European Journal of Medicinal Chemistry 281 (2025) 117021

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: www.elsevier.com/locate/ejmech

Review article

A novel life for antitumor combretastatins: Recent developments of hybrids, prodrugs, combination therapies, and antibody-drug conjugates

Marialuigia Fantacuzzi, Simone Carradori, Letizia Giampietro, Cristina Maccallini, Barbara De Filippis, Rosa Amoroso, Alessandra Ammazzalorso *

Department of Pharmacy, "G. d'Annunzio" University of Chieti-Pescara, Via dei Vestini 31, 66100, Chieti, Italy

ARTICLE INFO

Keywords:
Combretastatins
Hybrids
Prodrugs
ADC
Anticancer
Natural products

ABSTRACT

Since their discovery from natural sources, the potent cytotoxic effects of combretastatins were widely studied for the application in antitumor therapy. However, major pharmacokinetic issues as low water solubility and chemical instability of the double bond configuration prevented their use in therapy. A lot of efforts have been directed towards the search of novel strategies, allowing a safer use of combretastatins as anticancer agents. This review analyses the recent landscape in combretastatin research, characterized by the identification of hybrids, prodrugs, and novel combination treatments. Interestingly, the potent cytotoxic agent combretastatin A4 (CA4) was recently proposed as payload in the construction of novel antibody-drug conjugates (ADCs), allowing an efficient targeting of the cytotoxic agent to specific tumors.

1. Introduction

Since their isolation from the barks of the South African bush willow tree *Combretum caffrum,* combretastatins have been attracting a lot of interest due to their potent cytotoxic effects. These natural compounds are stilbene derivatives, classified in four families: family A comprises *cis*-stilbene-based compounds, family B dihydrostilbenes, whereas phenanthrenes and macrocyclic lactones are representative structures for families C and D, respectively (Fig. 1) [1–3]. Overall, two phenyl rings (one of which is trimethoxy substituted), linked by a double bond in *cis* configuration, represent the general features common to the combretastatins of series A. Natural combretastatins CA4 and CA1 are the most interesting derivatives given their biological activity: their structures are represented in Fig. 1, together with their prodrugs fosbretabulin, fosbretabulin tromethamine, and Oxi4503.

Extracts of *Combretum caffrum* have traditionally been used in South Africa as a folk remedy for the treatment of various conditions, including scorpion stings, cardiovascular disorders, and worm-related diseases.

Combretastatins exert their biological action by targeting microtubules and binding to the colchicine binding site (CBS) on tubulin in a similar orientation as colchicine [4]. The binding to CBS, which is primarily located at the β-subunit and at the α/β-subunit interface, impedes the adoption of the straight conformation, thereby inhibiting tubulin polymerization, and disrupting microtubule dynamics [5]. This ultimately leads to cell cycle arrest at the G2/M phase and subsequent apoptotic cell death. Fig. 2 presents a schematic illustration of the tubulin heterodimer, delineating the binding site of combretastatin on β-tubulin and the interacting site on α-tubulin. The most active combretastatin A-4 (CA4, 3′-hydroxy-3,4,4′,5-tetramethoxy-*cis*-stilbene, Fig. 1) is characterized by a 3,4,5-trimethoxy-substituted phenyl ring (A), a B phenyl ring substituted in 3′(OH) and 4' (OCH_3), and an ethylene bridge in *cis* configuration, responsible for the proper rigidity and the correct spatial orientation of aromatic rings. These structural features were found to be essential to determine a potent interaction in CBS and provide high levels of cytotoxicity [6]. In addition to the potent tubulin polymerization inhibition, CA4 can also induce significant anti-vascular and anti-angiogenic effects, by acting as a vascular disrupting agent (VDA) [7,8]. This important effect on tumor microvessels causes nutrient loss, oxygen deprivation and irreversible vascular damage, leading to haemorrhagic necrosis and cell death.

Despite the potent cytotoxic effects and the anti-angiogenic activity exerted by CA4 and its analogue CA1 (Fig. 1), these natural compounds suffer from some limitations, such as low water solubility and chemical instability of the double bond configuration. To overcome these problems, different medicinal chemistry strategies were applied to improve both the pharmacokinetic and pharmacodynamic profiles of natural combretastatins (Fig. 3) [9–13]. Briefly, the main approaches pursued include the formation of water-soluble prodrugs, the synthesis of

* Corresponding author. Department of Pharmacy, "G. d'Annunzio" University, Via dei Vestini 31, 66100, Chieti, Italy.
E-mail address: alessandra.ammazzalorso@unich.it (A. Ammazzalorso).

https://doi.org/10.1016/j.ejmech.2024.117021
Received 16 September 2024; Received in revised form 23 October 2024; Accepted 30 October 2024
Available online 31 October 2024

derivatives modified on the central bridge (by replacing the olefinic bond with cycles or heterocycles), heterocyclic derivatives (obtained replacing the phenyl rings A or B with heterocycles), and several hybrids and prodrugs designed to obtain a selective delivery to cancer cells. Some of these strategies were highly promising in the antitumor research, as evidenced by the development of fosbretabulin (Zybrestat®, Fig. 1), the CA4 phosphate derivative, that was subjected to extensive investigation in clinical studies alone or in combination with traditional chemotherapeutic agents or radiotherapy [14–17].

A summary of combretastatin derivatives that have entered clinical studies is shown in Table 1. The water-soluble prodrugs fosbretabulin, fosbretabulin tromethamine, and Oxi4503 (Fig. 1) were the most extensively studied compounds, undergoing to clinical trials with different therapeutic indications. Overall, they were tested for the treatment of multiple tumors and macular degeneration, alone or in combination with other drugs (pazopanib, everolimus, carboplatin/paclitaxel, bevacizumab, radiation therapy).

In 2006, the FDA has granted orphan drug designation to combretastatin A4 phosphate (CA4P) for the treatment of ovarian cancer, and in 2012 to OXi4503 for the treatment of acute myelogenous leukemia. Unfortunately, subsequent results from clinical trials did not allow for the further development of these agents, despite the promising activity in preclinical studies. To date, any combretastatin derivative (natural or synthetic) received the FDA approval.

Published results obtained in clinical studies are related to the evaluation of fosbretabulin in patients affected by age-related macular degeneration (NCT01570790), head and neck cancer (NCT00060242), and recurrent ovarian carcinoma (NCT01305213).

A prospective, interventional, dose-escalation clinical trial was undertaken to assess the safety, tolerability, and efficacy of intravenous infusion of CA4P in patients with neovascular age-related macular degeneration [18]. In this study (the first one of CA4P for an ophthalmic disease in humans) eight patients were treated with CA4P (27 or 36 mg/m^2) as weekly intravenous infusion for 4 consecutive weeks. Overall, the safety profile of intravenous CA4P was consistent with that reported in oncology studies for vascular disruptive agents. Clinical evidence supports the efficacy of CA4P in neovascular age-related macular degeneration; however, the results suggest that systemic CA4P does not represent an alternative treatment, when compared to the safe and efficacious intravitreal anti-vascular endothelial growth factor (VEGF) therapy. For these reasons, CA4P could be proposed as an adjunctive treatment, useful in combination approach by topical administration.

A phase II single-agent trial of fosbretabulin was conducted in patients with advanced anaplastic thyroid cancer [19]. Twenty-six patients received fosbretabulin (45 mg/m^2) as intravenous infusion. The results indicated that patients treated with fosbretabulin monotherapy had a median survival of 4.7 months, comparable to that observed for paclitaxel group. The administration of fosbretabulin did not induce significant change in the history of anaplastic thyroid cancer, but it was observed an encouraging survival of patients and an acceptable safety profile.

The combination of fosbretabulin tromethamine with bevacizumab was tested in a randomized Phase II clinical trial (NCT01315213), supposing that bevacizumab might prevent revascularization during and after treatment with a vascular disrupting agent [20]. The study enrolled 107 patients, treated with bevacizumab (15 mg/kg) or the combination of bevacizumab (15 mg/kg) plus fosbretabulin (60 mg/m^2) intravenously once every 3 weeks. The results highlighted that the proportion responding to bevacizumab was 28.2 % (39 patients with measurable disease) versus 35.7 % (42 patients treated with the combination). The hypertension was reported as the most common adverse event occurred

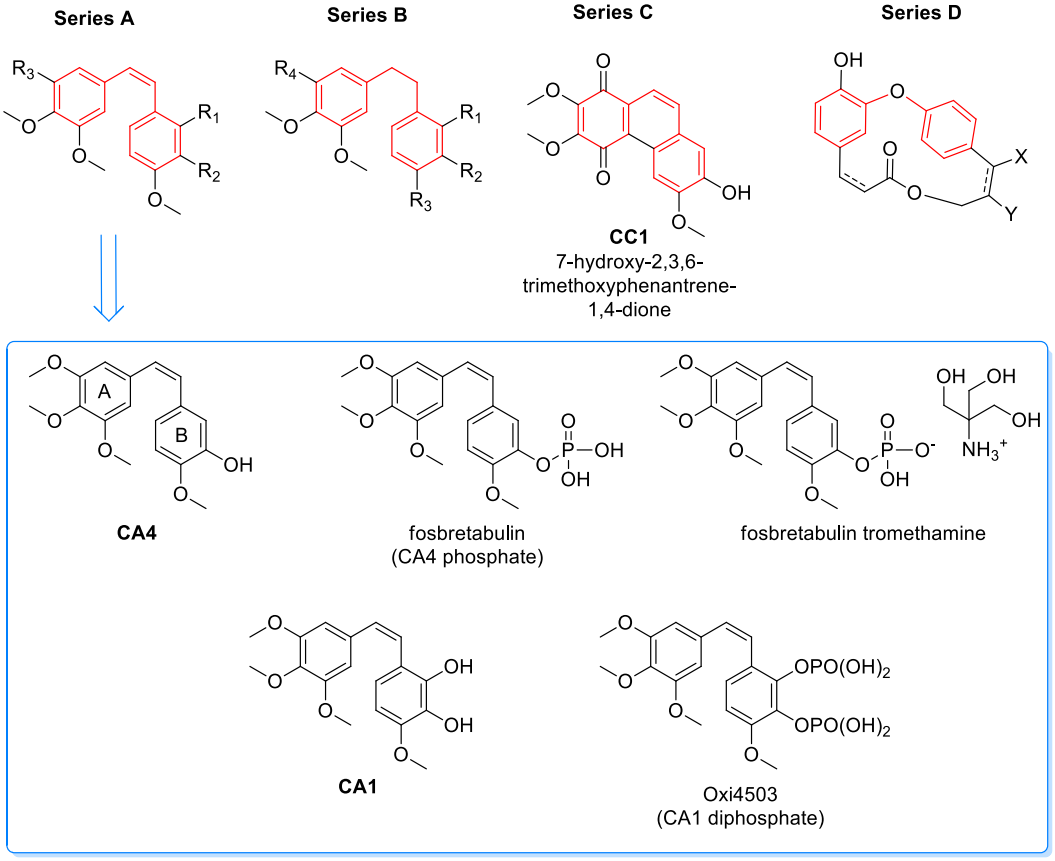

Fig. 1. Chemical structure of the A-D families of natural combretastatins. In series A, the structures of combretastatins A-4 (CA4) and A1 (CA1) are shown, together with their prodrugs fosbretabulin, fosbretabulin tromethamine, and Oxi4503.

in patients treated with the combination, with respect to the group treated with only bevacizumab. Overall, the option to add the combination fosbretabulin plus bevacizumab to classical chemotherapy is reputed a major opportunity to improve the progression-free survival of women with recurrent epithelial ovarian cancer.

In multiple clinical studies performed on fosbretabulin, cardiovascular side effects arose, limiting its safety profile [21]. Overall, the clinical experience suggests the need of a cardiovascular assessment of patients before the treatment with fosbretabulin and a careful management of blood pressure during the treatment. This strategy could mitigate the risk of cardiovascular adverse events, observed in several clinical trials.

In the last years, the interest in this class of natural compounds is greatly increasing, and several attempts have been done by medicinal chemists to improve the pharmacological potential of combretastatins. Several combretastatin derivatives endowed with improved pharmacokinetic properties and tumor targeting selectivity were identified in last years, leading to a library of molecules with a potential anticancer therapeutic application.

The aim of this review is to analyse the latest improvements (from 2018 to date) achieved in the combretastatin research, with a special attention to the development of hybrids, prodrugs, combination treatments, and their application in antibody-drug conjugates (ADCs). Derivatives obtained by classical modification of the stilbene scaffold will not be described in this review, while they have been widely reviewed elsewhere [9,10,22].

2. Combretastatin hybrids

The hybridization strategy allows for the combination of the main structural features of different bioactive compounds in a single molecule, thereby expanding the therapeutic application of single drugs. Multifunctional hybrids, whose activity is directed against multiple targets, represent powerful agents in the development strategies of several classes of drugs [23–25]. The combination of different structural features into a unique drug attracted the attention of researchers in the anticancer field, leading to the identification of multifunctional hybrids [26–28], in the attempt to overcome resistance mechanisms [29].

Combretastatin-Coxib hybrids were proposed by various research groups with the objective of enhancing anticancer efficacy (Fig. 4).

In the attempt to obtain a novel molecule active against colorectal cancer, the hybrid drug **1** was realized by combining the cyclooxygenase-2 (COX-2) selective non-steroidal anti-inflammatory drug (NSAID) rofecoxib with the CA4 structure [30]. The use of COX-2 inhibitors in colorectal cancer has been investigated, showing the ability to arrest cancer proliferation, increasing the chemotherapeutic efficacy [31,32]. The design of this hybrid allowed to retain the potent anti-microtubule and anti-angiogenesis activity of CA4, avoiding the isomerization of the *cis*-form to the biologically inactive *trans* one. The replacement of the olefinic bridge of CA4 by the five-membered heterocycle furanone results in a molecule that retains the COX-2 inhibiting property while maintaining the antitubulin action of the original drug. In colorectal cancer cell lines HT29, HCT116, SW620 and LoVo, the growth inhibition constants (IC_{50}) of **1** spanned from 258 to 642 nM; this molecule was also able to reduce the number and size of the tumor cell colonies. Experiments on the mechanism of action demonstrated for **1** the ability to inhibit tubulin polymerization in a concentration-dependent manner. In a mouse xenograft model using HT-29 colon cancer cells, **1** at 25 mg/kg strongly inhibited tumor growth along with a reduction of COX-2 levels. No organ toxicities were evident in xenografted mice, indicating the safety profile of this combretastatin-rofecoxib hybrid.

A similar strategy was reported by other authors, who used the 1,2-diphenyl substituted pyrazole ring of celecoxib as a scaffold to mimic the *cis*-1,2-diphenylethylene motif in CA4 [33]. The replacement of the double bond with five-membered heterocyclic rings retains both cytotoxic and antitubulin activities of the compounds. The cytotoxic properties of the celecoxib-CA4 hybrids were assayed in human cancer cell lines HT-29, Hep-G2, MCF-7. Generally, the presence of a nitrogen-bearing group together with a halogen-bearing group at *para*-position of both adjacent phenyl rings contribute to the increase of cytotoxicity. Among this series of celecoxib hybrids, further studied on compound **2** demonstrated to suppress prostaglandin E_2 (PGE_2) production, block the cell cycle progression at G2/M phase, and induce apoptosis mainly in early stage of MCF-7 cells via caspase-3 activation [34].

The hybrid compounds containing the scaffold 2,3-diphenyl-2*H*-indazole and the CA4 (**3**,**4**) were recently reported by a Mexican research group, in the attempt to increase the cytotoxic effect of two biologically active structures [35]. A strong cytotoxic effect was recorded in human cervix cancer cell HeLa, human non-small cell lung SK-LU-1, and human chronic myelogenous leukemia cells K562. The 3,4,5-trimethoxyphenyl and the 4-methoxy-3-hydroxyphenyl groups at positions 2 and 3 of the indazole were found essential to the observed cytotoxicity. Further experiments showed that the hybrid **3** induced G2/M arrest of HeLa cell cycle due to the disruption of the microtubule

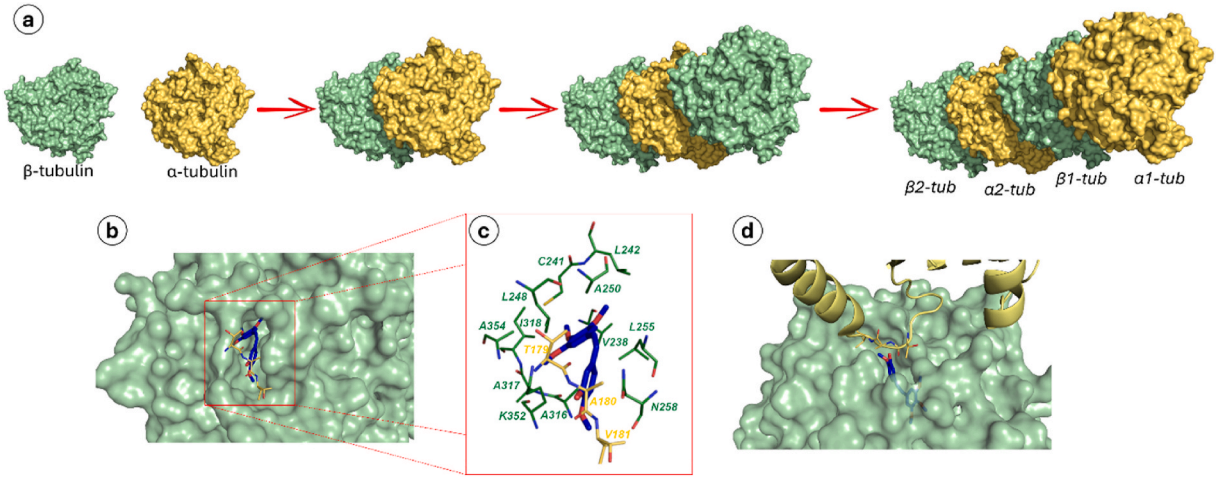

Fig. 2. a) Schematic representation of the α, β tubulin heterodimers, α-tubulin in yellow-orange cartoon and β-tubulin as light green cartoon; b) Binding site of combretastatin A4 in the active site of β-tubulin; c) in the red square enlargement, the interaction residues of the colchicine binding site (CBS) on β-tubulin (green lines) and the interactive residues on α-tubulin (yellow lines); d) a flip view of the α/β tubulin interface binding site (β-tubulin as green surface, α-tubulin as cartoon and the interaction residues as lines). The 3D structure of tubulin-combretastatin A4 has been retrieved from the PDB (PDB ID 5LYJ).

network.

Through a pharmacophore hybridization approach, a series of hybrids of discoipyrrole C and CA4 were designed and synthesized by Kurian et al. (Fig. 5) [36]. The alkaloids discoipyrroles, isolated from marine bacterium *Bacillus hunanensis,* are characterized by the 1*H*-pyrrol-3(2*H*)-one core, with a vicinal diaryl unit, geometrically restricted in a *cis*-orientation. The structural similarity to combretastatins prompted researchers to perform hybrids of these bioactive molecules, to modulate their cytotoxic profiles. The synthesized hybrids **5** were evaluated for their *in vitro* antiproliferative activities against the human cancer cell lines HeLa, the breast carcinoma MCF-7 and MDA-MB-231, and the non-small cell lung carcinoma A549. The derivative bearing the same substitution as CA4 (**5a**) was found effective in depolymerizing the interphase microtubules in HeLa cells and significantly increased the incidence of multipolar spindles. It also determined a 2.5-fold increase in the percentage of cells in the G2/M stage, hampering the cell cycle progression in HeLa cells. In breast cancer MDA-MB-231 and MCF-7 cells, **5a** was effective in reducing the number of colonies compared to the natural product discoipyrrole C, whereas CA4 induced a higher colony inhibition compared to **5a**.

An interesting study on some β-carboline-combretastatin hybrids (**6**, Fig. 5) has been reported, in which the tricyclic pyrido[3,4-*b*]indole ring system was connected to the CA4 scaffold through a carboxamide linker [37]. The newly synthesized hybrids were evaluated for their *in vitro* cytotoxicity against cervical cancer cells HeLa, prostate cancer line DU-145, and lung cancer line A549.**6a** and **6b** were found to be the most potent cytotoxic agents (IC_{50} value less than 2 μM), and they were further analysed in terms of apoptosis induction, DNA binding affinity, and topoisomerase II inhibition. Both compounds **6a-b** interfere with the catalytic activity of the topo-II enzyme and act as catalytic inhibitors. Treatment of A549 cells with **6a** resulted in G2/M cell cycle arrest, confirming the possibility for these novel hybrids to be further progressed into the preclinical development.

To improve cancer targeting and antitumor efficacy, a group of researchers proposed a novel family of hybrids, combining the combretastatin structure with the aziridine moiety (**7**, Fig. 5) [38]. The dual tubulin polymerization inhibition and DNA damage might enhance the activities of single compounds, resulting in an improved therapeutic effect. The aziridine ring, as in the structures of thiotepa and mitomycin, represents a chemical scaffold endowed with alkylating DNA properties. The novel hybrids retain the structural features of both pharmacophores, representing multitarget agents in the fight against cancer. Compound **7** was the most active derivative, showing strong cytotoxic effects (IC_{50} 0.16–1.40 μM) against four human cancer cell lines (ovarian A2780, colorectal HCT-116, cervical HeLa, lung A549). The study of the mechanism of action displays the ability to inhibit tubulin polymerization (IC_{50} 3.3 μM) with a better inhibitory activity than colchicine. Compound **7** significantly upregulated the expression of proapoptotic protein Bax and GADD45α, suggesting it could induce DNA damage. Additionally, compound **7** caused apoptosis of HeLa cells, upregulating the proapoptotic protein p53, and demonstrated a dose-dependent inhibition of cell colony formation and induced the cell cycle arrest at the G2/M phase. Furthermore, the antitumor activity of **7** was assessed in an *in vivo* A2780 cells xenograft model: the treatment with **7** dose-dependently inhibited tumor growth, and the efficacy obtained at 50 mg/kg was found to be comparable to the group treated with the antitumor drug paclitaxel.

Overall, the combretastatin hybrids discovered in the period analysed by this review were tested in preclinical models, with no derivative progressing into clinical evaluation.

3. Combretastatin prodrugs

In more recent years, to overcome some pharmacokinetic problems of CA4, the use of lipophilic prodrugs entrapped in liposomes has been proposed as a strategy for improving the antitumor efficacy of CA4 [39]. The hydroxy group of CA4 was esterified using acyl chlorides of various length (6–18 carbon atoms) to obtain the lipophilic prodrugs **8** (Fig. 6). The synthesized prodrugs were subsequently encapsulated in liposomes, which exhibited high encapsulation efficiency and good stability. Experiments on the drug release showed that longer modified acyl chains for CA4 were associated with slower rates of drug release and

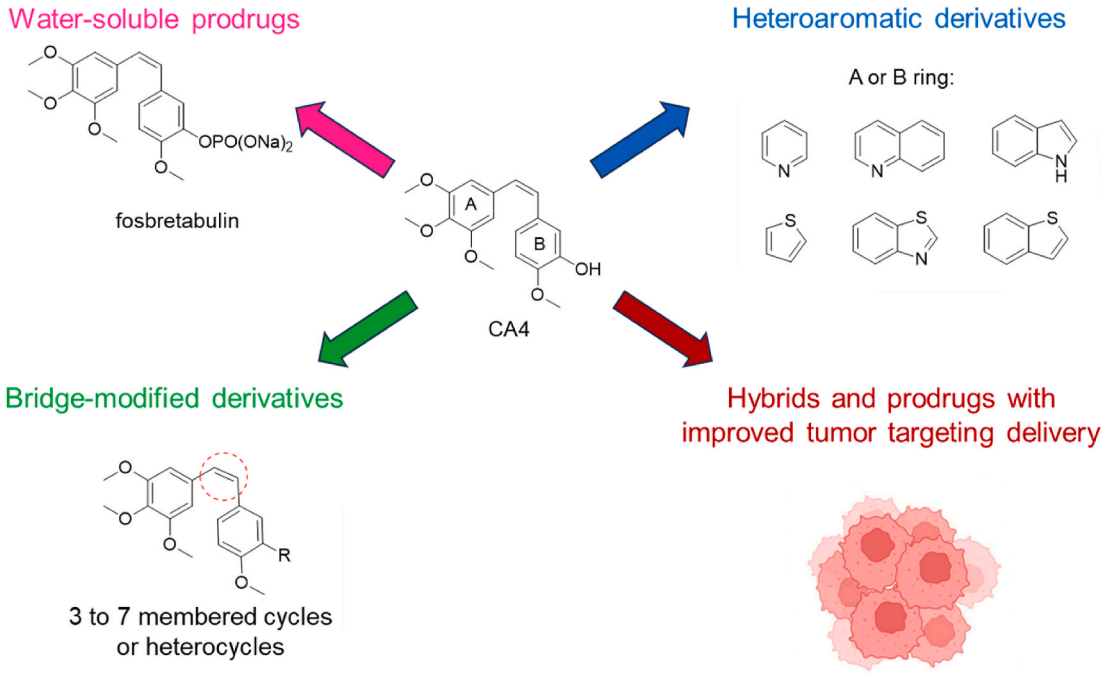

Fig. 3. General medicinal chemistry strategies applied to overcome solubility and instability issues of natural combretastatins. They include the formation of water-soluble prodrugs, the synthesis of derivatives modified on the central bridge, heterocyclic derivatives, and several hybrids and prodrugs designed to obtain a selective delivery to cancer cells.

Table 1
Clinical studies on combretastatin derivatives (ClinicalTrials.gov, accessed on 18/10/2024).

Drug	NCT Code	Conditions	Organization	Status
Fosbretabulin	NCT01570790	Age-related macular degeneration	Johns Hopkins University	Completed, results published [18]
	NCT01423149	Choroidal neovascularization, degenerative myopia	Mateon Therapeutics	Completed, results not published
	NCT00060242	Head and neck cancer	Case Comprehensive Cancer Center	Completed, results published [19]
	NCT02132468	Neuroendocrine tumors	Mateon Therapeutics	Completed with results, results not published
Fosbretabulin tromethamine	NCT02132468	Neuroendocrine tumors	Mateon Therapeutics	Completed with results, results not published
	NCT01305213	Recurrent fallopian tube carcinoma, recurrent ovarian carcinoma, recurrent primary peritoneal carcinoma	National Cancer Institute (NCI)	Completed, results published [20]
	NCT01023295	Polypoidal choroidal vasculopathy	Mateon Therapeutics	Completed, results not published
Oxi4503	NCT00960557	Neoplasm metastasis	Mateon Therapeutics	Completed, results not published

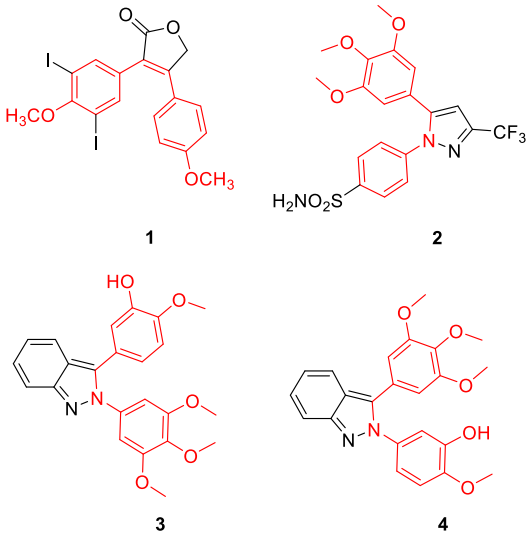

Fig. 4. Combretastatin hybrids with COX-2 inhibitors rofecoxib (**1**), celecoxib (**2**) and with the 2,3-diphenyl-2*H*-indazole scaffold (**3**,**4**). The combretastatin scaffold is indicated in red.

conversion. In cytotoxicity assays on MCF-7, S180, and HepG2 cells, the CA4 prodrugs with the longest carbon chain exhibited less cytotoxicity than the other acylated CA4 prodrugs. This aspect may be due to the easier release from the liposome and hydrolysis into the active parent drug of less lipophilic prodrugs, suggesting that the release of CA4 could significantly affect the *in vitro* cytotoxicity. When submitted to an *in vivo* study in S180 tumor-bearing mice, the long-chain acylated prodrugs showed an improved antitumor effect. This may be attributed to the longer circulation time *in vivo* of more lipophilic prodrugs. Worth of note, the stearyl derivative (C18) of CA4 exhibited higher antitumor efficacy than fosbretabulin, the ester prodrug of C4.

The most recent approaches aim at developing combretastatin prodrugs able to selectively target tumor site, possessing also theranostic properties or targeting specific tumor microenvironment. Photoremovable groups, bioreductive triggers, or chemical groups selectively targeting enzymes overexpressed in tumors have been applied to the development of novel CA4 prodrugs, leading to improved and more selective anticancer activity.

Authors from China reported the development of a photoresponsive hybrid prodrug (**9**, Fig. 6), bearing doxorubicin and CA4 covalently bonded to a photoremovable protecting group, aimed at combining the antitumor properties of two distinct compounds [40]. In this strategy, the light irradiation (365–405 nm) of the prodrug liberates bioactive drugs with a quasi-sequential release behaviour, avoiding the use of carriers, that could lead to biocompatibility problems and off target effects. Two *ortho*-nitrobenzyl functional groups were combined into an aromatic core, which was flanked by two hydrophilic side chains. CA4 was covalently linked through a carbonate group, whereas doxorubicin by a carbamate linker. The novel hybrid prodrug showed good stability and a satisfactory release of both active compounds by irradiation at proper wavelengths. In the breast cancer cell line MDA-MB-231, the prodrug exhibited significant cytotoxicity compared with doxorubicin and CA4 alone, suggesting a synergistic antitumor effect.

The synthesis of bioreductively activatable prodrug conjugates (BAPCs) represents a similar strategy to obtain a selective action of anticancer drugs in hypoxic regions of tumors [41,42]. An application of this intriguing strategy has been recently reported, with the identification of prodrug conjugates of CA4 and CA1 (compounds **10**–**11**, Fig. 7) [43].

In these conjugates, the combretastatin scaffolds are covalently linked to a bioreductive trigger (a nitrothiophene moiety) specifically

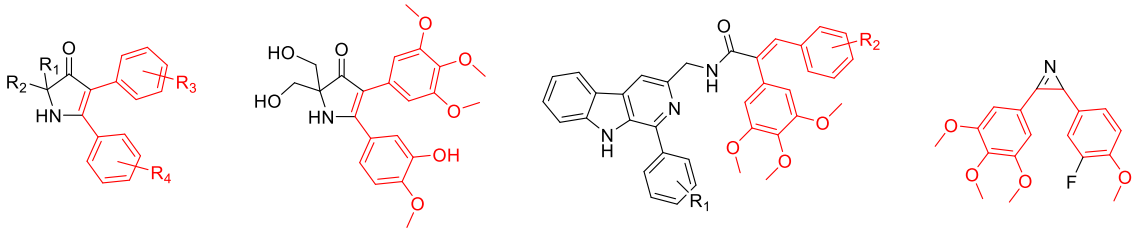

Fig. 5. Combretastatin hybrids containing the discoipyrrole C (**5**), the β-carboline (**6**) and the aziridine (**7**) scaffolds. The combretastatin scaffold is indicated in red.

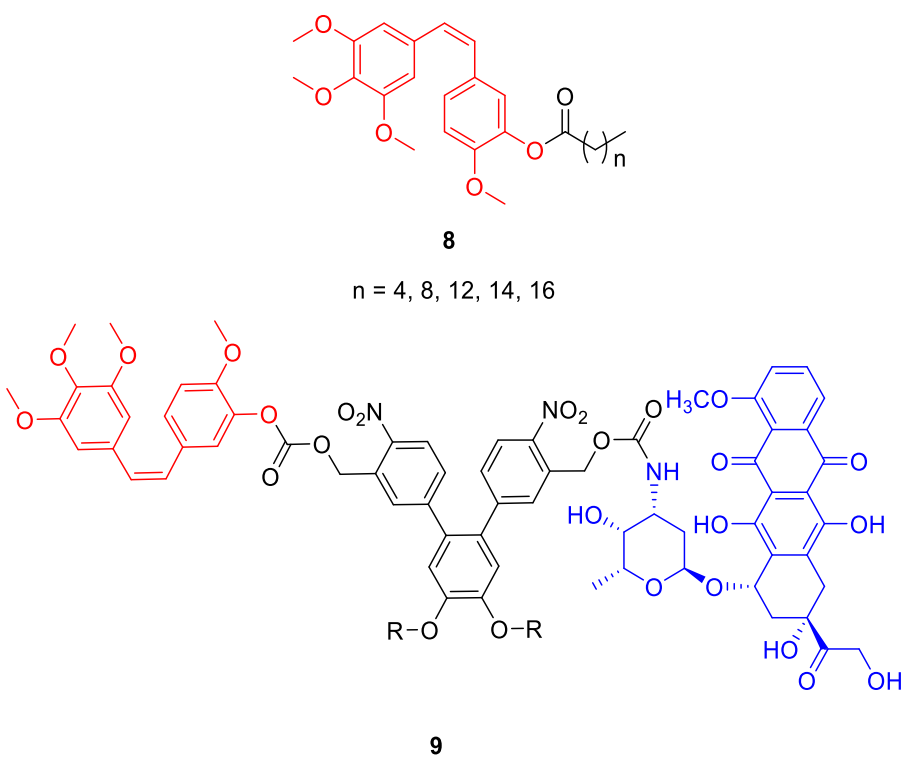

Fig. 6. Chemical structures of the acyl CA4 prodrugs (**8**) and of the photoresponsive hybrid prodrug **9**, bearing doxorubicin (blue) and CA4 (red).

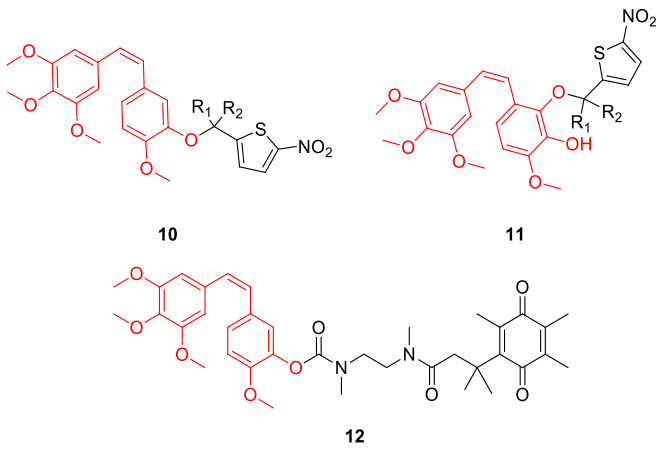

Fig. 7. Chemical structures of bioreductively activatable prodrug conjugates (BAPCs) of CA4 (**10**) and CA1 (**11**), NADPH quinone oxidoreductase 1 (NQO1) prodrug of CA4 (**12**). The CA scaffold is coloured in red.

designed to undergo enzyme-mediated cleavage under hypoxic conditions. The cytotoxicity of derivatives was tested in the A549 human cancer cell line; the gem-dimethyl CA4-BAPC (**10**) showed the greater resistance to cleavage in normoxic environments, releasing the parent anticancer agent selectively under hypoxic conditions (as evidenced by enhanced cytotoxicity). A preliminary *in vivo* study was undertaken on compound **10**, that was administered at a single dose (180 mg/kg, intraperitoneal) in orthotopic 4T1-luc breast tumors growing in the left frontal mammary fat pad of syngeneic BALB/c mice. Bioluminescence and histology experiments indicated an *in vivo* cleavage by NADPH-cytochrome P450 oxidoreductase and a subsequent vascular disruption effect by the released parent drug CA4.

The NADPH quinone oxidoreductase 1 (NQO1), a two-electron reductase responsible for the detoxification or the bioactivation of some quinones, is overexpressed in different solid tumors, and it represents a tumor-specific target in the anticancer therapy [44–46]. Prodrugs of CA4 based on NQO1-selective targeting have been proposed, consisting of three structural moieties: the parent drug CA4, a NQO1-responsive trigger group, and a self-immolating linker containing carbonate or carbamate groups [47]. The prodrug **12** (Fig. 7) was identified as the most promising derivative. The trimethyl quinone propionic acid serves as the trigger group undergoing an intramolecular cyclization following reductive activation of NQO1. Subsequently, the *N*-methylcarbamate undergoes self-cyclization, enabling the release of CA4. The cytotoxicity of novel prodrugs was tested against the NQO1-overexpressing A549 and HepG2 cells, hypoxia-exposed A549 and HepG2 cells, taxol-resistant A549 cells, normal human liver LO2 cells, and HEK293 cells. The most active and selective prodrug **12** was further evaluated for the effect on CA4 release in response to NQO1, the stability within plasma or buffers, and the antitumor effect. In HepG2 cells, **12** induced a marked apoptotic effect; in a xenograft liver tumor in mice, it determined a marked reduction of tumor size. The positive results obtained suggest that this NQO1-responsive prodrug is potentially useful for a selective tumor-targeting treatment.

Selenodiazole compounds display anticancer activity and radiosensitization effects, together with high bioavailability and general safety [48,49]. The idea of combining the antitumor potential of selenodiazoles with that of combretastatins led to the development of the combined prodrug of CA4 and azide selenodiazole (**13**, Fig. 8) [50]. Triphenylphosphine was used as a trigger to react with prodrug and achieve bioorthogonal cleavage reactions in the physiological environment. The triphenylphosphine-labile CA4-selenodiazole prodrug was synthesized by forming an ester from the hydroxy group of CA4 and the carboxylic group of the azide SeD. The obtained prodrug showed greater safety in circulating blood than CA4, and an anticancer efficacy highly superior to that of CA4. This applied prodrug strategy was found to not only be efficacious to improve the antitumor effect of CA4, but also to provide a possibility of strengthening the radiotherapeutic effect of X-rays. Seleno compounds could effectively enhance the

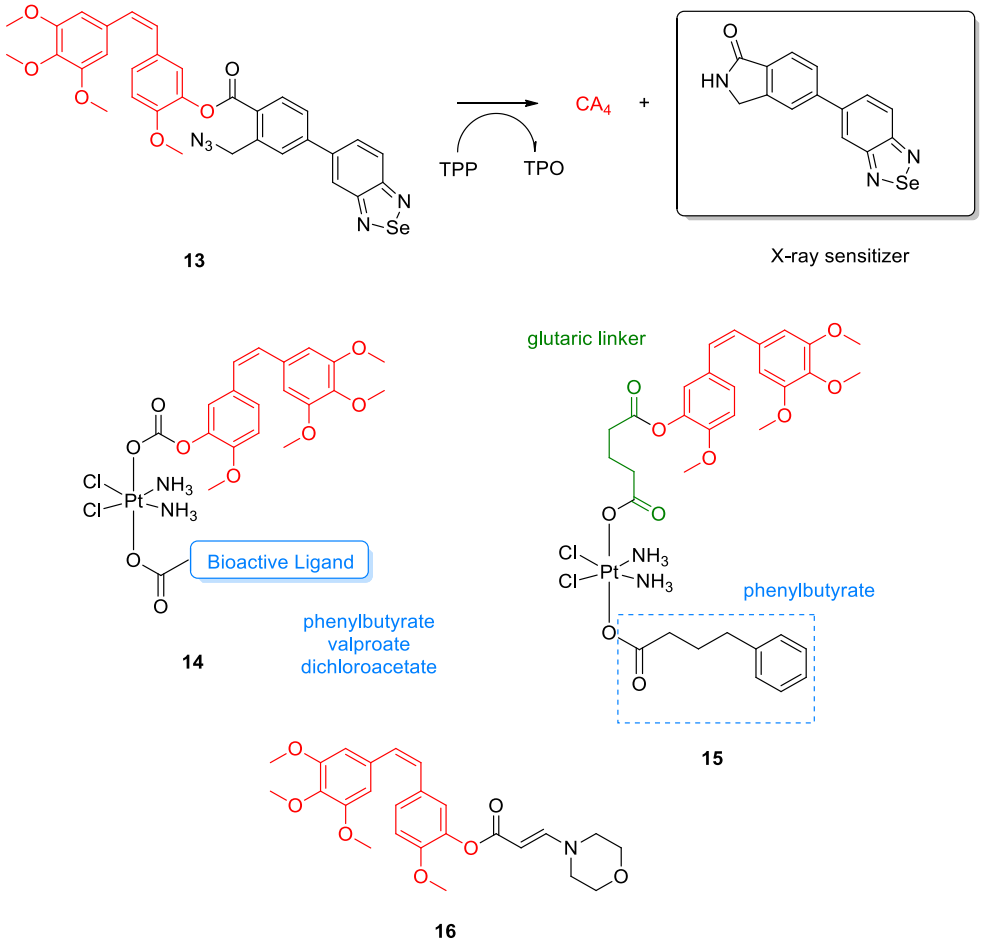

Fig. 8. Activation of the prodrug CA4-azide selenodiazole **13**, producing the CA4 and the X-ray sensitizer selenodiazole. Chemical structures of the prodrugs containing CA4, cisplatin, and other bioactive ligands (**14**, **15**), and of nonmitochondria-targeted prodrug **16**.

radio-sensitization of tumor cells for X-rays, resulting in an improved anticancer effect. As hypothesized, the prodrug **13** displayed excellent radio-sensitization properties and enhanced the inhibition effect toward cancer cell migration, invasion, and angiogenesis. This study provided a new approach for the rational design of the late-stage activation of multiple chemotherapeutic prodrugs that showed enhanced levels of bioorthogonal bioactivity for simultaneous anticancer and anti-angiogenesis therapies.

An interesting application of the prodrug strategy has been reported by a group of researchers, who synthesized Pt(IV) derivatives of cisplatin with CA4 (**14** and **15**, Fig. 8) [51]. The structure of CA4 was modified to be directly conjugated to Pt(IV), by adding linkers containing a carboxylic function so that it could be tethered to the OH of the Pt(IV). The linkers formed stable ether or ester linkages. After reduction of the Pt (IV) complex, the carbonate linker loses the CO_2, generating the bioactive molecule in its original unaltered form. Different bioactive ligands were added to these conjugates, selecting molecules with synergistic action to cisplatin, in the attempt to form triple-action prodrugs. The histone deacetylase (HDAC) inhibitors phenylbutyrate and valproate as well as the pyruvate dehydrogenase kinase (PDK) inhibitor dichloroacetate were added to form these novel prodrugs. The synthesized prodrugs were found relatively stable outside the cells, whereas underwent rapid activation inside the cells. They were tested for their antiproliferative activity on a panel of different cancer cells, both sensitive and resistant to cisplatin: the obtained IC_{50} were very similar to that of CA4, indicating that the cytotoxicity is probably due to CA4, and not to cisplatin. The results of these experiments suggest that these compounds are not multifunction cytotoxic agents, but rather prodrugs of CA4, able to effectively deliver it to cancer cells. Preliminary *in vivo* studies were carried out in Lewis lung carcinoma mice, showing an enhanced tumor growth inhibition and reduction of the acute toxicity (measured as body weight loss) compared to CA4. The authors of this study suppose that the main role of Pt(IV) is to act as a self-immolative carrier, that is activated by reduction inside the cancer cells, contributing to improve the CA4 activity and reduce its toxicity.

Nonmitochondria-targeted prodrugs of some anticancer drugs with different mechanisms of action have been proposed by American researchers [52]. CA4 was included in this group of compounds, and a prodrug (**16**, Fig. 8) was created by adding to the cytotoxic molecule a small protective group and a singlet oxygen (SO)-cleavable linker, in the attempt to minimize the increase of molecular weight and lipophilicity. These prodrugs are activated by SO, generated from visible or near IR light. The authors of this study hypothesized that it is not necessary to target mitochondria to potentiate the cytotoxic activity of drugs. The efficacy of these nonmitochondria-targeted prodrugs was assessed in monolayer cell cultures and in 3D spheroids; the prodrugs produced an enhanced cytotoxic effect when added to protoporphyrin IX-photodynamic therapy, also if high concentrations were needed. Results from these experiments suggest that the newly synthesized prodrugs could be used to enhance the effects of photosensitizers in photodynamic therapy when they are not localized or generated in mitochondria.

In addition to the anticancer field, the prodrug strategy was applied to combretastatins to obtain antiviral effects. The beneficial antiviral activity shown by inhibitors of tubulin polymerization against flaviviruses as Zika and Dengue virus (DENV) inspired the synthesis of

combretastatin and colchicine prodrugs, in the attempt to avoid the systemic cytotoxicity and deliver the effective drugs in target cells (as monocytes and hepatocytes) [53]. The applied strategy is based on prodrugs activated by human carboxylesterase-1 (hCE1), a key enzyme highly expressed in target cells. While this enzyme is described able to cleave the cyclopentanol esters of phenylglycine and leucine, the structure of combretastatin was derivatized to form phenylglycine- and leucine-cyclopentyl esters (**17**) (Fig. 9). The experiments demonstrated that only leucine-based derivatives of combretastatin were subjected to hydrolysis by hCE1, whereas the phenylglycine analogues were not cleaved by the enzyme. The combretastatin derivatives were less cytotoxic, but their antiviral potential was reduced compared the parent compound. This prodrug strategy was useful to reduce toxicity, but it did not lead to an improved biological activity.

The same research group reported a similar strategy to obtain combretastatin prodrugs with antiviral activity. They synthesized combretastatin-peptide hybrids (**18**), incorporating the cleavage site of the DENV protease to allow activation of the tubulin ligand within infected cells [54]. To attach a peptide sequence that is recognized by the DENV protease, the hydroxyl group of CA4 was replaced by an amino group. The amino acid residues incorporated in the tripeptide scaffold were Arg, Lys, Gly, Ala, β-Ala, and Ser. In *vitro* experiments, the synthesized hybrids were hydrolysed by the DENV protease at a rate comparable to its natural substrates. Both prodrugs and metabolites showed antiviral activity against DENV and Zika virus in hepatocyte-derived cells at sub-cytotoxic concentrations. However, this study did not provide clear relationships between prodrug activation and antiviral efficacy, and the therapeutic index of best compounds was too low to consider a further preclinical development.

4. Combretastatins in combination treatments

The antitumor efficacy of combretastatins inspired the researchers to realize drug combinations with other anticancer agents, to maximize the antitumor effects by a synergistic action. Through nanotechnological approaches, CA4 derivatives were synthesized and tested in combination with some antitumor agents, obtaining excellent results in breast and hepatic tumor models (Fig. 10).

A poly(l-glutamic acid)-CA4 conjugate (PLG-CA4) nanomedicine was developed by a group of Chinese authors to improve the potential of CA4 on tumor therapy [55]. This nanomedicine was mainly distributed around the tumor vessels, due to its low tissue penetration in solid tumor. CA4 nanoparticles (NPs) have been recently proposed as efficient tool to elevate hypoxia levels selectively in tumors, enhancing the antitumor efficacy of hypoxia-activated prodrugs [56]. To test this hypothesis, a combination of tirapazamine (a hypoxia-activated prodrug) plus CA4-NPs was used to treat highly metastatic 4T1 breast carcinoma. The CA4-NPs were successful to selectively destroy blood vessels and potentiate hypoxia inside tumors; this improved the therapeutic and antimetastatic effects of tirapazamine in the metastatic 4T1 mammary adenocarcinoma model. The combination of tirapazamine and CA4-NPs showed superior antitumor efficacy over the two monotherapies; in addition, this drug combination was able to completely suppress moderate (about 180 mm^3), but also large tumors (about 500 mm^3) with distant metastasis. These results appear of great interest, deserving further investigations of combination between hypoxia-activated prodrugs and CA4 nanomedicines for the treatment of other solid tumors.

A similar approach was described in a recent study, in which CA4-NPs were used to improve the hypoxia and facilitate the antitumor action of hypoxia-activated prodrugs. A hypoxia-activated prodrug of 6-diazo-5-oxo-L-norleucine (a potent glutamine antagonist) was developed as anticancer agent, showing improved therapeutic efficacy and tumor selectivity [57]. The combination of the glutamine antagonist prodrug HDON and CA4-NPs induced a synergistic anticancer effect, with the selective disruption of tumor blood vessels, the increased degree of hypoxia, and an increased expression of nitroreductases in the tumor microenvironment. The antitumor efficacy of HDON and CA4-NPs was assessed in MC38 murine colon cancer and 4T1 murine breast cancer models, achieving significant tumor suppression rates.

A combined treatment consisting of CA4 and an anti-PD-L1 agent was explored by Chinese researchers, who proposed a nanotechnological formulation of CA4 [58]. Nanoparticles of poly(l-aspartic acid)-poly(ethylene glycol)/combretastatin A4 were realized to improve the water solubility and prolong the half-life of CA4. This system offers the advantage of long-term circulation and drug accumulation around the tumor site. The active drug is gradually released from the nanocarriers in a controlled way, ensuring a constant drug concentration in the tumor. This nanodelivery system constructed by chemical bonding results more stable than physical enwrapping and may be sensitive to tumor acidic environment, resulting in a controlled release of the drug. In this study, the authors evaluated the use of CA4 nanoparticles in combination with an anti-PD-L1 antibody, to prevent immunosuppression related to severe hypoxic conditions, that could determine a high expression of hypoxia-inducible factor 1-alpha (HIF-1α) and the overexpression of PD-L1 [59]. Whereas CA4 nanoparticles arrest the tumor by disrupting the established central vasculature, the programmed death-ligand 1 (PD-L1) inhibitor can reactivate the T-cell immune response in the tumor microenvironment. This combination strategy was studied in subcutaneous Hepa1-6 hepatic tumor models on female C57BL/6 mice. Results from these *in vivo* experiments evidenced a marked anticancer effect of the combination strategy, confirming the potential value in the therapy of hepatocellular carcinoma.

The co-administration of CA4 and a multikinase inhibitor has been proposed as a powerful therapeutic strategy against hepatocellular carcinoma [60]. The authors of this study constructed a polymeric CA4 derivative [poly(L-glutamic acid)-graft-methoxy poly(ethylene glycol)/combretastatin A4, PLG-CA4], able to concentrate itself around tumor vessels after intravenous injection. Its low tissue penetration ability in solid tumors was improved by realizing a water-soluble form, consisting of polymeric CA4 sodium salt nanoparticles. This novel CA4 formulation was evaluated in subcutaneous and orthotopic H22 hepatic tumor models, where a significant disruption of tumor blood vessels was

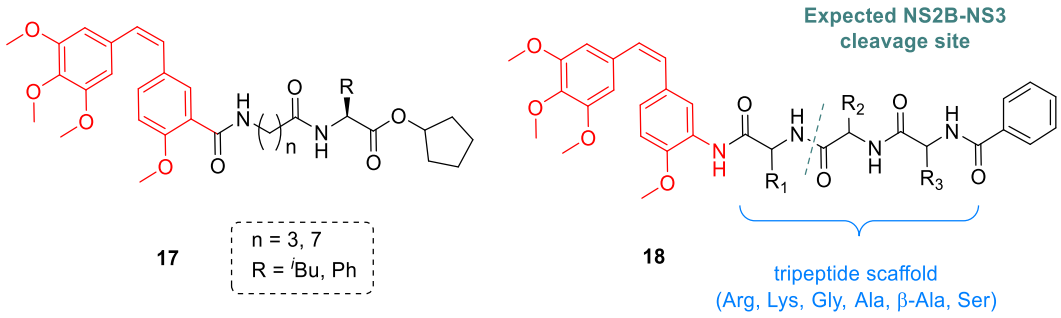

Fig. 9. Antiviral combretastatin prodrugs containing peptide scaffolds.

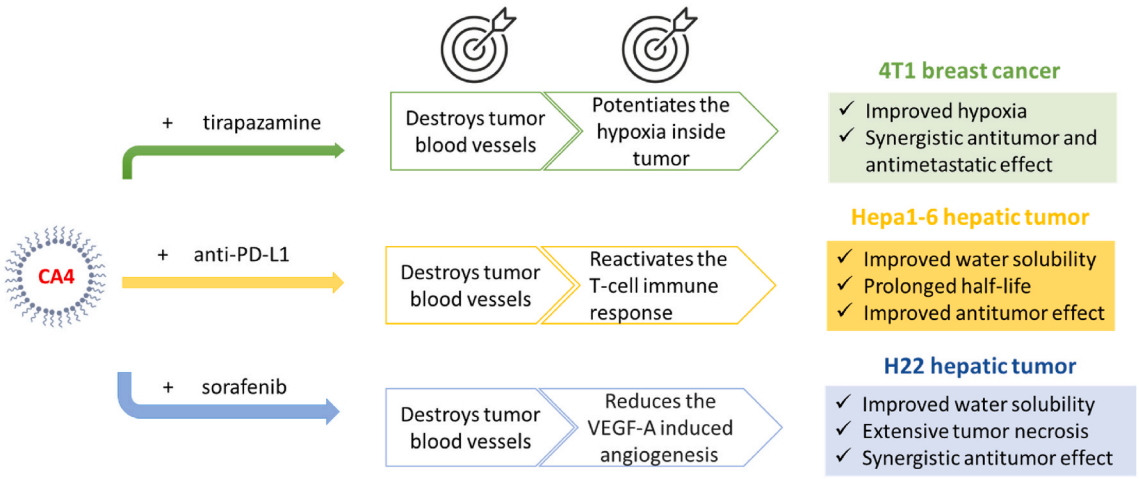

Fig. 10. Combination approaches of CA4 nanoparticles with tirapazamine, anti-PD-L1 antibody, and sorafenib improve the antitumor efficacy of CA4 through synergistic mechanisms of action.

observed. Together with beneficial effects, as the extensive tumor necrosis and the inhibited of tumor growth, an increased expression of vascular endothelial growth factor A (VEGF-A) was found. To overcome this problem, the authors of the study proposed the combined use of the polymeric CA4 nanoparticles with sorafenib, a well-established anticancer agent targeting VEGF-A. The combination of sorafenib (30 mg/kg) + CA4-NPs (30 mg/kg) determined an over 90 % tumor suppression rate in a hepatic H22 subcutaneous tumor model with low systemic toxicity. This cooperative mechanism of CA4-NPs plus sorafenib showed the potential to completely eradicate the whole tumor, suggesting that this synergistic approach is highly promising for the systemic treatment of hepatocellular carcinoma.

Novel therapeutic strategies are under investigation in the attempt to elicit the efficacy of chemotherapy, by eradicating tumor cells at the edge and central regions of a solid tumor. To this aim, a group of researchers demonstrated the synergic antitumor efficacy of a combined treatment consisting of the vascular disrupting agent CA4P and nanoparticle albumin-bound paclitaxel (nab-paclitaxel) in Walker 256 rat breast carcinoma cells [61]. The whole-body biodistribution and the intratumor uptake of paclitaxel were evaluated, with results showing that CA4P can improve the accumulation of nab-paclitaxel. The combined treatment of nab-paclitaxel and CA4P in xenograft model induced a stronger tumor growth inhibition than individual treatments. The improved efficacy of the co-administration may be attributed to the increased nab-paclitaxel retention, due to the trapping effect of CA4P within the tumor core. A further advantage of this co-treatment is that it is a multi-targeting strategy, as nab-paclitaxel acts on the proliferating cells at the tumor periphery, while CA4P targets endothelial cells, inducing necrosis at the core of the tumor. Overall, the results of this study hold great promise for a further evaluation of this combined treatment in chemotherapy.

In addition to CA4 nanoparticles combined with anticancer agents, other interesting combination strategies have been described, suggesting that multiple treatments based on combretastatins can improve classical or innovative anticancer therapies. Researchers from China proposed an innovative co-treatment combining CA4P plus chimeric antigen receptor T (CAR-T) therapy, with the aim of promoting the infiltration of CAR-T cells into solid tumors, enhancing the therapeutic efficiency of the treatment [62]. The combination of CAR-T cells that specifically target HER2 with CA4P was administered in mice transplanted with SKOV3 (HER2+) ovarian cancer cells; results showed an enhanced ability to suppress the growth of SKOV3 tumor cells in a cell-derived xenograft model. The combination treatment was also tested in HER2+ colorectal cancer and a patient-derived xenotransplantation model, obtaining positive results in terms of destruction of tumor vessels, promotion of T cell infiltration, and decreased expression of the HER2 antigen. Results from this study showed that CA4P effectively promotes CAR-T cell infiltration in *vivo* solid tumor models, producing a significant improvement of the antitumor ability of CAR-T cells targeting different solid tumors.

A novel combination approach was proposed against melanoma by the simultaneous inhibition of tumor vascularization and enhancement of macrophage-mediated antitumor responses [63]. To this aim, CA4 and the immunostimulatory adjuvant poly(I:C) (PIC) were co-delivered through a formulation based on an injectable metal-organic framework hydrogel. PIC is a synthetic double-stranded RNA molecule able to induce innate immune responses, by modulating tumor-associated macrophages (TAMs) towards an anti-tumor M1 phenotype. When administered in a subcutaneous melanoma model in mice, the combination produced a synergistic action, by inhibiting angiogenesis and disrupting tumor blood vessels. The simultaneous promotion of M1 macrophage infiltration and disruption of tumor vascularization led to a greater antimelanoma effect, proposing this new combination therapy as an innovative option in the immunotherapy of melanoma.

Another recent study examined whether the combination of immunotherapy and the antivascular agent CA4P will succeed in murine B16–F10 melanoma and triple-negative 4T1 breast tumor models [64]. Mice were treated with the stimulator of interferon genes (STING) pathway agonist cyclic GMP-AMP (cGAMP) and CA4P; in the group treated with the combination a significant therapeutic effect was obtained against 4T1 breast cancer, related to the tumor microenvironment polarization and the stimulation of the innate immune response. However, CA4P did not provide an additive antitumor effect in B16–F10 melanoma model, producing the same effect on growth inhibition obtained with the administration of only cGAMP. The authors underline that the tumor microenvironment plays a crucial role in the outcome of therapy in two tumor models analysed in this study.

A co-delivery system based on CA4P and cisplatin was developed for the local treatment of colon cancer [65]. The antitumor efficacy and safety of this novel drug co-loaded gel depot were investigated in a murine C26 xenograft model. Results obtained indicate that the gel depot enhances the tumor vascular disrupting effects through the sustained release of CA4P. In addition, the co-administration of CA4P and cisplatin led to the highest antitumor efficacy in the C26 bearing mice, after a single peritumoral injection. Results from this study confirmed the wide applicability of the biocompatible hydrogel and its suitability for biomedical application, holding potential for an enhanced local treatment of colon cancer.

The same combination therapy, studied by Dai et al. on human osteosarcoma-xenografted mice [66], demonstrated a significant tumor

growth inhibition and reduction of lung metastasis, compared with the monotherapy drug groups. Interestingly, the combined administration of CA4P and cisplatin led to synergistic effects at lower concentrations, promoting apoptosis and necrosis, inhibiting proliferation of osteosarcoma cells, without an increase of the systemic toxicity of chemotherapy.

Li et al. reported an interesting application of an anticancer treatment combining CA4 and a mild photothermal therapy (PTT) [67]. The authors realized a nanoplatform in which the CA4 was integrated into the protamine sulfate functionalized nanodiamonds hybrids; the near-infrared (NIR) laser irradiation was used to trigger the release of CA4 in Hep-G2 cells. In HepG-2 tumor-bearing mice, a highly synergistic anticancer efficacy was obtained by co-administration of nanodiamonds and exposure to NIR laser irradiation, leading to an enhanced photocytotoxicity.

The careful management of cardiotoxicity exerted by some anticancer drugs is an important problem to be solved. Various cardiotoxic effects could arise after administration of anticancer drugs, as heart failure, ischemic heart disease, hypertension, and other cardiac conditions [68,69]. The risk of cardiac injury has been reported from clinical trials of CA4P [70], and the bromodomain-containing protein 4 (BRD4) has been reported to be involved in the progression of heart failure [71]. A recent study performed by Japanese researchers focalized into the reduction of cardiotoxicity induced by CA4P in a combined treatment with the BRD4 inhibitor JQ1 [72]. The authors of this study selected a canine mammary carcinoma cell line (CHMp-13a) and tested the combined treatment CA4P + JQ1 *in vitro* and *in vivo*, using a mouse xenograft model. In the *in vitro* experiments, both compounds inhibited cell proliferation in a concentration-dependent way when added alone, and they exerted a synergistic effect when co-administered. In the xenograft model, a stronger inhibition of tumor growth was observed in the CA4P + JQ1 group compared to single treatments, suggesting that the enhanced effect of the combined use detected *in vitro* was reproduced *in vivo*. Moreover, the cardiotoxic effects were attenuated after the combined treatment, suggesting that the co-administration of CA4P and the BRD4 inhibitor allows to reach a security margin to maximize the distance between the effective concentration and cardiotoxic one.

5. Combretastatins as payloads in ADCs

Antibody-drug conjugates (ADCs) represent a new class of highly promising therapeutics for cancer treatment [73,74]. In the last years, a marked increase on the realization of these conjugates was observed, that led to the FDA approval of different ADCs and the clinical development for many others [75]. The clinical success of an ADC to address potent anticancer effects in a specific district is strongly related to the performance of their three components: the monoclonal antibody (mAb), the linker, and the cytotoxic payload. Research efforts were paid to the improvement of these components, and great success was obtained by a careful selection of the type of antibody, the proper linker, and the potent cytotoxic payload. Anyway, some problems related to pharmacokinetics and safety of ADCs remain to be solved, and the research is very active in this field of investigation, proposing technological solutions and next-generation ADCs [76–78].

To date, 13 ADCs have been approved by the FDA for their use in clinical oncology, with indication in leukemia, metastatic breast cancer, lymphoma, multiple myeloma, head and neck, gastric, lung, bladder, cervical, and ovarian cancer [79].

The choice of cytotoxic payloads in ADCs is a critical point, in the attempt to address a potent cytotoxic action, avoiding an uncontrolled general toxicity, ensuring low immunogenicity and high stability. For these reasons, large groups of cytotoxic compounds were explored, realizing conjugates containing natural compounds or their derivatives acting as tubulin inhibitors and DNA damaging agents. However, many of the traditional ADC payloads have important disadvantages and limitations, as inadequate efficacy, and the development of acquired drug resistance. To overcome these issues, novel payloads acting against different targets and endowed with reduced side effects are under development, as RNA inhibitors, immuno-agonists, apoptosis-promoting Bcl-xL inhibitors, PROTACs and others [80,81].

The use of CA4 as payload of ADC was proposed by Huang et al. [82]. They realized three different ADCs comprising the mAb cetuximab, targeting the epidermal growth factor receptor (EGFR), approved by the FDA in 2004 (Erbitux®) for the treatment of colorectal cancer. This chimeric monoclonal immunoglobulin G1 (IgG1) antibody targets and antagonizes EGF activity, resulting in the induction of apoptosis and the inhibition of cancer cell proliferation. The payload CA4 was connected to the mAb through three different linkers (one non-cleavable and two protease-cleavable, containing the sequence Val-Cit and Gly-Gly-Phe-Gly, respectively), whereas a divinylsulfonamide portion was used for the site-selective modification of antibody through disulfide re-bridging approach (Fig. 11). Three ACDs retained binding specificity, potency, and internalization ability comparable to that of cetuximab. The ADCs with cleavable linkers exhibited potent antiproliferative activities in a panel of lung and breast cancer cell lines. The *in vivo* antitumor activity of three ADCs was tested against NCI–H1975-GFP derived tumor xenograft model; all the derivatives strongly decreased tumor volume, without inducing significant changes of body weight in mice. This study demonstrates that CA4 is a viable payload option for ADC research, opening the way to the development of novel biological drugs containing combretastatins.

CA4 and cetuximab were used to obtain ADCs also by another research group, which combined the payloads CA4 and doxorubicin to cetuximab through a novel linker based on the 1–6 self-immolative *p*-hydroxybenzyl alcohol platform [83]. The pH-sensitive HMPO linker (5-(hydroxymethyl)pyrogallol orthoester derivative), obtained by chemical modification of gallic acid, resulted stable into biological fluids as blood, whereas hydrolysis at pH 5.5 determined a rapid release of drugs, confirming the validity of this approach for a specific delivery of the cytotoxic payload. The resulting ADCs exhibited improved cytotoxic activity in A431 and A549 cancer cells compared to the unconjugated antibody (Fig. 12).

A recent work reports on the use of a self-immolative phenoxysilyl linker, formed by the direct reaction of the phenolic group of CA4 with dichlorodiisopropylsilane, used to connect the CA4 to atezolizumab to construct a novel ADC (Fig. 12) [84]. The use of this linker allowed an increased conjugation efficiency and valuable improvements in the ADC action, probably related to a better penetration in target cells. The newly synthesized ADC exhibited good antiproliferative activity in PD-L1 positive cells (breast cancer MDA-MB-231, glioblastoma U-87, non-small-cell lung cancer Calu-1) displaying nanomolar IC_{50}s. Its superior efficacy compared to the atezolizumab-CA4 ADC with a non-cleavable linker is attributed to the linker features, that allow an improved and targeted release of the cytotoxic payload.

5.1. Conclusions and perspectives

Historically, plants and living organisms have represented precious sources of bioactive molecules, useful for the treatment of various diseases. Stilbenes, flavonoids, terpenoids, and combretastatins are interesting examples of bioactive compounds applied to the anticancer research, with a wide library of synthetic derivatives endowed with improved pharmacokinetic properties [85–88].

In this landscape, several medicinal chemist's efforts were directed towards the enhancement of tumor targeting of combretastatins, applying different strategies finalized at their selective delivery in the tumor microenvironment. The lack of selectivity in the cytotoxic action of CA4 represents a significant problem in chemotherapy, as witnessed by the discontinuation of clinical trials by CA4 derivatives. In this context, the most significant enhancements proposed in the last years see the application of systems, allowing a selective targeting of CA4 in specific tumor sites, encompassing the general toxicity associated with

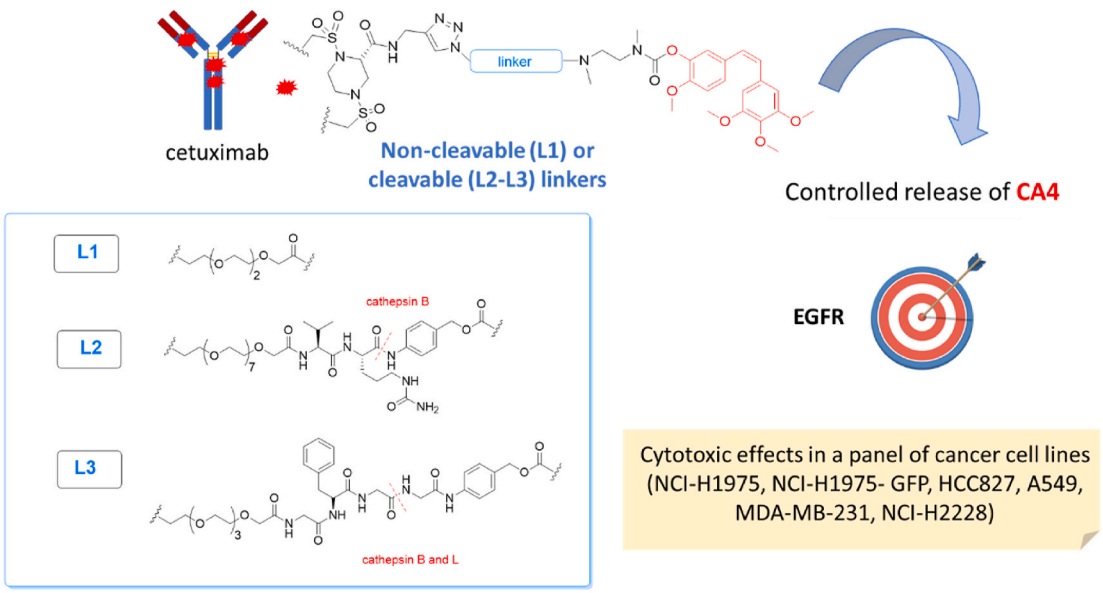

Fig. 11. The three novel ADCs targeting EGFR. They were synthesized connecting cetuximab to CA4 through a divinylsulfonamide portion and different linkers (non-cleavable or cleavable by proteases).

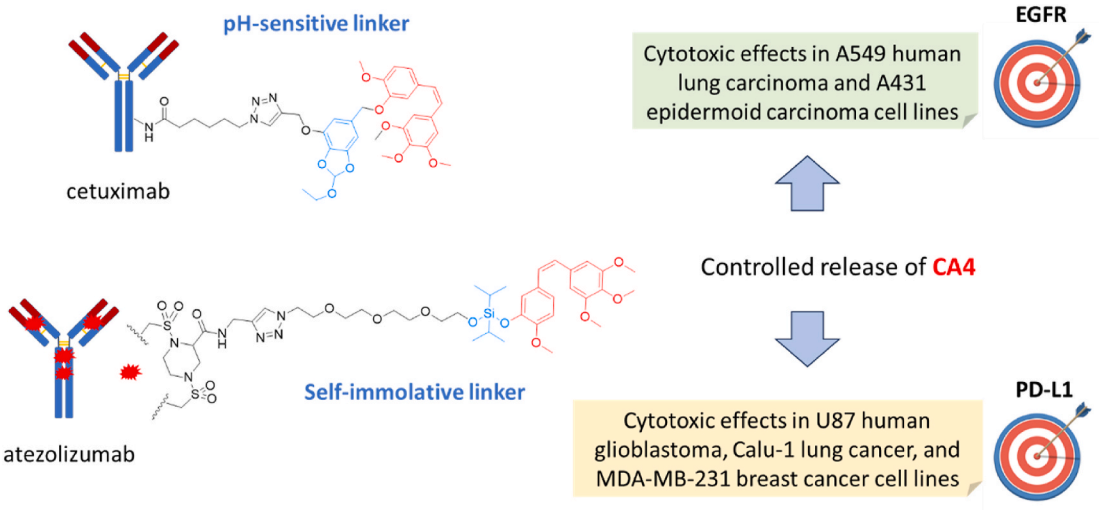

Fig. 12. Novel CA4-based ADCs targeting EGFR and PD-L1. The cetuximab was connected to CA4 through a pH-sensitive linker, whereas a self-immolative phenoxysilyl linker connected atezolizumab to the CA4 payload. Both the ADCs showed improved cytotoxic properties in cancer cells overexpressing the targets EGFR and PD-L1.

their use. CA4 prodrugs were created, able to release the cytotoxic agent in the tumor microenvironment, exploiting the tumor lower pH and hypoxia. Photoresponsive prodrugs and bioreductively activatable prodrugs represent interesting innovation in the therapeutic use of CA4, encompassing selectivity and toxicity issues.

The combination of combretastatins with other antitumor agents led to improved efficacy and tumor-targeting properties: CA4 nanoparticles with tirapazamine, anti-PD-L1 antibodies, and sorafenib determined an improved antitumor efficacy through synergistic mechanisms of action.

Combretastatins were also extensively studied in combination treatments with other anticancer agents, as cisplatin, including the innovative CAR-T approach and photothermal therapy. These studies hold great promises, deserving further evaluation in multiple cancer models, to widen the application field.

A recent and modern application of medicinal chemistry strategies is represented by the construction of ADCs based on CA4 as payload. This represents an exciting and promising approach, in the attempt to maximize the cytotoxicity of CA4 with a tumor-selective delivery, exploiting the high specificity of monoclonal antibodies. Three studies to date describe the construction of CA4-based ADCs, linked to cetuximab or atezolizumab through cleavable or non-cleavable linkers. Despite these conjugates require further biological evaluation, the idea to use CA4 as potent cytotoxic agent is a promising strategy. Preliminary results authorize a positive feeling for this novel class of CA4-based tumor-selective compounds as novel agents to treat tumors overexpressing EGFR and PD-L1.

Overall, the potent antivascular activity of CA4 and its selective delivery to tumors have greatly improved the anticancer efficacy of this drug. Future research should focus on enhancing the delivery of the treatment, with the aim of reducing off-target effects and systemic toxicity. The combined treatments of CA4 with other anticancer drugs sound really promising, with the possibility to achieve even more successful results in tumors through a therapy highly focused on specific features of cancer.

CRediT authorship contribution statement

Marialuigia Fantacuzzi: Writing – review & editing, Writing – original draft, Investigation, Data curation. **Simone Carradori:** Writing – review & editing, Supervision, Formal analysis, Data curation. **Letizia Giampietro:** Investigation, Data curation. **Cristina Maccallini:** Investigation, Data curation. **Barbara De Filippis:** Investigation, Data curation. **Rosa Amoroso:** Supervision, Formal analysis. **Alessandra Ammazzalorso:** Writing – review & editing, Writing – original draft, Supervision, Investigation, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Abbreviations

ADC	antibody-drug conjugate
AML	acute myeloid leukemia
BACPs	bioreductively activatable prodrug conjugates
BRD4	bromodomain-containing protein 4
CA1	combretastatin A-1
CA4	combretastatin A-4
CA4-NPs	combretastatin A-4 nanoparticles
CA4P	combretastatin A-4 phosphate
CAR-T	chimeric antigen receptor T
CBS	colchicine binding site
cGAMP	cyclic GMP-AMP
COX-2	cyclooxygenase-2
DENV	Dengue virus
EGFR	epidermal growth factor receptor
hCE1	human carboxylesterase-1
HDAC	histone deacetylase
HIF-1α	hypoxia-inducible factor 1-alpha
HMPO	5-(hydroxymethyl)pyrogallol orthoester
IgG1	immunoglobulin G1
mAb	monoclonal antibody
nab-paclitaxel	nanoparticle albumin-bound paclitaxel
NIR	near-infrared
NQO1	NADPH quinone oxidoreductase 1
NSAID	non-steroidal anti-inflammatory drug
PCV	polypoidal choroidal vasculopathy
PDK	pyruvate dehydrogenase kinase
PD-L1	programmed death-ligand 1
PGE_2	prostaglandin E_2
PIC	poly(I:C)
PLG-CA4	poly(l-glutamic acid)-CA4 conjugate
PTT	photothermal therapy
SO	singlet oxygen
STING	stimulator of interferon genes
TAMs	tumor-associated macrophages
VDA	vascular disrupting agent
VEGF-A	vascular endothelial growth factor A

Data availability

No data was used for the research described in the article.

References

[1] G.R. Pettit, S.B. Singh, M.L. Niven, E. Hamel, J.M. Schmidt, Isolation, structure, and synthesis of combretastatins A-1 and B-1, potent new inhibitors of microtubule assembly, derived from Combretum caffrum, J. Nat. Prod. 50 (1) (1987 Jan-Feb) 119–131, https://doi.org/10.1021/np50049a016.

[2] G.R. Pettit, S.B. Singh, J.M. Schmidt, M.L. Niven, E. Hamel, C.M. Lin, Isolation, structure, synthesis, and antimitotic properties of combretastatins B-3 and B-4 from Combretum caffrum, J. Nat. Prod. 51 (3) (1988 May-Jun) 517–527, https://doi.org/10.1021/np50057a011.

[3] G.R. Pettit, S.B. Singh, E. Hamel, C.M. Lin, D.S. Alberts, D. Garcia-Kendall, Isolation and structure of the strong cell growth and tubulin inhibitor combretastatin A-4, Experientia 45 (2) (1989 Feb 15) 209–211, https://doi.org/10.1007/BF01954881.

[4] C.M. Lin, H.H. Ho, G.R. Pettit, E. Hamel, Antimitotic natural products combretastatin A-4 and combretastatin A-2: studies on the mechanism of their inhibition of the binding of colchicine to tubulin, Biochemistry 28 (17) (1989 Aug 22) 6984–6991, https://doi.org/10.1021/bi00443a031.

[5] R. Gaspari, A.E. Prota, K. Bargsten, A. Cavalli, M.O. Steinmetz, Structural basis of cis- and trans-combretastatin binding to tubulin, Chem 2 (1) (2017) 102–113, https://doi.org/10.1016/j.chempr.2016.12.005.

[6] C.M. Lin, S.B. Singh, P.S. Chu, R.O. Dempcy, J.M. Schmidt, G.R. Pettit, E. Hamel, Interactions of tubulin with potent natural and synthetic analogs of the antimitotic agent combretastatin: a structure-activity study, Mol. Pharmacol. 34 (2) (1988 Aug) 200–208.

[7] G.G. Dark, S.A. Hill, V.E. Prise, G.M. Tozer, G.R. Pettit, D.J. Chaplin, Combretastatin A-4, an agent that displays potent and selective toxicity toward tumor vasculature, Cancer Res. 57 (10) (1997 May 15) 1829–1834.

[8] M. Su, J. Huang, S. Liu, Y. Xiao, X. Qin, J. Liu, C. Pi, T. Luo, J. Li, X. Chen, Z. Luo, The anti-angiogenic effect and novel mechanisms of action of Combretastatin A-4, Sci. Rep. 6 (2016 Jun 24) 28139, https://doi.org/10.1038/srep28139.

[9] G.Ş. Karatoprak, E. Küpeli Akkol, Y. Genç, H. Bardakci, Ç. Yücel, E. Sobarzo-Sánchez, Combretastatins: an overview of structure, probable mechanisms of action and potential applications, Molecules 25 (11) (2020 May 31) 2560, https://doi.org/10.3390/molecules25112560.

[10] E.C. McLoughlin, N.M. O'Boyle, Colchicine-binding site inhibitors from chemistry to clinic: a review, Pharmaceuticals 13 (1) (2020 Jan 3) 8, https://doi.org/10.3390/ph13010008.

[11] A. Ammazzalorso, T.V. Hansen, Combretastatin derivatives as tubulin inhibitors: a fascinating journey from nature to drug discovery strategies, Flavonoids and Phenolics Medicinal Chemistry Lessons From Nature 1 (2022) 112, https://doi.org/10.2174/9789815079098122010007.

[12] M. Mustafa, S. Anwar, F. Elgamal, E.R. Ahmed, O.M. Aly, Potent combretastatin A-4 analogs containing 1,2,4-triazole: synthesis, antiproliferative, anti-tubulin activity, and docking study, Eur. J. Med. Chem. 183 (2019 Dec 1) 111697, https://doi.org/10.1016/j.ejmech.2019.111697.

[13] F. Yang, X.E. Jian, P.C. Diao, X.S. Huo, W.W. You, P.L. Zhao, Synthesis, and biological evaluation of 3,6-diaryl-[1,2,4]triazolo[4,3-a]pyridine analogues as new potent tubulin polymerization inhibitors, Eur. J. Med. Chem. 204 (2020 Oct 15) 112625, https://doi.org/10.1016/j.ejmech.2020.112625.

[14] G.R. Pettit, C. Temple Jr., V.L. Narayanan, R. Varma, M.J. Simpson, M.R. Boyd, G. A. Rener, N. Bansal, Antineoplastic agents 322. synthesis of combretastatin A-4 prodrugs, Anti Cancer Drug Des. 10 (4) (1995 Jun) 299–309.

[15] J.A. Sosa, R. Elisei, B. Jarzab, J. Balkissoon, S.P. Lu, C. Bal, S. Marur, A. Gramza, R. B. Yosef, B. Gitlitz, B.R. Haugen, F. Ondrey, C. Lu, S.M. Karandikar, F. Khuri, L. Licitra, S.C. Remick, Randomized safety and efficacy study of fosbretabulin with paclitaxel/carboplatin against anaplastic thyroid carcinoma, Thyroid 24 (2) (2014 Feb) 232–240, https://doi.org/10.1089/thy.2013.0078.

[16] E.B. Garon, J.D. Neidhart, N.Y. Gabrail, M.R. de Oliveira, J. Balkissoon, F. Kabbinavar, A randomized Phase II trial of the tumor vascular disrupting agent CA4P (fosbretabulin tromethamine) with carboplatin, paclitaxel, and bevacizumab in advanced nonsquamous non-small-cell lung cancer, OncoTargets Ther. 9 (2016 Nov 30) 7275–7283, https://doi.org/10.2147/OTT.S109186.

[17] J. Kou, J. Liu, X. Gu, N. Liu, The efficacy of fosbretabulin disodium combined with radiofrequency ablation in lung cancer, Radiat. Res. 198 (5) (2022 Nov 1) 467–474, https://doi.org/10.1667/RADE-21-00242.1.

[18] M.A. Ibrahim, D.V. Do, Y.J. Sepah, S.M. Shah, E. Van Anden, G. Hafiz, J. K. Donahue, R. Rivers, J. Balkissoon, J.T. Handa, P.A. Campochiaro, Q.D. Nguyen, Vascular disrupting agent for neovascular age related macular degeneration: a pilot study of the safety and efficacy of intravenous combretastatin A-4 phosphate, BMC Pharmacol Toxicol 14 (2013 Jan 14) 7, https://doi.org/10.1186/2050-6511-14-7.

[19] C.J. Mooney, G. Nagaiah, P. Fu, J.K. Wasman, M.M. Cooney, P.S. Savvides, J. A. Bokar, A. Dowlati, D. Wang, S.S. Agarwala, S.M. Flick, P.H. Hartman, J.D. Ortiz, P.N. Lavertu, S.C. Remick, A phase II trial of fosbretabulin in advanced anaplastic thyroid carcinoma and correlation of baseline serum-soluble intracellular adhesion molecule-1 with outcome, Thyroid 19 (3) (2009 Mar) 233–240, https://doi.org/10.1089/thy.2008.0321.

[20] B.J. Monk, M.W. Sill, J.L. Walker, C.J. Darus, G. Sutton, K.S. Tewari, L.P. Martin, J. M. Schilder, R.L. Coleman, J. Balkissoon, C. Aghajanian, Randomized phase ii evaluation of bevacizumab versus bevacizumab plus fosbretabulin in recurrent ovarian, tubal, or peritoneal carcinoma: an nrg oncology/gynecologic oncology group study, J. Clin. Oncol. 34 (19) (2016 Jul 1) 2279–2286, https://doi.org/10.1200/JCO.2015.65.8153.

[21] R. Grisham, B. Ky, K.S. Tewari, D.J. Chaplin, J. Walker, Clinical trial experience with CA4P anticancer therapy: focus on efficacy, cardiovascular adverse events, and hypertension management, Gynecol Oncol Res Pract 5 (2018 Jan 5) 1, https://doi.org/10.1186/s40661-017-0058-5.

[22] M. Hawash, Recent advances of tubulin inhibitors targeting the colchicine binding site for cancer therapy, Biomolecules 12 (12) (2022 Dec 10) 1843, https://doi.org/10.3390/biom12121843.

[23] X. Li, X. Li, F. Liu, S. Li, D. Shi, Rational multitargeted drug design strategy from the perspective of a medicinal chemist, J. Med. Chem. 64 (15) (2021 Aug 12) 10581–10605, https://doi.org/10.1021/acs.jmedchem.1c00683.

[24] J. Zhou, X. Jiang, S. He, H. Jiang, F. Feng, W. Liu, W. Qu, H. Sun, Rational design of multitarget-directed ligands: strategies and emerging paradigms, J. Med. Chem. 62 (20) (2019 Oct 24) 8881–8914, https://doi.org/10.1021/acs.jmedchem.9b00017.
[25] V. Ivasiv, C. Albertini, A.E. Gonçalves, M. Rossi, M.L. Bolognesi, Molecular hybridization as a tool for designing multitarget drug candidates for complex diseases, Curr. Top. Med. Chem. 19 (19) (2019) 1694–1711, https://doi.org/10.2174/1568026619666190619115735.
[26] A.K. Singh, A. Kumar, H. Singh, P. Sonawane, H. Paliwal, S. Thareja, P. Pathak, M. Grishina, M. Jaremko, A.H. Emwas, J.P. Yadav, A. Verma, H. Khalilullah, P. Kumar, Concept of hybrid drugs and recent advancements in anticancer hybrids, Pharmaceuticals 15 (9) (2022 Aug 28) 1071, https://doi.org/10.3390/ph15091071.
[27] E. Sflakidou, G. Leonidis, E. Foroglou, C. Siokatas, V. Sarli, Recent advances in natural product-based hybrids as anti-cancer agents, Molecules 27 (19) (2022 Oct 6) 6632, https://doi.org/10.3390/molecules27196632.
[28] F. Gao, Z. Sun, F. Kong, J. Xiao, Artemisinin-derived hybrids and their anticancer activity, Eur. J. Med. Chem. 188 (2020 Feb 15) 112044, https://doi.org/10.1016/j.ejmech.2020.112044.
[29] M. Szumilak, A. Wiktorowska-Owczarek, A. Stanczak, Hybrid drugs-a strategy for overcoming anticancer drug resistance? Molecules 26 (9) (2021 Apr 29) 2601, https://doi.org/10.3390/molecules26092601.
[30] S.R. Punganuru, H.R. Madala, C.M. Mikelis, A. Dixit, V. Arutla, K.S. Srivenugopal, Conception, synthesis, and characterization of a rofecoxib-combretastatin hybrid drug with potent cyclooxygenase-2 (COX-2) inhibiting and microtubule disrupting activities in colon cancer cell culture and xenograft models, Oncotarget 9 (40) (2018 May 25) 26109–26129, https://doi.org/10.18632/oncotarget.25450.
[31] E.R. Rayburn, S.J. Ezell, R. Zhang, Anti-inflammatory agents for cancer therapy, Mol. Cell. Pharmacol. 1 (1) (2009) 29–43, https://doi.org/10.4255/mcpharmacol.09.05.
[32] J. Sheng, H. Sun, F.B. Yu, B. Li, Y. Zhang, Y.T. Zhu, The role of cyclooxygenase-2 in colorectal cancer, Int. J. Med. Sci. 17 (8) (2020 Apr 27) 1095–1101, https://doi.org/10.7150/ijms.44439.
[33] T.H.N. Thi, Y.T. Thi, L.A. Nguyen, N.B. Vo, Q.A. Ngo, Design, synthesis and biological activities of new pyrazole derivatives possessing both coxib and combretastatins pharmacophores, Chem. Biodivers. 16 (6) (2019 Jun) e1900108, https://doi.org/10.1002/cbdv.201900108.
[34] Q.A. Ngo, T.H.N. Thi, M.Q. Pham, D. Delfino, T.T. Do, Antiproliferative and antiinflammatory coxib-combretastatin hybrids suppress cell cycle progression and induce apoptosis of MCF7 breast cancer cells, Mol. Divers. 25 (4) (2021 Nov) 2307–2319, https://doi.org/10.1007/s11030-020-10121-2.
[35] J. Pérez-Villanueva, F. Matadamas-Martínez, L. Yépez-Mulia, V. Pérez-Koldenkova, M. Leyte-Lugo, K. Rodríguez-Villar, F. Cortés-Benítez, A.P. Macías-Jiménez, I. González-Sánchez, A. Romero-Velásquez, J.F. Palacios-Espinosa, O. Soria-Arteche, Synthesis and cytotoxic activity of combretastatin a-4 and 2,3-diphenyl-2H-indazole hybrids, Pharmaceuticals 14 (8) (2021 Aug 19) 815, https://doi.org/10.3390/ph14080815.
[36] J. Kurian, A. Ashtam, A. Kesavan, S.V. Chaluvalappil, D. Panda, M.K. Manheri, Hybridization of the pharmacophoric features of discoipyrrole c and combretastatin A-4 toward new anticancer leads, ChemMedChem 18 (16) (2023 Aug 15) e202300081, https://doi.org/10.1002/cmdc.202300081.
[37] C. Jadala, M. Sathish, T.S. Reddy, V.G. Reddy, R. Tokala, S.K. Bhargava, N. Shankaraiah, N. Nagesh, A. Kamal, Synthesis and in vitro cytotoxicity evaluation of β-carboline-combretastatin carboxamides as apoptosis inducing agents: DNA intercalation and topoisomerase-II inhibition, Bioorg. Med. Chem. 27 (15) (2019 Aug 1) 3285–3298, https://doi.org/10.1016/j.bmc.2019.06.007.
[38] S. Lin, Y. Liang, J. Cheng, F. Pan, Y. Wang, Novel diaryl-2H-azirines: antitumor hybrids for dual-targeting tubulin and DNA, Eur. J. Med. Chem. 214 (2021 Mar 15) 113256, https://doi.org/10.1016/j.ejmech.2021.113256.
[39] Y. Gu, J. Ma, Z. Fu, Y. Xu, B. Gao, J. Yao, W. Xu, K. Chu, J. Chen, Development of novel liposome-encapsulated combretastatin A4 acylated derivatives: prodrug approach for improving antitumor efficacy, Int. J. Nanomed. 14 (2019 Nov 7) 8805–8818, https://doi.org/10.2147/IJN.S210938.
[40] W. Liu, L. Liang, L. Zhao, H. Tan, J. Wu, Q. Qin, X. Gou, X. Sun, Synthesis and characterization of a photoresponsive doxorubicin/combretastatin A4 hybrid prodrug, Bioorg. Med. Chem. Lett 29 (3) (2019 Feb 1) 487–490, https://doi.org/10.1016/j.bmcl.2018.12.017.
[41] W.A. Denny, Nitroaromatic hypoxia-activated prodrugs for cancer therapy, Pharmaceuticals 15 (2) (2022 Feb 2) 187, https://doi.org/10.3390/ph15020187.
[42] B.A. Winn, Z. Shi, G.J. Carlson, Y. Wang, B.L. Nguyen, E.M. Kelly, RD 4th Ross, E. Hamel, D.J. Chaplin, M.L. Trawick, K.G. Pinney, Bioreductively activatable prodrug conjugates of phenstatin designed to target tumor hypoxia, Bioorg. Med. Chem. Lett 27 (3) (2017 Feb 1) 636–641, https://doi.org/10.1016/j.bmcl.2016.11.093.
[43] B.A. Winn, L. Devkota, B. Kuch, M.T. MacDonough, T.E. Strecker, Y. Wang, Z. Shi, J.L. Gerberich, D. Mondal, A.J. Ramirez, E. Hamel, D.J. Chaplin, P. Davis, R. P. Mason, M.L. Trawick, K.G. Pinney, Bioreductively activatable prodrug conjugates of combretastatin a-1 and combretastatin A-4 as anticancer agents targeted toward tumor-associated hypoxia, J. Nat. Prod. 83 (4) (2020 Apr 24) 937–954, https://doi.org/10.1021/acs.jnatprod.9b00773.
[44] K. Zhang, D. Chen, K. Ma, X. Wu, H. Hao, S. Jiang, NAD(P)H:Quinone oxidoreductase 1 (NQO1) as a therapeutic and diagnostic target in cancer, J. Med. Chem. 61 (16) (2018 Aug 23) 6983–7003, https://doi.org/10.1021/acs.jmedchem.8b00124.
[45] E.T. Oh, H.J. Park, Implications of NQO1 in cancer therapy, BMB Rep 48 (11) (2015 Nov) 609–617, https://doi.org/10.5483/bmbrep.2015.48.11.190.
[46] X. Cui, L. Li, G. Yan, K. Meng, Z. Lin, Y. Nan, G. Jin, C. Li, High expression of NQO1 is associated with poor prognosis in serous ovarian carcinoma, BMC Cancer 15 (2015 Apr 9) 244, https://doi.org/10.1186/s12885-015-1271-4.
[47] C. Zhang, Y. Qu, X. Ma, M. Li, S. Li, Y. Li, L. Wu, NQO1-selective activated prodrugs of combretastatin A-4: synthesis and biological evaluation, Bioorg. Chem. 103 (2020 Oct) 104200, https://doi.org/10.1016/j.bioorg.2020.104200.
[48] S. Garbo, S. Di Giacomo, D. Łażewska, E. Honkisz-Orzechowska, A. Di Sotto, R. Fioravanti, C. Zwergel, C. Battistelli, Selenium-containing agents acting on cancer-a new hope? Pharmaceutics 15 (1) (2022 Dec 28) 104, https://doi.org/10.3390/pharmaceutics15010104.
[49] A. Úriz, C. Sanmartín, D. Plano, R. de Melo Barbosa, C.A. Dreiss, G. González-Gaitano, Activity enhancement of selective antitumoral selenodiazoles formulated with poloxamine micelles, Colloids Surf. B Biointerfaces 170 (2018 Oct 1) 463–469, https://doi.org/10.1016/j.colsurfb.2018.06.009.
[50] L. Hou, W. Huang, J. Cheng, X. Deng, H. Lai, Z. Chen, Z. Zhan, P. Feng, Y. Li, F. Yang, T. Chen, The investigation and bioorthogonal anticancer activity enhancement of a triphenylphosphine-labile prodrug of seleno-combretastatin-4, Chem. Commun. 56 (92) (2020 Nov 19) 14495–14498, https://doi.org/10.1039/d0cc05498d.
[51] C. Schmidt, T. Babu, H. Kostrhunova, A. Timm, U. Basu, I. Ott, V. Gandin, V. Brabec, D. Gibson, Are Pt(IV) prodrugs that release combretastatin A4 true multi-action prodrugs? J. Med. Chem. 64 (15) (2021 Aug 12) 11364–11378, https://doi.org/10.1021/acs.jmedchem.1c00706.
[52] K.M.M. Rahman, P. Thapa, R. Hurst, S. Woo, Y. You, Singlet oxygen activatable prodrugs of paclitaxel, SN-38, MMC and CA4: nonmitochondria-targeted prodrugs, Photochem. Photobiol. 98 (2) (2022 Mar) 389–399, https://doi.org/10.1111/php.13589.
[53] M. Richter, V. Boldescu, D. Graf, F. Streicher, A. Dimoglo, R. Bartenschlager, C. D. Klein, Synthesis, biological evaluation, and molecular docking of combretastatin and colchicine derivatives and their hCE1-Activated prodrugs as antiviral agents, ChemMedChem 14 (4) (2019 Feb 19) 469–483, https://doi.org/10.1002/cmdc.201800641.
[54] M. Richter, M.M. Leuthold, D. Graf, R. Bartenschlager, C.D. Klein, Prodrug activation by a viral protease: evaluating combretastatin peptide hybrids to selectively target infected cells, ACS Med. Chem. Lett. 10 (8) (2019 Jul 18) 1115–1121, https://doi.org/10.1021/acsmedchemlett.9b00058.
[55] T. Liu, D. Zhang, W. Song, Z. Tang, J. Zhu, Z. Ma, X. Wang, X. Chen, T. Tong, A poly (l-glutamic acid)-combretastatin A4 conjugate for solid tumor therapy: markedly improved therapeutic efficiency through its low tissue penetration in solid tumor, Acta Biomater. 53 (2017 Apr 15) 179–189, https://doi.org/10.1016/j.actbio.2017.02.001.
[56] S. Yang, Z. Tang, C. Hu, D. Zhang, N. Shen, H. Yu, X. Chen, Selectively potentiating hypoxia levels by combretastatin a4 nanomedicine: toward highly enhanced hypoxia-activated prodrug tirapazamine therapy for metastatic tumors, Adv. Mater. 31 (11) (2019 Mar) e1805955, https://doi.org/10.1002/adma.201805955.
[57] M. Zheng, H. Xu, Y. Huang, J. Sun, H. Zhang, Z. Lv, Z. Liu, Z. Tang, X. Chen, Hypoxia-activated glutamine antagonist prodrug combined with combretastatin A4 nanoparticles for tumor-selective metabolic blockade, J. Contr. Release 365 (2024 Jan) 480–490, https://doi.org/10.1016/j.jconrel.2023.11.054.
[58] B. Zhao, Z. Dong, W. Liu, F. Lou, Q. Wang, H. Hong, Y. Wang, Co-administration of combretastatin A4 nanoparticles and anti-PD-L1 for synergistic therapy of hepatocellular carcinoma, J. Nanobiotechnol. 19 (1) (2021 May 1) 124, https://doi.org/10.1186/s12951-021-00865-w.
[59] Y. Zhao, X.X. Wang, W. Wu, H. Long, J. Huang, Z. Wang, T. Li, S. Tang, B. Zhu, D. Chen, EZH2 regulates PD-L1 expression via HIF-1α in non-small cell lung cancer cells, Biochem. Biophys. Res. Commun. 517 (2) (2019 Sep 17) 201–209, https://doi.org/10.1016/j.bbrc.2019.07.039.
[60] Y. Wang, H. Yu, D. Zhang, G. Wang, W. Song, Y. Liu, S. Ma, Z. Tang, Z. Liu, K. Sakurai, X. Chen, Co-administration of combretastatin A4 nanoparticles and sorafenib for systemic therapy of hepatocellular carcinoma, Acta Biomater. 92 (2019 Jul 1) 229–240, https://doi.org/10.1016/j.actbio.2019.05.028.
[61] M. Gao, D. Zhang, N. Yao, Q. Jin, C. Jiang, J. Zhang, F. Wang, Enhancing intratumoral biodistribution and antitumor activity of nab-paclitaxel through combination with a vascular disrupting agent, combretastatin A-4-phosphate, Cancer Chemother. Pharmacol. 84 (6) (2019 Dec) 1187–1194, https://doi.org/10.1007/s00280-019-03953-9.
[62] C. Deng, J. Zhao, S. Zhou, J. Dong, J. Cao, J. Gao, Y. Bai, H. Deng, The vascular disrupting agent CA4P improves the antitumor efficacy of CAR-T cells in preclinical models of solid human tumors, Mol. Ther. 28 (1) (2020 Jan 8) 75–88, https://doi.org/10.1016/j.ymthe.2019.10.010.
[63] X. Xiao, Y. Zheng, T. Wang, X. Zhang, G. Fang, Z. Zhang, Z. Zhang, J. Zhao, Enhancing anti-angiogenic immunotherapy for melanoma through injectable metal-organic framework hydrogel co-delivery of combretastatin A4 and poly(I:C), Nanoscale Adv. 6 (12) (2024 May 7) 3135–3145, https://doi.org/10.1039/d4na00079j.
[64] J. Czapla, A. Drzyzga, S. Matuszczak, T. Cichoń, M. Rusin, M. Jarosz-Biej, E. Pilny, R. Smolarczyk, Antitumor effect of anti-vascular therapy with STING agonist depends on the tumor microenvironment context, Front. Oncol. 13 (2023 Aug 15) 1249524, https://doi.org/10.3389/fonc.2023.1249524.
[65] S. Yu, S. Wei, L. Liu, D. Qi, J. Wang, G. Chen, W. He, C. He, X. Chen, Z. Gu, Enhanced local cancer therapy using a CA4P and CDDP co-loaded polypeptide gel depot, Biomater. Sci. 7 (3) (2019 Feb 26) 860–866, https://doi.org/10.1039/c8bm01442f.
[66] G. Dai, D. Zheng, G. Liu, Q. Song, Synergistic anticancer effects of cisplatin combined with combretastatin A4 phosphate on human osteosarcoma-xenografted

mice, Cells Tissues Organs 210 (4) (2021) 293–300, https://doi.org/10.1159/000517446.
[67] Y. Li, J. Lu, X. Deng, X. Wang, F. Jia, S. Zhong, X. Cui, Z. Pan, L. Shao, Y. Wu, Self-assembling combretastatin A4 incorporated protamine/nanodiamond hybrids for combined anti-angiogenesis and mild photothermal therapy in liver cancer, Nanotechnology 32 (46) (2021 Aug 23), https://doi.org/10.1088/1361-6528/ac1be0.
[68] E.T. Yeh, C.L. Bickford, Cardiovascular complications of cancer therapy: incidence, pathogenesis, diagnosis, and management, J. Am. Coll. Cardiol. 53 (24) (2009 Jun 16) 2231–2247, https://doi.org/10.1016/j.jacc.2009.02.050.
[69] G. Curigliano, D. Cardinale, S. Dent, C. Criscitiello, O. Aseyev, D. Lenihan, C. M. Cipolla, Cardiotoxicity of anticancer treatments: epidemiology, detection, and management, CA A Cancer J. Clin. 66 (4) (2016 Jul) 309–325, https://doi.org/10.3322/caac.21341.
[70] I.M. Subbiah, D.J. Lenihan, A.M. Tsimberidou, Cardiovascular toxicity profiles of vascular-disrupting agents, Oncol. 16 (8) (2011) 1120–1130, https://doi.org/10.1634/theoncologist.2010-0432.
[71] Q. Duan, S. McMahon, P. Anand, H. Shah, S. Thomas, H.T. Salunga, Y. Huang, R. Zhang, A. Sahadevan, M.E. Lemieux, J.D. Brown, D. Srivastava, J.E. Bradner, T. A. McKinsey, S.M. Haldar, BET bromodomain inhibition suppresses innate inflammatory and profibrotic transcriptional networks in heart failure, Sci. Transl. Med. 9 (390) (2017 May 17) eaah5084, https://doi.org/10.1126/scitranslmed.aah5084.
[72] H. Orihara, M. Ma, Y. Nagashima, R. Tochinai, S.I. Sekizawa, D. Kato, M. Shinada, S. Aoki, N. Fujita, T. Nakagawa, Y. Tsuru, Y. Tatewaki, T. Mutoh, Y. Taki, R. Nishimura, M. Kuwahara, Co-administration of JQ1, a bromodomain-containing protein 4 inhibitor, enhances the antitumor effect of combretastatin A4, a microtubule inhibitor, while attenuating its cardiotoxicity, Biomed. Pharmacother. 160 (2023 Apr) 114353, https://doi.org/10.1016/j.biopha.2023.114353.
[73] Z. Fu, S. Li, S. Han, C. Shi, Y. Zhang, Antibody drug conjugate: the "biological missile" for targeted cancer therapy, Signal Transduct. Targeted Ther. 7 (1) (2022 Mar 22) 93, https://doi.org/10.1038/s41392-022-00947-7.
[74] S. Ponziani, G. Di Vittorio, G. Pitari, A.M. Cimini, M. Ardini, R. Gentile, S. Iacobelli, G. Sala, E. Capone, D.J. Flavell, R. Ippoliti, F. Giansanti, Antibody-drug conjugates: the new frontier of chemotherapy, Int. J. Mol. Sci. 21 (15) (2020 Jul 31) 5510, https://doi.org/10.3390/ijms21155510.
[75] J.T.W. Tong, P.W.R. Harris, M.A. Brimble, I. Kavianinia, An insight into FDA approved antibody-drug conjugates for cancer therapy, Molecules 26 (19) (2021 Sep 27) 5847, https://doi.org/10.3390/molecules26195847.
[76] H. Shim, Bispecific antibodies and antibody-drug conjugates for cancer therapy: technological considerations, Biomolecules 10 (3) (2020 Feb 26) 360, https://doi.org/10.3390/biom10030360.
[77] C.M. Mckertish, V. Kayser, Advances and limitations of antibody drug conjugates for cancer, Biomedicines 9 (8) (2021 Jul 23) 872, https://doi.org/10.3390/biomedicines9080872.
[78] C.M. Yamazaki, A. Yamaguchi, Y. Anami, W. Xiong, Y. Otani, J. Lee, N.T. Ueno, N. Zhang, Z. An, K. Tsuchikama, Antibody-drug conjugates with dual payloads for combating breast tumor heterogeneity and drug resistance, Nat. Commun. 12 (1) (2021 Jun 10) 3528, https://doi.org/10.1038/s41467-021-23793-7.
[79] M. Kesireddy, S.R. Kothapalli, S.G. Gundepalli, S. Asif, A review of the current FDA-approved antibody-drug conjugates: landmark clinical trials and indications, Pharmaceut. Med. 38 (1) (2024 Jan) 39–54, https://doi.org/10.1007/s40290-023-00505-8.
[80] Z. Wang, H. Li, L. Gou, W. Li, Y. Wang, Antibody-drug conjugates: recent advances in payloads, Acta Pharm. Sin. B 13 (10) (2023 Oct) 4025–4059, https://doi.org/10.1016/j.apsb.2023.06.015.
[81] L. Conilh, L. Sadilkova, W. Viricel, C. Dumontet, Payload diversification: a key step in the development of antibody-drug conjugates, J. Hematol. Oncol. 16 (1) (2023 Jan 17) 3, https://doi.org/10.1186/s13045-022-01397-y.
[82] R. Huang, Y. Sheng, Z. Xu, D. Wei, X. Song, B. Jiang, H. Chen, Combretastatin A4-derived payloads for antibody-drug conjugates, Eur. J. Med. Chem. 216 (2021 Apr 15) 113355, https://doi.org/10.1016/j.ejmech.2021.113355.
[83] F. Migliorini, E. Cini, E. Dreassi, F. Finetti, G. Ievoli, G. Macrì, E. Petricci, E. Rango, L. Trabalzini, M. Taddei, A pH-responsive crosslinker platform for antibody-drug conjugate (ADC) targeting delivery, Chem. Commun. 58 (75) (2022 Sep 20) 10532–10535, https://doi.org/10.1039/d2cc03052g.
[84] D. Wei, Y. Mao, H. Wang, S. Qu, J. Chen, J. Li, B. Jiang, H. Chen, A mild phenoxysilyl linker for self-immolative release of antibody-drug conjugates, Chin. Chem. Lett. (2023) 108091, https://doi.org/10.1016/j.cclet.2022.108091.
[85] B. De Filippis, A. Ammazzalorso, M. Fantacuzzi, L. Giampietro, C. Maccallini, R. Amoroso, Anticancer activity of stilbene-based derivatives, ChemMedChem 12 (8) (2017 Apr 20) 558–570, https://doi.org/10.1002/cmdc.201700045.
[86] D.M. Kopustinskiene, V. Jakstas, A. Savickas, J. Bernatoniene, Flavonoids as anticancer agents, Nutrients 12 (2) (2020 Feb 12) 457, https://doi.org/10.3390/nu12020457.
[87] S. Kamran, A. Sinniah, M.A.M. Abdulghani, M.A. Alshawsh, Therapeutic potential of certain terpenoids as anticancer agents: a scoping review, Cancers 14 (5) (2022 Feb 22) 1100, https://doi.org/10.3390/cancers14051100.
[88] M. Fantacuzzi, M. Gallorini, N. Gambacorta, A. Ammazzalorso, Z. Aturki, M. Balaha, S. Carradori, L. Giampietro, C. Maccallini, A. Cataldi, O. Nicolotti, R. Amoroso, B. De Filippis, Design, synthesis and biological evaluation of aromatase inhibitors based on sulfonates and sulfonamides of resveratrol, Pharmaceuticals 14 (10) (2021 Sep 27) 984, https://doi.org/10.3390/ph14100984.