Pharmacological activities of natural triterpenoids and their therapeutic implications†

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This article reviews the pharmacological activity of naturally occurring triterpenoids excluding the degraded triterpenoids and cucurbitacins which have been reviewed recently. The therapeutic activities discussed include anticancer, anti-inflammatory, antiulcerogenic, antimicrobial and antiviral activity. 227 references are cited.

1 Introduction

To date the terpene group has been identified in approximately 30 000 identified compounds. Terpenes can be divided according to the number of structural molecules of isoprenes into mono-, sesqui-, di-, sester-, tri-, tetra-, and poly-terpenes and, in association with sterols, these create extensive groups of isoprenoids. Over the past decade, coming to the foreground of interest due to their diverse biological effects are a large group of cyclic triterpenoid-like substances which comprise more than 4000 different identified compounds so far: free triterpenoids, triterpenic glycosides (saponins), phytosterols and/or their precursors. These aside, there is another group of plant steroidal saponins structurally related to cholesterol and steroid hormones but without their hormonal effects.

The triterpenoids have a range of unique and potentially usable biological effects and reference to the use of plants with high saponin/triterpenoid content can be found in the first written herbariums. Here alluded to are plants (*Panax ginseng, Ganoderma lucidum*, *Platycodon grandiflorum*, Indian frankincense-resin from the tree *Boswellia serrata*) that were highly prized as panaceas *par excellence* due to their wide-ranging effects. Triterpenoids are also found in a variety of common European plants and fruits.[1–5] Since 1985 Connolly and Hill have regularly reviewed the scientific literature dealing with triterpenoid isolation and identification from such natural sources.[6]

From a biological point of view, the most important triterpenoid structures are oleanane, ursane, lupane and dammarane-euphane triterpenoids. Evidence for the intense interest in these related substances is the number of citations in the database Pubmed. These exceed 154 067 for the keyword terpenes and 8054 for triterpenes, as of January 13, 2006. Triterpenoids are studied for their anti-inflammatory, hepatoprotective, analgesic, antimicrobial, antimycotic, virostatic, immunomodulatory and tonic effects. They are used in the prevention and treatment of hepatitis, parasitic and protozoal infections and above all, for their cytostatic effects. The disadvantage of using triterpenoids, in fact, is the toxicity associated with their haemolytic and cytostatic properties. Hand in hand with ongoing extraction and isolation of natural material therefore, is the development of synthetic derivates with lower toxic and higher therapeutic potential.

2 Anticancer effects of triterpenoids

2.1 The lupane group

Betulinic acid (1a). As early as 1976 Trumbull and co-workers[7] published work describing the cytotoxic activities of an extract of *Vauquelinia corymbosa* on the lymphocytic leukaemia cell line P-388. Uvaol (**3e**), ursolic acid (**3a**) and betulinic acid (3β-hydroxylup-20(29)-en-28-oic acid, **1a**) were identified as effective

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Jan Sarek was born in Ostrava, Czech Republic in 1974. He obtained his MSc degree in organic chemistry in 1997 and his PhD degree (2002) from Charles University. He has been director of the natural products group at the department of Organic and Nuclear Chemistry at Charles University since 2003. His main interest is research into terpenoids with biological activity. He is the author of more than 10 publications and 4 international patents. In 2005, he was awarded a Dean's prize for exceptional results in transferring the outcome of research to a commercial application; some of the synthesised betulinines are in preclinical trials as potential cytostatics.

Petr Dzubak

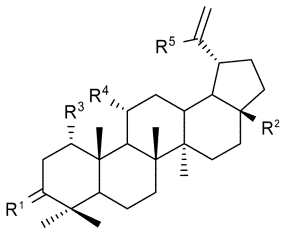
Marian Hajduch

Jan Sarek

substances. A series of publications by other researchers followed, which elucidated the effects of similar extract mixtures, containing betulin (**1h**) and acid **1a**.[8,9] However, it was only at the beginning of the 1990s that the cytostatic effects of acid **1a** were verified.[10] In 1995, Pisha *et al.*[11] published a key paper reporting the cytotoxic effects of acid **1a** on the human melanoma cell line. This caused a wave of interest in acid **1a** and its derivatives. According to their findings the IC_{50} of acid **1a** on human melanoma cell lines MEL-1, 2 and 4, ranged from 0.5–1.6 μg ml^{-1}. At the same time, dose dependent fragmentation of DNA with induction of apoptosis 24 hours after administration occurred. Eventually, effects were also found for neuroblastoma cell lines with IC_{50} = 14–17 μg ml^{-1} (ref. 12) and, according to more recent work,[13] the spectrum of sensitive lines has enlarged to include cell lines of non-neuroectodermal origin, regardless of p53 mutation: ovarian A2780, OVCAR-5, GROV-1, large cell lung cancer H460, epidermoid carcinoma A431, melanoma Me665/2/21, Me665/2/60, small cell lung cancer POGB and POGB/DX (doxorubicin resistant). The IC_{50} ranged from 1.5–4.5 μg ml^{-1}. In the same study, a control was followed on non-tumor human cell lines showing the lower toxicity of acid **1a** to HDFC (normal dermal fibroblasts), IC_{50} = 10.2 ± 1.5 μg ml^{-1}, and PBL (peripheral blood lymphocytes), IC_{50} = 50 μg ml^{-1}, in comparison with doxorubicin (HDFC $IC_{50} = 0.38\,\mu g\,ml^{-1}$; PBL $IC_{50} = 0.02\,\mu g\,ml^{-1}$). Differentiation of normal human keratinocytes into corneocytes was observed after treatment with subcytotoxic concentrations of acid **1a**.[14] In other *in vitro* testing on primary cancer cells isolated from glioblastoma multiforme, betulinic acid (**1a**) was markedly more effective than cisplatin, doxorubicin, vincristin and radiation.[15] The synthesis and cytotoxic behaviour of 3β-acetoxylupane derivates with oxidative modification on the E ring have also been described. The most potent of these has a value of $IC_{50} = 0.2$ μM. It is intriguing that they are not only effective against the majority of tested cancer cell lines including cells which are p53 mutated and/or pRb negative, regardless of histogenetic origin, they are also less toxic to non-cancer cells.[16] Cytotoxic effects are also described for the phthalates, esters, hydroxy and A seco derivatives of betulinic acid (**1a**).[17–20] The synthesis and cytotoxic activity of the 3-*O*-acyl, 3-hydrazone, 2-bromo, 20,29-dibromo derivates and antiangiogenic activity of the 3-*O*-acyl, 3-benzylidene, 3-hydrazone, 3-hydrazine, 17-carboxyacroyl esters of betulinic acid (**1a**) have been described and a number of these compounds, which have been tested against cancer cells, have an $IC_{50} < 1$ μg ml^{-1}.[21–23]

Further derivatives can also be produced by biotransformation using bacteria (*B. megaterium*, ATCC13368).[24] Some derivatives produced in this way 3-oxolup-20(29)-en-28-oic acid (**1b**), 11α-hydroxy-3-oxolup-20(29)-en-28-oic acid (**1c**) and 1α-hydroxy-3-oxolup-20(29)-en-28-oic acid (**1d**) show an ED_{50} against line MEL-2 in lower concentrations than ED_{50} for acid **1a**, even as low as 0.1 μg ml^{-1}. The bacterial model of acid **1a** transformation with the aid of cytochrome P450, helps us to understand the

metabolism of these compounds in mammalian organisms. This study[24] underlined the limited value of *in vitro* experimentation and the importance of metabolic studies for better comprehension of biological effects *in vivo.*



1a,	R^1= β–OH,H;	R^2= COOH;	R^3= H;	R^4= H;	R^5= CH_3
1b,	R^1= O;	R^2= COOH;	R^3= H;	R^4= H;	R^5= CH_3
1c,	R^1= O;	R^2= COOH;	R^3= H;	R^4= OH;	R^5= CH_3
1d,	R^1= O;	R^2= COOH;	R^3= OH;	R^4= H;	R^5= CH_3
1e,	R^1= β–OH,H;	R^2= CH_3;	R^3= H;	R^4= H;	R^5= CH_3
1f,	R^1= O;	R^2= CH_3;	R^3= H;	R^4= H;	R^5= CHO
1g,	R^1= O;	R^2= CH_2OH;	R^3= H;	R^4= H;	R^5= CH_3
1h,	R^1= β–OH,H;	R^2= CH_2OH;	R^3= H;	R^4= H;	R^5= CH_3
1i,	R^1= β–OAc,H;	R^2= CH_2OAc;	R^3= H;	R^4= H;	R^5= CH_3

Our current knowledge of the apoptosis induced by acid **1a** and its derivatives is that it is mediated by increasing permeability of the mitochondrial membrane. This disturbs the transmembrane potential, causing the release of cytochrome c and AIF (apoptosis inducing factor), with the resulting activation of caspases 3 and 8. Subsequent cleavage of the substrates and nuclear fragmentation occur.[25] Inhibition of mitochondrial membrane permeability by enhanced expression of Bcl-2 or Bcl-Xl proteins suppresses activation of caspases and other markers of apoptosis on cell lines of neuroectodermal origin.[26] The importance of the mitochondrial pathway in induction of apoptosis by acid **1a** has also been described in studies on plant cells.[27]

After treatment with acid **1a** (24 hours) cytotoxic effects against cell lines of human glioma were determined; IC_{50} = 20 μM for LN-229, 25 μM for U87, MG and T98G, 70 μM for LN-18, and 100 μM for LN-308. At the same time, an increased level of the proapoptotic protein Bax was observed. Other alterations comprise induction of reactive oxygen species (ROS), which are essential for activation of caspase 3.[28] On the other hand, clones of these cell lines expressing high levels of Bcl-2, showed reduced ROS, without activation of acid **1a** induced apoptosis. This finding agrees with the fact that the antiapoptotic properties of Bcl-2 are connected with its antioxidative mechanism. This underlines the importance of producing ROS in the induction of apoptosis by acid **1a**. In connection with this, the protective effect of α-DL-tocopherol in betulinic acid (**1a**) induced apoptosis in the SK-N-MC cell line was shown.[29] Mapping the activation pathways failed to reveal enhanced production of protein p53, which is a direct transactivator of the bax gene,[30] although the level of protein p21 (natural cycline dependent kinase inhibitor) was increased. Nevertheless, Rieber and Strasberg Rieber[31] describe an enhanced ratio of p53 : p21 after treatment with acid **1a** in cell lines of metastatic (C8161) and non-metastatic (C8161/neo 6,3) human melanoma. These observations suggest, that acid **1a** cytotoxicity must involve mechanisms affecting expression of the bax gene. Analysis of the cell cycle in lines T98G and LN-229 failed to reveal any changes due to acid **1a**, despite raised levels of p21. The authors ascribe this to damage at the Rb cell cycle checkpoint as a result of homozygous deletion of p16 in both cell lines.[28] The antiproliferative effects were observed to be closely associated with inhibition of topoisomerase I and MAPK activation.[32,33] A further finding was a threefold increase in expression of the antiapoptotic protein Mcl-1 in lines MES20, MES21, 518A2, A375 and Neo-II-tr after acid **1a** (5 μg ml^{-1}) treatment. The effects of radiation (2Gy) and acid **1a** (2.5 μg ml^{-1}) were additive, including effects on cell colony growth, when appropriately timed.[34] Synergistic effects of acid **1a** and VP16, paclitaxel and actinomycin D in cancer lines of neuroblastoma SHEP were also discovered.[35]

Acid **1a** is also an inhibitor of the enzyme aminopeptidase N which takes part in the angioproliferative and metastatic activity of tumors with an IC_{50} = 7.3 ± 1.4 μM ml^{-1}.[36] It is true however, that another published study failed to confirm the antiangioproliferative activities of acid **1a** and its relation to aminopeptidase N. This study associated the antiangiogenic effects of acid **1a** in concentrations below the cytotoxic level with changes in mitochondrial potential and function.[37]

An interesting, and from a clinical perspective, important property of acid **1a** is its greater effect in an environment with a pH lower than 6.8, which is the case for the majority of tumors.[38,39] Cancer cells subjected to hyperthermia also show increased sensitivity to acid **1a**, whereas the sensitivity of non-cancer cells remains unchanged.[40]

Further, acid **1a** has the ability to change the concentration of Ca^{2+} ions, which is also an important signalling factor and inducer of apoptosis after its release from the endoplasmic reticulum. In approximately 5 minutes, the basal concentration of Ca^{2+} ions in kidney tubule cells incubated with acid **1a** (250 nM) increased threefold.[41]

Another interesting property of betulin (**1h**) is its paradoxically stimulative estrogenic effect in low concentration – measured as the ability to increase the proliferation of estrogen dependent cancer cell line MCF-7, even at a concentration of 23 nM.[42]

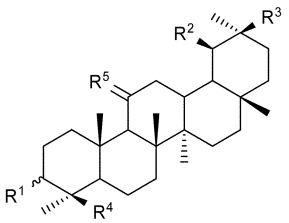
The antiproliferative effects of betulinic acid (**1a**) have been discussed in a series of reviews.[43,44]

Lupeol (1e). Lupeol (lup-20(29)-en-3β-ol, **1e**), is found in a range of fruits (*e.g.* mango)[45] and medicinal herbs.[9] It manifests chemopreventative effects: it suppresses benzoylperoxide (BPO) induced skin toxicity by way of activating a series of antioxidant enzymes inactivating BPO, such as catalase, glutathione peroxidase, glucose-6-phosphate 1-dehydrogenase, glutathione-disulfide reductase and glutathione transferase. At the same time they show direct antioxidant activity. On the basis of these findings, using lupeol (**1e**) in the case of illnesses induced by free radicals has been proposed.[46]

Another biological activity of lupeol (**1e**) is its antiangiogenic effect, even at doses below 30 μg ml^{-1}.[47] Derivatives of lupeol: 3-oxo-lup-20(29)-en-30-al (**1f**) and 28-hydroxy-lup-20(29)-en-3-one (**1g**) (*Acacia mellifera*) have cytotoxic effects against cancer lines NSCLC-N6 at CI_{50} = 15 μg ml^{-1} and 11 μg ml^{-1}.[48]

2.2 The ursane and oleanane group

Boswellic acids (2a,2b). Derivatives of boswellic acids (**2a**,**2b**) are triterpenoids found in high concentrations in the resin of the Indian tree *Boswellia serrata*, commonly known as Indian incense/frankincense. This resin has been an effective component since the days of Hippocrates and Dioscurides in mixtures



2a, R^1= α–OH,H; R^2= CH_3; R^3= H; R^4= CO_2H; R^5= H,H
2b, R^1= α–OH,H; R^2= H; R^3= CH_3; R^4= CO_2H; R^5= H,H
2c, R^1= α–OAc,H; R^2= CH_3; R^3= H; R^4= CO_2H; R^5= H,H
2d, R^1= α–OAc,H; R^2= H; R^3= CH_3; R^4= CO_2H; R^5= H,H
2e, R^1= α–OAc,H; R^2= H; R^3= CH_3; R^4= CO_2H; R^5= O
2f, R^1= β–OH,H; R^2= CH_3; R^3= H; R^4= CH_3; R^5= H,H
2g, R^1= β–OH,H; R^2= H; R^3= CH_3; R^4= CH_3; R^5= H,H
2h, R^1= β–OAc,H; R^2= CH_3; R^3= H; R^4= CH_3; R^5= O

with anti-inflammatory effects. It is widely used for its anti-inflammatory and antiarthritic effects in Indian medicine, which follows the Ayurvedic tradition.

Derivatives of boswellic acid: 3α-acetoxy-α-boswellic acid (**2c**), 3α-acetoxy-β-boswellic acid (**2d**) and 3α-acetoxy-11-oxo-β-boswellic acid (**2e**) were cytotoxic to glioma cell lines U87MG and U373MG, and leukaemic lines HL-60 and CCRF-CEM.[49,50] In animal models an increased passage of cells to apoptosis, inhibition of tumor growth and extension of survival after implantation of cell lines of malignant glioma C6 were observed, depending on dose.[51] Also described was induction of differentiation in leukaemic cell lines HL-60, U937 and ML-1 after application of **2c** and **2d** in concentrations lower than 24.2 μM, while cytotoxicity occurred at a concentration of 38.8 μM. Derivatives of boswellic acid induce differentiation in leukaemic cell lines in low concentrations and in higher concentrations they lead to apoptosis.[52]

The antiproliferative effects of these derivatives are connected to their ability to inhibit both topoisomerase I and IIα, whose activity is critical for the proliferation of cancer cells. The dual effect places them on the level of clinically used inhibitors of topoisomerases (Table 1). Furthermore, the effect of a combination of **2c** and **2d** 1 : 1 on the inhibition of activity and the secretion of matrix metalloproteinases in human fibrosarcoma line HT-1080 and differentiation, including inhibition of migration, in a cell line of mouse melanoma B16F10 has been described.[53] Boswellic acid, like acid **1a**, induces the expression of p21, by p53 independent mechanisms. Protein p21 induction does not appear decisive for these cytotoxic effects. Levels of Bcl-2 and Bax proteins remained unchanged during apoptosis.[54]

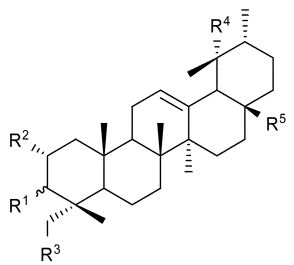
A considerable advantage of boswellic acid derivatives is their lipophilicity, enabling them to penetrate the blood–brain barrier and hence they have the potential for use in the treatment of malignancies of the CNS. In this regard, data from *in vitro* studies show inhibitory effects against glioma cell lines that are superior to clinically used camptothecins and etoposides.[50,55] This information has led to initiation of the first clinical trials. Derivatives of boswellic acid are suitable for the palliative treatment of progressive or relapsing brain tumors, due to, among other things, their described anti-edematous properties.[56]

Table 1 Overview of inhibitory activity of selected triterpenoids on DNA topoisomerases and polymerases

	Compound						
IC_{50}/μM	**1a**	**2a**	**9a**	**9b**	**2c**	**2d**	**2e**
Topoisomerase I	5	250	60	—	10	10	30
Topoisomerase II	—	200	25	—	3	10	30
DNA_α polymerase	—	38	58	27	—	—	—
DNA_β polymerase	—	42	79	53	—	—	—

By no means insignificant are the improved subjective state and minimal adverse effects in cases of incurable childhood brain tumors.[57]

Ursolic acid (3a). Ursolic acid (3β-hydroxyurs-12-en-28-oic acid, **3a**) is widespread, especially in higher plants (*e.g. Rosmarinus officinalis*, *Glechoma hederaceae*). It shows inhibitory activity against mammalian DNA polymerases α and β, and human DNA topoisomerases I and II (Table 1). Ursolic acid (**3a**) also inhibits bovine DNA polymerase α, rat polymerase β, HIV reverse transcriptase and plant DNA polymerase II (β-like) although less so. At the same time it lowers transcription of COX-2 by inhibition of the signal pathways PKC and AP-1. In a number of tumors, the expression of PKC and AP-1 is elevated and targeting these molecules is one of the aims of antiproliferative therapy.[58] *In vitro*, acid **3a** is cytotoxic to cancer lines NUGC-3 (LD_{50} = 30 μM),[59] HCT-15 (ED_{50} = 3.4 μg ml^{-1}), UISO (ED_{50} = 3.2 μg ml^{-1}) and OVCAR-5 (ED_{50} = 3.2 μg ml^{-1}),[60] A549 (ED_{50} = 4.2 μg ml^{-1}), SK-OV-3 (ED_{50} = 3.6 μg ml^{-1}), SK-MEL-3 (ED_{50} = 4.6 μg ml^{-1}), XF498 (ED_{50} = 4.5 μg ml^{-1}), HCT15 (ED_{50} = 4.4 μg ml^{-1}),[61,62] HONE-1 and (KB IC_{50} = 8.8 μM).[63] A recent paper shows that, as with betulinic acid (**1a**), apoptosis is induced in a cancer line of human melanoma M4Beu (ED_{50} = 20 μM) *via* the internal mitochondrial pathway with resulting activation of effector caspases.[64] Like oleanolic acid (**4a**), ursolic acid (**3a**) induces differentiation of the teratocarcinoma line F9 and expression for the differentiation specific genes laminin B1 and collagen IV.[65] Another effect which has been described is its antiangiogenic activity.[66] However, more detailed observation revealed angiogenic effects such as degradation of the extracellular matrix by matrix metalloproteinase 2 and urokinase.[67] In the same way, acid **4a** inhibits cancer growth in a mouse model and has similar radioprotective effects against hematopoetic tissue. Its influence is stronger with the application of preirradiation. It is probably distinguished from acid **4a** by its mechanisms of action.[68] Acid **3a** has inhibitory effects on 12-*O*-tetradekanoylphorbol-13-acetate (TPA)-mediated activation of EBV.[69,70]

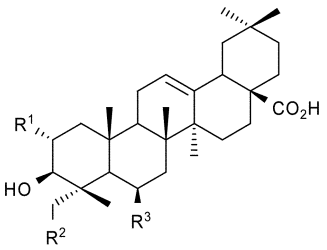


3a, R^1= α–OH; R^2= H; R^3= H; R^4= H; R^5= CO_2H
3b, R^1= β–OH; R^2= H; R^3= H; R^4= OH; R^5= CO_2H
3c, R^1= α–OH; R^2= H; R^3= H; R^4= OH; R^5= CO_2H
3d, R^1= α–OH; R^2= H; R^3= OH; R^4= OH; R^5= CO_2H
3e, R^1= β–OH; R^2= H; R^3= H; R^4= H; R^5= CH_2OH
3f, R^1= β–OH; R^2= OH; R^3= OH; R^4= H; R^5= CO_2H

Pomolic acid (3b). Another natural triterpenoid, pomolic acid (**3b**) (*Chrysobalanus icaco*, *Licania tomentosa*), is cytotoxic to

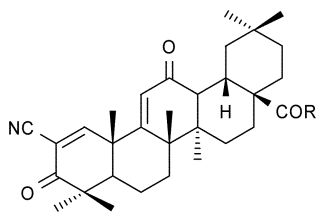
leukaemia cell lines HL-60, K562 and K562-Lucena 1, which is an MDR variant of this line expressing Pgp. In the case of cell line HL-60, apoptosis is induced *via* the mitochondrion-dependent apoptotic pathway.[71,72]

Oleanolic acid (4a). Oleanolic acid (3β-hydroxyolean-12-en-28-oic acid, **4a**) is present in high concentrations in the ginseng root,[73] along with acid **3a** in *Sambucus chinensis*[74] and is found in more than 120 plants.[75] As in the case of acid **3a**, acid **4a** reveals a range of antitumor effects. It induces differentiation in the teratocarcinoma cell line F9.[65] Its antimutagenic characteristics[76] and antiangiogenic activity[66] have also been described. Its cytotoxic activity has been established in lines: A549, SK-OV-3, SK-MEL-3, HCT15, HONE-1 and KB for ED_{50} ranging from 12.1–18.5 μg ml^{-1}.[61,63] The anticancer activity of acid **4a** isolated from rhizomes of *Astilbe chinensis* towards the HeLa cancer cell line has been described (IC_{50} = 6.49 μg ml^{-1}).[77] In addition, acid **4a** stimulates the release of NO and TNF-α, including induction of iNOS activity and TNF-α expression in macrophages. This effect is mediated by the ability to activate DNA binding to the transcription complex NF-κB and thereby its transactivation.[78]



4a, R^1= H; R^2= H; R^3= H
4b, R^1= OH; R^2= OH; R^3= OH
4c, R^1= OH; R^2= OH; R^3= H

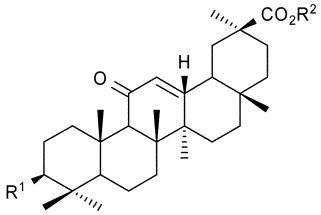
Derivatives of oleanolic acid (**4a**), that are formally derived from 2-cyano-3,12-dioxooleana-1,9(11)-dien-28-oic acid (CDDO, **5a**), show a range of interesting effects. This multifunctional molecule induces monocytic differentiation in human myeloid cells,[79] CLL B cells,[80] adipogenic differentiation of mouse fibroblasts 3T3-L1, neuronal differentiation of rat cells PC12 and inhibition of proliferation in a series of human cancer lines.



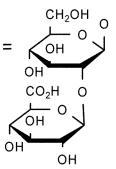
5a, R = OH
5b, R = OCH_3
5c, R = 

At concentration (IC_{50}) ranges from 0.03–1 μM, CDDO **5a** blocks *de novo* synthesis of iNOS and COX-2 at lower concentrations than 1 μM. It simultaneously inhibits creation of the products NO and PGE2, in concentrations as low as 1 pM.[81,82] Induction of apoptosis in cell lines Saos-2, U2OS, U-937, HL-60 and AML cells isolated from bone marrow, proceed *via* caspase-8 dependent cleavage of Bid protein. This results in the release of cytochrome c from the mitochondria and activation of the full caspase cascade.[83–85] Meanwhile, the role of intracellular Ca^{2+} in apoptosis induction by CDDO **5a** remains to be resolved.[86] An interesting explanation for adipogenic differentiation of 3T3-L1 fibroblasts is direct transactivation of PPARγ (Peroxisome proliferator-activated receptor gamma) with CDDO **5a**. This subsequently forms a heterodimer with retinoid X receptor (RXR) and this leads to activation of gene transcription. Binding and transactivation studies show that CDDO **5a** is a partial agonist for PPARγ. PPARγ is transcriptionally active in a series of human cancer lines. Its transactivation with the help of CDDO **5a** leads to inhibition of proliferation and subsequently, to induction of apoptosis. As a result of evaluation of its cytotoxicity in mammalian cancer models *in vivo*, CDDO **5a** has become a candidate for breast cancer treatment.[87] The methyl ester **5b** is in contrast, antagonistic to PPARγ. This may be due to the diverse effects of this compound.[88] The methyl ester of CDDO, **5b**, shows strong synergism with ATRA and RXR specific ligand LG100268 (cytotoxicity and differentiation) in myeloid leukaemic cell lines.[89] The methyl ester **5b** is effective against a line of human lung cancer in concentrations lower than 0.5 μM. Methyl ester **5b**, like CDDO **5a**, leads to release of cytochrome c, activation of caspases 3, 6, 7 and subsequent apoptosis regardless of the expression of Bcl-2.[90] CDDO **5a** induces apoptosis in cells of skin T cell lymphoma, like mycosis fungoides and Sezary syndrome, whereas synergistic effects were observed with bexaroten, which is a ligand of PPAR-γ. This observation suggests that inhibition of PPAR-γ is not, in this case, the only mechanism to induce apoptosis.[91] Another derivative of CDDO, CDDO-imidazolide **5c**, induces apoptosis in line CLL B at a concentration of 0.5 μM.[92] A combination of CDDO-imidazolide **5c** with proteazome inhibitor bortezomib, shows synergistic effects and induces apoptosis in cells of multiple myeloma patients who are resistant to therapy with bortezomib.[93]

Glycyrrhetic acid (6a) and glycyrrhizin (6b). Glycyrrhizin (**6b**), the 3β-*O*-glycoside of glycyrrhetic acid (**6a**), is one of the major components of liquorice, a common species of *Glycyrrhizia glabra.*



6a, R^1 = OH; R^2 = H

6b, R^1 =  R^2 = H

6c, $R^1 = NaO_2C(CH_2)_2CO_2$; R^2 = Na

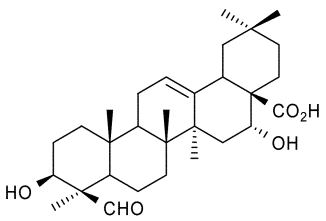
Glycyrrhizin (**6b**) can be hydrolyzed by the bacterial glucuronidase in the gastro-intestinal tract to its aglycone (acid **6a**), which exists as 18α- and 18β-isomers, whereas the 18α-epimer is more bioactive. Acid **6a** is a specific inhibitor of 11β-hydroxysteroid dehydrogenase, which under normal conditions is the chief factor implicated in controlling the levels of circulating cortisol. Acid **6a** also has an antimutagenic effect in models of

mutagenesis induced by benzo[*a*]pyrene, 2-aminofluorene, aflatoxin B1, Trp-P-2, Trp-P-1, 2-acetylaminofluorene and 2-(2-furyl)-3-(5-nitro-2-furyl)akrylamide.[94–96] This effect is probably due to inhibition of the monoxygenase system of cytochrome P450. Long-term administration of glycyrrhizin (**6b**) effectively protects patients with chronic hepatitis C from developing hepatocellular cancer. Similar conclusions were achieved in a study, which evaluated the influence of glycyrrhizin (**6b**) on the development of hepatic cancer after stimulation with diethylnitrosamine in a murine model.[97] Furthermore, it was found, that glycyrrhizin (**6b**) inhibited lung and liver tumorigenesis in mice. Studies supported by the National Cancer Institute (NCI) have demonstrated the chemopreventative effect of the 18β-isomer of acid **6a** and carbenoxolone (disodium salt of 3β-hemisuccinate of glycyrrhetic acid, **6c**). The 18β-isomer of acid **6a** was effective in models of carcinogenesis of rat mammary gland, rat colon and mouse liver *in vivo* and in mouse breast organ culture *in vitro.* Acid **6a** shows *in vitro* synergistic anti-proliferative effects in combination with glucocorticoids in lines MCF-7 and ZR-75-1, whereas the effects of particular compounds were weak. One disadvantage of using glycyrrhizin (**6b**) and acid **6a** is inhibition of 11β-hydroxysteroid dehydrogenase (11β-HSD), which leads to an excess of endogenous cortisol with resulting hypertension, hypercalemia and suppression of the renin-aldosterone axis (hyperaldosteronism): serious side effects frequently observed in individuals consuming high quantities of liquorice products. For details see the review by Wang and Nixon (2001).[98]

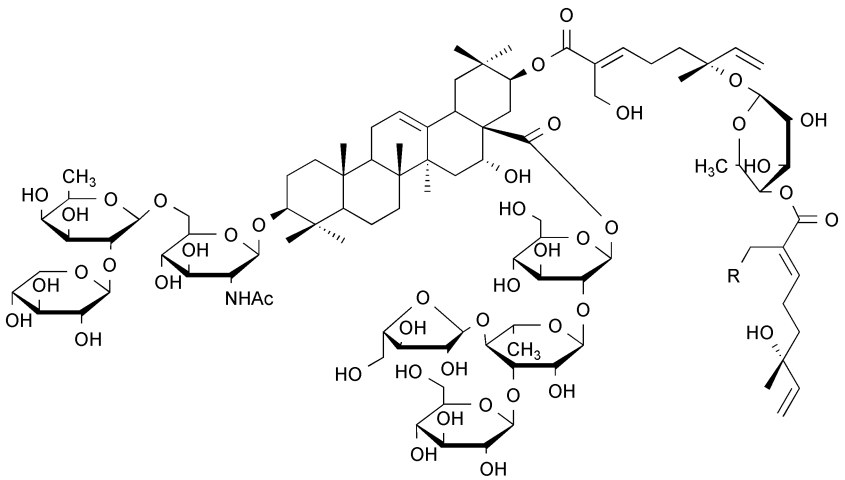
The latest mechanism to explain the anticancer action is the fact that acid **6a** causes mitochondrial swelling, which together with changes in the mitochondrial potential and release of pro-apoptogenic proteins leads to the death of transformed cells.[99]

Avicins (7a,7b). Avicins D (**7a**) and G (**7b**), the triterpenoid saponins isolated from *Acacia victoriae* have preventive effects: they protect cells against H-ras gene mutation and changes in karyotype in aneuploid models of chemically induced carcinogenesis in mice.[100] Apart from their protective effects, the avicins inhibit activation of NF-κB in the Jurcat cell line. This transcription factor regulates transcription of a series of genes involved in the immunity and inflammatory pathway, among whose products are the cytokines, adhesive molecules and proteins that partake in apoptosis. Avicins do not influence the degradation of IκB, which is essential for the release of NF-κB from cytoplasm, but decrease nuclear localization of its subunit 65p and inhibit the NF-κB bond to DNA responsive elements in nuclear extracts. Avicin-dependent inhibition can be reversed with DTT which suggests that it is mediated by modification of the sulfhydryl group, critical for activation of NF-κB. Probably as a consequence of the regulation of NF-κB, avicins lower the levels of iNOS and COX-2, including their reactive products.[101] A mixture of avicins D (**7a**) and G (**7b**), and the methanolic extract of *Acacia victoriae*-F094, inhibit the growth of the Jurcat cell line with an IC_{50} = 0.16–0.47 μg ml^{-1}. Early after treatment with avicins, increased concentration of cytochrome c in cytosol occurs. In systems with isolated mitochondria, it has been confirmed that this is by mechanisms independent of caspase 3. Release of cytochrome c leads subsequently to caspase 3 activation and cleavage of the substrate PARP (poly(ADP-ribose) polymerase). It is interesting to note that the changes in mitochondrial potential do not occur immediately following release of cytochrome c, but take up to 16 hours. Avicins also surprisingly lead to a decrease in the production of ROS.[102] Similar results were obtained by Mujoo and co-workers,[103] where IC_{50} for extract F035 for cancer cell lines ranged from 0.72–6.5 μg ml^{-1}. On the other hand, cell lines resistant to this treatment have also been described.[103]

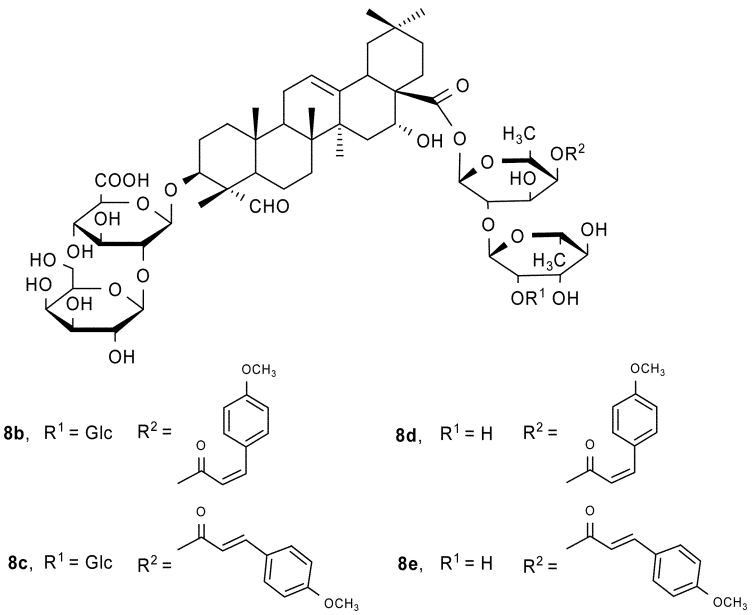
Jenisseensosides (8a–8e). Jenisseensosides A (**8b**), B (**8c**), C (**8d**) and D (**8e**) are triterpenoid saponins (glucosides of quillaic acid **8a**) isolated from the plant *Silene jenisseensis* (A, B) or *fortunei* (C, D), which in low concentrations stimulate the proliferation of the Jurcat cell line. In higher doses they regress to apoptosis.[104] In



8a



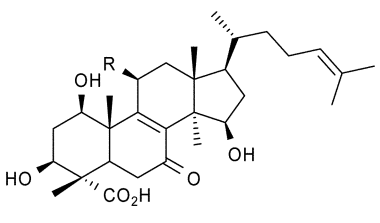
7a, R = OH
7b, R = H



addition they potentiate the toxicity of cisplatin by increasing its accumulation in cell line HT-29 by about 60–65%.[105]

2.3 The dammarane and euphane group

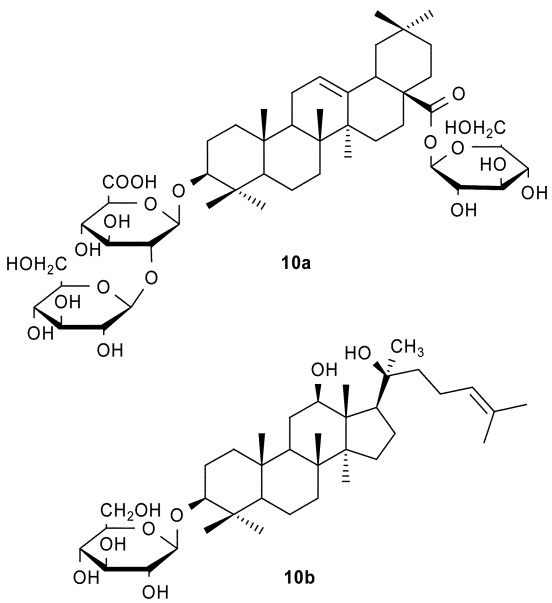
Fomitellic acids (9a,9b). Fomitellic acids A (**9a**) and B (**9b**), products of basidiomycete *Fomitella fraxinea* are inhibitors of mammalian DNA polymerases α and β, and human DNA topoisomerases I and II (Table 1). Acids **9a** and **9b** show inhibitory effects against DNA polymerase α, rat polymerase β, mild inhibition of plant DNA polymerase II (β-like), HIV reverse transcriptase as well as showing cytotoxic effects on cancer cell lines NUGC-3 (acid **9a** $LD_{50} = 38\ \mu M$) and PC-12 (acid **9a** $LD_{50} = 23\ \mu M$, acid **9b** $LD_{50} = 62\ \mu M$). Differentiation was observed using subcytotoxic concentrations.[59,106–108]



9a, R = OH
9b, R = H

Ginsenosides (10a–10e). The best-known glucosides with the fundamental structure of tetracyclic triterpenoids are dammaranes isolated from *Panax ginseng* and known as ginsenosides R(*x*). Only ginsenoside Ro (**10a**) is of the oleanane type.

Panax ginseng has been successfully tested for its chemopreventative effects not only on models of breast, colon, liver and cervical cancers but also against tumors of the CNS, in animals and in patients with oesophageal and endometrial precancers.[109–112] A study has been in progress since 2003 with the aim of evaluating the preventive activity of ginseng on the progression to hepatocellular cancer in patients with chronic hepatitis C.[113] Also underway are three epidemiological studies which show that *Panax ginseng* lowers the risk of the majority of tumors.[114] Further, a series of studies assessed the effects of extracts or effective substances on cell lines. These showed that extracts of ginseng inhibit the synthesis of DNA, lower the mutation frequency induced by methyl-mesylate and increase the rate of repair after a mutagenic incident. Included was a decline in transformation of normal cells into neoplastic ones.[115] The effects of methanolic extract of red ginseng on the accumulation and hence enhancement of mitomycin c cytotoxicity on Ehrlich's cancer cells has been described.[116] Over 25 ginsenosides which are present in red and white ginseng, have been identified.[117]

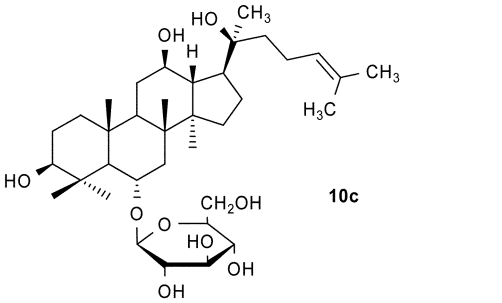


10a

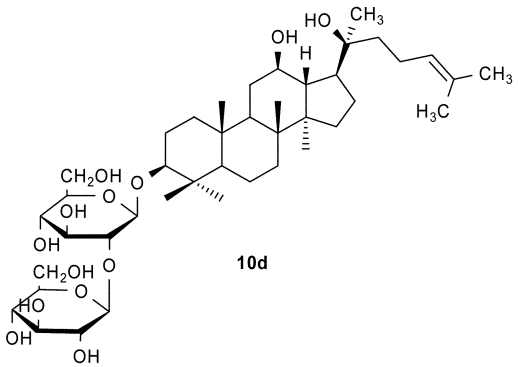
10b

The inhibitory activity of ginsenoside Rh-2 (**10b**) on the proliferation of human and murine cancer lines has been described.[118] Also published was the activation of adenylate cyclase after administration of Rh-1 **10c** and the renewed production of melanin in melanoma cells which may relate to their differentiation.

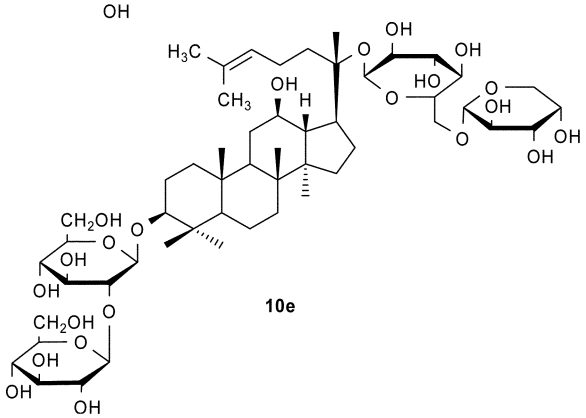
Ginsenosides were also tested in relation to specific inhibition of cancer invasion, where the most effective was ginsenoside Rg-3 (**10d**) (one of the major ginsenosides included in heat-treated ginseng). Rg-3 **10d** also inhibits TPA induced expression of pro-inflammatory enzyme COX-2 and ornithinedecarboxylase in mouse skin and in human breast epithelial line (MCF-10A). These enzymes play an important role in the origin of tumors. Rg-3 **10d** further inhibits TPA-induced activation of NF-κB and kinase ERK, which regulates activation of NF-κB. These observations suggest that the anticancer effects of heat-treated ginseng and Rg-3 **10d** are probably mediated by suppression of intracellular signal steps responsible for activation of NF-κB and the resulting inductance of COX-2. COX-2 expression and its relation to carcinogenesis, is well described for colon tumors and hence the chemopreventive effects of ginseng are a probable consequence of these activities.[119,120] Also described is inhibition of tumor angiogenesis after administration of Rb-2 **10e**. Rh-2 **10b** inhibited cancer cell growth and improved survival in nude mice with implanted ovarian cancer line HRA. In 2000, at the 4th Annual General Meeting of the Chinese Association of Clinical Oncology, the results for phase I clinical trials, which assessed the effects of a new anticancer preparation "Shenyi Jiaonang", whose chief component is Rg-3 ginsenoside (**10d**) (95%) were presented.[69] The results were very promising.[121] Currently a number of institutions are investigating the cytotoxic effects of semisynthetic derivatives of this interesting group of triterpenoids.[122]



10c



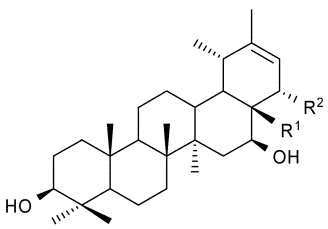
10d



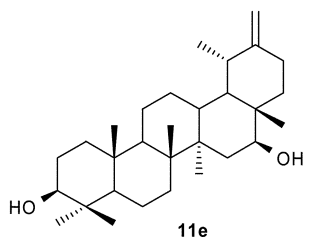
10e

2.4 The taraxastane and other group

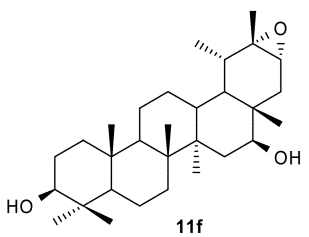
In the nonhydrolysable lipid fraction of *Chrysanthemum morifolium* extract, the following compounds have been identified as representatives of the taraxastane group: faradiol (**11a**), heliantriol B_0 (**11b**), heliantriol C (**11c**), 22α-methoxyfaradiol (**11d**), arnidiol (**11e**) and faradiol α-epoxide (**11f**). These compounds inhibit the activation of EBV-EA, which is comparable to the effects of acid (**11a**), a well-known anticancer promoter. Examination of the cytotoxic activities of faradiol (**11a**), heliantriol B_0 (**11b**), heliantriol C (**11c**), arnidiol (**11e**) and faradiol α-epoxide (**11f**) on a panel of 60 human cancer cell lines shows that arnidiol (**11e**) is broadly cytotoxic. The GI_{50} is, with the exception of two cell lines (RPMI-8226 and SR), sub 10 μM. Arnidiol (**11e**) was effective against the leukaemic line HL-60 with a GI_{50} of 0.47 μM. Faradiol (**11a**) was toxic to leukaemic cell lines CCRF-CEM, K-582 and SR as well as against a cell line of non-small cell lung cancer EKVX, Heliantriol B_0 (**11b**) was effective against renal cancer line RXF 393 and breast cancer line MCF-7.[123]



11a, $R^1 = CH_3$; $R^2 = H$
11b, $R^1 = CH_2OH$; $R^2 = H$
11c, $R^1 = CH_3$; $R^2 = OH$
11d, $R^1 = CH_3$; $R^2 = OCH_3$



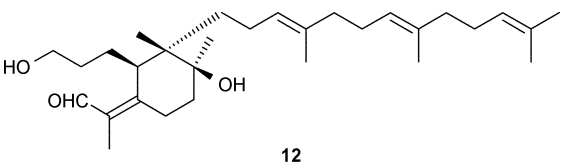
11e



11f

Iridal (12). Iridals (*e.g.* iridal **12**), which are triterpenoids that have been isolated from *Iris germanica*, show cytotoxicity towards human cancer lines A2780 and K562. All of the isolated iridals have marked cytotoxic effects on both lines, though K562 shows much more sensitivity towards these related substances.

IC_{50} for A2780 ranges from 3.59–0.17 μg ml^{-1} and for K562, 0.4–0.09 μg ml^{-1}. These compounds were less effective against multidrug resistant lines (MDR). Their IC_{50} range for K562 MDR+ was below 0.95 μg ml^{-1} and for A2780 MDR+ under 5.17 μg ml^{-1}.[124]



12

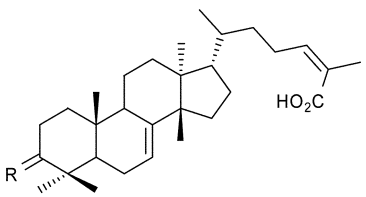
3 Further biological effects of triterpenoids

3.1 Anti-inflammatory effects

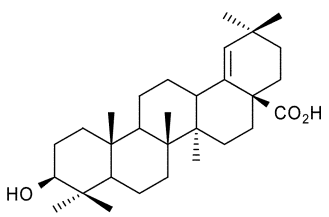
The anti-inflammatory effects of triterpenoids are largely ascribed to their ability to inhibit arachidonate 5-lipoxygenase (5-LO) and human leukocyte elastase (HLE) as well as their potential for modulating the immune response by affecting complement and antibody production. 5-LO is a pivotal enzyme in the synthesis of leukotrienes. Leukotrienes are signal molecules in disorders that are distinguished by the inflammatory response and hypersensitivity, such as asthma, arthritis, ulcerative colitis, Crohn's disease and disorders of the cardiovascular system *e.g.* shock and ischaemia of the myocardium.[125] Inhibition of the production of leukotriene B4 (LTB4) is observed after administration of *Boswellia serrata* extract, acid **4a** and its derivates (IC_{50} = 17 μM).[126,127] In the case of acid **2e**, the chief component in the extract, the inhibition of 5-LO is noncompetitive and calcium-dependent. Noninhibitory or only partially inhibitory analogues of boswellic acid: α-amyrin (**2f**), β-amyrin (**2g**), 3β-acetoxy-11-oxo-α-amyrin (**2h**), acids **2i** and **2b**, have paradoxically, the ability to reverse this inhibition.[128,129] Studies are ongoing to evaluate the effect of boswellic acid on rejection after allogeneic heart transplants in mice. Although not successful in inhibiting the rejection completely, a significant extension of survival of the graft was noted. The results were fully comparable to the effects of high doses of steroids or with toxic doses of other anti-inflammatory drugs such as acetylsalicylic acid.[130]

An indirect influence on the induction of COX-2, matrix metalloproteinase 9 and cyclin D1 is observed with acid **1a** and similarly for acid **3a**. Direct suppression of transcription factor NF-κB has been reported even in the face of its induction by various stimuli (TNF, H_2O_2, LPS, PMA, Il-1, OA, cigarette smoke).[131,132] Another factor, which plays a part in the inflammatory response, is increased activity of HLE, which can be part of the pathogenesis of pulmonary emphysema, cystic fibrosis, chronic pulmonary fibrosis, acute respiratory distress syndrome, glomerulonephritis and rheumatoid arthritis.[133] One of the first well-described inhibitors of HLE was acid **3a** (Ki = 4–6 μM). Ursolic acid (**3a**) is a compound which is widespread and occurs in relatively high concentrations in the plant kingdom. For example the peel of a ripe, average size apple may contain more than 50 mg of acid **3a**.[4] The inhibition of HLE by acid **3a** may account for its broad anti-inflammatory, antiarthritic and antiulcerous properties.[134,135] Similar effects were observed for acids **2b**, **2e** and **3a**.[136] Derivatives of boswellic and oleanolic acids (CDDO, **5a**) are unique inhibitors of HLE, 5-LO, and matrix metalloproteinases, without the adverse effects of steroids and non-steroidal antiphlogistic agents.[137,138] In contrast to these agents, reduced degradation of glycosaminoglycans in arthritic conditions in tissues, reduction in pain and inflammatory markers (CRP) have been found. These effects were observed in clinical studies after administration of *Boswellia serrata* extract (extract B15) and in the combination of rosemary extract, reduced iso-alpha acids and oleanolic acid (**4a**).[139,140] On the other hand, in a double blind study evaluating the effects of extract B15 in a patient suffering from rheumatoid arthritis, there was no improvement.[141] Clinical studies, which explore these anti-inflammatory effects in nonspecific bowel diseases such as Crohn's disease[142] and ulcerative colitis, are ongoing.[143,144] The effects are, in this case, comparable to sulfasalazin and mesalazin, where the gain : risk ratio tips the scales in favor of derivatives of boswellic acid. Short-term studies to evaluate the effect of extracts of *Boswellia serrata* in patients suffering from bronchial asthma also showed an extract : placebo effectivity ratio of 70% : 27%.[145] One advantage of *Boswellia serrata* extract is its virtually zero toxicity. A promising immunosuppressive profile is shown by (17α)-23-(*E*)-dammara-20,23-diene-3β,25-diol (*Borassus flabellifer*) *in vitro* as well as in *in vivo*.[146]

Another enzyme that takes part in the inflammatory response is phospholipase A2. The inhibitory activity of betulin (**1h**) and betulinic acid (**1a**) against this enzyme have been described as well as of other triterpenoids (masticadienonic acid (**13a**), masticadienolic acid (**13b**), schinol (**13c**) and morolic acid (**14**), and ganoderic acids R (**15a**), S (**15b**) and T (**15c**) Xi_{50} = 0.016–0.08 mol).[127,147,148] The observed phospholipase A2 binding to immobilized betulinic acid (**1a**) and computer modeling of binding sites suggest interaction among these enzymes and betulin (**1h**)



13a, R = O
13b, R = α-OH,H
13c, R = β-OH,H



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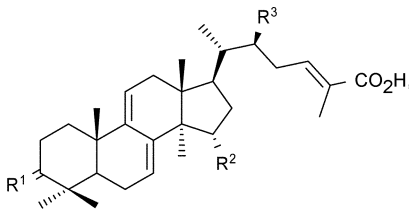
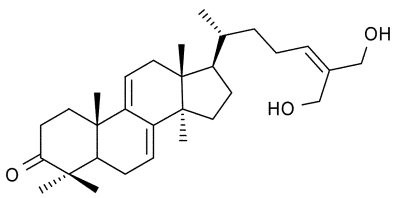


15a, R^1= α–OAc,H; R^2= H; R^3= OAc;
15b, R^1= O; R^2= H; R^3= H;
15c, R^1= α–OAc,H; R^2= OAc; R^3= OAc;

and/or acid **1a**.[147,149] A further binding interaction which has been observed with an influence over the elaboration of the inflammatory response is a bond between ursolic acid (**3a**) and TGF-β1 in its binding site for the TGF-β receptor which leads to antagonistic effects.[150]

The complement is another system which may be influenced by triterpenoids. An inhibitory effect of oleanolic acid (**4a**) on complement-mediated inflammatory responses has been described,[151] which according to this research relates to its ability to inhibit the C3-convertase classical complement course, including inhibition of single components of the complement system (IC_{50} = 72.3 μM).[152] Similar activities, albeit in higher concentrations, are also shown by its derivatives (IC_{50} = 488–633 μM).[153,154] Other triterpenoids also have the ability to inhibit the classic course of the complement system: ganoderiol F (**16**, IC_{50} = 4.8 μM), ganodermanondiol (**17a**, IC_{50} = 41.7 μM) and ganodermanontriol (**17b**, IC_{50} = 17.2 μM) isolated from the mushroom *Ganoderma lucidum*, which in the Far East has been used since time immemorial for its anti-inflammatory properties.[155] Also described is reduced inflammatory cell infiltration and reduced quantity of exudates in experimentally induced pleuritis after administration of acid **4a**.[156]



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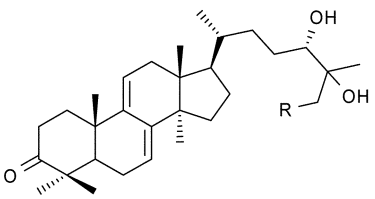
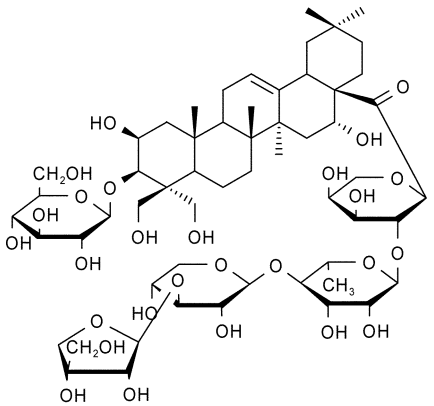


17a, R = H
17b, R = OH

Lowering the production of prostaglandin PGE_2 by inhibition of the activity of cyclooxygenase-2 (COX2) is another therapeutic approach to suppression of the inflammatory response. This inhibitory effect is accomplished by the triterpenoid glycoside, platycodin D (**18**, IC_{50} = 10 μM) isolated from the root of *Platycodon grandiflorum*,[157] ursolic acid (**3a**, IC_{50} = 130 μM, selectivity COX2/COX1 = 0.6) and oleanolic acid (**4a**, IC_{50} = 295 μM, selectivity 0.8).[58,158]

Apart from this, acid **4a** stimulates the release of NO and TNF-α, including induction of iNOS activity and TNF-α gene expression in macrophages. This effect is mediated by its ability to activate DNA binding against the NF-κB transcription complex and thereby its transactivation.[159] At the same time it is an extremely effective inducer of the enzyme phase 2 inflammatory response (NAD(P)H-dehydrogenase (quinone), oxidoreductase and hemeoxygenase 1) in sub-nanomolar concentrations.[160] Similarly, 18β-glycyrrhetic acid (**6a**) influences the expression of iNOS in macrophages.[161] On the other hand, 2-cyano-3,12-dioxooleana-1,9(11)-dien-28-oic acid (CDDO, **5a**) and its derivatives, show the ability to suppress induction and *de novo* formation of iNOS and



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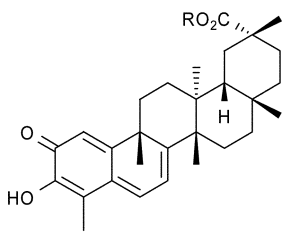
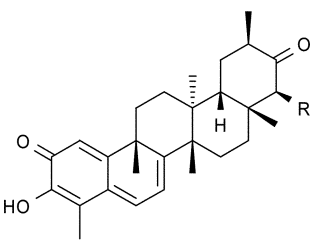
inducible COX-2 by inflammatory cytokines (IFN-γ, IL-1 a TNF-α).[82,162] Oleanolic (**4a**) and ursolic (**3a**) acids competitively inhibit isoforms of cytochrome P450. Acid **4a** is specific against CYP1A2 and CYP3A4, whereas acid **3a** is specific for CYP2C19. It seems that the inhibitory effect of acid **4a** against CYP1A2 may relate to its anti-inflammatory and anticancer effects.[163]

Lupeol (**1e**), betulin (**1h**), uvaol (**3e**), α-amyrin (**2f**), ursolic acid (**3a**), 19α-hydroxyursolic acid (**3c**) and 19α,24-dihydroxyursolic acid (**3d**), show suppressive effects on the induction of ROS. It is commonly known that production of ROS by neutrofils closely correlates to various inflammatory conditions, especially in dermatology. This finding is in harmony with the anti-inflammatory effects of these compounds described above.[164–166]

Tripterin (**19a**) and its related triterpenoid derivatives pristimerin (**19b**) and tingenone (**20a**) have been identified as compounds that inhibit the release of IL-1 even in concentrations of IC_{50} = 40 nM.[167] Interleukin-1 is the main mediator in the pathogenesis of acute and chronic inflammatory disorders. For this reason the search for small molecules, which are effective inhibitors of IL-1 is an attractive topic. Continuing studies of derivatives of tripterin (**19a**) also demonstrate its influence on cytokinines (I L-1α, IL-1β, TNF-α, IL-6, IL-8 and PGE2).[168] Other triterpenoids, *e.g.* tingenin B (**20b**) and its derivatives, show strong



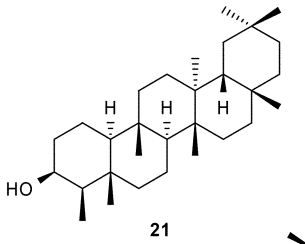
19a, R = H
19b, R = CH_3



20a, R = H
20b, R = OH

inhibitory activities against the production of IL-1α and IL-1β. The concentration that fully (100%) inhibited the production of IL-1α and IL-1β was as low as 1 μg ml^{-1}.[169]

The binding of the triterpenes: asiatic (**3f**), oleanolic (**4a**) and betulinic (**1a**) acids, β-amyrin (**2g**), friedelin (**21**), β-hederin (**22a**) and β-escin (**23**) on the human receptors for endothelin A (ETA) and angiotensin 1 (AT1) has been proven.[170] The relevance to inflammatory and cardiovascular disorders will be further examined.



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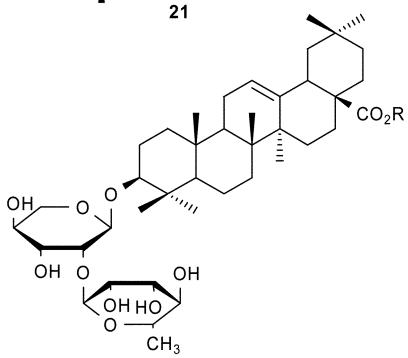
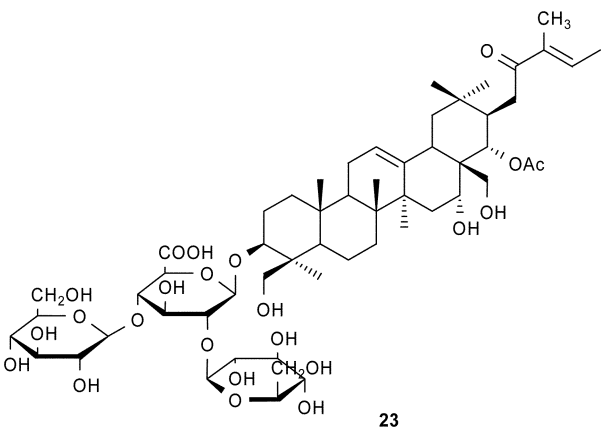


22a, R = H
22b, R = CH_3



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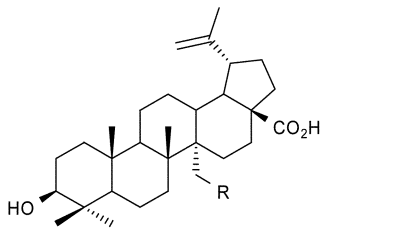
3.2 Antiulcerogenic effects

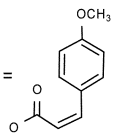
The antiulcerogenic effects[171] of glycyrrhetic (**6a**), oleanolic (**4a**) and ursolic (**3a**) acids, which are derivates of β-amyrin (**2g**) and α-amyrin (**2f**), have been known for a long time and are closely associated with their anti-inflammatory effects. Among the best known of these related substances used clinically is 18β-glycyrrhetic acid (enoxolon, **6a**), which was later replaced by its soluble succinate sodium salt (carbenoxolon **6c**) synthesized in 1960 in the Biorex Laboratories. Today, intensive research and the synthesis of new, effective derivates that lack inhibitory effects on the enzyme cortisone 5β-reductase are underway. Cortisone 5β-reductase, which is a key to intervening in the metabolism of cortisol and aldosterone, is linked to the chief adverse effects of glycyrrhetic acid (**6a**), largely hyperaldosteronism and the most serious consequences of this, hypertensive encephalopathy. For details see the review by Farina *et al.*[172]

3.3 Antimicrobial and antiplasmodial effects

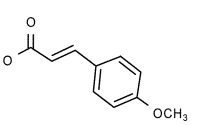
On screening the biological effects of compounds isolated from *Tovomita krukovii*, betulinic acid (**1a**) showed effective inhibition of the secreted aspartic proteinase (SAP) in a concentration 6.5 μg ml^{-1}. Importantly, the *Candida albicans* SAP is one of the most virulent factors in candida infection.[173] Apart from these effects, betulinic acid (**1a**) has been described as an active antiplasmodic substance in *Triphylophyllum peltatum* and *Ancistrocladus heyneanus*. Betulinic acid (**1a**) has EC_{50} = 10.46 μg ml^{-1} against *Plasmodium falciparum in vitro*, just about the same value as for chloroquin-resistant species.[174] A lower inhibitory concentration of IC_{50} = 1.5–3.8 μg ml^{-1}, was achieved in *in vitro* tests of other triterpenes: messagenic acid A (**24a**), messagenic acid B (**24b**) and uncarinic acid E (**25**).[175] A very likely mechanism for their action is modification of the erythrocyte membrane by incorporating active substances into the lipid bilayer.[176]

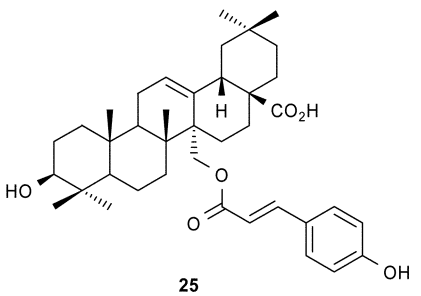


24a, R =



24b, R =





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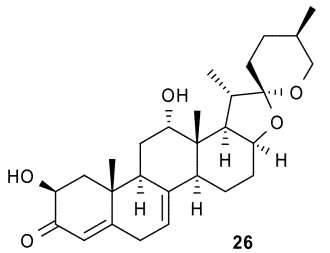
Unfortunately, under *in vivo* conditions, reduction in parasitemia was ineffective up to a concentration of 250 mg kg^{-1} day^{-1}.[177] In a study using saponins isolated from creeping ivy (*Hedera helix*), Majester-Savornin *et al.* increased our understanding of the relationship between structure and antileishmanic activities.[178] Monodesmosides extracted from *Hedera helix*, damaged host macrophages in concentrations between 5 and 25 μg ml^{-1}, but the level of toxicity was the same as that of glucantim. Oketch-Rabah *et al.* (1997) isolated the steroid muzanzagenin (**26**) from *Asparagus africanus.* This had antileishmanic and antiplasmodic properties with an EC_{50} range of tens of μM.[179,180] Antiplasmodic activity was also described for the iridals.[181]

Antibacterial activity against *Escherichia coli*, *Bacillus subtilis* and *Shigela sonnei* was found for arjulonic (**4b**), terminolic (**4c**) and asiatic (**3f**) acids and their esters.[182]



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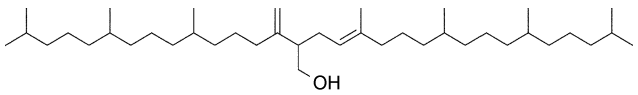
A series of triterpenoids (hopane, lupane, oleanane and ursane derivatives) isolated from *Chrysanthemum morifolium*, have antimycobacterial effects in the range MIC = 4–128 μg ml^{-1}, whereas the effects are difficult to relate to the structures of single substances.[183] For more details see the review by Cantrell *et al.* (2001).[184]

Triterpenoids with antifungal activity have been described, such as trianthenol (**27**) and betulinic acid (**1a**).[185,186]



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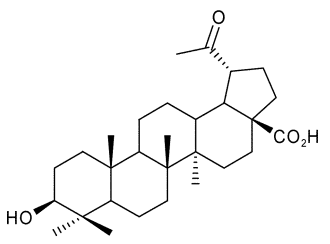
3.4 Anticariogenic activity

Oleanolic acid (**4a**) was identified as an effective substance in the fungus *Ganoderma lucidum* used in conventional Oriental medicines for the prevention of tooth decay. This activity is connected to inhibition of glucosyltransferase in *Streptococcus mutans*, a primary cariogenic bacterium.[187] Another triterpenoid that shows anticariogenic activities is glycyrrhizin (**6b**).[188]

3.5 Antiviral and anti-HIV effects of triterpenoids

Betulinic acid (**1a**) has inhibitory action against HIV replication in H9 lymphocytes with an $EC_{50} = 1.4\ \mu M$ and a therapeutic index (TI) = 9.3.

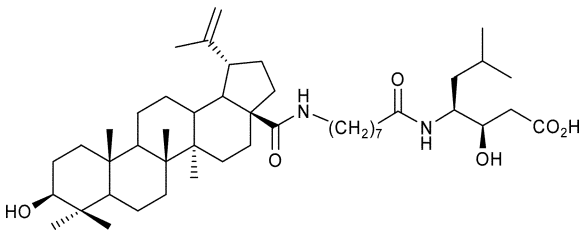
The related platanic acid (**28**) is active to a lesser degree, with a slightly higher EC_{50} value (6.5 μM). 3β-*O*-(3′,3′-Dimethylsuccinyl)betulinic acid (**1j**) (called PA-457 in the patent literature)[189,190] and 20(29)-dihydro derivatives of betulinic acid were indeed much more effective at $IC_{50} = 10.3$ nM and had a better therapeutic index >2500 than AZT used for the treatment of HIV. It has been suggested that the compound acts by disrupting a late step in Gag processing involving conversion of the capsid precursor p25 to mature capsid protein p23. At the same time the effects of these molecules against resistant variants of the virus HIV-1 have also been described. Compound **1j** and several



28

other very effective derivatives of betulinic acid (**1a**) have shown promising results in early clinical trials.[191–193]

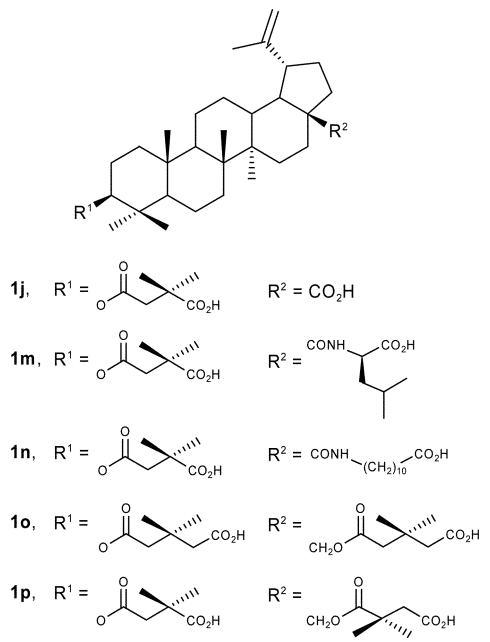
Further 3β-*O*-acyl derivatives are effective inhibitors of HIV replication with values of EC_{50} ranging from 0.04 to 2.3 nM and TI with intervals 292–2344. In a study of the mechanisms involved, selected derivatives of betulinic acid (**1a**) demonstrated complete inhibition of viral syncitia production ($IC_{100} = 20$–40 μM). One derivative inhibited HIV-induced membrane fusion with $IC_{100} = 20\ \mu g\ ml^{-1}$.[43,194–197] Another very effective derivative of betulinic acid (**1k**, known as IC-9564) blocks entry of the HIV-1 virus into the cell.[198,199]



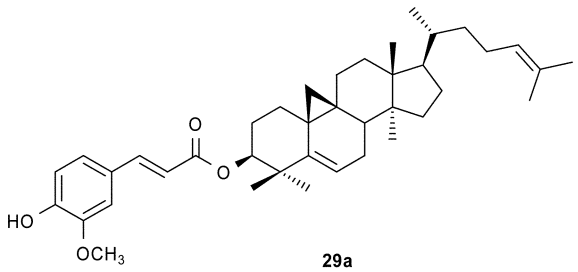
1k

Derivatives with a side chain on C3, inhibit HIV-1 maturation while, on the other hand derivatives with a side chain on C28 block the entry of HIV-1 into the cells. In an effort to combine these two effects, bifunctional derivatives with a side chain on C3 and C28 at the same time have been synthesized.

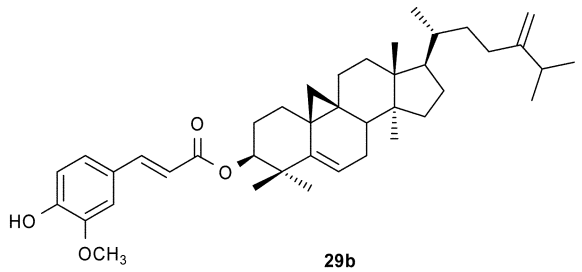
The most effective molecules **1n** and **1o** (also named LH15, LH55) were up to 20 times more effective ($IC_{50} = 2.6$ nM) than **1j** or IC-9564 (**1k**).[200,201] For comparison and mechanism of action see the review by Aiken and Chen (2005).[202] Betulin (**1h**), in comparison with betulinic acid (**1a**), is less effective (approximately 16 times), although its derivatives are extremely effective: betulin 3β,28-bis(3′,3′-dimethylhemiglutarate (**1o**, $EC_{50} = 0.66$ nM and TI = 21515), betulin 3β-(3′,3′-dimethylhemisuccinate)-28-(2″,2″-dimethylhemisuccinate) (**1p,** $EC_{50} = 0.87$ nM and TI = 42400).[203] Two further natural triterpenoid acids – oleanolic (**4a**) and pomolic (**3b**) – have values of $EC_{50} = 1.7\ \mu g\ ml^{-1}$ and $1.4\ \mu g\ ml^{-1}$ for HIV replication in H9 lymphocytes (TI = 12.8). A group of



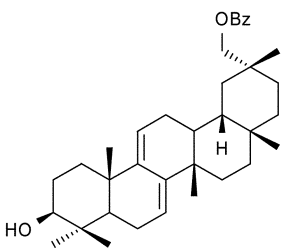
triterpenoids show inhibitory effects on HIV-1 reverse transcriptase, such as: cycloartenol ferulate (**29a**, IC_{50} = 2.2 μM), 24-methylenecycloartenol ferulate (**29b**, IC_{50} = 1.9 μM), lupenon (**1s**, IC_{50} = 2.1 μM), betulin diacetate (**1i**, IC_{50} = 1.4 μM) and karounidiol-29-benzoate (**30**, IC_{50} = 2.2 μM).[204]



29a



29b



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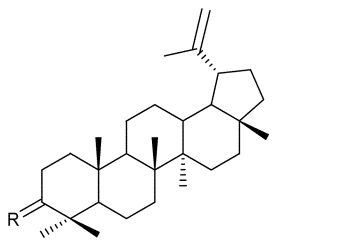
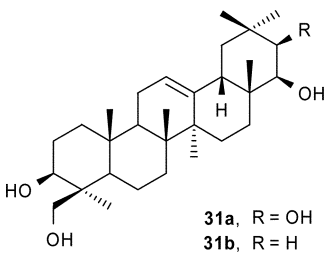
Another enzyme which can inhibit HIV maturation is HIV-1 protease. Triterpenes in this case inhibit dimerization, which is necessary for protease activity. IC_{50} for ursolic acid (**3a**), uvaol (**3e**), oleanolic acid (**4a**), α-hederin (**22b**) and betulinic acid (**1a**) are: 2, 3.5, 1, 5 and 2.5 μM respectively.[205]

Aside from anti HIV activities, antiviral effects have been described for ursolic acid (**3a**) against HSV-1, ADV-8, CVB1 and EV71 in the range EC_{50} = 0.4–6.6 μg ml^{-1}.[206]

3.6 Hepato- and cardio-protective effects

Ursolic (**3a**) and oleanolic acid (**4a**) have been investigated for some time for their hepatoprotective effects. The first study, centered on oleanolic acid (**4a**), was published as early as 1975.[207] The mechanism of effect is complex. It includes suppression of enzymes that play a role in liver damage such as cytochrome P450, cytochrome b5, CYP1A and CYP2A, and an increase in antioxidant substances such as glutathione, metalothionein, zinc, glutathione-*S*-transferase and glucuronosyltransferase, with simultaneous protective effects on liver mitochondria.[208] For more details see the review by Liu (1995).[209] Oleanolic acid (**4a**) protects mouse liver from hepatotoxic tetrachloromethane (inhibits expression of P450 2E1),[210] acetaminophen (AA) (increased glucuronosyltransferase activities, glucuronidisation and increased secretion of AA in urine),[211] bromobenzene, phaloidin, cadmium (induces production of metalothionein)[212] and at the same time it lowers the hepatotoxicity of D-galactosamine, endotoxin, thioacetamide, furosemide and colchicine. Oleanolic acid (**4a**) had no effect on the toxicity of dimethylnitrosamines, α-amanitine, chloroform and allyl alcohol.[213] Only ginsenoside Ro (**10a**), provided better protective effects including reduced formation of fibrotic tissue, in experimental models of acute and chronic hepatitis, than oleanolic acid (**4a**).[214] Ginsenoside Rh-2 (**10b**) shows more marked hepatoprotection than the parent molecule ginsenoside Rg-3 (**10d**) isolated from red ginseng.[215] Hepatoprotective activity is not peculiar to these related substances. It is also found in a range of other triterpenoids: ursolic acid (**3a**), betulin (**1h**), friedelin (**21**), soyasapogenols A and B (**31a**, **31b**), uvaol (**3e**), α-hederin (**22b**), glycyrrhizin (**6b**), glycyrrhetic acid (**6a**), lupeol (**1e**) and lupeol linoleate (**1r**) show significant protective activities under different conditions, against AA, CCl_4, $CdCl_2$ induced hepatotoxicity or in the case of cyclophosphamide, cardiotoxicity, in mouse or rat models.[211,216–220]





31a, R = OH
31b, R = H

The mechanism of induction of metalothionein in hepatic tissue and hence the hepatoprotective effects of ursolic acid (**3a**), may be caused by stimulation by TNF-α and IL-6, released from activated macrophages after treatment with ursolic acid (**3a**).[221] This goes hand in hand with the observation that ursolic acid (**3a**) stimulates the release of MIF (macrophage migration inhibitory factor) *via* ERK2 (extracellular signal regulated kinase). MIF shows a range of biological functions including the inductance of TNF-α.[222] Also noted were the protective effects of betulin (**1h**), betulinic (**1a**) and oleanolic (**4a**) acids against ethanol induced cytotoxicity in HepG2 cells.[223] A similar protective effect of ursolic acid (**3a**) was observed in *in vivo* models of ethanol induced hepatic damage in rats.[224]

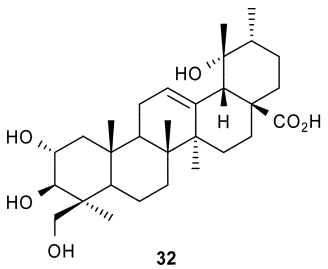
3.7 Analgesic effects

The bark of the Brazilian tree *Ocotea suaveolens* is used for pain relief in traditional medicine, and also as a tonic and for the treatment of asthma. 24-Hydroxytormentic acid (**32**) isolated from the bark shows antinociceptive properties without the side effects of *e.g.* muscle relaxation and sedation in mouse models (IC_{50} = 12.5 mg kg^{-1}).[225] Likewise, α- and β-amyrin acetate, and nimbin

Table 2 The most important triterpenoids in the plant kingdom, overview of effects (excluding cytotoxicity)

Latin name	Isolated active compound	Effect of active compound	Ref.
Boswellia serrata	Boswellic acids (**2a**,**2b**)	5-LO inhibition	128,129
Boswellia carteri	Derivates of boswellic acid	HLE inhibition	136
		Inhibition of graft rejection	130
		Antiarthritic effect	139
	Extract-B15	Crohn's disease treatment	142
		Ulcerative colitis treatment	143,144
		Bronchial asthma treatment	145
Ganoderma lucidum	Oleanolic acid (**4a**)	C3 convertase inhibition	151,152
Plantago major		COX-2 inhibition	58,158
Olea europaea		Antiulcerogenic effect	171,172
Panax ginseng		Inhibition of HIV-1 protease	205
		Hepatoprotective activity	207,209–213
Malus domestica	Ursolic acid (**3a**)	HLE inhibition	4,134,135
Calluna vulgaris		COX-2 inhibition	226
Arctostaphyllos uva-ursi		Inhibition of the production of reactive oxygen species	164–166
Sambucus chinensis		Antiulcerogenic effect	172
Solanum incanum		Inhibition of HIV-1 protease	205
Crataeva nurvala		Hepatoprotective activity	211,216–220
		Analgesic effect	226,227
Anemone raddeana	Betulinic acid (**1a**)	Phospholipase A2 inhibition	147
Lycopodium cernuum		SAP inhibition	173
Syzygium claviflorum		Antiplasmodial activity	174,177
		HIV replication activity	41,193
Betula alba	Betulin (**1h**)	Phospholipase A2 inhibition	147
Anemone raddeana		Hepatoprotective activity	211,216–220
Schinus terebinthifolius	Masticadienolic acid (**13b**)	Phospholipase A2 inhibition	127,148
Pistacia terebinthus			
Schinus terebinthifolius	Masticadienonic acid (**13a**)	Phospholipase A2 inhibition	127,148
Pistacia terebinthus			
Pistacia terebinthus	Morolic acid (**14**)	Phospholipase A2 inhibition	127
Schinus terebinthifolius	Schinol (**13c**)	Phospholipase A2 inhibition	148
Ganoderma lucidum	Ganoderiol F (**16**)	Complement inhibition	155
	Ganodermanontriol (**17b**)		
	Ganodermanondiol (**17a**)		
Platycodon grandiflorum	Platycodin D (**18**)	COX-2 inhibition	157
Anemone raddeana	Lupeol (**1e**)	Inhibition of the production of reactive oxygen species	164,165
Crataeva nurvala		Hepatoprotective activity	211,216–220
Diospyros kaki	Uvaol (**3e**)	Inhibition of reactive oxygen species production	166
		Inhibition of HIV-1 protease	205
Diospyros kaki	α-Amyrin (**2f**)	Inhibition of reactive oxygen species production	166
Glycyrrhizia glabra		Analgesic effect	226,227
Glycyrrhizia glabra	Glycyrrhetic acid (**6a**)	Antiulcerogenic effect	172
		Anticariogenic activity	188
		Hepatoprotective activity	211,216–220
Asparagus africanus	Muzanzagenin (**26**)	Antileishmania activity	179
		Antiplasmodial activity	180
Iris germanica	Iridal (**12**)	Antiplasmodial activity	181
Trianthema portulacastrum	Trianthenol (**27**)	Antifungal activity	185
Syzygium claviforum	Platanic acid (**28**)	HIV-1 replication inhibition	43,194–197
Panax ginseng	Ginsenoside R_0 (**10a**)	Hepatoprotective activity	214
Ocotea suaveolens	24-Hydroxytormentic acid (**32**)	Analgesic effect	133

(**33**), ursolic (**3a**) and 23-hydroxyursolic acids have analgesic properties (PD_{50} = 18.6–50 mg kg^{-1}), which are comparable and in one case outclass the activity of acetylsalicylic acid.[226,227]



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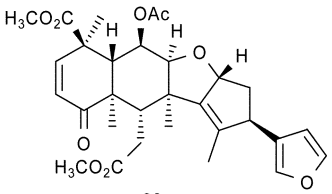

33

4 Conclusions

The triterpenoids represent a promising and expanding platform for biologically active natural compounds whose potential is currently only partly exploited by the pharmaceutical industry. A number of recently studied triterpenoids compare very favourably with drugs in current use (Table 2). We anticipate further progress as improvements in the methods of isolation and identification of natural triterpenoids open the way to targeted pharmacological modelling and to the resulting synthetic modifications. Research and development of drugs based on the triterpenoids is therefore a key program in a number of research institutions and pharmaceutical companies.

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