Anti-Cancer Potential of Sesquiterpene Lactones: Bioactivity and Molecular Mechanisms

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Abstract: Sesquiterpene lactones (SLs) are the active constituents of a variety of medicinal plants used in traditional medicine for the treatment of inflammatory diseases. In recent years, the anti-cancer property of various SLs has attracted a great deal of interest and extensive research work has been carried out to characterize the anti-cancer activity, the molecular mechanisms, and the potential chemopreventive and chemotherapeutic application of SLs. In this review, we attempt to summarize the current knowledge of the anti-cancer properties of SLs by focusing on the following important issues. First, we discuss the structure-activity relationship of SLs. All SLs contain a common functional structure, an α-methylene-γ-lactone group, and this important chemical characteristic means that the thiol-reactivity of SLs is an underlying mechanism responsible for their bioactivities. Second, we assess the experimental evidence for the anti-cancer function of SLs obtained from both *in vitro* cell culture and *in vivo* animal models. Various SLs have been demonstrated to execute their anti-cancer capability via inhibition of inflammatory responses, prevention of metastasis and induction of apoptosis. Thirdly, we outline the molecular mechanisms involved in the anti-cancer activity of SLs, in particular, the SL-thiols reaction, the effect of SLs on cell signaling pathways such as nuclear transcription factor-kappaB (NF-κB) and mitogen-activated protein kinases (MAPK). Finally, we recapitulate some important SLs with regards to their anti-cancer activities and their potential in anti-cancer drug development. Taken together, many SLs are emerging as promising anti-cancer agents with potential applications in both cancer chemotherapy and chemoprevention.

Key Words: Sesquiterpene lactones, anti-cancer, apoptosis, thiols, anti-inflammatory, NF-κB.

INTRODUCTION

Sesquiterpene lactones (SLs) are a large and diverse group of natural products present in more than 100 families of flowering plants. Among them, the greatest number of SLs are derived from the *Compositae* (Asteraceae) family, with over 3, 000 reported structures. Some of the important medicinal plants from this family such as *Arnica montana*, *Artemisia annua* and *Tanacetum parthenium* contain SLs as the main active component [1]. Most SLs are isolated from the leaves or flowering heads of plants where they can constitute up to 5% of dry weight [2].

To date, significant effort has been made to examine the pharmacological property of SLs and their potential as pharmaceutical agents. The pharmacological activities described for SLs include anti-microbial, anti-viral, anti-inflammatory and anti-tumor. In the search of the molecular mechanisms involved, it is generally accepted that the covalent binding of SLs to free sulfhydryl groups in proteins leads to the disruption of the functions of various macromolecules. As a result, SLs may interfere with some key biological processes, such as cell signaling, cell proliferation, cell death/apoptosis and mitochondrial respiration, all of which constituting the molecular basis for their diverse pharmacological activities.

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In this review, we intend to summarize the anti-cancer activity of SLs, covering the following important issues: (i) structure-bioactivity relationship of SLs, (ii) effects of SLs on cancer cells *in vitro*, (iii) evidence for the anti-cancer activity of SLs from *in vivo* animal cancer models, (iv) the molecular mechanisms underlying the anti-cancer property of SLs, and finally (v) the anti-cancer potential of some representative SLs.

1. STRUCTURE AND BIOACTIVITY RELATIONSHIP

As indicated in the prefix "sesqui", SLs are 15-carbon terpenoids. According to their carbocyclic skeletons, SLs can be chemically classified into four major groups: 1) germacranolides (with a 10-member ring), including some common SLs such as costunolide and parthenolide; 2) eudesmanolides (6/6-bicyclic compounds), including santamarine and a-santonine; 3) guanianolides, including arglabine and artabsine; and 4) pseudoguainolides (both 5/7-bicyclic compounds), including helenalin and parthenin [3]. The chemical structures of representative SLs are shown in Fig. (**1A**). Among them, germacranolides represent biogenetically the most primitive group and all other SLs are derived from this group. The suffix "olide" indicates the presence of a lactone group. All SLs contain an α-methylene-γ-lactone ring either *cis*- or *trans*- fused to the C_6-C_7 or C_8-C_7 position of the carboxylic skeleton (Fig. **1B**). The terms *cis* or *trans* refer to the orientation of the 5- or 7-membered rings situated on opposite sides of the bond linking C_1 and C_5.

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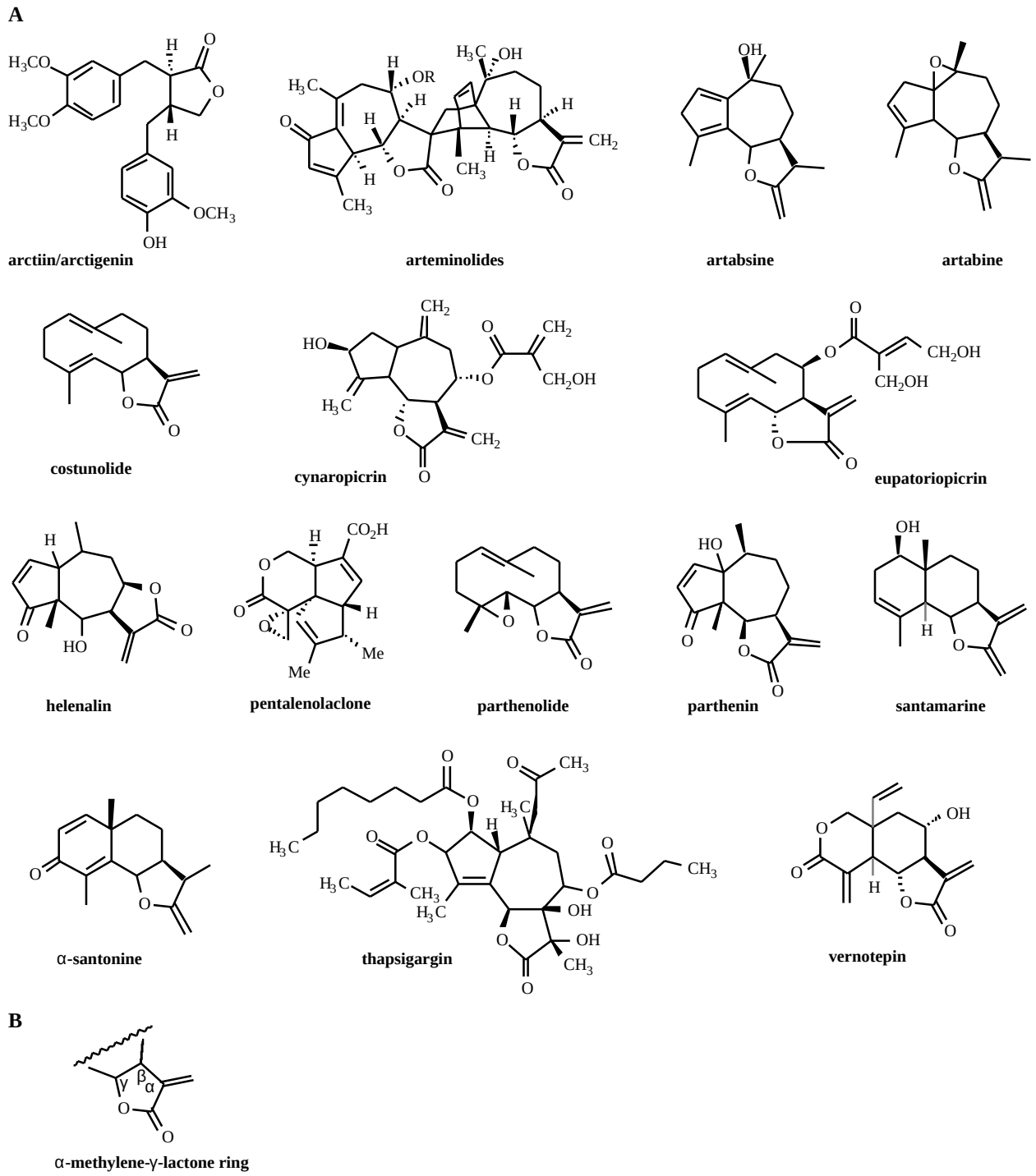

Fig. (1). Chemical structures of representative SLs.

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 the vitally important intracellular cell redox balance [4-6].

The relationship between chemical structure and bioactivity of SLs has been studied in several systems, especially with regards to cytotoxicity, anti-inflammatory and anti-tumor activity. It is believed that the exo-methylene group on the lactone is essential for cytotoxicity beacuse structural modifications such as saturation or addition to the methylene group resulted in the loss of cytotoxicity and tumor inhibition [7]. 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 [8]. It was later demonstrated that the presence of additional alkylating groups greatly enhanced the cytotoxicity of SLs [4, 9]. Furthermore, it was established that the α-methylene-γ-lactone moiety and α, β-unsaturated cyclopentenone ring (or an α-epoxycyclopentenone) present in SLs are essential for their *in vivo* anti-tumor activity [10].

When comparing the cytotoxicity of 21 helenanolide-type SLs, Beekman and coworkers noted that the absence of both the α-methylene-β-lactone and the cyclopentenone structures greatly diminishes their cytotoxic effects [11]. Other observations included a positive correlation between lypophilicity and cytotoxicity as well as an inverse relationship between the steric hindrance caused by the tigloyl group and cytotoxicity [11]. In addition, the reactivity of the two potential Michael addition sites of helenanolide-type SLs to physiological thiols, glutathione and cysteine in aqueous solution was also investigated by ^{1}H NMR spectroscopy [12]. It was found that the chemical environment of the target sulfhydryl group plays an important role in bioactivity. However, no correlation between the rate of cysteine addition and bioactivity was detected in their system.

Another fairly comprehensive study was conducted by Rungeler and coworkers in which the structure-activity relationship of 28 SLs was tested on tumor necrosis factor (TNF)-stimulated nuclear transcription factor-kappaB (NF-κB) activation [13]. Those selected SLs represented nearly all major skeletal classes, including germaranolides, eudesmanolides, guaianolides, and pseudoguaianolides. One interesting finding was that the majority of SLs with potent NF-κB inhibitory activity possess a highly reactive α-methylene-β-lactone group and some SLs have α, β, - or α, β, γ, δ-unsaturated carbonyl groups which further enhance their bioactivity. Based on computer molecular modeling, the authors proposed a molecular mechanism of action which explains the specific action of SLs on NF-κB activation: interfering with the DNA binding activity of p65 via direct alkylation of the cysteine residues [13]. A very recent and more comprehensive study by Siedle *et al.* [14] examined the quantitative structure-activity relationships among 103 different SLs for their NF-κB inhibiting properties. Their findings support the importance of α, β-unsaturated carbonyl function, which is in accordance with the previous study on 28 SLs [13]. In addition, a lead structure for synthesis of a NF-κB-inhibiting compound was suggested, although it is rather difficult to separate the wanted therapeutic effects from the unwanted side effects, such as cytotoxicity [14]. In addition to the effect on NF-κB, the structural requirements of 11 SLs that were capable of inhibiting lipopolysaccharide (LPS)-induced nitric oxide (NO) synthesis in RAW 264.7 macrophages were also studied [15]. It was found that the cytotoxicity of these SLs correlated with the presence of an α-methylene-β-lactone moiety, but the ability to inhibit NO synthesis did not show any association with either cytotoxicity or the ability to suppress LPS-stimulated NF-κB activation [15].

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. A good understanding of the chemical structure-bioactivity relationship helps to lay the foundation for the development of more effective SL analogues for potential clinical application.

2. ANTI-CANCER ACTIVITY OF SESQUITERPENE LACTONES: EVIDENCE FROM *IN VITRO* STUDIES

2.1. Inhibition of Cell Proliferation, Disruption of Cell Cycle, and Promotion of Cell Differentiation

It has been well established that tumor development is closely associated with dysregulation of the cell cycle control mechanisms through either over-expression or activation of cyclin-dependent kinases (CDK) and/or genetic loss/inhibition of CDK inhibitors [16], resulting in uncontrolled cell cycling and unremitting cell proliferation. Thus targeting cell cycle regulators is an important and promising approach for cancer therapy [17]. Effects of SLs on cell cycle have been demonstrated in a number of cancer cells. One SL, parthenolide (chemical structure shown in Fig. (**1A**)), has been reported to arrest cell cycle progression at the G2/M checkpoint, especially at low concentrations in an invasive sarcomatoid hepatocellular carcinoma cell line (SH-J1) [5]. In addition to G2/M arrest, cynaropicrin (chemical structure shown in Fig. (***1A***)), has been reported to induce cell cycle arrest at G1/S phase in various leukocyte cancer cell lines [18].

A number of studies focused on the effect of SLs on cell differentiation, a process known to be important in oncogenesis such as the development of leukemia [19]. Costunolide (chemical structure shown in Fig. (**1A**)) has been found to promote the differentiation of leukemia cells [20]. More importantly, several SLs, including parthenolide, costunolide and yomogin, work synergistically with other chemotherapeutic drugs to potentiate the differentiation of leukemia cells [21-23]. This promotion of differentiation by SLs suggests the potential of SLs as promising anti-cancer agents in leukemia therapy.

2.2. Induction of Apoptosis in Cancer Cells

Apoptosis, or programmed cell death, refers to a highly regulated and conserved cell death process in eukaryotic cells which is morphologically and biochemically distinct from necrotic cell death. Dysregulation of the apoptotic process has been shown to be implicated in tumor development and chemoresistance [24, 25]. Apoptotic cell death signaling involves two major pathways: the cell death receptor pathway and the mitochondrial pathway. In the cell death receptor pathway, the cell death signal is originated from the ligation of cell death ligands, such as Fas ligand, TNF and TNF-related apoptosis-inducing ligand (TRAIL), to their respective cell death receptors [26]. This ligation

triggers the recruitment and activation of an initiator caspase, caspase 8. Activation of caspase 8 directly targets the effector caspase, such as caspase 3, which executes the apoptotic processes [26]. On the other hand, the mitochondrial pathway is triggered by intracellular cell death signals such as DNA damage, and it involves the release of mitochondrial apoptotic proteins, such as cytochrome c and Smac, which work in coordination with caspase 9 and Apaf-1 to activate caspase 3 [24]. It has been well established that apoptosis is critical in maintaining tissue homeostasis and many cancer therapeutic agents act through induction of cancer cell apoptosis [16, 27].

It has been known for a long time that many SLs are cytotoxic to cancer cells [4, 9, 10]. Studies using *in vitro* cell culture systems demonstrated that SLs are capable of inducing apoptotic cell death in various cancer cells. As shown in Table **1**, many SLs are strong apoptosis inducers, in the range of micromolar concentrations, to many types of cancer cells. Similar to other anti-cancer agents, this ability to induce apoptosis in cancer cells is one of the important mechanisms involved in the anti-tumor property of SLs. Although the detailed molecular mechanisms involved in SL-induced apoptosis have not been well elucidated, it is believed that the α-methylene-β-lactone structure commonly found in the SLs is essential for their apoptogenic activity. Upon entry to cells, the thiol-reactive SLs quickly conjugate with GSH and deplete intracellular thiols, leading to the disruption of cellular redox status and induction of oxidative stress [5, 6, 28]. The excessive reactive oxygen species (ROS) and disrupted redox status then cause the initiation of the mitochondria-dependent apoptosis pathway. The involvement of mitochondria in SL-induced apoptosis has been observed in several representative SLs such as helenalin (chemical structure shown in Fig. (**1A**)) [29], costunolide [30] and parthenolide [6]. SLs trigger mitochondrial membrane transition, loss of mitochondrial membrane potential and release of pro-apoptotic mitochondrial proteins, e.g. cytochrome c and Smac, subsequently leading to caspase activation and apoptotic cell death.

To further evaluate the role of mitochondria in SL-induced apoptosis, a number of studies have examined the regulatory role of Bcl-2 family members in SL-induced apoptosis. It appears that the anti-apoptotic Bcl-2 proteins are not directly involved in SL-induced apoptosis based on following observations: (i) treatment with SLs has no effect on the expression level of Bcl-2 and Bcl-xL proteins [5, 6], and (ii) overexpression of Bcl-2 and Bcl-xL fails to prevent SL-induced apoptosis [28, 29]. In contrast, SL treatment significantly affects the pro-apoptotic Bcl-2 proteins, causing Bid cleavage, Bax mitochondrial translocation and Bak dimerization, and eventually promoting apoptotic cell death in human cancer cells [31].

In addition to mitochondria, other cell death regulators are also implied in SL-induced apoptosis. For example, thapsigargin (chemical structure shown in Fig. (**1A**)), a potent inhibitor of Ca^{2+}-activated ATPase on ER, induces ER stress and Ca^{2+} burst which then trigger cytochrome c-independent apoptosis in cancer cells [32]. Moreover, a recent study on cynaropicrin demonstrated that, in addition to ROS, protein kinase C (in particular PKCδ) may also play an important regulatory role in SL-induced apoptosis [18]. Finally, it is also worth noting that, under some circumstances, treatment with SLs could lead to atypical apoptosis or even necrotic cell death in human cancer cells [33].

2.3. Sensitization Activity

In cancer chemotherapy, combinations of different chemotherapeutic agents are an effective approach to overcome chemoresistance in certain cancer types. Several recent studies provide evidence that SLs sensitize human cancer cells to chemotherapeutic drugs. For instance, pretreatment with parthenolide, a potent NF-κB inhibitor, significantly increased the paclitaxel-induced apoptosis of breast cancer MDA-MB-231 cells [34]. Similar sensitization effects by parthenolide were also observed in other cancer cell models [35, 36]. This sensitization effect by SLs is mainly as a result of: (i) depletion of intracellular thiols and ROS generation; (ii) inhibition of the anti-apoptotic NF-κB signaling path-

Table 1. Induction of Apoptosis by Sesquiterpene Lactones in Human Cancer Cells

SLs	Treatments	Cell types	Outcomes	Ref.
Costunolide	(1-10μM) up to 12 hrs	human leukemia cells HL-60	ROS generation, mitochondrial permeability transition, apoptosis	[30]
Costunolide	(1-10μM) up to 10 hrs	human leukemia cells U937	depletion of thiols, induction of apoptosis	[28]
Cynaropicrin	(10-20μM) up to 12 hrs	human leukemia cells U937	G_1/S arrest, cleavage of PKCdelta, apoptosis	[18]
Helenalin	(10-50 μM) up to 24 hrs	human leukemia cells Jurkat	Induction of a CD-95 and Bcl-2/X_L independent apoptosis	[29]
Parthenolide	(1-10μM) up to 48 hrs	human invasive sarcomatoid hepatocellular carcinoma cells SH-J1	depletion of thiols, induction of apoptosis, overexpression of GADD153	[5]
Parthenolide	(5-50μM) up to 24 hrs	human colorectal cancer cells COLO205	thiols depletion, Bid cleavage, Bak dimeriztion, mitochondrial changes, apoptosis	[6,31]
Parthenolide	Paclitaxel, with pretreatment of parthenolide for 4 hrs	human breast cancer cells MDA-MB-231	sensitization of breast cancer cells to paclitaxel- induced apoptosis	[34]

way; and (iii) modulation of the MAPK signaling pathways. Of these, the most important mechanism is the inhibition of NF-κB. In some cancer cells, the NF-κB signaling pathway is constitutively activated and consequently leads to overexpression of a variety of NF-κB regulated anti-apoptotic proteins, such as Inhibitors of Apoptosis (IAPs), Bcl-2, and FLICE inhibitory proteins (FLIPs) [37]. Such a mechanism well explains the sensiti-zation effect of parthenolide on paclitaxel-induced apoptosis in breast cancer cells with constitutively high level of NF-κB activation [34]. Some cell death ligands, such as TNF, are known to trigger the death receptor pathway with simultaneous activation of the anti-apoptotic NF-κB pathway [38]. Parthenolide pretreatment is able to block NF-κB activation and then sensitizes TNF-mediated apoptotic cell death [36]. In addition to TNF, a similar sensitization effect by parthenolide has also been found in TRAIL-induced apoptosis, via modulation of the c-Jun N-terminal kinase (JNK) signaling pathway [35]. Ssustained activation of JNK has been shown to play a critical role in the sensitization effect of parthenolide in TNFα-induced apoptosis [36].

2.4. Anti-Metastasis Activity

Another important aspect of the anti-cancer activity of SLs is their suppressive effect on cancer cell metastasis. It is known that cell adhesion molecules (ICMs) are among the essential molecules that facilitate neoplastic cell adhesion and migration [39], and parthenolide has been found to inhibit the expression of ICMs in *in vitro* cell culture studies [40, 41]. Moreover, costunolide, another cytotoxic SL which inhibits NF-κB, is able to inhibit endothelial cell proliferation and angiogenesis induced by endothelial growth factor [42]. One of the underlying mechanisms responsible for the above activities is the inhibitory effect of SLs on the NF-κB signaling pathway, since ICMs are known to be one of the target genes of NF-κB [43, 44]. Taken together, these data imply that the anti-metastatic activity of SLs is an important aspect of their anti-cancer function.

2.5. Anti-Inflammatory Effect

It has been suggested that chronic inflammation promotes cancer development in many cancers, such as breast, colorectal, and liver [45]. Many anti-inflammatory drugs, e.g. the nonsteroidal anti-inflammatory drugs (NSAIDs), have been confirmed to reduce the risk of colon cancer formation in both animal cancer models as well as in epidemiological investigations [46]. Anti-inflammatory acitivty is a prominent bioactivity observed in many types of SLs. This activity is often associated with the ability of SLs to inhibit NF-κB and thus to down regulate many of the NF-κB-dependent inflammatory responsive genes such as interleukins (IL) [47], cyclooxygenase (COX) [48], and inducible nitric oxide synthase (iNOS) [49].

Interleukins are a big family of cytokines that play a pivotal role in inflammatory processes. Parthenolide, a potent inhibitor of NF-κB, significantly suppressed the expression and secretion of various interleukins including IL-2, IL-4, IL-8 and IL-12 [47, 50, 51]. In addition, parthenolide also suppresses IL-6 secretion and signaling via the inhibition of signal transducers and activators of transcription (STATs) phosphorylation and activation [52]. On the other hand, NO is another important regulatory molecule involved in the inflammatory response. Synthesis and release of NO are mediated by iNOS. Many SLs have been found to suppress iNOS expression through inhibition of NF-κB [53, 54]. For instance, it has been demonstrated that parthenolide suppresses the gene expression of iNOS in several cell lines under stimulation by LPS, interferon-α (INFα) or 12-*o*-tetradecanoylphorbol-13-acetate (TPA) [53]. Similar effects were also observed in other SLs, such as cynaropicrin, a SL from *Saussurea lappa*, which suppresses NO release in LPS-stimulated lymphocytes [55].

Cyclooxygenases (COX) are the rate limiting enzymes in prostaglandin synthesis. There are two isoforms of COX: COX-1 and COX-2. In contrast to constitutively active COX-1, COX-2 is highly inducible by pro-inflammatory cytokines and tumor promoters and plays a central role in inflammation [56]. The critical role of COX-2 during tumor formation, particularly in colorectal cancer, has been well established. COX-2 promotes cancer development by suppressing apoptosis, facilitating angiogenesis, and enhancing metastatic potential of colorectal cancer cells [57]. Parthenolide has been found to suppress the COX-mediated pathway by either directly inactivating COX-2 enzyme activity via interaction with sulphydryl groups on enzymes, or by indirectly inhibiting COX-2 transcription through NF-κB [58-60]. It is thus believed that a suppressive effect of SLs on COX-2 is one of the underlying mechanisms for the anti-cancer activity of SLs.

3. ANTI-CANCER ACTIVITY OF SESQUITERPENE LACTONES: EVIDENCE FROM *IN VIVO* ANIMAL MODELS

As summarized in Table **2**, a number of SLs have been tested for their anti-cancer activity using various *in vivo* animal models (Table **2**). An early report by Mew *et al.* (1982) demonstrated that parthenin (chemical structure shown in Fig. (**1A**)), the main bioactive SL in *Parthenium hysterophorus* L. (Asteraceae), was capable of significantly increasing the overall survival of female DBA/2J mice bearing solid tumors induced by injection of mastocytoma, leukemia and rhabdomyosarcoma cells [61]. Parthenin is known to possess remarkable cytotoxicity *in vitro* with inhibitory effects on DNA, RNA and protein synthesis, as well as on some key enzymes such as succinate dehydrogenase [62]. The nude mice xenograft model has also been used to test the anti-cancer effect of arteminolides (chemical structure shown in Fig. (**1A**)), which are mainly from the genus *Artemisia,* one of the largest genera of the *Compositae* family [63]. Many *Artemisia* species are frequently used for the treatment of malaria, hepatitis, cancer, inflammation and bacterial infections [64, 65]. Four bioactive components from the aerial parts of *Artemisia argyi*, arteminolides A, B, C and D, have been reported to have an inhibitory effect on farnesyl-protein transferase (FPTase), an important protein in Ras-dependent oncogenic signaling [66]. Among them, arteminolide C was found to be effective in inhibiting tumor cell growth in a dose-dependent manner using a nude mice xenograft model inoculated with human lung and colon tumor cells, with no obvious effect on the body weight of those mice [67].

Eupatoriopicrin (chemical structure shown in Fig. (**1A**)) is the principal SL found in *Eupatorium cannabinum* L., a native plant commonly found in Europe. Eupatoriopicrin has been reported to be cytotoxic to malignant cells and to prolong the life expectancy of mice bearing human tumor cells [68]. In a later investigation, Woerdenbag and coworkers noted that intraperitoneally administered eupatoriopicrin possessed a potent cell growth inhibitory effect on Lewis Lung tumor cells in female C578L mice [69].

A few studies have been conducted using primary animal cancer models and data from those studies support the cancer chemopreventive value of SLs. Arctiin and arctigenin (both chemical structures shown in Fig. (**1A**)) are the two major active constituents in the aerial part of *Saussurea medus*, a medicinal plant widely used for the treatment of rheumatoid diseases and for enhancing physical strength, in the Northwestern part of China and Nepal. When administered either topically or orally, arctiin is found to reduce the number of skin tumors in a two-stage carcinogenesis model induced by 7, 12-dimethylbenz[a]anthracene (DMBA) as an initiator and 12-*o*-tetradecanoyl phorbol-13-acetate (TPA) as a promoter [70]. Also, arctigenin is found to be effective on the two-stage carcinogenesis model of mouse pulmonary tumors induced by 4-nitroquinoline-N-oxide as an initiator and glycerol as a promoter [70].

Parthenolide is the principle SL from feverfew (*Tanacetum parthenium*), an important medicinal plant with a long history of treatment for fever, inflammation and migraine in Europe and northern America [71]. Recently, we have examined the anti-cancer property of parthenolide in UVB-induced skin cancer in female SKH-1 hairless mice. Mice fed a diet containing 1 mg/day of parthenolide showed significantly delayed onset of carcinogenesis, as well as a reduced number and size of skin papilloma induced by UVB exposure [72]. Moreover, the mechanistic studies provided evidence that the chemopreventive property of parthenolide is mediated through its inhibitory effect on activator protein (AP)-1 via suppression of UVB-induced JNK and p38 activation [72].

4. MOLECULAR MECHANISMS INVOLVED IN THE ANTI-CANCER ACTIVITY OF SLS

4.1. Thiol Depletion, Disruption of Redox Status and Induction of Oxidative Stress

Thiols (mercaptanes) constitute a class of organic compounds characterized by a sulphydryl (C-SH). In cells, the biological thiols (or biothiols) can be classified into low molecular weight free thiols, such as glutathione (GSH), and high molecular weight protein thiols [75]. Important functions of biothiols include (i) maintaining the spatial structure of key regulatory proteins and the bioactivity of many cellular enzymes, (ii) balancing the intracellular reduction/oxidation status (redox), and (iii) acting as antioxidants [75-78].

As discussed previously, SLs contain an α-methylene-β-lactone moiety which is highly reactive with cellular thiols, resulting in alkylation of sulphydryl residues through Michael type addition. Many types of SLs (vernolepin, helenalin, elephantopin, eriofertopin, costulide, repin and parthenolide) have been shown to significantly deplete intracellular thiols [5, 6, 13, 28, 79-81]. For instance, in cultured cancer cells, more than half of the intracellular GSH is quickly depleted by SLs due to a direct conjugation of SL molecules to GSH [13]. Interestingly, this fast covalent conjugation between GSH and SL molecules is reversible. Under physiological pH, the SL molecules are readily released from their GSH-adducts and then react with their protein targets [82], causing the depletion of protein thiols [6]. The interaction between SLs and their protein targets leads to changes in spatial structure and binding capability of proteins and thus inhibits their bioactivity [83]. Therefore it is believed that depletion of protein thiols by SLs has important implications in the bioactivity of SLs.

The biochemical processes within the cell involve a great number of thiol-bearing enzymes, which play a critical role in maintenance of normal cell function. Due to the high thiol reactivity of many SLs, those thiol-bearing enzymes become the direct molecular targets of SLs. The earlier evidence

Table 2. The Anti-Cancer Properties of Sesquiterpene Lactones Investigated Using *In Vivo* Animal Models

Type of SLs	Animal models	Outcomes	Ref.
Arctigenin	2-stage skin tumors in mice	Reduce the number of tumors	[70]
Arctiin	2-stage pulmonary tumors in mice	Reduce the number of tumors	[70]
Arteminolides	Nude mice xenograft	Reduce the volume of tumors	[67]
Eupatoriopicrin	Lewis lung tumor system in mice	Reduce the size of tumor	[69]
Costunolide	Rat intestinal carcinogenesis model induced by AOM	Reduce tumor incidence	[73]
Costunolide	Hamster buccal pouch carcinogenesis model induced by DMBA	Reduce tumor burden	[74]
Parthenin	Tumor cells induced solid tumor model in mice	Increase overall survival	[61]
Parthenolide	UVB-induced skin cancer in mice	Delay the onset of carcinogenesis and reduce size and number of tumors	[72]

Abbreviation used: AOM: azoxymethane; DMBA: 7,12 dimethylbenz(a)anthracene

from *in vivo* tumor-bearing mice indicated that injection with SLs (helenalin and tenulin) led to inhibition of DNA polymerase activity, suppression of protein synthesis, and blockage of the mitochondrial respiratory chain [4, 11]. The α-methylene-γ-lactone moiety in SLs is known to be the essential structure responsible for the inhibitory activities of SLs [11]. The following studies further indicated that inhibition of nucleic acid synthesis by many of SLs is the direct result of the inactivation of thiol-bearing enzymes during the multiple steps of nucleic acid metabolism, including DNA polymerase, inosine monophosphate dehydrogenase, purine synthetase, dihydrofolate reductase and ribonucleoside reductase [84-87]. In addition, it has been shown that deoxyribonucleoside triphosphate (dNTPs) pools are also depleted by SLs which would account in part for the observed inhibition effects on DNA synthesis [88]. Moreover, some SLs have also been found to inhibit a number of critical enzymes in the process of protein synthe-sis and aerobic carbohydrate metabolism. For instance, glucose metabolism is inhibited by SL pentalenolactone (chemical structure shown in Fig. (**1A**)) through inactivation of glyceraldehyde-3-phosphate dehydrogenase activity [89].

Another important consequence of intracellular thiol depletion is the disruption of the cellular redox balance and induction of oxidative stress. In aerobic organisms, ROS are constantly produced during aerobic respiration. To avoid the potential oxidative damage, a highly regulated antioxidant defense system, which consists of a range of enzymatic proteins and non-enzymatic molecules (such as GSH), has evolved. The intracellular reduction/oxidation status (redox) is a precise balance between levels of ROS generation and endogenous thiol buffers existing in the cell [90]. Enhanced ROS production and/or impaired antioxidant defense function will lead to oxidative stress, a status closely associated with many pathological processes in the cell [91]. In cancer cells treated with parthenolide or costunolide, elevated levels of ROS have been observed and found to be closely associated with apoptotic cell death [5, 6, 30]. It is well known that the mitochondrial respiratory chain is the major production site of intracellular ROS. Both the mitochondrial structural integrity and function are subject to a preferential redox condition. The severe oxidative stress conferred by SL-induced thiol depletion results in a disruption of the integrity of mitochondria and triggers mitochondrial permeability transition and release of mitochondrial pro-apoptotic proteins (cytochrome c, Smac, etc.) which then promote apoptosis [5, 6].

4.2. Inhibition on Nuclear Transcription Factor NF-κB Pathway

NF-κB is a ubiquitous nuclear transcription factor responsive to diverse stimuli such as TNF, UV, interleukins, endotoxins, etc. NF-κB activation plays a pivotal role in regulating inflammatory responses, cell growth/ differentiation and apoptosis [92]. Based on the understanding that NF-κB is an important factor in cell survival and that elevated NF-κB activation is often found in various cancer cells, NF-κB becomes a legitimate target in cancer chemotherapy [93]. So far there has been convincing evidence showing that NF-κB is the main molecular target for many SLs [92, 94-97]. Bork *et al*. (1997) were the first to examine the inhibitory activity of ethanol extracts from 54 Mexican Indian medicinal plants on NF-κB activation [94]. Subsequent studies from several other groups suggest that the SLs target multiple steps of the NF-κB signaling pathway to inhibit its activation. Among these SLs, the mechanisms of the inhibitory activity of parthenolide have been most extensively studied. Hehner *et al*. (1998) reported that parthenolide inhibits NF-κB activation induced by several stimuli (TPA, TNF, ligation of T cell receptor and hydrogen peroxide) by blocking the degradation of phosphorylated IκB, without interfering with the DNA binding activity of NF-κB [58]. They further demonstrated that parthenolide directly acts on the IKK complex (IKC) to inhibit IKK activity induced by TNF, while other TNF-inducible MAPK signaling pathways (p38 and JNK) were not affected by parthenolide [95]. Additional supporting evidence for the above observations came from a study by Kwok *et al*. (2001) that identified the exact position of a cysteine in IKKβ that reacts with parthenolide. They found that a single amino substitution in the activation loop (C179A) of IKKβ abolished parthenolide's IKKβ inhibitory activity [98].

On the other hand, NF-κB protein p65 has also been proposed as a molecular target of parthenolide. In this instance, the inhibitory activity of parthenolide is probably due to the alkylation and cross-linking of two cysteine residues (cys 38 and cys 120) located on the p65 subunit of NF-κB [14]. The direct modification of p65 by parthenolide at cys38 via alkylation leads to the suppression of NF-κB-DNA binding activity [83, 99]. Therefore, it appears that SLs such as parthenolide have multiple molecular targets along the NF-κB signaling pathway to ensure a more effective inhibitory effect. Furthermore, overexpression of upstream modulators such as TNF receptor associated factor 2 (TRAF2) and mitogen-activated protein kinase 1 (MEKK1) attenuated the inhibitory activity of parthenolide on TNF-induced NF-κB activation [95], highlighting the possibility that parthenolide may also disrupt the signaling pathway upstream of IKK. Our recent study supported this hypothesis by demonstrating that parthenolide inhibits TNF-mediated NF-κB activation via disruption of the recruitment of the IKK complex to TNF receptor, which then blocked subsequent NF-κB signaling events [36].

4.3. Inhibition of MAPK Signaling Pathway

In addition to NF-κB, SLs such as parthenolide have also been found to target MAPK signaling pathways, which play critical roles in extracellular signal transduction [100, 101]. The effects of SLs on MAPK signaling were first studied in macrophages in the context of their anti-inflammatory activity, by Hwang and his colleagues. They observed a dramatic inhibition of LPS-induced MAPK activation, including ERK1/2 and p38, by parthenolide [59]. The effect of parthenolide on ERK activation was supported by subsequent studies in which parthenolide inhibited the activation of ERK in primary rat microglia, leading to the inhibition of iNOS and NO release [102]. In addition to parthenolide, another SL costulide has also been found to inhibit the expression of interleukin-1β via suppression of MAPK (JNK and p38) in LPS-stimulated macrophages [103]. Although most of the effects of SLs on MAPK were studied in the context of their anti-inflammatory activity, there is preliminary evidence

suggesting that suppression of the MAPK signaling pathways may contribute to their anti-cancer property. For instance, a recent study in our laboratory demonstrated that pretreatment with non-cytotoxic doses of parthenolide prevents UVB-induced activation of JNK and p38, and sensitizes the cancer cell to UVB-induced apoptosis [72].

On the other hand, SLs have also been found to activate MAPK. Thapsigarin, a potent inhibitor of ER Ca^{2+}-ATPases, can transiently induce the phosphorylation of both p38 and ERK in M1 myeloid leukemic cells, a process involved in thapsigargin-induced apoptosis [104]. Interestingly, it was noted in our recent studies that pretreatment with non-cytotoxic concentrations of parthenolide converts TNF-induced transient JNK activation into a sustained JNK activation and promotes apoptosis, most probably via suppression of the NF-κB pathway [36].

4.4. Inhibition of the Key Enzymes Involved in Cancer Development

It has been well established that abnormal expression of hormones is an important etiologic factor in certain types of cancer development such as breast cancer [105, 106]. Some key enzymes catalyzing the corresponding hormone synthesis were found to be the molecular targets of SLs. Several studies have indicated that SLs, such as guaianolides, could inhibit aromatase, a core catalytic enzyme during estrogen biosynthesis [107, 108]. Interestingly, these SLs act as type II ligands to the hemo iron in the active site of aromatase, rather than through their potent nucleophilic reactivity to inhibit aromatase enzyme activity [107, 108]. Thus, understanding the potent inhibitory activity of SLs on estrogen synthesis is important for understanding the chemopreventive potential of these SLs on estrogen-dependent cancers.

5. REPRESENTIVE SESQUITERPENE LACTONES

5.1. Costunolide

Costunolide is the active component from the crude extract of the root of *Saussurea lappa clarks*, a traditional Chinese medicinal herb [109]. The anti-cancer property of costunolide was first reported in a rat intestinal carcinogenesis model induced by azoxymethane (AOM) [73], and supported by a subsequent study using a DMBA-induced hamster buccal pouch carcinogenesis model [74]. Following these two *in vivo* experiments, considerable effort has been devoted to understanding the mechanisms responsible for the anti-cancer activity of costunolide. First, costunolide is a potent apoptosis inducer in cancer cells, via multiple pathways. It has been reported that costunolide readily depletes intracellular GSH and disrupts the cellular redox balance [28]. It triggers an intracellular ROS burst which leads to mitochondrial dysfunction: loss of mitochondrial membrane potential, onset of mitochondrial membrane transition, and release of mitochondrial pro-apoptotic proteins [30]. 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 [110], while overexpression of Bcl-2 protein attenuated costunolide-induced apoptosis [28]. Second, costunolide suppresses NF-κB activation via prevention of IκB phosphorylation [96], a process also responsible for the strong anti-inflammatory activity of costunolide [53]. Third, costunolide is capable of promoting leukemia cell differentiation [20], inhibiting endothelial cells angiogenesis [42], and disrupting nuclear microtubule architecture in cancer cells [111]. Taken together, costunolide acts on multiple stages of cancer development, with multiple molecular targets, making costunolide a promising candidate as an anti-cancer agent.

5.2. Parthenolide

Parthenolide is the major SL responsible for the bioactivity of feverfew (*Tanacetum parthenium*), a traditional herbal plant which has been used for the treatment of fever, migraine and arthritis for centuries [71]. One well-explored bioactivity of parthenolide is its potent anti-inflammatory effect, which is mainly achieved through its strong inhibitory effect on NF-κB activation. It has been well established that parthenolide acts on multiple steps along the NF-κB signaling pathway [36, 83, 95, 98]. By suppressing NF-κB, parthenolide inhibits a group of NF-κB-regulated pro-inflammatory cytokines, such as interleukins and prostaglandins [50, 52, 59]. Recently, the potential anticancer activity of parthenolide has been pursued in a number of laboratories including our own. There are three lines of evidence supporting the notion that parthenolide is a potent anti-cancer agent. First, parthenolide is capable of inducing apoptosis in many types of human cancer cells. Fig. (**2**) summarizes the various mechanisms known to be involved in parthenolide-induced apoptosis, namely (i) depletion of intracellular GSH and protein thiols and disruption of the redox balance; (ii) induction of ER stress and calcium release; (iii) enhancement of caspase 8 activation and Bid cleavage; (iv) changes in pro-apoptotic Bcl-2 proteins via the mitochondrial apoptosis pathway; (v) suppression of the NF-κB signaling pathway; and (vi) sustained JNK activation. The above pathways may act individually or synergistically to induce apoptosis, making parthenolide a potent apoptosis inducer in cancer cells. For instance, the LD_{50} was found to be as low as 5 μM in human cancer cells [5, 6, 31, 112]. Second, parthenolide is able to sensitize human cancer cells to apoptosis induced by paclitaxel [34], TNF [36] and TRAIL [35]. Although the detailed mechanisms responsible for this activity are not fully understood, most probably some of the pathways summarized in Fig. (**2**) are involved. Third, a recent report from our laboratory suggested that parthenolide possesses strong chemopreventive activity in UVB-induced skin cancer in female SKH-1 hairless mice. Dietary parthenolide (1 mg/day/mouse) significantly delayed skin cancer development and reduced both the number and size of skin papillomas compared to the UVB control group [72]. Further *in vitro* investigation provided evidence that the chemopreventive property of parthenolide is mediated through its inhibitory effect on AP-1 and MAPK [72].

5.3. Helenalin

Helenalin is another SL, from *Arnica* species, which has been reported to possess cytotoxicity and anti-cancer activity. Earlier studies demonstrated its potent activity to inhibit nucleic acid and protein synthesis [113, 114]. Similar to other anti-cancer SLs, mechanisms of action mainly involve (i) thiol depletion [80], (ii) inhibition of NF-κB [80,

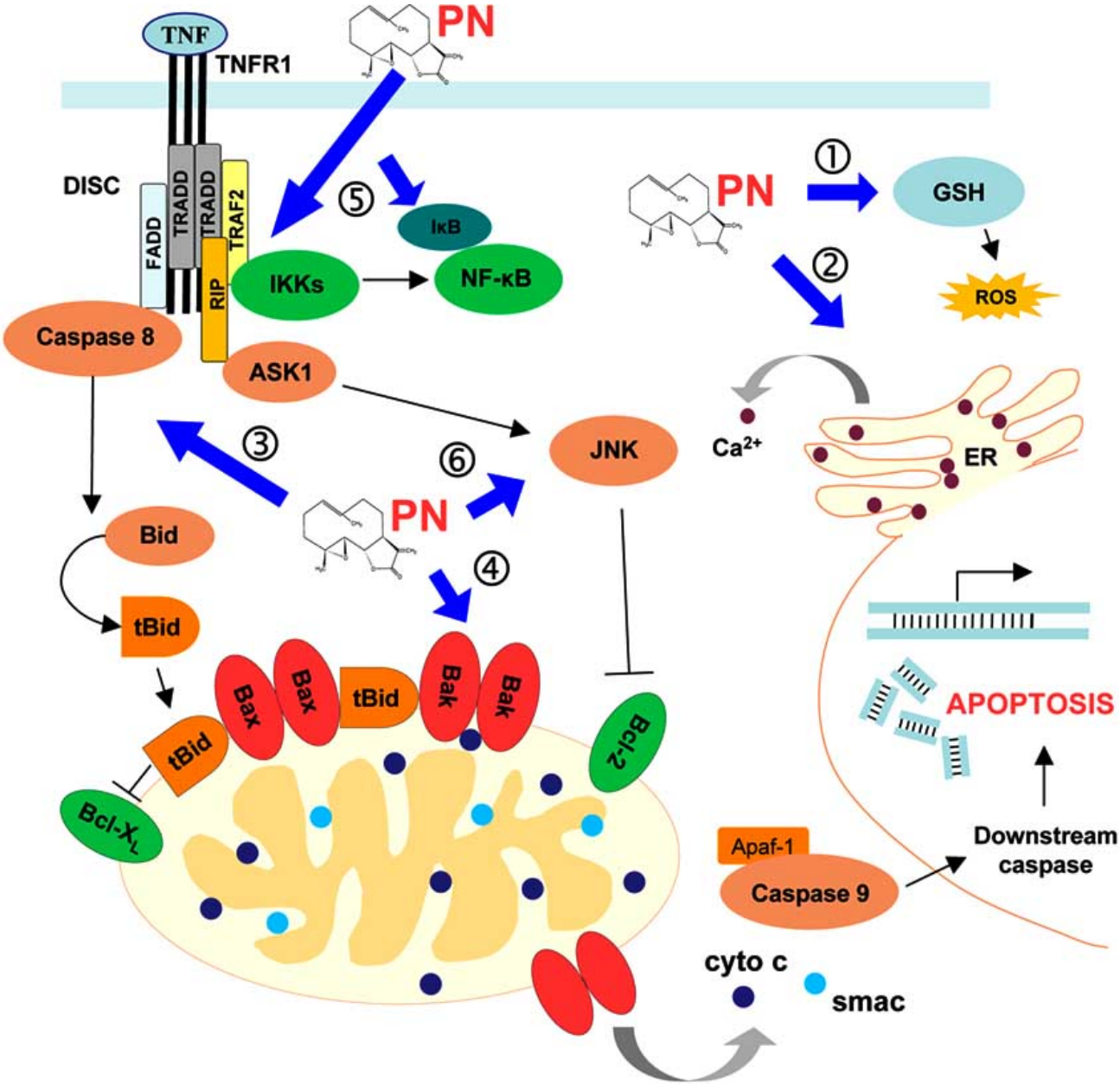

Fig. (2). Mechanisms involved in parthenolide (PN)-induced apoptosis. PN is capable of either directly inducing apoptosis or sensitizing cancer cells to apoptosis via the following pathways: ① Depletion of intracellular GSH and protein thiols and disruption of the redox balance; ② Induction of ER stress and calcium release; ③ Enhancement of caspase 8 activation and Bid cleavage; ④ Changes of pro-apoptotic Bcl-2 proteins via the mitochondrial apoptosis pathway; ⑤ Suppression of NF-κB signaling pathway; and ⑥ Sustained JNK activation.

97], and (iii) induction of apoptosis [29]. These prominent bioactivities make helenalin another potential anti-caner agent.

5.4. Arctiin and Arctigenin

The aerial part of *Saussurea medusa* has been used for the treatment of rheumatoid diseases and for enhancing physical strength in the Northwestern part of China and Nepal. Two major constituents, arctiin and arctigenin, have been shown to have strong anti-tumor activity. When administered either topically or orally, arctiin is found to reduce the number of tumors in a two-stage carcinogenesis test of mouse skin tumors induced with DMBA as an initiator and TPA as a promoter [70]. In addition, arctigenin is also found to be as effective on a two-stage carcinogenesis test of mouse pulmonary tumors induced by 4-nitro-quinoline-N-oxide as an initiator and glycerol as a promoter [70]. Recently, arctigenin was found to induce apoptotic cell death in human colorectal adenoma and carcinoma cells, with an IC_{50} at 16.5 μM) [115]. The main mechanism involves loss of mitochondrial membrane potential and down regulation of the anti-apoptotic protein Bcl-xl.

5.5. Arteminolides

The genus *Artemisia* is one of the largest genera of the *Compositae* family with more than 350 species reported [63]. Many *Artemisia* species are used for the treatment of malaria, hepatitis, cancer, inflammation and bacterial infections [64, 65]. Four bioactive components from the aerial parts of *Artemisia argyi*, arteminolides A-D, have been reported to have an inhibitory effect on farnesyl-protein transferase (FPTase), an important protein in Ras-dependent oncogenic signaling [66]. Subsequent investigation found that arteminolide C inhibits tumor growth in a dose-dependent manner in a nude mice xenograft model injected with human lung and colon tumors [67].

6. SUMMARY AND FUTURE WORK

SLs are an important group of natural products obtained from many species of medicinal plants. Accumulating evidence from both *in vitro* cell studies and *in vivo* animal cancer models has demonstrated the potent anti-cancer activity of SLs. With increasing knowledge of the molecular mechanisms responsible for their anti-cancer activity, it is believed that SLs are promising candidates for development of anti-cancer drugs. The future challenge in the study of SLs as anti-cancer agents is mainly to identify a SL product with high specificity and efficacy on cancer treatment with the following two applications: (i) as a sole cancer therapeutic agent, or (ii) as a chemosensitizer in combination with other anti-cancer drugs. One important approach might be through structural modification or entire synthesis of SL products. A successful example is DMXAA (5, 6-dimethylxanthenone-4-acetic acid), a synthetic low molecular weight flavone derivative with a striking anti-tumor activity [116]. Collective efforts are needed from oncologists, pharmacologists and organic chemists to achieve this goal.

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