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# Influence of the spacer on the photoreactivity of flurbiprofen-tyrosine dyads

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ABSTRACT

The photoreactivity of diastereomeric dyads containing (*S*)- or (*R*)-flurbiprofen (FBP) and (*S*)-Tyr, either directly linked (**1**) or separated by a cyclic spacer (**3**) has been investigated. The main feature is a remarkable intramolecular quenching of FBP fluorescence, especially in **1**. The process is clearly configuration dependent, being more efficient for the (*R,S*)- diastereomer in **1** and for the (*S,S*)- analogue in **3**. Noteworthy, exciplex emission is detected in the 380–500 nm region in the case of **3**. Fluorescence decay kinetics from the femtosecond to the nanosecond time-domains provides evidence for the dynamic nature of the quenching. In agreement with the steady-state and time-resolved observations, molecular modelling points to a more favourable geometric arrangement of the two interacting chromophores in **1** than in **3**.



## 1. Introduction

The binding of drugs to proteins constitutes an active research field in connection with drug transport and distribution through the organism, which is ultimately related to the therapeutic action. The interactions existing between drugs and proteins can modulate properties of the former, such as solubility in plasma, susceptibility to some reagents or in vivo half-life [1–4]. In this context, a better knowledge on the drug/protein complexation process can shed light on the structural bases leading to the design of new therapeutic agents [5,6].

Transport proteins, such as serum albumins, are in charge of the binding and transport of endogenous and exogenous target molecules through the blood stream [7]. Human serum albumin (HSA) contains 585 amino acid residues, one Trp, 17 disulfide bridges, and one free Cys [8,9]. Small organic molecules usually bind to HSA in the so-called site I and site II, following Sudlow's classification [10]; the only Trp residue is located in site I, while site II is mainly made-up of Tyr, His and Arg [7]. The role played by different amino acids when drugs are incorporated within the protein cavities has been widely investigated, especially for Tyr and Trp, which have become universal fluorescent markers for protein studies [11–15]. In addition, it is known that chemical modifications on these amino acids result in a decrease of drugs affinity for the protein [16,17]. For instance, the presence of a Tyr residue influences the esterase activity of bovine serum albumin (BSA) [18].

Flurbiprofen (FBP, Scheme 1) is a nonsteroidal anti-inflammatory drug (NSAID) prescribed for treatment of osteoarthritis, rheumatoid arthritis, tendinitis, bursitis, sunburns and prevention of migraine headache [19–24]. Although it is prescribed as a racemic mixture, its pharmacological effect is attributed to the (*S*)-enantiomer [25,26]. Its photobehaviour has been characterised either in aqueous or organic media [27], and binding to HSA has been investigated by spectroscopic techniques, namely fluorescence and laser flash photolysis [28–31]. Upon FBP complexation, a dynamic fluorescence quenching of $^1$FBP* and $^1$Trp* is observed, due to energy and electron transfer processes [29]. Concerning $^3$FBP*, the triplet lifetime ($\tau_T$) lengthens within the protein cavities, as a result of the protection provided by the microenvironment that prevents from attack by oxygen [28,30,31]. Enantioselectivity has been observed in the photobehaviour of (*R*)- and (*S*)-FBP/HSA complexes [29,30,32].

The use of dyads that contain a drug covalently linked to an amino acid is a useful tool to mimic processes that occur in the protein binding sites of the real supramolecular complexes. In this context, light-induced events such as energy transfer, electron transfer, or exciplex formation on drug-amino acid dyads have

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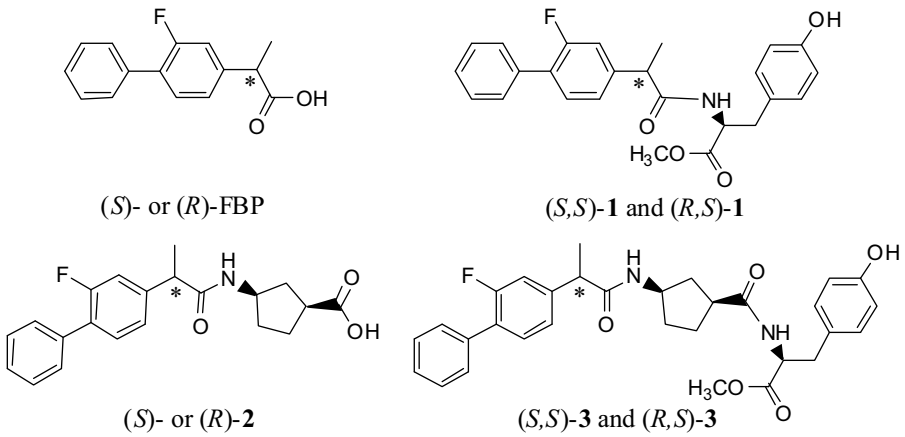


**Scheme 1.** Chemical structures of the investigated systems.

provided the key to demonstrate the mechanistic pathways responsible for photoallergy or to estimate the preferred binging site in HSA [29,33–37].

We have recently reported a thorough photophysical study on FBP/HSA mixtures, comparing their photobehaviour with that of FBP-Trp dyads [29]; stereoselective dynamic fluorescence quenching has been noticed in the model systems, although the kinetics of the involved processes are slower than in the real drug/protein complexes. Interestingly, an inversed stereodifferentiation has been noticed in the dyads with respect to that found for FBP@HSA complexes; this can be explained because the relative conformations of the FBP and Trp moieties are not necessarily the same in the dyads and within the protein.

In order to improve the picture on FBP/HSA interactions in the excited states, here we describe the photobehaviour of a series of diastereomeric dyads composed of FBP and Tyr, a fluorescent close-lying amino acid that may also influence the binding process. The design envisages the two subunits either directly linked or separated by means of a rigid spacer (Scheme 1). A comparative study on the different dyads would provide useful information on how the distance and conformational arrangement between the chromophores may affect the photophysical properties.

## 2. Experimental

### 2.1. Materials and methods

(*S*)- and (*R*)-Flurbiprofen, (*S*)-tyrosine methyl ester hydrochloride (TyrMe), (1*S*,3*R*)-3-amino-cyclopentanecarboxylic acid, 1-hydroxybenzotriazole (BtOH), *N*,*N*′-dicyclohexylcarbodiimide (DCC), *N*-hydroxysuccinimide (NHS) and 1-(3-dimethylaminopropyl)-*N*-ethylcarbodiimide hydrochloride (EDC) were purchased from Sigma-Aldrich. Their purity was checked by $^1$H NMR and HPLC analysis. Spectrophotometric HPLC or reagent grade solvents were obtained from Scharlab and used without further purification. The $^1$H-NMR and $^{13}$C-NMR spectra were recorded in $CDCl_3$ at 400 and 100 MHz, respectively, using a Bruker AVANCE III instrument; chemical shifts are reported in ppm. Steady state absorption spectra were recorded in a JASCO V-630 spectrophotometer. The X-ray structure was determined at Unidade de Raios X, at the Universidade de Santiago de Compostela. Crystallographic data (excluding structure factors) for the (*R*,*S*)-**1** structure have been deposited at the Cambridge Crystallographic Data Centre as supplementary publication number CCDC 662961.

Exact mass values were obtained using an Ultra Performance Liquid Chromatography (UPLC) ACQUITY UPLC system (Waters Corp.) with a conditioned autosampler at 4 °C. The separation was accomplished on an ACQUITY UPLC BEH C18 column (50 mm × 2.1 mm i.d., 1.7 μm), which was maintained at 40 °C. The analysis was performed using acetonitrile and water (80:20 v/v containing 0.01% formic acid) as the mobile phase with a flow rate of 0.5 mL/min and an injection volume of 5 μL. The Waters ACQUITY$^{TM}$ XevoQToF Spectrometer (Waters Corp.) was connected to the UPLC system via an electrospray ionization interface. This source was operated in positive ionization mode at 100 °C with the capillary voltage at 1.5 kV and a temperature of desolvation of 300 °C. The cone and desolvation gas flows were 40 and 800 L/h, respectively. The collision gas flow and collision energy applied were 0.2 mL/min and 12 V, respectively. All data collected in Centroid mode were acquired using Masslynx$^{TM}$ software (Waters Corp.). Leucine-enkephalin was used at a concentration of 500 pg/μL as the lock mass generating an $[M+H]^+$ ion (*m*/*z* 556.2771) and fragment at m/z 120.0813 with a flow rate of 50 μL/min to ensure accuracy during the MS analysis.

Steady-state fluorescence spectra were obtained using a JASCO spectrofluorometer system provided with a monochromator in the wavelength range 200–900 nm, with an excitation wavelength of 267 nm at 22 °C. Solutions were placed into 10 mm × 10 mm quartz cells. The absorbance of the samples at the excitation wavelength was kept below 0.1. Fluorescence quantum yields were determined using FBP in MeCN as reference, with $\varphi_F = 0.17$ (air) or $\varphi_F = 0.21$ ($N_2$) [27].

### 2.2. Equipment

Time-resolved fluorescence measurements were performed using the fluorescence upconversion (FU) and time-correlated single photon counting (TCSPC) techniques. The excitation source was the third harmonic (267 nm) of a mode-locked Ti-Sapphire laser, delivering ~120 fs pulses whose repetition rate was 76 and 4.75 MHz for FU and TCSPC, respectively (in the latter case set by a pulse-picker).

For the FU measurements, a home-built setup was used. This has been described in detail earlier [38,39]. Briefly, a 1 mm type I BBO sum-frequency crystal was used, providing an instrumental response function of about 350 fs (fwhm). We judge that the time resolution of the setup is better than 100 fs after deconvolution, depending on the signal-to-noise ratio. The average excitation power used was 40 mW. The power density cannot be measured precisely within the excitation volume but we estimate it to $0.2 \pm 0.1$ GW/cm$^2$ for a 40 mW output from the tripler unit (assuming a 40 micron diameter of the focused beam). Solutions (about 30 mL) were kept flowing through a 0.4 mm quartz cell,