Review

TUBULIN-INTERACTIVE STILBENE DERIVATIVES AS ANTICANCER AGENTS

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Abstract: Microtubules are dynamic polymers that occur in eukaryotic cells and play important roles in cell division, motility, transport and signaling. They form during the process of polymerization of α- and β-tubulin dimers. Tubulin is a significant and heavily researched molecular target for anticancer drugs. Combretastatins are natural *cis*-stilbenes that exhibit cytotoxic properties in cultured cancer cells *in vitro*. Combretastatin A-4 (3'-hydroxy-3,4,4', 5-tetramethoxy-*cis*-stilbene; CA-4) is a potent cytotoxic *cis*-stilbene that binds to β-tubulin at the colchicine-binding site and inhibits tubulin polymerization. The prodrug CA-4 phosphate is currently in clinical trials as a chemotherapeutic

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agent for cancer treatment. Numerous series of stilbene analogs have been studied in search of potent cytotoxic agents with the requisite tubulin-interactive properties. Microtubule-interfering agents include numerous CA-4 and *trans*-resveratrol analogs and other synthetic stilbene derivatives. Importantly, these agents are active in both tumor cells and immature endothelial cells of tumor blood vessels, where they inhibit the process of angiogenesis. Recently, computer-aided virtual screening was used to select potent tubulin-interactive compounds. This review covers the role of stilbene derivatives as a class of anti-tumor agents that act by targeting microtubule assembly dynamics. Additionally, we present the results of molecular modeling of their binding to specific sites on the α- and β-tubulin heterodimer. This has enabled the elucidation of the mechanism of stilbene cytotoxicity and is useful in the design of novel agents with improved anti-mitotic activity. Tubulin-interactive agents are believed to have the potential to play a significant role in the fight against cancer.

Key words: Tubulin polymerization, Tubulin-interactive agents, Stilbenes, Combretastatins

INTRODUCTION

The high incidence of cancer and the high cost of its treatment are important factors driving the search for new and effective chemotherapeutic substances with multi-target activity and no toxicity to normal cells. Microtubules are believed to be one of the more successful targets for cancer chemotherapy. In 1990, the tubulin-interacting agent paclitaxel was introduced as a clinically approved anticancer chemotherapy agent. Since then, the interest in tubulin as a molecular target for anticancer drugs has significantly increased. Over 25% of new drug candidates that entered clinical trials as anticancer agents between 2005 and 2007 exert their effects through interaction with tubulin [1].

Almost all tubulin-interacting agents are natural products or analogs thereof that are well suited to binding to the complex three-dimensional surface of the tubulin polymer. Studies on their interaction with tubulin-binding sites have provided information on the mechanism of anti-mitotic activity of potential drugs used for cancer treatment and allowed scientists to develop new and efficient chemotherapeutics. This review focuses on the anti-carcinogenic properties of natural and modified stilbenes with respect to their interaction with the cellular protein tubulin.

The interest in stilbenes as tubulin-interactive agents was inspired by the work of Pettit, who first isolated combretastatins and determined their anti-mitotic activity in the 1980s [2]. Combretastatins are natural *cis*-stilbenes that are isolated from the bark of the African willow tree (*Combretum caffrum*). The most prominent representative of this group of compounds, combretastatin A-4 (3'-hydroxy-3,4,4',5-tetramethoxy-*cis*-stilbene; CA-4; Fig. 3), exerts high anti-mitotic and anti-angiogenic activities [see 3 for a review]. Presently, phase III

clinical trials are underway to test the anticancer effectiveness of a phosphate prodrug of CA-4 (CA-4P, also known as fosbretabulin; trade name Zybrestat). CA-4P is used alone or in combination with traditional chemotherapeutic agents or with radiotherapy [4].

Microtubules, which are composed of αβ-tubulin heterodimers (Fig. 1), are dynamic polymers of eukaryotic cells. A microtubule is a tube constructed from parallel linear polymers (protofilaments) in which the heterodimers are assembled head to tail in a polar fashion (Fig. 2A). These protofilaments interact laterally to form the cylindrical and helical microtubule wall (Fig. 2B) [9].

Microtubules play important roles in cell division, cell motility, cellular transport, maintaining cell polarity, and cell signaling. Microtubule polymerization is a complex process involving a cooperative assembly of αβ-tubulin dimers followed by GTP hydrolysis [5]. Nogales *et al.* reported on the crystal structure of αβ-tubulin heterodimers (Fig. 1) [6]. Each subunit of the heterodimer has one GTP molecule bound to it. The α-subunit binds to GTP in an irreversible manner while the GTP bound to the β-subunit is exchangeable and hydrolyzes during polymerization [6]. Impaired microtubule function leads to abnormal cell morphology and may pose a challenge to cell survival, leading to apoptosis.

There are three distinct phases of microtubule polymerization: nucleation, polymer growth and steady-state equilibrium. In the nucleation phase, an oligomer consisting of 6 to 12 αβ-tubulin dimers is formed. In the polymer growth phase, GTP binds to αβ-tubulin dimers, which leads to microtubule elongation and the formation of the protofilament. This is followed by the steady-state phase, when the addition and dissociation of tubulin subunits is balanced and the net molecular mass of the polymer does not change.

Microtubule dynamics have been investigated *in vitro* using light microscopy of reconstituted microtubules and in live cells using time-lapse video microscopy. Two types of dynamics have been observed: treadmilling and dynamic instability. Treadmilling is a flow of subunits from one end of the microtubule (the minus end) to the other (the plus end); it does not change the average length of the microtubules. Dynamic instability is the process of alternate growth and shortening of microtubule ends. It was first reported by Mitchison and Kirschner [7]. They termed the transition from a growing phase to a shortening phase as a catastrophe and a transition from a shortening phase to a growing phase as a rescue. GTP hydrolysis is necessary for switching between catastrophe and rescue (Fig. 2C, step 4).

It has been postulated that GTP hydrolysis changes the conformation of a protofilament from a slightly curved to a more profoundly curved tubulin-GDP structure [8]. Additionally, microtubules are stabilized and destabilized by microtubule-associated proteins (MAPs). Specialized MAPs named plus-and-tracking proteins (+TIPs) interact with the microtubule ends and with each other to regulate microtubule dynamics [see 9 for a review].
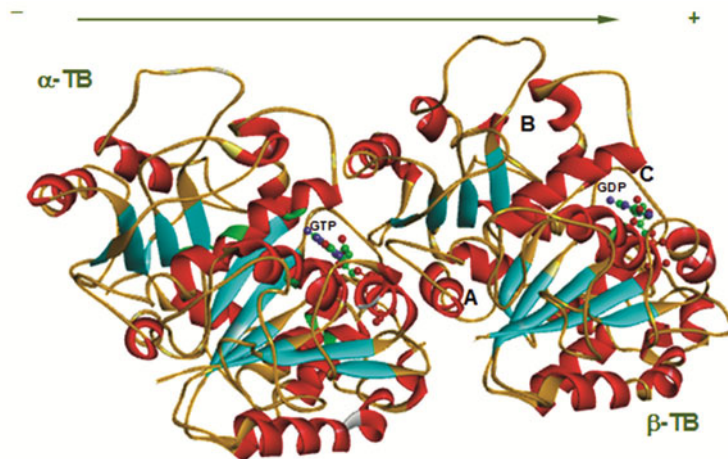
Fig. 1. Ribbon diagram of the tubulin dimer showing α-tubulin with bound GTP (left) and β-tubulin containing GDP (right) with three major binding sites: the colchicine-binding site (A), the paclitaxel-binding site (B), and the vinca alkaloid domain (C). The arrow indicates the direction of the protofilament and microtubule axis.
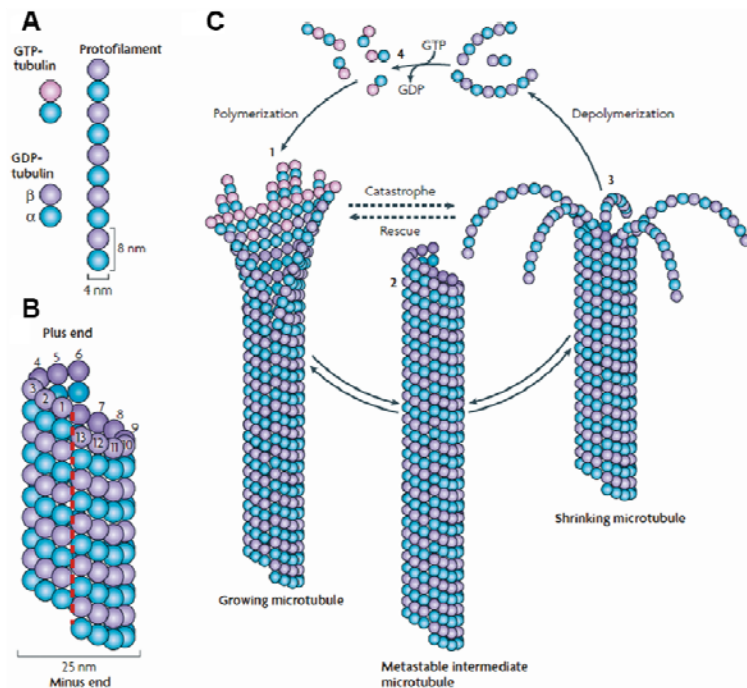
Fig. 2. Microtubule structure and dynamic instability. A – Protofilament structure. B – Microtubule structure. C – Illustration of dynamic instability. 1 – growing microtubule, 2 – metastable intermediate microtubule, 3 – shrinking microtubule, 4 – GTP hydrolysis. Reprinted from [9], with permission from Nature Publishing Group, license No. 3134720753071, Apr 23, 2013.

Microtubule dynamics play an essential role in mitosis [10, 11]. The plus ends of the microtubules undergo a series of random elongations and shortenings in the cytoplasm to search and bind the kinetochores of the chromosomes [12]. Metaphase microtubules are highly dynamic. At the onset of anaphase, their dynamics ensure proper chromosomal segregation [13]. The movement of the chromosomes towards the spindle pole is provoked by tension due to the action of motor proteins and shortening of the microtubules [14]. Since the dynamics of tubulin polymerization play important roles in basic cellular functions, microtubules are a target for drugs used in the treatment of several diseases, including cancer [15, 16]. Many microtubule-targeted agents are still in clinical trials.

Microtubule-interfering agents (MIA), a group that includes known anticancer drugs, exert polymerizing and depolymerizing effects on tubulin assembly. Both effects impair the proper function of microtubules and stop the cell cycle at the G2/M phase. This is accompanied by the activation of the signaling pathways leading to apoptosis. The mechanism combining these two phenomena has not yet been fully explained.

The best known depolymerizing agents (inhibitors) include colchicine, vinblastine and vincristine, but other chemotherapeutic drugs are polymerizing agents (enhancers), such as paclitaxel, docetaxel and the polyisoprenyl benzophenones. Studies on the mechanism of anti-mitotic activity of colchicine and other depolymerizing agents have provided evidence that these agents inhibit cell proliferation by perturbing microtubule dynamics [17]. However, anti-mitotic agents show affinity to different binding sites at the αβ-tubulin dimer. Recently, molecular modeling has been applied to explain the mechanism and the mode of ligand binding to a target protein. In the case of tubulin polymerization inhibitors, the determination of the crystallographic structure of the tubulin heterodimer made this possible [6].

Depolymerizing agents bind to the colchicine-binding site located at the interface between the α and β subunits of the tubulin dimer [18] and to the vinblastine-binding site close to the exchangeable GTP site on β-tubulin (Fig. 1) [19]. Additionally, it was demonstrated that unique binding sites may exist. Depolymerizing agents, such as benomyl, estramustine and LY290181 ([2-amino-4-(3-pyridyl)-4*H*-naphtho(1,2-b)pyran-3-carbonitrile]) do not compete with colchicine and vinblastine for tubulin binding. This suggests that these agents bind to tubulin at distinct sites [16]. Using fluorescence spectroscopy, Chakraborti *et al.* demonstrated that curcumin, which is known for its anti-carcinogenic properties, binds tubulin at a distance 32Å away from the colchicine-binding site. Docking studies suggested that the curcumin-binding site is localized close to the vinblastine-binding site [20].

The vinca alkaloids were the first natural products to enter clinical use. Vinblastine and vincristine have been used in clinical oncology since the 1960s. Vindesine and vinorelbine are semi-synthetic derivatives of vinblastine used in combination chemotherapy of leukaemia, lymphoma, non-small cell lung cancer

and non-metastatic breast cancer [21]. Although extensively studied, colchicine is not clinically used for cancer treatment because of its toxicity to normal cells. Numerous drugs, such as paclitaxel, docetaxel and polyisoprenyl benzophenones, are active tubulin polymerization enhancers. They stabilize microtubules and promote their polymerization. The paclitaxel-binding site on tubulin was characterized by Nogales *et al.* [22]. It is located on the inner surface of the microtubules in a deep hydrophobic pocket on β-tubulin (Fig. 1). In tumor cells *in vitro*, paclitaxel potently suppresses the microtubule dynamic [15]. Paclitaxel binding to its site induces conformational changes in the tubulin structure, presumably increasing its affinity for neighboring subunits.

Most microtubule-targeting drugs promote the apoptosis of cancer cells. However, the mechanism by which microtubule-interfering agents induce apoptosis is poorly understood. Generally, the action of MIAs causes the cells to arrest at the G2/M stage of the cell cycle. Li *et al.* [23] tried to explain the relationship between the mitotic block and the apoptotic signaling pathway caused by 2',3,4,4',5-pentamethoxy-*trans*-stilbene (PMS). They suggested that Bcl-2 and/or Bcl-xL hyperphosphorylation by CDC2 or JNK, BAD phosphorylation on serine 128 by CDK1, and Bim activation by JNK could be the mediators linking MIA-induced mitotic arrest and apoptosis. Moreover, it was postulated that MAPKs (ERK, p38, and JNK) did not play a critical role in the PMS-induced apoptotic response because the pharmacological inhibition of MAPKs did not affect the cytotoxicity and pro-apoptotic activity of PMS [23]. Activation of caspase-8 was observed during paclitaxel-induced apoptosis [24], but the relationship between the mitotic block and caspase-8 activation remains unknown.

Regarding the mechanism of anti-carcinogenic action of MIAs, a strong correlation between the anti-tubulin activity of these agents and their vascular-disrupting action should be considered. In oncology, vascular-targeting interventions with the use of small molecule agents are believed to be efficient and worth further development. Compounds that interact with the tumor vasculature are known as vascular-targeting agents (VTAs) and are divided into two groups: a) angiogenesis-inhibiting agents (AIA) that prevent neovascularization in tumors and b) vascular-disrupting agents (VDAs) that influence already established tumor vessels [25]. Bevacizumab (Avastin), a humanized monoclonal antibody, is used in oncology as an anti-angiogenic VTA, but no VDAs have been approved by the US Food and Drug Administration (FDA) to date. The interest in the development of new VDAs is still growing and many VDAs are currently undergoing human clinical trials.

There is a strong correlation between the vascular-disrupting activity of anticancer agents and their ability to inhibit tubulin assembly into microtubules *in vitro* [26]. By influencing tubulin polymerization, VDAs damage the proliferating endothelial cells of the cancer vasculature, thus decreasing the blood flow in the tumor tissue and leading to the death of cells in the central part of the tumor. Clinically relevant small-molecule VDAs interact with tubulin at

the colchicine-binding site on β-tubulin. The ability of the best known polymerizing agent, paclitaxel, to induce vascular damage has not yet been reported.

VDAs are used in combination with standard radiotherapy and chemotherapy of solid tumors [27]. As angiogenesis also plays a crucial role in retinal and choroidal neovascularization leading to vision loss, preclinical studies have been performed on the potential curative activity of CA-4 in these diseases, and the results are promising [28-30].

STILBENE DERIVATIVES AS TUBULIN-INTERACTIVE AGENTS

Cancer chemotherapy agents should have good bioavailability, high cytotoxicity towards cancer cells, negligible cytotoxicity towards normal cells, and a well-recognized potential for multi-targeted action. The search for new anticancer drugs is based on chemical modification of active compounds of natural origin or synthetic ones that show anti-carcinogenic activity in experimental model systems. Phenol compounds of plant origin, including *cis-* and *trans-*stilbene derivatives, are a very promising group of potential chemotherapeutic agents.

Combretastatin A4 and analogs

CA-4 (Fig. 3) was isolated from the root bark of the South African willow tree *Combretum caffrum* Kuntze [2]. Initially, the isolation was based on bioassay techniques that used the astrocyte reversal system. In 1987, further anti-neoplastic agents were isolated from this African tree (Fig. 3), namely combretastatin A-1 (CA-1), A-2 (CA-2), A-3 (CA-3), B-1 (CB-1) and B-2 (CB-2), and their potency as inhibitors of microtubule assembly was demonstrated [31, 32]. In 1989, the cytotoxic activity of CA-4 and its anti-tubulin activity were described. It was found that CA-4 and CA-1 inhibit the growth of murine lymphocytic leukemia cells and human colon cancer cells [33].
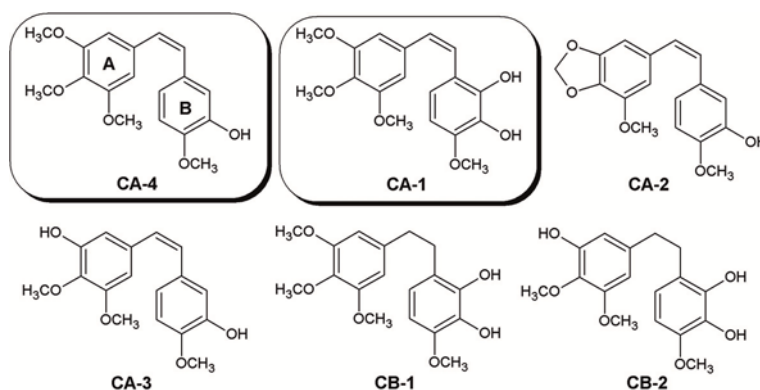

Fig. 3. The chemical structures of natural combretastatins: combretastatin A-4 (CA-4), combretastatin A-1 (CA-1), combretastatin A-2 (CA-2), combretastatin A-3 (CA-3), combretastatin B-1 (CB-1), combretastatin B-2 (CB-2).

Both CA-4 and CA-1 and their corresponding phosphate prodrug salts (CA-4P and CA-1P; Fig. 4) are of increasing therapeutic interest, but CA-4 seems to be more potent as a depolymerizing agent. Its anti-mitotic and cytotoxic properties have been reviewed by several authors [3, 34, 35]. Moreover, CA-4 shows a strong anti-angiogenic activity related to selective inhibition of the formation of new blood vessels in the cancer tissue [36, 37].

CA-4 is one of the best-known inhibitors of tubulin polymerization *in vitro*. CA-4 binds to tubulin at the colchicine-binding site, leading to the formation of abnormal mitotic spindles as a result of the disturbance of the dynamic equilibrium of microtubule formation [35]. The IC_{50} value (concentration that inhibits tubulin polymerization to 50%) of CA-4 was determined in several laboratories and ranged from 0.53 to 2.4 μM [33, 38-40].

CA-4 exerts a potent cytotoxicity against many human cancer cell lines, including multi-drug resistant (MDR) cancer cells [35]. However, CA-4 does not display the expected activity *in vivo* due to its poor bioavailability caused by its low solubility and the instability of its *cis* conformation, which easily changes to form the *trans*-isomer [41]. Phosphates as prodrugs of combretastatins show better aqueous solubility and importantly, they are metabolized to their active forms by phosphatase, which exerts high activity in the tumor environment. The anti-neoplastic activity of CA-4P was shown in preclinical studies with tumor models [see 34 for a review]. Disodium CA-4-3-O-phosphate (CA-4P; fosbretabulin, Zybrestat; Fig. 4) has been moved into clinical trials by the biopharmaceutical company OXiGENE, Inc. (Massachusetts). Clinical studies aiming to assess the dose-limiting toxicity and anti-tumor effectiveness of CA-4P were conducted in 2012. The dose-limiting toxicities were assessed in phase I trials, yielding in a maximum tolerated dose in the range 60-68 mg/m^2 [34]. In clinical trials, a significant reduction in tumor blood flow was observed at doses equal to or below the maximum tolerated dose. In solid tumors, CA-4P exerted a therapeutic effect on the inner part of the tumor tissue, leaving a rim of viable tumor cells at the periphery. Therefore, CA-4P is proposed in combination with other therapies that are more effective at treatment of the outer tumor region [34, 21].

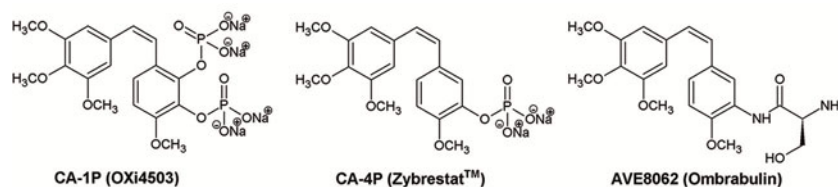CA-1P (OXi4503)   CA-4P (Zybrestat™)   AVE8062 (Ombrabulin)

Fig. 4. Chemical structure of the combretastatin A1 and A4 prodrugs that are currently in clinical trials.

The FDA has agreed to a Special Protocol Assessment (SPA) Phase III clinical trial (FACT 2) of Zybrestat for the treatment of highly aggressive and treatment-resistant anaplastic thyroid cancer. The trial will assess the effectiveness of CA-4P

in combination with traditional chemotherapeutic agents or radiotherapy. The FACT 2 study is designed as a placebo-controlled, double-blind study of 300 subjects randomized 1:1 to receive carboplatin and paclitaxel plus CA-4 or carboplatin and paclitaxel plus a placebo (www.clinicaltrials.gov).

CA-1 and its prodrug disodium CA-1P-2,3-diphosphate (CA-1P, OXi4503; Fig. 4) are cytotoxic against several human cancer lines [42] and the cell lining of tumor vasculature [43]. Oxi4503 is in several phase I/II clinical trials involving patients with solid tumors and the safety and dose-limiting toxicities of Oxi4503 are being estimated. The OXiGENE website, www.oxigene.com, is a good resource for current information on the progress of their clinical trials.

To increase the bioavailability of CA-4, a bioreductively activated prodrug delivery system was proposed. A series of CA-4 nitrothiophene ether-linked conjugates was synthesized and a promising new lead was found. This compound was a gem-substituted derivative (dimethyl α-carbon substitution). It exerted aerobic metabolic stability and displayed the efficiency of hypoxia-related release of CA-4 [44]. Investigations are ongoing into formulations that improve the bioavailability of MIAs, including nanoparticles, which are suggested to provide effective and selective delivery of cytotoxic agents to cancer cells [45].

Based on the results of preclinical studies, CA-4 derivatives were suggested to be effective for retinal neovascularization and choroidal neovascularization [28-30, 46]. In 2007, positive phase II human trial results for ophthalmic formulations of CA-4 used against age-related macular degeneration were announced by OXiGENE. Information on currently ongoing clinical studies is presented at the official websites www.cancer.gov and www.clinicaltrials.gov.

One second-generation CA-4-P analog that is suitable for therapeutic intervention and has entered clinical trials is a serinamide of 3'-amino-3,4,4', 5-tetramethoxy-cis-stilbene (ombrabulin; AVE8062; Fig. 4). Preclinical studies have proved the high anti-mitotic and vascular disrupting potency of this compound [47, 48].

Concerning the synthesis and biological evaluation of structurally modified CA-4 analogs, it is worth referring to the latest studies of Pettit et al. They synthesized iodocombstatin and diiodocombstatin phosphate prodrugs in order to obtain an enhanced concentration of the drug in the thyroid carcinoma tissue. Both series of compounds displayed significant inhibition of the growth of human breast, central nervous system, lung and prostate cancer and leukemia cell lines. Two analogs (3-iodo-4,4',5-trimethoxy-3'-hydroxy-cis-stilbene and 3,5-diiodo-4,4'-dimethoxy-3'-hydroxy-cis-stilbene) efficiently inhibited tubulin polymerization [49].

Summarizing, CA-4 exerts significant anti-mitotic and anti-angiogenic activities, and introduction of additional substituents to the CA-4 backbone is expected to improve its bioavailability and enhance its cytotoxic, anti-mitotic and pro-apoptotic activities. The achievements in this field have stimulated scientists to further their efforts to find new agents with clinical benefits.

Resveratrol analogs

Resveratrol (3,4',5-trihydroxy-*trans*-stilbene; 1; Fig. 5) is the best-known representative of the *trans*-stilbenes. It is a natural polyphenol with well-documented anti-oxidative, anti-inflammatory and pro-apoptotic activity [50]. It occurs in the human diet as a constituent of peanuts, grapes and red wine. Its chemopreventive activity is supposed to be related to its ability to arrest cell cycle progression or to initiate tumor cell death by apoptosis [51]. Surprisingly, this compound does not interact with tubulin. The anti-proliferative activity of resveratrol may be explained by a mechanism that directly involves modulation of specific signaling pathways.

However, both *cis*- and *trans*-stilbenes demonstrate anticancer properties in numerous experimental models *in vitro* and *in vivo*. It is assumed that the mechanism of their action is different. SAR analysis indicated the significance of the *cis* configuration of the olefinic bridge and 3,4,5-trimethoxyphenyl moiety in relation to the cytotoxic and anti-tubulin properties of stilbenes [3].

Substitution of the resveratrol molecule hydroxy groups with methoxy substituents improves the bioavailability of the compound by inhibiting conjugation reactions with sulphuric or glucuronic acids [51]. A methylated derivative of *cis*-resveratrol, 3,4',5-trimethoxy-*cis*-stilbene (Fig. 5, compound 2) displays 100-fold more potent anti-proliferative activity than the parent compound in completely arresting the growth of human colon cancer Caco-2 cells at 0.4 μM. The cytotoxic effect of compound 2 is explained in terms of the depletion of the intracellular pool of polyamines and suppression of tubulin polymerization [52]. The compound only partially inhibited colchicine binding to tubulin, indicating that *cis*-resveratrol derivative binds to a specific site that is not identical with the colchicine-binding site, but colchicine binding may be influenced by allosteric interaction.
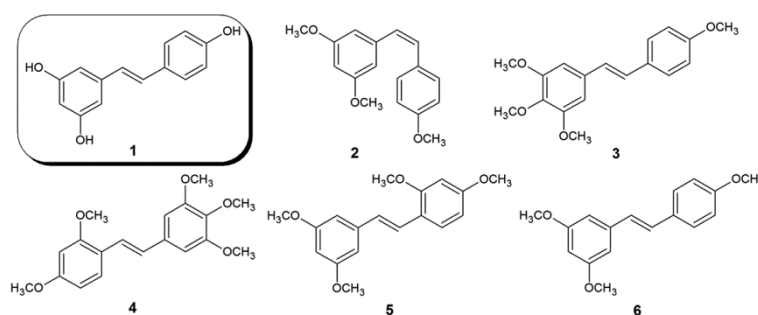

Fig. 5. The chemical structure of resveratrol and its analogs: 3,4',5-trihydroxy-*trans*-stilbene (resveratrol; 1), 3,4',5-trimethoxy-*cis*-stilbene (2), 3,4,4',5-tetramethoxy-*trans*-stilbene (DMU-212; 3), 2,3',4,4',5'-pentamethoxy-*trans*-stilbene (PMS; 4), 2',3,4',5-tetramethoxy-*trans*-stilbene (TMS; 5), and 3,4',5-trimethoxy-*trans*-stilbene (6).

In most studies, *trans* isomers of stilbene derivatives enhanced the process of tubulin polymerization, while *cis* isomers – analogs of CA-4 promoted the process of depolymerization, inhibiting the formation of microtubules. Mazue *et al.* [53] studied the anti-mitotic activity of a series of both *cis*- and *trans*-resveratrol analogs. Using a molecular docking method as a complement to experimental studies on the proliferation inhibition of the human colorectal tumor SW480 cell line, they showed that the *cis* configuration associated with the substitution of hydroxy groups with methoxy groups is crucial and increases inhibition efficacy. The docked structures of *cis*-polymethoxy isomers overlap with the docked structure of CA-4 at the tubulin colchicine-binding site, while *trans*-polymethoxy isomers do not fit CA-4. However, isomers of both conformations induced polyploidy resulting from the blocking of cell divisions at the mitosis level [53].

3,4,4',5-tetramethoxy-*trans*-stilbene (MR-4; DMU-212; 3; Fig. 5) is one of the most active resveratrol analogs that has been studied over the last ten years. It was reported to be 4 times more potent than resveratrol as an inhibitor of the growth of HCA-7 human colon cancer cells [54]. Moreover, it is also a potent inhibitor of adenoma development in the Apc^{Min+} mouse, a model of human intestinal carcinogenesis [55]. Other authors reported its higher cytotoxicity against two human breast cancer cell lines, MCF-7 and MDA-MB-435, in comparison with *trans*-resveratrol. The use of DMU-212 at a dose of 2.5 μM resulted in a microtubule-stabilizing effect comparable to that of 10 μM paclitaxel [56].

Another polymethoxy derivative of resveratrol, 2,3',4,4',5'-pentamethoxy-*trans*-stilbene (PMS; 4; Fig. 5) was found to be a potent inducer of apoptosis in colon cancer cells thanks to its targeting of microtubules [23]. However, for this *trans* isomer, enhancement of tubulin polymerization followed by G2/M mitotic arrest and caspase-dependent apoptosis was observed. PMS displayed a more potent anti-mitogenic effect on colon cancer cells (Caco-2, HT-29 and SW1116) when compared with 2',3,4',5-tetramethoxy-*trans*-stilbene (TMS; 5; Fig. 5). The potent cytotoxic effect of TMS was shown earlier using hormone-resistant breast cancer cells [57]. Recently, it was reported that PMS effectively suppressed colitis-associated colon carcinogenesis in mice (AOM/DSS animal model). The authors explained the chemopreventive effect of PMS in terms of suppression of cell proliferation, promotion of apoptosis, inactivation of β-catenin and downregulation of iNOS [58].

Other synthetic stilbene derivatives as tubulin-interactive agents
In the last decade, new anti-proliferative agents that could be used in cancer therapy were designed and synthesized. Looking at the literature up to 2005, one can find 102 references related to chemistry-focused studies that can be classified into three categories, depending whether there is a one-atom, two-atom or three-atom bridgehead as the linker between the two aryl rings of the combretastatins [59]. Here, we survey the studies of novel classes of anti-mitotic

agents based on combretastatin A-4 as the lead, focusing on compounds with a two-atom linker.

Recently, numerous structural modifications of CA-4 have been made leading to the identification of groups and positions that are important from the point of view of its anti-tumor activity [35, 60, 61, 62]. In general, these are modifications to the A ring, B ring and/or the double bond of CA-4. SAR studies of CA-4 have underlined that the presence of a 3,4,5-trimethoxy-substituted A-ring and 4-methoxy-substituted B-ring separated by a double bond with the *cis*-configuration are fundamental for anti-proliferative activity [3]. It has also been reported that the 3-hydroxy group on the B-ring is not necessarily determinant for potent activity [63, 64]. Cushman *et al.* [64] synthesized a series of 4'-methoxy-3,4,5-trimethoxy-*cis*-stilbene derivatives where the methoxy group in the B-ring was replaced with a variety of other substituents. The most potent tubulin polymerization inhibitor, 4'-methoxy-3,4,5-trimethoxy-*cis*-stilbene, influenced tubulin assembly with an IC_{50} of 2.0 μM comparable to CA-4. Amino derivatives of CA-4 with an OH-NH_2 isosteric substitution (Fig. 6, compound 7) and with an amino group in the ortho position in the B ring (Fig. 6, compound 8) are worth mentioning as very potent tubulin polymerization inhibitors and highly cytotoxic against the NCI-H460 and DU-145 cell lines [65, 66]. The trifluorostilbene 3'-amino-4'-methoxy-3,4,5-trifluoro-*cis*-stilbene (Fig. 6, compound 9) was exceptionally active as an inhibitor of tubulin polymerization with an IC_{50} of 2.9 μM [60].
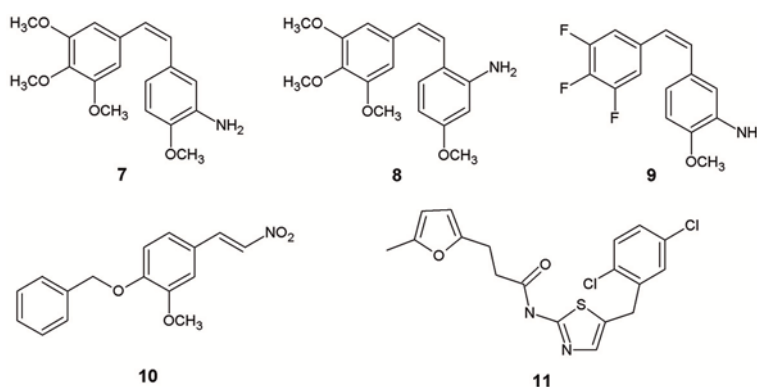
Fig. 6. Structures of potent tubulin polymerization inhibitors.

The activity of CA-4 is restricted by isomerization of the active *cis*-stibene configuration into the corresponding inactive *trans* analog. The search for chemically more stable CA-4 analogs has led to design and synthesis of numerous series of derivatives with modification on the double bond. The rationale of the design of active compounds was to retain the appropriate geometry of the two adjacent aryl groups required for potent bioactivity of chemically stable *cis*-restricted derivatives of CA-4. These were obtained by
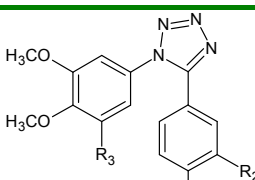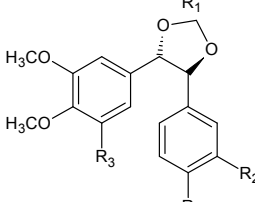
incorporating the olefinic double bond into vicinally diaryl-substituted five-member aromatic heterocyclic rings (Table 1), such as pyrazole [67], imidazole [67-69], thiazole [70-72], furazan (1,2,5-oxadiazole) [73], furan [74, 75], thiophene [76, 77], isoxazole [78, 79], oxazole [67, 68, 80, 81], 1,2,3-thiadiazole [39], triazole [82, 83, 84, 85], 1,2,3,4-tetrazole [70, 86] and dioxolane [87]. Replacement of the olefinic bond with a five-member heterocyclic ring allowed the retention of the correct geometric orientation of the two phenyl rings of CA-4, placing them at an appropriate distance for efficient interaction with the colchicine-binding domain on tubulin [35]. Below, we present numerous examples of modifications described in the last few years, focused mainly on the double bond of CA-4.

Table 1. Heterocycle-based CA-4 and CA-1 analogs with examples of the most potent tubulin inhibitors.

General structure	Substituted heterocycle	Reference	Example
			Tubulin assembly – IC$_{50}$ (μM)
	2,3-disubstituted pyrazole	[67]	R$_1$ = OCH$_3$, R$_2$ = NH$_2$, R$_3$ = OCH$_3$* not determined
	1,2-disubstituted imidazole	[67]	R$_1$ = N(CH$_3$)$_2$, R$_2$ = H, R$_3$ = OCH$_3$ 35
	1,5-disubstituted imidazole	[67]	R$_1$, R$_2$ = -N(CH$_3$)CHCH- R$_3$ = OCH$_3$ 1.8 CA-4 (1.2)
	4,5-disubstituted imidazole	[67]	R$_1$ = OCH$_3$, R$_2$ = NH$_2$, R$_3$ = OCH$_3$ 0.68 CA-4 (1.2)

General structure	Substituted heterocycle	Reference	Example
			Tubulin assembly – IC$_{50}$ (µM)
	4,5-disubstituted N-methylimidazole	[67]	R$_1$, R$_2$ = -N(CH$_3$)CHCH- R$_3$ = OCH$_3$ 2.0 CA-4 (1.2)
	2-amino-4,5-disubstituted thiazole	[71]	R$_1$ = OC$_2$H$_5$, R$_2$ = H, R$_3$ = OCH$_3$ 0.44 CA-4 (1.2)
	furazan (1,2,5-oxadiazole)	[73]	R$_1$ = OCH$_3$, R$_2$ = OH, R$_3$ = OCH$_3$* not determined
	3,4-disubstituted furan	[74]	R$_1$ = OCH$_3$, R$_2$ = NH$_2$, R$_3$ = OCH$_3$* not determined
	2,3-disubstituted furan	[75]	R$_1$ = OCH$_3$, R$_2$ = OH, R$_3$ = OCH$_3$* not determined
	2,3-disubstituted thiophene	[76, 77]	R$_1$ = OCH$_3$, R$_2$ = NH$_2$, R$_3$ = OCH$_3$, X = H* not determined

General structure	Substituted heterocycle	Reference	Example
			Tubulin assembly – IC$_{50}$ (µM)
	5-amino-3,4-disubstituted isoxazole	[78]	R$_1$ = OCH$_3$, R$_2$ = H, R$_3$ = OCH$_3$ 1.8 CA-4 (1.2)
	4,5-disubstituted isoxazole	[79]	R$_1$ = OCH$_3$, R$_2$ = NH$_2$, R$_3$ = OCH$_3$* not determined
	4,5-disubstituted oxazole	[80, 81]	R$_1$ = SCH$_3$, R$_2$ = H, R$_3$ = OCH$_3$ (**13**)* R$_1$ = OCH$_3$, R$_2$ = F, R$_3$ = Cl (**14**)* not determined
	4,5-disubstituted 1,2,3-thiadiazole	[39]	R$_1$ = OCH$_3$, R$_2$ = OH, R$_3$ = OCH$_3$ (**12**) 0.7 CA-4 (0.81)
	1,5-disubstituted 1,2,4-triazole	[83]	R$_1$ = OC$_2$H$_5$, R$_2$ = H, R$_3$ = OCH$_3$ 0.76 CA-4 (1.2)
	1,5-disubstituted 1,2,3-triazole	[82, 84, 85]	R$_1$ = OCH$_3$, R$_2$, R$_4$ = NH$_2$, R$_3$ = OCH$_3$ (**15**) R$_1$ = OCH$_3$, R$_2$ = NH$_2$, R$_3$ = OCH$_3$ (**16**) **15** (5.2) **16** (4.5) CA-1 (3.5)

General structure	Substituted heterocycle	Reference	Example	
			Tubulin assembly – IC_{50} (μM)	
	1,5-disubstituted 1,2,3,4-tetrazole	[86]	$R_1 = OC_2H_5$, $R_2 = H$, $R_3 = OCH_3$ 1.1 CA-4 (1.3)	
	(S,S)-dioxolane	[87]	$R_1 = OCH_3$, $R_2 = OH$, $R_3 = OCH_3$ 4-6 CA-4 (3-4)	

* The most cytotoxic compound in the series.

Wu *et al.* [39] designed and synthesized a series of eighteen 4,5-disubstituted-1,2,3-thiadiazole compounds. Six of them, the most active in cytotoxicity assays, were tested for their anti-tubulin effect. They displayed inhibiting potency on tubulin polymerization similar to CA-4. Experimental analysis led to the selection of the most promising compound, 4-(3,4,5-trimethoxyphenyl)-5-(3-hydroxy-4-methoxy)-1,2,3-thiadiazole (Table 1, compound 12), which also demonstrated relatively low toxicity to normal cells.

More recently, Schobert *et al.* [80] found two compounds in a series of oxazole-bridged CA-4 analogues with modified A and/or B rings. These compounds inhibited the growth of chemoresistant HT-29 colon carcinoma cells with sub-nanomolar efficacy. HT-29 cells overexpress MRP-1 and MRP-3 drug transporters. The most potent CA-4 analogs differed from the parent compound with groups substituted in the B ring: 4-(3,4,5-trimethoxyphenyl)-5-(4-methylthiophenyl)oxazole (Table 1, compound 13); or in both the A and B rings: 4-(5-chloro-3,4,-dimethoxyphenyl)-5-(3-fluoro-4-methoxyphenyl)oxazole (Table 1, compound 14). Interestingly, the authors observed that some of the newly synthesized derivatives were cytotoxic against HT-29 cells only when the cells were sensitized by the selective MRP-1 inhibitor MK-571 [80].

A series of *cis*-restricted 1,5-disubstituted 1,2,3-triazole derivatives of combretastatin A-1 were synthesized and evaluated for anti-tubulin and anti-angiogenic effects [82, 84]. The analogs, 1-(3,4,5-trimethoxyphenyl)-5-(2,3-diamino-4-methoxyphenyl)-1,2,3-triazole (Table 1, compound 15) and 1-(3,4,5-trimethoxyphenyl)-5-(3-amino-4-methoxyphenyl)-1,2,3-triazole (Table 1, compound 16) displayed an inhibitory effect on tubulin polymerization comparable to that of CA-1 with respective IC_{50} of 5.2 µM and 4.5 µM versus 3.5 µM for CA-1 [82]. 1,4-disubstituted 1,2,3-triazole analogs of CA-4 were significantly less cytotoxic [85].

Pettit *et al.* [88] synthesized a series of twenty-three β-*trans*-nitrostyrenes. All but eight displayed inhibitory action towards tubulin polymerization with an IC_{50} of less than 10 μM. (4-benzyloxy-3-methoxyphenyl)-β–*trans*-nitrostyrene (Fig. 6, compound 10), with its bulky hydrophobic substituent, was the most active tubulin polymerization inhibitor in the series with an IC_{50} of 2.5 μM. Its structure resembles the structure of combretastatins, in that there is a two-atom bridge between the two phenyl rings. Analogs of compound 10 with different substitution patterns in their phenyl rings seem to be worth further study. *trans*-3,4,5-trimethoxynitrostyrene was found to be a potent inhibitor with an IC_{50} of 4.5 μM, but it displayed relatively weak activity as an inhibitor of colchicine binding to tubulin. Moreover, it did not cause mitotic arrest in the mammalian cells that were treated with a cytotoxic concentration of the compound. It was suggested that a different cellular target is responsible for the cytotoxic activity of this nitrostyrene.

Tubulin-binding stilbenes as VDAs
CA-4 was the first small molecule VDA shown to have anti-vascular effects at low doses [89]. In tumors treated with CA-4-P, selective and extensive vasculature damage occurred, causing hemorrhagic necrosis and tumor growth delay [37]. Several CA-4 derivatives were described and patented as small molecule VDAs [42], including AVE 8062 and Oxi-4503 (Fig. 4), which demonstrate strong anti-tumor effects due to their vasculature-disrupting activity [90, 91]. Ombrabulin enhances the efficacy of standard therapies (radiation plus cisplatin and radiation plus cetuximab) in head and neck squamous cell carcinoma (HNSCC) xenograft models. A specific action of ombrabulin towards intratumoral vessels rather than to the peritumoral vasculature was demonstrated by means of immunochemical staining of HNSCC tumors [92]. The design of CA-4 analogs as VDAs was the subject of a concise review by Rajak *et al.*, who paid special attention to *cis*-restricted isomers [93].
Resveratrol's anti-angiogenic activity has been shown in *in vitro* assays and preclinical animal models [94, 95]. However, this polyphenol is a weak tubulin-interfering agent. Its anti-angiogenic activity is probably involved with the inhibition of the expression of HIF-1α and VEGF through multiple mechanisms, such as inhibition of Akt and MAP kinases, inhibition of protein translation regulators, and enhancement of proteosomal degradation of HIF-1α protein [96].
The anti-angiogenic and vascular-targeting activity of a series of *trans*-resveratrol derivatives was evaluated by Belleri *et al.* [97]. The prevention of new blood vessel formation and the disruption of the neovasculature by 3,4', 5-trimethoxy-*trans*-stilbene (Fig. 5, compound 6) was established with the use of a broad spectrum of *in vitro* and *in vivo* assays. Compound 6 exerted 30 to 100 times more potent anti-angiogenic activity in comparison to resveratrol in terms of inhibiting endothelial cell proliferation, sprouting, collagen gel invasion, and morphogenesis. *In vivo*, compound 6 caused the rapid stasis of blood flow and regression of intersegmental vessels in the trunk of zebrafish embryos [97].

More recently, these results were confirmed by Alex *et al.*, and it was postulated that the anti-angiogenic and vascular-targeting activities of compound 6 result from the downregulation of VEGFR2 expression and cell-cycle arrest at the G2/M phase [98]. It remains unexplained why the *trans*-isomer of compound 6 affects tubulin polymerization in the same way as methoxystilbenes in the *cis* configuration, which share high structural similarity with CA-4. The tubulin-interfering activity of *trans*-isomers that leads to cell cycle arrest [56, 99] still needs to be elucidated.

Cai *et al.* [62] synthesized six new stilbene derivatives and screened for cytotoxicity in different human tumor cell lines. They found that 3,4', 5-trimethoxy-*cis*-stilbene-3'-O-phosphate disodium (M410) is the most potent cytotoxic agent against multi-drug resistant tumor cells. M410 was a potent inhibitor of bovine brain tubulin polymerization *in vitro*. In experiments with human umbilical vein endothelial cells (HUVECs), rapid depolymerization of cellular microtubules, changes in cell shape and mitosis arrest were observed. In experiments on nude mice *in vivo*, M410 inhibited the growth of human colon carcinoma xenografts and reduced microvessel density in tumor tissues [62].

Most recently, it was shown that the *cis* isomer of CA-1P significantly impaired tumor blood flow leading to secondary tumor cell death and more than 95% tumor necrosis 24 h post drug exposure. It was suggested that CA-1P may be metabolized to orthoquinone intermediates leading to the formation of cytotoxic free radicals [99]. Treatment with the *trans*-isomer had no effect on the blood flow parameters. However, the combination of the *trans*-isomer with CA-4 increased the anti-tumor efficacy of the latter agent to near that of *cis*-CA-1P. These findings indicate that while the predominant *in vivo* effect of CA-1P is due to microtubule disruption and vascular shutdown, the formation of oxiquinones – toxic free radicals – could yield additional cytotoxic effects *in vivo*. Orthoquinones that bind to cellular nucleophiles form free radicals and have the potential to induce cancer cell death. Thus, the *trans*-CA-4P can contribute to the enhanced potency of the two isomers given to mice [100]. It has to be pointed out that the phosphorylated isomer did not show cytotoxicity *in vitro*, unlike the dephosphorylated orthoquinone species [101].

Molecular modeling and virtual screening
Molecular modeling makes an important contribution to the study of interactions between anti-mitotic agents and tubulin. The usefulness of this chemoinformatic approach for predicting the affinity of unknown compounds to molecular receptors has been confirmed in the virtual screening studies of tubulin inhibitors [102]. In order to create a tool for virtual screening, Nguyen developed a pharmacophore model [103] that was used and refined by other research groups [104]. Kim *et al.* screened a library of compounds with a 3,4,5-trimethoxyphenyl motif selected from a chemical database [105]. Three compounds showed strong anti-proliferative activity against HL60 cell lines and were able to inhibit tubulin polymerization *in vitro*.

Recently, in the colchicine-binding domain, three zones of interaction were identified and specific amino acids responsible for the recognition and binding of known MIAs were determined [106]. Advanced *in silico* studies with the use of pharmacophore models were included in order to identify the most active *cis*-stilbene derivatives worthy of further synthesis. This strategy allowed a reduction in synthesis costs. However, in most of the studies, molecular docking is used to provide a rationale for experimental observations [61, 107, 108]. For example, Romagnoli *et al.* [107] synthesized and evaluated 2-amino-3-(3',4',5'-trimethoxybenzoyl)-6-substituted-4,5,6,7-tetrahydrothieno[2,3-c]pyridine derivatives as anti-mitotic agents and inhibitors of tubulin polymerization. All of the compounds synthesized possessed a 3,4,5-trimethoxyphenyl motif. The most potent inhibitors of tubulin polymerization were docked in the colchicine-binding site. Docking studies revealed that the trimethoxyphenyl moiety is situated in the same pocket on β-tubulin as the structurally analogous A ring of the co-crystallized DAMA-colchicine. The carbonyl group of the carbamate of 2-amino-3-(3',4',5'-trimethoxybenzoyl)-6-ethoxycarbonyl-4,5,6,7-tetrahydrothieno[2,3-c]pyridine overlaps the carbonyl group of the C ring of DAMA-colchicine. 1,2,3-triazole analogs of CA-1 could be successfully docked into the colchicine-binding site of α,β-tubulin [82].

Kim *et al.* [109] synthesized a series of 43 stilbene derivatives and evaluated their cytotoxicity against human lung tumor cells (A5490). They used comparative molecular field analysis (CoMFA) to predict the biological activity of the designed molecules. With the use of the CoMFA pharmacophore model, they obtained a very good correlation between the calculated and observed IC_{50} for the cytotoxic effect of synthesized compounds.

Virtual screening is supposed to be a useful approach to discover new molecules with anti-proliferative activity through their ability to inhibit tubulin polymerization. Chiang *et al.* [110] identified novel tubulin inhibitors using a computational technique. Their complex approach included the generation and validation of a ligand-based pharmacophore model. This model was further employed for virtual screening of a chemical database containing approximately 130,000 compounds. Of the four compounds with anti-proliferative activity found *in silico*, one of them, compound 11 (Fig. 6), was identified as a potent anti-proliferative agent in KB cells with an IC_{50} of 187 nM. Moreover, this indole inhibited tubulin polymerization with an IC_{50} of 4.4 μM and induced cell cycle arrest in the G2/M phase. Molecular docking revealed binding of compound 11 to the colchicine-binding site via two hydrogen bonds with Thr179 and Asn101 at distances of 2.89 and 3.05 Å, respectively. Interestingly, the chemical structure of this compound is distinct from colchicine and CA-4 analogs and it does not possess a trimethoxyphenyl moiety, which was reported as an important pharmacophore for the inhibition of tubulin assembly. However, its 2,5-dichlorobenzyl group occupies a similar region in tubulin as the trimethoxyphenyl of colchicine.

The challenge for further *in silico* studies will be the polymorphism of α- and β-tubulin subunits. For the β subunit, eight isoforms exerting 90% similarity have been identified and for the α subunit, at least six different isoforms are known [106]. They are characterized by different tissue distribution in normal cells, and in cancer cells, they may interact differently with chemotherapeutics. It is suggested that mutations leading to stabilization of microtubules may confer a resistance to drugs that act as destabilizers, such as vincristine and vinblastine, while the expression of isoforms causing destabilization of microtubules gives resistance to stabilizing agents, e.g. paclitaxel.

Various isoform structures were obtained with the use of homology modeling. A set of novel colchicine derivatives with selective affinity to the colchicine domain of the αβIII isoform was synthesized. Their cytotoxic action correlated with the expression levels of specific tubulin isotypes [45, 111]. The discovery of novel tubulin-binding agents that could selectively bind specific isoforms will be the aim of further research with the use of molecular modeling. Interesting research was reported on the poor affinity of paclitaxel to tubulin derived from the yew tree (*Taxus baccata*), which is a producer of this cytotoxin. The analysis indicated that the paclitaxel-binding site of yew tree tubulin contains several amino acid substitutions compared to human tubulin, all of which reduce the binding affinity of paclitaxel to yew tree tubulin [112].

SUMMARY

Intense research is being carried out to discover and elucidate the mechanism of action of anticancer therapeutic agents and to find the compounds that have superior therapeutic activity and bioavailability as future drugs. Tubulin is the focus of large-scale studies as a crucial target for anticancer drugs. The well-recognized role of tubulin-interactive agents may help to develop new and efficient chemotherapeutics in the fight against cancer. The role of the combretastatin CA-4 and its numerous analogs seems to be significant.

Taking into account the synergistic effect, the use of combretastatins in complex therapy is proposed in clinical trials (adjuvant therapy). Recently, it has been postulated that a multi-targeted management of tumors by combining anti-angiogenic, vascular-disrupting and conventional chemotherapies and/or radiotherapy might be effective. Improvements in targeted drug delivery will contribute to the success of natural and synthetic products. Studies of the interaction between a drug and tubulin with the use of virtual docking and modeling create new perspectives for the design of synthetic analogs with improved properties and effectiveness in comparison to the parent compound.

REFERENCES

1. Butler, M.S. Natural products to drugs: natural product-derived compounds in clinical trials. **Nat. Prod. Rep.** 25 (2008) 475-516.
2. Pettit, G.R., Cragg, G.M., Herald, D.L., Schmidt, J.M. and Lobavanijaya, P. Antineoplastic agents. Part 84. Isolation and structure of combretastatin. **Can. J. Chem.** 60 (1982) 1374-1376.
3. Tron, G.C., Pirali, T., Sorba, G., Pagliai, F., Busacca, S. and Genazzani, A. Medicinal chemistry of combretastatin A-4: present and future directions. **J. Med. Chem.** 49 (2006) 3033-3044.
4. Siemann, D.W., Chaplin, D.J. and Walicke, P.A. A review and update of the current status of the vasculature-disabling agent combretastatin-A4 phosphate (CA4P). **Exp. Opin. Invest. Drugs** 18 (2009) 189-197.
5. Desai, A. and Mitchison, T.J. Microtubule polymerization dynamics. **Ann. Rev. Cell Dev. Biol.** 13 (1997) 83-117.
6. Nogales, E., Wolf, S.G., and Downing, K.H. Structure of the $\alpha\beta$-tubulin dimer by electron crystallography. **Nature** 391 (1998) 199-203.
7. Mitchison, T. and Kirscher, M. Microtubule assembly nucleated by isolated centrosomes. **Nature** 312 (1984) 232-237.
8. Wang, H.W. and Nogales, E. Nucleotide-dependent bending flexibility of tubulin regulates microtubule assembly. **Nature** 435 (2005) 911-915.
9. Akhmanova, A., and Steinmetz, M.O. Tracking the ends: a dynamic protein network controls the fate of microtubule tips. **Nat. Rev. Mol. Cell Biol.** 9 (2008) 309-322.
10. Kline-Smith, S.L. and Walczak, C.E. Mitotic spindle assembly and chromosome segregation: refocusing on microtubule dynamics. **Mol. Cell** 15 (2004) 317-327.
11. Kwon, M. and Scholey, J.M. Spindle mechanics and dynamics during mitosis in Drosophila. **Trends Cell Biol.** 14 (2004) 194-205.
12. Rieder, C.L., Davison, E.A., Jensen, L.C., Cassimeris, L. and Salomon, E.D. Oscillatory movements of monooriented chromosomes and their position relative to the spindle pole result from the ejection properties of the aster and half-spindle. **J. Cell Biol.** 103 (1986) 581-591.
13. Higuchi, T. and Uhlmann, F. Stabilization of microtubule dynamics at anaphase onset promotes chromosome segregation. **Nature** 433 (2005) 171-176.
14. Rieder, C.L., Schultz, A., Cole, R. and Sluder, G. Anaphase onset in vertebrate somatic cells is controlled by a checkpoint that monitors sister kinetochore attachment to the spindle. **J. Cell Biol.** 127 (1994) 1301-1310.
15. Jordan, M.A. and Wilson, L. Microtubules as a target for anticancer drugs. **Nat. Rev. Cancer** 4 (2004) 253-265.
16. Singh, P., Rathinasamy, K., Mohan, R. and Panda, D. Microtubule assembly dynamics: an attractive target for anticancer drugs. **IUBMB Life** 60 (2008) 368-375.

17. Bhattacharyya, B., Panda, D., Gupta, S., and Banerjee, M. Anti-mitotic activity of colchicine and the structural basis for its interaction with tubulin. **Med. Res. Rev.** 28 (2008) 155-183.

18. Ravelli, R.B., Gigant, B., Curmi P.A., Jourdain, I., Lachkar, S., Sobel, A. and Knossow, M. Insight into tubulin regulation from a complex with colchicine and a stathmin-like domain. **Nature** 428 (2004) 198-202.

19. Gigant, B., Wang, C., Ravelli, R.B.G., Roussi, F., Steinmetz, M.O., Curmi, P.A., Sobel, A. and Knossow, M. Structural basis for the regulation of tubulin by vinblastine. **Nature** 435 (2005) 519-522.

20. Chakraborti, S., Das, L., Kapoor, N., Das, A., Dwivedi, V., Poddar, A., Chakraborti, G., Janik, M., Basu, G., Panda, D., Chakrabarti, P., Surolia, A. and Bhattacharyya, B. Curcumin recognizes a unique binding site of tubulin. **J. Med. Chem.** 54 (2011) 6183-6196.

21. Kingston, D.G.I. Tubulin-interactive natural products as anticancer agents. **J. Nat. Prod.** 72 (2009) 507-515.

22. Nogales, E., Wolf, S.G., Khan, I.A., Luduena, R.F. and Downing, K.H. Structure of tubulin at 6.5 Å and location of the taxol-binding site. **Nature** 375 (1995) 424-427.

23. Li, H., Wu, W.K.K., Zheng, A., Che, C.T., Yu, L., Li, Z.J., Wu, Y.C., Cheng, K.-W., Yu, J., Cho, C.H. and Wang, M. 2,3',4,4',5'-Pentamethoxy-*trans*-stilbene, a resveratrol derivative, is a potent inducer of apoptosis in colon cancer cells via targeting microtubules. **Biochem. Pharmacol.** 78 (2009) 1224-1232.

24. Goncalves, A., Braguer, D., Carles, G., Andre, N., Prevot, C. and Briand, C. Caspase-8 activation independent of CD95/CD95-L interaction during paclitaxel-induced apoptosis in human colon cancer (HT29-D4). **Biochem. Pharmacol.** 60 (2000) 1579-1584.

25. Siemann, D.W., Bibby, M.C., Dark, G.G., Dicker, A.P., Eskens, F.A., Horsman, M.R., Marmé, D. and LoRusso, P.M. Differentiation and definition of vascular-targeted therapies. **Clin. Cancer Res.** 11 (2005) 416-420.

26. Mason, R.P., Zhao, D., Liu, L., Trawick, M.L. and Pinney, K.G. A perspective on vascular disrupting agents that interact with tubulin: preclinical tumor imaging and biological assessment. **Integr. Biol (Camb.)** 3 (2011) 375-387.

27. Siemann, D.W. The unique characteristics of tumor vasculature and preclinical evidence for its selective disruption by tumor-vascular disrupting agents. **Cancer Treat. Rev.** 37 (2011) 63-74.

28. Jockowich, M.E., Suarez, F., Alegret, A., Pina, Y., Hayden, B., Cebulla, C., Feuer, W. and Murray, T.G. Mechanism of retinoblastoma tumor cell death after focal chemotherapy, radiation, and vascular targeting therapy in a mouse model. **Invest. Ophthalmol. Vis. Sci.** 48 (2007) 5371-5376.

29. Nambu, H., Nambu, R., Melia, M. and Campochiaro, P.A. Combretastatin A-4 phosphate supresses development and induces regression of choroidal neovascularization. **Invest. Ophthalmol. Vis. Sci.** 44 (2003) 3650-3655.

30. Ma, L., Liu, Y.L., Ma, Z.Z., Dou, H.L., Xu, J.H., Wang, J.C., Zhang, X. and Zhang, Q. Targeted treatment of choroidal neovascularization using integrin-mediated sterically stabilized liposomes loaded with combretastatin A4. **J. Ocul. Pharmacol. Ther.** 25 (2009) 195-200.

31. Pettit, G.R. and Singh, S., Antineoplastic agents. Part 130. Isolation, structure and synthesis of combretastatins A-2, A-3, and B-2. **Can. J. Chem.** 65 (1987) 2390-2396.

32. Pettit, G.R., Singh, S.B., Niven, M.L., Hamel, E. and Schmidt, J.M. Antineoplastic agents. Part 123. Isolation, structure, and synthesis of combretastatin A-1 and B-1, potent new inhibitors of microtubule assembly, derived from Combretum caffrum. J**. Nat. Prod.** 50 (1987) 119-131.

33. Pettit, G.R., Singh, S.B., Niven, M.L., Hamel, E., Lin, C.M., Alberts, D.S. and Garcia-Kendall, D. Isolation and structure of the strong cell growth and tubulin inhibitor combretastatin A-4. **Experientia** 45 (1989) 209-211.

34. Pinney, K.G., Pettit, G.R., Trawick, M.L., Jelinek, C. and Chaplin, D.J. The discovery and development of the combretastatins. in: Anticancer Agents from Natural Products, (Cragg, G.R., Kingston, D.G.I. and Newman, D.J. Eds.) 2nd edition, CRC Press/Taylor & Francis, Boca Raton, FL, 2012, 27-63.

35. Chaudhary, A., Pandeya, S.N., Kumar, P., Sharma, P., Gupta, S., Soni, N., Verma, K.K. and Bhardwaj, G. Combretastatin A-4 Analogs as Anticancer Agents. **Mini-Rev. Med. Chem.** 7 (2007) 1186-1205.

36. Tozer, G.M., Kanthou, C., Parkins, C.S. and Hill, S.A. The biology of the combretastatins as tumour vascular targeting agents. **Int. J. Exp. Pathol.** 83 (2001) 21-38.

37. Thorpe, E.P. Vascular targeting agents as cancer therapeutics. **Clin. Cancer Res.** 10 (2004) 415-427.

38. Xia, Y., Yang, A.-Y., Xia, P., Bastow, K.F., Tachibana, Y., Kuo, S.-C., Hamel, E., Hacki, T. and Lee, K.-H. J. Antitumor agents. 181. Synthesis and biological evaluation of 6,7,2',3',4'-substituted-1,2,3,4-tetrahydro-2-phenyl-4-quinolones as a new class of anti-mitotic antitumor agents. **Med. Chem.** 41 (1998) 1155-1162.

39. Wu, M., Sun, Q., Yang, C., Chen, D., Ding, J., Chen, Y., Lin, L. and Xie, Y. Synthesis and activity of combretastatin A-4 analogues: 1,2,3-thiadiazoles as potent antitumor agents. **Bioorg. Med. Chem. Lett.** 17 (2007) 869-873.

40. Sriram, M., Hall, J.J., Grohmann, N.C., Strecker, T.E., Wootton, T., Franken, A., Trawick, M.L. and Pinney, K.G. Design, synthesis and biological evaluation of dihydronaphthalene and benzosuberene analogs of the combretastatins as inhibitiors of tubulin polymerization in cancer chemotherapy. **Bioorg. Med. Chem.** 16 (2008) 8161-8171.

41. Pettit, G.R., Toki, B.E., Herald, D.L., Boyd, M.R., Hamel, E., Pettit, R.K. and Chapuis, J.-C. J. Antineoplastic agents. 410. Asymetric hydroxylation of trans-combretastatin A-4. **Med. Chem.** 42 (1999) 1459-1465.

42. Cai, S.X. Small molecule vascular disrupting agents: potential new drugs for cancer treatment. **Recent Pat. Anticancer Drug Discov.** 2 (2007) 79-101.

43. Salmon, H.W. and Siemann, D.W. Effect of the second generation vascular disrupting agent OXi4503 on tumor vascularity. **Clin. Cancer Res.** <u>12</u> (2006) 4090-4094.

44. Thomson, P., Naylor, M.A., Everett, S.A., Stratford, H.R.L., Lewis, G., Hill, S., Patel, K.B., Wardman, P. and Davis, P.D. Synthesis and biological properties of bioreductively targeted nitrothienyl prodrugs of combretastatin A-4. **Mol. Cancer Ther.** <u>5</u> (2006) 2886-2894.

45. Calligaris, D., Verdier-Pinard, P., Devred, F., Villard, C., Braguer, D. and Lafitte, D. Microtubule targeting agents: from biophysics to proteomics. **Cell. Mol. Life Sci.** <u>67</u> (2010) 1089-1104.

46. Griggs, J., Skepper, J.N., Smith, G.A., Brindle, K.M., Metcalfe, J.C. and Hesketh, R. Inhibition of proliferative retinopathy by the antivascular agent combretastatin A-4. **Am. J. Pathol.** <u>160</u> (2002) 1097-1103.

47. Delmonte, A. and Sessa, C. AVE8062: A new combretastatin derivative vascular disrupting agent. **Expert Opin. Investig. Drugs** <u>18</u> (2009) 1541-1548.

48. Kim, T.J., Ravoori, M., Landen, C.N., Kamatt, A.A., Han, L.Y., Lu, C., Lin, Y.G., Merritt, W.M., Jennings, N., Spannuth, W.A., Langley, R., Gershenson, D.M., Coleman, R.L., Kundra, V. and Sood, A.K. Antitumor and antivascular effects of AVE8062 in ovarian carcinoma. **Cancer Res.** <u>67</u> (2007) 9337-9345.

49. Pettit, G.R., Rosenberg, H.J., Dixon, R., Knight, J.C., Hamel, E., Chapuis, J.C., Pettit, R.K., Hogan, F., Sumner, B., Ain, K.B. and Trickey-Platt, B. Antineoplastic agents. 548. Synthesis of iodo- and diiodocombstatin phosphate prodrugs. **J. Nat. Prod.** <u>75</u> (2012) 385-393.

50. Baur, J.A. and Sinclair, D.A. Therapeutic potential of resveratrol: the in vivo evidence. **Nat. Rev. Drug Discov.** <u>5</u> (2006) 493-506.

51. Szekeres, T., Fritzer-Szekeres, M., Saiko, P. and Jaeger, W. Resveratrol and resveratrol analogues – structure-activity relationship. **Pharm. Res.** <u>27</u> (2010) 1042-1048.

52. Schneider, Y., Chabert, P., Stutzmann, J., Coelho, D., Fougerousse, A., Gosse, F. Launay, J.-F., Brouillard, R. and Raul, F. Resveratrol analog (Z)-3,5,4'-trimethoxystilbene is a potent anti-mitotic drug inhibiting tubulin polymerization. **Int. J. Cancer** <u>107</u> (2003) 189-196.

53. Mazué, F., Colin, D., Gobbo, J., Wegner, M., Rescifina, A., Spatafora, C., Fasseur, D., Delmas, D., Meunier, P., Triangli, C. and Latruffe, N. Structural determinants of resveratrol for cell proliferation inhibition potency. Experimental and docking studies of new analogs. **Eur. J. Med. Chem.** <u>45</u> (2010) 2972-2980.

54. Sale, S., Verschoyle, R.D., Boockock, D., Jones, D.J.N., Wilsher, N., Potter, G.A., Farmer, P.B., Steward, W.P. and Gescher, A.J. Pharmacokinetics in mice and growth-inhibitory properties of the putative cancer chemopreventive agent resveratrol and the synthetic analogue *trans*-3,4,5,4'-tetramethoxystilbene. **Br. J. Cancer** <u>90</u> (2004) 736-744.

55. Sale, S., Tunstall, R.G., Ruparelia, K.C., Potter, G.A., Steward, W.P. and Gescher, A.J. Comparison of the effects of the chemopreventive agent

resveratrol and its synthetic analog *trans*-3,4,5,4'-tetramethoxystilbene (DMU-212) on adenoma development in the ApcMin+ mouse and cyclooxygenase-2 in human-derived colon cancer cells. **Int. J. Cancer** <u>115</u> (2005) 194-201.

56. Ma, Z., Molavi, O., Haddadi, A., Lai, R., Gossage, R.A. and Lavasanifar, A. Resveratrol analog *trans* 3,4,5,4'-tetramethoxystilbene (DMU-212) mediates antitumor effects via mechanism different from that of resveratrol. **Cancer Chemother. Pharmacol.** <u>63</u> (2008) 27-35.

57. Park, H., Aiyar, S.E., Fan, P., Wang, J., Yue, W., Okouneva, T., Cox, C., Jordan, M.A., Demers, L., Cho, H., Kim, S., Song, R.X.-D. and Santen, R.J. Effects of tetramethoxystilbene on hormone-resistant breast cancer cells: biological and biochemical mechanisms of action. **Cancer Res.** <u>67</u> (2007) 5717-5726.

58. Li, H., Wu, W.K.K., Li, Z.J., Chan, K.M., Wong, C.C.M., Ye, C.G., Yu, L., Sung, J.J.Y., Cho, C.H. and Wang, M. 2,3',4,4',5'-Pentamethoxy-*trans*-stilbene, a resveratrol derivative, inhibits colitis-associated colorectal carcinogenesis in mice. **Br. J. Pharmacol.** <u>160</u> (2010) 1352-1361.

59. Hsieh, H.P., Liou, J.P. and Mahindroo, N. Pharmaceutical design of anti-mitotic agents on combretastatins. **Curr. Pharm. Des.** <u>11</u> (2005) 1655-1677.

60. Hall, J.J., Sriram, M., Strecker, T.E., Tidmore, J.K., Jelinek, C.J., Kumar, G.D.K., Hadimani, M.B., Pettit, G.R., Chaplin, D.J., Trawick, M.L. and Pinney, K.G. Design, synthesis, biochemical, and biological evaluation of nitrogen-containing trifluoro structural modifications of combretastatin A-4. **Bioorg. Med. Chem. Lett.** <u>18</u> (2008) 5146-5149.

61. Dyrager, C., Wickström, M., Fridén-Saxin, M., Friberg, A., Dahlén, K., Wallén, E.A.A., Gullbo, J., Grøtli, M. and Luthman, K. Inhibitors and promoters of tubulin polymerization: synthesis and biological evaluation of chalcones and related dienones as potential anticancer agents. **Bioorg. Med. Chem.** <u>19</u> (2011) 2659-2665.

62. Cai, Y.-C., Zou, Y., Ye, Y.-L., Sun, H.-Y., Su, Q.-G., Wang, Z.-X., Zeng, Z.-L. and Xian L.-J. Anti-tumor activity and mechanisms of a novel vascular disrupting agent, (Z)-3,4',5-trimethoxylstilbene-3'-O-phosphate disodium (M410). **Invest. New Drugs** <u>29</u> (2011) 300-311.

63. Hatanaka, T., Fujita, K., Ohsumi, K., Nakagawa, R., Fukuda, Y., Nihei, Y., Suga,Y., Akiyama, Y. and Tsuji, T. Novel B-ring modified combretastatin analogues: syntheses and antineoplastic activity. **Bioorg. Med. Chem. Lett.** <u>8</u> (1998) 3371-3374.

64. Cushman, M., Nagarathnam, D., Gopal, D., Chakraborti, A.K., Lin, C.M. and Hamel, E. Synthesis and evaluation of analogues of (Z)-l-(4-methoxyphenyl)-2-(3,4,5 trimethoxyphenyl)ethene as potential cytotoxic and anti-mitotic agents. **J. Med. Chem.** <u>35</u> (1992) 2293-2360.

65. Pinney, K.G., Meija, M.P., Villalobos, V.M., Rosenquist, B.E., Pettit, G.R., Verdier-Pinard, P. and Hamel, E. Synthesis and biological evaluation of aryl

azide derivatives of combretastatin A-4 as molecular probes for tubulin. **Bioorg. Med. Chem.** <u>8</u> (2000) 2417-2425.

66. Monk, K.A., Siles, R., Hadimani, M.B., Mugabe, B.E., Ackley, J.F., Studerus, S.W., Edvardsen, K., Trawick, M.L., Garner, C.M., Rhodes, M.R., Pettit, G.R. and Pinney, K.G. Design, synthesis, and biological evaluation of combretastatin nitrogen-containing derivatives as inhibitors of tubulin assembly and vascular disrupting agents. **Bioorg. Med. Chem.** <u>14</u> (2006) 3231-3244.

67. Wang, L., Woods, K.W., Li, Q., Barr, K.J., McCroskey, R.W., Hannick, S.M., Gherke, L., Credo, R.B., Hui, Y.H., Marsh, K, Warner, R., Lee, J.Y., Zielinski-Mozng, N., Frost, D., Rosenberg, S.H. and Sham, H.L. Potent, orally active heterocycle-based combretastatin A-4 analogues: synthesis, structure-activity relationship, pharmacokinetics, and in vivo antitumor activity evaluation. **J. Med. Chem.** <u>45</u> (2002) 1697-1711.

68. Schobert, R., Biersack, B., Dietrich, A., Effenberger-Neidnicht, K., Knauer, S. and Mueller, T. 4-(3-Halo/amino-4,5-dimethoxyphenyl)-5-aryloxazoles and N-methylimidazoles that are cytotoxic against combretastatin A resistant tumor cells and vascular disrupting in a cisplatin resistant germ cell tumor model. **J. Med. Chem.** <u>53</u> (2010) 6595-6602.

69. Bonezzi, K., Taraboletti, G., Borsotti, P., Bellina, F., Rossi, R. and Giavazzi, R. Vascular disrupting activity of tubulin-binding 1,5-diaryl-1H-imidazoles. **J. Med. Chem.** <u>52</u> (2009) 7906-7910.

70. Ohsumi, K., Hatanaka, T., Fujita, K., Nakagawa, R., Fukuda, Y., Nihei, Y., Suga, Y., Morinaga, Y., Akiyama, Y. and Tsuji, T. Syntheses and antitumor activity of *cis*-restricted combretastatins: 5-membered heterocyclic analogues. **Bioorg. Med. Chem. Lett.** <u>8</u> (1998) 3153-3158.

71. Romagnoli, R., Baraldi, P.G., Brancale, A., Ricci, A., Hamel, E., Bortolozzi, R., Basso, G. and Viola, G. Convergent synthesis and biological evaluation of 2-amino-4-(3',4',5'-trimethoxyphenyl)-5-aryl thiazoles as microtubule targeting agents. **J. Med. Chem.** <u>54</u> (2011) 5144-5153.

72. Romagnoli, R., Baraldi, P.G., Salvador, M.K., Camacho, M.E., Preti, D., Tabrizi, M.A., Bassetto, M., Brancale, A., Hamel, E., Bortolozzi, R., Basso, G. and Viola, G. Synthesis and biological evaluation of 2-substituted-4-(3',4',5'-trimethoxyphenyl)-5-aryl thiazoles as anticancer agents. **Bioorg. Med. Chem.** <u>20</u> (2012) 7083-7094.

73. Tron, G.C., Pagliai, F., Sel Grosso, E., Genazzani, A.A. and Sorba, G. Synthesis and cytotoxic evaluation of combretafurazans. **J. Med. Chem.** <u>48</u> (2005) 3260-3258.

74. Pirali, T., Busacca, S., Beltrami, L., Imovilli, D., Pagliali, F., Miglio, G., Massarotti, A., Verotta, L., Tron, G.C., Sorba, G. and Genazzani, A.A. Synthesis and cytotoxic evaluation of combretafurans, potential scaffolds for dual action of antitumoral agents. **J. Med. Chem.** <u>49</u> (2006) 5372-5376.

75. Theeramunkong, S., Caldarelli, A., Massarotti, A., Aprile, S., Caprioglio, S., Zaninetti, R., Teruggi, A., Pirali, T., Grosa, G. and Tron, G.C.

Regioselective Suzuki coupling of dihaloheteroaromatic compounds as a rapid strategy to synthesize potent rigid combretastatin analogues. **J. Med. Chem.** <u>54</u> (2011) 4977-4986.

76. Zhang, W., Yang, Q., Wu, Y., Wu, L., Li, W., Qiao, F., Bao, K. and Zhang, L. Preparation of 2,3-diarylthiophene derivatives as antitumor agents. CN patent 101429189, 2009.

77. Qiao, F., Zuo, D., Shen, X., Qi, H., Wang, H., Zhang, W. and Wu, Y. DAT-230, a novel microtubule inhibitor, exhibits potent anti-tumor activity by inducing G2/M phase arrest, apoptosis in vitro and perfusion decrease in vivo to HT-1080. **Cancer Chemother. Pharmacol.** <u>70</u> (2012) 259-270.

78. Liu, T., Dong, X., Xue, N., Wu, R., He, Q., Yang, B. and Hu, Y. Synthesis and biological evaluation of 3,4-biaryl-5-aminoisoxazole derivatives. **Bioorg. Med. Chem.** <u>17</u> (2009) 6279-6285.

79. Sun, C.-N., Lin, L.-G., Yu, H.-J., Cheng, C.-Y. and Tsai, Y.-C. Synthesis and cytotoxic activities of 4,5-diarylisoxazoles. **Bioorg. Med. Chem. Lett.** <u>17</u> (2007) 1078-1081.

80. Schobert, R., Effenberger-Neidnicht, K. and Biersack, B. Stable combretastatin A-4 analogues with sub-nanomolar efficacy against chemoresistant HT-29 cells. **Int. J. Clin. Pharmacol. Ther.** <u>49</u> (2011) 71-72.

81. Biersack, B., Effenberger, K., Schobert, R. and Ocker, M. Oxazole-bridged combretastatin A analogues with improved anticancer properties. **ChemMedChem.** <u>3</u> (2010) 420-427.

82. Akselsen, O.W., Odlo, K., Cheng, J-J., Maccari, G., Botta, M. and Hansen, T.V. Synthesis, biological evaluation and molecular modeling of 1,2,3-triazole analogs of combretastatin A-1. **Bioorg. Med. Chem.** <u>20</u> (2012) 234-242.

83. Romagnoli, R., Baraldi, P.G., Cruz-Lopez, O., Lopez-Cara, C., Carrion, M.D., Brancale, A., Hamel, E., Chen, L., Bortolozzi, R., Basso, G. and Viola, G. Synthesis and antitumor activity of 1,5-disubstituted 1,2,4-triazoles as *cis*-restricted combretastatin analogs. **J. Med. Chem.** <u>53</u> (2010) 4248-4258.

84. Odlo, K., Hentzen, J., Fournier dit Chabert, J., Ducki, S., Gani, O.A.B.S.M., Sylte, I., Skrede, M., Flørenes, V.A. and Hansen, T.V. 1,5-disubstituted 1,2,3-triazoles as *cis*-restricted analogues of combretastatin A-4: synthesis, molecular modeling and evaluation as cytotoxic agents and inhibitors of tubulin. **Bioorg. Med. Chem.** <u>16</u> (2008) 4829-4838.

85. Odlo, K., Fournier-Dit-Chabert, J., Ducki, S., Gani, O.A.B.S.M., Sylte, I. and Hansen, T.V. 1,2,3-Triazole analogs of combretastatin A-4 as potential microtubule-binding agents. **Bioorg. Med. Chem.** <u>18</u> (2010) 6874-6885.

86. Romagnoli, R., Baraldi, P.G., Salvador, M.K., Preti, D., Tabrizi, M.D., Brancale, A., Fu, X.H., Li, J., Zhang, S.Z., Hamel, E., Bortolozzi, R., Basso, G. and Viola, G. Synthesis and evaluation of 1,5-disubstituted tetrazoles as rigid analogues of combretastatin A-4 with potent antiproliferative and antitumor activity. **J. Med. Chem.** <u>54</u> (2012) 475-488.

87. Shirai, R., Takayama, H., Nishikawa, A., Koiso, Y. and Hashimoto, Y. Asymetric synthesis of anti-mitotic combretadioxolane with potent

antitumor activity against multi-drug resistant cells. **Bioorg. Med. Chem. Lett.** 8 (1998) 1997-2000.

88. Pettit, R.K., Pettit, G.R., Hamel, E., Hogan, F., Moser, B.R., Wolf, S., Pon, S., Chapuis, J-C. and Schmidt, J.M. E-combretastatin and E-resveratrol structural modifications: Antimicrobial and cancer cell growth inhibitory β-E-nitrostyrenes. **Bioorg. Med. Chem.** 17 (2009) 6606-6612.

89. Dark, G.G., Hill, S.A., Prise, V.E., Tozer, G.M., Pettit, G.R. and Chaplin, D.J. Combretastatin A-4, an agent that displays potent and selective toxicity toward tumor vasculature. **Cancer Res.** 57 (1997) 1829-1834.

90. Hori, K., Saito, S., Nihei, Y., Suzuki, M. and Sato, Y. Antitumor effects due to irreversible stoppage of tumor tissue blood flow: evaluation of a novel combretastatin A-4 derivative, AC7700. **Jpn. J. Cancer Res.** 90 (1999) 1026-1038.

91. Sheng, Y., Hua, J., Pinney, K.G., Garner, C.M., Kane, R.R., Prezioso, J.A., Chaplin, D.J and Edvardsen, K. Combretastatin family member OXI4503 induces tumor vascular collapse through the induction of endothelial apoptosis. **Int. J. Cancer** 111 (2004) 604-610.

92. Clémenson, C., Jouannot, E., Merino-Trigo, A., Rubin-Carrez, C. and Deutsch, E. The vascular disrupting agent ombrabulin (AVE8062) enhances the efficacy of standard therapies in head and neck squamous cell carcinoma xenograft models. **Invest. New Drugs** 31 (2013) 273-284.

93. Rajak, H., Dewangan, P.K., Patel, V., Jain, D.K., Singh, A., Veerasamy, R., Sharma, P.C. and Dixit, A. Design of combretastatin A-4 analogs as tubulin targeted vascular disrupting agent with special emphasis on their *cis*-restricted isomers. **Curr. Pharm. Des.** 19 (2013) 1923-1955.

94. Brakenhielm, E., Cao, R. and Cao, Y. Suppression of angiogenesis, tumor growth and wound healing by resveratrol, a natural compound in red wine and grapes. **FASEB J.** 15 (2001) 1798-1800.

95. Tseng, S.H., Lin, S.M., Chen, J.C., Su, Y.H., Huang, H.Y., Chen, C.K., Lin, P.Y. and Chen, Y. Resveratrol suppresses the angiogenesis and tumor growth of gliomas in rats. **Clin. Cancer Res**. 10 (2004) 2190-2202.

96. Kundu, J.K. and Surh, Y.-J. Cancer chemopreventive and therapeutic potential of resveratrol: mechanistic perspectives. **Cancer Lett.** 269 (2008) 243-261.

97. Belleri, M., Ribatti, D., Nicoli, S., Cotelli, F., Forti, L., Vannini, V., Stivala, L.A. and Presta, M. Antiangiogenic and vascular-targeting activity of the microtubule-destabilizing *trans*-resveratrol derivative 3,5,4'-trimethoxystilbene. **Mol. Pharmacol.** 67 (2005) 1451-1459.

98. Alex, D., Leon, E.C., Zhang, Z.-J., Yan, G.T.H., Cheng, S.H., Leong, C.-W., Li, Z.-H., Lam, K.-H., Chan, S.-W. and Lee, S.M.-Y. Resveratrol derivative, *trans*-3,5,4'-trimethoxystilbene, exerts antiangiogenic and vascular-disrupting effects in zebrafish through the downregulation of VEGFR2 and cell-cycle modulation. **J. Cell. Biochem.** 109 (2010) 339-346.

99. Folkes, L.K., Christlieb, M., Madej, E., Stratford, M.R.L. and Wardman, P. Oxidative metabolism of combretastatin A-1 produces quinone intermediates with the potential to bind to nucleophiles and to enhance oxidative stress via free radicals. **Chem. Res. Toxicol.** 20 (2007) 1885-1894.

100. Rice, L., Pampo, C., Lepler, S., Rojiani, A.M. and Siemann, D.W. Support of a free radical mechanism for enhanced antitumor efficacy of the microtubule disruptor OXi4503. **Microvasc. Res.** 81 (2011) 44-51.

101. Madlambayan, G.J., Meacham, A.M., Hosaka, K., Mir, S., Jorgensen, M., Scott, E.W., Siemann, D.W. and Cogle, C.R. Leukemia regression by vascular disruption and anti-angiogenic therapy. **Blood** 116 (2010) 1539-1547.

102. Peláez, R., López, J.L. and Medarde, M. Application of chemoinformatic tools for the analysis of virtual screening studies of tubulin inhibitors. **Advances in Soft Computing** 44 (2007) 411-441.

103. Nguyen, T.L., McGrath, C., Hermone, A.R., Burnett, C.J., Zharevitz, D.W., Day, B.W., Wipf, P., Hamel, E. and Gussio, R. A common pharmacophore for a diverse set of colchicine site inhibitors using a structure-based approach. **J. Med. Chem.** 48 (2005) 6107-6116.

104. Massarotti, A., Theeramunkong, S., Mesenzani, O., Caldarelli, A., Genazzani, A.A. and Tron, G.C. Identification of novel antitubulin agents by using a virtual screening approach based on 7-point pharmacophore model of the tubulin colchicine site. **Chem. Biol. Drug Des.** 78 (2011) 913-922.

105. Kim, N.D., Park, E.-S., Kim, Y.H., Moon, S.K., Lee, S. S., Ahn, S.K., Yu, D.-Y., No, K.T. and Kim, K.-H. Structure-based virtual screening of novel tubulin inhibitors and their characterization as anti-mitotic agents. **Bioorg. Med. Chem.** 18 (2010) 7092-7100.

106. Massarotti, A., Coluccia, A., Silvestri, R., Sorba, G. and Brancale, A. The tubulin colchicine domain: a molecular modeling perspective. **Chem. Med. Chem.** 7 (2012) 33-42.

107. Romagnoli, R., Baraldi, P.G., Carrion, M.D., Cruz-Lopez, O., Cara, C.L., Tolomeo, M., Grimaudo, S., Di Cristina, A., Pipitone, M.R., Balzarini, J., Kandil, S., Brancale, A., Srkar, T. and Hamel, E. Synthesis and biological evaluation of 2-amino-3-(3',4',5'-trimethoxybenzoyl)-6-substituted-4,5,6,7-tetrahydrothieno[2,3-c]pyridine derivatives as anti-mitotic agents and inhibitors of tubulin polymerization. **Bioorg. Med. Chem. Lett.** 18 (2008) 5041-5045.

108. Ruan, B.-F., Lu, X., Tang, J.-F., Wei, Y., Wang, X.-L., Zhang, Y.-B., Wang, L.-S. and Zhu, H.-L. Synthesis, biological evaluation, and molecular docking studies of resveratrol derivatives possessing chalcone moiety as potential antitubulin agents. **Bioorg. Med. Chem.** 19 (2011) 2688-2695.

109. Kim, S., Min, S.Y., Lee, S.K., Cho, W.-J. Comparative molecular field analysis study of stilbene derivatives active against A549 lung carcinoma. **Chem. Pharm. Bull.** 51 (2003) 516-521.

110. Chiang, Y.K., Kuo, C.C., Wu, Y.S., Chen, C.T., Coumar, M.S., Wu, J.S., Hsieh, H.P., Chang, C.Y., Jseng, H.Y., Wu, M.H., Leou, J.S., Song, J.S.,

Chang, J.Y., Lyu, P.C., Chao, Y.S. and Wu, S.Y. Generation of ligand-based pharmacophore model and virtual screening for identification of tubulin inhibitors with potent anticancer activity. **J. Med. Chem.** 52 (2009) 4221-4233.

111. Tseng, C.Y., Mane, J.Y., Winter, P., Johnson, L., Huzil, T., Izbicka, E., Luduena, R.F. and Tuszynski, J.A. Quantitative analysis of the effect of tubulin isotype expression on sensitivity of cancer cell lines to a set of novel colchicine derivatives. **Mol. Cancer** 30 (2010) 131-150.

112. Tuszynski, J.A., Craddock, T.J., Mane, J.Y., Barakat, K., Tseng, C.Y., Gajewski, M., Winter, P., Alisaraie, L., Patterson, J., Carpenter, E., Wang, W., Deyholos, M.K., Li, L., Sun, X., Zhang, Y. and Wong, G.K. Modeling the yew tree tubulin and a comparison of its interaction with Paclitaxel to human tubulin. **Pharm. Res.** 29 (2012) 3007-3021.