. Furthermore, expression of class III  $\beta$ -tubulin might also generate more dynamic microtubules and counteract the preassembling activity of taxanes at the plus ends of microtubules [28]. A recent study showed that the aberrant expression of class III  $\beta$ -tubulin caused drug resistance of microtubule-binding agents binding to taxane or *Vinca* alkaloid binding sites but showed less effect on agents binding to the colchicine-binding site [29].

### Natural Products with Microtubule-Binding Activity

#### ▼ Natural products binding to the colchicine-binding site

**Colchicinoids:** Colchicine is a highly soluble alkaloid isolated from the meadow saffron, *Colchicum autumnale*. It is a well-studied tubulin-binding agent. In fact, in the past, tubulin was even referred to as “colchicine binding protein”. Colchicine could cause microtubule depolymerization by forming a stable complex with unpolymerized tubulin heterodimers. However, it would cause severe toxicity at the doses required for exhibiting anticancer effects. Therefore, colchicine is only used in therapy for gout but not for cancer. Recently, ZD6126, a synthesized colchicine derivative, entered clinical trials as a vascular-targeting drug [30]. It is a water-soluble phosphate pro-drug and would be converted *in vivo* into *N*-acetylcolchinol (ZD6126 phenol), which binds to the colchicine-binding site on tubulin. ZD6126 affects endothelial cell morphology and disrupts newly formed vessels. In an animal tumor model, it selectively induced tumor vascular damage and massive tumor necrosis at well-tolerated doses [31]. Results of a phase 1 clinical trial of ZD6126 indicated

that it was well tolerated and the side effects included mild but manageable gastrointestinal adverse events and dose-related cardiac toxicities [32].

**Combretastatins:** Combretastatin A-4 (CA4) was first isolated from the South African willow, *Combretum caffrum* in 1982. One of the advantages of CA4 is that it is not recognized by the ABC transporters so CA4 does not induce MDR mediated by ABC transporters. Since CA4 has very limited water solubility, water-soluble pro-drugs such as combretastatin A4 phosphate (CA4P) have been synthesized. Several combretastatins, including CA4, CA4P and AC-7700 (AVE-8062), are now in clinical studies. Like ZD2126, these reagents also target endothelial cells and cause disruption of the endothelial cytoskeleton by acting at the colchicine-binding site of the  $\beta$ -subunit of endothelial tubulin [33]. In addition to this, CA4P is also able to interfere with vascular endothelial-cadherin signaling. By inducing regression of unstable emerging tumor neovessels, CA4P demonstrated the ability to selectively target and disrupt tumor vasculature [34].

#### Natural products binding to the *Vinca* alkaloid-binding site

***Vinca* alkaloids:** *Vinca* alkaloids were originally isolated from the periwinkle plant *Catharanthus roseus*, also known as *Vinca rosea*. *Vinca* alkaloids are dimeric asymmetrical compounds with two multi-ringed subunits, vindoline and catharantine, linked by a carbon-carbon bridge. Vincristine (Oncovin), vinblastine (Velban) and vindesine are the first generation of *Vinca* alkaloids with antitumor activity. Vinblastine and vincristine were approved for clinical use in 1961 and 1963, respectively. The success of natural vincristine and vinblastine has led to the development of semisynthetic agents, including vindesine, vinorelbine and vinflunine. *Vinca* alkaloids have a well-established role in treating a variety of malignancies including hematological and lymphatic neoplasms as well as solid tumors such as breast cancer, testicular cancer, choriocarcinoma and non-small cell lung cancer. However, the use of *Vinca* alkaloids is restricted by drug resistance mediated by P-gp and side effects including myelosuppression and neurotoxicity [35–37].

Vinflunine (Javlor) is now still in a phase III clinical trial. It is the first fluorinated agent in the *Vinca* alkaloids family and is obtained by semisynthesis using superacid chemistry to selectively introduce two fluorine atoms at the 20 position of the catharanthine moiety. Interestingly, its non-fluorinated counterpart shows no similar antitumor activity which suggests the essential contribution of the fluorine atoms to the antitumor activity. Similar to other *Vinca* alkaloids, vinflunine suppresses microtubule dynamics by interacting with the *Vinca* alkaloid binding site on tubulin. However, compared with other *Vinca* alkaloids, vinflunine has the weakest affinity for tubulin and the binding of vinflunine to tubulin is more readily reversible. These differences might lead to specific effects of vinflunine on cell killing and the relatively low toxicity of vinflunine. Vinflunine also induces drug resistance mediated by P-gp but the potency of vinflunine to induce drug resistance is far weaker than that of vinorelbine. And, vinflunine is effective on human tumor cell lines with an atypical (non-Pgp dependent) multidrug-resistant phenotype. Besides, vinflunine has vascular-disrupting and antiangiogenic activities both *in vitro* and *in vivo* [38, 39]. In clinical trials, the main side effects induced by vinflunine, myelosuppression and constipation, are apparently more manageable compared to those of other *Vinca* alkaloids [40]. In all, compared with vinorelbine, vinflunine shows better functions in efficacy, tolerability and range of activity [41–43].

**Hemiasterlins:** Hemiasterlins are a family of natural peptides previously isolated from marine sponges (*Cymbastela* sp., *Hemiasterella minor*, *Siphonochalina* sp., and *Auletta* sp.). They are tripeptides composed of three sterically congested amino acids that are responsible for their activities. The three unusual amino acids in hemiasterlin A are trimethyltryptophan, *tert*-leucine and *N*-methylhomo-vinyllogous valine. By binding to the *Vinca* alkaloid-binding site of tubulin, hemiasterlins are highly potent in the suppression of microtubule depolymerization [44,45]. The total synthesis of hemiasterlins and analogues has been accomplished. The synthesis of hemiasterlin was first reported in 1997 and a potent derivative talbotulin (HTI-286, SPA-110), wherein a phenyl group replaces the 3-substituted indole ring, was synthesized in 2003 [46]. HTI-286 showed potent *in vivo* cytotoxicity and antimetabolic activity comparable to those of vincristine and paclitaxel and also had the advantage of circumventing drug resistance mediated by P-gp [47]. Extensive structure–activity relationship studies of HTI-286 analogues had been conducted and other superior analogues such as HTI-042 were discovered [48]. Both HTI-286 and hemiasterlin are now in clinical trials.

### Natural products binding to the taxane-binding site

**Taxanes:** Taxanes, specifically paclitaxel (Taxol), was initially extracted from the bark of the Pacific Yew, *Taxus brevifolia*. The anticancer activity of paclitaxel was found during a National Cancer Institute screen of plant extracts in the 1970s. The sample used was an extract collected by the U.S. Department of Agriculture in 1962 [49]. Paclitaxel was approved for clinical use in 1992. Now, paclitaxel is obtained by a semisynthetic method from 10-deacetylbaccatin III, which is extracted from the needles of the European yew tree, *Taxus baccata*. Docetaxel (Taxotere), a semisynthetic taxane approved for clinical use in 1996, was also synthesized from 10-deacetylbaccatin III. Paclitaxel and docetaxel are both hydrophobic compounds with a taxane ring core, esterification at the C-13 position with a complex ester group, and an unusual fourth ring at the C-4,5 position. Paclitaxel and docetaxel both have the tricyclic taxane skeleton while the distinction between them is the different substituents at C-10 and on the C-13 side chain [50]. By binding to  $\beta$ -tubulin, taxanes promote the polymerization of tubulin, stabilize microtubules, and perturb microtubule dynamics, which eventually leads to apoptosis. The binding mechanism of taxanes with microtubules has been well studied. They bind to polymerized microtubules within the lumen of the polymer and stabilize GDP-bound  $\beta$ -tubulin protofilaments by straightening them into a conformation resembling the more stable GTP-bound structure [51–53]. Since docetaxel has a higher affinity for the taxane-binding site than paclitaxel, its effects on cancer cells are also different from that of paclitaxel. For example, paclitaxel induces cell cycle arrest at the G2-M phase while docetaxel exerts its maximum cell-killing effect on cells in the S phase. Peripheral neuropathy, one of the side effects of taxanes, appeared less frequently and less severely under docetaxel treatment than under paclitaxel treatment [54]. At present, both paclitaxel and docetaxel are widely used in the treatment of solid tumors including breast cancer, ovarian cancer and lung cancer. The clinical use of the taxanes is limited mainly by drug resistance and toxicity. The resistance to taxanes is multifactorial and might involve the overexpression of the membrane efflux pump P-gp, tubulin mutations and increased microtubule dynamics associated with altered microtubule-associated protein (MAP) expression [55]. Taxane treatment has been shown to induce P-gp expression, leading to acquired drug resistance. Efforts have been

continually conducted to find Pgp inhibitors for combination therapy with taxanes [56,57]. And, another important cause of taxane resistance is the unnormal expression of the class III isotype of  $\beta$ -tubulin [58,59]. The toxic effects of taxanes include neutropenia, mucositis, neuropathy and hypersensitivity reactions. The hypersensitivity reactions are mainly caused by formulations used to improve the solubility of these drugs. Due to the poor solubility of these drugs, they have to be administered in formulations including surfactants such as polyoxyethylated castor oil (Cremophor) or polysorbate.

New members of the taxanes family with higher activity and lower toxicity are being continuously developed. Derivatives of paclitaxel overcoming transport-based resistance for taxanes have been designed and synthesized. XRP9881 (Larotaxel) and TPI287 are semisynthetic paclitaxel derivatives that circumvent the taxane resistance mediated by P-gp because they are poor substrates for P-gp. XRP9881 (Larotaxel) and TPI287 are now both in phase II clinical trials [4].

To ameliorate the toxicity caused by a vehicle, new formulations of taxanes are being developed and several of them are currently progressing through the clinic [60]. New formulations such as albumin, nanoparticles, emulsions, liposomes, and polyglutamates are being developed to handle the poorly soluble taxanes. For example, ABI-007 (Abraxane) is a novel albumin-stabilized, nanoparticle (mean particle size of about 130 nm) form of paclitaxel for injectable suspension. It shows superior efficacy and less toxicity than Cremophor-containing paclitaxel. The increased solubility of ABI-007 could strongly decrease the time required for drug administration, from 3 h to 30 min [61,62]. ABI-007 was approved by the FDA in January 2007 for the treatment of metastatic breast cancer and is still undergoing further clinical trials against other solid tumors. ANG1005 (Angiochem) is another modified formulation of paclitaxel. Containing paclitaxel molecules conjugated to a receptor-targeting peptide, ANG1005 is selectively transported across the blood-brain barrier and as such shows efficacy against intracerebral tumors in mice [63]. ANG1005 is now in early stage clinical trials.

**Epothilones:** Epothilones belong to a new family of non-taxane microtubule-stabilizing agents. Naturally occurring macrolides, such as epothilone A and epothilone B, were isolated as secondary metabolites from the myxobacterium *Sorangium cellulosum* in the early 1990s [64]. The common structure of the epothilones is a 16-membered ring macrolide that is covalently attached to a methylthiazole side chain. Based on the presence of or absence of an epoxide group in the C-12 to C-13 position of the macrolide ring, naturally occurring epothilones can be classified as epoxides (epothilones A, B, E, and F) or olefins (epothilones C and D). The myxobacterial origin of epothilones makes them relatively easy to be cultured and isolated on a large scale [65].

Ixabepilone (aza-epothilone B, BMS-247550), the newest epothilone approved for clinical use in 2007, was semisynthesized from epothilone B by the substitution of an azide group for oxygen at position 16 of the macrolide ring. Several members of the epothilone family, including patupilone (EPO 906, a natural epothilone B), KOS-862 (epothilone D), BMS-310705 (a second-generation epothilone B), ZK-EPO (a third-generation epothilone B) and KOS-1584 (a second-generation epothilone D) are under clinical trials [66]. Since epothilones compete with paclitaxel in tubulin-binding, it is proposed that they might target at or near the same binding site of taxanes on  $\beta$ -tubulin. Therefore, these two compound families have almost identical targets and mechanisms of action. The marked difference in chemical structure supports the

difference in function between epothilones and taxanes. The most important difference is that epothilones can overcome drug resistance mediated by P-gp. Therefore, compared with taxanes, epothilones exhibit improved pharmacological and resistance profiles both *in vitro* and *in vivo* [67–69].

### Natural products binding to other sites on tubulin

Natural products with binding sites not belonging to the three well-known tubulin-binding sites (colchicine-binding site, *Vinca* alkaloid-binding site and taxane-binding site) have been found. Among them, laulimalides, pelorusides, taccalonolides and halichondrins could serve as good examples.

**Laulimalides:** Laulimalides, also known as fijianolides, are a family of polyketide natural products of marine origin. They can be isolated from several species of marine sponges. Laulimalide (fijianolide B) and its isomer isolaulimalide (fijianolide A) were isolated almost contemporaneously by two groups from the marine sponges *Cacospongia mycofijiensis*, respectively *Hyatella* sp. and a nudibranch predator *Chromodoris lochi* in 1988. From the sponge *Fasciospongia ramosa*, a third isomer named neolaulimalide as well as laulimalide and isolaulimalide were isolated in 1996. Further studies led to the isolation of six additional fijianolides (D – I) from *Cacospongia mycofijiensis* [70].

Laulimalides are microtubule stabilizers. Like taxanes, laulimalide could block normal mitotic spindle formation and cause condensation of disorganized chromosomal material in the center of cells. The fact that they can show synergism with taxanes indicates that they have a non-taxane binding site on tubulin [71]. Besides, laulimalides show antiangiogenic activities and they are effective on P-gp overexpressing cell lines [72, 73]. However, the clinical usefulness of laulimalides might be limited by their high toxicity. A number of laulimalide derivatives are continuing to be synthesized and subjected to pharmacological evaluation. Compounds with retained efficacy but with decreased toxicity might hopefully be found in the future [74–76].

**Pelorusides:** Peloruside A is a polyoxygenated 16-membered macrolide of marine origin. In 2000, it was isolated as a secondary metabolite from the New Zealand marine sponge *Mycale hentscheli* [77]. Its 16-membered macrolide ring is similar to that of the epothilones. Peloruside A shows toxicity at nanomolar concentrations and it works in a manner similar to paclitaxel by stabilizing the polymerized form of microtubules. Like laulimalide, peloruside A also has a non-taxane binding site and showed synergism with taxanes [78–80]. Recent studies indicate that peloruside A might bind within a pocket on the exterior of  $\beta$ -tubulin at a previously unknown ligand site, rather than on  $\alpha$ -tubulin as suggested in earlier studies. The effects of peloruside A arise from interactions with the  $\alpha/\beta$ -tubulin intradimer interface as well as protofilament contacts [81]. A number of synthetic studies about peloruside A have already been reported [82]. Peloruside B, possessing the 3-de-O-methyl variant of peloruside A, was just recently isolated from the New Zealand marine sponge *Mycale hentscheli*. The bioactivity of peloruside B was comparable to that of peloruside A in promoting microtubule polymerization and arresting cells in the G2/M phase of mitosis [83].

**Taccalonolides:** Taccalonolides are a new class of plant-derived natural steroids with microtubule-stabilizing activity. They are the first plant-derived microtubule-stabilizing agents to be identified since paclitaxel and the first natural steroids to exhibit this activity. Taccalonolide A was first isolated from the tropical plant *Tacca plantaginea* in 1987 and taccalonolide E was isolated in 1991. Similar to other microtubule stabilizers, taccalonoids in-

duce the formation of abnormal mitotic spindles leading to mitotic arrest, Bcl-2 phosphorylation, and initiation of apoptosis [84]. However, results of *in vitro* studies showed that taccalonoids fail to modulate tubulin assembly or to bind microtubules. These results suggested a distinct mechanism of taccalonoids compared with all other microtubule-targeting agents [85]. Recent studies showed that the advantages of taccalonoids might include efficacy in cell lines and tumors with taxane-resistance mediated by Pgp or class III  $\beta$ -tubulin. Therefore, with a distinct structure and mechanism, taccalonolides have advantages over the taxanes in their ability to circumvent multiple drug resistance mechanisms [86, 87].

**Halichondrins:** Halichondrins are large polyether macrolides first from the western Pacific sponge *Halichondria okadai* and subsequently from several unrelated sponges belonging to the *Axinella* family. All of the members of the halichondrin family possess an unusual 2,6,9-trioxatricyclo[3.3.2.0]decane ring system, as well as a 22-membered macrolactone ring, two exocyclic olefins, and an array of polyoxygenated pyran and furan rings that define three major classes of halichondrins, i.e., halichondrin A, B, and C [88]. The total syntheses of halichondrin B and norhalichondrin B as well as analogues such as E7389 have been accomplished. E7389 (eribulin mesylate) is currently in phase III clinical trials for the treatment of metastatic breast cancer. Phase I and phase II clinical trials have demonstrated that eribulin was active in heavily pretreated individuals while maintaining a tolerable therapeutic index. Its most frequent adverse effects were neutropenia and fatigue. Since halichondrins exhibit activity as a noncompetitive inhibitor of vinblastine binding to tubulin and has no effect on colchicine binding, halichondrins were considered to bind tubulin at or near the *Vinca* alkaloid-binding site. Eribulin treatment resulted in a decrease in dynamicity by suppressing the growth parameters at microtubule plus ends without affecting microtubule shortening parameters [89]. Interestingly, recent studies suggested that eribulin might bind tubulin and microtubules through a novel action. Eribulin was shown to bind to a single site on soluble tubulin with a low affinity and to a very small number of sites at microtubule ends with a high affinity. And, binding of vinblastine to microtubules inhibited eribulin binding at low eribulin concentrations but also appeared to open additional, low-affinity binding locations for eribulin, at either one or both microtubule ends [90].

### Conclusions

Microtubule-binding natural products have contributed a lot to the improvement in cancer therapy. However, the clinical use of this kind of anticancer agent is limited by induced drug-resistance and side effects, including those caused by toxic formulations. Studies related to structure modification of existing compounds are continually being conducted and have successfully introduced a number of synthesized derivatives into clinical use. More importantly, by reviewing the development of microtubule-binding natural products, it seems that the future of these kind of natural products might mainly reside in (1) new formulations, (2) new targets, and (3) new sources. Improving the formulations of the old good drugs, especially the taxanes, has led to the approval of a new formulation of paclitaxel, ABI-007, for clinical use. And, identifying vascular endothelial cells as a new target of microtubule-binding agents has led to clinical trials of a number of agents such as ZD6126 and CA4P. Furthermore, natu-

ral products with novel microtubule-binding sites such as laulimalides, pelorusides and halichondrins are all isolated from marine sources. These examples suggest the increasing importance of marine sources, an under-explored source, for the discovery of medically useful natural products. With the advances in new formulations, new targets and new sources, it is reasonable to believe that microtubule-binding natural products are and will continue to be important drugs for cancer therapy.

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