

Synthesis, Cytotoxicity, and Antioxidative Activity of Minor Prenylated Chalcones from *Humulus lupulus*

Susanne Vogel and Jörg Heilmann*

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

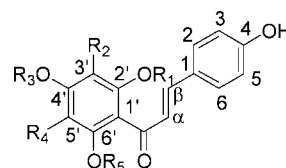
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The minor hop (*Humulus lupulus*) chalcones 3'-geranylchalconaringenin (**3**), 5'-prenylxanthohumol (**4**), flavokawin (**5**), xanthohumol H (**8**), xanthohumol C (**9**), and 1'',2''-dihydroxanthohumol C (**10**) were synthesized. The non-natural chalcones 3'-geranyl-6'-*O*-methylchalconaringenin (**2**), 3'-methylflavokawin (**6**), and 2'-*O*-methyl-3'-prenylchalconaringenin (**7**) were also synthesized. Cytotoxicity was investigated in HeLa cells, and these compounds all had IC₅₀ values comparable to xanthohumol (8.2–19.2 μM). The ORAC-fluorescein assay revealed potent antioxidative activity for **7** and **8** with 5.2 and 4.8 Trolox equivalents, respectively.

Hop (*Humulus lupulus* L., Cannabaceae) cones contain many structurally related prenylated chalcones, with xanthohumol (**1**) being the most abundant one. The minor compounds include xanthogalenol, 4'-*O*-methylxanthohumol, 5'-prenylxanthohumol (**4**), and xanthohumols B, C (**9**), D, E, and H (**8**).^{1,2} Due to the good availability of **1**, which can be isolated from hop cones or synthesized in good yields,^{3,4} its pharmacological characterization advanced significantly, and it has been shown to exhibit an interesting spectrum of pharmacological effects. In addition to antiproliferative activity against different cancer cell lines,^{5–7} **1** also exhibited apoptotic^{6,8} and chemopreventive activity due to protective effects against carcinogens or pro-carcinogens.^{9,10} Several in vitro studies substantiated effects on enzymes and transcription factors involved in the genesis of cancer,^{8,11–15} and in vivo growth inhibition of a vascular tumor has been reported.¹³ Pharmacological data concerning the minor related compounds are scarce due to limited availability via isolation. Recently we described a synthetic route to the minor hop compounds desmethylxanthohumol, xanthogalenol, and 4'-methylxanthohumol,³ and Lee and Xia reported the synthesis of **9**.¹⁶ Manageable synthetic approaches are needed to provide quantities of minor hop chalcones and potential phase I metabolites available for pharmacological testing. Here we report on the synthesis of 3'-geranylchalconaringenin (**3**), 5'-prenylxanthohumol (**4**), xanthohumol H (**8**), xanthohumol C (**9**), 1'',2''-dihydroxanthohumol C (**10**), and the non-prenylated chalcone flavokawin (**5**). The structurally related non-natural compounds 3'-geranyl-6'-*O*-methylchalconaringenin (**2**), 3'-methylflavokawin (**6**), and 2'-*O*-methyl-3'-prenylchalconaringenin (**7**) were synthesized to analyze the relevance of varying substitution for the pharmacological activity of prenylated chalcones. Compounds **8** and **10** have been isolated from hops,² but were also reported as metabolites from rat feces after oral application of **1**.¹⁷ The cytotoxic and antioxidative activity of all compounds was determined against HeLa cells and in the ORAC assay, respectively.

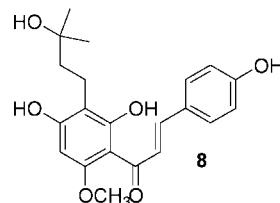
Results and Discussion

The general strategy utilized 6-hydroxy-2,4-dimethoxymethylacetophenone (**11**), prepared from 2,4,6-trihydroxyacetophenone, as the starting compound for all nine chalcones. By changing the sequence of the standard reactions, reflux with prenyl or geranyl bromide in acetone–K₂CO₃, Claisen rearrangement in *N,N*-dimethylaniline, methylation with dimethyl sulfate by using the phase transfer catalyst tetrabutylammonium iodide, MOM protection and deprotection, most of the B ring fragments were synthesized. A

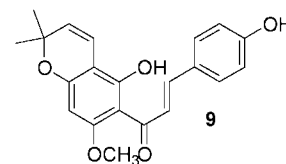


Compound	R ₁	R ₂	R ₃	R ₄	R ₅
1	H	pre	H	H	CH ₃
2	H	ger	H	H	CH ₃
3	H	ger	H	H	H
4	H	pre	H	pre	CH ₃
5	H	H	CH ₃	H	CH ₃
6	H	CH ₃	CH ₃	H	CH ₃
7	CH ₃	pre	H	H	H

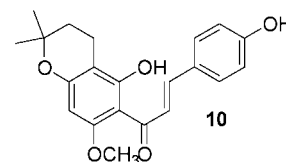
pre = prenyl, ger = geranyl



Xanthohumol H



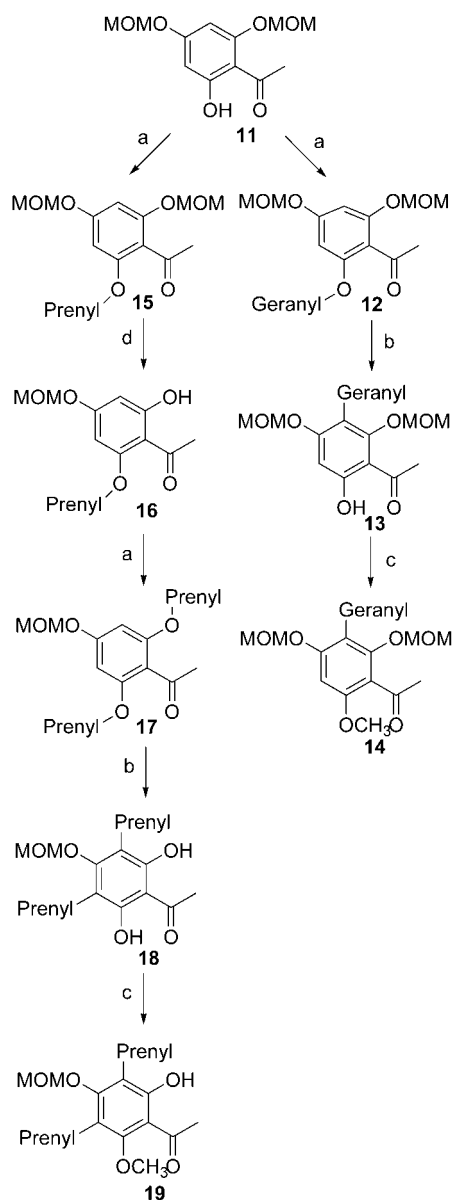
Xanthohumol C



1'',2''-Dihydroxanthohumol C

further concluding reaction was necessary only for xanthohumol H (**8**), xanthohumol C (**9**), and 1'',2''-dihydroxanthohumol C (**10**) to synthesize the ring fragments **25**, **27**, and **28** (Schemes 1 and 2,

* Corresponding author. Tel: +49 941 943 4759. Fax: +49 941 943 4990. E-mail: joerg.heilmann@chemie.uni-regensburg.de.

Scheme 1. Key Steps of the Synthetic Route for Compounds 2–4^a

^a (a) Acetone, K₂CO₃, geranyl or prenyl bromide, 24 h (reflux); (b) *N,N*-dimethylaniline, 3 h (reflux), argon atmosphere, 200 °C; (c) dimethyl sulfate, NaOH, CH₂Cl₂–H₂O, 3:2, tetrabutylammonium iodide (phase transfer catalyst), 24 h (room temperature); (d) acetone, 3 N HCl, 4 h (0 °C).

Experimental Section). For **4** a detour via deprotection of **15** to **16** was necessary to enable the double Claisen rearrangement of the diether **17**. The complete ¹H and ¹³C NMR data of **4** have not been reported, probably due to its isolation in very limited amounts,²² and are given in the Experimental Section. For **8** we report here the hitherto unpublished ¹³C NMR data. All assignments were justified by 2D NMR (¹H, ¹H COSY, ¹H, ¹³C HSQC, ¹H, ¹³C HMBC, and ¹H, ¹H NOESY) experiments.

Cytotoxicity of all synthesized chalcones was tested against a HeLa cell line using the MTT cell proliferation assay and compared to the positive control **1** (Table 1).^{18,19} Activity of all chalcones was similar, with 3'-geranyl-6'-*O*-methylchalconaringenin (**2**) being the most (IC₅₀ 8.2 ± 1.2 μM) and flavokawin (**5**) the least toxic compound (IC₅₀ 19.2 ± 1.2 μM).

To assay the antioxidative activity of the chalcones, the ORAC (oxygen radical absorbance capacity)-fluorescein assay was used, generating peroxy radicals by the application of 2,2'-azobis(2-

methylpropionamide) dihydrochloride (AAPH) as free radical initiator.²⁰ All compounds showed moderate to high activities of 1.7 to 5.2 Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) equivalents in a concentration range between 0.1 and 1.0 μM (Table 1). For the most active compounds, **5**, **7**, and **8** (4.0 ± 0.5, 5.2 ± 0.8, and 4.8 ± 0.1 Trolox equivalents, respectively), this is comparable to other strong antioxidants such as ferulic acid (4.4 ± 0.2 Trolox equivalents, concentration range 0.4–1.3 μM), but lower in comparison to the very potent quercetin (10.5 ± 0.4 Trolox equivalents, concentration range 0.2–0.6 μM) and caffeic acid (6.6 ± 0.2 Trolox equivalents, concentration range 0.2–1.0 μM).²⁰

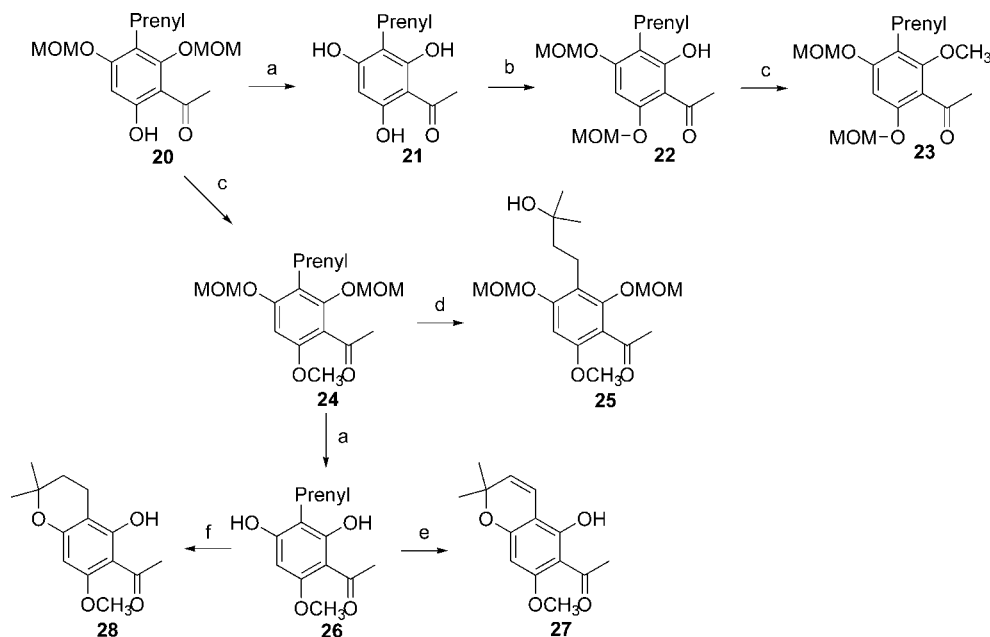
Interestingly, some minor secondary metabolites and phase I metabolites of **1** are more active in comparison to **1** itself. This makes their broad pharmacological characterization as well as the synthesis of further metabolites and structurally related non-natural derivatives worthwhile. Compound **6** was synthesized as a first representative of a set of chalcones with other substituents on 3', and ongoing investigations will focus on the synthesis of non-natural chalcones with increased antioxidant activity. Of special interest is the pharmacological activity of **8**, which is not only a minor secondary metabolite in hop cones but also a phase I metabolite of **1** detected in rat feces after oral application of 1000 mg/kg **1**.¹⁷ Introduction of an OH group increased the activity in comparison to **1**, and since this reaction often occurs in the metabolism of **1** (at different positions of the molecule),^{17,21} synthesis and pharmacological testing of these compounds could be interesting. It is also noteworthy that the presence of only 6'-*O*- and 4'-*O*-methylated prenylated chalcones has been reported in hop cones, whereas 2'-*O*-methyl derivatives generally do not occur. The biological activity of **7**, which we named xanthoflorianol, showed antioxidant activity in the ORAC test significantly higher in comparison to **1**, xanthogalenol, and 4'-*O*-methylxanthohumol.³ Thus, the present investigation revealed a strategy for the synthesis of various prenylated hop chalcones, xanthohumol phase I metabolites, and structurally related non-natural chalcones, and all investigated compounds showed interesting pharmacological activity.

Experimental Section

General Experimental Procedures. 2,4,6-Trihydroxyacetophenone hydrate (98%), MOM-Br (tech. 90%), prenyl (96%) and geranyl bromide (95%), and 2,3-dichloro-5,6-dicyano-1,4-benzochinone (DDQ, 98%) were obtained from Aldrich. Dimethyl sulfate (99%) was purchased from Merck, and *N,N*-dimethylaniline (99%) from Janssen. Melting points were measured on a Büchi melting point B-545 apparatus (uncorrected). UV spectra were recorded on a Cary 50 Scan (Varian; in MeOH, UVA-sol). All ¹H and ¹³C NMR experiments were recorded in acetone-*d*₆ (Deutero GmbH, purity 99.8%) or CDCl₃ (Deutero GmbH, purity 99.8%) on a Bruker Avance 300 (operating at 300.13 MHz for ¹H and 75.47 MHz for ¹³C) at 300.0 K and referenced against residual non-deuterated solvent. The 2D spectra were measured on a Bruker Avance 400 (operating at 400.13 MHz for ¹H and 100.61 MHz for ¹³C) at 300.0 K. HR- and LREIMS (70 eV) were measured on a MAT 710A. 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 F₂₅₄ 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).

Preparation of Compounds: MOM Protection. A mixture of 2,4,6-trihydroxyacetophenone (4.7 g, 1 equiv), anhydrous K₂CO₃ (24.2 g, 7 equiv), and MOM bromide (dropwise addition of 5.0 g, 2.5 equiv) was stirred and refluxed in acetone (160 mL) for 3 h. The reaction mixture was cooled to room temperature and filtered. The filtrate was evaporated and the residue subjected to CC with petroleum ether–EtOAc, 1:1, as eluent to yield intermediate **11** (68%, colorless oil, ¹H and EIMS identical to literature data.⁴ The same procedure was applied for protection of 4-hydroxybenzaldehyde (2.5 g, 1 equiv) using 1.3 equiv of MOM bromide and 4 equiv of anhydrous K₂CO₃ to the protected aldehyde in 90% yield.³

Aldol Coupling. Respective ring fragments (1 equiv) were stirred in cold (0 °C) EtOH–H₂O, 3:2, or EtOH–THF, 1:1 (for **7** and **9**),

Scheme 2. Key Steps of the Synthetic Route for Compounds **7–10**^a

^a (a) 3 N HCl, MeOH, 15 min (reflux); (b) acetone, K₂CO₃, Br-CH₂-O-CH₃, 3 h (reflux); (c) dimethyl sulphate, NaOH, CH₂Cl₂-H₂O, 3:2, tetrabutylammonium iodide (phase transfer catalyst), 24 h (room temperature); (d) Hg(OAc)₂-H₂O, tetrahydrofuran, NaBH₄, 3 N NaOH, 60 min (<30 °C); (e) benzene, dioxane (both dried), DDQ, 3 h (reflux); (f) tetrahydrofuran, formic acid, 3 h (reflux).

Table 1. Cytotoxic (HeLa cells, 150.000 cells/mL, 72 h incubation, IC₅₀ values in μ M \pm SD, $n = 8$) and Antioxidative (Trolox equivalents, concentration range 0.1–1 μ M) Activity of Chalcones **1–10**

compound	IC ₅₀ (μ M)	Trolox equiv
1	9.4 \pm 1.4	2.3 \pm 0.2
2	8.2 \pm 1.2	3.4 \pm 0.2
3	18.2 \pm 1.7	2.3 \pm 0.1
4	9.7 \pm 2.1	1.9 \pm 0.1
5	19.2 \pm 1.2	4.0 \pm 0.5
6	12.2 \pm 1.5	2.1 \pm 0.4
7	10.4 \pm 1.5	5.2 \pm 0.8
8	9.2 \pm 1.5	4.8 \pm 0.1
9	12.5 \pm 1.7	1.8 \pm 0.1
10	15.4 \pm 1.4	1.7 \pm 0.2

under an argon atmosphere together with the MOM-protected 4-hydroxybenzaldehyde (1.1 equiv) and KOH (dropwise addition of 50 equiv) initially for 1 h in an ice bath and afterward for 72 h at room temperature. The reaction mixture was poured into ice–water acidified with 3 N HCl and extracted three times with CH₂Cl₂ or EtOAc. The organic phases were combined, washed with water, dried over Na₂SO₄, and evaporated. The residue was subjected to CC (different petroleum ether–EtOAc mixtures: 1:1, 3:2, 2:1, and 3:1, respectively) and yielded the respective protected chalcones.³

Deprotection. For deprotection, all compounds were dissolved in MeOH, and 3 N HCl was added to give a ratio of 5:1 MeOH–3 N HCl. After 15 min under reflux the reaction mixture was poured into ice–water and extracted three times with EtOAc or CH₂Cl₂. The organic phases were combined, washed with water, and dried over Na₂SO₄. After evaporation, CC of the residue on silica gel using CH₂Cl₂–EtOAc (6:1 and 4:1) or petroleum ether–EtOAc (1:1, 1:2, and 1:3) gave the corresponding chalcones.³

3'-Geranyl-2',4,4'-trihydroxy-6'-methoxychalcone (3'-geranyl-6'-O-methylchalconaringenin) (2): yield 53%; dark yellow, amorphous powder; mp 107–112 °C; UV (MeOH) (log ϵ) λ_{\max} 370 nm (4.51); ¹H NMR (acetone-*d*₆, 300 MHz) δ 1.61 (3H, s, H₃-10''), 1.64 (3H, s, H₃-9''), 1.78 (3H, s, H₃-4''), 1.98 (4H, m, H₂-5'',6''), 3.31 (2H, d, $J = 6.9$, H₂-1''), 3.93 (3H, s, -OCH₃-6'), 5.08 (1H, t, $J = 6.9$, H-7''), 5.27 (1H, t, $J = 7.1$, H-2''), 6.14 (1H, s, H-5'), 6.91 (2H, d, $J = 8.5$, H-3,5'), 7.61 (2H, d, $J = 8.5$, H-2,6), 7.75 (1H, d, $J = 15.6$, H- β), 7.89 (1H, d, $J = 15.6$, H- α), 9.04 (1H, s, OH), 14.70 (1H, s, OH-2'); ¹³C NMR (acetone-*d*₆, 75 MHz) δ 193.3 (CO), 166.5 (C-2'), 162.8 (C-4'), 161.9 (C-6'),

160.6 (C-4), 143.2 (C- β), 134.8 (C-3''), 131.6 (C-8''), 131.3 (C-2,6), 128.2 (C-1), 125.5 (C- α), 125.2 (C-7''), 123.9 (C-2''), 116.8 (C-3,5), 108.9 (C-3'), 106.3 (C-1'), 91.6 (C-5'), 56.2 (OCH₃-6'), 40.6 (C-4''), 27.5 (C-6''), 25.8 (C-10''), 21.8 (C-1''), 17.7 (C-9''), 16.3 (C-4''); EIMS (pos mode) m/z 422 [M]⁺ (78), 407 [M - CH₃]⁺ (6), 379 [M - C₃H₇]⁺ (8), 353 [M - C₅H₉]⁺ (49), 299 (75), 233 (87), 179 (100); HREIMS m/z 422.2093 (calcd for C₂₆H₃₀O₅ 422.2093).

Geranyl bromide (2.54 g, 1.5 equiv), K₂CO₃ (4.31 g, 4 equiv), and **11** (2 g, 1 equiv) were refluxed in 150 mL of acetone for 24 h with stirring to give **12** after cooling to room temperature, filtration, and CC (petroleum ether–EtOAc, 6:1) of the evaporated filtrate (yield 78%). Compound **12** (1.4 g) was refluxed and stirred for 3 h (200 °C) in *N,N*-dimethylaniline (4 mL) under argon atmosphere. After cooling to room temperature, the reaction mixture was acidified with 3 N HCl and extracted three times with EtOAc. The organic phases were combined, washed with a solution of Na₂CO₃, dried over Na₂SO₄, and evaporated. CC of the residue with petroleum ether–EtOAc, 4:1, yielded **13** (23%). A mixture of **13** (220 mg, 1 equiv), dimethyl sulfate (1.1 equiv, dropwise addition), tetrabutylammonium iodide (0.1 equiv), and NaOH (1.4 equiv) was stirred in 10 mL of CH₂Cl₂–H₂O, 3:2, for 24 h at room temperature. Separation of organic and aqueous phases, extraction of the aqueous phase with CH₂Cl₂, and CC of the residue of (all dried) the combined and evaporated CH₂Cl₂ phases with petroleum ether–EtOAc, 2:1, yielded **14** (89%).

3-Geranyl-2,4-dimethoxymethyl-6-methoxyacetophenone (14): yellowish oil; ¹H NMR (CDCl₃, 300 MHz) δ 1.57 (3H, s, H₃-10''), 1.64 (3H, s, H₃-9''), 1.74 (3H, s, H₃-4''), 2.06 (4H, m, H₂-5'',6''), 2.50 (3H, s, COCH₃), 3.31 (2H, d, $J = 6.8$, H₂-1''), 3.47 (3H, s, MOM-CH₃), 3.48 (3H, s, MOM-CH₃), 3.79 (3H, s, OCH₃), 4.89 (2H, s, OCH₂O), 5.05 (1H, t, $J = 6.7$, H-7''), 5.14 (1H, t, $J = 6.7$, H-2''), 5.20 (2H, s, OCH₂O), 6.55 (1H, s, H-5). Aldol coupling of **14** with MOM-protected 4-hydroxybenzaldehyde and deprotection resulted in **2** after CC with petroleum ether–EtOAc, 1:1.

2',4,4',6'-Tetrahydroxy-3'-geranylchalcone (3'-geranylchalconaringenin) (3): yield 25%; yellow-orange, amorphous powder; mp 74–79 °C; UV (MeOH) λ_{\max} 365 nm; ¹H and ¹³C NMR identical with literature data;²² EIMS (pos mode) m/z 408 [M]⁺ (40), 365 [M - C₃H₇]⁺ (2), 323 (100), 285 (81), 203 (53), 165 (60); HREIMS m/z 408.1931 (calcd for C₂₅H₂₈O₅ 408.1937). Aldol coupling of **13** with MOM-protected 4-hydroxybenzaldehyde and deprotection yielded **3** after CC with petroleum ether–EtOAc, 1:2.

3-Geranyl-6-hydroxy-2,4-dimethoxymethylacetophenone (13): yellowish oil; ¹H NMR (CDCl₃, 300 MHz) δ 1.57 (3H, s, H₃-10''), 1.65

(3H, s, H₃-9''), 1.75 (3H, s, H₃-4''), 2.08 (4H, m, H₂-5'', 6''), 2.70 (3H, s, COCH₃), 3.31 (2H, d, *J* = 6.6, H₂-1''), 3.45 (3H, s, MOM-CH₃), 3.50 (3H, s, MOM-CH₃), 4.95 (2H, s, OCH₂O), 5.06 (1H, t, *J* = 6.7, H-7''), 5.15 (1H, t, *J* = 6.3, H-2''), 5.20 (2H, s, OCH₂O), 6.47 (1H, s, H-5), 12.95 (1H, s, OH).

2',4',4'-Trihydroxy-6'-methoxy-3',5'-diprenylchalcone (5'-prenyl-xanthohumol) (4): yield 61%; yellow-orange, amorphous powder; mp 62–68 °C; UV (MeOH) λ_{\max} 370 nm; ¹H (acetone-*d*₆, 300 MHz) δ 1.65 (3H, s, prenyl-CH₃), 1.68 (3H, s, prenyl-CH₃), 1.77 (3H, s, prenyl-CH₃), 1.79 (3H, s, prenyl-CH₃), 3.38 (4H, m, H₂-1'', 1'''), 3.69 (3H, s, OCH₃-6'), 5.19 (2H, m, H-2'', 2'''), 6.95 (2H, d, *J* = 8.5, H-3,5), 7.63 (2H, d, *J* = 8.5, H-2,6), 7.83 (1H, d, *J* = 15.4, H- β), 7.90 (1H, d, *J* = 15.4, H- α), 8.03 (1H, s, OH), 8.97 (1H, s, OH), 13.89 (1H, s, OH-2''); ¹³C NMR (acetone-*d*₆, 75 MHz) δ 193.8 (CO), 163.2 (C-2',4'), 160.9 (C-4), 159.9 (C-6'), 144.4 (C- β), 132.3 and 131.4 (C-3'',3'''), 131.4 (C-2,6), 127.9 (C-1), 124.4 (C- α), 124.1 and 123.3 (C-2'',2'''), 116.9 (C-3,5), 115.0 (C-5'), 112.4 (C-3'), 109.4 (C-1'), 63.5 (OCH₃-6'), 25.9 (C-5'',5'''), 23.3 and 23.2 (C-1'',C-1'''), 18.1 (C-4'',4'''); EIMS (pos mode) *m/z* 422 [M]⁺ (100), 407 [M - CH₃]⁺ (39), 379 [M - C₃H₇]⁺ (22), 351 (35), 287 (45), 247 (49), 231 (77); HREIMS *m/z* 422.2091 (calcd for C₂₆H₃₀O₅ 422.2093).

Prenylbromide (2.83 g, 1.5 equiv), K₂CO₃ (6.99 g, 4 equiv), and **11** (3.24 g, 1 equiv) were refluxed in 150 mL of acetone for 24 h with stirring to give **15** (colorless oil; ¹H NMR and EIMS identical to literature data⁴) after cooling to room temperature, filtration, and CC (petroleum ether–EtOAc, 2:1) of the evaporated filtrate (yield 91%). Then, 10 drops of 3 N HCl were successively added to a cooled (0 °C) solution of **15** (1.16 g, 1 equiv) in 20 mL of acetone (pH = 2) and stirred for 4 h in an ice bath. After evaporation the residue was distributed between EtOAc and H₂O, and the aqueous layer was extracted three times with EtOAc. The organic phases were combined, washed with water, dried over Na₂SO₄, and evaporated to give known **16** after CC (petroleum ether–EtOAc, 4:1) in 68% yield. Prenylbromide (1.07 g, 1.5 equiv) was added to a refluxing mixture of K₂CO₃ (2.64 g, 4 equiv) and **16** (1.34 g, 1 equiv) in 50 mL of acetone. Stirring for 24 h, cooling to room temperature, filtration, and CC (petroleum ether–EtOAc, 4:1) of the evaporated filtrate yielded **17** (79%). Compound **17** (1.29 g) was refluxed in *N,N*-dimethylaniline (4 mL) under argon to yield **18** (40%) after CC with petroleum ether–EtOAc, 6:1. Compound **18** was methylated according to intermediate **13**, resulting in **19** (CC with petroleum ether–EtOAc, 5:1) in 71% yield.

2-Hydroxy-4-methoxymethyl-6-methoxy-3,5-diprenylacetophenone (19): yellowish oil; ¹H NMR (CDCl₃, 300 MHz) δ 1.69 (6H, s, 2 \times prenyl-CH₃), 1.78 (6H, s, 2 \times prenyl-CH₃), 2.71 (3H, s, COCH₃), 3.34 (4H, d, *J* = 6.3, 2 \times prenyl-CH₂), 3.58 (3H, s, MOM-CH₃), 3.70 (3H, s, OCH₃), 4.98 (2H, s, OCH₂O), 5.20 (2H, t, *J* = 6.4, 2 \times CH=). Aldol coupling of **19** with MOM-protected 4-hydroxybenzaldehyde and deprotection yielded pure **4** after CC with petroleum ether–EtOAc, 1:1.

2',4-Dihydroxy-4',6'-dimethoxychalcone (flavokawin) (5): yield 70%; yellow-orange, amorphous powder; mp 179–182 °C; UV (MeOH) λ_{\max} 365 nm; ¹H and ¹³C NMR identical with literature data;^{1,23} EIMS (pos mode) *m/z* 300 [M]⁺ (100), 207 (42), 181 (64); HREIMS *m/z* 300.0998 (calcd for C₁₇H₁₆O₅ 300.0998). A mixture of 2,4,6-trihydroxyacetophenone (2.12 g, 1 equiv), tetrabutylammonium iodide (841 mg, 0.2 equiv), NaOH (1.14 g, 2.5 equiv), and dimethyl sulfate (3.16 g, 2.2 equiv) was stirred in 15 mL of CH₂Cl₂–H₂O, 3:2, for 24 h at room temperature. Separation of phases, extraction of the aqueous phase with CH₂Cl₂, and CC of the residue with petroleum ether–EtOAc, 2:1, yielded 2-hydroxy-4,6-dimethoxyacetophenone and 2-hydroxy-4,6-dimethoxy-3-methylacetophenone (30% each). Coupling of 2-hydroxy-4,6-dimethoxyacetophenone with MOM-protected 4-hydroxybenzaldehyde yielded the corresponding protected chalcone (83%) after CC with petroleum ether–EtOAc, 2:1. Deprotection resulted in pure **5** after CC with the same eluent mixture.

2',4-Dihydroxy-4',6'-dimethoxy-3'-methylchalcone (6): yield 73%; yellow-orange, amorphous powder; mp 173–177 °C; UV (MeOH) λ_{\max} (log ϵ) 365 nm (4.48); ¹H NMR (acetone-*d*₆, 300 MHz) δ 1.97 (3H, s, H₃-3'), 3.96 (3H, s, OCH₃-4'), 4.05 (3H, s, OCH₃-6'), 6.31 (1H, s, H-5'), 6.92 (2H, d, *J* = 8.8, H-3,5), 7.62 (2H, *J* = 8.8, H-2,6), 7.76 (1H, d, *J* = 15.6, H- β), 7.90 (1H, d, *J* = 15.6, H- α), 8.99 (1H, s, OH-4), δ 14.33 (1H, s, OH); ¹³C NMR (acetone-*d*₆, 75 MHz) δ 193.7 (CO), 165.0 (C-2'), 164.6 (C-4'), 162.3 (C-6'), 160.7 (C-4), 143.4 (C- β), 131.3 (C-2,6), 128.0 (C-1), 125.4 (C- α), 116.8 (C-3,5), 106.7 (C-1'), 105.8 (C-

3'), 87.8 (C-5'), 56.4 (OCH₃-6'), 56.1 (OCH₃-4'), 7.5 (CH₃); EIMS (pos mode) *m/z* 314 [M]⁺ (100), 221 (21), 194 (41); HREIMS *m/z* 314.1152 (calcd for C₁₈H₁₈O₅ 314.1154).

Coupling of 2-hydroxy-3-methyl-4,6-dimethoxyacetophenone with MOM-protected 4-hydroxybenzaldehyde yielded the MOM-protected chalcone after CC with petroleum ether–EtOAc, 2:1 (59% yield). Deprotection resulted in pure **6** after CC with petroleum ether–EtOAc, 1:1.

4,4',6'-Trihydroxy-2'-methoxy-3'-prenylchalcone (2'-O-methyl-3'-prenylchalconaringenin, xanthofloranol) (7): yield 41%; yellow-orange, amorphous powder; mp 57–60 °C; UV (MeOH) λ_{\max} (log ϵ) 370 nm (4.50); ¹H NMR (acetone-*d*₆, 300 MHz) δ 1.67 (3H, s, H₃-5''), 1.79 (3H, s, H₃-4''), 3.33 (2H, d, *J* = 6.9, H₂-1''), 3.72 (3H, s, OCH₃-2'), 5.24 (1H, t, *J* = 6.9, H-2''), 6.25 (1H, s, H-5'), 6.95 (2H, d, *J* = 8.5, H-3,5), 7.64 (2H, *J* = 8.5, H-2,6), 7.82 (1H, d, *J* = 15.6, H- β), 7.89 (1H, d, *J* = 15.6, H- α), 8.99 (1H, s, OH), 9.47 (1H, s, OH), 13.50 (1H, s, OH-6); ¹³C NMR (acetone-*d*₆ 75 MHz) δ 193.3 (CO), 165.6 (C-6'), 163.8 (C-4'), 162.3 (C-2'), 160.8 (C-4), 144.4 (C- β), 131.3 (C-2,6), 131.3 (C-3''), 127.9 (C-1), 124.3 (C- α), 123.9 (C-2''), 116.9 (C-3,5), 115.5 (C-3'), 109.4 (C-1'), 100.2 (C-5'), 63.4 (OCH₃-2'), 25.8 (C-5''), 23.0 (C-1'), 18.0 (C-4''); EIMS (pos mode) *m/z* 354 [M]⁺ (100), 339 [M - CH₃]⁺ (20), 235 (31), 234 (38), 219 (60), 179 (100); HREIMS *m/z* 354.1463 (calcd for C₂₁H₂₂O₅ 354.1467). 6-Hydroxy-2,4-dimethoxymethyl-3-prenylacetophenone (**20**) was synthesized according Vogel et al.³ **20** (450 mg; colorless oil, ¹H and EIMS identical to literature data⁴) was dissolved in 45 mL of MeOH and deprotected to yield **21** (40%; yellow solid, ¹H and EIMS identical to literature data²⁴). MOM protection of **21** gave, probably due to the strong hydrogen bond for the OH at position 2 (δ_{H} 14.0), the 2-hydroxy-4,6-dimethoxymethyl-3-prenylacetophenone **22** (45% yield), which was methylated according to **13** to give 2-methoxy-4,6-dimethoxymethyl-3-prenylacetophenone (**23**, 50% yield).

2-Methoxy-4,6-dimethoxymethyl-3-prenylacetophenone (23): yellowish oil; ¹H NMR (CDCl₃, 300 MHz) δ 1.66 (3H, s, prenyl-CH₃), 1.76 (3H, s, prenyl-CH₃), 2.51 (3H, s, COCH₃), 3.29 (2H, d, *J* = 5.8, H₂-1'), 3.46 (3H, s, MOM-CH₃), 3.47 (3H, s, MOM-CH₃), 3.71 (3H, s, OCH₃), 5.13 (2H, s, OCH₂O), 5.16 (3H, m, OCH₂O and H-2'), 6.70 (1H, s, H-5). Aldol coupling and deprotection yielded **7** after CC with petroleum ether–EtOAc, 1:1.

Xanthohumol H (8): yield 65%; yellow-orange, amorphous powder; mp 212–214 °C; UV (MeOH) λ_{\max} 370 nm; ¹H identical with literature data;² ¹³C NMR (acetone-*d*₆, 75 MHz) δ 193.3 (CO), 166.4 (C-2'), 163.2 (C-4'), 161.9 (C-6'), 160.6 (C-4), 143.1 (C- β), 131.3 (C-2,6), 128.2 (C-1), 125.5 (C- α), 116.8 (C-3,5), 110.1 (C-3'), 106.2 (C-1'), 92.0 (C-5'), 70.8 (C-3''), 56.1 (OCH₃-6'), 43.2 (C-2''), 29.7 (C-1'',5''), 17.9 (C-4''); EIMS (pos mode) *m/z* 372 [M]⁺ (49), 354 [M - H₂O]⁺ (13), 339 [M - H₂O - CH₃]⁺ (12), 179 (100); HREIMS *m/z* 372.1569 (calcd for C₂₁H₂₄O₆ 372.1573).

Intermediate **20** was methylated according to the procedure for **13** to give **24** in 89% yield (450 mg; yellowish oil, ¹H and EIMS identical to literature data⁴). THF (10 mL) and intermediate **24** (590 mg, 1 equiv) were added to a solution of Hg(OAc)₂ (2.22 g, 4 equiv) in 5 mL of H₂O and stirred (reaction temperature <30 °C, stirring until the dropwise addition of 3 N NaOH did not result in precipitation of Hg). Subsequently, 5 mL of 3 N NaOH and a solution of 99 mg NaBH₄ in 5 mL of 3 N NaOH were carefully added and stirred for a further 60 min. After separation of the precipitated Hg and saturation with NaCl, the reaction mixture was extracted three times with diethyl ether. The organic phases were combined, dried over Na₂SO₄, and evaporated. CC of the residue with EtOAc as eluent yielded **25** (70% yield).

3-(3-Hydroxy-3-methylbutyl)-6-methoxy-2,4-dimethoxymethylacetophenone (25): colorless oil; ¹H NMR (CDCl₃, 300 MHz) δ 1.25 (6H, s, 2 \times CH₃), 1.66 (2H, dt, *J* = 6.9, 8.4, H₂-2'), 1.77 (1H, s, OH), 2.47 (3H, s, COCH₃), 2.68 (2H, dt, *J* = 6.9, 8.4, H₂-1'), 3.48 (3H, s, MOM-CH₃), 3.50 (3H, s, MOM-CH₃), 3.78 (3H, s, OCH₃), 4.91 (2H, s, OCH₂O), 5.20 (2H, s, OCH₂O), 6.53 (1H, s, H-5). **25** was coupled with protected 4-hydroxybenzaldehyde and deprotected to give pure **8** (CC with petroleum ether–EtOAc, 1:2).

Xanthohumol C (9): yield 87%; yellow-orange, amorphous powder; mp 90–98 °C; UV (MeOH) λ_{\max} 370 nm; ¹H and ¹³C NMR identical with literature data;²² EIMS (pos mode) *m/z* 352 [M]⁺ (34), 337 [M - CH₃]⁺ (37), 217 (100); HREIMS *m/z* 352.1312 (calcd for C₂₁H₂₀O₅ 352.1311). Intermediate **24** was deprotected to yield 2,4-dihydroxy-6-methoxy-3-prenylacetophenone (**26**, 69% white powder, identical to

literature data²⁵). DDQ (354 mg, 1 equiv) and **26** (390 mg, 1 equiv) were dissolved in dried benzene and 10 drops of dried dioxane. The reaction mixture was heated under reflux for 3 h, cooled to room temperature, and filtered. Evaporation and CC of the residue with petroleum ether–EtOAc, 6:1, resulted in **27** (90% yield; yellow solid, identical to literature data²⁵). Intermediate **27** was coupled with protected 4-hydroxybenzaldehyde and deprotected to yield pure **9** after CC with petroleum ether–EtOAc, 1:1.

1'',2''-Dihydroxanthohumol C (2'',2''-dimethyl-3'',4''-dihydro-(2H)-pyrano[2'',3'':3',4']-2',4'-dihydroxy-6'-methoxychalcone¹⁷ (10): yield 96%; yellow-orange, amorphous powder; mp 104–114 °C; UV (MeOH) λ_{max} 370 nm; ¹H and ¹³C NMR identical with literature data;^{2,17} EIMS (pos mode) m/z 354 [M]⁺ (100), 261 (25), 234 (32), 179 (74); HREIMS m/z 354.1465 (calcd for C₂₁H₂₂O₅ 354.1467). Intermediate **26** (100 mg, 1 equiv) was dissolved in 2 mL of THF and 3 mL of formic acid and stirred for 3 h under reflux. The reaction mixture was poured into ice–water and extracted three times with ethyl acetate. The organic phases were combined, washed with water, dried over Na₂SO₄, and evaporated to give **28** after CC (petroleum ether–EtOAc, 6:1) in 60% yield (white solid, identical to literature data²⁵). **28** was coupled with protected 4-hydroxybenzaldehyde (yield 73%) and deprotected to yield pure **10** after CC with petroleum ether–EtOAc, 1:1.

Cell Culture and Determination of Cytotoxicity. HeLa cells (ATCC CCL17) were cultured at 37 °C in a humidified incubator with 5% CO₂. Culture medium was MEM (Biochrom AG) supplemented with 10% FCS and 2 mM L-glutamine. Cytotoxicity was evaluated with the colorimetric MTT assay as described by Mosman et al.¹⁸ (modified according Heilmann et al.¹⁹). Tests were performed in duplicate and all experiments repeated three times ($n = 8$). IC₅₀ values were calculated from eight different concentrations, and data are reported as mean \pm SD. Maximal observed (absolute) standard deviation was about 15%. Positive control measurements were performed with xanthohumol.

ORAC-Fluorescein Assay. The ORAC-fluorescein assay was performed according to Davalos et al.²⁰ and Vogel et al.³ 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 μ L) was incubated in different concentrations (chalcones, 0.1–1.0 μ M; Trolox, 1–8 μ M) together with a fluorescein solution (120 μ L) at 37 °C for 15 min. The reaction was started by addition of 60 μ L of AAPH (2,2'-azobis(2-methylpropionamide) dihydrochloride; final concentration, 12 mM), yielding a final volume of 200 μ L. After addition of AAPH, the fluorescence was recorded every minute in a Tecan 96-plate reader (λ_{ex} 485 nm, λ_{em} 536 nm, 37 °C) for 80 min. Reaction mixtures were prepared in quadruplicate, and at least four independent assays were performed for each sample. Samples were measured at five different concentrations (0.1–1.0 μ M). Eight calibration curves using 1–8 μ M Trolox as antioxidant were also carried out in each assay. Controls were measured without antioxidant as well as without AAPH and antioxidant. ORAC values were expressed as Trolox equivalents (mean \pm SD) by using the standard curve calculated for each assay. The regression coefficient between AUC and antioxidant concentration was calculated for all samples ($r^2 > 0.93$). Further positive control measurements were performed with xanthohumol.

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