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Short communication

Apoptotic effect of chalcone derivatives of 2-acetylthiophene in human breast cancer cells

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ABSTRACT

Background: A variety of chalcones have demonstrated cytotoxic activity toward several cancer cell lines. This study aimed to investigate the cytotoxicity of four chalcones derivatives of 2-acetylthiophene in human breast cancer cell lines.
Methods: MCF-7 and MDA-MB-231 cells were treated with synthesized chalcones and the cytotoxicity was evaluated by tetrazolium dye (MTT), live/dead, and DAPI assays.
Results: Chalcones significantly decreased MCF-7 and MDA-MB-231 cells viability *in vitro* in a dose dependent manner. After 48 h treatment, the IC_{50} values ranging from 5.52 to 34.23 μM. Chalcone 3c displayed the highest cytotoxic activity from all the tested compounds. Cytotoxic effects of compounds were confirmed in the live/dead assay. In addition, DAPI staining revealed that these compounds induce death by apoptosis.
Conclusion: The data speculate that chalcone derivatives of 2-acetylthiophene may represent a source of therapeutic agents for human breast cancer.

Introduction

Breast cancer affects 1.38 million women worldwide per year [1] making it the most common cause of cancer death among women (522,000 deaths in 2012), especially in less developed countries [2]. Approximately 60% of all patients with breast cancer ultimately undergo chemotherapy. However, only a minority of patients on chemotherapy show long-term remission and their suboptimal response rate accounts for high rate of failure and selection of chemoresistant tumors [3]. Further, the existing drugs for breast cancer treatment are invariably associated with several drawbacks such as poor oral bioavailability, non-selectivity and poor pharmacodynamics properties, limiting their clinical utility [4]. Triple Negative Breast Cancer (TNBC) is a subgroup of tumors that do not clinically express significant levels of estrogen receptor (ER), progesterone receptor (PR) and lack of human epidermal growth factor receptor 2 (HER-2) overexpression. This type of cancer accounts for approximately 15–20% among breast cancer cases and is associated with a poor prognostic factor for disease-free and overall survival. TNBC is responsible for a disproportionate number of deaths and no effective specific targeted therapy is readily available for it [5]. Therefore, the development of agents with better pharmacological profiles and the identification of new molecular targets that can suppress breast cancer cells is still of urgent concern.

Chalcones are ketones α,β-unsaturated with one aromatic ring bonded at carbonyl and another aromatic ring bonded an olefin function [6]. These molecules can be found in natural products, belonging to the flavonoid family, or obtained by synthesis process. Many studies have been presented in the literature with references to structural modifications of the chalcone template [6–10]. The biological properties of chalcones are equally wide-ranging. These molecules have displayed an impressive array of biological activities, among which anti-malarial [11], anti-microbial [12], anti-inflammatory [13] and anti-oxidant [14]. Furthermore, a variety of chalcones has demonstrated cytotoxic activity toward several cancer cell lines. [9,15–17]. Additionally, minimal toxicity has been observed, and pharmacokinetic studies indicate that these compounds metabolize rapidly [18].

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In recent years, our research group has been studying new *in vitro* therapeutic strategies against several tumor cell lines [10,19–21]. As a follow up of our studies in this area, and in light of the antitumour potential of several chalcone derivatives, this work aimed to evaluate the cytotoxic potential of chalcone derivatives of 2-acetylthiophene in human breast cancer cell lines.

Materials and methods

Apparatus and analysis

NMR spectra were recorded on a Bruker DPX 400 spectrometer (400.13 MHz for ^{1}H and 100.48 MHz for ^{13}C) at 300 K. Melting points (mp) were determined using capillary tubes on a Fisatom 430 apparatus with mercury thermometer. The progress of the reactions was monitored on a Shimadzu 2010 Gas Chromatograph with Flame Ionization Detector (GC-FID), equipped with a split/splitless injector and a HP-1 30 m × 0.32 mm × 0.25 μm column. Mass Spectra and purity of compounds were assessed on a Shimadzu GC–MS-QP 2010SE Gas Chromatography coupled to Mass Spectrometry (GC-MS) equipped with an AOC-20i automatic injector and a Rtx-5MS 30 m × 0.25 mm × 0.25 μm column.

Synthesis of chalcones

Solvents and chemicals used in the synthesis were obtained from Sigma-Aldrich Co., St. Louis, MO, USA, and used without further purification. Derivatives of 3-phenyl-1-(thiophen-2-yl) prop-2-en-1-one **3a–d** were synthesized based on the methodology previously reported by our research group [10]. A solution of potassium hydroxide (5%, 10 mL) was slowly added to a mixture of 2-acetyl thiophene **1** (0.01 mol) and substituted benzaldehydes **2a–d** (0.01 mol) in ethanol (50 mL) (Fig. 1). The mixture was stirred for 6 h. The precipitated solid was filtered, washed with water, dried and recrystallized with ethanol. All compounds were confirmed by Gas Chromatography coupled to Mass Spectrometry (GC–MS).

*Data of chalcones **3a–d***

*(E)-3-Phenyl-1-(thiophen-2-yl)prop-2-en-1-one (**3a**)*

Yield: 76%; purity: 96%; mp 145–146 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.13–7.15 (m, 1H, Ph-H), δ 7.36–7.42 (m, 3H, Ph-H), δ 7.40 (d, 1H, *J* 15.56 Hz, Hα), 7.59–7.64 (m, 3H, Ph-H), 7.82 (d, 1H, *J* 14.78 Hz, Hβ), 7.13-7.15 (m, 1H, Ph-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 121.48, 128.13, 128.33, 128.80, 130.44, 131.73, 133.76, 134.52, 143.84, 145.36, 181.85; GC–MS *m/z*, observed: 214.10; $C_{13}H_{10}OS$ $[M]^+$ requires: 214.05.

*(E)-3-(4-Bromophenyl)-1-(thiophen-2-yl)prop-2-en-1-one (**3b**)*

Yield: 90%; purity: 100%; mp 132–133 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.12–7.14 (m, ^{1}H, Ph-H), 7.35 (d, 1H, *J* 15.59 Hz, Hα), 7.42-7.50 (m, 4H, Ph-H), 7.63–765 (m, 1H, Ph-H), 7.71 (d, 1H, *J* 15.59 Hz, Hβ), 7.81–7.83 (m, 1H, Ph-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 122.03, 124.72, 128.21, 129.71, 131.87, 132.04, 133.48, 134.02, 142.45, 145.22, 181.58; GC–MS *m/z*, observed: 294.00 [M+2], 292.00; $C_{13}H_9BrOS$ $[M]^+$ requires: 291.96.

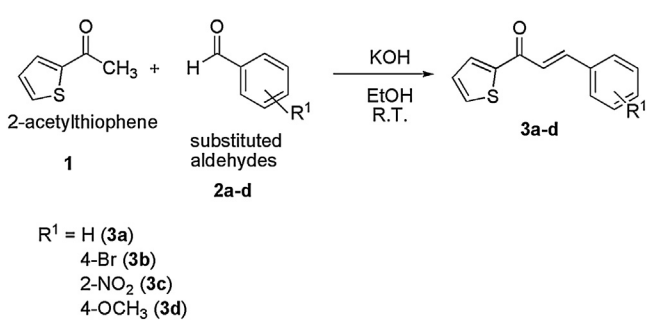

Fig. 1. Synthesis of Chalcones **3a–d.**

*(E)-3-(2-nitrophenyl)-1-(thiophen-2-yl)prop-2-en-1-one (**3c**)*

Yield: 51%; purity: 100%; mp 140–142 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.45–8.24 (m, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 136.8, 134.2, 133.3, 130.0,128.8, 128.6, 124.8, 124.6, 122.4; 148.9, 141.5,137.7; 189.6; GC–MS *m/z*, observed: 227.05; $C_{13}H_9NO_3S$ $[M]^+$ requires: 259.03.5

*(E)-3-(4-Methoxyphenyl)-1-(thiophen-2-yl)prop-2-en-1-one (**3d**)*

Yield: 78%; purity: 97%; mp 70–72 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.81 (s, 3H, CH_3), 6.89-6.91 (m, 2H, Ph-H), 7.13-7.15 (m, 1H, Ph-H), 7.23 (d,1H, *J* 15.51 Hz, Hα), 7.55-7.63 (m, 3H, Ph-H), 7.79 (d, 1H, *J* 15.59 Hz, Hβ), 7.81-7.83 (m, 1H, Ph-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 55.33, 114.36, 119.22, 127.35, 128.10, 130.19, 131.40, 133.41, 119.22, 143.78, 145.70, 161.65, 181.98; GC–MS: *m/z*, observed: 244.10; $C_{14}H_{12}O_2S$ $[M]^+$ requires: 244.06.

Cell culture

Human breast cancer cell lines MCF-7 and MDA-MB-231 were obtained from the Rio de Janeiro Cell Bank (PABCAM, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil) and routinely cultured in our laboratory. MCF-7 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) from Vitrocell Embriolife (Campinas, Brazil) and MDA-MB-231 cells were cultured in LEIBOVITZ L-15 medium from Vitrocell Embriolife (Campinas, Brazil) supplemented with 10% FBS and 0.2 mg mL^{-1} sodium bicarbonate. The cells were grown at 37 °C in an atmosphere of 95% humidified air while MCF-7 cells were grown at 5% of CO_2. MDA-MB-231 cells were grown without any CO_2.

Cytotoxicity

The cytotoxic potential of synthetized chalcones was evaluated by measuring the reduction of soluble MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] to water-insoluble formazan. Cells were seeded at a density of 2×10^4 cell per well in a volume of 100 μL in 96-well plates, and grown in ideal conditions for 24 h before being used in the cell viability assay. Then, cells were treated with different concentrations of chalcones 3a-d (5–80 μM) for 24, 48 and 72 h. These molecules were previously dissolved in dimethyl sulfoxide (DMSO) and added to the medium supplemented with 10% FBS to the desired concentrations. The final DMSO concentration in the medium does not exceed 0.2%, and an additional group was exposed to an equivalent concentration of this solvent. Thereafter, the incubation medium was removed, and subsequently 180 μL of medium and 20 μL MTT (5 mg MTT/mL solution) were added to each well. The plates were incubated for an additional 3 h, and the medium was discarded. DMSO was added to each well, and the formazan was solubilized on a shaker for 15 min at 100 g. The absorbance was read on a microplate reader (Victor ×5, PerkinElmer, USA) at a test wavelength of 492 nm. Cell inhibitory growth was determinate as follows: inhibitory rate = $(1 - Abs_{492treatedcells}/Abs_{492controlcells}) \times 100\%$ [10]. All observations were validated by at least three independent experiments in triplicates for each experiment.