Medicinal chemistry

Ngameni et al., Med chem 2013, 3:3
DOI: 10.4172/2161-0444.1000144

Research Article Open Access

Synthesis and Evaluation of Anticancer Activity of *O-allylchalcone* Derivatives

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Abstract

A large number of novel *O*-allylchalcones were synthesized by Claisen Schmidt condensation reaction of *O*-allylvanillin **3** with appropriate substituted acetophenones 4a-h. These model chalcones 5a-h and their precursor *O*-allylvanillin were screened for their *in vitro* cytotoxic activity against four human cancer cell lines. The most potent compound in this series with the IC_{50} values below or around 10 µM were 5f against THP-1 cells (10.42 µM) and 5g against THP-1 (4.76 µM), DU-145 (5.21 µM), HL60 (7.90 µM), Hep-G2 (10.12 µM) and MCF-7 (10.32 µM).

Keywords: Synthesis; *O*-allylchalcones; Anticancer; Structure–activity relationship

Introduction

There is a currently a good deal of interest in the health benefits of phytochemicals, in particular prenylated and allylated flavonoids. Chalcones (1,3-diaryl-2-propen-1-ones) and their derivatives are important intermediates of flavonoid synthetic pathway. Chalcones, one of the major classes of natural products with widespread distribution in fruits, vegetables, spices, tea and soy based foodstuff have also been the subject of great interest for their interesting pharmacological activities [1]. Chemically they can be considered open-chain flavonoids in which the two aromatic rings are joined by a three-carbon α,β-unsaturated carbonyl system. Chalcones have also been reported to possess many useful biological and pharmacological properties, including antibacterial [2,3], antimalarial [4,5], antifungal [6], antiviral [7,8], anti-inflammatory [9,10], and anticancer [11,12] properties. A good safety profile, possibility of oral administration [13] and easy synthesis are the major factors contributing to the increasing interest in exploring the pharmacological activities of chalcones. Chalcones comprise one of the main classes of natural small molecules with very promising anticancer activity, related to their ability to inhibit tubulin polymerization [14]. Most of the anticancer agents, of natural or synthetic origin exhibit enone function in their structure [15,16]. Also, synthesized chalcones holding allylic substitutions were recently reported as potent antimicrobial and antioxidant agents [17,18]. In addition, the substitution of ring B with electron withdrawing groups like methoxy or hydroxy group improve the antiproliferative activity against human colon HT-29 cancer cell line [19].

Prompted by all these observations, we report herein the synthesis of novel *O*-allylchalcones, bearing various substituents with potent activity against Human Hep-G2 hepatocarcinoma, breast carcinoma MCF-7, prostate carcinoma DU-145, and acute monocytic leukemia THP-1 and HL-60 cell lines. The structure–activity relationships are also discussed.

Materials and Methods

Chemistry

IR spectra were determined with a Perkin Elmer FT-IR spectrophotometer. ^1H and ^{13}C NMR spectra were recorded with Bruker WM-300 in the $CDCl_3$ at 300 and 75 MHz, respectively using TMS as the internal standard. All chemical shifts are reported on δ scale. Mass spectra were obtained using a Varian MAT-311A. Thin-layer chromatography (TLC) was carried out using Merck silica gel 60 F-254 plates (layer thickness 0.25 mm) and all solvents were distilled prior to use.

Synthesis

Compounds 5a-h were synthesized by the condensation reaction of compound 3 with different substituted acetophenones 4a-h. The main intermediate 3 was prepared from vanillin 1 and allylbromide 2 in the presence of potassium carbonate in anhydrous acetone.

Biology

Cytotoxicity assay: Cell lines and treatment: The effect of synthesized compounds on cell growth was determined on five human tumor cells including Hep-G2 hepatocarcinoma, breast carcinoma MCF-7, prostate carcinoma DU-145, and acute monocytic leukemia THP-1 and HL-60 cell lines, obtained from National Cancer Institute, USA. THP-1 and HL-60 were maintained in RPMI medium while Hep-G2, MCF-7 and DU-145 were cultured in MEM medium. All media used were supplemented with 10% fetal bovine serum (FBS), 100 IU/mL penicillin. The cell lines were maintained under standard cell culture conditions at 37°C and 5% CO_2 in a humidified environment.

The cytotoxicity of the samples against the five studied human cell lines was determined using Sulphorhodamine B (SRB) assay as previously described [20]. The cells were incubated at 37 °C in an atmosphere of 5% CO_2 and 95% relative humidity in a CO_2 incubator. Doxorubicin was used as positive reference. Suitable controls with

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Received June 17, 2013; **Accepted** July 26, 2013; **Published** July 28, 2013

Citation: Ngameni B, Kuete V, Ambassa P, Justin K, Marlyse ML, et al. (2013) Synthesis and Evaluation of Anticancer Activity of *O-allylchalcone* Derivatives. Med chem 3: 233-237. doi:10.4172/2161-0444.1000144

equivalent concentration of DMSO were also included. The optical density (OD) was recorded using a 96 well plate reader, and growth inhibition was calculated [20]. A preliminary study was first carried out with compounds (Table 1, 100 µM) and doxorubicin (at 50 µM) to detect if samples were able to inhibit the proliferation of more that 50% of the cells. Then samples were serially diluted and tested against other cell lines for IC_{50} determination. IC_{50} is the concentration of sample required to inhibit 50% of the cell proliferation after 72 h incubation and was calculated by plotting the percentage survival versus the concentration, using Microsoft Excel. For all samples, each compound concentration was tested thrice in triplicates.

Experimental

4-Allyloxy-3-methoxybenzaldehyde or *O*-allylvanillin (3)

To 0.304 g (1.99 mmol) of vanillin in acetone (8 mL) was added K_2CO_3 (0.1203 g) followed by allylbromide (0.12 mL, d = 1.43, 0.1772 g, 1.46 mmol). The reaction mixture was heated to reflux for 4 hours or left at room temperature for 48 hours. At the end of the reaction, the solvent was evaporated under reduced pressure and the residue is diluted in water (40 ml x3). The aqueous mixture was extracted with Ethyl Acetate (EA) (3 × 60 mL) and the extract was dried by anhydrous Na_2SO_4. After evaporation of the solvent and purification by column chromatography on silica gel eluting with Hexane-Ethyl Acetate (Hex-EA) system of increasing polarity, product 3 was obtained (725 mg, yield 70% in Hex-EA 87.5:12.5). ^{1}H NMR (600 MHz, CDCl3, Me_4Si) δ 3.79 (3H; s), 4.45 (2H; t; *J*=5.7 Hz), 5.23(1H; dd; *J*=1.5 and 14,4 Hz), 5.34 (1H; dd; *J*=1.5 and 14.4 Hz), 5.95 (1H; dd; *J*=5.4 and 1.5 Hz), 6.84 (1H; d; *J*=8.4 Hz), 7.28 (1H; dd; *J*=7.8 and 1.5 Hz), 7.30 (1H; d; *J*=1.5 Hz), 9.70 (1H; s); ESIMS m/z 193.2 $[M + H]^+$. HREIMS (m/z): 192.0776 $[M^+]$ (calcd for $C_{11}H_{12}O_3$, 192.0786).

4-allyloxy-3-methoxychalcone (5a)

To a solution of acetophenone (0.18 mL, 3.41 mmol, d=1.0266) in methanol (30 mL) was added first *O*-allylvanillin (110 mg, 0.57 mmol) and then an aqueous solution of KOH (50%, 1 mL / mmol of acetophenone) or 3.41 mL. The reaction mixture was refluxed at 70°C for 5 hours or left at room temperature for 15 hours. At the end of the reaction the mixture was diluted with water (30 mL) and extracted with CH_2Cl_2 (3×70 mL) and then the extract was washed with water (50 mL) and saturated with NaCl solution. The organic phase was dried with Na_2SO_4 and the solvent evaporated under reduced pressure. After purification of the residue by Column Chromatography and Thin Layer Chromatography preparative on silica gel (Hex-EA 9:1), compound 5a was obtained pure (103.2 mg, yield 28% in Hex-EA 80:20). IR ($CHCl_3$): υ_{max} cm^{-1}: 1654, 1579, 1257. ^{1}H NMR (300 MHz, $CDCl_3$, Me_4Si): δ 3.70 (3H; s), 4.33 (2H; d ; *J*=6.5 Hz), 5.47 (1H; dd; *J*=17.5 and 10.9 Hz), 5.55 (1H; dd; *J*=17.5 and 0.9 Hz), 6.18 (1H; m), 6.99 (1H; d; *J*= 8.4 Hz), 7.20 (1H; dd ; J=8.4 and 1.5 Hz), 7.27 (1H; d; J=1.5 Hz), 7.48 (1H; d; J=15.9 Hz), 7.50 (1H; m; J=8.1 and 2.1 Hz), 7.52 (1H; dd; J=8.1 and 1.5 Hz), 7.7 (1H; d; J=15.6 Hz), 7.81 (1H; dd; J=8.4 and 1.5 Hz); ^{13}C NMR (75 MHz, $CDCl_3$, Me_4Si) δ 59.9; 69.5; 110.4; 112.8; 118.4; 120.0; 122.9; 127.4; 128.3; 129.7; 132.5; 132.6; 138.4; 144.9; 149.5; 150.5; 190.5; ESIMS m/z 295.3 $[M + H]^+$. HREIMS (m/z): 294.1260 $[M^+]$ (calcd for $C_{19}H_{18}O_3$, 294.1256).

4-allyloxy-2',3-diméthoxychalcone (5b)

To a solution of 2-methoxyacetophenone (27, 84 µl, d=1.090) in ethanol (5 mL) was added first *O*-allylvanillin (38.8 mg, 0.20 mmol) and secondly an aqueous solution KOH (50%, 1 mL/mmol of acetophenone) or 0.20208 mL. The mixture was refluxed at 70°C for 5 hours or left at room temperature for 15 hours. We obtained the product **5b** (42.4 mg, yield 65% in Hex-EA 87.5:12.5) after separation of the residue of the reaction. IR ($CHCl_3$): υ_{max} cm^{-1}: 2368.9, 2333.6, 1653.5, 1594.8, 1250.8, 1019.5; ^{1}H NMR (300 MHz, $CDCl_3$, Me_4Si): δ 3.93 (3H; s), 3.95 (3H; s), 4.70 (2H; d; J=1.2 Hz), 5.36 (1H; dd; J=13.5 and 12.6 Hz), 5.45 (1H; dd; J=13.5 and 1.8 Hz), 6.10 (1H; m), 6.91 (1H; d; J=8,4 Hz), 7.04 (1H; d; J=15.9 Hz) , 7.10 (1H; d; J=8.7 and 1.5 Hz), 7.15 (1H; d; J=1.8 Hz), 7.16 (1H; dd; J=8.7 and 1.8 Hz), 7.23 (1H; m) , 7.49 (1H; dd; J=9.0 and 1.5 Hz), 7.58 (1H; d; J=16.2 Hz), 7.62 (1H; d; J=7.8 and 1.5 Hz); ^{13}C NMR (75 MHz, $CDCl_3$, Me_4Si): δ 55.3; 55.5; 69.3; 110.1; 111.1; 112.4; 117.9; 120.2; 122.2; 124.8 ; 127.8; 129.1; 129.6; 132.0; 132.3; 143.4; 149.0; 149.7; 157.4; 192,8; ESIMS m/z 325 $[M + H]^+$. HREIMS (m/z): 324.1365 $[M^+]$ (calcd for $C_{20}H_{20}O_4$, 324.1362).

4-allyloxy-3-methoxy-2', 4'-diméthylchalcone (5c)

To a solution of 2,4-dimethoxyacetophenone (48.46 µl, 0.33 mmol, d=0.997) in ethanol (7 mL) were added first *O*-allylvanillin (62.6 mg, 0.33 mmol), and secondly an aqueous solution of KOH (50%, 1 mL / mmol) or 0.32604 mL. The mixture was left at room temperature for 23 hours. After separation and purification of the residue of the reaction, the product 5c was obtained (53.5 mg, yield 82% in Hex-EA 85:15). IR ($CHCl_3$): υ_{max} cm^{-1}: 2917.5, 2369.6, 1590.9, 1508.7, 1260.7, 1139.2; 1014.7;^{1}H NMR (300 MHz, $CDCl_3$, Me_4Si): δ 2.55 (3H; s), 2.60 (3H; s), 4.10 (3H; s), 4.81 (2H; d; J=1.5 Hz), 5.48 (1H; dd; J=9.0 and 1.5 Hz), 5.61 (1H; dd; J=15.9 and 1.5 Hz), 6.24 (1H; m), 7.05 (1H; d; J=9.0 Hz), 7.20 (1H; d; J=15.9 Hz), 7.26 (2H; d; J=1.8 Hz), 7.28 (1H; dd; J=8.4 and 1.8 Hz), 7.29 (1H; dd; J=8.1 and 1.8 Hz), 7.58 (1H; d; J=8.1 Hz), 7.59 (1H; d; J=15.9 Hz); ^{13}C NMR (75 MHz, CDCl 3, Me_4Si) δ 19.8; 20.9; 55.4; 69.2; 109.8; 112.3; 117.9; 122.3; 124.4; 125.5; 127.4; 127.9; 131.6; 132.2; 135.9; 136.7; 140.11; 145.0; 149.0; 149.8; 195.7; ESIMS m/z 323 $[M + H]^+$. HREIMS (m/z): 322.1566 $[M^+]$ (calcd for $C_{21}H_{22}O_3$, 322.1569).

4-allyloxy-3',3-diméthoxychalcone (5d)

To a solution of 3-methoxyacetophenone (71.5 µl, 0.52 mmol, d=1.094) in ethanol (7 ml) was added first *O*-allylvanillin (100 mg, 0.52 mmol) and secondly a KOH solution (50%, 1 mL/mmol) or 0.52 mL. The mixture is stirred in a nitrogen atmosphere at room temperature for 21 hours. After separation and purification of the residue of the reaction by CC, the product 5d was obtained (64.6 mg, yield 38% in Hex-EA 85:15). IR spectrum ($CHCl_3$): υ_{max} cm^{-1}: 2917.2, 2361.7, 1658.3, 1576.3, 1508.7, 1260.5, 1140.2, 1029.9 ; ^{1}H NMR (300 MHz, $CDCl_3$, Me_4Si): δ 3.75 (3H; s) , 3.81 (3H; s) , 4.53 (2H; d; J=5.1 Hz), 5.20 (1H; dd; J=15.9 and 1.2 Hz), 5.31 (1H; dd; J=15.9 and 9.6 Hz) , 5.92 (1H; m), 6.75 (1H; d; J=8.4 Hz), 6.98 (1H; d; J=1.5 Hz), 7.06 (1H; d; J=1.8 Hz) , 7.07 (1H; dd; J=8.4 and 1.8 Hz), 7.21 (1H; ddd; J=9.3, 1.6 and 1.5 Hz), 7.27 (1H; d; J=15.9 Hz), 7.29 (1H; dd; J=9.3 and 8.1 Hz), 7.46 (1H; dd; J=8.1 and 1.5 Hz), 7.63 (1H; d; J=15.6 Hz); ^{13}C NMR (75 MHz, $CDCl_3$, Me_4Si): δ 55.8; 56.4; 70.1; 110.8; 113.2; 113.3; 118.8; 119.31; 120.4; 121.3; 123.4; 128.4; 129.9; 133.04; 140.2; 145.4; 149.9; 150.8; 160.2; 190.6; ESIMS m/z 649.3 $[2M + H]^+$, 325.2 $[M + H]^+$. HREIMS (m/z): 324.1358 $[M^+]$ (calcd for $C_{20}H_{20}O_4$, 324.1362).

4-allyloxy-3-methoxy-3', 4'-diméthylchalcone (5e)

To a solution of 3,4-dimethylacetophenone (77.11 µl, 0.52 mmol, d= 1.001; n_D^{20}: 1.538) in ethanol (7 mL) was first added *O*-allylvanillin (100 mg, 0.52 mmol), and secondly a 0.52 mL solution of KOH (50%, 1 mL/mmol) that is 0.52 mL. The mixture was stirred at room temperature for 26 hours. After separation and purification of the residue of the reaction, we obtained the compound 5e (68.8 mg, yield 41% in Hex-EA 85:15). IR spectrum ($CHCl_3$): υ_{max} cm^{-1}: 2919.0, 2361.3, 1653.5, 1508.7, 1259.7, 1136.7; ^{1}H NMR (300 MHz, $CDCl_3$, Me_4Si) δ 2.2 (6H; s), 3.8 (3H; s), 4.50 (2H; d; J=5.6 Hz), 5.20 (1H; dd; J=17.3 and 9.6 Hz) , 5.23 (1H; dd; J=17.3 and 0.5 Hz), 5.9 (1H; m), 6.74(1H; d; J=8.6

Hz) , 7.02(1H;dd ; J=8.6 and 1.7 Hz) , 7.05 (1H;d; J=1.7 Hz), 7.10 (1H;d ; J=8.7 Hz), 7.24 (1H; d; J=15.3 Hz), 7.59 (1H; d; J=1.8 Hz), 7.60 (1H;d ; J=15.6 Hz), 7.64 (1H; dd; J=8.7 and 1.8 Hz), ^{13}C NMR (75 MHz, $CDCl_3$, Me_4Si) δ 20.0; 20.3; 56.2; 69.9; 110.9; 113.3; 118.6; 120.5; 122.9; 126.4; 128.4; 129.8; 129.9; 132.9; 136.5; 137.2; 142.4; 144.6; 149.7; 150.5; 190.5; ESIMS m/z 323,3 $[M + H]^+$. HREIMS (m/z): 322.1562 $[M^+]$ (calcd for $C_{21}H_{22}O_3$, 322.1569).

4-allyloxy-3-methoxy-3'-methylchalcone (5f)

To a solution of 3-methylacetophenone (70.88 µl, 0.52 mmol, d = 0.986; n_D^{20}= 1.529) in ethanol (7 mL) was first added *O*-allylvanillin (100 mg, 0.52 mmol), and secondly a 0.52 mL solution of KOH (50%, 1 mL/mol) that is 0.52 mL. The mixture was stirred at room temperature for 24 hours. After separation and purification of the residue of the reaction product was obtained **5f** (96.3 mg, yield 60% in Hex-EA 88:12). IR spectrum ($CHCl_3$): υ_{max} cm^{-1}: 2927.1, 2367.7, 1655.3, 1578.9, 1506.4, 1256.9, 1132.1; ^{1}H NMR (300 MHz, $CDCl_3$, Me_4Si) δ 2,31 (3H; s), 3.82 (3H; s), 4.54 (2H; d; J=1.4 Hz), 5.20 (1H; dd; J=13.0 and 6.6 Hz) , 5.30 (1H; dd; J=13.0 and 1.5 Hz), 5.96 (1H;m), 6.77 (1H; d; J=8.7 Hz), 7.04 (1H; d; J=1.8 Hz), 7.06 (1H; dd; J=8.7 and 1.8 Hz), 7.10 (1H; d; J=15.9 Hz), 7.23 (1H; dd; J=8.4 and 8.1 Hz) , 7.27 (1H; ddd; J=8.1; 1.8 and 1.5 Hz) , 7.6 (1H; ddd; J=8.4; 1.8 and 1.5 Hz), 7.62 (1H; d; J=15.9 Hz), 7.65 (1H; dd; J=1.8 and 1.5 Hz), ^{13}C NMR (75 MHz, $CDCl_3$, Me_4Si) δ 21.0; 55.6; 69.3; 110.1; 112.5; 118.0; 119.9; 122.4; 125.2; 127.6; 128.0; 128.5; 131.1; 132.9; 137.9; 138.1; 144.4; 149.1; 149.9; 190.4; ESIMS m/z 309,3 $[M + H]^+$. HREIMS (m/z): 308.1407 $[M^+]$ (calcd for $C_{20}H_{20}O_3$, 308.1412).

4-allyloxy-3-methoxy-2'-methoxychalcone (5g)

To a solution of 2-methylacetophenone (139.769 µl, 1.04 mmol, d = 1.026, n_D^{20}= 1.5318) in ethanol (7 mL) was first added to *O*-allylvanillin (60 mg, 0.31 mmol), and secondly a 1.04166 mL solution of KOH (50%, 1 mL/mol). The reaction mixture was stirred room temperature for 24 hours. After separation and purification of the residue of the reaction, the product 5g was obtained (41.9 mg, yield 44% in Hex-EA 92:8). IR spectrum ($CHCl_3$): υ_{max} cm^{-1}: 2365.7, 2336.6, 1633.9, 1590.9, 1508.7, 1262.9, 1143.8; ^{1}H NMR (300 MHz, $CDCl_3$, Me_4Si) δ 2.35 (3H; s), 3.83 (3H; s), 4.57 (2H; d; J=1.6 Hz), 6.79 (1H; d; J=1.8 Hz) , 5.23 (1H; dd; J=14.1 and 5.7 Hz), 5.34 (1H; dd; J=14.1 and 1.8 Hz), 5.90 (1H; m), 6.81 (1H; d; J=1.8 Hz), 6.92 (1H; d; J=16.2 Hz), 7.18 (1H; dd; J=9.0 and 1.5 Hz), 7,20 (1H; dd; J=8.4 and 1.8 Hz) , 7.21 (1H; m; J=9.0 and 2.1 Hz), 7.29 (1H; m; J=9.0 and 2.1 Hz), 7.30 (1H; d; J=15.9 Hz), 7.40 (1H; d; J=9.0 and 2.1 Hz), ^{13}C NMR (75 MHz, $CDCl_3$, Me_4Si) δ 20.02; 55.9; 69.7; 110.2; 112.8; 118.4; 122.9; 124.9; 125.4; 127.6; 127.8; 130.1; 131.1; 132.6; 136.6; 139.3; 146.2; 149.3; 150.4; 196.8; ESIMS m/z 309.3 $[M + H]^+$. HREIMS (m/z): 308.1403 $[M^+]$ (calcd for $C_{20}H_{20}O_3$, 308.1412).

4-allyloxy-3, 4'-diméthoxychalcone (5h)

To a solution of 4-methylacetophenone (151.8 µl, 1.02 mmol) in ethanol (5 mL) was first added to *O*-allylvanillin (75 mg, 0.39 mmol), and secondly a 1.0107 mL solution of KOH (50%, 1 mL/mol). The reaction mixture was stirred at room temperature for 24 hours. After separation and purification of the residue of the reaction, the product 5h was obtained (93.7 mg, yield 74% in Hex-EA 75:25). IR spectrum ($CHCl_3$): υ_{max} cm^{-1}: 2361.5, 2336.9, 1653.5, 1600.1, 1506.8, 1256.7; ^{1}H NMR (300 MHz, $CDCl_3$, Me_4Si) δ 3.98 (3H; s), 4.00 (3H; s), 4.77 (2H; d; J=12.6 Hz), 5.43 (1H; dd; J=12.6 and 1.0 Hz), 5.50 (1H; dd; J=12.6 Hz), 6.2 (1H; m), 6.99 (1H; d; J=8.1 Hz), 7.09 (2H; d; J=10.1 Hz), 7.26 (1H; d; J=2.1 Hz) ,7.30 (1H; dd; J=8.1 and 2.1 Hz), 7.51 (1H; d; J=15.9 Hz), 7.85 (1H; d; J=15.6 Hz), 8.12 (2H; d; J=10,1 Hz); ^{13}C NMR (75 MHz, $CDCl_3$, Me_4Si) δ 55.4; 55.9; 69.7; 110.5; 112.9; 113.7(x2); 118.4; 119.8; 122.4; 128.2; 130.9 (x2); 131.3; 132.7; 144.0; 149.5; 150.2; 163.2; 188.7; ESIMS m/z 325.1 $[M + H]^+$. HREIMS (m/z): 324.1358 $[M^+]$ (calcd for $C_{20}H_{20}O_4$, 324.1362).

Statistical analysis

The one-way ANOVA at 95% confidence level was used for statistical analysis.

Results and Discussion

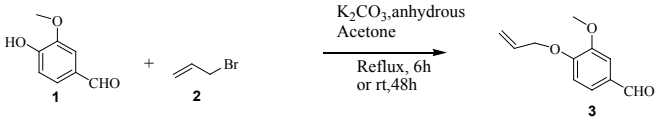
Chemistry (synthesis)

The synthesis of chalcones 5a-h was accomplished by a one-pot Claisen-Schmidt condensation [21,22] between the appropriate *O*-allylvanillin 3 and substituted acetophenones 4a-h, as shown in Scheme 2. *O*-allylvanillin 3 was prepared via the nucleophilic substitution of vanillin 1 and allylbromide 2 in the presence of potassium carbonate in anhydrous acetone (Scheme 1) [22].

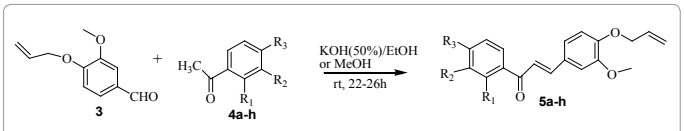
In all the chalcones synthesized, only the trans double bond (on the basis of coupling constant) was obtained. All synthesized compounds were characterized by spectral data (mass, UV, IR and NMR) and were consistent with the structures proposed. The purity of these compounds was ascertained by TLC and spectral analysis.

Biological studies

These synthesized compounds were evaluated for their *in vitro* anticancer activity using Sulforhodamine B assays [20]. A preliminary assay against leukemia THP-1 cell line showed that compounds 3,



Scheme 1: Synthesis of the intermediate *O*-allylvanillin **3.**



a: R_1= R2= R3= H
b: R_1= MeO-,R_2= R_3= H
c: R_1= R_3= CH_3-, R_2= H
d: R_1= R_3= H, R_2= MeO-
e: R_1= H, R_2= R_3= CH_3-
f: R_1= R_3= H, R_2= CH_3-
g: R_1= CH_3-, R_2= R_3= H
h: R_1= R_2= H, R_3= MeO-

Scheme 2: Synthesis of compounds **5a-h.**

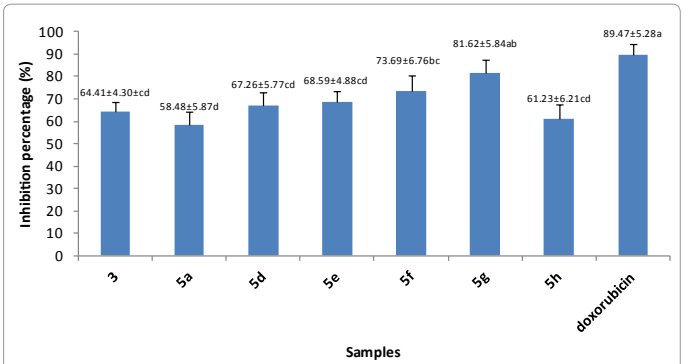


Figure 1: Inhibitory percentage (%) of compounds at 100 µM and doxorubicin (50 µM) on leukemia THP-1 cancer cell line. Values mean ± SD of three experiments; Data with different superscript letters (a, b, c...) are significantly different (P < 0.05).

Tested samples	Cell lines and IC_{50} values (μM)				
	THP-1	HL60	Hep-G2	DU-145	MCF-7
3	74.76 ± 3.27	63.52 ± 5.2	90.99 ± 7.72	-	90.11 ± 7.26
5a	12.80 ± 1.34	23.52 ± 2.11	-	-	77.37 ± 7.12
5d	25.19 ± 1.94	20.81 ± 1.97	43.75 ± 3.42	83.73 ± 6.43	56.54 ± 3.78
5e	27.03 ± 2.03	28.70 ± 2.37	33.22 ± 3.07	37.70 ± 2.71	28.98 ± 1.91
5f	10.42 ± 0.68	13.50 ± 1.14	19.94 ± 2.15	12.23 ± 1.19	17.28 ± 2.02
5g	4.76 ± 0.51	7.90 ± 0.64	10.12 ± 0.88	5.21 ± 0.28	10.32 ± 0.86
5h	27.78 ± 3.04	37.59 ± 3.16	53.28 ± 5.32	36.48 ± 3.09	45.12 ± 3.27
Doxorubicin	1.44 ± 0.09	2.17 ± 0.26	4.31 ± 0.36	2.59 ± 0.20	6.00 ± 0.72

(-): > 100 μM

Table 1: Cytotoxicity of the studied compounds towards cancer cell lines.

5a, 5d, 5e, 5f, 5g and 5h (at 100 μM) as well as doxorubicin at 50 μM were able to inhibit the proliferation of more than 50% cells (Figure 1). These samples were consequently tested in other cell lines and the results are summarized in Table 1. It appeared that compounds 5d-h displayed cytotoxic activities with IC_{50} values below 100 μM against the five cancer cell lines. In the US NCI screening program, a compound is generally considered to have *in vitro* cytotoxic activity, if the IC_{50} value following incubation between 48 and 72 h is less than 4 μg/ml or 10 μM [23]. In the present study, IC_{50} values below or around 10 μM were displayed by compounds 5f against THP-1 cells (IC_{50} of 10.42 μM) and 5g against THP-1 (IC_{50} of 4.76 μM), DU-145 (IC_{50} of 5.21 μM), HL60 (IC_{50} of 7.90 μM), Hep-G2 (IC_{50} of 10.12 μM) and MCF-7 (IC_{50} of 10.32 μM). Also the IC_{50} values obtained with doxorubicin were below 10 μM against the five cancer cell lines tested. The cytotoxicity of compounds 5g can be considered good with regards to the US NCI standard. When regarding the structure activity relationship, it appeared that the number and position of methyl group in cycle A of the synthesized chalcones influenced their activities, compound 5g with the $-CH_3$ group in position C-2 being more active on almost the five cell lines than compounds 5a (without any methyl group) and 5f bearing $-CH_3$ group in C-3 (Table 1). However, 5e with three $-CH_3$ groups was less active than compound 5g and 5f (only one $-CH_3$ group), but more active than 5a without a $-CH_3$ group, clearly confirming the influence of the methylation on the activity of the chalcones studied. Also, when comparing the activity of the two most cytotoxic compounds 5g and 5f with those of the methoxylated compounds 5d and 5h, it appeared that a single methylation induced an increase in activity compared to a single methoxylation of the chalcones studied. In addition, it is also clear that, the position of $-CH_3$ and that of $-OCH_3$ groups influence the antiproliferative activities of compounds 5d and 5h. Although the compounds studied did not show very good cytotoxicity, the study provides additional information on structure-activity relationships with chalcones, that could allow future synthesis of more potent derivatives. In future, mechanistic studies such as the effects of compound 5g on cell cycle distribution, induction of apoptosis, caspases, and the effects on mitochondrial membrane potential will be carried out to explain the mode of action on this compound.

Conclusion

In conclusion, we report here a series of new *O*-allylchalcone derivatives prepared by a Claisen-Schmidt condensation reaction [22] and their ability to kill tumor cells *in vitro*. The mechanisms of cytotoxicity underlying this process remain to be fully elucidated. Previous studies reported in the literature reveal that, flavonoids such as chalcones are known microtubule inhibitors with antimitotic activity [14]. Detailed mechanistic studies and lead optimization of these *O*-allylchalcone derivatives are under investigation. It is intended that results from these studies will assist in elucidating their precise mechanisms of action and provide an approach to develop new potent *O*-allylchalcone hybrid prototypes for further optimization and development to get new leads for the treatment of cancer.

Acknowledgement

BN, RR and MT are grateful to the Agence Universitaire de la Francophonie (AUF), Natural Sciences and Engineering Research Council of Canada (NSERC) and MATSUMAE for their financial support of this research and for a travel grant to the Department of Chemistry, Université du Québec à Montréal (Canada) and Department of Chemistry, University of Yamagata (Japan), respectively.

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