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

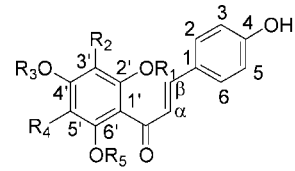
Susanne Vogel and Jörg Heilmann*

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

Received March 25, 2008

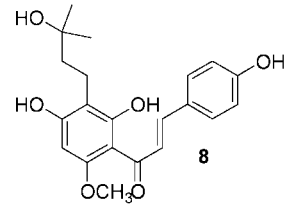
The minor hop (*Humulus lupulus*) chalcones 3′-geranylchalconaringenin (**3**), 5′-prenylxanthohumol (**4**), flavokawin (**5**), xanthohumol H (**8**), xanthohumol C (**9**), and 1″,2″-dihydroxanthohumol C (**10**) were synthesized. The non-natural chalcones 3′-geranyl-6′-*O*-methylchalconaringenin (**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 IC_{50} 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.

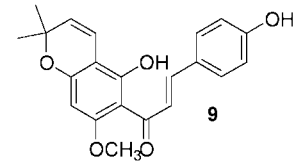


Compound	R_1	R_2	R_3	R_4	R_5
1	H	pre	H	H	CH_3
2	H	ger	H	H	CH_3
3	H	ger	H	H	H
4	H	pre	H	pre	CH_3
5	H	H	CH_3	H	CH_3
6	H	CH_3	CH_3	H	CH_3
7	CH_3	pre	H	H	H

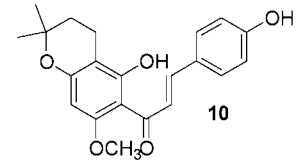
pre = prenyl, ger = geranyl



Xanthohumol H



Xanthohumol C



1′′,2′′-Dihydroxanthohumol C

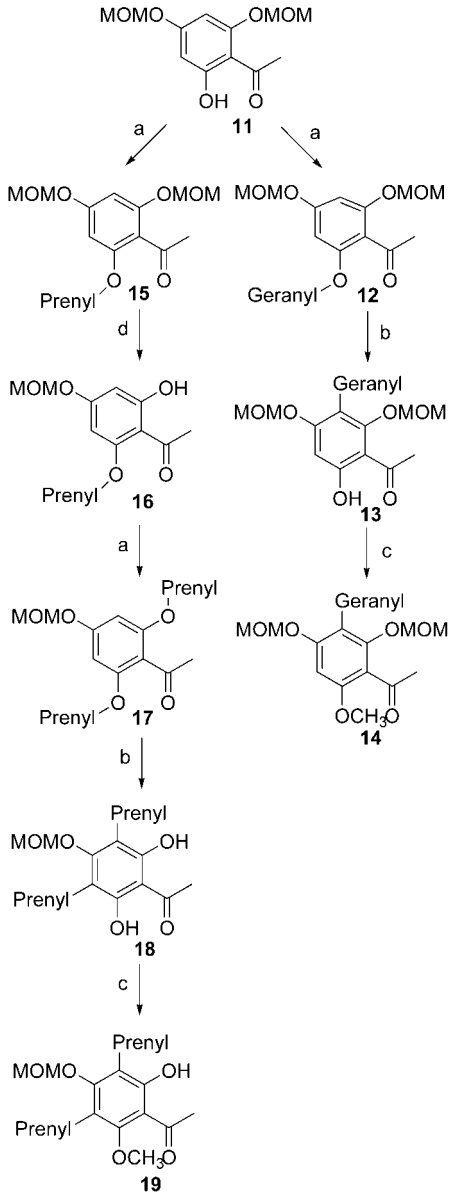
Results and Discussion

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−K_2CO_3, 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 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.

10.1021/np800188b CCC: $40.75
Published on Web 07/09/2008

Scheme 1. Key Steps of the Synthetic Route for Compounds **2**−**4**[a]



[a] (a) Acetone, K_2CO_3, geranyl or prenyl bromide, 24 h (reflux); (b) *N*,*N*-dimethylaniline, 3 h (reflux), argon atmosphere, 200 °C; (c) dimethyl sulfate, NaOH, CH_2Cl_2−H_2O, 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 ^{1}H and ^{13}C 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 ^{13}C NMR data. All assignments were justified by 2D NMR (^{1}H,^{1}H COSY, ^{1}H,^{13}C HSQC, ^{1}H,^{13}C HMBC, and ^{1}H,^{1}H 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 (IC_{50} 8.2 ± 1.2 μM) and flavokawin (**5**) the least toxic compound (IC_{50} 19.2 ± 1.2 μM).

To assay the antioxidative activity of the 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 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.

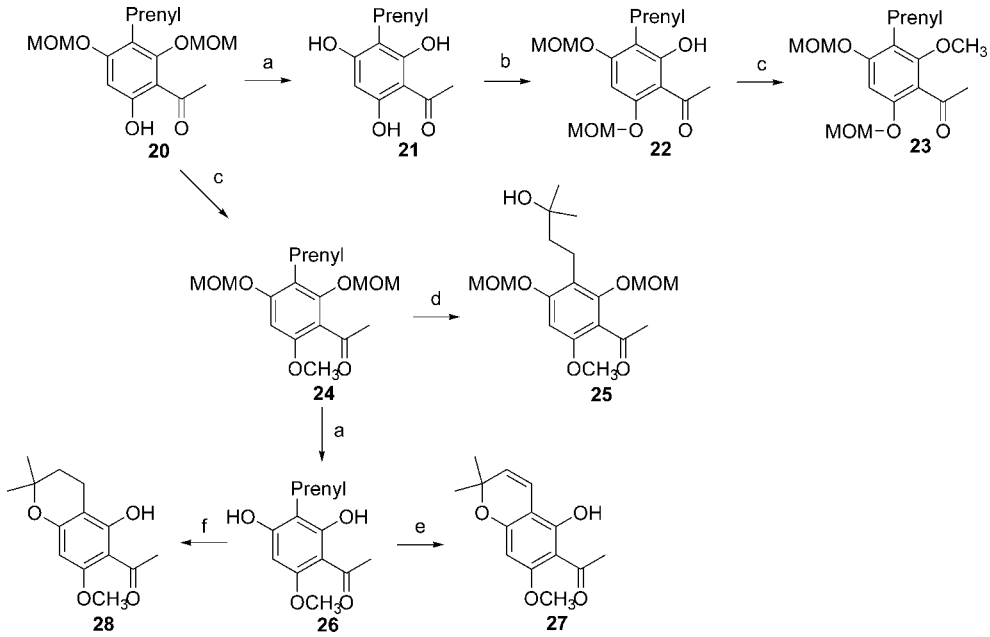
Experimental Section

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-dicyan-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 ^{1}H and ^{13}C NMR experiments were recorded in acetone-d_6 (Deutero GmbH, purity 99.8%) or $CDCl_3$ (Deutero GmbH, purity 99.8%) on a Bruker Avance 300 (operating at 300.13 MHz for ^{1}H and 75.47 MHz for ^{13}C) 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 ^{1}H and 100.61 MHz for ^{13}C) 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 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).

Preparation of Compounds: MOM Protection. A mixture of 2,4,6-trihydroxyacetophenone (4.7 g, 1 equiv), anhydrous K_2CO_3 (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, ^{1}H 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 K_2CO_3 to the protected aldehyde in 90% yield.[3]

Aldol Coupling. Respective ring fragments (1 equiv) were stirred in cold (0 °C) EtOH−H_2O, 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, K_2CO_3, Br−CH_2−O−CH_3, 3 h (reflux); (c) dimethyl sulphate, NaOH, CH_2Cl_2−H_2O, 3:2, tetrabutylammonium iodide (phase transfer catalyst), 24 h (room temperature); (d) $Hg(OAc)_2$−H_2O, tetrahydrofuran, $NaBH_4$, 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, IC_{50} values in μM ± SD, $n = 8$) and Antioxidative (Trolox equivalents, concentration range 0.1−1 μM) Activity of Chalcones **1**−**10**

compound	IC_{50} (μM)	Trolox equiv
1	9.4 ± 1.4	2.3 ± 0.2
2	8.2 ± 1.2	3.4 ± 0.2
3	18.2 ± 1.7	2.3 ± 0.1
4	9.7 ± 2.1	1.9 ± 0.1
5	19.2 ± 1.2	4.0 ± 0.5
6	12.2 ± 1.5	2.1 ± 0.4
7	10.4 ± 1.5	5.2 ± 0.8
8	9.2 ± 1.5	4.8 ± 0.1
9	12.5 ± 1.7	1.8 ± 0.1
10	15.4 ± 1.4	1.7 ± 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 CH_2Cl_2 or EtOAc. The organic phases were combined, washed with water, dried over Na_2SO_4, 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 CH_2Cl_2. The organic phases were combined, washed with water, and dried over Na_2SO_4. After evaporation, CC of the residue on silica gel using CH_2Cl_2−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); ^{1}H NMR (acetone-d_6, 300 MHz) δ 1.61 (3H, s, H_3-10″), 1.64 (3H, s, H_3-9″), 1.78 (3H, s, H_3-4″), 1.98 (4H, m, H_2-5″,6″), 3.31 (2H, d, $J = 6.9$, H_2-1″), 3.93 (3H, s, −OCH_3-6′), 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, O*H*), 14.70 (1H, s, O*H*-2′); ^{13}C NMR (acetone-d_6, 75 MHz) δ 193.3 (*C*O), 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 (O*C*H_3-6′), 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 $[M - CH_3]^+$ (6), 379 $[M - C_3H_7]^+$ (8), 353 $[M - C_5H_9]^+$ (49), 299 (75), 233 (87), 179 (100); HREIMS *m*/*z* 422.2093 (calcd for $C_{26}H_{30}O_5$ 422.2093).

Geranylbromide (2.54 g, 1.5 equiv), K_2CO_3 (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 Na_2CO_3, dried over Na_2SO_4, 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 CH_2Cl_2−H_2O, 3:2, for 24 h at room temperature. Separation of organic and aqueous phases, extraction of the aqueous phase with CH_2Cl_2, and CC of the residue of (all dried) the combined and evaporated CH_2Cl_2 phases with petroleum ether−EtOAc, 2:1, yielded **14** (89%).

3-Geranyl-2,4-dimethoxymethyl-6-methoxyacetophenone (14): yellowish oil; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.57 (3H, s, H_3-10″), 1.64 (3H, s, H_3-9″), 1.74 (3H, s, H_3-4″), 2.06 (4H, m, H_2-5″,6″), 2.50 (3H, s, COCH_3), 3.31 (2H, d, $J = 6.8$, H_2-1″), 3.47 (3H, s, MOM-CH_3), 3.48 (3H, s, MOM-CH_3), 3.79 (3H, s, OCH_3), 4.89 (2H, s, OCH_2O), 5.05 (1H, t, $J = 6.7$, H-7″), 5.14 (1H, t, $J = 6.7$, H-2″), 5.20 (2H, s, OCH_2O), 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; ^{1}H and ^{13}C NMR identical with literature data;[22] EIMS (pos mode) *m*/*z* 408 $[M]^+$ (40), 365 $[M - C_3H_7]^+$ (2), 323 (100), 285 (81), 203 (53), 165 (60); HREIMS *m*/*z* 408.1931 (calcd for $C_{25}H_{28}O_5$ 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; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.57 (3H, s, H_3-10″), 1.65

(3H, s, H_3-9″), 1.75 (3H, s, H_3-4″), 2.08 (4H, m, H_2-5″,6″), 2.70 (3H, s, COCH_3), 3.31 (2H, d, J = 6.6, H_2-1″), 3.45 (3H, s, MOM-CH_3), 3.50 (3H, s, MOM-CH_3), 4.95 (2H, s, OCH_2O), 5.06 (1H, t, J = 6.7, H-7″), 5.15 (1H, t, J = 6.3, H-2″), 5.20 (2H, s, OCH_2O), 6.47 (1H, s, H-5), 12.95 (1H, s, O*H*).

2′,4,4′-Trihydroxy-6′-methoxy-3′,5′-diprenylchalcone (5′-prenylxanthohumol) (4): yield 61%; yellow-orange, amorphous powder; mp 62−68 °C; UV (MeOH) λ_{max} 370 nm; ^{1}H (acetone-d_6, 300 MHz) δ 1.65 (3H, s, prenyl-CH_3), 1.68 (3H, s, prenyl-CH_3), 1.77 (3H, s, prenyl-CH_3), 1.79 (3H, s, prenyl-CH_3), 3.38 (4H, m, H_2-1″,1‴), 3.69 (3H, s, OCH_3-6′), 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, O*H*), 8.97 (1H, s, O*H*), 13.89 (1H, s, O*H*-2′); ^{13}C NMR (acetone-d_6, 75 MHz) δ 193.8 (*C*O), 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 (OCH_3-6′), 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 − CH_3]$^+$ (39), 379 [M − C_3H_7]$^+$ (22), 351 (35), 287 (45), 247 (49), 231 (77); HREIMS m/z 422.2091 (calcd for $C_{26}H_{30}O_5$ 422.2093).

Prenylbromide (2.83 g, 1.5 equiv), K_2CO_3 (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; ^{1}H NMR and EIMS identical to literature data[4]) 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 H_2O, and the aqueous layer was extracted three times with EtOAc. The organic phases were combined, washed with water, dried over Na_2SO_4, 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 K_2CO_3 (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; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.69 (6H, s, 2 × prenyl-CH_3), 1.78 (6H, s, 2 x prenyl-CH_3), 2.71 (3H, s, COCH_3), 3.34 (4H, d, J = 6.3, 2 × prenyl-CH_2), 3.58 (3H, s, MOM-CH_3), 3.70 (3H, s, OCH_3), 4.98 (2H, s, OCH_2O), 5.20 (2H, t, J = 6.4, 2 × C*H*═). 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; ^{1}H and ^{13}C 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 $C_{17}H_{16}O_5$ 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 CH_2Cl_2−H_2O, 3:2, for 24 h at room temperature. Separation of phases, extraction of the aqueous phase with CH_2Cl_2, 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); ^{1}H NMR (acetone-d_6, 300 MHz) δ 1.97 (3H, s, H_3-3′), 3.96 (3H, s, OCH_3-4′), 4.05 (3H, s, OCH_3-6′), 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, O*H*-4), δ 14.33 (1H, s, O*H*); ^{13}C NMR (acetone-d_6, 75 MHz) δ 193.7 (*C*O), 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 (OCH_3-6′), 56.1 (OCH_3-4′), 7.5 (CH_3); EIMS (pos mode) m/z 314 [M]$^+$ (100), 221 (21), 194 (41); HREIMS m/z 314.1152 (calcd for $C_{18}H_{18}O_5$ 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, xanthoflorianol) (7): yield 41%; yellow-orange, amorphous powder; mp 57−60 °C; UV (MeOH) λ_{max} (log ε) 370 nm (4.50); ^{1}H NMR (acetone-d_6, 300 MHz) δ 1.67 (3H, s, H_3-5″), 1.79 (3H, s, H_3-4″), 3.33 (2H, d, J = 6.9, H_2-1″), 3.72 (3H, s, OCH_3-2′), 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, O*H*), 9.47 (1H, s, O*H*), 13.50 (1H, s, O*H*-6); ^{13}C NMR (acetone-d_6 75 MHz) δ 193.3 (*C*O), 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 (OCH_3-2′), 25.8 (C-5″), 23.0 (C-1″), 18.0 (C-4″); EIMS (pos mode) m/z 354 [M]$^+$ (100), 339 [M − CH_3]$^+$ (20), 235 (31), 234 (38), 219 (60), 179 (100); HREIMS m/z 354.1463 (calcd for $C_{21}H_{22}O_5$ 354.1467). 6-Hydroxy-2,4-dimethoxymethyl-3-prenylacetophenone (**20**) was synthesized according Vogel et al.[3] **20** (450 mg; colorless oil, ^{1}H and EIMS identical to literature data[4]) was dissolved in 45 mL of MeOH and deprotected to yield **21** (40%; yellow solid, ^{1}H and EIMS identical to literature data[24]). 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; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.66 (3H, s, prenyl-CH_3), 1.76 (3H, s, prenyl-CH_3), 2.51 (3H, s, COCH_3), 3.29 (2H, d, J = 5.8, H_2-1′), 3.46 (3H, s, MOM-CH_3), 3.47 (3H, s, MOM-CH_3), 3.71 (3H, s, OCH_3), 5.13 (2H, s, OCH_2O), 5.16 (3H, m, OCH_2O 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; ^{1}H identical with literature data;[2] ^{13}C NMR (acetone-d_6, 75 MHz) δ 193.3 (*C*O), 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 (OCH_3-6′), 43.2 (C-2″), 29.7 (C-1″,5″), 17.9 (C-4″); EIMS (pos mode) m/z 372 [M]$^+$ (49), 354 [M − H_2O]$^+$ (13), 339 [M − H_2O − CH_3]$^+$ (12), 179 (100); HREIMS m/z 372.1569 (calcd for $C_{21}H_{24}O_6$ 372.1573).

Intermediate **20** was methylated according to the procedure for **13** to give **24** in 89% yield (450 mg; yellowish oil, ^{1}H and EIMS identical to literature data[4]). 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 H_2O 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 $NaBH_4$ 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 Na_2SO_4, 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; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.25 (6H, s, 2 × CH_3), 1.66 (2H, dt, J = 6.9, 8.4, H_2-2′), 1.77 (1H, s, O*H*), 2.47 (3H, s, COCH_3), 2.68 (2H, dt, J = 6.9, 8.4, H_2-1′), 3.48 (3H, s, MOM-CH_3), 3.50 (3H, s, MOM-CH_3), 3.78 (3H, s, OCH_3), 4.91 (2H, s, OCH_2O), 5.20 (2H, s, OCH_2O), 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; ^{1}H and ^{13}C NMR identical with literature data;[22] EIMS (pos mode) m/z 352 [M]$^+$ (34), 337 [M − CH_3]$^+$ (37), 217 (100); HREIMS m/z 352.1312 (calcd for $C_{21}H_{20}O_5$ 352.1311). Intermediate **24** was deprotected to yield 2,4-dihydroxy-6-methoxy-3-prenylacetophenone (**26**, 69% white powder, identical to

literature data[25]). 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 data[25]). 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-(2*H*)-pyrano[2″,3″:3′,4′]-2′,4-dihydroxy-6′-methoxychalcone[17] (10): yield 96%; yellow-orange, amorphous powder; mp 104−114 °C; UV (MeOH) λ_{max} 370 nm; ^{1}H and ^{13}C 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 $C_{21}H_{22}O_5$ 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 Na_2SO_4, and evaporated to give **28** after CC (petroleum ether−EtOAc, 6:1) in 60% yield (white solid, identical to literature data[25]). **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% CO_2. 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 ($n = 8$). IC_{50} values were calculated from eight different concentrations, and data are reported as mean ± 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 μL) was incubated in different concentrations (chalcones, 0.1−1.0 μM; Trolox, 1−8 μM) together with a fluorescein solution (120 μL) at 37 °C for 15 min. The reaction was started by addition of 60 μL of AAPH (2,2′-azobis(2-methylpropionamide) dihydrochloride; final concentration, 12 mM), yielding a final volume of 200 μL. 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 μM). Eight calibration curves using 1−8 μM 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 ± 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.

Acknowledgment. We are grateful to Mrs. G. Brunner and Mr. S. Pitzl (both University of Regensburg, Chair of Pharmaceutical Biology) for technical assistance. Special thanks are given to Dr. T. Burgemeister for measuring the 1D and 2D NMR spectra and to Mr. J. Kiermaier (both University of Regensburg, Department of Analytics, NWF IV) for mass spectra. Prof. Dr. S. Mahboobi (University of Regensburg, Chair of Pharmaceutical Chemistry) and Dr. B. Kraus (University of Regensburg, Chair of Pharmaceutical Biology) are gratefully acknowledged for stimulating discussions.

References and Notes

(1) Stevens, J. F.; Taylor, A. W.; Nickerson, G. B.; Ivancic, M.; Henning, J.; Haunold, A.; Deinzer, M. L. *Phytochemistry* **2000**, *53*, 759–775.
(2) Chadwick, L. R.; Nicolic, D.; Burdette, J. E.; Overk, C. R.; Bolton, J. L.; van Breemen, R. B.; Fröhlich, R.; Fong, H. H. S.; Farnsworth, N. R.; Pauli, G. F. *J. Nat. Prod.* **2004**, *67*, 2024–2032.
(3) Vogel, S.; Ohmayer, S.; Brunner, G.; Heilmann, J. *Bioorg. Med. Chem.* **2008**, *16*, 4286–4293.
(4) Khupse, R. S.; Erhardt, P. W. *J. Nat. Prod.* **2007**, *70*, 1507–1509.
(5) Delmulle, L.; Bellahcene, A.; Dhooge, W.; Cornhaire, F.; Roelens, F.; Huvaere, K.; Heyerick, A.; Castronovo, V.; De Keukeleire, D. *Phytomedicine* **2006**, *13*, 732–734.
(6) Pan, L.; Becker, H.; Gerhäuser, C. *Mol. Nutr. Food Res.* **2005**, *49*, 837–843.
(7) 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.
(8) Colgate, E. C.; Miranda, C. L.; Stevens, J. F.; Bray, T. M.; Ho, E. *Cancer Lett.* **2007**, *246*, 201–209.
(9) 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.
(10) Plazar, J.; Zegura, B.; Lah, T. T.; Filipic, M. *Mutat. Res.* **2007**, *632*, 1–8.
(11) 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.
(12) Delmulle, L.; Berghe, T. V.; De Keukeleire, D.; van den Abeele, P. *Phytother. Res.* **2008**, *22*, 197–203.
(13) Albini, A.; Dell'Eva, R.; Vene, R.; Ferrari, N.; Buhler, D. R.; Noonan, D. M.; Fascina, G. *FASEB J.* **2006**, *20*, 527–529.
(14) Monteiro, R.; Faria, A.; Azevedo, I.; Calhau, C. *J. Steroid Biochem. Mol. Biol.* **2007**, *105*, 124–130.
(15) Gerhäuser, C *Eur. J. Cancer* **2005**, *41*, 1941–1954.
(16) Lee, Y. R; Xia, L. *Synthesis* **2007**, *20*, 3240–3246.
(17) Nookandeh, A.; Frank, N.; Steiner, F.; Ellinger, R.; Schneider, B.; Gerhäuser, C.; Becker, H. *Phytochemistry* **2004**, *65*, 561–570.
(18) Mosman, T. *J. Immunol. Methods* **1983**, *65*, 55–63.
(19) Heilmann, J.; Wasescha, M. R.; Schmidt, T. J. *Bioorg. Med. Chem.* **2001**, *9*, 2189–2194.
(20) Davalos, A.; Gomez-Cordoves, C.; Bartolome, B. *J. Agric. Food. Chem.* **2004**, *52*, 48–54.
(21) Nikolic, D.; Li, Y.; Chadwick, L. R.; Pauli, G. F.; van Breemen, R. B. *J. Mass. Spectrom.* **2005**, *40*, 289–299.
(22) Stevens, J. F.; Ivancic, M.; Hsu, V. L.; Deinzer, M. L. *Phytochemistry* **1997**, *44*, 1575–1585.
(23) Wollenweber, E.; Roitman, J. N. *Z. Naturforsch.* **1991**, *46c*, 325–330.
(24) Boubakir, Z.; Beuerle, T.; Liu, B.; Beerhues, L. *Phytochemistry* **2005**, *66*, 51–57.
(25) Jain, A. C.; Zutshi, M. K. *Tetrahedron* **1972**, *28*, 5589–5593.

NP800188B