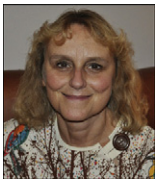
ELSEVIER

This review gives a brief description of the occurrence and biological activity of α-methylene-γ- and δ-lactones and lactams isolated from plants and their diverse synthetic analogs.

Natural and synthetic α-methylenelactones and α-methylenelactams with anticancer potential

Anna Janecka[1], Anna Wyrębska[1], Katarzyna Gach[1], Jakub Fichna[1] and Tomasz Janecki[2]

[1] Department of Biomolecular Chemistry, Medical University of Lodz, Lodz, Poland
[2] Institute of Organic Chemistry, Technical University of Lodz, Lodz, Poland



Anna Janecka was born in Lodz (Poland). She graduated in chemistry at the Technical University of Lodz and obtained her PhD from the Medical University of Lodz. Then she held postdoctoral fellowships first with Professor Karl Folkers at the University of Texas in Austin (USA) and then with Professor Cyril Bowers at Tulane University Medical Center (New Orleans, USA). In 1996 she was appointed professor at the Medical University of Lodz, where she currently works. Her main research interests focus on the synthesis and biological evaluation of opioid peptide analogs and anticancer activity of α-alkylidene lactones and lactams.

α-Methylene-γ- and δ-lactones, as well as α-methylene-γ- and δ-lactams, are plant-derived compounds often used in traditional medicine for the treatment of inflammatory diseases. In recent years, the anticancer properties of these compounds and the molecular mechanisms of their action have been studied extensively. In the search for modern anticancer drugs, various synthetic analogs of α-methylene-γ- and δ-lactones and lactams have been synthesized and tested for their cytotoxic activity. In this review, we give a brief description of the occurrence and biological activity of such compounds isolated from plants and their diverse synthetic analogs.

Throughout history, plants have always been an excellent source of pharmaceutical agents used in traditional medicine. More recently, a large number of important drugs have been obtained from plants, either directly, by extracting an active component or, more often, by structural modifications of natural compounds or the synthesis of their analogs with improved pharmacological properties. Many of the compounds used for cancer chemotherapy, such as the *Vinca* alkaloids, paclitaxel, camptothecin and etoposide, were originally derived from plant sources [1], and plants continue to be viewed as major sources for the development of new anticancer drugs.

A vast number of biologically significant natural products are characterized by the α-methylene-γ-lactone structural motif. Such products are abundant in plants of the Asteraceae (Compositae) family and have a broad spectrum of biological activities, ranging from anti-inflammatory, allergenic, phytotoxic, antibacterial and antifungal to cytotoxic and/or anticancer. The first α-methylene-γ-lactones **1** (Fig. 1) were isolated from plants of the Compositae family over 100 years ago, but real interest in their synthesis came during the 1960s and 1970s. However, in the years that followed, these compounds were not given much attention as possible drug candidates. It was not until the beginning of the 21st century that the great renaissance of interest in the biological properties, as well as in the synthesis, of α-methylene-γ-lactones occurred. Furthermore, other structurally similar systems containing the same, very characteristic *exo*-methylene moiety conjugated with a carbonyl group, such as α-methylene-δ-lactones **2**, α-methylene-γ-lactams **3** and α-methylene-δ-lactams **4** (Fig. 1), have become the frequent target of synthetic, as well as biological, studies.

Corresponding author:. Janecka, A. (anna.janecka@umed.lodz.pl)

1359-6446/06/$ - see front matter doi:10.1016/j.drudis.2012.01.013

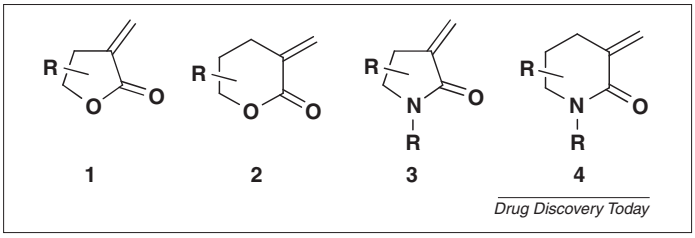


FIGURE 1

General structures of α-methylene-γ- and -δ-lactones and lactams.

Over the past 10 years, several excellent reviews describing the anticancer activity of some selected α-methylene-γ-lactones, in particular parthenolide **5**, have been published [2–6]. In this review, we describe the main groups of natural compounds isolated from plants that have a α-methylene-γ- or δ-lactone or lactam motif, as well as their synthetic analogs. We also discuss the anticancer activity of these compounds and the molecular mechanisms of their action in cancer cells.

Occurrence and biological activity of natural α-methylene-γ-lactones

Classification of α-methylene-γ-lactones

Natural products with an α-methylene-γ-lactone motif can be chemically classified into three categories, according to the carbocyclic skeletons attached to the lactone ring [7]. The largest and most diverse category is the sesquiterpene lactones (SLs), which are widely spread in plants of the Compositae. SLs are 15-carbon terpenoids, consisting of three isoprene (5C) units. A lactone ring is either *cis*- or *trans*-fused to the carbocyclic skeleton. SLs can be further classified into the following major groups: germacranolides, eudesmanolides, eremophilanolides, guaianolides, pseudoguaianolides, xanthanolides and carabranolides. The second category is the 20-carbon terpenoids, termed 'diterpenes', and the third category contains non-terpenoid α-methylene-γ-lactones [8]. Examples of important representatives of these groups with anticancer potential are given in Table 1 and their structures are shown in Fig. 2.

However, not all SLs are α-methylene-γ-lactones. The latter contain an exocyclic double bond, which is not always present in SLs. Well-known examples of SLs that are not α-methylene-γ-lactones are artemisinin and thapsigargin. Compounds that do not have an α-methylene-γ-lactone motif are not discussed in this review.

Mode of action of α-methylene-γ-lactones

Compounds containing an *exo*-methylene moiety conjugated with a carbonyl group can react as Michael-type acceptors with mercapto groups in cysteine residues of enzymes, other functional proteins and free intracellular glutathione, leading to the formation of covalent adducts. Such alkylation of cellular thiols is an important reaction that results in the disruption of some major processes in the cell. Agents that can alkylate cellular thiols are known for their potential anticancer properties [9]. Cytotoxic action of α,β-unsaturated lactones includes inhibition of proliferation, induction of apoptosis, sensitizing tumor cells to antineoplastic agents, reversing drug resistance and inhibition of metastasis.

However, these Michael-type acceptors are in their nature 'multi-targeted', which can result in diminished therapeutic value compared with compounds with only a single target. Toxicity toward normal cells and possible allergenic reactions are an additional limitation.

The question that therefore arises is whether selection of α-methylene-γ-lactones with specific activities is possible. The reaction of such compounds with mercapto groups of biological nucleophiles depends on the number and type of α,β-unsaturated carbonyl sites, the concentration of the thiols and the character of the mercapto structures. The chemical environment of the target mercapto group has a crucial role, especially in more sterically demanding situations (e.g. in hydrophobic pockets of the enzymes). More flexible α-methylene-γ-lactones will have increased chances in reacting in such environments. The presence of positively charged or hydrogen bond-donating structural elements in the right steric position in relation to the mercapto group on the target cell will strongly influence the rate of the addition. Therefore, some α-methylene-γ-lactones show selectivity toward cancer cells, which we discuss below.

Ongoing research on the reactivity of different α-methylene-γ-lactones toward a wide variety of polypeptides should allow optimization of the lead structures with respect to a desired biological effect.

Biological activity of α-methylene-γ-lactones

Structure–activity relationship studies

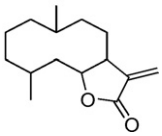
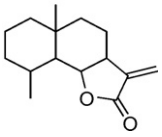
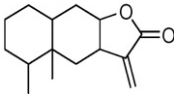
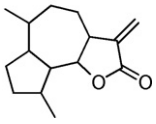
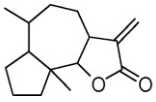
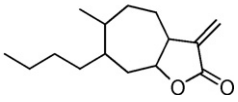
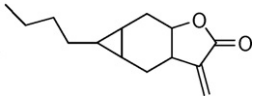
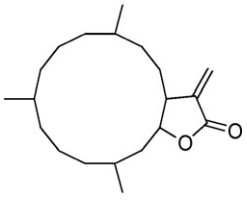
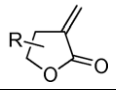
The α,β-unsaturated carbonyl system present in α-methylene-γ-lactones is responsible for their cytotoxic activity. Given that all α-methylene-γ-lactones share the same structural motif, the differences in activity can be explained by different numbers of alkylating structural elements. Compounds with two alkylating centers are termed 'bifunctional'. Other than the α-methylene-γ-lactone alkylating center, there are also α-methylene-δ-lactones, conjugated cyclopentenones, conjugated side-chain esters and epoxides [5]. For example, in helenalin **13**, which is a bifunctional SL with two alkylating centers, an endocyclic α,β-unsaturated ketone might cause more cytotoxicity than would the exocyclic α-methylene-γ-lactone [10]. In addition, parthenolide **5** has two sites for potential nucleophilic attack by a cysteine side-chain, because as well as its α-methylene-γ-lactone moiety, it also has an epoxide moiety as a second site.

In general, compounds with two alkylating centers are not only more potent inhibitors of tumor cell proliferation, but also more toxic. The presence of only one alkylation site yields significant cytotoxicity, but lower toxicity. Other factors, such as lipophilicity, molecular geometry and the chemical environment of the target sulfhydryl group, can also influence the activity of α-methylene-γ-lactones [5,11]. They are usually lipophilic compounds and, therefore, can easily penetrate cell membranes and show high cytotoxicity *in vitro*. However, high lipophilicity often results in lower drug bioavailability *in vivo* [12]. Different modifications of natural α-methylene-γ-lactones have been designed to change their lipophilic character and thus improve their pharmacological properties [13].

Conformational flexibility is another factor that can affect bioactivity [13,14]. Flexible helenalin **13** with a *cis*-fused lactone ring is more toxic than is rigid mexicanin **14** with a *trans*-fused lactone ring [13]. Stereochemistry of some chiral groups can also have an important role in defining the bioactivity [15]. Non-covalent interactions, such as hydrogen bonds between oxygen

TABLE 1

Major categories of α-methylene-γ-lactones with anticancer potential

Category	Group/skeleton	Ring size	Examples	Origin
Sesquiterpene lactones	Germacranolides 	10-membered	Parthenolide **5** Costunolide **6** Isocostunolide **7** Deoxyelephantopin **8**	*Tanacetum parthenium* *Magnolia sieboldii* *Inula helenium* *Elephantopus scaber*
	Eudesmanolides 	6/6-fused bicyclic	Santamarine **9**	*Tanacetum vulgare*
	Eremophilanolides 	6/6-fused bicyclic	Tsoongianolide E **10**	*Senecio tsoongianus*
	Guaianolides 	5/7-fused bicyclic	Arglabin **11** Artabsin **12**	*Artemisia myriantha* *Artemisia absinthium*
	Pseudoguaianolides 	5/7-fused bicyclic	Helenalin **13** Mexicanin **14** Cynaropicrin **15**	*Arnica montana* *Helenium mexicanum* *Cynara scolymus*
	Xanthanolides 	7-membered	Xanthatin **16**	*Xanthium italicum*
	Carabranolides 	6/3 tricyclic	Carabranolide **17**	*Carpesium faberi*
Diterpenes	Cembranolides 	14-membered (20 carbon)	Michaolide A **18** Crassumolide A **19**	*Lobophytum michaelae* *Lobophytum crassum* (soft coral)
Non-terpenoid α-methylene-γ-lactones	Butanolides 	Mono- and polycyclic	Subamolide D (*Z*) **20** Subamolide E (*E*) **21**	*Cinnamomum subavenium*

atoms of α-methylene-γ-lactones and amino acid residues adjacent to the reactive cysteine in a target protein, can precede alkylation and increase bioactivity [5].

Biochemical mechanisms involved in the anticancer activity of α-methylene-γ-lactones

Inhibition of transcription factors and gene expression

The transcription factor nuclear factor-κB (NF-κB) is an extracellular signal-activated transcription factor that usually resides in the cytoplasm of resting cells owing to its association with inhibitor of κB proteins (IκB). On exposure of cells to cytokines and growth factors, such as interleukin-1 (IL-1), tumor necrosis factor (TNF) or epidermal growth factor (EGF), a series of signaling events triggers phosphorylation and degradation of inhibitors of κB (IκB). NF-κB, liberated from inhibitors, translocates to the nucleus, binds specific response elements in the promoter region of target genes and activates the expression of many genes involved in cytokine production, cell proliferation and differentiation, cellular adhesion, inflammatory processes and apoptosis

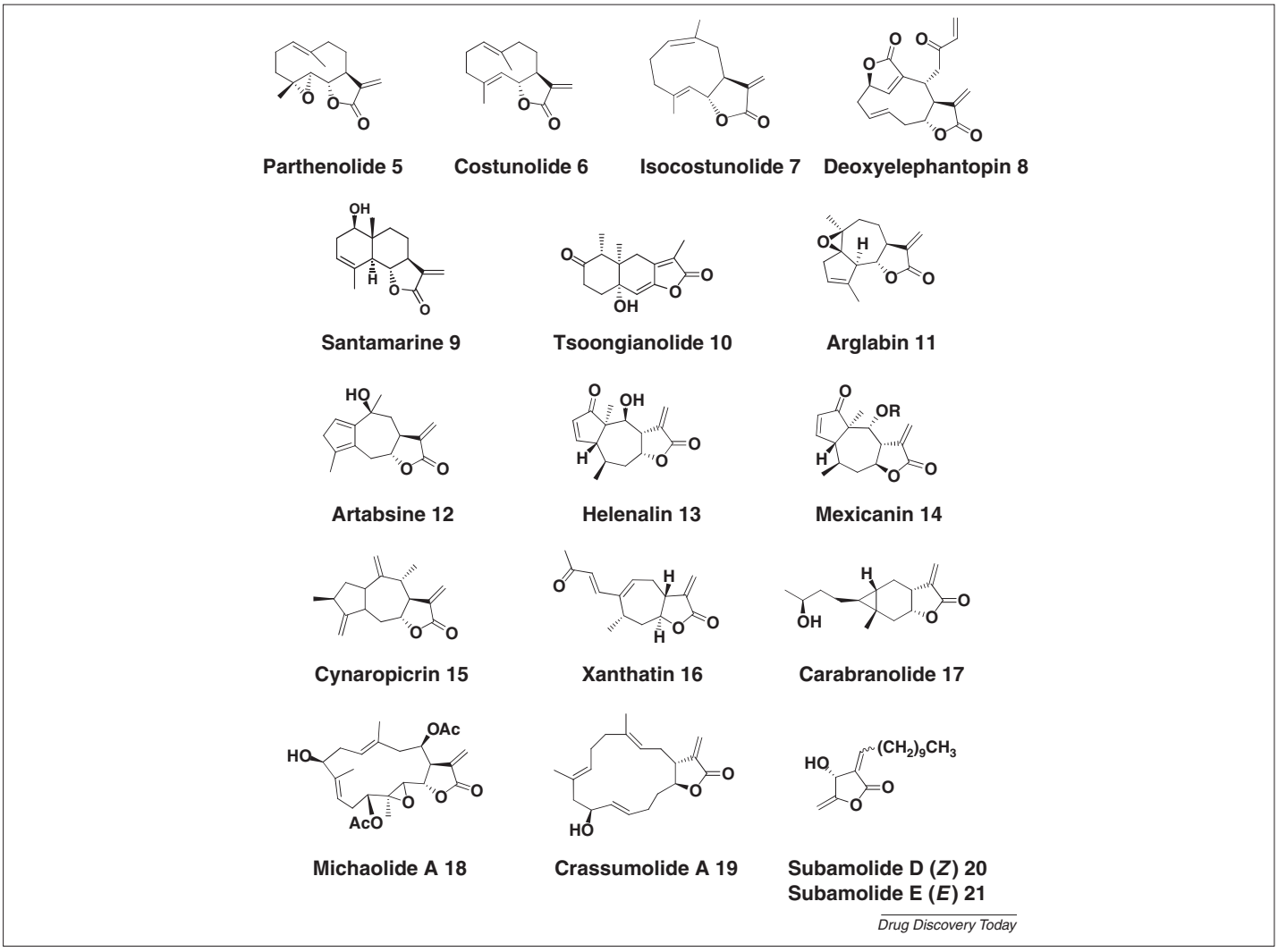


FIGURE 2

Structures of selected α-methylene-γ-lactones with anticancer potential.

[16]. Constitutive activation of NF-κB is observed in several cancers, including Hodgkin's disease [17], breast cancer [18] and colon cancer [19], and mediates the cellular transformation, proliferation, invasion, angiogenesis and metastasis of cancer. It is probable that constitutively active NF-κB contributes to chemotherapeutic resistance by upregulating the expression of anti-apoptotic genes. In human cancers, hyperactivation of NF-κB is usually linked with simultaneous inactivation of p53, a protein that regulates the cell cycle and, thus, functions as a tumor suppressor that is involved in preventing cancer. Recently, several α-methylene-γ-lactones have been shown to have the ability to inhibit NF-κB and activate the pro-apoptotic function of p53; therefore, these compounds can be placed among novel anticancer agents termed 'double-edged swords' [20]. They can impair the activity of NF-κB either by its alkylation or by the degradation of its inhibitory protein IκB [3,4,21–23]. Mechanistic studies demonstrated that α-methylene-γ-lactones, such as parthenolide **5** [24] and helenalin **13** [25] covalently block the mercapto group of cysteine at the active site of p65 NF-κB through their highly electrophilic α-methylene-γ-lactone ring. As a result, administration of these compounds blocks DNA binding of NF-κB and the lack of NF-κB activity renders cancer cells prone to apoptosis or to becoming sensitized to cytokine- and anticancer drug-induced cell death [26,27].

Inhibition of MAPK signaling pathway

Mitogen-activated protein kinases (MAPK) are enzymes that respond to extracellular stimuli (e.g. mitogens, osmotic stress, heat shock and pro-inflammatory cytokines) and regulate various cellular activities, such as gene expression, mitosis, differentiation, proliferation and cell survival and/or apoptosis [28]. An increasing body of evidence suggests that MAPKs, as well as NF-κB are involved in the anticancer activities of α-methylene-γ-lactones [29]. Two α-methylene-γ-lactones that have been found to target MAPK signaling pathways are parthenolide **5** [30] and costunolide **6** [31]. Recently, it was found that costunolide **6** dramatically increased c-Jun N-terminal kinase (JNK) activation and that this activation mediated costunolide **6**-induced apoptosis in human leukemia cells [20]. Furthermore, *in vivo* costunolide **6** was found to inhibit tumor growth in Lewis lung carcinoma in mice in a dose-dependent manner. However, in high doses, costunolide **6** failed to extend survival and decreased the body weight of animals, which indicated that it was toxic [20].

Induction of oxidative stress

There has been much recent interest in targeting intracellular redox signaling pathways as a therapeutic approach for cancer [32]. The redox potential in the cell is altered when thiol concentrations are lowered, causing oxidative stress and leading to the formation of reactive oxygen species (ROS), which initiate apoptosis via the mitochondrial-dependent pathway. Some evidence suggests that, in contrast to the oxidative extracellular redox state of normal cells, malignant cells exist in a reduced extracellular environment [33]. In tumor cells exposed to α-methylene-γ-lactones, a decrease in cellular glutathione levels has been observed both *in vitro* and *in vivo* [26]. Parthenolide **5**-mediated oxidative stress resulted mainly from reduced glutathione depletion and ROS generation. Tumor cell sensitivity to parthenolide **5** seems to correlate well with glutathione metabolism [34].

The recent data also suggest that the redox status of extracellular protein mercapto groups (i.e. exofacial thiols) affects cell behavior [35]. For example, the redox status of exofacial thiols on T-cells regulates their activation and proliferation [36]. Therefore, agents such as α-methylene-γ-lactones, which can affect redox reactions through alkylation of thiol groups, can modulate exofacial protein thiol levels. Parthenolide **5** action in normal cells was shown to augment cell viability and protect them from UVB- [37] or ROS-induced apoptosis [34,38]. By contrast, parthenolide **5** is toxic to abnormal cells and was shown to exert antilymphoma activity by modifying the redox state of crucial exofacial thiols [39]. Interestingly, parthenolide **5** was relatively tumor selective, having no effect on normal hematopoietic stem cells or T cells [40]. The observed tumor selectivity of parthenolide **5** could be the result, at least in part, of the difference in extracellular redox state between normal and malignant cells. One hypothesis is that the exofacial thiol targets of parthenolide **5** are in a reduced state on cancer cells and, thus, are susceptible to the drug, but are in an oxidized form on normal cells and unable to interact with parthenolide **5** [39]. Thus, parthenolide **5** is the only small molecule that has been reported so far to target several cancer and cancer stem cells selectively while sparing normal counterparts [12,41]. In addition, costunolide **6** readily depletes intracellular glutathione and disrupts the cellular redox balance [42].

Microtubule-interfering activity

Microtubules are key components of the cytoskeleton of eukaryotic cells. They are involved in cell division, being the principal components of the mitotic spindle. The building block of microtubules is the α,β-tubulin heterodimer. In most eukaryotic cells, the α-chain of tubulin can be cyclically modified by enzymatic cleavage of the C-terminal tyrosine residue by tubulin carboxypeptidase (TCP) and by re-addition of this amino acid by tubulin tyrosine ligase (TTL). Inhibition of TTL activity leads to defective spindle positioning during mitosis. Suppression of TTL and resulting accumulation of detyrosinated tubulin are frequent in human cancers and are associated with increased tumor aggressiveness.

Given that drugs that target microtubules are among the most commonly used anticancer agents, new natural microtubule-targeting compounds are especially interesting. Parthenolide **5** was shown to display microtubule-interfering properties by influencing tubulin and/or microtubule functions through tubulin binding. Indeed, two potential sites of alkylation have been identified on the β-tubulin molecule: the mercapto groups of cysteine residues at position 239 and 354, in the vicinity of colchicine binding site [43]. Of these, the integrity of Cyc239 is probably essential to the microtubule assembly process. In human breast cancer MCF-7 cells, parthenolide **5** exerted stimulatory activity on tubulin assembly by inducing the formation of well-organized microtubule polymers [44]. In HeLa cells, parthenolide **5**, but not the closely related costunolide **6**, showed strong TCP inhibitory activity [45]. Costunolide **6** has an α,β-unsaturated carbonyl structure and differs from parthenolide **5** by the lost of an epoxide function. Therefore, it seems that not only the presence of the *exo*-methylene group, but also the epoxide is necessary for the TCP inhibitory activity of parthenolide **5**. The interaction with the tubulin and/or microtubule system might represent an additional new mechanism by which parthenolide **5** can exert its activity.

Modulation of DNA methylation

DNA methylation of cytosine residues in the context of the sequence 5′-cytosine-gunosine (CpG) in gene promoter regions is an epigenetic mechanism that controls gene transcription, genome stability and genetic imprinting. This process is regulated by DNA methyltransferases in the presence of *S*-adenosyl-methionine (SAM), which serves as a methyl donor for the methylation of cytosine residues at the C-5 position to yield 5-methylcytosine. Hypermethylation of promoter CpG-rich regions of tumor suppressor genes results in their transcriptional silencing and is a hallmark of various tumors [46]. Methylation inhibitors have proven to be effective in restoring gene expression and normal patterns of differentiation and apoptosis in malignant cells [46]. Modulation of DNA methylation using such inhibitors represents a novel strategy for chemotherapy. Currently, effective DNA methylation inhibitors are mainly limited to decitabine and 5-aza-cytidine, which show unfavorable toxicity profiles in clinical settings. Recently, it was shown that parthenolide **5** inhibits DNA methyltransferase 1 *in vitro* and *in vivo* and downregulates its expression through γ-methylene lactone alkylation of the mercapto residue of Cys1226 at the catalytic domain of the enzyme [47].

Anticancer potential of α-methylene-γ-lactones

Induction of apoptosis

Apoptosis, or programmed cell death, is required for proper tissue homeostasis. Apoptotic cell death can be induced by two major pathways: the cell death receptor pathway or the mitochondrial pathway. Defects in these signaling pathways contribute to carcinogenesis and chemoresistance. Chemotherapeutic drugs have been shown to use apoptotic pathways to mediate their cytotoxic effect. α-Methylene-γ-lactones are promising compounds that, in many cell lines, are able to increase or restore the ability of tumor cells to undergo apoptosis. The most thoroughly tested α-methylene-γ-lactones, such as parthenolide **5** [26,34,48–55], costunolide **6** [42,56,57], isocostunolide **7** [58], helenalin **13** [59] and deoxyelephantopin **8** [60], were shown to induce apoptosis effectively in numerous cell lines in a dose-dependent manner. The induction of apoptosis was associated with inhibition of NF-κB [51,53,54], mitochondrial dysfunction and increase of ROS [34,42,53,54]. Several studies showed that the pro-apoptotic proteins from the Bcl-2 family, such as Bid, Bax and Bak, were involved in the apoptosis induced by these compounds [26,52,58].

Cell cycle arrest and inhibition of proliferation

The cell cycle is a collection of highly ordered processes that results in the duplication of eukaryotic cells. The sequential phases G0/G1, S, G2 and M represent the major cell cycle states of cells. Intrinsic mechanisms that check completion of each phase, called checkpoints, ensure the correct order of cell cycle events. Effects of α-methylene-γ-lactones on cell cycle have been demonstrated in several cancer cells. Activated $CD4^+$ T cells exposed to helenalin **13** were shown to undergo inhibition of proliferation by induction of G2–M cell cycle arrest [61]. Parthenolide **5** has been reported to arrest cell cycle progression at the G2–M [34] or G0–G1 [49] checkpoints and deoxyelephantopin **8** at the G2–M phase [60]. Cynaropicrin **15** can induce G1–S arrest in various leukocyte cancer cell lines [62], the action of costunolide **6** leads to G1 arrest of the cell cycle and subsequent apoptotic cell death in human prostate cancer cells [56] and isocostunolide **7** caused a marked loss of the G0–G1 phase in A2058 cells [58].

Suppression of metastasis

Another important aspect of the anticancer activity of α-methylene-γ-lactones is their suppressive effect on cancer metastasis. A characteristic feature of cancer is dissemination into distant tissues. Cancer cells can invade beyond the constraints of the normal tissue from which they originate by a multistep biochemical process, which includes detachment of cancer cells from the primary tumor, migration, adhesion and invasion of cancer cells into the blood vessels, extravasation out of the vessels and, finally, growth of a secondary tumor. It is known that cell adhesion molecules (CAMs) are among the essential molecules that facilitate neoplastic cell adhesion and migration. Parthenolide 5 was shown to inhibit the expression of CAMs *in vitro* [63] and inhibit migration and invasion of pancreatic cancer *in vitro* and *in vivo* [52].

Tumors cannot grow beyond a certain size, generally 1–2 mm, without oxygen and other essential nutrients, which are provided by blood. Therefore, tumors induce blood vessel growth (angiogenesis) by secreting various growth factors. The formation of new blood vessels from pre-existing ones is also essential for the spread of metastatic cells throughout the body. Inhibitors of angiogenesis that block angiogenic signals have also received considerable interest as a valuable addition to cytostatic and cytotoxic chemotherapy. Costunolide **6** was found to inhibit endothelial cell proliferation and angiogenesis induced by endothelial growth factor [64]. Parthenolide **5** inhibits the expression of matrix metalloproteinase-9 and urokinase plasminogen activator, proteolytic enzymes capable of degrading the extracellular matrix and responsible for the migration of cancer cells *in vitro*, as well as osteolytic bone metastasis *in vivo* [65].

The recent data showing the anticancer potential of α-methylene-γ-lactones in the *in vitro* studies are summarized in Table 2.

TABLE 2

Anticancer potential of α-methylene-γ-lactones

α-Methylene-γ-lactone	Mode of action	Cell line	Refs
Parthenolide	Induction of apoptosis	Leukemia AML	[12,40]
		Chronic lymphocytic leukemia CCL	[95]
		Cholangiocarcinoma SCK, JCK, Che-CK, Choi-CK	[26]
		Hepatoma Hep 3B, PLC/PRF/5, SK-HEP-1	[34]
		Hepatocellular carcinoma HepG2, Hep3B, PLC	[49]
		Hepatocellular carcinoma HepG2, Hep3B, SK-HEP-1	[50]
		Mammary adenocarcinoma MDA-MB-231, MDA-MB-436	[48]
		Mammary adenocarcinoma MCF7	[100]
		Melanoma A375, 1205Lu, WM793	[51]
		Pancreatic adenocarcinoma BxPC-3	[52]
		Prostate adenocarcinoma PC-3	[53]
		Non-small lung cancer A549	[54]
		Bladder cancer UMUC-3	[54]
	G2/M cell cycle arrest	Hepatoma Hep 3B, PLC/PRF/5, SK-HEP-1	[34]
	G0/G1 cell cycle arrest	Hepatocellular carcinoma HepG2, Hep3B, PLC	[49]
	Inhibition of CAMs	Murine Ba/F3 proB lymphoma	[63]
	Inhibition of MMP-9 and uPA	Rat breast cancer W 256	[65]
	Inhibition of migration and invasion	Pancreatic adenocarcinoma BxPC-3	[52]
Costunolide	Induction of apoptosis	Prostate cancer LNCaP, PC-3 and DU-145	[56]
		Promonocytic leukemia U 937	[42]
		B cell leukemia NALM-6	[57]
	G1 cell cycle arrest	Prostate cancer LNCaP, PC-3 and DU-145	[56]
	Inhibition of angiogenesis	Umbilical vein endothelial cells HUVECs	[64]
Isocostunolide	Induction of apoptosis	Melanoma A 2058	[58]
	G0/G1 cell cycle arrest	Melanoma A 2058	[58]
Helenalin	Induction of apoptosis	Activated CD4+ T cells	[61]
	G2/M cell cycle arrest	Activated CD4+ T cells	[61]
Deoxyelephantopin	Induction of apoptosis	Mammary adenocarcinoma TSA	[60]
	G2/M cell cycle arrest	Mammary adenocarcinoma TSA	[60]
Cynaropicrin	G1/S cell cycle arrest	Leukemia Eol 1, U 937, Jurkat T cells	[62]

Occurrence and biological activity of natural α-methylene-δ-lactones and α-methylene-γ- and δ-lactams

In contrast to α-methylene-γ-lactones **1**, α-methylene-δ-lactones **2** and γ- and δ-lactams **3** and **4** are less abundant in nature. The first α-methylene-δ-lactones, such as vernolepin **22** or vernomenin **23**, were isolated during the 1960s from *Vernonia hymenolepis* and contained both the α-methylene-γ-lactone and the α-methylene-δ-lactone moieties [66]. Both compounds showed significant cytotoxicity against human carcinoma of the nasopharynx (KB) cells [66]. In addition, some natural compounds with α-methylene-δ-lactone moiety alone have also been isolated. Of these, crassin **24** showed *in vitro* activity against KB cells [67].

α-Methylene-γ-lactams **3** are much less common in nature. Examples of a few naturally occurring compounds of this structure include pukeleimid E **25**, isolated from *Lyngbya majuscula* [59], and two imidazole alkaloids, anatin **26** and isoanatin **27** found in the leaves of *Cynometra*, a plant used in African folk medicine as a remedy for pain [68]. Compounds containing this moiety show important biological activities, such as cytotoxic [69], antitumor [70] and anti-inflammatory activities [71].

By contrast, natural α-methylene-δ-lactams are almost unknown. Very recently, a new humantenine-type alkaloid, gelegamine B **28**, was isolated from *Gelsemium elegans*, a liana growing in Southeast Asia [72]. The cytotoxicity of this compound was tested against HL-60 human leukemia and A-549 human lung cancer cell lines, but no obvious effects were recorded.

Examples of plant-derived α-methylene-δ-lactones, α-methylene-γ-lactams and α-methylene-δ-lactams are summarized in Table 3 and their structures are given in Fig. 3.

Synthetic α-methylene-γ- or δ-lactones or lactams with anticancer properties

Analogs of compounds isolated from plants

The functionalization of known compounds isolated from plants might allow for selective modulation of their reactivity with biological nucleophiles, thus allowing for more specific interactions and better selectivity as drug candidates.

Compounds that have the ability to alkylate DNA have had an important role in the development of anticancer drugs. Well-known examples of such drugs are *cis*-platin, chlorambucil and cyclophosphamide. However, these drugs have no particular affinity to DNA. α-Methylene-γ-lactones are strong alkylating agents, but also have no affinity to DNA. Hybrid molecules containing α-methylene-γ-lactone skeleton conjugated with a moiety that can bind to DNA seem to be an interesting solution. Certain α-methylene-γ-lactones bearing purine or pyrimidine bases were synthesized (e.g. **29**) [73]. These compounds showed strong growth-inhibitory activity against leukemia cell lines.

A natural antibiotic, distamycin A, is known to bind to the DNA minor grove, preferentially to the AT-rich sequences [74]; it can therefore be used as a DNA sequence-selective vector for alkylating agents. A series of distamycin A hybrids with different synthetic α-methylene-γ-lactones (e.g. **30**) was synthesized and tested against several cancer cell lines. It was shown that the cytotoxicity of the hybrids was much greater than that of the α-methylene-γ-lactones alone. Several analogs were also able to induce apoptosis in HL-60 cells [75].

α-Methylene-γ-lactones are very lipophilic compounds and lipophilicity often results in lower drug bioavailability *in vivo*. In the search for SLs with improved solubility and bioavailability, a series of parthenolide **5** derivatives was obtained through the diastereoselective addition of several primary and secondary amines to the exocyclic double bond [76,77]. These studies have generated an amino derivative of parthenolide **5**, dimethylaminoparthenolide (DMAPT, **31,** also referred to as LC-1) [77], When formulated as a fumarate salt, DMAPT demonstrates more than 1000-fold greater solubility in water relative to parthenolide **5**. Furthermore, pharmacokinetic studies in the rat indicate that DMAPT has an oral bioavailability of approximately 70%. Most recently, Shanmugan *et al.* [53] showed in experiments performed on mice that DMAPT might have activity as a single agent in men with castration-resistant prostate cancer.

Examples of the structures discussed above are given in Fig. 3.

Purely synthetic compounds with an α-methylene-γ or δ-lactone or -lactam skeleton

As a result of the biological importance of natural products with α,β-unsaturated carbonyl structures, purely synthetic compounds containing either 5- or 6-membered lactone or lactam ring with an exocyclic methylene group have been of interest to organic chemists for several years, resulting in the development of new improved methods for their synthesis, which are described in recent reviews [8,78]. Elford *et al.* [79] synthesized a library of functionalized γ-lactones and γ-lactams, containing saturated or unsaturated side chains at the α-position of the ring. These compounds were evaluated for their ability to inhibit homoserine transacetylase (HTA). This enzyme catalyzes the transfer of an acetyl group from acetyl-CoA to the hydroxyl group of homoserine [80], which is the first step in the biosynthesis of methionine from aspartic acid. HTA is found in fungi, gram-positive and gram-negative bacteria, but not in higher eukaryotes. This is an important enzyme for microorganisms in methionine-poor environments, such as blood. Therefore, inhibition of HTA can be deleterious to bacteria or fungi. Two of the polysubstituted α-methylene-γ-lactams (**32**, **33**) displayed moderate inhibitory activity against HTA and should be further tested for their antimicrobial and also anticancer potential.

Propanoic acid derivatives are important substances within the group of nonsteroidal anti-inflammatory therapeutic agents [81]. Introduction of an α-methylene-γ-lactone group into 3-(4-hydroxyphenyl)propanoic acid resulted in compound **34**, which

TABLE 3

Examples of plant-derived α-methylene-δ-lactones, α-methylene-γ-lactams and α-methylene-δ-lactams

Category	Example	Origin
α-Methylene-δ-lactones	Vernolepin **22** Vernomenin **23** Crassin **24**	*Vernonia hymenolepis* *Pseudoplexaura porosa*
α-Methylene-γ-lactams	Pukeleimid E **25** Anatin **26** Isoanatin **27**	*Lyngbya majuscula* *Cynometra cauliflora*
α-Methylene-δ-lactams	Gelegamine B **28**	*Gelsemium elegans*

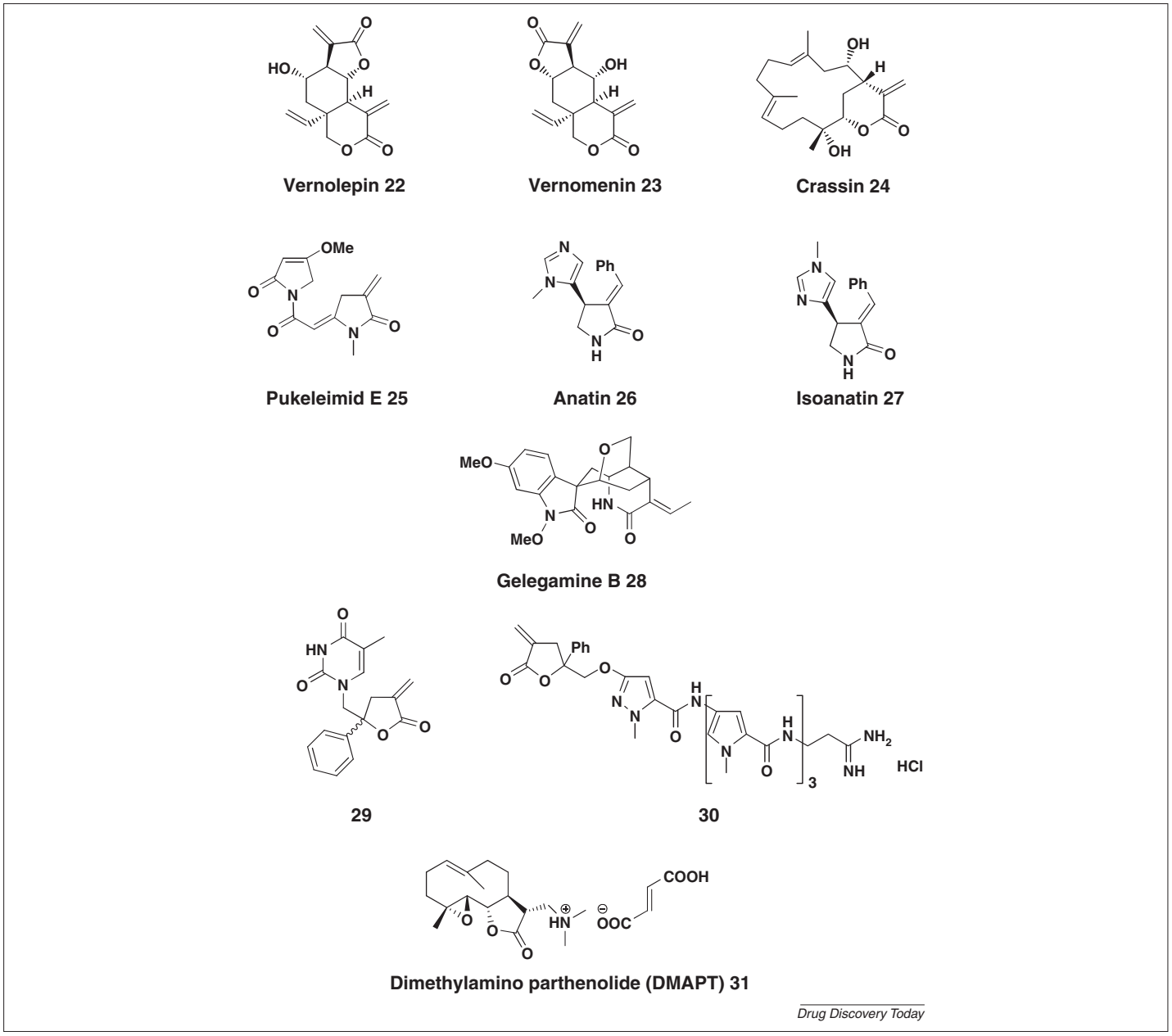


FIGURE 3

Examples of plant-derived and synthetic α-methylene-δ-lactones and α-methylene-γ- and δ-lactams.

inhibited proliferation and induced apoptosis in leukemia HL-60 cells [82].

In the search for highly cytotoxic, yet structurally simple α-methylene-γ-lactones, Janecki and co-workers performed diastereo- and enantioselective synthesis of several series of differently substituted α-methylene-γ-lactones and lactams **35**–**41** and tested their cytotoxicity against the human leukemia cell lines HL-60 and NALM-6. These studies revealed that α-methylene-γ-lactones **35** with 1-hydroxyalkyl substituents in the γ position are highly to moderately cytotoxic against these cell lines [83,84]. Straightforward correlation between lipophilicity and cytotoxicity of these lactones was observed, with the more lipophilic compounds also being more cytotoxic. Moreover, it was found that enantiomeric lactones **35** differ significantly in their cytotoxicity against the HL-60 cell line [84], with those having an S-configuration on both stereogenic centers being significantly more active. By contrast, simple, γ-alkyl substituted α-methylene-γ-lactone **36** had only moderate cytotoxicity [85]. In addition, a series of γ-aryl- [86] and β-aryl- [87] substituted α-methylene-γ-lactones **37**, **39** and γ-lactams **38**, **40** were synthesized and their cytotoxicity was tested. The β-aryl- and γ-aryl-substituted methylene-γ-lactones **37** and **39** displayed considerable activity. These studies showed that the presence of an aromatic substituent in γ-lactone ring is crucial for cytotoxic activity. In the series of β-methyl-γ-aryl-γ-lactones **37**, *trans*-configured lactones were significantly more potent than were corresponding *cis* stereoisomers. Comparing the cytotoxic activity of α-methylene-γ-lactams **38** and **40** versus α-methylene-γ-lactones **37** and **39** with the same substitution pattern revealed considerably less cytotoxicity of the former. Comparisons were also made between α-methylene-γ- and -δ-lactones with the corresponding

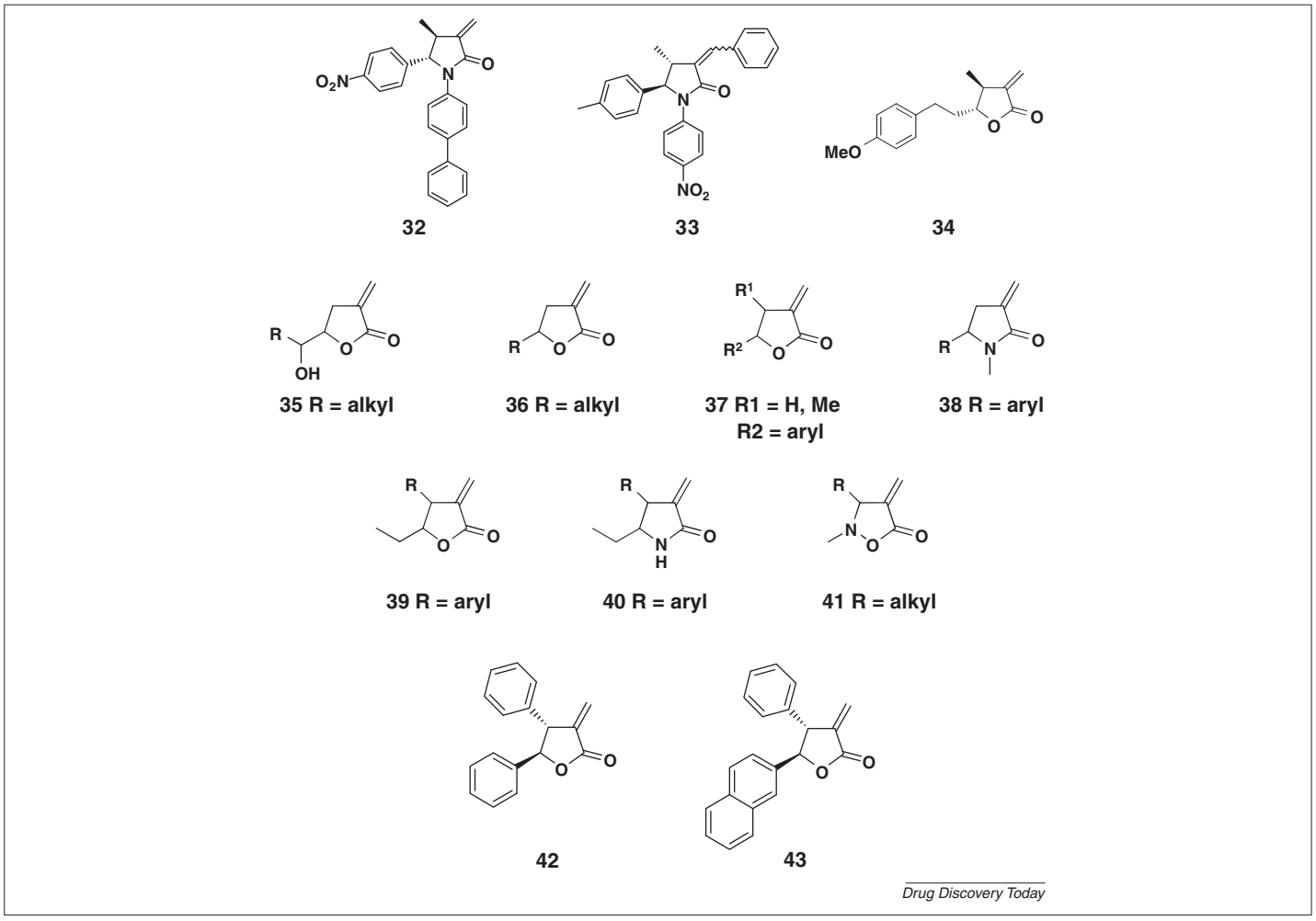


FIGURE 4

Examples of synthetic compounds with α-methylene-γ-lactones and lactams.

substitution patterns. In this case, similar cytotoxicities were observed, which indicates that the size of the ring is not crucial [88]. Finally, a series of 4-methyleneisooxazolidin-5-ones **41**, where one of the carbon atoms in the lactone ring was replaced by a nitrogen atom, was also synthesized and the cytotoxicity tested [84,89]. These compounds were found to be strongly cytotoxic against several cancer cell lines and induced apoptosis in HL-60 cells [90].

A series of simple, racemic alkyl- and aryl-substituted α-methylene-γ-lactones was recently synthesized by Ramachandran *et al.* [91] and screened against three human pancreatic cell lines (Panc-1, MIA PaCa-2 and BxPC-3). Structure activity–relationship studies revealed that *trans*-β,γ-diaryl-α-methylene-γ-lactones are the most active and enabled two new compounds to be selected, *trans*-β,γ-diphenyl- and *trans*-γ-2-naphthyl-β-phenyl-α-methylene-γ-lactones (**42** and **43**, respectively), as potent inhibitors of the three examined cell lines. Structures of compounds discussed here are given in Fig. 4.

Preclinical *in vivo* tests

Thousands of α-methylene-γ-lactones have been isolated from plants so far and, although many of them showed some anticancer activity in different *in vitro* tests, only a few have been tested in cancer clinical trials [5]. Parthenolide **5** is the most extensively studied α-methylene-γ-lactone and currently actively pursued as a potential anticancer treatment [50,55,92–94]. As discussed above, parthenolide **5** can generate oxidative stress, which leads to the activation of caspases and then to apoptosis. It also inhibits NF-κB signaling, which results in the reduced expression of anti-apoptotic proteins, interacts with the tubulin and/or microtubule system and inhibits methylation of DNA in cancer cells. The *in vitro* antiproliferative activity of parthenolide **5** is characterized by an IC_{50} value of between 1 and 10 μM in nearly all tested cell lines, both of leukemia [40,95] and multiple solid tumor origin [26,48,96]. Additionally, parthenolide **5** can sensitize resistant cancer cells to antitumor agents [48]; for example, it can function well in combination with paclitaxel [44], sulindac [96] or arsenic trioxide [97].

Despite widely documented anticancer activity *in vitro*, clinical development of parthenolide **5** was hampered by its poor water solubility and bioavailability. The breakthrough came with the development of a less lipophilic amino-derivative, DMAPT [77,98], which is readily water soluble and is 70% orally bioavailable [12]. Pharmacology and toxicology studies confirmed the ability of DMAPT to generate ROS, as well as inhibit NF-κB DNA binding and *in vitro* proliferation of cancer cells [12,53,54]. Thus, it maintains the

basic characteristics of its parent compound. Moreover, as it mentioned above, both parthenolide **5** and DMAPT have been shown to leave normal hematopoietic stem and progenitor cells unharmed [12]. DMAPT was further characterized *in vivo* [12] using mouse xenograft models and spontaneous acute canine leukemias. In animals treated with DMAPT, a rapid decrease in the percentage of $CD34^+$ cells was detected, accompanied by an increase in the frequency of differentiated cells. These data indicate that DMAPT mediates *in vivo* biological changes in leukemia cells that lead to their impairment and death.

Most recently, the *in vivo* suppression of prostate cancer by DMAPT was also observed by Shanmugam *et al.* [53]. The cells of androgen-independent prostate cancer cell lines were implanted into nude female mice (low testosterone levels). Administration of DMAPT resulted in significant tumor suppression, as evidenced by a decrease in final tumor volume as compared with control. DMAPT was shown to be more effective in inhibiting tumor growth than was bicalutamide and docetaxel, two medications used currently to treat prostate cancer. The same group also characterized DMAPT *in vivo* activity on two tobacco-associated cancers, lung and bladder cancer, using nude mice implanted with non-small lung cancer A549 or UMUC-3 bladder cancer cells. Significant suppression of tumors following oral administration of DMAPT was observed in both cases [54]. Based on these preclinical findings, oral bioavailability and a favorable toxicology profile, DMAPT entered phase I clinical testing in 2009.

Another α-methylene-γ-lactone, arglabin **11**, has been used successfully in Kazakhstan for the treatment of breast, colon, ovarian and lung cancers ([99] and citations therein).

Concluding remarks

α-Methylene-γ-lactones, in particular of the sesquiterpene type, have proven to be promising candidates for treatment of various types of cancer. However, isolation of these compounds from natural sources limits their availability. The poor bioavailability of SLs, which is because of their lipophilic character, is also a serious drawback. The first big success was achieved by introducing DMAPT into clinical trials. To achieve further progress in obtaining new drugs with an α-methylene-γ- or δ-lactone or -lactam motif, chemical modifications of the known structures or new designs are necessary.

References

1 Cragg, G.M. and Newman, D.J. (2005) Plants as a source of anti-cancer agents. *J. Ethnopharmacol.* 100, 72–79
2 Zhang, S. *et al.* (2005) Anti-cancer potential of sesquiterpene lactones: bioactivity and molecular mechanisms. *Curr. Med. Chem. Anticancer Agents* 5, 239–249
3 Pajak, B. *et al.* (2008) Molecular basis of parthenolide-dependent proapoptotic activity in cancer cells. *Folia Histochem. Cytobiol.* 46, 129–135
4 Koprowska, K. and Czyz, M. (2010) Molecular mechanisms of parthenolide's action: old drug with a new face. *Postepy Hig. Med. Dosw.* 64, 100–114
5 Ghantous, A. *et al.* (2010) What made sesquiterpene lactones reach cancer clinical trials? *Drug Discov. Today* 15, 668–678
6 Merfort, I. (2011) Perspectives on sesquiterpene lactones in inflammation and cancer. *Curr. Drug Targets* doi:BSP/CDT/E-Pub/00262
7 Picman, A.K. (1986) Biological activities of sesquiterpene lactones. *Biochem. Syst. Ecol.* 14, 255–281
8 Kitson, R.R.A. *et al.* (2009) The renaissance of alpha-methylene-gamma-butyrolactones: new synthetic approaches. *Angew. Chem. Int. Ed.* 48, 9426–9451
9 Pati, H.N. *et al.* (2007) Cytotoxic thiol alkylators. *Mini Rev. Med. Chem.* 7, 131–139
10 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
11 Scotti, M.T. *et al.* (2007) Quantitative structure–activity relationship of sesquiterpene lactones with cytotoxic activity. *Bioorg. Med. Chem.* 15, 2927–2934
12 Guzman, M.L. *et al.* (2007) An orally bioavailable parthenolide analog selectively eradicates acute myelogenous leukemia stem and progenitor cells. *Blood* 110, 4427–4435
13 Beekman, A.C. *et al.* (1997) Structure–cytotoxicity relationships of some helenanolide-type sesquiterpene lactones. *J. Nat. Prod.* 60, 252–257
14 Schmidt, T.J. *et al.* (1996) Helenanolide-type sesquiterpene lactones .2. 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
15 Recio, M.C. *et al.* (2000) In vivo activity of pseudoguaianolide sesquiterpene lactones in acute and chronic inflammation. *Life Sci.* 66, 2509–2518
16 Karin, M. and Lin, A. (2002) NF-kappaB at the crossroads of life and death. *Nat. Immunol.* 3, 221–227
17 Horie, R. and Watanabe, T. (2003) The biological basis of Hodgkin's lymphoma. *Drug News Perspect.* 16, 649–656
18 Wu, J.T. and Kral, J.G. (2005) The NF-kappaB/IkappaB signaling system: a molecular target in breast cancer therapy. *J. Surg. Res.* 123, 158–169
19 Braeuer, S.J. *et al.* (2006) Constitutively activated nuclear factor-kappaB, but not induced NF-kappaB, leads to TRAIL resistance by up-regulation of X-linked inhibitor of apoptosis protein in human cancer cells. *Mol. Cancer Res.* 4, 715–728
20 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
21 Oka, D. *et al.* (2007) Sesquiterpene lactone parthenolide suppresses tumor growth in a xenograft model of renal cell carcinoma by inhibiting the activation of NF-kappaB. *Int. J. Cancer* 120, 2576–2581
22 Kishida, Y. *et al.* (2007) Parthenolide, a natural inhibitor of nuclear factor-kappaB, inhibits lung colonization of murine osteosarcoma cells. *Clin. Cancer Res.* 13, 59–67
23 Ma, G.G. *et al.* (2007) Inhibition of NF-kappaB-mediated transcription and induction of apoptosis by melampolides and repandolides. *Cancer Chemother. Pharmacol.* 60, 35–43
24 Garcia-Pineres, A.J. *et al.* (2004) Role of cysteine residues of p65/NF-kappaB on the inhibition by the sesquiterpene lactone parthenolide and N-ethyl maleimide, and on its transactivating potential. *Life Sci.* 75, 841–856
25 Rungeler, P. *et al.* (1999) Inhibition of transcription factor NF-kappaB by sesquiterpene lactones: a proposed molecular mechanism of action. *Bioorg. Med. Chem.* 7, 2343–2352
26 Kim, J.H. *et al.* (2005) Susceptibility of cholangiocarcinoma cells to parthenolide-induced apoptosis. *Cancer Res.* 65, 6312–6320
27 Sweeney, C.J. *et al.* (2005) The sesquiterpene lactone parthenolide in combination with docetaxel reduces metastasis and improves survival in a xenograft model of breast cancer. *Mol. Cancer Ther.* 4, 1004–1012
28 Pearson, G. *et al.* (2001) Mitogen-activated protein (MAP) kinase pathways: regulation and physiological functions. *Endocr. Rev.* 22, 153–183
29 Nam, N.H. (2006) Naturally occurring NF-kappaB inhibitors. *Mini Rev. Med. Chem.* 6, 945–951
30 Hung, T.M. *et al.* (2008) Cholinesterase inhibitory and anti-amnesic activity of alkaloids from *Corydalis turtschaninovii*. *J. Ethnopharmacol.* 119, 74–80
31 Kang, J.S. *et al.* (2004) Costunolide inhibits interleukin-1beta expression by down-regulation of AP-1 and MAPK activity in LPS-stimulated RAW 264.7 cells. *Biochem. Biophys. Res. Commun.* 313, 171–177
32 Schumacker, P.T. (2006) Reactive oxygen species in cancer cells: live by the sword, die by the sword. *Cancer Cell* 10, 175–176
33 Chaiswing, L. *et al.* (2008) Extracellular redox state regulates features associated with prostate cancer cell invasion. *Cancer Res.* 68, 5820–5826
34 Wen, J. *et al.* (2002) Oxidative stress-mediated apoptosis. The anticancer effect of the sesquiterpene lactone parthenolide. *J. Biol. Chem.* 277, 38954–38964
35 Ou, W. and Silver, J. (2006) Role of protein disulfide isomerase and other thiol-reactive proteins in HIV-1 envelope protein-mediated fusion. *Virology* 350, 406–417

36 Gelderman, K.A. *et al.* (2006) T cell surface redox levels determine T cell reactivity and arthritis susceptibility. *Proc. Natl. Acad. Sci. U. S. A.* 103, 12831–12836
37 Won, Y.K. *et al.* (2004) Chemopreventive activity of parthenolide against UVB-induced skin cancer and its mechanisms. *Carcinogenesis* 25, 1449–1458
38 Yao, H. *et al.* (2007) Parthenolide protects human lens epithelial cells from oxidative stress-induced apoptosis via inhibition of activation of caspase-3 and caspase-9. *Cell Res.* 17, 565–571
39 Skalska, J. *et al.* (2009) Modulation of cell surface protein free thiols: a potential novel mechanism of action of the sesquiterpene lactone parthenolide. *PLoS One* 4, e8115
40 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
41 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
42 Choi, J.H. and Lee, K.T. (2009) Costunolide-induced apoptosis in human leukemia cells: involvement of c-jun N-terminal kinase activation. *Biol. Pharm. Bull.* 32, 1803–1808
43 Bai, R. *et al.* (2000) Mapping the binding site of colchicinoids on beta-tubulin. 2-chloroacetyl-2-demethylthiocolchicine covalently reacts predominantly with cysteine 239 and secondarily with cysteine 354. *J. Biol. Chem.* 275, 40443–40452
44 Gao, Z.W. *et al.* (2010) Paclitaxel efficacy is increased by parthenolide via nuclear factor-kappaB pathways in *in vitro* and *in vivo* human non-small cell lung cancer models. *Curr. Cancer Drug Targets* 10, 705–715
45 Fonrose, X. *et al.* (2007) Parthenolide inhibits tubulin carboxypeptidase activity. *Cancer Res.* 67, 3371–3378
46 Yoo, C.B. and Jones, P.A. (2006) Epigenetic therapy of cancer: past, present and future. *Nat. Rev. Drug Discov.* 5, 37–50
47 Liu, Z. *et al.* (2009) Modulation of DNA methylation by a sesquiterpene lactone parthenolide. *J. Pharmacol. Exp. Ther.* 329, 505–514
48 Nakshatri, H. *et al.* (2004) Antitumor agent parthenolide reverses resistance of breast cancer cells to tumor necrosis factor-related apoptosis-inducing ligand through sustained activation of c-Jun N-terminal kinase. *Oncogene* 23, 7330–7344
49 Ralstin, M.C. *et al.* (2006) Parthenolide cooperates with NS398 to inhibit growth of human hepatocellular carcinoma cells through effects on apoptosis and G0–G1 cell cycle arrest. *Mol. Cancer Res.* 4, 387–399
50 Carlisi, D. *et al.* (2011) Parthenolide sensitizes hepatocellular carcinoma cells to TRAIL by inducing the expression of death receptors through inhibition of STAT3 activation. *J. Cell. Physiol.* 226, 1632–1641
51 Czyz, M. *et al.* (2010) Cell context-dependent activities of parthenolide in primary and metastatic melanoma cells. *Br. J. Pharmacol.* 160, 1144–1157
52 Liu, J.W. *et al.* (2010) Parthenolide induces proliferation inhibition and apoptosis of pancreatic cancer cells *in vitro*. *J. Exp. Clin. Cancer Res.* 29, 108
53 Shanmugam, R. *et al.* (2010) A water-soluble parthenolide analogue suppresses *in vivo* prostate cancer growth by targeting NFkappaB and generating reactive oxygen species. *Prostate* 70, 1074–1086
54 Shanmugam, R. *et al.* (2011) A water soluble parthenolide analog suppresses *in vivo* tumor growth of two tobacco-associated cancers, lung and bladder cancer, by targeting NF-kappaB and generating reactive oxygen species. *Int. J. Cancer* 128, 2481–2494
55 Sahler, J. *et al.* (2011) The Feverfew plant-derived compound, parthenolide enhances platelet production and attenuates platelet activation through NF-kappaB inhibition. *Thromb. Res.* 127, 426–434
56 Hsu, J.L. *et al.* (2011) Costunolide induces apoptosis through nuclear calcium(2+) overload and DNA damage response in human prostate cancer. *J. Urol.* 185, 1967–1974
57 Kanno, S. *et al.* (2008) Costunolide-induced apoptosis is caused by receptor-mediated pathway and inhibition of telomerase activity in NALM-6 cells. *Biol. Pharm. Bull.* 31, 1024–1028
58 Chen, C.N. *et al.* (2007) Isocostunolide, a sesquiterpene lactone, induces mitochondrial membrane depolarization and caspase-dependent apoptosis in human melanoma cells. *Cancer Lett.* 246, 237–252
59 Cardellina, J.H. and Moore, R.E. (1979) Structures of pukeleimide-A, pukeleimide-B, pukeleimide-D, pukeleimide-E, pukeleimide-F, and pukeleimide-G. *Tetrahedron Lett.* 22, 2007–2010
60 Huang, C.C. *et al.* (2010) Deoxyelephantopin, a novel multifunctional agent, suppresses mammary tumour growth and lung metastasis and doubles survival time in mice. *Br. J. Pharmacol.* 159, 856–871
61 Berges, C. *et al.* (2009) Helenalin suppresses essential immune functions of activated CD4+ T cells by multiple mechanisms. *Mol. Immunol.* 46, 2892–2901
62 Cho, J.Y. *et al.* (2004) Cytotoxic and pro-apoptotic activities of cynaropicrin, a sesquiterpene lactone, on the viability of leukocyte cancer cell lines. *Eur. J. Pharmacol.* 492, 85–94
63 Schnyder, B. *et al.* (2002) Phytochemical inhibition of interleukin-4-activated Stat6 and expression of VCAM-1. *Biochem. Biophys. Res. Commun.* 292, 841–847
64 Jeong, S.J. *et al.* (2002) Costunolide, a sesquiterpene lactone from *Saussurea lappa*, inhibits the VEGFR KDR/Flk-1 signaling pathway. *Cancer Lett.* 187, 129–133
65 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
66 Kupchan, S.M. *et al.* (1968) Vernolepin, a novel elemanolide dilactone tumor inhibitor from *Vernonia hymenolepis*. *J. Am. Chem. Soc.* 90, 3596–3597
67 Weinheimer, A.J. *et al.* (1979) Naturally occurring cembranes. *Fortschr. Chem. Org. Naturst.* 36, 285
68 Naito, T. *et al.* (1993) Total syntheses of (+/−)-anantine and (+/−)-isoanantine via thiyl radical addition-cyclization reaction. *Chem. Pharm. Bull.* 41, 217–219
69 Belaud, C. *et al.* (1985) Synthesis of potential cytotoxic alpha-methylene gamma-lactams. *Synth. Commun.* 15, 1233–1243
70 Kornet, M.J. (1979) Synthesis of alpha-methylenebeutyrolactams as potential antitumor agents. *J. Pharm. Sci.* 68, 350–353
71 Ikuta, H. *et al.* (1987) Synthesis and anti-inflammatory activities of 3-(3,5-di-tert-butyl-4-hydroxybenzylidene)pyrrolidin-2-ones. *J. Med. Chem.* 30, 1995–1998
72 Zhang, Z. *et al.* (2009) Gelegamines A–E: five new oxindole alkaloids from *Gelsemium elegans*. *Tetrahedron* 65, 4551–4556
73 Lee, K.H. *et al.* (1999) Synthesis and anticancer evaluation of certain alpha-methylene-gamma-(4-substituted phenyl)-gamma-butyrolactone bearing thymine, uracil, and 5-bromouracil. *Bioorg. Med. Chem. Lett.* 9, 241–244
74 bu-Daya, A. *et al.* (1995) DNA sequence preferences of several AT-selective minor groove binding ligands. *Nucleic Acids Res.* 23, 3385–3392
75 Baraldi, P.G. *et al.* (2004) Design, synthesis, and biological evaluation of hybrid molecules containing alpha-methylene-gamma-butyrolactones and polypyrrole minor groove binders. *J. Med. Chem.* 47, 2877–2886
76 Nasim, S. and Crooks, P.A. (2008) Antileukemic activity of aminoparthenolide analogs. *Bioorg. Med. Chem. Lett.* 18, 3870–3873
77 Neelakantan, S. *et al.* (2009) Aminoparthenolides as novel anti-leukemic agents: discovery of the NF-kappaB inhibitor, DMAPT (LC-1). *Bioorg. Med. Chem. Lett.* 19, 4346–4349
78 Albrecht, A. *et al.* (2011) Recent advances in the synthesis of alpha-alkylidene-substituted delta-lactones, gamma-lactams and delta-lactams. *Eur. J. Org. Chem.* 15, 2747–2766
79 Elford, T.G. *et al.* (2009) Diversity-oriented synthesis and preliminary biological screening of highly substituted five-membered lactones and lactams originating from an allyboration of aldehydes and imines. *J. Comb. Chem.* 11, 155–168
80 Born, T.L. *et al.* (2000) Enzyme-catalyzed acylation of homoserine: mechanistic characterization of the *Haemophilus influenzae* met2-encoded homoserine transacetylase. *Biochemistry* 39, 8556–8564
81 Josa, M. *et al.* (2001) Pharmacokinetic/pharmacodynamic modeling of antipyretic and anti-inflammatory effects of naproxen in the rat. *J. Pharmacol. Exp. Ther.* 297, 198–205
82 Gonzalez, A.G. *et al.* (2002) Synthesis and antiproliferative activity of a new compound containing an alpha-methylene-gamma-lactone group. *J. Med. Chem.* 45, 2358–2361
83 Janecki, T. *et al.* (2002) New stereocontrolled synthesis and biological evaluation of 5-(1′-hydroxyalkyl)-3-methylidenetetrahydro-2-furanones as potential cytotoxic agents. *J. Med. Chem.* 45, 1142–1145
84 Janecki, T. *et al.* (2005) Enantioselective synthesis and cytotoxic evaluation of 4,5-dihydro-5-[aryl(hydroxy)methyl]-3-methylidenefuran-2(3H)-ones. *Chem. Biodivers.* 2, 1256–1265
85 Janecki, T. *et al.* (2005) Novel synthesis, cytotoxic evaluation, and structure–activity relationship studies of a series of alpha-alkylidene-gamma-lactones and lactams. *J. Med. Chem.* 48, 3516–3521
86 Albrecht, A. *et al.* (2008) Synthesis and cytotoxic activity of gamma-aryl substituted alpha-alkylidene-gamma-lactones and alpha-alkylidene-gamma-lactams. *Bioorg. Med. Chem.* 16, 4872–4882
87 Albrecht, A. *et al.* (2010) A convenient synthesis and cytotoxic evaluation of beta-aryl-alpha-methylidene-gamma-lactones and beta-aryl-alpha-methylidene-gamma-lactams. *New J. Chem.* 34, 750–761
88 Albrecht, L. *et al.* (2010) Synthesis and cytotoxic evaluation of beta-alkyl or beta-aryl-delta-methyl-alpha-methylene-delta-lactones. Comparison with the corresponding gamma-lactones. *Eur. J. Med. Chem.* 45, 710–718

89 Janecki, T. *et al.* (2006) 4-Methylideneisoxazolidin-5-ones – a new class of alpha-methylidene-gamma-lactones with high cytostatic activity. *Bioorg. Med. Chem. Lett.* 16, 1430–1433

90 Rozalski, M. *et al.* (2007) Synthesis and biological evaluation of 4-methylideneisoxazolidin-5-ones – a new class of highly cytotoxic alpha-methylidene-gamma-lactones. *Eur. J. Med. Chem.* 42, 248–255

91 Ramachandran, P.V. *et al.* (2010) Tailored alpha-methylene-gamma-butyrolactones and their effects on growth suppression in pancreatic carcinoma cells. *Bioorg. Med. Chem. Lett.* 20, 6620–6623

92 Sohma, I. *et al.* (2011) Parthenolide, an NF-kappaB inhibitor, suppresses tumor growth and enhances response to chemotherapy in gastric cancer. *Cancer Genomics Proteomics* 8, 39–47

93 Zanotto-Filho, A. *et al.* (2011) NFkappaB inhibitors induce cell death in glioblastomas. *Biochem. Pharmacol.* 81, 412–424

94 Hassane, D.C. *et al.* (2010) Chemical genomic screening reveals synergism between parthenolide and inhibitors of the PI-3 kinase and mTOR pathways. *Blood* 116, 5983–5990

95 Steele, A.J. *et al.* (2006) The sesquiterpene lactone parthenolide induces selective apoptosis of B-chronic lymphocytic leukemia cells *in vitro*. *Leukemia* 20, 1073–1079

96 Yip-Schneider, M.T. *et al.* (2005) Parthenolide and sulindac cooperate to mediate growth suppression and inhibit the nuclear factor-kappa B pathway in pancreatic carcinoma cells. *Mol. Cancer Ther.* 4, 587–594

97 Wang, W. *et al.* (2009) A novel combination therapy with arsenic trioxide and parthenolide against pancreatic cancer cells. *Pancreas* 38, e114–e123

98 Yip-Schneider, M.T. *et al.* (2008) Effect of celecoxib and the novel anti-cancer agent, dimethylamino-parthenolide, in a developmental model of pancreatic cancer. *Pancreas* 37, e45–e53

99 Zhangabylov, N.S. *et al.* (2004) Sesquiterpene lactone arglabin influences DNA synthesis in P388 leukemia cells *in vivo*. *Pharm. Chem. J.* 38, 651–653

100 Riggins, R.B. *et al.* (2005) The nuclear factor kappa B inhibitor parthenolide restores ICI 182,780 (Faslodex; fulvestrant)-induced apoptosis in antiestrogen-resistant breast cancer cells. *Mol. Cancer Ther.* 4, 33–41