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Chapter

Cytotoxic and Antitumoral Activities of Compounds Isolated from Cucurbitaceae Plants

Carlos Alberto Méndez-Cuesta, Ana Laura Esquivel Campos, David Salinas Sánchez, Cuauhtemoc Pérez González and Salud Pérez Gutiérrez

Abstract

The WHO says that annual cases of cancer will increase from 14 million in 2012 to 22 million in the next two decades. Cancer is the second cause of death in the world; in 2015, it caused 8.8 million deaths. On the other hand, it is necessary to consider that 70% of the total deaths due to this disease occur in developing countries, who have the least resources to acquire the drugs of choice for the treatment of this disease. Although there are treatments and these are effective, there are currently cases of resistance to drugs used to treat this disease, which has led to the search for new sources of drugs or compounds effective against the cancer being active; plants are the possible sources to achieve this. Cucurbitaceae is a family of plants widely distributed on the planet which has been used traditionally for the treatment of this disease and from they have been isolated different cucurbitanes. These compounds possess a wide biological activity, antidiabetic, anti-inflammatory, hepatoprotective, or cytotoxic and antitumoral effects. The aim of this review is to present 51 cucurbitacin compounds and 2 with different structures isolated from Cucurbitaceae plants with cytotoxic or antitumoral activity.

Keywords: cucurbitacin, Cucurbitaceae, cytotoxic, cancer, natural product

1. Introduction

Cancer is an abnormal growth of cells, which begin to divide without stopping and can form solid tumors. Cancer is a collection of more than 100 different diseases with genetic changes, which can be inherited or be caused by environmental exposure to chemicals, tobacco smoke, or radiation, such as UV rays from the sun.

Cancer is the second leading cause of death in the US and is responsible for approximately 1 out of every 4 deaths. Globally, nearly 1 in 6 deaths is due to cancer. Approximately, 70% of deaths from cancer occur in low- and middle-income countries. There were 14.1 million new cancer cases, 8.2 million cancer deaths, and 32.6 million people living with cancer (within 5 years of diagnosis) in 2012 worldwide, based on World Health Organization (WHO) estimates. A total of 57% (8 million) of new cancer cases, 65% (5.3 million) of cancer deaths, and 48% (15.6 million) of 5-year prevalent cancer cases occurred in less-developed regions [1, 2].

The most common treatments for cancer are surgery, chemotherapy, and radiation therapy, and in many cases, they are used in combination [3]. These treatments can be effective but can cause side effects, such as anemia, appetite loss, fatigue, and alopecia [4].

1.1 Generalities of the Cucurbitaceae family

Plants are an important source of compounds currently used in cancer chemotherapy. The Cucurbitaceae family, also called cucurbits, contains 120 genera with 825 species that are widely distributed in tropical and temperate regions [5], and those with edible fruits were the first cultivated plants in Europe and America. Many species of the Cucurbitaceae family are used as human food [6]. Most of the species in this family are annual vines, and some are lianas, thorny shrubs, or trees. The most important genera of this family are *Cucurbita* (squash, pumpkin, zucchini), *Lagenaria* (calabash), *Citrullus* (watermelon), *Cucumis* (cucumber, various melons), and *Luffa* (luffa).

1.2 Cucurbitanes of the Cucurbitaceae family with cytotoxic effects

Some cucurbitanes have been isolated from different species of the Cucurbitaceae family. These compounds exhibit an extensive range of biological actions, specifically antidiabetic, anti-inflammatory, cytotoxic, hepatoprotective, cardiovascular, and antiparasitic effects [7].

Cucurbitacins are characteristic compounds in many species of cucurbits. These compounds are tetracyclic triterpenes arising from a rearrangement of the protostane cation and are unsaturated and polyfunctional oxygenated compounds and occur most often as glycosides. They are particularly toxic and bitter chemicals, and their cytotoxicity contributes to their toxicity [8]. Cucurbitanes are found in many plants.

The most significant mechanisms of the apoptotic effects of cucurbitacins are their ability to modify the mitochondrial transmembrane potential and transcriptional activity via nuclear factors or genes and their ability to activate or inhibit pro- or antiapoptotic proteins.

Similar to other plant-derived compounds, cucurbitacins exert toxic effects in different cancer cell lines by inducing apoptosis. The main mechanism of this induction is the ability to modify the mitochondrial transmembrane potential [6].

Therefore, some compounds obtained from Cucurbitaceae could be useful scaffolds for developing new drugs. We consider it necessary to review the main chemicals from this genus with potential anticancer activity.

2. Cucurbitacins tetracyclic triterpenoids

Cucurbitacins are primarily tetracyclic triterpenoids (**Figure 1**) that compose a class of biochemical compounds contained in plants of the family Cucurbitaceae, which include the Thai medicinal plants *Trichosanthes cucumerina* L. and *T. kirilowii* Maximowicz, the leaves and fruits of the Tunisian plant *Ecballium elaterium*; the fruits of *Cucurbita pepo* cv. dayangua; the roots of *Cayaponia tayuya* (Tayuya), which has long been used in folk medicines from Brazil, Peru, and Colombia; *C. racemosa* Cong., the roots of *Hemsleya amabilis*, an ancient Chinese remedy, *Cucumis melo* L., *Momordica balsamina* L. (balsam pear), *Cucurbita andreana* (winter squash), and *Citrullus colocynthis* (bitter cucumber). Below, the most important cucurbitacins that have shown interesting cytotoxicity and anticancer activity are listed.
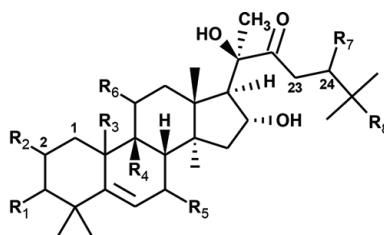
1': R_1= Ketone, R_2= -OH(S), R_3= -H(R), R_4= -CH$_3$(R), R_5= -H, R_6= Ketone, R_7= H, R_8= -OAc, 1-2= Simple, 23-24= Double(E)
2': R_1= Ketone, R_2= -OH(S), R_3= -H(R), R_4= -CH$_3$(R), R_5= -H, R_6= Ketone, R_7= H, R_8= -OAc, 1-2= Simple, 23-24= Simple
3': R_1= -OH(S), R_2= -OH(S), R_3= -H(R), R_4= -CH$_3$(R), R_5= -H, R_6= Ketone, R_7= H, R_8= -OH, 1-2= Simple, 23-24= Simple
4': R_1= Ketone, R_2= -OH(S), R_3= -H(R), R_4= -CH$_3$(R), R_5= -H, R_6= Ketone, R_7= H, R_8= -OAc, 1-2= Double, 23-24= Double(E)
5': R_1= Ketone, R_2= -OH(S), R_3= -H(R), R_4= -CH$_3$(R), R_5= -H, R_6= Ketone, R_7= H, R_8= -OH, 1-2= Simple, 23-24= Simple
6': R_1= Ketone, R_2= -OH(S), R_3= -H(R), R_4= -CH$_3$(R), R_5= -H, R_6= Ketone, R_7= H, R_8= -OH, 1-2= Double, 23-24= Double(E)
7': R_1= Ketone, R_2= -OH(S), R_3= -H(R), R_4= -CH$_3$(R), R_5= -H, R_6= Ketone, R_7= H, R_8= -OH, 1-2= Simple, 23-24= Double(E)
8': R_1= Ketone, R_2= -OH(S), R_3= -H(R), R_4= -OH(R), R_5= -H, R_6= Ketone, R_7= H, R_8= -OAc, 1-2= Simple, 23-24= Double(E)
9': R_1= -OH(R), R_2= -OH(S), R_3= -H(S), R_4= -CH$_3$(R), R_5= -H, R_6= -H, R_7= -H, R_8= -OH, 1-2= Simple, 23-24= Simple
10': R_1= Ketone, R_2= -Glucose-1-yl(S), R_3= -H(R), R_4= -CH$_3$(R), R_5= -H, R_6= Ketone, R_7= H, R_8= -(1-Ribose)Purine-6-yl, 1-2= Double, 23-24= Simple
11': R_1= Ketone, R_2= -Glucose-1-yl(S), R_3= -H(R), R_4= -CH$_3$(R), R_5= -H, R_6= Ketone, R_7= H, R_8= -1H-Purine-6-yl, 1-2= Double, 23-24= Simple
12': R_1= -OH(S), R_2= -OH(S), R_3= -H(R), R_4= -CH$_3$(R), R_5= -H, R_6= Ketone, R_7= H, R_8= -OAc, 1-2= Simple, 23-24= Simple
13': R_1= -OH(S), R_2= -OH(S), R_3= -H(R), R_4= -CH$_3$(R), R_5= -OH(R), R_6= Ketone, R_7= H, R_8= -OAc, 1-2= Simple, 23-24= Double(E)

Figure 1.
General structure of cucurbitacin tetracyclic triterpenoid.

2.1 Cucurbitacin B (1')

Cucurbitacin B (CuB) is an oxygenated tetracyclic triterpenoid compound (**1'**). The growth-inhibiting effect of CuB was evaluated on MCF-7 and MDA-MB-231 breast cancer cells and B16F10 melanoma cells by the MTT assay. This compound had antiproliferative effects against breast cancer cells in a dose-dependent manner, and the IC_{50} values for MCF-7 and MDA-MB-231 were 4.12 and 3.68 μM, respectively [9].

CuB potently suppressed the growth of four types of NSCLC cells (H1299, A549, HCC-827, and H661), inhibiting the proliferation of all the cell lines with IC_{50} values between 0.05 and 0.130 μM. The mean tumor volume at the end of the study in CuB-treated mice was 200 ± 111 mm^3, compared to 684 ± 321 mm^3 in the control group (average reduction of 70% in tumor volume (p < 0.05). No visible sign of toxicity was observed in CuB-treated mice [10]. CuB could suppress human NSCLC cell growth in vitro through its effects on the PI3Kinase and MAPK pathways, which lead to programmed cell death induction, as well as inhibition of cell migration and cell invasion [11]. Additionally, CuB induces cell cycle arrest in A-549 cells and causes DNA double strand breaks. It also produces DNA damage and G2/M phase arrest; this damage could be due to an increase in reactive oxygen species (ROS) formation [12].

The cytotoxic effect of CuB was tested on HeLa and U2OS cells, and the IC_{50} values were 12.2 and 17.07 μM, respectively. The inhibition of tubulin polymerization in vitro was observed with an IC_{50} > 1 mM [13]. CuB from the leaves of Tunisian *E. elaterium* exhibited an anticancer effect and displayed anti-integrin activity in human glioblastoma U87 cells, with no cytotoxicity observed at concentrations up to 500 nM. CuB affected the adhesion and migration of U87 cells to fibronectin with IC_{50} values of 86.2 and 84.6 nM, respectively. Time-lapse video-microscopy showed that CuB significantly reduced U87 cell motility and affected directional persistence. CuB also inhibited cell proliferation with an IC_{50} value of 70.1 nM, as determined using the crystal violet assay. Moreover, CuB inhibited in vitro human microvascular endothelial cell (HMEC) angiogenesis at concentrations up to 10 nM. Interestingly, this work demonstrated for the first time that this effect was specifically mediated by α5β1 integrins. These findings reveal a novel mechanism of action for cucurbitacin B, which displays potential as a specific anti-integrin drug [14].

2.2 23,24-Dihydrocucurbitacin B (DHCB) (2′)

C. tayuya (Cucurbitaceae) is a climbing lignified plant with a large tuber that has long been used in folk medicine as an anti-inflammatory, antitumor, and antirheumatic agent. DHCB (2′) was isolated from the roots of *C. tayuya* and assessed in isogenic colon cancer cell lines HCT116 and Hke-3 by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay. This compound induced apoptosis in both cell lines with IC_{50} values of 9.8 and 4.7 μM, respectively [15].

DHCB inhibited the viability of human cervical cancer cell lines with an IC_{50} of 40–60 μM, but its cytotoxic effects were less pronounced in normal epithelial fr2 and HerEpiC cells, where the IC_{50} was 125 μM. The underlying mechanisms were studied, and the results showed that DHCB induced apoptosis in HeLa cells and caused ROS-mediated shifts in the $\Delta\Psi$m. Additionally, DHCB caused cell cycle arrest in HeLa cells at the G2/M checkpoint. The phosphoinositide 3 kinase/protein kinase B/mechanistic target of rapamycin (PI3K/AKT/mTOR) cascade may play an important role in cancer tumorigenesis and progression and resistance to chemotherapy. The results indicated that DHCB decreased the expression of important proteins in the PI3K/Akt/mTOR cascade [16].

2.3 23,24-Dihydrocucurbitacin F (DHCF) (3′)

DHCF (3′) has been isolated from the roots of *H. amabilis*, an ancient Chinese remedy for bacillary dysentery, gastroenteritis, and cancer. While the toxicity of other cucurbitacins has been explored in several types of cancer, little data exist on the effect of DHCF on human cancers, including prostate cancer (PCa). Human PCa DU145, PC3, and LNCaP cells were treated with graded doses of DHCF in vitro, and the antiproliferative activity of this compound was determined using the MTS assay. DHCF inhibited the proliferation of all three PCa cell lines in a dose-dependent manner. The IC_{50} values of DHCF in DU145, PC3, and LNCaP cells were 15, 7, and 5 μM, respectively [17].

2.4 Cucurbitacin E (4′)

The inhibition of breast cancer metastasis in mouse models by CuE was reported. To evaluate the effect of CuE on the proliferation and apoptosis of inoculated 4T1 and MDA-MB231 cells in vivo, the expression of proliferating cell nuclear antigen (PCNA) and cleaved caspase-3 was tested by immunohistochemical analysis [18]. CuE targets the dissemination of breast cancer cells from the primary tumor but not the outgrowth of established micrometastases in target organs (lung, liver, between others). CuE exerts no significant effect on tumor cell apoptosis or proliferation in vivo [19].

CuE demonstrated cytotoxic activity against human oral squamous cell carcinoma SAS cells with an IC_{50} of 3.69 μM and induced the apoptosis of SAS cells after 24 h of treatment, but not MRC-5 or HS68 cells, which showed a dose-dependent reduction. Microscopic examination showed that following exposure to CuE (2.5 μM) for 6–24 h, the cells displayed a remarkable change in their morphology, and CuE induced the death of cancer cells [20].

The inhibitory effect of CuE on the proliferation of Bcap37 and MDA-MB-231 cells was assessed by the MTT assay. Breast cancer cells were treated with various concentrations (0, 0.1, 1, 10, and 100 μM) of CuE or DMSO as a control for 24, 48, and 72 h. The MTT method was then used to determine the number of viable cells. The data indicated that CuE inhibited cell growth in a concentration- and time-dependent manner (ANOVA, $p < 0.05$). After treatment with 0.1 μM CuE for

24 h, the growth of Bcap37 and MB-231 cells was significantly inhibited. At a CuE concentration of 100 µM, most of the cancer cells detached from the dish [21].

Additionally, CuE was evaluated on the chondrosarcoma SW 1353 cancer cell line, and the IC_{50} values indicated higher toxicity in this cell line than in the previously test lines (MTT assay). The amount of CuE that induced a mortality of 50% was calculated after 6, 12, and 24 h of treatment, and the results were 13.55, 12.65, and 9.16 µM, respectively [22]. The cytotoxic effect of CuE was tested on HeLa and U2OS cells, and the IC_{50} values were 6.43 and 15.07 nM, respectively. The inhibition of tubulin polymerization in vitro had an IC_{50} of 566.91 nM [13].

The effects of CuE from *E. elaterium* fruit on the expression of the BAX, caspase-3, LC3, and VEGF and c-MYC genes in the AGS cell line were investigated. The sub-G1 accumulation of AGS cells treated with CuE was increased compared to that of untreated cells. Moreover, the treatment of AGS cells with CuE-induced cell death. Additionally, the effects of CuE on the mRNA expression levels of the LC3, VEGF, BAX, caspase-3, and c-MYC genes were evaluated using qRT-PCR. LC3 mRNA levels were increased approximately 20-fold after treatment with CuE at a concentration of 0.1 µg/mL for 24 h. However, BAX, caspase-3, and c-MYC mRNA levels at these concentrations were not changed by the treatment [23, 24].

2.5 Cucurbitacin R (5′)

One additional cucurbitacin was discovered in the roots of *C. tayuya* and was identified as cucurbitacin R (CCR) (5′). This compound was experimentally assessed in isogenic colon cancer cell lines HCT116 and Hke-3 by the MTT assay and induced the apoptosis of both HCT116 and Hke-3 cells with IC_{50} values 37 and 27 µM, respectively [15].

2.6 Cucurbitacin I (6′)

CuI, also known as elatericin B or JSI 124, has been isolated from different plants, such as *M. balsamina* L. (balsam pear), *C. tayuya* (tayuya), *Cucurbita andreana* (winter squash), and *C. colocynthis* (bitter cucumber). This compound was tested in isogenic colon cancer cell lines HCT116 and Hke-3 and was found to induce apoptosis in both lines, with IC_{50} values of 0.29 and 0.09 µM, respectively [15].

The cytotoxicity IC_{50} values of CuI in SW 1353 cells after 6, 12, and 24 h of treatment were 7.93, 8.31, and 5.06 µM, respectively [22]. The cytotoxic activity of CuI against SW-480 human colon cancer cells was tested. In this case, CuI diminished cell proliferation in a concentration-dependent manner and increased apoptosis, enhancing cycle arrest at the G2/M phase [25].

The cytotoxicity of CuI was tested in HeLa and U2OS cells, and the IC_{50} values were 44.77 and 23.47 nM, respectively. The inhibition of tubulin polymerization in vitro had an $IC_{50} > 1$ mM [13]. The effects of CuI purified from *E. elaterium* fruit on the expression of the BAX, caspase-3, LC3, and VEGF and c-MYC genes in the AGS cell line were investigated. The sub-G1 accumulation of AGS cells treated with CuI was increased compared to that of untreated cells. Moreover, treatment of AGS cells with CuI-induced cell death. Additionally, the effects of CuI on the mRNA expression levels of the LC3, VEGF, BAX, caspase-3, and c-MYC genes were evaluated using qRT-PCR. After treatment, at a concentration of 0.5 µg/mL for 24 h, LC3 mRNA levels were increased approximately 25-fold, and VEGF mRNA levels were increased approximately 4.4-fold. However, BAX, caspase-3, and c-MYC mRNA levels were not considerably changed after treatment with CuI [23, 24].

2.7 Cucurbitacin D (7′)

CuD was evaluated in the chondrosarcoma SW 1353 cancer cell line. Its IC_{50} values against SW 1353 cells after 6, 12, and 24 h of treatment were 16.48, 13.03, and 13.14 μM, respectively [22].

The effects of CuD purified from *E. elaterium* fruit on the expression of the BAX, caspase-3, LC3, VEGF, and c-MYC genes in the AGS cell line were investigated. The sub-G1 accumulation of AGS cells treated with CuD was increased compared to that of untreated cells. Moreover, the treatment of AGS cells with CuD induced cell death. Additionally, the effects of cucurbitacin D on the mRNA expression levels of the LC3, VEGF, BAX, caspase-3, and c-MYC genes were evaluated using qRT-PCR. LC3 mRNA levels were increased approximately 23-fold after treatment with CuD at a concentration of 0.3 μg/mL for 24 h. However, the BAX, caspase-3, and c-MYC mRNA levels were not considerably changed after treatment. Regarding the effects of CuD and CuE on LC3 mRNA expression, CuD's effect was significantly greater than that of CuE [23].

2.8 Cucurbitacin A (8′)

The antiproliferative effects of Cucurbitacin A (CuA) on A-549 cells were determined by using the MTT assay. This compound exhibited a potent cytotoxic effect on A-549 cells. The assay was carried out at different concentrations of CuA (0, 10, 20, 40, 100, 150, and 200 μM) with incubation for 24 and 48 h. CuA showed inhibitory effects on cell proliferation in a dose- and time-dependent manner. However, the effect of the incubation time was more pronounced at higher doses of the compound. CuA also induced morphological changes in these cells, featuring chromatin condensation, cell shrinkage, and apoptotic body formation. G2/M phase cell cycle collapse was also induced by CuA along with inhibition of the expression levels of m-TOR/PI3K/Akt proteins [26].

2.9 2 β,3β,16α,20(*R*),25-Pentahydroxy-22-oxocucurbita-5-en (RPO) (9′)

This compound is a cucurbitacin isolated from *Cayaponia racemosa* Cong. The anticancer activity of RPO was evaluated with in vitro and in vivo models. Cucurbitacin (9′) reduced the number of viable HL-60 leukemia cells; however, there was no change in the number of nonviable cells at 5 μg/mL. This compound had no effect on normal proliferating lymphocytes at the concentrations tested (IC_{50} > 25 μg/mL). Morphological analysis of RPO-treated cells showed typical apoptotic features, such as heavy deposition of granules in the cytoplasm (eosinophilia), DNA fragmentation and irregularities in the plasma membrane, and intense vacuolization and disruption of the plasma membrane. Acridine orange/ethidium bromide staining confirmed these findings, revealing an increased number of apoptotic cells. In the sarcoma 180 tumor model, compound (9′) showed 52 or 62% antitumor activity when administered alone (25 mg/kg/day) or in association with the chemotherapeutic agent 5-FU (10 + 10 mg/kg/day), respectively. Moreover, treatment with compound (9′) either alone or in combination with 5-FU caused an increase in spleen weight and morphological alterations related to immunostimulatory properties [27].

2.10 Cucurbitaglycosides A (10′) and B (11′)

Phytochemical investigation of the fruits of *Cucurbita pepo* cv. dayangua led to the isolation of cucurbitaglycosides A (10′) and B (11′). This was the first report of
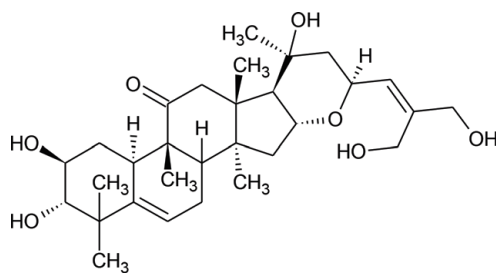
Figure 2.
2β,3β,20(S),26,27-Pentahydroxy-16α,23(S)-epoxycucurbita-5,24-dien-11-one.

cucurbitane triterpenoids with a purine unit. Cucurbitaglycosides A and B showed cytotoxic activity against the human epithelial carcinoma cell line HeLa with IC_{50} values of 17.2 and 28.4 μg/mL, respectively [28].

2.11 Hemslecin A (12′)

Three cucurbitane triterpenoids were isolated from *H. amabilis*, and the main compound was Hemslecin A (HA). This compound showed significant cytotoxic activity against HeLa cells with an IC_{50} value of 0.389 μM. Additionally, 7β-hydroxycucurbitacin F-25-O-acetate (13′) and 2β,3β,20(S),26,27-pentahydroxy-16α,23(S)-epoxycucurbita-5,24-dien-11-one (14′) (**Figure 2**) were isolated and showed less cytotoxic activity (IC_{50} values of 12.3 and 387 μM, respectively) than HA [29].

3. Cucurbitane-type triterpene glycosides

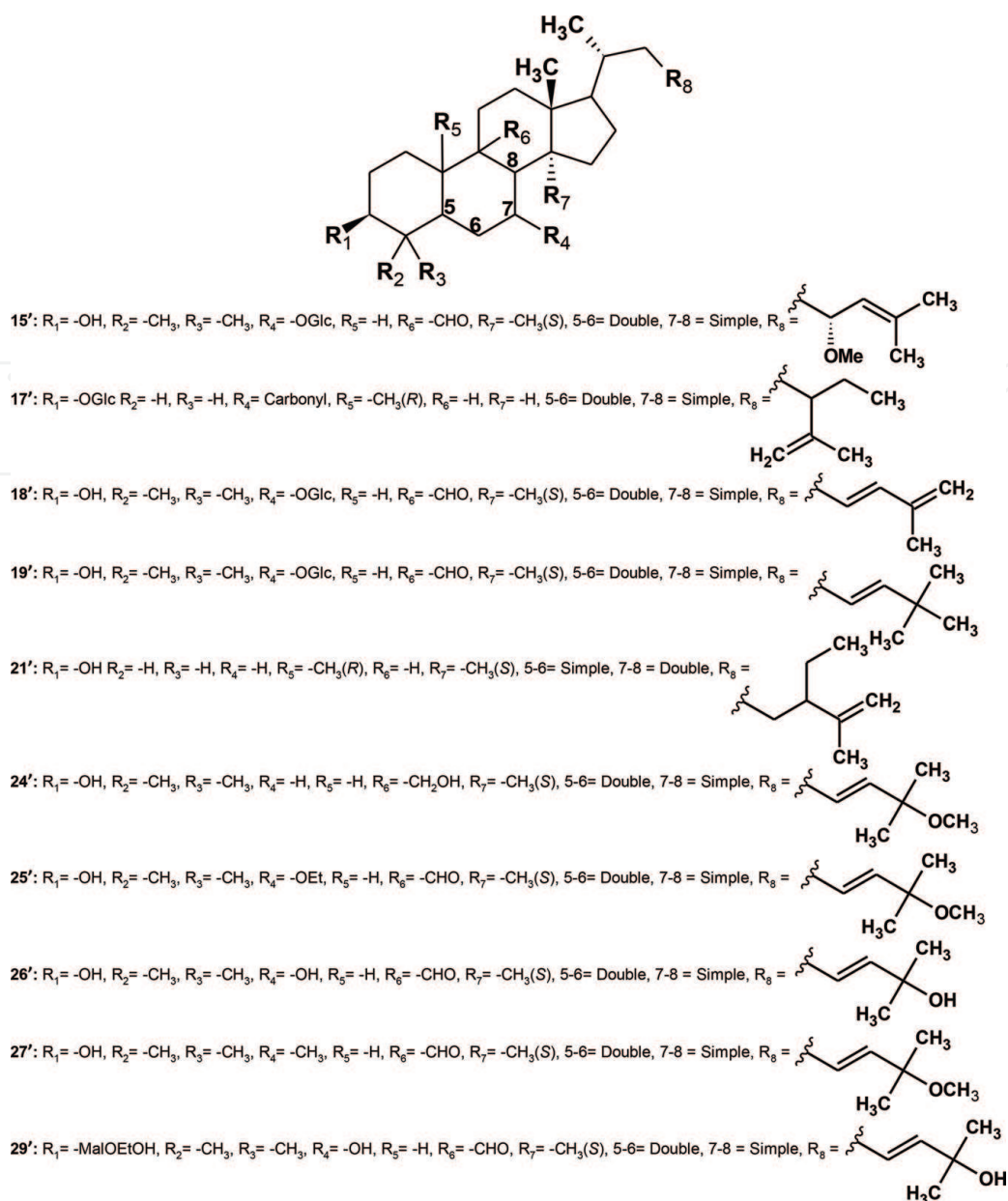Several new cucurbitane-type triterpene glycosides (**Figures 3** and **4**) have been isolated from the fruit pulp of *Momordica charantia* L., and their cytotoxic activity has been evaluated.

Two new cucurbitane-type triterpene glycosides, charantagenins D (15′) and E (16′), and one new sterol, 7-oxo-stigmasta-5, 25-diene-3-O-β-D-glucopyranoside (17′), were isolated from the fruit of *M. charantia* L. together with another six known compounds (18′–23′). The cytotoxic activities of the major isolated compounds were evaluated against the lung cancer cell line A549, glioblastoma cell line U87, and hepatoma carcinoma cell line Hep3B using in vitro MTT assays. Two new cucurbitane-type triterpenes, 25-methoxycucurbita-5,23(E)-diene-3β,19-diol (24′) and 7β-ethoxy-3β-hydroxy-25-methoxycucurbita-5,23(E)-dien-19-al (25′), together with three known cucurbitane-type triterpenes, 3β,7β,25-trihydroxycucurbita-5,23(E)-dien-19-al (26′), (23E)-3β-hydroxy-7β,25-dimethoxycucurbita-5,23-dien-19-al (27′), and 3β-hydroxy-25-methoxycucurbita-6,23(E)-dien-19,5β-olide (28′), were isolated from the fruit pulp of *M. charantia*. Their cytotoxic activity was evaluated against human hepatoma SK Hep-1 cells with etoposide as a positive control (IC_{50} = 3.7 μM). The results are shown in **Table 1** [19, 30].
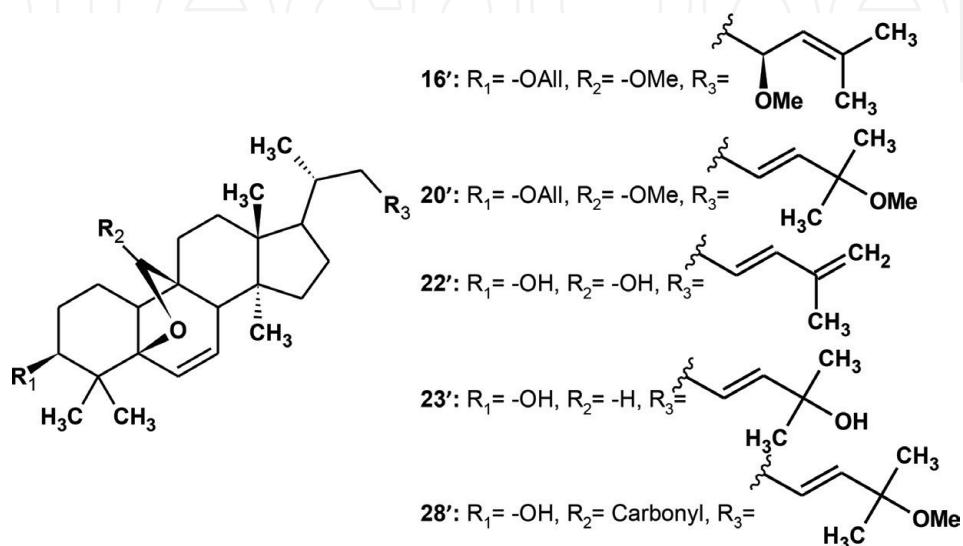
3.1 Momordicin VII (29′)

Several new cucurbitane triterpenoids were isolated from the stems and leaves of *M. charantia* and tested against five human cancer cell lines (HL-60, SMMC-7721, A-549, MCF-7, and SW480). Only momordicin VII (29′) was slightly active, with IC_{50} values of 16.2, 20.3, 20.5, 16.9, and 14.3 μM, respectively [31].

15′: R₁= -OH, R₂= -CH₃, R₃= -CH₃, R₄= -OGlc, R₅= -H, R₆= -CHO, R₇= -CH₃(S), 5-6= Double, 7-8 = Simple, R₈ =

17′: R₁= -OGlc R₂= -H, R₃= -H, R₄= Carbonyl, R₅= -CH₃(R), R₆= -H, R₇= -H, 5-6= Double, 7-8 = Simple, R₈ =

18′: R₁= -OH, R₂= -CH₃, R₃= -CH₃, R₄= -OGlc, R₅= -H, R₆= -CHO, R₇= -CH₃(S), 5-6= Double, 7-8 = Simple, R₈ =

19′: R₁= -OH, R₂= -CH₃, R₃= -CH₃, R₄= -OGlc, R₅= -H, R₆= -CHO, R₇= -CH₃(S), 5-6= Double, 7-8 = Simple, R₈ =

21′: R₁= -OH R₂= -H, R₃= -H, R₄= -H, R₅= -CH₃(R), R₆= -H, R₇= -CH₃(S), 5-6= Simple, 7-8 = Double, R₈ =

24′: R₁= -OH, R₂= -CH₃, R₃= -CH₃, R₄= -H, R₅= -H, R₆= -CH₂OH, R₇= -CH₃(S), 5-6= Double, 7-8 = Simple, R₈ =

25′: R₁= -OH, R₂= -CH₃, R₃= -CH₃, R₄= -OEt, R₅= -H, R₆= -CHO, R₇= -CH₃(S), 5-6= Double, 7-8 = Simple, R₈ =

26′: R₁= -OH, R₂= -CH₃, R₃= -CH₃, R₄= -OH, R₅= -H, R₆= -CHO, R₇= -CH₃(S), 5-6= Double, 7-8 = Simple, R₈ =

27′: R₁= -OH, R₂= -CH₃, R₃= -CH₃, R₄= -CH₃, R₅= -H, R₆= -CHO, R₇= -CH₃(S), 5-6= Double, 7-8 = Simple, R₈ =

29′: R₁= -MalOEtOH, R₂= -CH₃, R₃= -CH₃, R₄= -OH, R₅= -H, R₆= -CHO, R₇= -CH₃(S), 5-6= Double, 7-8 = Simple, R₈ =

Figure 3.
Structure of cucurbitane-type triterpene glycosides 15′, 17′-19′, 21′, 24′-29′.

16′: R₁= -OAll, R₂= -OMe, R₃=

20′: R₁= -OAll, R₂= -OMe, R₃=

22′: R₁= -OH, R₂= -OH, R₃=

23′: R₁= -OH, R₂= -H, R₃=

28′: R₁= -OH, R₂= Carbonyl, R₃=

Figure 4.
Structure of cucurbitane-type triterpene glycosides 20′, 22′, 23′, 28′.

| Cancer cell line	IC$_{50}$ values (μM)
A549 lung cancer cell line	**15'** = 1.07
	16' = 3.82
	17', 22', 23' = >100
	18' = 4.46
	19' = 4.89
	20' = 5.32
	21' = 15.10
U87 glioblastoma cell line	**15'** = 1.08
	16' = 67.32
	17',18',22',23' = >100
	19' = 0.60
	20' = 0.19
	21' = 8.65
Hep3B hepatoma carcinoma cell line	**15'** = 14.01
	16'–19',21'–23' = >100
	20' = 19.30
SKHep1 human hepatoma cell line	**24'** = 33.1
	25' = 24.3
	26' = >50
	27' = >13
	28' = 38.7

Table 1.
Cytotoxic activities of cucurbitane-type triterpene glycosides **15'–28'**.

4. Multiflorane-type triterpenes

Multiflorane-type triterpenes (**Figure 5**), a new class of cucurbitacins, were isolated from seeds of *Cucurbita maxima*, along with three known compounds from *Benincasa hispida* (Thunb.) Cogn., fruits that are widely consumed in China and tropical countries, and these compounds were found to exhibit interesting cytotoxicity activity.

Three new multiflorane-type triterpenes, 7α-methoxymultiflor-8-ene-3α, 29-diol-3-acetate-29-benzoate (**30'**), 7-oxomultiflor-8-ene-3α,29-diol-3-acetate-29-benzoate (**31'**), and multiflora-7,9(11)-diene-3α,29-diol-3-p-hydroxybenzoate-29-benzoate (**32'**), were isolated from seeds of *C. maxima*, along with three known compounds. These compounds exhibited cytotoxicity against HL-60 and P388 cells. Compound (**30'**) did not show significant cytotoxic activity, with an IC$_{50}$ > 100 μM in both lines, but compounds 31 and 32 showed cytotoxic activity against HL-60, with IC$_{50}$ values of 7.1 and 7.1 μM, respectively. The IC$_{50}$ values for P388 were 55.9 and 92.6 μM, respectively [32].

Analysis of *Benincasa hispida* (Thunb.) Cogn. fruits yielded three new triterpenoids, 3α,29-O-di-trans-cinnamoyl-D:C-friedooleana-7,9(11)-diene (**33'**), oleanolic acid 28-O-β-D-xylopyranosyl-[β-D-xylopyranosyl-(1→4)]-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside (**34'**), and oleanolic acid 28-O-β-D-glucopyranosyl-(1→3)-β-D-xylopyranosyl-[β-D-xylopyranosyl-
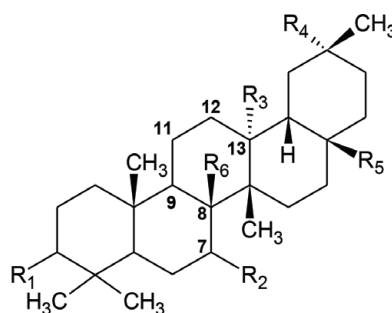
30′: R$_1$= -OAc, R$_2$= -OCH$_3$, R$_3$= -CH$_3$, R$_4$= -Benzoate, R$_5$= -CH$_3$, R$_6$= -, 7-8= Simple, 8-9=Double, 9-11= Simple, 11-12= Simple, 12-13= Simple

31′: R$_1$= -OAc, R$_2$= -Carbonyl, R$_3$= -CH$_3$, R$_4$= -Benzoate, R$_5$= -CH$_3$, R$_6$= -, 7-8= Simple, 8-9=Double, 9-11= Simple, 11-12= Simple, 12-13= Simple

32′: R$_1$= -(*p*-Hydroxy)benzoate, R$_2$= -H, R$_3$= -CH$_3$, R$_4$= -Benzoate, R$_5$= -CH$_3$, R$_6$= -, 7-8= Double, 8-9= Simple, 9-11= Double, 11-12= Simple, 12-13= Simple

33′: R$_1$= -*trans*-cinnamoyl, R$_2$= -H, R$_3$= -CH$_3$, R$_4$= --*trans*-cinnamoyl, R$_5$= -CH$_3$, R$_6$= -, 7-8= Double, 8-9= Simple, 9-11= Double, 11-12= Simple, 12-13= Simple

34′: R$_1$= -OH, R$_2$= -H, R$_3$= -H, R$_4$= --CH$_3$, R$_5$= -COO-Xyl-[(1→4)-Xyl]-(1→3)-Rha-(1→2)-Ara, R$_6$= -CH$_3$, 7-8= Simple, 8-9= Simple, 9-11= Simple, 11-12= Simple, 12-13= Double

35′: R$_1$= -OH, R$_2$= -H, R$_3$= -H, R$_4$= --CH$_3$, R$_5$= -COO-Glu-(1→3)-Xyl-[(1→4)-Xyl]-(1→3)-Rha-(1→2)-Ara, R$_6$= -CH$_3$, 7-8= Simple, 8-9= Simple, 9-11= Simple, 11-12= Simple, 12-13= Double
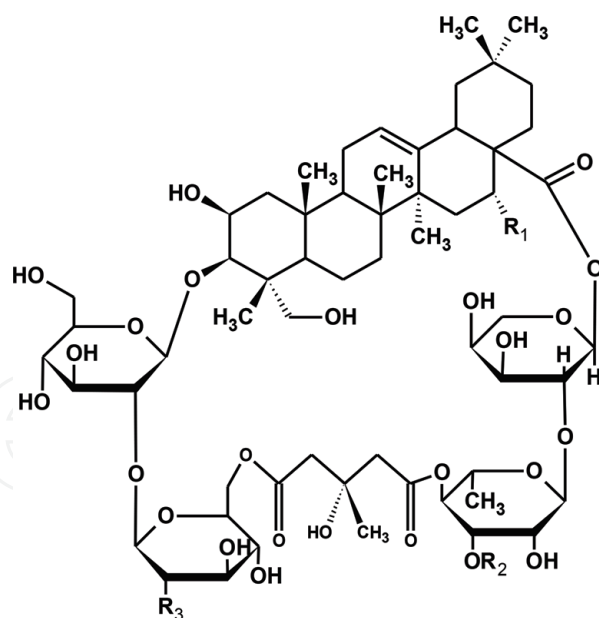
Figure 5.
Multiflorane-type triterpenes.

(1→4)]-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside (**35′**). The cytotoxic activities of compounds (**33′−35′**) were assessed by the MTT assay. These compounds showed no significant cytotoxic activity against HeLa human cervical, HL-60 human hepatoma, and SMMC-7721 human hepatoma cell lines [33].

5. Cyclic bisdesmosides

Cyclic bisdesmosides, new compounds analogous to cucurbitacins, share the tetracyclic triterpenoid core but contain carbohydrates to form a bicycle (**Figure 6**). These compounds were isolated from *Actinostemma lobatum* MAXIM and *Bolbostemma paniculatum* (Maxim) Franquet.

Two new cyclic bisdesmosides elucidated as lobatoside L (**36′**) and lobatoside M (**37′**) and four known cyclic bisdesmosides (**38′–41′**) were isolated from *A. lobatum* Maxim. The inhibitory effects of these six compounds on human cancer cell growth (including the esophageal squamous carcinoma cell line ECA109, lung cancer cell line A549, and gastric cancer cell line MGC-803) were determined using the MTT assay. The six compounds exhibited cytotoxicity against all the cell lines tested, and compounds (**36′, 38′,** and **40′**) showed significant activity in a dose-dependent manner against all the cell lines. The IC$_{50}$ values for compounds (**36′** and **40′**) against ECA-109 cells were 8.25 and 3.71 μM, respectively. The rest of the results are shown in **Table 2** [34].

Tubeimoside I (**42′**) isolated from *B. paniculatum* (Maxim) Franquet exhibited cytotoxic activity against HepG2 human cancer cells. The IC$_{50}$ values at different times of exposure (24, 48, and 72 h) were 15.5, 11.7, and 9.2 μM, respectively. This compound induced shrinkage, nuclear condensation, fragmentation, and cell cycle arrest in phase G2/M. The inhibition of growth in this case is mediated by a cascade of apoptosis [35].

Figure 6.
Cyclic bisdesmosides structure.

| Cancer cell lines	IC$_{50}$ values (μM)
Esophageal squamous carcinoma cell line ECA-109	36′ = 8.25
	37′ = 74.14
	38′ = 22.37
	39′ = 71.97
	40′ = 3.71
	41′ = 83.53
Lung cancer cell line A549	36′ = 26.52
	37′ = 69.28
	38′ = 27.27
	39′ = 77.79
	40′ = 35.12
	41′ = 77.02
Gastric cancer cell line MGC-803	36′ = 35.33
	37′ = 84.71
	38′ = 36.85
	39′ = 90.49
	40′ = 35.21
	41′ = 96.55

Table 2.
Cytotoxic activities of cyclic bisdesmosides 36′–41′.

6. Gypenosides

The gypenosides (**Figure** 7) were isolated from *Gynostemma pentaphyllum* and from the aerial parts of *G. pentaphyllum*.

Gypenoside L (**43′**) and gynogenin (**44′**), 20(*S*)-dammar-24-en-2a,3b,12b,20-tetrol, were isolated from *G. pentaphyllum* and tested against A-549 lung carcinoma cells. The IC_{50} values were 34.94 and 12.54 µg/mL, respectively [36]. Some gypenosides were isolated from the aerial parts of *C. pentaphyllum* (**45′–51′**), and their structures were elucidated with spectroscopic and chemical methods. Their cytotoxic activity was determined against different human cancer cell lines, A549, HT-29, MCF-7, and SK-OV-3. All the compounds showed low activity, with IC_{50} values between 62.8 and 19.6 µM [37].
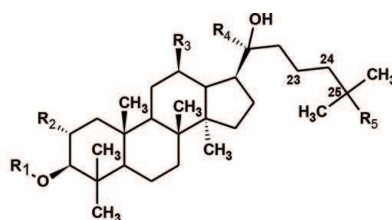
Gypenoside L (43′): R_1= -S^1, R_2= -OH, R_3= -OH, R_4= -CH$_3$, R_5= -, 23-24= Simple, 24-25= Double
Gynogenin (44′): R_1= -OH, R_2= -OH, R_3= -OH, R_4= -CH$_3$, R_5= -, 23-24= Simple, 24-25= Double
45′: R_1= -S^2, R_2= -H, R_3= -H, R_4= -S^3, R_5= -, 23-24= Simple, 24-25= Double
46′: R_1= -S^2, R_2= -H, R_3= Carbonyl, R_4= -CH$_3$, R_5= -, 23-24= Simple, 24-25= Double
47′: R_1= -S^4, R_2= -H, R_3= Carbonyl, R_4= -Glu-1, R_5= -, 23-24= Simple, 24-25= Double
48′: R_1= -S^2, R_2= -H, R_3= Carbonyl, R_4= -Glu-1, R_5= -OH, 23-24= Double, 24-25= Simple
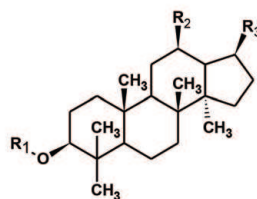
49′: R_1= -S^2, R_2= -OH, R_3= -S^5
50′: R_1= -S^2, R_2= Carbonyl, R_3= -S^5
51′: R_1= -S^2, R_2= -H, R_3= -S^6
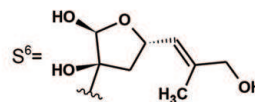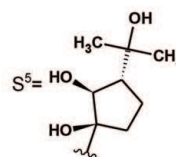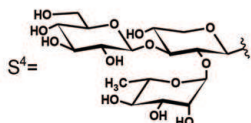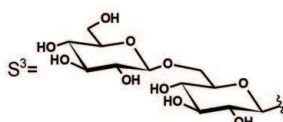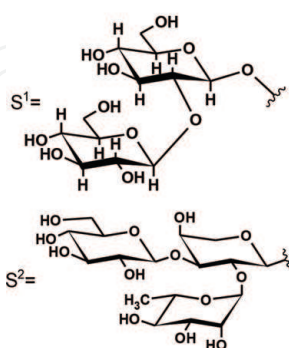
Figure 7.
Gypenosides structure.

7. Other substances of interest

In addition to the cucurbitacins, other substances isolated from the Cucurbitaceae family have been identified, including proteins isolated from the sarcocarp of *Cucurbita moschata* (pumpkin) and from the root of *T. kirilowii* Maxim

Figure 8.
Overall structure of cucurmosin in stereo view.

(Cucurbitaceae). Additionally, the protein from the root of *T. kirilowii* Maxim has been demonstrated to possess important cytotoxicity activity.

7.1 Cucurmosin

A novel type-1 ribosome-inactivating protein (RIP) designated cucurmosin was isolated from the sarcocarp of *Cucurbita moschata* (pumpkin). Its structure contains two domains: a large N-terminal domain composed of seven α-helices and eight β-strands and a smaller C-terminal domain consisting of three α-helices and two β-strands (**Figure 8**).

Cucurmosin was tested for its cytotoxicity against human leukemia cells (K562), murine melanoma cells (B16), lung adenocarcinoma cancer cells (A549), and peripheral blood lymphocytes using the standard MTT assay. The IC_{50} values of cucurmosin were 88.1, 63.4, and 359.3 nM in human leukemia cells (K562), murine melanoma cells (B16), and lung adenocarcinoma cancer cells (A549), respectively, while its IC_{50} in normal cells (peripheral blood lymphocytes) was higher than 1.4 μM [38].

7.2 Trichosanthin

Trichosanthin was isolated from the roots of *T. kirilowii* Maxim and has been used in traditional Chinese medicine. This compound was tested against HepG-2 and WRL 68 cells, and the IC_{50} values obtained were 10.38 and 15.45 μmol/L, respectively [39].

Tianhua, an extract of *T. kirilowii*, was analyzed to determine its mechanism of action against lung cancer cells (A549) using the MTS assay. This compound induced apoptosis via an anti-telomerase effect but had no effect on stimulating peripheral lymphocytes to produce interferon (IFN)-γ; tianhua inhibited the metastatic ability of cells and inhibited the growth of cancer cells in vivo [40].

8. Conclusion

The aim of this review is to present 51 cucurbitacin compounds and two compounds with different structures isolated from Cucurbitaceae plants, their chemical structures, their biological activities, and the mechanisms by which these compounds reduce the proliferation of cancer cells.

Conflict of interest

The authors declare that there is note conflict of interest.

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