BJP BRITISH JOURNAL OF PHARMACOLOGY
BPS BRITISH PHARMACOLOGICAL SOCIETY

BJP British Journal of Pharmacology

DOI:10.1111/j.1476-5381.2010.01020.x
www.brjpharmacol.org

RESEARCH PAPER

# Inhibition of microsomal prostaglandin $E_2$ synthase-1 as a molecular basis for the anti-inflammatory actions of boswellic acids from frankincense

U Siemoneit[1], A Koeberle[1], A Rossi[2], F Dehm[1], M Verhoff[1], S Reckel[3], TJ Maier[4], J Jauch[5], H Northoff[6], F Bernhard[3], V Doetsch[3], L Sautebin[2] and O Werz[1]

[1]*Pharmaceutical Institute, University of Tuebingen, Tuebingen, Germany,* [2]*Department of Experimental Pharmacology, University of Naples Federico II, Naples, Italy,* [3]*Institute of Biophysical Chemistry and Center for Biomolecular Magnetic Resonance, University of Frankfurt, Frankfurt, Germany,* [4]*Institute of Pharmaceutical Chemistry, University of Frankfurt, Frankfurt, Germany,* [5]*Institute of Organic Chemistry, University of Saarland, Saarbrücken, Germany, and* [6]*Institute for Clinical and Experimental Transfusion Medicine, University Medical Center Tuebingen, Tuebingen, Germany*

**Correspondence**
Dr Oliver Werz, Department for Pharmaceutical Analytics, Pharmaceutical Institute, University of Tuebingen, Auf der Morgenstelle 8, D-72076 Tuebingen, Germany. E-mail: oliver.werz@uni-tuebingen.de

**Keywords**
inflammation; prostaglandin; boswellic acid; microsomal prostaglandin $E_2$ synthase; arachidonic acid

**Received**
16 February 2010
**Revised**
30 June 2010
**Accepted**
9 August 2010

## BACKGROUND AND PURPOSE
Frankincense, the gum resin derived from *Boswellia* species, showed anti-inflammatory efficacy in animal models and in pilot clinical studies. Boswellic acids (BAs) are assumed to be responsible for these effects but their anti-inflammatory efficacy *in vivo* and their molecular modes of action are incompletely understood.

## EXPERIMENTAL APPROACH
A protein fishing approach using immobilized BA and surface plasmon resonance (SPR) spectroscopy were used to reveal microsomal prostaglandin $E_2$ synthase-1 (mPGES1) as a BA-interacting protein. Cell-free and cell-based assays were applied to confirm the functional interference of BAs with mPGES1. Carrageenan-induced mouse paw oedema and rat pleurisy models were utilized to demonstrate the efficacy of defined BAs *in vivo*.

## KEY RESULTS
Human mPGES1 from A549 cells or *in vitro*-translated human enzyme selectively bound to BA affinity matrices and SPR spectroscopy confirmed these interactions. BAs reversibly suppressed the transformation of prostaglandin (PG)$H_2$ to $PGE_2$ mediated by mPGES1 ($IC_{50}$ = 3–10 μM). Also, in intact A549 cells, BAs selectively inhibited $PGE_2$ generation and, in human whole blood, β-BA reduced lipopolysaccharide-induced $PGE_2$ biosynthesis without affecting formation of the COX-derived metabolites 6-keto $PGF_{1\alpha}$ and thromboxane $B_2$. Intraperitoneal or oral administration of β-BA (1 mg·kg$^{-1}$) suppressed rat pleurisy, accompanied by impaired levels of $PGE_2$ and β-BA (1 mg·kg$^{-1}$, given i.p.) also reduced mouse paw oedema, both induced by carrageenan.

## CONCLUSIONS AND IMPLICATIONS
Suppression of $PGE_2$ formation by BAs via interference with mPGES1 contribute to the anti-inflammatory effectiveness of BAs and of frankincense, and may constitute a biochemical basis for their anti-inflammatory properties.

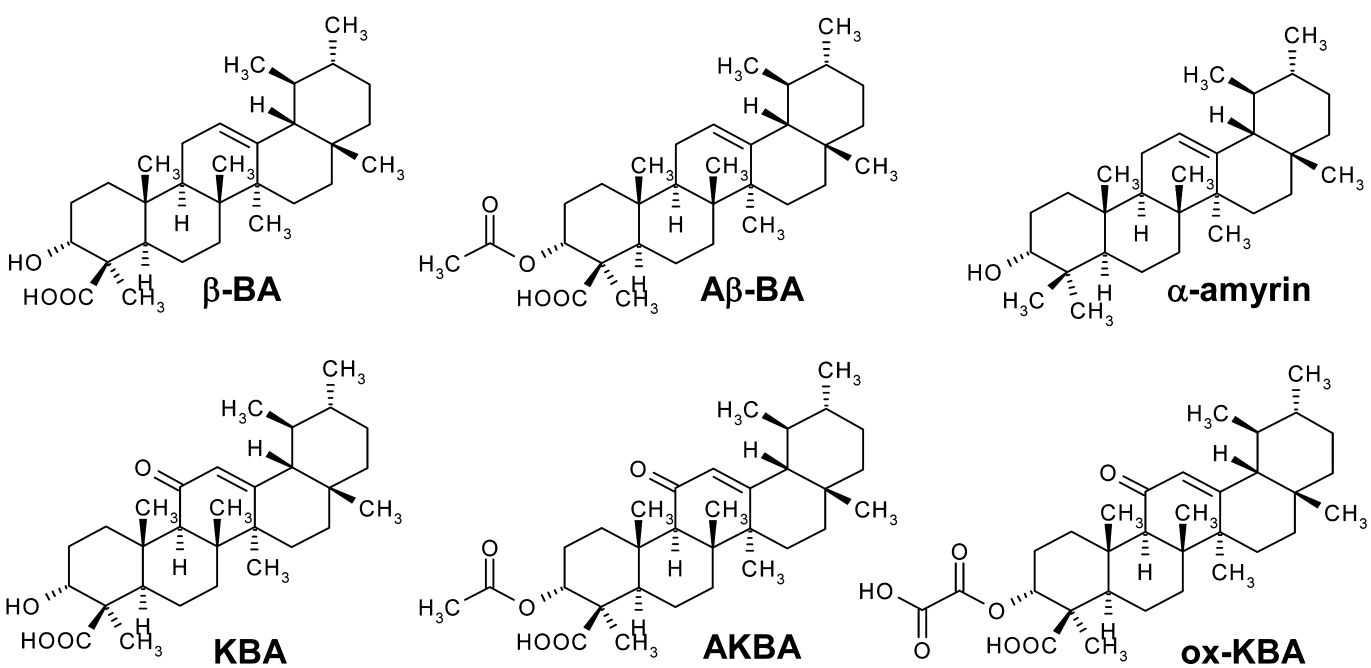
### Abbreviations

5-LOX, 5-lipoxygenase; Aβ-BA, 3-O-acetyl-β-boswellic acid; AA, arachidonic acid; AKBA, 3-O-acetyl-11-keto-β-boswellic acid; BA, boswellic acid; COX, cyclooxygenase; FCS, fetal calf serum; GSH, glutathione; 12-HHT, 12(S)-hydroxy-5-*cis*-8,10-*trans*-heptadecatrienoic acid; HLE, human leukocyte elastase; IL-1β, interleukin-1β; KBA, 11-keto-β-boswellic acid; LPS, lipopolysaccharide; MAPK, mitogen-activated protein kinase; mPGES, microsomal prostaglandin $E_2$ synthase; NFκB, nuclear factor κB; NSAID, non-steroidal anti-inflammatory drugs; OA, osteoarthritis; PBS, phosphate-buffered saline; PG, prostaglandin; RA, rheumatoid arthritis; RU, resonance unit; SPR, surface plasmon resonance; $TXB_2$, thromboxane $B_2$

## Introduction

Extracts of the gum resins from *Boswellia* species (frankincense) are commonly used in folk medicine to treat various inflammatory disorders. The pentacyclic triterpenes boswellic acids (BAs; Figure 1) are assumed to be the pharmacological active principles of frankincense extracts, and based on data obtained from experiments using cellular and animal models (Poeckel and Werz, 2006), there is accumulating evidence for anti-inflammatory and anti-tumorigenic effects of BAs. Pilot clinical studies have suggested some efficacy of frankincense preparations in the treatment of osteoarthritis (OA), rheumatoid arthritis (RA), inflammatory bowel diseases, asthma and cancer (see Ammon, 2006; Poeckel and Werz, 2006). Molecular mechanisms responsible for these therapeutics effects have been mainly attributed to the interference of 3-*O*-acetyl-11-keto-β-BA (AKBA) with signalling pathways including the nuclear factor-κB route (Syrovets *et al.*, 2005), mitogen-activated protein kinase pathway and $Ca^{2+}$ signalling (Poeckel *et al.*, 2006a) as well as targeting human leukocyte elastase (HLE) (Safayhi *et al.*, 1997), 5-lipoxygenase (5-LOX) (Safayhi *et al.*, 1992), platelet-type 12-lipoxygenase (12*S*-LOX) (Poeckel *et al.*, 2006b) and COX-1 (Siemoneit *et al.*, 2008). However, *in vivo* studies confirming the pharmacological relevance of these proposed target interactions are still missing, and the efficacy of defined BAs in *in vivo* models of inflammation remains to be assessed. Recently, we showed that the serine protease cathepsin G is a high affinity and pharmacologically relevant target of BAs (Tausch *et al.*, 2009).

Prostaglandins (PGs) are important lipid mediators derived from arachidonic acid (AA) that control not only numerous physiological events such as blood pressure, blood clotting and sleep, but also inflammation (Funk, 2001). $PGE_2$ is a key player in pyresis, pain and inflammatory responses (Smith,




### Figure 1

Chemical structures of boswellic acids (BAs) and α-amyrin. 3-*O*-Acetyl-β-boswellic acid (Aβ-BA); 3-*O*-acetyl-11-keto-β-boswellic acid (AKBA); β-boswellic acid (β-BA); 11-keto-β-boswellic acid (KBA); 3-*O*-oxaloyl-11-β-keto-boswellic acid (ox-KBA).

1989), and the beneficial therapeutic effects of non-steroidal anti-inflammatory drugs (NSAIDs) are essentially attributed to the suppression of $PGE_2$ (Funk, 2001). The biosynthetic pathway to $PGE_2$ includes the release of AA from membrane phospholipids by phospholipases $A_2$ followed by conversion via COX-1 and –2 to $PGH_2$ and its subsequent isomerization by $PGE_2$ synthases (PGES). mPGES1 is induced by pro-inflammatory stimuli such as interleukin-1β (IL-1β) or lipopolysaccharide (LPS), and receives $PGH_2$ preferentially from COX-2 (Murakami *et al.*, 2002). Thus, inflammation, pain, fever and different types of cancer are closely linked to the increased $PGE_2$ formation originating from up-regulated mPGES1 (Samuelsson *et al.*, 2007). Data from studies using mPGES1-deficient mice indicate that suppression of mPGES1 may provide an efficient pharmacological approach for the treatment of inflammatory diseases (Trebino *et al.*, 2003), avoiding effects on the formation of physiologically important and homeostatic PGs.

Here, we have demonstrated that BAs were direct inhibitors of mPGES1, and particularly β-BA (the most abundant BA in frankincense) was highly effective *in vitro* and *in vivo*. Moreover, we analysed and compared for the first time the anti-inflammatory efficacy of the four major β-configured BAs *in vivo*. As pathophysiological $PGE_2$ formation is mainly related to mPGES1 (Samuelsson *et al.*, 2007), our findings may provide a molecular mechanism contributing to the anti-inflammatory efficacy of BAs or frankincense preparations.

## Methods

### *Animals*

All animal care and experimental procedures complied with Italian regulations on the protection of animals used for experimental and other scientific purpose (Ministerial Decree 116192), and with the European Economic Community regulations (Official Journal of E.C. L 358/1 12/18/1986). Male adult CD1 mice (25–35 g, Harlan, Milan, Italy) and Wistar Han rats (200–220 g, Harlan) were housed in a controlled environment and provided with standard rodent chow and water.

### *Cells and cell viability assay*

A549 cells were cultured as described (Koeberle *et al.*, 2008) and cell viability was measured using the colorimetric MTT dye reduction assay in a 96-well format using a multi-well scanning spectrophotometer (Victor$^3$ plate reader, PerkinElmer, Rodgau-Juegesheim, Germany) as recently reported (Koeberle *et al.*, 2008). Neither α-amyrin nor any of the five BAs (30 μM each) significantly reduced cell viability within 5 h, compared with the effects of dimethyl sulphoxide (DMSO) as vehicle (data not shown), excluding possible acute cytotoxic effects of the compounds in the cellular assays.

### *Cell-free expression of human mPGES1*

Human mPGES1 was obtained by the continuous-exchange cell-free expression system according to Schwarz *et al.* (2007). This system comprises a reaction mixture that contains *Escherichia coli* S30 extract (derived from the A19 strain), T7 polymerase, tRNAs, pyruvate kinase and the template DNA for human mPGES1 (cloned in the pBH4 vector derived from pET19b, Novagen, Gibbstown, NJ, USA). The reaction mixture was dialysed against the feeding mixture that supplies amino acids, energy equivalents acetyl phosphate, and phosphoenol pyruvate as well as nucleotides. Reactions are incubated at 30°C for up to 20 h. Protein synthesis takes place in the reaction mixture and up to 1.5 mg of mPGES1 per mL are obtained in the precipitate. mPGES1 was resuspended in 50 mM potassium phosphate buffer pH 7.4, 1 mM GSH, 10% glycerol and 2% (w/v) LysoFos12 choline (Anatrace, Maumee, OH, USA) for 2 h at 30°C, and insoluble parts were removed by centrifugation (10 000× *g* 10 min, 10°C).

### *Protein pull-down assays using immobilized BAs*

For immobilization of BAs, β-BA or KBA were linked to EAH Sepharose 4B beads via the C3-OH group using glutaric acid as linker as described previously (Poeckel *et al.*, 2006b). For protein pull-down experiments, $1 \times 10^7$ A549 cells were lysed in 375 μL lysis buffer (50 mM HEPES pH 7.4, 200 mM NaCl, 1 mM EDTA, 1% Triton X-100, 2 mM phenylmethanesulfonyl fluoride, 10 $\mu g \cdot mL^{-1}$ leupeptin and 120 $\mu g \cdot mL^{-1}$ soybean trypsin inhibitor). After sonification ($3 \times 8$ s) and centrifugation (12 000× *g*, 10 min, 4°C), 125 μL of the Sepharose slurries (50%, v/v) were added to the lysates and incubated at 4°C overnight, with continuous rotation. For pull-down experiments with isolated mPGES1, 200 ng of the purified enzyme was diluted into 500 μL lysis buffer containing 1000-fold excess of *E. coli* (BL21 strain) protein, and 100 μL of the Sepharose slurries (50%, v/v) were added. Beads were extensively washed three times with 10 volumes of binding buffer (HEPES pH 7.4, 200 mM NaCl, 1 mM EDTA) and precipitated proteins were denatured by the addition of sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) sample loading buffer (20 mM Tris-HCl, pH 8, 2 mM EDTA, 5% (m/v) SDS, 10% β-mercaptoethanol). After boiling (95°C,

6 min), proteins were separated by SDS-PAGE and analysed by Western blotting, using specific antibodies against COX-2 (Biomol Intern., Hamburg, Germany) and mPGES1 (Cayman Chemical, Ann Arbor, MI, USA). Ponceau S-staining of the membranes after blotting assured equal protein loading of bound proteins to the beads.

### *Surface plasmon resonance spectroscopy*

A BIAcore X device (GE Healthcare Bio-Sciences, Freiburg, Germany) was used. *In vitro*-translated mPGES1 (100 μg·mL$^{-1}$) in 10 mM Na-acetate pH 6.0 was coupled to a carboxymethylated dextran surface (CM-5 chip, GE Healthcare) using standard amine coupling procedure according to the manufacturer's instructions. Flow cell 1 on the chip was not altered (reference) whereas on flow cell 2, mPGES1 (236 fmol·mm$^{-2}$) was immobilized corresponding to 4700 resonance units (RU) and equilibrated by a continuous flow of assay buffer (10 mM HEPES, 150 mM NaCl, 0.01% surfactant P20, and 1% DMSO, pH 7.4) The stock solution of 3-*O*-oxaloyl-KBA (in DMSO) was diluted into assay buffer. Measurements were performed at 25°C and at a flow rate of 30 μL·min$^{-1}$. After recording association, the liquid phase was replaced by assay buffer, and the dissociation was monitored. The binding profiles were obtained after subtracting the response signal of the untreated reference cell 1, and sensograms were processed by using automatic correction for non-specific bulk refractive index effects using BIAEVALUATION Version 3.1 software (GE Healthcare Bio-Sciences).

To obtain dissociation constants from the equilibrium binding data, two different fitting models were adopted. First, the change in the equilibrium amount of compound bound as a function of the concentration of compound was fitted to the equation (Eqn 1) for a simple 1:1 binding model:

$$R_{eq} = (R_{max} \times [compound]/(K_D + [compound]) \quad (1)$$

where $R_{eq}$ is the equilibrium response, $R_{max}$ is the maximum response and $K_D$ is the dissociation constant. A Scatchard analysis was also used to determine $K_D$ (represented by the negative reciprocal of the slope). Analysis employing the BIAEVALUATION software version 3.1 was performed to determine kinetics. The integrated rate equation describing a 1:1 Langmuir interaction was fitted simultaneously to the entire concentration range for 3-*O*-oxaloyl-KBA. This fit yielded the association rate $k_a$, the dissociation rate $k_d$ and the dissociation constant $K_D$ (Roden and Myszka, 1996; Karlsson and Falt, 1997). The quality of the fit was determined by the $\chi^2$-values as well as the magnitude and distribution of the residuals.

### *Stimulation of A549 cells and isolation of microsomes*

Preparation of A549 cells was performed as described (Koeberle *et al.*, 2009). In brief, cells were incubated for 16 h at 37°C and 5% $CO_2$, and after changing the medium, mPGES1 expression was induced by IL-1β (1 ng·mL$^{-1}$). After 72 h, cells were frozen in liquid nitrogen, ice-cold homogenization buffer (0.1 M potassium phosphate buffer pH 7.4, 1 mM phenylmethanesulfonyl fluoride, 60 μg·mL$^{-1}$ soybean trypsin inhibitor, 1 μg·mL$^{-1}$ leupeptin, 2.5 mM GSH and 250 mM sucrose) was added, and after 15 min, cells were resuspended and sonicated on ice (3 × 20 s). The homogenate was subjected to differential centrifugation at 10 000× *g* for 10 min and at 174 000× *g* for 1 h at 4°C. The pellet (microsomal fraction) was resuspended in 1 mL homogenization buffer and the protein concentration was determined by the Coomassie protein assay.

### *Determination of PGES1 activity in microsomes of A549 cells*

Microsomal membranes of A549 cells were diluted in potassium phosphate buffer (0.1 M, pH 7.4) containing 2.5 mM GSH (100 μL total volume), and $PGE_2$ formation was initiated by the addition of $PGH_2$ (20 μM, final concentration). After 1 min at 4°C, the reaction was terminated with 100 μL of stop solution (40 mM $FeCl_2$, 80 mM citric acid and 10 μM of 11β-$PGE_2$); $PGE_2$ was separated by solid phase extraction and analysed by reversed phase-high performance liquid chromatography (RP-HPLC) as described (Koeberle *et al.*, 2009).

### *Determination of $PGE_2$ and 6-keto $PGF_{1\alpha}$ formation in intact A549 cells*

The expression of mPGES1 in A549 cells was induced as described previously. After trypsinization, cells were washed twice with phosphate-buffered saline (PBS), resuspended in PBS ($4 \times 10^6$ mL$^{-1}$) containing $CaCl_2$ (1 mM) and pre-incubated with the indicated compounds at 37°C for 10 min. Prostanoid formation was started by the addition of ionophore A23187 (2.5 μM), AA (1 μM) and [$^3$H]AA (18.4 kBq). The reaction was stopped after 15 min at 37°C, and the samples were put on ice. For quantification of radiolabelled $PGE_2$, samples were extracted, fractionated by HPLC and then analysed by liquid scintillation counting (Koeberle *et al.*, 2008). 6-Keto $PGF_{1\alpha}$ was determined by High Sensitivity EIA Kits (Assay Designs, Ann Arbor, MI, USA) according to the manufacturer's protocols.

### *Determination of prostanoid formation in human whole blood*

Human venous blood from healthy adult donors, who had not received any medication for at least

2 weeks (Blood Center, University Hospital Tuebingen, Germany), was freshly withdrawn and collected in monovettes containing 16 IE heparin $mL^{-1}$ (Sarstedt, Nümbrecht, Germany). For determination of $PGE_2$ and 6-keto $PGF_{1\alpha}$, aliquots of whole blood (0.8 mL) were mixed with CV4151 (1 μM) and with aspirin (50 μM). For determination of $TXB_2$, aliquots of whole blood (0.5 mL) were used without the addition of CV4151. A total volume of 1 mL was adjusted with sample buffer (10 mM potassium phosphate buffer pH 7.4, 3 mM KCl, 140 mM NaCl and 6 mM D-glucose). After pre-incubation with the indicated compounds for 5 min at room temperature, the samples were stimulated with LPS (10 $\mu g \cdot mL^{-1}$) for 5 h at 37°C. Prostanoid formation was stopped on ice, the samples were centrifuged (2300× *g*, 10 min, 4°C), and 6-keto $PGF_{1\alpha}$ and $TXB_2$ were quantified in the supernatant using High Sensitivity EIA Kits (Assay Designs), according to the manufacturer's protocols. $PGE_2$ was determined as described (Koeberle *et al.*, 2008). In brief, the supernatant was acidified with citric acid (30 μL, 2 M), and after centrifugation (2300× *g*, 10 min, 4°C), solid phase extraction and RP-HPLC, analysis of $PGE_2$ was performed to isolate $PGE_2$. The $PGE_2$ peak (3 mL), identified by co-elution with the authentic standard, was collected, and acetonitrile was removed under a nitrogen stream. The pH was adjusted to 7.2 by the addition of 10 × PBS buffer pH 7.2 (230 μL) before $PGE_2$ contents were quantified using a $PGE_2$ High Sensitivity EIA Kit (Assay Designs) according to the manufacturer's protocol.

For determination of the COX product 12(*S*)-hydroxy-5-*cis*-8,10-*trans*-heptadecatrienoic acid (12-HHT), freshly drawn human blood (2 mL) was pre-incubated with the indicated compounds at 37°C for 10 min, and 30 μM $Ca^{2+}$ ionophore A23187 was added. After 10 min, the reaction was stopped on ice, and the samples were centrifuged (600× *g*, 10 min, 4°C). Aliquots of the resulting plasma (500 μL) were then mixed with 2 mL of methanol, and 200 ng prostaglandin $B_1$ was added as internal standard. The samples were cooled to –20°C for 2 h and centrifuged again (600× *g*, 15 min, 4°C). The supernatants were collected and diluted with 2.5 mL PBS and 75 μL HCl 1N, and formed 12-HHT was extracted and analysed by HPLC as described (Siemoneit *et al.*, 2008).

## *Carrageenan-induced paw oedema*

Mice were divided into groups ($n = 10$ for each group) and lightly anaesthetized with enflurane (4%) mixed with $O_2$, 0.5 $L \cdot min^{-1}$, and $N_2O$, 0.5 $L \cdot min^{-1}$. Each group of animals received subplantar administration of saline (0.05 mL) or λ-carrageenan type IV (1% w/v) (0.05 mL) in saline. The paw was marked in order to immerse it to the same extent in the measurement chamber. The volume was measured by using a hydroplethysmometer, specially modified for small volumes (Ugo Basile, Milan, Italy) immediately before subplantar injection and 2, 4 and 6 h thereafter. The assessment of paw volume was performed always under double blind conditions and by the same operator. The increase in paw volume was calculated by subtracting the initial paw volume (basal) to the paw volume measured at each time point.

## *Carrageenan-induced pleurisy*

Rats were anaesthetized with enflurane (4%) mixed with $O_2$, 0.5 $L \cdot min^{-1}$, $N_2O$, 0.5 $L \cdot min^{-1}$, and submitted to a skin incision at the level of the left sixth intercostal space. The underlying muscle was dissected, and saline (0.2 mL) or λ-carrageenan type IV (1% w/v) (0.2 mL) was injected into the pleural cavity. The skin incision was closed with a suture, and the animals were allowed to recover. At 4 h after the injection of λ-carrageenan, the animals were killed by inhalation of $CO_2$. The chest was carefully opened, and the pleural cavity was rinsed with 2 mL saline solution containing heparin (5 $U \cdot mL^{-1}$). The exudate and washing solution were removed by aspiration, and the total volume was measured using an adjustable-volume pipette (P1000, range volume 1000–200 μL or P200, range volume 200–30 μL). Any exudate that was contaminated with blood was discarded. The amount of exudate was calculated by subtracting the volume injected (2 mL) from the total volume recovered. Leukocytes in the exudate were resuspended in PBS and counted with an optical light microscope in a Burker's chamber after vital staining with Trypan blue.

The amounts of $PGE_2$ and 6-keto $PGF_{1\alpha}$ in the supernatant of centrifuged exudate (800× *g*, 10 min) were assayed by radioimmunoassay ($PGE_2$) and EIA (6-keto $PGF_{1\alpha}$), respectively (Cayman Chemical), according to the manufacturer's protocol. The results are expressed as ng per rat and represent the mean ± SE of 10 rats.

## *Experimental design of animal experiments*

For i.p. administration, the test compounds were dissolved in DMSO and diluted with saline, achieving a final DMSO concentration of 2 or 4%. For oral administration, the compounds were dissolved in water containing 0.5% (w/v) carboxymethylcellulose, 10% Tween-20 (v/v), and 1% sesame oil (v/v).

For carrageenan-induced paw oedema in the treated group of animals, β-BA (0.25 and 1 $mg \cdot kg^{-1}$) or indomethacin (5 $mg \cdot kg^{-1}$, reference compound) were given i.p. 30 min before carrageenan. The

vehicle-treated group of mice received DMSO 2% (i.p.).

For carrageenan-induced pleurisy in the treated group of animals, the BAs (1 mg·kg$^{-1}$, each) and indomethacin (5 mg·kg$^{-1}$, reference compound) were given either i.p. or p.o. 30 min before carrageenan. The vehicle-treated group of rats received DMSO 4% (i.p.) or water containing 0.5% CMC, 10% Tween-20 and 1% sesame oil (p.o.). DMSO (4%, i.p.) itself did not affect exudate volume as compared with saline (0.30 ± 0.071 and 0.34 ± 0.02 mL respectively).

### *Data analysis*

Data are expressed as mean ± SE. The program Graphpad Instat (Graphpad Software Inc., San Diego, CA, USA) was used for statistical comparisons of the data by one-way analyses of variance for independent or correlated samples followed by Tukey HSD *post hoc* tests. Where appropriate, Student's *t*-test for paired and correlated samples was applied. A $P$ value of <0.05 (*) was considered significant. $IC_{50}$ values of compounds are approximations determined by graphical analysis (linear interpolation between the points between 50% activity).

### *Materials*

BAs were prepared as previously described (Jauch and Bergmann, 2003). All BAs and synthetic derivatives were at least 95% pure. Structures were confirmed by $^{1}$H-nuclear magnetic resonance (NMR) spectroscopy, $^{13}$C-NMR spectroscopy, H,H-correlated spectroscopy (COSY), heteronuclear multiple quantum coherence (HMQC), heteronuclear multiple bond correlation, HMQC-COSY, and nuclear Overhauser and exchange spectroscopy as well as electrospray ionization mass spectrometry in the negative mode. α-Amyrin was from Extrasynthèse (Genay, France); EAH-Sepharose 4B was from GE Healthcare Bio-Sciences (Freiburg, Germany); thromboxane synthase inhibitor CV4151 was a gift by Dr Stefan Laufer (Tuebingen, Germany); DMEM/High glucose (4.5 g·L$^{-1}$) medium, penicillin, streptomycin, trypsin/EDTA solution, were from PAA (Coelbe, Germany); $PGH_2$ was from Larodan (Malmö, Sweden); 11β-$PGE_2$, MK-886, [5, 6, 8, 9, 11, 12, 14, 15-$^{3}$H] AA ([$^{3}$H]AA), were from BioTrend Chemicals GmbH (Cologne, Germany); Ultima Gold™ XR was from Perkin Elmer (Boston, MA, USA); λ-carrageenan type IV isolated from *Gigartina aciculaire* and *Gigartina pistillata* was from Sigma-Aldrich (Milan, Italy); [$^{3}$H-$PGE_2$], PerkinElmer Life Sciences (Milan, Italy); $PGE_2$ antibody, Sigma-Aldrich. AA, LPS, fetal calf serum and all other chemicals were obtained from Sigma-Aldrich (Deisenhofen, Germany) unless stated otherwise. Nomenclature for the receptors and molecular targets studied here follows Alexander *et al.* (2009).

## Results

### *Identification of mPGES1 as a BA-binding protein*

A target fishing approach using immobilized BAs (Poeckel *et al.*, 2006b) was applied in order to investigate whether or not BAs interact with mPGES1. Lysates of IL-1β-treated A549 cells, expressing mPGES1 (Jakobsson *et al.*, 1999), were incubated with resins composed of β-BA or KBA, linked via the C3-OH moiety to glutaric acid and coupled to EAH Sepharose 4B beads yielding β-BA-Seph or KBA-Seph respectively. EAH Sepharose 4B beads without ligand (Seph) were used as the negative control. Beads were extensively washed and the bound proteins were detached by the addition of SDS-PAGE sample loading buffer followed by separation by SDS-PAGE and Western blot analysis, using specific antibodies against mPGES1 and COX-2. Substantial amounts of mPGES1 were bound to BA-Seph beads but hardly any was bound to Seph beads without ligand (Figure 2A). In contrast, COX-2 was not detected in these precipitated proteins. Furthermore, *in vitro*-translated mPGES1 (200 ng purified

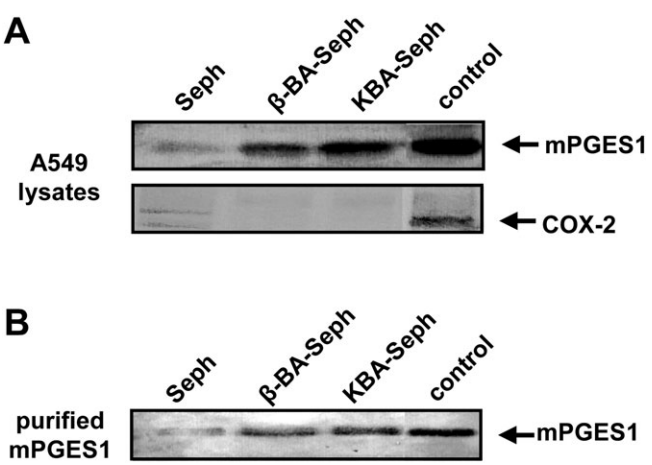



**Figure 2**

Boswellic acids (BAs) bind to microsomal prostaglandin $E_2$ synthase 1 (mPGES1). (A) Supernatants of A549 cell lysates were incubated with β-BA-Seph, KBA-Seph or with Seph, as indicated. (B) Purified, *in vitro*-translated mPGES1 (200 ng) was incubated with β-BA-Seph, KBA-Seph or Seph beads. Precipitated proteins were separated by SDS-PAGE, and visualized by Western blotting, using specific antibodies against mPGES1 (A,B) or COX-2 (A). An aliquot of the supernatant was used as positive control. Similar results were obtained in three additional experiments. KBA, 11-keto-β-boswellic acid; SDS-PAGE, sodium dodecyl sulphate polyacrylamide gel electrophoresis.

protein) in the presence of 1000-fold excess of *E. coli* protein was precipitated by both KBA-Seph and β-BA-Seph, but was minimally precipitated by Seph beads (Figure 2B).

To characterize the interaction of BAs with mPGES1 in more detail, surface plasmon resonance (SPR) spectroscopy studies were carried out. The progress of interaction, binding of analyte (association) and dissociation from the immobilized mPGES1 was monitored as a sensogram, expressing changes in binding responses as RU. Unfortunately, no consistent binding patterns were obtained using naturally occurring BAs as analytes, presumably due to their high lipophilicity leading to concentration-dependent aggregation and super-stoichiometric binding behaviour (Giannetti *et al.*, 2008). Neither the addition of bovine serum albumin as carrier protein nor the variation of commercial assay buffers (with or without detergent) or changes in temperature improved the quality of the recorded sensograms. Hence, we used the more hydrophilic synthetic derivate 3-*O*-oxaloyl-KBA (10 μM) to obtain more valuable and reproducible sensograms, which indicated specific reversible binding to mPGES1 (Figure 3A). α-Amyrin (a pentacyclic triterpene which lacks the C4-COOH moiety of BAs; Figure 1) failed to bind mPGES1 up to concentrations of 30 μM (Figure 3A). In order to determine equilibrium-binding constants, 3-*O*-oxaloyl-KBA was analysed at concentrations ranging from 0.5 to 25 μM. The equilibrium response ($R_{eq}$) was calculated, and fitting the data to the 1:1 binding model (Eqn. 1) and Scatchard plot analysis yielded $K_D$ values of 13 and 5.2 μM respectively (Figure 3B).

Kinetic data were estimated using BIAEVALUATION 3.1 software. Assuming the simple relationship $k_a/k_d = K_D$ for 3-*O*-oxaloyl-KBA, a $K_D$ value of 23 μM was calculated that essentially matches the $K_D$ obtained from the equilibrium binding data (Figure 3C). Nevertheless, these kinetic parameters should be regarded as rough determinations rather than absolute values. Together, these data support a direct physical interaction between BAs and mPGES1.

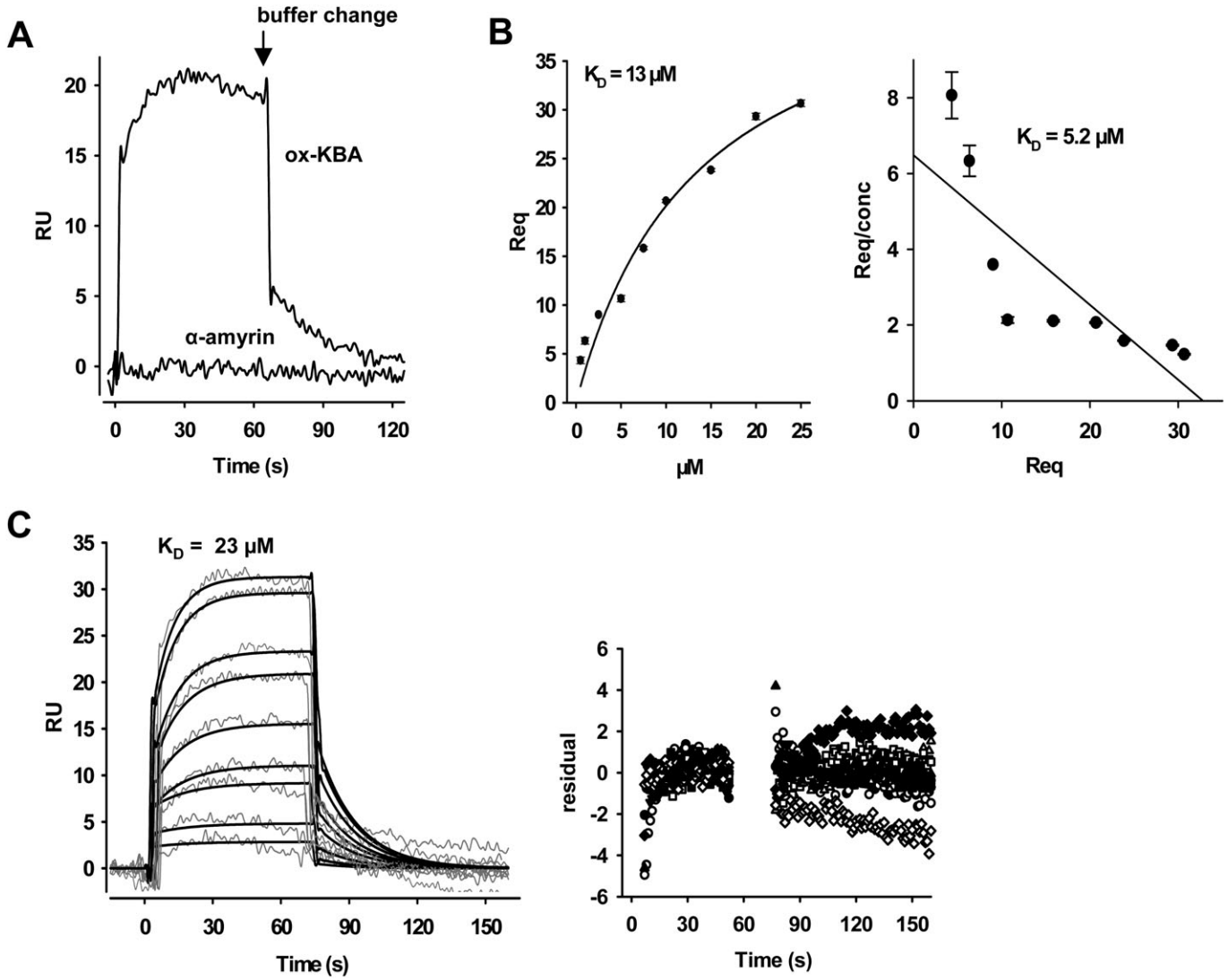
## *BAs inhibit the catalytic activity of mPGES1 in a cell-free assay*

Next, we investigated whether BAs may affect the catalytic activity of mPGES1. Isolated microsomes of IL-1β-treated A549 cells were pre-incubated with BAs, and $PGE_2$ formation was induced by the addition of 20 μM $PGH_2$. The mPGES1 inhibitor MK-886 was used as reference drug (Claveau *et al.*, 2003; Koeberle *et al.*, 2008) and blocked $PGE_2$ formation with an $IC_{50}$ = 2 μM (not shown). AKBA, β-BA and KBA concentration dependently suppressed $PGE_2$ formation with $IC_{50}$ values of 3, 5, and 10 μM respectively. As previously observed for other mPGES1 inhibitors (Koeberle *et al.*, 2008; Koeberle and Werz, 2009), about 20–30% activity still remained even at high concentrations of BAs (100 μM, Figure 4A) or of MK-886 (30 μM, not shown), suggesting mPGES1-independent basal formation of $PGE_2$. The synthetic derivative 3-*O*-oxaloyl-KBA suppressed $PGE_2$ formation ($IC_{50}$ = 5 μM, not shown), which is in good agreement with the SPR data. Aβ-BA was less potent ($IC_{50} \geq 30$ μM) and α-amyrin was entirely inactive up to 100 μM (data not shown).

To assess whether the inhibition of mPGES1 by BAs occurs in a reversible fashion, washout experiments were performed. Microsomal preparations of A549 cells were pre-incubated with BAs (10 μM, each), and MK-886 (3 μM) served as a control for a reversible mPGES1 inhibitor (Koeberle *et al.*, 2008). Because all four BAs should act by a common mode, only the most active representatives (i.e. AKBA and β-BA) were tested. MK-886 at 3 μM or BAs at 10 μM efficiently blocked $PGE_2$ formation (Figure 4B). Upon 10-fold dilution, a significant loss of potency was observed and the inhibition was comparable with the effect of BAs at 1 μM or MK-886 at 0.3 μM. Hence, BAs may inhibit mPGES1 in a reversible manner, as did MK-886.

## *Effects of BAs on prostanoid biosynthesis in intact cells*

The treatment of A549 cells with IL-1β for 72 h results in the co-expression of COX-2 and mPGES1 (Thoren and Jakobsson, 2000), whereas COX-1 is essentially absent (Asano *et al.*, 1996). We used this model to assess whether BAs inhibit $PGE_2$ formation also in intact cells, and whether BAs selectively inhibit COX-2-derived $PGH_2$ transformation to $PGE_2$ without affecting the biosynthesis of other COX-2-derived prostanoids (i.e. 6-keto $PGF_{1\alpha}$). The formation of prostanoids was induced by the stimulation of A549 cells with 2.5 μM A23187 plus 1 μM AA and $^3$[H]AA (18.4 kBq). The use of A23187 and exogenous AA to induce prostanoid formation excludes effects of BAs on receptor-coupled signal transduction and/or on endogenous substrate supply for COX-2. In agreement with the effects in the cell-free mPGES1 activity assay, AKBA, KBA and β-BA (30 μM, each) significantly inhibited $PGE_2$ synthesis to the same extent as MK-886 (30 μM), whereas Aβ-BA or α-amyrin were barely or not active (Figure 5A). More detailed analysis showed that AKBA, KBA and β-BA suppressed $PGE_2$ synthesis in a concentration-dependent manner ($IC_{50}$ approximately 20–30 μM, Figure 5B). Nevertheless, the



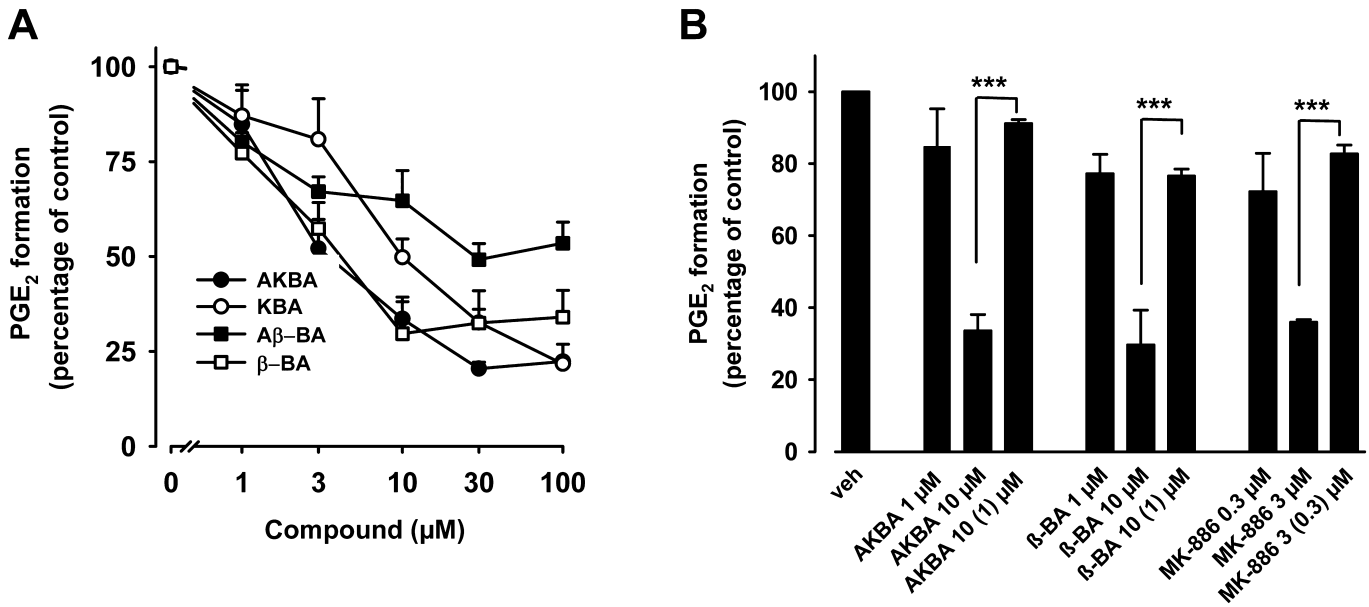

## Figure 3

Analysis of the binding of boswellic acids to microsomal prostaglandin $E_2$ synthase 1 (mPGES1) by surface plasmon resonance spectroscopy. *In vitro*-translated mPGES1 was coupled to a CM5 biosensor chip and 3-*O*-oxaloyl-KBA was used as analyte. Specific binding profiles were obtained after subtracting the signal [response units (RU)] from the untreated control cell. (A) Binding of 3-*O*-oxaloyl-KBA (ox-KBA) and α-amyrin (10 µM each) to mPGES1. (B) Binding curves for 3-*O*-oxaloyl-KBA. The equilibrium responses ($R_{eq}$) for 3-*O*-oxaloyl-KBA at different concentrations were plotted versus the concentration of the compound. (C) Kinetic analysis of 3-*O*-oxaloyl-KBA-binding to mPGES1. Representative sensograms for the injection of 0.5 µM up to 25 µM 3-*O*-oxaloyl-KBA are shown. A general analysis was applied to fit the data to a 1:1 binding model (bold lines), and the quality of the fit is displayed by the plots of the residuals. Results are representative for at least three independent experiments. KBA, 11-keto-β-boswellic acid.

suppression of $PGE_2$ formation by BAs was not complete and 30 µM MK-886 also caused only 47% inhibition (Figure 5A). Importantly, the concomitant generation of 6-keto $PGF_{1\alpha}$ was unaffected by all BAs, α-amyrin and by MK-886 (Figure 5A), whereas the COX-2 inhibitor celecoxib (5 µM) blocked the formation of both $PGE_2$ and 6-keto $PGF_{1\alpha}$ to the same extent, as expected. Also, celecoxib was much more efficient in the suppression of $PGE_2$ formation, compared with BAs or MK-886 (Figure 5A).

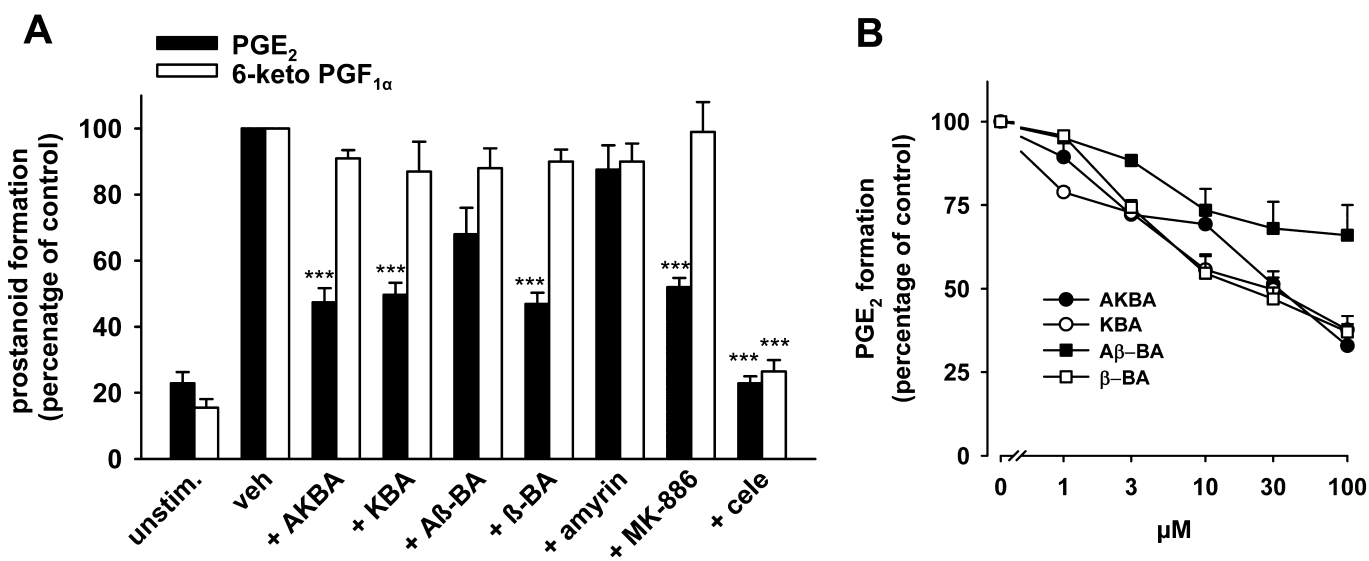
### *Effects of BAs on prostanoid formation in human whole blood*

In order to estimate the efficacy of BAs to interfere with (COX-2/mPGES1-derived) $PGE_2$ formation in a more complex biological test system, human whole blood assays were performed. Heparinized blood was pre-incubated with BAs for 10 min, prior to stimulation with LPS (10 $\mu g \cdot mL^{-1}$) for 5 h (Koeberle *et al.*, 2008). β-BA significantly reduced $PGE_2$ synthesis (46% inhibition) at 10 µM comparable with MK-886 at 30 µM (45% inhibition), whereas the



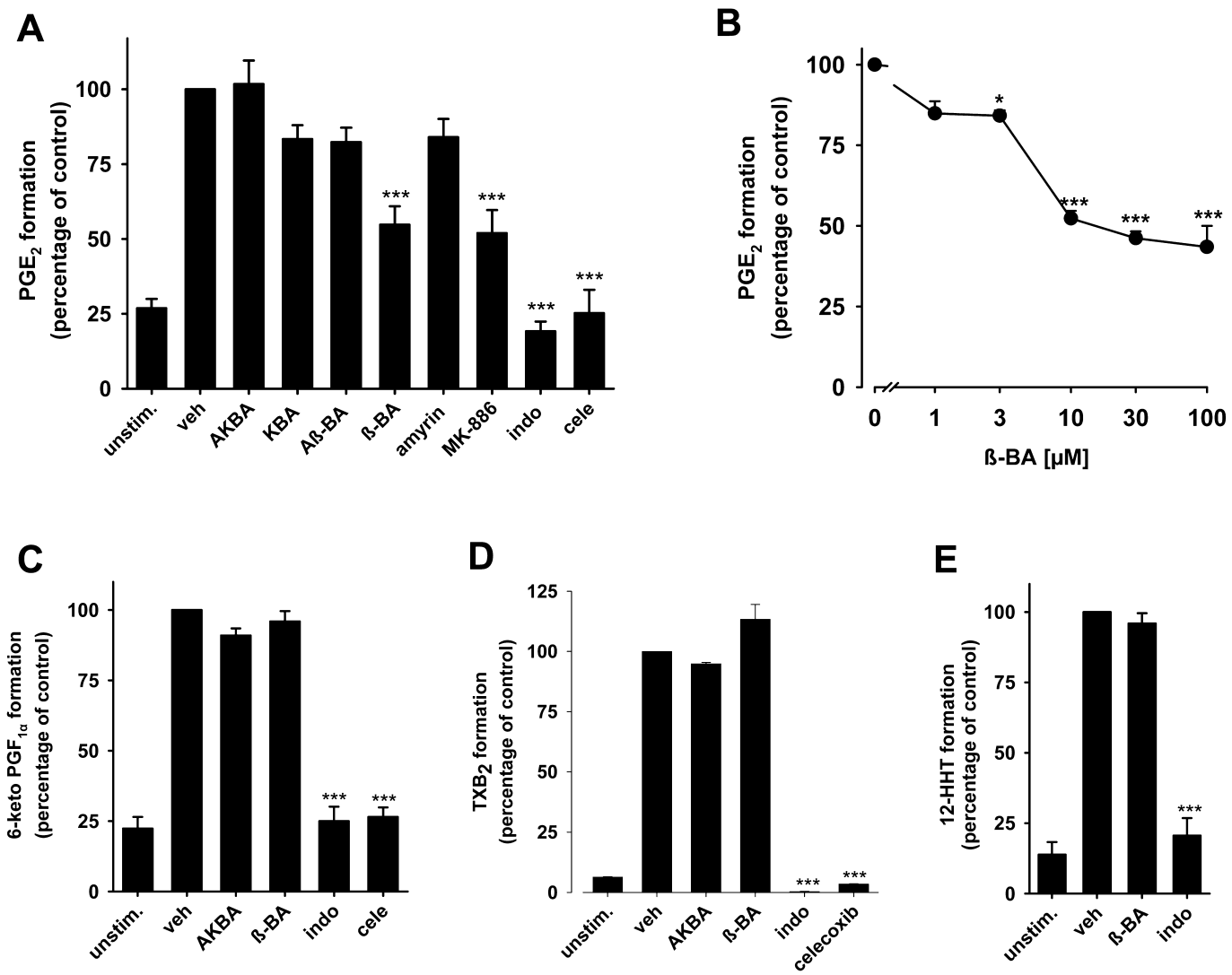

## Figure 4

Effects of boswellic acids (BAs) on the activity of microsomal prostaglandin $E_2$ synthase 1 (mPGES1) in a cell-free assay. (A) Concentration-response analysis. Microsomal preparations of IL-1β-stimulated A549 cells were pre-incubated with vehicle (DMSO) or BAs for 15 min at 4°C. $PGH_2$ was added and after 1 min, the reaction was stopped and $PGE_2$ was analysed by RP-HPLC as described. The 100% value corresponds to 944 ± 118 pmol $PGE_2$ formed. (B) Reversibility of mPGES1 inhibition. Microsomal fractions of IL-1β-stimulated A549 cells were pre-incubated with 3 μM MK-886 or 10 μM BAs. An aliquot was diluted 10-fold to obtain an inhibitor concentration of 0.3 and 1 μM respectively. For comparison, microsomal preparations were pre-incubated with 0.3 μM MK-886, 1 μM BA or with vehicle (veh, DMSO), and then, 20 μM $PGH_2$ was added (no dilution). After 1 min, $PGE_2$ formation was analysed by RP-HPLC. Data are given as mean + SE, $n = 3–4$, ***$P < 0.001$ versus vehicle (DMSO) control. DMSO, dimethyl sulphoxide; RP-HPLC, reversed phase-high performance liquid chromatography.




## Figure 5

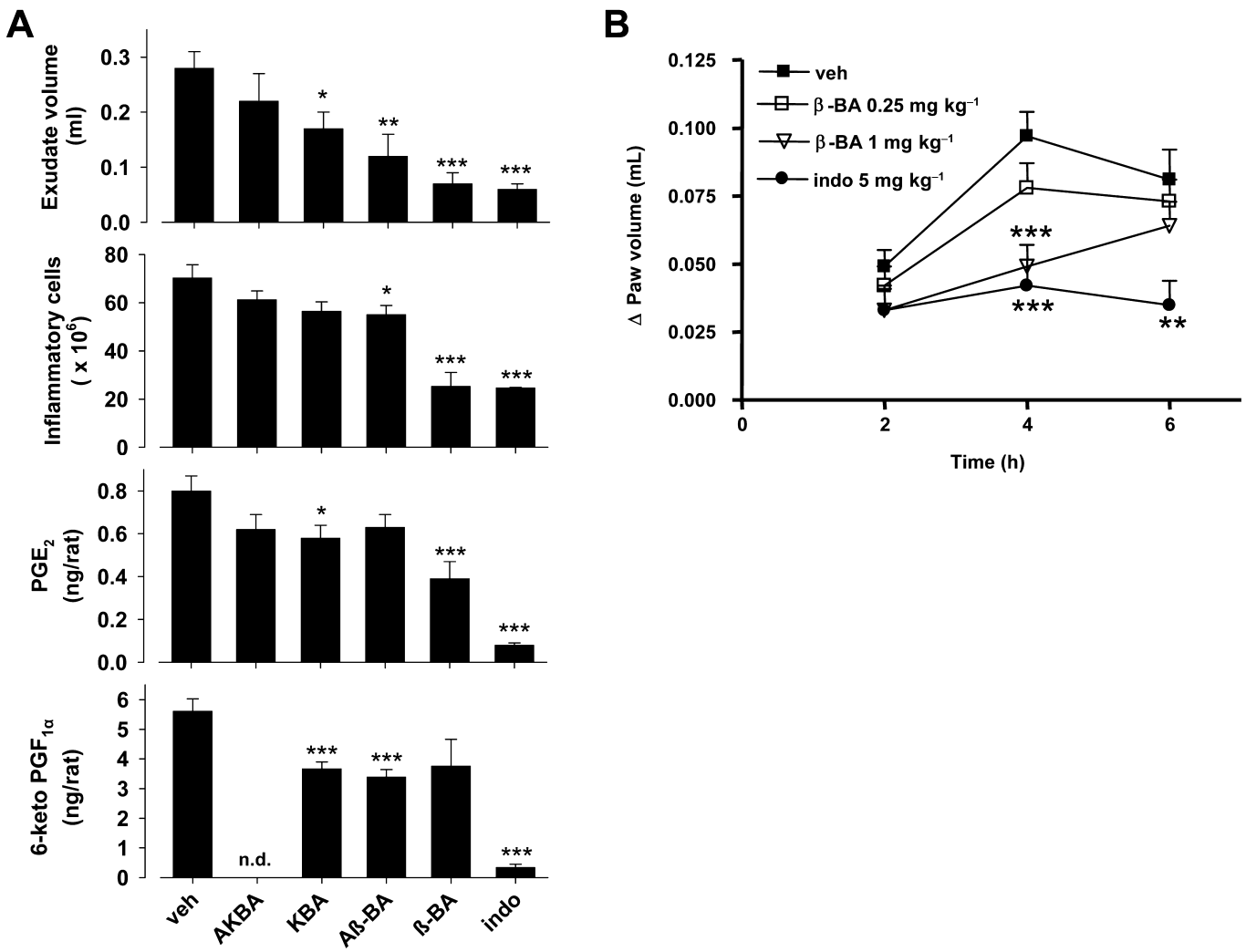
Effects of boswellic acids (BAs) on $PGE_2$ and 6-keto $PGF_{1\alpha}$ formation in intact A549 cells. IL-1β-treated A549 cells were pre-incubated (A) with vehicle (veh, DMSO), BAs (30 μM each), α-amyrin (30 μM), MK-886 (30 μM) or celecoxib (cele, 5 μM) (B) with BAs at the indicated concentrations. After 10 min at 37°C, 2.5 μM A23187 plus 1 μM AA and [$^3$H]AA (18.4 kBq) were added (or left untreated = unstim.) and after another 15 min, formed [$^3$H]$PGE_2$ was analysed as described in the Methods. 6-Keto $PGF_{1\alpha}$ was analysed using High Sensitivity EIA Kits; the 100% value corresponds to 87 ± 11 pg $10^6$ cells. Data are given as mean + SE, $n = 3–5$. ***$P < 0.001$ versus vehicle (DMSO) control. AA; arachidonic acid; DMSO, dimethyl sulphoxide.




## Figure 6

Effects of boswellic acids (BAs) on prostanoid biosynthesis in human whole blood. Heparinized human whole blood, treated with 1 µM CV4152 and 50 µM aspirin, was pre-incubated with (A) BAs and α-amyrin (10 µM, each) or vehicle (veh, DMSO) for 10 min at RT and then 10 µg·mL$^{-1}$ LPS was added (or left untreated = unstim.). After 5 h at 37°C, $PGE_2$ was separated by RP-HPLC and quantified by EIA. Controls: MK-886 (30 µM), indomethacin (indo, 50 µM), and celecoxib (cele, 20 µM). (B) Concentration-response of β-BA. (C,D) 6-keto $PGF_{1\alpha}$ and $TXB_2$ formation. 6-Keto $PGF_{1\alpha}$ (C) was directly determined in blood plasma from samples above (see A) incubated with β-BA or AKBA (10 µM, each), indo (50 µM), cele (20 µM) or veh (DMSO). Inhibition of $TXB_2$ formation (D) was assessed in heparinized human whole blood without CV4152 and aspirin. Both 6-keto $PGF_{1\alpha}$ and $TXB_2$ were measured by EIA. The 100% values corresponds to 221.8 ± 19.7 pg·mL$^{-1}$ $PGE_2$, 382.5 ± 22.3 pg·mL$^{-1}$ 6-keto $PGF_{1\alpha}$, and 37.9 ± 4.4 ng·mL$^{-1}$ $TXB_2$ respectively. (E) 12-HHT formation. Heparinized human blood was pre-incubated with β-BA (50 µM), indo (20 µM) or veh (DMSO) for 10 min, and A23187 (30 µM) was added (or left untreated = unstim.). After 10 min at 37°C, 12-HHT was analysed by HPLC. The 100% value corresponds to 148.8 ± 16.7 ng·mL$^{-1}$ 12-HHT. Data are given as mean + SE, $n$ = 4–5; *$P$ < 0.05; ***$P$ < 0.001 versus vehicle (0.1% DMSO) control. β-BA, β-boswellic acid; AKBA, 3-O-acetyl-11-keto-β-boswellic acid; DMSO, dimethyl sulphoxide; RP-HPLC, reversed phase-high performance liquid chromatography.

other BAs and α-amyrin failed in this respect (Figure 6A). Concentration-response experiments revealed an $IC_{50}$ value of 10 µM for β-BA (Figure 6B), but even at high concentrations (100 µM), about 40% $PGE_2$ still remained. Indomethacin (50 µM) and celecoxib (20 µM) efficiently inhibited $PGE_2$ formation in whole blood. In contrast, the concomitant formation of the COX-2-derived 6-keto $PGF_{1\alpha}$ (Figure 6C) or $TXB_2$ (Figure 6D) was not affected by β-BA or AKBA (and also not by Aβ-BA or KBA, not shown), implying that the suppressive effect on $PGE_2$ synthesis is not related to reduced generation of the common precursor $PGH_2$ (e.g. by inhibition of $PLA_2$ or COX), but instead is due to select inhibition of $PGH_2$ transformation to $PGE_2$. Indomethacin and celecoxib efficiently suppressed the formation of all three prostanoids as expected. Moreover, β-BA (50 µM), in contrast to indomethacin, failed to




## Figure 7

Effects of boswellic acids (BAs) in animal models *in vivo*. (A) Carrageenan-induced pleurisy in rats. Thirty minutes before intrapleural injection of carrageenan, rats ($n = 10$ for each experimental group) were treated i.p. with BAs (1 mg·kg$^{-1}$ each), indomethacin (5 mg·kg$^{-1}$) or vehicle (veh, DMSO 4%). Exudate volume, $PGE_2$ and 6-keto $PGF_{1\alpha}$ levels as well as inflammatory cell accumulation in pleural cavity were assessed 4 h after carrageenan injection. Data are expressed as mean ± SE, $n = 10$. *$P < 0.05$; **$P < 0.01$; ***$P < 0.01$ versus vehicle; n.d. = not determined. (B) Carrageenan-induced mouse paw oedema. Animals ($n = 10$ for each experimental group) were treated i.p. with 0.25 and 1 mg·kg$^{-1}$ β-BA, 5 mg·kg$^{-1}$ indomethacin (indo) or veh (2% DMSO) 30 min before carrageenan subplantar injection. Data are given as mean + SE, *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ versus vehicle control. DMSO, dimethyl sulphoxide.

affect COX-1-mediated generation of 12-HHT in whole blood stimulated with A23187 (Figure 6E).

### *Effects of BAs on carrageenan-induced rat pleurisy and mouse paw oedema*

Although the anti-inflammatory efficacy of undefined mixtures of BAs in animal models are well documented (Poeckel and Werz, 2006; Singh *et al.*, 2007), it is still unclear which of the BAs is responsible for the beneficial actions *in vivo*. Therefore, we assessed the effects of the four major β-configured BAs in the well-recognized carrageenan-induced rat pleurisy model. An injection of carrageenan into the pleural cavity of rats (DMSO 4% group) elicited an acute inflammatory response within 4 h characterized by the accumulation of exudate that contained large numbers of inflammatory cells (Figure 7A). β-BA (1 mg·kg$^{-1}$) given i.p. 30 min prior to carrageenan potently inhibited the inflammatory response, as demonstrated by the significant attenuation of exudate formation (75%) and cell infiltration (64%), being as effective as the reference drug indomethacin (5 mg·kg$^{-1}$, i.p.) (Figure 7A). Aβ-BA and KBA (1 mg·kg$^{-1}$ i.p., each) were markedly less efficient and AKBA (1 mg·kg$^{-1}$ i.p.) caused no significant effects. For β-BA, the $PGE_2$ levels in the

## Table 1

Effect of β-BA on carrageenan-induced pleurisy in rats

| Treatment | Exudate volume (mL) | Inflammatory cells × $10^6$ | $PGE_2$ (ng·rat$^{-1}$) |
|---|---|---|---|
| Vehicle | 0.19 ± 0.020 | 47.1 ± 3.0 | 2.15 ± 0.20 |
| β-BA | 0.029 ± 0.016*** | 29.6 ± 2.8*** | 1.21 ± 0.14** |
| 1 mg·kg$^{-1}$ | 85% | 37% | 44% |
| Indo | 0 | 17.0 ± 4.9** | n.d. |
| 5 mg·kg$^{-1}$ | 100% | 64% | 100% |

**$P < 0.01$; ***$P < 0.001$ versus vehicle.

Thirty minutes before intrapleural injection of carrageenan, rats ($n = 11$ for each experimental group) were treated *per os* with 1 mg·kg$^{-1}$ β-BA, 5 mg·kg$^{-1}$ indomethacin (indo) or vehicle (water containing 0.5% CMC, 10% Tween-20 and 1% sesame oil). Exudate volume and $PGE_2$, as well as inflammatory cell accumulation in the pleural cavity were assessed 4 h after carrageenan injection. Data are expressed as mean ± SEM, $n = 11$.

β-BA, β-boswellic acid; n.d., not detectable, under the limit of detection of the assay (0.0625 ng·mL$^{-1}$).

exudates were reduced by 51%, whereas the amount of 6-keto $PGF_{1\alpha}$ was only slightly, and non-significantly, reduced. The other BAs hardly reduced $PGE_2$ levels (<28%). Nevertheless, KBA and Aβ-BA significantly inhibited the generation of 6-keto $PGF_{1\alpha}$ by 35 and 40%. Indomethacin almost completely suppressed formation of $PGE_2$ as well as 6-keto $PGF_{1\alpha}$ (Figure 7A).

Next, β-BA was analysed for its anti-inflammatory efficacy *in vivo* after oral administration. As shown in Table 1, β-BA (1 mg·kg$^{-1}$) caused potent inhibition of exudate formation and prevented infiltration of inflammatory cells, along with reduced levels of $PGE_2$. The reference compound indomethacin (5 mg·kg$^{-1}$), given p.o., completely prevented the formation of $PGE_2$ associated with strong inhibition of exudate formation and cell infiltration (Table 1).

Finally, we explored the efficacy of β-BA in carrageenan-induced paw oedema, another rodent model of acute inflammation, to assess the pathophysiological role of mPGES1 in inflammation *in vivo* (Guay *et al.*, 2004). The injection of carrageenan into the mouse paw produced a marked increase of paw volume, with a maximal effect after 4 h. Pre-treatment (30 min) of mice with β-BA attenuated the inflammatory response at 4 h (Figure 7B). Thus, for mice treated (i.p.) with 0.25 and 1 mg·kg$^{-1}$ of β-BA, the peak of the response to carrageenan at 4 h was reduced by 20 and 50% respectively. Indomethacin (5 mg·kg$^{-1}$) caused 57% inhibition of the carrageenan response.

## Discussion and conclusions

The modes of action and molecular targets of BAs and frankincense preparations are still incompletely understood. Most studies have focused on AKBA or KBA as the pharmacologically active principles and numerous targets (5-LOX, 12*S*-LOX, COX-1, HLE, IκB kinases and topoisomerases) have been proposed, but the interference with these targets *in vivo* has been largely neglected and thus, the pharmacological relevance is still unclear. Recently, we presented the identification of cathepsin G as a pharmacologically relevant target of all major BAs (Tausch *et al.*, 2009). $PGE_2$ is a key player in inflammation and pain, and mPGES1 is regarded as a potential target for the development of anti-inflammatory therapeutics (Samuelsson *et al.*, 2007). Here, we present mPGES1 as a molecular target of BAs, and we provide evidence for a functional interaction of β-BA with mPGES1 *in vivo* which may contribute to its anti-inflammatory effectiveness.

The mPGES-1 bound to immobilized β-BA and KBA in a pull-down assay and SPR spectroscopy data confirmed a direct interaction. Pull-down assays using immobilized BAs were previously applied to demonstrate a direct interference of BAs with COX-1, 12*S*-LOX and cathepsin G (Poeckel *et al.*, 2006b; Siemoneit *et al.*, 2008; Tausch *et al.*, 2009), supporting the suitability of this methodology for target identification. The SPR-based ligand-analyte studies revealed $K_D$ values of 5.2–23 μM for 3-*O*-oxaloyl-KBA, and these values fit the $IC_{50}$ (5 μM) of 3-*O*-oxaloyl-KBA in the mPGES1 cell-free assay, suggesting specific binding to mPGES1 and hence a direct relation between enzyme binding and interference with mPGES1 activity.

AKBA, KBA and β-BA at low micromolar concentrations inhibited mPGES1-mediated $PGE_2$ formation in cell-free assays, and also concentration dependently blocked $PGE_2$ biosynthesis in intact A549 cells. Previous studies showed that BAs (up to 100 μM) hardly inhibited isolated COX-2

(Siemoneit *et al.*, 2008) and all BAs (up to 30 μM) failed to reduce the formation of the COX-2-derived products 6-keto $PGF_{1\alpha}$ and $TXB_2$ in intact A549 cells and in human whole blood. Therefore, we conclude that impaired $PGE_2$ biosynthesis is the result of the selective inhibition of the transformation of $PGH_2$ to $PGE_2$ by interference of BAs with mPGES1, rather than with COX-2 or other distal events (such as AA release). In particular β-BA, the major BA present in frankincense that reaches the highest plasma levels (up to 10.1 μM) among the BAs in treated humans (Buchele and Simmet, 2003; Tausch *et al.*, 2009), suppressed $PGE_2$ formation in human whole blood, again without significant reduction of 6-keto $PGF_{1\alpha}$ or $TXB_2$ levels. Interference with COX-1 was excluded as 12-HHT formation in whole blood was unaffected by β-BA, although BAs (in particular AKBA and KBA) may inhibit COX-1 in cell-free and cell-based models at higher concentrations (Siemoneit *et al.*, 2008).

β-BA at a dose of 1 $mg \cdot kg^{-1}$ given i.p. or p.o. reduced the inflammatory reaction in two *in vivo* models of acute inflammation, carrageenan-induced mouse paw oedema and rat pleurisy, being about as effective as 5 $mg \cdot kg^{-1}$ indomethacin. During carrageenan-induced oedema formation, $PGE_2$ levels are significantly elevated (Harada *et al.*, 1982; Guay *et al.*, 2004), and COX inhibitors prevent the inflammatory response (Gemmell *et al.*, 1979). Results from studies using carrageenan-induced paw oedema and i.p. application of undefined mixtures of BAs concur with our data, although much higher doses (e.g. 125 $mg \cdot kg^{-1}$) were used in those studies (Singh *et al.*, 2007). Whether or not BAs and related triterpenes present in frankincense extracts synergize or antagonize each other remains to be investigated. In the early phase of carrageenan-induced pleurisy, $PGE_2$ plays a central role (Kawamura *et al.*, 2000); and in fact, exudates from β-BA-treated rats showed markedly lower $PGE_2$ levels. Thus, lowering $PGE_2$ by inhibition of mPGES1 may contribute to the anti-inflammatory properties of β-BA. However, compared with indomethacin, β-BA was less potent in reducing $PGE_2$ levels, but still efficiently suppressed exudate formation and infiltration of inflammatory cells. It is possible that other anti-inflammatory features of β-BA, such as inhibition of cathepsin G (Tausch *et al.*, 2009), may contribute to the overall anti-inflammatory effects. The inhibition of cathepsin G may also explain the slight but still significant reduction of oedema formation and cell infiltration upon pretreatment with Aβ-BA, which was the least potent inhibitor of mPGES1 and failed to suppress $PGE_2$ formation in whole blood. Similarly, interference with other pro-inflammatory components, such as cytokines and transcription factors (Syrovets *et al.*, 2005; Kunnumakkara *et al.*, 2009), may suppress COX-2 induction, explaining the reduced 6-keto $PGF_{1\alpha}$ levels in the exudates of KBA- and Aβ-BA-treated rats.

Initially, the inhibition of 5-LOX activity and formation of leukotrienes (LTs) by BAs was proposed as an anti-inflammatory mechanism (Poeckel and Werz, 2006). Thus, AKBA and KBA blocked 5-LOX with $IC_{50}$ values of 1.5–50 μM depending on the experimental settings (Safayhi *et al.*, 1992; Siemoneit *et al.*, 2009). However, in whole blood assays, AKBA and KBA failed to inhibit 5-LOX product synthesis, and the $LTB_4$ plasma levels of human healthy volunteers treated with standard doses of frankincense were not affected (Siemoneit *et al.*, 2009). Similarly, AKBA and KBA failed to suppress $PGE_2$ formation in human whole blood and in rats, despite significant inhibition of mPGES1 in the cell-free assay. The failure of KBA and AKBA to suppress LT and $PGE_2$ formation *in vivo* could be related to the marginal permeability of AKBA and moderate absorption of KBA (Kruger *et al.*, 2009), resulting in poor bioavailability (Kruger *et al.*, 2008) with fairly low plasma concentrations (0.3 and <0.1 μM) (Buchele and Simmet, 2003; Tausch *et al.*, 2009). The marked loss of activity of AKBA in whole blood might be related to its strong plasma protein binding (Siemoneit *et al.*, 2009). Together, the 11-keto moiety may hamper the cellular and biological availability of respective BAs in a physiological environment and thus compromise the overall anti-inflammatory effectiveness of AKBA *in vivo*. In contrast, β-BA (at 1 $mg \cdot kg^{-1}$) showed comparably high efficacy in the pleurisy model after p.o. or i.p. administration, suggesting clearly different and better bioavailability of this BA.

Our data favour a role of β-BA as the most relevant anti-inflammatory BA acting at least in part via inhibition of $PGE_2$ formation. Pilot clinical studies indicated some therapeutic efficacy of frankincense, mainly in OA and RA (Ammon, 2006; Sengupta *et al.*, 2008). Clinical studies using 5-LOX inhibitors (i.e. zileuton) and studies on 5-LOX- or 5-LOX-activating protein-deficient mice, exclude a prominent role of LTs in OA or RA (Werz and Steinhilber, 2006). However, $PGE_2$ is a key mediator accounting for typical disease symptoms in OA and RA (Smith, 1989); and in patients suffering from RA or OA, a pivotal role for mPGES1 has been demonstrated (Westman *et al.*, 2004; Li *et al.*, 2005). Moreover, data from animal arthritis models support the relevance of mPGES1 to inflammatory joint diseases (Claveau *et al.*, 2003; Trebino *et al.*, 2003; Guay *et al.*, 2004). Hence, the inhibition of $PGE_2$ biosynthesis by β-BA may contribute to the beneficial effects of frankincense preparations observed in clinical

studies and animal models of OA and RA (Ammon, 2006). Such speculations are favoured by the close correlation between steady-state plasma levels of β-BA (6.4–10.1 μM) in humans obtained after oral administration of frankincense preparations [containing 18.2% β-BA, 10.5% Aβ-BA, 6.1% KBA and 3.7% AKBA (Sterk *et al.*, 2004)] in clinical trials (Buchele and Simmet, 2003; Tausch *et al.*, 2009) and the effective concentrations of β-BA (≥3 μM) to suppress $PGE_2$ synthesis in human whole blood.

The mPGES1 inhibitors might be alternatives to NSAIDs with reduced side effects (Koeberle and Werz, 2009). Less gastric and cardiovascular complications were evident in mPGES1-deficient mice, compared with COX-1- or COX-2-deficient mice or mice treated with NSAIDs (Cheng *et al.*, 2006; Wang *et al.*, 2006). In fact, the mPGES1 inhibitor MF63 relieved pyresis and inflammatory pain in animal models, without NSAID-like gastric toxicity (Xu *et al.*, 2008). BAs showed gastric ulcer protective effects in different experimental models (Singh *et al.*, 2008), and a 90-day placebo-controlled study supports evidence for the safety of *Boswellia serrata* extracts in OA patients (Sengupta *et al.*, 2008).

In conclusion, we have shown that BAs directly and functionally interfered with mPGES1. The formation of $PGE_2$ was selectively suppressed by β-BA in cell-based assays and in pleural exudates *in vivo*, and β-BA clearly exhibited anti-inflammatory effectiveness in the carrageenan-induced mouse paw oedema and rat pleurisy after i.p. or p.o. administration of fairly low doses. As the effective concentrations of β-BA, under physiologically relevant conditions, were in the range of β-BA levels in plasma of humans treated with frankincense, an interference with mPGES1 might represent a reasonable molecular mechanism contributing to some of the anti-inflammatory properties of frankincense extracts and rationalize its therapeutic use.

## Acknowledgements

We thank Gertrud Kleefeld for expert technical assistance. We would like to acknowledge the financial support from Pharmasan GmbH (Freiburg, Germany), Medeon GmbH, Berlin, Aureliasan GmbH (Tuebingen, Germany) and the Deutsche Forschungsgemeinschaft.

## Conflicts of interest

None.

## References

Alexander SPH, Mathie A, Peters JA (2009). Guide to receptors and channels (GRAC), 4th edn. Br J Pharmacol 158 (Suppl. 1): S1–S254.

Ammon HP (2006). Boswellic acids in chronic inflammatory diseases. Planta Med 72: 1100–1116.

Asano K, Lilly CM, Drazen JM (1996). Prostaglandin G/H synthase-2 is the constitutive and dominant isoform in cultured human lung epithelial cells. Am J Physiol 271: L126–L131.

Buchele B, Simmet T (2003). 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 795: 355–362.

Cheng Y, Wang M, Yu Y, Lawson J, Funk CD, Fitzgerald GA (2006). Cyclooxygenases, microsomal prostaglandin E synthase-1, and cardiovascular function. J Clin Invest 116: 1391–1399.

Claveau D, Sirinyan M, Guay J, Gordon R, Chan CC, Bureau Y *et al.* (2003). Microsomal prostaglandin E synthase-1 is a major terminal synthase that is selectively up-regulated during cyclooxygenase-2-dependent prostaglandin E2 production in the rat adjuvant-induced arthritis model. J Immunol 170: 4738–4744.

Funk CD (2001). Prostaglandins and leukotrienes: advances in eicosanoid biology. Science 294: 1871–1875.

Gemmell DK, Cottney J, Lewis AJ (1979). Comparative effects of drugs on four paw oedema models in the rat. Agents Actions 9: 107–116.

Giannetti AM, Koch BD, Browner MF (2008). Surface plasmon resonance based assay for the detection and characterization of promiscuous inhibitors. J Med Chem 51: 574–580.

Guay J, Bateman K, Gordon R, Mancini J, Riendeau D (2004). Carrageenan-induced paw edema in rat elicits a predominant prostaglandin E2 (PGE2) response in the central nervous system associated with the induction of microsomal PGE2 synthase-1. J Biol Chem 279: 24866–24872.

Harada Y, Tanaka K, Uchida Y, Ueno A, Oh-Ishi S, Yamashita K *et al.* (1982). Changes in the levels of prostaglandins and thromboxane and their roles in the accumulation of exudate in rat carrageenin-induced pleurisy – a profile analysis using gas chromatography-mass spectrometry. Prostaglandins 23: 881–895.

Jakobsson PJ, Thoren S, Morgenstern R, Samuelsson B (1999). Identification of human prostaglandin E synthase: a microsomal, glutathione-dependent, inducible enzyme, constituting a potential novel drug target. Proc Natl Acad Sci USA 96: 7220–7225.

Jauch J, Bergmann J (2003). An efficient method for the large-scale preparation of 3-O-acetyl-11-oxo-beta-boswellic acid and other boswellic acids. Eur J Org Chem 24: 4752–4756.

Karlsson R, Falt A (1997). Experimental design for kinetic analysis of protein-protein interactions with surface plasmon resonance biosensors. J Immunol Methods 200: 121–133.

Kawamura M, Hatanaka K, Saito M, Ogino M, Ono T, Ogino K *et al*. (2000). Are the anti-inflammatory effects of dexamethasone responsible for inhibition of the induction of enzymes involved in prostanoid formation in rat carrageenin-induced pleurisy? Eur J Pharmacol 400: 127–135.

Koeberle A, Werz O (2009). Inhibitors of the microsomal prostaglandin E(2) synthase-1 as alternative to non steroidal anti-inflammatory drugs (NSAIDs) – a critical review. Curr Med Chem 16: 4274–4296.

Koeberle A, Siemoneit U, Buehring U, Northoff H, Laufer S, Albrecht W *et al*. (2008). Licofelone suppresses prostaglandin E2 formation by interference with the inducible microsomal prostaglandin E2 synthase-1. J Pharmacol Exp Ther 326: 975–982.

Koeberle A, Pollastro F, Northoff H, Werz O (2009). Myrtucommulone, a natural acylphloroglucinol, inhibits microsomal prostaglandin E(2) synthase-1. Br J Pharmacol 156: 952–961.

Kruger P, Daneshfar R, Eckert GP, Klein J, Volmer DA, Bahr U *et al*. (2008). Metabolism of boswellic acids in vitro and in vivo. Drug Metab Dispos 36: 1135–1142.

Kruger P, Kanzer J, Hummel J, Fricker G, Schubert-Zsilavecz M, Abdel-Tawab M (2009). Permeation of Boswellia extract in the Caco-2 model and possible interactions of its constituents KBA and AKBA with OATP1B3 and MRP2. Eur J Pharm Sci 36: 275–284.

Kunnumakkara AB, Nair AS, Sung B, Pandey MK, Aggarwal BB (2009). Boswellic acid blocks signal transducers and activators of transcription 3 signaling, proliferation, and survival of multiple myeloma via the protein tyrosine phosphatase SHP-1. Mol Cancer Res 7: 118–128.

Li X, Afif H, Cheng S, Martel-Pelletier J, Pelletier JP, Ranger P *et al*. (2005). Expression and regulation of microsomal prostaglandin E synthase-1 in human osteoarthritic cartilage and chondrocytes. J Rheumatol 32: 887–895.

Murakami M, Nakatani Y, Tanioka T, Kudo I (2002). Prostaglandin E synthase. Prostaglandins Other Lipid Mediat 68–69: 383–399.

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

Poeckel D, Tausch L, George S, Jauch J, Werz O (2006a). 3-O-Acetyl-11-keto-boswellic acid decreases basal intracellular Ca2+ levels and inhibits agonist-induced Ca2+ mobilization and mitogen-activated protein kinase activation in human monocytic cells. J Pharmacol Exp Ther 316: 224–232.

Poeckel D, Tausch L, Kather N, Jauch J, Werz O (2006b). Boswellic acids stimulate arachidonic acid release and 12-lipoxygenase activity in human platelets independent of Ca2+ and differentially interact with platelet-type 12-lipoxygenase. Mol Pharmacol 70: 1071–1078.

Roden LD, Myszka DG (1996). Global analysis of a macromolecular interaction measured on BIAcore. Biochem Biophys Res Commun 225: 1073–1077.

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

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

Samuelsson B, Morgenstern R, Jakobsson PJ (2007). Membrane prostaglandin E synthase-1: a novel therapeutic target. Pharmacol Rev 59: 207–224.

Schwarz D, Klammt C, Koglin A, Lohr F, Schneider B, Dotsch V *et al*. (2007). Preparative scale cell-free expression systems: new tools for the large scale preparation of integral membrane proteins for functional and structural studies. Methods 41: 355–369.

Sengupta K, Alluri KV, Satish AR, Mishra S, Golakoti T, Sarma KV *et al*. (2008). 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 10: R85.

Siemoneit U, Hofmann B, Kather N, Lamkemeyer T, Madlung J, Franke L *et al*. (2008). Identification and functional analysis of cyclooxygenase-1 as a molecular target of boswellic acids. Biochem Pharmacol 75: 503–513.

Siemoneit U, Pergola C, Jazzar B, Northoff H, Skarke C, Jauch J *et al*. (2009). On the interference of boswellic acids with 5-lipoxygenase: mechanistic studies in vitro and pharmacological relevance. Eur J Pharmacol 606: 246–254.

Singh S, Khajuria A, Taneja SC, Khajuria RK, Singh J, Qazi GN (2007). Boswellic acids and glucosamine show synergistic effect in preclinical anti-inflammatory study in rats. Bioorg Med Chem Lett 17: 3706–3711.

Singh S, Khajuria A, Taneja SC, Khajuria RK, Singh J, Johri RK *et al*. (2008). The gastric ulcer protective effect of boswellic acids, a leukotriene inhibitor from Boswellia serrata, in rats. Phytomedicine 15: 408–415.

Smith WL (1989). The eicosanoids and their biochemical mechanisms of action. Biochem J 259: 315–324.

Sterk V, Buchele B, Simmet T (2004). Effect of food intake on the bioavailability of boswellic acids from a herbal preparation in healthy volunteers. Planta Med 70: 1155–1160.

Syrovets T, Buchele B, Krauss C, Laumonnier Y, Simmet T (2005). Acetyl-boswellic acids inhibit lipopolysaccharide-mediated TNF-alpha induction in monocytes by direct interaction with IkappaB kinases. J Immunol 174: 498–506.

Tausch L, Henkel A, Siemoneit U, Poeckel D, Kather N, Franke L *et al*. (2009). Identification of human cathepsin G as a functional target of boswellic acids from the anti-inflammatory remedy frankincense. J Immunol 183: 3433–3442.

Thoren S, Jakobsson PJ (2000). Coordinate up- and down-regulation of glutathione-dependent prostaglandin E synthase and cyclooxygenase-2 in A549 cells. Inhibition by NS-398 and leukotriene C4. Eur J Biochem 267: 6428–6434.

Trebino CE, Stock JL, Gibbons CP, Naiman BM, Wachtmann TS, Umland JP *et al*. (2003). Impaired inflammatory and pain responses in mice lacking an inducible prostaglandin E synthase. Proc Natl Acad Sci USA 100: 9044–9049.

Wang M, Zukas AM, Hui Y, Ricciotti E, Pure E, FitzGerald GA (2006). Deletion of microsomal prostaglandin E synthase-1 augments prostacyclin and retards atherogenesis. Proc Natl Acad Sci USA 103: 14507–14512.

Werz O, Steinhilber D (2006). Therapeutic options for 5-lipoxygenase inhibitors. Pharmacol Ther 112: 701–718.

Westman M, Korotkova M, af Klint E, Stark A, Audoly LP, Klareskog L *et al*. (2004). Expression of microsomal prostaglandin E synthase 1 in rheumatoid arthritis synovium. Arthritis Rheum 50: 1774–1780.

Xu D, Rowland SE, Clark P, Giroux A, Cote B, Guiral S *et al*. (2008). MF63 [2-(6-chloro-1H-phenanthro[9,10-d]imidazol-2-yl)-isophthalonitrile], a selective microsomal prostaglandin E synthase-1 inhibitor, relieves pyresis and pain in preclinical models of inflammation. J Pharmacol Exp Ther 326: 754–763.