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-butyroxy-β-boswellic acid
- BSE: Boswellia serrata extract
- catG: cathepsin G
- C-KβBA: 3-cinnamoyl-11-keto-β-boswellic acid
- Cmax: maximum concentration
- CEMB: 2-cyano, 3-eneone 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
- 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
- macro: maximum concentration
- CEMB: 2-cyano, 3-eneone 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
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* These authors contributed equally to this work.
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 at least 20% 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, pharmacokinetics 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
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: \( \beta \)-BA (1), \( \alpha \)-BA (2), \( \beta \)-ABA (3), \( \alpha \)-ABA (4), KBA (5), and AKBA (6). Their structures are shown in Fig. 1. BAs (Fig. 1) exist as ursane (\( \alpha \)) and oleanane (\( \beta \)) types depending on the position of two methyl groups on C-19/C-20 (\( \alpha \)-types: geminal groups on C-20; \( \beta \)-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 \( \beta \)-types exert stronger bioactivities compared with the corresponding \( \alpha \)-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-

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

<table>
<thead>
<tr>
<th>Dosage of BSE</th>
<th>Methods</th>
<th>Concentrations of BAs determined in plasma (( \mu )M) or brain* (ng/g)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1600 mg/day (n = 1)*</td>
<td>HPLC&lt;sup&gt;a,h&lt;/sup&gt;</td>
<td>NA NA NA NA 1.7 NA [80]</td>
<td></td>
</tr>
<tr>
<td>333 mg/day for 7 d (n = 12)*</td>
<td>HPLC&lt;sup&gt;c&lt;/sup&gt;</td>
<td>10.1 3.5 2.4 4.0 0.3 0.1 [82]</td>
<td></td>
</tr>
<tr>
<td>3 × 800 mg/day for 4 wk (n = 3)*</td>
<td>HPLC-ESI/MS&lt;sup)b,e&lt;/sup&gt;</td>
<td>6.35 ± 0.52 NA 4.9 ± 0.5 NA 0.3 ± 0.1 0.04 ± 0.01 [41]</td>
<td></td>
</tr>
<tr>
<td>4200 mg/day (n = 14)*</td>
<td>HPLC-ESI/MS&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.19–26.20 [0.08–10.59] [0.26–12.31] [0.14–5.99] [0.01–0.52] [0.0–0.03] [83]</td>
<td></td>
</tr>
<tr>
<td>240 mg/kg (n = 3)*</td>
<td>HPLC-ESI/MS&lt;sup&gt;f&lt;/sup&gt;</td>
<td>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]</td>
<td></td>
</tr>
<tr>
<td>240 mg/kg (n = 6)*</td>
<td>HPLC-ESI/MS&lt;sup&gt;g&lt;/sup&gt;</td>
<td>1066.6 485.1 163.7 43.0 11.6 37.5</td>
<td></td>
</tr>
<tr>
<td>240 mg/kg (n = 9)*</td>
<td>HPLC-APCI/MS&lt;sup&gt;h&lt;/sup&gt;</td>
<td>NA NA NA NA 0.4 0.2 [85]</td>
<td></td>
</tr>
<tr>
<td>240 mg/kg (n = 9)*</td>
<td>HPLC-APCI/MS&lt;sup&gt;i&lt;/sup&gt;</td>
<td>NA NA NA NA 99 95</td>
<td></td>
</tr>
<tr>
<td>500 mg/day (n = 6)*</td>
<td>HPTLC&lt;sup&gt;d&lt;/sup&gt;</td>
<td>NA NA NA NA 0.05–0.13 [87]</td>
<td></td>
</tr>
</tbody>
</table>

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<sub>max</sub>.

**Fig. 1** Chemical structures of the main boswellic acids. The characteristic structure of \( \alpha \)-types is marked in blue. (Color figure available online only.)
Selective 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 it is directly bound to this enzyme in vitro [37]. 5-Loxin (standardized BSE enriched with 30% AKBA) was found to prevent MMPs that, in comparison to AKBA and KBA, completely abrogated the TNF-α (type-II)-induced OA in Wister rats revealed significant antiarthritic and antiulcer activities [67]. BOBA (10) is the butyryloxy 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-kB, and the DNA repair system in apoptosis [64]. α-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].

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-eneone 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-kB, and the DNA repair system in apoptosis [64]. α-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 502713) cell lines, and compound 15 showed cytotoxicity (73–99%) against prostate (DU-145) and colon (SW-620, 502713, HT-29) cell lines [68].
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 IC50 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 Hsp90 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 IC50 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 cancers [69].

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.)
cancer cell lines by inhibiting the NF-κB 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–KβBA (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 7β,16α-dihydroxy-3-acety-11-keto-β-boswellic acid (26) were screened from metabolites of the biotransformation of AKBA by Cunninghamella blakesleana and exhibited IC50 values of 25.8 and 12.5 µM, respectively, by the stronger inhibition of NO production induced by LPS and cytotoxicity in RAW 264.7 macrophages [77].

3-Acetyl-α-boswellic acid analogues/derivatives

3α-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. 3α-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 7β-16α-dihydroxy-3-acety-11-keto-β-boswellic acid (29) were screened from metabolites of the biotransformation of AKBA by C. blakesleana and exhibited IC50 values of 1.5 and 10.5 µM, respectively, compared with the IC50 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.
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 Cmax 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 1.7 µM was obtained after 1 h. A linear quantification ranges of plasma concentration of three soft gelatin capsules containing 800 mg of BSE by triple quadrupole LC-ESI/MS was 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 α-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 87.0–11948.5, 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, α-BA, β-ABA, α-ABA, KBA, and AKBA was established, and the detection limit of the method for KBA was approximately 0.040 µg/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 Cmax of KBA determined by HPLC reached 2.72 × 10⁻¹ ± 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 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 α-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 87.0–11948.5, 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, α-BA, β-ABA, α-ABA, KBA, and AKBA was from 0.5 to 12000 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.
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 Boswellia® 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 (IC50 = 2.8 to 8.8 µM) but fail to inhibit 5-LOX product synthesis in whole human blood. Meanwhile, a single dose (800 mg) oral administration of BSE (H15) to human healthy volunteers failed to suppress the LTB4 plasma levels [32]. Similarly, KBA and AKBA were found to significantly inhibit mPGES1 in the cell-free assay (AKBA: IC50 = 3 µM; KBA: IC50 = 10 µM), but they all failed to suppress PGF2α 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 relevant. Frankincense usually contains much more β-BA and β-ABA than KBA and AKBA, which correlates to the considerable plasma levels of β-BA and β-ABA (Table 1) [82, 88], and both of them presented IC50 values greater than those determined for catG inhibition (β-BA: IC50 = 0.8 µM; β-ABA: IC50 = 1.2 µM) [41]. β-BA results in the greatest inhibition of PGE2 (IC50 = 3 µM) and LPS (IC50 = 1.8 µM), which implies the interaction of β-BA with catG and indicates that PGF2α 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 PBEC 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 II 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 LTB4 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 Boswellic capsules (400 mg of BSE containing 2.77% KBA and 2.64% AKBA/capsule) in the fasted and fed conditions were deter-
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 IC50s for cag and 5-LOX after a significant but clinically infeasible dose increase of 10- to 15-fold Boswel lan 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.

### Table 2

<table>
<thead>
<tr>
<th>Dosage</th>
<th>Methods</th>
<th>Condition</th>
<th>Cmax of BAs determined in human plasma (µM)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 × BSE-018 capsules/day (n = 12)</td>
<td>HPLC&lt;sup&gt;a&lt;/sup&gt;</td>
<td>fasted</td>
<td>0.41 [0.09–3.96] α-BA ND ND 0.17 [0.05–0.52] KBA 0.01 [0.00–0.08] [93]</td>
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<tr>
<td>2 × Boswel lan capsules/day (n = 15)</td>
<td>HPLC-MS&lt;sup&gt;b&lt;/sup&gt;</td>
<td>fasted</td>
<td>2.45 [0.90–4.67] β-ABA 0.69 [0.09–2.93] ND 0.23 [0.09–0.80] KBA 0.48 [0.21–0.88] AKBA 0.05 [0.02–0.51] [94]</td>
<td></td>
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</tbody>
</table>

<sup>a</sup> = not analyzed; ND = not detectable; <sup>b</sup> = mean ± SD

### Table 3

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

<table>
<thead>
<tr>
<th>Formula</th>
<th>Enhanced activities</th>
<th>Indicators</th>
<th>Conditions</th>
<th>Cmax of BAs in human plasma (µM)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>BAs-Phosphatidylcholine</td>
<td>Anti-inflammatory, hypolipidemic</td>
<td>NF&lt;sup&gt;a&lt;/sup&gt;</td>
<td>NA NA NA NA NA</td>
<td>0.161 ± 0.035 [95]</td>
<td></td>
</tr>
<tr>
<td>AKBA-Polymeric nanomicelles</td>
<td>Anti-inflammatory, anti-arthritis</td>
<td>Skin permeation&lt;sup&gt;a&lt;/sup&gt;</td>
<td>NA NA NA NA NA</td>
<td>0.488 ± 0.036 [96]</td>
<td></td>
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<tr>
<td>BSE-Phospholipid and pluronic f127</td>
<td>Pharmacokinetic&lt;sup&gt;a&lt;/sup&gt;</td>
<td>NF&lt;sup&gt;c&lt;/sup&gt;,&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1066.6 ± 781.6 485.1 ± 363.8 163.7 ± 248.9 43.0 ± 55.7 11.6 ± 12.6 37.5 ± 56.8</td>
<td>[25]</td>
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<td></td>
<td></td>
<td></td>
<td>F&lt;sup&gt;c&lt;/sup&gt;,&lt;sup&gt;d&lt;/sup&gt;</td>
<td>5908.2 ± 2275.6 2443.9 ± 914.4 156.1 ± 82.7 60.1 ± 25.4 13.6 ± 4.2 203.8 ± 86.4</td>
<td></td>
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<td></td>
<td>F vs. NF&lt;sup&gt;c&lt;/sup&gt;,&lt;sup&gt;d&lt;/sup&gt;</td>
<td>6-fold 6-fold 6-fold 6-fold 26-fold 14-fold</td>
<td></td>
</tr>
<tr>
<td>BSE-Phytosome&lt;sup&gt;e&lt;/sup&gt; (Soy lecithin)</td>
<td>Pharmacokinetic&lt;sup&gt;a&lt;/sup&gt;</td>
<td>NF&lt;sup&gt;c&lt;/sup&gt;,&lt;sup&gt;d&lt;/sup&gt;</td>
<td>[0.0–98.2] [241.3–380.6] [35.5–318.9] [39.9–93.1] [0.0–44.9] [0.0–45.0]</td>
<td>[26]</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>F&lt;sup&gt;c&lt;/sup&gt;,&lt;sup&gt;d&lt;/sup&gt;</td>
<td>[0.0–1376.8] [219.93–850.0] 4.5–317.7 10.9–100.9 [22.4–477.8] [0.0–586.1]</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>F vs. NF&lt;sup&gt;c&lt;/sup&gt;,&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2.2 ± 0.3 1.1 ± 0.4 5.0 ± 2.1 1.0 ± 0.2 0.6 ± 0.3 0.6 ± 0.1</td>
<td></td>
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</tbody>
</table>

<sup>a</sup> = rats; F = formulation; NF = non-formulation; NA = not analyzed; | = not investigated; Cmax<sup>a</sup> = concentrations of BAs in plasma; Cmax<sup>b</sup> = concentrations of BAs in brain; Jss<sup>a</sup> = permeation rate at steady state; IC50<sup>b</sup> = permeability coefficient; a = mean ± SD; b = the relevant literature provided only multiple comparison, there was no accurate numerical reference; c = [range]; d = Cmax

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].
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-vinylpyrrolidione, 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 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.

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