Genus *Bupleurum*: a review of its phytochemistry, pharmacology and modes of action

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Abstract

Objectives *Radix Bupleuri* represents one of the most successful and widely used herbal drugs in Asia for treatment of many diseases over the past 2000 years. Thorough studies have been carried out on many species of this genus and have generated immense data about the chemical composition and corresponding biological activity of extracts and isolated secondary metabolites. In this work, we review the chemistry and pharmacology of the genus *Bupleurum* and explore the relationships between the pharmacological effects and the chemical composition of these drugs.

Key findings Early studies on the genus *Bupleurum* had focused only on the traditional uses of the plants in the treatment of inflammatory disorders and infectious diseases. After chemical profiling, several groups of secondary metabolites were characterized with relevant biological activity: triterpene saponins (saikosaponins), lignans, essential oils and polysaccharides. As a result, present interest is now focused on the bioactivity of the isolated triterpene saponins acting as immunomodulatory, anti-inflammatory and antiviral agents, as well as on the observed ant-iulcer activity of the polysaccharides and anti-proliferative activity of different lignans. Many saikosaponins exhibited very potent anti-inflammatory, hepatoprotective and immunomodulatory activities both *in vivo* and *in vitro*.

Conclusions Further investigations and screenings are required to explore other *Bupleurum* species, to evaluate the clinical safety and possible interactions with other drugs or herbs. Standardization of *Bupleuri* extracts is crucial for them being integrated into conventional medicine due to large chemical and biological variations between different species and varieties.

Keywords biological activity; *Bupleurum*; mode of action; secondary metabolites

Introduction

The family Apiaceae, or carrot family, is one of the most widely studied families of flowering plants comprising about 450 genera with 3540 species.[1,2] Members of this family are herbs, less often shrubs or trees, with global distribution, especially in temperate regions.[1] Many of the genera provide us with economically important food items, herbs and spices, such as carrot, anise, fennel and caraway. Many medicinally important apiaceous plants (almost 250 species) have been used for centuries in folk medicine in the treatment of various ailments.[3] These plants provide us with many potentially active compounds from all classes of secondary metabolites, including essential oils, phenolics (flavonoids, coumarins and lignans), triterpene saponins, alkaloids and polyacetylenes. These bioactive metabolites can serve as lead compounds in the treatment of many serious diseases.[4]

The name of the genus *Bupleurum* originates from the Latin word *boupleuron* (*bous* = ox and *pleura/on* = rib/s) describing the shape of the roots, which are the commonly used part of the plant.[5] Most *Bupleurum* species are perennial herbs, up to 150 cm in height with compound umbels. The flowers are bisexual, yellowish or rarely purplish with five stamens and the fruits occur mostly as cremocarps. Leaves are simple, long, slender and alternate with entire margin. The genus is represented by 180–190 species, which are widely distributed in the Northern Hemisphere and commonly used in Eurasia and North Africa for their medicinal properties.[2] Embedded in Asian traditional medicine, several *Bupleurum* species have been used either alone or in combination with other ingredients for the treatment of common cold, inflammatory disorders, hepatitis, cancer and fever in the form of over-the-

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counter herbal teas or in different pharmaceutical preparations.[6,7] *Bupleurum* species are officially listed in the Chinese and Japanese Pharmacopoeias in addition to the WHO monographs of the commonly used medicinal plants of China and Korea.[8,9] However, none of the *Bupleurum* plants were selected by the German Commission E, the British Pharmacopoeia 2009 or the British National Formulary 57.[10]

Historical and traditional uses of *Bupleurum*

The genus *Bupleurum* is a major component of Oriental folk medicine. Preparations containing the roots of *Bupleurum* species have been prescribed for more than 2000 years in China where the first record about their use appeared in *Shen-Nong's* Herbal.[11] Inspired by the role in regulating metabolism and controlling Yin/Yang as mentioned in the old Chinese literature, *Bupleurum* was widely known in Korea and Japan for the treatment of fever, pain and inflammation associated with influenza and the common cold.[4,10] In addition, *Bupleurum* species were also used as analgesics in the treatment of distending pain in the hypochondriac region of the chest and against amenorrhoea. Many *Bupleuri* extracts have been used for improvement and protection against chronic hepatitis, nephrotic syndrome and autoimmune diseases.[12] Other uses include improvement of cholecystitis and wound healing and treatment of deafness, dizziness, vomiting, dry throat and diabetes. However, these effects are not supported by experimental or clinical data. Moreover, combinations of *Bupleurum* with ginseng and *Astragalus* are used against haemorrhoids, anal or uterine complications and diarrhoea.[8,9,12]

Chemical diversity of the secondary metabolites in the genus *Bupleurum*

Thorough phytochemical investigations of approximately 50 *Bupleurum* species led to the isolation and identification of almost 250 natural compounds from all major phytochemical classes. Nevertheless, the genus still holds other therapeutically relevant species probably containing other bioactive substances that have not yet been explored. In general, the chemical combinations and the ratio between components vary from one species to another but most of the secondary metabolites isolated belong to the classes of phenolics, lignans, terpenoids (triterpenoids and sterols), mono- and sesquiterpenes (essential oils) and polyacetylenes. In addition, minor components, including phenylpropanoids, polysaccharides and a few alkaloids, have also been reported. In the following sections we will summarize the current knowledge on the chemical structures of compounds isolated from the genus *Bupleurum*. A compilation of all isolated compounds with their original sources will be provided in supplementary materials.

Secondary metabolites present in essential oils

Like many members of the family Apiaceae, all representatives of the genus *Bupleurum* produce essential oils. About 200 components of essential oils from 20 species have been

documented. Li and coworkers[13,14] examined the compositions of the essential oils obtained from the roots of 10 different species from China and they were found to consist mainly of a series of aliphatic aldehydes and acids, such as hexanal, heptanal (*E*)-2-nonenal (*E*,*E*)-2,4-decadienal, hexanoic acid, heptanoic acid, octanoic acid and hexadecanoic acid. These aldehydes are characteristic of the Chinese species. This was confirmed by our work on *B. marginatum* in which β-caryophyllene, β-caryophyllene oxide and spathulenol, in addition to the aforementioned aldehydes, represent the major components of the oil.[15] In contrast, essential oils from European species are characterized by the presence of a high abundance of α/β-pinene, limonene and 1,8-cineole rather than aliphatic aldehydes. This difference can be used to distinguish between oils from Italy or Spain and those from China.[16–19]

Triterpene saponins

Saikosaponin triterpenes generally constitute the main class of secondary metabolites in the genus *Bupleurum* amounting to up to 7% of the total dry weight in roots. To date, more than 120 glycosylated oleanane-type and ursane-type saponins have been isolated from *Bupleurum* species.[20–23] The aglycones of these saikosaponins are closely related oxygenated pentacyclic triterpenoidal structures that can be distinguished only by the positions and numbers of the double bonds in rings C and D and oxygenation patterns in positions 16, 23, 28 and 30 (Figure 1). These saponins generally bear one (monodesmosidic) or, less often, two (bidesmosidic) carbohydrate chains that are directly attached to the hydroxyl groups in position 3 for monodesmosidic saponins and to positions 3 and 28 or 30 in the case of the bidesmosidic saponins. The according carbohydrate chains are composed mainly of fucose, rhamnose, xylose, galacatose and glucose moieties. Generally, the bidesmosides are stored in plant vacuoles and are considered prodrugs. They are hydrolysed by the metabolizing enzymes when the plant is wounded to give the monodesmosides, which are the pharmacologically active forms.[24] Saikosaponins with the aglycone structure I represent the most abundant triterpene saponins found in *Bupleurum* species.[25] Of these, saikosaponins A, C and D, which had been isolated for the first time from *B. falcatum* roots, constitute the most common saponins in such species as *B. kunmingense*, *B. marginatum* and *B. wenchuanense*.[10,26] Variations in component abundance, as well as total saponin content, are commonly encountered between different plants and especially from different localities.[27] In addition, other saikosaponins with different aglycones or sugar chains have been also isolated from other *Bupleurum* species, as shown in the supplementary materials.[28–32] Most of these saponins are potentially active compounds and exhibit a wide range of pharmacological actions, which are discussed later.

Sterols

This class of secondary metabolites was apparently of least interest for many phytochemists. Only 14 compounds have been identified from few species. For example, the phytosterol composition of *B. falcatum* was identified to be β-sitosterol,
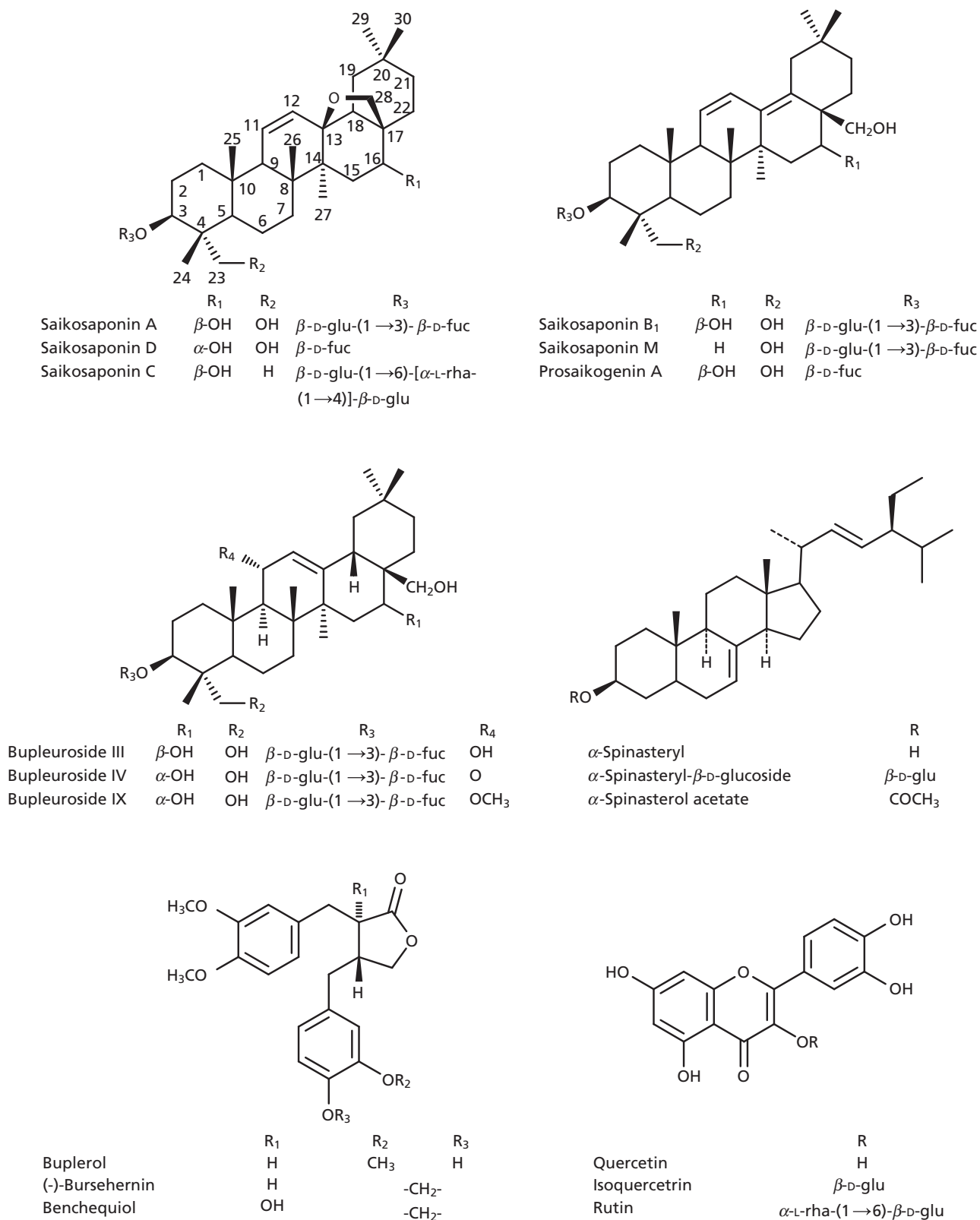
	R_1	R_2	R_3
Saikosaponin A	β-OH	OH	β-D-glu-(1 →3)- β-D-fuc
Saikosaponin D	α-OH	OH	β-D-fuc
Saikosaponin C	β-OH	H	β-D-glu-(1→6)-[α-L-rha-(1→4)]-β-D-glu

	R_1	R_2	R_3
Saikosaponin B$_1$	β-OH	OH	β-D-glu-(1 →3)-β-D-fuc
Saikosaponin M	H	OH	β-D-glu-(1 →3)-β-D-fuc
Prosaikogenin A	β-OH	OH	β-D-fuc

	R_1	R_2	R_3	R_4
Bupleuroside III	β-OH	OH	β-D-glu-(1 →3)- β-D-fuc	OH
Bupleuroside IV	α-OH	OH	β-D-glu-(1 →3)- β-D-fuc	O
Bupleuroside IX	α-OH	OH	β-D-glu-(1 →3)- β-D-fuc	OCH$_3$

	R
α-Spinasteryl	H
α-Spinasteryl-β-D-glucoside	β-D-glu
α-Spinasterol acetate	COCH$_3$

	R_1	R_2	R_3
Buplerol	H	CH$_3$	H
(-)-Bursehernin	H	-CH$_2$-	
Benchequiol	OH	-CH$_2$-	

	R
Quercetin	H
Isoquercetrin	β-D-glu
Rutin	α-L-rha-(1 →6)-β-D-glu

Figure 1   Representatives from different classes of compounds isolated from *Bupleurum* spp.

	R_1	R_2
Morinin G	H	OCH$_3$
5-(1-Ethoxyallyl)-1,2,3-trimethoxybenzene	OCH$_3$	OC$_2$H$_5$

	R_1	R_2
Aesculetin	OH	OH
Scopletin	OCH$_3$	OH
Scoparone	OCH$_3$	OCH$_3$

	n
Hexanal	3
Heptanal	4

	R
Bupleurynol	H
Bupleurotoxin	OH
Acetylbupleurotoxin	OAc
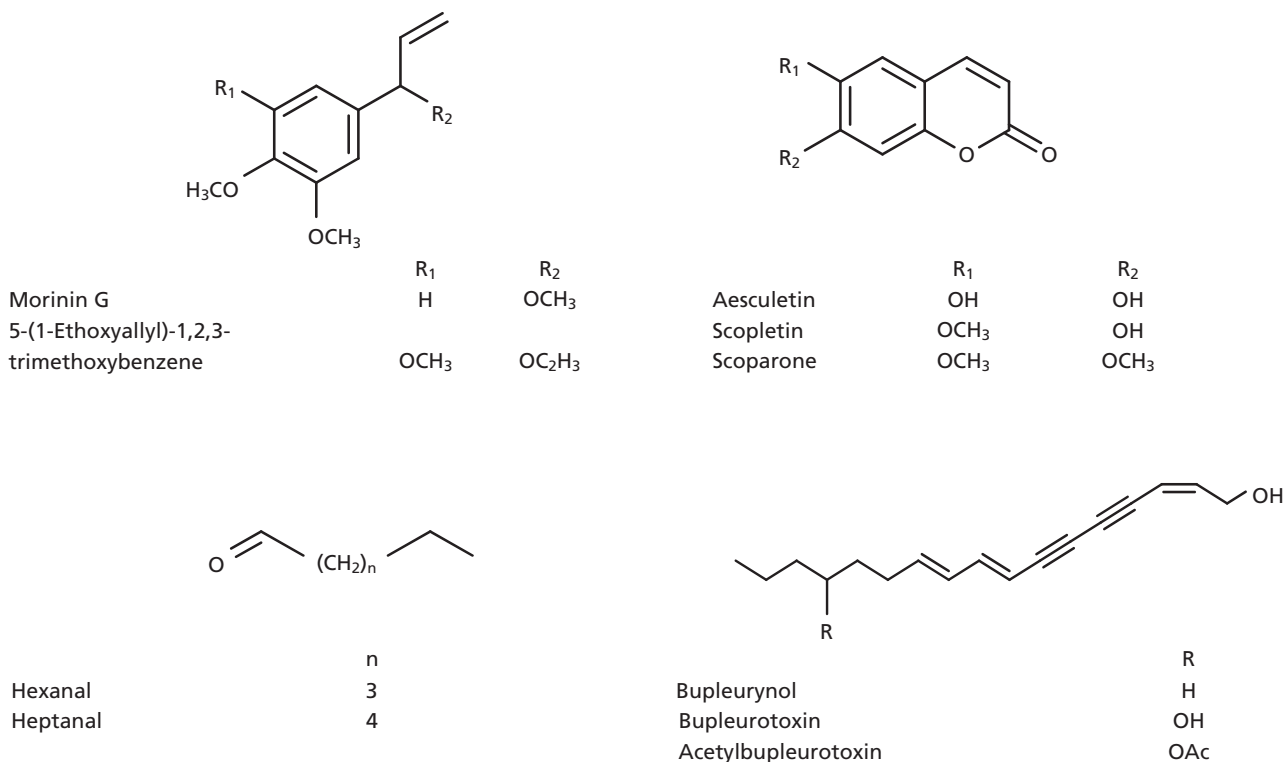
Figure 1 *(Continued)*

stigmasterol, Δ^7-stigmastenol, Δ^{22}-stigmastenol and α-spinasterol,[33] while aerial parts of *B. flavum* contain mainly betulin, betulinic acid, epibetulin and jasminol.[34] *B. marginatum* roots have been shown to contain β-sitosterol and α-spinasterol; lupeol, cycloeucalenol and erythrodiol have been isolated from *B. fruticosum*.[35,36] In general, betulin and α-spinasterol are the most commonly occurring sterol components in *Bupleurum* spp.

Lignans

Lignans are the second most common class of secondary metabolite within this genus with almost 50 isolated compounds. Four major subclasses of lignans can be distinguished depending on the pattern of additional bridging between the two β-linked phenylpropanoid units. The dibenzylbutyrolactone derivatives (Figure 1) are the most common compounds but arylnaphthalenes, aryltetralinelactones and tetrahydrofurofuranes are also present. This class of secondary metabolite has been extensively studied in *B. salicifolium* and about 32 compounds were isolated from different plant parts.[37–42] Arylnaphthalene derivatives (i.e. chinensin and isodiphyllin) are common in *B. fruticescens*, *B. handiense* and *B. marginatum*,[43–45] while tetrahydrofurofurane derivatives are only found in *B. salicifolium* and *B. wenchuanense*.[38,46] The majority of isolated lignans occur as aglycones and only two glycosides (phillyrin and wenchuanensin) have been isolated from *B. wenchuanense*.[46]

Flavonoids and related chromones

Formerly it was believed that most flavonoids in the genus are derivatives of the flavonol aglycones kaempferol, isorhamne-tin or quercetin. Recently, however, some other aglycones like apigenin, acacetin, chrysin, luteolin and tamarixetin have been characterized.[34,47–49] To date about 30 flavonoids have been isolated with the diglycoside rutin being the most common. In addition, narcissin, the other diglycoside form of isorhamnetin, is present in *B. flavum* and *B. fruticosum*.[10,34] Flavonoids, including the minor compounds, are widely used as chemotaxonomical markers to distinguish between different *Bupleurum* species and different geographical sources.[47] The chromone derivatives eugenin,[50] saikochromone A[51] and saikochromoside A[52] have been isolated from *B. scorzonerifolium*, *B. falcatum* and *B. chinense*, respectively, and the isoflavonoid derivative saikoisoflavonoside A[53] from *B. scorzonerifolium*.

Coumarins

Coumarins are characteristic constituents of the family Apiacae and 14 compounds have so far been reported in the genus *Bupleurum*, especially simple alpha-keto benzopyranes (Figure 1) from *B. fruticescens* and *B. fruticosum*. Herniarin, scopoletin, isoscopoletin, scoparone and limettin have been isolated from the aerial parts of *B. fruticescens*[45,54] and virgatenol, capensin, fraxetin, prenyletin and aesculetin from *B. fruticosum*.[55,56] Furthermore, complex pyranocoumarin derivatives, such as anomalin and praeruptorin A, have been isolated from *B. falcatum* and *B. marginatum* roots.[35,57]

Polyacetylenes

About 25 bioactive polyacetylenes (polyines) have been reported from seven different species. Barrero and cowork-

ers[49,58] have identified bupleurynol, 2(*E*),9(*Z*)-octadecadiene-4,6-diyne-1,18-diol with its mono- and diacetate derivatives and oenanthetol with its aldehyde and acetate forms, from the aerial part of *B. acutifolium*, and 5(*E*),7(*E*)-pentadecadiene-9,11,13-triyn-2-one and 8(*Z*)-decene-4,6-diyn-1-ol from the aerial part of *B. spinosum*. Saikodiyne A, B and C were isolated for the first time from the roots of *B. falcatum* (the prefix 'saiko' is derived from the Japanese name of the plant[59]), falcarinol and diynene were isolated from the acetone extract of *B. rigidum* roots.[20]

Other compounds

Other minor compounds are present in the genus, such as phenylpropanoid derivatives and polysaccharides. The phenylpropanoid derivatives represent the fourth class of phenolics in this genus of which 14 different compounds have been described from aerial parts of *B. fruticosum*. Morinins D, G and L, in addition to ferulic acid and its derivatives, were the main compounds.[60,61] No data could be found regarding the other parts of *B. fruticosum* or other plants from the same genus.

Rare monosaccharides, like ribitol, xylose and arabinose, and pectic polysaccharides, like bupleuran 2IIb and bupleuran 2IIC, have been isolated from *B. falcatum* and many other species; these compounds exert anti-ulcer, anti-inflammatory, anti-infective and immunomodulatory effects in autoimmune diseases as discussed later.[62–64]

Free organic and fatty acids, like pinellic acid, angelic acid, petroselic acid and lignoceric acid, have also been identified in many species.[10]

Other secondary metabolites, such as tannins, anthocyanidins and alkaloids, are absent in the genus, although a recent report claimed the presence of an indole-type alkaloidal glycoside (chaihuxinoside B) in the aerial parts of *B. chinense*.[65]

Pharmacological properties of *Bupleurum*

Additive or synergistic effects may be expected from the wide range of combinations of secondary metabolites in many plants of the genus *Bupleurum*. In-vitro and in-vivo studies on *Bupleurum* extracts or isolated components (mainly saikosaponins) revealed significant anti-inflammatory, anti-ulcer and immunomodulatory activity. Other activity includes hepatoprotective, antitussive, antispasmodic, diaphoretic, antioxidant and antimicrobial effects. Some lignans of *Bupleurum* species may be useful as anti-mitotic agents in the treatment of cancer by their inhibition of microtubule formation due to their high structural similarity to podophyllotoxin. Although the biological activity of certain *Bupleurum* essential oils and saponins had been compiled early in 2006,[10] a vast body of scientific literature on the pharmacological activity of other species and classes of secondary metabolites have since emerged.[63,66–74] A summary of some of the relevant literature is given in Table 1 and discussed in the following section.

Immunomodulatory activity

An early study on the effect of water-soluble extracts and purified derivatives from the roots of *B. falcatum* upon the immune response of BALB/c mice used heterologous erythrocytes and bacterial lipopolysaccharide as T-dependent or T-independent antigens, respectively.[83] Saikosaponins A and D, but not B_1, B_2 or C, suppressed the response of plaque-forming cells to heterologous erythrocytes by stimulating T and B cells in a dose-dependent manner. The activity of saikosaponin A was higher than that of D and the optimal dose for this activity was 1 mg/kg. Further studies on the effect of other saikosaponins and saikogenins on macrophage activation in mice showed that 10 μg intraperitoneally administered saikosaponin D (ssD) potently activated peritoneal macrophages in terms of enhancement of phagocytic activity.[89] In addition, an increase in the cellular level of acid phosphatase, induction of cytostatic activity and expression of Ia antigen on the cell surface of spleen cells were observed. Moreover, saikosaponin D modulated lymphocyte activity by suppressing the T-cell response and inducing the B-cell response to different mitogens and up-regulating the interleukin (IL)-2/IL-4 production in cell cultured thymocytes by affecting their post-receptor signal transduction.[90,91]

A recent study with ssD on mouse T lymphocytes activated through the NF-κB, nuclear factor of activated T-cells (NF-AT) and activator protein 1 (AP-1) signalling pathways, cytokine secretion and IL-2 receptor expression revealed that ssD suppressed stimulated human T-cell proliferation and activation *in vitro*. The inhibitory effect of ssD on phorbol myristate acetate (PMA)-induced T-cell activation was associated with down-regulation of NF-κB signalling. In addition, ssD at 15 μM concentration decreased the production of pro-inflammatory cytokines IL-6, tumour necrosis factor (TNF)-α and interferon (IFN)-γ.[68]

The immunomodulatory effect of some *B. fruticosum* extracts and isolated compounds has been investigated extensively against several markers of the immune response.[98,101] A petroleum ether extract (100 μg/ml) caused 80–100% release of IL-1β and IL-6 and prostaglandin (PG) E_2 synthesis in human monocytes. However TNF-α was inhibited using 10 μg/ml. Morinin L and one other phenylpropanoid derivative isolated from the ethyl acetate fraction were tested for their possible effect on many cytokines and immune mediators. Inhibition of the transcriptional activity of an NF-κB-controlled reporter gene was observed by both compounds. This inhibition took place at a post-degradation level. Further work with the same compounds indicated that they could prevent the release of IL-1, IL-6, IL-8 and TNF-α and PGE$_2$ synthesis. Similar results were obtained with different extracts from *B. scorzonerifolium* tested against the IL-2 secretion in stimulated human peripheral blood T cells. The isolated compounds wogonin, eugenin and saikochromone A inhibited the secretion at a dose of 10 μg/ml by 99, 72 and 71%, respectively.[50]

The acidic polysaccharide fraction from *B. smithii* inhibited the complement activation (IC50 340 and 81 μg/ml) for both classical and alternative pathways, respectively. This inhibition was mediated through interaction with C1s, C3 and C4 complements, which are the major components of the innate immune system.[64]

Anti-inflammatory activity

It is usually difficult to separate the inflammatory process from the immune response; drugs with immunomodulatory

Table 1 Pharmacological activity of different species of the genus *Bupleurum* and their isolated compounds

Plant species	Extract/substances	Main pharmacological activities	Source
B. chinense	Saikosaponin mixture	(−) Hepatitis B replication	[75,76]
	Alcoholic extract	(−) *Helicobacter pylori* activity	[77]
	Saikosaponin C	(−) Hepatitis B replication	[75]
B. falcatum	Acidic polysaccharide fraction	(−) Gastric lesions	[78]
	Saikosaponin mixture	(−) Acute and chronic inflammation	[79]
	Bupleuran 2IIc	(−) Gastric lesions	[80–82]
		(+) Gastric mucosa regeneration	
	Saikosaponin A	(+) B cells	[83–88]
		(−) T cells	
		(−) PGE_2	
		(−) sGOT and sGPT levels	
		(−) Lipid peroxidation	
		(−) Asthmatic bronchoconstriction	
	Saikosaponin D	(+) Macrophage phagocytosis	[75,84–87,92–94]
		(−) T cells	
		(+) Acid phosphatase	
		(+) IL-2/IL-4 production	
		(−) NF-κB	
		(−) IL-6, TNF-α, IFN-γ	
		(+) PGE_2 production	
		(−) sGOT, sGPT and TGF-β1 levels	
		(−) Lipid peroxidation	
		Cytotoxic against HepG2 cell line	
		(−) Measles and herpes viruses	
	Saikosaponin B1	(+) PGE_2 production	[84,92]
	Saikosaponin B2	(+) PGE_2 production	[73,84,92]
		(−) Human coronavirus (HCoV) replication	
	Saikogenin D	(−) PGE_2 production	[95]
B. fruticescens	Butanol extract	(−) Acute inflammation	[96]
	Methanol extract	(−) 5-LOX, COX-1	[97]
	Essential oil	(−) Acute inflammation	[17]
	Fruticesaponin B	(−) Acute inflammation	[96]
B. fruticosum	Petroleum ether extract	(−) IL-1β, IL-6, TNF-α, PGE_2	[98]
	Essential oil	(−) Acute and chronic inflammation	[18,99]
		Oxytocin antagonism	
		Antifungal	
	Saikosaponin fraction	Hepatoprotective against DMN	[100]
	Morinin L	(−) IL-1, IL-6, IL-8, TNF-α, NF-κB	[101]
	Buddlejasaponin IV	Hepatoprotective against DMN	[100]
B. gibraltaricum	Methanol extract	(−) Acute inflammation	[102]
	Essential oil	(−) Acute and chronic inflammation	[16,19]
		Oxytocin antagonism	
		Antibacterial, antifungal	
	Saikosaponin I	(−) Acute inflammation	[102]
B. kaoi	Saponin-rich fraction	Hepatoprotective against CCL_4	[103–106]
		(−) sGOT, sGPT, AST and ALT levels	
		(+) IFN-γ level	
		Cytotoxic against A549 cell line	
	Water extract	(+) IFN-γ and GSH levels	[66,105]
		(−) Coxsackie B1 replication	
	Polysaccharide-rich fraction	(−) sGOT and sGPT levels	[105]
		(+) IFN-γ and GSH levels	
B. marginatum	Essential oil	(−) 5-LOX	[15]
		(−) PGE_2 production	
		Antibacterial	
B. rigidum	Buddlejasaponin IV	(−) Acute inflammation	[107,108]
		(−) 5-LOX, COX-1	
		(−) Vesicular stomatitis virus (VSV)	
	Buddlejasaponin I	(−) Acute inflammation	[107]
	Sandrosaponin I	(−) 5-LOX, COX-1	

Table 1   *(Continued)*

Plant species	Extract/substances	Main pharmacological activities	Source
B. rotundifolium	Butanol extract	(−) Acute inflammation	[109]
	Rotundioside F	(−) Acute inflammation	[109]
	Rotundioside A, H, I, J	Cytotoxic against MK-1, HeLa and B16F10 cell lines	[30,110]
B. salicifolium	Polyacetylenes derivatives	Antibacterial	[111,112]
B. scorzonerifolium	Acetone extract	Cytotoxic against A549 cell line	[113–115]
	Wogonin, eugenin, saikochromone A	(−) IL-2	[50]
	Saikosaponin B3, bupleurosides III, VI, IX and XIII and scorzonerosides A, B, C	Hepatoprotective against DMN, LPS	[29,116]
		(−) sGOT and sGPT levels	
B. smithii	Acidic polysaccharide fraction	(−) Complement system	[64]
B. wenchuanense	3′-*O*-Acetyl Saikosaponin A 3′-*O*-Acetyl Saikosaponin D	Cytotoxic against P-388, KB cell lines	[32]

(−), decrease, inhibit, reduce, down-regulate. (+), increase, activate, up-regulate.

activity usually interfere in inflammation. A wide range of *Bupleurum* preparations has been shown to affect artificially induced acute and chronic inflammation in animal models and, in addition, exert a marked in-vitro activity on inflammatory mediators resulting from the inhibition of either lipoxygenases or cyclooxygenase.

B. falcatum has been extensively investigated for its anti-inflammatory activity. In 1975, a saikosaponin mixture (mainly saikosaponin A, C and D) isolated from the roots was tested by intramuscular and oral administration to female albino rats. Both routes of administration of the saikosaponin mixture demonstrated significant activity against acute and chronic inflammation, saikosaponin D being particularly active against chronic inflammation at a dose of 2 μg/g.[79]

Ten years later, another study measured the possible effects of isolated saponins on PGE_2 production in rat peritoneal macrophages.[84] Interestingly, saikosaponins B_1, B_2 and D increased the production of the inflammatory mediator, while saikosaponins A and C were inhibitory. The maximal increase was observed with 10 μg/ml ssD, and the maximal inhibition after 8 h pre-incubation with 100 μg/ml ssA. These results were confirmed in 1999 for the saikosaponins B_1, B_2 and D, which led to a spontaneous increase in PGE_2 production in C6 rat glioma cells (IC50 15.0, 14.4 and 11.0 μM, respectively).[92] Surprisingly, saikosaponins A and C significantly increased the amount of inflammatory mediators after 10 min of drug administration, indicating the importance of the incubation time. To clarify the possible mode of action, the aglycone of ssD, saikogenin D, was also examined for its effect on PGE_2 production and intracellular free calcium ion ($[Ca^{2+}]i$) concentration in C6 rat glioma cells.[95] Saikogenin D inhibited PGE_2 production in a concentration-dependent manner with an IC50 value of about 3 μM. On the other hand, saikogenin D elevated $[Ca^{2+}]i$ with an EC50 value of about 35 μM. These results suggest that saikogenin D possesses a dual effect by inhibition of PGE_2 production with an elevation

of $[Ca^{2+}]I$, which is attributed to Ca^{2+} release from intracellular stores. This key finding could explain the latent inhibition of PGE_2 by ssD after an 8-h pretreatment, which might undergo a gradual cellular hydrolysis to saikogenin D being responsible for this action.

Extracts of *B. fruticescens*, as well as certain isolated betulinic acid derivatives (fruticesaponin A, B and C), were examined for both oral and topical anti-inflammatory activity. The orally administered *n*-butanol extract (100 mg/kg) caused an inflammatory inhibition by 43% in carrageenan-induced acute oedema in mouse hindpaw after 5 h. Moreover, tetradecanoyl phorbol acetate (TPA)-induced mouse ear oedema was inhibited by 74% after 4 h of topical application of the tested drug (0.5 mg/ear). Fruticesaponin B was the most active betulinic acid derivative in both oral and topical application but the maximum oral activity was observed after 3 h.[96] These results were confirmed by testing the methanol extract on different in-vitro enzymes involved in inflammation: a significant inhibition of 5-lipoxygenase (5-LOX) activity was observed, which reduced both leukotriene-B_4 and 5(*S*)-HETE production (IC50 112 μg/ml and 95 μg/ml, respectively). In addition, at a 200 μg/ml dose, the extract inhibited cyclooxygenase-1 (COX-1) and elastase activity by 90% and 54%, respectively.[97]

Another study with different *Bupleurum* species from Spain showed that the methanol extract of *B. gibraltaricum* and its isolated saponin (saikosaponin I) decreased carrageenan-induced inflammatory oedema in a rat hindpaw model by 50% at 5 h after intraperitoneal injection of 150 and 50 mg/kg methanolic extracts and the isolated substance, respectively.[102]

Buddlejasaponin I, IV and the sulfated saikosaponin (sandrosaponin I), which were isolated from *B. rigidum*, exhibited a very potent in-vivo anti-inflammatory effect (1 mg/ear dose) on mouse ear oedema induced by PMA compared with the equivalent dose of indometacin. The effect of these compounds on other inflammatory parameters

revealed that buddlejasaponins I and IV exhibited potent leukotriene-C_4 inhibition (IC50 2.4 and 2.6 μM, respectively). The results are very close to those of the known reference inhibitor nordihydroguaiaretic acid (NDGA, IC50 = 2.0 μM). In the PGE_2 release assay, all tested saikosaponins exhibited less potency towards the inhibition of COX with IC50 values higher than 45 μM compared with indometacin with an IC50 of 6.5 μM. Moreover, inhibition of thromboxane B2 formation was also observed with IC50 values of 27, 4.6 and 2.4 μM for buddlejasaponin I, IV and sandrosaponin I, respectively. These results revealed that inhibition of arachidonic acid metabolism is one of the biochemical mechanisms exerted by saikosaponins in their putative antiphlogistic activity.[107]

The *n*-BuOH fraction of *B. rotundifolium* extract and the isolated saponins were evaluated for their possible topical effects on acute and chronic ear oedema in mice provoked by TPA. The extract caused a 73% inhibition of the acute oedema (0.5 μg/ear). The most active triterpene derivative was rotundioside F, which exerted a potent activity against acute inflammation with an IC50 value of 0.099 μM/ear.[109]

The essential oils of *Bupleurum* also exhibit a positive anti-inflammatory effect. For instance, the anti-inflammatory activity of intraperitoneally and orally administered essential oils of *B. gibraltaricum* and *B. fruticosum* and their major components were evaluated against both acute inflammation using carrageenan-induced oedema model and chronic proliferative inflammation by evaluating the granuloma formation.[16,18] The two oils showed a dose-dependent activity in both systems but the effect was more obvious in the acute test and by the intraperitoneal route. In addition, *B. gibraltaricum* oil was more active than *B. fruticosum* oil and exhibited about one-third of the activity shown by indometacin in the paw oedema model. Both oils had the advantage of sustaining the anti-inflammatory activity for a longer time compared with the control drug. This activity was attributed to the main components Δ^3-carene, β-pinene and α-pinene, which caused a highly significant reduction of the paw oedema at the 33 mg/ml dose 3 h after oral administration. The absence of Δ^3-carene in *B. fruticosum* oil is apparently the reason for the reduced activity compared with *B. gibraltaricum* oil.[18]

Essential oils of *B. gibraltaricum* from different localities differed mainly in the composition of Δ^3-carene, β-pinene and α-pinene components. *Bupleurum* oils rich in Δ^3-carene and β-pinene were active against acute inflammation while those with lower concentrations failed to exert such activity.[117]

Comparable results were also obtained with *B. fruticescens*; orally administered oil and its major components α-pinene and β-caryophyllene had a significant in-vivo effect on both carrageenan- and PGE_2-induced oedema. The activity of the oil itself was much higher than that of the individual components; this was attributed to a synergistic effect of the compounds.[17]

A recent in-vitro study was carried out by our group to evaluate the activity of *B. marginatum* oil against both soybean 5-LOX and PGE_2 production in MIA-PaCa-2 cancer cells.[15] The oil was able to inhibit 5-LOX with an IC50 value of 63.64 μg/ml and to exert a 26% inhibition in the PGE_2 production in the cancer cells treated with a 25 μg/ml dose.

Anti-ulcer activity

Triterpene saponins like glycyrrhizin, which resemble many of the saikosaponins, exhibit potent anti-inflammatory and anti-ulcer effects.[118] The anti-ulcer effects of saikosaponins have not been studied so far and in most cases the anti-ulcer properties of *Bupleurum* spp. appear to be attributed only to pectic polysaccharides.

The effects of the acidic polysaccharide fraction from the roots of *B. falcatum* on induced gastric lesions in mice using different routes of administration have been evaluated. A protective effect was observed after administration by all the routes using a concentration range of 25–100 mg/kg.[78] The major acidic polysaccharide with anti-ulcer activity was identified as bupleuran 2IIc after a bioassay-guided fractionation of the sugar fraction. This polysaccharide, which is composed of galacturonic acid units, reduced the HCl/ethanol-induced gastric lesions in mice more potently than the known anti-ulcer drug sucralfate through scavenging the free oxygen radicals at a 100 mg/kg dose.[80,81] Similar results were obtained using acetic acid-induced ulcers in rats in which the repair process closely resembles that of the human peptic ulcer, where an orally administered acidic polysaccharide-rich fraction at a dose of 200 mg/kg twice daily reduced the lesions to almost 52% and regeneration of the mucosa was clearly seen.[82]

Antioxidant and hepatoprotective activity

The high concentrations of triterpene saponins and polyphenolics significantly contribute to the observed hepatoprotective effects of *Bupleurum* spp. The administration of saikosaponins, especially saikosaponin A and D, showed interesting effects on liver function, such as decreasing the activity of glucose-6-phosphatase and NADPH-cytochrome C reductase and significantly increasing 5′-nucleotidase activity. Moreover, an inhibitory effect on D-galactosamine-induced hepatic necrosis was observed in the form of significant reduction in many hepatic injury markers such as serum GOT and GPT, total and direct bilirubin and cholesterol levels after pretreatment of animals with 5 mg/kg for four successive days.[85] Similar results were obtained in other studies in which the effects of saikosaponin D on acute and chronic hepatic injury provoked by chloroform and enhanced by phenobarbitone were evaluated in rats. In addition to the reduction of hepatic enzyme levels, a significant inhibition of lipid peroxidation in the liver and a general improvement in liver weight were observed.[86,87]

The components of another Chinese species, *B. scorzonerifolium*, were studied by Yoshikawa and coworkers, where saikosaponin B_3, bupleurosides III, VI, IX and XIII and scorzonerosides A, B and C reduced the cytotoxicity of both D-galactosamine and lipopolysaccharides in primary cultured rat hepatocytes as shown in the form of a decrease in sGOT and sGPT levels.[29,116]

Similar results were obtained for the saponin fraction of *B. fruticosum* and its isolated compound buddlejasaponin IV. Both the saponin fraction and the isolated compound were

ineffective against hepatic damage caused by chloroform whereas they showed substantial hepatoprotective effects against D-galactosamine, similar to the widely used natural hepatoprotective compound silybin.[100]

An in-vitro study of the extract and its saponin-rich fractions of a native Taiwanese species, *B. kaoi*, revealed significant protection of primary hepatocytes against chloroform damage.[103,104] The results demonstrated that oral pretreatment of rats with the extracts at concentrations below 0.5 mg/ml 3 days before a single dose of CCl$_4$ significantly lowered the serum levels of hepatic enzyme markers aspartae aminotransferase (AST) and alanine aminotransferase (ALT). In addition, pathological examination showed that lesions, including ballooning degeneration, necrosis and hepatitis, were partially healed by treatment with *B. kaoi* extracts. The extracts were also able to suppress the elevated hepatic enzyme activity of superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase, which resulted from the high oxidative stress induced by CCl$_4$. In another hepatic injury model, the hepatoprotective effects of a water extract and both polysaccharide- and saponin-enriched fractions of *B. kaoi* were evaluated against dimethyl nitrosamine (DMN)-induced hepatic fibrosis in rats.[105] Administration of the plant extracts markedly counteracted the harmful effects of DMN as indicated by reduction of sGOT, sGPT collagen content and elevation of the hepatic glutathione (GSH), albumin and interferon-γ levels in the serum and liver homogenates. Another study using a tea preparation from the leaves of *B. kaoi* revealed a significant in-vitro scavenging activity against both DPPH and superoxide anion radicals with IC50 values of 0.36 and 4.35 mg/ml, respectively, in addition to the inhibition of lipid peroxidation and the subsequent malondialdehyde (MDA) formation.[119] Furthermore, a leaf infusion decreased the hepatotoxicity of paracetamol and CCl$_4$ in rat liver cells, as indicated by increased viability of intoxicated primary hepatocytes. The results are in agreement with a study carried out in 2009 with saikosaponin A.[120]

In another recent study, the therapeutic effect of saikosaponin D on liver fibrosis and cirrhosis was evaluated.[93] The results clearly demonstrated that saikosaponin D significantly reduced collagen I deposition in the liver. Moreover, it reduced the concentration of transforming growth factor β1 (TGF-β1) in the liver, which is used as a key marker for liver fibrosis induced by DMN in addition to its role in reducing the elevated level of oxidative stress enzymes.

Cytotoxicity and anti-tumour activity

Many *Bupleurum*-containing herbal drugs have been traditionally used in the treatment of tumours and cancer. In this review we will focus on the anti-tumour and antiproliferative effects of *Bupleurum* extracts and their isolated components in cell culture systems.

The cytotoxicity of different extracts from *B. scorzonerifolium* was assessed against A549 human lung cancer cells.[113] The acetone extract showed a dose-dependent antiproliferative effect with an IC50 value of 59 μg/ml after a 24-h treatment. The mechanism of this anti-proliferative activity was assumed to be mediated through cell cycle arrest in the G2/M phase, induction of tubulin polymerization, induction of apoptosis via kinase 1/2 (ERK 1/2) activation in addition to activation of caspase-3/9 and suppression of telomerase activity in the A549 cell line.[114,115] Saponin-enriched fractions from *B. kaoi* exhibited similar properties on the same cell line and the IC50 value was 196 μg/ml.[106]

The cytotoxic effects of many *Bupleurum* saponins, especially those with an epoxy bridge, were very potent against many cancer cells. For example, saikosaponin D exerted very potent activity against the HepG2 cell line with an IC50 value of 12.5 μg/ml. The mechanism of cytotoxicity was attributed to the induction of apoptosis via activation of caspases 3 and 7 and the subsequent activation of poly-ADP-ribose polymerase (PARP), which leads to DNA fragmentation.[75]

The cytotoxic activity of both oleanane-type and ursane-type triterpene saponins isolated from *B. rotundifolium* by Fujioka and coworkers were examined in human gastric adenocarcinoma (MK-1), human cervical carcinoma (HeLa) and murine melanoma (B16F10) cell lines.[30,110] Among the monodesmosidic ursane-type saponins, rotundifoliosides A, H, I and J exhibited very potent cytotoxic activity with IC50 values in the range 11–71 μM. The highest activity was observed with rotundifolioside J, which had IC50 values of 16, 21 and 11 μM against the aforementioned cell lines, respectively.[110] The monodesmosidic oleanane saikosaponins also demonstrated different degrees of cytotoxicity but those that contained the epoxy bridge in their skeleton were highly active, with IC50 values in the range 6.6–37.3 μM.[30] This high reactivity is attributed to the presence of the epoxy group which can covalently bond with the SH or amino groups in proteins leading to conformational changes and loss of functionality. This will be discussed in detail later.[24]

Some of the saikosaponins isolated from *B. wenchuanense* exhibited a potent activity against leukaemia P-388 cells and nasopharynx carcinoma KB cells. The most active were the 3′-*O* acetyl derivatives of saikosaponin A and D with IC50 values of 0.5 and 6.3 μg/ml for the former and 1.2 and 6.3 μg/ml for the latter.[32] The corresponding deacetylated forms had lower cytotoxic activity against HepG2 with IC50 values of 50 and 20 μg/ml, respectively.[121] This reduction in the cytotoxicity is due to the increase in the polarity of deacetylated forms and therefore the molecules cannot penetrate the biomembrane to exert their action.[24]

Antiviral activity

Extracts from *Bupleurum* have been evaluated *in vitro* for their possible activity against such viruses as herpes simplex, measles, hepatitis B and C viruses and many others. In general, the saikosaponins isolated from *Bupleurum* spp. exhibited a more potent antiviral activity than the total extracts and the individual saikosaponins showed a certain degree of selectivity towards different types of viruses.

A total aqueous extract of *B. kaoi* was tested against Coxsackie B virus type 1 (CVB$_1$) and the results indicate that the extract has both direct and indirect effects on CVB$_1$ infection. The extract was able to neutralize CVB$_1$-induced cytopathic effects in a human neonatal foreskin fibroblast cell line (CCFS-1/KMC) with an IC50 value of 12.38 μg/ml. In addition, viral replication was completely inhibited at 200 μg/ml after 48 h treatment through induction of type I interferon expression.[66]

Chang and coworkers showed that a crude saikosaponin mixture obtained from *B. chinense* inhibited DNA replication of hepatitis B virus (HBV) and significantly reduced the HBV antigen level in transfected HepG2 cells.[75,76] The main active saikosaponin responsible for the activity was saikosaponin C; it showed a potent inhibition of both HBeAg secretion (IC50 of 11 μg/ml) and HBV-DNA expression (IC50 of 13.1 μg/ml). This activity was much higher than that obtained by the well-known antiviral drug lamivudine, which is the most widely accepted drug for treatment of chronic HBV.[75] In a recent study, other members of the saikosaponin group were identified from a *B. chinense* extract but only saikosaponin D was able to inhibit HBV-DNA replication.[122]

The antiviral effects of saikosaponin D isolated from the roots of *B. falcatum* against herpes simplex, poliovirus and measles were investigated.[94] Saikosaponin D at a 5 mM concentration directly inactivated both measles virus and herpes simplex virus while a 500 mM concentration of the drug resulted in complete loss of viral infectivity. However, saikosaponin D was ineffective against the replication of measles virus, herpes virus, and polio virus.

Saikosaponins isolated from *B. rigidum* were also evaluated for their in-vitro antiviral activity against herpes simplex type 1 (HSV-1), vesicular stomatitis virus (VSV) and poliovirus type 1. Buddlejasaponin IV exhibited a potent virucidal activity against VSV only at concentrations in the range 20–25 μg/ml without being toxic at this range to the cell line used and even more potent than the reference substance dextran sulfate.[108]

The possible effects of saikosaponins and their mode of action against human coronavirus (HCoV), which is the main cause of severe acute respiratory syndrome (SARS), were also examined. All the tested saikosaponins exhibited antiviral activity at concentrations of 0.25–25 mM, and the strongest activity was observed for saikosaponin B_2 with an IC50 of 1.7 mM. This activity was attributed to the inhibition of viral replication at early stages of cell infection.[73]

Antibacterial and antifungal activity

Extracts, essential oils and some of the other isolated compounds from the *Bupleurum* spp. exhibit substantial antimicrobial activity against Gram-positive bacteria whereas they are almost inactive towards Gram-negative bacteria or yeasts. Different essential oils obtained from aerial parts of *B. gibraltaricum* were tested against different bacteria and the minimum inhibitory concentration (MIC) values were determined. *Micrococcus luteus* was the most sensitive bacterium with a MIC value as low as 3 μg/ml, while the Gram-negative *Escherichia coli*, *Pseudomonas fluorescens* and *Candida albicans* were less sensitive.[54] The antifungal activity of the same essential oil was also evaluated against *Plasmopara halstedii*; the oil could reduce the sporulation frequency of the fungus after an 11-day treatment at all tested concentrations and a complete inhibition was obtained at a concentration of 5.0 ml/l.[19]

Comparable antifungal and antibacterial activity was observed with the essential oil of *B. fruticosum* collected in Italy, which showed a significant effect against the Gram-positive pathogens *Streptococcus faecalis*, *Staphylococcus albus* and, to a lesser extent, *Staphylococcus aureus*.[99]

We have examined the antimicrobial and antifungal activity of the essential oil and hexane–ether extract of *B. marginatum* on 12 different microorganisms including three methicillin-resistant *Staphylococcus aureus* (MRSA) strains. Both oil and extract showed a significant antimicrobial activity against Gram-positive pathogens (*Streptococcus pyogenes* and *Streptococcus agalactiae*) with MIC values in the range 0.125–0.50 mg/ml. In addition, a measurable inhibition of MRSA strains was recorded with an MIC value of 4 mg/ml. In contrast, the tested Gram-negative microorganisms and yeasts were not susceptible to any of the tested samples.[15]

Another study was conducted to evaluate the possible action of many plant extracts, including both ethanol and aqueous extracts of the aerial parts of *B. chinense,* on the growth of the Gram-negative microorganism *Helicobacter pylori,* which is the major causative agent of peptic ulcers. The alcoholic extract was more active than the aqueous extract with an MIC value of 60 μg/ml.[77]

Some of the commercially available *Bupleurum* preparations, like 'Chaihu injection', have been tested *in vitro* for possible antimicrobial activity. Slight inhibition of *Staphylococcus aureus* was observed but no effects were observed against *Staphylococcus albus*, *Neisseria gonorrhoeae*, *Diplococcus pneumoniae,* haemolytic *Streptococcus* or *Pseudomonas aeruginosa.*[123]

Also, other isolated secondary metabolites from *B. salicifolium* have been assessed for their anti-infective activity: one of the isolated polyacetylenes (8S-heptadeca-2(Z)-9(Z)-diene-4,6-diyne-1,8-diol) exhibited significant inhibitory activity against three Gram-positive bacteria, *Staphylococcus aureus*, *Bacillus subtilis* and *Micrococcus luteus*, with MIC values of 10, 5–10 and 10–15 μg/ml, respectively. However, it was inactive against Gram-negative bacteria (*E. coli*, *Salmonella* spp., *Pseudomonas aeruginosa*) and the yeast *Candida albicans*.[111,112]

Kumazawa and coworkers examined *in vivo* the protective effect of many individual saikosaponins isolated from *Bupleuri Radix* against *Pseudomonas aeruginosa* and *Listeria monocytogenes* infections in mice. The protective effect was attributed to the immunomodulatory action of different saikosaponins on macrophages which enhances the resistance of the mice to the bacterial infection.[124]

In general, *Bupleurum* has not been used in traditional medicine to treat infections. Research on antimicrobial properties and modes of action are limited but we can anticipate a detergent effect of saikosaponins on the bacterial and fungal biomembrane which would make bacterial or fungal cells leaky and could lead to their death.[24]

Miscellaneous biological activity

Ocete and coworkers examined the antispasmodic activity of essential oils obtained from both *B. gibraltaricum* and *B. fruticosum* on the rat uterus model. Both oils were able to antagonize uterine contractions induced by oxytocin and acetylcholine to different degrees. *B. gibraltaricum* essential oil was more active due to its higher content of Δ^3-carene (33% of the total oil) and was able at low doses (1.1 μg/ml and
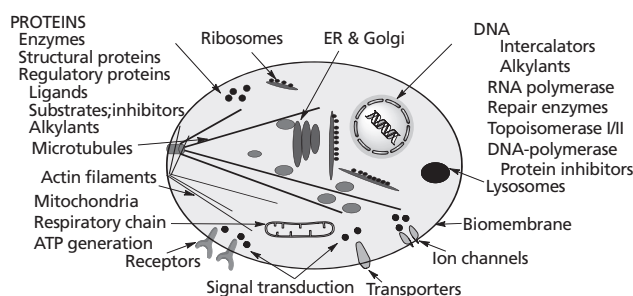
PROTEINS
Enzymes
Structural proteins
Regulatory proteins
Ligands
Substrates;inhibitors
Alkylants
Microtubules

Ribosomes   ER & Golgi

DNA
Intercalators
Alkylants
RNA polymerase
Repair enzymes
Topoisomerase I/II
DNA-polymerase
Protein inhibitors
Lysosomes

Actin filaments
Mitochondria
Respiratory chain
ATP generation
Receptors

Biomembrane

Ion channels

Signal transduction   Transporters

Figure 2   The main molecular targets of many secondary metabolites in the mammalian cell. ER, endoplasmic reticulum.

2.2 μg/ml) to competitively and noncompetitively antagonize the oxytocin-induced contraction.[16,18] Saikosaponin A inhibited the passive cutaneous anaphylaxis reaction in rats and suppressed asthmatic bronchoconstriction in sensitized guinea-pigs at a dose of 1 mg/kg. In addition, weak inhibitory activity on histamine-induced tracheal contraction in guinea-pigs and on histamine release induced by A-23187 in rat mast cells were observed. These results indicate that some saikosaponins might be useful in treatment of allergic asthma.[88]

Mode of action

Generally, it is very difficult to attribute the pharmacological activity of a multi-component mixture, as in plant extracts consisting of a diversity of secondary metabolites, to only a single compound of an extract.[125] Secondary metabolites are able to interfere with many molecular targets in the cells and in the following section we will try to distinguish the main molecular targets of secondary metabolites isolated from the genus *Bupleurum* in order to understand their modes of action.

The major types of molecular target in eukaryotic cells that are relevant in this context include the biomembrane, proteins and nucleic acids (DNA, RNA) as summarized in Figure 2.

Some of the isolated secondary metabolites from *Bupleurum* act directly on the biomembrane, such as the most common triterpene saponins (saikosaponins), the sterol fraction, the polyacetylenes and the small lipophilic molecules from essential oils. Both saikosaponins and sterol glycosides are amphiphilic molecules that function as detergents and exist mostly in their monodesmosidic forms. The monodesmosides are anchored with their lipophilic moiety in the lipophilic membrane bilayer after complexing with cholesterol, while the hydrophilic sugar part remains outside the cell and can interact with other glycoproteins or glycolipids (Figure 3). As a result, loss of the membrane integrity and fluidity occurs with the subsequent leakage of many polar molecules out of the cells or the entry of unwanted molecules into a cell. Therefore, many saponins are cytotoxic against a wide range of cells (cancer, bacteria and fungi).[24,126,127]

In addition, other lipophilic secondary metabolites such as mono- and sesquiterpenes, which are the main essential oil components, can dissolve in biomembranes resulting in disturbance of the close interaction between membrane lipids and proteins thus changing the conformation of membrane proteins. These membrane proteins include ion channels, trans-

porters for nutrients and intermediates, receptors, and proteins of signal transduction, and the cytoskeleton.[126] A change of protein conformation usually leads to a loss of function. Moreover, at higher concentrations, these secondary metabolites interact with the lipophilic inner core of biomembranes represented by fatty acids and cholesterol leading to disturbance of the membrane fluidity as shown in Figure 3. This type of interaction between the secondary metabolites and the biomembranes could explain the antimicrobial, antiviral and spasmolytic effects of many *Bupleurum* preparations.

Proteins represent the most important molecular targets of many secondary metabolites. Proteins have multiple functions in a cell, ranging from enzymes, transporters, ion channels, receptors, microtubules, histones to regulatory proteins (signal molecules, transcription factors etc.). Many secondary metabolites (such as coumarins, lignans, phenylpropanoids etc.) interact with proteins unselectively through formation of either noncovalent or covalent bonds that, in turn, interfere with the protein conformation leading to loss of activity in most cases.[125,126,128]

Phenolics are a major class of secondary metabolite present in all species of the genus *Bupleurum* in the form of phenylpropanoids, flavonoids, coumarins and lignans. These compounds are characterized by possessing one or more phenolic hydroxyl groups. The phenolic hydroxyl groups can partly dissociate under physiological conditions resulting in O^- ions. The polyphenols interact with proteins by forming hydrogen bonds with electronegative atoms of the peptide or ionic bonds with positively charged side chains of basic amino acids, respectively.[125,126] A single of these noncovalent bonds would be quite weak but because several of them are formed concomitantly the effect is much stronger and a change in protein conformation is likely to occur, which then may lead to protein inactivation. However, the formation of covalent bonds can also occur as shown in Figure 4.

Several types of secondary metabolite carry reactive substituents like epoxide, aldehyde, triple bonds or exocyclic methylene groups that can covalently bind to amino and sulphydryl groups of proteins.[129] This alkylation also leads to a conformational change and thus loss of activity. Secondary metabolites of *Bupleurum* with such properties are polyacetylenes with their reactive triple bonds and aldehyde-containing monoterpenes. This type of interaction can explain the inhibition of many enzymes such as lipoxygenases and cyclooxygenases and hence, the anti-inflammatory, immunomodulatory and hepatoprotective activity of many *Bupleurum* extracts can be rationalized. Furthermore, the presence of flavonoids and other phenolics with their radical scavenging properties may assist in reducing the oxidative stress inside the cell by direct quenching of the unwanted free radicals with their damaging role in many serious diseases like cancer, atherosclerosis and inflammation.

Some secondary metabolites probably exhibit more selective modes of action that are usually related to a particular kind of molecule rather than the whole extract. For example, many lignans with their high structural similarity to podophyllotoxin exhibit strong anti-miotic and anti-tumour effects by inhibiting microtubule formation in addition to inhibiting topoisomerase and thus blocking cell division in the late G2 phase.
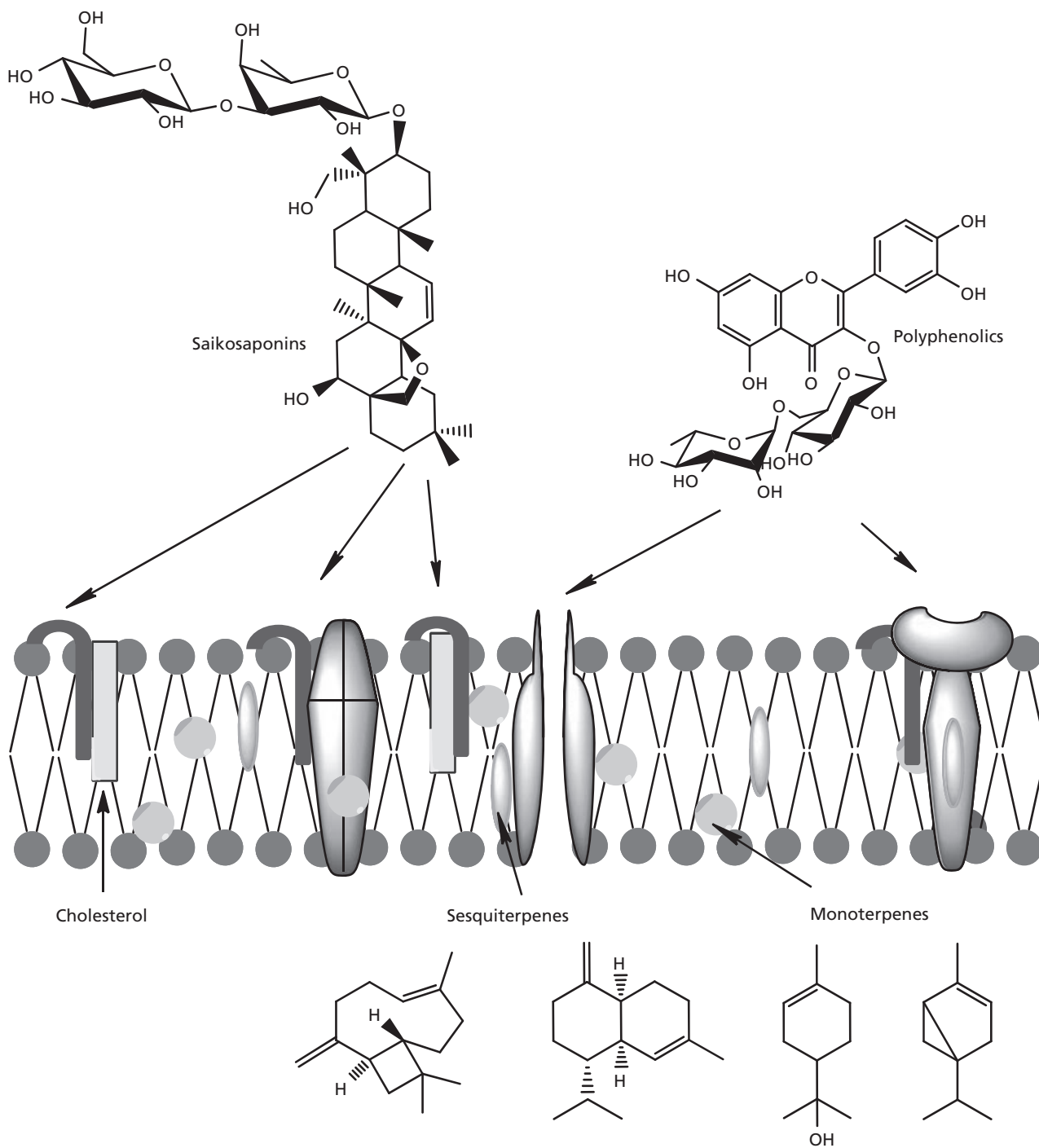
Figure 3   Interactions of some representatives compounds from *Bupleurum* with the cell membrane and membrane proteins.

Conclusions

The genus *Bupleurum* provides many efficacious herbal drugs that contain different lead compounds with a wide variety of biological effects. Substantial information on chemical and biological aspects is available but many species have not been evaluated for their biological activity and their chemical compositions remain to be investigated. Our chemical survey indicates the presence of new saponins and lignans with potential

therapeutic activity, waiting to be explored. Many dated reports on biological and medicinal properties refer to *Bupleuri* radix, which comprises many different species. Considering the differences in the chemical compositions between different species or even the same species from different localities, we can assume the pharmacological properties to vary quite significantly. Therefore, authentication of all the drugs should be undertaken carefully by chemical fingerprinting or DNA barcoding to ensure the quality of the drug and
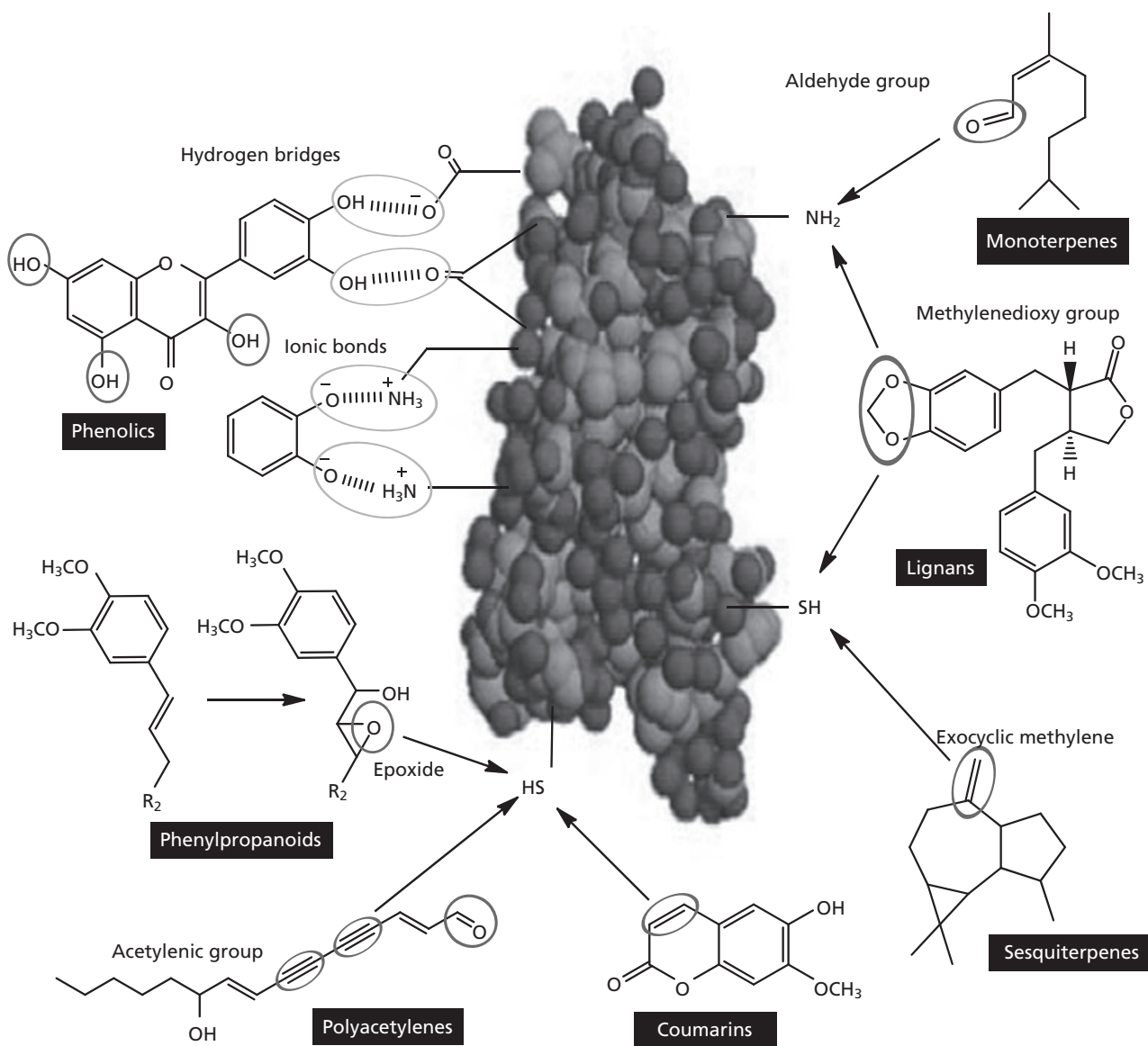
Figure 4   Possible protein conformational changes due to formation of covalent bonds with plant secondary metabolites.

hence the corresponding biological activity. Finally, studies on standardization of the drug and preclinical trials are required for an integration and acceptance of many *Bupleurum* extracts in conventional medicine.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1 Chemical structures of the isolated triterpene saponins.
Table S2 Chemical structures of the isolated sterols.

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