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Review Article

Natural, semisynthetic and synthetic microtubule inhibitors for cancer therapy

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Abstract

Paclitaxel, docetaxel and vinca alkaloids like vinblastine are compounds interacting with the mitotic spindle by binding to β-tubulin. They are used as therapeutics in standard chemotherapy regimens and in combination with new drugs like the HER2 targeting antibody trastuzumab. But toxicity, drug resistance, complex galenic formulations and limited bioavailability are restricting their clinical use in cancer therapy. Thus, new tubulin targeting antimitotic agents with better tolerability and efficacy against late-stage resistant tumors are urgently needed. This review summarizes tubulin targeting drugs in late preclinical and early clinical development, as well as compounds with interesting structural features. Pharmacological and clinical phase I/II data are presented for new semisynthetic paclitaxel analogues and natural compounds of diverse structure such as epothilones, combretastatins, colchinoids or dolastatins. Finally, synthetic compounds of low molecular weight but cytotoxic activity equal to paclitaxel are presented. With a clear focus on clinical efficacy and superiority to current antimitotic agents, this timely review attempts to give a perspective on the progress of an important class of anticancer drugs.

Introduction

The cell division cycle regulating chromosome replication/segregation and cell division is of fundamental importance for any living organism and in diseases such as cancer. Limitless replicative potential, self-sufficiency in growth signals and insensitivity to antigrowth signals are acquired capabilities of malignant cancer cells, leading to uncontrolled cell cycling (1). The M-phase or mitosis is the most important part of the cell division cycle and includes condensation of nuclear chromatin and disruption of the nuclear envelope, organization of a mitotic spindle, and chromosome segregation. Key players within the cell cycle, namely cyclin-dependent kinases (cdks), cdk inhibitors, microtubules and microtubule associated proteins (MAPs), have been selected as targets for the discovery of new antimitotic cancer drugs. New targets are emerging, with the family of mitotic kinases as regulators of mitotic progression and kinesins as mitotic motor proteins being the most prominent examples (2). The present review focuses on tubulin as the essential structural subunit of the mitotic spindle and, as a key player in mitosis, a target for anticancer drug development.

The mitotic spindle is a dynamic assembly of distinct proteins, namely microtubules, motor proteins of the kinesin and dynein families, MAPs and catastrophe factors (Fig. 1A) (3). It is self-organizing, using energy from nucleotide hydrolysis to segregate sister chromatids accurately into daughter cells (4). Microtubules are hollow tubes consisting of α- and β-tubulin heterodimers that polymerize parallel to a cylindrical axis (Fig. 1B) (5). Each tubulin monomer consisting of approximately 450 amino acids is formed by a core of 2 β-sheets surrounded by α-helices building 3 functional domains, namely the amino terminal region with the nucleotide binding pocket, an intermediate domain with the paclitaxel binding site and the carboxyl terminal domain constituting a putative binding surface for motor proteins and MAPs (Fig. 1C) (6, 7). There are 3 or more and 4 or more isotypes of α- and β-tubulin in humans, respectively, sharing about 40% amino acid homology (8). The functional importance of distinct tubulin isotype expression is not well defined but may be of importance for understanding the activity and toxicity profile of tubulin inhibitors in human cancer therapy. Tubulin is subject to various posttranslational modifications, namely acetylation tyrosination/detyrosination, phosphorylation, polyglutamylation and polyglycylation (8). Interestingly, histone deacetylase 6 (HDAC6) was

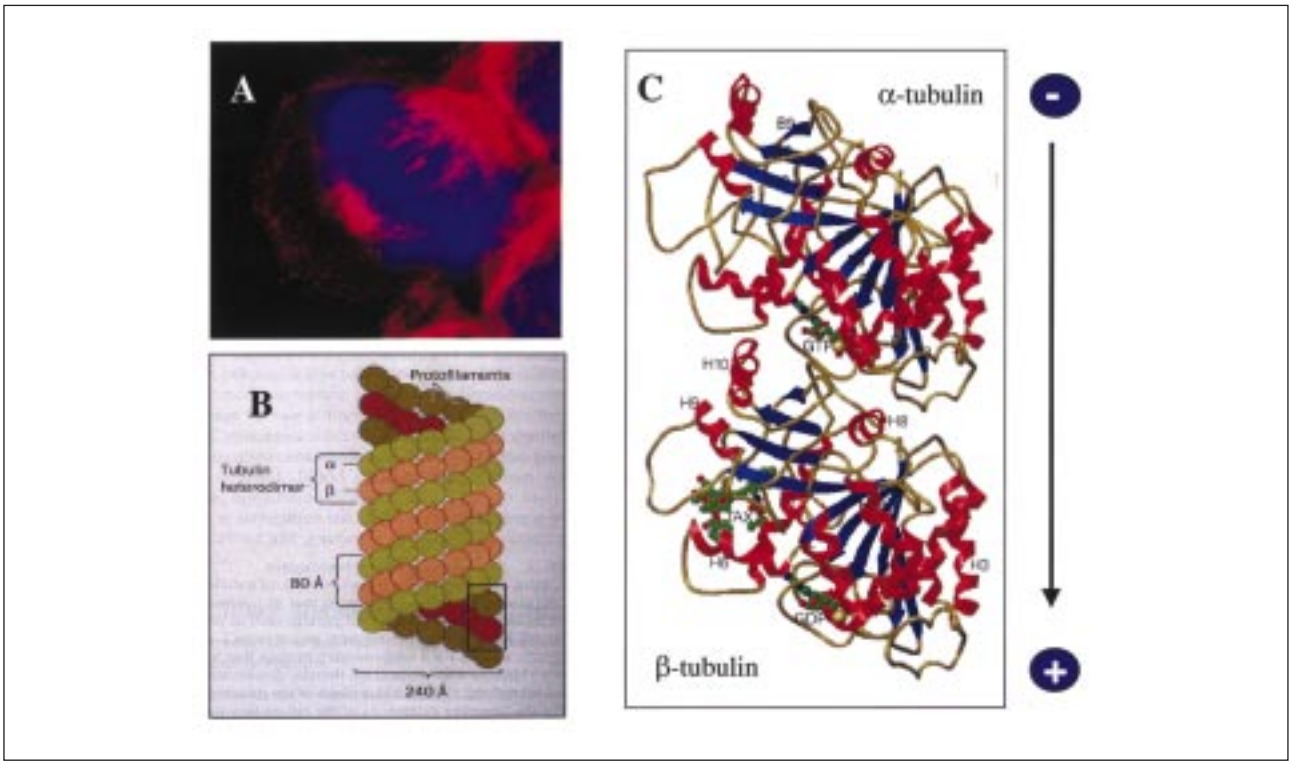


Fig. 1. Tubulin structure, microtubule organization and the mitotic spindle. The mitotic spindle is shown in (A) by staining cells for α-tubulin (antibody B-5-1-2 and a second antibody labeled with Cy3; red) and DNA (DAPI, blue). In (B) the organization of a tubulin protofilament with αβ-tubulin heterodimers as the core structural elements is schematically shown. The X-ray structure of the αβ-tubulin heterodimer at 3.7 Å resolution is shown in (C) (adapted from 6). Tubulin heterodimers are added to the "plus-end" and lost from the opposite end, the "minus end", a controlled process called treadmilling. GTP/GDP and Taxol (TAX) bound to αβ-tubulin and β-tubulin, respectively, are highlighted in green.

recently described as a microtubule-associated deacetylase (9). Mitotic microtubules are highly dynamic structures, switching between growing and shortening states, a process known as dynamic instability (3, 10). Tubulin subunits are added to one end (the "plus end") and lost from the opposite end (the "minus end") in a balanced process called treadmilling (Fig. 1). MAPs bind to and stabilize microtubules by reducing the catastrophe rate or increasing the polymerization rate (11, 12). Besides their function in mitosis, microtubules are involved in the organization and guidance of vesicular and organelle transport, as an organizing principle in cell morphogenesis and as structural elements in cilia and flagella.

Colchicine (**1**), vincristine (**2**) and vinblastine (**2a**), natural compounds isolated from the meadow saffron *Colchicum autumnale* and the periwinkle *Catharanthus roseus*, respectively, were the first antiproliferative, tubulin binding agents discovered (13, 14). Colchicine and the vinca alkaloids bind to β-tubulin and interfere with the dynamic instability of microtubules by destabilization, arresting mitotic cells in the M-phase of the cell division cycle, finally leading to apoptotic cell death (Fig. 2A/B). As part of the National Cancer Institute's program to identify natural antimitotic compounds, Paclitaxel (**3**) was identified and purified from the bark of the pacific yew *Taxus brevifolia* (15). Paclitaxel binds to a distinct side within β-tubulin (Fig. 1C) (16) but, in contrast to colchicine, interferes with dynamic instability by stabilizing microtubules (Fig. 2E/F) (17).

Paclitaxel, the semisynthetic paclitaxel analogue docetaxel (**4**), vincristine and vinblastine are standard agents in cancer therapy. Despite limited availability and poor solubility, paclitaxel (Taxol®; Bristol-Myers Squibb) was approved for second-line treatment of metastatic carcinoma of the ovary in 1992 and as first-line therapy in combination with cisplatin or carboplatin in 1998. In addition to treatment of ovarian carcinoma, paclitaxel is used for second-line therapy of breast cancer and in combination with trastuzumab (Herceptin®) in metastasizing breast cancer as well as in combination with cisplatin in non-small cell lung cancer (NSCLC) (18). The worldwide annual sales of paclitaxel, docetaxel and the vinca alkaloids in 1999 were USD 1480, 490 and 320 million, respectively (source: DataMonitor, Cytotoxic Therapies 2000). Although paclitaxel, docetaxel and the vinca alkaloids are broadly used cancer drugs, there are severe drawbacks. For taxanes, a narrow therapeutic window, marginal oral bioavailability, poor solubility, toxicity

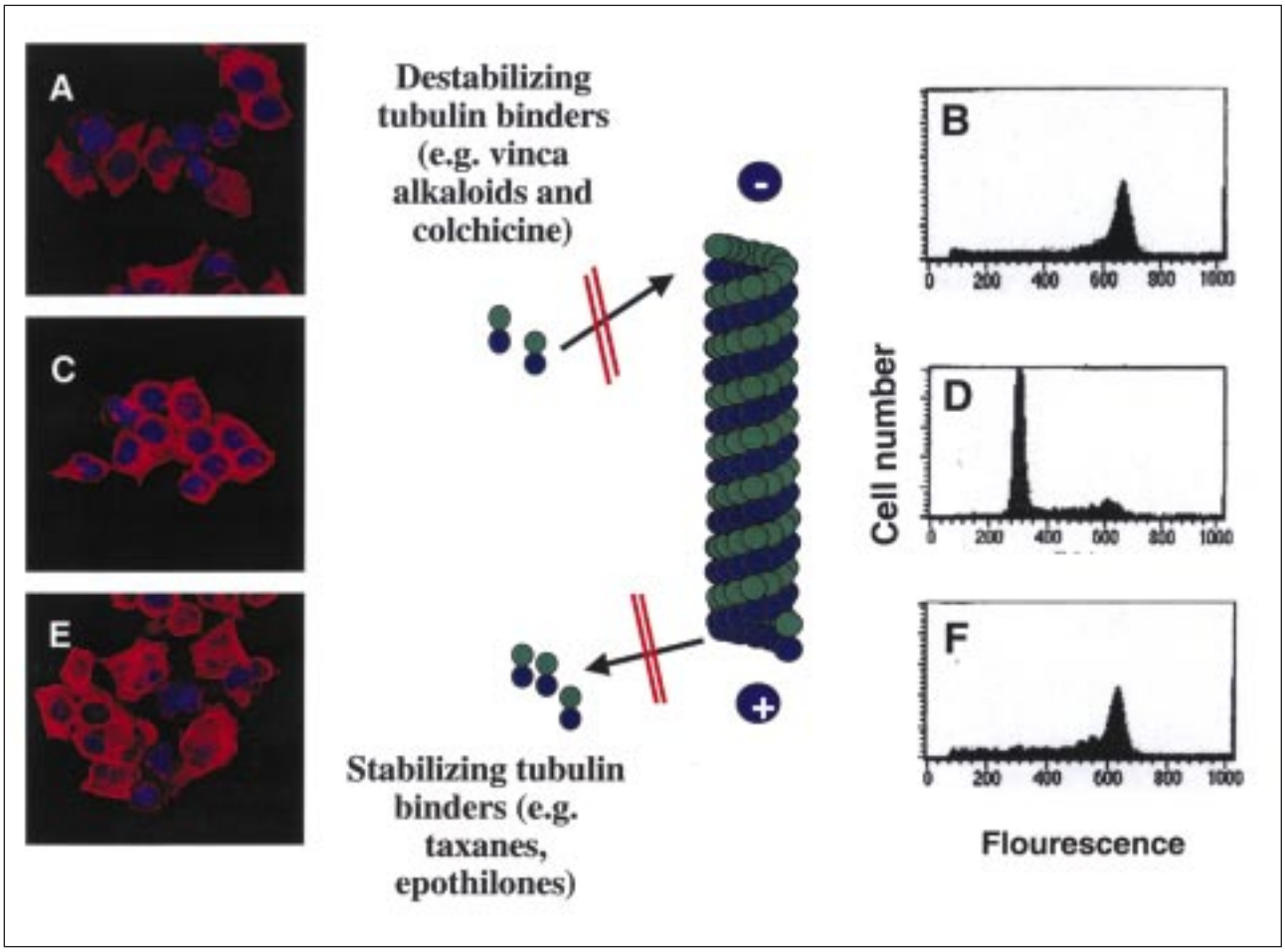
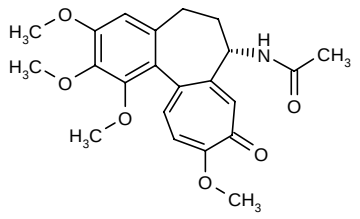


Fig. 2. Effect of tubulin-binding agents on microtubule structure and cell cycling. The effect of 1 nM vincristine as a destabilizing agent (A and B) and 7 nM paclitaxel as a stabilizing agent (E and F) on microtubule organization and cell cycle distribution compared to DMSO-treated HeLa cervical carcinoma cells (C and D) are shown. Tubulin organization was studied by nuclear DNA staining with DAPI (blue) and tubulin visualization using an antibody specific for α-tubulin (clone B-5-1-2) and a Cy3 conjugated goat-anti-mouse antibody (red). Cells were analyzed by confocal microscopy and representative selections of cells treated with vincristine (A), DMSO (C) or paclitaxel (E) are shown. Vincristine as well as paclitaxel induce a strong cell cycle arrest in G_2/M and apoptotic cells in sub-G_1 as determined by flow cytometry (histograms in B and F, control in D; for details see 98).

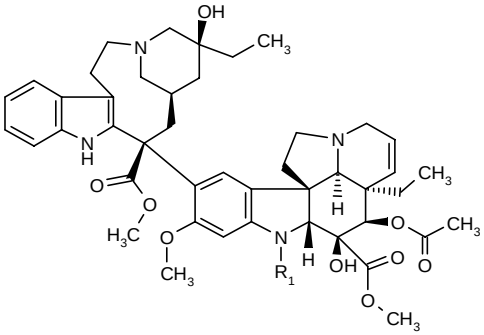
problems related to galenic formulations containing polyoxyethylated castor oil (Cremophor EL), a complex synthesis and, most important, the development of drug resistance restrict their clinical use. As a consequence, tremendous efforts in academic research and the pharmaceutical industry aimed at finding new tubulin-interacting compounds with a superior safety and efficacy profile were initiated (19-21). Currently, more than 30 compounds are in advanced preclinical and early clinical development. These compounds are classified into semisynthetic paclitaxel analogues, natural and semisynthetic compounds of diverse structure and synthetic, low-molecular-weight compounds. In the following sections, the chemistry, pharmacology and clinical profile of these compound classes are summarized and discussed in the context of their potential use in cancer therapy.

Semisynthetic paclitaxel analogues

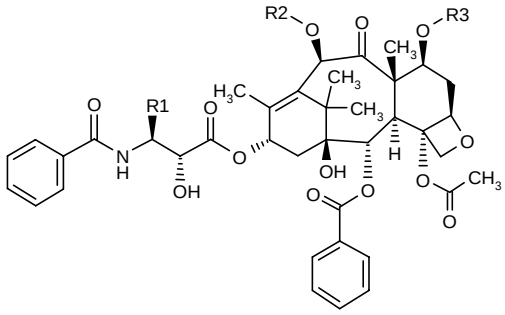
The clinical use of paclitaxel and docetaxel, although highly potent and effective cancer drugs, is restricted mainly by low oral bioavailability, drug resistance and toxicity related to the mechanism of action (neutropenia and peripheral neurotoxicity) as well as to the galenic formulation with Cremophor EL. In a recent study with docetaxel, coadministration of ciclosporin as a P-glycoprotein (Pgp) modulator and cytochrome P450 3A4 (CYP3A4) substrate, enhanced oral bioavailability from 8 ± 6% without ciclosporin to 90 ± 44% with a single dose of 15 mg/kg ciclosporin (22). To avoid using Cremophor EL, new formulations of paclitaxel with higher aqueous solubility are currently in clinical development, namely TaxoExtra (SuperGen Inc.) and SP-1010C (Supratek Pharmaceuti-



(**1**) Colchicine



(**2**) R = CHO Vincristine
(**2a**) R = CH_3 Vinblastine



(**3**) R1 = Ph, R2 = $COCH_3$, R3 = H Taxol INN: Paclitaxel
(**4**) R1 = ^tBuO, R2 = H, R3 = H Taxotere INN: Docetaxel

cals). Paclitaxel conjugates with new features have been described, namely polyglutamate paclitaxel/CT-2103 (Xiotax®, Cell Therapeutics), polyethylenglycol paclitaxel (Enzon) and DHA-paclitaxel (Taxoprexin®, Protarga). The molecular alterations leading to clinical resistance to paclitaxel and docetaxel are complex and the subject of intensive studies. MDR1/Pgp overexpression, mutations within β-tubulin and increased expression of specific tubulin isotypes have been correlated with drug resistance in patients (23-26). Numerous new semisynthetic paclitaxel analogues have been synthesized to improve the aqueous solubility, oral bioavailability and to circumvent drug resistance. Selected compounds which are currently in clinical development are reviewed below (Table I) (27).

IDN-5109

Through substitutions in the 14β-hydroxy-10-deacetylbaccatin III (14-OH-DAB) synthon, a diterpene present in the needles of *Taxus wallichiana*, IDN-5109 [13-(*N*-boc-β-isobutylisoserinyl)-14-hydroxhybaccatin-1,14-carbonate] (**5**) was selected as a potent paclitaxel analogue (28). IDN-5109 is a poor substrate of Pgp, hence it is highly active against MDR-positive cancer cell lines and has oral bioavailability despite high Pgp expression in the gastrointestinal tract (28). After oral administration of IDN-5109 to tumor-bearing nude mice, regression of MX-1 breast and 1A9 ovarian carcinoma was described (29, 30). Compared to the i.v. route, oral bioavailability was about 50% with a long terminal half-life of > 6 h (30). IDN-5109 was first described by Indena, licensed to Bayer AG in March 2000 and is currently in phase I clinical trials. Thus far no clinical data on the toxicity and efficacy profile have been published.

BMS-184476 and BMS-188797

Bristol-Myers-Squibb, the company which developed paclitaxel, published data on several paclitaxel derivatives namely BMS-184476 (**6**) and BMS-188797 (**7**) (31). In a preclinical study in comparison to paclitaxel, both analogues displayed similar or superior efficacy in nude mice xenograft models, namely A2780 ovarian, HCT116/pk colon and L2987 lung carcinoma (32). Paclitaxel resistance was only partially overcome and both analogues are considered Pgp substrates. Both compounds are currently in phase I clinical trials and data for BMS-184476 have been published (33). BMS-184476 was administered as a 1-h i.v. infusion every 3 weeks. The maximum tolerated dose (MTD) was estimated at 60 mg/m^2 with severe neutropenia and diarrhea/mucositis as the dose-limiting toxicities (DLTs). BMS-184476 showed linear pharmacokinetics with a clearance of 200 ± 89 ml/min/m^2, a volume of distribution of 402 ± 231 l/m^2 and a terminal half-life of 40.8 ± 21.8 h (33).

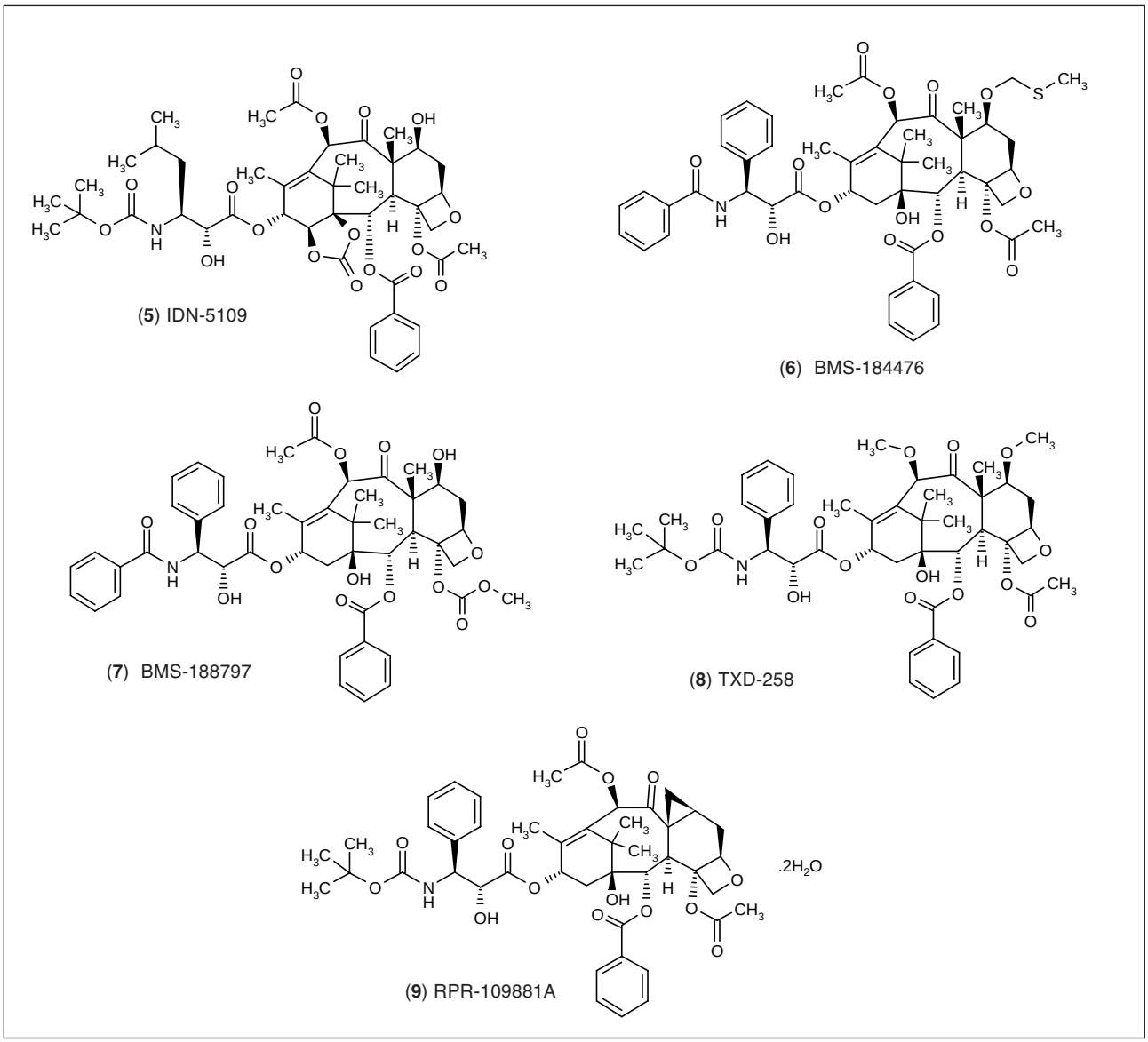
TXD-258 and RPR-109881A

Two taxane analogues are in clinical development by Aventis Pharma, *i.e.*, TXD-258 (**8**) and RPR-109881A (**9**). TXD-258, after i.v. and p.o. administration, has been reported to be effective against various human tumor xenografts, including MDR-positive and taxane-resistant models, and to cross the blood-brain barrier (34, 35). TXD-258 is currently in phase I/II clinical trials, but thus far no data have been published. RPR-109881A was first described in 1994 and phase I clinical data have been reported recently (36). Patients treated with RPR-109881A (i.v. administration on days 1 and 8 in a 21-day cycle) experienced severe diarrhea and granulocytopenia as DLTs with a MTD of 45 mg/m^2.

Table I: Microtubule-stabilizing taxane analogs.

Compound	Source	Taxane cross-resistance	Administration route	MTD[1]	Status	Ref.
IDN-5109	Bayer/Indena	No	p.o.	n.p.	Phase I	29
BMS-184476	Bristol-Myers Squibb	Yes	i.v.	60 mg/kg	Phase I	33
TXD-258	Aventis Pharma	Partial	i.v./p.o.	n.p.	Preclinical	34

n.p. = not published. [1]Established in clinical phase I studies.



Natural compounds of diverse structure

Many tubulin-binding compounds from various natural sources and semisynthetic analogues thereof have been described within the last two decades. Natural compounds like combretastatin A-4, cryptophycin 52, dolastatin 10 and dolastatin 15 destabilize tubulin, whereas epothilones, laulimalide, peloruside A, eleotherobine and (+)-discodermolide – similar to paclitaxel – stabilize microtubules. Chemically, microtubule-stabilizing agents are categorized as terpenoids (taxanes, eleutherobin/sarcodictyin), macrolides (epothilones, laulimalide, peloru-

Table II: Tubulin-binding natural and semisynthetic compounds of diverse structure.

Compound	Source	Toxicity *in vitro* (IC_{50})	Administration route	MTD[1]	Status	Ref.
Epothilone B	Novartis	0.2-0.8 μM	i.v.	n.p.	Phase II	129
BMS-247550	Bristol-Myers Squibb	3.9 nM (mean)	i.v.	6 mg/m^2 (Qdx5 every 3 wks)	Phase II	46
Eleutherobin	Bristol-Myers Squibb	10-60 nM	n.p.	n.p.	Phase I	49
(+)-Discodermolide	Novartis	8-36 nM	n.p.	n.p.	Phase I	55
Vinflunine	Pierre Fabre	18 nM	i.v.	400 mg/m^2	Phase II	74
Cryptophycin 52	Eli Lilly	13-232 pM	i.v.	1.5 mg/m^2 (single dose)	Phase II (suspended)	38
Combretastatin A-4	Oxigene		i.v.	≤ 60 mg/m^2	Phase I	82
ZD-6126	AstraZeneca	n.p.	i.v.	Dose escalation ongoing	Phase I	90, 91
Dolastatin 10	NCI	0.5 nM (L1210 model)	i.v.	400 and 325 μg/m^2 (s.d.)	Phase II	39, 93

n.p. = not published. [1]Established in clinical phase I studies.

side A) and polyhydroxylated alkatetraene lactones (discodermolide). Data on the efficacy of these new tubulin-stabilizing agents currently in phase I/II clinical trials are expected to be published soon.

The suspension of a phase II study with cryptophycin 52 (37), the lack of efficacy of dolastatin 10 (38, 39) and the failure of various other compounds clearly illustrates that the efficacy and toxicity profiles are crucial for clinical success. In the subsequent sections, selected compounds with comprehensive preclinical and clinical data or interesting structural features are reviewed (Table II).

Epothilones

Epothilone A (**10**) and B (**10a**) (EPO-906), developed by Novartis, are 16-membered macrolides isolated from the mycobacterium *Sorangium cellulosum*. Epothilones were originally isolated and structurally resolved by G. Hoefle and colleagues in 1996 (40). Epothilone A and B act by stabilizing microtubules. They compete with paclitaxel for binding to β-tubulin, exhibiting slightly higher *in vitro* cytotoxicity against taxane-resistant tumor cell lines (41, 42). Unlike paclitaxel, epothilone A and B have no endotoxin-like properties (43). A closely related analogue is desoxyepothilone B/epothilone D (KOS-862) developed by Kosan BioSciences in collaboration with Roche and currently being evaluated in clinical phase I studies (44). Phase I data on epothilone B (bolus injection and continuous infusion) have also been published, with alopecia, neuropathy and nausea reported as the most prominent toxicities (45). Currently, phase II clinical trials in patients with breast, ovarian, NSCLC and ovarian cancer are scheduled or ongoing for epothilone B and D.

BMS-247550

BMS-247550 (ixabepilone) (**10b**) is a semisynthetic analogue of epothilone B developed by Bristol-Myers Squibb currently in phase II clinical trials. Like epothilone B, it is a stabilizing tubulin antagonist with broad antitumor activity (46). BMS-247550 is active against paclitaxel-sensitive and -nonsensitive cell lines, including MDR/Pgp overexpressing HCT116 colorectal carcinoma and A2789Tax ovarian carcinoma cells with defined β-tubulin mutations ($F^{270} \rightarrow V$ or $A^{364} \rightarrow T$) (25). BMS-247550 has a potent tubulin polymerization capacity (2.5-fold more potent than paclitaxel), a broad cytotoxicity (mean IC_{50} = 3.9 nM) and is efficacious *in vivo* after i.v. and p.o. administration (46). A MTD of 6 mg/m^2 with neutropenia as the DLT (without G-CSF cotreatment) was determined in a 1-h infusion on day 1-5 every 3 weeks schedule, with objective responses in patients with breast and cervical cancer refractory to prior taxane therapy (47). In this clinical trial with infusion doses ranging from 7.4-59.2 mg/m^2, the formation of microtubule bundles in peripheral blood mononuclear cells detected 1 h after infusion correlated well with the plasma AUC, and cell death occurred 23 h after peak microtubule bundle formation (48).

Eleutherobin

The antimitotic diterpene eleutherobin (**11**) and sarcodictyins A-D (**12a-d**) are structurally related, natural compounds isolated from the marine soft coral *Eleutherobia sp.* and *Sarcodictyon roseum*, respectively (49), whose chemical syntheses have been published (50). Eleutherobin is a competitive inhibitor of paclitaxel binding to tubulin (K_i = 2.1 ± 0.3 μM) thereby enhancing tubulin assembly and stability. The *in vitro* cytotoxicity against human tumor cell lines (IC_{50} = 10-60 nM) is comparable to that of epothilone A, with sarcodictyin A and B being at least 10 times less potent (49). Eleutherobin is a substrate of Pgp, whereas there are conflicting results concerning the cross-resistance to paclitaxel-resistant ovarian carcinoma cell lines with the mutated β-tubulin isotype

M40 (25, 49, 51). Eleutherobin, identified by researchers at the Scripps Institution of Oceanography in La Jolla, was licensed to Bristol-Myers Squibb and recently entered clinical trials although no data have published to date.

(+)-Discodermolide

(+)-Discodermolide (**13**), a lactone-bearing polyhydroxylated alkatetraene isolated from the sponge *Discodermia dissoluta*, was described as a stabilizing tubulin inhibitor with superior activity compared to paclitaxel (52). The chemical synthesis in 23 steps with 10.3% overall yield was recently reported (53). (+)-Discodermolide competes with [^{3}H]-paclitaxel for binding to tubulin (K_i = 0.4 μM) and is a poor Pgp substrate and effective against paclitaxel-resistant ovarian carcinoma cells with mutated β-tubulin isotypes (54). In a study using the taxane-resistant NSCLC cell line A549-T12, which requires paclitaxel for normal cell division, (+)-discodermolide could not substitute for paclitaxel (55). Quite unexpectedly, further experiments showed a strong synergism between the two compounds. This was not observed with epothilones or eleutherobin; therefore, new combination regimens with (+)-discodermolide are feasible in the clinic (56, 57). Discodermolide is currently being developed in a phase I clinical trial by Novartis.

Laulimalide

Laulimalide (**14**), a cytotoxic macrolide from the marine sponge *Cacospongia mycofijensis*, was first identified in 1988 (58) and was defined as a microtubule-stabilizing agent in a mechanism-based screening of marine invertebrates extracts. Laulimalide, a poor Pgp substrate, is a potent antiproliferative compound with IC_{50} values of 5.7 and 11.5 nM in MDA-MB425 human breast and SKOV-3 ovarian cancer cell lines, respectively (59). The tubulin-stabilizing activity (EC_{50} = 4.3 μM) was slightly weaker as compared to paclitaxel (EC_{50} = 1.4 μM). The total chemical synthesis of laulimalide in 27 steps with 2.9% overall yield was recently described (60) but no further preclinical data have been published up to now.

Cryptophycin 52

Cryptophycins are highly cytotoxic cyclic peptolides (depsipeptides) originally identified in blue-green algae (cyanobacteria) belonging to *Nostocaceae* (61). Cryptophycin 52 (LY-355703) (**15**), a cryptophycin A analogue with a stabilized ester linkage developed by Eli Lilly (62, 63), was selected from diverse synthetic analogues displaying superior potency, stability and amenability of clinical formulation. Proliferation of diverse tumor cell lines was inhibited (IC_{50} = 13-232 pM) and activity was minimally affected by Pgp or MRP overexpression (64). Cryptophycin 52 binds reversibly to tubulin *in vitro* with high affinity (K_d = 47 nM), presumably overlapping with the vinblastine binding site (65). At low concentrations of 3-30 pM, cryptophycin 52 arrests proliferating HeLa cervical carcinoma cells in G_2/M by suppressing microtubule dynamic instability without visible alterations of the spindle apparatus (65). The compound is concentrated more than 700-fold within cells, thus explaining the discrepancy between *in vitro* binding and cellular activity. In xenograft models after i.v. administration of up to 5 mg/kg, cryptophycin 52 was effective as a monotherapy and in combination with doxorubicin, paclitaxel and 5-FU (66). In a clinical phase I study, cryptophycin 52 was administered as a single i.v. infusion, displaying linear kinetics from 0.1-1.92 mg/m^2, with an estimated MTD of 1.5 mg/m^2 (67). Preliminary data from a phase II clinical trial with stage IIIb and IV NSCLC patients were published recently, showing severe toxicities at 1.5 mg/m^2 without objective tumor responses which led to trial suspension (38).

Rhizoxin

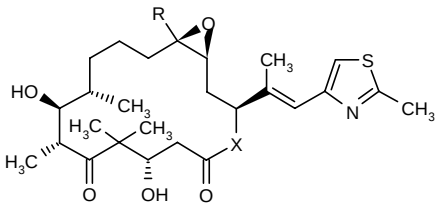
The antitumor macrolide rhizoxin (FR-900216) (**16**), originally isolated from the fungus *Rhizopus chinensis*, was first described in 1987 with data showing binding to the maytansine binding site of tubulin (68). Various phase I/II clinical trials showed a rapid systemic clearance after i.v. infusion with marginal or no antitumor activity at tolerated doses. As a consequence, clinical development of rhizoxin was discontinued by Fujisawa in 2001.

Isohomohalichondrin B

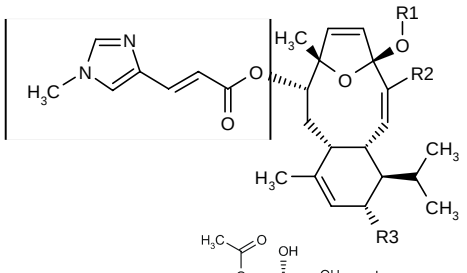
Isohomohalichondrin B (**17**), isolated from the sponge *Lissodendoryx sp.*, was first described as a highly cytotoxic drug (IC_{50} = 0.05-0.1 nM against human prostate cancer cell lines) acting as a destabilizing tubulin inhibitor (69, 70). Isohomohalichondrin B is currently under preclinical development by PharmaMar, Spain.

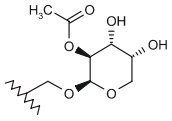
Vinflunine

Vinflunine (F-12158) (**18**) is a bifluorinated analogue of vinorelbine (a vinca alkaloid approved for the treatment of NSCLC, metastatic breast and ovarian cancer) clinically developed by Pierre Fabre (71). In comparison to vinorelbine, MDR-related drug resistance developed less readily and the overall response rate *in vivo* of vinflunine (64%) was superior (72, 73). In a clinical phase I trial, vinflunine was administered i.v. every 21 days showing an elimination half-life of 26.1 ± 4 h, a MTD of 400 mg/m^2, as well as 3 partial responses. Phase II studies with a dose of 350 mg/m^2 are currently ongoing (74).

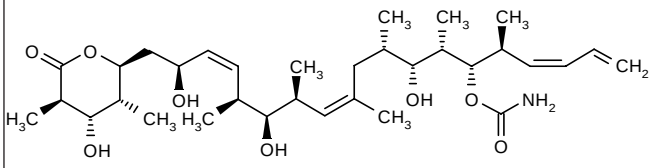


(**10**) X = O, R = H Epothilone A
(**10a**) X = O, R = CH_3 Epothilone B
(**10b**) X = NH, R = CH_3 BMS-247550

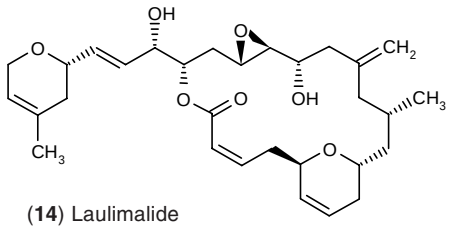




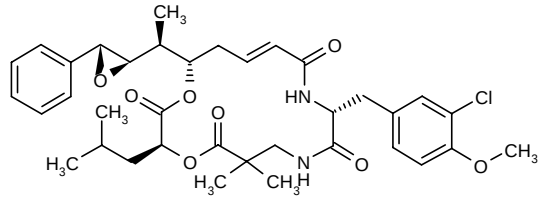
(**11**) R1 = CH_3, R2 = R3 = H Eleutherobin	Urocanoyl residue E
(**12a**), R1 = H, R2 = $COOCH_3$, R3 = H Sarcodictyin	E
(**12b**), R1 = H, R2 = $COOCH_2CH_3$, R3 = H Sarcodictyin B	E
(**12c**), R1 = H1, R2 = $COOCH_3$, R3 = OH Sarcodictyin C	E
(**12d**), R1 = H, R2 = $COOCH_3$, R3 = OH Sarcodictyin D	Z



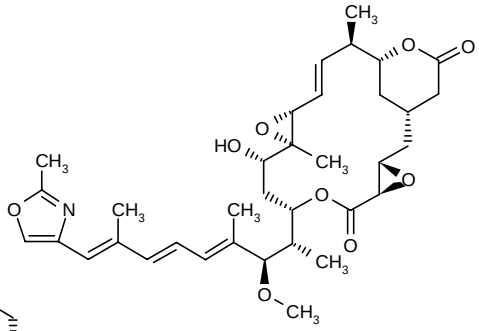
(**13**) Discodermolide



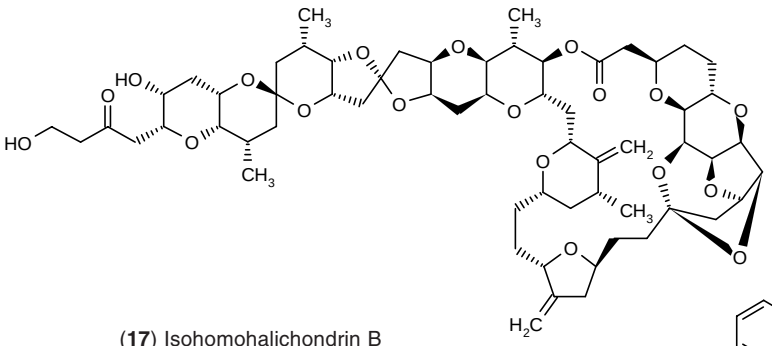
(**14**) Laulimalide



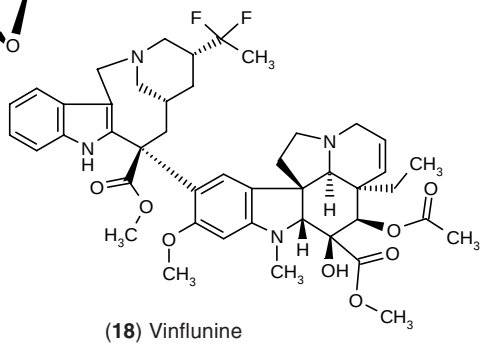
(**15**) Cryptophycin 52 (LY-355703)



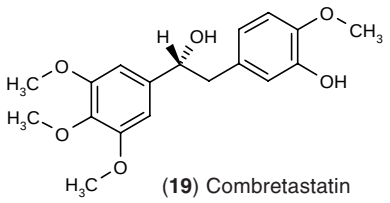
(**16**) Rhizoxin (FR-900216)



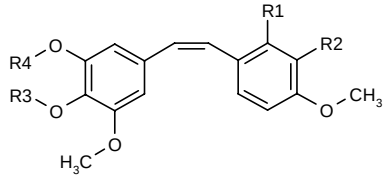
(**17**) Isohomohalichondrin B



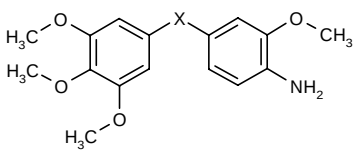
(**18**) Vinflunine



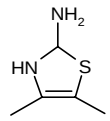
(**19**) Combretastatin



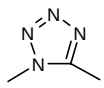
(**19a**) R1 = R2 = OH, R3 = R4 = CH_3
Combretastatin A-1
(**19b**) R1 = H, R2 = OH, R3 = R4 = CH_3
Combretastatin A-4
(**19c**) R1 = H, R2 = OH; R3, R4 = -CH_2-
Combretastatin A-2
(**19d**) R1 = H, R2 = NH_2, R3 = R4 = CH_3
AC-7739



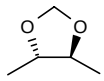
(**19e**) X =



(**19f**) X =



(**19g**) X =



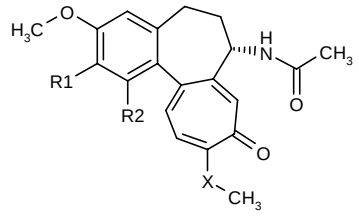
Combretastatins

Combretastatin (**19**) as well as combretastatin A-1 (**19a**), A-4 (**19b**) and A-2 (**19c**) have been identified in extracts from the South African tree *Combretum caffrum* as antimitotic agents interfering with the colchicine binding site of tubulin (75, 76). Medicinal chemistry efforts were aimed at improvement of water solubility and *in vivo* activity. The most important discovery was the introduction of an amino group instead of the phenolic hydroxy group as shown at the amino stilbene AC-7728 (**19d**), which increased water solubility and efficacy (20). Based on the structural features of (**19**), heterocombretastatins (*e.g.*, **19e-g**) as synthetic analogues were prepared, inhibiting tumor cell proliferation with an IC_{50} up to 0.3 μM (77, 78).

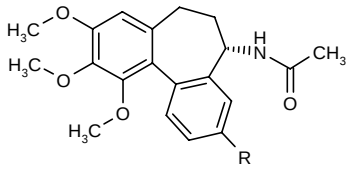
The water soluble prodrug combretastatin A-4 (CA4P) was licensed from Arizona State University to Oxigene in 1997. Like ZD-6126 (**20**) (see below), CA4P acts as a tumor vascular targeting agent leading to tumor necrosis by shutdown of blood flow (79-81). CA4P was investigated in 3 phase I clinical trials and results from the Ireland Cancer Trial were published in 2002 (82). Dose escalation after 10 min or subsequently after a 60-min infusion of CA4P in 25 patients showed a MTD of < 60 mg/m^2 with manageable short-term acute toxicities. Most strikingly, the traditional cytotoxic side effects such as myelosuppression or alopecia were not observed, but tumor pain occurred in 10% of treatment cycles. Interestingly, 1 patient with a therapy refractory thyroid carcinoma had a complete response. CA4P currently is being evaluated in phase II clinical studies, monitoring tumor blood flow as the critical parameter for optimal dosing of drugs targeting the tumor vasculature.

Colchicine and ZD-6126

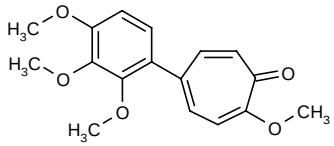
Colchicine (**1**) has been used in the treatment of gout and other inflammatory diseases since the 6th century A.D. (83). Colchicine binds to a high affinity binding site in β-tubulin, thereby inducing conformational changes in αβ heterodimer tubulin structure and hindering assembly. A natural analogue derived from *Colchicum cornigerum* is cornigerine (**1a**) which binds to β-tubulin with about 3-fold higher affinity (84). Many efforts were aimed at reducing the high toxicity of colchicines. With thiocolchicine (**1b**), a selected analogue with potent antimitotic activity inhibiting tubulin polymerization with an IC_{50} value of 2.1 μM, no advantage concerning tolerability was achieved. The colchicine binding site on β-tubulin has very stringent structural requirements; the 3 methoxy groups in the A-ring seem to be essential for full binding affinity whereas the 7-membered ring and the C-7 side chain appear not to be crucial for tubulin binding, as shown by the high activity of allocolchicine (**1c**) (IC_{50} = 1.4 μM) and (**1d**) (85). Further potent antimitotic allocolchicinoids have been synthesized (86) and 2-methoxy-5-(2',3'4'-trimethoxyphenyl)tropone (MTPT) (**1e**) also has colchicine-type substructure (87).



(**1**) R1 = R2 = OCH_3, X = O
Colchicine
(**1a**) R1, R2 = -OCH_2O-, X = O
Conigerine
(**1b**) R1 = R2 = OCH_3, X = S
Thiocholchicine



(**1c**) R = CO_2CH_3
Allocolchicine
(**1d**) R = $COCH_3$



(**1e**) 2-Methoxy-5-(2',3',4',trimethoxy-phenyl)tropone
(MTPT)

ZD-6126 (ANG-45) (**20**) is the water-soluble phosphate prodrug of *N*-acetylcolchinol, a novel tubulin binding agent directly targeting tumor vasculature. It is currently being developed by AstraZeneca in phase I clinical trials and was in-licensed from Angiogene Pharmaceuticals Inc. in 1999. In preclinical studies, single doses of ZD-6126 (200 mg/kg i.p.) induced hemorrhage and necrosis in the PC-14PE6 NSCLC nude mice metastasis tumor model with some selectivity towards tumor endothelial cells seen (88). In a second preclinical study, ZD-6126 showed significant effects on tumor vasculature in different nude mice xenograft models and was well tolerated at doses up to 16-fold below the MTD (about 400 mg/kg i.p. or i.v.). In the Calu-6 NSCLC model, 100 mg/kg ZD-6126 (i.p. for 5 days) in combination with 4 mg/kg cisplatin had more than additive effects on tumor growth delay (89). Phase I clinical trials to define the MTD and DLT and validate the use of circulating endothelial cells as a surrogate marker are ongoing. Preliminary results showed reasonable tolerability after a 10-min single-dose infusion of up to 112 mg/$m^{2.}$ Rapid clearance of *N*-acetylcolchinol was observed ($t_{1/2}$ = 2-3 h) and a 2-fold increase in circulating endothelial cells was seen 4-6 h after the ZD-6126 infusion (90, 91).

Dolastatins and semisynthetic analogues

The dolastatins are highly cytotoxic cyclic pentapeptides isolated from the sea hare *Dolabella auricularia* that were first described in 1990 (92). They include dolastatin 10 (NSC-376128) (**21**), the analogue auristatin (TZT-1027) (**21a**), as well as dolastatin 15 (**22**) and the analogues cematodin (LU-103793) (**22a**) and ILX-651 (LU-223651) (**22b**). Dolastatin 10, currently under development by the NCI, acts as a destabilizing tubulin inhibitor (IC_{50} = 1.2 μM for inhibition of tubulin polymerization), noncompetitively inhibiting vinca alkaloid binding to tubulin (92). In a phase I study in patients with advanced solid tumors, dolastatin 10 was administered as an i.v. bolus every 3 weeks, with granulocytopenia as the DLT and a MTD of 400 mg/m^2 and 325 mg/m^2 for patients with minimal or heavy pretreatment, respectively (93). Peripheral sensory neuropathy developed but was not dose-limiting. In phase II studies conducted in patients with metastatic prostate and colorectal carcinoma, dolastatin 10 was well tolerated at doses of 400-450 μg/m^2 but lacked clinical efficacy (38, 39). Little data has been published on ILX-651 which is being developed by Ilex Oncology. Preliminary data from a phase I study was recently reported (94), showing reasonable tolerability up to 13 mg/m^2 (q.d. for 5 days every 3 weeks) but a short plasma half-life and evidence of drug accumulation were seen.

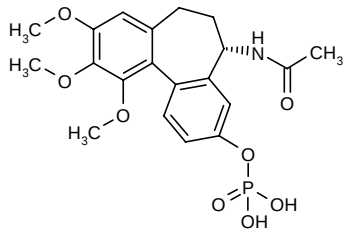
Tryprostatins

Tryprostatin A and B (**23a, 23b**) are indole alkaloid-based fungal products isolated from *Aspergillus fumigatus*. Tryprostatin A inhibits MAP-dependent tubulin polymerization (at high concentrations of about 250 μM) and M-phase progression (95). Tryprostatin A interacts with the function of MAP2 and Tau in microtubule assembly and therefore might be a lead structure for antimitotic agents with a target associated with but distinct from tubulin.

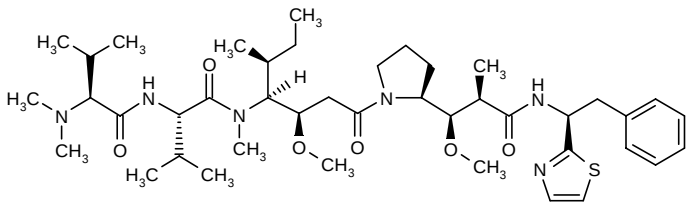
RPR-112378

RPR-112378 (**24**), extracted from the Indian plant *Ottelia alismoides*, was identified in a screening program for new antimitotic drugs by a research group at Rhône-Poulenc Rorer/Aventis Pharma and shown to be a potent destabilizing inhibitor (IC_{50} = 1.2 μM for tubulin polymerization *in vitro*) and to compete with colchicine for binding (96). RPR-112378 is cytotoxic with IC_{50} = 20 nM in KB (HeLa) human cervical carcinoma cell line. No further preclinical data have been published on this new class of compounds.

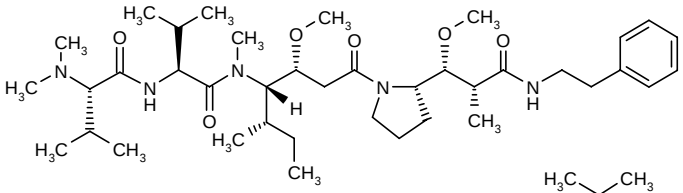
Natural Compounds



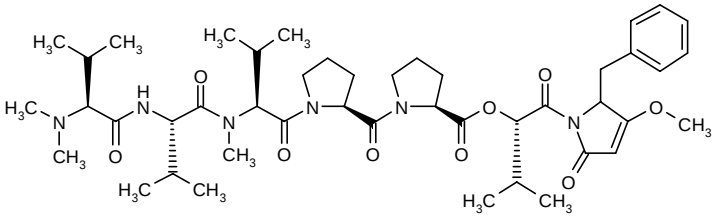
(**20**) ZD-6126



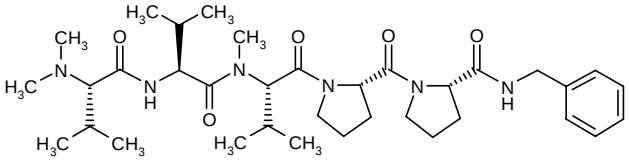
(**21**) Dolastatin 10 (NSC-376128)



(**21a**) Auristatin PE (TZT-1027)

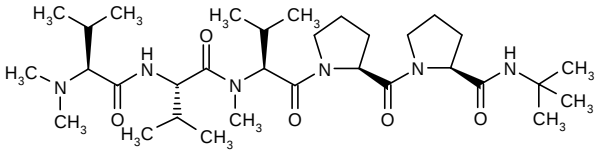


(**22**) Dolastatin 15



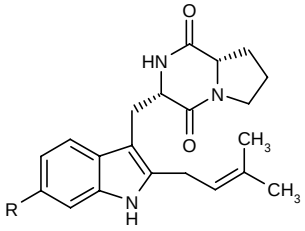
.HCl

(**22a**) Cematodin (LU-103793)

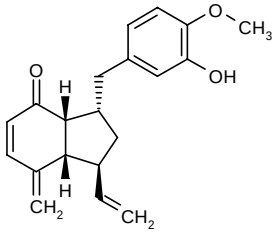


.HCl

(**22b**) ILX-651/LU-223651



(**23a**) R = OCH_3
Tryprostatin A
(**23b**) R = H
Tryprostatin B



(**24**) RPR-112378

Synthetic, low-molecular-weight compounds

From a synthetic point of view, compounds of low molecular weight (small molecules) are very attractive as a drug format. Within the last years, various synthetic small-molecule tubulin inhibitors have been described. Most, if not all, compete with colchicine for binding to β-tubulin, thereby acting as destabilizing agents. Regarding their core structure, these compounds can be grouped into heterocombretastatins, sulfonamides, phenstatins, indoles and quinolones. All compounds, except T-128067, are currently in preclinical development. Thus, their superiority to natural compounds and semisynthetic analogues in terms of bioavailability, potency and toxicological profile must be demonstrated (Table III).

D-24851

N-(Pyridin-4-yl)-[1-(4-chlorbenzyl)-indol-3yl]glyoxylamid (D-24851) (**25**), currently in preclinical development by Baxter Oncology, was identified by random

Table III: Synthetic small-molecule tubulin inhibitors.

Compound	Source	Toxicity *in vitro* (IC_{50})	Administration route	MTD[1]	Status	Ref.
D-24851	Baxter Oncology	36-285 nM	n.p.	n.p.	Preclinical	96
D-64131	Baxter Oncology	24-144 nM	n.p.	n.p.	Preclinical	98
A-289099	Abbott	7 nM	n.p.	n.p.	Preclinical	112
Indanocine	Salmedix	≤ 20 nM (mean)	n.p.	n.p.	Preclinical	113
E-7010	Abbott	0.2-40 ng/ml	p.o.	480 mg/m^2 (single dose)	Phase I	108
T-138067	Tularik	11-165 nM	i.v.	220/385 mg/m^2	Phase II	102
CI-980	Pfizer		i.v.	3.75 mg/m^2/d	Phase II (discontinued)	115

n.p. = not published. [1]Established in clinical phase I studies.

screening for cytotoxic compounds. Its mechanism of action was subsequently elucidated. D-24851 acts as a destabilizing β-tubulin binding agent (97) and is highly cytotoxic *in vitro* and *in vivo,* with efficacy against tumor cell lines with various resistance phenotypes. Most notably, D-24851 has oral bioavailability and no neurotoxic effects (*i.e.*, deficits in motor function and decreases in nerve conduction velocity) at effective doses in rats. No data on D-24851 analogues have been published thus far. Clinical trials are expected to start soon (Baxter Oncology company web page).

2-Aroylindoles

2-Aroylindoles, with (5-methoxy-1*H*-2-indolyl)-phenylmethanone (D-64131) (**26**) as the lead structure, were described as a new class of synthetic microtubule inhibitors (98, 99). D-64131 and analogues compete with [^{3}H]-colchicine for binding to tubulin and arrest dividing cells in G_2/M. The proliferation of tumor cells from 12 out of 14 different organs and tissues was inhibited with mean IC_{50} values of 62 nM for D-64131 and 24 nM for D-68144 (**26a**), comparable to that of paclitaxel (mean IC_{50} = 10 nM) (98). Having no cross-resistance against cell lines with different resistance phenotypes, oral bioavailability and good tolerability at effective doses in nude mice, this class of compounds is currently in preclinical development by Baxter Oncology.

2-Phenylindoles

2-Phenylindoles (**27**), colchicine site binding cytotoxic compounds possessing an indole skeleton, have been described (100). The modification of 3,4,5-trimethoxybenzo[b]thiophene led to 6-methoxy-2-(4-methoxyphenyl)-indoles (**27a**) which retained the high cytotoxic activity (IC_{50} = 35 and 160 nM against MDA-MB231 and MCF7 breast cancer cell lines, respectively). 3,4,5-Trimethoxybenzo[b]thiophene represents a fragment of the tetracyclic tubulin inhibitor 12-formyl-5,6-dihydroindole[2,1*a*]-isochinoline and shows comparable activities (101).

Diarylindoles and heterocombretastatins

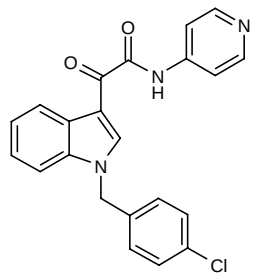
As already mentioned, synthetic microtubule inhibitors based on the structure of the natural compound combretastatin A-4 were synthesized and named heterocombretastatins (77, 78). The most active compounds published thus far (**28, 29**) showed antiproliferative activity *in vitro* against cancer cell lines with IC_{50} = 0.3-1.3 μM. No further preclinical data have been published.

T-138067 and T-900607

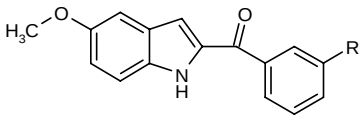
2-Fluoro-1-methoxy-4-[pentafluorophenylsulfonamide]benzene (T-138067) (**30**) and its analogue T-900607 (**30a**), developed by Tularik Inc., irreversibly bind tubulin by covalent modification of Cys-239 within β_1, β_2 and β_4 tubulin (102). They are potent antimitotic agents with high cytotoxic activity *in vitro* against tumor cells especially with the MDR resistance phenotype and after i.p. dosing in nude mice *in vivo* (102). In phase I clinical trials with T-138067, a weekly 3-h infusion schedule was safe; neutropenia, diarrhea and peripheral neuropathy were typical toxicities seen at doses > 250 mg/kg (103). In ongoing phase II clinical trials, the drug was reasonably tolerated at doses of 165 mg/m^2 in hepatocellular carcinoma (HCC) patients and 330 mg/m^2 (3-h infusion once weekly) in NSCLC patients; no responses in the lung cancer study and 2 partial responses in the HCC trial were reported (104, 105).

E-7010

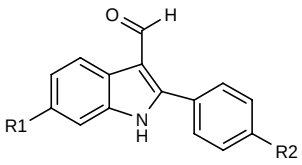
E-7010 (ABT-751) (**31**) is a sulfonamide analogue identified by Esai and licensed for clinical development to Abbott in 2000. E-7010 binds to the colchicine binding site of β-tubulin, has potent antiproliferative activity *in vitro* (IC_{50} = 0.2-40 ng/ml) against 26 tumor cell lines, is active in cell lines with various resistance phenotypes including Pgp overexpression, and was effective (25-100 mg/kg daily p.o.) in nude mice xenograft models (106, 107). Phase I studies published in 1998 determined the MTD (480 mg/m^2, single dose) with peripheral neuropa-



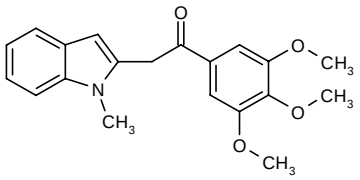
(**25**) N-(Pyridin-4-yl)-[1-(4-chlor-benzyl)-indol-3-yl]glyoxyl-amid (D-24851)



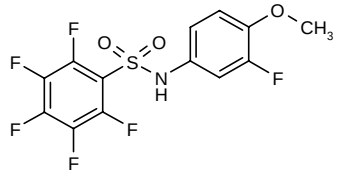
(**26**) R = H, D-64131
(**26a**) R = OCH_3, D-68144
(**26b**) R = F



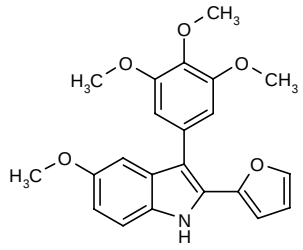
(**27**) 2-Phenylindoles
(**27a**) R1 = R2 = OCH_3



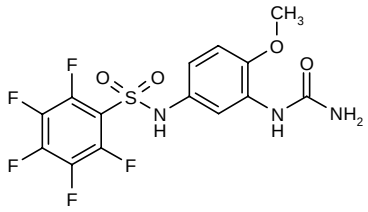
(**29**) Heterocombretastatins e.g.



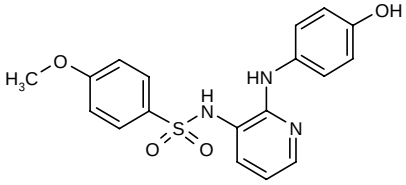
(**30**) T-138067



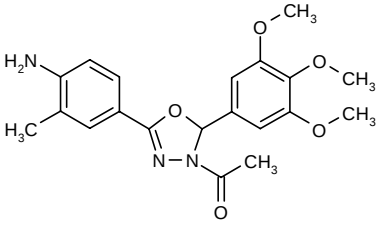
(**28**) Diarylindoles, e.g.



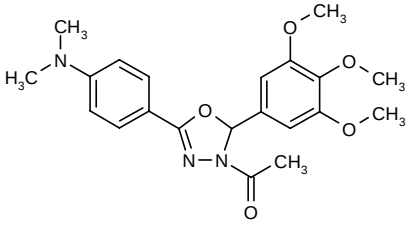
(**30a**) T-900607



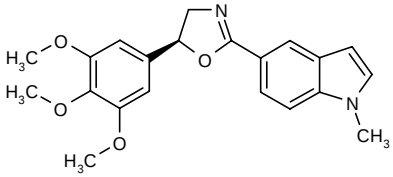
(**31**) E-7010



(**32**) A-105972



(**32a**) A-204197



(**33**) (*S*)-enantiomer: A-289099
Racemate: A-259745

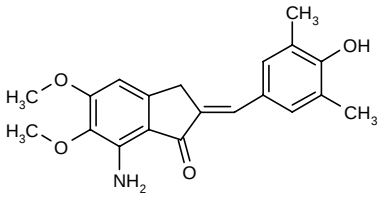
thy and intestinal paralysis as DLTs in the single-dose as well as the 5-day, repeated-dose study (108). New analogues with higher cytotoxicity have been published recently by Abbott but have the disadvantage of short half-lives ($t_{1/2}$ < 1 h) (109). No further development activities have been published on E-7010 and analogues up to now.

Oxadiazolines and 2-indolyloxazolines

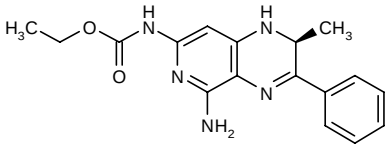
The oxadiazoline derivatives A-105972 (**32**) and A-204197 (**32a**) were reported by Abbott Laboratories (110, 111). Both compounds are synthetic small-molecule tubulin inhibitors competing with colchicine for binding to β-tubulin. A-105972 has broad cytotoxic activity (IC_{50} = 3-158 nM) independent of Pgp expression. Nevertheless, systemic bioavailability was poor (111). Similar data were subsequently published for A-204197 with cytotoxicity against cell lines with colchicine, paclitaxel and vinblastine resistance (110). Further efforts led to the discovery of the 2-indolyloxadiazoline A-289099 (**33**) which has oral bioavailability up to 44% and *in vivo* activity in the syngeneic M5076 murine ovarian sarcoma tumor model (110).

Indanocine/SDX-103

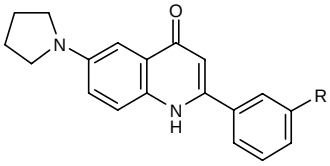
Another class of microtubule-binding compounds is represented by (*E*)-2-benzylidene-1-tetralones and (*E*)-2-benzylidene-1-indanones (**34**). A selected derivative, indanocine (NSC-698666), and the water soluble prodrug SDX-103 are in preclinical development by Salmedix



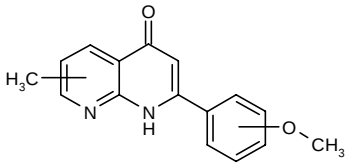
(**34**) Indanones, e.g., Indanocine NSC-698666



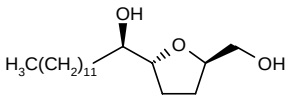
(**35**) Carbamates, e.g., CI-980 (NSC-613826)



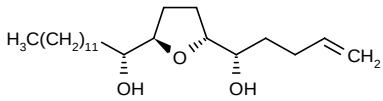
(**36**) 2-Phenyl-4-quinolones, R1 = OCH_3 or Cl e.g. R2 = N-pyrrolidine



(**36a**) 2-Phenyl-1,8-naphthyridin-4-ones



(**37**) WHI-261



(**37a**) COBRA 1

(113, 114). Indanocine induces apoptotic cell death in stationary and MDR tumor cells, but no data are available on the *in vivo* activity or bioavailability of the agent.

CI-980

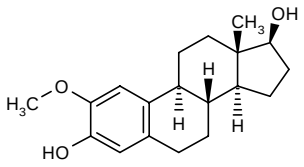
The carbamate derivative CI-980 (NSC-613862) (**35**) was described in 1994 as a colchicine-like tubulin destabilizing cytotoxic agent with activity at low nM concentrations (115, 116). CI-980 was developed clinically by Pfizer/Parke-Davis in phase I/II trials since 1991 for the treatment of various cancers (*e.g.*, malignant glioma and melanoma, SCLC and platin-refractory ovary carcinoma). Development was discontinued in 2001 because of lack of clinical efficacy against various cancers including recurrent glioma, SCLC and disseminated malignant melanoma (117-119).

Quinolones

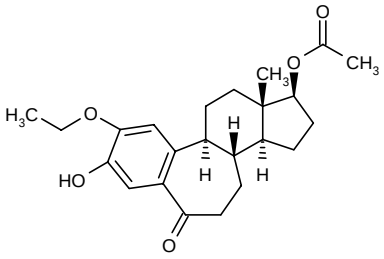
Quinolones and related structures also represent a promising group of compounds affecting tubulin. The synthetic 2-phenyl-4-quinolones (**36**), structurally related to naturally occurring and antimitotic flavonoids, displayed promising activity and impressive differential cytotoxicity against human tumor cell lines, comparable to that of colchicine (85, 120-122). The structurally related 2-phenyl-1,8-naphthypyridin-4-ones (**36a**), containing an additional nitrogen in position 8 of the aromatic system, also exhibited potent cytotoxicity.

COBRA-0 and COBRA-1

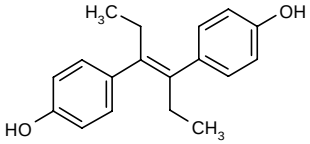
Based on the structure of α- and β-tubulin, a new hydrophobic binding pocket was identified in α-tubulin, and a synthetic molecule with the monotetrahydrofuran (THF) moiety attached to a C_{12} aliphatic chain was synthesized. This compound was named COBRA-0 (WHI-261) (**37**) and like the analogue COBRA-1 (**37a**) bound to α-tubulin and exhibited cytotoxicity at concentrations of 100 μM or higher (123). Although not a suitable anticancer drug, this molecular modeling guided approach led to the discovery of a unique tubulin-binding compound.



(**38**) 2-Methoxyestradiol (NSC-659853)



Ring expanded homologes of **38**, posessing structural similarity to colchicine



(**39**) Diethylstilbestrol

Synthetic steroids with colchicine-type substructure

2-Methoxyestradiol (NSC-659853) (**38**), a cytotoxic human metabolite developed by Entremed Inc., binds to the colchicine site of tubulin with reasonable affinity (IC_{50} = 4.7 μM). Based on the hypothesis that the colchicine structure confers efficacy, a series of B-ring expanded 2-ethoxyestradiol analogues were synthesized, in which the B-ring of the steroid was replaced by the B-ring of colchicines. While the resulting analogues showed significant affinity to the colchicine binding site consistent with the proposed structural resemblance, derivatives having a ketone at C-6 surprisingly resembled paclitaxel (124). Further investigations on 2-methoxyestradiol have been performed and the agent was shown to have antiproliferative effects on hormone-dependent and hormone-independent breast cancer cells (125) as well as antiangiogenic activity (126). Furthermore, the pharmacological profile was reported to be especially dependent on steric and electronic influences of the substituent in position 2 (127). Phase I trials in patients with different solid tumors are in preparation. Finally, another tubulin-binding compound diethylstilbestrol (**39**,) which was originally developed as an synthetic estrogen, was described. The antineoplastic effect observed by women treated with diethylstilbestrol has been shown to be caused by depolymerization of microtubules (128).

Summary and perspectives

Interfering with mitosis using tubulin-binding drugs is a clinically validated approach to treat cancer patients. Paclitaxel and docetaxel are standard therapeutics in first-line and second-line therapy of advanced ovarian carcinoma and second-line therapy of mammary carcinoma or NSCLC with new indications planned. Today, more than 30 new compounds targeting tubulin, either stabilizing or destabilizing microtubule dynamics, are in late preclinical or early clinical development. Many of these are natural or semisynthetic compounds of complex structure, but surprisingly simple synthetic molecules are also potent antimitotic agents. Although we cannot predict which compounds are effective as cancer drugs or superior to established therapeutics, tubulin inhibitors are still a hot topic more than 30 years after the discovery of paclitaxel. By understanding the mechanism of action of tubulin-targeting drugs including signaling pathways to the mitotic spindle apparatus, new targets can be studied and guide us to new cancer drugs with presumably better tolerability and efficacy.

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