# Binding interactions of Biginelli-type compounds with serum albumin and DNA

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**Abstract:** Tetrahydropyrimidines (THPMs), synthesized *via* the classical Biginelli multicomponent reaction, represent a valuable class of heterocyclic compounds with diverse pharmacological potential. In our previous work, we reported the synthesis of a novel chlorine-containing tetrahydropyrimidine derivative with pronounced *in vitro* cytotoxicity against K562 leukemia cells. Building on these findings, the present study explores its interaction with DNA and human serum albumin (HSA) in order to gain insight into its potential mechanism of action and pharmacokinetic behavior. DNA binding was assessed by competitive studies with ethidium bromide (EB). The addition of compound **A** to the EB–DNA complex resulted in a concentration-dependent decrease of the fluorescence emission, indicating that compound **A** is capable of displacing EB. The calculated Stern–Volmer constant ($K_{SV}$ = 6.76 × $10^3$ $M^{-1}$) supports intercalation as the probable binding mode, although additional interactions such as groove binding cannot be excluded. The binding to HSA was examined by monitoring intrinsic fluorescence changes. Analysis using the Scatchard model provided a binding constant ($K_b$ = 8.51 × $10^4$ $M^{-1}$), which is within the typical range for reversible ligand–protein complexes, and $n \approx 1$ suggests the presence of a single dominant binding site for compound **A** on HSA. These findings imply that HSA may act as a potential carrier protein, affecting the distribution and bioavailability of this compound.

**Keywords**: Biginelli reaction, tetrahydropyrimidines, DNA, HSA, anticancer

## 1. Introduction

Pietro Biginelli reported one of the first multicomponent reactions of urea, ethyl acetoacetate, and benzaldehyde in 1891 [1]. This straightforward condensation provides tetrahydropyrimidines (THPMs, formerly known as dihydropyrimidines, DHPMs). Although the reaction was initially overlooked for many years, the synthesis of monastrol and approved anticancer activity as well as the inhibition of Eg5 protein [2], made the Biginelli reaction popular again. A variety of starting building blocks and catalysts, together with the simplicity of the procedure, makes the Biginelli reaction a

highly relevant tool in synthetic chemistry [3]. Moreover, tetrahydropyrimidines have a wide range of biological activities, such as anticancer, antimicrobial, antioxidant, anti-HIV, and many others [3,4].

Deoxyribonucleic acid (DNA) is a fundamental pharmacological target for many anticancer agents, since its interaction with small molecules can alter replication and transcription processes, ultimately inhibiting cancer cell proliferation. Classical chemotherapeutics, such as doxorubicin and cisplatin, exert their activity by binding to DNA, which highlights the importance of evaluating new compounds for their DNA-binding properties. In addition to DNA binding, the interaction of bioactive compounds with plasma proteins, particularly human serum albumin (HSA), plays a key role in drug distribution, transport, and bioavailability. Since HSA is the most abundant carrier protein in blood plasma, studying drug–HSA interactions provides valuable information about the pharmacokinetic profile of new molecules. Therefore, this short study explores the potential of THPM to interact with DNA and HSA.

## 2. Methodology

### *2.1. Materials*

Compound **A** was synthesized as previously described [5]. Calf thymus DNA (ct-DNA), ethidium bromide (EB), and human serum albumin (HSA) were purchased from commercial suppliers and used without further purification. Stock solutions were prepared in 10 mM PBS buffer (pH = 7.4). The concentration of DNA was determined spectrophotometrically measuring absorbance at 260 nm.

### *2.2. Fluorescence spectroscopy and data analysis*

DNA-binding studies were performed using EB as a fluorescent probe. Fluorescence spectra were recorded upon titration of the EB–DNA system with increasing concentrations of compound **A** (up to $1 \times 10^{-4}$ M). Quenching experiments with HSA were performed under similar conditions by titrating HSA solution with compound **A** (up to $2 \times 10^{-5}$ M) and monitoring changes in emission at $\lambda = 322$ nm.

Stern–Volmer constants ($K_{SV}$) were obtained from Stern–Volmer plots according to Eq. (1) where $I_0$ is emission intensity before adding **A**, I is emission intensity after adding **A**, while [Q] is equimolar concentration of quencher (compound **A**).

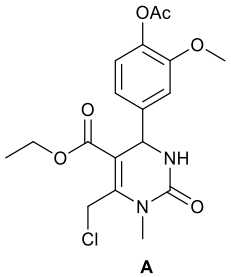
$$I_0/I = 1 + K_{SV}[Q] \quad (1)$$

Binding constants ($K_b$) and the number of binding sites per albumin ($n$) were determined from the double logarithmic Scatchard equation (Eq. (2)) and corresponding plot.

$$\log[(I_0 - I)] = \log K_b + n\log[Q] \quad (2)$$

## 3. Results and Discussion

In previous research, selective *in vitro* cytotoxicity was demonstrated for a novel series of chlorine-containing THPMs, particularly against chronic myelogenous leukemia K562 cells [5]. Compound **A** (Figure 1) exhibited an $IC_{50}$ value of 1.76 ± 0.09 μM, and was therefore selected for further experiments [5]. Its ability to bind to DNA and HSA was investigated using fluorescence spectroscopy.




**A**

**Figure 1**. Structure of compound **A**.

Ethidium bromide (EB) was used as a typical intercalator in these experiments. Spectra in the 550–750 nm region (Figure 2) revealed that the fluorescence signal of EB–DNA at 610 nm decreased with rising concentrations of compound **A**, suggesting that compound **A** may compete with EB and intercalate into DNA. Calculation of the Stern–Volmer constant ($K_{sv}$) using equation (1) supports an intercalative binding mode ($K_{sv}$ = 6.76 × $10^3$ $M^{-1}$), although additional modes of interaction such as groove binding or external electrostatic interactions cannot be excluded.

Moreover, the addition of compound **A** to HSA solution also resulted in enhanced emission at $\lambda$ = 322 nm. The binding constant ($K_b$) and the number of binding sites per albumin molecule ($n$) were determined using the double logarithmic Scatchard equation (2) and corresponding plot. $K_b$ values in the range of $10^4$–$10^6$ are characteristic of reversible ligand–protein binding. Thus, the determined constant (8.51 × $10^4$ $M^{-1}$) confirms moderate and reversible binding of the studied compound to BSA, indicating that BSA may serve as a carrier for compound **A**. The binding parameter $n$ indicates that compound **A** associate with BSA at approximately one site per protein molecule.

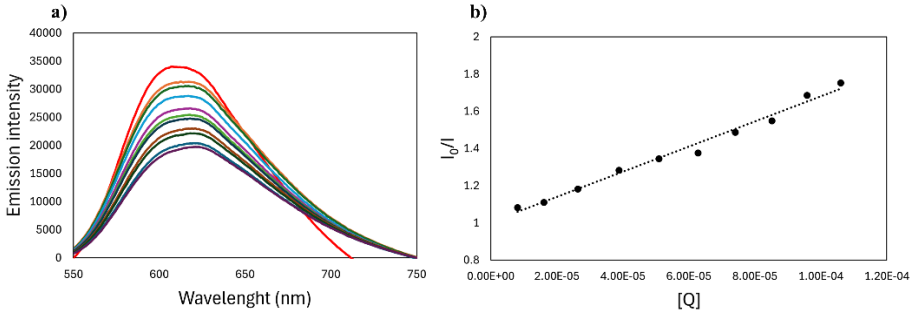



**Figure 2.** Emission spectra of EB-DNA in the absence (red lines) and presence of **A**. pH = 7.4; [EB] = [DNA] = 30 μM; $\lambda_{ex}$ = 527 nm; (b) Stern–Volmer plot.

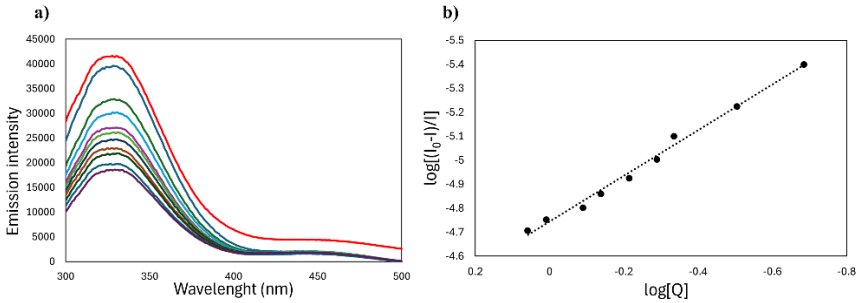



**Figure 3.** a) Emission spectra of HSA in the absence (red lines) and presence of quencher **A**. pH = 7.4; [HSA] = 2 μM; $\lambda_{ex}$ = 285 nm; (b) Scatchard plot.

## 4. Conclusions

These results indicate that compound **A** exhibits a moderate affinity toward both DNA and HSA. The observed increase in fluorescence intensity upon addition to DNA suggests at least partial intercalation, although additional binding modes may also be present. The binding to HSA, on the other hand, confirms the ability of the compound to interact with plasma proteins, which is relevant for its distribution and transport *in vivo*. Overall, the obtained data point to the potential of compound **A** as a biologically active tetrahydropyrimidine derivative, and further studies are warranted to clarify its exact binding mechanism and biological implications.

## Acknowledgment

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