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10. Sesquiterpene lactones: Structural diversity and their biological activities

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Abstract. Sesquiterpenes lactones (SLs) have been isolated from numerous genera of the family Asteraceae (compositae) and can also be found in other angiosperm families. They are described as the active constituents of a variety of medicinal plants used in traditional medicine for the treatment of inflammatory diseases. They are known to possess wide variety of biological and pharmacological activities such as antimicrobial, cytotoxic, anti-inflammatory, antiviral, antibacterial, antifungal activities, effects on the central nervous and cardiovascular systems as well as allergenic potency. Their wide structural diversity and potential biological activities have made further interest among the chemists. The present chapter will be highlighted on the recent developments on the SLs and their diverse biological activities.

1. Introduction

Sesquiterpene lactones (SLs) constitute a large and diverse group of biologically active plant chemicals that have been identified in several plant families such as Acanthaceae, Anacardiaceae, Apiaceae, Euphorbiaceae, Lauraceae, Magnoliaceae, Menispermaceae, Rutaceae, Winteraceae and Hepatideae *etc* [1]. However, the greatest numbers are found in the *Compositae*

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(Asteraceae) family with over 3000 reported different structures [2]. Sesquiterpene lactones are a class of naturally occurring plant terpenoids that represent a diverse and unique class of natural products and are important constituent of essential oils, which are formed from head-to-tail condensation of three isoprene units and subsequent cyclization and oxidative transformation to produce a *cis* or *trans*-fused lactone. These secondary compounds are primarily classified on the basis of their carbocyclic skeletons into pseudoguainolides, guaianalides, germanocranolides, eudesmanolides, heliangolides and hyptocretenolides *etc* (Figure 1).

The suffix "olide" refers to the lactone function and is based on costunolide, a germanacranoride which is related to the ten-membered carbocyclic sesquiterpene, germacrone. However, SLs exhibit variety of other skeletal arrangements. An individual plant species generally produces one skeletal type of SLs concentrated primarily in the leaves and flower heads. The percentage of SLs per dry weight may vary from 0.01% to 8%. Losses of livestock intoxicated by plants containing SLs are well known. In fact, they have been shown to exhibit a wide range of biological activities.

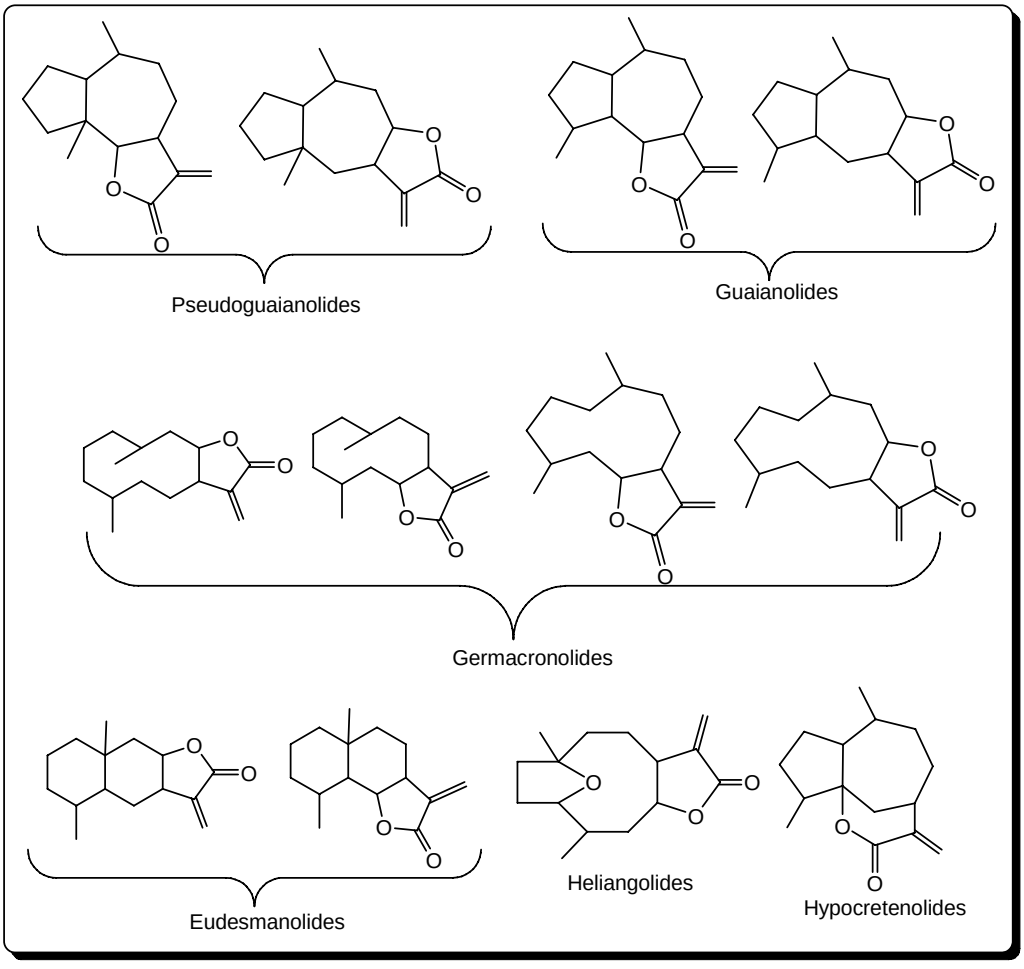


Figure 1. Basic skeletons of sesquiterpenes lactones.

An important usual feature of the SLs is the presence of a γ-lactone ring (closed towards either C-6 or C-8) containing in many cases, an α-methylene group. Among other modifications, the incorporation of hydroxyls or esterified hydroxyls and epoxide ring are common. A few SLs occur in glycoside form and some contain halogen or sulfur atoms [3].

Majority of SLs have shown cytotoxic activity (KB and P388 leukemia *in vitro*) and activity against *in vivo* P388 leukemia. Structure activity relationship studies showed that various cytotoxic SLs react with thiols, such as cystiene residues in the protien, by rapid Michael type of addition. These reactions are mediated chemically by α,β-unsaturated carbonyl system present in the SLs. These studies support the view that SLs inhibit tumor growth by selective alkylation of growth regulatory biological macromolecules such as key enzymes, which controls cell division, thereby inhibiting a variety of cellular functions, which directs the cell into apoptosis. Differences in activity between individual SLs may be explained by different number of alkylating structural elements. However, other factors, such as lipophilicity, molecular geometry, and chemical environment or the target sulfhydryl may also influence the activity of sesquitepenes lactones.

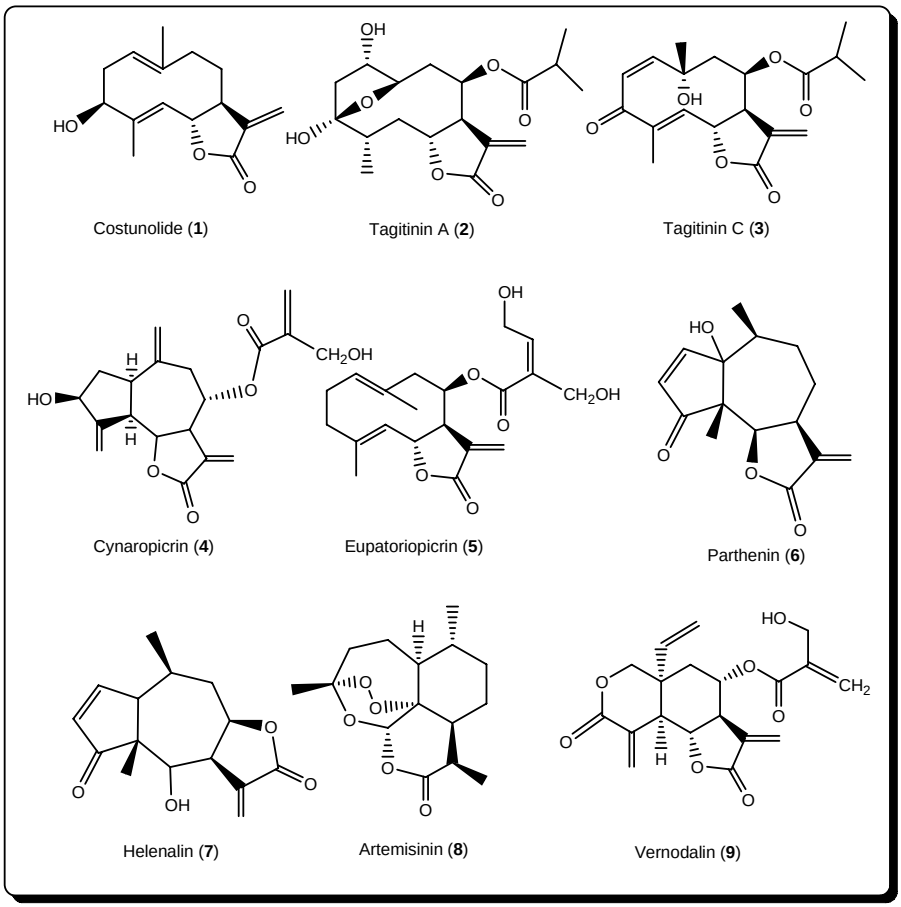


Figure 2. Structurally diverse sesquiterpenes lactones (SLs **1**-**9**).

Some of structurally diverse sesquiterpenes lactones have been shown in **Figure 2** and **3**. Distribution of different structural classes of sesquiterpene lactones have been depicted in **Table 1**.

2. Biological activity of sesquiterpene lactones

[A] Anticancer activity

In recent years, many researchers over the world have reported that sesquiterpenes lactones possess potential anticancer activity. Some of the important compounds of this class have been discussed below:

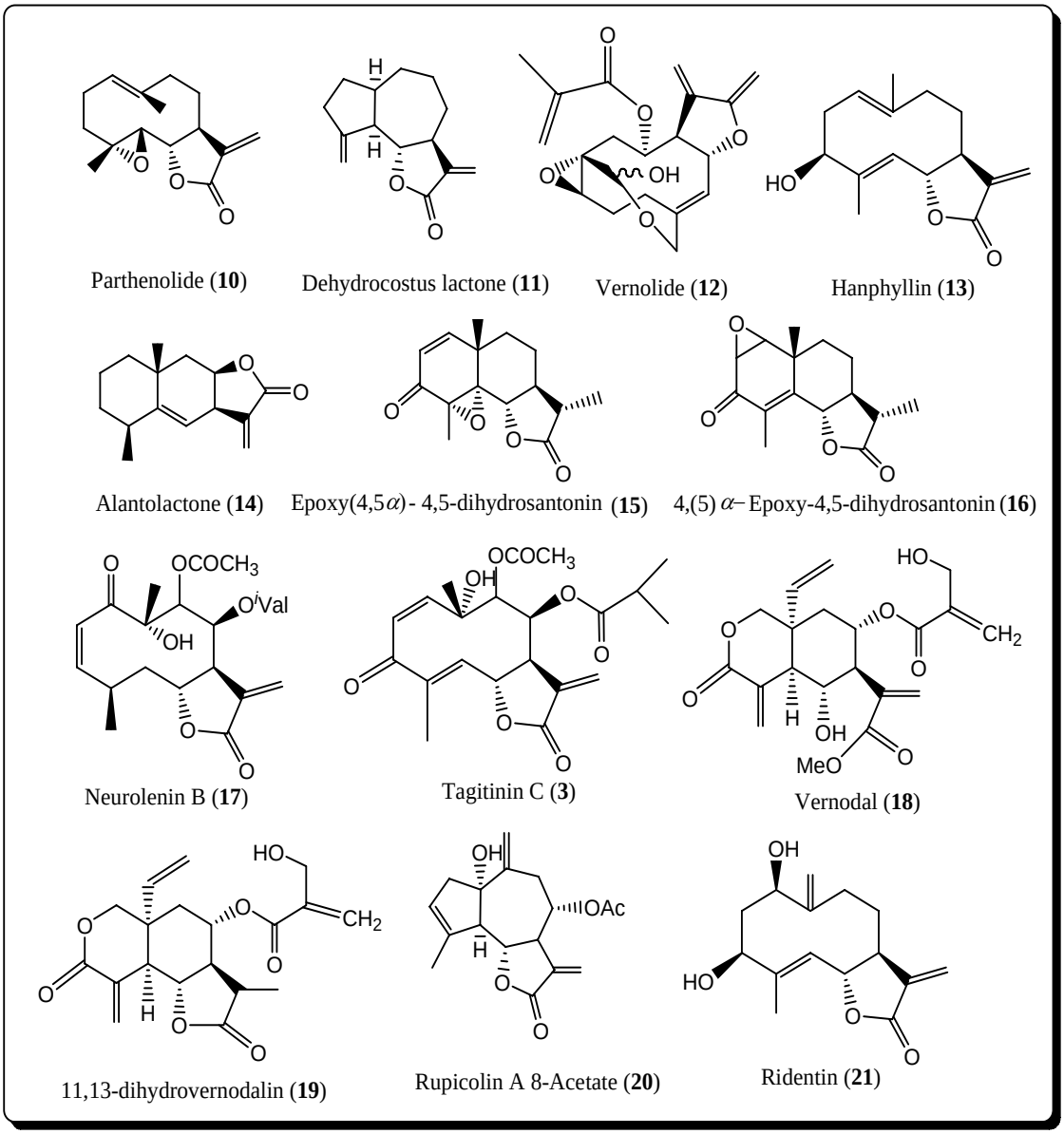


Figure 3. Structurally diverse sesquiterpenes lactones (**10-21**).

Table 1. Distribution of different structural classes of sesquiterpene lactones in the family-Compositae.

Tribes (No. of genera)	**No. of genera with sesquiterpene lactones**	**Type of lactones present**
Eupatorieae (50)	4	Germacranolides Elemanolides Guaianolides Ambrosanolides Seco-Ambrosanolides
Vernonieae (50)	4	Germacranolides Elemanolides Guaianolides
Astereceae (100)	1	Germacranolides Guaianolides Elemanolides
Inuleae (100)	5	Guaianolides Xanthanolides Ambrosanolides Helenanolides Seco-Eudesmanolides Seco-Ambrosanolides Germacranolides
Heliantheae (250)	24	Elemanolides Guaianolides Eudesmanolides Xanthanolides Ambrosanolides Helenanolides Seco-Eudesmanolides Seco-Ambrosanolides Seco-Helenanolides
Senecioneae (50)	4	Germacranolides Xanthanolides Eremophilanolides Helenanolides Bakkenolides
Anthemideae (50)	10	Germacranolides Elemanolides Guaianolides Helenanolides Cadinanolides Chrymoranolides
Arcototeae-Calenduleae (50)	1	Guaianolides

Table 1. Continued

Cynareae (50)	8	Germacranolides Elemanolides Guaianolides Eudesmanolides
Mutisieae (55)	1	Eudesmanolides
Lactucae (75)	7	Germanocranolides Eudesmanolides Guaianolides

I. Costunolide

Costunolide (**1,** Figure 2) is an active component from the crude extract of *Saussurea lappa* roots, a traditional Chinese medicinal herb [3]. The anticancer property of costunolide was first reported in a rat intestinal carcinogenesis model induced by azoxymethane and supported by a subsequent study using a DMBA induced hamster buccal pouch carcinogenesis model [4]. Following these two *in vivo* experiments, considerable efforts have been devoted to understad the mechanism responsible for the anti-cancer activity of costunolide. First, costunolide is a potent apoptotic inducer in cancer cells, *via* multiple pathways. It has been reported that costunolide readily depletes intracellular GSH and disrupts the cellular redox balance [5]. It triggers an intracellular reactive oxygen species (ROS) burst which leads to mitochondrial dysfunction: loss of mitochondrial membrane potential, onset of mitochondrial membrane transition, and release of mitochondrial pro-apoptotic proteins [6].

The apoptosis-inducing activity of costunolide was found to be closely associated with Bcl-2, based on observations that costunolide treatment decreased the anti-apoptotic Bcl-2 protein expression [7], while over expression of Bcl-2 protein attenuated costunolide-induced apoptosis [12]. Second, costunolide suppresses NF-kB activation *via* prevention of IkB phosphorylation [8], a process also responsible for the strong anti-inflammatory activity of costunolide [9]. Third, costunolide is capable of promoting leukemia cell differentiation [10], inhibiting endothelial cells angiogenesis [11], and disrupting nuclear microtubule architecture in cancer cells [12].

II. Parthenolide

Parthenolide (**10,** Figure 3), is the major SL responsible for bioactivity of feverfew (*Tanacetum parthenium*), a traditional herb plant which has been used for the treatment of fever, migraine and arthritis for centuries [13]. One well-explored bioactivity of parthenolide is its potent anti-inflammatory

effect, which is mainly achieved through its strong inhibitory effect on NF-kB activation. It has been well established that parthenolide acts on multiple steps along the NF-kB signaling pathway [14]. By suppressing NF-kB parthenolide inhibits a group of NF-kB regulated pro-inflammatory cytokines, such as interleukins and prostaglandins [15]. The anticancer activity of parthenolide has been pursued in a number of laboratories. A large number of studies have been undertaken to investigate the mechanism of action of parthenolide at molecular levels in the different phases of carcinogenesis. The data were obtained using different tumor cell systems. Parthenolide induced apoptosis in pre-B acute lymphoblastic leukemia lines, including cells carrying chromosomal translocations [16]. Parthenolide induced rapid apoptotic cell death distinguished by loss of nuclear DNA, externalization of cell membrane phosphatidyl-serine, and depolarization of mitochondrial membranes at concentrations ranging from 5 to 100 μM. Steele *et al*. investigated the *in vitro* actions of parthenolide on cells isolated from patients with chronic lymphocytic leukemia. Brief exposure to the sesquiterpene lactone (one to three hours) was sufficient to induce caspase activation and commitment to cell death. The mechanism of cell killing was *via* parthenolide induced generation of ROS, resulting in turn in a pro-apoptotic Bax conformational change, release of mitochondrial cytochrome C and caspase activation. Other studies also demonstrated that parthenolide-mediated apoptosis correlated well with ROS generation. Parthenolide strongly induced apoptosis in four multiple myeloma cell lines, although there are considerable differences in susceptibility to the sesquiterpene lactone. KMM-1 and MM1S sensitive to parthenolide possess less catalase activity than the less sensitive KMS-5 and NCI-H929 cells. These findings indicate that parthenolide-induced apoptosis in multiple myeloma cells depend on increased ROS and that intracellular catalase activity is a crucial determinant of their sensitivity to parthenolide. Chen *et al.* also reported the anti-proliferative and apoptosis-inducing effects of parthenolide on human multiple myeloma cells, mediated by an enhancement of caspase-3 activity [17].

III. Helenalin

Helenalin (**7**, Figure 2) is another SL, from *Arnica* species, which has been reported to possess cytotoxicity and anti-cancer activity [18]. Earlier studies demonstrated its potent activity to inhibit nucleic acid and protein synthesis [19]. Similiar to other anticancer SLs, mechanism of action mainly involve: (i) thiol depletion, (ii) inhibition of NF-kB, and (iii) induction of apoptosis [20]. These prominent bioactivities make helenalin another potential anti-cancer agent.

IV. Artemisinin and its derivatives

Given the high accumulation of iron in cancer cells, researchers Henry Lai and Narendra Singh became interested in possible artemisinin (**8**, Figure 2) activity against malignant cells and have used artemisinin against numerous cancer cells *in vitro* [21]. 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 high rates of iron flux *via* transferrin receptors when compared to normal cells, cancer cells are also particularly sensitive to oxygen radicals. Artemisinin becomes cytotoxic in the presence of ferrous ion. Since iron influx is naturally high in cancer cells, artemisinin and its analogs can selectively kill cancer cells *in vivo* [22]. 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. In recent years, in order to search for potential anticancer agents many researchers have directed their efforts in synthesizing various kinds of artemisinin dimers, trimers, tetramers wherein several of which have shown potential anticancer activity and are in the various phases of clinical trials [23].

[B] Anti-inflammatory activity

Sesquiterpenes lactones have displayed potential anti-inflammatory activity through NF-kB pathway. Since NF-kB plays a central role in most disease processes, and since it can regulate the expression of many key genes involved in inflammatory as well as in a variety of human cancers [24], NF-kB represents a relevant and promising target for the development of new chemopreventive and chemotherapeutic agents. Some of the important SLs have displayed anti-inflammatory activity are as follows:

I. Costunolide

Costunolide (**1,** Fig. 2) is a closely related sesquiterpene lactone analogue of parthenolide present in several plants such as *Magnolia grandiflora*, *Tanacetum parthenium*. Koo *et al*. showed that costunolide also dose-dependently inhibited LPS-induced NF-kB activation. In this assay system, costunolide even exhibited more potent inhibitory activity than parthenolide. Detailed mechanism studies revealed that, similar to parthenolide, costunolide also significantly inhibited the degradation of IkB-α and IkB-β. In addition, costunolide also inhibited the phosphorylation of IkB-α. These

accumulative results indicate that costunolide inhibits NF-kB activation by preventing the phosphorylation of IkB, and therefore, sequestering the complex in an inactive form [8].

II. Parthenolide

Parthenolide (**10,** Figure 3) is a sesquiterpene lactone present in several medicinal plants that have been used in folk medicine for their anti-inflammatory and analgesic properties. Several *in vitro* studies have shown that a great part of the anti-inflammatory action of this compound appears to be related to its ability to inhibit the NF-kB pathway. *In vitro* studies have proven that the sesquiterpene lactone parthenolide does not interfere with the generation of oxygen radicals [25], whereas it specifically inhibits activation of the NF-kB pathway by targeting IKK [26] and/or preventing the degradation of IkB-α and IkB-β [25]. Furthermore, parthenolide has recently been reported to exert beneficial effects during endotoxic shock in rats through inhibition of NF-kB DNA binding in the lung [27]. These effects of parthenolide may also accounts for its inhibition of pro-inflammatory mediator genes, such as the gene for the inducible nitric oxide synthase after endotoxin stimulation in rat smooth muscle cells [28] and the gene for IL-8 in immune-stimulated human respiratory epithelial cells [29]. In addition, parthenolide has also been demonstrated to protect against myocardial ischemia and reperfusion injury in the rat by selective inhibition of IKK activation and IkBα degradation [30].

III. Helenalin

Since different types of sesquiterpene lactones showed inhibition of NF-kB activation at similar concentrations, this effect seems to be characteristic for many of the sesquiterpene lactones with an exomethylene group like parthenolide and costunolide. Exomethylene groups of α,β-unsaturated carbonyl compounds can react by Michael type addition to sulfhydryl groups of cysteine residues in the DNA binding domain of the NF-kB subunit [31]. Recently, Lyu *et al*. provided evidence that a sesquiterpene lactone, helenalin (**7**, Fig. 2), containing two functional groups, namely α,β-unsaturated carbonyl group and α-methylene-δ-lactone ring, exerts its effect by direct alkylation of the p65 subunit of NF-kB without inhibition of IkB degradation [32]. *In vitro* studies also demonstrated that helenalin selectively modifies the p-65 subunit of NF-kB at the nuclear level, therefore inhibiting its DNA binding [33]. However, costunolide differs from helenalin in a

number of functional groups and inhibits degradation of IkB by inhibiting phosphorylation of IkB. Therefore, another functional group other than the exomethylene group and the molecular geometry of sesquiterpene lactone compounds appear to be important factors to determine the mode of NF-kB inhibition. However, the epoxide group in parthenolide is not likely important because parthenolide is at least less effective to inhibit both NF-kB activation and NO production.

[C] Anti-malarial activity

I. Artemisinin and its derivatives

In 1972, a group of Chinese researchers isolated a new anti-malarial drug (+)- artemisinin (**8**, Figure 4), a sesquiterpene lactone of the amorphene sub-group of cadinene from the hexane extract of a traditional Chinese medicinal plant *Artemesia annua* (Asteraceae) - a plant which has been used for the treatment of fever and malaria since ancient times [23]. Artemisinin is a sesquiterpene lactone containing an endoperoxide linkage in it. This highly oxygenated sesquiterpene lactone peroxide, unlike most other anti-malarials, lacks nitrogen containing heterocyclic ring systems and was found to be superior plasmocidal and blood schizontocidal agent to conventional anti-malarial drugs, such as chloroquine, quinine *etc* against malaria strains, without obvious adverse effects in patients.

Artemisinin is active at nanomolar concentrations *in vitro* both against chloroquine sensitive and resistant *P. falciparum* strains. However, the practical values of artemisinin, nevertheless, are impaired (i) poor solubility either in oil or water; (ii) high rate of parasite recrudescence after treatment; (iii) short-plasma half life (3-5h) and poor oral activity. However, a low level of resistance has

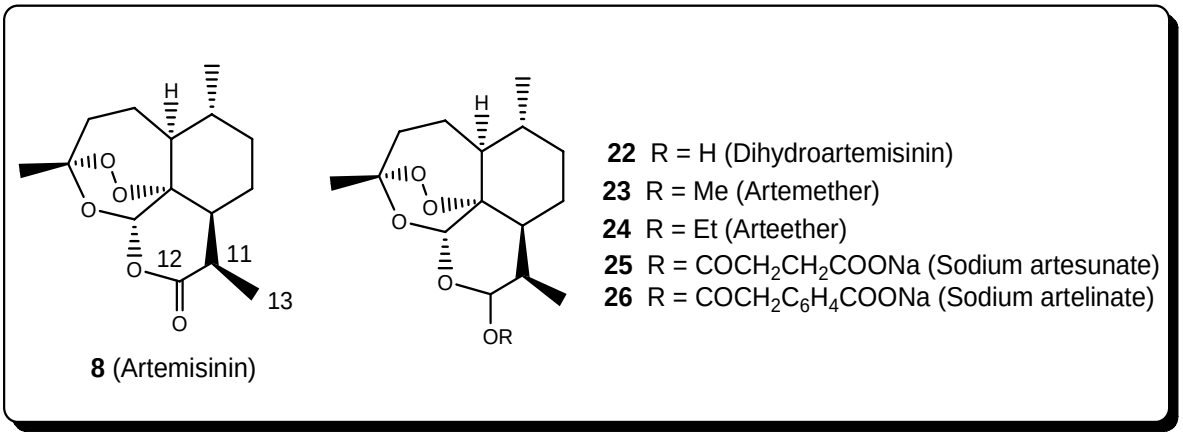


Figure 4. Structure of artemisinin and its analogs.

recently been observed using artemisinin, which disappeared as soon as the drug-selection pressure has been withdrawn. However, artemisinin with an endoperoxide linkage is a sensitive molecule for large scale derivatization. Fortunately, it was found that the carbonyl group of artemisinin **8**, can be easily reduced to dihydroartemisinin **22** in high yields using sodium borohydride, which has in turn led to the preparation of a series of semi-synthetic first-generation analogues included oil soluble artemether **23**, arteether **24**, water soluble sodium artesunate **25**, and sodium artelinate **26**.

These three analogs become very potent anti-malarial drugs effective against chloroquine-resistant strains of *P. falciparum*. Artemether **23** has been included in the WHO lists of Essential Drugs for the treatment of severe MDR malaria. In this family, the Walter Reed Institute of research has patented a stable, water-soluble derivative called artelinic acid **26** which is now being tested in animals. A key advantage of these endoperoxides containing anti-malarial agents, which have been used for nearly two decades, is the absence of drug resistance. It has been realized through the structure-activity relationship (SAR) of artemisinins that mainly endoperoxide affects the antimalarial activity. In order to increase antimalarial potency of these molecules, researchers around the world become interested to synthesize artemisinin dimers, trimers and tetramers in recent years. Many of them have shown promising antimalarial activity than artemisinin and their first generation analogs.

II. Miscellaneous antimalarials

Antimalarial activity of sesquiterpenes lactones from *Neurolena lobata* has been documented (Figure 5) [34]. Germacranolide sesquiterpenes lactones like neurolenin B (**17**, IC_{50} = 0.62 μM) more potent than furanoheliangolides lobatin B (IC_{50} = 16.51 μM). Among the germacranolides, the shift of the double bond from the 2,3-position (neurolenin B) into the 3,4-position (lobatin A) led to dramatic decrease in the activity suggesting that one of the structural requirements is the presence of α/β-unsaturated keto function. Additionally, a free hydroxyl group at C-8 increased the antiplasmodial activity, while a free hydroxyl group at C-9 decreased the activity.

Goffin *et al.* investigated the antiplasmodial properties of *Tithonia diversifolia* against three strains of *P. falciparum*, and sesquiterpene lactone (Fig. 5/Fig 3) Tagitinin C (**3**) was found to be active against FCA strain (IC_{50} = 0.33 μg/mL) [35]. Jenett-Siems *et al.* reported four sesquiterpenes, vernodalol (**18**), 11β,13-dihydrovernodalin 11β,13-dihydrovernolide (**19**) and 11β,13,17,18-tetrahydrovernolide from *Vernonia colorata.* Among these, vernodalol (**18**) and 11β,13-dihydrovernodalin (**19**) exhibited the strongest antiplasmodial activity (IC_{50} = 4.8 and 1.1 μg/mL) respectively). Among the sesquiterpene lactones obtained from *Artemisia afra*, 1-desoxy-1α-peroxy-rupicolin A-8-*O*-acetate

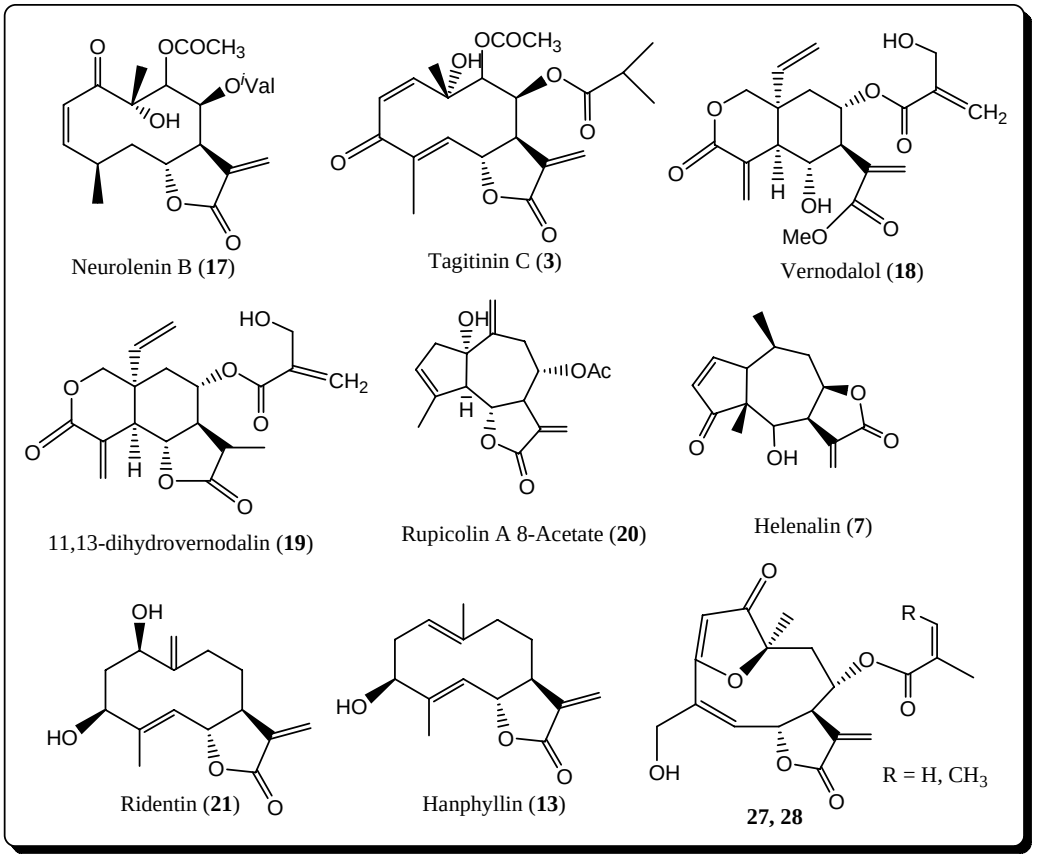


Figure 5. Structures of some of anti-malarials sesquiterpene lactones.

(**20**), 1β,4β-dihydroxy-bishopsolicepolide and rupicolin A-8-*O*-acetate (**20**) possessed *in-vitro* antiplasmodial activity (IC_{50} = 10.8-17.5 μg/mL) [36]. Passreiter *et al.* have isolated sesquiterpene lactones of the pseudoguaianolide type from *Arnica Montana*, helenalin (**7**), dihydrohelenalin and their acetates showing activities against *P. falciparum in vitro* (IC_{50} = 0.23 to 7.41 μM) [37]. Inhibitory effect upon the growth of *P. falciparum* has been reported for sesquiterpene lactones (**27)** and (**28)** isolated from *Camchaya calcarea* (IC_{50} = 1.2 and 0.3 μg/mL) respectively [38].

[D] Antiviral activity

In spite of an effective and safe vaccine therapy against hepatitis B virus (HBV), viral infection by HBV caused a global health problem in the world, especially the third world. Moreover, because direct antiviral therapy against HBV infection is not yet perfectly developed, it is important to discover the lead compounds for novel anti-HBV agents from the potential library. Recently, there was a report about anti-HBV activity of artemisinin (**8**) and artesunate (**25**) based on the screening by using HBV-transferred $HepG_2$ 2.2.15 cell [39], which is derived from hepatoblastoma $HepG_2$ cell

[40]. This screening method is a useful *in vitro* model for evaluation of novel anti-HBV drugs, as well as to study several steps of the HBV biology [41]. Artemisinin (**8**), artesunate (**25**), and a variety of purified compounds from traditional Chinese medicine remedy were investigated by measuring the release of surface protein (HBsAg) and HBV-DNA after drug exposure (0.01-100 μM) for 21 days [39]. As a result, artesunate (**25**) strongly inhibited the HAsAg secretion with an IC_{50} of 2.3 μM and IC_{90} of 16 μM, respectively, whereas artemisinin (**8**) had a mild inhibition activity. To evaluate an enhancement in viron production, the amount of the HBV-DNA release to the HepG2 2.2.15 culture medium during different treatments was measured, and it was significantly reduced. In addition, it was discovered that, for artesunate (**25**), toxicity in host cell was shown in drug concentration of 20 μM and therapeutical index (TI) calculated from IC_{50} of HBV-DNA release was 40. When comparing to TI value (500) of lamivudine as positive control, the value of artesunate (**25**) is quite low, but reasonable value for further investigation. Finally, artesunate (**25**) was tried in combination treatment with lamivudine. When both compounds were administered together in concentration of 20 nM each, no toxicity was observed, but a synergic inhibitory effect in HBsAg release was found. It means that it is possible to be potential antiviral agent against infection of lamivudine-resistance HBV strains, frequent problem in clinical treatment [42]. This result was quite similar to potency previously reported for human cytomegaloviruses [39].

Anti-viral activity of various sesquiterpene lactones was reported by Hsieh and their coworkers against hepatitis C virus (Fig. 6) [43]. They have tested a series of 10 compounds such as parthenolide (**10,** EC_{50} = 2.21 μM), costunolide (**1**, EC_{50} = 2.69 μM), dehydrocostus lactone (**11,** EC_{50} = 3.08 μM), Helenalin (**7**, EC_{50} = 1.25 μM), alantolactone (**14**, EC_{50} = 2.03 μM), Epoxy-dihydrosantonin (**15,** EC_{50} = >10 μM), artemisinin, and two other conjugated lactones. Wherein they found the best anti-HCV activity was shown by helenalin. They have further derivatized a series of parthenolide analogs **29** wherein they found that best activity was realized while putting a piperidine moiety (R = piperidine, EC_{50} = 1.64 μM).

[E] Antibacterial activity

There has been an overwhelming amount of evidence indicating that certain SLs are effective in exerting antibacterial activity. Rabe *et al.* showed that *Vernonia colorata,* a member of the Compositae found in west, central and South Africa possess SLs with antibacterial activity primarily against Gram-positive species and lower activity towards Gram-negative species [44]. The SLs vernodalin (**30**), vernolide (**12**) (Fig. 7) and 11β,13-dihydroovernolide were isolated and screened against *Staphylococcu aureus*

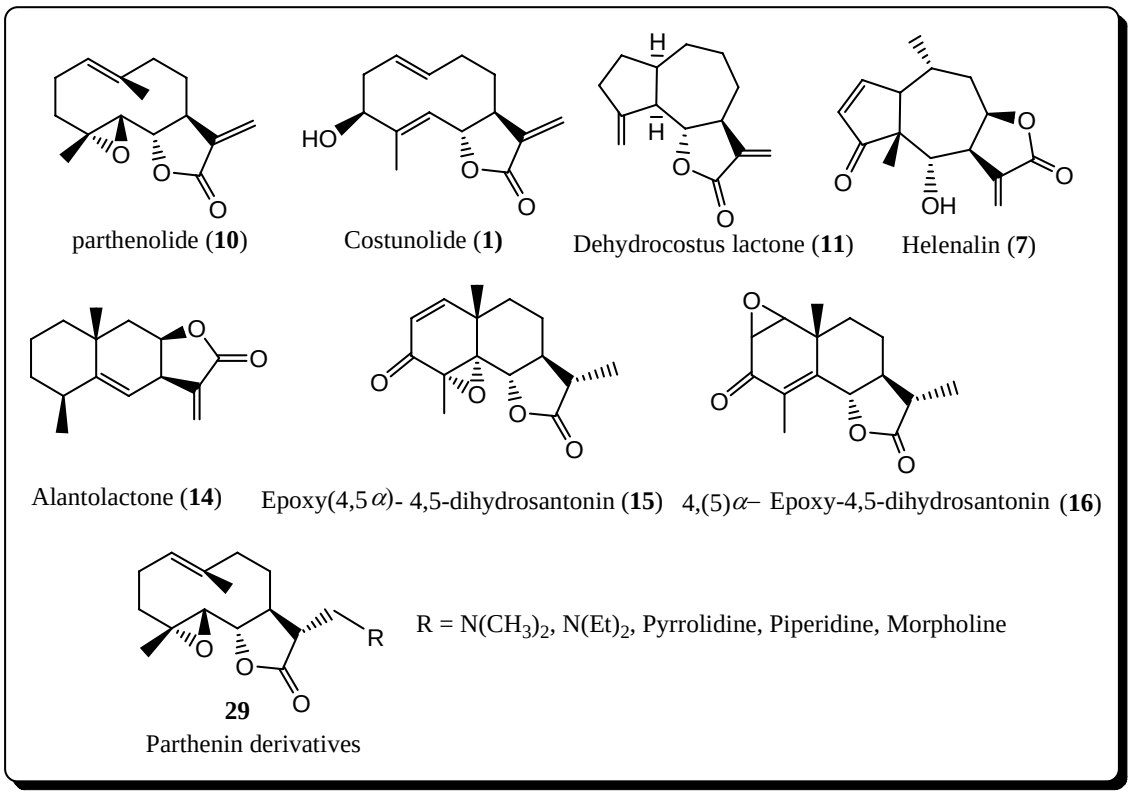


Figure 6. Antiviral sesquiterpene lactones.

and *Bacillus subtilis* (Gram-positive species) and *Escherichia coli* and *Klebsiella pneumoniae* (Gram-negative species).

11*β*,13-Dihydroovernolide is a novel SLs in that it has never been isolated from a *Vernonia* species before. All three of the compounds screened had very low inhibitory action against the Gram-negative bacteria. However, *S. aureus* and *B. subtilis* showed the most sensitivity towards all of the SLs screened. It needs to be noted, however, that although 11*β*,13-dihydrovernolide is a novel SL, it had the lowest activity against the Gram-positive species compared to vernolide and vernodalin which had MIC values of 0.1-0.5 mg/mL.

Taylor and Towers isolated, characterised and screened three SLs belonging to the pseudoguaianolides class of SLs from *Centipeda minima*, a member of the Compositae [45]. This plant is used throughout Southeast Asia to treat colds, coughs, and sinus infections. Three SLs, 6-*O*-methylacrylylplenolin (**31)**, 6-*O*-angeloylplenolin (**32)**, and 6-*O*-isobutyroylplenolin (**33)** (Figure 8) were isolated, with 6-*O*-methylacrylylplenolin being novel, and were then screened for antibacterial activity against *B. subtilis* and *S. aureus.* All three of the SLs screened had significant activity against the bacteria with 6-*O*-isobutyroylplenolin being the most bioactive. Both 6-*O*-isobutyroylplenolin and 6-*O*-methylacrylylplenolin exhibited MIC value of 150 *μ*g/mL against *B. subtilis.* 6-*O*-Angeloylplenolin was less active with a MIC of 300 *μ*g/mL. All three

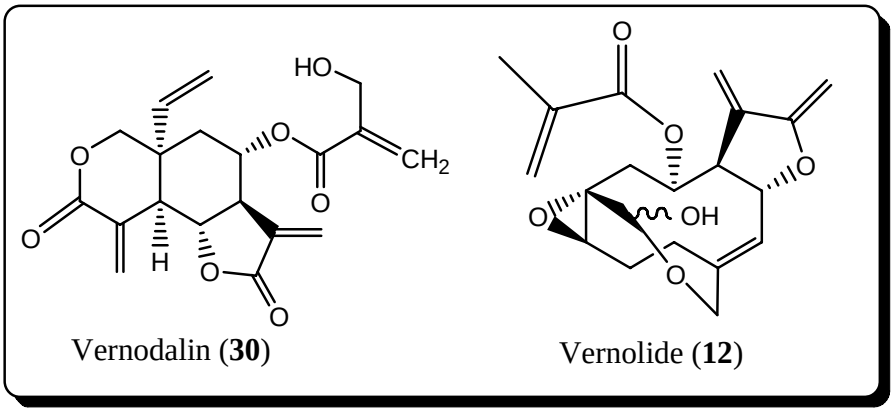


Figure 7. Antibacterial sesquiterpenes lactones (**30, 12**).

SLs showed activity against both methicillin-resistant and methicillin-sensitive strains of *S. aureus.* Both 6-*O*-isobutyroylplenolin and 6-*O*-methylacrylylplenolin had a MIC of 300 μg/mL against methicillin-resistant *S. aureus,* while 6-*O*-angeloylplenolin was less active with a MIC of 600 μg/mL against this strain. With respect to the methicillin-sensitive strain of *S. aureus,* 6-*O*-methylacrylylplenolin and 6-*O*-angeloylplenolin had MIC values of 75 μg/mL while 6-*O*-isobutyroylplenolin had a MIC of 38 μg/mL indicating that this SL is more bioactive against methicillin-sensitive *S. aureus* than the other SLs screened.

Further amplifying the possibility for the use of SLs found in plant oils, Wang and coworkers recently discovered four new SLs in a plant species known as *Ligulariopsis shichuana,* which is a new genus of the Compositae [46]. The four SLs isolated and characterised were: (a) 3β-acetoxy-9β-angeloyloxy-1β, 10β-epoxy-8α-hydroxyeremophil-7(11)-en-8β-(12)-olide (**34)**; (b) 3β-senecioyl-oxy-1β,10β-epoxy-8α-hydroxyeremophil-7(11)-en-8β-(12)-olide (**35)**; (c) 6β-angeloyloxy-8α-hydroxyeremophil-1(10),7(11)-dien-8β-(12)-olide (**36)**; and (d) 1-oxo-6β-senecioyloxy-8α-hydroxyeremophil-7(11),9(10)-dien-β-(12)-olide (**37)**

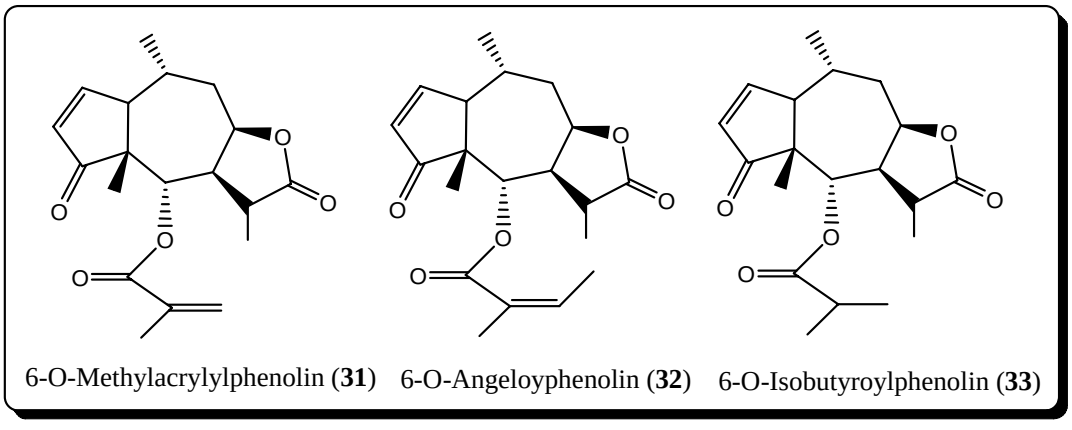


Figure 8. Antibacterial sesquiterpene lactones (**31-33**).

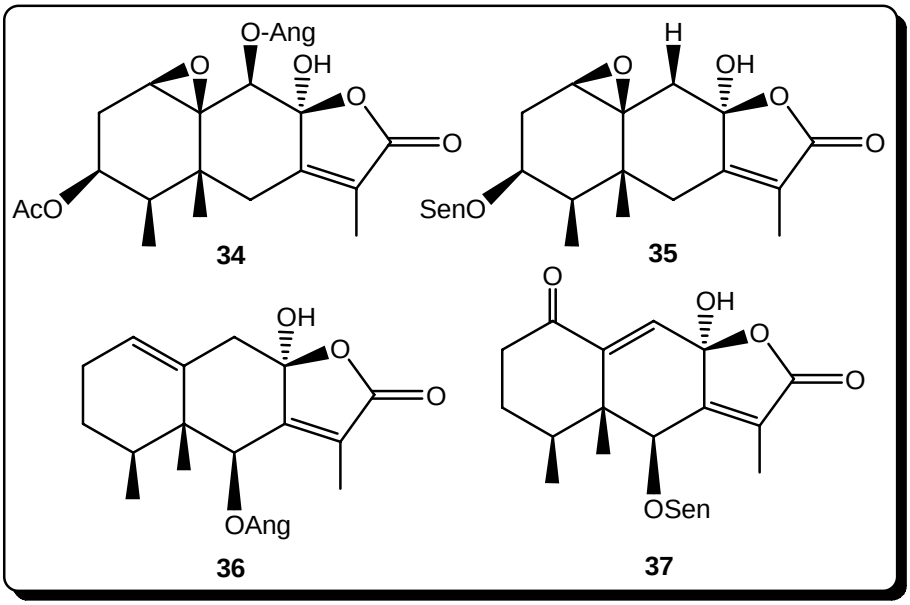


Figure 9. Antibacterial sesquiterpenes lactones (**34**-**37**).

(Fig. 9). Only compounds **34** and **35** were screened for their antibacterial activity. Compound **34** showed moderate activity towards both the Gram-positive and Gram-negative species *B. subtilis* and *E. coli*, respectively. Compound **35,** while exhibiting moderate activity towards *E. coli*, showed much stronger activity against *B. subtilis* at MIC concentrations up to 100 μg/mL.

Finally, there have been reports that other SLs, such as helenalin **7**, showed inhibitory action against *Mycobacterium tuberculosis* as well as activity against *Corynebacterium diptheriae* [47]. Helenalin **7**, a mixture of alantolactone **14** and isoalantolactone **38**, is derived from the plant species *Inula helenium* (Fig. 10). Helenalin **7** has primarily been utilised as an antiseptic for the urinary tract [47]. However, helenalin **7** was also shown to inhibit both Gram-positive and Gram-negative bacterial growth, with the former showing more sensitivity [48]. As one can see, there is certain hope for those essential oils containing SLs in therapeutics. The preclinical data implicates that SLs are effective in reducing bacterial growth which gives strength to the idea that SLs could be potentially used in the medical treatment of both Grampositive and Gram-negative bacterial infections.

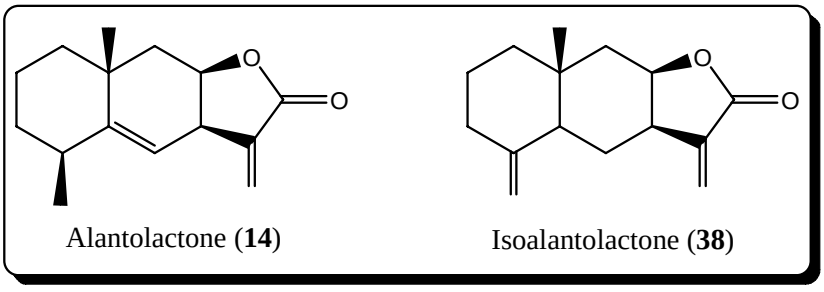
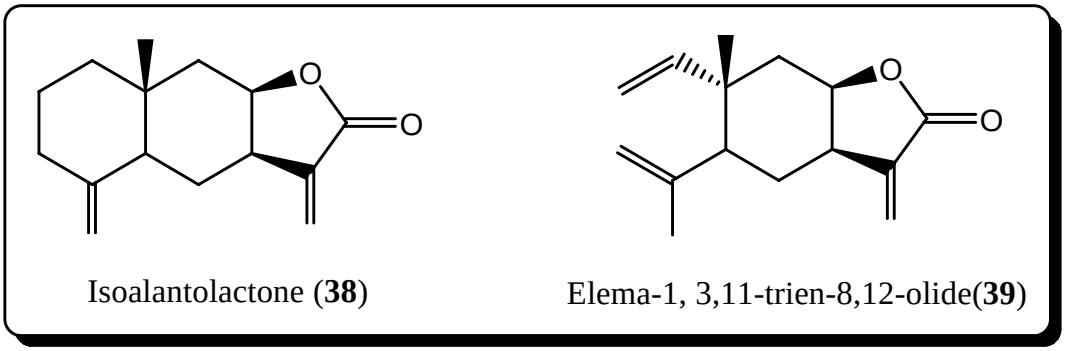


Figure 10. Antibacterial sesquiterpene lactones (**14, 38**).

[F] Antifungal activity

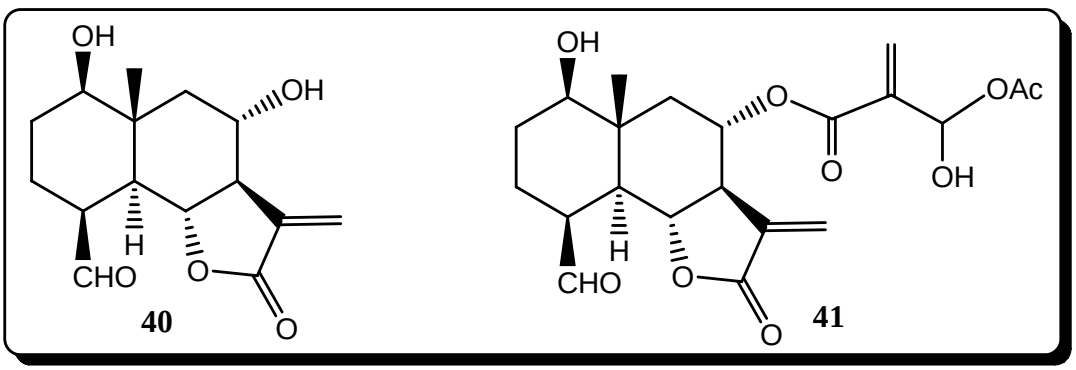
There certainly exists a vast amount of empirical data supporting that certain SLs found in essential oils have the potential to act as antibacterial agents. It also needs to be shown that certain SLs also possess antifungal activities. The following section will focus on studies implicating the SLs for probable use as antifungal agents.

Calera *et al.* isolated, characterised, and screened two bioactive SLs from the roots of yellow flowered perennial herb *Ratibida mexicana*. This plant is found primarily along the Sierra Madre Occidental in the northwestern part of Mexico [49]. Indian tribes find that the roots are useful in alleviating headaches, colds and rheumatism. The two SLs isolated from this plant are isoallolantolactone (**38)** and elema-1,3,11-trien-8,12-olide (**39)** (Fig. 11). The *in vitro* antifungal screen revealed that both SLs inhibited the radial growth of *Helminthosporium* with the MIC being 650 μg/mL for both SLs. Pythium growth was far more sensitive to isoallolantolactone (**38)** with a MIC of 125 μg/mL. *Fusarium* was also screened for sensitivity against isoallolantolactone (**38)** and elema-1,3,11-trien-8,12-olide, with again isoallolantolactone (**39)** showing the most bioactivity by inhibiting 45% of radial growth at 200 μg/mL for this particular fungus.



Isoalantolactone (**38**) Elema-1, 3,11-trien-8,12-olide(**39**)

Figure 11. Antifungal activity of sesquiterpene lactones (**38, 39**).



40 **41**

Figure 12. Antifungal activity of sesquiterpene lactones (**40-41**).

Two new eudesmanolides were isolated from the aerial parts of *Centaurea thessala spp. drakiensis* and *C. attica spp. attica*, plants which are primarily used in folk medicine in the Mediterranean region [50]. The two novel eudesmanolides isolated were 8α-hydroxy-4-epi-sonchucarpolide (**40)** and the 8α-(4-acetoxy-3-hydroxy-2-methylenebutanoyloxy) derivative **(41)** of the 8α-hydroxy-4-epi-sonchucarpolide, also known as 40-acetoxymalacitanolide (Fig. 12).

A variety of fungal species showed sensitivity towards 8α-hydroxy-4-epi-sonchucarpolide (**40**) and 40-acetoxymalacitanolide (**41**) [50]. 8α-Hydroxy-4-epi-sonchucarpolide **40**, when compared to 40-acetoxymalacitanolide **41**, showed higher activity against all the fungal species screened, with the exception of one species. The MIC values for 8α-hydroxy-4-epi-sonchucarpolide **40** were considerably lower indicating that sensitivity is much higher for this compound. The exception was for *Cladosporium cladosporioides*; this species showed higher sensitivity towards 40-acetoxymalacitanolide, with a MIC value of 0.06 μg/mL while the MIC value for 8α-hydroxy-4-epi-sonchucarpolide was 0.5 μg/mL. In addition, both SLs had indentical MICs against *Penicillium funiculosum*, showing no disparity between these two SLs in their inhibitory action against this particular species. The authors of this paper speculated that the differences in activity between these two SLs could be attributed to the different skeletal types and functional groups present on the compounds. Finally, it needs to be mentioned that both SLs, possessed greater antifungal activity that miconazole, a commercial fungicide used as the positive control.

3. Structural-activity relationships (sar) of sesquiterpene lactones

It is generally believed that the bioactivity of SLs is mediated by alkylation of nucleophiles through their α, β- or α, β, γ-unsaturated carbonyl structures, such as α-methylene-γ-lactones or α,β-unsaturated cyclopentenones. These structure elements react with nucleophiles, especially the cysteine sulfhydryl groups by Michael-type addition. Therefore, it is widely accepted that thiol groups such as cysteine residues in proteins, as well as the free intracellular GSH, serve as the major targets of SLs. In essence, the interaction between SLs and protein thiol groups or GSH leads to reduction of enzyme activity or causes the disruption of GSH metabolism and vitally important intracellular cell redox balance.

The relationship between chemical structure and bioactivity of SLs has been studied in several systems, especially with regards to cytotoxicity, anti-inflammatory and antitumor activity. It is believed that the *exo*-methylene

group on the lactone is essential for cytotoxicity because structural modifications such as saturation or addition to the methylene group resulted in the loss of cytotoxicity and tumor inhibition. However, it has also been shown that the factor responsible for the cytotoxicity of SLs might be the presence of the O=C-C=CH_2 system, regardless of lactone or cyclopentenone. It was latter demonstrated that the presence of additional alkylating groups greatly enhanced the cytotoxicity of SLs. Furthermore, it was established that the α-methylene-γ-lactones and α,β-unsaturated cyclopentenone ring (or α-epoxycyclopentenone) present in SLs essential for their *in vivo* anti-tumor activity. It has been confirmed through various published reports that the various kinds of biological activities displayed by SLs is due to presence of either α-methylene-γ-lactones and α,β-unsaturated cyclopentenone ring. In summary, the differences in activity among individual SLs may be explained by differences in the number of alkylating elements, lipophilicity, molecular geometry, and the chemical environment of the target sulfhydryl group.

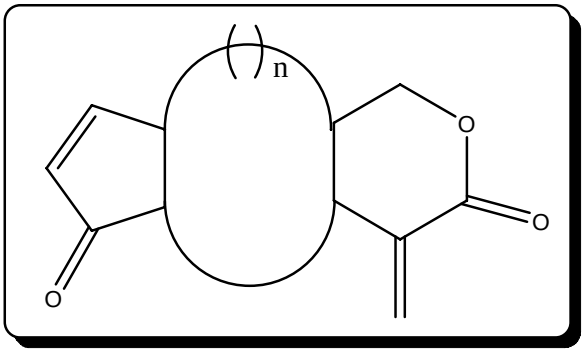


Figure 13. General structure of sesquiterpene lactones.

4. Conclusions

Sesquiterpene lactones are an important group of natural products obtained from many species of medicinal plants. Their structural diversity and diverse potential biological activities such as anticancer, anti-inflammatory, anti-tumor, anti-malarial, antiviral, antibacterial, antifungal *etc.* have made further interest among the chemists to the drug discovery research. Although, the exact mechanism of action of SLs are not well known but it has been documented through the various published reports that the biological activity displayed by majority of sesquiterpene lactones is due to the presence of α-methylene-γ-lactones and α,β-unsaturated cyclopentenone ring. The present chapter deals an overview on the various kinds of biologically activity of structurally diverse sesquiterpene lactones

which may be useful for the chemists/pharmacologists working in the area of drug discovery of the relevant subject.

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