

**The luminescent Chalcones. Its potential use as a luminescent and antitumoral agents**  
**Chalconas luminiscentes. Su uso potencial como agentes luminiscentes y antitumorales**

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### Abstract

**Background:** Hepatocellular carcinoma (HCC) is one of the most diagnosed cancers worldwide. Chemoprevention of HCC can be achieved using natural or synthetic compounds that reverse, suppress, detect, or prevent cancer progression. **OBJECTIVES:** In this study, both the antiproliferative effects and luminescent properties of 2'-hydroxychalcones were evaluated.

**Methods:** Cell viability was evaluated using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) colorimetric assay, spectroscopy assays, and density functional theory (DFT) calculations were used to determine the luminescent properties of 2'-hydroxychalcones.

**Results:** Cytotoxic effects of 2'-hydroxychalcones were observed over the HepG2 and EA.hy926 cells. Since the chalcone moiety could be used as a fluorescent probe, these compounds may be helpful in cancer diagnosis and tumor localization. They may enable tumor observation and regression through the fluorescence during treatment; therefore, the compounds are a potential candidate as novel anticancer agents acting on human hepatomas.

**Conclusions:** This report describes the chalcones' use as a specific luminescent biomarker in tumor cells. We also report the cellular uptake of 2'-hydroxychalcones, their cellular distribution, and the mechanisms that may be responsible for their cytotoxic effects.

**Keywords:** 2'-hydroxychalcones; anticancer agent; luminescent biomarker; Cytotoxic effects

### Resumen

**Antecedentes:** El carcinoma hepatocelular (CHC) es uno de los cánceres más diagnosticados en todo el mundo. La quimio prevención del CHC se puede lograr utilizando compuestos naturales o sintéticos que reviertan, supriman, detecten o prevengan la progresión del cáncer.

**Objetivos:** En este estudio, se investigó tanto los efectos antiproliferativos como las propiedades luminiscentes de las 2'-hidroxicalconas.

**Métodos:** La viabilidad celular se evaluó usando el ensayo colorimétrico (MTT), los ensayos de espectroscopia y los cálculos DFT se usaron para determinar las propiedades luminiscentes de las 2'-hidroxichalconas.

**Resultados:** Se observaron efectos citotóxicos sobre las líneas celulares del tipo HepG2 y EA.hy926. Dado que la estructura de la 2'-hidroxichalcona puede ser usada como sonda fluorescente, estos compuestos pueden ser útiles en el diagnóstico del cáncer y la localización del tumor, ya que pueden permitir la observación a través de la fluorescencia y la regresión del tumor durante el tratamiento, por lo que son candidatas potenciales como nuevos agentes anticancerígenos que podrían actuar sobre hepatomas humanos.

**Conclusiones:** Este trabajo describe el uso de las 2'-hidroxichalconas como un biomarcador luminiscente específico para células tumorales. También informamos la captación celular de 2'-hidroxichalconas, su distribución celular y los mecanismos que pueden ser responsables de sus efectos citotóxicos.

**Palabras Claves:** 2'-hidroxichalconas; agente anticancerígeno; biomarcador luminiscente; efectos citotóxicos.

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## Introduction

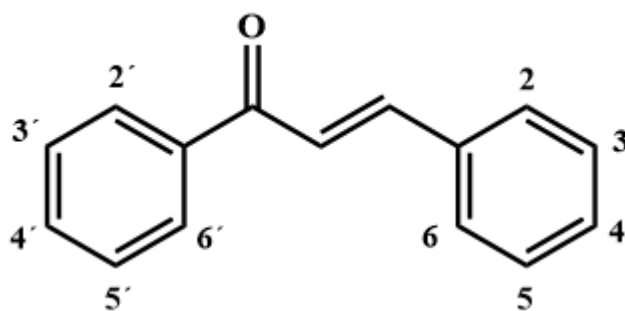
Chalcones are precursors of flavonoids and isoflavonoids present in edible plants. Their derivatives have attracted increasing attention due to their numerous potential pharmacological applications. They have displayed a broad spectrum of pharmacological activities that derive from the changes introduced in their structure by adding different substituents in their aromatic rings. These variations proved to be helpful for developing new medicinal agents, having improved potency and lesser toxicity (1–3).

Chalcones are important structural units, most of which exhibit significant biological activity. The wide range of biological activity associated with many Chalcone derivatives isolated from natural products has stimulated interest in developing synthetic strategies to synthesize heterocyclic systems based on the chalcone core. Along with efficient methodologies implemented for their preparation, investigations have also focused on studying their reactivity and assessing their possible biological activities (4–6). Particularly in the pharmaceutical field, chalcones and their derivatives have been used in different applications for treating cancer (6–12).

Chalcones can be used as a fluorescent probe because of their pi-conjugated propenone core (13–15). Therefore, the potential use of luminescent chalcones in biological and medical fields depends on the knowledge of their mode of action.

Up to date, luminescent biomarker properties of chalcones were almost unknown. Therefore, to promote the use of chalcones as biomarkers in biological and medical fields, more studies concerning their cellular uptake, subcellular localization, and cytotoxicity (3,6–8,10,11,14) should be undertaken.

In this study, we evaluated both the antitumoral activity and the luminescent properties of a set of synthetic chalcone derivatives (Figure 1) to assess their potential as biomarker agents.



Chalcones	2'	4'	5'	2	3	4	5
1	OH	OCH <sub>3</sub>	H	OCH <sub>3</sub>	OCH <sub>3</sub>	H	H
2	OH	H	H	OCH <sub>3</sub>	H	H	OCH <sub>3</sub>
3	OH	H	H	OCH <sub>3</sub>	H	OCH <sub>3</sub>	H
4	H	H	Br	H	OCH <sub>3</sub>	OCH <sub>3</sub>	H

**Figure 1: Chemical structures of the studied chalcones**

## Materials and Methods

### Synthesis of Chalcones

The synthesis of the compounds 1, 2, 3, 4 has been previously described as follows: Chalcones were prepared following a previously described methodology (8,11,16) by adding, in a dropwise manner, a solution of the corresponding substituted benzaldehyde, (7.34 mmol in 98 ethanol, 20 mL) to a stirred mixture of 2'-hydroxyacetophenone solution (7.34 99 mmol, in ethanol, 20 mL) and potassium hydroxide solution (2g in 10 mL distilled water). The mixture was allowed to react overnight at room temperature. Then, it was diluted with distilled water (200 mL), neutralized with hydrochloric acid, and extracted four times with ethyl acetate (50 mL). The compounds were crystallized from ethanol. All these compounds have been described before (8,11,16).

### Cell culture

The HepG2 hepatocellular carcinoma cell line (HB 8065; American Type Culture Collection, ATCC), derived from a human hepatoblastoma (17), was maintained in Dulbecco's modified Eagle's (DMEM)-high glucose (GIBCO) with 10% heat-inactivated fetal bovine serum (FBS), and 50 U mL<sup>-1</sup> penicillin-streptomycin (Sigma). HUVEC-derived endothelial cell line (EA.hy926) was kindly provided by C-J Edgell. It was grown in DMEM-low glucose (GIBCO) supplemented with 10% heat-inactivated FBS, 2 mmol L<sup>-1</sup>, and 50 U mL<sup>-1</sup> penicillin-streptomycin (Sigma). All cell cultures were grown at 37 °C in a 5%: 95% CO<sub>2</sub>: air atmosphere.

## Cell viability assay

Cell viability was evaluated using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) colorimetric assay (Invitrogen, Eugene, Oregon, USA). Cell viability was quantified by the amount of MTT reduction assay (18) HepG2 and EA.hy926 were exposed to different concentrations of chalcones for 48 hours. After treatment cells were co-incubated with MTT (0.5 mg/mL) for 4 hours, and then solubilized with an acidified (0.04 N HCl) isopropanol/dimethyl sulfoxide (DMSO) solution in equal proportions. Optical density was measured at 540 nm. All experiments were performed as triplicates. Cell survival (%) data were plotted and adjusted to a sigmoidal best-fit curve, where:  $EC_{50}$  is the chalcone concentration to reach the half-maximal cell survival.

## Epifluorescence microscope in live cells

HepG2 and EA.hy926 cells cultured in 6-well plates were exposed to different concentrations of chalcones for 24 hours. The cells were washed twice using phosphate-buffered saline (PBS) and visualized using a FLOID® Cell Imaging Station ( $N = 3-5$ ).

UV-Vis absorption measurements

Electronic spectroscopic data were collected at room temperature on a MeOH solution using a Jasco V-630 UV-vis spectrophotometer. Measurements were recorded between 1000-190 nm with a data interval of 0.1 nm.

## Fluorescence measurements

Emission and excitation spectra were recorded on a JASCO spectrofluorometer (FP-6200) using a 1 cm path length JASCO spectrofluorometer (FP6200) using a quartz cuvette of 1 cm path length. Excitation wavelengths for all complexes were set to 347, 405, 460, and 362 nm. Those wavelengths correspond to the maximum absorption in the UV-Vis spectra. The fluorometric characterization was carried out at room temperature. All emission spectra measurements were recorded between 250 and 750 nm.

## Computational Details

The geometry optimizations and computational properties of the Chalcone family were performed using density functional theory (DFT) implemented in the Amsterdam density functional package (ADF2012) (19).

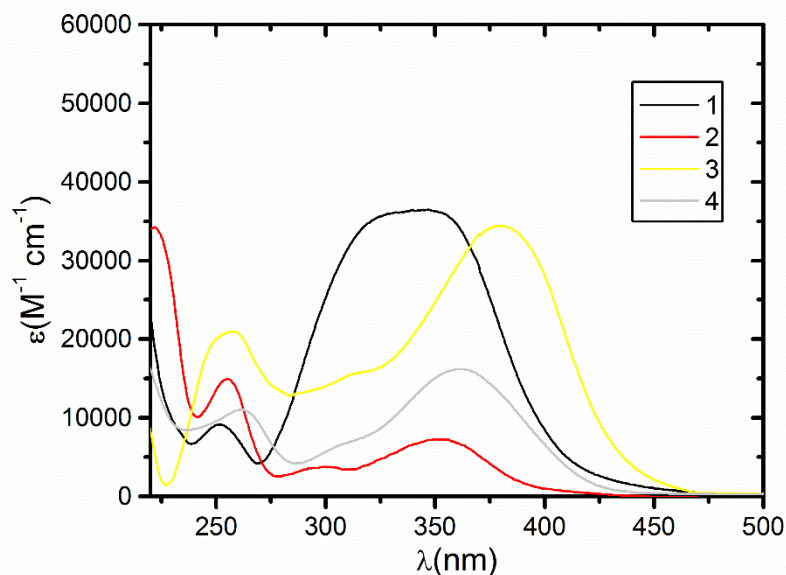
The computations of the ground and excited states were carried out using the ZORA Hamiltonian incorporating scalar relativistic corrections (20). Triple- $\zeta$  Slater basis set plus two

polarization functions (TZ2P) <sup>(21)</sup> and the nonlocal correction for the exchange and correlation (XC) incorporated via the general gradient approximation (GGA) within the functional BLYP were employed. The molecular structures were fully optimized without symmetry constriction. The excitation energies were estimated by Time-Dependent Density Functional Theory (TDDFT) <sup>(22)</sup>. This methodology is based on the linear response formalism within the iterative Davison procedure implemented in the ADF code. The calculations performed by the first-principles method let to obtain accurate excitation energies and oscillator strengths for the calculated complexes. Solvation effects were simulated by a ‘‘Conductor-like Screening Model’’ (COSMO) <sup>(23)</sup> using methanol as solvent.

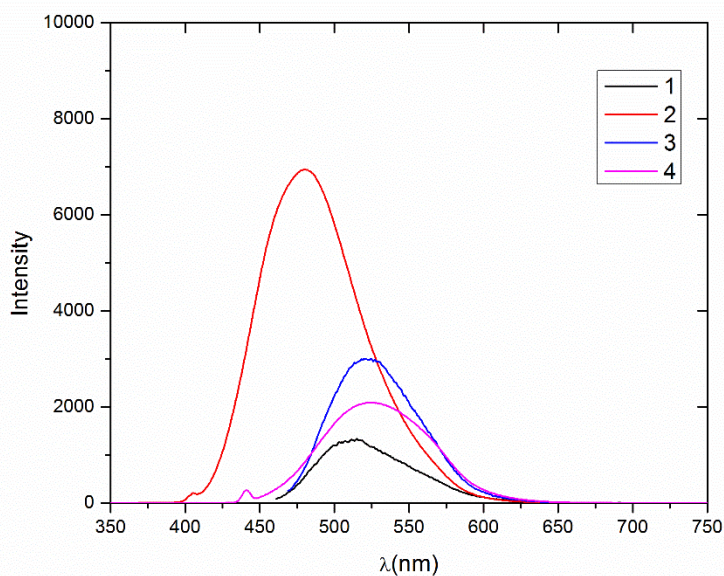
## Results and Discussions

A luminescent biomarker is a possible indicator of the biological state of the disease. It is characteristic of a specific state and, therefore, can be used as a marker for a target disease. These biomarkers are usually appropriated to study cellular processes and monitor or recognize disruption or alterations in the cellular processes of cancer cells. Luminescent biomarkers, specifically cancer biomarkers, indicate cancer presence, and detecting them can verify the existence of that specific disease <sup>(24,25)</sup>.

The optical properties of the compounds indicate that all the structures allow  $\sigma \rightarrow \sigma^*$  or  $\pi \rightarrow \pi^*$  transitions in the UV-Vis region with high extinction coefficients (Figures 2 and 3). The frontier orbitals are the highest occupied MO (HOMO) and the lowest unoccupied MO (LUMO). We focused on these two orbitals because they are the closest in energy. These orbitals are intimately involved in chemical reactivity because they are the most available to electrophiles and nucleophiles, respectively. Another key change has to do with the frontier orbitals, the  $\pi$  (HOMO) and  $\pi^*$  (LUMO) orbitals.



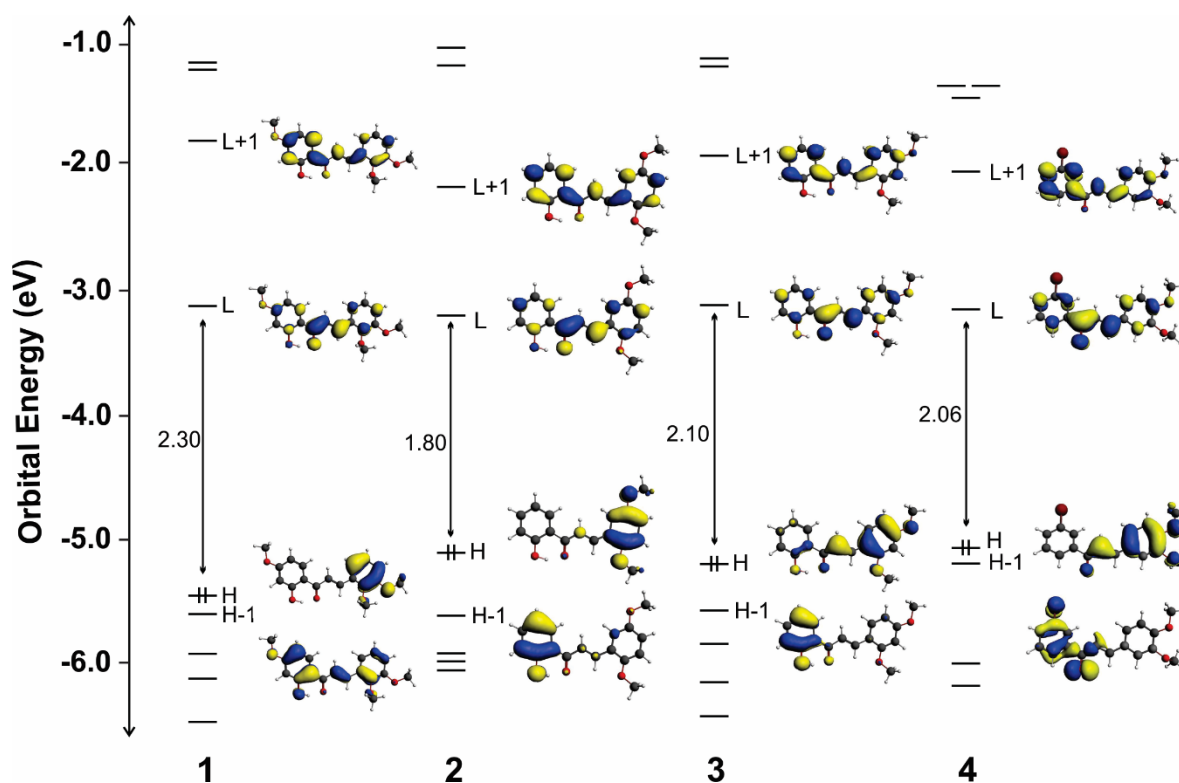
**Figure 2. Experimental UV-Vis absorption spectra of the compounds 1 to 4.**



**Figure 3. Experimental emission spectra of compounds 1, 2, 3 and 4.**

The HOMO represents the ability to donate an electron, and LUMO as an electron acceptor represents the ability to get an electron. This electronic absorption corresponds to the transition from the ground to the first excited state. This is mainly described by one electron excitation from the highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO) are the main orbitals taking part in chemical stability. Also, 3D plots of HOMOs and LUMOs for the title compounds are shown in Figure 4 and considered all occupied and unoccupied energy levels in a diagram that shows how the energy changed among the levels.

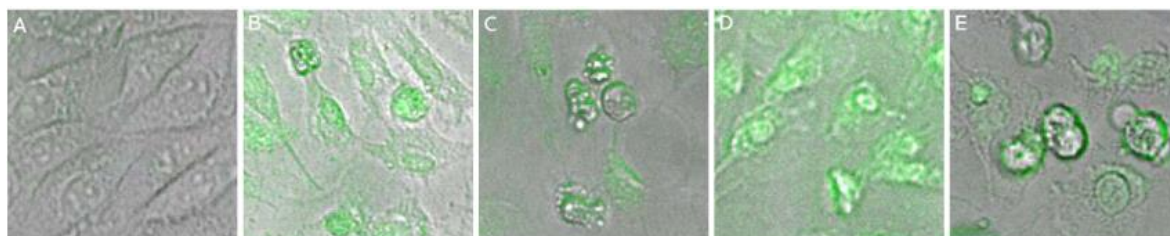
Figure 4 shows the orbital energy levels of the frontier Kohn-Sham molecular orbitals (MOs) and the isosurfaces plots of the lowest unoccupied molecular orbital (LUMO+1 and LUMO) and the highest occupied molecular orbitals (HOMO and HOMO-1). In the case of the LUMO energy of the studied chalcones, these lie at approximately -3.1 eV. In chalcones 3 and 4, the HOMO energy is approximately -5.2 eV. Chalcone 1 displays the most stable HOMO showing the largest HOMO-LUMO gap, and chalcone 2 displays the less stable HOMO showing the shortest HOMO-LUMO gap. In all compounds, the HOMOs are confined to the p-orbitals of the same ring, but 3 and 4 show significance of the bridge orbitals. HOMO-1 orbitals are confined to the p-orbitals over the other ring. On the contrary, the LUMO orbitals are extended over the whole molecules with few compositions of the substituents.



**Figure 4. DFT calculated Isosurfaces of the frontier molecular orbitals and energy levels diagram.**

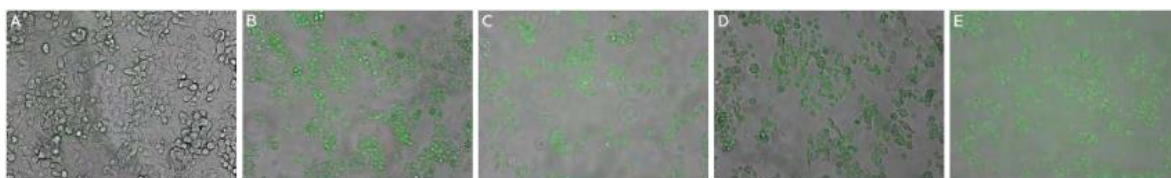
The biological properties of chalcones and the potential use of luminescent chalcones depends on the investigation of their cellular uptake, subcellular localization, and cytotoxicity.

Considering that EA.hy926 and HepG2 cells showed a cytotoxic response to the chalcones, we exposed these cells to a value close to IC<sub>50</sub> for 24 hours and observed by a FLOID® Cell Imaging Station the intrinsic fluorescence. Cell membrane penetration of the EA.hy926 and HepG2 cells for 24 hours showed chalcones inside the cells, localized mainly in the cytoplasm. The HepG2 and EA.hy926 cells showed similar intracellular accumulation of chalcones, whereas cells treated with the vehicle did not exhibit green fluorescence (Figures 5a and 6a). Cells treated with chalcones for 24 hours showed intracellular green marks that were attributed to the presence of the chalcones (Figures 5b,c,d,e, and 6b,c,d,e). These results demonstrated that chalcones entered to the cells.



**Figure 5. Incorporation and morphologic changes in EA.hy926 cells exposed to chalcones. EA.hy926 cells were grown for 24 h, then treated with chalcones for 24 h. Figures show representative phase-contrast images from at least three separated experiments of EA exposed to vehicle (control) (A), 10 μM of compound 1(B), 10 μM of compound 2 (C), 10 μM of compound 3 (D), 5 μM of compound 4 (E).**



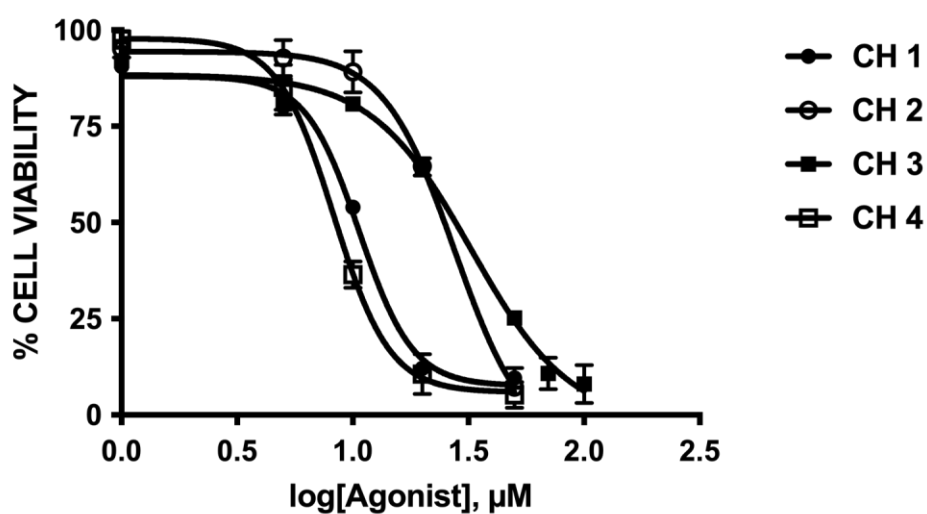


**Figure 6. Incorporation and morphologic changes in HepG2 cells exposed to chalcones. HepG2 cells were grown for 24 h, then treated with chalcones for 24 h. Figures show representative phase-contrast images from at least three separates experiments of HepG2 exposed to vehicle (control) (A), 15  $\mu\text{M}$  of compound 1(B), 30  $\mu\text{M}$  of compound 2 (C), 30  $\mu\text{M}$  of compound 3(D), 10  $\mu\text{M}$  of compound 4 (E).**

The current report describes for the first time the use of chalcones as a specific luminescent biomarker agent against tumor cells. We studied the cytotoxicity of chalcones in different cell cultures from tumoral cell lines (table 1 and figure 7) to assess its antitumor activities. We also report the cellular uptake of luminescent chalcones and their subcellular distribution (Figures 5 and 6).

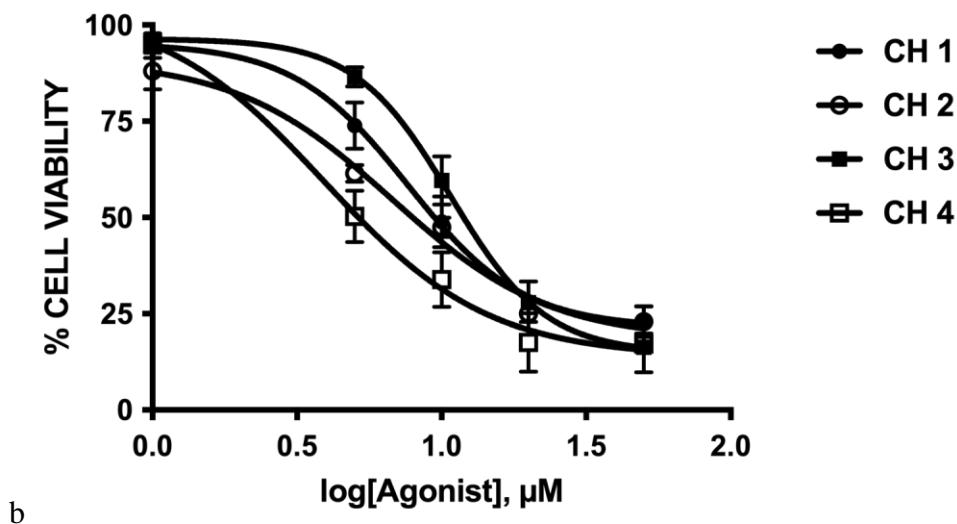
**Table 1. The  $\text{IC}_{50}$  value on EA.hy926 and HepG2 incubated with different chalcones for 48 hours. Cell viability was measured using the MTT assay. Data area expressed as mean  $\pm$  SEM from the three independent experiment, each performed in triplicate.**

Chalcone	EA.hy926 $\text{IC}_{50}/\mu\text{M}$	HepG2 $\text{IC}_{50}/\mu\text{M}$
1	$7.66 \pm 0.46$	$10.65 \pm 0.98$
2	$6.76 \pm 1.89$	$27.61 \pm 2.51$
3	$10.73 \pm 0.11$	$31.26 \pm 2.42$
4	$3.92 \pm 1.34$	$8.27 \pm 0.19$



a





**Figure 7. Effect of Chalcones on growth of (a) HepG2 and (b) EA.hy926 cells, which were treated with various concentrations of chalcones (0.5-2 mg/mL) for 48h.**

## Conclusion

Based on their fluorescent properties, the chalcones may be helpful for cancer diagnostics, tumor localization, and may enable the observation through the fluorescence of tumor regression during treatment. The findings reported here represent the first attempts to use chalcones as biomarkers in cancer disease. We are currently planning new experiments to shed light on the complex mechanisms involved.

## References

1. Rahman MA, Chalcone : A Valuable Insight into the Recent Advances and Potential. *Chem. Sci.* 2011, 2011, 1–16, DOI: <https://doi.org/10.4172/2150-3494.1000021>
2. Kumar V, Kumar S, Hassan M, Wu H, Thimmulappa RK, Kumar A, Sharma SK, Parmar VS, Biswal S, Malhotra S V Novel chalcone derivatives as potent Nrf2 activators in mice and human lung epithelial cells. *J. Med. Chem.* 2011, 54, 4147–59, DOI: <https://doi.org/10.1021/jm2002348>.
3. Marquina S, Maldonado-Santiago M, Sánchez-Carranza J N, Antúnez-Mojica M, González-Maya L, Razo-Hernández RS, Alvarez L, Design, synthesis and QSAR study of 2'-hydroxy-4'-alkoxy chalcone derivatives that exert cytotoxic activity by the mitochondrial apoptotic pathway. *Bioorganic Med. Chem.* 2019, 27, 43–54, DOI: <https://doi.org/10.1016/J.BMC.2018.10.045>.
4. Tadigoppula N, Korthikunta V, Gupta S, Kancharla P, Khaliq T, Soni, A, Srivastava RK, Srivastava K, Puri S K, Raju K S R, et al. Synthesis and insight into the structure-activity relationships of chalcones as antimalarial agents. *J. Med. Chem.* 2013, 56, 31–45, DOI: <https://doi.org/10.1021/jm300588j>.
5. Shenvi S, Kumar K, Hatti K S, Rijesh K, Diwakar L, Reddy G C, Synthesis, anticancer and antioxidant activities of 2,4,5-trimethoxy chalcones and analogues from asaronaldehyde: structure-activity relationship. *Eur. J. Med. Chem.* 2013, 62, 435–42, DOI: <https://doi.org/10.1016/j.ejmech.2013.01.018>.

6. Zarate X, Schott E, Escobar C A, Lopez-Castro R, Echeverria C, Alvarado-Soto L, Interaction of Chalcones with CT-DNA by Spectrophotometric Analysis and Theoretical Simulations. *Quim. Nova* 2016, 39, 914–918.
7. Dickson J, Flores L, Stewart M, LeBlanc R, Pati H N, Lee M, Holt H, Synthesis and Cytotoxic Properties of Chalcones: An Interactive and Investigative Undergraduate Laboratory Project at the Interface of Chemistry and Biology. *J. Chem. Educ.* 2006, 83, 934, DOI: <https://doi.org/10.1021/ed083p934>.
8. Echeverria C, Santibañez J F, Donoso-Tauda O, Escobar C , Ramirez-Tagle R , Structural antitumoral activity relationships of synthetic chalcones. *Int. J. Mol. Sci.* 2009, 10, 221–31, DOI: <https://doi.org/10.3390/ijms10010221>.
9. Detsi A, Majdalani M, Kontogiorgis C, Hadjipavlou-Litina D, Kefalas P, Natural and synthetic 2'-hydroxy-chalcones and aurones: synthesis, characterization and evaluation of the antioxidant and soybean lipoxygenase inhibitory activity. *Bioorg. Med. Chem.* 2009, 17, 8073–85, DOI: <https://doi.org/10.1016/j.bmc.2009.10.002>.
10. Boumendjel A, Boccard J, Carrupt P A, Nicolle E, Blanc M, Geze A, Choisnard L, Wouessidjewe D, Matera E L, Dumontet C, Antimitotic and antiproliferative activities of chalcones: forward structure-activity relationship. *J. Med. Chem.* 2008, 51, 2307–10, DOI: <https://doi.org/10.1021/jm0708331>.
11. Ramirez-Tagle R, Escobar C, Romero V, Montorfano I, Armisen R, Borgna V, Jeldes E, Pizarro L, Simon F, Echeverria C, Chalcone-Induced Apoptosis through Caspase-Dependent Intrinsic Pathways in Human Hepatocellular Carcinoma Cells. *Int. J. Mol. Sci.* 2016, 17, 260, DOI: <https://doi.org/10.3390/ijms17020260>.
12. Suwito H, Jumina M, Pudjiastuti P, Fanani M Z, Kimata-Arigo Y, Katahira R, Kawakami T, Fujiwara T, Hase T, et al. Design and synthesis of chalcone derivatives as inhibitors of the ferredoxin - Ferredoxin-NADP<sup>+</sup> reductase interaction of Plasmodium falciparum: Pursuing new antimalarial agents. *Molecules* 2014, 19, 21473–21488, DOI: <https://doi.org/10.3390/MOLECULES191221473>.
13. Jagtap AR, Satam VS, Rajule RN, Kanetkar VR, Synthesis of highly fluorescent coumarinyl chalcones derived from 8-acetyl-1,4-diethyl-1,2,3,4-tetrahydro-7H-pyrano(2,3-g)quinoxalin-7-one and their spectral characteristics. *Dye. Pigment.* 2011, 91, 20–25, DOI: <https://doi.org/10.1016/j.dyepig.2011.01.011>.
14. Asiri A, Marwani H, Green Synthesis, Characterization, Photophysical and Electrochemical Properties of Bis-chalcones. *Int. J. Electrochem.* 2014, 9, 799–809.
15. Xue Y, Mou J, Liu Y, Gong X, Yang Y, An L, An ab initio simulation of the UV/Visible spectra of substituted chalcones. *Cent. Eur. J. Chem.* 2010, 8, 928–936, DOI: <https://doi.org/10.2478/s11532-010-0058-3>.
16. Zarate X, Schott E, Escobar CA, Lopez-Castro R, Echeverria C, Alvarado-Soto L, Ramirez-Tagle, R. Interaction of chalcones with CT-DNA by spectrophotometric analysis and theoretical simulations. *Quim. Nova* 2016, 39, DOI: <https://doi.org/10.5935/0100-4042.20160114>.
17. Javitt B, Hep G2 as a resource cholesterol, for metabolic and bile studies: lipoprotein , acids. *FASEB* 1990, 4, 161–168, DOI: <https://doi.org/10.1096/fasebj.4.2.2153592>
18. Mosmann T, Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J. Immunol. Methods* 1983, 65, 55–63.
19. te Velde G , Bickelhaupt F M, Baerends E J, Fonseca Guerra C, van Gisbergen S J A , Snijders J G , Ziegler T, Chemistry with ADF, *Journal of Comp Chem* , 2001, 22, 931–967, DOI: <https://doi.org/10.1002/jcc.1056>.
20. van Lenthe E, Baerends E J, Snijders J G , Van Relativistic total energy using regular approximations. *J. Chem. Phys.* 1994, 101, DOI: <https://doi.org/10.1063/1.467943>

21. Snijders J G, Vernooijs P, Baerends E J, Roothaan-Hartree-Fock-Slater atomic wave functions: Single-zeta, double-zeta, and extended Slater-type basis sets for 87Fr-103Lr. *Atomic Data and Nuclear Data Tables* 26, (6) 1981, 26, 483–509, DOI: [https://doi.org/10.1016/0092-640X\(81\)90004-8](https://doi.org/10.1016/0092-640X(81)90004-8)
22. Casida M E, Huix-Rotllant M, Progress in time-dependent density-functional theory. *Annu. Rev. Phys. Chem.* 2012, 63, 287–323, DOI: <https://doi.org/10.1146/annurev-physchem-032511-143803>.
23. Pye C C, Ziegler T, van Lenthe E, Louwen J N, An implementation of the conductor-like screening model of solvation within the Amsterdam density functional package — Part II. COSMO for real solvents 1. *Can. J. Chem.* 2009, 87, 790–797, DOI: <https://doi.org/10.1139/V09-008>.
24. Choi Y E, Kwak J W, Park J W, Nanotechnology for early cancer detection. *Sensors (Basel)*. 2010, 10, 428–55, DOI: <https://doi.org/10.3390/s100100428>.
25. Sharma R, Kumar R, Kodwani R, Kapoor S, Khare A, Bansal R, Khurana S, Singh S, Thomas J, Roy B, et al. A Review on Mechanisms of Anti Tumor Activity of Chalcones. *Anticancer. Agents Med. Chem.* 2015, 16, 200–211, DOI: <https://doi.org/10.2174/1871520615666150518093144>.
26. Ramirez-Tagle R, Escobar C A, Romero V, Montorfano I, Armisén R, Borgna V, Jeldes E, Pizarro L, Simon F, Echeverria C, Chalcone-induced apoptosis through caspase-dependent intrinsic pathways in human hepatocellular carcinoma cells. *Int. J. Mol. Sci.* 2016, 17, DOI: <https://doi.org/10.3390/ijms17020260>.