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RESEARCH ARTICLE

Inhibition of Caco-2 and MCF-7 cancer cells using chalcones: synthesis, biological evaluation and computational study

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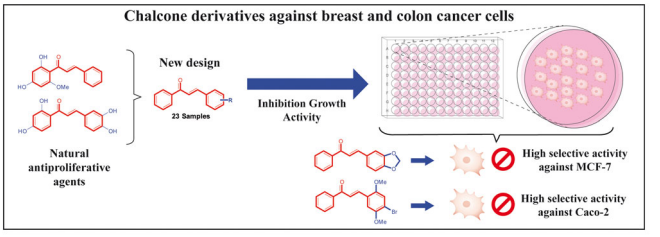
ABSTRACT

Cancer is the second death cause worldwide, with breast and colon cancer among the most prevalent types. Traditional treatment strategies have several side effects that inspire the development of novel anticancer agents derived from natural sources, like chalcone derivatives. For this investigation, twenty-three chalcones (**4a-w**) were synthesized and evaluated as antiproliferative agents against MCF-7 and Caco-2 cells, finding three and two compounds with similar or higher antiproliferative activity than daunorubicin, while only two chalcones showed better selectivity indexes than daunorubicin on MCF-7. From these results, we developed good-performance QSAR models ($r > 0.850$, $q^2 > 0.650$), finding several structural features that could modify chalcone activity and selectivity. According to these models, chalcones **4w** and **4t** have high potency and selectivity against Caco-2 and MCF-7, respectively, which make them attractive candidates for hit-to-lead development of ROS-independent pro apoptotic agents.



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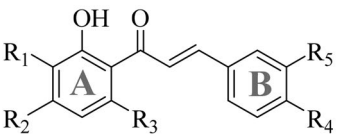


(**1a**) R_1= Prenyl, R_2=R_4= OH, R_3=R_5= H
(**1b**) R_1=R_4=R_5= H, R_2= OH, R_3= OMe
(**1c**) R_1= Prenyl, R_2= OMe, R_3=R_4=R_5= H
(**1d**) R_1=R_3=R_4=R_5= H, R_2= O-Prenyl
(**1e**) R_1= Prenyl, R_2=R_4= OH, R_3= OMe, R_5= H

Figure 1. Natural chalcones with antiproliferative effects on breast and colon cancer cell lines.

1. Introduction

According to the World Health Organization, cancer is the second cause of death worldwide (WHO 2015), with breast and colon cancer among the most prevalent cancer types in Latin America (Grosse et al. 2014). Despite the advances in chemotherapeutics, severe side effects reduce their effectiveness (Mai et al. 2014; Wang et al. 2017). Inspired by this, scientists around the world have studied nature for new sources of anticancer agents (Horneber et al. 2012), finding a wide range of chalcone derivatives with antiproliferative activity against cancer cells, including colon and breast cancer (see Figure 1), showing specific molecular mechanism for these effects (Michalkova et al. 2021). For example, isobavachalcone (**1a**) has high antiproliferative activity against MDA-MB-231 cells,(Kuete et al. 2015) and induce apoptosis through AKT/GSK-3β/β-catenin pathway inhibition;(Li et al. 2019) cardamonin (**1 b**) has high activity against MDA-MB-231 cells (Kuete et al. 2015) and inhibits breast cancer growth by repressing HIF-1α;(Jin et al. 2019) derricin (**1c**) and derricidin (**1d**) have antiproliferative activity on HT-29 cells by modulating the Wnt/β-catenin axis;(Fonseca et al. 2015) and xanthohumol (**1e**) has antiproliferative activity against HT-29 cells (Fonseca et al. 2015) by increasing caspases-3/9 activation and Bax/Bcl-2 ratio (Liu et al. 2019).

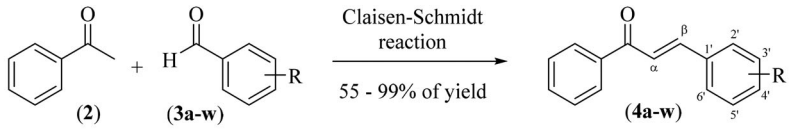
According to the previous examples of natural chalcones, we simplified the A ring of these compounds (see Scheme 1) and assessed their antiproliferative activity against MCF-7 and Caco-2 cells. Additionally, we performed a Quantitative Structure-Activity Relationship (QSAR) analysis to identify the structural features that could modify antiproliferative activity and selectivity. Finally, we proposed a cell death mechanism to explain the antiproliferative effects of these chalcone derivatives.

2. Results and discussion

2.1. Chemistry

Chalcones (**4a-w**) were obtained with moderate to excellent yields (55 – 99%) through a Claisen-Schmidt reaction between acetophenone (**2**) and the corresponding benzaldehydes (**3a-w**) (Scheme 1).

In order to check if the desired compounds were obtained, spectroscopic analysis was performed (see Supplementary Material). Briefly, ^{1}H-NMR spectra showed two doublet signals at downfield (δ∼ 7.5 to 8.0 ppm) with a coupling constant of *J*∼ 16.0 Hz, which corresponds to the *trans* α and β hydrogens of the chalcone core. In addition, spectroscopic evidence of all synthetic compounds was compared with previous reports from our research team (Mellado et al. 2018, 2020, 2021).



(**4a**) R= H
(**4b**) R= 2'-Me
(**4c**) R= 2'-OMe
(**4d**) R= 2'-OC_6H_{13}
(**4e**) R= 3'-Me
(**4f**) R= 3'-OMe
(**4g**) R= 4'-Me
(**4h**) R= 4'-OH
(**4i**) R= 4'-OMe
(**4j**) R= 4'-NMe_2
(**4k**) R= 2'-OH-3'-OMe
(**4l**) R= 2'-OC_6H_{13}-3'-OMe
(**4m**) R= 2'-OH-3'-NO_2
(**4n**) R= 2',5'-$(OAc)_2$
(**4o**) R= 2',5'-$(OMe)_2$
(**4p**) R= 2',5'-$(OC_6H_{13})_2$
(**4q**) R= 3'-OMe-4'-OH
(**4r**) R= 3',4'-$(OMe)_2$
(**4s**) R= 3'-OMe-4'-OC_6H_{13}
(**4t**) R= 3'-(OCH_2O)-4'
(**4u**) R= 2'-OH-3'-OMe-5'-NO_2
(**4v**) R= 2',4',5'-$(OMe)_3$
(**4w**) R= 2',5'-$(OMe)_2$-4'-Br

Scheme 1. Synthetic chalcone obtained from Claisen-Schmidt reaction.

2.2. Antiproliferative activity and selectivity

Antiproliferative activity of all chalcone derivatives (**4a-w**) was carried out using the Alamar Blue reduction method. These measurements showed that all of the analysed chalcones (except **4p**) were active against both MCF-7 and Caco-2 cells (**Table S1**), relating these effects to the presence of α,β-unsaturated Michael acceptor fragments (Abonia et al. 2012), which can give the capability to act as alkylating agents (Ahn and Sok 1996). For these assays, four drugs, three intercalating agents and one antimetabolite, were used as positive controls: daunorubicin, oxaliplatin, *cis*-platin, and 5-fluorouracil. Among them, daunorubicin showed the higher antiproliferative activity and selectivity against cancer cells. For this reason, daunorubicin was used to compare the activity and selectivity of chalcone derivatives. In Caco-2, three compounds showed similar antiproliferative activity than daunorubicin (IC_{50}= 3.9 μM, compounds **4f**, **4k**, and **4w**), having all an electron-donor group in the B ring position 3′. Regarding this position, a bulky substituent on 3′ improved the antiproliferative activity (IC_{50} Me > OMe), while similar substituents on position 4′ reduced activity (IC_{50}> 100 μM, compounds **4 h-j**). Therefore, these effects could be related to a high electron density on any fragment of this ring (see QSAR section). About the selectivity index (SI) on Caco-2 cells, daunorubicin showed a SI = 4.4, and only two chalcone showed similar SI to this agent (**4 b** and **4d**, **Table S1**).

Analysing the effect of chalcone derivatives on MCF-7, compound **4t** showed higher antiproliferative activity than daunorubicin, and chalcone **4f** had similar effects. These compounds have an electron-donor substituent at positions 3′ and 4′. Moreover, compound **4f** has a 3′-OMe substituent that contributes to its antiproliferative activity against MCF-7. However, when this -OMe substitution position is changed, the antiproliferative activity decreases significantly (**4c**: 6.8-foldin 2′-substitution, **4i**: 7.1-foldin 4′-substitution). Interestingly, the most potent compound on MCF-7 is **4t** (IC_{50}= 2.3 μM, **Table S1**), being 1.6-times more active than daunorubicin. This chalcone has a dioxomethylene substitution on 3′,4′-positions, and its potent activity is similar to the previously reported (Mai et al. 2014). Regarding daunorubicin selectivity against MCF-7, we observed a SI = 4.6, while two chalcone derivatives had better indexes than daunorubicin (**4f** and **4t**, SI= 6.7 and 9.1, respectively; **Table S1**).

Due to the poly substitution on chalcone ring B, the structural features that improved antiproliferative activity and selectivity against Caco-2 and MCF-7 cell lines are detailed in the following section.

2.3. Quantitative structure-activity relationship (QSAR)

In order to understand the relationship between antiproliferative activity and SI with the structural features of chalcones, we performed a QSAR study according to previous reports made by our research group (see **Supplementary material**) (Montenegro et al. 2019; López et al. 2020; Luczywo et al. 2021), obtaining models that had proper statistical values in gas and condensed phases (**Tables S2–S5**), which are described by the following equations:

$$pIC_{50\ Caco-2} = 0.11 - 0.21\boldsymbol{\omega} - 14.8\boldsymbol{C_{6'}} \quad \text{(Eq. 1)}$$

$$pIC_{50\ MCF-7} = 6.62 - 0.09\mathbf{RB} + 0.43\boldsymbol{\mu} \quad \text{(Eq. 2)}$$

According to these models, antiproliferative activity on Caco-2 cells is related to $C_{6'}$ atomic charge and global electrophilicity index (ω, **Eq. 1**), being the latter descriptor used in previous reports to relate chalcones antiproliferative activity against NALM-6 cells (Kupcewicz et al. 2014) and this relationship could be explained by the presence of α,β-unsaturated Michael acceptor fragments (Abonia et al. 2012). Moreover, **Eq. 1** showed that increasing negative charges on $C_{6'}$ could improve antiproliferative activity (*e.g.*,**4f**, **4k**, **4n-o**, **4q-t**, **Table S1**). With this information, new calculations were made using compound **4w** as a template, obtaining that electron-donating groups substituted on *meta*-position increase electronic density around $C_{6'}$ (**Table S10**).

On the other hand, when we analysed the antiproliferative effect of chalcones on MCF-7, we found relationships with rotatable bonds (RB) amount and chemical potential (μ, **Eq. 2**). RB has been used earlier for QSAR modelling on human P-glycoprotein inhibitors (Parveen et al. 2014). However, in our case, chalcone activity against MCF-7 has a stronger relationship with μ, which corresponds to the electron-donating capacity from HOMO to LUMO (Lopez et al. 2013). As a descriptor, μ has been used previously to relate chalcones antiproliferative effects on HL-60 and WM-115 cells (Kupcewicz et al. 2014). Following **Eq. 2** parameters, we calculated a series of new B ring-modified chalcones in order to increase the antiproliferative effect on MCF-7, obtaining that electron-donating substituents on position 6′ can achieve the desired effects for μ (**Table S11**).

QSAR analysis for SI in both Caco-2 and MCF-7 cells showed proper models for gas and condensed phases (**Table S6–S9**), obtaining the following equations for each cell lines:

$$SI_{Caco-2} = -0.28 + 19.8\boldsymbol{C_4} - 3.85\boldsymbol{C_{6'}} - 11.8\boldsymbol{C_\alpha} \quad \text{(Eq. 3)}$$

$$SI_{MCF-7} = -17.96 + 8.36\mathbf{Vol} * \gamma - 7.59\boldsymbol{C_{2'}} - 58.0\boldsymbol{C_\alpha} \quad \text{(Eq. 4)}$$

In **Eq. 3** was found that Caco-2 SI is related only to C_4, $C_{6'}$, and C_α partial atomic charges, being this descriptor associated with the nature of the substituents bonded to a specific atom (Gross et al. 2002). These results are similar to those found by our group on chalcones selectivity against SH-SY5Y cells (Mellado et al. 2018). In this equation can also be observed that C_4 and C_α descriptors have major relevance (5.1 and

3.1-fold more important than $C_{6'}$, respectively), which drives the conclusion that any improvement on these characteristics could enhance the SI against Caco-2. With these parameters, new molecules were designed, finding that electron-donating substituents on R_3 increased SI against Caco-2 (**Table S12**).

About the MCF-7/SI relationship, **Eq. 4** shows that SI is correlated with atomic charges on $C_{2'}$ and C_α and molecular volume (MV), being the later descriptor a steric feature linked with xenobiotic-receptor interaction (Karcher and Devillers 1990). Comparing these variables, we found that $C_{2'}$ and C_α atomic charges are ~900-times more important than MV. $C_{2'}$ and C_α descriptors have negative slopes, so increasing the negative characteristic of these values, could enhance chalcones SI on MCF-7 cells. With these results, and using compound **4t** as template, new molecules were calculated, finding that halogen substitutions on $R_{5'}$ and $R_{6'}$ could increase $C_{2'}$ and C_α negative character (**Table S14**).

The structural features obtained for Caco-2 and MCF-7 in 2 D-QSAR models are summarized in **Figure S2**.

Finally, in order to describe if the evaluated chalcones produced their effects by apoptosis induction, pan-caspase activity, caspase 3/7 activity, Bcl-2/Bax mRNA levels, DNA fragmentation and ROS generation were measured using the most selective compound (**4t**, SI= 9.1 on MCF-7) (**Figure S3–S7**). According to these assays, compound **4t** induced caspases activation, increased executor caspases (3/7) activity, reduced Bcl-2/Bax mRNA ratio, and promoted an apoptotic DNA fragmentation pattern on MCF-7 cells, which is in agreement with the proapoptotic effects described for other chalcones with structural similarity (Hsu et al. 2006). However, this chalcone did not show any effect on ROS generation, being this in contrast with the typical effects described for chalcones on this activity (Villena et al. 2021). These interesting findings highlight compound **4t** as a ROS-independent proapoptotic agent with a promising applicability on the treatment of breast cancer.

3. Experimental

Methodological aspects of this paper are detailed as **Supplementary Material.**

4. Conclusion

Inspired by natural compounds, twenty-three chalcones were synthesized and assessed as antiproliferative agents against Caco-2 and MCF-7 cells, finding three and two compounds with similar or higher antiproliferative activity than doxorubicin. While two chalcones showed better selectivity indexes than doxorubicin on MCF-7 cells. From these bioactivity results, we developed QSAR-models with proper statistical values ($r > 0.850$, $q^2 > 0.650$), finding several structural features that could modify chalcone's antiproliferative activity and selectivity. Our findings suggest that **4w** and **4t** are attractive candidates for hit-to-lead development of ROS-independent proapoptotic compounds against Caco-2 and MCF-7 cancer cell lines, respectively.

Disclosure statement

No potential conflict of interest was reported by the authors.

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