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Original Article

Interaction of 3-(1H-tetrazol-5-yl) Coumarin With Bovine Serum Albumin and Calf Thymus DNA: Deciphering the Mode of Binding by In Vitro Studies

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Abstract

Background: Coumarins comprise a large family of heterocyclic compounds with a benzo-a-pyrone moiety.

Objectives: This study aimed to analyze the binding affinity of 3-(1H-tetrazol-5-yl) coumarin to bovine serum albumin (BSA) and calf thymus DNA (Ct-DNA) using fluorescence spectroscopy. The quenching of fluorescence was recognized during the interaction between 3-(1H-tetrazol-5-yl) coumarin and BSA, followed by a static mechanism.

Methods: The hydrogen bonds, hydrophobic interactions, and Vander Waals forces were regarded as the principal part in the 3-(1H-tetrazol-5-yl) coumarin and BSA complexation process. The fluorescence spectral characteristics demonstrated an enhancement in fluorescence intensity of the 3-(1H-tetrazol-5-yl) coumarin in the presence of ct-DNA solution.

Results: The experimental results indicated that the 3-(1H-tetrazol-5-yl) coumarin binds to DNA via interjection, hydrogen bonds, and Vander Waals forces. This work illustrated that BSA fluorescence was quenched by 3-(1H-tetrazol-5-yl) coumarin via a static mechanism and the ct-DNA fluorescence enhancement by 3-(1H-tetrazol-5-yl) coumarin was a static process. The secondary structure of proteins changed upon drug binding.

Conclusion: It is deduced that 3-(1H-tetrazol-5-yl) coumarin represents a higher binding affinity to DNA compared to BSA. This finding can be useful in designing more effective new drugs with fewer side effects.

Keywords: 3-(1H-tetrazol-5-yl) coumarin, Bovine serum albumin, Calf thymus DNA (Ct-DNA), Binding, Spectroscopy

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Background

Coumarins have different synthetic and plant (*Dipteryx odorata* Wild) origins. These compounds belong to the heterocyclic groups and have many uses in medicine and biology (1, 2).

Warfarin and acenocoumarol are coumarin derivatives, which have blood thinning and anticoagulant properties, respectively (1,3). Other biological properties of coumarin derivatives include anti-inflammatory, anti-cancer, and anti-oxidant activities (4,5).

The 1H- and 2H-tetrazoles are marked as isosteric replacements of carboxylic acids with ameliorated characteristics in pharmacokinetics and drug metabolism (6-8). 3-(1H-tetrazol-5-yl) coumarin (3-(1H-tetrazol-5-yl)-2H-1-benzopyran-2-ones) as imidazocoumarin is a coumarin derivative which is successfully synthesized via domino Knoevenagel condensation, Pinner reaction, and 1,3-dipolar cycloaddition of substituted salicylaldehydes (2-hydroxybenzaldehydes), malononitrile (propanedinitrile), and [BMIm]N3 in the solvent-free condition (8,9).

The interaction between biomacromolecules, e.g., drug-albumin or drug-DNA complexes, has been an interesting research field in biology, chemistry, and medicine (10,11). Serum albumin is a major soluble protein that serves as a carrier protein for fatty acids and hydrophobic steroid hormones (non-specific binding) (12). In order to identify new compounds and drugs, it is important and necessary to have fundamental information about the mode, function, structure, and characteristics of drug-protein or

drug-DNA interactions.

Bovine serum albumin (BSA; 583 a.a) consists of a distinct secondary structure of a single polypeptide chain with negatively charged natural proteins. The binding of drugs to macromolecules such as plasma proteins and DNA is a vital pharmacological feature since it influences the absorption, metabolism, and distribution of the drug (13,14). The study of the mechanism of interaction of 3-(1H-tetrazol-5-yl) coumarin with BSA and calf thymus DNA (Ct-DNA) is helpful in understanding the pharmacological properties of 3-(1H-tetrazol-5-yl) coumarin, which will assist in designing more efficacious drug candidates (15). Our research group has studied the synthesis of new coumarin derivatives and the interaction of these molecules with serum albumins and DNA.

BSA and Ct-DNA were used as the standard models to recognize physiological interactions and relate their activity. The results of the study may be helpful in reforming the existing drugs or more effective compounds.

Materials and Methods

Chemicals

3-(1H-tetrazol-5-yl)coumarin was synthesized by Dr. Khaghaninejad at Arak University, and the stock solution was prepared in sterile distilled water at a concentration of 1 mM and stored at -4°C until further use. Dilution of the drug was prepared instantly before use in the proper buffer. All chemicals and reagents were of analytical grade. Double-stranded Ct-DNA was obtained from Sigma-Aldrich Corporation (Sigma), dissolved in distilled water, and dialyzed overnight against 10 mM Tris–HCl (pH 7.2) at 4°C. Then, its concentration was determined using an extinction coefficient of 6600 $M^{-1}cm^{-1}$ at 260 nm. The interaction buffer was 10 mM Tris–HCl (pH 7.2). BSA (Sigma) was obtained from a local market without further purification to prepare a stock solution (1 mM) and was stored in a dark flask at 4°C.

Spectroscopy Measurements

UV Absorbance

An invariable amount of BSA (1000 μM) and Ct-DNA (1000 μM) was incubated in the presence and absence of different concentrations of 3-(1H-tetrazol-5-yl) coumarin (0, 0.25 μM, 0.5 μM, 1 μM, 2 μM, and 4 μM) in Tris–HCl (10 mM: pH 7.2) for 1 hour at 25°C in the dark. Different concentrations of 3-(1H-tetrazol-5-yl) coumarin were incubated with the study samples under equal experimental conditions. These component-treated samples and the controls were then subjected to the spectroscopic analysis using a Shimadzu UV-260 spectrophotometer (Cary Eclipse, Bio-Varian and Australia). Difference spectra were generated for Ct-DNA–3-(1H-tetrazol-5-yl) coumarin and BSA–3-(1H-tetrazol-5-yl) coumarin complexes between 200 and 700 nm versus different concentrations of the 3-(1H-tetrazol-5-yl) coumarin in the same buffer.

Fluorescence

The calculations were accomplished using a fluorescence spectrophotometer (Cary Eclipse, Bio-Varian and Australia) equipped with a 1 cm optical path quartz cell and thermostatically controlled cell holder at atmosphere temperature. The excitation and emission slits were set at 10/10 nm to decrease the intensity of the signal corresponding to the experiment and excitation was set at 278 nm (BSA), and the intensity was decreased to 258 nm (Ct-DNA). Then, the spectra were drawn between 200 and 700 nm. A constant concentration of Ct-DNA (1 μM) and BSA (1 μM) was incubated in the absence and presence of different concentrations of 3-(1H-tetrazol-5-yl) coumarin (0, 0.25 μM, 0.5 μM, 1 μM, 2 μM, and 4 μM) in 10 mM Tris–HCl (pH 7.2) for 1 hour at 25°C in the dark. The fluorescence emission spectra were then measured at three various temperatures (298K, 310K, and 315K) for Ct-DNA and BSA. The fluorescence quenching efficacy was evaluated by the classical Stern-Volmer equation to obtain the Stern-Volmer constant (Ksv).

$$F_o/F = 1 + Ksv\ [3\text{-}(1H\text{-tetrazol-}5\text{-yl})\ coumarin].$$

where F_o and F are fluorescence intensities before and after the addition of the quencher, respectively (16). Ksv value is obtained as the slope of F_o/F versus [3-(1H-tetrazol-5-yl) coumarin] linear plot. The association binding constant (K_b) was calculated from the slope and the number of binding sites (n) was determined from the intercept of the modified Stern-Volmer plot $\log [(Fo - F)/F] = \log K_b + n \log [3\text{-}(1H\text{-tetrazol-}5\text{-yl})\ coumarin]$.

Enthalpy and entropy as thermodynamic parameters were obtained from the following equation, which is known as Van 't Hoff equation.

$$LnK = -\Delta H/R + \Delta S/R$$

where R is the gas constant) and K_b is the binding constant at the related temperature (T). Furthermore, ΔG (free energy change) was calculated using $\Delta G = \Delta H - T\Delta S = -RTlnKb$ (17, 18).

Circular Dichroism

CD spectra of Ct-DNA, BSA, and their complexes with 3(1H-tetrazol-5-yl) coumarin were obtained using a CD spectrometer (AVIV 215, USA). The spectra afar as backbone CD (from about ~190 to 260 nm) and near –UV as aromatic CD region (260 to 320 nm) were produced in a 10 nm path-length quartz cell. Different concentrations of 3(1H-tetrazol-5-yl) coumarin (0, 0.25 μM, 0.5 μM, 1 μM, 2 μM, and 4 μM) were incubated with a fixed concentration of Ct-DNA and BSA (1 μM) in 0.01 M Tris–HCl (pH 7.2) in the dark for 60 minutes using CD spectrometer model 215. The instrument was set at a scan speed of 20 nm/min and a response time of 0.3330 at constant room temperature. The CD spectra of 3(1H-tetrazol-5-yl) coumarin at different concentrations were obtained from

the sample experiments, and the results were described as molar ellipticity and expressed as [θ], in deg × cm^2 × $dmol^{-1}$ (19).

Results

UV-Vis absorption spectroscopy is a simple and powerful technique that is used for analyzing the ligand–macromolecule interaction (20). To realize the pharmacological mechanism of drugs and derived compounds, it is important and necessary to evaluate the interaction between the drug and its new derivatives and macromolecules such as protein and nucleic acid.

Figure 1 shows the UV spectrum of 3(1H-tetrazol-5-yl) coumarin in 0.01 M Tris–HCl (pH 7.2). It displays main peaks at 210 and short peaks at 208, 280, 320, and 439 nm. UV absorbance changes of BSA and Ct-DNA at 260 and 280 nm upon increasing drug concentration also represent hyperchromicity. Absorbance changes of BSA at concentrations of the drug (1 μM) enhanced in the absorbance at 280 nm are observed with higher concentrations. Absorbance changes of Ct-DNA were the same as those of BSA (Figure 2).

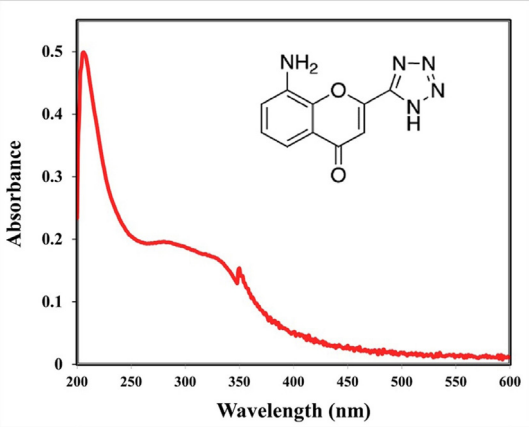

Figure 1. UV Absorption Spectrum of 3(1H-Tetrazol-5-yl) Coumarin in 10 mM Tris–HCl, pH 7.2. The spectrum shows a main peak at 210 and short peaks at 208, 280, 320 and 439 nm. Also chemical formula of 3(1H-tetrazol-5-yl) coumarin is shown

Quenching of BSA Chromophores With 3(1H-Tetrazol-5-yl) Coumarin

Fluorescence spectroscopy is a potent tool for elucidating the interaction between ligand and biomacromolecules (21). To study the binding of 3(1H-tetrazol-5-yl) coumarin to BSA, various amounts of 3(1H-tetrazol-5-yl) coumarin (0, 0.25 μM, 0.5 μM, 1 μM, 2 μM, and 4 μM) were added to a fixed concentration of and BSA (1 μM) and the fluorescence intensity was recorded. Excitation was recorded at 278 nm and emission was recorded at 310-420 nm for BSA. The results are depicted in Figure 3A. As is seen, the gradual addition of 3(1H-tetrazol-5-yl) coumarin to BSA solution reduced the fluorescence emission intensity without any red shift in the emission maxima (F_{max}) as the drug concentration enhanced.

Figure 3B displays Stern–Volmer plots for the quenching of 3(1H-tetrazol-5-yl) coumarin with BSA. The Ksv value was obtained as the slope of F_o/F versus [3(1H-tetrazol-5-yl) coumarin] linear plot for BSA (Table 1).

These experiments were performed at different temperatures (298K, 310K, and 315K), which revealed the mechanism of extinction or single static or dynamic quenching existed in the binding reaction of 3(1H-tetrazol-5-yl) coumarin with BSA. The thermodynamic parameters were dependent on temperature. The quenching mechanism was determined using the Stern-Volmer equation. Figure 3B shows a Stern-Volmer diagram at different temperatures for the quenching effect of 3(1H-Tetrazol-5-yl) coumarin and BSA. This diagram with a positive slope shows a linear relationship between drug concentrations and the extinction rate of the BSA emission spectrum. The Stern-Volmer constant (K_{SV}) was obtained from the slope of the Stern-Volmer equation curve at different temperatures.

Quenching constant (Ksv) is a strong parameter for quenching mechanisms, which can be classified into 3 groups: 1) dynamic quenching, when the fluorophore

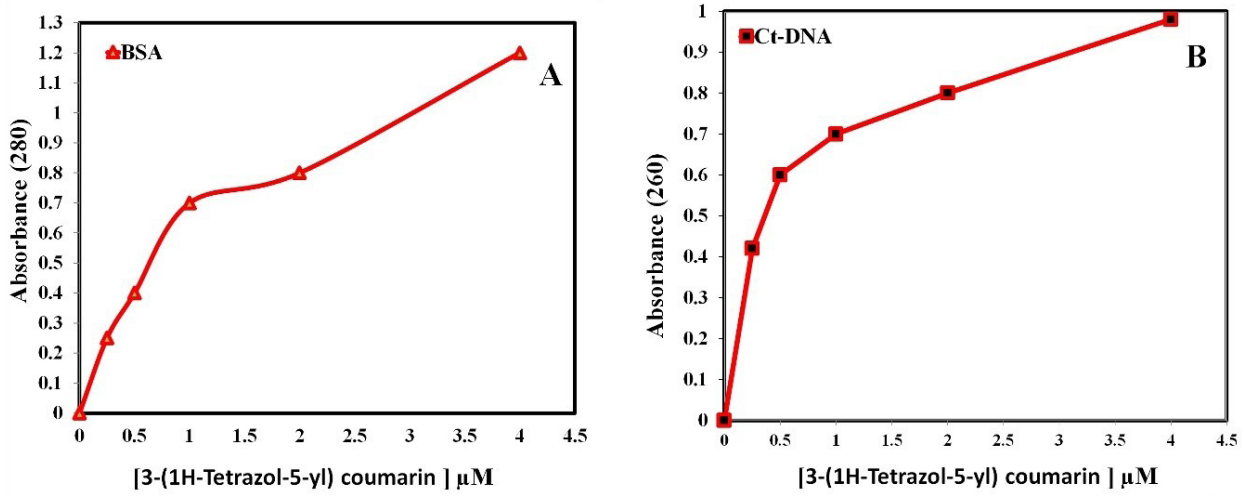

Figure 2. Represents UV Absorbance Changes of BSA in the Absence and Presence of Different Concentration of 3(1H-tetrazol-5-yl) Coumarin Measured at 280 (A) and 260 nm (B)

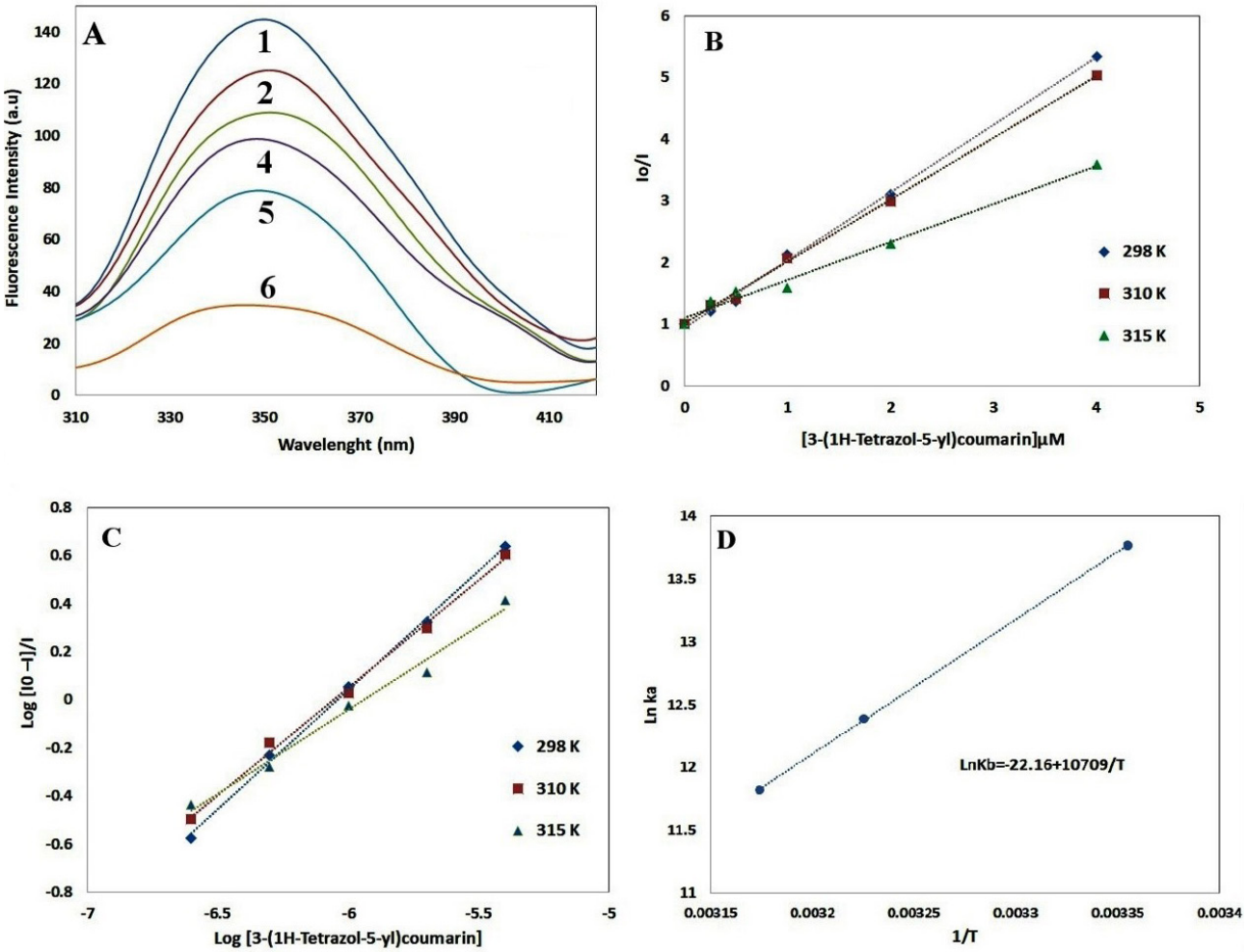

Figure 3. Fluorescence Emission Intensity of BSA in the Absence and Presence of Different Concentrations of 3(1H-Tetrazol-5-yl) Coumarin After Excitation at 278 nm. All samples were prepared in reaction buffer (10 mM Tris–HCl, pH 7.2) and the incubation time after drug addition was 1 hours. Spectra 1–6 are 0, 0.25, 0.5, 1, 2 and 4 µM of 3(1H-tetrazol-5-yl) coumarin, respectively. (A) Fluorescence emission intensity of DNA after excitation at 278 nm (n = 4). (B) Stern-Volmer plot showing quenching of 3(1H-tetrazol-5-yl) coumarin with the target chromophores at different temperatures (298 K, 310 K and 315 K) (n = 4). (C) Binding constant Kb and the number of binding sites n at different temperatures (298 K, 310 K and 315 K). (D) The plot of lnKb versus1/T of the 3(1H-tetrazol-5-yl) coumarin -BSA system; λex = 278 nm, λem = 310–600 nm

Table 1. Binding Parameters for Interaction Between BSA and 3-(1H-tetrazol-5-yl) Coumarin

Drug	Macromolecule	Temperature (K)	Ksv × 10^5 (M^{-1})	Kq × 10^{14} ($M^{-1}S^{-1}$)	n	Kb × 10^5 (M^{-1})	ΔH (kJ/mol)	ΔS (J/mol K)	ΔG (kJ/mol)
3-(1H-tetrazol-5-yl) coumarin	BSA	298	10.9	1.82	0.989	9.5	-89.034	-184.23	-34.13
		310	10.3	1.67	0.892	2.53			-31.92
		315	6.1	1.03	0.695	1.36			-31.00

collides with the quencher. The quenching mechanism is directly proportional between Ksv and temperature; 2) static quenching, when a complex is formed between the quencher and fluorophore and there is an inverse correlation between Ksv and temperature; 3) mixed quenching, both of the above-mentioned mechanisms are involved, which means that the complex is formed and the collision occurs between the quencher and the fluorophore. Therefore, the relationship between F0/F and [Q] is probably non-linear. As depicted in Figure 3B, the values of K_{SV} ranged from 10.9 to 6.1×10^5 M^{-1} with the increase of temperature, indicating that the quenching mechanism may be static and can result in a decrease in the complex stability.

The data on emission quenching at different temperatures can provide a lot of information in determining the type of the 3(1H-tetrazol-5-yl) coumarin-BSA interaction and the 3(1H-tetrazol-5-yl) coumarin-Ct-DNA interaction through the Stern-Volmer equation:

$$F_0/F = 1 + K_{SV}[Q] = 1 + K_q \tau_0 [Q] \quad \text{Eq. (A.1)}$$

Here, F_0 and F are fluorescence intensities of BSA before and after the addition of 3(1H-tetrazol-5-yl) coumarin and Kq is the constant of bimolecular quenching. τ_0 is the fluorescence lifetime without the quencher (it is reported to be about 6×10^{-9} s for BSA) (22). [Q] is the concentration of quencher (Eq. A.1).

As shown in Table 1, with the increase of temperature,

the Stern-Volmer constant or K_{SV} decreased and the value of Kq (from the equation $Kq = K_{SV}/\tau_0$) was greater than 2.0×10^{10}L $M^{-1}s^{-1}$, which corresponds to the static quenching complex rather than the dynamic quenching mechanism. Therefore, in this study, the quenching mechanism was due to the formation of the complex between 3(1H-tetrazol-5-yl) coumarin and BSA rather than the collision between 3(1H-tetrazol-5-yl) coumarin and BSA (23).

Since 3(1H-tetrazol-5-yl) coumarin shows a significant binding affinity to BSA, assuming that the binding sites in the biomolecule are independent and similar, it is possible that the binding constant (K_b) and the number of binding sites (n) are calculated by the intercept and slope of the double logarithm regression curve of the log $(F_o\text{-}F)/F$ *versus* values *on* the x-axis of (*log molar*) the drug based on equation (A. 2).

The findings displayed that the values at room temperature were about one, which indicates a binding site of 3(1H-tetrazol-5-yl) coumarin on BSA (Table 1).

The number of binding sites (n) was 0.98 for BSA (Figure 3C), which has been presented in Table 1. Protein-ligand binding can be divided into 4 different types based on non-covalent interactions including hydrophobic forces, hydrogen bonds, electrostatic interactions, and Van der Waals interactions (24). Entropy and entropy change as thermodynamic parameters are very important and necessary to prove binding forces. The affinity of drug receptor is one of the essential factors for analyzing the effects of the drug, which is related to the binding constant (K_b) between the drug and the receptor. Generally, the affinity of drug receptor becomes weak as the K_b value decreases. However, the decrease of the value of K_b with the rising temperature indicated that the stability of the 3(1H-tetrazol-5-yl) coumarin–BSA complex decreased with the increasing temperature. And the decreased tendency of n value may imply that the capacity of the 3(1H-tetrazol-5-yl) coumarin binding on the BSA was decreased with the rise in temperature. The positive ΔS^0 and ΔH^0 values for the drug–macromolecule interaction are typically regarded as the evidence of hydrophobic and electrostatic interaction and negative ΔS^0 and ΔH^0 values for a drug–macromolecule interaction are generally regarded as the evidence of Van der Waals interaction and hydrogen bonds (24,25).

The Van 't Hoff equation (equation A.3) is used to characterize the acting forces (enthalpy and entropy changes) between 3(1H-tetrazol-5-yl) coumarin and BSA. Therefore, by determining the amount of entropy changes, it is possible to calculate the Gibbs free energy changes (ΔG^0) at different temperatures (Equation A.4).

$$\log\left[(F_O - F) / F\right] = \log Kb + n\log[Q] \qquad \text{Eq. (A.2)}$$

$$\text{LnKb} = -\frac{\Delta H}{RT} + \frac{\Delta S}{R} \qquad \text{Eq. (A.3)}$$

$$\Delta G = \Delta H - T\Delta S = -RT\ln Kb \qquad \text{Eq. (A.4)}$$

Where R indicates the gas constant, Kb is the binding constant, and T is the temperature (in kelvin, K). The ΔH° was obtained from the slope and ΔS° was obtained from the intercept of the linear Van 't Hoff plot (Eq. A.2).

The amount of the thermodynamic parameters of 3(1H-tetrazol-5-yl) coumarin binding to BSA was obtained from the Van 't Hoff equation. Then, the amount of ΔG^0 was calculated, which is summarized in Table 1. According to Table 1, the values of ΔG^0 for drug binding were negative, which indicates that the binding process was spontaneous. Enthalpy and entropy values for interaction between BSA and 3(1H-tetrazol-5-yl) coumarin were negative, indicating the significant contribution of Van der Waals and hydrogen bonds in the interaction between BSA and 3(1H-tetrazol-5-yl) coumarin.

Fluorescence Spectroscopic Studies of the Interaction Between Ct-DNA and 3(1H-Tetrazol-5-yl) Coumarin

To study the binding of 3(1H-tetrazol-5-yl) coumarin to Ct-DNA, various amounts of 3(1H-tetrazol-5-yl) coumarins were added to a fixed concentration of Ct-DNA (1 µM) and the fluorescence intensity was calculated. Excitation was recorded at 258 nm and emission was recorded at 480-560 nm for Ct-DNA (Figure 4A).

The obtained results are depicted in Figure 4A. The gradual addition of 3(1H-tetrazol-5-yl) coumarin to Ct-DNA solution increased the fluorescence emission intensity. Other studies also showed an increase in the intensity of biomacromolecule emission using other compounds (26-29). Enhancements of fluorescence show that the 3(1H-tetrazol-5-yl) coumarin interacted with Ct-DNA and the efficiency of quantum of 3(1H-tetrazol-5-yl) coumarin was increased simultaneous with quenching process; the constant of enhancement can be obtained by the following equation (30):

$$F_0/F = 1\text{-}K_E[E] \qquad \text{Eq. (A.5)}$$

Equation (4) can be rewritten as follows if its dynamic mechanism is part of the enhancing process:

$$F_0/F = 1\text{-}K_D[E] = 1\text{-}K_B\,\tau_0 \qquad \text{Eq. (A.6)}$$

Where k_D is the dynamic enhancement constant, k_B is the biomacromolecular enhancement constant, and τ_0 is the fluorophore lifetime in the absence of the enhancer. Accordingly, the constants can be calculated using equation (A.6) (Figure 4B, Table 2). The data show an increase in emission intensity at 520 nm, which is used to estimate n and K_f from these variable constants using the below equation (Figure 4B):

$$\log(F_0\text{-}F)/F = \log K_f + n\log[3(1H\text{-tetrazol-5-yl})\,\text{coumarin}] \qquad \text{Eq. (A.7)}$$

Based on this equation, the fluorescence intensity of the fluorophore in the absence of the drug is called F_0 and the fluorescence intensity in the presence of the drug is called F, in this case, the value of F is greater than F_0, according to

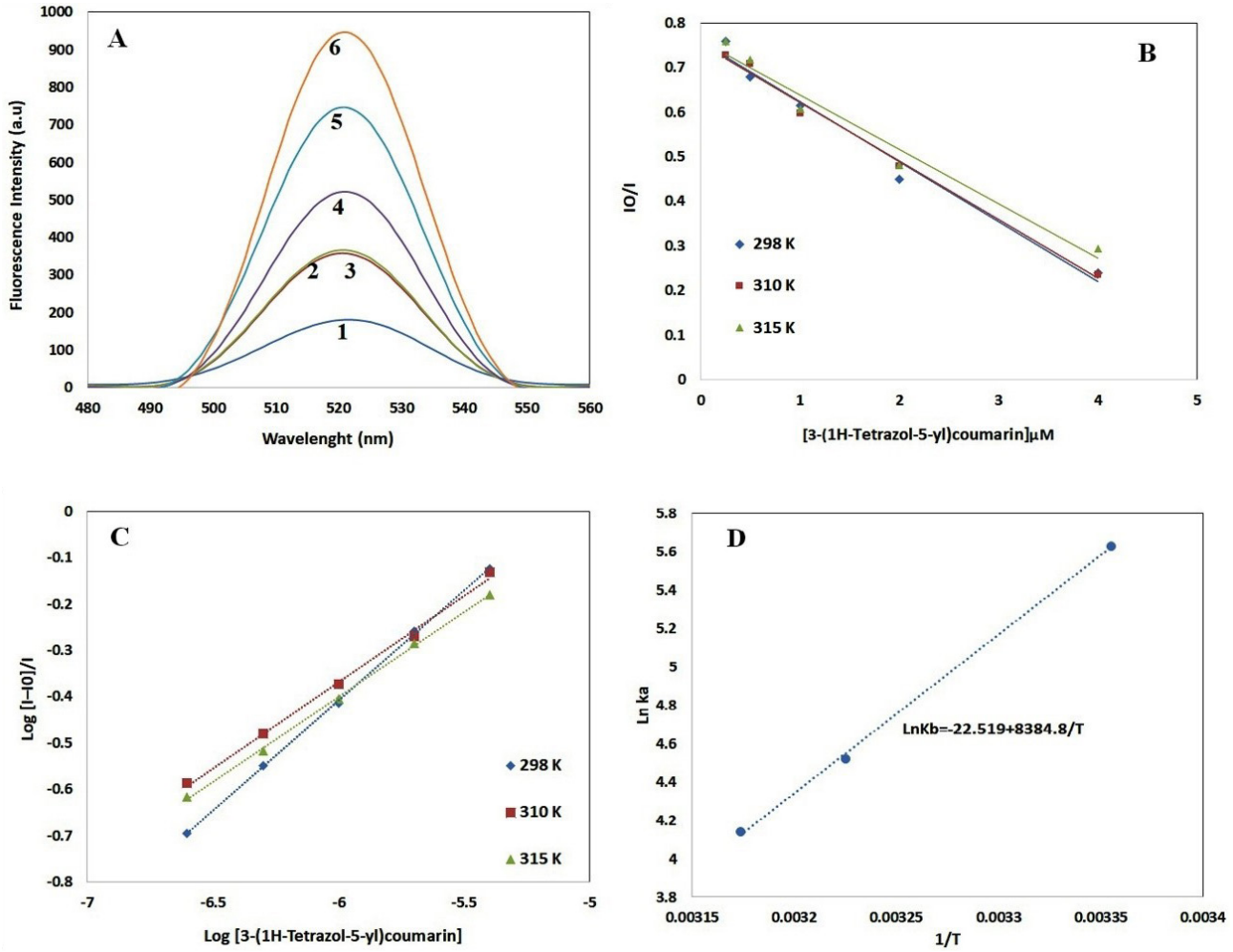

Figure 4. Fluorescence Emission Intensity of Ct-DNA in the Absence and Presence of Various Concentrations of 3(1H-tetrazol-5-yl) Coumarin After Excitation at 258 nm. All samples were prepared in reaction buffer (10 mM Tris–HCl, pH 7.2) and the incubation time after drug addition was 60 min. Spectra 1–6 are 0, 0.25, 0.5, 1, 2 and 4 µM of 3(1H-tetrazol-5-yl) coumarin, respectively. (A) Fluorescence emission intensity of DNA after excitation at 258 nm (n = 4). (B) Stern-Volmer plot showing quenching of 3(1H-tetrazol-5-yl) coumarin with the target chromophores at different temperatures (298 K, 310 K and 315 K) (n = 4). (C) Binding constant Kb and the number of binding sites n at different temperatures (298 K, 310 K and 315 K). (D) The plot of lnKb versus1/T of the 3(1H-tetrazol-5-yl) coumarin –ct-DNA system; λex = 258 nm, λem = 310–600 nm

Table 2. Binding Parameters for Interaction Between Ct-DNA and 3-(1H-Tetrazol-5-yl) Coumarin

Drug	Macromolecule	Temperature (K)	$K_D \times 10^5$ (M^{-1})	$Kb \times 10^{13}$ ($M^{-1}S^{-1}$)	n	$K_F \times 10^2$ (M^{-1})	ΔH (kJ/mol)	ΔS (J/mol K)	ΔG (kJ/mol)
3-(1H-tetrazol-5-yl) coumarin	Ct-DNA	298	1.39	1.39	0.47	2.79	-69.71	-187.22	-13.92
		310	1.31	1.31	0.37	0.73			-11.67
		315	1.22	1.22	0.36	0.63			--10.75

which equation A.7 becomes the following equation:

$$\log (F\text{-}F_0)/F = \log K_F + n \log [3(1H\text{-tetrazol-5-yl}) \text{ coumarin}] \quad \text{Eq. (A.8)}$$

Based on the above-mentioned equation, Figure 4C was drawn, where (n) is the number of binding sites at different temperatures, which decrease with increasing temperature, indicating the change in the accessibility of the fluorophore to the enhancer (Table 2). As a result, it is suggested that it is probably a complex static mechanism.

Structural Alteration of BSA and DNA upon 3(1H-tetrazol-5-yl) Coumarin Binding

The high affinity of 3(1H-tetrazol-5-yl) coumarin to BSA and Ct-DNA motivated us to explore the possible effect of 3(1H-tetrazol-5-yl) coumarin on the secondary structure of BSA compared to Ct-DNA. In the CD spectrum of free DNA, there were two positive peaks at 220 and 275 nm and a negative peak at 245 nm, which was similar B-conformation of DNA as shown in Figures 5B and 5C (31). Upon the interaction of 3(1H-tetrazol-5-yl) coumarin with and Ct-DNA, the ellipticity at the positive peak significantly reduced (without any significant shift); however, at 245 nm and two positive peaks, it became more negative (without any significant shift) for Ct-DNA.

The CD spectrum shows the n→π* transition usually with two negative bands at 208 and 222 nm in BSA (32).

To better understand the changes of the second structure and the binding mechanism of 3(1H-tetrazol-5-yl) coumarin to BSA and Ct-DNA (constant concentration), the CD spectrum was used in the absence and presence

of 3(1H-tetrazol-5-yl) coumarin (with different concentrations) (Figure 5) (33,34).

Double negative bands at 208 and 222 nm can be seen in the natural structure of BSA, which increases the concentration of 3(1H-tetrazol-5-yl) coumarin, causing these two bands to become more negative, which indicates the reduction of the helix content in BSA. In this way, the interaction of different concentrations of 3(1H-tetrazol-5-yl) coumarin causes changes in the structure of BSA.

In the natural spectrum of Ct-DNA without the presence of 3(1H-tetrazol-5-yl) coumarin, two positive and negative bands are observed at 275 nm and 245 nm, respectively (31).

With the increase in the concentration of 3(1H-tetrazol-5-yl) coumarin, this spectrum undergoes changes, as a result of which it probably becomes more negative at 245 nm with the increase of the concentration of 3(1H-tetrazol-5-yl) coumarin, which indicates a change in the right-handed helicity in B-DNA. Moreover, it is more negative at 275 nm with increasing 3(1H-tetrazol-5-yl) coumarin concentration, which probably indicates helix instability and non-stacking.

Various biological activities of heterocyclic coumarin derivatives have been identified, which include anti-tumor, anti-HIV, anti-inflammatory, and anti-coagulant activities. Coumarin is also known as a fat-reducing agent with triglyceride-reducing activity. In this study, for the first time, spectroscopic methods are used to study the interaction of 3(1H-tetrazol-5-yl) coumarin as one of the coumarin derivatives with BSA and Ct-DNA.

Discussion

Therefore, the goal of this study was to define the binding affinity of 3(1H-tetrazol-5-yl) coumarin to Ct-DNA and BSA to elucidate the possible role of DNA and protein in this binding process. The results indicate that 3(1H-tetrazol-5-yl) coumarin interacts with BSA with higher affinity than Ct-DNA. An increase in the UV absorbance of Ct-DNA and BSA shows the induction of unfolding at low concentrations of 3(1H-tetrazol-5-yl) coumarin; however, at higher concentrations, the interaction of 3(1H-tetrazol-5-yl) coumarin is accompanied by DNA and BSA compaction/aggregation. Moreover, the reduction in fluorescence emission intensities in BSA suggests that 3(1H-tetrazol-5-yl) coumarin affects aromatic amino acid residues of BSA. The temperature dependence of the quenching by 3(1H-tetrazol-5-yl) coumarin showed that it follows a static mechanism, indicating that the BSA-3(1H-tetrazol-5-yl) coumarin interaction is static. The Ct-DNA-3(1H-tetrazol-5-yl) coumarin interaction is also a static mechanism. One could eventually argue that the fluorescence quenching (for BSA), instead of being caused by direct interaction with 3(1H-tetrazol-5-yl) coumarin, could alternatively be due to a major conformational change of the protein induced by 3(1H-tetrazol-5-yl) coumarin, leading to a complete shift in the microenvironment of one of the Trp residues. Stern-Volmer quenching effect also confirmed the higher affinity of 3(1H-tetrazol-5-yl) coumarin to BSA in solution compared with Ct-DNA. This is in agreement with the findings of a study conducted by Sharma et al on the binding of coumarin analogs with BSA, confirming that the mechanism of fluorescence quenching was static (35). In addition, the investigation by Sindhu et al showed that the 7-diethylamino-4-methyl coumarin has the ability to interact with serum albumin mainly through a static quenching process (36). Sarwar et al confirmed formation of coumarin–DNA complex through the analysis of UV absorbance spectra and steady state fluorescence (37). The spectroscopic data obtained by Bayraktutan and Onganer revealed that the fluorescence of BSA was quenched by the addition of C35 via a static mechanism (38).

Our results were in disagreement with the findings of the study conducted by Akbay et al in which BSA fluorescence was quenched by the coumarins with dynamic mechanisms through the formation of C3-BSA complexes (39). Basic thermodynamic parameters such as the number of binding sites and binding constants at different

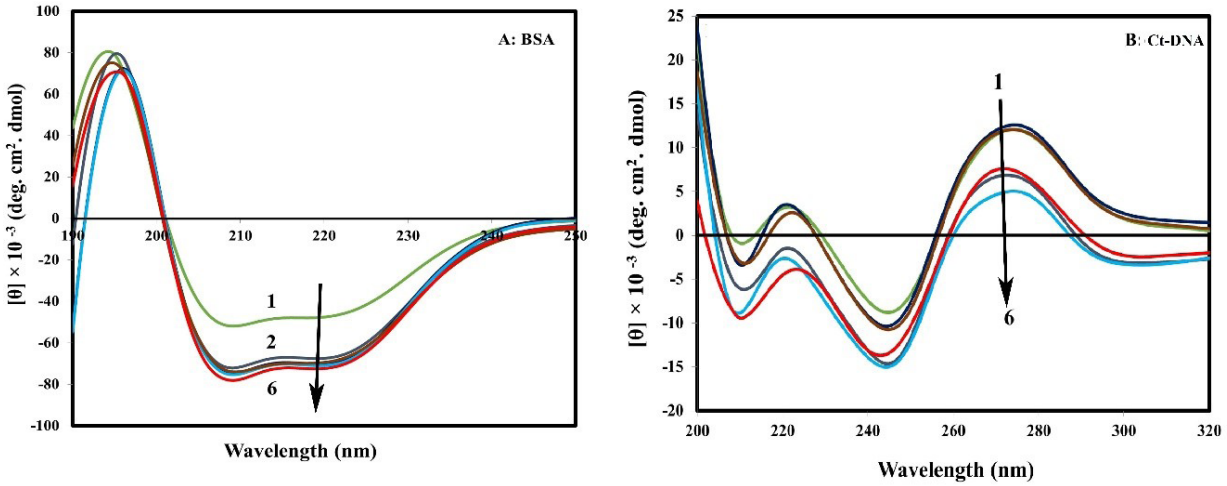

Figure 5. CD Profiles Obtained for BSA (A) and Ct-DNA (B) in the Absence and Presence of Various Concentrations of 3(1H-Tetrazol-5-yl) Coumarin in 10 mM Tris–HCl (pH=7.2). Numbers 1–6 are 0, 0.25, 0.5, 1, 2 and 4 μM of 3(1H-tetrazol-5-yl) coumarin respectively (n=3)

temperatures were calculated. The results indicate that van der Waals interactions and hydrogen bonds are the predominant intermolecular forces in stabilizing the BSA-3(1H-tetrazol-5-yl) coumarin complex and the interaction between Ct-DNA and 3-(1H-tetrazol-5-yl) coumarin. The procedures for the fluorescence determination of 3(1H-tetrazol-5-yl) coumarin have been developed based on the fluorescence quenching of BSA and Ct-DNA.

A significant reduction in ellipticity at 275 nm suggests alteration of DNA helicity and base stacking and possibly conversion of B-DNA into A-DNA conformation. The result implies that in BSA, the structure of BSA is considerably altered and the content of their secondary structures that are mainly in the form of α-helix is reduced.

In our laboratory conditions, a change in 245 nm and 275 nm is observed, which corresponds to the binding of different concentrations of the 3-(1H-tetrazol-5-yl) coumarin to Ct-DNA, and in this way, the mechanism of action of 3-(1H-tetrazol-5-yl) coumarin in binding to Ct-DNA is determined.

Conclusion

In summary, it can be concluded that this study can provide useful information about the binding of 3-(1H-tetrazol-5-yl) coumarin with biomolecules such as BSA and Ct-DNA. This information provides a better understanding of the effect of 3-(1H-tetrazol-5-yl) coumarin on protein and DNA function during its transport and distribution in the blood, which can help in its effectiveness and drug development.

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Validation: Javad Sargolzaei.
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Writing – review & editing: Javad Sargolzaei, Soheila Khaghaninejad.

Conflict of Interests

The authors declare no potential conflict of interest relevant to this article.

Ethical Issues

Not applicable.

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