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Preliminary Evaluation of Cytotoxicity for Small Chalcones on Breast and Colorectal Cancer Cell Lines: Synthesis and Structure-Activity Relationship

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Abstract

Chalcones are a wide-range of natural and synthetic compounds with several pharmacological applications, highlighting their antiproliferative action on multiple cancer cell lines. In this study, their synthesis and preliminary evaluation as cytotoxic agent on breast cancer (MCF-7) and colorectal cancer (Caco-2) cell lines, as well as, their evaluation on non-cancer human fibroblast is shown. The synthesis showed high yields (55% a 99%), while cytotoxic assays showed high activity and selectivity on MCF-7, mainly for chalcones with a 3',4'-(OCH_2O) substitution pattern (compound 5g IC_{50}= 2.3μM; SI= 9.1). In the case of Caco-2, the best result were obtained with chalcones with a 3'-OMe substitution pattern (compound 5c IC_{50}= 18.0μM; SI= 2.0). This evaluation, although preliminary, suggests that the substitution pattern 3',4' - (OCH_2O) for MCF-7 and the 3'-OMe substitution for Caco-2 are the avenue to follow for new antiproliferative agents development.

Keywords: Chalcones; Antiproliferative activity; MCF-7; Caco-2; Selectivity index

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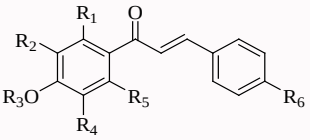
Introduction

Cancer is one of the principal causes of mortality in the society around the world [1]. According to the World Health Organization (WHO) estimation, for 2030 the deaths caused by cancer will increase by 44% with respect to 2008 [2]. The disease is treated with surgery, radiation, chemotherapy, hormonal and biological therapy [3-5]. The chemotherapeutic agents used for several cancer types, *e.g.* breast and colon [6,7], have high toxicity levels, limiting their use [8-10]. In addition, in Chile cancer is the second cause of death with the highest prevalence being for stomach, breast, prostatic, lung, vesicle, and colon [11].

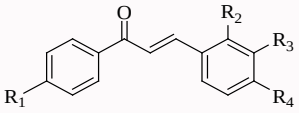
Breast cancer is the most common invasive cancer in female worldwide. It accounts for 16% of all female cancers and 22.9% of invasive cancers in women, 18.2% of all cancer deaths worldwide including both in males and females are from breast cancer [12]. On the other hand, colorectal cancer is one of the most common cancer worldwide, with between one and two million new cases being diagnosed every year, thus making colorectal cancer the third most common cancer and the fourth most common cause of cancer-related death, with 700,000 deaths per year, exceeded only by lung, liver and stomach cancers [13,14].

On the other hand, natural products have been used as a model to develop new compounds with potential application for several pharmacological treatments [15]. In this sense, phenolic compounds have attracted scientists attention due to their wide-range of *in vitro* pharmacological activities, *e.g.* flavonoids and structurally related compounds [16]. Actually, chalcones are natural compounds belonging to the flavonoid family [16,17]. These compounds have attracted a great interest due to their wide range of pharmacological activities, especially their anti-inflammatory, analgesic, anti-pyretic, anti-mutagenic, anti-leishmania, anti-fungal activities, and cytotoxic effects on cancer cell lines [18-21]. Within the later, several natural (1a-d) and synthetic chalcones (2a-g) with antiproliferative activity on MCF-7 and Caco-2 have been reported (Figure 1) [22-29].

Despite the available information related to the pharmacological applications of chalcones as antiproliferative agents on cancer cell lines, some of them have complex structures obtained after several synthetic steps (*e.g.*1a-d) decreasing their industrial scalability to become a drug in the oncology market. In addition, the structure-activity studies have allowed to obtaining compounds



(**1a**) R_1=R_5=R_6= OH, R_2= Prenyl, R_3=R_4= H
(**1b**) R_1=R_5=R_6= OH, R_2= Prenyl, R_3=Me, R_4= H
(**1c**) R_1= OH, R_2=R_4= Me, R_3=R_6= H, R_5= OMe
(**1d**) R_1=R_5= H, R_2=R_4=R_6= OMe, R_3= Me



(**2a**) R_1=R_2=R_3= H, R_4= Cl
(**2b**) R_1=R_2=R_3= H, R_4= Br
(**2c**) R_1=R_2=R_3= H, R_4= OMe
(**2d**) R_1=R_2=R_3= H, R_4= SMe
(**2e**) R_1=R_2=R_3= H, R_4= NHAc
(**2f**) R_1=R_2=R_4= H, R_3= Me
(**2g**) R_1= Me, R_2= OH, R_3=R_4= H

Figure 1: Natural and synthetic chalcones with antiproliferative activity on MCF7 and Caco-2 cell lines.

with defined and improved properties. Due to this information, this report shows the synthesis of eight small chalcones evaluated on two cancer cell lines (MCF-7, breast cancer; Caco-2, colorectal cancer) and on human fibroblasts (non-tumoral primary culture), this is accompanied with their preliminary structure-activity relationship study.

Materials and Methods

General

Melting Point was measured in Fischer Scientific apparatus. Infrared spectra were recorded in Buck Scientific M500. Recorded range 600cm^{-1} to 4000cm^{-1}, all samples was registered on ATR system (Attenuated Total Reflectance). ^{1}H and ^{13}C-NMR, 2D-HSQC and 2D-HMBC spectra were recorded on a Bruker Advance 400 Digital NMR spectrometer, operating at 400.13MHz for ^{1}H and 100.6MHz for ^{13}C, respectively. Chemical shifts are reported in δ (ppm downfield from the TMS resonance) and coupling constants (*J*) are given in Hz. GC-MS was carried out Agilent Technologies 6890 model with automatic ALS, mass detector HP MD 5973 in spliless mode (5min).

Synthesis

All reactives and solvents with analytical grade were acquired from Sigma-Aldrich and Merck.

General procedure for chalcone synthesis (5a-h): To a dry 100-mL round-bottomed flask, the acetophenone 3 (250mg, 2.08mmol) and corresponding benzaldehyde 4a-h (1.2 molar equivalents) were added. Both reactive were solubilized in ethanol (5mL), NaOH saturated solution (in ethanol 20mL) was added and the mixture was stirred 48h. Then, 5% HCl solution was added until pH ≈ 7 to stop the reaction and extracted with EtOAc (3 x 30 mL). The organic layer was dried with Na_2SO_4, filtered and separated with a flash column using a mix hexane: EtOAc with increasing polarity, obtaining compounds 5a-h in yields between 55 to 99%.

Bioactivity

Cell culture: Human breast cancer MCF-7, human colorectal adenocarcinoma CACO-2, and human fibroblasts in primary culture were maintained in Dulbecco's Modified Eagle Medium (DMEM; Corning, Manassas, USA) supplemented with 10% fetal calf serum (FCS; Biological Industries, Belt Haemek, Israel), 2mM glutamine (Gibco, Carlsbad, USA) and penicillin 10U/L/streptomycin, 100µg/mL (Gibco, Carlsbad, USA). Cells were grown in T-75 culture flasks in a Thermo-Forma incubator with a humidified atmosphere at 37°C and 5.0% CO_2.

Cytotoxicity assay: The cytotoxic effect of synthetic compounds was evaluated according to Ahmed et al., based on the reduction of resazurin by viable cells to resorufin [30]. Initially, the cells were seeded on wells of 96 wells plates at a density of 5000 (MCF-7) or 15000 (Caco-2 and fibroblasts) cells/cm^2 and incubated for 24h. Then, both cell types were exposed to the synthetic chalcones at concentrations from 200µM to 1.0µM, using 0.1% DMSO in DMEM as the solvent. All assays were tested in triplicate wells and repeated at least twice. Control cells were incubated with 1% DMSO in DMEM. After 48h cell viability was assessed by adding a 4mg /L resazurin® (Sigma-Aldrich, St. Louis, USA) solution in cell culture media and measuring fluorescence after 4h at an excitation and emission wavelength of 544 and 590 nm, respectively using a plate reader (Thermo scientific Appliskan).

Results and Discussion

The synthetic chalcones were obtained from acetophenone and benzaldehydes using Claisen – Schmidt condensation in alkaline media with moderate to high yield (Table 1). In addition, each compound was analyzed spectroscopically (IR, NMR, and MS), as well as by comparison with previous reports in the literature [31-37].

Comparing the simplest chalcone on MCF-7 (5a) with the mono-substitution, the methoxy group position has a high relevance on antiproliferative activity because the 3'-OMe (5c) group has 6.2 folds more activity, while 2'-OMe (5b) and 4'-OMe (5d) substituent have similar activity on the same cell line (Table 1). In addition, considering di-substituted chalcones, 3',4'-OMe substitution (5f) has similar activity than (5a), while compounds with 2',5'-OMe (5e) and 3',4'-dioxomethylen (5g) have more activity than the simplest chalcone (between 2 to 14 folds, respectively, Table 1). Finally, the tri-substitution (5h) on MCF-7 cell line has no more activity than the simplest chalcone (Table 1). The antiproliferative effect on cancer cell line is an important property of potential anti-cancer drugs; however, the selectivity index (SI) is an important factor for developing new chemotherapeutic agents due to the undesirable side effect of typical antineoplastic drugs on the normal bystander cells [10]. In this context, the compound 5g has a selectivity index comparable to the more complex chalcones, obtained after several synthetic steps (Figure 2) [38-43].

On the other hand, in Caco-2 cell line the simplest chalcone has low activity on this cell line (compound 5a, IC_{50}= 123.4µM, Table 1). Comparing this result with mono-substitution with the methoxy group, the activity is improved on 3'-OMe with 6.9 folds of increase in activity than H, while the 2'-OMe substitution give a compound with 1.8 folds more activity than the original compound (Table 1). Considering the di-substitution, there is between 3.3 to 5.5 folds more activity in comparison with H (Table 1), however comparing the tri-substitution with the same target, the activity of compound 5h is only 1.9 folds more active (Table 1). Comparing the antiproliferative

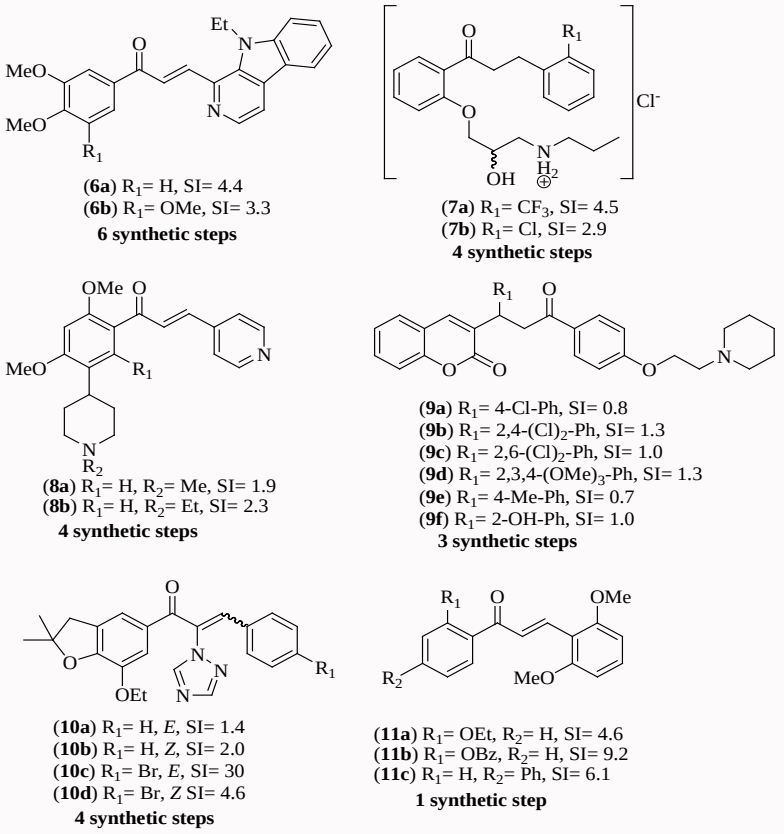


Figure 2: Comparison of the complex chalcone structure with similar or less selectivity index on MCF-7 cell line than small chalcones evaluated in this work.

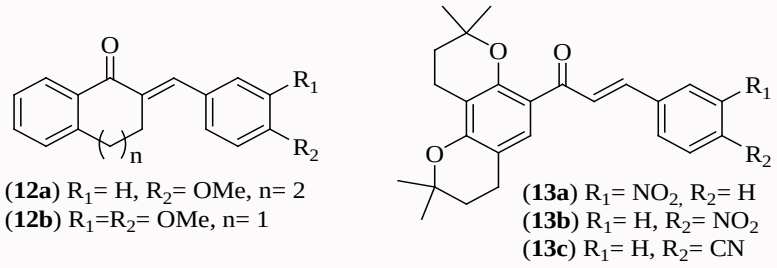
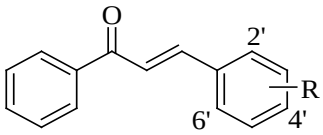


Figure 3: Synthetic chalcone analogues with antiproliferative activity on Caco-2 cell line.

Table 1: Synthetic chalcone obtention and their antiproliferative effect on fibroblasts, MCF-7 and CACO-2 cell lines.



Comp.	Substituent	Yield	Ref.	Fibroblast (IC_{50} µM)	MCF-7 (IC_{50} µM)	Caco-2 (IC_{50} µM)	SI[a] MCF-7	SI[b] Caco-2
5a	**H**	99 %	[31]	41.84 ± 1.2	33.1 ± 1.5	123.4 ±5.0	1.3	0.3
5b	**2'-OMe**	97 %	[32]	36.19 ± 1.1	36.7 ± 0.4	68.5 ±1.0	1.0	0.5
5c	**3'-OMe**	55 %	[33]	35.66 ± 0.2	5.3 ± 1.2	18.0 ±0.3	6.7	2.0
5d	**4'-OMe**	99 %	[31]	44.66 ± 1.9	38.1 ± 2.0	108.1 ±0.9	1.2	0.4
5e	**2',5'-$(OMe)_2$**	68 %	[34]	18.67 ± 0.2	16.3 ± 0.2	22.2 ±1.1	1.1	0.8
5f	**3',4'-$(OMe)_2$**	79 %	[35]	21.64 ± 0.4	30.1 ± 2.4	37.4 ±2.3	0.7	0.6
5g	**3',4'-(OCH_2O)**	95 %	[36]	20.47 ± 1.0	2.3 ± 0.6	22.3 ±1.3	9.1	0.9
5h	**2',4',5'-$(OMe)_3$**	74 %	[37]	40.56 ± 0.8	32.8 ± 1.1	62.7 ± 0.1	1.2	0.6

[a]SI MCF-7= IC_{50} fibroblast/IC_{50} MCF-7; [b]SI Caco-2= IC_{50} fibroblast/IC_{50} Caco-2.

activity of small chalcones on the Caco-2 cell line for structural related compounds, benzosuberone 12a and tetralone 12b (Figure 3) showed better antiproliferative activity in comparison with the compounds evaluated herein, however, the authors do not report selectivity index [44], the same happens for bis-dihydrochromane-chalcone (13a-c, Figure 3) [45].

Conclusion

The eight chalcones were synthesized using Claisen-Schmidt reaction in alkaline media with moderate to excellent yield. In addition, these compounds were evaluated on two cell lines: MCF-7 (breast cancer), Caco-2 (colorectal cancer) and a primary culture of fibroblast (non-cancer cell). In MCF-7 the substitution on position 3' with methoxy group is very important to increase its cytotoxic activity, as well as the 3',4'-(OCH_2O) substitution. In addition, these compounds exhibit low activity on the non-cancer cell line, which indicates that they could have a good selectivity. On the other hand, for the Caco-2 cell line, the mono- and di-substitution 3' and 3',4' results in very cytotoxic compounds, however, the same effects are shown on fibroblast, due to this, they required selectivity is not obtained.

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