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A new horizon for the steroidal alkaloid cyclovirobuxine D (huangyangning) and analogues: Anticancer activities and mechanism of action

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Abstract— For a long time, huangyangning was a standard therapy for cardiovascular and cerebrovascular illnesses in China. Its active ingredient is the steroidal alkaloid cyclovirobuxine D (Cvb-D). It is on the Chinese pharmacopeia list. This little-known alkaloid has recently come to light as having anticancer effects in both laboratory and animal models. The medicine inhibits phosphorylation of proteins such as EGFR, ERK, Akt, and mTOR, among others, by activating several signaling pathways. Consequently, Cvb-D has anti-metastatic and anti-proliferative effects. Cvb-D and similar natural compounds derived from Buxus species have been studied for their potential anticancer properties in this review. Current knowledge on the molecular targets of Cvb-D is limited; nevertheless, theories have been developed by examining the drug's effects on signaling pathways and drawing comparisons to other drugs. Potential upstream targets of Cvb-D include the proteins EGFR and CTHRC1, which are involved in its anti-proliferative effect. As a further, more audacious hypothesis, the metastasis-associated protein S100A4 is proposed as a possible co-target for Cvb-D. With its well-established anti-cardiovascular effectiveness and promising safety profile, Cvb-D has the potential to be the subject of more mechanistic investigations into its anticancer effects.

Introduction

An evergreen shrub endemic to Southern China, Buxus microphylla var. Sinica (Chinese Boxwood, Buxaceae, 小叶黄杨) is beneficial for absorbing and containing air pollutants, such as tiny particulate matter PM2.5.1 The Aside from being a popular decorative tree, it has a long history of usage in traditional Chinese medicine (Huang Yang Mu Ye, xiaoyehuangyang) for the treatment of cardiovascular diseases in China.2 The buxmicrophyllines and other cycloartane type alkaloids are present in B. microphylla extracts, among other triterpenoid alkaloids.Three to five different cancer cell lines have been shown to be significantly cytotoxic by buxmicrophylline B and R.four, six Other chemicals have been extracted from the plant's roots and leaves.as well as cyclomicrobuxinine and buxbodine B found in plants. However, cyclovirobuxine D (Cvb-D, Fig. 1), the principal alkaloid derived from B. microphylla, has been in use for over 30 years in

medicine since its discovery in 1964,7 and thorough characterization in 1979,8.10 Cvb-D is a pregnane-derived steroidal alkaloid that has a low water solubility but a satisfactory bioavailability when made into disintegrating tablets.11 The CFDA of China authorized huangyangning dispersible tablets (Fig. 1) in 2009 to treat various cerebrovascular and cardiovascular diseases, including coronary heart disease, angina pectoris, arrhythmia, heart failure, hypertension, and cardiac neurosis. Cvb-D is the active ingredient in these tablets.12 The 2015 Chinese Pharmacopoeia includes it. Cvb-D modulates blood pressure by expanding blood vessels and increasing coronary flow. In addition to lowering oxygen consumption by the heart, it has an antiarrhythmic action. This medicine has a lengthy history of usage in preventing acute cerebral ischemia and myocardial ischemia.Multiple pharmacological investigations in animal models (13–15) and clinical trials have shown Cvb-D's therapeutic impact on heart failure caused by myocardial infarction.2 The compound known as JLX001, which is the Cvb-D dihydrochloride salt The drug is now in development by Zhejiang Jingxin Pharmaceutical Co. of Shaoxing, China, with the goal of treating ischemic stroke.16 Just recently, research has shown that JLX001 may effectively reduce cerebral ischemia damage by blocking platelet activation and thrombus formation.17 As its principal route of action, JLX001 inhibited oxidative stress and inflammation in animal models via modulating the TLR2/4-NF-kB and AMPK-ULK1 signaling pathways. The brain is protected against ischemia damage by this investigational medication.18, 19 When it comes to human clinical trials, JLX001 is an intriguing candidate. Cancer therapy is one of many new possible therapeutic indications for Cvb-D that has emerged in recent years. This article provides a summary of Cvb-anticancer D's characteristics and the current understanding of its action mechanism.

Anticancer activity of cyclovirobuxine D

Human breast cancer cells were used in an in vitro investigation to establish that Cvb-D had an anticancer impact. At an IC50 of 10 mM, the drug was discovered to decrease MCF7 cell viability. After 24 hours of treatment, it was discovered to induce autophagy, which is marked by the appearance of autophagosomes/autolysosomes and the up-regulation of autophagy markers like microtubule-associated protein LC3-II and autophagy protein ATG5.20 Because Cvb-D at low concentrations (1e10 mM) reduced Akt phosphorylation and suppressed mTOR phosphorylation without altering their intracellular expression levels, it was hypothesized that this inhibition of the Akt/mTOR axis was responsible for autophagic activation.20 Another in vitro investigation using two gastric cancer cell lines found that Cvb-D dose- and time-dependently decreased cell proliferation of MGC-803 and MKN2 cells; nevertheless, rather high doses (about 50–60 mM at 72 h) were necessary to achieve a 50% reduction in cell growth. Concentrations lower than 10 mM were more effective in inhibiting colony development. At very high doses (i.e., 30–120 mM), the effect was followed by a disruption of the cell cycle and the activation of apoptosis as shown by mitochondrial damages, caspase-3 cleavage, Bcl-2 down-regulation, and Bax up-regulation.21 In their study, the scientists used a murine macrophage RAW264.7 cell line—which does not contain tumors—to demonstrate that the medication blocks the JAK-STAT pathway.22

There has been an analogous in vitro investigation with glioblastoma cell lines.23 Cvb-D caused apoptosis, cell cycle arrests (in the S and G0/G1 phases), and inhibition of glioma cell growth in T98G and Hs683 models. However, drug doses ranging from 100 to 200 mM were required to induce a significant amount of apoptosis. The sensitivity of these glioma cells to Cvb-D seems to be low. Nevertheless, this provides more evidence that the medication may inhibit cell proliferation and promote cell death in response to aggressive malignant cells. Using hepatocellular carcinoma (HCC) cells, researchers have recently shown that Cvb-D may inhibit cell proliferation and induce cell death.24 With an IC50 of around 15–17 mM, Cvb-D treatment inhibited the proliferation of HepG2 and HCCLM3 cells in a dose-dependent manner, whereas lower drug concentrations resulted in impaired colony formation. At doses ranging from 10 to 40 mM, Cvb-D was seen to trigger a significant death of HCC cells, just as it did in the gastric cell line. The potency is moderate. However, the fact that the research also

documented the drug's action in living organisms makes it noteworthy. Mice with HepG2 tumors showed an increase in the rate of tumor cell death and a significant decrease in tumor volume after receiving intraperitoneal administration of Cvb-D (10 mg/kg, every two days, for 14 days). The medicine effectively reduced tumor development in mice and seemed to be well-tolerated (treated animals did not lose weight). Thus, the anticancer effect is associated with a medication-induced suppression of the EGFR-FAK-AKT/ERK1/2-Slug signaling pathway, as shown by biochemical tests indicating the drug could diminish phosphorylation of EGFR, Akt, and ERK1/2 in HCC cells in vitro.24

A comparable study using cells from colorectal cancer was reported not long ago.Cell colonies are formed and DLD-1 and LoVo cell proliferation is reduced by 25 Cvb-D with an IC50 of 23–26 mM. Vimentin and N-cadherin are important indicators of the drug-induced epithelial-mesenchymal transition (EMT), and the medication decreased their expression in both cell types in a dose-dependent manner while boosting E-cadherin's expression. In order to decrease tumor cell invasion and dissemination via blood and lymphatic vessels, the medication protects the extracellular matrix. By inhibiting matrix metalloproteinases MMP2 and MMP9 and downregulating effector proteins including Snail, Slug, and ZEB, Cvb-D can control EMT. Cvb-D also decreased cancer cell motility and induced cell death by changing the expression of the up-regulated Bax protein and the down-regulated Bcl-2 protein, respectively. There was a clear reduction in Akt and Erk-1/2 phosphorylation, which points to the PI3K/AKT/ERK signaling pathway as the primary mechanism by which the medication exerts its anticancer effects. It is worth noting that a key protein involved in the anticancer effect was identified: CTHRC1, which stands for collagen triple helix repeat containing 1. This protein is often overexpressed in cancerous cells. Cancer cell motility, colony formation, invasion, and proliferation are all facilitated by this protein, making it an important mediator of oncogenesis.Cvb-D can significantly downregulate CTHRC1 expression in DLD-1 and LoVo cells, which in turn causes phospho-AKT and phospho-ERK to disappear. Cvb-D may have an upstream target in CTHRC1, according to the authors' findings.25 This anticancer

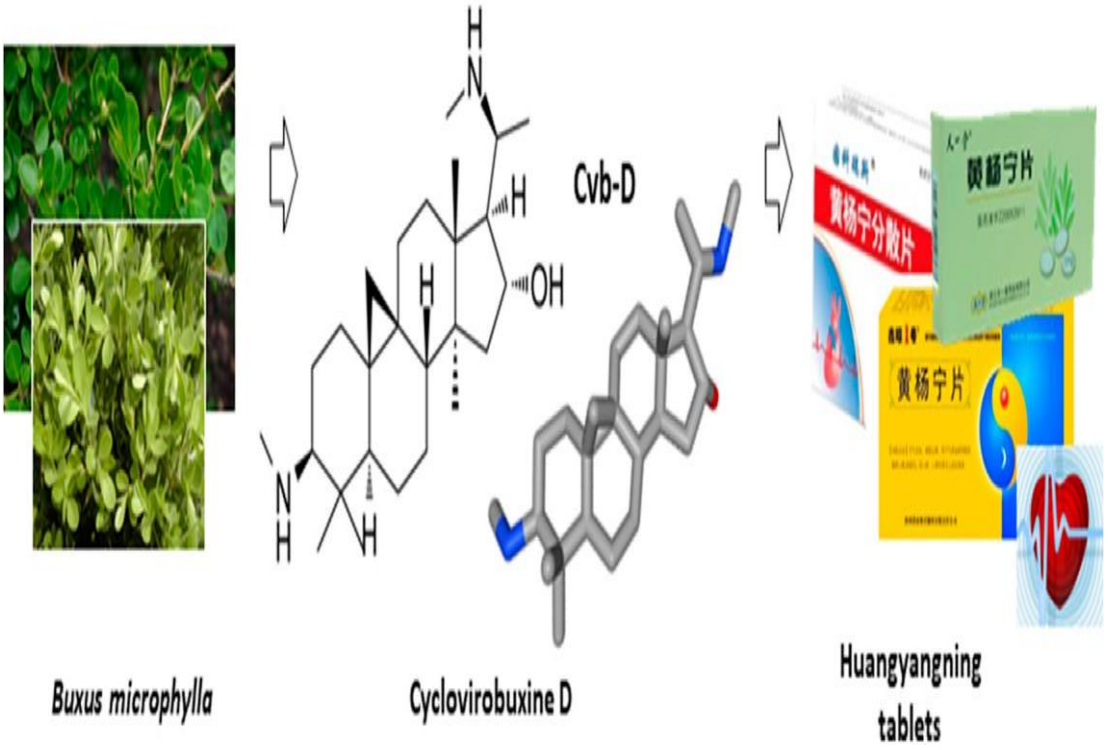

Fig. 1. Structure of cyclovirobuxine D (Cvb-D) isolated from *Buxus microphylla*. Cvb-D is the active pharmaceutical ingredient of the drug Huangyangning tablets used in China to treat cardiovascular diseases. Cvb-D is also known as cyclovirobuxinum D or bebuxine ($C_{26}H_{46}N_2O$) and is listed in the Pharmacopoeia of the People's Republic of China (edition 2015).

Cvb-D's activity was studied in vivo by xenografting DLD-1 cancer cells into mice. The tumor volume was significantly decreased and the residual tumor showed evident symptoms of drug-induced apoptosis after four weeks of intraperitoneal injection of Cvb-D (15 mg/kg/day). This new research not only backs up Cvb-D's anticancer claims in colon cancer, but it also lays out crucial molecular details, such as how the secreted glycoprotein CTHRC1 is a potential target for Cvb-D and a major driver of its anticancer impact. It may also function as an effector, changing its state in reaction to Cvb-D's influence on a target farther upstream. So far, five separate investigations have shown that Cvb-D has anticancer effects in various animal models (Table 1). Two in vitro + in vivo investigations with hepatocellular and colorectal cancer cells, as well as three in vitro investigations with gastric, breast, and glioma cell lines20,21,23.24,25 Although more research is needed, the current findings support the idea of Cvb-D as a potential new cancer treatment. But details of how it works are all over the place. Specifically, Cvb-D's probable molecular targets are still mostly unknown (see below). Different things that Cvb-D does

To treat or prevent many cardiovascular illnesses, Cvb-D is an effective medicine. Cardiomyocytes damaged by oxidation or hypoxia are more likely to survive after taking this medicine.27 Additionally, it activates an antioxidant response mediated by Nrf 2 that protects against diabetic cardiomyopathy. Based on a molecular modeling investigation, it has been suggested that Cvb-D directly binds to Nrf2, a transcription factor. This might explain why drugs can increase Nrf2's nuclear translocation and reduce oxidative stress.28 Similarly, doxorubicin, an anticancer medication, may cause cardiotoxicity; however, Cvb-D can protect against this. In vitro, it decreased cardiac oxidative damages and mitochondrial biogenesis impairment by doxorubicin-induced myocardial cell death.29 Cvb-D is not the only one with this quality. Cyclobuxine and buxaustroines A-N, two other alkaloids derived from the Chinese shrub Buxus austro-yunnanensis, have shown cardioprotective effects (Fig. 2).30

Other anticancer Buxus natural products

In vitro studies have shown that extracts from many Buxus plants, including Buxus papillosa, Buxus hildebrandtii, and Buxus sempervirens, have substantial antiproliferative activity against various cancer cells.no. 31

33 The ability of an acetonic B. sempervirens extract to suppress the growth of cancer cells and to induce apoptosis and autophagy in cancer cells is particularly noteworthy.33 While alkaloids and cyclovirobuxines are present in these extracts, they are far from the only natural compounds they contain.

More than 250 members make up the enormous and diverse Buxus alkaloid family to which Cvb-D belongs; these members all have a core structure that is triterpenoid-steroidal pregnane tetracyclic (Fig. 2).34 The members of this family may be categorized into two groups based on the number of B rings they contain: those with a 7-membered ring and those with a 6-membered ring. The first group consists of derivatives of the 9(10e19)-abeo-4,4,14a-trimethyl-5a-pregnane system.Similar to the other natural products shown in Figure 2, including cyclovirobuxeine A, buxandrine, and E-buxenone, Cyclovirobuxines A–D belong to the second category. Buxmicrophylline B and N-acetyldihydrocyclomicrophylline F, both derived from B. microphylla, have shown cytotoxic effects on cancer cells, such as HL60 leukemia cells and human tumor HepG2 cells (with sub-micromolar activity against both types of cells).5

Not all Buxus alkaloids are cytotoxic; nevertheless, those that are either mildly or Additional beneficial characteristics may be shown by non-cytotoxic chemicals. One such cycloartane alkaloid is O-tigloylcyclovirobuxeine-B, which has a strong anti-parasitic action against the malaria parasite Plasmodium falciparum but very mild cytotoxic effects on L6 rat cells.36,37 It has also been shown to have anti-leishmanial and anti-fungal bioactivities.38 A number of Buxus alkaloids, including buxamine C, have been shown to inhibit acetyl-cholinesterase (AChE), an enzyme critical to the breakdown of the neurotransmitter

acetylcholine. Molecular modeling has led to the hypothesis that the drug interacts directly with this enzyme.39 However, in several instances, the level of 40AChE inhibition is low; for example, buxidine and buxandrine have IC50 values more than 100 mM.40 Although buxmicrophylline C and buxbodine B, both derived from Buxus macowanii, are more effective AChE inhibitors, their IC50 values remain quite high, exceeding 10 mM.41

Two chemicals with anticancer properties are isolated from the compound family. One of them is KBA01, a triterpenoid alkaloid derived from B. microphylla that has shown promising antiproliferative effects against several cancer cell lines. It was much more effective than other cancer cell lines against the highly sensitive HT29 colon cancer cells (CRC; IC50 = 5 mM).The p53 protein has an oncogenic mutation (R273H mutation) in 42 HT29 cells. Similar to Cvb-D in structure (Fig. 2), KBA01 was discovered to engage in a chaperone-mediated cascade that ultimately leads to the death of tumor suppressor protein p53.

Table 1

Anticancer activities of cyclovirobuxine D in different models.

Cancer models *[cell lines]*	Cyclovirobuxine D activities	References
Breast cancer *[MCF7]*	- Inhibition of cell proliferation - Inhibition of Akt/mTOR axis - Induction of autophagy	20
Gastric cancer *[MGC-803, MKN2]*	- Inhibition of cell proliferation and colony formation - Blockade of the JAK-STAT pathway - Cell cycle arrest and induction of apoptosis	21
Glioblastoma *[T98G, Hs683]*	- Inhibition of cell proliferation - Cell cycle block and induction of apoptosis	23
Hepatocellular carcinoma *[HepG2, HCCLM3]*	- Inhibition of cell proliferation and colony formation - Inhibition of the EGFR/AKT/ERK1/2 signaling pathway - Cell cycle arrest and induction of apoptosis - Reduction of tumor *in vivo*	24
Colorectal cancer *[DLD-1, LoVo]*	- Inhibition of cell proliferation and colony formation - Regulation of the epithelial-mesenchymal transition - Reduction of cancer cell mobility (with reduced expression of CTHRC1) - Induction of apoptosis *in vitro* and *in vivo* - Inhibition of tumor growth *in vivo*	25

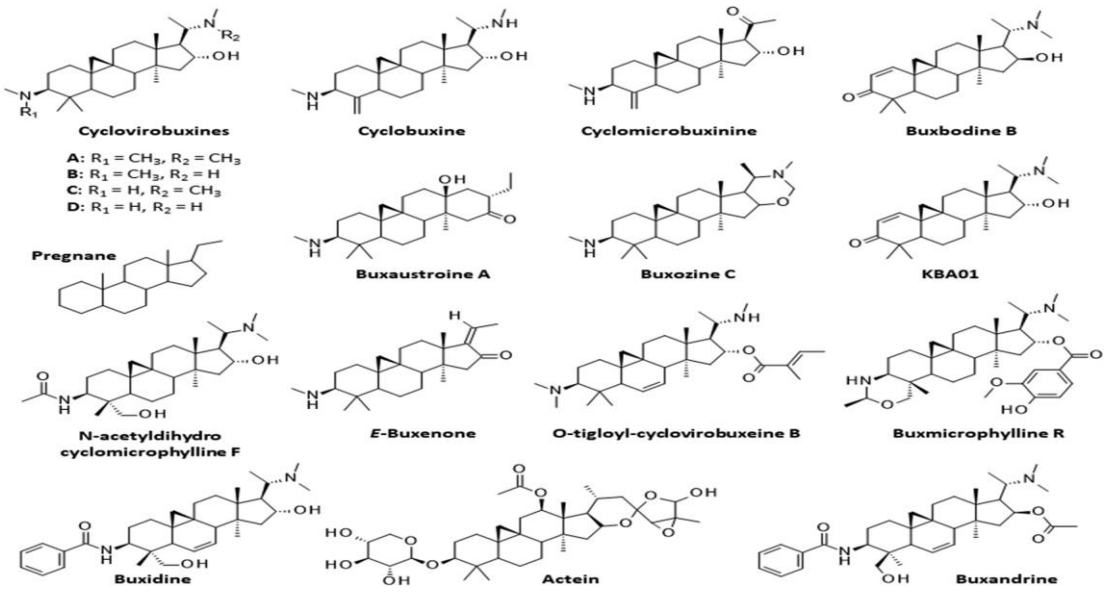

Fig. 2. Structures of several *Buxus* alkaloids and other natural products cited in this review.

drug-induced degradation of the mutant p53 protein was not seen, despite the aided mechanism. An attractive anticancer strategy that works in tandem with existing medications is to target the proteasomal degradation of the tumor suppressor protein p53. When combined with anticancer drugs like doxorubicin and 5-fluorouracil, the natural product lipoic acid leads to the synergistic killing of colorectal cancer cells in a p53-dependent manner. It also induces ubiquitination and proteasomal degradation of p53 in various colorectal cancer cell lines.43 The anticancer effects of other medications that may cause the mutant p53 protein to degrade are encouraging.44 A number of malignancies, including colorectal and osteosarcoma, have shown promise when treated with therapeutic strategies that target mutant p53.45

Another alkaloid from Buxus that deserves particular notice for its biological activities is E-buxenone, which is derived from Buxus hyrcana. It has been shown to inhibit the proliferation of T cells triggered by phytohemagglutinin. Curiously, a molecular modeling research suggested that E-buxenone and its analog buxidin directly interact with IL-2, and both compounds were discovered to reduce IL-2 and IL-4 production in a dose-dependent manner (Fig. 2).46 Although experimental confirmation of this in silico prediction is still pending, E-buxenone and buxidin's immunosuppressive characteristics may be therapeutic in a variety of diseases.

Cvb-D molecular targets?

The cyclovirobuxine literature review and associated

product brought attention to an underappreciated category of all-natural goods. Cvb-D seems to have helpful anticancer characteristics in addition to its well-known vascular protective benefits. Several supplementary in vitro and in vivo experimental models have now shown its anti-tumor efficacy. Although the amount of activity is not very high, it is noticeable in models of colon and liver cancer.24, 26 To fully understand the drug's potential, more in vivo trials using other models and in conjunction with current anti-tumor medications are necessary. In order to guide the selection of medication combinations and develop optimum protocols, however, a better understanding of its mechanism of action and its targets is required. As of right now, this is the only restriction on this newly revealed anticancer drug. Where does Cvb-D aim its molecular attacks?

We may go to research in an effort to shed light on this mystery.

carried out using Cvb-D or other naturally

occurring compounds belonging to the same chemical class. In the first scenario, target suggestions obtained from in silico research are our exclusive option because to the lack of drug-target structural investigations. Above, we noted that the drug's protective impact against oxidative stress in cardiomyocytes might be explained by a modeled direct interaction of Cvb-D with the Nrf 2-Keap 1 complex.28 The medication may be able to fit into a binding pocket in the Nrf2- Keap1 complex, as revealed by the docking research. This would lower the complex's binding free energy and disrupt the

treatments for cancer. We think that Cvb-D's anticancer activity can't be explained by this target interaction alone; other targets are required. The anticancer impact was shown to be mediated in part by the secreted protein CTHRC1 (Collagen triple helix repeat containing-1), according to another research (Fig. 3).25 The antiproliferative action of Cvb-D was diminished when CTHRC1 was knocked down using siRNA. Several studies shown that Cvb-D inhibited colorectal cell proliferation via a CTHRC1-dependent pathway. Even while this doesn't prove that CTHRC1 is a molecular target of Cvb-D, it does show that it is involved in its action.25 Recent research has identified this protein as a key regulator of both primary tumor development and the metastasis of cancer cells to other tissues.48 out of 49 Colorectal cancer, 50 osteosarcoma, 51 HCC, and endometriosis are among the diseases for which it is being evaluated as a potential predictive biomarker and a potential therapeutic target.53 Intriguingly, a cervical cancer model showed a strong decrease of tumor cell metastasis when a particular monoclonal antibody was used to directly block CTHRC1.54 Similarly, a xenograft model revealed that the protein enhanced the metastatic spread of epithelial ovarian cancer cells.55 Undoubtedly, CTHRC1 promotes metastasis; hence, it may be very beneficial to enhance cancer therapies by down-regulating it using antibodies or medications such as Cvb-D. At this time, no medication has been identified that interacts directly with CTHRC1. Cvb-D may be the first small molecule ligand for this receptor. The second strategy involves searching for complementary natural substances in

the same class of chemicals and the targets that they

connection between Nrf2 and Keap1. Consequently, Cvb-D would encourage Nrf2 nuclear translocation and activate its downstream signaling pathways, resulting in oxidative stress attenuation.28 Additional tiny compounds that can

There are known compounds that disrupt Nrf2-Keap1 complexes, such as quercetin (3,40-

Such compounds as diglucoside, esculin, and salvianolic acid A)47 do not have strong

interact with. The structural and functional diversity of saponins is astounding. Of the various subclasses, there are as many as eleven primary classes (dammaranes, tirucallanes, lupanes, hopanes, ole-ananes, taraxasteranes, ursanes, cycloartanes, lanostanes, cucurbitanes, and steroids).56 Together, these classes provide a wide range of potential applications.

many substances that may be compared to Cvb-D. Anthracite, argentatin A, actein, (23 R, 24 E)-23-acetoxymangiferonic acid, and many other anticancer drugs are members of the cycloartanes, the saponin class most closely related to Cvb-D. Regrettably, the majority of these drugs have had their anticancer effects studied, but not their molecular targets. An example of a rare situation where a target has been suggested is the derivative cycloartane-3,24,25-triol. It was discovered that out of 451 kinases tested, MRCKa (myotonic dystrophy protein kinase-like alpha) inhibits serine/threonine protein kinase activity with a Kd50 of 0.26 mM.60 Because of its function in p53-dependent autophagy, this target is intriguing.61, 62 Aiming to combat cancer, researchers are diligently seeking small-molecule inhibitors that specifically target Cdc42-binding MRCK kinases.63 Examining Cvb-D's action on this kinase may provide some intriguing insights. There are likely several targets involved in the action mechanism of Cvb-D, and it is challenging to identify the target of any natural substance. The related cycloartane actein (from the medicinal plant Cimicifuga racemosa) was used in a recent target-fishing investigation that used system chemical biology approaches. The analysis revealed eight possible targets, one of which being the metastasis-associated calcium-binding protein S100A4.64.65

dollars Hepatocellular carcinoma66 and colorectal cancers associated with herpes simplex virus have recently had S100A4 suggested as a potential therapeutic target.67, 68 Specifically, S100A4 controls how polyploid large cancer cells migrate and invade.69 Cvb-D has shown significant action in vivo models of HCC and colon cancer, as previously mentioned. Hence, research into the possibility of Cvb-D binding to S100A4 is warranted. Although they share some structural features, the glycosylated cycloartane actein is structurally different from Cvb-D (Fig. 2). We should expect a comparable level of mechanical complexity, with several goals contributing to it.

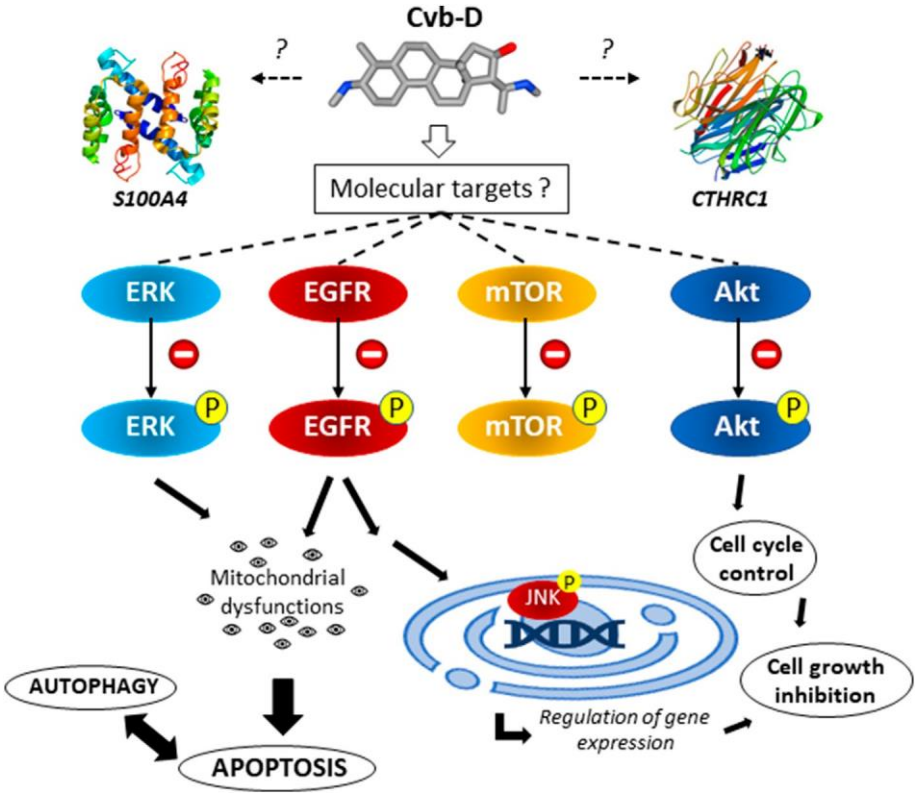

Figure 3 shows a schematic of the mechanism of action of cyclovirobuxine D (Cvb-D). Several proteins, including as ERK1/2, EGFR, mTOR, and Akt, are phosphorylated when exposed to Cvb-D. Because of these consequences, mitochondrial disturbances trigger the intrinsic apoptotic pathway. Additionally, it triggers the transcription of genes that play a role in regulating the cell cycle and promoting cell proliferation. Cvb-D also inhibits the Akt/mTOR axis, which allows certain cancer cells to initiate autophagy. Currently, Cvb-D's upstream targets remain a mystery; nevertheless, there is talk of a possible direct effect on EGFR as well as the proteins S100A4 and CTHRC1.

anti-cancer effects. Since S100A4 is an essential ligand of the EGFR receptor, an interaction between Cvb-D and S100A4 may account for the drug's subsequent effects, most noticeably the drug-induced suppression of EGFR phosphorylation seen in vitro.70, 71 The function of S100A4 in cancer metastasis72 has been shown, and a small poly- aromatic compound called amlexanox has been found to suppress cancer cell proliferation by disrupting the interaction between S100A4 and EGF.73 In addition, part of the p53-dependent effects shown with Cvb-D may be explained by the fact that S100A4 forms complexes with p53,74. Therefore, we suggest looking into the possibility of Cvb-D interacting with S100A4 and the consequent EGFR antagonist effect. Additionally, it would be beneficial to study how Cvb-D may interact with EGFR, since this receptor has been shown to be directly occupied by a number of natural products that inhibit EGF binding to EGFR, such as cucurbitacin D and analogs, and protopanaxadiol.From 75 to 78

In conclusion, it has been shown after a thorough literature review that proteins CTHRC1, S100A4, and EGFR are the three most promising anticancer target candidates for Cvb-D (Fig. 3). Naturally, there can be other ones. To find other proteins that Cvb-D can target, it might be helpful to use reverse screening methods79,80. However, it may result in a large number of contenders. To illustrate the point, twelve signal transduction pathways and thirty-three critical target proteins were uncovered when the anticancer medicine epigallocatechin-3-gallate's potential targets were identified using a reverse docking approach.81

Conclusion and prospects

Historically, cyclovirobuxine D, a triterpenoid alkaloid, has been used to treat cardiovascular diseases in traditional Chinese medicine. The anti-arrhythmia and vasodilatory actions of the medicine Huangyangning have made it famous. No organ damage or general toxicity was seen in rats fed for eight weeks continuously with a dose 50-to-200 times higher than the human dosage, according to a recent toxicology research.18 Despite the lack of published clinical research supporting the charges, the medicine seems to be safe and effective, and it has been seen in Chinese hospitals for decades. But the Huangyangning pills' active pharmaceutical ingredient (API), cyclovirobuxine D, has a history of use in the treatment of cardiovascular disorders. Since 2009, the CFDA has authorized the medicine, and many pharmaceutical firms offer the active pharmaceutical ingredient (API). To speed up the drug's dissolving rate, researchers are creating new Cvb-D formulations including Cvb-D nanosuspensions.82

Cvb-D has recently opened up new avenues of potential use outside the cardiovascular sector, namely in the fight against cancer. Preliminary evidence of activity in vivo has been reported lately, and other investigations have shown that the medication has antiproliferative effects against various cancer cell types in vitro. Though the activity level isn't very high, the antitumor effect—which results in the death of cancer cells and the suppression of metastasis—is evident. Cvb-D regulates cell cycle progression, inhibits metastasis, and activates signaling pathways similar to those of other steroidal alkaloids. These pathways include up-or down-regulation of apoptotic (Bax, Bcl 2, caspases) and autophagic (LC3, AKT, mTOR) proteins.No. 83 Unfortunately, the chemical effects that initiate these reactions remain mostly unknown. Little is known about the significance or molecular processes that underlie the anticancer action. However, other proteins might be suggested as possible targets; for example, EGFR and CTHRC1 are known to be involved in Cvb-D activity, and here we make a more daring assumption about S100A4.

This all-natural product needs to be promoted and studied immediately.

enhance its anticancer efficacy and mode of action in living organisms. With any luck, this evaluation will serve as a catalyst for further Cvb-D testing in other models, perhaps with more suitable medication compositions and combinations. Cvb-D has a lengthy history of usage and a high safety profile; it may one day be a cancer therapy medicine or a molecular tool to alter the activity of the proteins CTHRC1 and S100A4, neither of which have particular pharmacological effectors at this time.

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Declaration of competing interest

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CRediT authorship contribution statement

Christian Bailly: Conceptualization, investigation, project administration, supervision, writing – original draft, and writing – review & editing. Jihong Zhang: Writing – review & editing.

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