Antiproliferative activity and synthesis of 8-prenylnaringenin derivatives by demethylation of 7-O- and 4’-O-substituted isoxanthohumols

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Abstract Several analogues of 7-O- and 4’-O-substituted isoxanthohumol and 8-prenylnaringenin, the strongest known phytoestrogen and potential anticancerogenic agent, were synthesized. Acyl, alkyl, and allyl derivatives of isoxanthohumol underwent the demethylation process using MgI$_2$ $\times$ 2Et$_2$O in anhydrous THF with the yields of 61–89%. Some of the compounds approached the international criteria of antiproliferative activity (4 µg/ml) for synthetic agents against the human cancer cell lines.

Keywords Hop flavonoids • Phytoestrogens • Antitumor agents • Antiproliferative activity • MCF-7 • HT-29 • CCRF/CEM

Introduction

Hops (Humulus lupulus L.) are used in the brewing industry to add flavor and bitterness to beer. They consist of many prenylated chalcones and flavonones (Stevens and Page, 2004). Among them, xanthohumol (1) has received much attention in recent years as an anti-cancer (Colgate et al., 2007; Drenzek et al., 2011; Okano et al., 2011), antioxidant (Delmulle et al., 2006; Jacob et al., 2011), and anti-HIV (Cos et al., 2008) agent. It is readily accessible from carbon dioxide-extracted-hops (spent hop) where its content ranges up to 1% of dry matter. Spent hop is an important by-product of the process of hop extraction in the beer brewing industry, which is usually used as a fertilizer or as an animal feed in the U.S. However, in order to increase the added value of spent hops, hop processing industries have been looking for an alternative utilization of spent hops (Faltermeyer et al., 2006; Oosterveld et al., 2002). Other flavonoids, isoxanthohumol (2) and 8-prenylnaringenin (3) are also present in hops, but in ten to one hundred times lower concentrations than the content of 1 (Stevens et al., 2000). Compound (3) is the potential drug in menopausal hormone therapy and the strongest phytoestrogen known in the nature (Borrelli and Ernst, 2010; Böttner, 2008; Chadwick et al., 2006; Hyun et al., 2008; Overk et al., 2008). The compounds (1–3) have also anti-breast cancer activity (Brunelli et al., 2007; Monteiro et al., 2007; Wesolowska et al., 2010a, b). Prenylflavonoid (3) can be synthesized in high yield from xanthohumol (1). It requires the cyclization of 1 to isoxanthohumol (2) in basic conditions and demethylation of 2–3 with MgI$_2$ $\times$ 2Et$_2$O (Aniol et al., 2008).

Wilhelm and Wessjohann, (2006) studied demethylation of 2–3 with AlBr$_3$, BBr$_3$ or MeAlCl$_2$ in collidine; ZnBr$_2$, CuI, ZnBr$_2$/CuI Yb$_2$(SO$_4$)$_3$/KI or CuI, Sm(OTf)$_3$/KI, CeCl$_3$/LiI. Product (3) was not detected or obtained with low yield. Hydroxyl groups of 2 were also protected with chlorotriisopropylsilane, demethylated with AlBr$_3$ and deprotected with (n-Bu)$_4$NF to obtain 8-prenylnaringenin (3) with 73% yield. The best result was obtained for Sc(OTf)$_3$/KI (92%).

Magnesium iodide etherate was previously applied in the regioselective demethylation of 5-acetyl-4,6-dimethoxy-2-isopropenyl-2,3-dihydrobenzofuran (Yamaguchi et al., 1987) and substituted 2,6-dimethoxybenzaldehydes (Yamaguchi et al., 1999).

Only a few studies can be found in the literature that reported 8-prenylnaringenin and isoxanthohumol derivative synthesis. Methylation of 8-prenylnaringenin (3) with...
Me$_2$SO$_4$ resulted in the formation of di-O-methyl derivatives of 1 and 2 (Jain et al., 1978). The synthesis of 7,4'-di-O-acetyl-8-prenylnaringenin was carried out using 7,4'-di-O-acetyl naringenin as a substrate via its 4-O-prenyl ether, which underwent the Claisen–Cope rearrangement (Gester et al., 2001). The preparation of chiral 7,4'-dimethyl- or diacetyl- isoxanthohumols and 8-prenyl naringenins was achieved by reducing a carbonyl group to a hydroxyl group with a mixture of formic acid and a base in the presence of a chiral catalyst. Separation of the non- transferred enantiomers (2S) or (2R) of the reduced 8-prenyl naringenin diacetyl derivative and splitting the acyl residues in enantiomers by enzyme catalyst solvolysis gave (2S)-8-prenyl naringenin or (2R)-8-prenyl naringenin. The second enantiomers (2R) or (2S) of 8-prenyl naringenin diacetyl derivative was recovered by oxygenation of a hydroxyl group (Metz and Schwab, 2007). Starting from 3, several carboxylic acid haptenes of this compound were also synthesized. Five linkers \(-\text{CH}_2\text{COOH}, n = 1, 3, 5, 6, \text{ and } 9\) were coupled to the C7-OH or C4'-OH group of 8-prenyl naringenin to obtain five derivatives (Schaef er et al., 2005).

In this article, we report methods of synthesis of 7-O- and 4'-O-substituted alkyl, alkenyl and acyl isoxanthohumol derivatives and their demethylation using magnesium iodide etherate. This research is connected with utilization of the spent hop, obtained after extraction with supercritical carbon dioxide. This waste product of the hop industry is rich in xanthohumol, the starting compound in the synthesis of all the compounds described in this article.

### Materials and methods

#### Chemistry

#### General

All the reactions were carried out under a dry nitrogen atmosphere. The organic solvents were dried and purified according to the standard procedures. The reagents were purchased from Fluka. Isoxanthohumol (2) was obtained from xanthohumol (1) by dissolving in 1% NaOH and acidification of the reaction mixture as it was described previously (Aniol et al., 2008). Analytical thin-layer chromatography was carried out on DC-Alufolien Kieselgel 60 F$_{254}$ silica gel (0.2 mm; Merck) with chloroform: methanol (96:4) as the developing solvent. Visualization was effected with a solution of 10 g Ce (SO$_4$)$_2$ and 20 g phosphomolybdic acid in 1 l of 10% H$_2$SO$_4$, followed by heating. Preparative column chromatography was accomplished using silica gel (Kiesel 60, 230–400 mesh; Merck) columns. Proton NMR spectra were recorded on a Bruker AMX 300 instrument at 300 MHz with acetone-$d_6$ as the solvent and TMS as an internal standard. The infrared (IR) spectra in KBr were recorded on a Mattson IR 300 spectrometer.

#### Synthesis of isoxanthohumol derivatives

**7,4’-Di-O-methylisoxanthohumol (4) and 7-O-methylisoxanthohumol (5)** A mixture of isoxanthohumol (100 mg, 0.282 mmol), anhydrous K$_2$CO$_3$ (232 mg, 1.68 mmol), and methyl iodide (0.5 ml) in 5 ml of anhydrous acetone was stirred for 12 h at room temperature. Acetone was evaporated and the resultant reaction mixture was treated with 10 ml of a saturated NaCl solution and extracted with Et$_2$O (3 x 10 ml). The organic phase was dried over anhydrous Na$_2$SO$_4$, concentrated and was subjected to column chromatography (CHCl$_3$:MeOH, 99:1) to provide 74.9 mg (69.4%) of light yellow solid (mp = 37–39°C, $R_f$ = 0.60, CHCl$_3$:MeOH, 98:2) of 7,4’-di-O-methylisoxanthohumol (4) and 9.1 mg (8.8%) of white solid (mp = 181–184°C, $R_f$ = 0.21, CHCl$_3$:MeOH, 98:2) of 7-O-methylisoxanthohumol (5). $^1$H NMR and IR spectroscopic data were in agreement with those reported in the literature (Metz and Schwab, 2007; Stevens et al., 2000).

**7-O-n-pentylisoxanthohumol (6) and 7,4’-di-O-n-pentyl-8-isoxanthohumol (7)** The reaction was carried out exactly in the same way as it is described for compounds (4 and 5) but 1 ml of n-pentyl iodide was used instead of methyl iodide. The product (33.5 mg, 27.6%) 7-O-n-pentylisoxanthohumol (6) was obtained as a pale yellow solid (mp = 140–142°C, $R_f$ = 0.61, CHCl$_3$:MeOH, 97:3). The $^1$H NMR (300 MHz, acetone-$d_6$) for compound (6): $\delta$ (ppm): 0.93 (t, 3H, $J$ = 7.1 Hz, C-7-O(CH$_2$)$_2$(CH$_3$)), 1.33–1.54 (m, 4H, C-7-O(CH$_2$)$_2$CH$_2$CH$_2$CH$_3$)), 1.61 (d, 6H, $J$ = 1.3 Hz, CH$_3$-4") and CH$_3$-5")$^5$; 1.78–1.87 (m, 2H, 2H, C7-OCH$_2$(CH$_2$)$_2$(CH$_3$)); 2.63 (dd, 1H, $J$ = 16.4 Hz, C-4 = 181–184°C, $R_f$ = 0.21, CHCl$_3$:MeOH, 98:2) of 7-O-methylisoxanthohumol (5). $^1$H NMR and IR spectroscopic data were in agreement with those reported in the literature (Metz and Schwab, 2007; Stevens et al., 2000).
7,4′-Di-O-allylisoxanthohumol (8) The reaction was carried out similarly as it is described for compounds (4 and 5) but 1 ml of allyl bromide and 6 ml of anhydrous THF were used instead methyl iodide and acetone. The product was purified by column chromatography (CHCl3:MeOH, 99:3.0:7.0) to give 100.2 mg of 7, 4′-di-O-allylisoxanthohumol (8) as a light yellow solid (mp = 79–83°C, Rf = 0.85, CHCl3:MeOH, 95:5) with 81.2% yield. 1H NMR (300 MHz, acetone-d6) δ (ppm): 1.61 (d, 6H, J = 1.4 Hz, CH3-4′ and CH3-5′); 2.66 (dd, 1H, J = 16.3 Hz, J = 1.3 Hz, CH-3); 2.95 (dd, 1H, J = 16.3 Hz, J = 12.5 Hz, CH-3); 3.28 (2H, J = 7.2 Hz, CH2-1′); 3.84 (3H, C-5–OCH3); 4.61 and 4.73 (2H, J = 5.2 Hz, J = 1.7 Hz, CH2-6′); 5.18 (t sept, 1H, J = 1.7 Hz, J = 1.4 Hz, CH-2′); 5.25 and 5.29 (two dq, 2H, J = 10.4 Hz, J = 1.5 Hz and J = 10.4 Hz, J = 1.5 Hz, trans-C-7′ and trans-C-4′-OCH2CH=CH2); 5.42 (dd, 1H, J = 12.5 Hz, J = 3.1 Hz, CH-2′); 5.41 and 5.47 (two dq, 2H, J = 8.8 Hz, J = 1.7 Hz, J = 8.8 Hz, 1.7 Hz, cis-C-7′- and cis-C-4′-OCH2CH=CH2); 6.09 and 6.11 (two ddt, 2H, J = 10.4 Hz, J = 8.8 Hz, J = 5.2 Hz and J = 10.4 Hz, J = 8.8 Hz, 5.2 Hz, 5.2 Hz, C-7′- and 1C-4′-OCH2CH=CH2); 6.36 (s, 1H, CH-6); 7.01 (dd, 2H, J = 8.7 Hz, CH-3′ and CH-5′); 7.48 (2H, J = 8.7 Hz, CH-2′ and CH-6′). IR (KBr) cm−1: 3080, 2985, 2962, 2915, 2852, 1678, 1604, 1574, 1515, 1272, 1116, 1018, 932, 821, C27H32O8 (434.54): calcd. C 74.63, H 6.96; found C 74.70, H 6.85; J = 6.3 Hz, CH2-1′); 3.80 (3H, C-5–OCH3); 5.09 (t sept, 1H, J = 7.1 Hz, J = 1.4 Hz, CH-2′); 5.59 (dd, 1H, J = 12.9 Hz, J = 2.9 Hz, CH-2); 6.49 (s, 1H, CH-6); 7.21 (d, 1H, J = 8.6 Hz, CH-3′ and CH-5′); 7.62 (d, 2H, J = 8.5 Hz, CH-2′ and CH-6′). IR (KBr) cm−1: 2964, 2927, 1759, 1687, 1593, 1510, 1477, 1369, 1213, 1170, 1093, 837. C25H26O7 (438.48): calcd. C 68.48, H 5.98; found C 68.58, H 6.10.

7,4′-Di-O-palmitoylisoxanthohumol (10) To a solution of 100 mg (0.282 mmol) of isoxanthohumol and 0.28 ml (2.1 mmol) of Et3N in 5.7 ml of anhydrous THF was added dropwise palmitoyl chloride (155 mg, 0.594 mmol). After 12 h of stirring at room temperature the reaction medium was shaken with 30 ml of cold water (~0°C), extracted with diethyl ether (3 × 10 ml), dried over anhydrous Na2SO4, and concentrated. The resulting residue was purified by column chromatography (hexane:Et2O:MeOH, 5:1:5) to give 191.2 mg (81.6% yield) of 7,4′-di-O-palmitoylisoxanthohumol (10) as white crystals (mp = 71–73°C, Rf = 0.86, CHCl3:MeOH, 95:5). 1H NMR (300 MHz, acetone-d6) δ (ppm): 0.87 (t, 6H, J = 6.9 Hz, C-7′- and C-4′-OOC(CH2)3(CH3)2); 1.28 (s, 44H, C-7′- and C-4′-OOC(CH2)3(CH3)2); 1.40 (m, 4H, J = 6.9 Hz, C-7′- and C-4′-OOC(CH2)3(CH2)3(CH3)2); 1.59 (d, 6H, J = 1.2 Hz, CH3-4′ and CH3-5′); 1.73 (kwintet, 4H, J = 7.3 Hz, C-7′- and C-4′-OOCCH2CH2(CH2)3(CH3)2); 2.60 and 2.64 (two t, 4H, J = 7.3 Hz, C-7′- and C-4′-OOCCH2CH2(CH2)3(CH3)2); 2.78 (dd, 1H, J = 16.3 Hz, J = 3.0 Hz, CH-3′); 3.07 (dd, 1H, J = 16.3 Hz, J = 12.9 Hz, CH-3); 3.19 (d, 2H, J = 6.7 Hz, CH2-1′); 3.80 (s, 3H, C-5–OCH3); 5.08 (t sept, 1H, J = 6.7 Hz, J = 1.2 Hz, CH-2′); 5.60 (dd, 1H, J = 12.9 Hz, J = 3.0 Hz, CH-2); 6.47 (s, 1H, CH-6); 7.20 (d, 2H, J = 8.5 Hz, CH-3′ and CH-5′); 7.62 (d, 2H, J = 8.5 Hz, CH-2′ and CH-6′). IR (KBr) cm−1: 3184, 2919, 2850, 1759, 1688, 1589, 1510, 1468, 1376, 1265, 1139, 1102, 844, 721. C33H38O7 (831.24): calcd. C 76.58, H 9.94; found C 76.48, H 9.94.

Demethylation of isoxanthohumol derivatives

**General procedure** Each time 50 mg of compounds (4–10) were demethylated.

A solution of I2 (3 eq., 99.5 mg, 0.393 mmol) in anhydrous Et2O (3.5 ml) and Mg (6 eq., 19.1 mg, 0.786 mmol), taken in the round-bottomed flask and protected from light, was stirred at room temperature until the reaction mixture turned colorless (1.5 h). The resulting mixture of magnesium iodide etherate was separated from unreacted Mg and transferred via syringe under N2 into the two-neck flask (50 ml), equipped with condenser, containing 50 mg of substrate [4 (1 eq., 0.131 mmol)-10] in anhydrous THF.
(9 ml). The reaction mixture was stirred and refluxed for 12 h and afterward the solvent was evaporated under reduced pressure. Then, 1 ml of THF and saturated solution of NH₄Cl (10 ml) were added and the whole mixture was extracted with CH₂Cl₂ (3 × 5 ml). The combined extracts were dried over anhydrous Na₂SO₄ and the solvent was removed under reduced pressure to give crude product. After purification by column chromatography on silica gel (see Table 1) the products (11–15) were obtained.

7,4'-di-O-methyl-8-prenylnaringenin (11) Yield 61.3%, mp = 105–107°C, Rₜ = 0.32 (CHCl₃; hexane, 7:3), light-white solid. The ¹H NMR and IR spectroscopic data were in agreement with those reported in the literature (Cano et al., 2006; Siddiqui et al., 2003).

7-O-pentyl-8-prenylnaringenin (12) Yield 84.8%, mp = 132–134°C, Rₜ = 0.67 (CHCl₃; MeOH), 97:3, white crystals. ¹H NMR (300 MHz, acetone-d₆) δ (ppm): 0.93 (t, 3H, J = 7.3 Hz, C-7–O(CH₂)₃CH₃); 1.41 (m, 2H, C-7–O(CH₂)₃CH₂CH₃); 1.49 (m, 2H, C-7–O(CH₂)₂CH₂CH₂CH₃); 1.61 (d, 6H, J = 1.4 Hz, CH₃–4’ and CH₃–5’); 1.82 (m, 2H, C-7–OCH₂CH₂(CH₂)₃CH₃); 2.79 (dd, 1H, J, 1H = 17.0 Hz, J = 3.0 Hz, CH-3); 3.16 (dd, 1H, J = 17.0 Hz, J = 12.6 Hz, CH-3); 3.22 (d, 2H, J = 7.2 Hz, CH₂–1’); 4.08 (t, 2H, J = 6.3 Hz, C-7–OCH₂(CH₂)₂CH₃); 5.15 (t, 1H, J = 7.2 Hz, J = 1.4 Hz, CH-2’); 5.46 (dd, 1H, J = 12.6 Hz, J = 3.0 Hz, CH-2); 6.12 (s, 1H, CH-6); 6.90 (d, 2H, J = 8.5 Hz, CH-3’ and CH-5’); 7.41 (d, 2H, J = 8.5 Hz, CH-2’ and CH-6’); 8.51 (s, 1H, C-4’–OH); 12.24 (s, 1H, C-5–OH).

IR (KBr) cm⁻¹: 3260, 2955, 2926, 2855, 1638, 1616, 1592, 1520, 1467, 1364, 1229, 1094, 832, C₂₅H₂₀O₅ (410.51); calcd.: C 73.15, H 7.37; found C 73.32, H 7.54.

7,4'-Di-O-allyl-8-prenylnaringenin (13) Yield 78.9%, mp = 103–105°C, Rₜ = 0.84 (CHCl₃; MeOH, 99.3:0.7), pale yellow solid. ¹H NMR (300 MHz, acetone-d₆) δ (ppm): 1.60 (d, 6H, J = 1.3 Hz, CH₃–4’ and CH₃–5’); 2.82 (dd, 1H, J = 17.1 Hz, J = 3.1 Hz, CH-3); 3.18 (dd, 1H, J = 17.1 Hz, J = 12.5 Hz, CH-3); 3.24 (d, 2H, H = 7.2 Hz, CH₂–1’); 4.59 and 4.65 (two ddd, 4H, J = 5.1 Hz, J = 17.5 Hz, J = 1.5 Hz, CH-7 and C-4’–OCH₂CH₂CH₂CH₃); 5.16 (t, 1H, J = 7.2 Hz, J = 1.3 Hz, CH-2’); 5.25–5.31 (m, 2H, trans-C-7’ and trans-C-4’–OCH₂CH₂CH₂CH₃); 5.51 (dd, 1H, J = 12.5 Hz, J = 3.1 Hz, CH-2); 5.39–5.48 (m, 2H, cis-C-7’ and cis-C-4’–OCH₂CH₂CH₂CH₃); 6.02–6.16 (m, 2H, C-7’ and C-4’–OCH₂CH₂CH₂; 6.12 (s, 1H, CH-6); 7.02 (d, 2H, J = 8.8 Hz, CH-3’ and CH-5’); 7.50 (d, 2H, J = 8.8 Hz, CH-2’ and CH-6’). IR (KBr) cm⁻¹: 2967, 2911, 2857, 1636, 1587, 1517, 1448, 1378, 1255, 1178, 1118, 1021, 921, 829. C₂₆H₂₆O₅ (420.51); calcd.: C 74.26, H 6.71; found: C 74.09, H 6.88.

7,4'-Di-O-acetyl-8-prenylnaringenin (14) Yield 88.4%, mp = 139–140°C, Rₜ = 0.84 (CHCl₃; MeOH, 98:2), white solid. ¹H NMR and IR spectroscopic data were in agreement with those reported in the literature (Gester et al., 2001; Huempel et al., 2005; Metz and Schwab, 2007; Schaefer et al., 2005).

7,4'-Di-O-palmitoyl-8-prenylnaringenin (15) Yield 74.6%, mp = 67–69°C, Rₜ = 0.91 (hexane:Et₂O:MeOH, 5:5:0.1), white crystals. ¹H NMR (300 MHz, acetone-d₆) δ (ppm): 0.87 (t, 6H, J = 6.9 Hz, C-7– and C-4’–OOC(CH₂)₁₄–CH₃); 1.29 (s, 44H, C-7– and C-4’–OOC(CH₂)₃(CH₂)₂₁–CH₃); 1.40 (m, 4H, J = 6.9 Hz, C-7– and C-4’–OOC(CH₂)₃(CH₂)₁₁–CH₃); 1.60 (d, 6H, J = 1.3 Hz, CH₃–4’ and CH₃–5’); 1.73 (quintet, 4H, J = 6.9 Hz, C-7– and C-4’–OOC(CH₂)₃(CH₂)₁₂–CH₃); 2.60 and 2.64 (two t, 4H, J = 7.4 Hz, C-7– and C-4’–OOC(CH₂)₃(CH₂)₁₃–CH₃); 2.96 (dd, 1H, J = 17.2 Hz, J = 3.0 Hz, CH-3); 3.17 (d, 2H, J = 6.8 Hz, CH₂–1’); 3.32 (dd, 1H, J = 17.2 Hz, J = 13.1 Hz, CH-3); 5.07 (t, 1H, J = 6.8 Hz, J = 1.3 Hz, CH-2’); 5.71 (dd, 1H, J = 13.1 Hz, J = 3.0 Hz, CH-2); 6.30 (s, 1H, CH-6); 7.22 (d, 2H, J = 8.5 Hz, CH-3’ and CH-5’); 7.65 (d, 2H, J = 8.5 Hz, CH-2’ and CH-6’); 11.87 (s, 1H, C-5–OH). IR (KBr) cm⁻¹: 3437, 2918, 2850, 1751, 1648, 1624, 1592, 1512, 1469, 1379, 1264, 1149, 1077, 840, 722. C₅₂H₄₀O₇ (817.21); calcd.: C 76.43, H 9.87; found: C 76.22, H 10.01.

**Table 1** Eluents for column chromatography for the purification of compounds (11–15)

| Compound | 11 | 12 | 13 | 14 | 15 |
|----------|----|----|----|----|----|
| Eluent   | CHCl₃; hexane 70:30 | CHCl₃; MeOH 99.2:0.8 | CHCl₃ 100 | CHCl₃; MeOH 99.5:0.5 | CHCl₃; Et₂O 90:10 |

Antiproliferative activity

The human cell lines of breast cancer (MCF-7), colon adenocarcinoma (HT-29), and leukemia (CCRF/CEM) were obtained from American Type Culture Collection (Rockville, Maryland, USA) and maintained in the Cell Culture Collection at the Institute of Immunology and Experimental Therapy, Wroclaw, Poland. The cells at the density of 10⁵/ml were cultivated in 96-well plates (Sarstedt, Germany) in 100 µl of culture medium at 37°C in humid atmosphere containing 5% CO₂. In the case of MCF-7 cell lines, the culture medium...
consisted of Eagle’s medium (IIET, Wroclaw, Poland) with addition of 10% fetal bovine serum (FBS, Sigma-Aldrich Chemie GmbH, Steinheim, Germany), 100 μg/ml streptomycin (Jelfa, Jelenia Góra, Poland), 100 U/ml penicillin (Jelfa, Jelenia Góra, Poland), 2 mM L-glutamine (Gibco, Warsaw, Poland), 1.0 mM sodium pyruvate, 1% amino acid, and 0.8 mg/l insulin. The cells of HT-29 line were cultured in the RPMI 1640 and Opti-MEM (1:1) (both from Gibco) medium with addition of 5% FBS, 100 μg/ml streptomycin, 100 U/ml penicillin, and 2 mM L-glutamine. CCRF/CEM culture medium consisted RPMI 1640, 10% FBS, 100 μg/ml streptomycin, 10 U/ml penicillin and 2 mM L-glutamine.

The compounds were dissolved in acetone (1–4, 8, and 10) or absolute ethanol (5–7, 9, 11–13) to the concentration of 10 mg/ml, stored at 4°C, and diluted in the culture medium to obtain concentrations from 0.1 to 100 μg/ml. The controls contained acetone or ethanol at the appropriate concentrations. The solutions of the synthesized compounds in 100 μl of culture medium were added after 24 h of incubation. The sulphorhodamine B (SRB, Sigma-Aldrich Chemie GmbH, Steinheim, Germany) assay for MCF-7 and HT-29 cells and the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium (MTT) assay (Sigma–Aldrich, Germany) for CCRF/CEM cells were executed. Assays were performed after 72 h of continuous exposure of the cultivated cells to varying concentrations of test compounds according to the methods described by Skehan et al. (1990) and Marcinkowska et al. (1998), using a Multiskan RC photometer.

**Results and discussion**

**Chemistry**

The main goal of this research was investigation of the demethylation reaction of substituted isoxanthohumols (4–10) to provide 8-prenylnaringenins (11–15). The investigated reactions are shown in Fig. 1 and the results are summarized in Table 2.
Xanthohumol, the substrate in the isoaxanthohumol synthesis, was isolated from carbon dioxide-extracted-hops (Marynka variety), purified and transformed into isoxanthohumol as described previously (Aniol et al., 2008).

As model substrates for demethylation, methyl, n-pentyl, allyl, acetyl, and palmitoyl derivatives of 2 were selected. They had different chain lengths. It was assumed that the reactivity of homologous series of compounds should be similar, as well as reactivity of monosubstituted isoxanthohumol derivatives in comparison to disubstituted. For this reason, alkylating and acylating agents were used in high quantity to obtain disubstituted derivatives of 2 as a goal of synthesis.

Methyl ethers (4 and 5) were synthesized using excess of methyl iodide with 69.4 and 8.8% yield, respectively (Table 2, Entries 1a and 1b). During the course of reaction, it was observed that the formation of 7-O-methyl compound (5), which was methylated to get a dimethyl compound (4). There was a characteristic shift of the signal for C-6 proton of substrate (2) from 6.21 to 6.36 ppm for compound (5) on the NMR spectrum. It was caused by the substitution of C-7-OH group by a methoxy group. The chemical shifts of C-3’, C-5’, and C-2’- protons were exactly the same in both the compounds (δ = 6.89 and 7.38 ppm, respectively). The formation of products of cleavage of C ring leading to xanthohumol derivatives, as reported for methylation of 8-prenylnaringenin with Me2SO4 (Jain et al., 1978). In case of prenylation (Table 2, Entries 2a and 2b), the order of alkylation was the same as that of compounds (4 and 5). The first product, 7-O-pentylisoxanthohumol (6) was formed with 27.6% yield (δ = 6.34 (CH-6), 6.89 (CH-3’, CH-5’) and 7.38 ppm (CH-2’, CH-6’), and 7, 4’-O-dipentylisoxanthohumol (7) with 13.6% yield. The best yield of alkylation was observed during the synthesis of the diallyl compound (8, Table 2, Entry 3). Diacyl compounds (9 and 10) were obtained with 74.1 and 81.6% yield, respectively (Table 2, Entries 4 and 5).

Demethylation reactions were carried out according to published procedure (Aniol et al., 2008). Each time 50 mg of substrate was taken. The rest of the reagents were used proportionally in molar quantities. Demethylation of trimethoxy derivative (4) confirmed that the reaction of methyl-aryl ethers with magnesium iodide etherate occurred mainly at ortho-position in relation to acyl group. The main product of demethylation (11) was obtained with yield of 61.3% (Table 2, Entry 6) but during the reaction course, the formation of complicated mixture of by-products was observed, which was confirmed by TLC and HPLC. This reaction was not as clean as that of demethylation of isoaxanthohumol (Aniol et al., 2008). The 1H NMR spectrum of 11 showed the lack of signal of C-8- OCH3 protons at 3.86 ppm, and the presence of signal at 12.25 ppm for the proton of C-8–OH group involved in a strong intramolecular hydrogen bond. A quite similar effect as above was observed for the rest of the synthesized 8-prenylnaringenin derivatives. All the spectra were recorded within 1–2 h after the sample preparation in...
acetone-\textit{d}_{6}. When the spectrum was accumulated on the next day or later the signals for the hydroxyl protons disappeared because of the hydrogen deuterium exchange. Compound (11) was also isolated from \textit{Azadirachta indica} (Siddiqui et al., 2003) and \textit{Esenbeckia berlandieri} ssp. \textit{Acapulcensis} (Cano et al., 2006). Substrate (4) in the above reaction was present in hops in low quantity (Faltermeier et al., 2006; Oosterveld et al., 2002). For testing whether the demethylation depends on chain length of alkyl group, pentyl derivative of isoxanthohumol (6) was synthesized.

Demethylation of 7-O-pentylisoxanthohumol (6) to product (12) occurred with high yield of 84.8% (Table 2, Entry 7).

Cleavage of allyl ethers of alcohols and phenols was observed using Lewis acids such as the CeCl$_3$–NaI system (Bartoli et al., 2001; Thomas et al., 1999). Compound (8) was synthesized to check whether its demethylation was affected by deallylation. There was a possibility that MgI$_2$, composed with magnesium (typical Lewis acid) and iodine (strong nucleophile) could be similar in action to CeCl$_3$–NaI system. We did not observe the allyl–aryl ether cleavage and the desired product (13) were obtained with good 78.9% yield (Table 2, Entry 7). As in the case of alkyl ethers of isoxanthohumol, for testing whether the yield of demethylation depends on chain length of acyl group, diacetyl and dipalmityl derivatives of isoxanthohumol (9 and 10) were synthesized. These compounds, as representatives of esters, commonly applied as prodrugs, underwent demethylation with magnesium iodide etherate (Table 2, Entries 9 and 10). The products, 8-prenylnaringenins (14 and 15) were obtained with 88.4 and 74.6% yield, respectively. Thus, introduction of acyl, allyl or acyl group into isoxanthohumol moiety did not significantly influence the demethylation reaction and all the synthesized compounds were stable during the course of reactions. Nevertheless, during the optimization of the isoxanthohumol demethylation (Aniol et al., 2008) to 8-prenylnaringenin the instability of reagents was observed, which could be associated with the known low stability of flavonoids.

Investigations conducted by a group of Wilhelm and Wessjohann (2006) showed that demethylation of such compounds as isoxanthohumol was very difficult to carry out. Among the 17 demethanlyzing agents only Sc(OTf)$_3$/KI system worked with high yield. Our previous investigations demonstrated that this system could be replaced with MgI$_2$ × 2Et$_2$O to obtain 8-prenylnaringenin with 93% of yield. Now, we showed that this cheap, non toxic, easy to prepare and use agent could be applied in demethylation of acyl, alkyl, and allyl derivatives of isoxanthohumol.

\textbf{Antiproliferative activity, in vitro}

The synthesized compounds were examined for their antiproliferative activity in vitro against the human cell lines of breast cancer (MCF-7), colon adenocarcinoma (HT-29), and leukemia (CCRF/CEM). The results presented in Table 2 are expressed as the concentration in µg/ml leading to 50% inhibition of tumor cells proliferation (ID$_{50}$-dose) in comparison with the untreated ones. Acetone or ethanol, which was used as solvents, did not show any inhibitory effect on cell proliferation, even in the largest concentrations used. Xanthohumol (1), isoxanthohumol (2), and 8-prenylnaringenin (3), studied previously against selected tumor cell lines (Brunelli et al., 2007, 2009; Monteiro et al., 2007; Zanoli and Zavatti, 2008), were used as reference compounds. The two newly synthesized compounds (8 and 12) exhibited higher antiproliferative activity than the most active xanthohumol (1) against CCRF/CEM (2.7 µg/ml) and MCF-7 (3.9 µg/ml) cell lines and approaching the cytotoxic activity criterion ID$_{50}$ ≤ 4 µg/ml for new anticancer synthetic substances. The conducted investigations showed that, 7,4′-di-O-methyl-, 7,4′-di-O-pentyl-, and 7,4′-di-O-allyl- derivatives of isoxanthohumol (4, 7, 8) were significantly more active than parental isoxanthohumol (2) (9.4–32.6 µg/ml) against all investigated cells (2.7–6.6 µg/ml). On the other hand, diacetyl derivatives (9: 16.9–32.1 µg/ml and 10: ID$_{50}$ > 100 µg/ml) did not show any significant activity. Among the 8-prenylnaringenin derivatives, the most active compound was 7-O-pentyl-8-prenylnaringenin (12). This compound possessed the activity against the cells of MCF-7 (3.9 µg/ml), HT-29 (10.0 µg/ml), and CCRF/CEM (4.8 µg/ml) more than three times higher than 8-prenylnaringenin (3), 19.4, 33.2, 24.2 µg/ml, respectively. The rest of the derivatives of 8-prenylnaringenin (11, 13–15) possessed low activity or were inactive (ID$_{50}$ > 100 µg/ml).

\textbf{Conclusion}

In conclusion, the presented simple methodology of demethylation of isoxanthohumol derivatives via the formation of magnesium iodide etherate, offers an easy transformation route for 8-prenylnaringenin derivatives synthesis using xanthohumol as a starting material, which can be applied to several functional groups. Although the yields obtained (61.3–88.4%) were not as good as in case of demethylation of unsubstituted isoxanthohumol, the method was still easy, cheap and could be carried out in mild conditions. The synthesized compounds showed antiproliferative activity against the human cell lines of breast cancer (MCF-7), colon adenocarcinoma (HT-29), and leukemia (CCRF/CEM). The most active compound possessed activity of 2.7 µg/ml against leukemia cell lines. The developed demethylation protocol could be used in the synthesis of various potentially bioactive 8-prenylnaringenin derivatives and can be of use in the combinatorial
chemistry to prepare libraries of such compounds. It would also help in proper utilization of the spent hops, the waste product of hop industry.

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