Synthesis and Evaluation of Anticancer Activity of O-allylchalcone Derivatives

Bathélymé Ngameni1*, Victor Kuete2, Pantaleon Ambassa3, kamga Justin1, Moungang Luciane Marylyse4, Abdou Tchoukoua3, René Roy5, Bonaventure Tchaleu Ngadjui1,3 and Murayama Tetsuya6

1Department of Pharmaceutical Sciences and Traditional Pharmacopoeia, Faculty of Medicine and Biomedical Sciences, University of Yaoundé I, Cameroon
2Department of Biochemistry, Faculty of Science, University of Dschang, Cameroon
3Department of Organic Chemistry, Faculty of Science, University of Yaoundé I, Cameroon
4Department of Biology and Animal Physiology, Faculty of Science, University of Yaoundé I, Cameroon
5Department of Chemistry, Université du Québec à Montréal, Québéc, Canada
6Department of Chemistry, Faculty of Agriculture, University of Yamagata, Japan

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 IC50 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 currently a good deal of interest in the health benefits of phytochemicals, in particular prenylated and allylated flavonoids. Chalcones (1,3-diaryl-2-propan-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 subjected to a great deal of 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 13C NMR spectra were recorded with Bruker WM-300 in the CDCl3 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% CO2 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% CO2 and 95% relative humidity in a CO2 incubator. Doxorubicin was used as positive reference. Suitable controls with

*Corresponding author: Bathélymé Ngameni, Department of Pharmaceutical Sciences and Traditional Pharmacopoeia, Faculty of Medicine and Biomedical Sciences, University of Yaoundé I, Cameroon

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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 than 50% of the cells. Then samples were serially diluted and tested against other cell lines for IC₅₀ determination. IC₅₀ 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 aceton (8 mL) was added K₂CO₃ (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 15 hours. We obtained the product 5c (68.8 mg, yield 38% in Hex-EA 87.5:12.5) after separation and purification of the residue of the reaction, we obtained the compound 5e (68.8 mg, yield 28% in Hex-EA 80:20). IR (CHCl₃): υ max cm⁻¹: 2917.2, 2365.8, 1653.5, 1594.8, 1250.8, 1019.5; ¹H NMR (300 MHz, CDCl₃, MeSi): 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; dd; J=16.2 Hz), 7.62 (1H; d; J=7.8 and 1.5 Hz); ¹C NMR (75 MHz, CDCl₃, MeSi): δ 55.3; 55.5; 69.3; 90.1; 111.1; 112.4; 117.9; 120.2; 122.3; 124.8; 127.9; 128.1; 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 C₁₃H₁₂O₂, 324.1362).

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

To a solution of 3,4-dimethylacetophenone (77.11 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/ml) or 0.52mM. The mixture is stirred in a nitrogen atmosphere at room temperature for 21 hours. After separation and purification of the residue by column chromatography on silica gel eluting with Hexane-Ether (Hex-EA) system of increasing polarity, product 5c was obtained (53.5 mg, yield 82% in Hex-EA 85:15). IR (CHCl₃): υ max cm⁻¹: 2919.2, 2361.7, 1653.8, 1576.3, 1508.7, 1260.5, 1140.4, 1029.2; ¹H NMR (300 MHz, CDCl₃, MeSi): 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), 5.45 (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), 7.29 (1H; dd; J=8.4 and 1.8 Hz), 7.58 (1H; d; J=8.1 and 1.8 Hz), 7.59 (1H; d; J=15.9 Hz); ¹C NMR (75 MHz, CDCl₃, MeSi): δ 19.8; 20.9; 55.4; 69.2; 109.8; 112.3; 117.9; 122.3; 124.4; 125.7; 127.4; 137.1; 132.0; 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 C₁₃H₁₂O₂, 322.1569).

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

To a solution of 3-methoxyacetophenone (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/ml) 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 (CHCl₃): υ max cm⁻¹: 2917.2, 2365.9, 1659.9, 1508.7, 1260.7, 1139.2; ¹H NMR (300 MHz, CDCl₃, MeSi): 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), 5.45 (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), 7.29 (1H; dd; J=8.4 and 1.8 Hz), 7.58 (1H; d; J=8.1 and 1.8 Hz), 7.59 (1H; d; J=15.9 Hz); ¹C NMR (75 MHz, CDCl₃, MeSi): δ 19.8; 20.9; 55.4; 69.2; 109.8; 112.3; 117.9; 122.3; 124.4; 125.7; 127.4; 137.1; 132.0; 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 C₁₃H₁₂O₂, 322.1569).
To a solution of 3-methylacetophenone (70.88 μl, 0.52 mmol, d = 0.986; n, 292.159) in ethanol (7 mL) was first added to O-allylvanillin (60 mg, 0.31 mmol), and secondly a 1.026 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 5g was obtained (41.9 mg, yield 44% in Hex-EA 92:8). IR spectrum (CHCl₃), ν max cm⁻¹: 2365.7, 2336.6, 1633.9, 1590.9, 1508.7, 1262.9, 1143.8; H NMR (300 MHz, CDCl₃, Me₄Si) 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), 5.61 (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=19.0 and 2.1 Hz). ¹³C NMR (75 MHz, CDCl₃, Me₄Si) 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; 131.1; 132.6; 134.8; 144.2; 149.1; 149.9; 190.4. ESIMS m/z 323.3 [M + H]⁺. HREIMS (m/z): 322.1562 [M⁺] (calcd for C₁₀H₈O₃, 322.1569).

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

To a solution of 2-methylacetophenone (139.769 μl, 1.04 mmol, d = 1.026, n, 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 at room temperature for 24 hours. After separation and purification of the residue of the reaction, the product 5f was obtained (41.9 mg, yield 44% in Hex-EA 92:8). IR spectrum (CHCl₃): ν max cm⁻¹: 2365.7, 2336.6, 1633.9, 1590.9, 1508.7, 1262.9, 1143.8; H NMR (300 MHz, CDCl₃, Me₄Si) 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), 5.20 (1H; dd; J=13.0 and 6.6 Hz), 5.30 (1H; d; J=13.0 and 1.5 Hz), 5.96 (1H;m), 6.79 (1H; d; J=1.8 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=8.7 and 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), 7.7 (1H; d; J=15.9 Hz). ESIMS m/z 309.3 [M + H]⁺. HREIMS (m/z): 308.1407 [M⁺] (calcd for C₂₀H₁₈O₃, 308.1412).

4-allyloxy-3-methoxy-3'-methoxymethylchalcone (5g)

To a solution of 2-methylacetophenone (139.769 μl, 1.04 mmol, d = 1.026, n, 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 at 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 (CHCl₃), ν max cm⁻¹: 2365.7, 2336.6, 1633.9, 1590.9, 1508.7, 1262.9, 1143.8; H NMR (300 MHz, CDCl₃, Me₄Si) 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), 5.20 (1H; dd; J=13.0 and 6.6 Hz), 5.30 (1H; d; J=13.0 and 1.5 Hz), 5.96 (1H;m), 6.79 (1H; d; J=1.8 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=8.7 and 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), 7.7 (1H; d; J=15.9 Hz). ESIMS m/z 309.3 [M + H]⁺. HREIMS (m/z): 308.1407 [M⁺] (calcd for C₂₀H₂₀O₄, 309.1436).

Results and Discussion

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, 4 and 5 were the most potent. The inhibitory activity of the synthesized chalcones 3 is shown in Table 1.
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 subsequently tested in other cell lines and the results are summarized in Table 1. It appeared that compounds 5d-h displayed cytotoxic activities with IC50 values below 100 µM against the five cancer cell lines. In the US NCI screening program, a compound is classified as active if the IC50 value is generally considered to have inhibited more than 50% cell growth. For example, doxorubicin displayed cytotoxic activities with IC50 values of 7.90 µM against Hep-G2 and 10.12 µM against MCF-7. In the NCI screening program, a compound is classified as active if the IC50 value is generally considered to have inhibited more than 50% cell growth. For example, doxorubicin displayed cytotoxic activities with IC50 values of 7.90 µM against Hep-G2 and 10.12 µM against MCF-7.

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 potential O-allylchalcone hybrid prototypes for further optimization and development to get new leads for the treatment of cancer.

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

| Tested samples | Cell lines and IC50 values (µM) | THP-1 | HL60 | Hep-G2 | DU-145 | MCF-7 |
|----------------|---------------------------------|-------|------|--------|--------|-------|
| 3              | 74.76 ± 3.37                    | 63.52 ± 5.2 | 90.99 ± 7.72 | - | 90.11 ± 7.26 |
| 5a             | 12.60 ± 1.34                    | 23.62 ± 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

Conclusions

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 making 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 potential O-allylchalcone hybrid prototypes for further optimization and development to get new leads for the treatment of cancer.

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