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Review Article

Combretastatin A-4 analogs: Past, present, and future directions

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ABSTRACT

Microtubules are protein biopolymers created by polymerizing heterodimers of α and β-tubulins. Microtubule disruption induces G2/M cell cycle arrest and abnormal mitotic spindle formation. Their significance in cell division makes microtubules an appealing target for anticancer drug discovery. Several naturally occurring compounds, such as vinblastine, paclitaxel, combretastatin, and colchicine exert their activity by changing tubulin dynamics, such as polymerization and depolymerization. Tubulin, an essential tumor therapy target, is one of the hotspots in the area of antineoplastic drugs, and it is of a significance importance to design novel inhibitors for this target. Both natural and synthetic scaffolds are the main tubulin inhibitors sources. In this article, the main structural features and motifs tubulin polymerization inhibitors are reviewed. Thus, it provides a theoretical basis for target optimization of new inhibitors of tubulin polymerization. In addition, we highly recommend implementing PROTAC-based multi-acting tubulin polymerization inhibotrs for obtaining better potency.

1. Introduction

The leading goal is to design a drug that will only target cancer cells and destroy or render them benign and not affect normal cells. The increased cell growth and division of cancer cells present an attractive and achievable target for drug design by making them more selective to rapidly dividing cells. The most interesting biochemical target is tubulin and the formation of microtubules, which plays a vital role in cell

division. Targeting tubulin polymerization and microtubule formation will impede the replication of the dividing cancer cells and lead to cell death. It is essential to understand the crucial role of tubulin in the cell's life cycle. Microtubules (**Figure 1**) are tube-shaped protein polymers formed from α- and β-tubulin heterodimers [1, 2]. Microtubules are vital for cell division, and without them, cell division cannot occur. In eukaryotic cells, microtubules form a dynamic network essential in cellular processes such as mitosis, maintenance of cell shape, cytoplasmic organelle movement, and cell replication. The microtubule assembly requires the association of two guanosine triphosphate (GTP) molecules for each tubulin heterodimer. One of them binds to an exchangeable site on the β-tubulin subunit, It can be hydrolyzed to guanosinediphosphate (GDP), which is essential for microtubule elongation, and the other GTP molecule binds to α-tubulin and is stable to hydrolysis. The dynamic behavior of microtubules is necessary for normal cell function and growth and is affected by several factors, including the intracellular GTP/GDP ratio, the ionic microenvironment, and the presence of stabilizing microtubule-associated proteins [3].

1.1. Anticancer agents

Chemotherapeutic interference with tubulin/microtubule polymerization dynamics has two pivotal anticancer effects: i) inhibition of cancer cell proliferation through interruption of mitotic spindle formation which leads to apoptosis, and ii) disruption of cell signaling pathways involved in regulating and maintaining the cytoskeleton of endothelial cells in tumor vasculature [4].

1.1.1. Natural products as anticancer drugs

Numerous anticancer drugs were developed from natural products (**Figure 2**) and lead to developing large number of their analogues to improve their pharmacologic and the pharmacokinetic profiles [5].

Microtubule-targeted agents constitute a very important class of anticancer agents targeting three different sites on the α- and β-tubulin subunit: the paclitaxel, the colchicine, the vinca alkaloid. The agents that target the paclitaxel binding site such as paclitaxel 1 are known to stabilize the microtubule cytoskeleton against depolymerization, thus promoting tubulin assembly causing an increase in the mass of microtubules in cells leading to suppression of microtubule polymerization dynamics causing cell cycle arrest [6]. The agents which bind to the vinca alkaloid domain like vinblastine 2, or to the colchicine binding site like colchicine 3 [7], combretastatin A-4 4 [8-10], steganacin 5, and podophyllotoxin 6 induce depolymerization of tubulin thus preventing tubulin assembly. Hence, this class destabilizes microtubules by inhibiting tubulin polymerization. These compounds are microtubule binding agents which bind to specific binding sites. Podophyllotoxin was found to be very toxic in clinical trials so several studies were done to synthesize podophyllotoxin analogues to overcome this drawback. As a result, etoposide 7 (**Figure 2**) was developed and is currently being used to treat testicular and small cell lung cancers [11, 12]. It was found that it forms a ternary complex with DNA topoisomerase II, an enzyme involved in the unfolding of DNA in cell replication, rather than a microtubule interaction [12]. The eukaryotic cell cycle is generally divided into four stages known as G1, S, G2 and M. Gap-phase 1 (G1) is a preparation period for DNA replication. This is followed by S-phase where replication of the DNA and chromosomes takes place. The cell cycle then enters G2 phase which is the second gap-phase, where the integrity of new DNA is checked. Then, M phase starts leading to segregation of chromosomes and the cell division. In addition to these phases, there is G0 which is reversible quiescent phase used to describe the cell cycle in which cells exist in a quiescent state [4, 13].

2. Combretastatin A4: a potent anticancer agent

Combretastatin A4 (CA-4), **4** is a natural product with low molecular weight and simple molecular structure discovered three decades ago. It was isolated from the stem wood of the South African tree combretum caffrum [8-10]. CA-4 showed structural similarity with most

tubulin polymerization inhibitors resulting in higher matching with colchicine binding site of tubulin [14]. CA-4 has dual mechanism on tumors; the first is by binding to tubulin dimers and preventing microtubule polymerization leading to apoptosis, and the second is by disrupting the VE-cadherin that is responsible for cell-cell interaction resulting in ischemic necrosis of tumor tissues [4, 15, 16]. The problem of low solubility of CA-4 was solved by synthesis of more soluble prodrugs, phosphate disodium (CA-4P, fosbretabulin) and a serinamido derivative (AVE-8062, ombrabulin) that showed promising results in clinical trials on the anaplastic thyroid carcinoma [17-20]. Unlike most anticancer drugs, CA-4P showed less bone marrow toxicity and alopecia [21, 22]. SAR studies of CA-4 have shown some structural features which are highly essential for activity. The trimethoxy group in ring A, the cisoid configuration at the bridge, and presence of methoxy group in para position on ring B are all essential for the cytotoxic activity of CA-4 (**Figure 3**) [23, 24].

2.1. Early modifications on CA-4

Several studies have been conducted to determine the structural features responsible for the anticancer activity of CA-4, and to improve its pharmacokinetic profile. These studies have focused on modifying three structural moieties; ring A, ring B, and double bond.

2.1.1. Modifications on ring A

It was thought that the presence of the trimethoxybenzene moiety is essential for the cytotoxic and tubulin inhibitory activity. This was based on two reasons; (i) the recurrence in nature of this moiety in other antitubulin drugs (e.g. podophyllotoxin, steganacin, and colchicine **(Figure 2)** [25] (ii) the potency of CA-4 over CA-3 **14 (Figure 4)**, where the meta methoxy group is replaced with a hydroxyl group [26]. A significant loss in potency was reported when a simple aromatic ring (phenyl) was present or when deletions of the *meta* or *para* position methoxy groups were performed [27]. Similarly, loss of cytotoxic activity was observed when the methoxy groups were substituted with bulkier (e.g. ethoxy) groups **9** [24]. This latter observation suggested that the steric factor plays a pivotal role in accommodating the ring inside the active site **(Figure 5)**. In an attempt to exploit the hydrophobic interactions that might occur in the binding site of tubulin, the trimethoxybenzene was replaced with trimethylbenzene **10** or naphthalene **11** however, this lead to decreasing cytotoxic activity [24, 28]. Pettit and colleagues [29] synthesized series of fluorcombretastatins **12** where the methoxy group at the *meta* position was replaced with a fluorine group. The compounds showed antitubulin activity comparable to that of CA-4 but there was only a slight loss of cytotoxic activity. An isocombretaquinazoline derivative **13** devoid of the 3,4,5-trimethoxyphenyl ring showed comparable results to CA-4 with potent vascular-disrupting activity at micromolar levels [30]. These results indicated that the trimethoxy phenyl moiety is very important moiety for this scaffold but may not be crucial for the cytotoxic activity.

2.1.2. Modifications on ring B

Several structural modifications were done to establish the most active structure for this ring **(Figure 6)**. By substitution of the phenolic group of ring B with fluorine **14** [31] or bromine **15** [20] atoms, the produced compounds showed significant cytotoxic activity and more metabolic stability. Replacement of ring B with a quinolone **16** resulted in 70-fold less cytotoxic activity than CA-4 [32], while substitution of the methoxy group with a dimethylamino moiety at the para position showed 10-fold less cytotoxic potency **17** [33].

Exchanging the para methoxy group with an ethoxy **18** or propoxy group lead to loss in activity [27], suggesting that steric effects may influence the activity of these compounds.

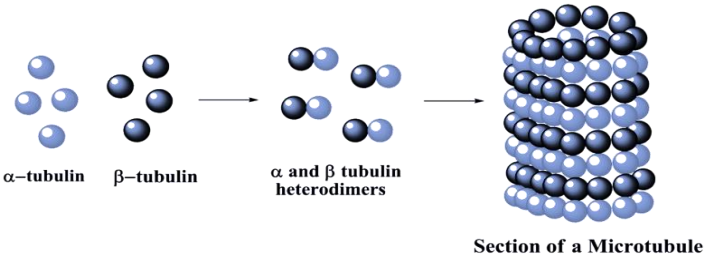

Figure 1: Schematic representation of the structure and formation of microtubules.

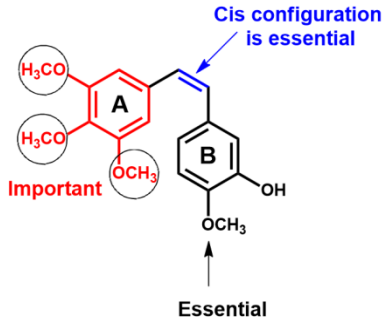

Figure 3: Structure activity relationship of CA-4

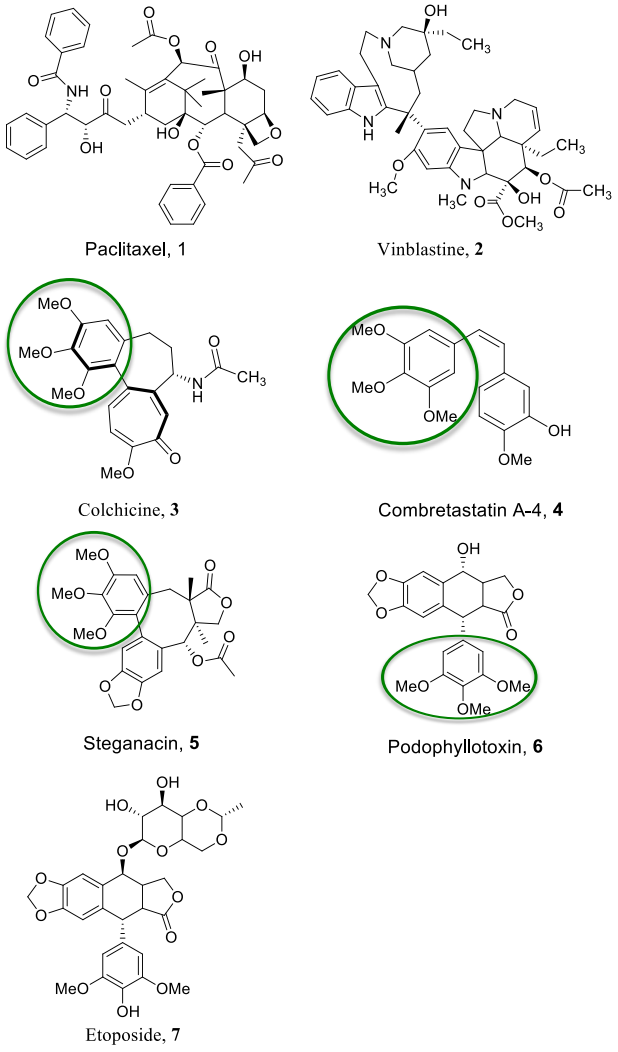

Figure 2: Chemical structures of some natural anticancer agents

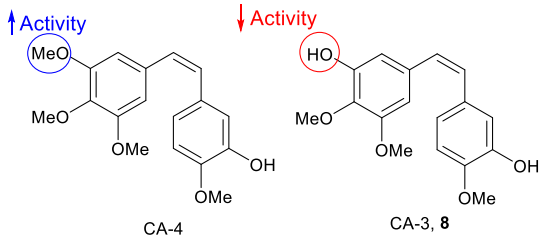

Figure 4: The increased potency of CA-4 over CA-3

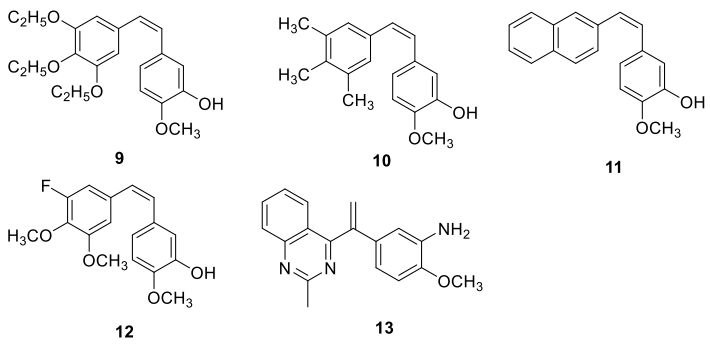

Figure 5: Selected analogues of CA-4 modified on the aromatic ring A

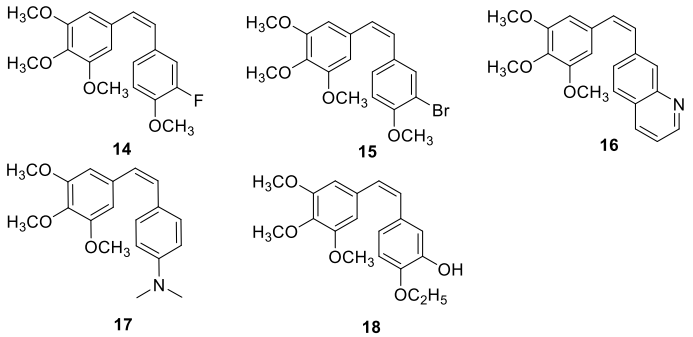

Figure 6: Selected analogues of CA-4 modified on the aromatic ring B

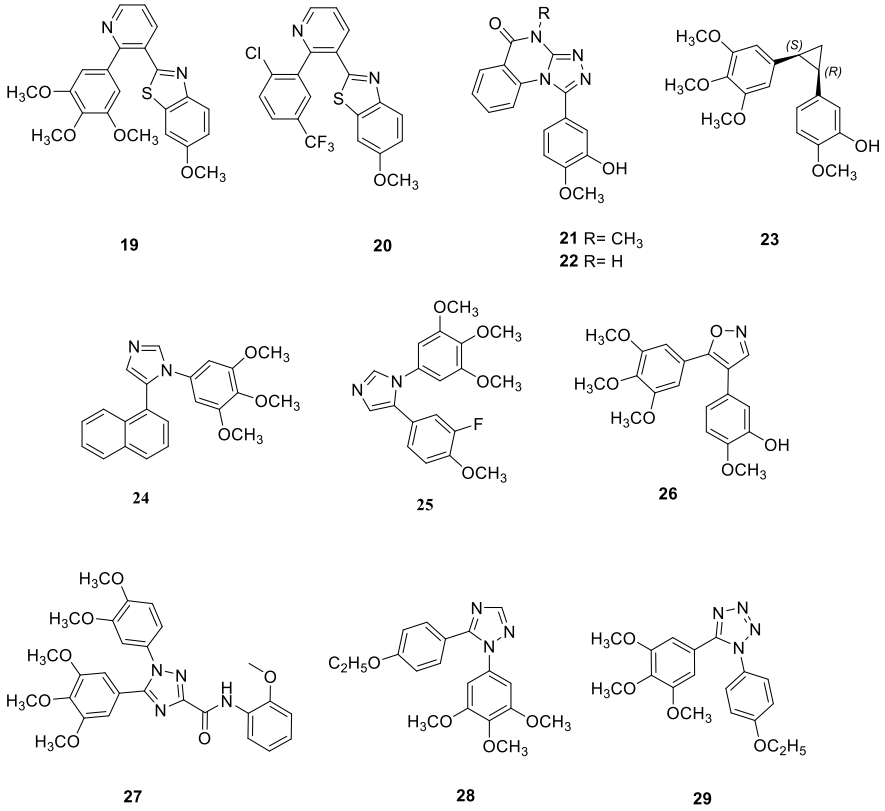

Figure 7: Selected analogues of CA-4 modified in the double bond

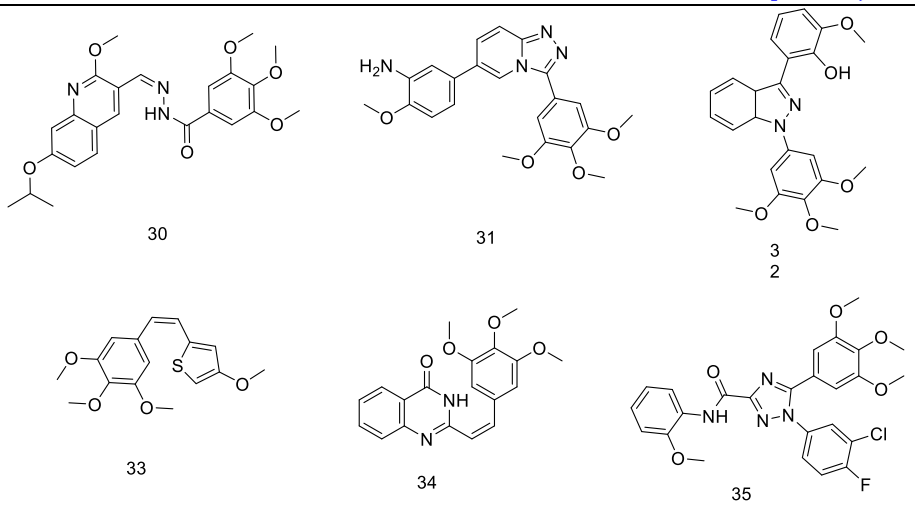

Figure 8: Recent advances on CA-4 analogs

2.1.3. Modifications of the double bond

The olefinic component of CA-4 has undergone major molecular changes (**Figure 8**). The main reason for this is the Z-stilbenic double bonds that can easily isomerize to the E-form because of storage and metabolism leading to dramatic reduction in activity [34]. Therefore, the chemical instability (isomerization) of CA-4 has been addressed by designing and synthesizing a series of *cis*-constrained derivatives of CA-4. Extensive studies have been conducted to stabilize CA-4 by replacing the double bond bridge with heterocyclic ring systems such as 1,2,3-triazole [35-37], 1,2,4-triazole [38, 39], tetrazole [40-42], thiadiazole [43], isoxazole [44, 45] and oxadiazole [46].

In a recent study [47], New *cis*-restricted CA-4 analogues were developed by incorporation of pyridine ring instead of the carbon-carbon double bond. They also replaced ring B of CA-4 by benzothiazole scaffold. Compound **19** with trimethoxy groups on ring A and a methoxy group on benzothiazole moiety was very potent mimic for CA-4 exhibiting IC_{50} of 0.06 μM against HeLa liver cancer cell line (**Figure 8**). Whereas, a nanomolar activity was observed for compound **20** with two electron withdrawing groups (CF_3 and Cl) on ring A and methoxy group on benzothiazole moiety against all the tested cancer cell lines with GI_{50} ranging from 0.04-0.09 μM. Interestingly, compound **20** exhibited superior antiproliferative activity compared to the standard CA-4, particularly against liver cancer and prostate cancer (HeLa and DU-145 cell lines respectively). Two conformationally restricted CA-4 analogues containing quinazolinone moiety exhibited potent antiproliferative activities against leukemia SR, lung cancer NCI-H522, and melanoma MDA-MB-435 cell lines. Specifically, compounds **21** and **22** displayed potent tubulin polymerization inhibitory activity with IC_{50} values of 4.26 and 0.15 μM, respectively [48].

From a series of *cis*-restricted cyclopropyl containing analogues of CA-4, compound **23** displayed similar tubulin polymerization inhibitory activity to CA-4 with IC_{50} of 0.30 μM [49]. Furthermore, two compounds **24** and **25** from a series of novel CA-4 like diaryl-1*H*-imidazoles showed similar cytotoxic activity as compared to CA-4 against lung cancer and colon cancer (NCI-H460 and HCT-15 cell lines, respectively) [50]. Kaffy and colleagues have prepared an isoxazole derivative **26** of CA-4 that exhibited greater tubulin polymerization inhibitory activity than

that of CA-4 (0.75 vs. 1.2 μM) [44]. Aly and colleagues [39] prepared 1,2,4-triazole-3-carboxamide derivatives acting as *cis*-restricted analogues of CA-4. The anilide of *o*-anisidine **27,** *m*-anisidine**,** and 3,5-difluoroaniline were the most active compounds against hepatocellular carcinoma HepG2, breast cancer MCF7, and leukemia HL-60 cell lines. These compounds produced a significant reduction in cellular microtubules at a concentration of 25 μg/ml. Synthesis of these compounds as combretastatin analogues exhibited promising results when using ring A as 3,4,5-trimethoxy phenyl moiety and ring B as 3,4-dimethoxy phenyl moiety connected together through 1,2,4-triazole ring instead of the carbon-carbon double bond of CA-4. Furthermore, 1-(3,4,5-trimethoxyphenyl)-5-aryl -1,2,4-triazoles and 1-(3,4,5-trimethoxyphenyl)-5-aryl-1,2,3,4-tetrazoles were developed as *cis*-restricted CA-4 analogues. Screening of their cytotoxic activity showed that compounds containing *para*-ethoxy group on ring B possessed potential cytotoxic activity. Compound **27** was more cytotoxic than the reference CA-4 against lung cancer A549, breast cancer MCF7, and jurkat leukemic T-cell lines, and showed more potent cytotoxic activity than the reference CA-4 against cervical cancer HeLa, lung cancer A549, breast cancer MCF7, colon cancer HT29, and jurkat leukemic T-cell lines. Compounds **28** and **29** possessed high tubulin polymerization inhibitory activity with the same IC_{50} values (0.76 μM) compared to CA-4 with IC_{50} value of 1.2 μM. [38, 42, 51].

2.2. Present modifications on CA-4

In the last five years, several CA-4 analogs have been reported as tubulin polymerization inhibitors with potential anticancer activity (**Figure 8**). Some of these reported CA-4 analogs retained the anticancer and the anti-tubulin activities, whereas some of these compounds only retained one of these activities. Ibrahim and coworkers reported the synthesis of CA-4 bearing a rigid hydrazone open linker. Interestingly, compound **30** retained both the anticancer and the anti-tubulin activity (**Figure 9**) [52]. The same result was reported by Yang and his research team, who reported the synthesis of novel 1,2,4-triazolo[4,3-a]pyridines. Compound **31** showed strong tubulin polymerization inhibition and was better than CA-4 against Hela cells. However, **31** did not show notable activity on A549, MCF-7, and T47D cell lines [53]. Contrarily, Jian et al. reported pyrazolo3,4-b-pyridine bridged CA-4 analogs (**32**). The prepared derivatives did not retain the anti-tubulin activity, nor the anticancer potency [54]. Faouzi and his team reported CA-4 analogs bearing thiophene instead of ring B (**33**) [55]. Unfortunately, none of the prepared derivatives possessed notable anti-tubulin or anticancer activity. Interestingly, Mcherbakov et al. reported photoswitchable quinazoline based CA-4 analogs (**34**) [56]. The idea was to convert the *E* configuration to *z*, which led to a 9-fold increase in the anticancer activity compared to the *E* form. Mustafa et al. has reported new 1,2,4-triazole bearing analogs and utilized an extension on the structure to target both α and β tubulin. The applied strategy resulted in a substantial increase in the anticancer activity and good affinity on tubulin compared to CA-4 (**35**) [57]. However, it is strongly believed that this scaffold could have another target which aided fortifying the anticancer activity. Recently, Hamze et al. reviewed the isoCA-4 analogs in the literature as a promising scaffold in the anticancer treatment [58]. The analysis of these results implies that the trials to prepare potent CA-4 have partially succeeded.

2.3. Future directions for CA-4 analogs

Several CA-4 analogs have been studied in the last decades. However, only small attempts showed promising results and retained the enzymatic and the anticancer activity. It could be best to merge the pharmacophores of the various anticancer mechanisms to develop chimeric chemical scaffolds, leading to preparing multi-acting inhibitors. Besides, PROTAC strategy should be studied in more

depth on tubulin degraders. The challenge towards tubulin degraders is located in the fact that the cereblon-recruiting PROTACs were not efficient tubulin degraders [59]. Hence, it could open an avenue towards medicinal chemists to optimize and find the appropriate E3 ligases as tubulin degraders.

3. Conclusions

Tubulin and microtubules play a vital role in chromosome segregation during cell division which makes them attractive targets for anticancer drug development. CA-4 is characterized by powerful inhibition of tubulin polymerization and anticancer activity. However, its therapeutic application is substantially hindered due to geometric isomerization. In this review, several combretastatin analogs were discussed. The analogs that retained the anticancer and the anti-tubulin activities have been analyzed. Also, the future of CA-4 analogs was discussed in brief.

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