Carnosol and Carnosic Acids from Salvia officinalis Inhibit Microsomal Prostaglandin E₂ Synthase-1

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ABSTRACT
Prostaglandin E₂ (PGE₂), the most relevant eicosanoid promoting inflammation and tumorigenesis, is formed by cyclooxygenases (COXs) and PGE₂ synthases from free arachidonic acid. Preparations of the leaves of Salvia officinalis are commonly used in folk medicine as an effective antiseptic and anti-inflammatory remedy and possess anticancer activity. Here, we demonstrate that a standard ethyl acetate extract of S. officinalis efficiently suppresses the formation of PGE₂ in a cell-free assay by direct interference with microsomal PGE₂ synthase (mPGES)-1. Bioactivity-guided fractionation of the extract yielded closely related fractions that potently suppressed mPGES-1 with IC₅₀ values between 1.9 and 3.5 µM. Component analysis of these fractions revealed the diterpenes carnosol and carnosic acid as potential bioactive principles inhibiting mPGES-1 activity with IC₅₀ values of 5.0 µM. Using a human whole-blood assay as a robust cell-based model, carnosic acid, but not carnosol, blocked PGE₂ generation upon stimulation with lipopolysaccharide (IC₅₀ = 9.3 µM). Carnosic acid neither inhibited the concomitant biosynthesis of other eicosanoids [6-keto PGF₁α, 12(S)-hydroxy-5-cis-8,10-trans-heptadecatrienoic acid, and thromboxane B₂] in human whole blood nor affected the activities of COX-1/2 in a cell-free assay. Together, S. officinalis extracts and its ingredients carnosol and carnosic acid inhibit PGE₂ formation by selectively targeting mPGES-1. We conclude that the inhibitory effect of carnosic acid on PGE₂ formation, observed in the physiologically relevant whole-blood model, may critically contribute to the anti-inflammatory and anticarcinogenic properties of S. officinalis.

Introduction
Leaves of Salvia officinalis (sage) are used as culinary herb and in folk medicine to treat sore throats, dyspepsia, and diverse inflammatory diseases in the Western world (Johnson, 2011). Among the multiple polyphenols identified in sage, the ß-diphenolic diterpenes carnosol and carnosic acid are most abundant (Johnson, 2011). In addition to their strong antioxidant character, carnosol and carnosic acid exert potent anti-inflammatory and anticarcinogenic properties (Johnson, 2011). They impair the proliferation of several cancer cell lines and induce apoptosis (Dörrie et al., 2001; Steiner et al., 2001; Visanji et al., 2006; Hussein et al., 2007; Khan et al., 2007; Johnson et al., 2008, 2010; Yesil-Celiktas et al., 2010; Tsai et al., 2011), reduce tumor growth in athymic nude mice implanted with prostate carcinoma cells (Johnson et al., 2010), interfere with carcinogen-induced mammary tumorigenesis in rats (Singleton et al., 1996), inhibit phorbol-12-myristate-13-acetate-induced ear inflammation and tumor promotion in mice (Huang et al., 1994), and decrease adenoma formation in APC(−/−) mice (Moran et al., 2005). On the molecular level, carnosol and carnosic acid may interfere with multiple signaling pathways that are deregulated during inflammation and cancer, including nuclear factor κB (Lo et al., 2002; Huang et al., 2005), p38

ABBREVIATIONS: COX, cyclooxygenase; 12-HHT, 12(S)-hydroxy-5-cis-8,10-trans-heptadecatrienoic acid; LPS, lipopolysaccharide; PG, prostaglandin; mPGES, microsomal PGE₂ synthase; PBS, phosphate-buffered saline; HPLC, high-performance liquid chromatography; DMSO, dimethyl sulphoxide; ANOVA, analysis of variance; ELISA, enzyme-linked immunosorbent assay; HSD, honestly significant difference; LOQ, limit of quantification; CV4151, (E)-7-phenyl-7-(3-pyridyl)-6-heptenoic acid; MD92, 2-(2-chlorophenyl)-1H-phenanthro[9,10-d]-imidazole; MK-886, 3-(4-chlorobenzyl)-3-t-butylthio-5-isopropylindo-2-yl]-2,2-dimethylpropanoic acid.
mitogen-activated protein kinase (Lo et al., 2002), extracellular signal-regulated kinase (Lo et al., 2002), phosphatidylinositol-3-kinase (Martin et al., 2004), protein kinase C (Subbaramaiah et al., 2002), cyclooxygenase (COX) (Laughton et al., 1991; Subbaramaiah et al., 2002), androgen and estrogen receptors (Johnson et al., 2010), the B-cell lymphoma-2 protein family (Dorrie et al., 2001), ß-catenin (Moran et al., 2005), and intracellular Ca²⁺ (Lee et al., 2006; Poeckel et al., 2008). However, only a few direct targets of carnosol and carnosic acid have been described so far. Raú et al. (2006) ascribed the long-term anti-inflammatory properties of carnosol and carnosic acid to the activation of peroxisome proliferator-activated receptor γ. Moreover, we proposed that the inhibition of human 5-lipoxygenase (Laughton et al., 1991; Poeckel et al., 2008) contributes to their immediate anti-inflammatory effects. Direct molecular targets explaining the anticarcinogenic effects of carnosol and carnosic acid are not described.

Exaggerative prostaglandin (PG) E₂ formation links carcinogenesis to inflammation (Rådmark and Samuelsson, 2010). During biosynthesis of PGE₂, arachidonic acid is transformed to PGH₂ by cyclooxygenases and further converted to PGE₂ by PGE₂ synthases (Koeberle and Werz, 2009). The isoenzymes COX-2 and microsomal PGE₂ synthase (mPGES)-1 are functionally coupled and responsible for the inducible PGE₂ formation under pathophysiological conditions (Koeberle and Werz, 2009). Both isoenzymes are induced by proinflammatory stimuli and overexpressed in various cancers (Koeberle and Werz, 2009). Accordingly, genetic or pharmacological inhibition of COX-2 or mPGES-1 reduces inflammation, fever, and pain in numerous cellular and animal studies as well as tumorigenesis, angiogenesis, and metastasis (Smith et al., 2000; Koeberle and Werz, 2009; Rådmark and Samuelsson, 2010).

Here, we identified an ethyl acetate extract of *S. officinalis* and its active principles carnosol and carnosic acid as direct inhibitors of mPGES-1. Carnosic acid, but not carnosol, inhibited PGE₂ biosynthesis in a physiologically relevant human whole-blood assay at low micromolar concentrations that can be achieved in vivo after oral administration. Neither the biosynthesis of other prostanoids than PGE₂ nor the activity of cell-free COX-1 or COX-2 was markedly affected, suggesting a preferred interference with mPGES-1 underlying the suppression of cellular PGE₂ biosynthesis.

### Materials and Methods

#### Solvents and Reagents

All solvents used for phytochemical work were obtained from VWR International (Darmstadt, Germany). Solvents for HPLC were provided by Merck (Darmstadt, Germany). Ultrapure water was produced by a Sartorius Arium 611 UV water purification system (Sartorius AG, Göttingen, Germany). Carnosol, carnosic acid, ursoic acid (Baricic et al., 2001), oleaenic acid (Werz, 2007), and rosmarinic acid (Werz, 2007) (Sigma-Aldrich, Germany) were dissolved in PBS, pH 7.4 and 1 mM CaCl₂ to obtain isolated human platelets. Carnosol, carnosic acid (4.5 g/l) medium, penicillin, streptomycin, trypsin/EDTA solution (PAA, Coelbe, Germany); PGH₂ (Larodan, Malmö, Sweden); 11β-PGE₂, PGE₂, 3-[1-(4-chlorobenzoyl)-3-tert-butythio-5-isopropylindol-2-yl]-2,2-dimethylpropanoic acid (MK-886), human recombinant COX-2, and ovine COX-1 (Cayman Chemical, Ann Arbor, MI). All other chemicals were obtained from Sigma-Aldrich (Deisenhofen, Germany) unless otherwise stated.

#### Plant Material

Dried, cut leaves of *S. officinalis* (2 kg) were purchased from Kottas Pharma GmbH (Vienna, Austria). A voucher specimen (JR-20100802-A1) is deposited at the Institute of Pharmacy/Pharmacoergony, University of Innsbruck (Innsbruck, Austria).

#### Isolation and Fractionation of *S. officinalis* and Sample Preparation

Ground leaves (94 g) of *S. officinalis* were extracted five times for 30 min with 600 ml of ethyl acetate in an ultrasonic bath at room temperature. The combined extracts were evaporated to dryness, yielding 14.62 g, and 13.9 g of the extract were separated by flash silica gel 60 (40–63 μm; 202 g; Merck) column chromatography (3.9 x 47.5 cm) using petroleum ether as eluent with stepwise (200 ml each) increasing amounts of dichloromethane, followed by ethyl acetate and finally acetone, yielding 11 fractions.

The ethyl acetate extract of *S. officinalis* and its fractions were solubilized in DMSO, stored at −20°C in the dark, and analyzed within 4 weeks. Fraction 1 was not completely soluble in DMSO at 0.3 μg/ml, even after extensive sonification. The insoluble pellet was removed by centrifugation.

#### HPLC Quantification

To determine the content of carnosol and carnosic acid, standard solutions were diluted to appropriate concentrations. The ethyl acetate extract of *S. officinalis* and its 11 fractions were dissolved in acetonitrile and, when necessary, in a mixture of acetonitrile and tetrahydrofuran (fractions 9 and 10). Every sample was analyzed by HPLC three times. The amount of carnosol and carnosic acid found in the samples was calculated as the percentage of the weight of the dry extract and fractions. Limits of quantification were determined as the signal-to-noise ratio of 10. HPLC-diode-array detection analyses were performed on a Shimadzu (Kyoto, Japan) UFLC-XR instrument, equipped with auto sampler, photo diode array, and on-line degasser and column thermostat. Stationary phase was Max RP 80A column (150 x 4.6 mm, 3.5-μm particle size; Phenomenex, Torrance, CA). Mobile phase was double-distilled water (A) and methanol containing 1% acetic acid (B). Flow rate was 1.0 ml/min with detection wavelength at 284 nm and solvent gradient at start 35% A, 65% B; 20 min 2% A, 98% B; stop 30 min; post time 10 min.

#### Cells

Human platelets were freshly isolated from leukocyte concentrates obtained at the Blood Center of the University Hospital Tuebingen (Tuebingen, Germany) according to Albert et al. (2002). In brief, leukocyte concentrates were prepared by centrifugation (4000g, 20 min, 20°C) from venous blood from healthy adult donors who did not take any medication for at least 7 days. Blood cells were immediately separated by dextran sedimentation and centrifugation on Nycoprep cushions (PAA). The supernatant was mixed with phosphate-buffered saline (PBS), pH 5.9 (3:2 v/v) and centrifuged (2100g, 15 min, room temperature). The pellet was washed with PBS, pH 5.9/0.9% NaCl (1:1, v/v) and resuspended in PBS, pH 7.4 and 1 mM CaCl₂ to obtain isolated human platelets.

Human lung adenocarcinoma epithelial A549 cells were cultured in DMEM/high-glucose (4.5 g/l) medium supplemented with heat-inactivated fetal calf serum (10%, v/v), penicillin (100 U/ml), streptomycin (100 μg/ml) at 37°C and 5% CO₂, harvested after 48 h, and frozen in liquid nitrogen. After reuptake of the cells in ice-cold homogenization buffer (0.1 M potassium phosphate buffer, pH 7.4, 1 mM phenylmethylsulphonyl fluoride, 90
μg/ml soybean trypsin inhibitor, 1 μg/ml leupeptin, 2.5 mM glutathione, and 250 mM sucrose) and incubation for 15 min, cells were sonicated on ice (3 × 20 s) and subjected to differential centrifugation at 10,000g for 10 min and 174,000g for 1 h at 4°C. The microsomal fraction (pellet) was resuspended in homogenization buffer, analyzed for its protein content by using a protein assay kit (Bio-Rad Laboratories GmbH, Munich, Germany), and diluted in potassium phosphate buffer (0.1 M, pH 7.4) containing 2.5 mM glutathione to a final concentration of 25 to 50 μg protein/ml. After preincubation with the test compounds (dissolved in DMSO) for 15 min at 4°C, the reaction (100 μl of total volume, 4°C) was initiated by the addition of ΔPGH2 (20 μM final concentration) and terminated after 1 min by the addition of 100 μl of stop solution (40 mM FeCl2, 80 mM citric acid, and 10 μM 11b-PGE2 as internal standard). PGE2 was extracted and analyzed by reversed phase-HPLC as described previously (Koeberle et al., 2008). The final concentration of DMSO in the reaction volume was always adjusted to 1%. Addition of ΔPGH2 to a reaction mix containing heat-inactivated microsomes yielded relative amounts of PGE2 of 8.9 ± 0.8 and 11.0 ± 1.4%, respectively. Results are expressed as percentage of the vehicle (DMSO)-treated control.

**Determination of 11b-PGE2 in Human Whole Blood**. Blood from healthy adult volunteers was obtained by venipuncture and collected in monovettes containing heparin (20 U/ml), and prostanoid formation was analyzed according to Koeberle et al. (2009b). In brief, for the determination of 11b-PGE2 and 6-keto-PGF1α, aliquots of whole blood (0.8 ml) were mixed with thromboxane synthase inhibitor CV4151 (1 μM) and aspirin (50 μM) and adjusted to a total volume of 1 ml with 10 mM potassium phosphate buffer, pH 7.4, 3 mM KCl, 140 mM NaCl, and 6 mM D-glucose. CV4151 and aspirin were omitted for the determination of 12-HHT and thromboxane B2 formation. Samples were preincubated with the test compounds for 5 min at room temperature and stimulated with lipopolysaccharide (LPS; 10 μg/ml) for 5 h at 37°C. After prostanooid formation was stopped on ice, the samples were centrifuged (2300g, 10 min, 4°C) and analyzed for 6-keto-PGF1α and thromboxane B2 by ELISA (Enzo Life Sciences). The 11 fractions obtained were then tested for their inhibitory properties (Johnson, 2011). Because PGE2 formation through the inducible PGE2 synthase mPGES-1 plays a critical role in the progression of both inflammation and cancer (Koeberle and Werz, 2009), we investigated whether an ethyl acetate extract of the leaves of *S. officinalis* may inhibit mPGES-1. The contents of carnosol and carnosic acid in the extract were determined to be 4.3 and 20.1%, respectively. The ethyl acetate extract was tested in a cell-free mPGES-1 activity assay. The direct substrate of mPGES-1, PGG2, is enzymatically converted in this assay to PGE2 by mPGES-1 from microsomal preparations of interleukin-1β-stimulated A549 cells. MK-886 (10 μM), used as reference, suppressed mPGES-1 activity by 74.5 ± 1.5% as expected (Koeberle et al., 2008). The ethyl acetate extract of *S. officinallis* concentration-dependently inhibited mPGES-1 with an IC50 of 5.0 μg/ml (Fig. 1).

**Bioactivity-Guided Fractionation and Characterization of the *S. officinallis* Extract**. To identify the active constituents of the ethyl acetate extract of *S. officinialis* responsible for the inhibition of mPGES-1, the extract was fractionated by means of silica gel column chromatography. The 11 fractions obtained were then tested for their inhibitory activity against ovine COX-1 and COX-2. Effects on ovine COX-1 and human COX-2 enzyme activity were determined as described previously (Koeberle et al., 2008). In brief, purified ovine COX-1 (50 units) or human recombinant COX-2 (20 units) were preincubated in 1 ml of reaction mixture containing 100 mM Tris buffer, pH 8, 5 mM glutathione, 5 μM hemoglobin, and 100 μM EDTA with the test compounds for 5 min at 4°C. After prewarming for 60 s at 37°C, the samples were incubated with arachidonic acid (COX-1, 5 μM; COX-2, 2 μM) for 5 min. COX-derived 12-HHT was extracted and then analyzed by HPLC as described previously (Albert et al., 2002).

**Results**

**An Ethyl Acetate Extract of Sage Inhibits mPGES-1.** Sage extracts have anti-inflammatory and anticarcinogenic properties (Johnson, 2011). Because PGE2 formation through the inducible PGE2 synthase mPGES-1 plays a critical role in the progression of both inflammation and cancer (Koeberle and Werz, 2009), we investigated whether an ethyl acetate extract of the leaves of *S. officinalis* may inhibit mPGES-1. The contents of carnosol and carnosic acid in the extract were determined to be 4.3 and 20.1%, respectively. The ethyl acetate extract was tested in a cell-free mPGES-1 activity assay. The direct substrate of mPGES-1, PGG2, is enzymatically converted in this assay to PGE2 by mPGES-1 from microsomal preparations of interleukin-1β-stimulated A549 cells. MK-886 (10 μM), used as reference, suppressed mPGES-1 activity by 74.5 ± 1.5% as expected (Koeberle et al., 2008). The ethyl acetate extract of *S. officinialis* concentration-dependently inhibited mPGES-1 with an IC50 of 5.0 μg/ml (Fig. 1).

**Fig. 1.** An ethyl acetate extract of *S. officinialis* inhibits mPGES-1 activity. Microsomal preparations of interleukin-1β-stimulated A549 cells were preincubated with an ethyl acetate extract of *S. officinialis* for 15 min at 4°C, and the reaction was started with 20 μM PGG2. After 1 min at 4°C, the reaction was terminated by using a stop solution containing FeCl3 and 11b-PGE2 (1 nmol) as internal standard. The concentration-response curve for the ethyl acetate extract of *S. officinialis* is shown. The 100% value corresponds to 246 ± 4 ng of PGE2. Data are given as mean ± S.E. (n = 3–5), *p < 0.05 or ***p < 0.001 versus vehicle (DMSO) control, ANOVA + Tukey HSD post hoc tests.
Inhibition of mPGES-1 by Carnosol and Carnosic Acid. Because carnosol or carnosic acid were enriched in the active fractions of the sage ethyl acetate extract (with the exception of fraction 2), we investigated the effect of these diterpenes on mPGES-1 activity in the microsomes of A549 cells. In fact, both compounds concentration-dependently inhibited mPGES-1 activity with IC$_{50}$ values of 5 µM each (Fig. 2B). It is noteworthy that the inhibition of mPGES-1 by the ethyl acetate extract of *Salvia officinalis* perfectly matches with the content and IC$_{50}$ values of carnosol and carnosic acid, suggesting a major role of carnosol and carnosic acid for the inhibition of mPGES-1 by the extract. Recently, we identified boswellic acid triterpenes as inhibitors of mPGES-1 (Siemoneit et al., 2011). However, triterpenes found in sage extracts, like ursolic acid or oleanolic acid (Baricevic et al., 2001; Ninomiya et al., 2004), hardly (ursolic acid) or at least less efficiently (oleanolic acid) suppressed mPGES-1 activity at a concentration of 10 µM (Fig. 2C). In addition, the polyphenol rosmarinic acid (10 µM) failed to inhibit mPGES-1 activity.

Carnosic Acid Suppresses PGE$_2$ Formation in LPS-Stimulated Whole Blood. Potent inhibition of PGE$_2$ biosynthesis in a cell-free assay is not necessarily preserved under more physiological conditions such as in whole blood. Therefore, we investigated whether carnosol and carnosic acid suppress PGE$_2$ formation in LPS-stimulated human whole blood, which emerged as a routine assay for selecting candidate COX-2 and mPGES-1 inhibitors for preclinical investigation (Hawkey, 1999; Koeberle and Werz, 2009). Carnosic acid concentration-dependently inhibited PGE$_2$ formation in human whole blood with an IC$_{50}$ value of 9.3 µM (Fig. 3A), whereas carnosol surprisingly did not show an effect up to 100 µM (data not shown). MDS2 (2 µM) and

### Table 1

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<tr>
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<td>17.5</td>
<td>&lt; LOQ</td>
<td>&lt; LOQ</td>
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N.I., no inhibition; LOQ, limit of quantification.

* Incompletely dissolved.

The content of carnosol and carnosic acid was determined by HPLC and is given as percentage of the fraction’s dry weight.
indomethacin (10 μM), used as mPGES-1 and COX reference inhibitors, respectively, suppressed PGE₂ formation as expected (Fig. 3A) and described previously (Koeberle et al., 2009a).

**Carnosic Acid Is a Selective Inhibitor of mPGES-1 within Prostanoid Biosynthesis.** If mPGES-1 would be the only target within prostanoid biosynthesis, carnosic acid should not interfere with the biosynthesis and release of other prostanoids than PGE₂. In fact, carnosic acid neither significantly suppressed the formation of 6-keto PGF₁α (a stable metabolite of PGI₂; Fig. 3B), 12-HHT (which can be considered as a biomarker of PGH₂ formation), nor thromboxane B₂ (Fig. 3D) in LPS-stimulated whole blood. Accordingly, carnosic acid failed to suppress COX-2-derived 6-keto PGF₁α formation from exogenous arachidonic acid in human lung epithelial A549 cells (up to 10 μM; data not shown). COX-1 activity, measured as 12-HHT formation in arachidonic acid-treated platelets, was inhibited (Fig. 4A) but only at high concentrations of carnosic acid (≥ 30 μM). Inhibition of COX-1 could not be confirmed in platelets for thromboxane B₂ as biomarker (Fig. 4B) or for the isolated enzyme (Fig. 4C). In addition, the activity of isolated COX-2 was only slightly suppressed by 30 μM carnosic acid (Fig. 4D), suggesting mPGES-1 as a preferential functional target of carnosic acid within prostanoid biosynthesis. The reference inhibitors indomethacin (10 μM; COX-1) and celecoxib (5 μM; COX-2) suppressed prostanoid formation (6-keto PGF₁α, 12-HHT, and thromboxane B₂) under the respective assay conditions as expected (Figs. 3 and 4B and data not shown) and described previously (Koeberle et al., 2008; Bauer et al., 2011).

**Discussion**

Anticarcinogenic and anti-inflammatory properties of sage extracts and its constituents carnosol and carnosic acid have been described for numerous cellular and animal models (Johnson, 2011). Although diverse mechanisms have been discussed, only a few direct molecular targets have been identified (Johnson, 2011). In particular, targets responsible for the anticancer effects of carnosol and carnosic acid are still elusive. Research over the last decade revealed a prominent role of mPGES-1-derived PGE₂ not only in inflammation but also for tumor progression, vascularization, and metastasis (Koeberle and Werz, 2009; Rådmark and Samuelsson, 2010). Accordingly, inhibitors of mPGES-1 (or the upstream enzyme COX-2) possess prominent anti-inflammatory and antitumorogenic activities (Koeberle and Werz, 2009). Previous studies have shown that carnosol and carnosic acid impair the proliferation of cancer cells, particularly from prostate, breast, and lung (Singletary et al., 1996; Hussein et al., 2007; Johnson et al., 2008, 2010; Yesil-Celiktas et al., 2010), prevent tumorigenesis in APC(Min/−) mice (Moran et al., 2005), and suppress phorbol ester-induced ear inflammation (Huang et al., 1994). Inducible PGE₂ formation occurs under all these experimental conditions. Prostate, breast, and lung cancer cells overexpress mPGES-1 (Koeberle and Werz, 2009), knockout of the mPGES-1 gene re-
duces intestinal cancer growth in APC-mutant mice (Nakanishi et al., 2008), and treatment with phorbol esters results in induction of mPGES-1 protein (Pham et al., 2006). From this, we speculated that an interference with mPGES-1 might contribute to the pleiotropic anticarcinogenic and anti-inflammatory activities of carnosol and carnosic acid. In fact, our study confirms carnosol and carnosic acid as active principles of sage selectively suppressing PGE₂ synthesis by the inhibition of mPGES-1. Neither the moderate inhibition of COX isoenzymes as described here and by others (Laughton et al., 1991; Subbaramaiah et al., 2002; Mengoni et al., 2011; Barni et al., 2012) nor a potential interference with other COX isoenzymes as described here and by others (Laughton et al., 1991; Subbaramaiah et al., 2002; Mengoni et al., 2011; Barni et al., 2012) could confirm an inhibition of COX product formation (i.e., 12-HHT) by carnosol and carnosic acid for human platelets stimulated with arachidonic acid. Substantially higher concentrations of carnosol or carnosic acid than for inhibition of mPGES-1 activity were required, however. Because arachidonic acid is directly converted to PGH₂ by COX, and PGH₂ can react nonenzymatically to 12-HHT, we were surprised that neither carnosol nor carnosic acid inhibited isolated COX isoenzymes. The discrepancy might be explained by an inhibition of thromboxane synthase that is abundantly expressed in platelets and can catalyze the conversion of PGH₂ to 12-HHT (Shen and Tai, 1986). Thus, 12-HHT is formed both nonenzymatically and through thromboxane synthase. However, the failure of carnosic acid to inhibit the formation of thromboxane B₂ (which is exclusively formed from PGH₂ by thromboxane synthase) precludes thromboxane synthase as a major target of carnosic acid. Together, these observations question a direct interaction of carnosol and carnosic acid with COX and suggest other intracellular points of at-

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Fig. 4. Effects of carnosol and carnosic acid on COX activity. Effects on 12-HHT (A) and thromboxane B₂ formation (B) in intact platelets and on the activity of isolated bovine COX-1 (C) and human recombinant COX-2 (D). Platelets (10⁶/ml) or COX isoenzymes were preincubated with carnosol or carnosic acid for 5 min, and 12-HHT (A, C, and D) or thromboxane B₂ formation (B) was initiated with arachidonic acid. After 5 min at 37°C, 12-HHT was determined by reversed-phase-HPLC as described (A, C, and D), and thromboxane B₂ was quantified by ELISA (B). Prostanoids formed in the absence of test compounds (100%, control) were: 92.2 ± 23.1 ng/ml 12-HHT (A) and 0.58 ± 0.03 µg/ml thromboxane B₂ (B) for intact platelets, 201.7 ± 54.0 µg/ml 12-HHT for COX-1 (C), and 32.9 ± 5.7 µg/ml 12-HHT for COX-2 (D). Data are given as mean ± S.E. (n = 3–5). *, p < 0.05; **, p < 0.01; or ***, p < 0.001 versus vehicle (DMSO) control, ANOVA + Tukey HSD post hoc tests.
might detrimentally compensate for PGE2 (Koeberle and other prostaglandins upon inhibition of mPGES-1, which is essential for the production of PGH2 toward the biosynthesis of thromboxane B2, or 12-HHT, eventually because of the overlapping cellular uptake, or metabolism underlie the different potencies of carnosol and carnosic acid in inhibiting PGE2 formation in LPS-stimulated human whole blood has yet to be answered. Oral administration of carnosic acid to mice resulted in peak plasma levels up to 44 μM (Satoh et al., 2008), implying that carnosic acid inhibits PGE2 formation in whole blood at pharmacologically highly relevant concentrations. Several studies described a redirection of PGH2 toward the biosynthesis of other prostanoids upon inhibition of mPGES-1, which might detrimentally compensate for PGE2 (Koeberle and Werz, 2009). For carnosol and carnosic acid, a redirection was not observed to 6-keto PGF1α, thromboxane B2, or 12-HHT, eventually because of the overlapping cellular inhibition of mPGES-1 and COX isoenzymes.

As dual inhibitors of mPGES-1 (as shown here) and 5-lipoxygenase (as reported previously (Poeckel et al., 2008)), carnosol and carnosic acid possess a beneficial pharmacological profile. Dual inhibition of inducible PGE2, and leukotriene biosynthesis is considered advantageous compared with interference with prostanoid formation alone. Combined inhibitors might not only exhibit an increased anti-inflammatory and anticarcinogenic potential but also seem to be associated with reduced side effects (Celotti and Laufer, 2008; Rädmark and Samuelsson, 2010). Particularly, gastrointestinal, renal, and cardiovascular intolerance have been reported for COX-1/2 inhibitors (Koeberle and Werz, 2009). Such side effects were not observed for the treatment with traditional remedies containing *S. officinalis* or reported within the limited number of preclinical studies using carnosol, carnosic acid, or plant extracts enriched in either of them.

Taken together, we 1) identified an ethyl acetate extract of *S. officinalis* to potentially inhibit mPGES-1, 2) ascribed this inhibition to carnosol and carnosic acid through bioactivity-guided fractionation, 3) confirmed the inhibition of mPGES-1 by carnosol and carnosic acid in cell-free and cellular assays, and 4) characterized the specificity of carnosol and carnosic acid for mPGES-1 within prostanoid biosynthesis. Our data suggest that the selective inhibition of mPGES-1-derived PGE2 formation may contribute to the anticarcinogenic and anti-inflammatory properties of carnosol and carnosic acid, which are major ingredients of the traditional herbal remedy *S. officinalis*.

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Authorship Contributions
Participated in research design: Rollinger, Stuppler, Werz, and Koeberle.
Conducted experiments: Bauer, Kuehnl, Scherer, and Koeberle.
Contributed new reagents or analytic tools: Rollinger, Northoff, Stuppler, and Werz.
Performed data analysis: Bauer, Kuehnl, and Koeberle.
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