



# An overview of tubulin modulators deposited in protein data bank

Haowei Guo<sup>1</sup> · Xingcong Li<sup>2</sup> · Ya Guo<sup>3</sup> · Linlin Zhen<sup>1</sup>

Received: 8 November 2018 / Accepted: 13 February 2019 / Published online: 7 May 2019  
© Springer Science+Business Media, LLC, part of Springer Nature 2019

## Abstract

Microtubules are cytoskeletal polymers of tubulin and composed of  $\alpha$ - and  $\beta$ -tubulin heterodimers, which are regarded as one of the most important, promising and successful targets for chemotherapeutic agents to treat cancer. With more and more tubulin-modulator co-crystal complex structures being solved, the mechanism of action (MOA) of microtubule targeting agents (MTAs) are well understood, which in turn inspired the development of more efficient tubulin modulators. By analyzing the reported tubulin modulators (most of them are inhibitors) that have been co-crystalized with tubulin and deposited in protein data bank (PDB), two new discovered inhibitor binding sites (maytansine and pironetin binding sites) on tubulin are elucidated, while the typical modulator binding sites (colchicine, taxanes, vinca alkaloids, and laulimalid binding sites) are also characterized. Among all the reported tubulin modulators, several inhibitors that bind to taxanes or vinca alkaloids binding sites have been approved by FDA to treat cancer. Thus, tubulin inhibitors discovery and design is still a hotspot in anticancer drug development. In this review, all the reported tubulin modulators that have co-crystal structures with tubulin in PDB are sorted out and classified according to their binding sites in tubulin. Besides, the ease or complexity of developing tubulin inhibitor on different binding sites on tubulin is also discussed. This work could contribute to discovering and designing new potent and specific modulators of tubulin.

**Keywords** Microtubules inhibitors · Anti-cancer target · New tubulin binding sites · Drug discovery · Protein data bank

## Introduction

Microtubules play an important role in various cellular processes, including mitosis, cell migration, cell shape maintenance, cell signaling and intracellular transport. These diverse physiologic functions were achieved by the dynamic transitions between the polymerization and depolymerization of  $\alpha$ ,  $\beta$ -heterodimer. Microtubules in tumor cells promote the frequency of cell mitosis process, resulting in uncontrolled growth of cancer cells. Thus tubulin is made to be an attractive target for antitumor therapy and

several microtubule inhibitors have been developed to clinically effective anti-cancer drugs.

Microtubule-targeted drugs were previously thought to work through increasing or decreasing the cellular microtubule mass. Although these effects might play a role in their chemotherapeutic actions, microtubule-targeted drugs can suppress microtubule dynamics without changing microtubule mass, resulting in mitotic block and apoptosis. Microtubule inhibitors induced cell apoptosis by disrupting microtubule dynamic and arresting cancer cells in G2/M phase. According to their MOA, microtubule targeting agents (MTAs) are classified into two major types: (i) microtubule destabilizing agents (MDAs); (ii) microtubule stabilizing agents, both of them inhibit the dynamic instability of tubulin polymerization (Negi et al. 2015). As shown in Fig. 1, based on their different binding sites on microtubule protein, MTAs are further classified into six types: (i) colchicine, (ii) vinca alkaloids, (iii) taxanes, (iv) laulimalid, (v) maytansine, and (vi) pironetin site-binding agents. Colchicine, vinca alkaloids, maytansine, and pironetin are MDAs, while taxanes and laulimalid are microtubule stabilizing agents. Both maytansine and pironetin could potentially inhibit tubulin assembly into microtubules

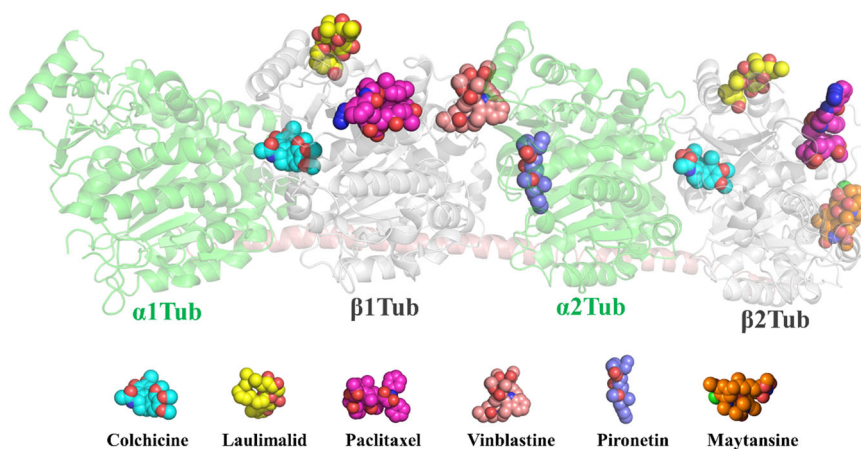
✉ Linlin Zhen  
simu1027@sina.com

<sup>1</sup> Department of Thyroid and Breast Surgery, The Affiliated Huaian No.1 People's Hospital of Nanjing Medical University, Huai'an 223300, P. R. China

<sup>2</sup> Department of Orthodontics, Nanjing Stomatological Hospital, Medical School of Nanjing University, Nanjing 210008, P. R. China

<sup>3</sup> Department of Pediatric Surgery, Huai'an Maternal and Child Health-Care Center, Huai'an 223002, P. R. China

**Fig. 1** Presently reported tubulin binding sites of microtubule targeting agents. Six different binding sites are deposited in the PDB



by binding to the sites that are distinct from the typical tubulin inhibitor binding domain (Fig. 1).

In this review, we will systematically summarize the reported tubulin modulators deposited in PDB according to their unique binding sites on microtubule. Besides, by summarizing the reported relative binding sites modulators, the ease or complexity of developing tubulin inhibitor on different binding sites is also discussed. This work further offers a convenient way for the rational design of potent MDAs for the development of more efficient cancer therapies.

## Material and methods

The binding sites of tubulin inhibitors were analyzed by PyMOL (The PyMOL Molecular Graphics System, Version 2.0 Schrödinger, LLC.).

## Results and discussion

### Microtubule destabilizing agents (MDAs)

According to the MOA, colchicine, vinca alkaloids, maytansine, and pironetin site-binding agents belong MDAs. Although they represent a successful class of anticancer drugs, the molecular MOA of several important MDAs on tubulin and microtubules has so far remained elusive. With more and more crystal structures of tubulin in complex with MTAs solved, the molecular MOA of MTAs were delineated.

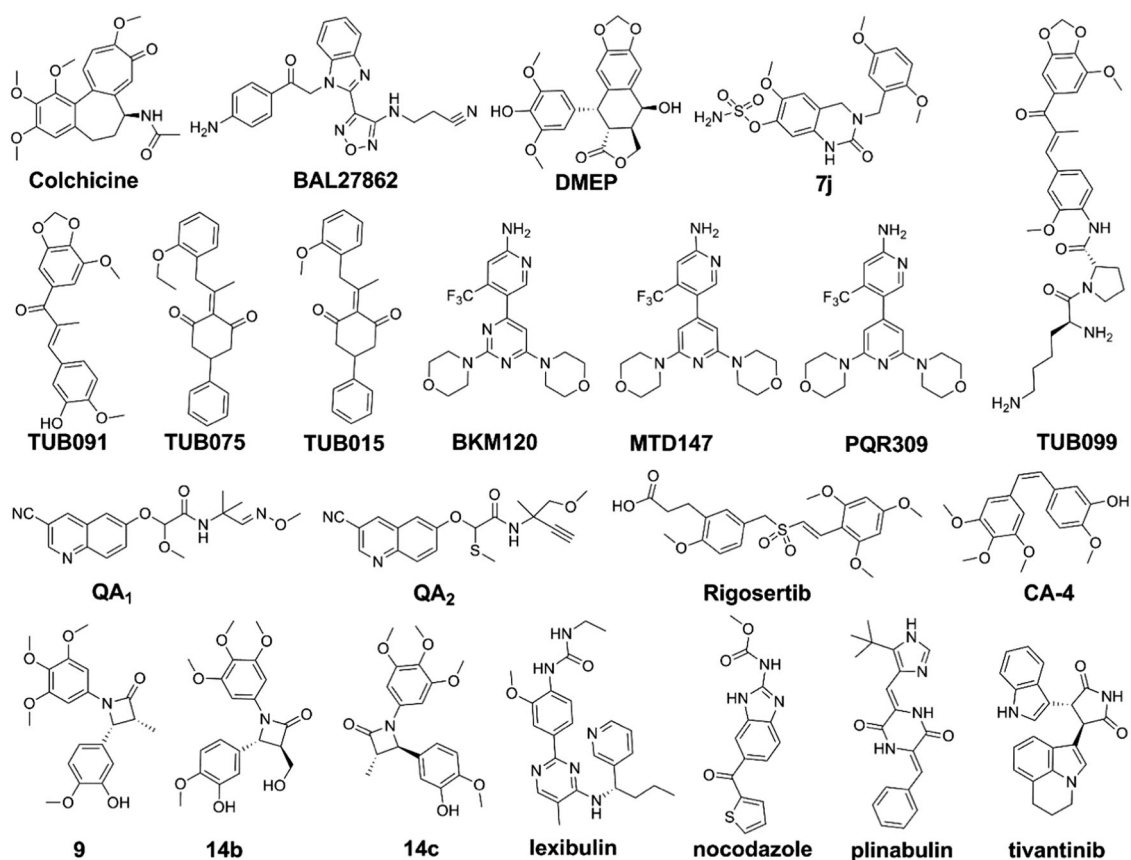
### Colchicine site-binding agents

Although there are no FDA approved drugs that specifically target this site for the treatment of cancer currently, MTAs that bind to colchicine site have received widely attention

and are of particular interest in antitumor therapy own to their dual mechanism of action as anti-mitotics and vascular disrupting agents during the last 10 years. Based on numerous tubulin-colchicine complex structures, the MOA was determined. The crystal structure of tubulin in complex with colchicine (see Fig. 2 for colchicine structure) was first reported in 2004 (Ravelli et al. 2004), this crystal structure (PDB code: 1SA0) sheds light on the mechanism of colchicine's activity: colchicine binds at a location where it prevents curved tubulin from adopting a straight structure, which inhibits assembly. The detailed interactions between colchicine and tubulin were shown in Fig. 3. Clearly, hydrophobic interaction was observed and contributed most to their binding, which indicated that colchicine binding site was a hydrophobic pocket. The mainly hydrophobic residues were Cys241, Leu242, Leu248, Ala250, Leu255, Met259, Val315, Ala316, Ile318, and Ile378 from  $\alpha$  tubulin, and Ala180, Val181 from  $\beta$  tubulin. Therefore, in the discovery of tubulin inhibitors targeting on colchicine binding site, researcher should focus on the hydrophobicity of the candidate compound.

BAL27862 (Fig. 2) is a novel microtubule-destabilizing drug that is currently undergoing phase I clinical trial evaluation. Prota et al. (2014c) determined the crystal structure of tubulin-BAL27862 (PDB code: 4O2A) by X-ray crystallography thus demonstrated that BAL27862 binds to the same site as colchicine. This compound displayed potent tubulin assembly inhibition activity with an  $IC_{50}$  of 1.4  $\mu$ M as well as a dissociation constant of  $244 \pm 30$  nM to unassembled tubulin (Prota et al. 2014c).

A novel class of chalcones, represented by TUB091 (Fig. 2), was shown to bind to the colchicine site of tubulin via X-ray crystallography (PDB code: 5JVD) (Canela et al. 2017). As at low nanomolar concentrations, TUB091 exhibited vascular disrupting effects in vitro and in the chicken chorioallantoic membrane (CAM) assay, it was identified as an interesting lead compound for vascular disrupting agents. Besides, this inhibitor could inhibit



**Fig. 2** Colchicine site-binding agents that have been co-crystallized with tubulin

cancer and endothelial cell growth, induce G2/M phase arrest and apoptosis at low nanomolar concentrations (1–10 nM). TUB099 (Fig. 2), a derivative of TUB091, exhibited potent antitumor activity in melanoma and breast cancer xenograft models, as well as anti-metastatic activity (Canela et al. 2017).

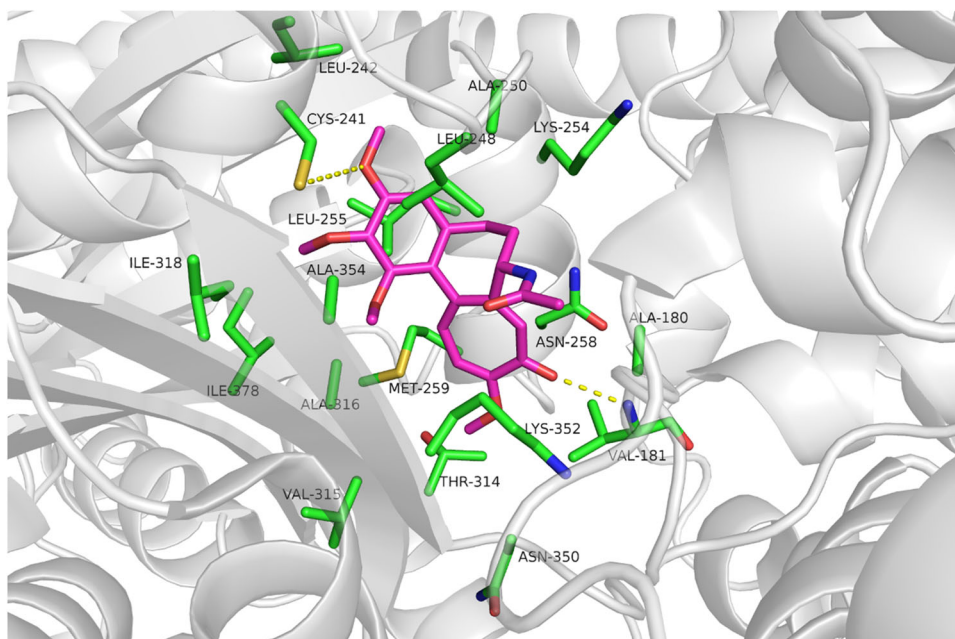
Cyclohexanediones derivatives (TUB015 and TUB075, Fig. 2) have been identified as a new class of colchicine-domain binders with a similar MOA to that of colchicine. Bueno et al. (2018) solved the crystal structures of tubulin-TUB015 (PDB code: 6FKL) and tubulin-TUB075 (PDB code: 6FKJ) by X-ray crystallography. Then structure-guided design was performed based on these complex structures, finally a new series of cyclohexanediones with a distal 2-substituted benzofurane as high-affinity ligands of the colchicine binding site were identified. As expected, these new compounds displayed potent antiproliferative activity against three human cancer cell lines [breast carcinoma (MDA-MB-231), lymphoblastic leukemia (CEM) and cervical carcinoma (HeLa) cells] and one endothelial cell [human microvascular endothelial cells (HMEC-1)] with  $IC_{50}$  values in the nM range (Bueno et al. 2018). MOA results on MDA-MB-231 cells suggested that these compounds arrested cell cycle progression at the G2/M phase

and induced apoptosis at sub-micro concentrations, which indicated that they are still colchicine site-binding agents.

Rigosertib (Fig. 2), a drug in phase III clinical trials for high-risk myelodysplastic syndrome with molecular target had not been elucidated. The combined CRISPRi/a-based chemical genetic screens was performed by Jost et al. (2017) to reveal that rigosertib is a MDA. Co-crystal structure (PDB code: 5OV7) were determined to give the direct binding of rigosertib with microtubule (Jost et al. 2017). Mutation assays further validated that rigosertib kills cancer cells by destabilizing microtubules. With rigosertib's molecular target identified, the rational and structure-guided development and selection of targeted patient groups and treatment applications will be facilitated.

Compound 7j (Fig. 2) was afforded by a preliminary structure-activity relationship on quinazolinone sulfamates. This compound showed inhibition activity on tubulin assembly ( $IC_{50} = 2.5 \mu\text{M}$ ) in vitro and colchicine binding (61% inhibition), as well as displayed potent antiproliferative activity against DU-145 human prostate and MDA-MB-231 human breast cancer cells with  $GI_{50}$  of 50 nM (Dohle et al. 2018). After co-crystallized with the  $\alpha\beta$ -tubulin heterodimer (PDB code: 5OSK), its sulfamate group was found interacting positively at the colchicine binding site (Dohle et al. 2018).

**Fig. 3** The detailed interactions between colchicine and tubulin. Tubulin and colchicine were shown as cartoon (white) and sticks (magenta), respectively. All the interactive residues were labeled and rendered as sticks (green). The yellow dashed lines indicated the H bond interaction



BKM120 (Buparlisib, Fig. 2) is a phosphoinositide 3-kinase (PI3K) inhibitor, which has been enlisted in numerous clinical studies. However, the off-target effect with microtubule polymerization inhibition was also reported. Therefore, its dominant antitumorigenic mechanism needs to be cautiously evaluated to avoid misconception of preclinical and clinical data. Bohnacker et al. (2017) separated the dual activity of BKM120 into discrete PI3K and tubulin inhibitor via their developed two BKM120 derivatives (MTD147 and PQR309, Fig. 2) that with only one atom different from BKM120. They confirmed that the antiproliferative activity of BKM120 is mainly result from microtubule-dependent cytotoxicity rather than through inhibition of PI3K by further analysis of the cellular growth arrest phenotypes and microtubule dynamics after compounds treatment (Bohnacker et al. 2017). Co-crystal structures of tubulin-BKM120 (PDB code: 5M7E), tubulin-MTD147 (PDB code: 5M7G), tubulin-MTD265 (PDB code: 5M8G), and tubulin-MTD265-R1 (PDB code: 5M8D) provided insights into the binding mode of action of this series of drugs (Bohnacker et al. 2017), thus confirmed these compounds are colchicine site-binding agents.

Podophyllotoxin derivatives are important colchicine binding site tubulin inhibitors, Niu et al. (2017) reported the first high-resolution (2.8 Å) structure of tubulin complexed with 4'-demethylepipodophyllotoxin (PDB code: 5XLT, see Fig. 2 for the structure of DMEP,) and revealed the detailed interactions between tubulin and DMEP. This work provides insights into the development of new podophyllotoxin derivatives as tubulin inhibitors targeting the colchicine site.

**Table 1** Anti-proliferative effect of QAs in A549, A2780, A2780AD cells

Compound	A549	A2780	A2780AD
QA <sub>1</sub>	60 ± 2	71 ± 14	141 ± 42
QA <sub>2</sub>	44.3 ± 11	104 ± 8	262 ± 85
Colchicine	55 ± 4	13.6 ± 2	663 ± 23

IC<sub>50</sub> (nM, mean ± standard error) values of QAs determined in lung carcinoma A549, in ovarian carcinoma A2780 and A2780AD

Quinolin-6-yloxyacetamides (QAs, represented by QA<sub>1</sub> and QA<sub>2</sub>, Fig. 2) constitute a chemical class that have fungal tubulin polymerize inhibition activity that were previously reported as fungicides (Lamberth et al. 2014). Sharma et al. (2017) found that QAs showed potent anti-proliferative effect on human cancer cells (Table 1), especially in inhibiting the proliferation of multidrug-resistant ovarian cancer cells (A2780AD) that overexpress P-glycoproteins. The crystal structure of the tubulin-QA complex (PDB code: 5O7A) showed that QAs bind to the colchicine site on tubulin (Sharma et al. 2017), which indicated that QAs are microtubule destabilization agents.

Combretastatin A4 (CA-4, Fig. 2) and its derivatives are microtubule-destabilizing agents which have attracted great attention due to their high potency, vascular disrupting and antiangiogenic activities (Tron et al. 2006). Although the cis conformation of CA-4 is more active than that of the trans conformation, it isomerize into trans form easily under natural light (Jiang et al. 2015). CA-4 was suggested to bind to the colchicine site of tubulin, but the exact binding mode of CA-4 on tubulin remain unclear because of the high-resolution structural information is lacking. Gaspari et al.

(2017) reported the high-resolution crystal structure of tubulin-cis-CA-4 (PDB code: 5LYJ). This structural data lay a unique basis for the design of combretastatin derivatives with improved activities for both medical and basic research applications.

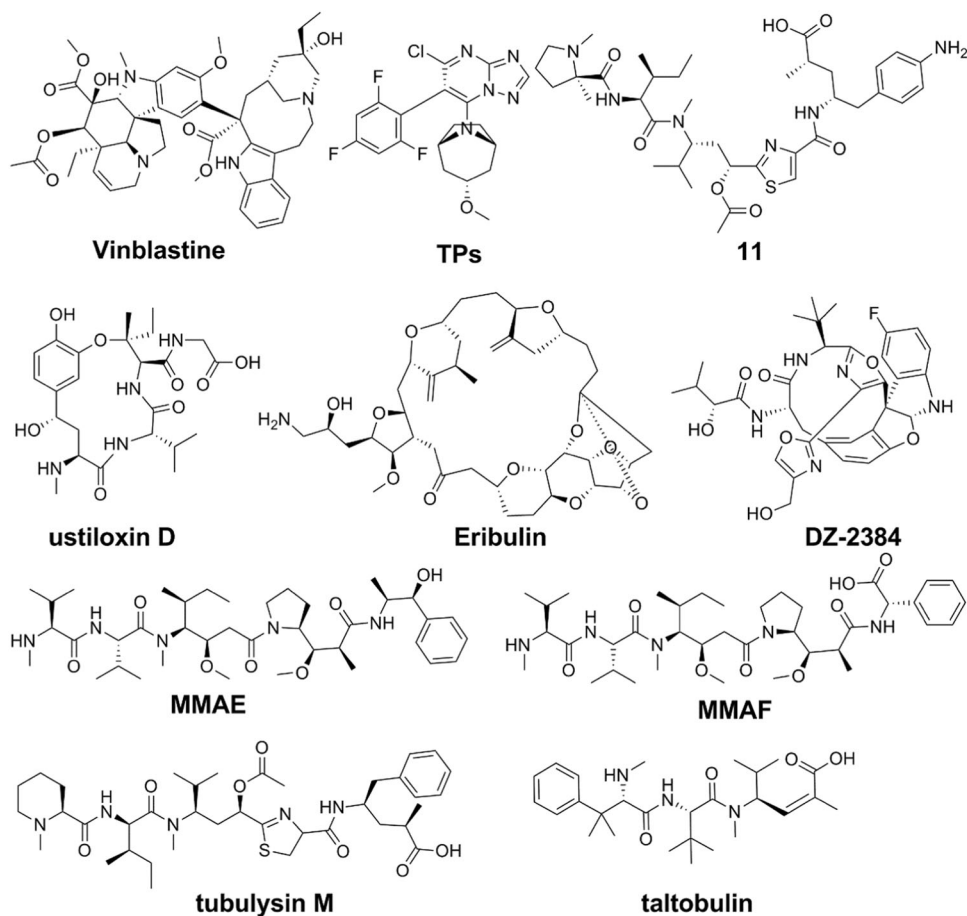
Due to the less stable property of CA-4, structural optimization was performed to improve its thermodynamically stability. Zhou et al. (2018; 2016) synthesized a series of chiral  $\beta$ -lactam bridged analogs (3-substituted 1,4-diaryl-2-azetidiones) CA-4, and evaluated their antitumor activities in vitro and in vivo. Among them, compound 9 (Fig. 2), 14b (Fig. 2), and 14c (Fig. 2) displayed potent tubulin polymerization inhibition with  $IC_{50}$  values of 3.5, 3.5 and 1.7  $\mu$ M, respectively (Zhou et al. 2018; 2016). Besides, these three compounds also exhibited potent anti-proliferative activity against four human cancer cell lines (A2780, HeLa, SKOV-3 and MDA-MB-231) with  $IC_{50}$  values of 0.001–0.021  $\mu$ M (Zhou et al. 2018; 2016). Finally, the cocrystal structures of tubulin in complex with represent compound 9, 14b, and 14c were determined by X-ray crystallography, which showed that all of these three compounds bind to the colchicine binding site (PDB code: 5GON, 5XAG, and 5XAF) (Zhou et al. 2018; 2016).

Wang et al. (2016b) determined the crystal structures of tubulin complexed with diverse colchicine binding site inhibitors [lexibulin (Fig. 2), nocodazole (Fig. 2), plinabulin (Fig. 2) and tivatinib (Fig. 2)]. High resolution structures revealed the detailed interactions between these inhibitors and tubulin (5CA0, 5CA1, 5C8Y and 5CB4, respectively) (Wang et al. 2016b). This co-crystal structures provide a solid structural basis and paid the way for developing new anti-cancer agents targeting the colchicine binding site.

### Vinca alkaloids site-binding agents

Vinca alkaloids are potent anti-tumor drugs that bind to tubulin at its inter-heterodimeric interface and have been widely used in chemotherapy. Vinblastine (Fig. 4) is one of vinca alkaloids and the crystal structure of vinblastine bound to tubulin was first resolved in 2005 with a resolution of 4.1 Å (PDB code: 1Z2B) by X-ray crystallography (Gigant et al. 2005). After that, three groups also successively determined the vinblastine-tubulin complex structure, with PDB code: 4EB6 (Ranaivoson et al. 2012), 5BMV (Wang et al. 2016a), and 5J2T (Waight et al. 2016), respectively.

**Fig. 4** Vinca alkaloids site-binding agents that have been co-crystalized with tubulin



Ustiloxins are vinblastine site-binding agents with a well established total synthesis. A 2.7 Å resolution structure of ustiloxin D bound to vinblastine binding site has been determined (PDB code: 3UT5, see Fig. 4 for the structure of ustiloxin D) (Ranaivoson et al. 2012). This finding precisely defines the interactions of ustiloxins with tubulin allows structure-based suggestions to be made for improved activity of tubulin vinblastine site-binding agents.

Antibody-drug conjugates (ADCs) have been successfully used in cancer therapy in recent years. Monomethyl auristatin E (MMAE, Fig. 4) and F (MMAF, Fig. 4) peptidyl microtubule inhibitors consisting of natural and unnatural amino acids, are extremely cytotoxic and have been widely used as a warhead in ADCs. Wang et al. (2016a) determined the high resolution crystal structures of tubulin in complex with three peptidyl microtubule inhibitors, which can be accessed under PDB codes 4ZHQ, 4ZI7, and 4ZOL, respectively [MMAE, taltobulin (HTI-286, Fig. 4), and tubulysin M (Fig. 4)]. Besides, Waight et al. (2016) also determined the crystal structures of tubulin in complex with MMAE (PDB code: 5IYZ) and MMAF (PDB code: 5J2U).

Eribulin (also known as E7389 and ER-086526, Fig. 4) is a marketed anticancer drug under the trade name Halaven for the treatment of certain patients with breast cancer and liposarcoma. It is a fully synthetic macrocyclic ketone analog of the natural product halichondrin B, derived from the marine sponge *Halichondria okadaei*. Hickford et al. (2009); Doodhi et al. (2016) first revealed that eribulin binds to a site on  $\beta$ -tubulin that is required for protofilament plus-end elongation using X-ray crystallography (PDB code: 5JH7) and demonstrated Eribulin binds predominantly to half of the vinca domain on  $\beta$ -tubulin.

DZ-2384 (Fig. 4) is a preclinical compound that displayed potent antitumor activity in models of multiple cancer types without neurotoxicity in rats at effective plasma concentrations. By analyzing the crystal structure of tubulin-DZ-2384 (PDB code: 5LOV), Wiczorek et al. (2016) demonstrated that DZ-2384 causes straightening of curved protofilaments, an effect proposed to favor polymerization of tubulin. Although DZ-2384 has a similar influence on microtubule growth rate with vinorelbine, they have different modulation, that is, DZ-2384 could increase the rescue frequency and preserve the microtubule network in nonmitotic cells and in primary neurons.

The tubulysins class of natural products have become popular payloads in the development of ADCs and small molecule drug conjugates (SMDCs) due to their potent cytotoxicity against many human cancer cells. Microtubule was the probable target of tubulysins. A series of novel tubulysin analogs were design and synthesized, among them, compound 11 (Fig. 4) exhibited much better

multidrug-resistant profile than the clinically used MMAE (Leverett et al. 2016). The co-crystal structure of 11 bound to tubulin (PDB code: 5KX5) (Leverett et al. 2016) was determined and utilized to design more analogs with improved activity.

[1,2,4]Triazolo[1,5-a]pyrimidines (TPs, Fig. 4) are a class of MTAs that belong to microtubule-stabilizing agents. Inexplicably, TPs were found to bind to vinblastine binding site on tubulin (PDB code: 5NJH) (Saez-Calvo et al. 2017), which was typically occupied by microtubule-destabilizing agents. This apparent discrepancy was addressed by Saez-Calvo et al. (2017) with combination of structural biology, cellular, and biochemical approaches. They demonstrated that TPs could promote longitudinal tubulin contacts in microtubules, different to typical microtubule-stabilizing agents that principally promote lateral contacts (Saez-Calvo et al. 2017). Besides, p-glycoprotein overexpression has no effect on TPs, which indicated they are promising MTAs against multidrug-resistant cancer cells.

### Microtubule stabilizing agents (MSAs)

Through promoting polymerization of tubulin and stabilizing the polymer, as well as preventing depolymerization, MSAs continued to be the efficacious anticancer chemotherapeutic agents that have been widely used for the treatment of cancer. With more and more tubulin-MSAs complex crystal structures determined, the molecular mechanisms by which MSAs stabilize microtubules and the interactions of MSAs with tubulin and microtubules at the molecular level are revealed.

### Taxanes site-binding agents

Paclitaxel (Taxol, Fig. 5) and its semisynthetic analog docetaxel (Fig. 5) were among the most popular anticancer drugs in the late 21st century. Paclitaxel was approved for clinical use in 1995, and has been widely used as anticancer chemotherapeutic agents for treatment of breast and ovarian cancer, non-small-cell lung cancer. Although the binding site of paclitaxel on tubulin was known with precision as the tubulin-paclitaxel complex electron crystal structure was determined (Nogales et al. 1995), the high-resolution (3.5 Å) cryo-electron microscopy (cryo-EM) reconstructions of microtubule stabilized by taxol were reported in 2017 with PDB code 5SYF (Kellogg et al. 2017). The clinical success of the taxanes, together with the poor solubility problems and development of drug resistance have stimulated the search for other drugs with similar MOA but with improved pharmacological profiles. As shown in Fig. 6, the interactions

between paclitaxel and tubulin were hydrophobic,  $\pi$ - $\pi$  stacking, and charged.

The marine natural product zampanolide (Fig. 5) and its analogs are covalent inhibitors targeting paclitaxel binding site. Zampanolide-ligated tubulin (PDB code: 5NG1) represented an assembly activated state of tubulin and the effect of zampanolide on tubulin association and the binding of tubulin ligands at other binding sites were well studied (Field et al. 2018). It was demonstrated that covalent binding of zampanolide to  $\beta$ -tubulin affects both the colchicine site and the exchangeable nucleotide binding site (Field et al. 2018).

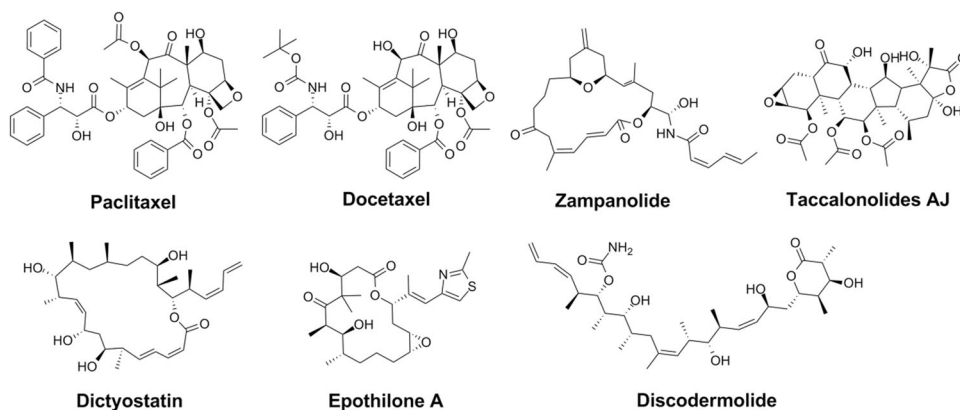
Dictyostatin (Fig. 5) is a potent anticancer macrolide that target tubulin, little is known about the details of its interaction with tubulin. Until the crystal structure of tubulin in complex with dictyostatin was available, the rational structure-based design of dictyostatin analogs with improved activity was able to carry out. Trigili et al. (2016)

solved the tubulin-dictyostatin complex structure (PDB code: 5MF4) using X-ray crystallography.

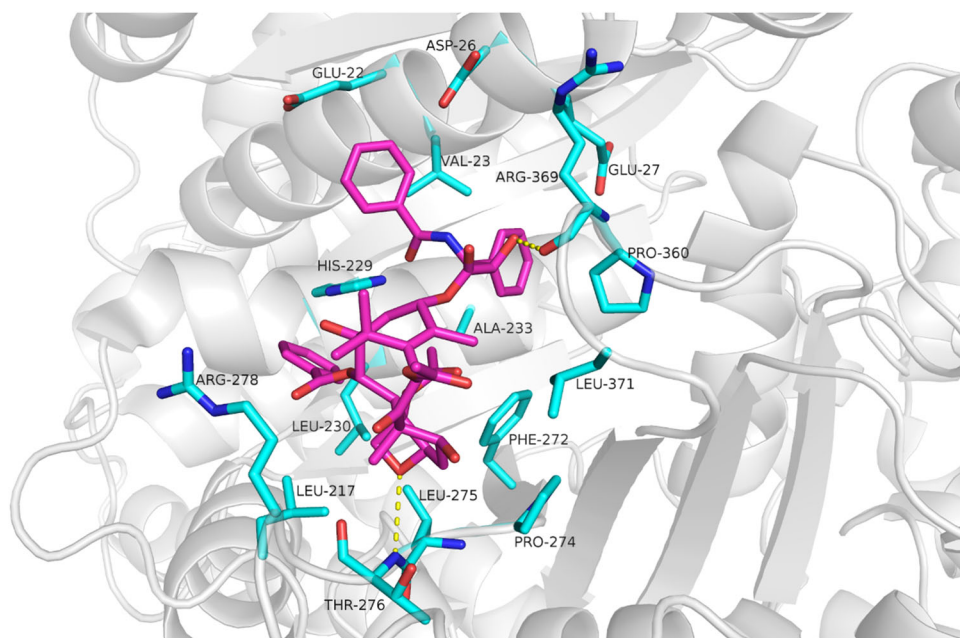
Taccalonolides AJ (Fig. 5) exhibited a paclitaxel comparable potent tubulin inhibition ( $IC_{50} = 4.2$  nM) and demonstrated a direct interaction with purified tubulin (Li et al. 2011). Although it has been demonstrated to covalently bind to microtubules (Risinger et al. 2013), the exact residue involved in taccalonolides AJ covalent binding has not been determined. Using X-ray crystallography, Wang et al. (2017) determined a 2.05 Å crystal structure of the tubulin-taccalonolides AJ complex (PDB code: 5EZY). The structure reveals that taccalonolides AJ covalently binds to  $\beta$ -tubulin D226 at the taxane-site.

Epothilone A (Fig. 5) was a new group of compound obtained from the *myxobacterium Sorangium cellulosum* with a similar action mechanism to paclitaxel. Prota et al. (2013) determined a high-resolution crystal structure of  $\alpha\beta$ -tubulin in complex with epothilone A (PDB code: 4I50),

**Fig. 5** Taxanes site-binding agents that have been co-crystallized with tubulin



**Fig. 6** The detailed interactions between paclitaxel and tubulin. Tubulin and paclitaxel were shown as cartoon (white) and sticks (magenta), respectively. All the interactive residues were labeled and rendered as sticks (cyan). The yellow dashed lines indicated the H bond interaction



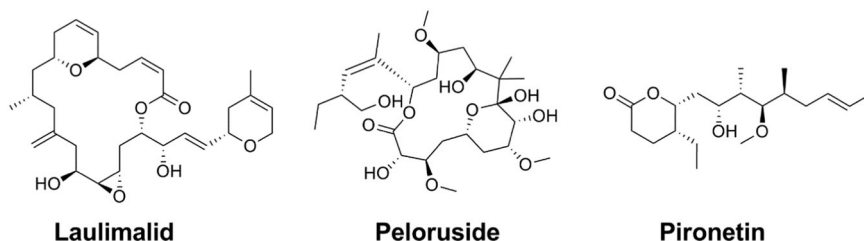
epothilone A was found to bind to the taxane pocket of  $\beta$ -tubulin and use its side chains to induce structuring of the M-loop into a short helix.

Discodermolide (Fig. 5), a potent antitumor polyketide from the marine sponge *Discoderma dissolute* (Gunasekera et al. 1990), binds to the taxane site of  $\beta$ -tubulin (Hung et al. 1996). Compared to paclitaxel, discodermolide exhibited more potent binding affinity with tubulin, more efficient in promoting tubulin polymerization and has potent cytotoxic activity against a number of human tumor cell lines, including paclitaxel-resistant ovarian and colon carcinoma cells (Huang et al. 2006; Kowalski et al. 1997). Prota et al. (2017) successfully crystallized and solved the high resolution structure of DDM and KS-1-199-32 in complex with tubulin (PDB code: 5LXT).

### Laulimalide site-binding agents

Laulimalide (Mooberry et al. 1999) (Fig. 7) and peloruside A (Hood et al. 2002; West et al. 2000) (peloruside, Fig. 7) are marine sponge products and are promising non-taxane-site MSAs for their desirable properties, such as they possess potent inhibition activity against the growth of multidrug-resistant cancer cells (Gaitanos et al. 2004; Pryor et al. 2002), and have synergistically effect with paclitaxel or epothilones on promoting the microtubule stability (Gapud et al. 2004; Hamel et al. 2006). To address how laulimalide and peloruside A promote tubulin assembly and microtubule stability, and how they synergize with taxane-site drugs, Prota et al. (2014b) determined crystal complex structures of tubulin-laulimalide (PDB code: 4O4H) and tubulin-peloruside A (PDB code: 4O4J) using X-ray crystallography. These two complex structures showed that laulimalide and peloruside A bind to non-taxane site on  $\beta$ -tubulin and use their respective macrolide core structures to interact with a second tubulin dimer across protofilaments. Meanwhile, they allosterically stabilize the taxane-site M-loop. Besides, ternary complexes structures of tubulin-laulimalide-epothilone A (PDB code: 4O4I) and tubulin-peloruside A-epothilone A (PDB code: 4O4L) are also solved, and a crosstalk between the laulimalide/peloruside and taxane sites via the M-loop of  $\beta$ -tubulin is found (Prota et al. 2014b).

**Fig. 7** Laulimalide and Pironetin site-binding agents that have been co-crystallized with tubulin



## New binding sites on tubulin

### Pironetin site-binding agents

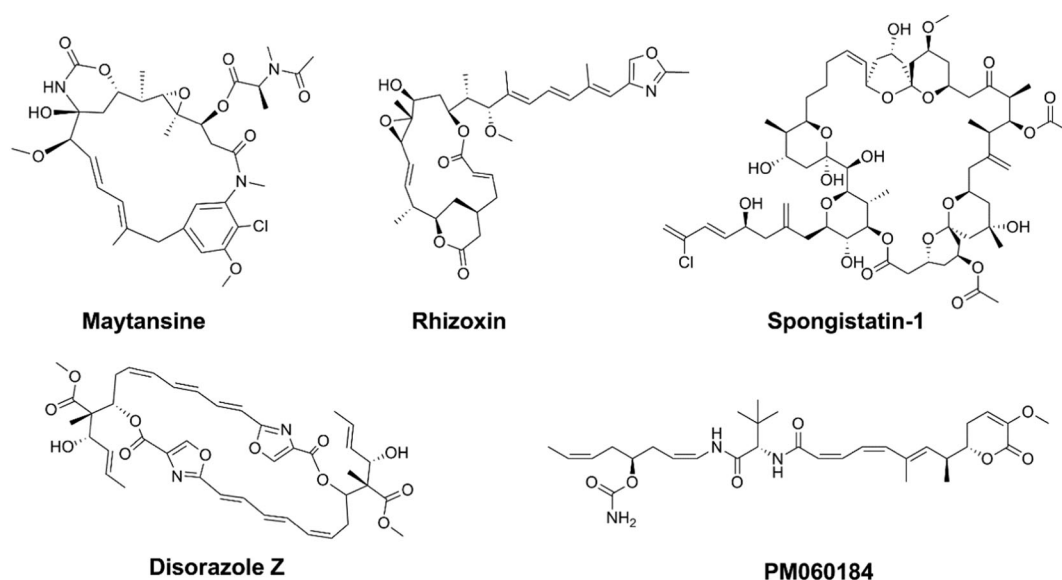
Pironetin (Fig. 7) was originally isolated from fermentation broths of *Streptomyces* strains and was subsequently found potent inhibitory activity on microtubule formation. Yang et al. (2016) and Prota et al. (2016) successively solved the crystal structure of the tubulin-pironetin complex (PDB code: 5FNV, 5LA6) and found that this compound covalently binds to Cys316 of  $\alpha$ -tubulin and acted as destabilized microtubule agent.

### Maytansine site-binding agents

Maytansine (Fig. 8) is part of an ADC and is approved for the treatment of advanced breast cancer (Kupchan et al. 1972; Verma et al. 2012). Prota et al. (2014a) determined the crystal complex structure of tubulin-maytansine (PDB code: 4TV8) by X-ray crystallography, established the exact tubulin-binding site of maytansine and clarified the specific interactions. The binding site of maytansine was a new tubulin-binding site and pharmacophore which can be used for the development of microtubule-destabilizing anticancer drugs. Besides, both the phase I drug PM060184 (Fig. 8) and the phase II drug rhizoxin (Fig. 8) were demonstrated to bind to the same site as maytansine by X-ray crystallography (PDB code: 4TV9 and 4TUY) (Prota et al. 2014a). This site is associated with a distinct molecular mechanism for the inhibition of microtubule formation, thus provide a structural basis for the structure-based design of more potent microtubule-destabilizing agents.

A quantitative fluorescence anisotropy displacement assay based on a fluorescein-labeled maytansine derivative was developed by Menchon et al. (2018) with the aim to identify and characterize more maytansine-site microtubule inhibitors. Using this assay, they discovered that the two natural products spongistatin-1 (Fig. 8) and disorazole Z (Fig. 8) bind to the maytansine site on  $\beta$ -tubulin. They confirmed their findings by determining the high-resolution crystal complex structures of tubulin-spongistatin-1 (PDB code: 6FII) and tubulin-disorazole Z (PDB code: 6FJM) using X-ray crystallography.





**Fig. 8** New binding sites tubulin inhibitors that have been co-crystallized with tubulin

## Conclusions

In this review, all the reported microtubule inhibitors that have co-crystal structures with tubulin are summarized. From the insights of developing tubulin inhibitors, we proposed that it is very difficult to develop pironetin site tubulin inhibitor as there is only one inhibitor targeting this site reported. As for the other newly discovered maytansine binding site, it is also intractable to target though it is a fascinating site with distinct molecular mechanism for the inhibition of microtubule formation. Laulimalid binding site is on the  $\beta$  tubulin surface while this binding pocket is not large enough for accommodating of commonly natural products, therefore, except for molecules that possess potent affinity could be developed targeting this site. Vinca alkaloids and paclitaxel binding sites are typical tubulin inhibitor binding sites that have been successfully used to develop anti-cancer agents. Although there are no colchicine-binding site inhibitors approved by FDA to treat cancer, developing inhibitors targeting this site (the intradimer interface) enjoyed great success in the past few years, which can be indicated by the diverse inhibitors reported. Thus, it is relatively easy to develop tubulin inhibitors targeting this site. However, all drugs in clinical use employ the maytansine, the vinca and the paclitaxel site tubulin inhibitors. We think the reason for this observation is these three binding sites are druggable target sites. Besides, all of these three sites are located or mainly located on  $\beta$  tubulin subunit, which may be the reason for their druggability sites.

As can be indicated by this review, almost all the tubulin inhibitors belong to natural product, so with the development of total synthesis, more and more potent tubulin inhibitors is expected. In addition, with the more and more

tubulin inhibitors deposited in PDB, unique binding sites on microtubule will be revealed, which could further promote tubulin inhibitors development.

Although vinblastine and paclitaxel is widely used to treat various cancers, the broad clinical application of MDAs, is hampered by their severe neurotoxicity and myelosuppression adverse effects and the development of resistance. Therefore, we believe that the direction of the future tubulin inhibitors development is to discover microtubules targeted drugs with distinct mechanism. Besides, use ADC approaches is also a feasible choice which have revived interest in the development of highly potent MDAs for therapeutic use.

## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

**Publisher's note:** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

## References

- Bohnacker T, Prota AE, Beauflis F, Burke JE, Melone A, Inglis AJ, Rageot D, Sele AM, Cmiljanovic V, Cmiljanovic N, Bargsten K, Aher A, Akhmanova A, Diaz JF, Fabbro D, Zvelebil M, Williams RL, Steinmetz MO, Wymann MP (2017) Deconvolution of Buparlisib's mechanism of action defines specific PI3K and tubulin inhibitors for therapeutic intervention. *Nat Commun* 8:14683
- Bueno O, Estevez Gallego J, Martins S, Prota AE, Gago F, Gomez-SanJuan A, Camarasa MJ, Barasoain I, Steinmetz MO, Diaz JF,

- Perez-Perez MJ, Liekens S, Priego EM (2018) High-affinity ligands of the colchicine domain in tubulin based on a structure-guided design. *Sci Rep* 8:4242
- Canela MD, Noppen S, Bueno O, Protá AE, Bargsten K, Saez-Calvo G, Jimeno ML, Benkheil M, Ribatti D, Velazquez S, Camarasa MJ, Diaz JF, Steinmetz MO, Priego EM, Perez-Perez MJ, Liekens S (2017) Antivascular and antitumor properties of the tubulin-binding chalcone TUB091. *Oncotarget* 8:14325–14342
- Dohle W, Jourdan FL, Menchon G, Protá AE, Foster PA, Mannion P, Hamel E, Thomas MP, Kasprzyk PG, Ferrandis E, Steinmetz MO, Leese MP, Potter BVL (2018) Quinazolinone-Based Anticancer Agents: Synthesis, Antiproliferative SAR, Antitubulin Activity, and Tubulin Co-crystal Structure. *J Med Chem* 61:1031–1044
- Doodhi H, Protá AE, Rodríguez-García R, Xiao H, Cústar DW, Bargsten K, Katrukha EA, Hilbert M, Hua S, Jiang K, Grigoriev I, Yang CH, Cox D, Horwitz SB, Kapitein LC, Akhmanova A, Steinmetz MO (2016) Termination of Protofilament Elongation by Eribulin Induces Lattice Defects that Promote Microtubule Catastrophes. *Curr Biol* 26:1713–1721
- Field JJ, Pera B, Gallego JE, Calvo E, Rodríguez-Salariés J, Saez-Calvo G, Zuwerra D, Jordi M, Andreu JM, Protá AE, Menchon G, Miller JH, Altmann KH, Diaz JF (2018) Zampanolide Binding to Tubulin Indicates Cross-Talk of Taxane Site with Colchicine and Nucleotide Sites. *J Nat Prod* 81:494–505
- Gaitanos TN, Buey RM, Diaz JF, Northcote PT, Teesdale-Spittle P, Andreu JM, Miller JH (2004) Peloruside A does not bind to the taxoid site on beta-tubulin and retains its activity in multidrug-resistant cell lines. *Cancer Res* 64:5063–5067
- Gapud EJ, Bai R, Ghosh AK, Hamel E (2004) Laulimalide and paclitaxel: a comparison of their effects on tubulin assembly and their synergistic action when present simultaneously. *Mol Pharmacol* 66:113–121
- Gaspari R, Protá AE, Bargsten K, Cavalli A, Steinmetz MO (2017) Structural Basis of cis- and trans-Combretastatin Binding to Tubulin. *Chem-US* 2:102–113
- Gigant B, Wang C, Ravelli RB, Roussi F, Steinmetz MO, Curmi PA, Sobel A, Knossow M (2005) Structural basis for the regulation of tubulin by vinblastine. *Nature* 435:519–522
- Gunasekera SP et al. (1990) Discodermolide: a new bioactive polyhydroxylated lactone from the marine sponge discodermia dissoluta. *Journal of Organic Chemistry* 55:4
- Hamel E, Day BW, Miller JH, Jung MK, Northcote PT, Ghosh AK, Curran DP, Cushman M, Nicolaou KC, Paterson I, Sorensen EJ (2006) Synergistic effects of peloruside A and laulimalide with taxoid site drugs, but not with each other, on tubulin assembly. *Mol Pharmacol* 70:1555–1564
- Hickford SJ, Blunt JW, Munro MH (2009) Antitumor polyether macrolides: four new halichondrins from the New Zealand deep-water marine sponge *Lissodendoryx* sp. *Bioorg Med Chem* 17:2199–2203
- Hood KA, West LM, Rouwe B, Northcote PT, Berridge MV, Wakefield SJ, Miller JH (2002) Peloruside A, a novel antimetabolic agent with paclitaxel-like microtubule-stabilizing activity. *Cancer Res* 62:3356–3360
- Huang GS, Lopez-Barcons L, Freeze BS, Smith 3rd AB, Goldberg GL, Horwitz SB, McDaid HM (2006) Potentiation of taxol efficacy and by discodermolide in ovarian carcinoma xenograft-bearing mice. *Clin Cancer Res* 12:298–304
- Hung DT, Chen J, Schreiber SL (1996) (+)-Discodermolide binds to microtubules in stoichiometric ratio to tubulin dimers, blocks taxol binding and results in mitotic arrest. *Chem Biol* 3:287–293
- Jiang J, Zheng C, Zhu K, Liu J, Sun N, Wang C, Jiang H, Zhu J, Luo C, Zhou Y (2015) Quantum chemistry calculation-aided structural optimization of combretastatin A-4-like tubulin polymerization inhibitors: improved stability and biological activity. *J Med Chem* 58:2538–2546
- Jost M, Chen Y, Gilbert LA, Horlbeck MA, Krenning L, Menchon G, Rai A, Cho MY, Stern JJ, Protá AE, Kampmann M, Akhmanova A, Steinmetz MO, Tanenbaum ME, Weissman JS (2017) Combined CRISPRi/a-Based Chemical Genetic Screens Reveal that Rigosertib Is a Microtubule-Destabilizing Agent. *Mol Cell* 68:210–223 e216
- Kellogg EH, Hejab NMA, Howes S, Northcote P, Miller JH, Diaz JF, Downing KH, Nogales E (2017) Insights into the Distinct Mechanisms of Action of Taxane and Non-Taxane Microtubule Stabilizers from Cryo-EM Structures. *J Mol Biol* 429:633–646
- Kowalski RJ, Giannakakou P, Gunasekera SP, Longley RE, Day BW, Hamel E (1997) The microtubule-stabilizing agent discodermolide competitively inhibits the binding of paclitaxel (Taxol) to tubulin polymers, enhances tubulin nucleation reactions more potently than paclitaxel, and inhibits the growth of paclitaxel-resistant cells. *Mol Pharmacol* 52:613–622
- Kupchan SM, Komoda Y, Court WA, Thomas GJ, Smith RM, Karim A, Gilmore CJ, Haltiwanger RC, Bryan RF (1972) Maytansine, a novel antileukemic ansa macrolide from *Maytenus ovatus*. *J Am Chem Soc* 94:1354–1356
- Lamberth C, Kessabi FM, Beaudegnies R, Quaranta L, Trah S, Berthon G, Cederbaum F, Knauf-Beiter G, Grasso V, Bieri S, Corran A, Thacker U (2014) Synthesis and fungicidal activity of quinolin-6-yloxyacetamides, a novel class of tubulin polymerization inhibitors. *Bioorg Med Chem* 22:3922–3930
- Leverett CA, Sukuru SC, Vetelino BC, Musto S, Parris K, Pandit J, Loganzo F, Varghese AH, Bai G, Liu B, Liu D, Hudson S, Doppalapudi VR, Stock J, O'Donnell CJ, Subramanyam C (2016) Design, Synthesis, and Cytotoxic Evaluation of Novel Tubulysin Analogues as ADC Payloads. *ACS Med Chem Lett* 7:999–1004
- Li J, Risinger AL, Peng J, Chen Z, Hu L, Mooberry SL (2011) Potent taccalonolides, AF and AJ, inform significant structure-activity relationships and tubulin as the binding site of these microtubule stabilizers. *J Am Chem Soc* 133:19064–19067
- Menchon G, Protá AE, Lucena-Agell D, Bucher P, Jansen R, Irschik H, Müller R, Paterson I, Diaz JF, Altmann KH, Steinmetz MO (2018) A fluorescence anisotropy assay to discover and characterize ligands targeting the maytansine site of tubulin. *Nat Commun* 9:2106
- Mooberry SL, Tien G, Hernandez AH, Plubrukarn A, Davidson BS (1999) Laulimalide and isolaulimalide, new paclitaxel-like microtubule-stabilizing agents. *Cancer Res* 59:653–660
- Negi AS, Gautam Y, Alam S, Chanda D, Luqman S, Sarkar J, Khan F, Konwar R (2015) Natural antitubulin agents: importance of 3,4,5-trimethoxyphenyl fragment. *Bioorg Med Chem* 23:373–389
- Niu L, Wang Y, Wang C, Wang Y, Jiang X, Ma L, Wu C, Yu Y, Chen Q (2017) Structure of 4'-demethylepipodophyllotoxin in complex with tubulin provides a rationale for drug design. *Biochem Biophys Res Commun* 493:718–722
- Nogales E, Wolf SG, Khan IA, Luduena RF, Downing KH (1995) Structure of tubulin at 6.5 Å and location of the taxol-binding site. *Nature* 375:424–427
- Protá AE, Bargsten K, Diaz JF, Marsh M, Cuevas C, Liniger M, Neuhaus C, Andreu JM, Altmann KH, Steinmetz MO (2014a) A new tubulin-binding site and pharmacophore for microtubule-destabilizing anticancer drugs. *Proc Natl Acad Sci USA* 111:13817–13821
- Protá AE, Bargsten K, Northcote PT, Marsh M, Altmann KH, Miller JH, Diaz JF, Steinmetz MO (2014b) Structural basis of microtubule stabilization by laulimalide and peloruside A. *Angew Chem Int Ed Engl* 53:1621–1625
- Protá AE, Bargsten K, Redondo-Horcajo M, Smith III AB, Yang CH, McDaid HM, Paterson I, Horwitz SB, Fernando Diaz J, Steinmetz

- MO (2017) Structural Basis of Microtubule Stabilization by Discodermolide. *Chembiochem* 18:905–909
- Prota AE, Bargsten K, Zurwerra D, Field JJ, Diaz JF, Altmann KH, Steinmetz MO (2013) Molecular mechanism of action of microtubule-stabilizing anticancer agents. *Science* 339:587–590
- Prota AE, Danel F, Bachmann F, Bargsten K, Buey RM, Pohlmann J, Reinelt S, Lane H, Steinmetz MO (2014c) The novel microtubule-destabilizing drug BAL27862 binds to the colchicine site of tubulin with distinct effects on microtubule organization. *J Mol Biol* 426:1848–1860
- Prota AE, Setter J, Waight AB, Bargsten K, Murga J, Diaz JF, Steinmetz MO (2016) Pironetin Binds Covalently to alphaCys316 and Perturbs a Major Loop and Helix of alpha-Tubulin to Inhibit Microtubule Formation. *J Mol Biol* 428:2981–2988
- Pryor DE, O'Brate A, Bilcer G, Diaz JF, Wang Y, Wang Y, Kabaki M, Jung MK, Andreu JM, Ghosh AK, Giannakakou P, Hamel E (2002) The microtubule stabilizing agent laulimalide does not bind in the taxoid site, kills cells resistant to paclitaxel and epothilones, and may not require its epoxide moiety for activity. *Biochemistry* 41:9109–9115
- Ranaivoson FM, Gigant B, Berritt S, Joullie M, Knossow M (2012) Structural plasticity of tubulin assembly probed by vinca-domain ligands. *Acta Crystallogr D Biol Crystallogr* 68:927–934
- Ravelli RB, Gigant B, Curmi PA, Jourdain I, Lachkar S, Sobel A, Knossow M (2004) Insight into tubulin regulation from a complex with colchicine and a stathmin-like domain. *Nature* 428:198–202
- Risinger AL, Li J, Bennett MJ, Rohena CC, Peng J, Schriemer DC, Mooberry SL (2013) Taccalonolide binding to tubulin imparts microtubule stability and potent in vivo activity. *Cancer Res* 73:6780–6792
- Saez-Calvo G, Sharma A, Balaguer FA, Barasoain I, Rodriguez-Salarichs J, Olieric N, Munoz-Hernandez H, Berbis MA, Wendeborn S, Penalva MA, Matesanz R, Canales A, Prota AE, Jimenez-Barbero J, Andreu JM, Lamberth C, Steinmetz MO, Diaz JF (2017) Triazolopyrimidines Are Microtubule-Stabilizing Agents that Bind the Vinca Inhibitor Site of Tubulin. *Cell Chem Biol* 24:737–750 e736
- Sharma A, Saez-Calvo G, Olieric N, de Asis Balaguer F, Barasoain I, Lamberth C, Diaz JF, Steinmetz MO (2017) Quinolin-6-Yloxyacetamides Are Microtubule Destabilizing Agents That Bind to the Colchicine Site of Tubulin. *Int J Mol Sci* 18:E1336
- Trigili C, Barasoain I, Sanchez-Murcia PA, Bargsten K, Redondo-Horcajo M, Nogales A, Gardner NM, Meyer A, Naylor GJ, Gomez-Rubio E, Gago F, Steinmetz MO, Paterson I, Prota AE, Diaz JF (2016) Structural Determinants of the Dictyostatin Chemotype for Tubulin Binding Affinity and Antitumor Activity Against Taxane- and Epothilone-Resistant Cancer Cells. *ACS Omega* 1:1192–1204
- Tron GC, Pirali T, Sorba G, Pagliai F, Busacca S, Genazzani AA (2006) Medicinal chemistry of combretastatin A4: present and future directions. *J Med Chem* 49:3033–3044
- Verma S, Miles D, Gianni L, Krop IE, Welslau M, Baselga J, Pegram M, Oh DY, Dieras V, Guardino E, Fang L, Lu MW, Olsen S, Blackwell K, Group ES (2012) Trastuzumab emtansine for HER2-positive advanced breast cancer. *N Engl J Med* 367:1783–1791
- Waight AB, Bargsten K, Doronina S, Steinmetz MO, Sussman D, Prota AE (2016) Structural Basis of Microtubule Destabilization by Potent Auristatin Anti-Mitotics. *PLoS ONE* 11:e0160890
- Wang Y, Benz FW, Wu Y, Wang Q, Chen Y, Chen X, Li H, Zhang Y, Zhang R, Yang J (2016a) Structural Insights into the Pharmacophore of Vinca Domain Inhibitors of Microtubules. *Mol Pharmacol* 89:233–242
- Wang Y, Yu Y, Li GB, Li SA, Wu C, Gigant B, Qin W, Chen H, Wu Y, Chen Q, Yang J (2017) Mechanism of microtubule stabilization by taccalonolide. *AJ. Nat Commun* 8:15787
- Wang Y, Zhang H, Gigant B, Yu Y, Wu Y, Chen X, Lai Q, Yang Z, Chen Q, Yang J (2016b) Structures of a diverse set of colchicine binding site inhibitors in complex with tubulin provide a rationale for drug discovery. *FEBS J* 283:102–111
- West LM, Northcote PT, Battershill CN (2000) Peloruside A: a potent cytotoxic macrolide isolated from the new zealand marine sponge *Mycale* sp. *J Org Chem* 65:445–449
- Wieczorek M, Tcherkezian J, Bernier C, Prota AE, Chaaban S, Rolland Y, Godbout C, Hancock MA, Arezzo JC, Ocal O, Rocha C, Olieric N, Hall A, Ding H, Bramoulle A, Annis MG, Zogopoulos G, Harran PG, Wilkie TM, Brekken RA, Siegel PM, Steinmetz MO, Shore GC, Brouhard GJ, Roulston A (2016) The synthetic diazonamide DZ-2384 has distinct effects on microtubule curvature and dynamics without neurotoxicity. *Sci Transl Med* 8:365ra159
- Yang J, Wang Y, Wang T, Jiang J, Botting CH, Liu H, Chen Q, Yang J, Naismith JH, Zhu X, Chen L (2016) Pironetin reacts covalently with cysteine-316 of alpha-tubulin to destabilize microtubule. *Nat Commun* 7:12103
- Zhou P, Liang Y, Zhang H, Jiang H, Feng K, Xu P, Wang J, Wang X, Ding K, Luo C, Liu M, Wang Y (2018) Design, synthesis, biological evaluation and cocrystal structures with tubulin of chiral beta-lactam bridged combretastatin A-4 analogues as potent antitumor agents. *Eur J Med Chem* 144:817–842
- Zhou P, Liu Y, Zhou L, Zhu K, Feng K, Zhang H, Liang Y, Jiang H, Luo C, Liu M, Wang Y (2016) Potent antitumor activities and structure basis of the chiral beta-lactam bridged analogue of combretastatin A-4 binding to tubulin. *J Med Chem* 59:10329–10334