Research Article

Identification of 3-hydroxy-β-damascone and related carotenoid-derived aroma compounds as novel potent inducers of Nrf2-mediated phase 2 response with concomitant anti-inflammatory activity

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Structural comparison of apple constituents with known inducers of phase two cytoprotective enzymes led to the identification of 3-hydroxy-β-damascone and related carotenoid derived aroma compounds as potent inducers of NAD(P)H:quinone reductase (QR) activity. Damascone-related compounds were found to be more potent inducers than ionone derivatives, with CD values (concentrations required to double the specific activity of QR in Hepa1c1c7 cell culture) in the range of 1.0–5.7 μM. QR induction by 3-hydroxy-β-damascone was shown to be mediated *via* transcription factor Nrf2 signaling in transient transfection experiments. We further identified aroma compounds as potent inhibitors of LPS-induced inducible nitric oxide synthase activity in Raw 264.7 cell culture. Again, damascone derivatives were most potent with half-maximal inhibitory concentration values of 1.8–7.9 μM. These results reveal previously unrecognized cancer chemopreventive potential of aroma compounds such as β-damascenone, 3-hydroxy-β-damascone, and related substances, which may contribute to the cancer protective efficacy of apple products and other dietary sources in animal models.

Keywords: Apples / Apple juice / Cancer chemoprevention / Carotenoid-derived aroma compounds / NAD(P)H:quinone oxidoreductase

Received: October 24, 2008; revised: March 5, 2009; accepted: March 8, 2009

1 Introduction

Epidemiologic evidence suggests that regular consumption of fruit, vegetables, and whole grains may reduce the risk for various types of cancer [1]. Apples (*Malus* sp., Rosaceae) belong to the most consumed fruit in Germany with an average annual *per* capita consumption of 18.4 kg [2]. Importantly, regular uptake of one or more apples a day has been associated with a significantly reduced risk for lung and colorectal cancer (reviewed in ref. [3]). Apples and apple products are a rich source of dietary phytochemicals and possess a wide range of biological activities which may contribute to cancer preventive activity (reviewed in ref. [3]).

In the framework of a German National Network program to investigate the effect of dietary components on intestinal health (Nutrition Net), we initiated a project to identify apple constituents with cancer chemopreventive potential. Polyphenol-enriched apple juice extracts were fractionated by column chromatography, and extracts and fractions were subjected to bioassay testing to identify compounds with antioxidative, anti-inflammatory, and anti-hor-

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Abbreviations: ARE, antioxidant response element; **CD,** concentration required to induce the specific activity of quinone reductase; **CO,** carbon monoxide; **CoPP,** Co-protoporphyrion; **CYP,** cytochrome P450; **HO-1,** heme oxygenase; **IC_{50},** half-maximal inhibitory concentration; **iNOS,** inducible nitric oxide synthase; **Keap1,** Kelch-like ECH-associated protein 1; **NO,** nitric oxide; **NQO1,** nicotinamide quinone oxidoreductase 1; **Nrf2,** nuclear factor E2-related protein 2; **QR,** NAD(P)H:quinone oxidoreductase; **Tox,** cytotoxic or antiproliferative activity

monal activities and potential to modulate carcinogen metabolism [4]. Modulation of enzymes involved in the detoxification of xenobiotics and carcinogens belongs to the best investigated mechanisms of cancer chemopreventive agents [5]. Food-based natural products have been shown to induce cytoprotective enzymes, such as NAD(P)H:quinone oxidoreductase 1 (QR, also known as nicotinamide quinone oxidoreductase 1 (NQO1)), glutathione S-transferases, superoxide dismutase, and heme oxygenase-1 (HO-1) [6]. Promoter regions of at least some of these inducible genes contain the antioxidant response element (ARE), which is activated upon binding of the nuclear factor E2-related protein 2 (Nrf2) transcription factor protein [7]. Nrf2 has been shown to be essential in the upregulation of these genes in response to oxidative stress, electrophiles, and natural products [8]. Natural inducers of ARE-dependent genes (ARE inducers) include diphenols and quinones, Michael reaction acceptors, isothiocyanates, and related sulfur compounds, 1,2-dithiole-3-thiones, hydroperoxides, and carotenoids and related polyenes [9]. Under basal conditions, Nrf2 is sequestered in the cytosol and maintained at low levels by interaction with the Kelch-like ECH-associated protein 1 (Keap1) and degradation by the proteasome. Upon stimulation by ARE inducers, Nrf2 is released from its interaction with Keap1, accumulates and translocates to the nucleus, where it leads to increased transcription of genes under control of the ARE. Decreased ubiquitination/proteasomal degradation of Keap1 and Nrf2, covalent or oxidative thiol modification of Keap1, and phosphorylation of Nrf2 have been proposed as three possibilities of ARE inducers to alter Nrf2-Keap1 interactions (reviewed in ref. [6]).

During the activity-guided fractionation of apple juice extracts, early eluting fractions (*e.g.*, fraction A 2.1 in ref. [4]) consistently enhanced the activity of QR in Hepa1c1c7 cell culture. Analyses with HPLC did not reveal the presence of phenolic compounds as potential ARE inducers in these fractions. Consequently, we started a systematic *in silico* search of apple constituents, which may contribute to the observed QR-inducing activity, focusing on structural similarities with known inducers (as summarized in ref. [9, 10]). As a result, we identified some norisoprenoid aroma compounds as potential ARE inducers (Fig. 1).

Norisoprenoids, including damascones, ionones, and their derivatives represent a group of carotenoid-derived compounds (apocarotenoids) that are widely spread in the flavor and fragrance industries [11].

Here, we describe QR-inducing potential as a novel bioactivity of damascones and ionones as well as some of their functionalized derivatives (Fig. 1). Phase 2 enzyme induction has repeatedly been shown to correlate with anti-inflammatory potency [12 – 14]. Consequently, we could further identify damascone-type aroma compounds as novel inhibitors of inducible nitric oxide synthase (iNOS) induction, using the murine macrophage cell line Raw 264.7 stimulated with bacterial LPSs as a model. A potential mechanistic correlation between induction of ARE-dependent genes and inhibition of iNOS induction is discussed.

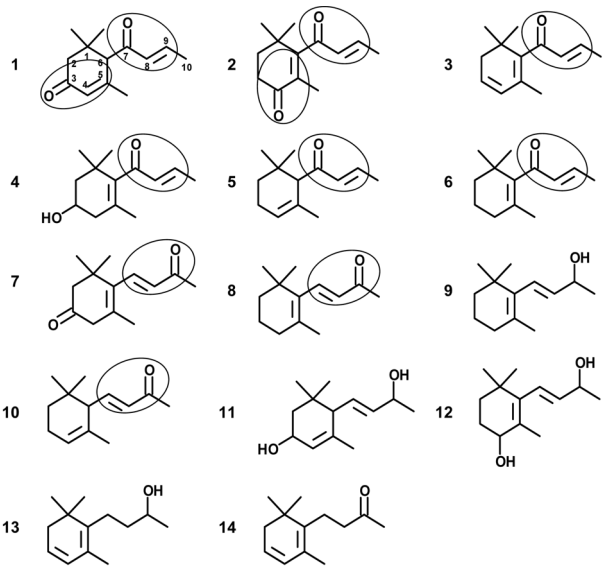

Figure 1. Chemical structures of damascones and ionones under study (*cf.* Table 1). Functional groups are highlighted by circles: most active compounds contain one or two Michael acceptor functionalities (α,β-unsaturated carbonyl moiety, in some cases in conjugation with additional double bonds).

2 Materials and methods

2.1 Chemicals

All cell culture media and supplements were obtained from Invitrogen (Eggenstein, Germany). Fetal calf serum was provided by PAA Laboratories (Pasching, Austria). β-Damascenone was purchased from Sigma – Aldrich (Steinheim, Germany). (±) α-Damascone was a product of Eurolabs Limited (London, UK). β-Damascone and α-ionone were from Extrasynthèse (Lyon, France). β-Ionone was purchased from Fisher Scientific (Nidderau, Germany) and (±)-E-β-ionol from Advanced Technology (Hong Kong, China). 3-Oxo-α-damascone, 4-oxo-β-damascone, and 7,8-dihydro-3,4-dehydro-β-ionone were synthesized in previous studies [15, 16]. 3-Hydroxy-β-damascone, 4-oxo-β-ionone, (±)-3-hydroxy-α-ionol, 4-hydroxy-β-ionol, as well as 7,8-dihydro-3,4-dehydro-β-ionol were available from a previous project [17]. All other chemicals were purchased from Sigma Chemical (Deisenhofen, Germany).

2.2 QR induction in mouse hepatoma cell culture

For the detection of phase 2 enzyme induction, QR activity was measured in cultured Hepa 1c1c7 murine hepatoma cells by the NADPH-dependent menadiol-mediated reduc-

tion of 3-(4,5-dimethylthiazo-2-yl)-2,5-diphenyltetrazolium bromide (MTT) to a blue formazan as described previously [18]. Protein was determined by crystal violet staining of an identical set of test plates. Compounds were dissolved in 100% DMSO (0.5% final concentration) and generally tested at non-toxic concentrations (cell staining >50% of solvent-treated control cells). Notably, some of the compounds were rather volatile and induced QR activity in untreated cells of neighboring wells. Therefore, untreated rows were routinely assigned between treated wells and especially in the vicinity of solvent controls. Induction of QR activity was calculated from the ratio of specific enzyme activities of compound-treated cells in comparison with a solvent control, and CD values (concentration required to double the specific QR activity in μM) were generated.

2.3 Inhibition of LPS-mediated inducible nitric oxide synthase (iNOS) induction in murine macrophages

Inhibition of LPS-mediated nitric oxide (NO) production by murine Raw 264.7 macrophages was determined *via* nitrite levels in culture supernatants by the Griess reaction as described previously [13] with minor modification. Briefly, Raw macrophages were plated at a density of 3.5×10^4 cells/well in 96-well plates. After a pre-incubation period of 24 h, inhibitors were added and iNOS expression was induced by addition of LPSs (final concentration 50 ng/mL). After 24 h, iNOS activity was quantified *via* nitrite levels in 100 μL of cell culture supernatants and compared to a nitrite standard curve. To determine cytotoxic effects of test compounds, residual cell culture medium was removed, cells were fixed at 4°C for 30 min with 50 μL ice-cold 10% trichloroacetic acid solution, washed five times with tap water and briefly dried. Cell numbers were estimated by sulforhodamin B staining [19].

2.4 Detection of Nrf2 activation with a pNQO1-ARE reporter construct

HT29 cells were cultivated in DMEM (high glucose) supplemented with 10% FBS. The *pNQO1-ARE* reporter plasmid was kindly provided by Dr. Akira Kobayashi (Nagoya University, Nagoya, Japan). This reporter responds strongly and selectively to Nrf2 driving firefly luciferase expression [20]. The promoterless pGL3 basic vector (Promega, Mannheim, Germany) served as control for basal luciferase activity. A plasmid encoding *Renilla* luciferase under control of the thymidine kinase promoter (pRL-TK, Promega, Mannheim, Germany) served for normalization of cell counts and transfection efficiency. HT29 cells were transfected with *pNQO1-ARE* (or pGL3-Basic for control) and pRL-TK (ratio 10:1) using Lipofectamine 2000 (Invitrogen, Karlsruhe, Germany), with a DNA:lipofectamine ratio of 1:6. After 5 h of transfection, cells were trypsinized and 2×10^4 cells were seeded *per* well of a 96 well plate (flat bottom, clear, Greiner Bio-One, Frickenhausen, Germany). Treatment was started the next day for 48 h. After 48 h of treatment with increasing concentrations of 3-hydroxy-β-damascone (4) (in presence of 100 U/mL catalase to exclude artifacts through hydrogen peroxide production in the culture medium), cells were washed twice with PBS and lysed in 20 μL passive lysis buffer (Promega, Mannheim, Germany). Luminescence was measured in a LumiStar Galaxy reader (BMG Labtechnologies, Durham, NC, USA) as described [21]. The firefly luciferase values were normalized to *Renilla* luciferase activity.

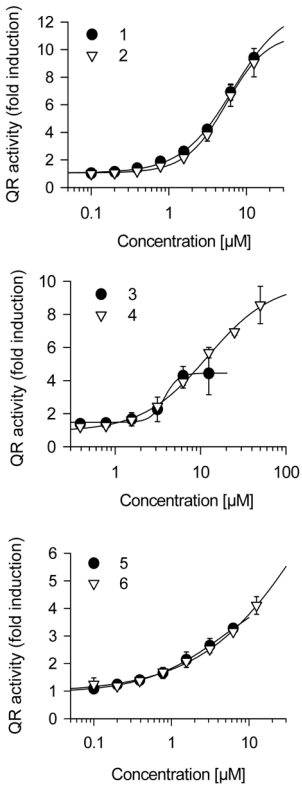

Figure 2. Induction of QR activity in Hepa1c1c7 murine hepatoma cells. Cells were incubated in the presence of 3-oxo-α-damascone (1), 4-oxo-β-damascone (2), β-damascenone (3), 3-hydroxy-β-damascone (4), (±)-α-damascone (5), and β-damascone (6) for 48 h. Data are means ± SD of three or more experiments. Specific activities of untreated controls were in the range of 15 – 36 nmoles/min/mg protein ($n = 16$). Sulforaphane was used as a positive control with a CD value of 0.27 ± 0.03 μM (mean ± SD, $n = 10$).

3 Results

3.1 Induction of QR activity

Based on structural similarities with known ARE inducers bearing a Michael-reaction acceptor moiety, we hypothe-

Table 1. Summary of potential chemopreventive activities of damascones and ionones

No.	Compound	CAS	QR induction		Inhibition of iNOS induction	
			CD (μM)[a]	Tox. IC_{50} (μM)[b]	IC_{50} (μM)[c]	Tox. IC_{50} (μM)[b]
1	3-oxo-α-Damascone	43126-29-0	1.0 ± 0.1[d]	>12.5 (61)	2.0 ± 0.9	33.0 (n = 2)
2	4-oxo-β-Damascone	53398-08-6	1.4 ± 0.1	>12.5 (77)	1.8 ± 0.9	29.3 (n = 2)
3	β-Damascenone	23726-93-4	1.5 ± 0.4	>12.5 (75)	3.0 ± 0.7	>50
4	3-Hydroxy-β-damascone	35734-61-3	2.2 ± 0.2	>50	7.9 ± 1.7	>50
5	(±)-α-Damascone	24720-09-0	5.5 ± 2.1	>50	4.9 ± 1.1	>50
6	β-Damascone	23726-91-2	5.7 ± 0.4	>50	6.5 ± 1.1	>50
7	4-oxo-β-Ionone	117048-10-9	18.8 ± 2.0	>50	>50 (27)	>50
8	β-Ionone	79-77-6	>50 (1.8)	>50	>50 (17)	>50
9	(±)-E-β-ionol	472-80-0	>50 (1.9)	>50	>50 (10)	>50
10	α-Ionone	127-41-3	>50 (1.5)	>50	>50 (8)	>50
11	(±)-3-Hydroxy-α-ionol	62660-03-1	>100	>100	>50 (4)	>50
12	4-Hydroxy-β-ionol	27185-80-4	>100 (1.8)	>100	>50 (2)	>50
13	7,8-Dihydro-3,4-dehydro-β-ionol	57069-86-0	>50 (1.6)	>100	>50 (8)	>50
14	7,8-Dihydro-3,4-dehydro-β-ionone	53398-08-6	>100 (1.1)	>100	>50 (9)	>50

a) CD, concentration required to double the specific activity of QR. Values in parenthesis indicate the fold induction *versus* control at the indicated concentration.
b) Tox, cytotoxic or antiproliferative activity; IC_{50} of cell viability. Values in parenthesis indicate the percentage of cell viability in comparison with the control at the indicated concentration.
c) IC_{50} of LPS-induced iNOS activity. Values in parenthesis indicate the percentage of inhibition at the indicated concentration.
d) Data are mean values ± SD with $n \geq 3$.

sized that carotenoid derived aroma compounds such as 3-hydroxy-β-damascone (4) and β-damascenone (3) as found, among others, in apple may account for potential to induce phase 2 enzyme activity. Using QR induction in murine Hepa1c1c7 cell culture as a model, we tested a series of 14 aroma compounds belonging to the main structural classes, *i.e.*, damascones (1 – 6) and ionones (7 – 14) (summarized in Table 1, structures in Fig. 1). We could demonstrate that damascone-related aroma compounds (1 – 6) potently induced QR activity up to ten-fold in a dose-dependent manner (Fig. 2). β-Damascenone (3), a character impact odorant of several fruits including apple [22], was identified as one of the most potent compounds from the tested series, with a CD value (concentration required to double the specific activity of QR) of 1.5 μM. The damascones (5) and (6) were found to be less active as 4-oxo-β-damascone (2). Of the ionone series, only 4-oxo-β-ionone (7) caused a dose-dependent QR induction, and we calculated a CD value of 18.8 μM. All other ionone and ionol-related compounds did not induce QR activity more than two-fold, even at concentrations up to 50 or 100 μM, respectively (Table 1).

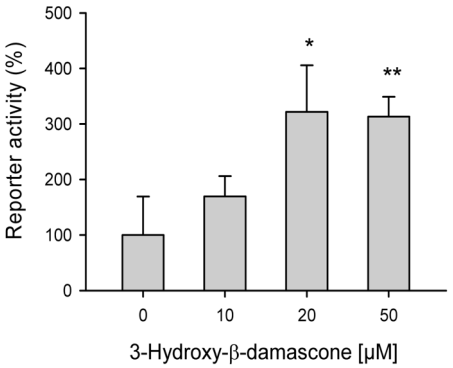

Figure 3. 3-Hydroxy-β-damascone (4) stimulates Nrf2 transcriptional activity. HT29 colon carcinoma cells were transiently transfected with *pNQO1-ARE*, a reporter for Nrf2 activity which is derived from the NQO1 promoter. *pNQO1-ARE*-transfected HT29 cells were treated with 3-hydroxy-β-damascone (4) at the indicated concentrations in presence of catalase for 48 h. Cells were extracted; firefly luciferase activity was measured and normalized to activity of a cotransfected renilla luciferase reference plasmid. Data (means ± SD, n = 8, $^*p < 0.05$, $^{**}p < 0.01$ for comparison with respective solvent control) are representative of two independent experiments with consistent results.

3.2 Activation of Nrf2 signaling

To confirm that damascone-type aroma compounds indeed belong to the class of ARE inducers, we performed transient transfection experiments with HT29 colon adenocarcinoma cells. A reporter construct *pNQO1-ARE* derived from the QR promoter was employed. Indeed, 3-hydroxy-β-damascone (4) stimulated reporter activity in a dose-dependent manner (Fig. 3). These results indicated that 3-hydroxy-β-damascone (4) can activate Nrf2 leading to ARE-mediated gene expression. Consistent with this finding early eluting fractions from apple extracts, which contain 3-hydroxy-β-damascone (4) [4], also potently activated Nrf2 reporter activity (data not shown).

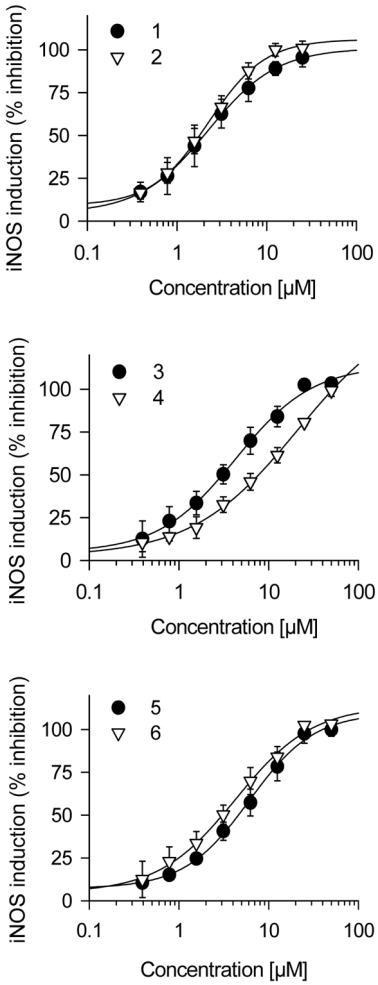

Figure 4. Inhibition of LPS-mediated NO production in Raw 267.4 macrophages. Cells were stimulated with LPSs (50 ng/mL) and incubated in the presence of 3-oxo-α-damascone (1), 4-oxo-β-damascone (2), β-damascenone (3), 3-hydroxy-β-damascone (4), (±)-α-damascone (5), and β-damascone (6) for 24 h. Data are means ± SD of three or more experiments. Nitrite levels in unstimulated controls were in the range of 3–12 nmoles nitrite/mg protein, after LPS-stimulation 26–104 nmoles nitrite/mg ($n = 16$). The IC_{50} value of curcumin used as a positive control was 6.5 ± 2.6 μM (mean ± SD, $n = 10$).

3.3 Inhibition of LPS-mediated induction of iNOS activity

Nrf2 signaling and phase 2 induction may play an important role in suppression of inflammation [14, 23]. We therefore tested whether the selected series of aroma compounds would possess anti-inflammatory potential in the murine macrophage cell line Raw 264.7 stimulated with bacterial LPSs to induce iNOS activity. As summarized in Table 1, only damascone-related aroma compounds inhibited LPS-mediated iNOS induction, with half-maximal inhibitory concentration (IC_{50}) values of 1.8–7.9 μM. The ionones

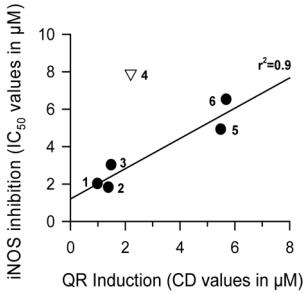

Figure 5. Correlation between QR induction and iNOS inhibition. CD values for the induction of QR activity in Hepa1c1c7 cells of compounds (1), (2), (3), (5), and (6) were plotted against IC_{50} values for the inhibition of LPS-mediated iNOS induction in Raw 264.7 murine macrophages. A linear correlation coefficient of 0.9 was computed.

were inactive at concentrations below 50 μM. The inhibitory potential of compounds (1–6) was dose-dependent in a concentration range of 0.4–50 μM (Fig. 4). With the exception of results obtained with 3-hydroxy-β-damascone (4), we observed a good linear correlation between QR inducing potential and inhibition of iNOS induction ($r^2 = 0.9$) (Fig. 5).

4 Discussion

The present report describes novel bioactivities of selected norisoprenoid aroma compounds. Here, we summarize the identification of damascones and their derivatives as a novel class of potential cancer chemopreventive phytochemicals, acting by coordinate upregulation of phase 2 cytoprotective enzymes and concomitant inhibition of pro-inflammatory enzyme induction, at concentrations in a range of 1–10 μM. These results may be of interest for further flavor research, since investigations of such physiological effects are largely missing.

β-Ionone (8), which was relatively inactive in our test systems, has previously been investigated as a chemopreventive agent in a series of studies. It demonstrated antimutagenic activity against aflatoxin and cyclophosphamide in the Ames test [24], potently inhibited pentoxyresorufin *O*-dealkylase activity as a measure of cytochrome P450 (CYP) 2B1 in liver microsomes *in vitro* at submicromolar concentrations [25], but lead to an induction of CYP2B1 mRNA and protein expression in rat liver *in vivo* [26]. It was also shown to inhibit cell proliferation and induce apoptosis in several cancer cell lines, although at relatively high concentrations in the range of 50 μM ([27–29] and cited references). Interestingly, recent reports indicate that β-ionone (8) prevented chemically-induced mammary and colon carcinogenesis in rat models when added to the diet at 0.1 and 0.2% [29–31].

In contrast, little information has so far been available on biological activities of damascone derivatives. Activity-guided fractionation of crude extracts of *Ipomoea pes-carprae* (L.) R. Br. (Convolvulaceae), which is used in Thailand against various types of inflammation including jellyfish sting dermatitis, identified β-damascenone (3) as an anti-spasmodic agent [32]. Since induced iNOS expression and NO have been associated with contact hypersensitivity and acute urticaria [33, 34], it is tempting to speculate that the ability of β-damascenone (3) to potently inhibit iNOS induction as shown here may also be involved in the anti-inflammatory effects of *I. pes-carprae.*

Our structure-activity analyses of a series of 14 norisoprenoid aroma compounds indicate that damascones (1 – 6) are significantly more potent in inducing QR activity and inhibiting iNOS induction than ionones (7 – 14). This is of interest, since both classes of compounds are characterized by an exocyclic double bond conjugated to a carbonyl group (Michael acceptor functionality), differing only in the orientation of the carbonyl moiety relative to the ring system. QR inducing potential of Michael acceptors has been attributed to sulfhydryl reactivity, *e.g*., with the cytosolic protein Keap1, which is sequestering transcription factor Nrf2 in the cytosol [35]. Using 3-hydroxy-β-damascone (4) as an example, we confirmed by transient transfection experiments that induction of QR was mediated *via* Nrf2 and ARE signaling. Further experiments have to clarify whether covalent or oxidative modification of cysteine thiols of Keap1, phosphorylation of Nrf2, or Nrf2/Keap1 stability are major targets of damascone-type compounds.

Two of the most active compounds, *i. e.*, 3-(oxo)-α-damascone (1) and 4-(oxo)-β-damascone (2) are characterized by double Michael reaction acceptor moieties (indicated in Fig. 1). This double functionality may explain highest reactivity with SH-groups and consequently highest QR inducing potential. Similarly, in β-damascenone (3) the carbon at position 3 is activated through the electron-withdrawing carbonyl group as a part of a vinylogous system, and may therefore also provide a second position for interaction with thiol groups. (±) α-Damascone (5) and β-damascone (6) demonstrated almost equal QR inducing potential, indicating that the double bond in position 4 or 5 may not contribute to the inducing activity. Apparently, in β-damascone (6), reactivity of carbon 5 as a Michael acceptor is reduced by sterical hindrance through the methyl group substitution, whereas additional hydroxyl-substitution in position 3 as in 3-hydroxy-β-damascone (4) increases QR-inducing potential.

Sterical hindrance may also account for the reduced activity of the ionones in comparison with the damascones under study. As an example, we determined CD values of 5.5 and 5.7 μM for QR induction by (±) α- and β-damascone (5 and 6), respectively, whereas α- and β-ionone (10 and 8) induced QR activity only 1.5- and 1.8-fold at a concentration of 50 μM. These results may indicate that interaction of the freely accessible carbon at position 9 as in damascone derivatives with SH-groups is facilitated in comparison to the less freely accessible carbon in position 7 as in the ionone series. All compounds lacking the α,β-unsaturated carbonyl moiety possessed only negligible activities at concentrations below 50 μM, underlining the importance of the Michael acceptor functionality for the bioactivities investigated in the present report.

With the exception of 3-hydroxy-β-damascone (4), we observed good correlation between potential of damascone-related compounds to induce QR activity, and their ability to inhibit LPS-mediated iNOS activation (Fig. 5). Our findings are in agreement with recent results of Liu *et al*. [14], who convincingly demonstrated correlation of both mechanisms by comparing activities of 19 compounds belonging to seven different chemical classes of phase 2 inducers [14]. These consistent observations let us to speculate whether both activities may be mechanistically linked. As a matter of fact, several recent reports have demonstrated a functional requirement for Nrf2 and the phase 2 enzyme HO-1 in the inhibition of iNOS induction. HO-1, which is induced in an ARE-dependent manner in parallel with other chemopreventive phase 2 enzymes [36], was shown to promote potent anti-inflammatory effects through release of carbon monoxide (CO), one of the main byproducts of oxidative degradation of heme through HO-1 [37]. Overexpression of HO-1 in transfection experiments, or treatment of Raw 264.7 macrophages with CO was sufficient to significantly reduce LPS-mediated pro-inflammatory cytokine production [37]. Moreover, iNOS mRNA expression induced by LPS in peritoneal macrophages was inhibited by the HO-1 inducer Co-protoporphyrin (CoPP). This effect was significantly lower in Nrf2 knockout macrophages, which had a strongly reduced capacity to induce HO-1 after CoPP treatment. These findings indicate that iNOS gene expression is regulated in a negative feedback manner by induction of HO-1 *via* Nrf2 [38]. HO-1 induction also contributed to the inhibition of LPS-mediated upregulation of iNOS expression in Raw 264.7 macrophages by a series structurally distinct compounds such as low dose 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 [39], the flavonoids 3-OH-flavone, baicalein, kaempferol, and quercetin [40], 2′-hydroxychalcone [41], the mushroom *Phellinus linteus* [42], propyl gallate [43], and low dose curcumin [44]. In these studies, blockage of HO-1 activity by an inhibitor, downregulation of HO-1 expression by siRNA, or co-treatment with hemoglobin as a scavenger of CO restored LPS-induced NO production reduced by these inhibitors. Consistently, we found that co-treatment of LPS-stimulated Raw 264.7 macrophages with 3-(oxo)-α-damascone (1), 4-(oxo)-β-damascone (2), or β-damascenone (3) at concentrations around the IC_{50} value, and 0.5 mg/mL hemoglobin as a CO scavenger almost completely prevented inhibition of iNOS induction. These preliminary results indicate that the anti-inflammatory potential of damascones may indeed be mediated by HO-1 induc-

tion. Further mechanistic analyses will have to demonstrate a functional requirement of HO-1 induction for inhibition of iNOS induction by damascone-type compounds.

Our study has identified damascone-related norisoprenoid aroma compounds as a novel class of potential cancer chemopreventive agents. In cellular systems, the compounds act by Nrf-2 dependent induction of phase 2 detoxifying enzymes, which may result in enhanced excretion of xenobiotics and carcinogens *in vivo*. In addition, a reduction of inflammatory mediators by inhibition of iNOS induction may prevent tumor promotion. Based on these data, further investigations of cancer preventive efficacy of damascone-type aroma compounds in animal models are warranted.

We are grateful to Gabriele Schulz-Raffelt for carrying out some initial Nrf2 reporter measurements, to Akira Kobayashi for providing the pNQO1-ARE plasmid, and to Christian Kliem for helpful discussion on structure-activity relationships. We also thank the German Federal Ministry for Education and Research (BMBF) for financial support of this study.

The authors have declared no conflict of interest.

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