Prospects of Boswellic Acids as Potential Pharmaceutics

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Abstract

Boswellic acids have long been considered the main bioactive components of frankincense, and many studies *in vitro* and in animals as well as several clinical studies have confirmed their various bioactivities. In particular, a large number of mechanistic studies have confirmed their anti-inflammatory and antitumor activities. However, not every boswellic acid exhibits a satisfactory pharmacological performance, which depends on the chemical structure and functional groups of the acid. To enhance the pharmacological values of boswellic acids, derivatization has been specifically applied with the aim of discovering more active derivatives of BAs. In addition, the preliminary pharmacokinetic studies of these compounds using various standard methods show their poor bioavailability in humans and rodents, which has led to questions of their pharmacological relevance and potentially limits their use in clinical practice and pharmaceutical development. To improve these effects, some approaches have shown some improvements in effectiveness, and the new formula compatibility approach is considered a very reasonable method for improving the bioavailability of boswellic acids.

Abbreviations

AKBA:	3-acetyl-11-keto-β-boswellic acid
Akt:	serine/threonine kinase
α-ABA:	3-acetyl-α-boswellic acid
β-ABA:	3-acetyl-β-boswellic acid
APCI:	atmospheric pressure chemical ionization
API:	atmospheric pressure ionization
ATM:	ataxia-telangiectasia mutated
BA:	boswellic acid
α-BA:	α-boswellic acid
β-BA:	β-boswellic acid
BA145:	3-O-n-butyryl-11-keto-b-boswellic acid
BA-PC:	a complex of boswellic acid prepared with phosphatidyl-choline
BCDD:	butyl2-cyano-3,11-dioxours-1,12-dien-24-oate
BKBA:	butyryloxy-11-keto-β-boswellic acid
BOBA:	3-α-butyryloxy-β-boswellic acid
BSE:	*Boswellia serrata* extract
catG:	cathepsin G
C–KβBA:	3-cinnamoyl-11-keto-β-boswellic acid
C_{max}:	maximum concentration
CEMB:	2-cyano, 3-enone of methyl boswellates
COX-1:	cyclooxygenase-1
COX-2:	cyclooxygenase-2
CXCR4:	CXC chemokine receptor type 4
CYP3A4:	cytochrome P450, family 3, subfamily A, polypeptide 4
DNA:	deoxyribonucleic acid
EGFR:	epidermal growth factor receptor
ERK2:	signal-regulated kinase 2
ESI:	electrospray ionization
GC-NICI/MS:	gas chromatography-negative ion chemical ionization-mass
HL-60 cell:	human promyelocytic leukemia cells
HLE:	human leukocyte elastase
HLM:	human liver microsomes
HKBA:	hexanoyloxy-11-keto-β-boswellic acid
HMEC:	human microvascular cell
HPTLC:	high-performance thin-layer chromatographic
Hsp:	heat shock protein-90
IBD:	inflammatory bowel disease
KBA:	11-keto-β-boswellic acid

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5-LOX:	5-lipoxygenase
LCMS:	liquid chromatography-tandem mass spectrometry
LPS:	lipopolysaccharide
LTB_4:	leukotriene B4
LT:	leukotriene
MAPK:	mitogen-activated protein kinase
MeSH:	medical subject headings
miRNA:	microRNA
MMP:	matrix metalloproteinase
mPGES1:	microsomal prostaglandin E2 synthase-1
MRP2:	multidrug resistant protein
mTOR:	mammalian target of rapamycin
NF-κB:	nuclear factor κB
NO:	nitric oxide
OA:	osteoarthritis
OATP1B3:	organic anion transporting polypeptides, member 1B3
PARP:	poly ADP-ribose polymerase
PBCEC:	porcine brain capillary endothelial cells
PGE2:	prostaglandin E2
Pgp:	P-glycoprotein
PI3K:	phosphoinositide-3-kinase
PKBA:	propionyloxy-11-keto-β-boswellic acid
p12-LO:	platelet-type 12-lipoxygenase
PMNL:	polymorphonuclear leukocytes
POBA:	3-α-propionyloxy-β-boswellic acid
PUMA:	p53 upregulated modulator of apoptosis
RLM:	rat liver microsomes
RNA:	ribonucleic acid
ROS:	reactive oxygen species
SAR:	structure-activity relationship
STAT:	signal transducer and activator of transcription protein
tid:	*ter in die* (Latin: three times a day)
2TNF-α:	tumor necrosis factor-α
TPD:	triterpenediol
VCAM-1:	vascular cell adhesion molecule-1
VEGF:	vascular endothelial growth factor
VLB:	human lymphocytic leukaemia cell line
Wnt:	Wingless-Int

Introduction

Frankincense has been considered throughout the ages to have a wealth of medicinal supporting properties. It has been widely used in India, China, and African countries, and has become popular in Western countries in the last two decades. Frankincense is the common name given to oleogum resins of *Boswellia* species (Burseraceae: approximately 20 species) [1,2]. In fact, the most important frankincense-producing species are *Boswellia serrata* in Northwestern India and *Boswellia carterii* in Africa (Northern Somalia, Sudan, Eritrea, and Ethiopia). Large amounts of experimental data from *in vitro* studies and animal models support the potential of different extracts or constituents from frankincense for the treatment of various diseases, and BAs are considered the most bioactive constituents of frankincense. Thus, BAs and their bioactivities, including their principal activities, i.e., anti-inflammatory and antitumor properties, as well as many other pharmacological activities, such as antiulcer, immunomodulatory, hypolipidemic, antibacterial, and antiobesity effects [3–11], have become major research priorities. Based on this background, extracts from frankincense and BAs have been considered a promising alternative based on several clinical trials, including trials for the treatment of IBD, Crohn's disease, ulcerative colitis [12,13], osteoarthritis [14–16], rheumatoid arthritis [17], asthma [18], photoaged skin [19], and peritumoral brain edema [20]. In addition, BAs-rich extracts from *B. serrata* have been introduced into medicinal commodities, such as H15 [20], 5-Loxin [14], and Aflapin [15,21]. Moreover, BSE was assigned orphan drug status in 2002 by the European Medicines Agency for the treatment of peritumoral edema.

The anti-inflammatory and antitumor activities of BAs are the most extensively researched activities in experimental and clinical studies [4,22], and different functional groups have the potential to influence the pharmacological mechanisms of BAs [23, 24]. Multiple mechanisms underlying their anti-inflammatory, antitumor, and other biological activities mainly contribute to AKBA, thereby resulting in the enrichment of AKBA in medicinal commodities, such as 5-Loxin (BSE enriched with 30% AKBA) [14] and Aflapin [21] (BSE enriched with at least 20% AKBA). Thus, many research studies have attempted to increase the diversity of the biological activities of other BAs, such as KBA and β-BA, which also have strong anti-inflammatory but poor antitumor activity. However, various pharmacokinetic studies using different standard analytical methods (**Table 1**) have revealed the low bioavailability of BAs, particularly AKBA and KBA, in human and animals, which results in questions regarding their pharmacological relevance for *in vivo* bioactivities. Despite the widespread use of preparations from frankincense, only limited studies have attempted to resolve the poor bioavailability of BAs. Nevertheless, the sparse approaches that have addressed this issue indicate that certain improvements have been achieved, particularly in the application of new formulas to enhance the pharmacological activities by increasing the availability of BAs in the plasma and brain [25,26].

In this review, we provide the first summary of the more active derivatives from different BAs. In addition, the available pharmacokinetic studies and approaches for enhancing the bioavailability of BAs are summarized exhaustively and systematically.

Methods

Data sources and search strategy

Primary studies were identified by searching electronic databases (PubMed, Web of Science, Wiley, and Medline), and the search terms (frankincense, boswellic acids, *Boswellia* extract, derivatives, pharmacokineticsm and systematic reviews) were mainly determined using a combination of MeSH and free-word terms in the individual databases. The results were initially screened by title to exclude any obviously irrelevant articles, and the potential hits were downloaded into NoteExpress 2 files. No language restrictions were applied. Publications up to the end of April 2014 were included in the review.

Selection criteria and data collection

The abstracts of the selected articles were examined independently by two reviewers who applied the selection criteria. All possible articles were retained, and their full text was critically

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Table 1 Plasma and brain concentrations of boswellic acids determined in different studies.

Dosage of BSE	Methods	Concentrations of BAs determined in plasma (μM) or brain* (ng/g)						Ref.
		β-BA	α-BA	β-ABA	α-ABA	KBA	AKBA	
1600 mg/day (n = 1)[H]	HPLC[a,h]	NA	NA	NA	NA	1.7	NA	[80]
333 mg/day for 7 d (n = 12)[H]	HPLC[b,h]	NA	NA	NA	NA	2.72 ± 0.18	ND	[81]
4 × 786 mg/day for 10 d (n = 1)[H]	HPLC[c]	10.1	3.5	2.4	4.0	0.3	0.1	[82]
3 × 800 mg/day for 4 wk (n = 3)[H]	HPLC-ESI/MS[b,e]	6.35 ± 0.52	NA	4.9 ± 0.5	NA	0.3 ± 0.1	0.04 ± 0.01	[41]
4200 mg/day (n = 14)[H]	HPLC-ESI/MS[d]	[0.19–26.20]	[0.08–10.59]	[0.26–12.31]	[0.14–5.99]	[0.01–0.52]	[0–0.03]	[83]
240 mg/kg (n = 3)[R]	HPLC-ESI/MS[f,h]	2.33 ± 1.02	1.19 ± 0.63	1.65 ± 0.11	0.48 ± 0.06	0.38 ± 0.15	0.33 ± 0.16	[84]
240 mg/kg (n = 6)[R]*	HPLC-ESI/MS[g]	1066.6	485.1	163.7	43.0	11.6	37.5	
240 mg/kg (n = 9)[R]*	HPLC-APCI/MS[h]	NA	NA	NA	NA	0.4	0.2	[85]
240 mg/kg (n = 9)[R]*	HPLC-APCI/MS[h]	NA	NA	NA	NA	99	95	
500 mg/day (n = 6)[H]	HPTLC[d]	NA	NA	NA	NA	NA	[0.05–0.13]	[87]

H = human; R = rats; NA = not analyzed; ND = not detectable; a = approximation; b = mean ± SE; c = absolute contents; d = [range]; e = steady state concentration; f = mean ± SD; g = mean; h = C_{max}

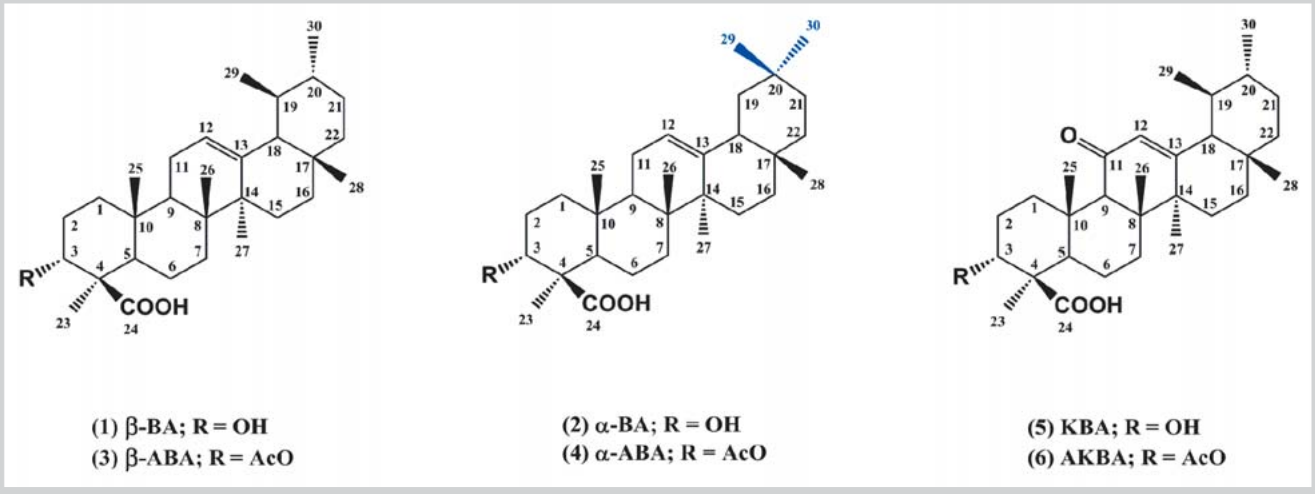

Fig. 1 Chemical structures of the main boswellic acids. The characteristic structure of α-types is marked in blue. (Color figure available online only.)

reviewed. Abstracts reporting incomplete data for evaluation were excluded. Publications with complete experiments, credible data, and a certain reputation were selected. The inclusion articles relevant to our subject (bioactive molecular targets, synthetic derivatives, pharmacokinetics, and the new technologies of preparation of BAs) were further selected.

Structure-Activity Relationships of Boswellic Acids and Synthetic Derivatives

Interaction of boswellic acids with molecular targets

BAs are generally defined as a bioactive mixture of four major beta- and two alpha-pentacyclic triterpene acids: β-BA (**1**), α-BA (**2**), β-ABA (**3**), α-ABA (**4**), KBA (**5**), and AKBA (**6**). Their structures are shown in **Fig. 1**. BAs (**Fig. 1**) exist as ursane (α) and oleanane (β) types depending on the position of two methyl groups on C-19/C-20 (α-types: geminal groups on C-20; β-types: vicinal groups on C-19/C-20). Further structural variety can be achieved by the presence or absence of a carbonyl group on C-11 and an acetoxy group or a hydroxyl group on C-3. A series of pharmacological studies indicated that β-types exert stronger bioactivities compared with the corresponding α-types [27]; moreover, different functional groups have a potential impact on the pharmacological activities of BAs.

BAs are pharmacologically active ingredients of frankincense with anti-inflammatory properties. Through a series of *in vitro* experiments, KBA and AKBA, possessing an active carbonyl group at C-11, have long been considered the major contributors to the anti-inflammatory effect of frankincense. KBA and AKBA are se-

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lective and non-redox inhibitors of 5-LOX, and AKBA was the most efficient BA tested, with IC_{50} values of 1.5–50 µM (concentration required for 50% inhibition of 1.5 µM). In addition, BAs that lack the 11-keto function inhibited 5-LOX incompletely and exhibited maximal inhibitory activity against 5-LOX (60% inhibition) at concentrations of approximately 15 µM, and this maximal activity could not be augmented even with an increase in concentration up to 50 µM [8, 23, 28–32]. SARs have shown that the 11-keto function is essential for 5-LOX activity, whereas the acetoxy group on position C-3 increases the affinity of AKBA to its effector site [23]. In addition to the intervention with LTs via the inhibition of 5-LOX, AKBA and KBA possess potential cyclooxygenase-1 inhibitory activity (AKBA: IC_{50} = 6 µM; KBA: IC_{50} = 14 µM), but β-BA and β-ABA showed weak inhibitory activity (IC_{50} > 100 µM) [33]. AKBA and KBA are able to activate p38 MAPK and p42/44 MAPK and stimulate Ca^{2+} mobilization in PMNL [34, 35]. In addition, AKBA is able to decrease the activity of HLE (IC_{50} = 15 µM) [36] and inhibit p12-LO (IC_{50} = 15 µM) and is directly bound to this enzyme *in vitro* [37]. 5-Loxin (standardized BSE enriched with 30% AKBA) was found to prevent MMPs and the inducible expression of mediators of apoptosis of TNF-α-inducible genes, resulting in anti-inflammatory effects, and completely abrogated the TNF-α-induced VCAM-1 expression in HMECs [38]. Obviously, compared with KBA, AKBA has an acetoxy group at C-3, which may increase the above activities.

The analysis of the relevant anti-inflammatory activity showed that, in comparison to AKBA and KBA, β-BA possessing a 3-hydroxyl function not only evoked moderate Ca^{2+} mobilization and activated p38 MAPK but also induced the phosphorylation of ERK2 and Akt [39]. β-BA acts selectively in part via the inhibition of PGE2 by directly and functionally interfering with mPGES1 in human whole blood (IC_{50} = 3 µM), but AKBA and KBA fail to suppress PGE2 synthesis [40]. β-BA is able to suppress LPS activity (IC_{50} = 1.8 µM) directly, whereas KBA and AKBA, possessing an active C-11 carbonyl group, and β-ABA, lacking a C-11 carbonyl group but possessing a C-3 acetoxy group, failed in this regard. The SAR indicated that the C-3 acetoxy group and the C-11-keto moiety may hamper the bioactivity, and the C-3 hydroxyl function may be a determinant for the inhibition of LPS [24]. Interestingly, β-ABA (IC_{50} = 1.2 µM) was shown to be equipotent to β-BA (IC_{50} = 0.8 µM) for the reduction of catG activities, whereas KBA (IC_{50} = 3.7 µM) was less potent, and catG was suppressed by AKBA at the lowest concentration (IC_{50} = 0.6 µM) [41].

AKBA possesses antiproliferative and apoptotic effects in various tumor cells by increasing caspase-8, caspase-9, and caspase-3 activities accompanied by the cleavage of PARP [42–44], activation of the PI3K/Akt pathway, and inhibition of the PI3K pathway [45]. AKBA is able to enhance apoptosis and inhibit the growth, metastasis, and angiogenesis of tumors. It was also correlated with the potential inhibition of the NF-κB system [46–48], downregulation of the expression of COX-2, MMPs, CXCR4, and VEGF [47–51], and the modulation of the Wnt/β-catenin [52], EGFR, and ATM/P53 signaling pathways [53]. AKBA has also been found to bind and inhibit topoisomerases I and Iiα [54]. With the application of gene chip technology, a panel of genes from diverse functional groups was significantly associated with sensitivity to β-BA and α-BA, as well as transcription factors and signal transduction [55]. β-BA, β-ABA, KBA, and AKBA were examined for their antitumor activity *in vitro*, and it was found that these inhibit the synthesis of DNA, RNA, and protein in human leukemia HL-60 cells in a dose-dependent manner with IC_{50} values ranging from 0.6 to 7.1 µM [56, 57]. In particular, AKBA induces the most pronounced inhibitory effects on DNA, RNA, and protein synthesis with IC_{50} values of 0.6, 0.5, and 4.1 µM, respectively [56]. In addition, AKBA exerts its antitumor effect in CRC cells by modulating specific miRNAs within the let-7 and miR-200 miRNA families [58] and demethylating and reactivating methylation-silenced tumor suppressor genes [59]. Moreover, AKBA also possesses potential antimicrobial [10, 11, 59, 60], antiulcer [50, 61], and lipolysis-inducing activities [9]. The other BAs exhibit weaker effects or even fail to present most of the aforementioned activities.

New synthetic boswellic acids analogues/derivatives

Despite KBA and β-BA possessing multiple mechanisms of anti-inflammatory activity, other pharmacological activities, especially the antitumor effect, were unsatisfactory. With the purpose of enhancing and extending more extensive activities of BAs, especially as KBA, β-BA, and the α-type of BAs, applications of chemical modification and biotransformation have been considered as a possible mean of transformation of BAs for bioactive derivatives. Structures of selected screened maternal and relevant derivatives are shown in **Fig. 2** (analogues/derivatives of α- or β-BA), **Fig. 3** (analogues/derivatives of KBA), and **Fig. 4** (analogues/derivatives of α-ABA and AKBA).

α- and β-Boswellic acid analogues/derivatives

β-CEMB (**7**) and α-CEMB (2-cyano, 3-enone of methyl boswellates) (**8**) are modified from β-BA and α-BA and show potent cytotoxic (a number of cancer cell lines with IC_{50}s ranging from 0.2 to 0.6 µM), growth inhibition, anti-inflammatory, and pro-differentiative activities. CEMB inhibited DNA synthesis and induced apoptosis in A549 cells (lung carcinoma cell line) at concentrations of 0.25 µM and 1 µM, respectively, and this action is mediated by caspase-8 [62].

POBA (**9**) is the propionyloxy derivative of β-BA and exhibits significant cytotoxic activity on a wide range of human cancer cell lines via potential suppression of the PI3K pathway [63].

BOBA (**10**) is the butyryloxy derivative of β-BA and provides evidence of PI3K-mediated apoptosis. This novel compound was developed in an attempt to discover anticancer therapeutics and has the potential to target the mitochondria-dependent pathway, NF-κB, and the DNA repair system in apoptosis [64].

3β,24-dihydroxyolean-12-ene (**11**) and 3α,24-dihydroxyurs-12-ene (**12**) are obtained by the reduction reaction from β-BA and α-BA. TPD, which is a mixture composed of **11** and **12**, produced oxidative stress in prostate cancer cells that triggers self-demise by the ROS- and NO-regulated activation of both the intrinsic and extrinsic signaling cascades [65].

The rhein-boswellic acid conjugate **13** is synthesized chemically by two molecules of α-BA and one molecule of rhein through a bioreversible ester linkage [66]. The results of the evaluation of the disease-modifying effects of the conjugate in collagenase (type-II)-induced OA in Wister rats revealed significant antiarthritic and antiulcer activities [67].

4-Amino analogues **14** and **15** were screened and prepared from β-BA, wherein the carboxyl group in the ursane nucleus was replaced by an amino function via the Curtius reaction. Compound **14** showed cytotoxicity (80–96%) on prostate (DU-145) and colon (SW-620 and 502 713) cell lines, and compound **15** showed cytotoxicity (73–99%) against prostate (DU-145) and colon (SW-620, 502 713, HT-29) cell lines [68].

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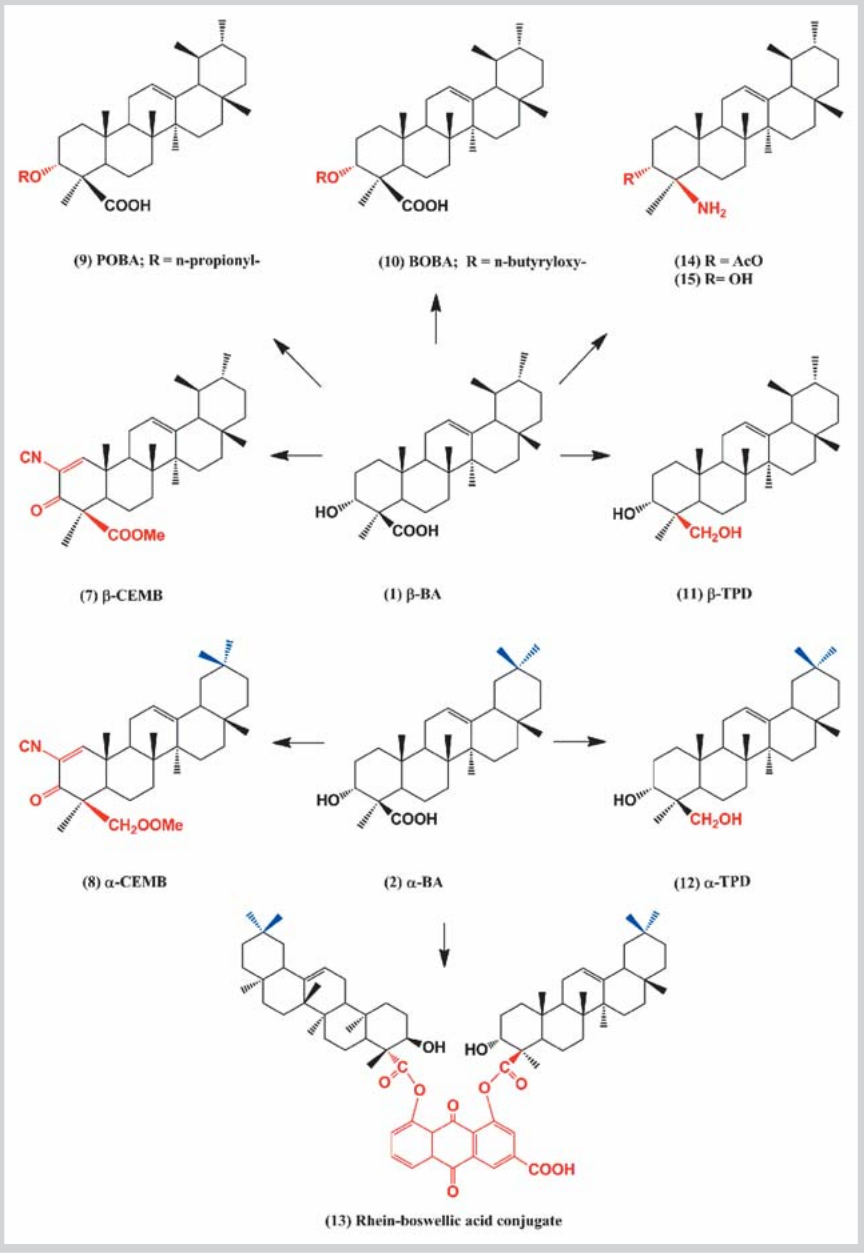

Fig. 2 Chemical structures of α- and β-boswellic acid analogues/derivatives. The characteristic structure of α-types is marked in blue. The introduced functional groups and their corresponding positions are marked in red. (Color figure available online only.)

11-Keto-β-boswellic acid analogues/derivatives

4-Amino analogues **16** and **17** were screened and prepared from KBA using the method mentioned above [68]. Compound **16** showed cytotoxicity (72%) against colon cell lines (SW-620), and compound **17** showed cytotoxicity (71–94%) against prostate (DU-145) and colon (SW-620, 502 713, and HT-29) cell lines. Furthermore, the results of the analysis of the cytotoxicity of analogues **14, 15**, and **17** at a concentration of 1 µM indicated that compound **17** is the most promising analogue that displays significant activity. Compounds **14** to **17** display improved cytotoxicity compared with the parent compound and also exhibit apoptotic activity by inducing DNA fragmentation.

An endoperoxide (**18**) was synthesized from KBA. It has been shown that this compound is an initiator of programmed cell death and has shown high antitumor activity against a panel of 15 human cancer cell lines. In fact, compound **18** induces apoptosis with an average IC_{50} value of 0.4–4.5 µM [69].

BCDD (**19**) is a cyano derivative of KBA and has been found to be highly cytotoxic against human papillomavirus (HPV)-positive cervical cancer cells, HeLa (HPV18) and SiHa (HPV16) cells. The detailed mechanism of the cytotoxicity in HeLa cells suggests that BCDD causes apoptosis, regulates the expression of p53/PUMA/p21 and affects other cell survival signaling events [70]. Moreover, it inhibits PI3K/AKT activity and enhances NF-κB and Hsp-90 in HL-60 cells, which may increase the sensitivity of cells to apoptosis [71].

HKBA (**20**), BKBA (**21**), and PKBA (**22**) are active derivatives synthesized from KBA [72, 73]. The IC_{50} values obtained for these compounds in the induction of apoptosis in HL-60 cells have been found to be 4.5, 7.7, and 8.7 µg/mL, respectively. In particular, HKBA presents better antiproliferative and proapoptotic potential against the HL-60 cell line than the previously known boswellic acids due to the inhibition of topoisomerases I and II [72].

BA145 (**23**) was synthesized from KBA and was screened as a promising lead that exhibited cytotoxicity against various human

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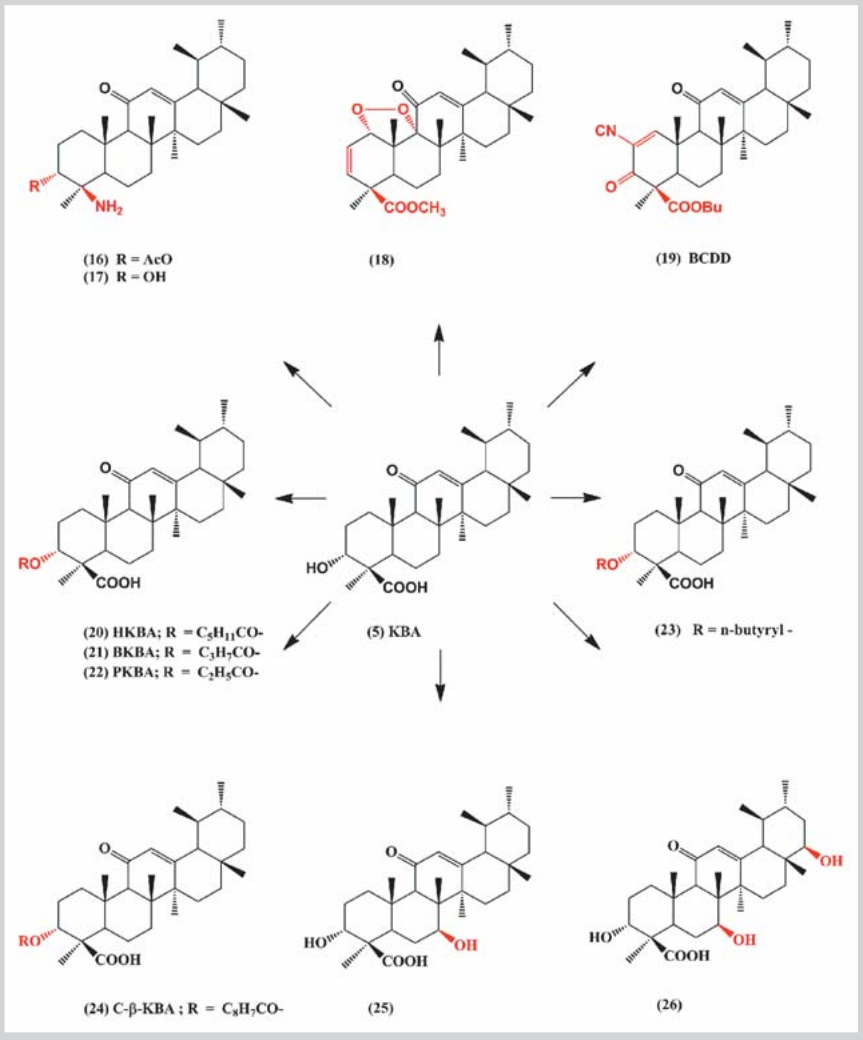

Fig. 3 Chemical structures of 11-keto-*β*-boswellic acid analogues/derivatives. The introduced functional groups and their corresponding positions are marked in red. (Color figure available online only.)

cancer cell lines by inhibiting the NF-*κ*B and STAT family of proteins [74]. A further study indicated that apoptosis induced by BA145 in HL-60 cells is completely dependent on caspases, and the MEK1/2 inhibitor PD98059 and the PI3K inhibitor LY294002 protect HL-60 cells by diminishing the activation of pre-executioner caspases-8 and -9 by BA145 [75].

C–K*β*BA (**24**) was synthesized from KBA and exhibited specific antiproliferative and proapoptotic effects in cancer cell lines *in vitro* and in PC-3 prostate cancer xenografts *in vivo*, even at low concentrations and after a single, rather short exposure. These effects have been attributed to its inhibition of the mTOR signaling pathway [76].

7-*β*-hydroxy-11-keto-*β*-boswellic acid (**25**) and 7*β*,22-*β*-dihydroxy-11-keto-*β*-boswellic acid (**26**) were screened from metabolites of the biotransformation of KBA by *Cunninghamella blakesleana* and exhibited IC_{50} values of 25.8 and 12.5 µM, respectively, by the stronger inhibition of NO production induced by LPS without decreasing cell viability in RAW 264.7 macrophages [77].

3-Acetyl-*α*-boswellic acid analogues/derivatives

3*α*-Acetyl-11-keto-*α*-boswellic acid (**27**) was synthesized by a radical-type reaction using bromine and *α*-ABA in an *α*-configuration in contrast with AKBA. 3*α*-Acetyl-11-keto-*α*-boswellic acid triggers apoptosis in chemoresistant and androgen-independent cancer cells *in vitro* and *in vivo* by the activation of caspase 3 and the induction of DNA fragmentation [78].

3-Acetyl-11-keto-*β*-boswellic acid analogues/derivatives

7-*β*-Hydroxyl-3-acety-11-keto-*β*-boswellic acid (**28**) and 7-*β*-16*α*-dihydroxy-3-acety-11-keto-*β*-boswellic acid (**29**) were screened from metabolites of the biotransformation of AKBA by *C. blakesleana* and exhibited IC_{50} values of 1.5 and 10.5 µM, respectively, compared with the IC_{50} value of 15.6 obtained for AKBA, due to the stronger inhibition of NO production induced by LPS and cytotoxicity in RAW 264.7 macrophages [79].

The above-described derivatives demonstrate considerable bioactivities, particularly antitumor activity, and are even stronger than their parents. As shown in ▶ **Fig. 2** to **4**, the addition of functional groups has mainly focused on the transformation of the hydroxyl group at C-3 and the carboxyl group at C-24, and the other nonfunctional groups of the carbon atoms were added to a hydroxyl group. Other advantages are that the simultaneous screening of derivatives can provide the active sites and selectable bioactive target structures. Investigations of the safety, pharmacokinetics, and other bioactivities of the derivatives remain to be conducted in the future.

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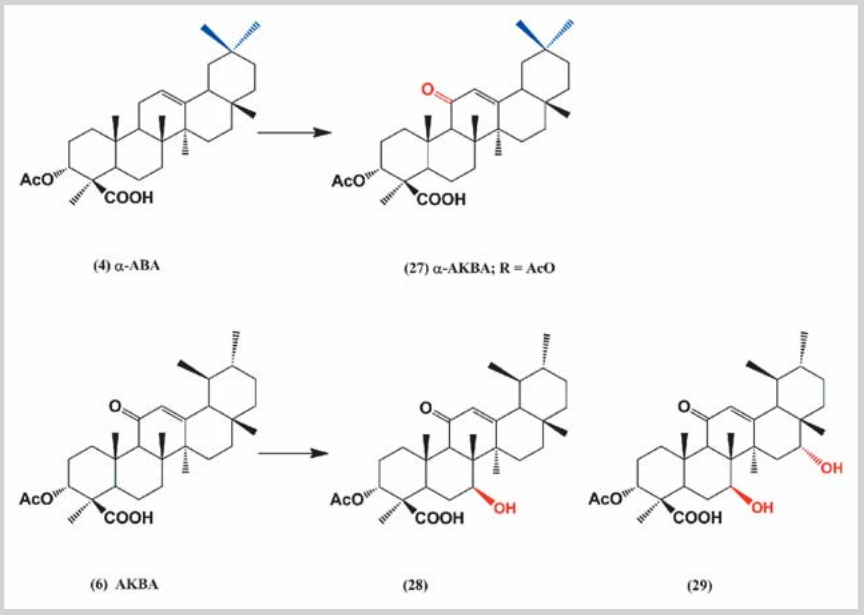

Fig. 4 Chemical structures of 3-acetyl-α-boswellic acid and 3-acetyl-11-keto-β-boswellic acid analogues/derivatives. The characteristic structure of α-types is marked in blue. The introduced functional groups and their corresponding positions are marked in red. (Color figure available online only.)

Available Pharmacokinetic Studies of Boswellic Acids

Despite the widespread use of preparations from frankincense and BAs, it is important to emphasize that the pharmacokinetic properties of a drug are very important for exerting an effective role *in vivo*. Pharmacokinetics is a subject that studies the kinetic process of absorption, distribution, metabolism, and excretion of drugs in the body. The concentration of the drug in the plasma and the target organ lesions is a very important pharmacokinetic parameter that can be used to measure whether the drug can achieve an effective concentration. The detection of the concentrations of BAs in the plasma and brain has been published in previous articles, and different methods have different sensitivity, accuracy, precision, and concentration ranges. In this review, we summarize the different methods used for measuring the concentration of BAs by administering different oral doses in animals (plasma and brain) and humans (plasma), and discuss the sensitivity and measurement ranges for the determination of different BAs. The determined concentrations of BAs in the plasma and brain, the subjects (animals or humans), dosage, and relational analysis methods are shown in **Table 1**.

High-performance liquid chromatography analysis

The concentrations of KBA in the plasma of a volunteer who was administered a single dose of 1600 mg of a BSE formulated as a hard gelatin capsule were determined by HPLC, and a C_{max} of approximately 1.7 µM was obtained after 1 h. A linear quantification range from 0.1 and 2.0 mg/mL for KBA in the plasma was established, and the detection limit of the method for KBA was approximately 0.040 µ/mL [80]. Twelve healthy adult men volunteers were administered the WokVel™ capsule (333 mg BSE) containing 18.51% β-BA, 6.93% α-BA, 8.58% β-ABA, 1.85% α-ABA, 6.44% KBA, and 2% AKBA for seven days, and the C_{max} of KBA determined by HPLC reached $2.72 \times 10^{-3} \pm 0.18$ µM after 4.55 ± 0.55 h [81]. The plasma levels of a patient with a brain tumor treated for ten days with a dose of BSE (4 × 786 mg/day) containing 19.22% β-BA, 13.78% α-BA, 10.04% β-ABA, 3.37% α-ABA, 6.66% KBA, and 3.81% AKBA were analyzed. The absolute contents of β-BA, α-BA, β-ABA, α-ABA, KBA, and AKBA were determined by HPLC to be 10.1 µM, 3.5 µM, 2.4 µM, 4.0 µM, 0.34 µM, and 0.1 µM, respectively. Linear quantification ranges of plasma levels of 0.02 to 1.1 µg/ml and 0.16 to 9 µg/ml were established for the six main BAs and the other six pentacyclic triterpenic acids [82].

Liquid chromatography-mass spectrometry analysis

LC-MS is considered a more sensitive detection method for the determination of trace amounts of BAs than HPLC. This is due to API, a type of soft ionization technique that includes APCI and ESI, which provides efficient ionization for very different molecules, including polar, labile, and high-molecular mass drugs and metabolites. The plasma concentrations of patients with Crohn's disease were measured for a period of four weeks after the administration of three soft gelatin capsules containing 800 mg of BSE by triple quadrupole LC-ESI/MS. β-ABA, β-BA, KBA, and AKBA were found at concentrations of 4.9 ± 0.5 µM, 6.35 ± 0.5 µM, 0.33 ± 0.1 µM, and 0.04 ± 0.01 µM, respectively. The calibration standards were used with the following concentrations ranges: β-BA, 0.125 to 1250 ng/mL; and Aβ-BA, KBA, and AKBA, 0.013 to 125 ng/mL [41]. Fourteen patients were administered a high dose of 4200 mg of BSE each day, and the presence of β-BA, α-BA, β-ABA, α-ABA, KBA, and AKBA was confirmed by HPLC-ESI/MS at the steady plasma concentrations (ng/mL) of 87.0–11 948.5, 36.7–4830.1, 131.4–6131.3, 73.4–2985.8, 6.4–247.5, and 0–15.5, respectively. The detection range of KBA and AKBA was from 5.0 to 3000 ng/mL, and that of β-BA, β-ABA, α-BA, and α-ABA was from 0.5 to 12 000 ng/mL [83]. As determined by HPLC-ESI/MS [83], the concentrations of KBA, AKBA, β-BA, β-ABA, α-BA, and α-ABA in the rat brain 8 h after the oral administration of 240 mg/kg of BSE were 11.6 ng/g, 37.5 ng/g, 1066.6 ng/g, 163.7 ng/g, 485.1 ng/g, and 43.0 ng/g, respectively [84]. The determinations of KBA and AKBA in the rat plasma and brain following the administration of a single oral dose of 240 mg/kg BSE were performed by triple quadrupole LC-APCI/MS. In the rat plasma, KBA and AKBA were detected to reach concentrations of 0.4 µM and 0.2 µM. A concentration of 0.3 µM was detected in the brain, corresponding to 99 KBA and 95 ng/g AKBA in the brain. The linearity was obtained for the entire calibration range

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of KBA and AKBA from 5 to 1500 ng/mL in the plasma, and of KBA and AKBA from 5 to 1000 ng/mL in the brain [85].

Gas chromatography-mass spectrometry analysis

The GC-NICI/MS analytical method was applied for the quantitative determination of KBA in human plasma, and the method revealed a validated lower limit of quantification of 0.0100 mg/mL using 1 mL of plasma. The calibration curves were linear in the measured range of 10.0 to 2000 ng/mL plasma, but the plasma concentrations obtained in humans after oral administration were not analyzed [86].

High-performance thin-layer chromatography analysis

A rapid and sensitive HPTLC method was developed and validated for the quantitative estimation of BAs in formulations containing BSE and AKBA in human plasma. The limits of detection and quantification for AKBA in the human plasma were found to be 8.75 and 29.15 ng/mL, respectively, and the plasma levels of AKBA in six human volunteers 2 h after the administration of a single oral dose of a Boswell® tablet containing 500 mg of BSE were in the range of 24.02 to 67.04 ng/mL plasma [87].

Questioning the pharmacological relevance

KBA and AKBA are direct 5-LOX inhibitors that efficiently suppress 5-LOX product synthesis in isolated neutrophils (IC_{50} = 2.8 to 8.8 µM) but fail to inhibit 5-LOX product synthesis in human whole blood. Meanwhile, a single dose (800 mg) oral administration of BSE (H15) to human healthy volunteers failed to suppress the LTB_4 plasma levels [32]. Similarly, KBA and AKBA were found to significantly inhibit mPGES1 in the cell-free assay (AKBA: IC_{50} = 3 µM; KBA: IC_{50} = 10 µM), but they all failed to suppress PGE_2 formation in human whole blood and in rats [40]. Moreover, fairly low plasma levels of KBA (0.01–0.52 µM) and AKBA (0–0.03 µM) were obtained even after a high oral administration of frankincense (4200 mg). The poor bioavailabilities of KBA and AKBA have limited their pharmacological efficacy *in vivo* and result in questions of their pharmacologically relevance. Frankincense usually contains much more β-BA and β-ABA than AKBA and KBA, which correlates to the considerable plasma levels of β-BA and β-ABA (**Table 1**) [82,88], and both of them presented IC_{50} values greater than those determined for catG inhibition (β-BA: IC_{50} = 0.8 µM; β-ABA: IC_{50} = 1.2 µM) [41]. β-BA results in the greatest inhibition of PGE_2 (IC_{50} = 3 µM) and LPS (IC_{50} = 1.8 µM), which implies the interaction of β-BA with catG and indicates that PGE_2 and LPS may be reasonable molecular targets related to anti-inflammatory activity.

Exploration of poor bioavailability

The possible factors responsible for the poor pharmacokinetics of BAs have also been investigated in several studies. A previous study revealed that the very low bioavailabilities of KBA and AKBA after the oral intake of *Boswellia* extract may be attributed to an altered absorption of these acids because they are substrates for Pgp in VLB and PBCEC cells, whereas β-BA, β-ABA, α-BA and α-ABA without a keto group have been found to not be subject to the efflux activity of Pgp [89]. The study of the metabolism of BAs in RLM, hepatocytes, and HLM showed that KBA induces the cells to strongly undergo extensive phase I metabolism, whereas AKBA does not [90]. In addition, β-BA also induces extensive phase I metabolism in HLM and RLM, but α-BA is extensively phase I metabolized in HLM but not in RLM, whereas the acetylated α-ABA, β-ABA, and AKBA are rather metabolically stable. The acetyl group at position 3 appears to impede phase I metabolism. All six BAs are not subjected to phase II metabolism; hence, the extensive phase I metabolism of KBA may be the main reason for its low plasma concentration [84]. The observed extensive phase I metabolism of KBA strongly contributes to its low bioavailability. The study of the permeation of KBA and AKBA in the Caco-2 model showed the poor permeability of AKBA and the moderate absorption of KBA and revealed that the poor absorption of AKBA from the gastrointestinal tract may contribute to its low bioavailability. In addition, this study suggested that the Pgp inhibition may be attributed to KBA and AKBA being allosteric inhibitors [91], rather than the Pgp inhibition of KBA and AKBA, as mentioned above, which is due to Pgp substrates [89]. Because the Caco-2 model is adapted to physiological conditions, the permeability of AKBA is improved compared with the results described above, and the explanation for this is assumed to be that the greater distribution of AKBA in different compartments may contribute to its low bioavailability [84]. Further investigations of the permeability-related hurdles in the oral delivery of KBA include the gastrointestinal instability, the CYP3A4-mediated intestinal metabolism, the accumulation within the enterocytes and saturable kinetics [92].

It is remarkable that their poor bioavailability has limited their practical and effective role *in vivo* because the most bioactive interactions of BAs are observed at relatively high concentrations *in vitro*.

Bioavailability Enhancement of Boswellic Acids

For a long time, AKBA and KBA have been believed to be responsible for the pharmacological activities of frankincense. However, in view of their fairly low plasma and brain levels and in consideration of their failure to inhibit 5-LOX in whole blood, the suppression of LTB_4 synthesis *in vivo* by administered frankincense extracts is questionable. Only β-BA, as the best inhibitor of PGE2 and LPS, has considerable bioavailability with a high content in frankincense. To make better use of all the potential pharmacological properties of different BAs based on the presented background, we summarize some of the approaches that have been used to address this problem.

Administering with a standardized meal

Some scholars have attempted to research how to resolve the problem of the poor bioavailability of BAs. The effect of food intake on bioavailability was analyzed, and the maximum concentrations of BAs in human plasma are presented in **Table 2**.

The plasma concentrations of BAs in 12 male volunteers who were administered a single dose of three BSE-018 capsules (262 mg of dry BSE containing 18.2% β-BA, 13.2% α-BA, 10.5 β-ABA, 3.3% α-ABA, 6.1% KBA, and 3.7% AKBA/capsule) in the fasted state and in a standardized high-fat meal (fed state) were determined by HPLC. The concomitant food intake significantly increased the mean area under the concentration-time curve and enhanced the plasma concentrations of β-BA, KBA, and AKBA in comparison to the starved volunteers. The pharmacokinetic profile of BSE-018 greatly varied with food intake, an effect that is likely due to the improved absorption of BAs in the presence of bile acids [93]. The plasma concentrations of KBA and AKBA in 15 male volunteers who were administered a single dose of two Boswelan capsules (400 mg of BSE containing 2.77% KBA and 2.64% AKBA/capsule) in the fasted and fed conditions were deter-

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Table 2 Maximum plasma concentrations of boswellic acids determined in human plasma under fasted and fed conditions.

Dosage	Methods	Condition	C_{max} of BAs determined in human plasma (μM)						Ref.
			β-BA	α-BA	β-ABA	α-ABA	KBA	AKBA	
3 × BSE-018 capsules/day (n = 12)	HPLC[a]	fasted	0.41 [0.09–3.96]	ND	ND	ND	0.17 [0.05–0.52]	0.01 [0.00–0.08]	[93]
		fed	2.45 [0.90–4.67]	0.69 [0.09–2.93]	ND	0.23 [0.09–0.80]	0.48 [0.21–0.88]	0.05 [0.02–0.51]	
2 × Boswelan capsules/day (n = 15)	HPLC-MS[b]	fasted	NA	NA	NA	NA	0.33 ± 0.13	0.05 ± 0.03	[94]
		fed	NA	NA	NA	NA	0.43 ± 0.20	0.06 ± 0.05	

NA = not analyzed; ND = not detectable; a = mean and [range]; b = mean ± SD

Table 3 Improvements in the bioactivities and investigatory parameters by the new formulations compared with non-formulations.

Formulation	Enhanced activities	Indicators	Conditions	$C_{plasma}^{\#}$ (μM), C_{brain}^{*} (ng/g), $Jss^{\&}$ (mg/cm² per h), and $K_p \times 10^{-2\triangle}$ (cm/h) of BAs						Ref.
				β-BA	α-BA	β-ABA	α-ABA	KBA	AKBA	
BAs-Phosphatidylcholine	Anti-inflammatory, hypolipidemic	\[R]	\	\	\	\	\	\	\	[95]
AKBA-Polymeric nanomicelles	Anti-inflammatory, anti-arthritis	Skin permeation[R]	NF[&]	NA	NA	NA	NA	NA	0.161 ± 0.035	[96]
			F[&]	NA	NA	NA	NA	NA	0.488 ± 0.036	
			NF[△]	NA	NA	NA	NA	NA	0.341 ± 0.098	
			F[△]	NA	NA	NA	NA	NA	1.036 ± 0.151	
BSE-Phospholipid and pluronic f127	\	Pharmacokinetic[R]	NF[*,a]	1066.6 ± 781.6	485.1 ± 363.8	163.7 ± 248.9	43.0 ± 55-.7	11.6 ± 12-.6	37.5 ± 56.8	[25]
			F[*,a]	5908.2 ± 2275.6	2443.9 ± 914.4	156.1 ± 82.7	60.1 ± 25.4	13.6 ± 4.2	203.8 ± 86.9	
			F vs. NF[#,b]	6-fold	6-fold	6-fold	6-fold	26-fold	14-fold	
BSE-Phytosome® (Soy lecithin)	\	Pharmacokinetic[R]	NF[*,c]	[0.0–98.2]	[241.3–380.6]	[35.5–318.9]	[39.9–93.1]	[0.0–44.9]	[0.0–45.0]	[26]
			F[*,c]	[0.0–1376.8]	[219.93–850.0]	4.5–317.7	10.9–100.9	[22.4–477.8]	[0.0–586.1]	
			NF[#,a,d]	2.2 ± 0.3	1.1 ± 0.4	5.0 ± 2.1	1.0 ± 0.2	0.6 ± 0.3	0.6 ± 0.1	
			F[#,a,d]	6.6 ± 4.1	2.2 ± 1.3	4.2 ± 2.5	1.45 ± 0.7	1.6 ± 0.6	1.2 ± 0.6	

R = rats; F = formulation; NF: non-formulation; NA = not analyzed; \ = not investigated; $C_{plasma}^{\#}$ = concentrations of BAs in plasma; C_{brain}^{*} = concentrations of BAs in brain; $Jss^{\&}$ = permeation rate at steady state; $K_p \times 10^{-2\triangle}$ = permeability coefficient; a = mean ± SD; b = the relevant literature provided only multiple comparison, there was no accurate numerical reference; c = [range]; d = C max

mined by HPLC-MS. This study confirmed the results of the study described above [93], which showed that food interacts with the bioavailability of KBA but not of AKBA. Moreover, the magnitude of the increase in the KBA plasma concentrations with food was much less pronounced. This study indicated that the *in vivo* KBA and AKBA plasma concentrations would start to increase with *in vitro* IC_{50}s for catG and 5-LOX after a significant but clinically infeasible dose increase of 10- to 15-fold Boswelan tid [94].

Although the plasma concentrations of BAs were increased when administered with a high-fat meal compared with the fasted state, as shown in ◉ **Table 2**, the problem of the low bioavailability of BAs has not been fundamentally resolved.

Administering with anionic drugs

The transporter-mediated uptake of drugs into and out of cells is another important determinant of drug disposition and bioavailability. A previous study revealed that KBA and AKBA are able to modulate the activity of organic anion transporters (OATP1B3) and the multidrug resistant protein MRP2, demonstrating that the combined administration of BAs with anionic drugs is a possible solution [91].

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New formula compatibility

The application of new formulations and formula technology to BAs has achieved some initial success in several *in vitro* and rat studies. The improvements in the pharmacological activity and investigatory parameters by the new formulations compared with non-formulations are displayed in **Table 3**.

BA-PC is a complex of BA prepared with phosphatidyl-choline. The complex shows better anti-inflammatory and hypolipidemic activity compared with BA alone, and this effect is attributed to the amphiphilic nature of BA-PC, which greatly enhances the water and lipid solubility of BAs. The combination of phospholipids with BAs provides a potential bioavailability enhancement and faster and improved absorption in the intestinal tract [95].

AKBA is loaded in the polymeric nanomicelles of N-isopropylacrylamide, N-vinylpyrrolidone, and acrylic acid, which offers the possibility of a controlled release, the protection of active substances, and nanometer sizes. In aqueous buffer (pH 7.4), the release of AKBA from the polymeric nanomicelles is 23 and 55 % after 2 and 8 h, respectively. The AKBA polymeric nanomicelle gel shows significantly enhanced anti-inflammatory and antiarthritic activities and a 3-fold increase compared with the AKBA gel containing the same amount of AKBA in *in vitro* skin permeation studies, which shows its great potential for transdermal drug delivery [96].

A chosen formulation composed of BSE/phospholipid/pluronic f127 (1 : 1 : 1 w/w/w) has been considered a basis for the production of a dispensable free-flowing powder that can be further processed into tablets or capsules. The increased solubility of KBA, AKBA, α-BA, β-BA, α-ABA, and β-ABA is approximately up to 54-fold greater compared with the non-formulated extract, and these exhibited the highest mass net flux in the permeability of the physiological Caco-2 cell model. As determined by the LC-MS method, the oral administration of this formulation to rats (240 mg/kg) resulted in 26- and 14-fold higher plasma levels of KBA and AKBA, respectively. In the brain, 5-fold higher levels of AKBA compared with the non-formulated extract were determined 8 h after oral administration [25].

Casperome™ is a formulation of BSE and Phytosome® (soy lecithin) at a 1 : 1 ratio. Compared with the non-formulated extract, Casperome™ provides significantly higher plasma levels, i.e., up to 7-fold for KBA and 3-fold for β-BA, expressed as the area under the plasma concentration-time curve. In the brain, there were increases in the concentrations of KBA and AKBA (35-fold) and β-BA (3-fold). It is noteworthy that up to 17-fold higher BA levels were observed in poorly vascularized organs [26].

The evidence described above led us to hypothesize that BAs will be further developed in the near future. Preparations have been designed to improve the bioavailability of BAs by enhancing absorption, and preliminary studies are positive.

Conclusions

The reputation of BAs as potential pharmaceutical candidates has found support in numerous studies. In particular, AKBA, as the most bioactive and representative BA, has been used as an enriched key component for the evaluation of the quality of frankincense used for the treatment of peritumoral edema [20] and therapeutic interventions against inflammatory diseases [15]. Based on potential pharmacological activities with multiple targets of BAs, we summarized various studies on the application of chemical modification and bio-derivatization approaches to different BAs for the purpose of obtaining more valuable active derivatives.

The available pharmacokinetic investigations using different analysis methods have revealed the poor bioavailability of BAs, particularly KBA and AKBA. Their plasma and brain concentrations have been found to be very low following the oral administration of even a high dose. Bioavailability has been a major hurdle in the translation of the preclinical potential of frankincense extracts and BAs into therapeutic effects. Therefore, we summarized some approaches for enhancing pharmacological activities by increasing the availability of BAs.

Above all, we anticipate further research on BAs and accurate pharmacological mechanisms for explaining the potential medicinal uses of frankincense. We also anticipate more novel approaches for enhancing the bioavailability and bioactivity of BAs in humans to increase the range of clinical applications and the development of more bioactive and bioavailable derivatives in the near future.

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Conflict of Interest

The authors declare that no conflicts of interest exist.

References

1 *Mathe C, Culioli G, Archier P, Vieillescazes C.* High-performance liquid chromatographic analysis of triterpenoids in commercial frankincense. Chromatographia 2004; 60: 493–499
2 *Hasson SS, Al-Balush MS, Sallam TA, Idris MA, Habbal O, Al-Jabri AA. In vitro* antibacterial activity of three medicinal plants-Boswellia (Luban) species. Asian Pac J Trop Biomed 2011; 1: 178–182
3 *Atta-ur-Rahman, Naz H, Fadimatou, Makhmoor T, Yasin A, Fatima N, Ngounou FN, Kimbu SF, Sondengam BL, Choudhary MI.* Bioactive constituents from Boswellia papyrifera. J Nat Prod 2005; 68: 189–193
4 *Moussaieff A, Mechoulam R.* Boswellia resin: from religious ceremonies to medical uses; a review of *in-vitro, in-vivo* and clinical trials. J Pharm Pharmacol 2009; 61: 1281–1293
5 *Siddiqui MZ. Boswellia serrata*, a potential anti-inflammatory agent: an overview. Indian J Pharm Sci 2011; 73: 255–261
6 *Ammon HP.* Boswellic acids in chronic inflammatory diseases. Planta Med 2006; 72: 1100–1116
7 *Ernst E.* Frankincense: systematic review. BMJ 2008; 337: a2813
8 *Zhang Y, Ning Z, Lu C, Zhao S, Wang J, Liu B, Xu X, Liu Y.* Triterpenoid resinous metabolites from the genus Boswellia: pharmacological activities and potential species-identifying properties. Chem Cent J 2013; 7: 153
9 *Liu JJ, Toy WC, Liu S, Cheng A, Lim BK, Subramaniam T, Sum CF, Lim SC.* Acetyl-keto-beta-boswellic acid induces lipolysis in mature adipocytes. Biochem Biophys Res Commun 2013; 431: 192–196
10 *Raja AF, Ali F, Khan IA, Shawl AS, Arora DS, Shah BA, Taneja SC.* Antistaphylococcal and biofilm inhibitory activities of acetyl-11-keto-beta-boswellic acid from *Boswellia serrata*. BMC Microbiol 2011; 11: 54
11 *Raja AF, Ali F, Khan IA, Shawl AS, Arora DS.* Acetyl-11-keto-beta-boswellic acid (AKBA); targeting oral cavity pathogens. BMC Res Notes 2011; 4: 406

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12 *Gerhardt H, Seifert F, Buvari P, Vogelsang H, Repges R.* Therapy of active Crohn disease with *Boswellia serrata* extract H 15. Z Gastroenterol 2001; 39: 11–17

13 *Madisch A, Miehlke S, Eichele O, Mrwa J, Bethke B, Kuhlisch E, Bastlein E, Wilhelms G, Morgner A, Wigginghaus B, Stolte M. Boswellia serrata* extract for the treatment of collagenous colitis. A double-blind, randomized, placebo-controlled, multicenter trial. Int J Colorectal Dis 2007; 22: 1445–1451

14 *Sengupta K, Alluri KV, Satish AR, Mishra S, Golakoti T, Sarma KV, Dey D, Raychaudhuri SP.* A double blind, randomized, placebo controlled study of the efficacy and safety of 5-Loxin for treatment of osteoarthritis of the knee. Arthritis Res Ther 2008; 10: R85

15 *Sengupta K, Krishnaraju AV, Vishal AA, Mishra A, Trimurtulu G, Sarma KV, Raychaudhuri SK, Raychaudhuri SP.* Comparative efficacy and tolerability of 5-Loxin and AflapinAgainst osteoarthritis of the knee: a double blind, randomized, placebo controlled clinical study. Int J Med Sci 2010; 7: 366–377

16 *Notarnicola A, Tafuri S, Fusaro L, Moretti L, Pesce V, Moretti B.* The "ME-SACA" study: methylsulfonylmethane and boswellic acids in the treatment of gonarthrosis. Adv Ther 2011; 28: 894–906

17 *Sander O, Herborn G, Rau R.* Is H15 (resin extract of *Boswellia serrata*, "incense") a useful supplement to established drug therapy of chronic polyarthritis? Results of a double-blind pilot study. Z Rheumatol 1998; 57: 11–16

18 *Gupta I, Gupta V, Parihar A, Gupta S, Ludtke R, Safayhi H, Ammon HP.* Effects of *Boswellia serrata* gum resin in patients with bronchial asthma: results of a double-blind, placebo-controlled, 6-week clinical study. Eur J Med Res 1998; 3: 511–514

19 *Pedretti A, Capezzera R, Zane C, Facchinetti E, Calzavara-Pinton P.* Effects of topical boswellic acid on photo and age-damaged skin: clinical, biophysical, and echographic evaluations in a double-blind, randomized, split-face study. Planta Med 2010; 76: 555–560

20 *Kirste S, Treier M, Wehrle SJ, Becker G, Abdel-Tawab M, Gerbeth K, Hug MJ, Lubrich B, Grosu AL, Momm F. Boswellia serrata* acts on cerebral edema in patients irradiated for brain tumors: a prospective, randomized, placebo-controlled, double-blind pilot trial. Cancer 2011; 117: 3788–3795

21 *Sengupta K, Kolla JN, Krishnaraju AV, Yalamanchili N, Rao CV, Golakoti T, Raychaudhuri S, Raychaudhuri SP.* Cellular and molecular mechanisms of anti-inflammatory effect of Aflapin: a novel *Boswellia serrata* extract. Mol Cell Biochem 2011; 354: 189–197

22 *Efferth T, Greten HJ.* Anti-inflammatory and anti-cancer activity of boswellic acids from frankincense (*Boswellia serrata* Roxb. et Colebr, B. carterii Birdw.). For Immunopathol Dis Therap 2011; 2: 303–313

23 *Sailer ER, Subramanian LR, Rall B, Hoernlein RF, Ammon HP, Safayhi H.* Acetyl-11-keto-beta-boswellic acid (AKBA): structure requirements for binding and 5-lipoxygenase inhibitory activity. Br J Pharmacol 1996; 117: 615–618

24 *Henkel A, Kather N, Monch B, Northoff H, Jauch J, Werz O.* Boswellic acids from frankincense inhibit lipopolysaccharide functionality through direct molecular interference. Biochem Pharmacol 2012; 83: 115–121

25 *Husch J, Gerbeth K, Fricker G, Setzer C, Zirkel J, Rebmann H, Schubert-Zsilavecz M, Abdel-Tawab M.* Effect of phospholipid-based formulations of *Boswellia serrata* extract on the solubility, permeability, and absorption of the individual boswellic acid constituents present. J Nat Prod 2012; 75: 1675–1682

26 *Husch J, Bohnet J, Fricker G, Skarke C, Artaria C, Appendino G, Schubert-Zsilavecz M, Abdel-Tawab M.* Enhanced absorption of boswellic acids by a lecithin delivery form (Phytosome®) of Boswellia extract. Fitoterapia 2013; 84: 89–98

27 *Poeckel D, Werz O.* Boswellic acids: biological actions and molecular targets. Curr Med Chem 2006; 13: 3359–3369

28 *Safayhi H, Mack T, Sabieraj J, Anazodo MI, Subramanian LR, Ammon HP.* Boswellic acids: novel, specific, nonredox inhibitors of 5-lipoxygenase. J Pharmacol Exp Ther 1992; 261: 1143–1146

29 *Safayhi H, Sailer ER, Ammon HP.* Mechanism of 5-lipoxygenase inhibition by acetyl-11-keto-beta-boswellic acid. Mol Pharmacol 1995; 47: 1212–1216

30 *Safayhi H, Sailer ER, Ammon HP.* 5-Lipoxygenase inhibition by acetyl-11-keto-beta-boswellic acid (AKBA) by a novel mechanism. Phytomedicine 1996; 3: 71–72

31 *Safayhi H, Boden SE, Schweizer S, Ammon HP.* Concentration-dependent potentiating and inhibitory effects of Boswellia extracts on 5-lipoxygenase product formation in stimulated PMNL. Planta Med 2000; 66: 110–113

32 *Siemoneit U, Pergola C, Jazzar B, Northoff H, Skarke C, Jauch J, Werz O.* On the interference of boswelliinc acids with 5-lipoxygenase: mechanistic studies *in vitro* and pharmacological relevance. Eur J Pharmacol 2009; 606: 246–254

33 *Siemoneit U, Hofmann B, Kather N, Lamkemeyer T, Madlung J, Franke L, Schneider G, Jauch J, Poeckel D, Werz O.* Identification and functional analysis of cyclooxygenase-1 as a molecular target of boswellic acids. Biochem Pharmacol 2008; 75: 503–513

34 *Altmann A, Fischer L, Schubert-Zsilavecz M, Steinhilber D, Werz O.* Boswellic acids activate p42(MAPK) and p38 MAPK and stimulate Ca(2+) mobilization. Biochem Biophys Res Commun 2002; 290: 185–190

35 *Altmann A, Poeckel D, Fischer L, Schubert-Zsilavecz M, Steinhilber D, Werz O.* Coupling of boswellic acid-induced Ca^{2+} mobilisation and MAPK activation to lipid metabolism and peroxide formation in human leucocytes. Br J Pharmacol 2004; 141: 223–232

36 *Safayhi H, Rall B, Sailer ER, Ammon HP.* Inhibition by boswellic acids of human leukocyte elastase. J Pharmacol Exp Ther 1997; 281: 460–463

37 *Poeckel D, Tausch L, Kather N, Jauch J, Werz O.* Boswellic acids stimulate arachidonic acid release and 12-lipoxygenase activity in human platelets independent of Ca^{2+} and differentially interact with platelet-type 12-lipoxygenase. Mol Pharmacol 2006; 70: 1071–1078

38 *Roy S, Khanna S, Shah H, Rink C, Phillips C, Preuss H, Subbaraju GV, Trimurtulu G, Krishnaraju AV, Bagchi M, Bagchi D, Sen CK.* Human genome screen to identify the genetic basis of the anti-inflammatory effects of Boswellia in microvascular endothelial cells. DNA Cell Biol 2005; 24: 244–255

39 *Poeckel D, Tausch L, Altmann A, Feisst C, Klinkhardt U, Graff J, Harder S, Werz O.* Induction of central signalling pathways and select functional effects in human platelets by beta-boswellic acid. Br J Pharmacol 2005; 146: 514–524

40 *Siemoneit U, Koeberle A, Rossi A, Dehm F, Verhoff M, Reckel S, Maier TJ, Jauch J, Northoff H, Bernhard F.* Inhibition of microsomal prostaglandin E2 synthase-1 as a molecular basis for the anti-inflammatory actions of boswellic acids from frankincense. Brit J Pharmacol 2011; 162: 147–162

41 *Tausch L, Henkel A, Siemoneit U, Poeckel D, Kather N, Franke L, Hofmann B, Schneider G, Angioni C, Geisslinger G, Skarke C, Holtmeier W, Beckhaus T, Karas M, Jauch J, Werz O.* Identification of human cathepsin G as a functional target of boswellic acids from the anti-inflammatory remedy frankincense. J Immunol 2009; 183: 3433–3442

42 *Liu JJ, Nilsson A, Oredsson S, Badmaev V, Zhao WZ, Duan RD.* Boswellic acids trigger apoptosis via a pathway dependent on caspase-8 activation but independent on Fas/Fas ligand interaction in colon cancer HT-29 cells. Carcinogenesis 2002; 23: 2087–2093

43 *Liu JJ, Nilsson A, Oredsson S, Badmaev V, Duan RD.* Keto-and acetyl-keto-boswellic acids inhibit proliferation and induce apoptosis in Hep G2 cells via a caspase-8 dependent pathway. Int J Mol Med 2002; 10: 501–505

44 *Lu M, Xia L, Hua H, Jing Y.* Acetyl-keto-beta-boswellic acid induces apoptosis through a death receptor 5-mediated pathway in prostate cancer cells. Cancer Res 2008; 68: 1180–1186

45 *Liu JJ, Duan RD.* LY294002 enhances boswellic acid-induced apoptosis in colon cancer cells. Anticancer Res 2009; 29: 2987–2991

46 *Cuaz-Pérolin C, Billiet L, Baugé E, Copin C, Scott-Algara D, Genze F, Büchele B, Syrovets T, Simmet T, Rouis M.* Antiinflammatory and antiatherogenic effects of the NF-κB inhibitor acetyl-11-keto-*β*-boswellic acid in LPS-challenged ApoE−/− mice. Arterioscler Thromb Vasc Biol 2008; 28: 272–277

47 *Takada Y, Ichikawa H, Badmaev V, Aggarwal BB.* Acetyl-11-keto-beta-boswellic acid potentiates apoptosis, inhibits invasion, and abolishes osteoclastogenesis by suppressing NF-kappa B and NF-kappa B-regulated gene expression. J Immunol 2006; 176: 3127–3140

48 *Park B, Prasad S, Yadav V, Sung B, Aggarwal BB.* Boswellic acid suppresses growth and metastasis of human pancreatic tumors in an orthotopic nude mouse model through modulation of multiple targets. PLoS One 2011; 6: e26943

49 *Pang X, Yi Z, Zhang X, Sung B, Qu W, Lian X, Aggarwal BB, Liu M.* Acetyl-11-keto-beta-boswellic acid inhibits prostate tumor growth by suppressing vascular endothelial growth factor receptor 2-mediated angiogenesis. Cancer Res 2009; 69: 5893–5900

50 *Liang Y, Li P, Huang Q, Zhao J, Liu X, Dai M.* Acetyl-11-keto-beta: boswellic acid regulates the activities of matrix metalloproteinases-1,-2,-9. Chin J Pathophysiol 2009; 25: 2004–2011

51 *Park B, Sung B, Yadav VR, Cho SG, Liu M, Aggarwal BB.* Acetyl-11-keto-beta-boswellic acid suppresses invasion of pancreatic cancer cells

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through the downregulation of CXCR4 chemokine receptor expression. Int J Cancer 2011; 129: 23–33
52 *Zhang YS, Xie JZ, Zhong JL, Li YY, Wang RQ, Qin YZ, Lou HX, Gao ZH, Qu XJ.* Acetyl-11-keto-beta-boswellic acid (AKBA) inhibits human gastric carcinoma growth through modulation of the Wnt/beta-catenin signaling pathway. Biochim Biophys Acta 2013; 1830: 3604–3615
53 *Yuan Y, Cui SX, Wang Y, Ke HN, Wang RQ, Lou HX, Gao ZH, Qu XJ.* Acetyl-11-keto-beta-boswellic acid (AKBA) prevents human colonic adenocarcinoma growth through modulation of multiple signaling pathways. Biochim Biophys Acta 2013; 1830: 4907–4916
54 *Syrovets T, Buchele B, Gedig E, Slupsky JR, Simmet T.* Acetyl-boswellic acids are novel catalytic inhibitors of human topoisomerases I and IIalpha. Mol Pharmacol 2000; 58: 71–81
55 *Eichhorn T, Greten HJ, Efferth T.* Molecular determinants of the response of tumor cells to boswellic acids. Pharmaceuticals 2011; 4: 1171–1182
56 *Shao Y, Ho CT, Chin CK, Badmaev V, Ma W, Huang MT.* Inhibitory activity of boswellic acids from *Boswellia serrata* against human leukemia HL-60 cells in culture. Planta Med 1998; 64: 328–331
57 *Huang MT, Badmaev V, Ding Y, Liu Y, Xie JG, Ho CT.* Anti-tumor and anticarcinogenic activities of triterpenoid, beta-boswellic acid. Biofactors 2000; 13: 225–230
58 *Takahashi M, Sung B, Shen Y, Hur K, Link A, Boland CR, Aggarwal BB, Goel A.* Boswellic acid exerts antitumor effects in colorectal cancer cells by modulating expression of the let-7 and miR-200 microRNA family. Carcinogenesis 2012; 33: 2441–2449
59 *Shen Y, Takahashi M, Byun HM, Link A, Sharma N, Balaguer F, Leung HC, Boland CR, Goel A.* Boswellic acid induces epigenetic alterations by modulating DNA methylation in colorectal cancer cells. Cancer Biol Ther 2012; 13: 542–552
60 *Jones R, Rubin G, Berenbaum F, Scheiman J.* Gastrointestinal and cardiovascular risks of nonsteroidal anti-inflammatory drugs. Am J Med 2008; 121: 464–474
61 *Golbabaei S, Bazl R, Golestanian S, Nabati F, Omrany ZB, Yousefi B, Hajiaghaee R, Rezazadeh S, Amanlou M.* Urease inhibitory activities of beta-boswellic acid derivatives. Daru 2013; 21: 2
62 *Ravanan P, Singh SK, Rao GS, Kondaiah P.* Growth inhibitory, apoptotic and anti-inflammatory activities displayed by a novel modified triterpenoid, cyano enone of methyl boswellates. J Biosci 2011; 36: 297–307
63 *Qurishi Y, Hamid A, Sharma PR, Wani ZA, Mondhe DM, Singh SK, Zargar MA, Andotra SS, Shah BA, Taneja SC, Saxena AK.* PARP cleavage and perturbance in mitochondrial membrane potential by 3-alpha-propionyloxy-beta-boswellic acid results in cancer cell death and tumor regression in murine models. Future Oncol 2012; 8: 867–881
64 *Qurishi Y, Hamid A, Sharma PR, Wani ZA, Mondhe DM, Singh SK, Zargar MA, Andotra SS, Shah BA, Taneja SC, Saxena AK.* NF-kappa B down-regulation and PARP cleavage by novel 3-alpha-butyryloxy-beta-boswellic acid results in cancer cell specific apoptosis and *in vivo* tumor regression. Anticancer Agents Med Chem 2013; 13: 777–790
65 *Bhushan S, Kumar A, Malik F, Andotra SS, Sethi VK, Kaur IP, Taneja SC, Qazi GN, Singh J.* A triterpenediol from *Boswellia serrata* induces apoptosis through both the intrinsic and extrinsic apoptotic pathways in human leukemia HL-60 cells. Apoptosis 2007; 12: 1911–1926
66 *Suneela D, Dipmala P.* Synthesis and pharmacokinetic profile of rheinboswellic acid conjugate. Bioorg Med Chem Lett 2012; 22: 7582–7587
67 *Dhaneshwar S, Dipmala P, Abhay H, Prashant B.* Disease-modifying effect of anthraquinone prodrug with boswellic acid on collagenase-induced osteoarthritis in Wistar rats. Inflamm Allergy Drug Targets 2013; 12: 288–295
68 *Shah BA, Kumar A, Gupta P, Sharma M, Sethi VK, Saxena AK, Singh J, Qazi GN, Taneja SC.* Cytotoxic and apoptotic activities of novel amino analogues of boswellic acids. Bioorg Med Chem Lett 2007; 17: 6411–6416
69 *Csuk R, Niesen-Barthel A, Barthel A, Kluge R, Strohl D.* Synthesis of an antitumor active endoperoxide from 11-keto-beta-boswellic acid. Eur J Med Chem 2010; 45: 3840–3843
70 *Khan S, Chib R, Shah BA, Wani ZA, Dhar N, Mondhe DM, Lattoo S, Jain SK, Taneja SC, Singh J.* A cyano analogue of boswellic acid induces crosstalk between p53/PUMA/Bax and telomerase that stages the human papillomavirus type 18 positive HeLa cells to apoptotic death. Eur J Pharmacol 2011; 660: 241–248
71 *Khan S, Kaur R, Shah BA, Malik F, Kumar A, Bhushan S, Jain SK, Taneja SC, Singh J.* A novel cyano derivative of 11-keto-beta-boswellic acid causes apoptotic death by disrupting PI3K/AKT/Hsp-90 cascade, mitochondrial integrity, and other cell survival signaling events in HL-60 cells. Mol Carcinog 2012; 51: 679–695
72 *Chashoo G, Singh SK, Mondhe DM, Sharma PR, Andotra SS, Shah BA, Taneja SC, Saxena AK.* Potentiation of the antitumor effect of 11-keto-beta-boswellic acid by its 3-alpha-hexanoyloxy derivative. Eur J Pharmacol 2011; 668: 390–400
73 *Chashoo G, Singh SK, Sharma PR, Mondhe DM, Hamid A, Saxena A, Andotra SS, Shah BA, Qazi NA, Taneja SC, Saxena AK.* A propionyloxy derivative of 11-keto-beta-boswellic acid induces apoptosis in HL-60 cells mediated through topoisomerase I & II inhibition. Chem Biol Interact 2011; 189: 60–71
74 *Kumar A, Shah BA, Singh S, Hamid A, Singh SK, Sethi VK, Saxena AK, Singh J, Taneja SC.* Acyl derivatives of boswellic acids as inhibitors of NF-kappa B and STATs. Bioorg Med Chem Lett 2012; 22: 431–435
75 *Pathania AS, Joshi A, Kumar S, Guru SK, Bhushan S, Sharma PR, Bhat WW, Saxena AK, Singh J, Shah BA, Andotra SS, Taneja SC, Malik FA, Kumar A.* Reversal of boswellic acid analog BA145 induced caspase dependent apoptosis by PI3K inhibitor LY294002 and MEK inhibitor PD98059. Apoptosis 2013; 18: 1561–1573
76 *Morad SA, Schmid M, Buchele B, Siehl HU, El GM, Lunov O, Syrovets T, Simmet T.* A novel semisynthetic inhibitor of the FRB domain of mammalian target of rapamycin blocks proliferation and triggers apoptosis in chemoresistant prostate cancer cells. Mol Pharmacol 2013; 83: 531–541
77 *Wang Y, Sun Y, Wang C, Huo X, Liu P, Wang C, Zhang B, Zhan L, Zhang H, Deng S, Zhao Y, Ma X.* Biotransformation of 11-keto-beta-boswellic acid by Cunninghamella blakesleana. Phytochemistry 2013; 96: 330–336
78 *Buchele B, Zugmaier W, Estrada A, Genze F, Syrovets T, Paetz C, Schneider B, Simmet T.* Characterization of 3alpha-acetyl-11-keto-alpha-boswellic acid, a pentacyclic triterpenoid inducing apoptosis *in vitro and in vivo*. Planta Med 2006; 72: 1285–1289
79 *Sun Y, Liu D, Xi R, Wang X, Wang Y, Hou J, Zhang B, Wang C, Liu K, Ma X.* Microbial transformation of acetyl-11-keto-β-boswellic acid and their inhibitory activity on LPS-induced NO production. Bioorg Med Chem Lett 2013; 23: 1338–1342
80 *Tawab MA, Kaunzinger A, Bahr U, Karas M, Wurglics M, Schubert-Zsilavecz M.* Development of a high-performance liquid chromatographic method for the determination of 11-keto-beta-boswellic acid in human plasma. J Chromatogr B Biomed Sci Appl 2001; 761: 221–227
81 *Sharma S, Thawani V, Hingorani L, Shrivastava M, Bhate VR, Khiyani R.* Pharmacokinetic study of 11-Keto beta-Boswellic acid. Phytomedicine 2004; 11: 255–260
82 *Buchele B, Simmet T.* Analysis of 12 different pentacyclic triterpenic acids from frankincense in human plasma by high-performance liquid chromatography and photodiode array detection. J Chromatogr B Analyt Technol Biomed Life Sci 2003; 795: 355–362
83 *Gerbeth K, Meins J, Kirste S, Momm F, Schubert-Zsilavecz M, Abdel-Tawab M.* Determination of major boswellic acids in plasma by high-pressure liquid chromatography/mass spectrometry. J Pharmaceut Biomed 2011; 56: 998–1005
84 *Gerbeth K, Husch J, Fricker G, Werz O, Schubert-Zsilavecz M, Abdel-Tawab M. In vitro* metabolism, permeation, and brain availability of six major boswellic acids from *Boswellia serrata* gum resins. Fitoterapia 2013; 84: 99–106
85 *Reising K, Meins J, Bastian B, Eckert G, Mueller WE, Schubert-Zsilavecz M, Abdel-Tawab M.* Determination of boswellic acids in brain and plasma by high-performance liquid chromatography/tandem mass spectrometry. Anal Chem 2005; 77: 6640–6645
86 *Kaunzinger A, Baumeister A, Cuda K, Häring N, Schug B, Blume HH, Raddatz K, Fischer G, Schubert-Zsilavecz M.* Determination of 11-keto-boswellic acid in human plasma. J Pharmaceut Biomed 2002; 28: 729–739
87 *Shah SA, Rathod IS, Suhagia BN, Patel DA, Parmar VK, Shah BK, Vaishnavi VM.* Estimation of boswellic acids from market formulations of *Boswellia serrata* extract and 11-keto beta-boswellic acid in human plasma by high-performance thin-layer chromatography. J Chromatogr B Analyt Technol Biomed Life Sci 2007; 848: 232–238
88 *Krohn K, Rao MS, Raman NV, Khalilullah M.* High-performance thin layer chromatographic analysis of anti-inflammatory triterpenoids from *Boswellia serrata* Roxb. Phytochem Anal 2001; 12: 374–376
89 *Weber CC, Reising K, Muller WE, Schubert-Zsilavecz M, Abdel-Tawab M.* Modulation of Pgp function by boswellic acids. Planta Med 2006; 72: 507–513
90 *Kruger P, Daneshfar R, Eckert GP, Klein J, Volmer DA, Bahr U, Muller WE, Karas M, Schubert-Zsilavecz M, Abdel-Tawab M.* Metabolism of boswellic acids *in vitro* and *in vivo*. Drug Metab Dispos 2008; 36: 1135–1142
91 *Kruger P, Kanzer J, Hummel J, Fricker G, Schubert-Zsilavecz M, Abdel-Tawab M.* Permeation of Boswellia extract in the Caco-2 model and pos-

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sible interactions of its constituents KBA and AKBA with OATP1B3 and MRP2. Eur J Pharm Sci 2009; 36: 275–284
92 *Bagul P, Khomane KS, Bansal AK.* Investigating permeability related hurdles in oral delivery of 11-keto-beta-boswellic acid. Int J Pharm 2014; 464: 104–110
93 *Sterk V, Buchele B, Simmet T.* Effect of food intake on the bioavailability of boswellic acids from a herbal preparation in healthy volunteers. Planta Med 2004; 70: 1155–1160
94 *Skarke C, Kuczka K, Tausch L, Werz O, Rossmanith T, Barrett JS, Harder S, Holtmeier W, Schwarz JA.* Increased bioavailability of 11-keto-beta-boswellic acid following single oral dose frankincense extract administration after a standardized meal in healthy male volunteers: modeling and simulation considerations for evaluating drug exposures. J Clin Pharmacol 2012; 52: 1592–1600
95 *Sharma A, Gupta NK, Dixit VK.* Complexation with phosphatidyl choline as a strategy for absorption enhancement of boswellic acid. Drug Deliv 2010; 17: 587–595
96 *Goel A, Ahmad FJ, Singh RM, Singh GN.* 3-Acetyl-11-keto-beta-boswellic acid loaded-polymeric nanomicelles for topical anti-inflammatory and anti-arthritic activity. J Pharm Pharmacol 2010; 62: 273–278

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