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Species-dependent binding of new synthesized bicalutamide analogues to albumin by optical biosensor analysis☆

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ABSTRACT

The binding of some novel bicalutamide analogues to human serum albumin (HSA) and rat serum albumin (RSA) was investigated by surface plasmon resonance (SPR) based optical biosensor technique. The serum protein binding of the bicalutamide analogues was determined and compared to that of the parent compound. Furthermore, HSA and RSA were used as target plasma proteins, in order to highlight possible differences among species when performing pharmacokinetic studies.

HSA and RSA were covalently immobilized on carboxymethyl dextran matrixes, using an amine coupling procedure. The anchoring method was validated by determining the dissociation constant (K_D) of a standard analyte to confirm that the binding properties of the proteins were maintained. The ranking of the bicalutamide analogues for their HSA and RSA bound fractions was used to compare the behaviour of the two albumins. Most of the bicalutamide analogues showed higher binding levels with respect to the lead compound, (R)-bicalutamide. Further, meaningful differences in the binding level to the two serum proteins were obtained. The dissociation constants (K_D) of the interaction between the lead compound, (R)-bicalutamide, and the two proteins were calculated. As a result, the K_D obtained with HSA was one order of magnitude higher than that obtained with RSA.

The observed differences in the HSA and RSA bonding of the bicalutamide analogues increase the knowledge on the possible low reliability in extrapolating the distribution data obtained on animals to humans. This work demonstrates that SPR based optical biosensor technique is well suited for the medium-high throughput screening of compounds' ligand binding to serum albumins.

1. Introduction

The process to generate a successful medicine is characterized by a high attrition rate. The main reason of these liabilities is related to molecular characteristics that make the ADMET (absorption, distribution, metabolism, excretion, toxicity) profile of a drug candidate unsuitable. Among all, binding to plasma protein plays an important role on the in vivo distribution of a drug. Human serum albumin (HSA) is the most abundant protein in plasma, with a concentration of about 600 μM [1]. This protein represents the major serum carrier being responsible for the transport of numerous endogenous factors and various xenobiotics, through the formation of non-covalent complexes at specific binding sites. The knowledge of drug–HSA interactions is of great importance for the pharmacokinetic, pharmacodynamic and toxicological evaluation of a new drug candidate [2–4]. Furthermore, the bound drug fraction acts as a reservoir for a longer drug action, increasing its half-life in the human body and thus maintaining its therapeutic level.

Since animal models are often used for pharmacokinetic in vivo experiments, the interest in studying the binding of new compounds to albumins from different species is growing. Despite the great similarity between the amino acid sequences of albumins from different animal species, the existence of significant interspecies differences has already been demonstrated. Hence this kind of investigation is very useful to evaluate the reliability of extrapolating to humans the distribution data obtained on animals in preclinical studies [5,6].

It is also clear how the characterization of interactions between proteins and drugs determines a growing need of methodologies to study any specific molecular event as well as the structural

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elements that drive the process. The most widely used techniques for protein binding measurements are equilibrium dialysis, ultrafiltration, affinity chromatography, capillary electrophoresis, spectroscopy (fluorescence, circular dichroism, nuclear magnetic resonance) and mass spectrometry [7–14].

Surface plasmon resonance (SPR) based optical biosensor technique is one of the most successful in the biomolecular interaction analysis field. Its use to characterize target macromolecules and biopharmaceuticals is well documented in the current literatures [15–20]. The optical biosensor is based on the SPR optical phenomenon. This device consists of three main components: the sensor surface, where one of the interacting compounds is attached to; the microfluidic system, to ensure reproducible sample consumption and an SPR detector, which measures the change in the refractive index close to the surface of the sensor chip, during complex association and dissociation. The SPR response observed is proportional to the mass of the analyte that is bound to the immobilized ligand. This instrument provides not only qualitative information about the specificity of the binding (Yes/No answer), but also many quantitative data, such as the amount of active analyte in the sample, the kinetic parameters of the interaction (k_{on}, k_{off}) and the affinity constants that characterize the complex formation. Thus, it is clear how this technique represents a useful tool in advanced drug discovery stages, where it becomes crucial to have a complete view of the binding mechanisms to understand different biological and pathological phenomena [2,4,9]. Since the sensitivity of this technique is directly related to the mass of the analyte under investigation, it is obvious that it is the technique of choice to study protein–protein interactions. However, recent improvements in the SPR biosensor technology have made its use suitable also for the detection of small molecule drugs (<200 Da).

The main advantage of this technique is the lack of a labelling step for both the ligand and the analyte, thus avoiding extra time and costs as well as misinterpretation of the collected data due to the occlusion of specific binding sites [20]. However, the immobilization always remains a critical step, because the immobilized compound may show a different behaviour from that observed in solution. On the other hand, the chip can be reused even for a long time after the immobilization, depending on the stability of the immobilized ligand. This parameter can also be monitored by tracking the surface's binding capacity and the baseline stability during the analysis. Furthermore, the SPR biosensor requires short time of analysis and very low sample consumption. This technique has already been successfully used for the screening of drugs or drug candidates for their binding to HSA. The serum protein immobilization process was proved to give binding data comparable with those obtained in solution [21]. In the present work the amine-coupling procedure was used to covalently immobilize both HSA and RSA

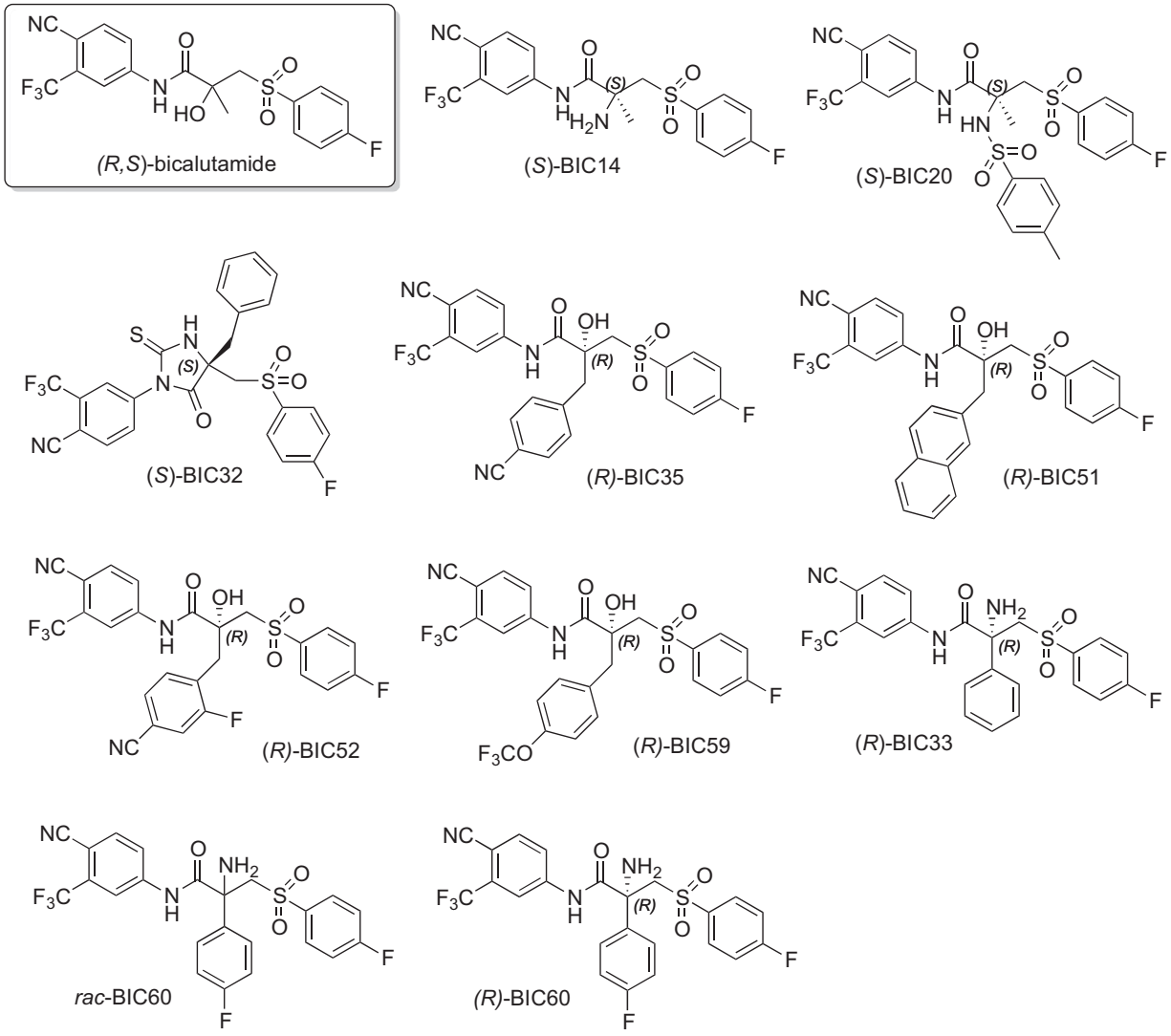

Fig. 1. Structures of (R)-bicalutamide and its analogues.