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# Evaluating the interaction between di-fluorinated chalcones and plasmatic albumin

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ABSTRACT

By using spectroscopic methods (fluorescence and circular dichroism), the interactions of three di-fluorinated chalcones (CH23F, CH25F and CH35F) with bovine serum albumin (BSA) in a PBS buffer solution (pH = 7.4), at 288 K, 293 K and 298 K, were probed. Molecular docking was performed to evaluate the main protein cavity for the BSA/chalcone interactions. Fluorescence quenching of the albumin by the di-fluorinated chalcones follow a combination of static and dynamic quenching mechanisms. The Stern-Volmer binding constant ($K_a$) values are in the range of $10^4\,M^{-1}$ indicating a moderate association between chalcones and BSA. Besides, circular dichroism data show that this association does not affect significantly the secondary structure of the albumin. The Förster resonance energy transfer (FRET) theory suggests that non-radiative energy transfer can occur from BSA to di-fluorinated chalcones ($r \approx 3$ nm). The thermodynamic parameters $\Delta H^\circ$ and $\Delta S^\circ$ indicate that hydrogen bonding and/or hydrophobic interactions play a major role in the association, which is entropically driven. The enthalpy and entropy change values for CH25F and CH35F are similar, but very different for CH23F. The negative $\Delta G^\circ$ values are consistent with a spontaneous association. The highest docking score suggests the Trp-212 site as the most probable binding site for the interaction between BSA with the di-fluorinated chalcones. Trp-212 residue interacts via hydrogen bonding with CH25F and CH35F. On the other hand, CH23F interacts via T-stacking with the Trp-212 residue and via hydrogen bonding with the Ser-343 residue. CH23F has the highest dipole moment value of all evaluated chalcones, having its fluorinated aromatic ring more exposed to the aqueous medium than inside the protein cavity. In addition to these results theoretical methods aimed to study the interaction BSA:CH2′3′F, BSA:CH2′5′F and BSA:CH3′5′F were also employed.


## 1. Introduction

Chalcone (1,3-diphenyl-prop-2-en-1-one) is an abundant natural product that can be obtained from many vegetable species such as *Pyracantha coccinea*, *Paratocarpus venenosa* Zoll and *Bidens pilosa*. Natural chalcones are commonly polyhydroxylated through a stereospecific reaction catalyzed by the enzyme *chalcone isomerase* [1]. Chalcones can exist in *cis* and *trans* isomeric forms, with the *trans* form being thermodynamically favourable. The importance of chalcone and its derivatives resides in the large diversity of processes in which they are involved. In nature, chalcones are precursors in the biosynthesis of flavones, flavanones, and chromanones. Since they belong to the class of flavonoids, chalcones also play an important role in defense against pathogens and insects [2,3].

The introduction of fluorine substituents into an organic molecule can readily change its physicochemical and biological properties. There are very few naturally occurring organic compounds that contain fluorine, so generally fluorine molecules are synthesized in the laboratory. Many fluorinated compounds are widely used as antidepressants, anti-inflammatory agents, antimalarial drugs, antipsychotics, antiviral agents, and general anesthetics [4]. The synthetic fluorine chalcone derivatives show high biological activities, such as: analgesic [5], antioxidant [6] antiviral, antiprotozoal, insecticidal [7], 5-lipoxygenase inhibition on rat basophilic leukemia-1 (RBL-1) cells, inhibitory action on Fe (3+)-ADP induced NADPH-dependent lipid peroxidation in rat liver

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microsomes [8], anti-inflammatory activity due to the inhibition of enzymatic catalysis of nitric oxide production [9,10] and mono-amine oxidase-B inhibitor [11–13].

Serum albumin is a single-chain protein synthesized in and secreted from liver cells. The protein has 582 amino acids, and a molecular mass of 66,500 Da. Due to a large number of acidic (Glu and Asp) and basic residues (Lys and Arg), the protein is highly soluble in aqueous media [14]. It is the most abundant soluble protein in blood plasma and among its multiple functions stands out its capacity of bio-distribution of a large variety of molecules in the body. Human Serum Albumin (HSA) is commonly substituted by Bovine Serum Albumin (BSA) in laboratory experiments due to the availability and lower cost of the later. The BSA binding capacity is similar to that of HSA, due the 76% identity and 88% similarity in amino acids sequences [15,16].

Recently several publications have dealt with the interaction between serum albumin and chalcone derivatives [17–22]. However, in none of the cases fluorinated chalcones were investigated. The study of the binding modes between the later compounds and albumin will provide basic information on the influence of bio-distribution of fluorinated chalcones in the human blood, contributing to the development of strategies to allow their safe therapeutic use.

In view of the biological importance of fluorinated chalcone derivatives, the present paper reports the results of studies, using spectroscopic techniques (fluorescence, UV–vis absorption, and circular dichroism), aiming to evaluate the interactions between BSA and three synthesized di-fluorinated chalcones ((*E*)-3-(2,3-difluorophenyl)-1-phenylprop-2-en-1-one – CH23F, (*E*)-3-(2,5-difluorophenyl)-1-phenylprop-2-en-1-one – CH25F, (*E*)-3-(3,5-difluorophenyl)-1-phenylprop-2-en-1-one – CH35F) through their corresponding binding parameters. Besides, the binding sites of BSA for the synthesized chalcones CH23F, CH25F, CH35F, and the non-synthesized compounds CH2′3′F, CH2′5′F and CH3′5′F molecules, in which the fluorine substitution is located in the ring B of chalcone (Fig. 1), were explored by molecular docking.

## 2. Experimental

### 2.1. Materials

Bovine Serum Albumin, phosphate buffer (pH = 7.4), 2,3-difluorobenzaldehyde, 2,5-difluorobenzaldehyde and 3,5-difluorobenzaldehyde were purchased from Sigma-Aldrich Chemical Company, St. Louis, USA. Ethanol (spectrophotometric grade) and acetophenone were obtained from Vetec Quimica Fina Ltda, R.J., Brazil and Carlo Erba, Val de Reuil, France, respectively.

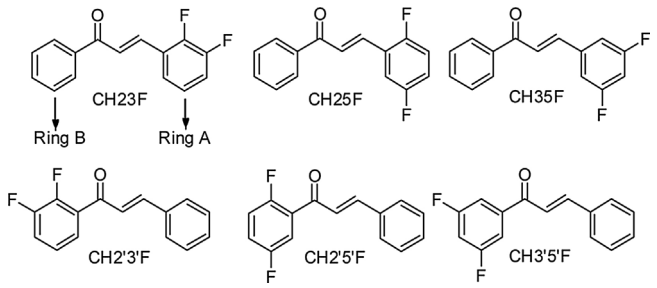


**Fig. 1.** Chemical structure for the fluorinated chalcones: (*E*)-3-(2,3-difluorophenyl)-1-phenylprop-2-en-1-one (CH23F), (*E*)-3-(2,5-difluorophenyl)-1-phenylprop-2-en-1-one (CH25F), (*E*)-3-(3,5-difluorophenyl)-1-phenylprop-2-en-1-one (CH35F), (*E*)-1-(2,3-difluorophenyl)-3-phenylprop-2-en-1-one (CH2′3′F), (*E*)-1-(2,5-difluorophenyl)-3-phenylprop-2-en-1-one (CH2′5′F), (*E*)-1-(3,5-difluorophenyl)-3-phenylprop-2-en-1-one (CH3′5′F).

The di-fluorinated chalcones (CH23F, CH25F and CH35F) were synthesized by an aldol condensation reaction in basic medium [23]. In a 50 mL round bottom flask an aqueous solution of KOH (10%, $2.60 \times 10^{-3}$ gL$^{-1}$) was added to a solution of ethanol–water (6:4 v/v, 10 mL). The corresponding difluorobenzaldehyde (550 mg, 5,2 mmol) and acetophenone (279 mg, 2.33 mmol) were added in small portions to the ethanol–water solution. The reaction mixture was stirred at low temperature (278 K) for 24 h. Finally, the reaction yielded a white solid. The final mixture was neutralized with hydrochloric acid (10%). The product was recrystallized three times from ethanol. Spectroscopic and spectrometric properties for all synthesized fluorinated chalcones are in full accord with the proposed structures [24–26] (see Supplementary material).

### 2.2. Apparatus and methods

#### 2.2.1. Fluorescence experiments

The fluorescence measurements were performed on Jasco Model J-815 spectrofluorimeter equipped with a 1.0 cm quartz cell and a Jasco PFD-425S15F thermostat system with 0.1 °C accuracy. All spectra were recorded with appropriate background corrections. The excitation wavelength of 280 nm was selected for the experiments, and fluorescence spectra were obtained in the range of 300–440 nm at 288 K, 293 K and 298 K. To a 3.0 mL solution, containing an appropriate concentration of BSA ($1.00 \times 10^{-5}$ M) at pH 7.4, were added successive aliquots from an ethanol stock solution of CH23F, CH25F or CH35F ($1.00 \times 10^{-3}$ M). The addition was done manually by using a 10 microliter syringe, and varying concentrations of 0.12; 0.24; 1.47; 1.96; 2.43; 2.91; 3.33; $3.84 \times 10^{-5}$ M were obtained for CH23F, CH25F and CH35F.

The quenching of BSA fluorescence in the presence of increasing CH23F, CH25F and CH35F concentrations was analyzed using the Stern–Volmer equation (Eq. (1)) [27]:

$$\text{(A)} \quad \frac{F_0}{F} = 1 + k_q \tau_0 [Q] = 1 + K_{SV}[Q] \qquad \text{(B)} \quad k_q = \frac{K_{SV}}{\tau_0}$$

where $F_0$ and F represent the fluorescence intensity values of the protein sample in the absence and the presence of the quencher, respectively, [Q] is the CH23F, CH25F or CH35F concentration, $K_{SV}$ is the Stern-Volmer quenching constant, $k_q$ is the bimolecular quenching constant and $\tau_0$ is the lifetime of the fluorophore in the absence of CH23F, CH25F and CH35F (tryptophan residue, $10^{-8}$ s) [28].

The tryptophan residue fluorophores in albumin are not all equally accessible to quenchers; if part of those fluorophores cannot be affected by quenchers, a modified Stern-Volmer equation can be used (Eq. (2)) [29]:

$$\frac{F_0}{F_0 - F} = \frac{1}{f[Q]K_a} + \frac{1}{f} \tag{2}$$

where $F_0$ and F are the steady-state fluorescence intensities in the absence and presence of quencher, respectively; $K_a$ is the modified Stern-Volmer binding constant; $f$ is the fraction of the initial fluorescence intensity that corresponds to fluorophore that is accessible to the quencher and [Q] the CH23F, CH25F and CH35F concentration

The thermodynamic parameters ($\Delta H^\circ$, enthalpy change and $\Delta S^\circ$, entropy change) for the BSA:CH23F, BSA:CH25F and BSA:CH35F interaction were obtained from the van't Hoff plot using Eq. (3) [30]:

$$\ln K_a = -\frac{\Delta H^0}{RT} + \frac{\Delta S^0}{R} \tag{3}$$

where T is the temperature (288 K, 293 K and 298 K) and $R$ is the gas constant (8.3145 Jmol$^{-1}$ K$^{-1}$). The value of the free energy change,