Sesquiterpenes: Natural Products That Decrease Cancer Growth

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Abstract: Despite recent advances in our understanding of the biological processes leading to the development of cancer, there is still a need for new and effective agents to help bring this disease under control. One of the oldest and most effective strategies for developing new chemotherapeutics is the isolation and evaluation of chemicals of natural origin. The importance of natural products for drug discovery has been impressive: One has to only look at the number of clinically active drugs that are used in cancer therapy to see how many are either natural products or are based on natural products. It is also apparent that materials from natural sources are excellent probes (indicators) for cellular targets that, when modulated, may have a deleterious effect upon the survival or proliferation of tumor cells. And the search goes on.

Sesquiterpenes are a class of naturally occurring molecules that have demonstrated therapeutic potential in decreasing the progression of cancer. These molecules are 15-carbon isoprenoid compounds that are typically found in plants and marine life. Although this class of compounds has frequently provided encouraging leads for chemotherapeutics, they have not been evaluated as potential anticancer agents. In this review, we provide a current overview of sesquiterpenoids that have potential as anticancer agents.

INTRODUCTION

Over three-quarters of the world's population rely mainly on plants and plant products for health care. Recent World Health Organization (WHO) studies indicate that over 30% of the world's plant species have at one time or another, been used for medicinal purposes. Of the 250, 000 higher plant species on Earth, more than 80, 000 species are medicinal [1]. Although traditional medicine is widespread throughout the world, it is an integral part of each individual culture. Its practice is based mainly on traditional beliefs handed down from generation to generation for hundreds or even thousands of years. Unfortunately, much of this ancient knowledge and many valuable plants are being lost at an alarming rate. The scientific study of traditional medicines and the systematic preservation of medicinal plants are thus of great importance.

Natural products have been regarded as important sources of potential chemotherapeutic agents. Over 50% of anticancer drugs approved by United States Food and Drug Administration since 1960, have originated from natural sources, especially terrestrial plants. Data from cytotoxicity bioassays indicate that more than 400 compounds were isolated from plants, marine organisms, and microorganisms between 1996 and 2003. Recently, interest of natural product research has slowly moved to plants and marine organisms. As a result, almost 50% of reported cytotoxic compounds–acetogenins, alkaloids and terpene skeletons–have been isolated from natural products as reported by the FDA [2].

CANCER

Cancer occurs as a result of alterations in normal growth and homeostasis. At the cellular level, these abnormalities include increased proliferation, evasion of apoptosis, sustained angiogenesis and the ability to invade and metastasize to regions distant from the primary site. Acquisition of these traits is a direct or indirect consequence of genomic instability, which confers selective advantages upon mutant cells. Tumor development is highly dependent on the interactions between incipient tumor cells and their neighbors, as well as with the microenvironment in which they exist. Conversion to the malignant phenotype, though dependent on the mutations acquired, ultimately leads in the absence of successful intervention, to the establishment of metastasis(es) and death. It is thus no surprise that cancer is a major cause of deaths worldwide [3].

Despite the marked success that has been attained in the treatment of cancer, a continuous demand still exists for new and effective anticancer agents. One of the oldest and most effective strategies that has emerged from the quest for chemotherapeutics has been the testing of natural substances of plant and animal origin for biological activity. Many of the more effective natural substances tested, belong to the class of molecules known as terpenes.

TERPENES

To date, over 30, 000 natural terpenoids have been identified. Many of these are found in plants, where they play important roles in the ecological chemistry of interactions with insects and pathogens. Terpenoids accumulate to high levels in some plant species and are significant components of essential oils that have found important uses in the flavor and fragrance industry. Many of these terpenoids have proved to be of immense pharmaceutical importance, and consequently, a substantial effort is underway to identify and characterize novel terpenes and assess their biological activity. Terpenes, or terpenoids, are classified according to their skeletal structure; sesquiterpenes represent a large fraction of these naturally occurring terpenes.

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SESQUITERPENES

Sesquiterpenoids are a group of 15-carbon compounds derived from the assembly of 3 isoprenoid units. Found mainly in higher plants, they are represented by several acyclic, mono-, bi-, tri-, and tetracyclic systems (Fig. **1**). Many of them have biological activity, including antimicrobial, antitumor, and cytotoxic properties. In plants, they play important ecological roles in interactions with insects and microbes and act as attractants, deterrents, antifeedants and phytoalexins.

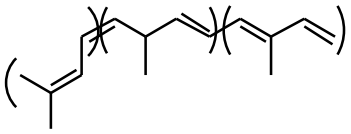

Fig. (1). General structure of sesquiterpene.

The tens of thousands of sesquiterpenes known today are derived metabolically from some 300 distinct C_{15}- hydrocarbon skeletons, which in turn are produced from the single substrate farnesyl diphosphate (FPP) by the action of sesquiterpene synthases. It is known that these enzymic cyclizations proceed by ionization of the diphosphate group of FPP to form an enzyme-bound allylic cation, which then undergoes isomerization before cyclization to yield new cations. Subsequent rearrangements and final quenching of the carbocations by elimination or by reaction with water yield the various C_{15} hydrocarbon and alcohol products that typically result from sesquiterpene synthase activity.

1) Acyclic sesquiterpenes

Since the early years of research into natural products from marine sources, marine algae and invertebrates have yielded an impressive number of novel sesquiterpenes with new carbocyclic skeletons and unusual functionalities. Acyclic sesquiterpenoids have been less frequently encountered, being mainly obtained from green algae of the order Caulerpales and from sponges. Data concerning the pharmacological potential of acyclic sesquiterpenoids are, in general, scarce. A rare exception is the extract from the gorgonian *Plexaurella grisea*, which contains five new acyclic sesquiterpenes (Fig. **2**), (3*E*, 5*E*)-3, 7, 11-trimethyl-9-oxododeca-1, 3, 5-triene (**3**), (3*Z*, 5*E*)-3, 7, 11-trimethyl-9-oxododeca-1, 3, 5-triene (**4**), (3*E*)-6-acetoxy-3, 11-dimethyl-7-methylidendodeca-1, 3, 10-triene (**5**), (3*E*, 5*E*)-7-hydroxy-3, 7, 11-trimethyldodeca-1, 3, 5, 10-tetraene (**6**), and (3*E*, 5*E*, 9*E*)-8, 11-diacetoxy-3, 7, 11-trimethyldodeca-1, 3, 5, 9-tetraene (**7**), and two new linear norsesquiterpenes, (2*E*, 4*E*, 7*Z*)-2, 6, 10-trimethylundeca-2, 4, 7, 9-tetraenal (**8**) and (2*E*, 4*E*)-2, 6, 10-trimethylundeca-2, 4, 9-trienal (**9**), in addition to the previously known compounds **1**, **2**, and **10** [4].

With the exception of compound **8**, which was inactive (IC_{50} > 10 μg/mL), the other new sesquiterpenes and the one norsesquiterpene exhibited a mild cytotoxic activity, with IC_{50} values of 2.5 to 5 μg/mL. Of these, norsesquiterpene **9** exhibited the greatest and selective activity against the P388 cell line, with an IC_{50} of 0.5 μg/mL.

2) Monocyclic sesquiterpenes

Bisabolanes are monocyclic sesquiterpenes possessing a 1, 4-functionalized cyclohexane skeleton. α-Bisabolol (**11**) (Fig. **3**) is a small, oily sesquiterpene alcohol, isolated from the essential oils of a variety of plants, shrubs, and trees. Only a few scientific reports describing the biological effects of α-bisabolol have thus far been published [5].

This compound was found to have a strong time- and dose-dependent cytotoxic effect on human and rat glioma

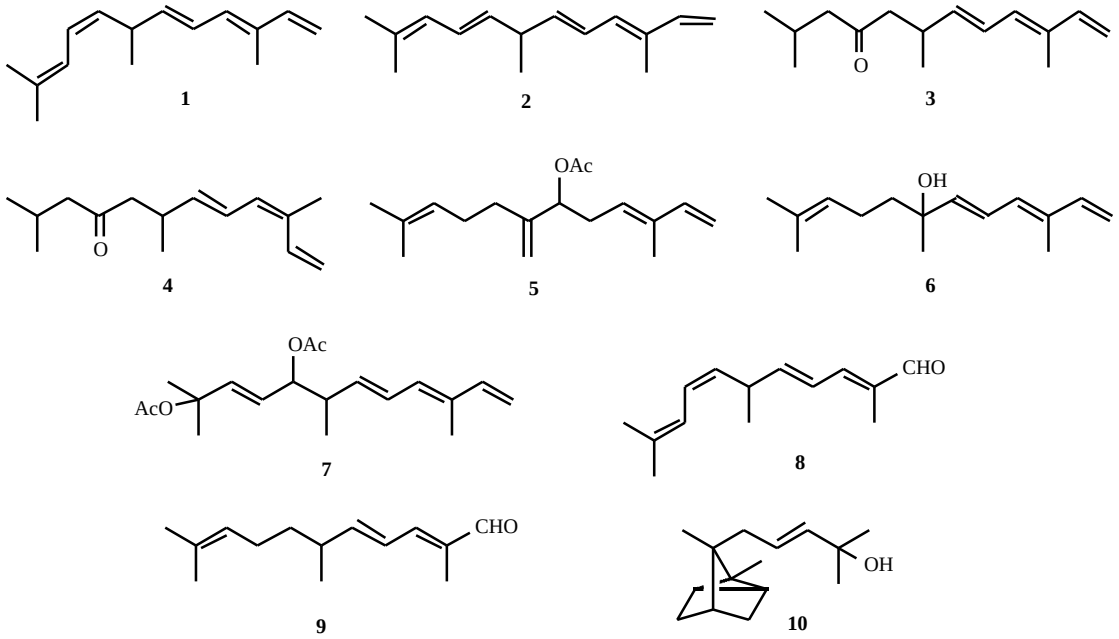

Fig. (2). Acyclic sesquiterpenes.

cells: A high concentration of α-bisabolol (10 μM) resulted in 100% cell death of glioma cells. The cytotoxicity triggered by α-bisabolol resulted from apoptotic induction through an intrinsic pathway. These experimental results suggested that α-bisabolol has promise as a novel compound that is able to inhibit glioma cell growth and survival.

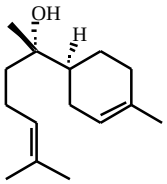

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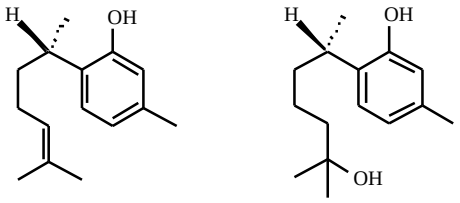
Fig. (3). α-Bisabolol.

Two other bisabolane type sesquiterpene phenols, (+)-curcuphenol (**12**) and (+)-curcudiol (**13**), have been isolated from a marine sponge *Didicus* sp. and found to have cytotoxic properties indicative of a p53-independent mechanism (Fig. **4**) [6].

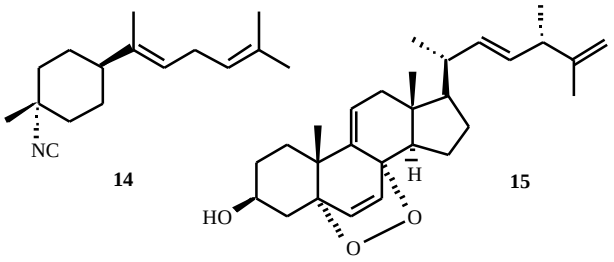


12 **13**

Fig. (4). Bisabolane-type sesquiterpene from the marine sponge *Didicus* sp.

The activity of (+)-curcuphenol has recently been tested on a panel of isogenic HCT-116 cells. It showed nearly identical activity in both p53+/+ and p53-/- cell lines. Another report has described a new bisabolane-type sesquiterpenoid, (*E*)-3-isocyanobisabolane-7, 10-diene (**14**), and a new epidioxyergostane-type steroid, 9(11)-dehydroaxinysterol (**15**) Fig. (**5**), both isolated from the Okinawan sponge of the genus *Axinyssa* [7]. Epidioxysterol (**15**) was found to show significant growth inhibitory effects against human cancer cell lines.

Compound **15** exhibited a strong growth inhibitory effect against some ovarian cancer cells, with an IC_{50} of 0.19 μg/ml



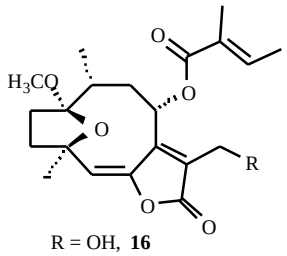
14 **15**

Fig. **(5)**. Bisabolane-type sesquiterpenes from the sponge of the genus *Axinyssa*.

($logGI_{50,}$ 26.20) in OVCAR-3 cells and an IC_{50} of 0.22 μg/ml ($logGI_{50,}$ 26.14) in OVCAR-8 cells.

Vernolides

Another class of monocyclic sesquiterpenoids with antitumor activity is the vernolides. Bioassay-directed fractionation of an ethanolic extract of stems of *Vernonia cinerea* has resulted in the isolation of two novel sesquiterpene lactones, vernolide-A (**16**) and B (**17**) (Fig. **6**) [8].

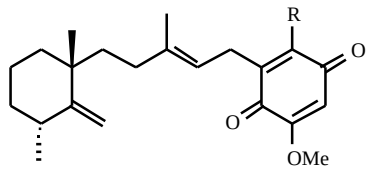


R = OH, **16**
R = OAc, **17**

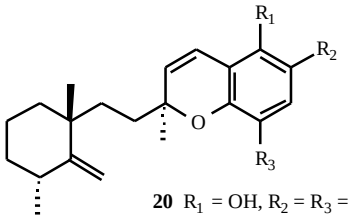
Fig. (6). Vernolides A and B.

Biological evaluation showed that **16** has potent cytotoxicity against human KB, DLD-1, NCI-661, and HeLa tumor cell lines (ED_{50} values of 0.02, 0.05, 0.53, and 0.04 μg/ml, respectively); **17** had marginal cytotoxicity (ED_{50} values of 3.78, 5.88, and 6.42 μg/ml for KB, NCI-661, and HeLa cells, respectively).

In addition to metachromins (**18**), (**19**) and (**26**), two new sesquiterpene hydroquinones, hippochromins A and B (**20**, **21**), have been isolated from the Taiwanese marine sponge *Hippospongia metachromia* (Fig. **7**) [9].



18 R = OH
19 R = OMe



20 R_1 = OH, R_2 = R_3 = OMe
21 R_1 = R_2 = OH, R_3 = OH
22 R_1 = R_3 = OH, R_2 = OH
23 R_1 = R_2 = OAc, R_3 = OMe
24 R_1 = R_3 = OAc, R_2 = OMe
25 R_1 = OAc, R_2 = R_3 = OMe

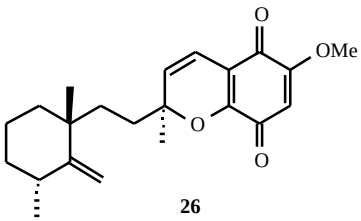


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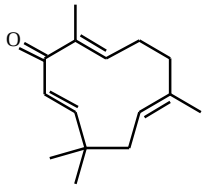
Fig. (7). Sesquiterpenes from the sponge *Hippospongia metachromia.*

When the cytotoxicities of these sesquiterpenes were evaluated *in vitro* against human tumor cell lines, **18** and **19**, hippochromin A diacetate **23**, and metachromin B monoacetate **25,** all exhibited potent cytotoxicities against human colon (COLO-205) tumor cells at concentrations of 0.1, 0.26, 0.22, and 0.53 μg/mL, respectively. These four sesquiterpenoids also inhibited the growth of nasopharyngeal (KB) tumor cells, with IC_{50} values of 1.8, 0.68, 3.06, and 1.32 μg/mL, respectively. However, the other isolated compounds were inactive.

Zerumbones

Another monocyclic sesquiterpene with potential relevance for the drug market is zerumbone. An active constituent of ginger root, is a monocyclic sesquiterpene with potent anticancer and anti-HIV activity. A sesquiterpene **27 (**Fig. **8**) from the edible plant *Zingiber zerumbet* has been found to suppress tumor promoter 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced Epstein-Barr virus activation in a potent manner [10].



27

Fig. (8). Zerumbone.

The anti-inflammatory and chemopreventive potential of **27** has been explored in a variety of cell culture experiments [10]. It inhibited the proliferation of several human colonic adenocarcinoma cell lines (LS174T, LS180, COLO205, and COLO320DM) in a dose-dependent manner, with the growth of normal human dermal (2F0-C25) and colon (CCD-18 Co) fibroblasts being affected to a lesser extent. This compound also induced apoptosis in COLO205 cells. Intriguingly, α-humulene, a structural analog lacking only the carbonyl group in **27**, was virtually inactive in all experiments conducted, indicating that the α, β-unsaturated carbonyl group of **27** may play a pivotal role in interactions with unidentified target molecule(s). Taken together, the results indicated that **27** is a good phytochemical with distinct potential for use in anti-inflammation, chemoprevention, and chemotherapy strategies.

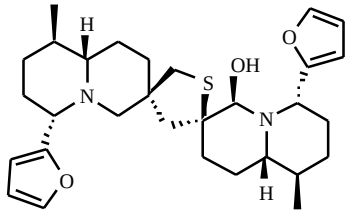
Further studies revealed that pretreatment(s) with zerumbone suppressed leukocyte infiltration and reduced proliferating cell nuclear antigen-labeling indices [11], indicating that zerumbone is a promising agent for the prevention of both tumor-initiating and -promoting processes through induction of anti-oxidative and phase II drug-metabolizing enzymes, as well as attenuation of proinflammatory signaling pathways.

A more recent and detailed study has provided biological evidence that **27** has a significant ability to suppress oxidative stress, possibly through induction of endogenous antioxidants such as the phase II xenobiotic metabolizing enzymes and glutathione reductase (GSH). Considering the importance of oxidative damage in carcinogenesis, the antioxidant effect of **27,** points to its potential use as a cancer chemopreventive agent targeting inflammation-related cancers such as skin and colon cancer [12].

3) Bicyclic Sesquiterpenes

Nupharidine

Potent antitumor activity has also been observed in compounds belonging to the Nupharidine family. A methanolic extract and its alkaloid fraction from the rhizomes of *Nuphar pumilum* have been shown to inhibit invasion of B16 melanoma cells across collagen-coated filters *in vitro* [13]. Dimeric sesquiterpene thioalkaloids with the 6-hydroxyl group, (6-hydroxythiobinupharidine, 6, 6'-dihydroxythiobinupharidine, and 6-hydroxythionuphlutine B) (**28**) (Fig. **9**), showed potent activity, with IC_{50} values of 0.029, 0.087, and 0.36 μM, respectively; other dimeric sesquiterpene thioalkaloids showed only weak activity.



28

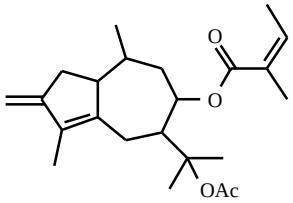
Fig. (9). Nupharidine-type sesquiterpene from *Nuphar pumilum.*

The alkaloid fraction (20 mg/kg/d, po) and the principal dimeric sesquiterpene thioalkaloid 6-hydroxythiobinupharidine (5 mg/kg/d, po) significantly inhibited lung tumor formation by more than 90% at 10 days after injection of B16 melanoma cells into mice.

Torilin

Dried ripe fruits of *Torilis japonica* have anti-fungal, antihelminthic, and anti-trichomonas effects. The bicyclic sesquiterpene torilin **29** (Fig. **10**) was isolated from the fruits of *Torilis japonica* and was found to have potent MDR-reversing activity in MDR cells *vs.* drug-sensitive cells. Torilin has also been found to potentiate the cytotoxicities of adriamycin, vinblastine, taxol and colchicine against multidrug-resistant KB-V1 and MCF7/ADR cells [14]. In addition, it significantly enhances the cytotoxicity of anticancer drugs such as doxorubicin, paclitaxel, colchicine, and vinblastine against MDR cells.

Another study has clearly elucidated the anti-angiogenic activity of torilin in chorioallantoic membrane (CAM) assays and mouse Matrigel plug assays [15]. Structural analysis has revealed that torilin is a guaiane-type sesquiterpene. Its anti-angiogenic activity has been demonstrated in CAM assays. It significantly inhibited the developmental neovascularization of chick embryo up to 54% at 50 μg/egg, but it had little anti-angiogenic activity below 20 μg. Vessels already formed by vasculogenesis during embryonic development were not affected by torilin, but newly synthesized vessels sprouted from pre-existing ones were markedly inhibited as the CAM extended.



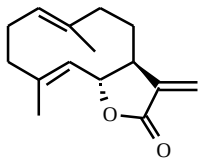
29

Fig. (10). Torilin.

When co-administered with anti-cancer drugs, torilin may strongly suppress tumor growth by increasing the cytotoxicity of tumors *via* its potent MDR-reversing activity, and also interfere with the survival of tumor cells through its strong anti-angiogenic activity. A later report from the same group [16] investigated the anti-invasive properties of this compound. Interestingly, it was found to completely block intravasation of HT1080 human fibrosarcoma cells inoculated on the CAM of chick embryo. The activity and expression of matrix metalloproteinase-9 (MMP-9), which is very important in tumor invasion and metastasis, were also decreased by torilin treatment. Therefore, it is possible that torilin may decrease the metastatic potential of tumor cells by inhibiting their attachment to endothelial cells and intravasation to blood vessels. Thus, torilin may strongly suppress tumorigenesis by inhibiting tumor invasion.

Costunolides

A major group of cytotoxic sesquiterpenes is the class germacranolides. A potent molecule in this series is costunolide, an active sesquiterpene lactone found in medicinal herbs with anti-inflammatory and potential anti-cancer activity. It was first shown in 1972 that an ethanolic extract of *Michelia champaca* and a petroleum ether extract of *Talauma ovata* have activity against human epidermoid carcinoma in the nasopharynx test system [17]. The active constituents in these two extracts are sesquiterpene lactones, identified as parthenolide and costunolide.



30

Fig. (11). Costunolide-type sesquiterpene.

The modifying effects of costunolide **30 (**Fig. **11**) on intestinal carcinogenesis were later examined in a rat model using azoxymethane. The results suggested that the natural sesquiterpene, found in plants that have been used as drugs in the Orient, could be a promising chemopreventive agent for human intestinal neoplasia [18].

Further evidence of chemoprevention by costunolide was provided in 2001 in a report investigating the effects of costunolide on cellular activation induced by a tumor-promoting phorbol ester, TPA [19]. iNOS promoter-dependent reporter gene activity was significantly increased by TPA, and the TPA-induced increase in reporter gene activity was efficiently reduced by costunolide, with an IC_{50} of approximately 2 μM. The addition of sulfhydryl (SH) compounds effectively abrogated the inhibitory effects of costunolide, suggesting the involvement of its reactivity with SH groups of target proteins and/or thiol-depleting properties.

Another report investigated the effect of costunolide on cellular differentiation in the human promyelocytic leukemia HL-60 cell culture system. Costunolide markedly increased the degree of HL-60 leukemia cell differentiation when simultaneously combined with 5 nM 1, 25-dihydroxyvitamin D_3 (1, 25-$(OH)_2D_3$) [20].

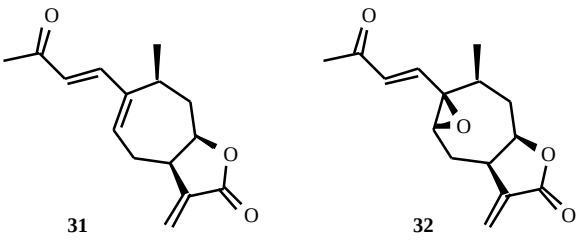
Costunolide by itself had very weak effects on the differentiation of HL-60 cells. Cytofluorometric analysis and cell morphological studies indicated that costunolide potentiated 1, 25-$(OH)_2D_3$-induced cell differentiation, predominantly into monocytes. Inhibitors of PKC, PI3-K, and ERK markedly inhibited HL-60 cell differentiation induced by costunolide in combination with 1, 25-$(OH)_2D_3$.

It has also recently been reported that costunolide may have anti-angiogenic properties. It selectively inhibited the endothelial cell proliferation induced by vascular endothelial growth factor (VEGF). Furthermore, it was also found to inhibit the VEGF-induced chemotaxis of human umbilical vein endothelial cells (HUVECs) in a dose-dependent manner [21]. From these results it was hypothesized that costunolides might inhibit angiogenesis by blocking the angiogenic factor signaling pathway. The results suggested that the molecule may prove useful for the development of a novel angiogenesis inhibitor.

A recent study also showed that costunolide exerts a dose-dependent antiproliferative activity in human breast cancer MCF-7 cells [22]. In addition, light microscopy observations indicated that costunolide affects nuclear organization and reorganized microtubule architecture. The antiproliferative and antimicrotubular effects of costunolide were not influenced by paclitaxel, a well-known microtubule-stabilizing anticancer agent. The microtubule-interacting activity of costunolide was confirmed by *in vitro* studies on purified microtubular protein. In fact, costunolide demonstrated polymerizing ability, by inducing the formation of well-organized microtubule polymers.

Xanthanolides

Two xanthanolide sesquiterpene lactones, 8-*epi*-xanthatin (**31**) and 8-*epi*-xanthatin epoxide (**32**) (Fig. **12**), isolated from the leaves of *Xanthium strumarium* (Compositae), demonstrated significant inhibition of the proliferation of cultured human tumor cells, i.e., A549 (non-small cell lung), SK-OV-3 (ovary), SK-MEL-2 (melanoma), XF498 (central nervous system), and HCT-15 (colon) cells *in vitro* [23]. They were also found to inhibit the farnesylation process of human lamin-B by farnesyltransferase (FTase) in a dose-dependent manner *in vitro* (IC_{50} values were calculated as 64 and 58 μM, respectively). Due to the relatively high concentrations of **31** and **32** required to obtain an FTase inhibition when compared to those necessary for a cytotoxic effect on tumor cells, it remains unclear whether a relationship between these two activities exists.



31 **32**

Fig. (12). Xanthanolide-type sesquiterpene from *Xanthium strumarium.*

In conclusion, the strong inhibitory effect of the leaves of *X. strumarium* (Compositae) on the proliferation of cultured human tumor cells *in vitro* may be predominantly due to the presence of large amounts of **31** and the oxidized artifact of **31**, 8-*epi*-xanthatin epoxide (**32**).

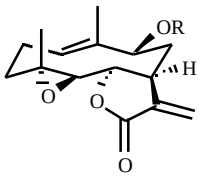
Three new xanthanolides, **33**-**35** Fig. (**13**), along with nine known compounds, have been isolated from the aerial parts of *Carpesium longifolium*. The structures of the new compounds were elucidated as 1β, 4β-epoxy-5β-hydroxy-10αH-xantha-11(13)-en-12, 8β-olide (**33**), 1β, 4β, 4 α, 5β-diepoxy-10α,H, 11α,H-xantha-12, 8β-olide (**34**), and 4-acetoxy-1β, 5β-epoxy-10αH-xantha-11(13)-en-12, 8β-olide (**35**) by spectroscopic methods. Parthenolide and michelenolide exhibited significant cytotoxic activity against cultured SMMC-7721 (human hepatoma) and HO-8910 (human ovarian carcinoma) cells [24].

Because of their abundance in the plant, parthenolide, 11β,13-dihydroparthenolide, michelenolide, and 5-hydroxy-4', 7-dimethoxydihydroflavone were evaluated for their cytotoxic activity against cultured SMMC-7721 (human hepatoma), HO-8910 (human ovarian carcinoma), and LO2 (human hepatocyte) cells using the MTT assay. Cytotoxic activity data indicated that parthenolide and michelenolide had significant cytotoxic activity against SMMC-7721 and HO-8910 cells. The α, β-unsaturated lactone is apparently the key active center in these compounds.

Parthenolides

Another series of germacranolides with potent antitumor activity is a group of sesquiterpenes called parthenolides. Initial studies performed in 1973 suggested that this group of molecules may have potent cytotoxic properties. The principal active component of this plant has been used conventionally to treat migraines, inflammation, and tumors. However, the antitumor effects of parthenolide and the mechanism(s) involved are poorly understood. Parthenolide has been shown to effectively inhibit hepatoma cell growth in a tumor cell-specific manner and to trigger apoptosis of hepatoma cells [25]. Parthenolide triggered apoptosis in invasive sarcomatoid hepatocellular carcinoma cells (SH-J1), as well as in other ordinary hepatoma cells at 5-10 μM, and also arrested cell growth (at G_2/M phase) at sublethal concentrations (1-3 μM). The sensitivity of tumor cells to parthenolide appears to result from the low expression level of the multifunctional detoxification enzyme glutathione *S*-transferase π in these cells. Parthenolide apparently mimics the effects of IκBα by inhibiting NF-κB DNA binding activity and Mn-superoxide dismutase (SOD) expression and by increasing paclitaxel-induced apoptosis of breast cancer cells. These results suggest that active ingredients of herbs with anti-inflammatory properties may be useful in increasing the sensitivity to chemotherapeutic drugs of cancers with constitutively active NF-κB.

The aerial structures of *Inula verbascifolia* subsp *methanea* have yielded three new epoxygermacranolides, compounds **36**-**38** (Fig. **14**), in addition to the previously known 9β-hydroxyparthenolide. The *in vitro* cytotoxic activity of compounds **36**-**38** was evaluated against six human solid tumor cell lines [26].

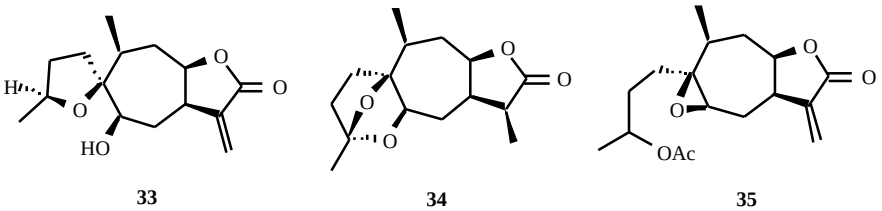


36 R = $COCH_2C(CH_3)(OH)CH_2CH_3$
37 R = $COCH_2C(CH_3)(OH)CH_3$
38 R = $COCHC(CH_3)CH(OH)CH_3$

Fig. (14). Germacranolide-type sesquiterpenes from *Inula verbascifolia* subsp. *Methanea.*

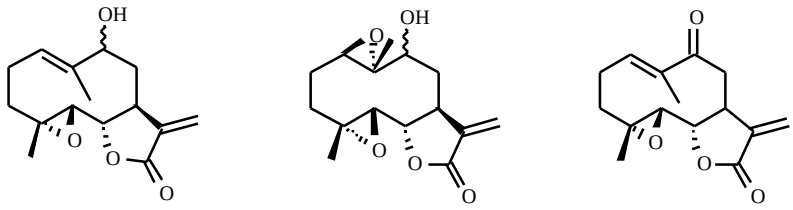
These three new compounds showed potent activity against three colon cancer cell lines and PC-3 androgen-insensitive cells, but only marginal or no significant activity against MCF-7 and LNCaP cells, which express estrogen and androgen receptors, respectively. The most active compound was **38**, with an IC_{50} of 0.39 μg/mL against the HCT-116 colon carcinoma cell line [26].

The aerial parts of *Anvillea garcinii* have yielded two new germacranolides, 9 α-hydroxy-1β, 10 α-epoxyparthenolide (**42**) and parthenolid-9-one (**43**), in addition to the known 9α-hydroxyparthenolide (**39**), 9β-hydroxyparthenolide (**40**), and 9β-hydroxy-1β, 10 α-epoxyparthenolide (**41**) (Fig. **15**) [27].



33 **34** **35**

Fig. (13). Xanthanolide-type sesquiterpenes from *Carpesium longi-folium.*



39
40

41
42

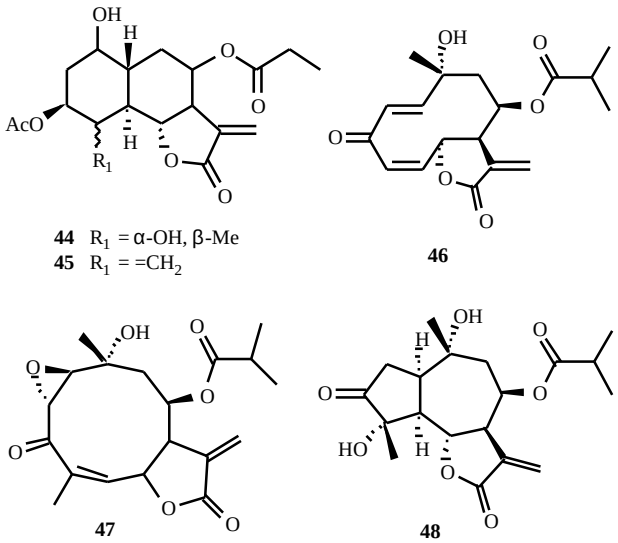
43

Fig. (15). Germacranolide-type sesquiterpenes from *Anvillea garcinii.*

Compounds **39-43** were evaluated by the National Cancer Institute (NCI) in an *in vitro* disease-oriented antitumor screen, which determines cytotoxic effects against a panel of approximately 60 human tumor cell lines. Compound **43** showed the highest activity (ED_{50} 0.05-4.38 μg/mL), consistent with the report by Kupchan and coworkers that increasing the degree of unsaturation increases the activity. Compounds **39-42** showed moderate to significant activity.

Tithofolinolide

Previous phytochemical investigations of *Tithonia diversifolia* have resulted in the isolation of cadinane, chromene, eudesmane, flavone and germacrane, and rearranged eudesmane derivatives. As part of an ongoing search for novel plant-derived cancer chemopreventive agents, the methanolic extract of the aerial structures of *T. diversifolia* was found to exhibit several significant biological activities in preliminary screening, including antiproliferative activity (in a sulforhodamine B assay with human colon cancer [Col2] cells), cellular differentiation-inducing activity with human promyelocytic leukemia (HL-60) cells, and inhibitory activity in a 7, 12-dimethylbenz[*a*]anthracene (DMBA)-induced mouse mammary organ culture assay system [28]. Among these isolates, **46** and **47** showed significant antiproliferative activity; **44**, **45**, and **48** induced HL-60 cellular differentiation; and **45** significantly inhibited (by 63 % at 10 μg/mL) lesion formation in the organ culture assay (Fig. **16**).



44 R_1 = α-OH, β-Me
45 R_1 = =CH_2

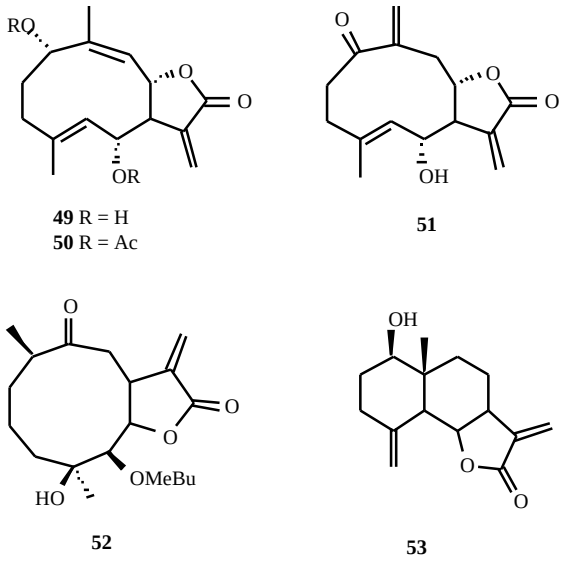
46

47

48

Fig. (16). Tithofolinalide from *T. diversifolia.*

It has also been reported that exposure to sesquiterpene lactones of the germacranolide type elicits apoptosis in HL-60 and U937 cells [29].



49 R = H
50 R = Ac

51

52

53

Fig. (17). Germacranolide-type sesquiterpenes.

All the sesquiterpene lactones tested (**49-53;** Fig. **17**) were found to inhibit the growth and cell viability of HL-60 and U937 cells in culture, as determined by the MTT dye-reduction assay. Compounds **50** and **52** were found to be the most potent, while **49** and **53** were least potent. The potency of these sesquiterpene lactones in inhibiting proliferation in HL-60 cells was: **50**>**52**~**51**>**49**~**53.** For U937 cells, the potency was: **52**>**50**>**51**~**49**>**53**, as determined by two independent experiments. In both cell lines, the germacranolide **50**, tatridin A diacetate, was the most potent apoptosis inducer among the assayed sesquiterpene lactones, while assayed sesquiterpene lactones displayed similar IC_{50} values.

In a bioassay-guided search for cytotoxic compounds from higher plants in South Korea have come four new sesquiterpenes of the germacranolide type, named cardivins A (**54**), B (**55**), C (**56**), and D (**57**) (Fig. **18**). These compounds have been isolated from the aerial parts of *Carpesium divaricatum* [30] and their structures elucidated by spectroscopic techniques. Compounds **54**, **55**, **56**, and **57** had cytotoxic effects on five human tumor cell lines, A-549 (non-small cell lung), SK-OV-3 (ovary), SK-MEL-2 (skin), XF-498 (central nervous system), and HCT-15 (colon).

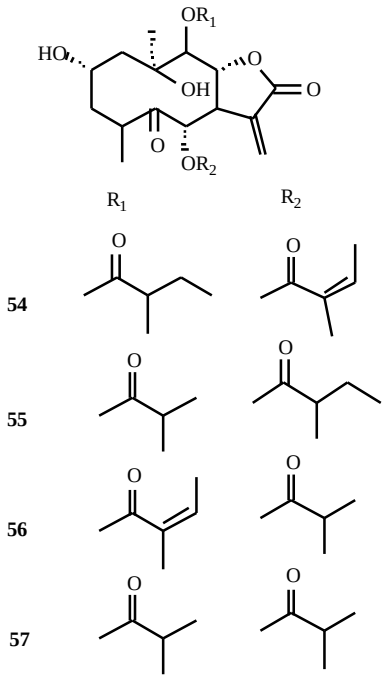


Fig. (18). Germacranolide-type sesquiterpenes from *Carpesium divaricatum.*

Thapsigargin

Thapsigargin (**58**) (Fig. **19**), a plant-derived sesquiterpene lactone and the skin-irritating principle of the Mediterranean herb *Thapsia garganica* (Apiaceae), is an extremely tight-binding inhibitor of intracellular calcium (SERCA) pumps. It has been shown that in contrast to its well-known inhibition of the catalytic cycle as a whole, thapsigargin binding actually activates phosphate-HOH oxygen exchange by enhancing the hydrolysis of the well-known phosphoenzyme E2P.

Thapsigargin (**58**) inhibits the sarcoplasmic and endoplasmic reticulum Ca^{2+}-ATPases (SERCA) [31]. Inhibition of SERCA causes an increase in the cytosolic Ca^{2+} concentration, which eventually leads to programmed cell death irrespective of the cell phase. Consequently, thapsigargin (**58**), in contrast to most chemotherapeutics, also affects slowly proliferating cells. The ubiquitous presence of SERCA makes thapsigargin (**58**) a general cell toxin.

Several cancer cell types are known to secrete characteristic proteases. For example, prostate cancer cells secrete the protease prostate specific antigen (PSA) and express prostate-specific membrane antigen (PSMA) on their surfaces. Both of these proteases have unique substrate specificity and are only active in close vicinity of these cells. Conversion of **58** into a derivative containing an anchoring point for a pep-

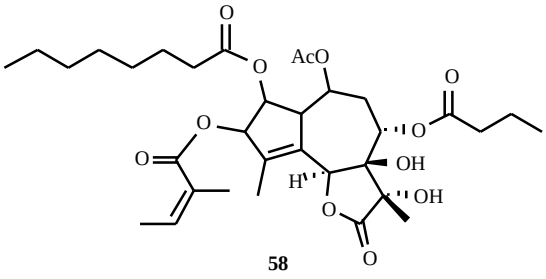
Table 1. Cytotoxicity (ED50, μg/mL)[a] of 1-4 Against Human Tumor Cell Lines

Compound	Cell Lines[b]				
	A-549	SK-OV-3	SK-Mel-2	XF-498	HCT-15
1	3.17	1.47	1.16	1.40	1.28
2	3.71	2.24	1.45	1.69	2.06
3	3.12	1.60	1.08	1.31	1.38
4	8.36	3.78	1.88	3.05	3.00
Doxorubicin	0.12	0.13	0.11	0.23	2.40

ED_{50} values of cardivins A-D (54-57) are shown in Table 1.

[a] ED_{50} value of compounds against each cancer cell line, which was defined as the concentration (μg/mL) that caused 50% inhibition of cell growth *in vitro*.

[b] Cell lines: A-549, non small cell lung cancer; SK-OV-3, ovarian cancer; SK-Mel-2, melanoma; XF-498, CNS cancer; and HCT-15, colon cancer.



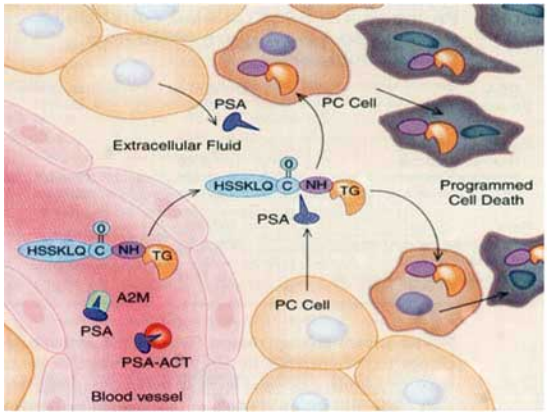


Fig. (19). Thapsigargin and the mechanism of action of its prodrug.

tide and coupling a substrate for PSA or PSMA to this anchoring point have enabled the preparation of prodrugs selectively targeted against prostate cancer cells. Denmeade and coworkers [32] coupled a chemically modified form of thapsigargin, L12ADT, to a peptide carrier that is a substrate for the prostate-specific antigen (PSA) protease, producing a water-soluble, cell-impermeant latent prodrug that is specifically activated extracellularly within metastatic prostate cancer sites by PSA. This group then analyzed the kinetics of PSA hydrolysis of the prodrug, the *in vitro* cytoxicity of the prodrug against PSA-producing LNCaP human prostate cancer and PSA non-producing HCT-116 human colon cancer cells, and the *in vivo* pharmacokinetics of the prodrug in mice.

The L12ADT peptide prodrug was hydrolyzed efficiently by PSA, was selectively toxic to PSA-producing prostate cancer cells *in vitro*, and was stable in human plasma. A single dose of 7 mg/kg resulted in a peak serum prodrug concentration of 15.4±1.1 μM and a half-life of approximately 2.8 h. Over 24 h, less than 0.5% of the free L12ADT was observed in plasma. The levels of prodrug and liberated L12ADT in prostate cancer xenograft tumors were approximately eight-fold and six-fold higher than the *in vitro* LD_{50} values. Prostate cancer xenograft tumors in mice treated with prodrug by i.v. administration were growth-inhibited without substantial host toxicity [32].

Prodrug administration in mice produced complete growth inhibition of established PSA-producing prostate cancer xenograft tumors but had no effect on PSA non-producing renal carcinoma xenograft tumors.

Cadinene

The soft corals of the genus *Xenia* are rich in terpenoids and steroids. As part of a search for bioactive substances from marine organisms, the Formosan soft coral *Xenia puerto-galerae* (family Xeniidae) was studied because CH_2Cl_2 extracts had shown significant cytotoxicity against A549 (human lung adenocarcinoma), HT-29 (human colon adenocarcinoma), and P388 (mouse lymphocytic leukemia) cell cultures [33]. Bioassay-guided fractionation resulted in the isolation of six new cadinene sesquiterpenoids, xenitorins A-F (**59**-**64**). Xenitorin A (**59**) exhibited cytotoxicity against A-549 cells, and xenitorin E (**63**) showed cytotoxicity against P388 and A-549 cells.

Xenitorin A (**59**) exhibited cytotoxicity against A-549 cells, with an ED_{50} of 0.79 μg/mL, and xenitorin E (**63**) showed cytotoxicity against P388 and A-549 cells, with ED_{50} values of 3.69 and 1.86 μg/mL, respectively. The ED_{50} values for xenitorins B-D (**60**-**62**) and xenitorin F (**64**) against P388 and A-549 cells were >50 μg/mL.

Parviflorene

A novel natural sesquiterpene–dimer-type compound, parviflorene A (**65**) (Fig. **21**), has been isolated from the extract of a tropical Zingiberaceous plant, *Curcuma parviflora*. Parviflorene A (**65**) possesses an unprecedented asymmetrical bis-cadinane skeleton [34].

Parviflorene A (**65**) exhibited cytotoxity against murine leukemia P388 cells, and the IC_{50} values against vincristine-resistant P388 cells in the presence and absence of 12.5 μg/mL of vincristine were 3.2 and 3.0 μg/mL, respectively, while the IC_{50} against a sensitive P388 strain was 3.2 μg/mL,

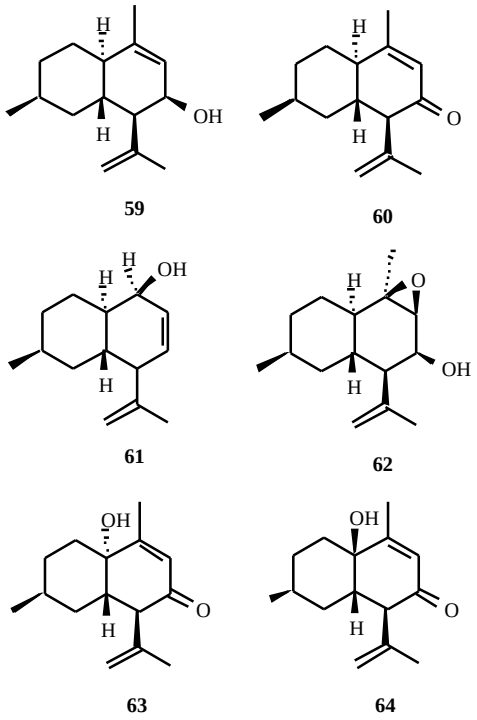


Fig. (20). Cadinene-type sesquiterpenes from *Xenia puerto-galerae.*

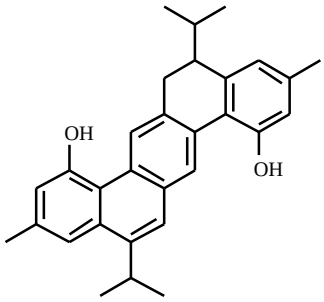


Fig. (21). Parviflorene A.

indicating that compound **65** had no effect in reversing multidrug resistance. Compound **65** also exhibited cytotoxicity against B16 melanoma cells, with an IC_{50} value of 4.1 μg/mL. Six new terpenoids (two maaliane-type sesquiterpenoids, **66** and **67**, one aromadendrane-type sesquiterpenoid, **68**, one noraromadendrane-type sesquiterpenoid, **69**, and two neodolabellane-type diterpenoids, **70** and **71)** have been isolated from the soft coral *Clavularia koellikeri* (Fig. **22**) [35]. Compound **71** exhibited a modest growth-inhibition effect *in vitro* toward tumor cells.

Compounds **70** and **71** are the rare neodolabellane-type diterpenoids, which include neodolabellin from *Clavularia koellikeri* and neodolabellenol from *Clavularia inflata.* Compound **71** exhibited a modest growth-inhibitory activity

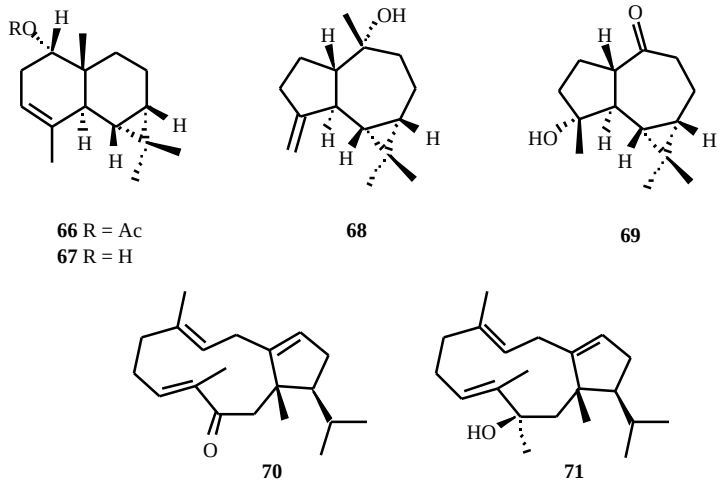


Fig. (22). Sesquiterpenes from *Clavularia koellikeri.*

in vitro against a number of cancer cell lines: lung cancer (NCI-H522, GI_{50} 5.2 μg/mL), melanoma (LOX-IMVI, GI_{50} 4.9 μg/mL), stomach cancer (MKN74, GI_{50} 5.2 μg/mL), and central nervous system cancer (SF-539 and SNB75, GI_{50} 4.9 μg/mL for both) cell lines, evaluated in the Japanese Foundation for Cancer Research 39-cell line assay.

Reticulidins

Two new sesquiterpenes, reticulidins A (**72**) and B (**73**) (Fig. **23**), containing the rare functional group $N{=}CCl_2$, have been isolated together with known congeners from the nudibranch *Reticulidia fungia* and their structures elucidated by spectroscopic data.

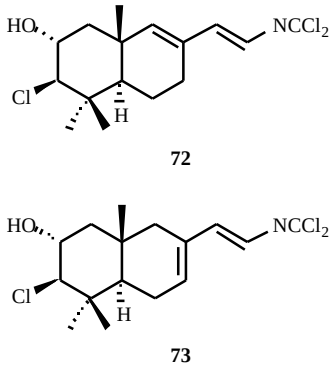


Fig. (23). Reticulidins from *Reticulidia fungia.*

Both reticulidins were moderately cytotoxic, with IC_{50} values 0.41 and 0.42 μg/mL, respectively, against KB cells, and 0.59 and 0.11 μg/mL against L1210 cells [36].

Suberosols

Screening for biologically active metabolites from the Taiwanese gorgonian *Subergorgia suberosa* yielded four new caryophyllene-type sesquiterpenes, suberosols A-D (**74-77**), along with two known metabolites, buddledins C (**78**) and D (**79**) (Fig. **24**). This coral has been found to contain a novel tricyclopentanoid cardiotoxin, subergorgic acid (**80**); four analogs of subergorgic acid; a cytotoxic sesquiterpene, suberosenone (**81**); and new 9, 11-secosterols. Cytotoxicity of metabolites **74-79** toward P-388 (mouse lymphocytic leukemia), A549 (human lung adenocarcinoma), and HT-29 (human colon adenocarcinoma) cancer cell lines has been reported [37].

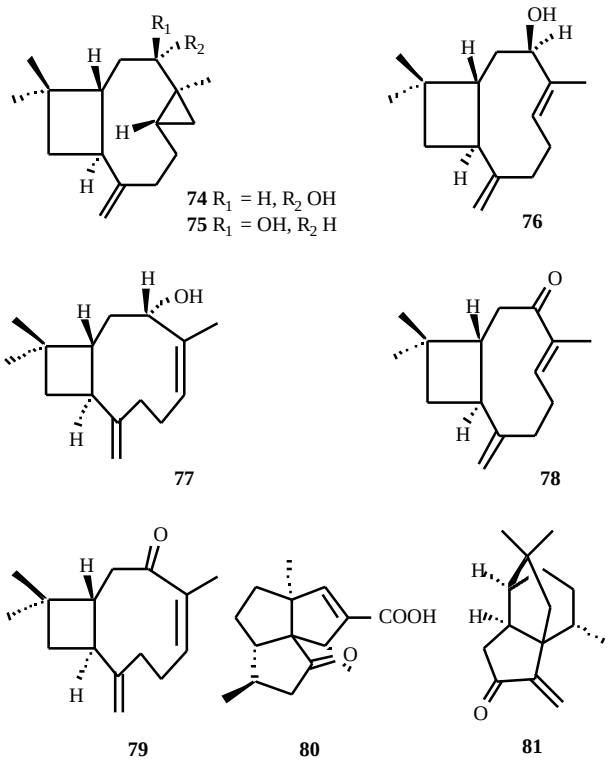


Fig. (24). Sesquiterpenes from *Subergorgia suberosa.*

The results revealed that metabolites **74**, **76**, and **77** have significant cytotoxicity against P-388 cancer cells; compound **78** was toxic to A549 cancer cells; and compounds **76-78** showed significant activity against HT-29 cells.

Aristolochic Acid

Aristolochia heterophylla (*Aristolochia shimada*) is a perennial shrub found in thickets and forests in mainland China and Taiwan. The fruit and root have been used in traditional Chinese medicine. Three new sesquiterpene esters of aristolochic acid, aristoloterpenate-II (**83**), -III (**84**) and –IV (**85**), as well as a known compound, aristoloterpenate-I (**82**), have been isolated from the root and stem of *A. heterophylla* (Fig. **25**) [38].

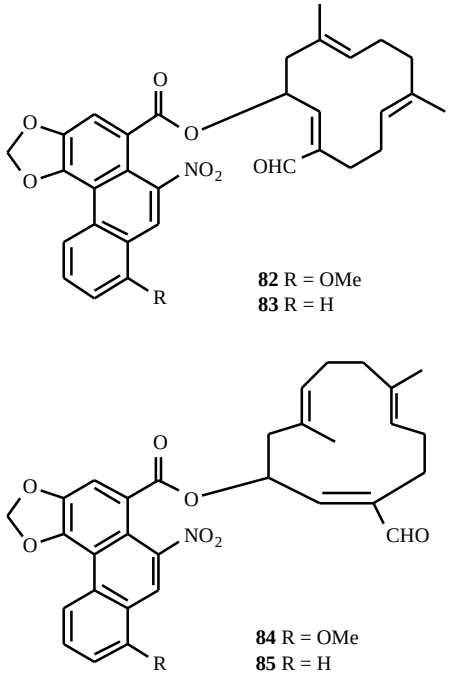
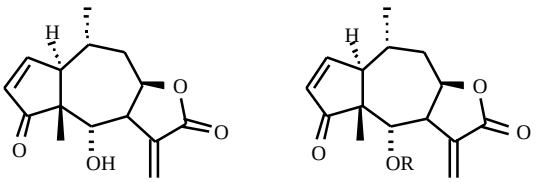


Fig. (25). Esters of aristolochic acid.

Compounds **82-85** had IC_{50} values of 4.83, 8.23, 5.44, and 7.53 μM, respectively, against hepatoma G2, 2, 2, and 15 cells.

Helenalins

The sesquiterpene lactone helenalin, found in several plant species of the Asteraceae family, is a potent anti-inflammatory and antineoplastic agent. Treatment of human platelets with helenalin (**86**) has been shown to cause irreversible inhibition of LTC_4 synthase in a concentration- and time-dependent manner, with an IC_{50} of 12μM after a 60-min preincubation (Fig. **26**) [39].



86 **87**

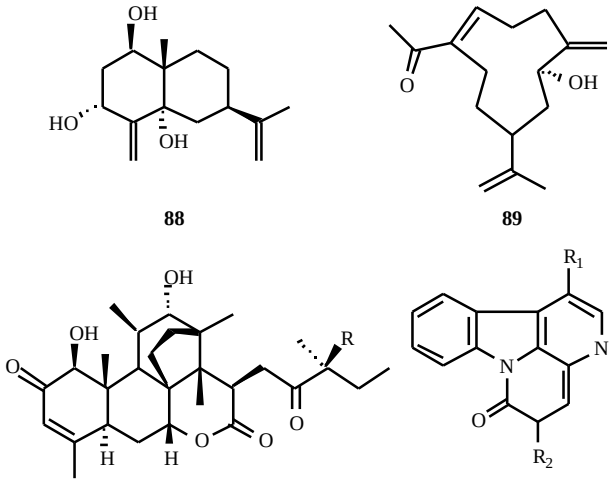
Fig. (26). Helenalin.

This effect was not related to decreased GSH levels or cytotoxicity.

Esters of helenalins have also been investigated [38], and the results of this research have suggested that a parent molecule can be made more cytotoxic by increasing the lipophilic character and/or by adding groups (such as conjugated esters) that might act as alkylating agents. The esters **87a-g** (Fig.**26**), as well as dimethylamine adducts of the esters, were synthesized and tested *in vitro* against cells derived from human epidermoid carcinoma of the larynx (H.Ep.-2). Six of the esters were tested *in vivo* against the Ehrlich ascites carcinoma, with the maximum effective dose being 38.2 pmol/kg/day [40]. It is of interest to note that both **5**-fluorouracil and helenalin are highly effective against the ascites tumor at the same concentration. Moreover, the fact that all survivors showed essentially 100% inhibition of the ascites tumor tends to suggest that each of these compounds could act as an effective inhibitor, but at a lower dose level than that for helenalin (Table **2**).

Leitneridanin

Two new sesquiterpenes, leitneridanin A (**88**) and leitneridanin B (**89**), and seven known compounds, lirioresinol B, (-)-pinoresinal, (+)-lariciresinol, quassimarin (**90**), simalikalactone D (**91**), 1-methoxycanthinone (**92**), and 5-methoxycanthinone (**93**), have been isolated from *Leitneria floridana* (Fig. **27**) [41]. *In vitro* biological evaluation showed that **90**-**93** suppressed the growth of a panel of human tumor cell lines (KB, A-549, HCT-8, CAKI-1, MCF-7, and SK-MEL-2). Compounds **90** and **91** were strongly active, with ED_{50} values in the range of 0.26-0.012 μg/mL.



88 **89**

Fig. (27). Leitneridanin-type sesquiterpenes from *Leitneria floridana.*

4) Tricyclic sesquiterpenes

Illudins

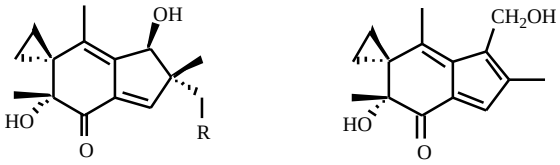
The illudins are a family of natural sesquiterpene compounds with anti-tumor activity that were originally isolated

Table 2. Results of Screening Test vs Ehrlich Ascites Carcinoma[a]

Compound	Dose μmol/kg/day	Mortality		Volume		Ascritocrit		Av TPCV T as %C
		C	T	T/C, ml	SDT	T/C	SDT	
87a[b]	38.2	2/10	1/10	0.16/6.83	0.31	0/0.228	0	0
87b[b]	53.5	2/10	6/10	0 /6.83	0	0/0.228	0	0
87c[b]	68.8	2/10	4/10	0.13/6.83	0.32	0.005/0.228	0.01	0.04
87d[c]	38.2	1/9	2/10	0 /4.64	0	0/0.226	0	0
87e[c]	38.2	1/9	7/10	0 /4.64	0	0/0.226	0	0
87f[c]	38.2	1/9	4/10	0 /4.64	0	0/0.226	0	0
87g[c]	38.2	2/10	9/10	0 /5.94	0	0/0.277	0	0
87h[c]	38.2	2/10	10/10					
87i[c]	38.2	2/10	8/10	0 /5.94	0	0/0.277	0	0
FU[d]	38.2	1/10	1/10	1.8/6.62	0.71	0.145/0.254	0.056	15.5

[a]T = Treated group, C = vehicle control group, TPCV = average total packed cell volume of tumor cells on the final day of assay, SD = standard deviation of TPCV of the treated group. The average SD of the control group was 1.27 ml, and the average SD of ascritocrit was 0.053.
[b]Vehicle was 0.9% NaCl-DMSO.
[c]Vehicle was 0.9% NaCl-DMSO; FU = 5 -fluorouracil.

from the mushrooms *Omphalotus illudens* (jack-o-lantern) or *Lampteromyces japonicus*. Although the isolation of illudin S (**94a**) and illudin M (**94b**) Fig. (**28**), antibiotic compounds derived from culture liquids of *Clitocybe illudens*, was reported earlier, their structures were first proposed in 1963 [42].



94a R = OH
94b R = H

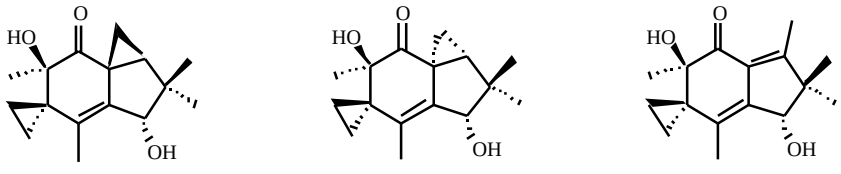
95

Fig. (28). Illudin S, illudin M, and irofulven.

Although many illudins are highly effective against various drug-resistant tumors both *in vivo* and *in vitro*, the extreme cytotoxicity of these compounds in the nanomolar range has severely restricted their practical use in cancer therapy. Recently, semisynthetic derivatives with a strongly improved therapeutic index have been reported. One of these, hydroxymethylacylfulvene (HMAF, Irofulven) (**95**) (Fig. **28**), is currently in clinical trials. The tissue specificity and tumor selectivity of the illudins has been attributed to the presence of an energy-dependent system mediating transport into the cells and subsequent metabolic activation to an unknown reactive intermediate. Inside the cells, strong inhibition of DNA synthesis occurs presumably as a result of DNA damage. Early genetic studies using UV-sensitive rodent mutant cell lines have indicated that nucleotide excision repair is involved in the removal of illudin-induced lesions [43].

Because of their unacceptable toxicity, natural illudins are of limited interest as antitumor agents. In an attempt to exploit their cytotoxic potential, several derivatives have been synthesized with the goal of producing compounds with an improved therapeutic index. Alkylation and oxidation reactions on the parent structure gave rise to less cytotoxic derivatives. All the compounds were less cytotoxic than illudin M and S but still very active [44].

As shown in Table **3**, compounds **96-98** (Fig. **29**) were still active but were three orders of magnitude less cytotoxic to A2780 cell lines than was the parent compound illudin M (**95**).



96 **97** **98**

Fig. (29). Illudin-type sesquiterpenes.

Studies of the mechanism of toxicity of illudins indicate that they behave as alkylating agents. Illudin S reacts spontaneously at room temperature with thiols, such as cysteine or glutathione, at an optimum pH of about 6, and its toxicity to myeloid leukemia cells (HL60) can be modulated by altering glutathione levels in cells. A Michael-type addition to the α, β-unsaturated ketone gives a cyclohexadiene intermediate, an extremely reactive alkylating agent, which is rapidly converted to a stable aromatic product [45].

Table 3. IC_{50} of Illudin Analogs Against the A2780 Cell Line

Compound	IC_{50} (nM)
GSH	260
96	0.0028
97	9.9
98	6.8
95	1.7

Illudin S has been found to undergo bioreductive activation with NADPH in a rat liver cytosol preparation. Addition of hydride to the α, β-unsaturated ketone presumably occurs, producing a highly unstable intermediate, as in the reaction with thiols (Fig. **30**). This intermediate is a potent alkylating agent. Aromatic products (**99** and **100**) were isolated from the enzyme-catalyzed reaction.

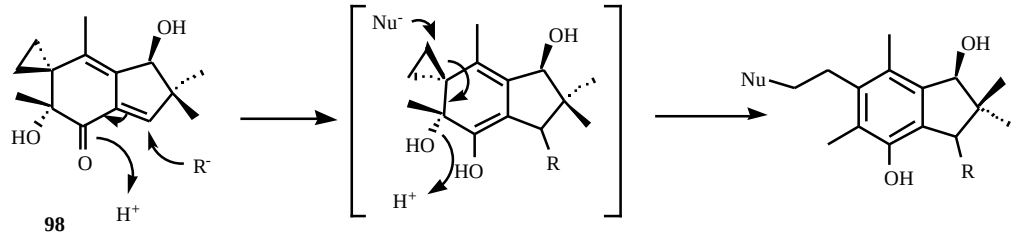
Illudioid : Irofulven

A direct result of the high cytotoxicity of illudin has been the development of the synthetic analog 6-hydroxymethylacylfulvene **95** (HMAF, MGI 114) [46]. Although illudins lack significant activity as antiproliferative agents in tumor-bearing animals, several of their properties, including their potent inhibition of DNA synthesis and unique interaction with DNA, have led to a structure-activity-based synthetic effort to obtain analogs with improved therapeutic potential. MGI 114 was selected for further development on the basis of its antitumor activity in numerous preclinical tests. It was evaluated against adult and pediatric human tumors taken directly from cancer patients and cultured in a human tumor colony-forming assay, in order to assess the antitumor spectra, concentration-response relationship, schedule dependence and activity of this agent against tumors considered resistant to conventional anticancer drugs. Human tumor colony-forming units were treated with MGI 114 at concentrations of 0.001, 0.01, 0.1 and 1 μg/ml, both at a 1 h exposure and at a continuous 14-day exposure. A response was defined as a colony survival of ≤50%. *In vitro* response rates in the range of 50-80% were observed against tumor colony-forming units originating from carcinomas of the colon, kidney, breast, lung cancer, ovary and melanoma. MGI 114 also demonstrated antitumor activity against neuroblastoma colony-forming units. Antitumor activity was not influenced by exposure time, as demonstrated by the similar response rates obtained with the 1 h and continuous exposures at all concentrations tested. However, there was a significant positive concentration-response relationship with exposure duration, with responses increasing from <10% at the lowest concentration to >70% at the highest concentration, except in the case of pediatric tumors subjected to 1 h exposure, for which this relationship was less apparent.

At the highest concentration tested, MGI 114 displayed substantial antiproliferative effects in the range of 70%, against tumor specimens resistant to classic cytotoxic agents that included irinotecan, paclitaxel, 5-fluorouracil, cisplatin, doxorubicin, and cyclophosphamide. These results indicate that MGI 114 exhibits a broad spectrum of antitumor activity against both adult and pediatric primary tumor colony-forming units in a concentration-dependent manner after both short and prolonged exposures. The substantial *in vitro* activity of MGI 114 at concentrations achievable in clinical trials, together with its activity against tumors resistant to classic standard cytotoxic drugs, justifies the further clinical evaluation of this unique agent [46].

MGI Pharma acquired the rights to develop acylfulvenes as anticancer drugs from the University of California in 1993. This company has sponsored preclinical investigations as well as Phase I trials that were designed to identify the highest dose of MGI 114 that can be safely administered to adults with solid tumors. The results indicated that significant doses of the drug can be administered to humans before a dose-limiting degree of bone narrow suppression is observed. Other drug-related toxicities reported include nausea, vomiting, fatigue, facial flushing, phlebitis and reversible kidney effects.

MGI Pharma has initiated its Phase II clinical program with the treatment of hormone-refractory prostate cancer patients. The company plans to begin Phase II studies in



R = GS or H (from NADPH)

Fig. (30). Mechanism of toxicity and antitumor activity of illudins.

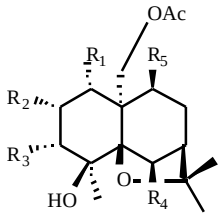
ovarian and pancreatic cancer patients this year. The National Cancer Institute has announced the start of its own Phase II trials, which will target renal, colorectal, lung, breast and ovarian cancer.

Dihydroagarofuran

The family Celastraceae is well-known for producing various dihydro-β-agarofuran derivatives, some of which exhibit insecticidal or insect antifeedant activity, antitumor activity, and antitumor promoting activity. Recently, it was reported that several dihydro- β-agarofuran compounds can reverse multidrug resistance (MDR) in cancer cells [47].

Plants of the genus *Tripterygium wilfordii* have been used in traditional Chinese medicine for centuries for the treatment of cancer. The presence of the anti tumor and anti leukemic diterpenoid triptolide and the cytotoxic triterpene tingenone in this genus accounts for much of this activity. However, the discovery of the anti-tumor properties of the macrolide maytansine from *Maytenus ovatus* promoted a widespread search for further Celastraceae-derived anticancer agents.

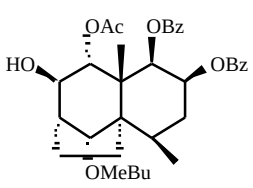
Six new dihydro-β-agarofuran [5, 11-epoxy 5β, 10α-eudesm 4-(14) ene] sesquiterpenes have been isolated from the seeds of *Euonymus nanoides*, including five with a novel substitution pattern: (1*R*, 2*S*, 4*S*, 5*R*, 7*R*, 9*S*, 10*R*)1α-benzoyloxy 2α, 15-diacetoxy 4β-hydroxy 9β-cinnamoyloxy β-dihydroagarofuran (**101**), 1α-(α-methyl) butanoyl 2α, 15-diacetoxy 4β-hydroxy 9β-(β)furoyloxy β-dihydroagarofuran (**102**), 1α, 2α-di(α-methyl)-butanoyl 4β-hydroxy 9β-(β)furoyloxy 15-acetoxy β-dihydroagarofuran (**103**), 1α-(α methyl) butanoyl 2α-(α-methyl) propynoyloxy 4β-hydroxy 9β-(β)furoyloxy 15-acetoxy β-dihydroagarofuran (**104**), and 1α, 2α, 9β tri-(β)furoyloxy 4β-hydroxy 15-acetoxy β-dihydroagarofuran (**105**). The other dihydroagarofuran sesquiterpene was 1α, 2α, 6β, 15-tetraacetoxy 3α-(α-methyl) butanoyl 4β-hydroxy 9β-(β)furoyloxy β-dihydroagarofuran (**106**) (Fig. **31**) [47]. These compounds have been evaluated for their *in vitro* antitumor effects (IC_{50} values: 27.71 - 50.69 μg/mL) and their structure-activity relationships have also been investigated.



101 R_1 = OBz R_2 = OAc R_3 = H R_4 = H R_5 = OCin
102 R_1 = OMeBu R_2 = OAc R_3 = H R_4 = H R_5 = OFu
103 R_1 = OMeBu R_2 = OMeBu R_3 = H R_4 = H R_5 = OFu
104 R_1 = OMeBu R_2 = OMeBu R_3 = H R_4 = H R_5 = OFu
105 R_1 = OFu R_2 = OFu R_3 = H R_4 = H R_5 = OFu
106 R_1 = OAc R_2 = OAc R_3 = OMeBu R_4 = OAc R_5 = OFu

Fig. (31). Dihydroagarofurane-type sesquiterpenes.

When compounds **101** - **106** were tested for *in vitro* antitumor activity against BEL 7402 (human liver carcinoma), they were found to have low inhibitory activity (IC_{50} values, 27.71 - 50.69 μg/mL) when compared to etoposide ($IC_{50,}$ 7.00 μg/mL); the order of activity was **104** > **101** > **103** > **105** > **106** > **102**. This pattern indicated that the inhibitory effects of the dihydro-β-agarofuran sesquiterpenes were related to their substitution patterns and substitution groups: The activities of **101**, **103**, **104 and 105,** which have five substituents, were stronger than that of compound **106,** which has seven substituents; the activity increased in the order **102**, **105**, **103** and**104,** which have the same substitution patterns: 1α, 2α, 4α, 9α, 15. The first to be found was celaglaumin (**107**) from the bark of *Celastrus glaucophyllus*, which was shown to exhibit weak activity against the murine leukemia cell line L1210 ($IC_{50,}$ 2.11 μg/mL) and the lymphatic leukemia cell line P388 ($IC_{50,}$ 4.12 μg/mL). More recently, Takaishi has evaluated a number of dihydroagarofuran sesquiterpenes using an Epstein-Barr virus early antigen (EBV-EA) screen and found that triptofordin F-2 (**108**) and triptogelin A-1 (**109**) from *Tripterygium wilfordii* show promising antitumor activity (Fig. **32**) [48]. Triptogelin A-1 (**109**) was shown to reduce both the incidence and frequency of skin tumors in mice without excessive cytotoxicity. From these studies it was deduced that the functionalities at C-6, C-14 and C-8 play an important part in the inhibition of EBV: An acetyl residue at C-6 confers greater activity than does an alcohol, while a nicotinoyl residue is optimal. The presence of C-14 as an acetoxymethyl group also appears to confer superior activity, when compared to a methyl group at that position.



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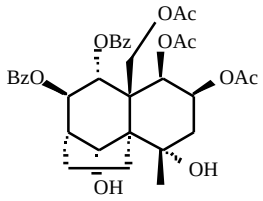

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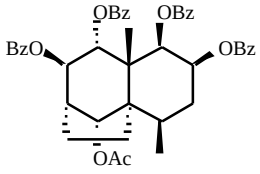

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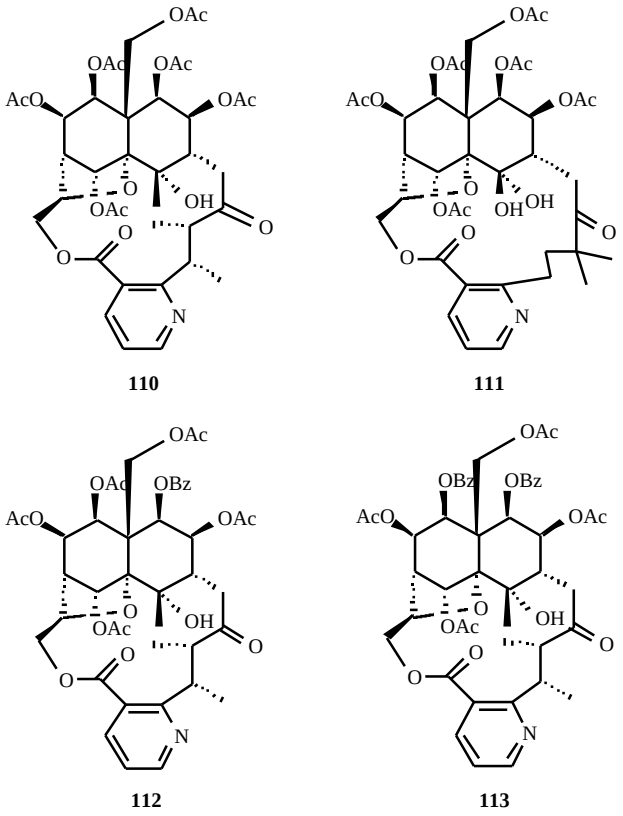
Fig. (32). Sesquiterpenoids from Celastrus glaucophyllus and *Tripterygium wilfordii*.

A series of dilactone-bridged dihydroagarofuran structures that includes emarginatine-A (**110**) and emarginatinine (**111**), isolated from the stems and branches of *Maytenus emarginata*, have also been shown to display *in vitro* cytotoxicity ($ED_{50,}$ 0.4–4.0 μg/mL) against the KB cell line, which is derived from a human epidermoid carcinoma of the nasopharynx. Emarginatine-A (**110**), which is based on the euonyminol skeleton and contains a C-3–C-13 evononic acid dilactone bridge, was slightly less active than emarginatinine (**111**) (ED_{50} = 4.0 *vs.* 2.1 μg/mL). Emarginatine-F (**112**) (Fig. **33**), which is based on the isoeuonyminol skeleton, showed

more potent activity against the human KB line ($ED_{50,}$ 0.51 μg/mL) and also showed significant activity against the human colon adenocarcinoma cell line HCT-8 ($ED_{50,}$ 1.29 μg/mL), the human medulloblastoma cell line TE-671 ($ED_{50,}$ 0.21 μg/mL), and the murine lymphocytic leukemia cell line P388 ($ED_{50,}$ 0.69 μg/mL). A related macrolide, celahinine A (**113**), was also found to be present in a crude ethanolic extract of *Celastrus hindsii*. This compound displayed potent *in vitro* cytotoxicity against human nasopharynx, cervix, and colon carcinoma cell lines.



110 **111** **112** **113**

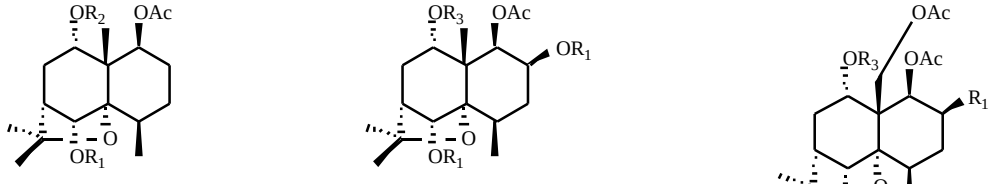
Fig. (33). Sesquiterpenoids from *Maytenus emarginata* and *Celastrus hindsii*.

Six new (**114-119**) and three known (**120-122**) sesquiterpene esters have been isolated from the roots of *Celastrus orbiculatus* [49]. The structures of the new compounds were identified as 1β-acetoxy-6α-furoyloxy-9benzoyloxydihydro-β-agarofuran (**114**), 1β-acetoxy-6α-benzoyloxy-9α-furoyloxydihydro-β-agarofuran (**115**), 1β-acetoxy-6α, 9α-difuroyloxydihydro-β-agarofuran (**116**), 1β, 2β-diacetoxy-6α-furoyloxy-9α benzoyloxydihydro-β-agarofuran (**117**), 1β-acetoxy2β, 6α-difuroyloxy-9α-benzoyloxydihydro-β-agarofuran (**118**), and 1β-acetoxy-2β, 6α, 9α -tribenzoyloxydihydro-β-agarofuran (**119**). Compounds **117**, **118**, and **120-122** were shown to be more active than verapamil in reversing vinblastine resistance in multidrug-resistant KB-V1 cells.

The relative MDR-reversing activity of each compound was determined by comparing the cytotoxicity enhancement factor (EF), the ratio of the IC_{50} values in the KB-V1 cells in the absence or presence of VLB. The penta-ester and all the tetra-esters except **119** exhibited strong reversal activity, with EF values of 35.7-87.0. Of the tetra-esters, **117**, **120**, and **125**, which had an additional acetoxy group at C-2, showed strong reversal activity with EFs of 66.2-87.0. The reversal activity of **118**, with a furoyloxy at C-2, was reduced to about 50% in comparison with **117**. The reversal activity of **119**, with a benzoyloxy at C-2, was markedly reduced to EF 5.9, the weakest activity among the compounds tested. Compounds **121** and **122**, with two acetoxy groups at C-1 and C-15, exhibited strong activity irrespective of the presence of the ester group at C-2. These results suggest that the polarity of C-1/C-2 or C-1/C-15 is an important factor in MDR-reversal activity.

Ten sesquiterpenoids with a dihydro-β-agarofuran skeleton, **126**–**135,** have been isolated from *Maytenus cuzcoina* (Celastraceae) (Fig. **35**). Their structures have been elucidated, and they have been tested for their inhibitory effects in EBV-EA activation induced by TPA in Raji cells, as a means of identifying potential cancer chemopreventive agents (Table **4**) [50].

Compounds **126-135** had strong anti tumor promoting activity even at a 10 mol ratio/TPA (100% inhibitory activity at 1000 mol ratio/TPA, and >70% and 25% even at 500 mol ratio/TPA and 100 mol ratio/TPA, respectively), without appreciably reducing the viability of the Raji cells (>70% viability at a 10 to 1000 mol ratio/TPA). Thus, while **126-135** were the most active of the compounds assayed (9.0% inhibition of induction at 10 mol ratio/TPA), they showed an insignificant inhibitory activity (87.4% at 1000 mol ratio/TPA). Compound **134,** with a skeleton based on 4β-

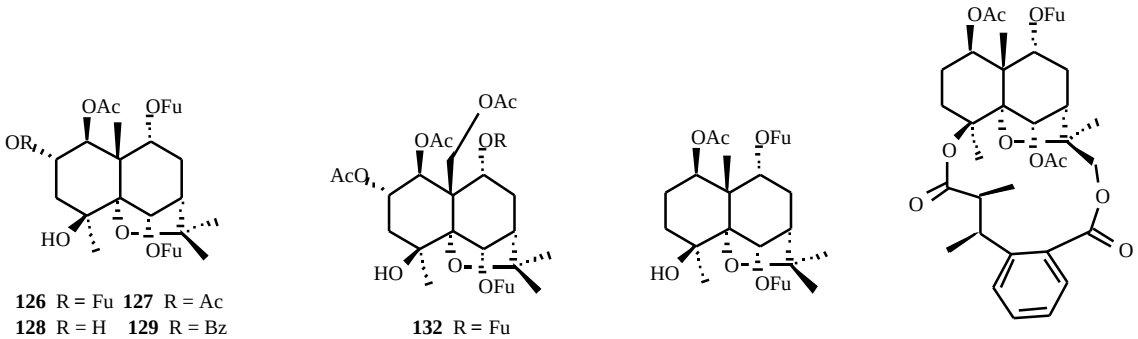


114 R_1 = Fu R_2 = Bz
115 R_1 = Bz R_2 = Fu
116 R_1 = Fu R_2 = Fu
123 R_1 = Bz R_2 = Bz
124 R_1 = Cin R_2 = Bz

117 R_1 = Ac R_2 = Fu R_3 = Bz
118 R_1 = Fu R_2 = Fu R_3 = Bz
119 R_1 = Bz R_2 = Bz R_3 = Bz
120 R_1 = Ac R_2 = Ac R_3 = Bz
125 R_1 = Ac R_2 = Bz R_3 = Bz

121 R_1 = H R_2 = Ac R_3 = Bz
122 R_1 = OBz R_2 = Ac R_3 = Bz

Fig. (34). Dihydroagarofurane-type sesquiterpenes from *Celastrus orbiculatus.*



126 R = Fu **127** R = Ac
128 R = H **129** R = Bz
130 R = Pr **131** R = MeBu

132 R = Fu
133 R = Bz

134

135

Fig. (35). Dihydroagarofurane-type sesquiterpenes from *Maytenus cuzcoina.*

Table 4. Percentage of Epstein-Barr Virus Early Antigen (EBV-EA) Induction in the Presence of Compounds 126–135 with Respect to a Positive Control

Concentration mol ratio/TPA	126	127	128	129	130	131	132	133	134	135
1000	0	0	0	11.7	6.3	0	0	12.6	10.5	26.4
	(70)	(70)	(70)	(60)	(70)	(70)	(70)	(60)	(70)	(70)
500	30.2	27.4	28.5	42.9	34.9	32.7	25.9	48.0	40.2	59.6
100	76.9	74.8	76.7	87.1	77.9	78.0	72.0	86.9	84.6	86.7
10	94.6	93.0	95.8	100	100	96.2	91.0	100	100	100

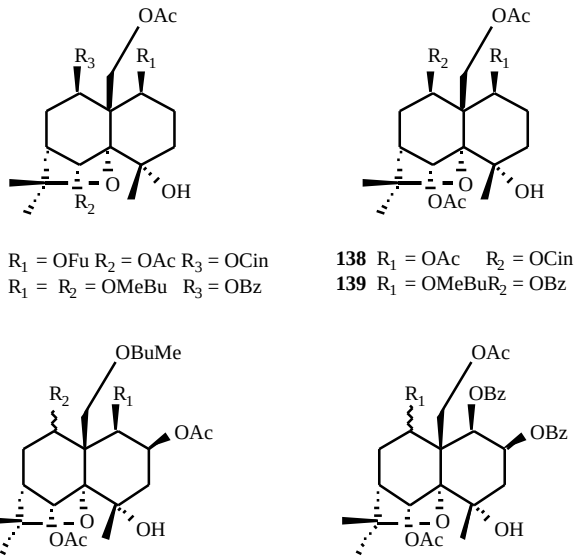
hydroxy-celorbicol, showed an inhibitory activity comparable to those of **126-135**.

The isolation, structure elucidation, and antitumor activity of four new sesquiterpene polyol esters, 6α, 13-bis(acetyloxy)-9β-(cinnamoyloxy)-1β-(furan-3-ylcarbonyl)oxy]-4α-hydroxy -β- dihydroagarofuran (**136**), 13 - (acetyl-oxy) - 9β-(benzoyloxy)-4α-hydroxy-1β, 6α- bis [(2-methylbutanoyl) oxy]-β-dihydroagarofuran (**137**), 1β, 6α, 13-tri(acetyloxy)-9β-(cinnamoyloxy)-4 α-hydroxy β-dihydroagarofuran (**138**), and 6 α, 13-bis(acetyloxy)-9 β-(benzoyloxy)- 4 α-hydroxy-1-[(2-methylbutanoyl)oxy] β-dihydroagarofuran (**139**), and of five known sesquiterpene polyol esters **140-144** (Fig. **36**) from the seed oil of *Euonymus nanoides* have also been reported [51].

All the new and previously known compounds were tested for *in vitro* antitumor activity against three human tumor cell lines, A-549 (lung carcinoma), HL-60 (leukemia neoplasm), BEL-7402 (liver carcinoma), and one mouse tumor cell line, P388 (leukemia neoplasm). IC_{50} values were determined for compounds **136-144** (Table **5**). These results indicate that the compounds are able to inhibit tumor activity, with IC_{50} values below 100 μM.

Drimanes

Fourteen new sesquiterpenes of the drimane series, dendocarbins A-N (**145-158**), have been obtained from the Japanese nudibranch *Dendrodoris carbunculosa*, together with two known compounds, isodrimeninol (**159**) and 11-epivaldiviolide (**160**). The structures were elucidated mainly from spectral data, and most of these sesquiterpenes were



136 R_1 = OFu R_2 = OAc R_3 = OCin
137 R_1 = R_2 = OMeBu R_3 = OBz

138 R_1 = OAc R_2 = OCin
139 R_1 = OMeBu R_2 = OBz

140 R_1 = OAc R_2 = a-OBz
141 R_1 = OFu R_2 = b-OMeBu
144 R_1 = OBz R_2 = b-OMeBu

142 R_1 = a-OAc
143 R_1 = b-OBz

Fig. (36). Dihydrofuran-type sesquiterpenes.

Table 5. ***In vitro*** **Antitumor Activities (IC_{50} [μM]) of Compounds 136-144**

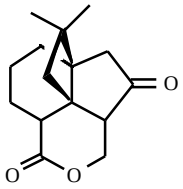
	P388	HL-60	A-549	BEl-7402
136	64.41	39.72	83.05	46.45
137	72.24	29.30	>100	52.72
138	>100	91.12	>100	-
139	68.06	45.59	>100	62.02
140	>100	98.24	-	>100
141	13.26	8.68	>100	12.08
142	91.53	54.46	>100	74.83
143	80.86	17.48	-	41.13
144	71.83	12.05	>100	6.23

found to exhibit cytotoxicity. In addition, isodrimeninol (**159**), the major sesquiterpene from this animal, was found to have a sharp peppery taste (Fig. **37**) [52].

All the sesquiterpene compounds isolated possessed the drimane skeleton. The ethoxy groups found in dendocarbins I (**153**), J (**154**), and K (**155**) were most likely introduced during extraction with EtOH. The cytotoxic activity of these sesquiterpenes against murine leukemia P388 cells was examined, and the IC_{50} values against adriamycin (ADR)- and vincristine (VCR)-resistant P388 cells (P388/ADR and P388/VCR, respectively) as well as those against sensitive P388 strain (P388/S), are presented in Table **5**. Although these sesquiterpene derivatives had no reversal effect on multidrug resistance, compounds **154** and **160** exhibited moderate cytotoxicity against both sensitive and resistant cell strains [52].

Quadrone

Quadrone (**161**) (Fig. **38**), a fungal metabolite from *Aspergillus terreus* reported in 1978, was found to exhibit inhibitory activity *in vitro* against human epidermoid carcinoma of the nasopharynx (KB), with an ED_{50} of 1.3 kg/mL and *in vivo* against P338 lymphocytic leukemia in mice. The reported biological activity and the deceptively challenging structural features of this novel sesquiterpene have combined to trigger intensive efforts directed at total synthesis. Two independent entries in the quadrone class that share a general strategic theme have recently been reported by Danishefsky and coworkers [53].



161

Fig. (38). Quadrone.

A novel cyclopentenedione, asterredione (**162**); two new terrecyclic acid A derivatives, (+)-5(6)-dihydro-6-methoxyterrecyclic acid A (**163**) and (+)-5(6)-dihydro-6-hydroxyterrecyclic acid A (**164**); and five known compounds, (+)-

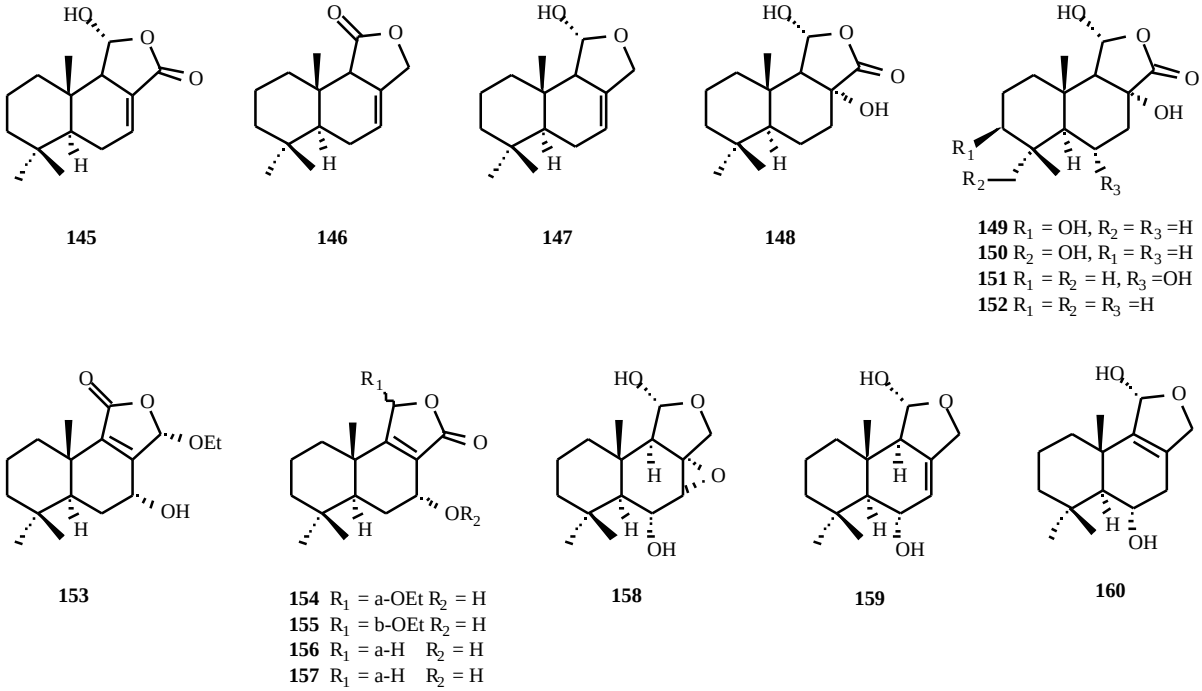


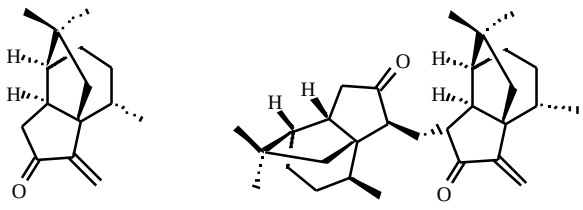
Fig. (37). Drimane-type sesquiterpenes from *Dendrodoris carbunculosa.*

terrecyclic acid A (**165**), (-)-quadrone (**166**), betulinan A (**167**), asterriquinone D (**168**), and asterriquinone C-1 (**169**), have been isolated by bioassay-guided fractionation from *Aspergillus terreus* occurring in the rhizosphere of *Opuntia versicolor* (Fig. **39**)[52]. Acid-catalyzed reaction of **163** under mild conditions afforded **165**, whereas under harsh conditions, **163** yielded **166** and (-)-isoquadrone (**170**). Catalytic hydrogenation and methylation of **165** afforded 5(6)-dihydro-terrecyclic acid A (**171**) and (+)-terrecyclic acid A methyl ester (**172**), respectively. All of these compounds were evaluated for cytotoxicity in a panel of three sentinel cancer cell lines, NCI-H460 (non-small cell lung cancer), MCF-7 (breast cancer), and SF-268 (CNS glioma), and were found to be moderately active. Cell cycle analysis of **163**, **165**, and **166** using the NCI-H460 cell line indicated that **165** is capable of disrupting the cell cycle through an apparent arrest of progression at the G_1 and G_2/M phases in this p53-competent cell line.

When compounds **162-172** were evaluated for *in vitro* cytotoxicity against a panel of three sentinel cancer cell lines, NCI-H460 (non-small cell lung), MCF-7 (breast), and SF-268 (CNS glioma), the IC_{50} values were found to range from 4.1 to 58.4 μM. Compound **164** showed selective activity toward the SF-268 cell line, whereas **167** and **169** were found to be more toxic to MCF-7 cells [54].

Suberosenone

A recent report [53] has described the isolation, structure elucidation, and *in vitro* antitumor activity of suberosenone (**173**) Fig. (**40**), a novel sesquiterpene related to quadrone and terrecyclic acid derivatives. Alertenone (**174**), a related dimer, was also isolated and identified [55].



173 **174**

Fig. (40). Suberosenone-type sesquiterpene from *Subergorgia suberosa.*

In comparative testing against 10 human tumor cell lines (A-549, HOP-92, SF-295, SF-539, SNB-19, LOX, M14, MALME-3M, OVCAR-3, and MCF7), suberosenone (**173**) exhibited the same potency and differential cytotoxicity observed earlier, with IC_{50} values of 0.002-1.6 μg/mL. However, alertenone (**174**) was surprisingly nontoxic; nine of the cell lines gave IC_{50} values of 35-45 μg/mL, while one was unresponsive at 100 μg/mL.

The suberosenone isolated in this study was then tested in a hollow-fiber assay for *in vivo* antitumor activity. A high dose of 40 mg/kg yielded cytotoxic activity against two of six human tumor cell lines implanted subcutaneously and intraperitoneally in mice. In the case of the H-522 lung tumor and U-251 CNS tumor lines, a 10-30% net cell growth

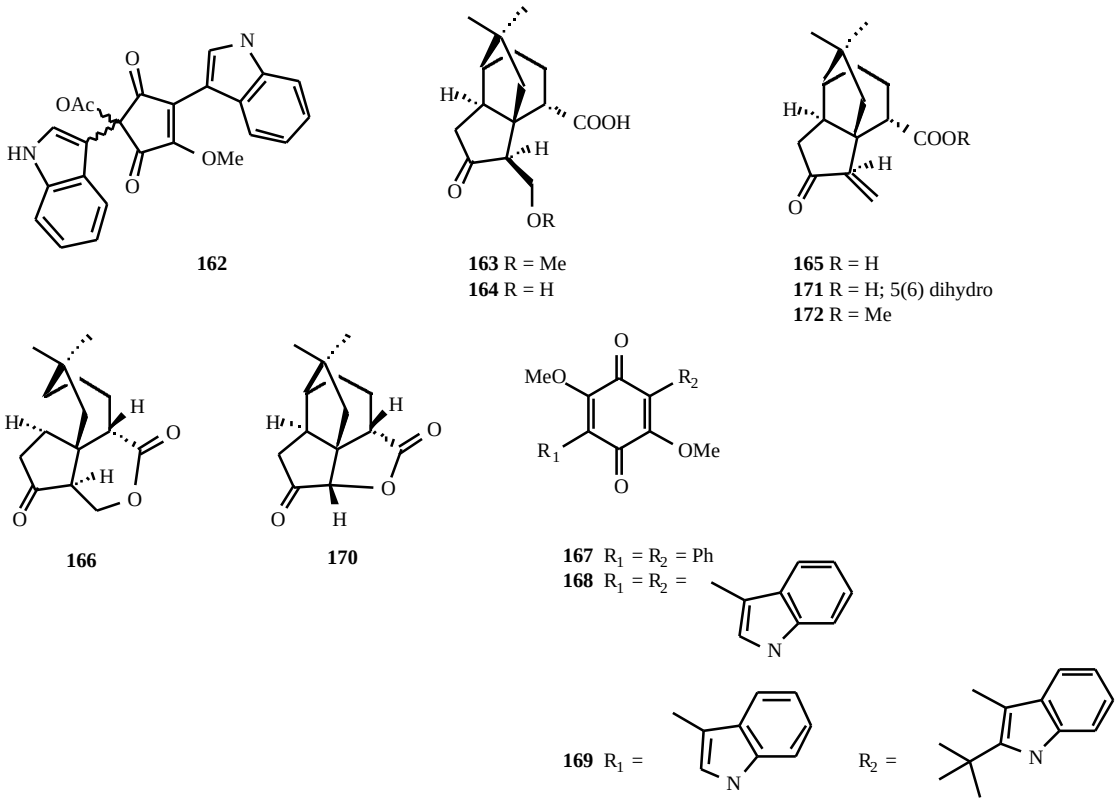


Fig. (39). Sesquiterpenes **162-172**.

versus controls was observed at multiple doses in both the subcutaneous and intraperitoneal models [55].

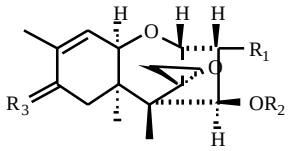
5) Tetracyclic Sesquiterpenes

Trichothecenes

Trichothecenes are highly functionalized sesquiterpenes based on the tetracyclic core. Interest in the trichothecenes continues because of their biological activity, the challenge associated with their total synthesis, curiosity about their biosynthesis, and the possibility for the re-engineering of their biosynthetic pathways. In addition, many of them have cytotoxic and antitumor activity. At the cellular level, trichothecenes inhibit protein synthesis in eukaryotic cell lines, resulting in inhibition of DNA synthesis.

Bioassay-guided fractionation resulted in the isolation of three novel 12, 13-epoxytrichothecenes, trichothecinols A–C (**175-177**) (Fig. **41**), together with three known analogs, trichothecin (**178**), trichodermol (**179**) and trichothecolone (**180**). Compounds **175-180** strongly inhibited EBV-EA activation by TPA in Raji cells, the EBV genome-carrying human lymphoblastoid cells. Furthermore, the most highly active compound, **175,** also suppressed TPA-induced tumor promotion initiated with 7, 12-dimethylbenz[*a*]anthracene (DMBA) in mouse skin two-stage carcinogenesis experiments. Therefore, trichothecinol A (**175**) was suggested as a promising candidate for further evaluation as a cancer-preventing agent. In addition, the isolated compounds were evaluated for their ability to inhibit the growth of several bacteria and fungi [56].

Trichothecenes **175-180** were all found to strongly inhibit TPA-induced EBV-EA activation. Among them, compounds **175**, **177** and **178** were amazingly potent inhibitors, with IC_{50} values (mol ratio/32 pmol TPA) of 0.51, 0.56, and 0.92, respectively. In particular, compounds **175** and **177** were 600-fold more potent than curcumin. No other cancer-preventive



175 R_1 = OH R_2 = COCH=CHMe R_3 = O
176 R_1 = H R_2 = COCH=CHMe R_3 = a-OH, b-H
177 R_1 = OH R_2 = COCH=CHMe R_3 = a-OH, b-H
178 R_1 = H R_2 = COCH=CHMe R_3 = O
179 R_1 = H R_2 = H R_3 = a-H, b-H
180 R_1 = H R_2 = H R_3 = O

Fig. (41). Trichothecene-type sesquiterpenes.

agents that have been evaluated in this *in vitro* assay are more effective than these four trichothecenes. Although the remaining trichothecenes **176** and **178-180** were much less potent than the other analogs, they were still 10 times more potent than curcumin. On the other hand, they did not show significant cytotoxicity against Raji cells; they maintained a high level of viability (70%) even at a 1000 mol ratio/TPA.

Strains of *Myrothecium verrucaria* and the related *Myrothecium roridum* have been intensely studied by Jarvis and others because of their content of the trichothecene class of sesquiterpenes and its macrocyclic analogs. In contrast to the previously described thichothecenes, there are few examples of 12, 13-deoxytrichothecenes [57].

Study of the sponge-derived strain of *Myrothecium verrucaria* proved to be rewarding because it elaborated a broad

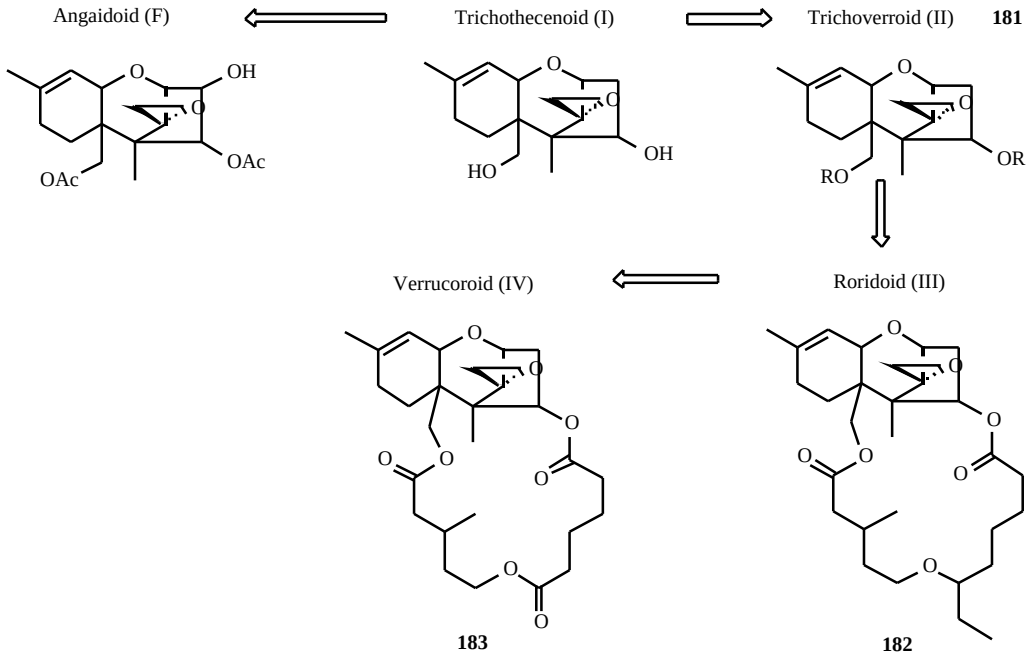


Fig. (42). Trichothecene-type sesquiterpenes.

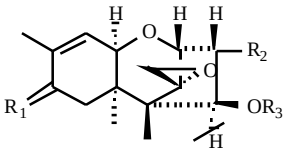
array of functionalized trichothecenes. Saltwater culturing provided compounds in the trichoverroid (**181**), roridoid (**182**), and verrucaroid (**183**) classes.

Bioassay-guided fractionation of an extract of *Holarrhena floribunda* stem has led to the isolation of three new trichothecenes, 8-dihydrotrichothecinol A (**184**), loukacinol A (**185**), and loukacinol B (**186**), and the known compounds trichothecolone (**187**), trichothecin (**188**), trichothecinol A (**189**), rosenonolactone (**190**), 6β-hydroxyrosenonolactone (**191**), and rosololactone (**192**) Fig. (**43**) [58].

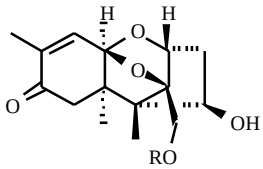
Evaluation of the cytotoxicity of compounds **184**-**189** and **185a** against KB, SK-MEL 30, A-459, and MCF-7 cancer cell lines revealed significant activity by compound **189** that was consistent with results previously reported by Iida and coworkers [58], indicating an antitumor-promoting effect of trichothecinol A. Compound **184** was also markedly cytotoxic, whereas the remaining compounds were only moderately active. These results agree with previous generalized conclusions about the structure-cytotoxicity relationship of trichothecenes and indicate that the C-3 α-hydroxy substituent in **184** and **189** is responsible for the increase in activity when compared to that of trichothecolone and trichothecin. A similar effect was observed in the brine shrimp assay, with LC_{50} values of 2.2 and 0.52 μg/mL for **188** and **189**, respectively.

In another report, 42 analogs and reaction products derived from T-2 toxin or neosolaniol were assayed for their cytotoxicity against cultured mouse lymphoma cells. Structure-activity relationships confirmed the stereospecific nature of the cytotoxic action of T-2 toxin (Fig. **44**): Cytotoxic activity was particularly susceptible to changes at C3, C4, C9, and C10, but was relatively unaffected by changes at C8, which appears to represent a region of steric tolerance in the interaction of T-2 with a cellular constituent. The most potent compounds were T-2, diacetoxyscirpenol, and a series of C8 ester analogs **193** and **194**-**199** (Fig. **44**) [59].

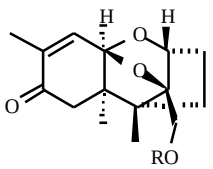
It appears that for the simple trichothecenes, natural evolution has produced the metabolites T-2 toxin and diacetoxyscirpenol, with maximum cytotoxicity. Modifications at C3, C4, C15, and C9-C10 generally decreased activity, with a marked dependence on stereochemistry being shown for substituents at C3 and C4. The only compounds synthesized that were equipotent with T-2 were a series of C8-modified ester analogs, suggesting that C8 corresponds to a region of steric tolerance in the interaction of trichothecenes with a cellular receptor. However, it is not known to what extent the mouse lymphoma cells metabolize trichothecenes, and this reaction could involve hydrolysis at C8. The lipophilicity of these compounds is likely to affect the compound's access to cells; however, decreased activity was observed with compounds containing more than two hydroxy groups.



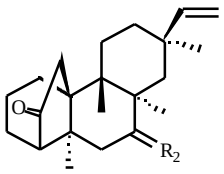
184 $R_1 = H_2$ R_2 = OH R_3 = COCH=CHMe
187 R_1 = O R_2 = H R_3 = H
188 R_1 = O R_2 = H R_3 = COCH=CHMe
189 R_1 = O R_2 = OH R_3 = COCH=CHMe



185 R = H
185a R = OH

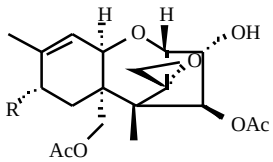


186 R = H
186a R = OH



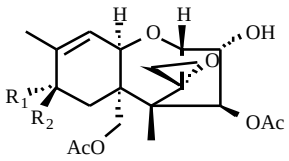
190 R_1 = H R_2 = O
191 R_1 = OH R_2 = O
192 R_1 = OH $R_2 = H_2$

Fig. (43). Trichothecene-type sesquiterpenes from *Holarrhena floribunda.*



R = H, $OC(O)CH_2CH(CH_3)_3$

T2 Toxin



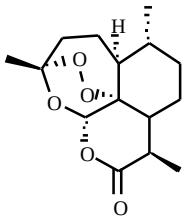
193 R_1 = OAc R_2 = H
194 R_1 = OCOEt R_2 = H
195 R_1 = OCOPr R_2 = H
196 R_1 = OCOBu R_2 = H
197 R_1 = OCOPe R_2 = H
198 R_1 = OCOPh R_2 = H
199 R_1 = H R_2 = OH

Fig. (44). Potent trichothecenes.

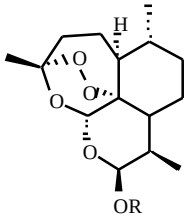
Artemisinins

Artemisinin (**200**) (Fig. **45**), the key ingredient obtained from *Artemisia annua*, has a long history of use as an antimalarial remedy. *Artemisia annua*, or "sweet wormwood, " is mentioned in the *Recipes For 52 Kinds Of Diseases* found in the Mawangdui Han Dynasty tomb, dating from 168 B.C. In that work, the herb is recommended for use for hemorrhoids. The major active principle was first isolated in l972, and investigators at the Walter Reed Army Institute of Research located and crystallized the active component in l984 [60].

Artemisinin and two synthetic derivatives, artemether (**204**) and sodium artesunate (**202**) (Fig. **45**), were evaluated in the l970s. A number of tropical countries have conducted trials of these compounds. In a 1979 study in China of 2, 099 patients infected with *P. viva* and *P. falciparum*, artemisinin had good therapeutic effects and improved or cured all the patients. Furthermore, artemisinin had no obvious side effects. Artemisinin is also effective in cerebral malaria. In one study, the body temperature of patients normalized within 72 h of treatment with the drug, and asexual parasites were eliminated within 72 h. However, there was a relapse rate of 21% [61].



200



201 R = $OCOCH_2CH_2COONa$
202 R = H
203 R = Me

Fig. (45). Artemisinin and its derivatives.

Artemisinin contains an internal peroxide group. Because of this group, reactive oxygen is already present in the molecule. This conclusion is in agreement with the observation that derivatives of artemisinin lacking the peroxide moiety are devoid of antimalarial activity [62].

Artemisinin has been shown to work through oxygen- and carbon-based free radical mechanisms. Its structure includes an endoperoxide bridge. Peroxides generate free radicals in a Fenton-type reaction when exposed to unbound ferrous iron. The malarial parasite, which grows in the erythrocytes, causes the accumulation of excess iron, which can be present in unbound form. Electron microscopy has confirmed the destruction of plasmodium membranes, which display a morphology typical of injury by free radicals.

Given the high accumulation of iron in cancer cells, researchers Henry Lai and Narenda Singh became interested in possible artemisinin activity against malignant cells and have used artemisinin against numerous cancer cell lines *in vitro*. Their article has mobilized interest in artemisinin as an addition to the anticancer arsenal [63].

There are a number of properties shared by cancer cells that favor the selective toxicity of artemisinin against cancer cell lines and against cancer *in vivo*. In addition to their higher rates of iron flux *via* transferrin receptors when compared to normal cells, cancer cells are also particularly sensitive to oxygen radicals [64].

A subsequent article by Singh and Lai [65] has demonstrated a selective toxicity of artemisinin and holotransferrin toward human breast cancer cells. In this article, rapid and complete destruction of a radiation-resistant breast cancer cell line was achieved when holotransferrin was used in the *in vitro* cell system to increase the iron uptake. The cancer cell line was completely nonviable within 8 h of combined incubation, with minimal effect on the normal cells.

Artemisinin becomes cytotoxic in the presence of ferrous iron. Since iron influx is naturally high in cancer cells, artemisinin and its analogs can selectively kill cancer cells *in vivo*. Furthermore, it is possible to increase or enhance iron flux in cancer cells by supplying conditions that lead to increased intracellular iron concentrations. However, intact *in vivo* systems do not need holotransferrin, since the body provides all the necessary iron transport proteins.

Efferth and coworkers have reported that the antimalarial artesunate (ART) (**201**) is also active against cancer [66], and they have described dramatic cytotoxic activity against a wide variety of cancers, including drug-resistant cell lines. ART, a semi-synthetic derivative of artemisinin, has been tested against 55 cell lines by the Developmental Therapeutics Program of the National Cancer Institute. ART was most active against leukemia and colon cancer cell lines, with mean GI_{50} values of 1.11 μM and 2.13 μM, respectively. Non-small cell lung cancer cell lines were least sensitive to ART (GI_{50}, 26.62 μM). Intermediate GI_{50} values were obtained for melanoma, breast, ovarian, prostate, CNS, and renal cancer cell lines. Most importantly, ART was found to be active in molar concentration ranges comparable to those of established antitumor drugs. When leukemia lines resistant to doxorubicin, vincristine, methotrexate, or hydroxyurea were tested, none of these drug-resistant lines showed resistance to ART. The explanation offered for this lack of resistance is the absence from ART of a tertiary amine that is present in virtually all other chemotherapy agents and is required for cellular transport systems to move the drug across the cell membrane.

Lai used holotransferrin in Molt-4 human leukemia lymphoblastoid cells and normal human lymphocytes to further sensitize the tumor cell lines to the oxidizing properties of dihydroartemisinin, which is metabolically generated from the parent compound *in vivo* [65].

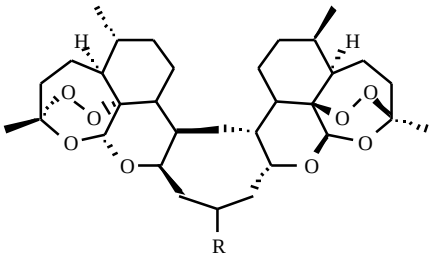
A significant decrease ($p<0.035$) in cell count was noted with artemisinin alone, and greater effects were noted when transferrin and dihydroartemisinin were used together. Combined treatment produced considerable tumor cell death when 1 μM dihydroartemisinin was incubated with the cells for 8h. Furthermore, there is reason to believe that artemisinin can work at lower concentrations *in vivo* than *in vitro*, because of the destruction of the artemisinin molecule *in vitro*.

Table 6. Growth Inhibition of Prostate Cancer Cell Lines[a]

Compound	IC_{50} (nM)			
	C1A	C2D	C2G	C2H
204	23.3	18.5	15.4	9.2
205	84.6	62.1	47.4	36.1
206	158.7	231.4	134.0	116.0
Gemzar	9.0	11.9	3.7	4.7
Doxorubicin (Adriamycin)	75.9	46.5	28.7	30.3

[a] MTT assay was used to determine growth inhibition of C1A, C2D, C2G, and C2H. IC_{50} was determined using calcusyn.

Posner *et al.* have examined the medicinal value of 1, 2, 4-trioxanes and trioxane dimers as both antimalarial and anticancer agents. They have identified orally active antimalarial and anticancer artemisinin-derived trioxane dimers with high stability and efficacy. The effect of semi-synthetic analogs **204**-**206** Fig. (**46**), on prostrate cancer was described in a recent report (Table **6**) [67]. Two of these new chemical entities have been shown in rodents to be more orally efficacious as antimalarials than either artelinic acid or the clinically used sodium artesunate. Also, they were found to be strongly inhibitory toward several human cancer cell lines.

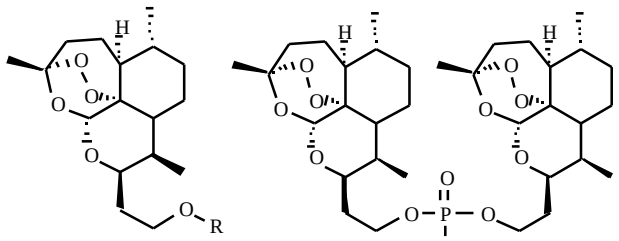


204 R = :CH_2
205 R = CH_2OH
206 R = COOH

Fig. (46). Dimers of artemisinin.

Another series of C-10 non-acetal dimers prepared from the key trioxane alcohol 10β-(2-hydroxyethyl)deoxoartemisinin (**207**) have been reported (Fig. **47**) [68]. All of the dimers displayed potent, low-nanomolar antimalarial activity. In contrast to their potent activity against malarial parasites, virtually all of the dimers had poor anticancer activity, with the exception of the trioxane phosphate ester dimers **210** and **211**, which had nanomolar growth inhibitory (GI_{50}) values versus a range of cancer cell lines in the NCI 60 human cell line screen. Further detailed studies of these dimers *in vitro* in HL60 cells demonstrated that both phosphate ester dimers (**210** and **211**) were more potent than the anticancer agent doxorubicin.

Interestingly, phosphate ester monomers **208** and **209**, active against *P. falciparum* malaria in the low nanomolar range, were inactive as anticancer agents even at millimolar concentrations. This observation emphasizes the importance of the two trioxane units for high antiproliferative activity and suggests that the nature of the linker in dimers of this type plays a crucial role in imparting potent anticancer activity.



207 R = H
208 R = $PO(OMe)_2$
209 R = $PO(OPh)_2$
210 R = Me
211 R = Ph

Fig. (47). Phosphate dimers of artemisinin.

CONCLUSION

In this review, we have provided a brief survey of the naturally occurring sesquiterpenes that have already displayed an intrinsic ability to attack tumors of various types. Although a few of these compounds have already been shown to have high potency and specificity, many others continue to be fruitful subjects for investigation. The initial indications regarding some of these molecules suggest that they have the potential to act as excellent and specific anticancer agents. The initial results need to be examined critically, and we believe that in the near future, many more derivatives of these naturally occurring sesquiterpenes will emerge as lead molecules and clinical agents. In conclusion, we predict that the age-old strategy of searching for compounds of natural origin will continue to yield rich dividends in the quest for specific and highly efficacious anticancer agents.

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