Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry 16 (2008) 4286–4293
Bioorganic & Medicinal Chemistry

Natural and non-natural prenylated chalcones: Synthesis, cytotoxicity and anti-oxidative activity

Susanne Vogel, Susanne Ohmayer, Gabi Brunner and Jörg Heilmann*

Institute of Pharmacy, Chair of Pharmaceutical Biology, University of Regensburg, Universitätsstr. 31, 93053 Regensburg, Germany

Received 5 October 2007; revised 18 February 2008; accepted 26 February 2008
Available online 29 February 2008

Dedicated to HD Dr. B. Liebermann on the occasion of his 65th birthday

Abstract—A general strategy for the synthesis of 3′-prenylated chalcones was established and a series of prenylated hydroxychalcones, including the hop (*Humulus lupulus* L.) secondary metabolites xanthohumol (**1**), desmethylxanthohumol (**2**), xanthogalenol (**3**), and 4-methylxanthohumol (**4**) were synthesized. The influence of the A-ring hydroxylation pattern on the cytotoxic activity of the prenylated chalcones was investigated in a HeLa cell line and revealed that non-natural prenylated chalcones, like 2′,3,4′,5-tetrahydroxy-6′-methoxy-3′-prenylchalcone (**9**, IC_{50} 3.2 ± 0.4 μM) as well as the phase 1 metabolite of xanthohumol (**1**), 3-hydroxyxanthohumol (**8**, IC_{50} 2.5 ± 0.5 μM), were more active in comparison to **1** (IC_{50} 9.4 ± 1.4 μM). A comparison of the cytotoxic activity of xanthohumol (**1**) and 3-hydroxyxanthohumol (**8**) with the non-prenylated analogs helichrysetin (**12**, IC_{50} 5.2 ± 0.8) and 3-hydroxyhelichrysetin (**13**, IC_{50} 14.8 ± 2.1) showed that the prenyl side chain at C-3′ has an influence on the cytotoxicity against HeLa cells only for the dihydroxylated derivative. This offers interesting synthetic possibilities for the development of more potent compounds. The ORAC activity of the synthesized compounds was also investigated and revealed the highest activity for compounds **12**, 4′-methylxanthohumol (**4**), and desmethylxanthohumol (**2**), with 4.4 ± 0.6, 3.8 ± 0.4, and 3.8 ± 0.5 Trolox equivalents, respectively.

1. Introduction

Xanthohumol is the most abundant prenylated chalcone in hop cones (*Humulus lupulus* L.) and has been shown to exhibit an interesting spectrum of pharmacological effects. Besides its remarkable anti-proliferative activity against different cancer cell lines,[1–3] xanthohumol also exhibited apoptotic activity[2,4] and showed chemopreventive effects.[5,6] Furthermore, several in vitro studies substantiated effects on enzymes and transcription factors involved in the genesis of cancer.[4,7–11] Very recently, in vivo growth inhibition of a vascular tumor has been reported.[9] Hop cones also contain several minor prenylated and structurally related chalcones like xanthogalenol, 5′-prenylxanthohumol, xanthohumol B, and C,[12] but the pharmacological data for these compounds are scarce due to the limited availability via isolation.[13] Recently, we described a synthesis for xanthohumol[14] and in parallel another synthetic approach was published by Khupse and Erhardt,[15] but no synthesis has been described for other prenylated hop chalcones, except for demethylxanthohumol.[16] Up to now, all compounds have to be isolated from hop cones. Here, we report on a synthetic strategy generally applicable for the synthesis of natural and non-natural prenyl chalcones. Furthermore, we investigated the cytotoxic and anti-oxidative activities of these compounds. Due to the fact that recent studies on the metabolism of xanthohumol identified some hydroxylated derivatives as potential phase I metabolites,[17,18] we also synthesized one of these compounds (**8**) to evaluate its biological activity in comparison to xanthohumol.

2. Results and discussion

2.1. Chemistry

2.1.1. Compounds 1, 6–13. MOM protection of 2,4,6-trihydroxyacetophenone with 2.5 equivalents MOM bromide yielded intermediate **1a**, which was refluxed for

Keywords: Synthesis of prenylated chalcones; Xanthohumol; Cytotoxic activity; Anti-oxidative activity.
* Corresponding author. Tel.: +49 941 943 4759; fax: +49 941 943 4990; e-mail: joerg.heilmann@chemie.uni-regensburg.de

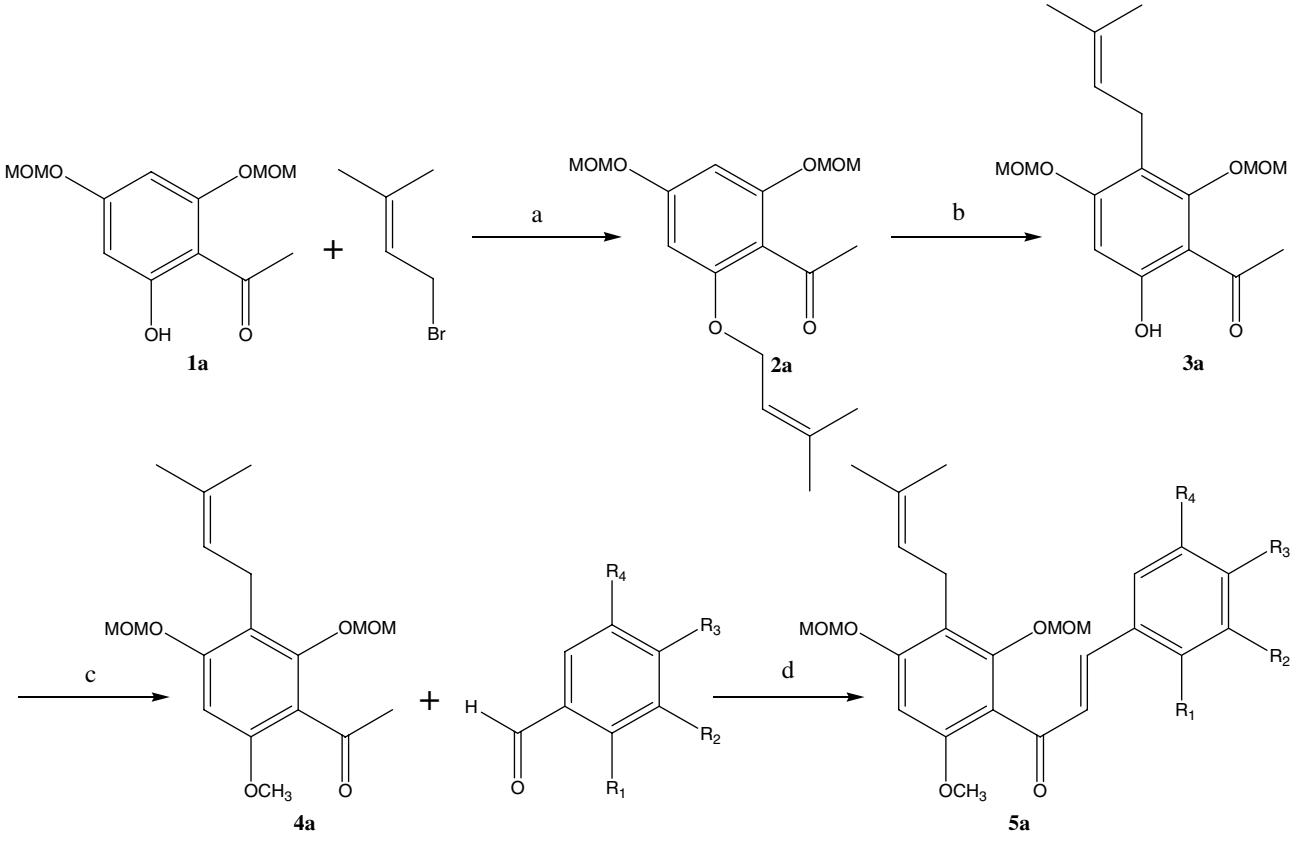
0968-0896/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2008.02.079

24 h with prenylbromide in acetone/K_2CO_3 to obtain compound **2a** in 91% yield. Claisen rearrangement of ether **2a** in *N*,*N*-dimethylaniline leads to the MOM protected and prenylated acetophenone (**3a**). Using the phase transfer catalyst tetrabutylammonium iodide, methylation with dimethylsulfate gave **4a**. Aldol coupling with the respective MOM protected benzaldehydes leads to the corresponding protected chalcones (**5a**, Scheme 1). Deprotection with 3 N HCL in MeOH (reflux) yielded the chalcones (**1**, **6**–**11**). Compounds **12** and **13** were synthesized by analogy, but without prenylation. In parallel, another approach for one of the key steps, the introduction of the prenyl side chain to the phenolic hydroxyl group via a Mitsunobu reaction (with triphenyl phosphine, diethyl diazodicarboxylate, 3-methyl-2-en-1-ol, and toluene) was suggested for the synthesis of xanthohumol, but looks less practicable relative to green chemistry and the yield (80% in comparison to 91%).[15] Furthermore, the authors did the methylation step without the transfer catalyst tetrabutylammonium iodide and obtained slightly lower yields for **4a** (82% in comparison to 89%). Besides xanthohumol, no other prenylated hop chalcones were synthesized in that study.

2.1.2. Compounds 2–5. Slight modification of the general strategy by omitting (or doubling) the methylation step, or by changing the sequence of the reactions, also enables the synthesis of compounds **2**–**5**.

2.2. Biology

The cytotoxic activity of all the synthesized chalcones was tested against a Hela cell line using a MTT cell proliferation assay.[19,20] In accordance with literature data on other cancer cell lines, xanthohumol (**1**) showed a remarkable cytotoxic activity with an IC_{50} value of 9.4 μM, whereas desmethylxanthohumol (**2**) is less active (IC_{50} 16.5 μM). Compounds with different methylation patterns (**3**–**5**) also showed lower cytotoxicity (Table 1). Interestingly, the variation of the hydroxyl group pattern of ring A (concerning number and position) resulted in various compounds showing higher activity in comparison to **1**. Chalcones **8** (2′,3,4,4′-tetrahydroxy-6′-methoxy-3′-prenylchalcone) and **9** (2′,3,4′,5-tetrahydroxy-6′-methoxy-3′-prenylchalcone) were the most active, exhibiting IC_{50} values of 2.5 and 3.2 μM, respectively. It is especially of interest that compound **8** is more active as xanthohumol (**1**) itself because it is reported to be one of the possible phase I metabolites of **1**.[17] A couple of other oxidized and/or conjugated xanthohumol metabolites have been identified showing, for example, a hydroxylated prenyl side chain or an additional substituted furan ring.[17,21] Biological and chemical characterization of these compounds has not been possible until now due to the limited amounts available. Thus, synthesis along this strategy and further pharmacological investigation of phases I and II metabolites is a worthwhile follow-up project. It is of further



Scheme 1. Key steps of the synthetic route for xanthohumol (**1**) and its derivatives varying in A Ring substitution. Reagents and conditions: (a) Acetone, K_2CO_3, 24 h (reflux); (b) *N*,*N*-dimethylaniline, 3 h (reflux), argon atmosphere; (c) dimethylsulfate, NaOH, DCM/H_2O 3:2, tetrabutylammonium iodide (phase transfer catalyst), 24 h (room temperature); (d) KOH, EtOH/H_2O 3:2, 1 h (ice), 72 h (room temperature).

Table 1. Cytotoxic (HeLa cells, 150.000 cells/mL, 72 h incubation, IC_{50} values in μM ± SD, $n = 8$) and antioxidative (Trolox equivalents, concentration range in brackets)[23] activity of chalcones (**1**–**13**)

	IC_{50} (μM)	Trolox equiv
1	9.4 ± 1.4	2.3 ± 0.2 (0.25–1.5)
2	16.5 ± 1.3	3.8 ± 0.5 (0.25–1.5)
3	28.3 ± 3,6	1.8 ± 0.3 (0.25–1.5)
4	11.8 ± 1.7	3.8 ± 0.4 (0.1–1.5)
5	25.6 ± 3.3	1.5 ± 0.2 (0.25–1.5)
6	5.9 ± 0.4	2.3 ± 0.2 (0.25–1.5)
7	4.8 ± 0.3	2.7 ± 0.1 (0.25–1.5)
8	2.5 ± 0.5	3.1 ± 0.3 (0.1–1.0)
9	3.2 ± 0.4	2.9 ± 0.4 (0.1–1.0)
10	30.3 ± 6.4	1.1 ± 0.3 (0.1–1.0)
11	17.1 ± 1.8	0.9 ± 0.1 (0.5–2.0)
12	5.2 ± 0.8	4.4 ± 0.6 (0.1–1.0)
13	14.8 ± 2.1	3.0 ± 0.2 (0.25–1.5)

interest that the cytotoxic activity of prenylated (**1** and **8**; IC_{50} 9.4 and 2.5 μM, respectively) and the corresponding non-prenylated chalcones (**12** and **13**; IC_{50} 5.2 and 14.8 μM, respectively) revealed that prenylation at 3′—has no significant effect concerning the cytotoxicity of the 4-hydroxy-chalcones, but is important for the 3,4-dihydroxy-structures. This offers interesting possibilities for changing the substitution on C-3′ for non-prenylated xanthohumol analogs with the aim to synthesize derivatives with still higher cytotoxic activity against tumor cells. Also the development of hybrid and bivalent molecules is possible using C-3′ as coupling position.

Literature data strongly suggested that the pharmacological importance of phenolic compounds in food is often related to their antioxidant activity.[22] To assay the antioxidant activity of the synthesized chalcones, the ORAC- (oxygen radical absorbance capacity) Fluorescein assay was used generating peroxyl radicals by the application of 2,2′-azobis (2-methylpropionamide) dihydrochloride (AAPH) as a free radical initiator.[23] All tested compounds showed a remarkable activity of 0.9 (**11**) to 4.4 (**12**) Trolox equivalents in a concentration range between 0.1 and 2.0 μM (Table 1). In comparison to other potent antioxidants like quercetin (10.5 ± 0.4 Trolox equiv, concentration range 0.2–0.6 μM),[23] this represents a relatively low to moderate activity. For the most active compounds **2** (3.8 ± 0.5 Trolox equiv), **4** (3.8 ± 0.4), and **12** (4.4 ± 0.6), the activity is comparable to that reported for ferulic and *p*-coumaric acid (4.47 ± 0.21 and 4.51 ± 0.23, concentration range 0.3–1.0 and 0.4–1.0, respectively).[23] Gerhäuser et al. reported for a concentration of 1 μM, an ORAC of 2.9 Trolox equivalents[5] for xanthohumol (**1**). This was nearly exactly confirmed by our experiments for a concentration range of 0.25–1.5 μM revealing 2.3 ± 0.2 Trolox equivalents. Interestingly, some minor compounds of hop cones like 4′-methylxanthohumol (**4**) were more active.

3. Conclusion

A general strategy for the synthesis of prenylated chalcones has afforded several analogs. Among these prenylated hop chalcones, not only does the most abundant hop chalcone xanthohumol (**1**) exhibit interesting activity, but also the minor compounds, the non-natural chalcones, and one of the phase 1 metabolites of **1** exhibit activity.

4. Experimental

4.1. Instruments and materials

All ^1H and ^{13}C NMR experiments were recorded on a Bruker Avance 300 (operating at 300.13 MHz for ^1H and 75.47 MHz for ^{13}C) at 296.1 K. The spectra were recorded in acetone-d_6, $CDCl_3$ or DMSO-d_6 (Firma: Deutero GmbH, purity 99.8%) and referenced against residual non-deuterated solvent. Melting points were measured on a Büchi Melting Point B-545 apparatus (uncorrected). HR- and LREIMS (70 eV) were measured on a MAT 710A. UV spectra were recorded on a Cary 50 Scan (Varian; all compounds in MeOH, purity >99.9%). Column chromatography (CC) was always performed with normal phase silica gel (Firma Merck, 0.063–0.200 mm), TLC analysis was done with Silica gel 60 F_{254} plates (Merck) using a UV lamp for detection (254 and 365 nm). The optical density in the MTT cytotoxicity assay was measured at 560 nm using a microplate reader (Tecan).

4.2. Preparation of compounds 1, 2, 4, and 6–13

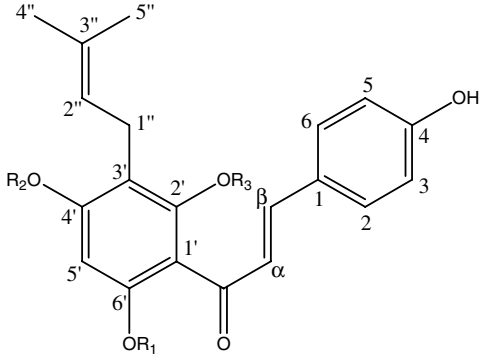
A mixture of 2,4,6-trihydroxyacetophenone (4.6 g, 1 equiv), anhydrous K_2CO_3 (7 equiv), and MOM bromide (2.5 equiv) was stirred and refluxed in acetone for 3 h. The reaction mixture was cooled to room temperature and filtered. The filtrate was evaporated and the residue subjected to CC with PE/EA 1:1 as an eluent to yield compound **1a** (68%). Prenylbromide (1.5 equiv), K_2CO_3 (4 equiv), and **1a** (2 g) were refluxed in acetone for 24 h with stirring gave **2a** after filtration and CC (PE/EA 2:1) of the evaporated filtrate (91%). Compound **2a** was refluxed and stirred in *N*,*N*-dimethylaniline under argone atmosphere to give **3a** (41%) after CC with PE/EA 6:1 as eluent. A mixture of **3a** (400 mg), dimethylsulfate (1.1 equiv), tetrabutylammonium iodide (0.1 equiv), and NaOH (1.4 equiv) was stirred in dichloromethane/water 3:2 for 24 h at room temperature. Separation of organic and aqueous phase, extraction of the aqueous phase with CH_2Cl_2, and CC of the residue of all dried, combined and evaporated CH_2Cl_2 phases with PE/EA 2:1 yielded **4a** (89%). Compound **4a** (300 mg) was stirred in ethanol/water (3:2) under argone atmosphere together with the respective protected benzaldehyde derivative (1.1 equiv) and KOH (50 equiv) initially for 1 h in an ice bath afterwards for 72 h at room temperature. The reaction mixture was poured into ice-water acidified with 3 N HCL and extracted three times with CH_2Cl_2 or ethyl acetate. The organic phases were combined, washed with water, dried on Na_2SO_4, and evaporated. The residue was subjected to CC (different PE/EA mixtures: 1:1, 3:2, 2:1 and 3:1, respectively) and yielded **5a** (yields between 53% and 86%). Compound **5a** was dissolved in methanol and

3 N HCl was added to give a ratio of 5:1 methanol/3 N HCl. After 15 min under reflux, the reaction mixture was poured into ice-water and extracted three times with ethyl acetate or dichloromethane. The organic phases were combined, washed with water, and dried on Na_2SO_4. After evaporation, CC of the residue on silica gel using CH_2Cl_2/EA (4:1 and 6:1) or PE/EA (1:1, 1:2 and 1:3) gave the corresponding chalcones **1**, **6**–**11** (yields between 40% and 78%). Compounds **12** and **13** were analogously prepared, omitting the prenylation steps. Intermediate **3a** was directly coupled with the protected 4-hydroxybenzaldehyde to give compound **2** after deprotection (yield 47%). For the preparation of compound **4**, intermediate **4a** was deprotected as described for compound **5a** and the resulting 2,4-dihydroxy-6-methoxy-3-prenylacetophenone (yield 58%) was again methylated with dimethylsulfate and tetrabutylammonium iodide as described above to give 2-hydroxy-4,6-dimethoxy-3-prenylacetophenone (yield 81%). Coupling with MOM protected 4-hydroxybenzaldehyde followed by deprotection yielded 2′,4-dihydroxy-4′,6′-dimethoxy-3′- prenylchalcone (**4**) (yield 63%).

4.2.1. 2′,4,4′-Trihydroxy-6′-methoxy-3′-prenylchalcone (xanthohumol, 1). Yield: 64%; Orange-yellow amorphous powder; mp 162–163 °C; UV λ_{max} 369.6 nm; EI-MS (pos. mode) *m*/*z*: 354 $[M]^+$ (70), 339 $[M-CH_3]^+$ (10), 311 $[M\text{-}C_3H_7]^+$ (48), 179 (100); ^{1}H NMR ($CDCl_3$) δ (ppm): 1.77 (3H, s, H_3-5″), 1.83 (3H, s, H_3-4″), 3.41 (2H, d, J = 7.1, H_2-1″), 3.90 (3H, s, OC*H*$_3$), 5.22 (1H, s, O*H*), 5.30 (1H, t, J = 7.1, H-2″), 5.94 (1H, s, O*H*), 6.19 (1H, s, H-5′), 6.86 (2H, d, J = 8.5, H-3 and H-5), 7.52 (2H, J = 8.5, H-2 and H-6), 7.74 (1H, d, J = 15.6, H-β), 7.80 (1H, d, J = 15.6, H-α), 14.65 (1H, s, O*H*-2′); ^{13}C NMR (acetone-d_6) δ (ppm): 193.3 (*C*O), 166.4 (C-2′), 162.8 (C-4′), 161.9 (C-6′), 160.6 (C-4), 143.2 (C-β), 131.2 (C-2 and C-6), 131.0 (C-3″), 128.2 (C-1), 125.5 (C-α), 124.0 (C-2″), 116.8 (C-3 and C-5), 108.9 (C-3′), 106.3 (C-1′), 91.7 (C-5′), 56.2 (O*C*H$_3$), 25.9 (C-5″), 22.1 (C-1″), 17.9 (C-4″).

4.2.2. 2′,4,4′,6′-Tetrahydroxy-3′-prenylchalcone (desmethylxanthohumol, 2). Yield 43%; Orange-yellow amorphous powder; mp 176–177 °C; UV λ_{max} 365.0 nm; EI-MS (pos. mode) *m*/*z*: 340 $[M]^+$ (70), 325 $[M-CH_3]^+$ (13), 297 $[M\text{-}C_3H_7]^+$ (20), 165 (100); ^{1}H NMR (DMSO-d_6) δ (ppm): 1.60 (3H, s, H_3-5″), 1.69 (3H, s, H_3-4″), 3.09 (2H, d, J = 6.6, H_2-1″), 5.13 (1H, t, J = 6.6, H-2″), 6.02 (1H, s, H-5′), 6.83 (2H, d, J = 8.8, H-3 and H-5), 7.52 (2H, J = 8.8, H-2 and H-6), 7.65 (1H, d, J = 15.6, H-β), 7.99 (1H, d, J = 15.6, H-α), 10.05 (1H, s, O*H*), 10.34 (1H, s, O*H*), 10.64 (1H, s, O*H*), 14.54 (1H, s, O*H*-2′); ^{13}C NMR (acetone-d_6) δ (ppm): 193.4 (*C*O), 165.9 (C-2′), 162.8 (C-4′), 160.5 (C-6′), 160.2 (C-4), 143.0 (C-β), 131.2 (C-2 and C-6), 130.8 (C-3″), 128.2 (C-1), 125.6 (C-α), 124.3 (C-2″), 116.8 (C-3 and C-5), 108.1 (C-3′), 105.7 (C-1′), 95.3 (C-5′), 25.9 (C-5″), 22.1 (C-1″), 17.9 (C-4″).[24]

4.2.3. 2′,4-Dihydroxy-4′,6′-dimethoxy-3′-prenylchalcone (4′-methylxanthohumol, 4). Yield 66%; Orange-yellow amorphous powder; mp 145 °C; UV λ_{max} 369.9 nm; EI-MS (pos. mode) *m*/*z*: 368 $[M]^+$ (73), 353 $[M-CH_3]^+$ (20), 325 $[M\text{-}C_3H_7]^+$ (65), 193 (100); ^{1}H NMR (acetone-d_6) δ (ppm): 1.62 (3H, s, H_3-5″), 1.75 (3H, s, H_3-4″), 3.26 (2H, d, J = 7.1, H_2-1″), 3.96 (3H, s, OC*H*$_3$), 4.04 (3H, s, OC*H*$_3$), 5.18 (1H, t, J = 7.1, H-2″), 6.30 (1H, s, H-5′), 6.92 (2H, d, J = 8.7, H-3 and H-5), 7.61 (2H, J = 8.7, H-2 and H-6), 7.75 (1H, d, J = 15.6, H-β), 7.88 (1H, d, J = 15.6, H-α), 9.11 (1H, s, O*H*-4), 14.33 (1H, s, O*H*-2′); ^{13}C NMR (acetone-d_6) δ (ppm): 193.8 (*C*O), 164.8 (C-4′), 164.4 (C-2′), 162.5 (C-6′), 160.8 (C-4), 143.5 (C-β), 131.3 (C-2 and C-6), 131.0 (C-3″), 128.0 (C-1), 125.4 (C-α), 123.9 (C-2″), 116.9 (C-3 and C-5), 110.0 (C-3′), 106.9 (C-1′), 88.0 (C-5′), 56.5 (O*C*H$_3$-6′), 56.2 (O*C*H$_3$-4′), 25.9 (C-5″), 22.0 (C-1″), 17.9. (C-4″).[12]



	R_1	R_2	R_3
Xanthohumol (**1**)	CH_3	H	H
Desmethylxanthohumol (**2**)	H	H	H
Xanthogalenol (**3**)	H	CH_3	H
4′-Methylxanthohumol (**4**)	CH_3	CH_3	H
4,6′-Dihydroxy-2′,4′-dimethoxy-3′-prenylchalcone (**5**)	H	CH_3	CH_3

4.2.4. 2,2′,4′-Trihydroxy-6′-methoxy-3′-prenylchalcone (6). Yield 40%; Orange-yellow amorphous powder; mp 84–85 °C; UV λ_{max} 365.0 nm; EI-MS (pos. mode) *m*/*z*: 354 $[M]^+$ (42), 293 (100), 179 (85); EI-HRMS (pos. mode) *m*/*z*: 354.1459 (calcd for $C_{21}H_{22}O_5$, 354.1467); ^{1}H NMR ($CDCl_3$) δ (ppm): 1.78 (3H, s, H_3-5″), 1.83 (3H, s, H_3-4″), 3.41 (2H, d, J = 7.4, H_2-1″), 3.89 (3H, s, OCH_3), 5.30 (1H, t, J = 7.4, H-2″), 5.60 (1H, s, O*H*), 5.94 (1H, s, O*H*), 6.23 (1H, s, H-5′), 6.85 (1H, m, H-5), 6.96 (1H, d, J = 15.9, H-β), 6.98 (1H, m, H-3), 7.25 (1H, m, H-4), 7.55 (1H, dd, J = 8.0, 1.6, H-6), 8.04 (1H, d, J = 15.9, H-α), 14.56 (1H, s, O*H*-2′); ^{13}C NMR (acetone-d_6) δ (ppm): 193.8 (*C*O), 166.4 (C-2′), 162.9 (C-4′), 162.0 (C-6′), 157.7 (C-2), 138.3 (C-β), 132.2 (C-4), 131.0 (C-3″), 129.6 (C-6), 128.3 (C-α), 124.0 (C-2″), 123.5 (C-1), 120.9 (C-5), 117.1 (C-3), 108.9 (C-3′), 106.4 (C-1′), 91.7 (C-5′), 56.1 (O*C*H$_3$), 25.9 (C-5″), 22.1 (C-1″), 17.9 (C-4″).

4.2.5. 2′,3,4′-Trihydroxy-6′-methoxy-3′-prenylchalcone (7). Yield 55%; Orange amorphous powder; mp 79–80 °C; UV λ_{max} 355.0 nm; EI-MS (pos. mode) *m*/*z*: 354 $[M]^+$ (64), 339 $[M-CH_3]^+$ (12), 311 $[M\text{-}C_3H_7]^+$ (40), 179 (100); EI-HRMS *m*/*z*: 354.1465 (calcd for $C_{21}H_{22}O_5$, 354.1467); ^{1}H NMR ($CDCl_3$) δ (ppm): 1.78 (3H, s, H_3-5″), 1.83 (3H, s, H_3-4″), 3.41 (2H, d, J = 7.4, H_2-1″), 3.90 (3H, s, OCH_3), 4.99 (1H, s, O*H*), 5.29 (1H, t, J = 7.4, H-2″), 5.95 (1H, s, O*H*), 6.23 (1H, s, H-5′), 6.86 (1H, ddd, J = 8.0, 2.7, 1.1, H-4), 7.07 (1H, m, H-2), 7.18 (1H, m, H-6), 7.27 (1H, t, J = 8.0, H-5), 7.70 (1H, d, J = 15.6, H-β), 7.85 (1H, d, J = 15.6, H-α), 14.52 (1H, s, O*H*-2′); ^{13}C NMR (acetone-d_6) δ (ppm): 193.3 (*C*O), 166.4 (C-2′), 163.2 (C-4′), 162.1 (C-6′), 158.8 (C-3), 142.6 (C-β), 137.9 (C-1), 131.1 (C-3″), 130.9 (C-5), 128.7 (C-α), 123.9 (C-2″), 120.9 (C-6), 118.2 (C-4), 115.3 (C-2), 108.9 (C-3′), 106.3 (C-1′), 91.8 (C-5′), 56.2 (O*C*H$_3$), 25.9 (C-5″), 22.1 (C-1″), 17.9 (C-4″).

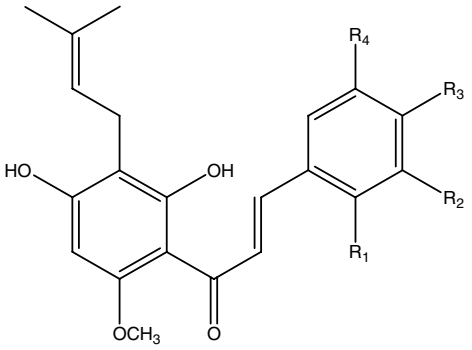
4.2.6. 2′,3,4,4′-Tetrahydroxy-6′-methoxy-3′-prenylchalcone (3-hydroxyxanthohumol, 8). Yield 42%; Orange amorphous powder; mp 127 °C; UV λ_{max} 380.0 nm; EI-MS (pos. mode) 370 $[M]^+$ (57), 355 $[M-CH_3]^+$ (9), 327 (31), 179 (100); EI-HRMS (pos. mode) *m*/*z*: 370.1416 (calcd for $C_{21}H_{22}O_6$, 370.1416); ^{1}H NMR ($CDCl_3$) δ (ppm): 1.77 (3H, s, H_3-5″), 1.83 (3H, s, H_3-4″), 3.40 (2H, d, J = 7.1, H_2-1″), 3.90 (3H, s, OCH_3), 5.29 (1H, t, J = 7.1, H-2″), 5.72 (1H, s, O*H*), 5.94 (1H, s, H-5′), 6.25 (1H, s, O*H*), 6.87 (1H, s, O*H*), 7.08 (1H, d, J = 8.2, H-5), 7.09 (1H, dd, J = 8.2, 1.9, H-6) 7.15 (1H, J = 1.9, H-2), 7.68 (1H, d, J = 15.6, H-β), 7.76 (1H, d, J = 15.6, H-α), 14.65 (1H, s, O*H*-2′); ^{13}C NMR (acetone-d_6) δ (ppm): 193.3 (*C*O), 166.4 (C-2′), 162.8 (C-4′), 161.9 (C-6′), 148.8 (C-4), 146.4 (C-3), 143.5 (C-β), 131.0 (C-3″), 128.8 (C-1), 125.6 (C-α), 124.0 (C-2″), 123.1 (C-6), 116.5 (C-5), 115.3 (C-2), 108.9 (C-3′), 106.3 (C-1′), 91.7 (C-5′), 56.2 (O*C*H$_3$), 25.9 (C-5″), 22.1 (C-1″), 17.9 (C-4″).

4.2.7. 2′,3,4′,5-Tetrahydroxy-6′-methoxy-3′-prenylchalcone (9). Yield 71%; Orange amorphous powder; mp 141–142 °C; UV λ_{max} 355.0 nm; EI-MS (pos. mode) 370 $[M]^+$ (73), 355 $[M-CH_3]^+$ (11), 327 $[M\text{-}C_3H_7]^+$ (22), 179 (100); EI-HRMS (pos. mode) *m*/*z*: 370.1416 (calcd for $C_{21}H_{22}O_6$, 370.1416); ^{1}H NMR ($CDCl_3$) δ (ppm): 1.78 (3H, s, H_3-5″), 1.83 (3H, s, H_3-4″), 3.40 (2H, d, J = 7.4, H_2-1″), 3.90 (3H, s, OCH_3), 5.05 (1H, s, O*H*), 5.29 (1H, t, J = 7.4, H-2″), 5.94 (1H, s, O*H*), 6.24 (1H, s, H-5′), 6.39 (1H, t, J = 2.1, H-4), 6.65 (2H, d, J = 2.1, H-2 and H-6), 7.60 (1H, d, J = 15.6, H-β), 7.80 (1H, d, J = 15.6, H-α), 14.50 (1H, s, O*H*-2′); ^{13}C NMR (acetone-d_6) δ (ppm): 193.3 (*C*O), 166.4 (C-2′), 163.1 (C-4′), 162.0 (C-6′), 159.9 (C-3 and C-5), 142.9 (C-β), 138.5 (C-1), 131.1 (C-3″), 128.6 (C-α), 123.9 (C-2″), 109.0 (C-3′), 107.6 (C-2 and C-6), 106.3 (C-1′), 105.6 (C-4), 91.8 (C-5′), 56.2 (O*C*H$_3$), 25.9 (C-5″), 22.1 (C-1″), 17.9 (C-4″).

4.2.8. 2′,3,4,4′,5-Pentahydroxy-6′-methoxy-3′-prenylchalcone (10). Yield 69%; Orange amorphous powder; mp 64–66 °C; UV λ_{max} 385.0 nm; EI-MS (pos. mode) *m*/*z*: 386 $[M]^+$(32), 371 $[M-CH_3]^+$ (4), 343 $[M\text{-}C_3H_7]^+$ (8), 179 (100), 153 (68); EI-HRMS (pos. mode) *m*/*z*: 386.1360 (calcd for $C_{21}H_{22}O_7$ 386.1366); ^{1}H NMR (DMSO-δ_6) δ (ppm): 1.61 (3H, s, H_3-5″), 1.70 (3H, s, H_3-4″), 3.14 (2H, d, J = 7.0, H_2-1″), 3.88 (3H, s, OCH_3), 5.13 (1H, t, J = 7.0, H-2″), 6.08 (1H, s, H-5′), 6.65 (2H, s, H-2 and H-6), 7.49 (1H, d, J = 15.4, H-β), 7.66 (1H, d, J = 15.4, H-α), 14.70 (1H, s, O*H*-2′); ^{13}C NMR (acetone-d_6) δ (ppm): 193.2 (C*O*), 166.4 (C-2′), 162.8 (C-4′), 161.9 (C-6′), 146.8 (C-3 and C-5), 143.9 (C-β), 136.7 (C-4), 130.9 (C-3″), 127.8 (C-1), 125.7 (C-α), 124.0 (C-2″), 108.9 (C-3′), 108.8 (C-2 and C-6), 106.3 (C-1′), 91.7 (C-5′), 56.2 (O*C*H$_3$), 25.9 (C-5″), 22.1 (C-1″), 17.9 (C-4″).

4.2.9. 2′,4′-Dihydroxy-3,4,6′-trimethoxy-3′-prenylchalcone (11). Yield 78%; Orange amorphous powder; mp 154–155 °C; UV λ_{max} 375.1 nm; EI-MS (pos. mode) *m*/*z*: 398 $[M]^+$ (80), 355 $[M\text{-}C_3H_7]^+$ (53), 179 (100); ESI-HRMS (pos. mode) *m*/*z*: 398.1726 (calcd for $C_{23}H_{26}O_6$, 398.1729); ^{1}H NMR (DMSO-d_6) δ (ppm): 1.61 (3H, s, H_3-5″), 1.70 (3H, s, H_3-4″), 3.14 (2H, d, J = 6.6, H_2-1″), 3.81 (3H, s, OCH_3), 3.83 (3H, s, OCH_3), 3.87 (3H, s, OCH_3), 5.14 (1H, t, J = 6.6, H-2″), 6.09 (1H, s, H-5′), 7.03 (1H, d, J = 8.5, H-5), 7.26 (1H, dd, J = 1.6, 8.5, H-6) 7.32 (1H, J = 1.6, H-2), 7.68 (1H, d, J = 15.6, H-β), 7.83 (1H, d, J = 15.6, H-α), 14.57 (1H, s, O*H*-2′); ^{13}C NMR ($CDCl_3$) δ (ppm): 193.2 (*C*O), 166.4 (C-2′), 162.9 (C-4′), 162.0 (C-6′), 152.6 (C-4), 150.6 (C-3), 143.1 (C-β), 131.0 (C-3″), 129.4 (C-1), 126.4 (C-α), 124.0 (C-2″), 123.6 (C-6), 112.6 (C-5), 111.6 (C-2), 108.9 (C-3′), 106.3 (C-1′), 91.7 (C-5′), 56.2 (3× O*C*H$_3$-3,4,6′), 25.9 (C-5″), 22.1 (C-1″), 17.9 (C-4″).

4.2.10. 2′,4,4′-Trihydroxy-6′-methoxy-chalcone (Helichrysetin, 12). Yield 76%; Orange-yellow amorphous powder; mp 253–254 °C; UV λ_{max} 365.0 nm; EI-MS (pos. mode) *m*/*z*: 286 $[M]^+$ (89), 285 $[M-H]^+$ (80), 167 (100); ^{1}H NMR (DMSO-d_6) δ (ppm): 3.87 (3H, s, OCH_3), 5.91 (1H, d, J = 2.2, H-3′), 6.01 (1H, d, J = 2.2, H-5′), 6.84 (2H, d, J = 8.8, H-3 and H-5), 7.57 (2H, J = 8.8, H-2 and H-6), 7.62 (1H, d, J = 15.6, H-β), 7.69 (1H, d, J = 15.6, H-α), 13.96 (1H, s, O*H*-2′); ^{13}C NMR (acetone-d_6) δ (ppm): 193.2 (*C*O), 169.1 (C-



	R_1	R_2	R_3	R_4
2,2′,4′-Trihydroxy-6′-methoxy-3′-prenylchalcone (**6**)	OH	H	H	H
2′,3,4′-Trihydroxy-6′-methoxy-3′-prenylchalcone (**7**)	H	OH	H	H
2′,3,4,4′-Tetrahydroxy-6′-methoxy-3′-prenylchalcone (**8**)	H	OH	OH	H
2′,3,4′,5-Tetrahydroxy-6′-methoxy-3′-prenylchalcone (**9**)	H	OH	H	OH
2′,3,4,4′,5-Pentahydroxy-6′-methoxy-3′-prenylchalcone (**10**)	H	OH	OH	OH
2′,4′-Dihydroxy-3,4,6′-trimethoxy-3′-prenylchalcone (**11**)	H	OCH_3	OCH_3	H

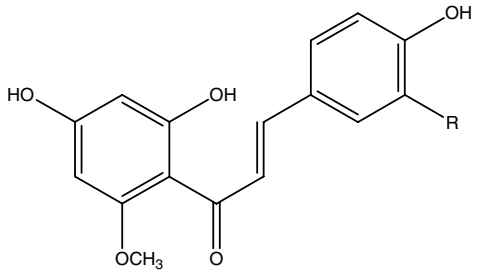
2′), 165.7 (C-4′), 164.3 (C-6′), 160.7 (C-4), 143.4 (C-β), 131.3 (C-2 and C-6), 128.1 (C-1), 125.2 (C-α), 116.8 (C-3 and C-5), 106.4 (C-1′), 97.0 (C-3′), 92.2 (C-5′), 56.4 (O*C*H$_3$).[25]

4.2.11. 2′,3,4,4′-Tetrahydroxy-6′-methoxy-chalcone (3-hydroxyhelichrysetin, 13). Yield 71%; Orange amorphous powder; mp 230 °C; UV λ_{max} 380.0 nm; EI-MS (pos. mode) *m*/*z*: 302 $[M]^+$ (72), 301 $[M-H]^+$ (40), 167 (100); ESI-HRMS *m*/*z*: 302.0783 (calcd for $C_{16}H_{14}O_6$, 302.0790); ^{1}H NMR (DMSO-d_6) δ (ppm): 3.88 (3H, s, O*C*H$_3$), 5.91 (1H, d, J = 2.2, H-3′), 6.01 (1H, d, J = 2.2, H-5′), 6.79 (1H, d, J = 8.8, H-5), 7.02 (1H, dd, J = 8.8, 2.2, H-6), 7.11 (1H, d, J = 2.2, H-2), 7.54 (1H, d, J = 15.6, H-β), 7.62 (1H, d, J = 15.6, H-α), 9.28 (1H, s, O*H*), 9.64 (1H, s, O*H*), 10.61 (1H, s, O*H*), 14.03 (1H, s, O*H*-2′); ^{13}C NMR (acetone-d_6) δ (ppm): 193.1 (CO), 169.1 (C-2′), 165.7 (C-4′), 164.3 (C-6′), 148.9 (C-4), 146.4 (C-3), 143.8 (C-β), 128.7 (C-1), 125.3 (C-α), 123.1 (C-6), 116.5 (C-5), 115.3 (C-2), 106.4 (C-1′), 97.0 (C-3′), 92.2 (C-5′), 56.4 (O*C*H$_3$).

4.3. Preparation of compounds 3 and 5

2,4,6-Trihydroxyacetophenone was stirred with dimethylsulfate (1.1 equiv (**3**) and 2.2 equiv (**5**)), NaOH (1.4 equiv) and tetrabutylammonium iodide (0.1 equiv) in CH_2Cl_2/H_2O 3:2 for 24 h at room temperature. After separation, the aqueous phase was washed with CH_2Cl_2 and all organic phases were combined. After drying over Na_2SO_4 and evaporation, the residue was subjected to CC and yielded pure 2,6-dihydroxy-4-methoxy-acetophenone and 6-hydroxy-2,4-dimethoxy-acetophenone using PE/EA 2:1 as an eluent. Subsequent prenylation, coupling with MOM-protected 4-hydroxybenzaldehyde, and terminal deprotection yielded **3** and **5**, respectively.

4.3.1. 2′,4,6′-Trihydroxy-4′-methoxy-3′-prenylchalcone (xanthogalenol, 3). Yield 54%; Orange-yellow amorphous powder; mp 95–97 °C; UV λ_{max} 365.0 nm; EI-MS (pos. mode) *m*/*z*: 354 $[M]^+$ (60), 339 $[M-CH_3]^+$ (21), 311 $[M\text{-}C_3H_7]^+$ (21), 179 (100); ^{1}H NMR (acetone-d_6) δ (ppm): 1.62 (3H, s, H$_3$-5″), 1.74 (3H, s, H$_3$-4″), 3.24 (2H, d, J = 7.1, H$_2$-1″), 3.84 (3H, s, O*C*H$_3$),



	R
Helichrysetin (**12**)	H
3-Hydroxyhelichrysetin (**13**)	OH

5.18 (1H, t, J = 7.1, H-2″), 6.17 (1H, s, H-5′), 6.92 (2H, d, J = 8.5, H-3 and H-5), 7.58 (2H, J = 8.5, H-2 and H-6), 7.78 (1H, d, J = 15.6, H-β), 8.12 (1H, d, J = 15.6, H-α), 8.91 (1H, s, O*H*), 9.84 (1H, s, O*H*), 14.05 (1H, s, O*H*-2′); ^{13}C NMR (acetone-d_6) δ (ppm): 193.8 (CO), 164.3 (C-4′ and C-2′), 160.8 (C-6′), 160.6 (C-4), 143.4 (C-β), 131.2 (C-2 and C-6), 130.8 (C-3″), 128.1 (C-1), 125.4 (C-α), 124.1 (C-2″), 116.8 (C-3 and C-5), 109.0 (C-3′), 106.1 (C-1′), 91.5 (C-5′), 55.9 (O*C*H$_3$), 25.9 (C-5″), 21.9 (C-1″), 17.8 (C-4″).[12]

4.3.2. 4,6′-Dihydroxy-2′,4′-dimethoxy-3′-prenylchalcone (2′-methylxanthogalenol, 5). Yield 67%; Orange-yellow oil; UV λ_{max} 375.1 nm; EI-MS (pos. mode) *m/z*: 368 $[M]^+$ (91), 353 $[M-CH_3]^+$ (21), 248 (61), 233 (100); ESI-HRMS (pos. mode) *m/z*: 368.1621 (calcd for $C_{22}H_{24}O_5$, 368.1624); ^{1}H NMR (acetone-d_6) δ (ppm): 1.66 (3H, s, H$_3$-5″), 1.78 (3H, s, H$_3$-4″), 3.30 (2H, d, J = 6.9, H$_2$-1″), 3.71 (3H, s, O*C*H$_3$), 3.91 (3H, s, O*C*H$_3$), 5.17 (1H, t, J = 6.9, H-2″), 6.34 (1H, s, H-5′), 6.95 (2H, d, J = 8.7, H-3 and H-5), 7.64 (2H, J = 8.7, H-2 and H-6), 7.84 (1H, d, J = 15.6, H-β), 7.89 (1H, d, J = 15.6, H-α), 8.98 (1H, s, O*H*-4), 13.63 (1H, s, O*H*-6′); ^{13}C NMR (acetone-d_6) δ (ppm): 193.5 (CO), 166.3 (C-2′), 165.3 (C-4′), 161.2 (C-6′), 161.0 (C-4), 144.8 (C-β), 131.5 (C-2 and C-6), 131.5 (C-3″), 127.8 (C-1), 124.2 (C-α), 123.7 (C-2″), 117.0 (C-3 and C-5), 116.4 (C-3′), 109.6 (C-1′), 96.9 (C-5′), 63.5 (O*C*H$_3$-2′), 56.5 (O*C*H$_3$-4′), 25.9 (C-5″), 23.0 (C-1″), 17.9 (C-4″).

4.4. Cell culture and determination of cytotoxicity

HeLa cells (ATCC CCL17) were cultured at 37 °C in a humidified incubator with 5% CO_2. Culture medium was MEM (Biochrom AG) supplemented with 10% FCS, 1 μg/mL amphotericin B, 100 U/mL penicillin, 100 U/mL streptomycin, and 2 mM L-glutamine. The cytotoxicity was evaluated with the colorimetric MTT assay as described by Mosman et al.[19] (modified according Heilmann et al.[20]). Every test was performed in duplicates and all experiments have been repeated three times (n = 8). IC_{50} values were calculated from eight different concentrations and data are reported as means ± SD. Maximal observed (absolute) standard deviation was about 15%. Positive control measurements were performed with helenalin (IC_{50} 0.7 ± 0.1 μM).

4.5. ORAC-Fluorescein assay

The ORAC-Fluorescein assay was performed according to Davalos et al.[23] in 96-well plates with fluorescein (final concentration 300 nM) as fluorescent probe and 75 mM phosphate buffer (pH 7.4) for all dilution steps and as reaction milieu. The antioxidant (chalcones or Trolox, 20 μL) was incubated in different concentrations (chalcones: 0.1–2.0 μM, Trolox: 1–8 μM) together with a fluorescein solution (120 μL) at 37 °C for 15 min. The reaction was started by the addition of 60 μL AAPH (2,2′-azobis (2-methylpropionamide) dihydrochloride, final concentration: 12 mM) yielding a final volume of 200 μL. After the addition of AAPH, the fluorescence was immediately recorded every minute in a Tecan 96-plate reader (λ_{ex} 485 nm, λ_{em} 536 nm, 37 °C) for 80 minutes. Reaction mixtures were prepared in quadruplicates and at least four independent assays were performed for each sample. Each sample was measured at five different concentrations (0.1–1.5 μM). Eight calibration curves using 1–8 μM Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) as antioxidant were also carried out in each assay. Controls were measured without antioxidant as well without AAPH and antioxidant. ORAC values were expressed as Trolox equivalents (means ± SD) by using the standard curve calculated for each assay. Regression coefficient between AUC and antioxidant concentration was calculated for all samples ($r^2 > 0.93$).

Acknowledgments

We are grateful to Mr. D. Lachmann and Mr. S. Pitzl (both Universität Regensburg) for excellent technical assistance. Special thanks are given to Dr. T. Burgemeister for measuring the 1D and 2D NMR spectra and to Mr. J. Kiermaier (both Zentrale Analytik der Universität Regensburg, NWF IV) for recording the MS spectra. Dr. Birgit Kraus (Chair of Pharmaceutical Biology, University of Regensburg) is gratefully acknowledged for allowing us to perform into the ORAC-FL test and stimulating discussions.

References and notes

1. Delmulle, L.; Bellahcene, A.; Dhooge, W.; Cornhaire, F.; Roelens, F.; Huvaere, K.; Heyerick, A.; Castronovo, V.; De Keukeleire, D. *Phytomedicine* **2006**, *13*, 732–734.
2. Pan, L.; Becker, H.; Gerhäuser, C. *Mol. Nutr. Food Res.* **2005**, *49*, 837–843.
3. Miranda, C. L.; Stevens, J. F.; Helmrich, A.; Henderson, M. C.; Rodriguez, R. J.; Yang, Y. H.; Deinzer, M. L.; Barnes, D. W.; Buhler, D. R. *Food Chem. Toxicol.* **1999**, *37*, 271–285.
4. Colgate, E. C.; Miranda, C. L.; Stevens, J. F.; Bray, T. M.; Ho, E. *Cancer Lett.* **2007**, *246*, 201–209.
5. Gerhäuser, C.; Alt, A.; Heiss, E.; Gamal Eldeen, A.; Klimo, K.; Knauft, J.; Neumann, I.; Scherf, H. R.; Frank, N.; Bartsch, H.; Becker, H. *Mol. Cancer Ther.* **2002**, *1*, 959–969.
6. Plazar, J.; Zegura, B.; Lah, T. T.; Filipic, M. *Mut. Res.* **2007**, *632*, 1–8.
7. Dietz, B. M.; Kang, Y. H.; Liu, G.; Eggler, A. L.; Yao, P.; Chadwick, L. R.; Pauli, G. F.; Farnsworth, N. R.; Mesecar, A. D.; van Breemen, R. B.; Bolton, J. L. *Chem. Res. Toxicol.* **2005**, *18*, 1296–1305.
8. Delmulle, L.; Berghe, T. V.; De Keukeleire, D.; van den Abeele, P. *Phytother. Res.* **2007**, epub.
9. Albini, A.; Dell'Eva, R.; Vene, R.; Ferrari, N.; Buhler, DR.; Noonan, D. M.; Fassina, G. *FASEB J.* **2006**, *20*, 527–529.
10. Monteiro, R.; Faria, A.; Azevedo, I.; Calhau, C. *J. Steroid Biochem Mol. Bio.* **2007**, *105*, 124–130.
11. Gerhäuser, C. *Eur. J. Cancer* **2005**, *41*, 1941–1954.
12. Stevens, J. F.; Taylor, A. W.; Nickerson, G. B.; Ivancic, M.; Henning, J.; Haunold, A.; Deinzer, M. L. *Phytochemistry* **2000**, *53*, 759–775.
13. Fröhlich, S.; Schubert, C.; Bienzle, U.; Jenett-Siems, K. *J. Antimicrob. Chemother.* **2005**, *55*, 883–887.

14. Vogel, S.; Heilmann, J. *Planta Med.* **2007**, *73*, 953.
15. Khupse, R. S.; Erhardt, P. W. *J. Nat. Prod.* **2007**, *70*, 1507–1509.
16. Diller, R. A.; Riepl, H. M.; Rose, O.; Frias, C.; Henze, G.; Prokop, A. *Chem. Biodivers.* **2005**, *2*, 1331–1337.
17. Nikolic, D.; Li, Y.; Chadwick, L. R.; Pauli, G. F.; van Breemen, R. B. *J. Mass. Spectrom.* **2005**, *40*, 289–299.
18. Nookandeh, A.; Frank, N.; Steiner, F.; Ellinger, R.; Schneider, B.; Gerhäuser, C.; Becker, H. *Phytochemistry* **2004**, *65*, 561–570.
19. Mosman, T. *J. Immunol. Methods* **1983**, *65*, 55–63.
20. Heilmann, J.; Wasescha, M. R.; Schmidt, T. J. *Bioorg. Med. Chem.* **2001**, *9*, 2189–2194.
21. Stevens, J. F.; Page, J. E. *Phytochemistry* **2004**, *65*, 1317–1330.
22. Crozier, A.; Yokota, T.; Jaganath, I. B.; Marks, S.; Saltmarsh, M.; Clifford, M. N. In *Plant Secondary Metabolites*; Crozier, A., Clifford, M. N., Ashihara, H., Eds.; Blackwell Publishing: Oxford, 2006; pp 208–288.
23. Davalos, A.; Gomez-Cordoves, C.; Bartolome, B. *J. Agric. Food. Chem.* **2004**, *52*, 48–54.
24. Stevens, J. F.; Ivancic, M.; Hsu, V. L.; Deinzer, M. L. *Phytochemistry* **1997**, *44*, 1575–1585.
25. van Puyvelde, L.; de Kimpe, N.; Costa, J.; Munyjabo, V.; Nyirankuliza, S.; Hakizamungo, E.; Schamp, N. *J. Nat. Prod.* **1989**, *52*, 629–633.