# Investigation of binding mode of novel 2,4-diketo esters to BSA

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DOI: 10.46793/ICCBI23.463M

**Abstract**: 2,4-Diketo esters are well-known for their wide pharmacological activities as well as their usage in a lot of synthetic transformations. The specific structure of 2,4-diketo esters with many carbonyl groups and the presence of keto-enol tautomerism give suitable chemical properties for many applications. Therefore, an affinity for one of the binding sites of bovine serum albumin (BSA) of 2,4-diketo esters possessing good antimicrobial activity, was examined. Binding modes of compounds **A** and **B** were determined using fluorescence spectroscopy. Eosin Y was used as a marker for Sudlow's Site I (subdomain IIA), while ibuprofen was used as a marker for Sudlow's Site II (subdomain IIIA). Obtained values of $K_a$ suggested that both compounds reversibly bind to BSA. Due to the values of $K_a$ in the presence of site markers, the site II is more likely to be occupied by compound **A**, and compound **B** uses other binding modes. The presented results will help to improve the research of the mechanism of the interaction between transport proteins and similar compounds.

**Keywords**: 2,4-Diketo esters, BSA, eosin Y, ibuprofen

## 1. Introduction

2,4-Diketo es te rs are an essential part in the structures of pharmacological and therapeutically important drugs, and are also common scaffolds in the naturally occurring molecules [1]. The presence of keto-enol tautomerism and several carbonyl groups in the molecule of 2,4-diketo ester are the characteristics that have been significant reasons for their application in many chemical syntheses. Therefore, 2,4-diketo esters are widely used as precursors in many reactions for obtaining different biologically active compounds such as 2,4-diketobutanoic acid derivatives, pyrazoles, isoxazoles, pyrrolidinones , quinoxalinones, benzoxazin-2-ones, and various other

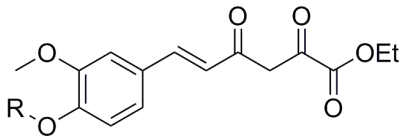
compounds [1]. Besides that, the presence of many oxygens and a highly electrophilic environment makes this type of compound excellent for coordination with different metals [1].

The research of the interaction with transport proteins helps in understanding the ability of the investigated compound to reach the target and it is an important first step during drug development. For that purpose, bovine serum albumin (BSA) is a widely used molecule as a representative transport protein that has similar physico-chemical properties to human serum albumin (HSA). Due to its low cost and easy availability, it is often used instead of HSA.

There are the three most important drug-binding sites on HSA: Sudlow's Site I (subdomain IIA), Sudlow's Site II (subdomain IIIA), and Heme site (subdomain IB) [2]. Drugs are placed at two main binding sites located in subdomain IIA (site IIA) and IIIA (site IIIA). Depending on drug properties, one of the two sites is favored. Bulky heterocyclic compounds with negative charge delocalized near the unpolar fragment usually bind to site IIA, while IIIA (indole-benzodiazepine site) binds the compounds that contain a peripheral negative charge [4].

## 2. Results and Discussions

The interaction with BSA for previously synthesised 2,4-diketo esters containing vanillin fragments that possess good antibacterial activity was investigated [3]. In this paper, we tried to explain and examine which binding site of BSA is preferred by selected compounds **A** and **B** (Figure 1). Experiments were performed u sing fluorescence emission titration and ibuprofen and eosin Y as site markers . Eosin Y is known as a marker for Sudlow's Site I (subdomain IIA), while ibuprofen is known as a marker for Sudlow's Site II (subdomain IIIA) [6].




**A**, R= $CH_3$
**B**, R= $C_2H_5$

**Figure 1**. Structures of selected compounds.

### *2.1 Determination of the binding site in the BSA*

Fluorescence spectroscopy is a frequently used technique in the investigation of the binding mode because it is quite simple and has good results in the examination of interaction between small organic molecules and macromolecules such as proteins and DNA.

The trpyptophan (Trp) residues of BSA showed fluorescent emission near 360 nm while excited at a 295 nm wavelength and the decrease of intensity of tryptophan emission spectrum was detected while the concentration of investigated compounds was increasing (Figure 2). Competitive experiments with site markers (eosin Y or ibuprofen)

were performed in an identical way as the experiments with BSA and compounds **A** and **B**, while concentration of site marker was the same as the concentration of BSA [4]. After the addition of site markers, they bind to BSA molecule, and the fluorescence intensity decreases.

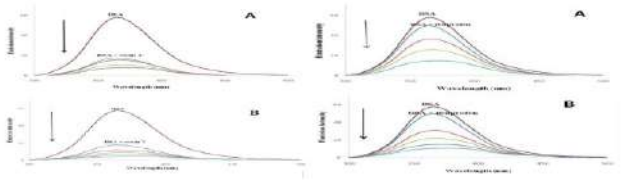


**Figure 2.** Emission spectra of BSA, BSA + eosin Y/ibuprofen in the absence and the presence of compounds **A** and **B**; [**A**] = 0-15 μm, [**B**] = 0-20 μM, [BSA] = [eosin Y] = [ibuprofen] = 10 μM; $\lambda_{ex}$ = 295 nm.

By examining the dependence of log[($F_0$ −F)/F] versus log[Q], we obtained the values of the binding constant ($K_a$) and the number of binding sites (n) (Figure 3). The values are presented in Table 1.

**Figure 3**. log[($F_0$ −F)/F] dependence of log[Q].

When the **A** or **B** is added to the ibuprofen–BSA system, the compound must compete with ibuprofen in order to bind to BSA. A similar phenomenon can be observed with eosin Y. According to the results from Table 1. the binding constant is mostly affected in the presence of ibuprofen in comparison with eosin Y for compound **A**, thus compound

**A** occupies site II of BSA [5]. In the case of compound **B** results suggested that **B** probably binds *via* some different mechanism and can bind to both sites.

**Table 1**. Binding parameters ($K_a$ and n) for **A** and **B**

| system | $K_a$ [$M^{-1}$] | n | R |
|---|---|---|---|
| **A** + BSA [4] | $1.9 \times 10^6$ | 1.37 | 0.998 |
| **A** + BSA + eosin Y | $6.25 \times 10^5$ | 1.26 | 0.998 |
| **A** + BSA + ibuprofen | $1.51 \times 10^7$ | 1.60 | 0.942 |
| **B** + BSA [4] | $5.0 \times 10^5$ | 1.20 | 0.996 |
| **B** + BSA + eosin Y | $5.43 \times 10^6$ | 1.33 | 0.988 |
| **B** + BSA + ibuprofen | $4.5 \times 10^6$ | 1.30 | 0.993 |

## 3. Conclusions

Both compounds reversibly bind to BSA and obtained values for the $K_a$ when site markers are added, suggest that compound **A** binds in the site II, while **B** binds using some other mechanism. The presented results will help in explaining the mechanism of distribution in mimicking physiological conditions and are a good starting point for our further research.

## Acknowledgment

This research is funded by the Ministry of Education and Ministry of Science, Technological Development and Innovation, Republic of Serbia, Grants: No. 451-03-47/2023-01/200378.

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