Saponins in Tumor Therapy

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Abstract: Saponins are plant glycosides with favorable anti-tumorigenic properties. Several saponins inhibit tumor cell growth by cell cycle arrest and apoptosis with IC_{50} values of up to 0.2 μM. We discuss diverse groups of saponins (dioscins, saikosaponins, julibrosides, soy saponins, ginseng saponins and avicins) investigated in relation to tumor therapy and focus on cellular and systemic mechanisms of tumor cell growth inhibition both *in vitro* and *in vivo*. The review also describes saponins in combination with conventional tumor treatment strategies, which result in improved therapeutic success. Some combinations of saponins and anti-tumorigenic drugs induce synergistic effects with potentiated growth inhibition.

Key Words: Saponin, cancer, tumor therapy, cytotoxicity, triterpenoid, steroid, glycoside.

DIVERSE STRUCTURES AND FUNCTIONS OF SAPONINS

Saponins are plant glycosides and possess great diversity in their structure. They are common in a variety of higher plants and usually found in roots, tubers, leaves, blooms or seeds. They contain a steroid, steroid alkaloid or triterpene core structure, the so-called aglycone. The steroidal aglycone consists of 27 C-atoms while the triterpenoidal aglycone has 30 C-atoms. The saponins with a steroid alkaloid as aglycone are similar to those with a steroidal aglycone but contain additional nitrogen atoms within the core structure. The detailed composition of the aglycone varies between different saponins from different sources, however, these variations are less pronounced compared to those within the glycan structures, which are attached to the aglycone. Usually, one or more sugar chains are covalently linked to the core structure. Glucose, galactose, glucuronic acid, xylose or rhamnose are among the sugars commonly found in saponins. However, even in one source the composition of the sugars may vary, resulting in saponins with different glycosylation [1]. Thus the diversity of saponins is a result of differences in the aglycone structure and the amount and composition of the sugar side chains.

Diverse functions have been described for distinct saponins. The effects observed are often specific for certain saponins due to the great variability of their structures. They are known as foaming substances due to the combination of the non-polar aglycone and their water-soluble side chains. This property is of interest for the beverage industry. Furthermore, saponins added to the food of ruminating animals increase growth, milk or wool production by eliminating protozoa, which predate on crucial bacteria in the first stomach (reviewed in [2]). Another prominent effect of saponins is their membrane permeabilizing property [3, 4]. The pore formation is ascribed to an interaction between saponin and membrane-bound cholesterol [5]. The amount of cholesterol in the membrane has been shown to be important for this interaction [6]. Although it has been shown that hemolysis of erythrocyte membranes by certain saponins is inhibited by depletion of cholesterol, for other saponins the effect is augmented [7]. Zhao *et al.* described the formation of cholesterol-saponin complexes for the saponin platycodin D while this saponin did not interact with triglycerides [8]. It was also demonstrated by Hu *et al.* that pore-formation by saponins with two sugar side chains is independent of membrane cholesterol while those without sugars are cholesterol-dependent [9]. The number of side chains influences both hemolytic activity and membrane permeability. Woldemichael *et al.* reported that saponins possessing two side chains induce less activity than those with only one sugar side chain [10]. Taking all information into consideration, the specific effects of saponins may be due to the combination of target membrane composition, the type of the saponin side chain(s) and the nature of the aglycone [1, 11, 12]. These results underline the saponins variability in biological functions due to their different structures. Saponins are often used to permeabilize membranes in order to make intracellular compartments accessible for antibodies [13]. Interestingly Lee *et al.* demonstrated that a certain saponin from ginseng only interacted with the extracellular side of the membrane [14]. In relation to the membrane-interacting functions of saponins Morein *et al.* described saponins as adjuvant additive [15]. The saponin from the bark of the tree *Quillaja saponaria* exhibited improved adjuvant effects in formulated immunostimulating complexes in comparison to simple mixtures of saponin and immunogen [16]. The saponin forms a cage-like structure together with membrane cholesterol, demonstrating that for this saponin cholesterol is essential for its adjuvant function [17].

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Saponins possess in addition many effects on tumor cells. Different cytotoxic properties have been described for a number of saponins promoting their potential as anti-cancer drugs or adjuvants. Analyses of saponins in tumor therapy from the last five years are presented in the following section. Special emphasis is given to those studies describing a mechanistic background of saponin-mediated anti-cancer effects.

SAPONINS AS ANTI-CANCER AGENTS

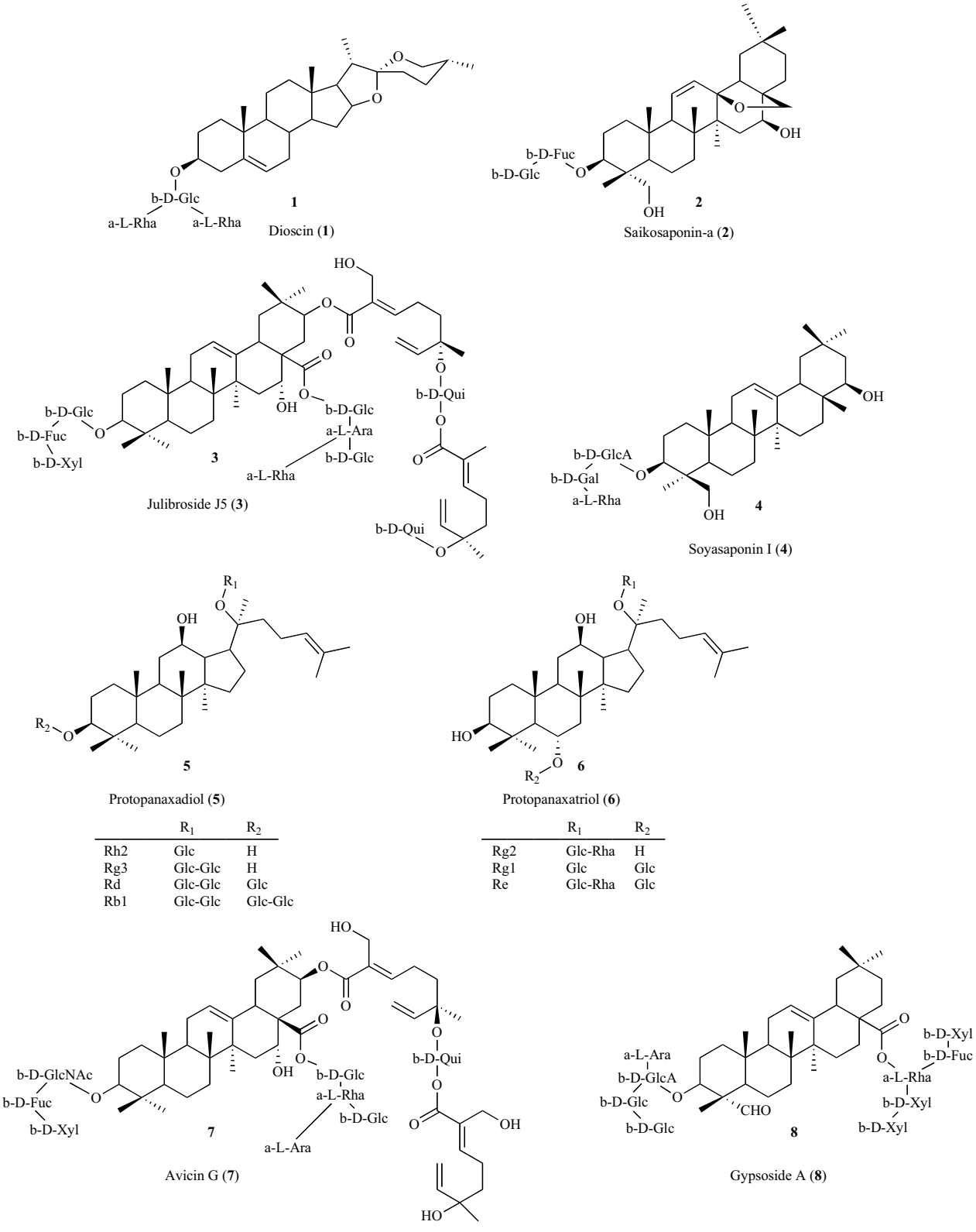
One of the first studies with saponins for the treatment of cancer was described by Ebbesen *et al.* in 1976 [18]. The saponin Quil A prolonged the survival of mice, which developed spontaneous leukemia (Table **1**). While many groups used saponins to permeabilize cellular membranes for microscopic studies, an increased interest in saponins as potential drugs for the treatment of cancer took place in the 1990s. A study investigating the growth inhibitory effect of two saikosaponins and two ginsenosides, saponins from *Panax ginseng*, showed that saikosaponin-a inhibited cell proliferation of human hepatoma cells while saikosaponin-c and the ginsenosides Rb1 and Rg1 had no effect [19]. Yu *et al.* identified a triterpenoidal saponin, tubeimoside 1, from the bulb of *Bolbostemma paniculatum* [20]. This saponin had a potent anti-tumorigenic effect in a mouse skin tumor model. The number of studies on saponins as anti-tumor drugs has increased drastically in the last decade, this review focuses on the studies of the last five years for certain groups of saponins. Most studies were performed *in vitro* with cell culture models, however, the number of mouse studies is increasing. A general problem of several cell culture studies presented in this review is the lack of control experiments to reveal the effects of the saponins on healthy tissue. Therefore one has to be careful when considering a certain saponin whose efficacy was only demonstrated on the basis of its high toxicity on tumor cell lines.

DIOSCINS

Dioscin (Fig. (**1**)) is a steroidal saponin produced by many plants of different genera. In 2002 Cai *et al.* described the cellular mechanisms of dioscin, purified from the root of *Polygonatum zanlanscianense* Pamp., that lead to cell death of tumor cells with a half maximal inhibitory concentration (IC_{50}) of 4.4 μM [21]. In cell culture experiments with HeLa cervix carcinoma cells dioscin induced apoptosis *via* the mitochondrial pathway. A reduction in the expression of the anti-apoptotic protein Bcl-2 together with caspase activation was observed. Dioscin was also isolated from rhizomes of *Smilacina atropurpurea* and induced cytotoxicity on several tumor cell lines with an IC_{50} of 1.9–6.8 μg/ml [22] indicating a high impact on tumor cells. Protodioscin and methyl protodioscin are structurally closely related to dioscin. Protodioscin from fenugreek (*Trigonella foenumgraecum*) induced cell death in the leukemic cell line HL-60 by apoptosis while the gastric cancer cell line KATO III was merely inhibited with no apoptosis observed thus demonstrating cell line-dependent results [23]. The natural derivative of protodioscin, methyl protodioscin, from the rhizome of *Dioscorea collettii var. hypoglauca* of the family *Dioscoreaceae*, inhibited many solid tumors with an $IC_{50} < 10$ μM while leukemia cell lines were relatively insensitive with IC_{50} values of 10–30 μM [24]. The higher cytotoxicity on solid tumors compared to leukemic cells is opposite to the effect of the related protodioscin, for which only one study with two cell lines demonstrated a higher impact on leukemic cells. Thus, further studies are necessary to determine which structural features or cell structures result in these contrasting results. The plant *Dioscorea collettii var. hypoglauca* is a traditional Chinese medicine used for the treatment of certain solid tumors, thus the observed treatment success may be attributed to the action of saponins like methyl protodioscin. Recently the mechanism of growth inhibition was studied, and cell cycle arrest and apoptosis induction observed [25, 26]. The

Table 1. Anti-Tumorigenic Effects of Saponins Observed in Mice

Saponin	Anti-Tumorigenic Effect	Reference
Quil A	prolonged survival in spontaneous leukemia model	[18]
tubeimoside 1	growth inhibitory effect on skin tumors	[20]
soyasaponin I	reduction of lung metastases	[50]
ginsenoside Rh2	reduction of human ovarian tumor cell growth suppressive effect on tumor induction enhanced anti-tumor activity of cisplatin and paclitaxel inhibition of tumor growth in combination with cyclophosphamide	[58] [59] [91, 95] [98]
ginsenoside Rg3	chemo-preventive and anti-mutagenic effect inhibition of tumor growth in combination with cyclophosphamide	[68] [97, 99]
ginsenoside Rp1	prevention and growth inhibition of papillomas; increased activity of detoxifying enzymes	[70]
ginsenoside Rb1	increased radiosensitivity of tumor cells	[102]
mixture of avicins	prevention of mutations after UV radiation protective effects against papilloma-inducing chemicals	[85] [86]
formosanin-C	potentiation of growth inhibitory effect of 5-fluorouracil	[90]



	R_1	R_2
Rh2	Glc	H
Rg3	Glc-Glc	H
Rd	Glc-Glc	Glc
Rb1	Glc-Glc	Glc-Glc

	R_1	R_2
Rg2	Glc-Rha	H
Rg1	Glc	Glc
Re	Glc-Rha	Glc

Fig. (1). Structures of exemplary saponins. Abbreviations for sugars: α-L-arabinose (α-L-Ara), β-D-fucose (β-D-Fuc), β-D-galactose (β-D-Gal), β-D-glucose (β-D-Glc), β-D-glucuronic acid (β-D-GlcA), β-D-N-acetylglucosamine (β-D-GlcNAc), β-D-quinovose (β-D-Qui = 6-deoxy-β-D-Glc), α-L-rhamnose (α-L-Rha), β-D-xylose (β-D-Xyl). Examples for several ginsenosides varying only in the residues R1 and R2 are presented in the tables beneath the structures of protopanaxadiol and -triol.

latter was shown to be a result of upregulation of pro-apoptotic Bax and downregulation of anti-apoptotic Bcl-2 protein in HepG2 liver carcinoma cells and K562 hematopoietic malignant cells (Fig. (**2**)). Three further saponins structurally closely related to dioscin and protodioscin are gracillin, methyl protogracillin and methyl protoneogracillin, which were also isolated from rhizomes of *Dioscorea collettii var. hypoglauca*. These saponins induced cytotoxicity in the μM range on tumor cell lines as observed for methyl protodioscin [27]. However, the cytotoxicities observed on leukemia cell lines were 6 to 11-fold lower for methyl protogracillin compared to its stereoisomer (R/S configuration at C-25) methyl protoneogracillin, emphasizing distinct structural requirements for potent anti-tumorigenic activity.

SAIKOSAPONINS

Saikosaponins are triterpenoidal saponins produced in plants of the genus *Bupleurum* and several derivatives are described for their anti-cancer effects. The Japanese drug saiko (Chinese Chai-hu, 柴胡) contains rhizomes of *Bupleurum kaoi* and serves as source for saikosaponins. The growth-inhibiting potency of saikosaponin-a (Fig. (**1**)) in hepatoma HuH-7 cells was published as early as 1993 by Okita *et al.* while saikosaponin-c did not alter cell proliferation [19]. Surprisingly, the authors stated that growth inhibition by saikosaponin-a was independent of the cell cycle while Wu *et al.* reported in a more extensive study that saikosaponin-a inhibited cell growth of HepG2 hepatoma cells by upregulating gene expression of the cyclin dependent kinase inhibitors p-15^{INK4b} and p-16^{INK4a}, both specific inhibitors of cyclin dependent kinase 4/6 [28]. Furthermore, a link to a possible involvement of the protein kinase C pathway was also described. Wen-Sheng *et al.* reported in 2003 that the mitogen-activated protein kinase 3 (MAPK3, "ERK1") signaling pathway is involved in $p15^{INK4b}$/$p16^{INK4a}$ expression mediated by saikosaponin-a [29] (Fig. (**2**)). MAPK8 ("JNK1") and MAPK14 ("p38") pathways were not altered by saikosaponin-a. A pro-apoptotic effect of saikosaponin-a was described in 2003 in the human breast cancer cell lines MDA-MB-231 and MCF-7 [30]. Of special interest is that the two cell lines exhibited different cell line-specific apoptotic characteristics: Apoptosis in MCF-7 cells was dependent on the activation of p21 inhibitor by p53 whereas in MDA-MB-231 cells this process was independent of p53. The latter displayed an increase in expression of the cyclin-dependent kinase inhibitor p21 and pro-apoptotic Bax as well as activation of caspase 3. In human lung cancer cells Fas-dependent apoptosis induction was observed after treatment with a saponin-enriched fraction from *Bupleurum kaoi*, however, this fraction contained several saikosaponins [31]. Chiang *et al.* analyzed the cytotoxicity of saikosaponin-a, -c and -d on hepatoma cells. While saikosaponin-a and -d reduced cell growth with an IC_{50} of about 10 μg/ml and induced apoptosis by activating the caspases 3 and 7, sai-

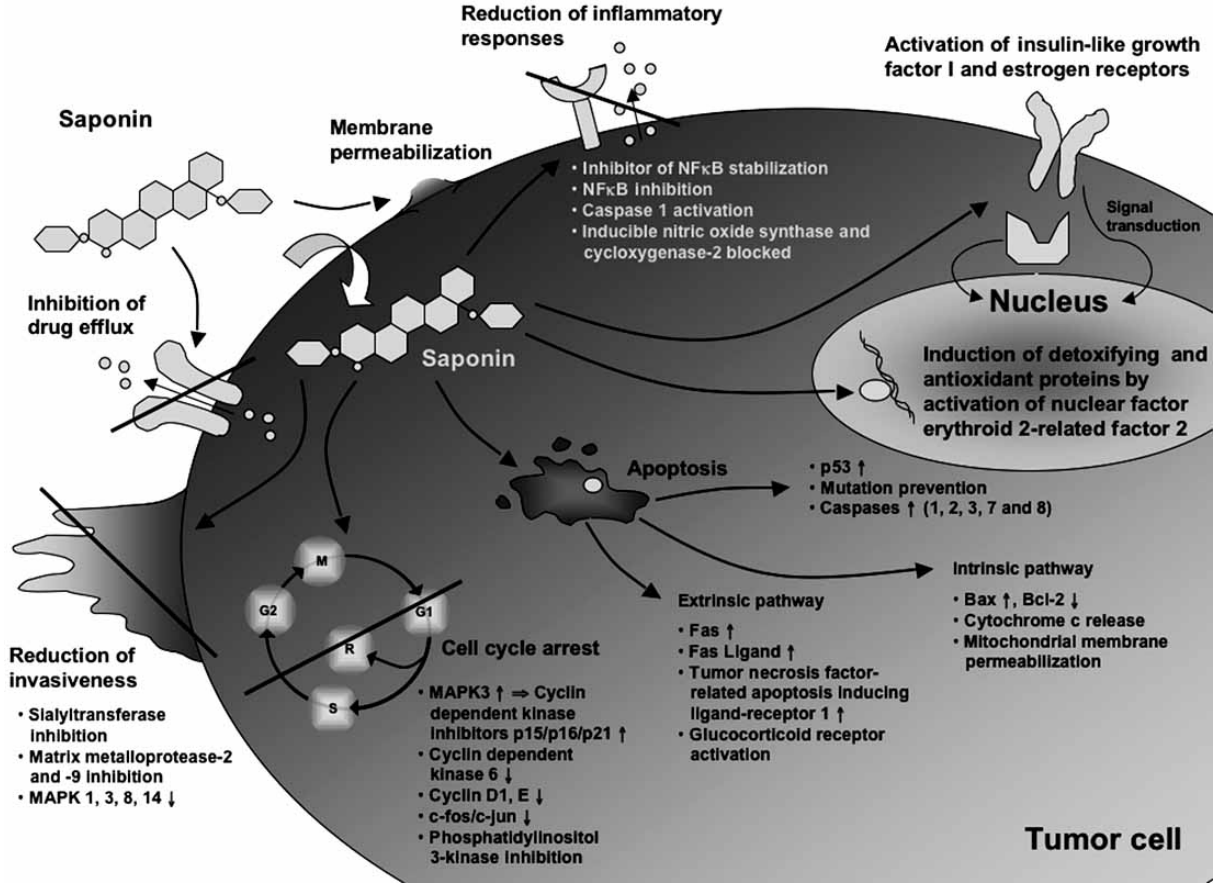


Fig. (2). Cellular effects of saponins. The schematic illustration depicts the different molecular pathways contributing to the anti-tumorigenic properties of various saponins. Note that a number of pathways are observed only for certain cell lines and certain saponins.

kosaponin-c was non-toxic at a concentration of 40 μg/ml [32]. However, saikosaponin-c reduced replication of hepatitis B virus in cell culture. In a further study, saikosaponin-c demonstrated a proliferating effect on endothelial cells and increased the expression of matrix metalloproteinase-2 and vascular endothelial growth factor, rendering this saponin inappropriate for cancer therapy [33]. Saikosaponin-d was intensely studied by Hsu and colleagues in 2004. This saponin induced apoptosis in two hepatoma cell lines and inhibited proliferation with IC_{50} values in the range of 2.6–4.3 μM [34]. In HepG2 cells treatment with saikosaponin-d resulted in accumulation in cell cycle phase G1, increased expression of cyclin-dependent kinase inhibitor p21 by p53 and increased expression of death receptor Fas and Fas ligand (Fig. (**2**)). Induction of apoptosis in Hep3B cells was also observed but cell cycle arrest and p21 upregulation were not detected. The ratio of pro-apoptotic Bax protein to anti-apoptotic Bcl-X_L protein increased in both cell lines, though Bax was only upregulated in HepG2 cells. A similar study on a lung tumor cell line revealed comparable effects of saikosaponin-d: Apoptosis was induced with an IC_{50} of 10.2 μM associated with p53/p21 upregulation, cell cycle arrest and Bax protein elevation [35]. The recent studies described both saikosaponin-a and -d as convenient drugs for tumor therapy by inhibiting cell growth, arresting cell cycle progression and inducing apoptosis. However, *in vivo* studies with different tumors are necessary to finally prove the anti-tumorigenic potential of saikosaponins.

JULIBROSIDES

The triterpenoidal julibrosides are isolated from the stem bark of *Albizia julibrissin*. These saponins contain three sugar side chains each with 2–4 sugar residues. Several derivatives have been tested on tumor cell lines in cell culture. The julibrosides J18 and J19 exhibited cytotoxicity against cervix carcinoma, hepatoma and breast cancer cell lines [36]. The julibrosides J28 and J21 exhibited cytotoxicity on a hepatoma cell line with an IC_{50} of about 10 μM [37] and 10 μg/ml, respectively [38]. The derivatives J5 (Fig. (**1**)), J8, J12 and J13 were analyzed for their cytotoxicity on a hepatocarcinoma cell line. All four saponins exhibited cytotoxicity with IC_{50} values in the range of 10 μg/ml [39]. However, J8 and J13 were most effective in killing the tumor cells since they reduced cell numbers by about 90%. Zheng *et al.* presented a more detailed study on julibroside J8, which inhibited proliferation of three cancer cell lines drastically at 100 μg/ml (46 μM) [40]. Apoptosis was observed in the cervix carcinoma cell line HeLa by induction of DNA fragmentation, upregulation of pro-apoptotic Bax, downregulation of anti-apoptotic Bcl-2 and caspase 3-activation (Fig. (**2**)). The 3 julibrosides J29, J30 and J31 exhibited significant cytotoxicity on three cancer cell lines [41]. At 10 μM all saponins inhibited growth of the cervix carcinoma cell line HeLa more than 60% while the hepatoma carcinoma cell line was inhibited by about 50%. All saponins except J31 were more cytotoxic than the chemotherapeutic drug doxorubicin (Adriamycin™). Cao *et al.* isolated 3 saponins from *Albizia gummifera*, which are structurally closely related to the julibrosides [42]. The saponins were incubated on 5 different tumor cell lines and inhibited cell growth notably with IC_{50} values of less than 1 μM. As for the saikosaponins, relevant *in vivo* data are not available for the julibrosides thus impeding estimation of their true potential.

SOY SAPONINS

Saponins from soy (*Glycine max*) have been intensely studied in recent years due to the great significance of soy on nutrition. Since Kerwin reviewed anti-cancer effects of soy saponins in 2004 [43], we will focus only on the latest publications on soy saponins. Most studies on soy saponins have been performed on human colon cancer cells, since soy saponins are expected to influence primarily the gastrointestinal system after they are taken up as part of the diet. Kim *et al.* analyzed a crude extract of soybean containing different saponins on human colon cancer cells [44]. The saponins inhibited cell growth and reduced inflammatory responses by mediating increased inhibition of the transcription factor nuclear factor-kappa B (NFκB), which mediates expression of inflammatory proteins. These effects are the result of interference with the degradation of the inhibitor of NFκB, IκBα (Fig. (**2**)). Thus, soy saponins may present a different mechanism for anti-tumorigenic effects compared to the saponins described above. Studies with crude saponin extracts may reveal interesting results but should be considered rather preliminary. However, it is also possible that components of the crude extract interact synergistically and thus induce effects not observed for pure saponins. Therefore investigations with purified saponins are indispensable for matching a result to the molecular action of a specific saponin. In a more detailed study with purified saponins, only the aglycones soyasapogenin A and B were potent growth inhibitors of colon cancer cell lines, while the acetylated and glycosylated variants, including soyasaponin I (Fig. (**1**)) and soyasaponin III, were inactive [45]. Thus, deglycosylation of soyasaponins by intestinal bacteria in the digestive tract may help to generate potent anti-cancer drugs. While the report of Oh *et al.* supports the hypothesis that soy saponins do not induce apoptosis [46], Yanamandra *et al.* described apoptosis induction and reduced invasiveness due to the effect of the group B saponins from soy, a mixture of 4 triterpenoidal saponins possessing the same aglycone but different glycosylations [47]. However, in relation to other studies very high amounts of saponin (up to 75 μg/ml) were needed to induce the observed effects and even under these conditions activation of the caspases 3 and 9 was weak. In a further study it was shown that group B saponins induced cell cycle arrest and macroautophagy in human colon cancer cells [48]. A significant increase in autophagic vacuoles was detected by incorporation of monodansylcadaverine. Recently, the MAPK1 ("ERK2") and MAPK3 signaling pathways were identified in this process of macrophage induction [49]. Soyasaponin I from the group B saponins was described as an inhibitor of sialyltransferases, enzymes responsible for hypersialylation of cell surface proteins and are associated with highly metastatic tumors. In an *in vivo* model, soyasaponin I treatment of metastatic tumor cells drastically reduced the amount of metastases found in the lungs of mice [50] (Table **1**). The inhibition of invasiveness may block the formation of metastases and is thus very important in tumor therapy. In accordance with the previous results is the outcome of an investigation by Hsu *et al.*, who found soyasaponin I decreased cell migration thus helping to

avoid metastases [51]. However, the saponin was unable to inhibit tumor cell proliferation and the cell cycle. Jun *et al.* described in an additional study a cancer protective property for soy saponins preventing DNA mutations and attachment of DNA mutagens [52]. The protective properties of soy saponins, as well as their ability to block metastases, makes them very promising drugs for tumor therapies.

GINSENG SAPONINS

The saponins produced by *Panax ginseng C. A. Meyer* (Korean ginseng, 인삼) and *Panax notoginseng* (Chinese ginseng, 三七) are called ginsenosides (Fig. (**1**)) and were analyzed in various studies to explore their anti-carcinogenic potential. The root of ginseng is a very well known drug in Korea and China for several diseases including cancer (reviewed in [53]). The ginsenosides contain a steroidal dammarane aglycone, which can either be a protopanaxadiol or a protopanaxatriol. Dammarane aglycones are found together with ginsenosides in the roots but they are also generated in the intestinal tract after saponin uptake. *Panax ginseng* produces several saponins and Wang *et al.* were able to identify 11 dammarane aglycones compounds and saponins in a preparation from the fruits [1]. All of these compounds were analyzed for their impact on apoptosis, proliferation and cell cycle progression. Of seven ginsenosides tested (Rh2, Rg1, Rg2, Rg3, Rd, Re and Rb1) only Rh2 was cytotoxic on several tumor cells with an IC_{50} in the range of 20–70 μM. However, in comparison with saponins from other groups the cytotoxicities are rather low. Two dammarane aglycones showed similar cytotoxicity while all other compounds were non-toxic. Annexin and propidium iodide staining revealed induction of apoptosis was strongest for the protopanaxadiol. The effect of the aglycones and ginsenosides on cell cycle arrest was rather small. The ginsenoside Rh2 was the focus of further studies and induction of apoptosis, cell cycle arrest and inhibition of cell growth was detected on many different tumor cell lines. Kim *et al.* studied Rh2 on a neuroblastoma cell line and demonstrated activation of caspases 1 and 3, increased expression of Bax (Bcl-2 remained unaltered) and activation of p53 [54] (Fig. (**2**)). In 2004, the ginsenosides Rh2 and Rg3 were identified as most potent among 11 ginsenosides to inhibit the growth of two prostate cancer cell lines with IC_{50} values of 4–14 μM [55]. In the study from Wang *et al.* described above, the authors reported lower IC_{50} values for the same ginsenosides on the same cell lines; especially for Rg3, which showed a 21 to 32-fold lower IC_{50} value [1], however, Kim *et al.* determined the inhibition of protein synthesis while Wang *et al.* detected growth inhibition. Kim *et al.* reported that both saponins induced cell detachment, which is possibly part of an apoptotic pathway. Cheng *et al.* described the induction of apoptosis by Rh2 on the human lung adenocarcinoma cell line A549 [56] that was later also analyzed by Wang *et al.* with comparable results. A more in depth study into the induction of apoptosis revealed the activation of the caspases 2, 3 and 8, cell cycle arrest, downregulation of cyclins D1, E and cyclin dependent kinase 6 and upregulation of the death receptor and tumor necrosis factor-related apoptosis-inducing ligand-receptor 1 (TRAIL-R I), which possibly plays a key role in initiating apoptosis. Jia *et al.* described the apoptosis induced by Rh2 to be mediated by glucocorticoid receptor activation [57]. As the growth inhibiting properties of Rh2 were promising, it was tested in an *in vivo* model. While Rh2 was able to reduce growth of human ovarian tumor cells in mice more effectively than cisplatin [58] and a suppressive effect on tumor induction was also shown [59] (Table **1**), the ginsenoside enhanced the metastatic potential of tumor cells in an *in vitro* assay rendering its potential use in human tumor therapy problematic [59]. Popovich *et al.* studied the ginsenosides Rh1, Rh2, Rg3, a protopanaxadiol and -triol aglycone on human leukemic cells [60]. Rh2 and the two aglycones showed high activity with IC_{50} values of about 15 μg/ml, while Rg3 and Rh1 had low cytotoxic activity with IC_{50} values greater than 200 μg/ml. Thus, Rh2 causes comparable cytotoxicities both on leukemia cells and solid tumor cells. Since Rh1 seems to be unable to inhibit tumor cell growth, other properties of the ginsenoside were investigated. Lee *et al.* identified Rh1 as a potential but weak ligand for estrogen receptors, however the binding to the receptor was specific and induced a functional signal [61] (Fig. (**2**)). Comparable results were published for ginsenoside Rg1, which stimulates growth of an estrogen receptor-positive human cell line [62]. A direct interaction between Rg1 and the estrogen receptors does not occur and the effect of the ginsenoside seems to be mediated by stimulation of insulin-like growth factor I receptor and intracellular cross talk with estrogen receptor signaling pathways [63]. While ginsenoside Rg3 exhibited moderate toxicity in the studies presented before [1, 60] other anti-carcinogenic effects were reported. Rg3 inhibited partly papilloma formation after treatment with the phorbol ester phorbol 12-myristate 13-acetate by inhibition of NFκB [64]. The ginseng root used in this study was steamed prior to preparation, a process known to increase the activity of ginseng root extracts and leads to the production of Rg3 and Rg5 [65]. Wang *et al.* also showed the production of Rg3 and Rg5 after steaming berries of *Panax ginseng* and reported an increased cytotoxic effect of the extract [66]. Therefore the assumption is acceptable that at least one of these saponins mediates increased growth inhibition. The main ginsenoside produced was Rg3, which showed moderate growth inhibition on human tumor cells with an IC_{50} value of about 150 μM (120 μg/ml). This result is different to the result from Kim *et al.* [55], who reported a high cytotoxicity of Rg3 but measured, as stated above, protein synthesis inhibition in contrast to growth inhibition detected by Wang *et al.* who, moreover, worked only with enriched saponin extracts with lower Rg3 content. Two ginsenosides present before steaming, Rb3 and Re, were not cytotoxic at concentrations up to 1000 μM. Kim *et al.* reported that Rg3 was able to reverse drug resistance of tumor cells [67]. A concentration of 320 μM of Rg3 inhibited efflux of vinblastine and rhodamine from multidrug resistant cells, however using Rg3 at a concentration high above the IC_{50} value will make it difficult to determine whether the effects are due to the saponin or the drug. The inhibition on drug efflux was furthermore described for doxorubicin-resistant tumor cells in mice. Panwar *et al.* described a chemo-preventive and anti-mutagenic effect of Rg3 in mice although Rg3 comprised only about 3% (w/w) of the tested saponin mixture [68] (Table **1**). A derivative of Rb3 mediated moderate cytotoxicity (IC_{50} 50–60 μg/ml) on human tumor cells [69], the ginsenoside Rp1 prevented development and growth of pa-

pillomas in mice after oral application and increased the activity of detoxifying enzymes [70] (Table **1**), Rd inhibited cell growth (IC_{50} 150 μg/ml) and induced apoptosis in human cervix carcinoma cells [71] but its cytotoxicity is weak compared to other saponins and apoptosis was only observed at concentrations considerably above the IC_{50}. The ginsenoside Rb2 inhibited invasiveness of human tumor cells by suppressing matrix metalloproteinase-2 activity [72] (Fig. (**2**)), however, this inhibition was not mediated by tissue inhibitors of metalloproteinases [73]. The same result was obtained for 20(S)-protopanaxadiol, which effected this inhibition at concentrations much lower than the IC_{50} value of 76 μM. Inhibition of matrix metalloproteinase-9 by another protopanaxadiol, named compound K, was demonstrated to be a result of reduced protein expression and appears to be mediated through repressing the kinases MAPK1, MAPK3, MAPK8 and MAPK14 in human glioma cells [74] (Fig. (**2**)). An influence on signal transduction by protopanaxadiols and -triols was also shown by Han *et al.*, who observed inhibition of epidermal growth factor-mediated cell proliferation with concomitant repression of c-fos and c-jun gene expression [75] (Fig. (**2**)). Notably, effects were much stronger for a total extract of ginsenosides, demonstrating that the extract may contain more potent saponins or exhibit synergistic effects. The often described effects of saponins on apoptosis-induction were also observed together with caspase activation and cytochrome c release [76, 77], as well as cell cycle arrest and inhibition of DNA synthesis with an IC_{50} of about 1 μM [78]. A multidrug resistance-reversal effect was also described for a protopanaxatriol [79], however the effect was rather small compared to the impact of Rg3 described above [67]. Two ginsenoside metabolites inhibited furthermore inflammatory processes by inactivating NFκB leading to blocked effects of inducible nitric oxide synthase and cyclooxygenase-2 [80, 81] (Fig. (**2**)). Numerous effects on tumor cells have been described for ginsenosides and even more results will be revealed in the next years. While the growth inhibiting effects of ginsenosides are weaker than for other saponins, they were used to inhibit the mobility of tumor cells and may therefore be used in combination with other saponins.

AVICINS

In 2001 Haridas *et al.* identified triterpenoidal saponins from *Acacia victoriae*, an Australian tree. These saponins were named avicins and contain three sugar side chains. Avicins D and G (Fig. (**1**)) induced growth inhibition of human T lymphocytes at very low concentrations of 0.3 and 0.2 μg/ml, respectively, and promoted apoptosis as shown by activation of caspases and cytochrome c release [82] (Fig. (**2**)). The avicins are thus currently the most potent growth inhibiting saponins described. The impact on other human tumor cell lines was lower, however they still exhibited good IC_{50} values in the range of 1–2.5 μM [83]. In this context, inhibition of phosphatidylinositol 3-kinase activity especially for avicin G was reported, while the MAPK pathway seemed to be unaffected. Avicin G inhibited furthermore the activation and DNA binding of NFκB [84]. This avicin reduced the expression of inducible nitric oxide synthase and cyclooxygenase-2 as described for ginsenosides metabolites. Avicin D induced the expression of nuclear factor erythroid 2-related factor 2 (Fig. (**2**)), a transcription factor, which mediates the expression of several detoxifying and antioxidant proteins [85] (Table **1**). The protective effect was underlined by UV radiation of mice treated with avicin D, where severe damage and mutations were prevented. A mixture of avicins showed additional protective effects against papilloma-inducing chemicals 7,12-dimethylbenzanthracene and phorbol 12-myristate 13-acetate in mice and reduced the number of mutations in the oncogene H-*ras* [86] (Table **1**). The pore forming ability of the avicins D and G at concentrations of 25 μg/ml was demonstrated by Li *et al.* [87]. While pore formation was strongly cholesterol dependent for avicin G, this was not so for the close derivate avicin D. The non-selective pores are too small for proteins but allow ion flux. The authors concluded that the pores might influence the membrane potential of mitochondria. The direct influence on mitochondria was corroborated by studies on rat mitochondria, where both avicin D and G induced permeabilization leading to decreased respiratory activity [88] and ATP efflux after inhibition of the voltage dependent anion channel in the outer mitochondrial membrane [89]. This is possibly the main reason for apoptosis induction by avicins and it is likely that further saponins induce pore formation in mitochondrial membranes to induce apoptosis (Fig. (**2**)).

KNOWLEDGE, GAPS AND FUTURE

The various cellular effects of saponins described above are summarized in a schematic illustration (Fig. (**2**)). Saponins can either act extracellularly on tumor cells or influence intracellular pathways. Among the extracellular effects of saponins are plasma membrane permeabilization and inhibition of drug efflux by direct inhibition of membrane proteins. Permeabilizing effects occur at high saponin concentrations (usually > 100 μg/ml with variations for different saponins) while intracellular effects may also take place at lower concentrations. Cell cycle arrest and apoptosis induction are the best studied events. These outcomes by saponin action were shown for nearly all saponins described in this review. Other intracellular effects like the inhibition of invasiveness for metastasizing cells, the reduction of inflammatory responses, the activation of insulin-like growth factor I and estrogen receptors, and the induction of detoxifying and antioxidant proteins were only observed in a few studies and the relevance for anti-tumor therapy remains to be cleared. The most effective saponins are the avicins, which inhibit tumor cell growth at about 1 μg/ml. Due to their high impact on tumor cells, their intracellular effects were studied in great detail, especially the mechanisms leading to induction of apoptosis. However, it is notable that all described effects of saponins on tumor cells are dependent on the type of tumor and the saponin. Some activated pathways have as yet only observed for specific saponins such as the stimulation of insulin-like growth factor I receptor described for the ginsenoside Rg1, while induction of apoptosis is a common feature of nearly all saponins. More detailed studies for the diverse saponins will surely discover a higher degree of analog mechanisms for structurally related saponins. Nevertheless, the focus of future research on saponins for tumor therapy will remain the discovery of new saponins from diverse plants and the analyses of structure-activity relationships to allow identification

of important structural components for the development of optimized synthetic saponins.

COMBINATIONS OF SAPONINS AND OTHER ANTI-CANCER AGENTS

The combined application of saponins with other anti-tumor drugs offers an interesting development in cancer treatment since in many reports additive or even synergistic effects between saponins and other drugs have been observed. These combinations will lead to essentially improved possibilities for the treatment of cancer. When intensified studies on saponins and their effect on cancer cells started in the 1990s, the first experiments with combinations of saponins and chemotherapeutics were initiated. Wu *et al.* reported in 1990 that formosanin-C, a saponin from *Paris formosana*, did not only increase natural killer cell activity and interferon production at a concentration of 2.5 mg/kg but potentiated additionally the growth inhibitory effect of 5-fluorouracil in mice [90] (Table **1**). When the ginsenoside Rh2 was used for treatment it did not inhibit the growth of human ovarian tumor cells in nude mice. However, a combined application with cisplatin resulted in an enhanced anti-tumor activity as a result of a synergistic action [91] (Table **1**).

Several approaches for the combined application of saponins and chemotherapeutics were investigated with the goal of achieving additional benefit of anti-tumorigenic effects on tumor cells. The well-known drug cisplatin was used in combination with different saponins. Gaidi *et al.* combined this chemotherapeutic with four triterpene jenisseensosides from *Silene* species and another triterpene saponin from *Achyranthes bidentata* [92]. While all jenisseensosides elevated the cytotoxicity of cisplatin synergistically, the *Achyranthes* saponin did not and in addition was non-toxic by itself on the colon cancer cell line used in the study. The authors concluded that the *p*-methoxycinnamoyl groups naturally present in the jenisseensosides where responsible for the enhancing potential, however, there are several further structural differences between the saponins that may be responsible for enhancing cisplatin activity. A study by Haddad *et al.* examined saponins isolated from *Albizia adianthifolia* and no potentiation of cisplatin cytotoxicity on human colon cancer cells was observed [93]. A further study with four saponins from the roots of *Muraltia heisteria* revealed that only the stereoisomeric compounds 3 and 4 inhibited tumor cell growth and none were able to enhance the effect of cisplatin [94]. Thus only certain saponins seem to be able to enhance cisplatin activity due to their specific structure.

A number of further chemotherapeutics were combined with saponins to enhance the anti-tumorigenic impact. The ginsenosides described above were successfully used in many studies with chemotherapeutics. Ginsenoside Rh2 potentiated the cytotoxicity of paclitaxel (Taxol™) *in vitro* [57] and *in vivo* [95] (Table **1**). The chemotherapeutic mitoxantrone (Novantrone™) also acted synergistically with Rh2 *in vitro* but the combination failed to reduce tumor growth *in vivo* [95]. The ginsenosides Rg1, Rg3, Rh1 and Rh2 and the aglycones protopanaxadiol and -triol were studied in combination with mitoxantrone and doxorubicin on drug insensitive cell lines [96]. While the enhancement of doxorubicin cytotoxicity was weak and maximal for protopanaxadiol and -triol, mitoxantrone cytotoxicity was enhanced 27 to 82-fold by the aglycones and Rh2 at a concentration of 20 μM. At high concentrations of 100 μM, the other ginsenosides enhanced mitoxantrone cytotoxicity only 2.5-fold. On tumor cells without upregulated breast cancer resistance protein all ginsenosides and aglycones were unable to increase mitoxantrone cytotoxicity, thus underlining the effect on multidrug resistance related proteins. The ginsenosides Rg3 and Rh2 were recently applied together *in vivo* in mice with the alkylating agent cyclophosphamide and inhibited tumor growth synergistically [97, 98] (Table **1**). In a further study Rg3 in combination with cyclophosphamide showed only an additive effect of both substances, possibly due to the shorter treatment regimen (only 10 days compared to > 50 days) and the lower dose of Rg3 (3 mg/kg compared to 10 mg/kg) [99].

Chemical derivatives of diosgenyl saponins were analyzed on primary B cell chronic leukemia tumor cells in combination with the cytostatic drug cladribine (Leustatin™) and enhanced the toxic effect of cladribine only slightly [100]. Besides the enhanced saponin-chemotherapeutic-mediated cell death, a combination of saponins and radiotherapy was described by Chen *et al.* [101]. An extract of *Panax notoginseng* and ginsenoside Rb1 increased radiosensitivity of tumor cells in mice (Table **1**). The saponins had to be injected 30 minutes prior to radiation to achieve maximal tumor cell growth inhibition. Rb1 was 100-fold more effective than the saponin extract without mediating dose-dependent toxicity to the bone marrow and thus presents an encouraging approach.

Heisler *et al.* examined the combination of a saponin extract from *Gypsophila paniculata*, named Saponinum album, and a targeted chimeric toxin composed of human epidermal growth factor and the plant ribosome-inactivating protein saporin [102]. Saponinum album consists mainly of the saponin gypsoside A (30%) and further saponins with the identical aglycone (40%). The combination of Saponinum album at a non-permeabilizing and non-toxic concentration of 1.5 μg/ml with the chimeric toxin enhanced cytotoxicity more than 3500-fold [103]. Furthermore, the highest increase in cytotoxicity of the chimeric toxin was observed on epidermal growth factor receptor expressing cells, thereby increasing the therapeutic window of the chimeric toxin. In relation to these observations further saponins from diverse plants and with different structures were analyzed for their ability to increase the cytotoxicity of chimeric toxins [12]. Among the saponins examined only Saponinum album and quillajasaponin increased the cytotoxicity of the chimeric toxin more than 1000-fold. Gypsoside A (Fig. (**1**)), the main component of Saponinum album, and quillajasaponin are both triterpene saponins with an aldehyde function at position C-4 that seems to be important for the synergistic action. Notably, only Saponinum album increased the specificity of the chimeric toxin for target cells, while quillajasaponin enhanced the impact of the chimeric toxin on both non-targeted and targeted cells similarly. The enhancement of cytotoxicity of several protein toxins was reported by Hebestreit *et al.* with maximal increase for the type I ribosome-inactivating proteins saporin and agrostin [104]. The combination of Saponinum album and chimeric toxins is to date the most powerful combination of saponins and anti-tumor drugs and

presents a very strong synergism with a currently unknown mechanism.

CONCLUSION

The number of newly isolated and described saponins is increasing constantly and many further saponins will be identified due to improved methods of purification and detection. The majority of the new saponins as well as several well-known saponins possess impressive anti-cancer effects and might help to develop improved anti-cancer regimens. The avicins have currently the highest impact on cancer cells with IC_{50} values for growth inhibition in the range of 1 µg/ml. Almost all saponins induce apoptosis in tumor cells, they are preferable drugs for the treatment of cancer, because eliminating tumor cells by apoptosis is helpful to lower side effects in patients by avoiding necrosis. Other actions like the inhibition of invasiveness as mediated by ginsenoside Rb2 and the group B soy saponins is furthermore valuable in order to prevent the development of metastases. The chemical modification of saponins might be a way to further increase their activity. However, a good understanding of structure-activity relationships is a prerequisite for modifications and currently not well established. Individual studies have shown relevance of the stereochemistry of methyl protoneogracillin or higher activity for deglycosylated derivatives of soy saponin but it is impossible to determine which functional groups within a saponin would result in the highest impact on tumor cells since currently only a few studies have examined structure-activity relationships. Furthermore, no standardized experimental procedures including tumor cells and incubation time have been used to allow quantitative comparisons of different saponins. The low content of a certain saponin in an analyzed sample is a further obstacle for detecting anti-tumorigenic effects. Special attention should be given to combinations of saponins and other anti-carcinogenic drugs, since these offer very efficient treatment regimens against cancer. Most important is the saponin-mediated potentiation of tumor growth inhibition and the possibility to circumvent drug resistance. The elucidation of structure-activity relationships between different saponins in combination with conventional drugs is much more complicated than for saponins alone. Thus, it is not surprising that no mechanistic processes for these effects are known, however, detailed information on this basis is necessary for a directed improvement of saponin-based tumor therapies in the future.

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ABBREVIATIONS

IC_{50} = Half maximal inhibitory concentration

MAPK = Mitogen-activated protein kinase

NFκB = Nuclear factor-kappa B

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