What made sesquiterpene lactones reach cancer clinical trials?

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Sesquiterpene lactones (SLs) are plant-derived compounds often used in traditional medicine against inflammation and cancer. This review focuses on the chemical and biological properties of SLs that lead to enhanced anticancer and anti-inflammatory effects. The chemical properties comprise alkylating center reactivity, lipophilicity, and molecular geometry and electronic features. SLs in clinical trials are artemisinin, thapsigargin and parthenolide and many of their synthetic derivatives. These drugs are selective toward tumor and cancer stem cells by targeting specific signaling pathways, which make them lead compounds in cancer clinical trials.

A large-scale screening program for antitumor agents, launched in 1960 by the National Cancer Institute (USA), evaluated 35,000 plant samples, from which emerged Taxol, the best-selling antitumor drug today. In fact, 67% of anticancer drugs are natural products or natural product derivatives [1]. More than 200 natural-product-derived drugs are in preclinical or clinical development [2]. Research on the natural compounds sesquiterpene lactones (SLs) began within few years of the NCI screening program. To date, approximately 1500 publications have reported SL anticancer and anti-inflammatory properties, more than 90% of which have been published since 1990 (Medline and EMBASE databases).

SLs are a colorless, bitter and stable subfamily of terpenoids, a class of plant secondary metabolites of lipophilic character. They are almost exclusively derived from Asteraceae but are also found in Umbelliferae and Magnoliaciae at concentrations often exceeding 1% of plant dry weight. SLs are 15-carbon (15-C) compounds consisting of three isoprene (5-C) units and a lactone group (cyclic ester) (Fig. 1a). They can be categorized, relative to their carbocyclic skeleton, into the following major groups: germacranolides (**1a, 1b**) (ten-membered ring); eudesmanolides (**2a, 2b**) and eremophilanolides (**3**) (all 6/6-bicyclic compounds); and guaianolides (**4a, 4b**), pseudoguaianolides (**5a, 5b**) and hypocretenolides (**6**) (all 5/7-bicyclic compounds) (Fig. 1a). Many members of these groups also bear open ring structures such as iso-seco-tanapartholides (**7**) (Fig. 1a). Interestingly, guaianolides and pseudoguaianolides share similar skeletons, differing simply by the position of a methyl group, which is at C-4 in guaianolides and at C-5 in pseudoguaianolides (Fig. 1a).

Extracts from plants rich in SLs have gained considerable interest for treating human diseases such as inflammation, headache and infections. The SL-derived drugs from thapsigargin (**8**), artemisinin (**9**), and parthenolide (**10**) (Fig. 1b) are now in cancer clinical trials (Table 1). Few reviews have focused on SL extraction, analysis and characterization in biological systems, particularly for defining their anticancer and anti-inflammatory activities [3,4].

In this review, we focus on the basic chemical properties and biological activities of SLs that make them lead compounds in cancer clinical trials. First, we summarize and analyze important structural features that give SLs enhanced antitumor and anti-inflammatory properties *in vitro* and *in vivo*. Second, we attempt to answer a crucial question: are there specific biochemical mechanisms exploited by SLs that enable them to selectively target tumor and cancer stem cells while sparing normal ones? (Box 1).

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SL structure–activity relationships in cancer and inflammation

SLs' biological activities can be affected by three major chemical properties: (i) alkylating center reactivity, (ii) side chain and lipophilicity and (iii) molecular geometry and electronic features. SL structure–activity is described in relation to the interrelated processes of cancer and inflammation (Table 2).

Alkylating center reactivity

In 1969, Hartwell and Abbott reviewed more than 50 SLs known to be cytotoxic to tumors and provided indirect evidence linking their biological activity to the α-methylene-γ-lactone (**11**) moiety (Fig. 1b) [5]. Further testing of 37 SLs on nasopharynx carcinoma cells established that it is the exo- but not the endocyclic α-methylene-γ-lactone that causes cytotoxicity [6]. The α-methylene-γ-lactone moiety reacted by Michael-type addition with biological nucleophiles (the most reactive of which are the thiol-containing cysteine residues in proteins), thus, forming stable adducts [7]. Other studies showed that in helenalin (**12**) analogs (Fig. 1b), an endocyclic α,β-unsaturated ketone might cause more cytotoxicity than the exocyclic α-methylene-γ-lactone [8–11]. Apparently, the unsaturated carbonyl or '-enone' ($O{=}C{-}C{=}CH_2$)

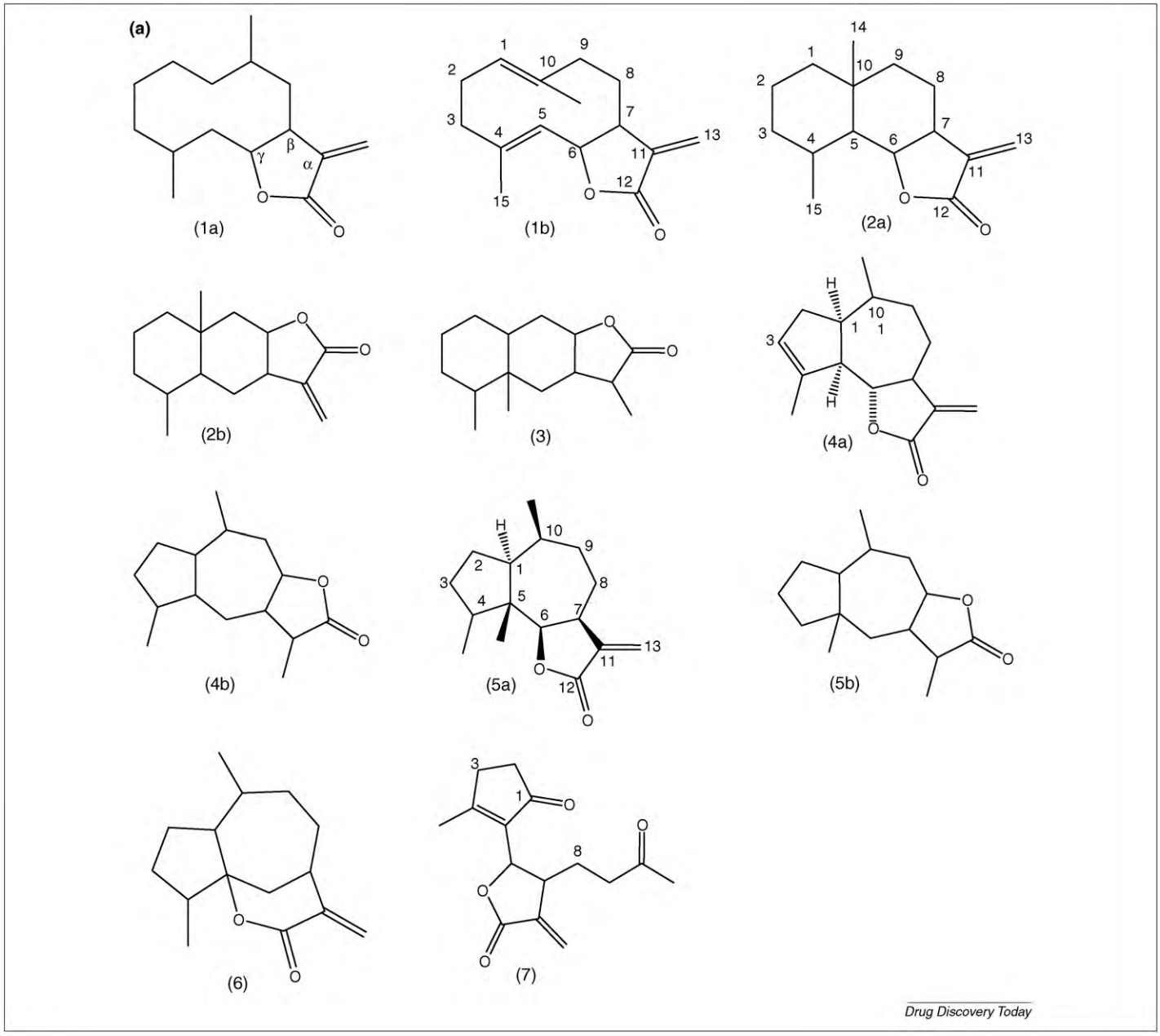

FIGURE 1

Chemical structures of sesquiterpene lactones and relevant moieties. **(a)** Different subgroups of sesquiterpene lactones (**1**)–(**7**). **(b)** Representative sesquiterpene lactones and chemical moieties (**8**)–(**23**). (**1a**–**1b**) Germacranolide isomers; (**2a**–**2b**) eudesmanolide isomers; (**3**) eremophilanolide; (**4a**–**4b**) guaianolide isomers; (**5a**–**5b**) pseudoguaianolide isomers; (**6**) hypocretenolide; (**7**) iso-seco-tanapartholide; (**8**) thapsigargin; (**9**) artemisinin; (**10**) parthenolide; (**11**) α-methylene-γ-lactone; (**12**) helenalin; (**13**) mexicanin I; (**14**) 11α, 13-dihydrohelenalin; (**15**) 11α,13-dihydroarnifolin; (**16**) 11α,13-dihydrochamissonolide; (**17**) concave facing convex sides; (**18**) repin; (**19**) epoxide; (**20**) halohydrin; (**21**) twist boat; (**22**) twist chair; (**23**) 3α,10α-dihydroxy-1α,5α,15α-H-guaia-11(13)-en-6α,12-olide.

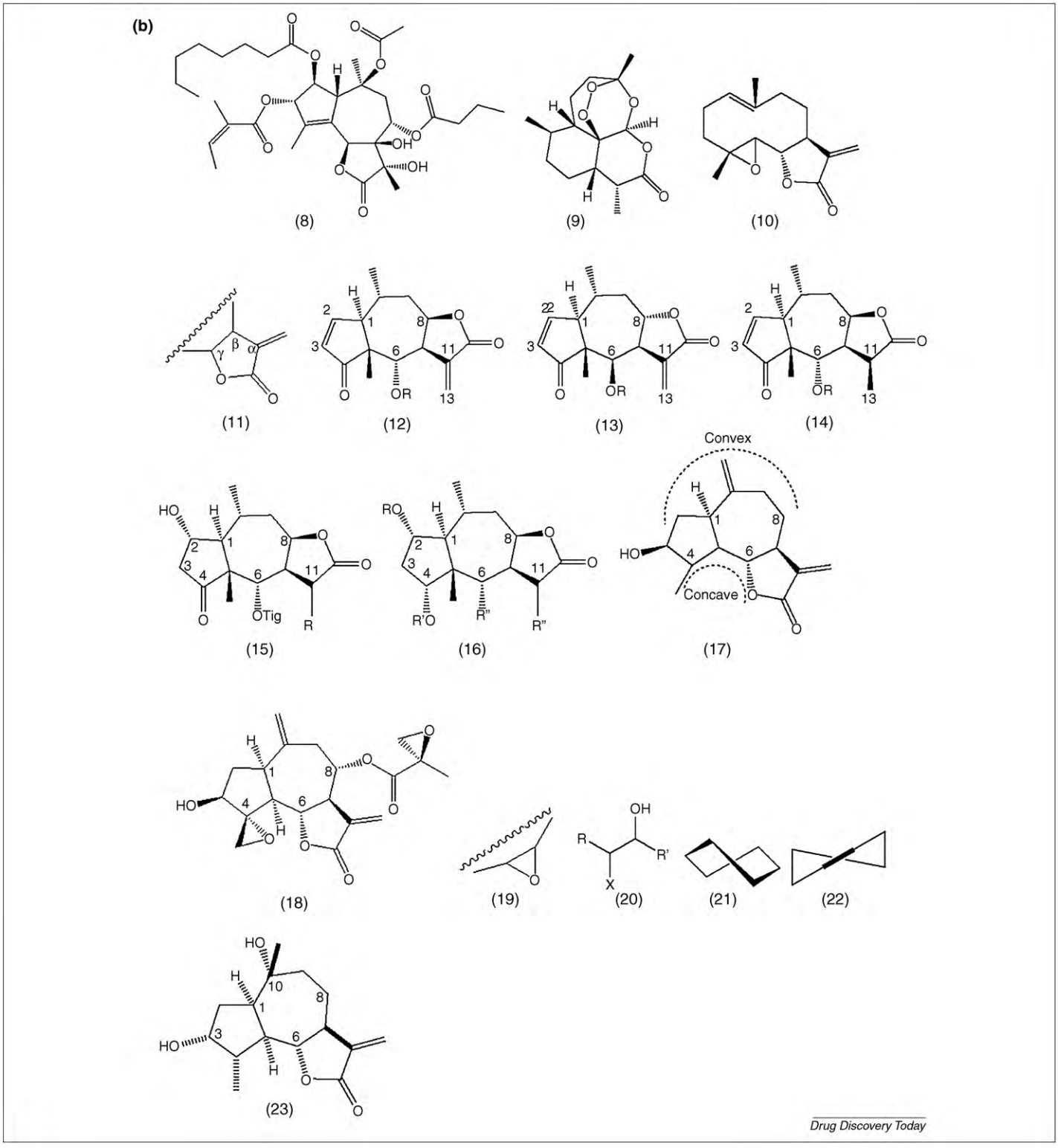

Fig. 1. (*Continued*).

systems, whether in lactone or cyclopentenone rings or in an ester side chain, increase SL toxicity toward tumor cells [6,8–11].

Subsequent studies reported that 'enone' systems, which increase SL cytotoxicity *in vitro*, are also essential for tumor inhibition *in vivo* [10,12,13]. In carcinosarcomas and chronic adjuvant arthritis, the α-methylene-γ-lactone, the β-unsubstituted cyclopentenone and the α-epoxycyclopentanone are essential for SL antitumorigenic and anti-inflammatory properties [12,13]. In carrageenan- or ethyl-phenylpropiolate-induced edema in rodents, the α-methylene-γ-lactone and unsaturated cyclopentenone, respectively, are decisive features of SL anti-inflammatory activities [13,14]. Some reports, however, have shown that SLs bind to blood proteins containing sulfhydryl groups, reducing their bioavailability and preventing them from achieving concentrations

TABLE 1

Sesquiterpene lactones in cancer clinical trials

Sesquiterpene lactone or derivative	Cancer or inflammation condition	Clinical study	Refs
Artemisinin	Lupus nephritis		[39]
	Metastatic breast cancer		ClinicalTrials.gov: NCT00764036
	Colorectal cancer		Isrctn.org: ISRCTN05203252
Artesunate	Nonsmall cell lung cancer		[40]
	Metastatic uveal melanoma		[41]
	Laryngeal squamous cell carcinoma		[42]
Artemether	Pituitary macroadenoma		[43]
Dimethyl-amino-parthenolide (LC-1)	AML, ALL and other blood-lymph tumors	Phase I clinical trial	http://www.globenewswire.com/newsroom/news.html?d=158480
Thapsigargin (G-202)	Advanced solid tumors	Phase I clinical trial	ClinicalTrials.gov: NCT01056029

that are crucial to cytotoxicity [15]. In blood, SLs also form glutathione adducts by interacting with red blood cells, which are known to contain high glutathione content [16,17].

From 1981 to 1991, to our knowledge, only one article documented SL structure–biological activity and focused on allergenic rather than on anti-inflammatory or anticancer properties [18]. Allergic contact dermatitis often develops from direct contact with plants rich in SLs. The most common SL allergens in plants include parthenin, helenalin, helenin and hymenin and have been reviewed by Picman [19]. Tests with many SLs showed that the interaction of the α-methylene-γ-lactone with cysteines is a prerequisite for such immunologic reactions [20]. After 1991, interest in SL structure–activity relationships was regained with the advent of novel methods, namely comparative molecular field analysis (CoMFA), quantitative fractional accessible molecular surface areas (Q_frASAs) and self-organizing map and molecular descriptors (SOMMD). CoMFA showed that the α-methylene-γ-lactone is not sufficient for the inhibition of serotonin release from blood platelets by parthenolide and its analogs [21]. Similarly, SLs that do not have the α-methylene-γ-lactone inhibit elastase and cathepsin release from granulocytes and neutrophils, respectively [22,23]. Moreover, an ester group at C-8 might be more important than the α-methylene-γ-lactone for SL cytotoxicity [24]: 11,13-β-dihydrolactucopicrin, lacking an α-methylene-γ-lactone but bearing an ester group at C-8, was more cytotoxic to nasopharyngeal and liver cancer cells than lactucin, having the same structure but with an α-methylene-γ-lactone and no ester at C-8 [24]. Forthcoming results show that it is the number and type of alkylating centers that are most important for antitumor activities [21,22,25,26].

BOX 1

Review criteria

The information was collected by searching PubMed, Medline, and EMBASE for articles published between 1950 and 23 March 2010, including electronic publications available ahead of print. Search terms included 'sesquiterpene', 'sesquiterpene lactone derivative', 'cancer' and 'inflammation' as MeSH terms and using explosion search. Full articles were acquired, and references were checked for additional publications. Articles discussing sesquiterpene lactone structure–activity in cancer and inflammation were selected based on their abstracts, and major findings were reported in the review. Completed and ongoing cancer clinical trials of sesquiterpene lactones were retrieved from http://www.clinicaltrials.gov, http://www.isrctn.org, Cochrane, Medline, and Google Scholar and updated on 8 April 2010. MeSH terms (when applicable) and text words of the drug name were searched. We apologize to those whose work could not be cited because of space limitations.

Typical alkylating centers are the α-methylene-γ-lactone, α-methylene-δ-lactone, conjugated cyclopentenone and conjugated side chain ester. SLs with two alkylating centers are termed 'bifunctional'. The cytotoxicity of helenanolide-type pseudoguaianolides was tested on human epidermoid laryngeal carcinoma and Ehrlich ascites tumor cells [9,25]. The bifunctional helenalin (**12**) and mexicanin I (**13**) series (Fig. 1b) were the most toxic. The presence of only one alkylation site still yielded considerable cytotoxicity, as in the 11α,13-dihydrohelenalins (**14**) having a cyclopentenone ring or in the 2,3-dihydrohelenalins (**15, 16**) that had a methylene (=CH_2) side chain at C-13 (Fig. 1b). 11α,13-Dihydroarnifolin (**15**) and 11α,13-dihydrochamissonolide (**16**) (Fig. 1b), lacking these alkylation centers, were the least toxic [9,25,26]. Similarly, compounds with two alkylating centers, the α-methylene-γ-lactone and the α,β-unsaturated aldehyde, were more potent to different tumor cells than their allylic alcohol isomers [27].

Side chain and lipophilicity

In general, higher lipophilicity can facilitate penetration through the cell membrane, thereby increasing SL cytotoxicity *in vitro*. For this type of positive correlation, however, there is sometimes a threshold limit set by steric hindrance [25]. Moreover, higher lipophilicity often dictates lower drug bioavailability *in vivo* [28]. This section discusses the effect of different side chains and lipophilicity on SL bioactivity.

In the bifunctional helenalin (**12**) and mexicanin I (**13**) analogs (Fig. 1b), the addition of a lipophilic character, particularly a lipophilic conjugated ester group at C-6, enhanced cytotoxicity against Ehrlich ascites carcinoma *in vitro* and *in vivo* [10,25]; however, there was a 'size optimum' of lipophilic ester groups beyond which SL toxicity decreased. By contrast, within the monofunctional 11α,13-dihydrohelenalins (**14**) (Fig. 1b), cytotoxicity was directly proportional to the size of the ester side chain at C-6, probably because it is distant from the molecules' alkylating center (the cyclopentenone at C-2) [25]. These findings show that for molecules possessing an α-methylene-γ-lactone, a 'size optimum' exists for the adjacent ester at C-6. Although larger ester groups can increase lipophilicity, these moieties, beyond a size

TABLE 2

Structure–cytotoxicity relationship of sesquiterpene lactones against cancer and inflammation

Sesquiterpene lactone skeleton (number investigated)[a]	Chemical parameter crucial for cytotoxicity	Biological system[b]	Analysis type	Refs
Alkylating center reactivity				
Germacranolide (**17**), guaianolide (**12**) and pseudoguaianolide (**8**)	α-Methylene-γ-lactone	KB-N cell line	QSAR[c]	[6]
Santanolide (**7**), xanthanolide (**3**), germacranolide (**2**), guaianolide (**2**) and pseudoguaianolide (**4**)	O=C–C=CH_2 system, whether in a lactone, cyclopentenone or side chain ester	WI-38, H.Ep.2 and W-18 cell lines	qSAR[d]	[8]
2,3-Dihydrohelenalin and Michael-type secondary amine adducts of helenalin[e] (**14**)	α-Methylene-γ-lactone; α,β-unsaturated ketone	H.Ep.2 cell line	qSAR	[9]
Germacranolide (**6**), pseudoguaianolide (**7**) and SL analogs (**4**)	α-Methylene-γ-lactone, β-unsubstituted cyclopentenone, α-epoxycyclopentanone and an allylic ester side chain in Walker 256 and Ehrlich ascites tumors	Ehrlich, Walker 256 and Lewis Lung carcinosarcomas and P-388 lymphocytic leukemia *in vitro* and *in vivo*	qSAR	[12]
Germacranolide (**7**), pseudoguaianolide (**17**) and SL analogs (**5**)	α-Methylene-γ-lactone; β-unsubstituted cyclopentenone in chronic adjuvant arthritis only	Carrageenan-induced edema and chronic adjuvant arthritis in rodents	qSAR	[13]
Germacranolide and eudesmanolide (**35**), guaianolide (**8**) and pseudoguaianolide (**11**)	α-Methylene-γ-lactone insufficient for inhibition of serotonin release	KB-3 cell line	QSAR, CoMFA	[21]
Germacranolide (**4**), guaianolide (**1**), pseudoguaianolide (**2**) and eudesmanolide (**2**)	α-Methylene-γ-lactone insufficient for inhibition of elastase release	Fresh-blood neutrophils	qSAR	[22]
Lactucin-like guaianolide (**4**)	Ester at C-8 more important than α–methylene-γ-lactone although both are crucial	KB-N and Bel 7402 cell lines	qSAR	[24]
Helenanolide (**21**)	Number and type of alkylating centers	Ehrlich ascites tumor cell line	qSAR, QSAR	[25,26]
Pseudoguaianolide (**7**)	Unsaturated cyclopentenone; an additional γ-lactone	Mouse ear edema	qSAR	[14]
Germacranolide (**8**), elemanolide (**5**) and guaianolide (**3**)	*Transformation of the allylic alcohol at C-15 to an α,β-unsaturated aldehyde*; conjugated ester side chain, regardless of lipophilicity, in compounds with the α-methylene-γ-lactone as the only alkylating center	KB-N, A549, MCF-7, MDA-MB-231, 1A9, HCT-8, U87-MG, PC-3 and KB-VIN cell lines	qSAR	[27]
Side chain and lipophilicity				
Santanolide (**7**), xanthanolide (**3**), germacranolide (**2**), guaianolide (**2**) and pseudoguaianolide (**4**)	Glucose carrier moiety	WI-38, H.Ep.2 and W-18 cell lines	qSAR	[8]
Germacranolide (**14**), guaianolide (**9**), pseudoguaianolide (**8**) and elemanolide (**6**)	8β-Angeloyloxy group; 6,12-lactonization	KB-N cell line	QSAR	[34]
Germacranolide (**22**), guaianolide (**16**), pseudoguaianolide (**9**), elemanolide (**6**) and eudesmanolide (**2**)	Angeloyloxy group at C-8; OH at C-5	KB-N cell line	SOMMD	[35]
Germacranolide (**7**), pseudoguaianolide (**17**) and SL analogs (**5**)	OH at C-6 of helenalin in carrageenan-induced edema; α-epoxy cyclopentenone in chronic adjuvant arthritis	Carrageenan-induced edema and chronic adjuvant arthritis in rodents	qSAR	[13]
Helenalin esters and related derivatives (**20**)	OH at C-6; ester side chain at C-6, particularly if lipophilic, conjugated and within a size limit	H.Ep.2 cell line, Ehrlich ascites carcinoma in CF-1 male mice		[10]
Germacranolide (**17**), guaianolide (**12**) and pseudoguaianolide (**8**)	OH or O-acyl groups adjacent to the α-methylene; activity of monofunctional, but not bifunctional, SLs correlates with lipophilicity	KB-N cell line	QSAR	[6]
Germacranolide and eudesmanolide (**35**), guaianolide (**8**) and pseudoguaianolide (**11**)	A concave hydrophilic region adjacent to the lactone and a convex hydrophobic region on the opposite side of the SL	KB-3 cell line	QSAR, CoMFA	[21]
Helenanolide (**21**)	Number of H-bond acceptors in SLs; chemical environment around sulfhydryl groups in a SL target; noncovalent interactions between SLs and their targets; ester side chain at C-6 within a limited size in molecules with an α-methylene-γ-lactone	Ehrlich ascites tumor cell line	qSAR, QSAR	[16,25,26]
Germacranolide (**8**), elemanolide (**5**) and guaianolide (**3**)	Conjugated ester side chain, if lipophilic, in bifunctional SLs and regardless of lipophilicity in monofunctional SLs	A549, MCF-7, MDA-MB-231, 1A9, HCT-8, U87-MG, PC-3, KB-N and KB-VIN cell lines	qSAR	[27]
Iso-seco-tanapartholide (**4**)	Small lipophilic groups	HaCaT cell line	qSAR	[31]

TABLE 2 (*Continued*)

Sesquiterpene lactone skeleton (number investigated)[a]	Chemical parameter crucial for cytotoxicity	Biological system[b]	Analysis type	Refs
Repin[f] and derivatives (**14**)	An epoxide, rather than a diol or allylhydroxyl, on the cyclic skeleton or on a side chain	A549, MCF-7, HCT-8, SK-Mel-2, 1A9, KB-N and KB-VIN cell lines	qSAR	[29]
Molecular geometry and electronic features				
Santanolide (**7**), xanthanolide (**3**), germacranolide (**2**), guaianolide (**2**) and pseudoguaianolide (**4**)	Angular santanolides are the least cytotoxic but the most selective toward virally transformed tumor versus normal cells	WI-38, H.Ep.2 and W-18 cell lines	qSAR	[8]
Helenanolide (**21**)	More flexible bifunctional helenalins; 7,8-cis-relative to transfused lactone ring; a bulky ester side chain in twist boat relative to twist chair conformation	Ehrlich ascites tumor cell line	qSAR, QSAR	[32,25]
Pseudoguaianolide (**7**)	β-OH parthenin relative to its α-OH optical isomer	Mouse ear edema	qSAR	[14]
Chinensiolide B[g] and reduced isomers (**4**)	3α-OH group relative to 3β-OH optical and 3-oxo isomers	HepG2, WI-38 and VA-13 cell lines	qSAR	[33]
Iso-seco-tanapartholide (**4**)	3β-OH group relative to 3α-OH optical isomer	HaCaT cell line	qSAR	[31]
Germacranolide (**2**), guaianolide (**6**), pseudoguaianolide (**23**), eudesmanolide (**8**) and carabranolide (**1**)	Cyclopentenone and methylene lactone but not the carbonyl oxygen and carbon atoms	KB-C cell line	qSAR, Q_frASA	[30]
Germacranolide (**14**), guaianolide (**9**), pseudoguaianolide (**8**) and elemanolide (**6**)	Double bonds relative to oxygen atoms Double bond at positions 3 or 10; SLs with highest activity are guaianolides followed by pseudoguaianolides	KB-N cell line	QSAR	[34]
Germacranolide (**22**), guaianolide (**16**), pseudoguaianolide (**9**), elemanolide (**6**) and eudesmanolide (**2**)	Double bond at position 3; SLs with highest activity are guaianolides followed by pseudoguaianolides	KB-N cell line	SOMMD	[35]

[a] Indicates the skeleton type and, in parentheses, the number of sesquiterpene lactones investigated for a given type.
[b] Cell line abbreviations: 1A9, human ovarian cancer; A549, human nonsmall cell lung cancer; Bel 7402, human liver cancer; HaCaT, human immortalized keratinocyte; HCT-8, human colon cancer; H.Ep.2, human epidermoid laryngeal carcinoma; HepG2, human primary liver cancer; KB-C and KB-3, human cervical carcinoma; KB-N, human nasopharyngeal carcinoma; KB-VIN, vincristine-resistant human nasopharyngeal carcinoma; MCF-7, estrogen receptor positive human breast adenocarcinoma; MDA-MB-231, estrogen receptor negative human breast adenocarcinoma; PC-3, human prostate cancer; SK-Mel-2, human melanoma; U87-MG, human glioblastoma–astrocytoma; VA-13 and WI-38, normal human lung fibroblasts; W-18, simian virus 40-transformed cells of human origin.
[c] QSAR, quantitative structure–activity analysis.
[d] qSAR, qualitative structure–activity analysis.
[e] Helenanolides are 10α-methylpseudoguaianolides.
[f] Repin is a guaianolide.
[g] Chinensiolide B is a guaianolide.

limit, cause steric hindrance onto the exocyclic methylene group, preventing it from approaching its target [25]. Another study addressed the effect of ester groups in monofunctional versus bifunctional SLs. In compounds bearing the α-methylene-γ-lactone as the only alkylating center, a conjugated ester side chain increased cytotoxicity regardless of its lipophilic properties [27]. If two alkylating centers were present, however, an ester side chain had to be lipophilic and conjugated to increase SL potency [27]. Interestingly, in both mono- and bifunctional SLs, an OH or O-acyl group adjacent to the α-methylene-γ-lactone enhanced cysteine addition reaction, although no direct correlation between cytotoxicity and reaction rate was observed [6]. Hence, although the presence of an OH decreases lipophilicity, its location, if appropriate, can enhance SL bioactivity.

In vivo structure–activity studies of SLs using carrageenan-induced edema showed that an OH group, at C-6 of helenalin (**12**) (Fig. 1b), is required for potency; esterification or elimination of this group drastically reduced anti-inflammatory activity [13]. Equally, a concave hydrophilic region adjacent to the lactone facing a convex hydrophobic region (**17**) (Fig. 1b) is imperative for SL's interaction with its target [21]. The number and, sometimes, the position, of H-bond acceptors influenced SL cytotoxicity [13]. Noncovalent interactions, such as hydrogen bonds forming between oxygen atoms in SLs and amino acid residues adjacent to the reactive cysteine in a target protein, can precede alkylation and increase SL bioactivity [16,25,26]. In addition, the chemical environment around the target's sulfhydryl groups, which are SL Michael addition sites, is important for bioactivity [16].

The effect of the OH group on SL cytotoxicity has produced contradictory outcomes depending on the position of the OH group. When the toxicity of repin (**18**) and its derivatives was assessed against human tumor cell lines (Table 2), it was found that the presence of a diol, rather than an epoxide (**19**), or of allylhydroxy abolished activity (Fig. 1b) [29]. Activity was retained, however, when the two epoxide moieties of repin were modified to halohydrins (**20**) (Fig. 1b) [29]. Another study addressed the epoxide effect on SL bioactivity. Out of 40 SLs, three compounds lacking α,β-unsaturated carbonyl groups but possessing an epoxide moiety were inactive toward cervical carcinoma cells [30].

We have recently shown that methoxy (–OCH_3) moieties and the absence of hydroxyl groups at C-1, -3 and -8 increased the cytotoxicity of bifunctional iso-seco-tanapartholides (**7**) (Fig. 1a) against immortalized keratinocytes [31]. These results are in line with earlier findings reporting enhanced activity of bifunctional SLs owing to small lipophilic side chains [25]. Methoxy groups at C-3 of iso-seco-tanapartholides were distant from the α-methylene-γ-lactone and, hence, could not sterically hinder its approach

toward nucleophiles. Moreover, iso-seco-tanapartholides bear open ring structures enabling conformational rotations that can minimize steric hindrance [31].

Molecular geometry and electronic features

Conformational flexibility has important effects on SL bioactivity [25,32]. Flexible bifunctional helenalins (**12**), with 7,8-*cis*-fused lactone ring, were more toxic than rigid mexicanin I (**13**) derivatives, with 7,8-*trans*-fused lactone ring (Fig. 1b) [25]. Within the 2,3-dihydrohelenalin derivatives (**15, 16**), flexibility accounted for the fivefold difference in cytotoxicity between two compounds having identical structures but one bearing a carbonyl, instead of hydroxyl, group at C-4 [25]. The less potent hydroxyl derivative had a twist boat (**21**) conformation, whereas the carbonyl counterpart had a twist chair (**22**) conformation (Fig. 1b) [32]. Both compounds have an O-tigloyl ester side chain at C-6. In twist boat form, this ester is in equatorial position with the molecule's sole alkylating center, causing more steric hindrance than in twist chair conformation [25].

Stereochemistry is also important in defining SLs' antitumorigenic properties. Studies of structurally related pseudoguaianolides showed that the β-OH isomer (parthenin) at C-1 is more active against ethyl-phenylpropiolate-induced mouse ear edema than the α-OH equivalent (hymenin) [14]. Another study also reported an effect of OH optical activity on SL bioactivity [33]. The guaianolide 3α,10α-dihydroxy-1α,5α,15α-H-guaia-11(13)-en-6α,12-olide (**23**) (Fig. 1b) exhibited the most toxicity toward several liver and lung cancer cells because its 3α-OH group is more crucial than the 3-oxo[5] or 3β-OH groups [33]. We have shown that an iso-seco-tanapartholide (**7**) (Fig. 1a) with a 3β-OH group is more cytotoxic to immortalized epidermal cells than its stereoisomer bearing a 3α-OH group [31], confirming the importance of OH optical activity in SL cytotoxicity.

An analysis of 37 SLs on nasopharyngeal carcinoma cells showed that guaianolides (**4a, 4b**) have the highest cytotoxicities, followed by pseudoguaianolides (**5a, 5b**) (Fig. 1a) [34]. Compounds from these two families had a double bond in the five-membered ring, at C-3, except for one guaianolide, which showed less activity than pseudoguaianolides [34]. A methylene, at C-10, was also present in the most potent SLs. Quantitative structure–activity relationship (QSAR) analyses showed that oxygen atoms have secondary importance to double bonds in cytotoxicity [34]. These findings were confirmed using SOMMD on 55 SLs from five different skeletons [35].

Another global approach toward SL structure–cytotoxicity analysis assessed the role of oxygen atoms and atomic charges in SL molecular surface areas. Forty SLs from five different skeletons were studied using Q_frASAs, a method that analyzes the overall distribution of negative and positive charges on a molecule's surface and can predict SL reactivity through covalent or non-covalent interactions [30]. The cyclopentenone and methylene lactone were found to be positive contributors to reactivity, whereas the carbonyl oxygen and carbon atoms of these two moieties were negative contributors. This might be a result of decreased reactivity of the β-carbon in these α,β-unsaturated carbonyl structures, in cases in which the carbonyl carbon is too easily accessible [30].

[5] Oxo refers to any compound containing an oxygen atom connected to another atom by a double bond.

SL biochemical mechanisms for selectively targeting cancer cells

Effective cancer treatment is through alleviation of tumor load and inhibition of cancer stem cells, which are implicated in cancer relapse and treatment resistance [36]. SLs in cancer clinical trials have properties that enable them to target tumor and cancer stem cells while sparing normal ones [36–38]. At present, the SL drugs in clinical trials are artemisinin from *Artemisia annua* L, thapsigargin from *Thapsia* (Apiaceae) and parthenolide from *Tanacetum parthenum* (feverfew) and/or many of their synthetic derivatives.

Clinical evidence indicates that artemisinin-derived drugs are promising for laryngeal carcinomas, uveal melanomas and pituitary macroadenomas and are in phase I–II trials against lupus nephritis and breast, colorectal and nonsmall cell lung cancers [39–43] (Table 1). Thapsigargin-derived drugs are undergoing phase I clinical trials for breast, kidney and prostate cancer treatments. The orally bioavailable parthenolide analog, dimethylamino-parthenolide, or LC-1 [28], is at present in phase I against acute myeloid leukemia (AML), acute lymphoblastic leukemia (ALL) and other blood and lymph node cancers (Table 1). The selectivity of thapsigargin, artemisinin and/or parthenolide toward tumor cells are attributed to their ability to target the sarco/endoplasmic reticulum (ER) calcium ATPase (SERCA) pump [44], particular proteases secreted by cancer cells [45], high iron content and cell surface transferrin receptors [46,47], nuclear factor-κB (NF-κB) signaling [48,49], MDM2 degradation and p53 activation [50], angiogenesis [51], metastasis [52], and epigenetic mechanisms [53,54] (Fig. 2).

Inhibition of SERCA pump

Most chemotherapeutics preferentially target fast-proliferating cancer cells by blocking pathways in mitosis; therefore, slow-proliferating cancers, such as prostate, are resistant to conventional chemotherapy and no treatment is available once metastases develop. Thapsigargins diffuse within the intracellular membranes and fit into the SERCA pump through lipophilic interactions, inhibiting it from transporting Ca^{2+} from the cytosol into the ER. This results in elevated cytosolic Ca^{2+} concentrations and subsequent ER stress and cell death [44]. However, toxicity was also noted in normal cells in response to SERCA pump inhibition [44]. Fortunately, when thapsigargin was used as a prodrug by conjugation with peptides unique to prostate-specific antigen enzyme, successful targeted therapy of prostate cancer was achieved [45].

Iron-dependent free radical generation

Rapidly proliferating cancer cells express elevated levels of transferrin receptors on their cell surface and have higher amounts of intracellular iron uptake than normal or slow-proliferating cells. The antimalarial drug artemisinin has an endoperoxide bridge that is cleaved upon binding to Fe(II), producing toxic C-4 and seco-C-4 free radicals that destroy tumor cells. Addition of holotransferrin and iron sources to artemisinin increases its antitumor properties and targeted delivery [47]. Artemisinin has shown antitumor properties *in vitro* and *in vivo* and promising clinical results [55].

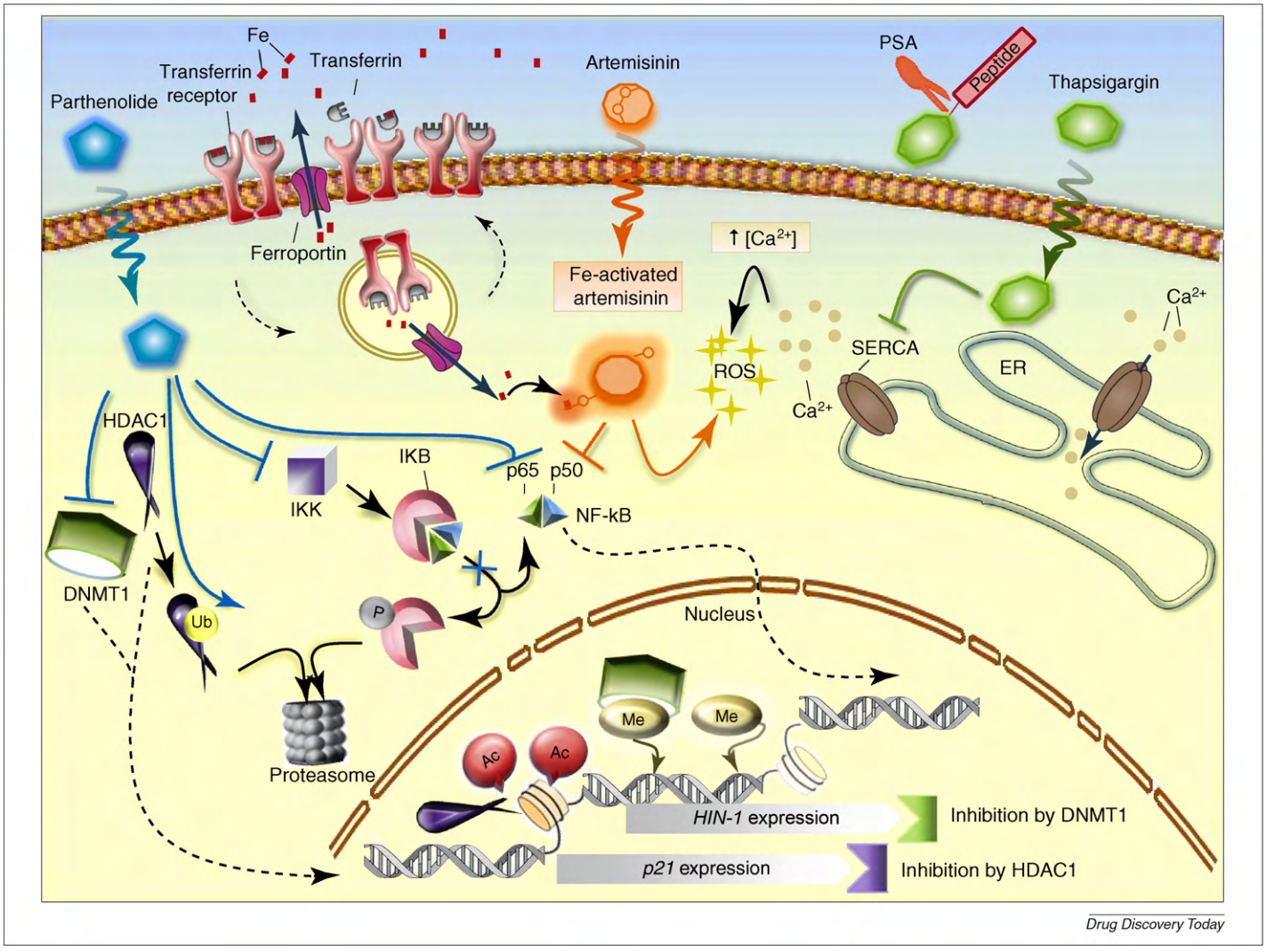

FIGURE 2

Sesquiterpene lactones target specific pathways in tumor and cancer stem cells. Sesquiterpene lactones in cancer clinical trials include or are derived from thapsigargin, artemisinin and parthenolide. Sesquiterpene lactones diffuse through the plasma membrane and selectively target the SERCA pump, high iron content and cell surface transferrin receptors, NF-κB signaling, and epigenetic mechanisms. Thapsigargin can diffuse into the SERCA pump blocking its ability to transfer Ca^{2+} from the cytosol into the ER. High cytosolic Ca^{2+} concentrations lead to the generation of ROS and subsequent cell death. Many cancers have high intracellular Fe content and elevated levels of transferrin receptors. Cytosolic Fe binds to artemisinin's endoperoxide bridge, causing its activation and the generation of toxic ROS. Parthenolide directly inhibits DNMT1 and causes ubiquitin-mediated proteasomal degradation of HDAC1, leading to expression of *HIN-1* and *p21*, respectively. Parthenolide directly inhibits the NF-κB p65 subunit and the IKK complex preventing IKK-mediated phosphorylation and proteasomal degradation of IκB. Artemisinin also inhibits NF-κB activity. Abbreviations: Ac, acetyl group; DNMT1, DNA methyl transferase; ER, sarcoplasmic/endoplasmic reticulum; HDAC1, histone deacetylase 1; IKK, IκB kinase; IκB, inhibitor of NF-κB; PSA, prostate-specific antigen; Me, methyl group; NF-κB, nuclear factor-κB; ROS, reactive oxygen species; SERCA, ER calcium ATPase; Ub, ubiquitin. Dashed and solid lines indicate translocation and activation, respectively.

Pharmacogenomic and molecular pharmacological approaches have identified key genes that determine the sensitivity or resistance of tumor cells to artemisinin [46]. These genes control different biological processes encompassing proliferation, angiogenesis, NF-κB signaling, p53-dependent and -independent apoptosis, and iron uptake and metabolism [46].

Inhibition of angiogenesis and metastasis

Antiangiogenic therapy is at the forefront of drug development. Artemisinins prevented angiogenesis by inhibiting human vein endothelial cell proliferation and vascular endothelial growth factor and receptor expression [56]. DNA microarray analysis correlated artemisinin's tumor inhibition to reduced expression of crucial angiogenesis-related transcripts, namely, vascular endothelial growth factor, fibroblast growth factor, several matrix metalloproteinases and hypoxia-inducing factor [57]. Thapsigargins also inhibited microvessel formation and proliferation of human artery endothelial cells [51]. Recently, parthenolide was found to inhibit the expression of matrix metalloproteinase-9 and urinary plasminogen activator and the migration of carcinoma cells *in vitro*, as well as osteolytic bone metastasis *in vivo* [52].

Regulation of NF-κB and p53 signaling pathways

NF-κB signaling is implicated in cancer development and progression, and its constitutive activity is enhanced in a variety of solid and liquid tumors. Elevated NF-κB signaling is associated with chemo- and radioresistance. Consequently, the pharmaceutical industry has invested tremendous resources to develop NF-κB

inhibitors as cancer therapeutics. Medicinal plants, rich in SLs and having anti-inflammatory properties, are a potential source of NF-κB inhibitors [48]. Parthenolide and artemisinin are established NF-κB inhibitors and render cancer cells sensitive to chemotherapy [49,55]. Parthenolide was found to directly modify the NF-κB p65 subunit [58] or to suppress the activity of the upstream IκB kinase complex leading to the stabilization of the NF-κB inhibitors, IκBα and IκBβ [59]. The nucleophilic attack by parthenolide occurs through α-methylene-γ-lactone ring and epoxide moieties that target specific nucleophiles but not others [60].

Several artemisinin-type compounds also inhibit NF-κB activity – namely, artesunate [61], artemisinin [62] and dihydroarteannuin [63]. Normal cells are usually not sensitive to these SLs because their basal NF-κB activity is often low or sometimes required for cell differentiation rather than oncogenesis [64,65]. For instance, NF-κB has two antagonistic functions in skin physiology. Whereas NF-κB in normal keratinocytes reduces the propensity of malignant transformation and induces differentiation, its activation serves as an important survival mechanism for neoplastic keratinocytes [66]. We have observed that parthenolide causes growth suppression and cell death in human and murine neoplastic keratinocytes at concentrations that are noncytotoxic to primary keratinocytes (A.G. *et al.*, unpublished).

Because of common and simultaneous inactivation of p53 and hyperactivation of NF-κB in human cancers, novel drugs described as ''double-edged swords'' have been recently designed or discovered to target both p53 and NF-κB pathways [67]. MDM2 and p53 activities are tightly controlled by a feedback regulatory mechanism that is often deregulated in human cancers, leading to MDM2 overexpression in several human cancers. As a result, small-molecule drugs have been designed to target MDM2 and compete for p53 binding [68]. Parthenolide inhibited NF-κB and activated p53 by promoting the ubiquitination and degradation of the p53-negative regulator MDM2 [50]. The ability to simultaneously target the NF-κB and p53 pathways has set SLs onto a higher scale as lead anticancer drugs that selectively kill cancer but not normal stem cells [28,36,38,69]. Cancer stem cells are the reason behind standard chemotherapy resistance and relapses that result in inefficient cancer eradication. Parthenolide is the only small molecule, to date, that has been reported to selectively target several cancer and cancer stem cells while sparing normal counterparts [28,38,69]. NF-κB signaling is elevated in leukemic stem cells but not in the normal hematopoietic stem cells. Parthenolide caused robust apoptosis of primary AML cells and effectively eradicated AML stem and progenitor cells *in vitro* without affecting hematopoietic stem cells [28,69]. The mechanism of action of parthenolide was found to be through the inhibition of NF-κB, the activation of proapoptotic p53 and an increase in reactive oxygen species [69]. Gleevec or Cytarabine, treatments commonly used for the most aggressive leukemias, are not as effective as parthenolide because they are not as specific. Promising antileukemic drugs should mediate cell death and inhibit leukemic stem-cell-specific activity, and parthenolide fulfills these criteria [64].

Parthenolide toxicity on two types of breast cancer stem cells – the side population and the breast mammosphere – was studied and showed that parthenolide is able to inhibit growth and colony formation of each of these populations and to concomitantly reduce NF-κB activity [36]. Recently, parthenolide has been shown to target prostate cancer stem cells whether isolated from prostate cancer cell lines or from patients [38]. In addition, parthenolide inhibited prostate cancer stem-cell-mediated tumor initiation and progression in mouse xenografts [38]. Although these findings are promising and suggest that parthenolide could be efficient against cancer stem cells, caution should be taken in the clinical setting. In fact, many questions remain unanswered in the cancer stem cell field regarding the origin of these cells, identity, specific molecular mechanisms, and their role in tumor progression and metastasis [36].

Modulation of the epigenetic code

Epigenetic mechanisms are frequently altered in cancer, and several tumor types display disrupted histone modification and DNA methylation patterns. Cancer cells show aberrant histone modifications, express elevated histone deacetylase 1 (HDAC1) activity and are more sensitive to the actions of HDAC inhibitors than normal cells, although the underlying mechanisms are not fully understood. Recently, parthenolide's chemotherapeutic properties were attributed to its epigenetic role [53,54]. It specifically depleted HDAC1 proteins through proteasomal-mediated degradation with no effect on class I/II HDACs [53]. For instance, parthenolide induces an HDAC1 inhibitor-like effect on the p21 gene promoter by increasing local histone H3 acetylation and subsequent p21 transcription [53]. Thus, parthenolide is a leading example of a small molecule that is a specific inhibitor of a single HDAC class. This specificity will, we hope, prove to have fewer side-effects in patients (particularly in the treatment of chronic diseases such as cancer) than those encountered with the use of sustained pan-HDAC inhibition.

Another recent study highlighted an epigenetic role for parthenolide in cancer, in particular in DNA methylation [54]. Several types of tumor cells express elevated levels of DNA methyltransferase (DNMT)1 and DNMT3b. Parthenolide induced global DNA hypomethylation *in vitro* and *in vivo* by specifically inhibiting DNMT1 activity through γ-methylene lactone alkylation of the thiolate of Cys1226 of the enzyme's catalytic domain [54]. Furthermore, parthenolide caused hypomethylation of the tumor suppressor HIN-1 gene promoter region and its reactivation *in vitro* [54]. At present, the nucleoside analogs decitabine and 5-azacytidine are the most commonly used DNA methylation inhibitors, which show unfavorable toxicity owing to their ability to trap chunks of DNMTs without demethylating specific genes. The discovery of novel epigenetic regulators like parthenolide – with abilities to inhibit specific DNMTs and HDACs, leading to more favorable toxicity – is essential to broaden the spectrum of epigenetic therapy.

Concluding remarks

SLs are promising candidates in cancer drug discovery, and their preferential selectivity toward tumor and cancer stem cells positions them as lead compounds in the clinic. Despite those favorable features, SLs have some drawbacks: their isolation relies on limited natural product resources, and they might have poor bioavailability owing to their extensive plasma protein interactions and hydrophobicity. Some SLs have poor toxicology profiles, and several studies are warranted to determine which structural moieties, if any, cause general unwanted toxicity toward normal cells. In addition, SLs can have allergenic properties and off-target

effects through interaction with the thiol groups of vitally important proteins and enzymes. This necessitates the formulation of more bioavailable SLs and the close collaboration of synthetic chemists, pharmacologists, cancer researchers and clinicians.

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References

1 Balunas, M.J. and Kingborn, A.D. (2005) Drug discovery from medicinal plants. *Life Sci.* 78, 431–441
2 Harvey, A.L. (2008) Natural products in drug discovery. *Drug Discov. Today* 13, 894–901
3 Zhang, S. *et al.* (2005) Anti-cancer potential of sesquiterpene lactones: bioactivity and molecular mechanisms. *Curr. Med. Chem. Anticancer Agents* 5, 239–249
4 Gurib-Fakim, A. (2006) Medicinal plants: traditions of yesterday and drugs of tomorrow. *Mol. Aspects Med.* 27, 1–93
5 Hartwell, J.L. and Abbott, B.J. (1969) Antineoplastic principles in plants: recent developments in the field. *Adv. Pharmacol. Chemother.* 7, 117–209
6 Kupchan, S.M. *et al.* (1971) Tumor inhibitors. 69. Structure–cytotoxicity relationships among the sesquiterpene lactones. *J. Med. Chem.* 14, 1147–1152
7 Kupchan, S.M. *et al.* (1970) Reactions of alpha methylene lactone tumor inhibitors with model biological nucleophiles. *Science* 168, 376–378
8 Lee, K.H. *et al.* (1971) Cytotoxicity of sesquiterpene lactones. *Cancer Res.* 31, 1649–1654
9 Lee, K.H. and Furukawa, H. (1972) Antitumor agents. 3. Synthesis and cytotoxic activity of helenalin amine adducts and related derivatives. *J. Med. Chem.* 15, 609–611
10 Lee, K.H. *et al.* (1973) Antitumor agents. 4. Cytotoxicity and *in vitro* activity of helenalin esters and related derivatives. *J. Med. Chem.* 16, 299–301
11 Lee, K.H. *et al.* (1977) Sesquiterpene antitumor agents: inhibitors of cellular metabolism. *Science* 196, 533–536
12 Hall, I.H. *et al.* (1978) Antitumor agents. XXX. Evaluation of alpha-methylene-gamma-lactone-containing agents for inhibition of tumor growth, respiration, and nucleic acid synthesis. *J. Pharm. Sci.* 67, 1235–1239
13 Hall, I.H. *et al.* (1979) Anti-inflammatory activity of sesquiterpene lactones and related compounds. *J. Pharm. Sci.* 68, 537–542
14 Recio, M.C. *et al.* (2000) *In vivo* activity of pseudoguaianolide sesquiterpene lactones in acute and chronic inflammation. *Life Sci.* 66, 2509–2518
15 Wagner, S. (2004) *In vitro* behaviour of sesquiterpene lactones and sesquiterpene lactone-containing plant preparations in human blood, plasma and human serum albumin solutions. *Planta Med.* 70, 227–233
16 Schmidt, T.J. (1997) Helenanolide-type sesquiterpene lactones – III. Rates and stereochemistry in the reaction of helenalin and related helenanolides with sulfhydryl containing biomolecules. *Bioorg. Med. Chem.* 5, 645–653
17 Schmidt, T.J. *et al.* (1999) Helenanolide type sesquiterpene lactones. Part 5: the role of glutathione addition under physiological conditions. *Bioorg. Med. Chem.* 7, 2849–2855
18 Hausen, B.M. and Schmalle, H.W. (1985) Structure–activity aspects of 4 allergenic sesquiterpene lactones lacking the exocyclic alpha-methylene at the lactone ring. *Contact Dermatitis* 13, 329–332
19 Picman, A.K. (1986) Biological activities of sesquiterpene lactones. *Biochem. Syst. Ecol.* 14, 255–281
20 Mitchell, J.C. *et al.* (1970) Allergic contact dermatitis from Frullania and Compositae. The role of sesquiterpene lactones. *J. Invest. Dermatol.* 54, 233–239
21 Marles, R.J. *et al.* (1995) Sesquiterpene lactones revisited: recent developments in the assessment of biological activities and structure relationships. In *Phytochemistry of Medicinal Plants* (Arnason, J.T., Mata, R., Romeo, J.T., eds), pp. 333–356, Plenum Press
22 Siedle, B. *et al.* (2003) The effect of sesquiterpene lactones on the release of human neutrophil elastase. *Biochem. Pharmacol.* 65, 897–903
23 Hall, I.H. *et al.* (1980) Mode of action of sesquiterpene lactones as anti-inflammatory agents. *J. Pharm. Sci.* 69, 537–543
24 Ren, Y. *et al.* (2005) Discovery, structural determination and anticancer activities of lactucin-like guaianolides. *Lett. Drug Des. Discov.* 2, 444–450
25 Beekman, A.C. *et al.* (1997) Structure–cytotoxicity relationships of some helenanolide-type sesquiterpene lactones. *J. Nat. Prod.* 60, 252–257
26 Schmidt, T.J. (1999) Quantitative structure–cytotoxicity relationships within a series of helenanolide type sesquiterpene lactones. *Pharm. Pharmacol. Lett.* 9, 9–13
27 Bruno, M. *et al.* (2005) Cytotoxic activity of some natural and synthetic sesquiterpene lactones. *Planta Med.* 71, 1176–1178
28 Guzman, M.L. *et al.* (2007) An orally bioavailable parthenolide analog selectively eradicates acute myelogenous leukemia stem and progenitor cells. *Blood* 110, 4427–4435
29 Bruno, M. *et al.* (2005) Cytotoxic activity of some natural and synthetic guaianolides. *J. Nat. Prod.* 68, 1042–1046
30 Schmidt, T.J. and Heilmann, J. (2002) Quantitative structure–cytotoxicity relationships of sesquiterpene lactones derived from partial charge (Q)-based fractional accessible surface area descriptors (Q_frASAs). *Quant. Struct. Act. Relat.* 21, 276–287
31 Ghantous, A. *et al.* (2009) Structure–activity relationship of seco-tanapartholides isolated from *Achillea falcata* for inhibition of HaCaT cell growth. *Eur. J. Med. Chem.* 44, 3794–3797
32 Schmidt, T.J. *et al.* (1996) Helenanolide type sesquiterpene lactones. II. The molecular conformations of arnifolin and some related helenanolides as determined by X-ray-crystallographic and NMR-spectroscopic analyses. *J. Mol. Struct.* 385, 113–121
33 Dai, J. *et al.* (2005) Biotransformation of chinensiolide B and the cytotoxic activities of the transformed products. *J. Mol. Catal. B: Enzym.* 33, 87–91
34 Scotti, M.T. *et al.* (2007) Quantitative structure–activity relationship of sesquiterpene lactones with cytotoxic activity. *Bioorg. Med. Chem.* 15, 2927–2934
35 Fernandes, M.B. *et al.* (2008) Use of self-organizing maps and molecular descriptors to predict the cytotoxic activity of sesquiterpene lactones. *Eur. J. Med. Chem.* 43, 2197–2205
36 Zhou, J. and Zhang, Y. (2008) Cancer stem cells: models, mechanisms and implications for improved treatment. *Cell Cycle* 7, 1360–1370
37 Jordan, C.T. (2006) Searching for leukemia stem cells – not yet the end of the road? *Cancer Cell* 10, 253–254
38 Kawasaki, B.T. *et al.* (2009) Effects of the sesquiterpene lactone parthenolide on prostate tumor-initiating cells: an integrated molecular profiling approach. *Prostate* 69, 827–837
39 Lu, L. (2002) Study on effect of *Cordyceps sinensis* and artemisinin in preventing recurrence of lupus nephritis. *Chin. J. Integr. Trad. West. Med.* 22, 169–171
40 Zhang, Z.Y. (2008) Artesunate combined with vinorelbine plus cisplatin in treatment of advanced non-small cell lung cancer: a randomized controlled trial. *Chin. J. Integr. Med.* 6, 134–138
41 Berger, T.G. *et al.* (2005) Artesunate in the treatment of metastatic uveal melanoma – first experiences. *Oncol. Rep.* 14, 1599–1603
42 Singh, N.P. and Verma, K.B. (2002) Case report of a laryngeal squamous cell carcinoma treated with artesunate. *Arch. Oncol.* 10, 279–280
43 Singh, N.P. and Panwar, V.K. (2006) Case report of a pituitary macroadenoma treated with artemether. *Integr. Cancer Ther.* 5, 391–394
44 Denmeade, S.R. and Isaacs, J.T. (2005) The SERCA pump as a therapeutic target: making a ''smart bomb'' for prostate cancer. *Cancer Biol. Ther.* 4, 14–22
45 Christensen, S.B. *et al.* (2009) A trojan horse in drug development: targeting of thapsigargins towards prostate cancer cells. *Anticancer Agents Med. Chem.* 9, 276–294
46 Efferth, T. (2006) Molecular pharmacology and pharmacogenomics of artemisinin and its derivatives in cancer cells. *Curr. Drug Targets* 7, 407–421
47 Nakase, I. *et al.* (2009) Transferrin receptor-dependent cytotoxicity of artemisinin–transferrin conjugates on prostate cancer cells and induction of apoptosis. *Cancer Lett.* 274, 290–298
48 Hehner, S.P. *et al.* (1998) Sesquiterpene lactones specifically inhibit activation of NF-kappa B by preventing the degradation of I kappa B-alpha and I kappa B-beta. *J. Biol. Chem.* 273, 1288–1297
49 Pajak, B. *et al.* (2008) Molecular basis of parthenolide-dependent proapoptotic activity in cancer cells. *Folia Histochem. Cytobiol.* 46, 129–135
50 Gopal, Y.N. *et al.* (2009) Parthenolide promotes the ubiquitination of MDM2 and activates p53 cellular functions. *Mol. Cancer Ther.* 8, 552–562
51 Shukla, N. *et al.* (2001) Thapsigargin inhibits angiogenesis in the rat isolated aorta: studies on the role of intracellular calcium pools. *Cardiovasc. Res.* 49, 681–689
52 Idris, A.I. *et al.* (2009) Pharmacologic inhibitors of IkappaB kinase suppress growth and migration of mammary carcinosarcoma cells *in vitro* and prevent osteolytic bone metastasis *in vivo*. *Mol. Cancer Ther.* 8, 2339–2347

53 Gopal, Y.N. *et al.* (2007) Parthenolide specifically depletes histone deacetylase 1 protein and induces cell death through ataxia telangiectasia mutated. *Chem. Biol.* 14, 813–823
54 Liu, Z. *et al.* (2009) Modulation of DNA methylation by a sesquiterpene lactone parthenolide. *J. Pharmacol. Exp. Ther.* 329, 505–514
55 Efferth, T. (2007) Willmar Schwabe Award 2006: antiplasmodial and antitumor activity of artemisinin – from bench to bedside. *Planta Med.* 73, 299–309
56 Chen, H.H. *et al.* (2004) Inhibitory effects of artesunate on angiogenesis and on expressions of vascular endothelial growth factor and VEGF receptor KDR/flk-1. *Pharmacology* 71, 1–9
57 Anfosso, L. *et al.* (2006) Microarray expression profiles of angiogenesis-related genes predict tumor cell response to artemisinins. *Pharmacogenomics J.* 6, 269–278
58 Garcia-Pineres, A.J. *et al.* (2001) Cysteine 38 in p65/NF-kappaB plays a crucial role in DNA binding inhibition by sesquiterpene lactones. *J. Biol. Chem.* 276, 39713–39720
59 Kwok, B.H. *et al.* (2001) The anti-inflammatory natural product parthenolide from the medicinal herb feverfew directly binds to and inhibits IkappaB kinase. *Chem. Biol.* 8, 759–766
60 Bork, P.M. *et al.* (1997) Sesquiterpene lactone containing Mexican Indian medicinal plants and pure sesquiterpene lactones as potent inhibitors of transcription factor NF-kappaB. *FEBS Lett.* 402, 85–90
61 Efferth, T. *et al.* (2002) mRNA expression profiles for the response of human tumor cell lines to the antimalarial drugs artesunate, arteether, and artemether. *Biochem. Pharmacol.* 64, 617–623
62 Aldieri, E. *et al.* (2003) Artemisinin inhibits inducible nitric oxide synthase and nuclear factor NF-kB activation. *FEBS Lett.* 552, 141–144
63 Li, W.D. *et al.* (2006) Dihydroarteannuin ameliorates lupus symptom of BXSB mice by inhibiting production of TNF-alpha and blocking the signaling pathway NF-kappa B translocation. *Int. Immunopharmacol.* 6, 1243–1250
64 Jordan, C.T. (2007) The leukemic stem cell. *Best Pract. Res. Clin. Haematol.* 20, 13–18
65 Sarkar, F.H. and Li, Y. (2008) NF-kappaB: a potential target for cancer chemoprevention and therapy. *Front. Biosci.* 13, 2950–2959
66 Ren, Q. *et al.* (2006) Malignant transformation of immortalized HaCaT keratinocytes through deregulated nuclear factor kappaB signaling. *Cancer Res.* 66, 5209–5215
67 Dey, A. *et al.* (2008) Double-edged swords as cancer therapeutics: simultaneously targeting p53 and NF-kappaB pathways. *Nat. Rev. Drug Discov.* 7, 1031–1040
68 Vassilev, L.T. *et al.* (2004) *In vivo* activation of the p53 pathway by small-molecule antagonists of MDM2. *Science* 303, 844–848
69 Guzman, M.L. *et al.* (2005) The sesquiterpene lactone parthenolide induces apoptosis of human acute myelogenous leukemia stem and progenitor cells. *Blood* 105, 4163–4169