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Synthesis of acetamide derivatives of 1,2,4-triazole bearing azinane and their binding interactions with bovine serum albumin using spectroscopic techniques

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Abstract: A new series of acetamide derivatives containing 1,2,4-triazole and azinane moieties has been synthesized and characterized using ^{1}H NMR, ^{13}C NMR, IR, and EI-MS spectroscopic analysis. The intermediate triazole was synthesized through a sequential synthesis of carboxylate and carbohydrazide. The bovine serum albumin (BSA) binding of the newly synthesized 1,2,4-triazole derivatives was evaluated along with thermodynamics, site-selective binding, and synchronous study. The results obtained by BSA binding as well as thermodynamic studies justify that all the compounds show spontaneous interaction with BSA and could be effectively distributed and eliminated from the body. Therefore, the triazole-based analogs might be a useful strategy for designing new drug systems.

Key words: Acetamides, 1,2,4-triazole, azinane, sulfonamide, bovine serum albumin binding

1. Introduction

Proteins are the most abundant macromolecules in cells and play a vital role in maintaining normal cell functions, which are in turn responsible for the origin, evolution, and metabolism of life. Among the different types of proteins in the body, human serum albumin (HSA) is the principal extracellular protein found in blood plasma, corresponding to a concentration of 42 mg/mL. It exhibits several crucial physiological and pharmacological functions such as maintaining osmotic blood pressure, buffering pH, and serving in the transportation and distribution of a variety of substances such as fatty acids, amino acids, hormones, and pharmaceuticals. Serum albumin has the ability to bind with most endogenous and exogenous compounds to form stable complexes. The binding of proteins and drugs greatly influences the absorption, distribution, metabolism, and excretion of typical drugs.[1]

Bovine serum albumin (BSA) has been extensively used as a model protein for binding studies with biologically active compounds due to its stability and solubility in aqueous media, its low speci?city, and its strong resemblance to HSA in terms of homology sequence (76%) and 3D structures.[1] The main regions of

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ligand binding sites on BSA are located in subdomains IIA and IIIA, namely site I and site II. It has been reported that some small molecules including hybrids based on azole heterocyclic systems bind exclusively to specific sites, either to the tryptophan residue (site I) or to the tyrosine residue (site II) on BSA molecules.[2]

Synthetic chemists are in continuous effort to design and synthesize versatile variety of hybrids based on azole heterocyclic systems.[3–5] Nitrogen-based heterocyclic systems have been reported to have various pharmacological applications.[6] The 1,2,4-triazole core has been found to possess interesting biological activities, namely antiinflammatory,[7] anticancer,[8] insecticidal,[9] antiviral,[10] antioxidant,[11] analgesic,[12] antimicrobial,[13] anticonvulsant, and antidepressant[14] activities. Triazole derivatives have also been used as fungicides in agrichemicals as well as in pharmaceutical applications for the treatment of topical and systematic fungal infections. The widespread use of triazole derivatives has generated extensive concerns regarding their distribution, free concentration, and metabolism within the body, which are affected by the ligand/protein interactions in the blood stream.[15]

Based on these findings, it was of interest to synthesize a new series of triazole derivatives and evaluate their interaction with BSA by studying the effect of varying substituents on the binding strength of the triazole derivatives. The binding mode and binding site of the triazoles to BSA molecules were also studied. These findings may provide basic data on the binding mechanism of the synthesized triazole derivatives with blood carrier proteins, which can be helpful for the security of human health when applied in pharmaceutical formulations.

2. Results and discussion

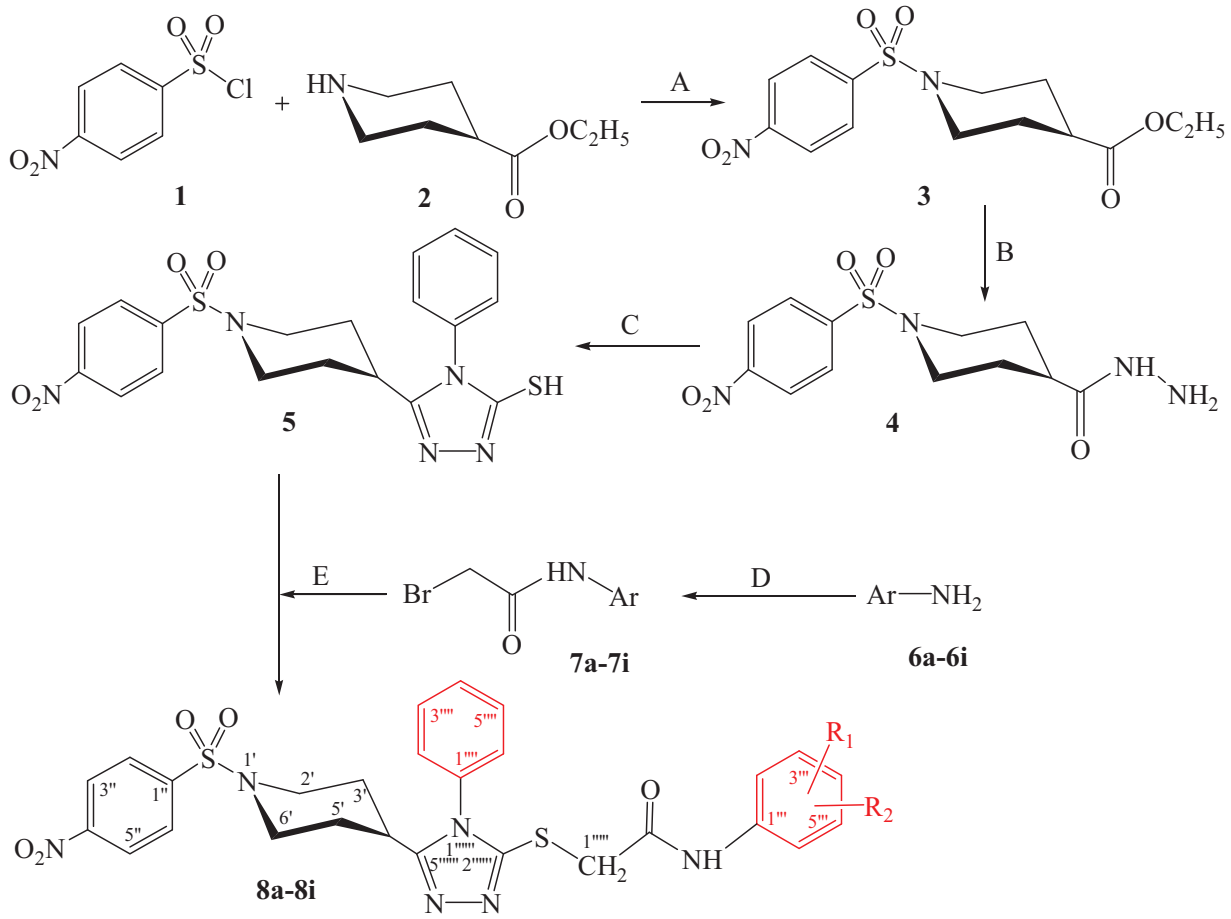
2.1. Chemistry

N-(substituted)-2-(5-(1-(4-nitrophenylsulfonyl)piperidin-4-yl)-4-phenyl-4*H*-1,2,4-triazol-3-ylthio)acetamides (**8a**–**8i**; Table 1; Scheme) were synthesized in good yield. Reaction of 4-nitrobenzene sulfonyl chloride with ethyl isonipecotate produced ethyl 1-[(4-nitrophenyl)sulfonyl]piperidin-4-carboxylate (**3**), which on refluxing with hydrazine monohydrate yielded 1-(4-nitrophenylsulfonyl)piperidin-4-carbohydrazide (**4**). Compound **4** was first refluxed with phenyl isothiocyanate and then cyclized by KOH into 5-(1-(4-nitrophenylsulfonyl)piperidin-4-yl)-4-phenyl-4*H*-1,2,4-triazole-3-thiol (**5**). Nine different compounds of *N*-(substituted)-2-(5-(1-(4-nitrophenylsulfonyl)piperidin-4-yl)-4-phenyl-4*H*-1,2,4-triazol-3-ylthio)acetamide (**8a**–**8i**) were synthesized by stirring compound **5** with *N*-aryl/aralkyl-2-bromoacetamides (**7a**–**8i**) in DMF at room temperature.

Table 1. Different aryl substitutions for **8a**–**8i**.

Comp.	R_1 & R_2	Comp.	R_1 & R_2
8a	R_1 =R_2 = H	**8f**	R_1 = 2Me; R_2 = 5Me
8b	R_1 = 2Et; R_2 = H	**8g**	R_1 = 2Me; R_2 = 6Me
8c	R_1 = 4Et; R_2 = H	**8h**	R_1 = 3Me; R_2 = 4Me
8d	R_1 = 2Et; R_2 = 6Me	**8i**	R_1 = 3Me; R_2 = 5Me
8e	R_1 = 2Me; R_2 = 3Me		

Compound **8e**, a yellowish amorphous solid, was synthesized in good yield with the molecular formula $C_{29}H_{30}N_6O_5S_2$ as established by EI-MS through molecular ion peak at m/z 606 and by ^{1}H NMR by counting the number of protons. In the IR spectrum the values calculated at 3340 cm^{-1}, 3075 cm^{-1}, 1678 cm^{-1}, 1665



Scheme. General scheme for the synthesis of *N*-(substituted)-2-(5-(1-(4-nitrophenylsulfonyl)piperidin-4-yl)-4-phenyl-4*H*-1,2,4-triazol-3-ylthio)acetamide (**8a–8i**). Reagents and conditions: (A) 10 % Na_2CO_3 (aq), H_2O, 8 h. (B) $N_2H_4 \bullet H_2O$, EtOH, reflux, 5 h. (C) i. PhNCS, MeOH, reflux, 5 h. ii. 10% KOH, reflux, 6 h. (D) $BrCH_2COBr$, 15% Na_2CO_3 (aq), H_2O, 1 h. (E) DMF, LiH, 2–4 h.

cm^{-1}, 1596 cm^{-1}, 1523 cm^{-1}, 1435 cm^{-1}, 1076 cm^{-1}, and 605 cm^{-1} were assigned to (N-H) stretching, (Ar C-H) stretching, (C=N) stretching, (C=O) stretching, (Ar C=C) stretching, (N=O) stretching, (S=O) stretching, (C-N) stretching, and (C-S) stretching, respectively. The spectrum of ^{1}H NMR showed signals at 7.50 (d, J =8.7 Hz, 2H) and 6.64 (d, J =8.6 Hz, 2H) due to aromatic protons of the 4-nitrophenylsulfonyl group. The signals at 7.56 (d, J =8.8 Hz, 2H), 7.47 (t, J =10.44 Hz, 1H), and 7.19 (d, J =7.62 Hz, 2H) were due to aromatic protons of the phenyl group attached with a triazole ring. The signals for protons of the *N*-substituted aromatic ring of acetamide were obtained at 7.60 (d, J =8.04 Hz, 1H), 7.19 (t, J =10.2 Hz, 1H), and 6.98 (d, J =7.5 Hz, 1H). An amidic proton showed the signal at 8.56 (s, 1H, N-H) and piperidine showed signals at 3.71–3.69 (m, 2H), 2.27–2.22 (m, 2H), 2.11 (br.s, 1H), and 1.83–1.81 (m, 4H). The signal at 3.94 (s, 2H) was due to a -CH_2 group directly attached with a heteroatom and the signals at 2.29 (s, 3H) and 2.18 (s, 3H) showed the presence of two methyl groups. In the ^{13}C NMR spectrum, peaks of aromatic quaternary carbons were obtained at δ 167.1158.1, 137.3, 135.7, 132.8, and 132.4 for the whole molecule and two quaternary carbons of the five-membered triazole ring appeared at 152.6 and 150.5. The carbonyl carbon of the acetamoyl

group appeared at 168.9. The signals of aromatic carbons of phenyl attached to the sulfonyl group resonated at 130.8 and 129.8 while the signals of two aromatic rings attached with the 1,2,4-triazole ring appeared at 127.6, 126.9, 125.7, 124.2, 122.8, and 113.9. Peaks of carbon atoms of the piperidine ring appeared at 45.5, 35.6, and 29.2. The carbons of methylene and two methyl groups appeared at 32.1, 20.6, and 13.8, respectively. On the basis of these accumulative spectral data, the structure of compound **8e** was determined as *N*-(2,3-dimethylphenyl)-2-(5-(1-(4-nitrophenylsulfonyl)piperidin-4-yl)-4-phenyl-4*H*-1,2,4-triazol-3-ylthio)acetamide.

2.2. Biological activities

2.2.1. BSA binding study

Triazole and its derivatives have been known for their wide range of biological activities, such as antibacterial, antispasmodic, antiinflammatory, antiplatelet agglomeration and proliferation inhibition, and induction of differentiation and apoptosis of tumor cells.[16] In this study, the interaction of selected triazole derivatives with BSA (BSA) was investigated in order to provide some useful information on the therapeutic effects of these compounds in pharmacology and pharmacodynamics.

The binding studies of selected triazole derivatives with BSA were evaluated in view of investigating the effect of various substituents attached to the triazole ring on the interactions with the globular protein. The binding of the triazole derivatives with BSA was carried out by fluorometric titrations of BSA with varying concentration of the triazole derivatives (0–5.57 $\times$ 10^{-4} M). The binding abilities and binding site of the synthesized derivatives were compared to that of fluorescent markers warfarin and ibuprofen. Fluorometric titrations of BSA with and without compounds **8a**–**8i** or markers were carried out at an emission of 336 nm (excitation: 295 nm). The emission spectra of BSA in the presence of the different synthesized derivatives are given in Figure 1. It was observed that BSA showed maximum emission at λ_{em} = 336 nm. Addition of the synthesized compounds quenched the fluorescence intensity of BSA, showing that the 1,2,4-triazole-based analogs were able to bind to BSA, causing some modification in the microenvironment around the amino acid residues situated in the subdomain of BSA leading to a hypochromic shift in the fluorescence spectra. The plots in Figures 2a–2e show the Stern–Volmer plots for the binding of BSA and compounds **8a**–**8i** and the K_{sv} values obtained from the slopes are summarized in Table 2.

Table 2. Stern–Volmer quenching constant and quenching rate constant of the different compound-BSA systems.

Entry	Compound	K_{sv} (L mol^{-1})	K_q (L mol^{-1}s^{-1}) ($\times$ 10^{10})
1	**8a**	902.5	9
2	**8c**	666.2	6
3	**8d**	406.8	4
4	**8h**	713.3	7
5	**8i**	1633	16
6	Ibuprofen	3502	35

Fluorescence quenching can be classified as either a static or dynamic process and is caused by different mechanisms including excited state reactions, molecular rearrangements, energy transfer, formation of ground state complexes, and collisional quenching.[17] The type of quenching mechanism is usually interpreted by the

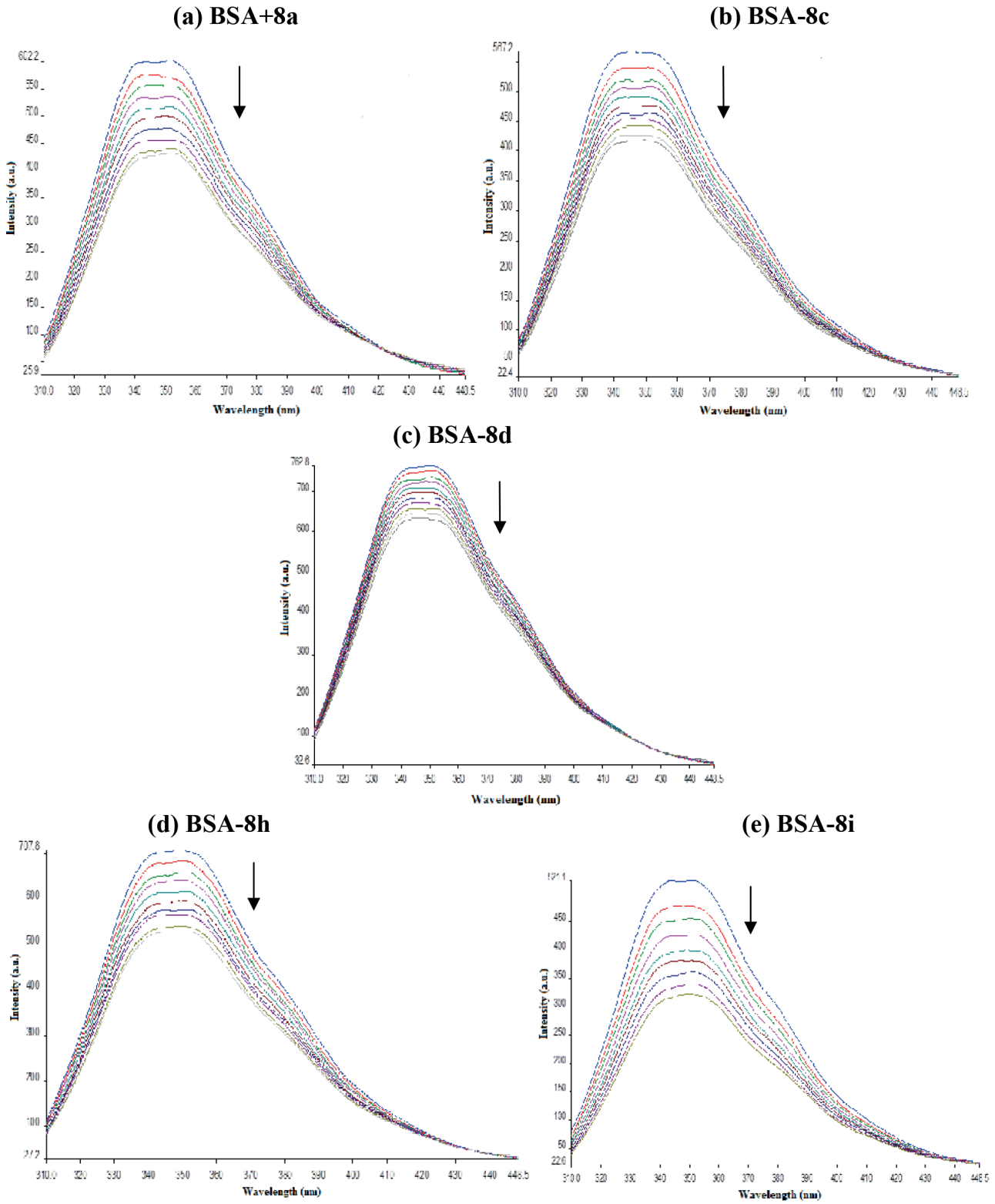


Figure 1. a–e) Fluorescence spectra of BSA in presence of different amounts of selected compounds (**8a**, **8c**, **8d**, **8h**, **8i**) at 295 nm and 298 K (inset graph corresponding to the Stern–Volmer plot).

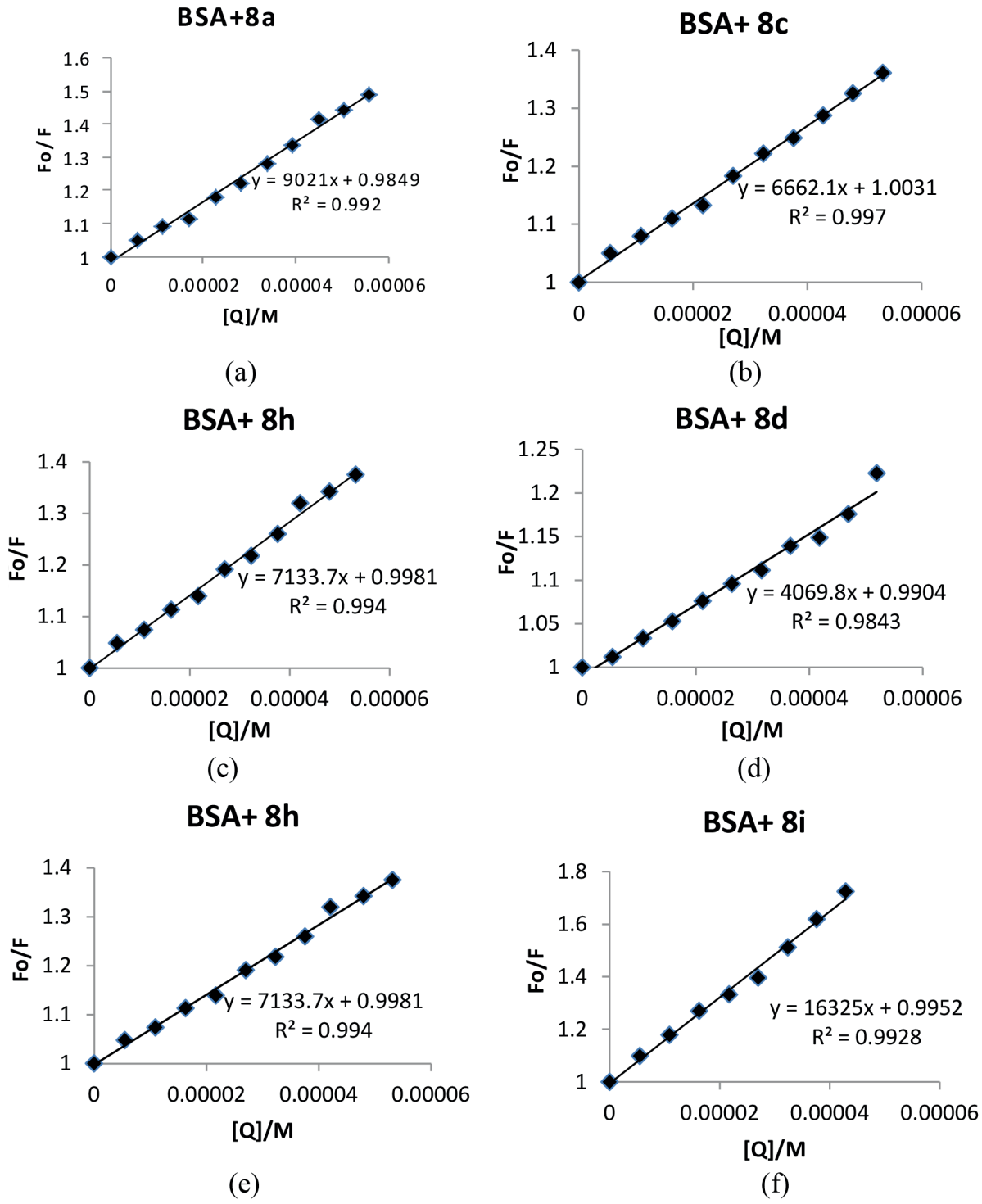


Figure 2. a–e) Stern–Volmer plots of the interactions of selected compounds (**8a**, **8c**, **8d**, **8h**, **8i**) with BSA at 298 K.

Stern–Volmer equation:[18]

$$F_o/F = 1 + K_{SV}[Q] = K_q\tau_o[Q] + 1, \quad (1)$$

where F_o and F are the fluorescence intensities of BSA before and after addition of quencher, respectively; K_{sv} is the Stern–Volmer quenching constant; [Q] is the concentration of the quencher; K_q is the apparent bimolecular quenching rate constant; and τ_o is the average lifetime of the biomolecule without the quencher and its value is 10^{-8} s.[19]

In all cases, the calculated K_q values were found to be larger than the maximum scattering collision quenching rate constant ($2 \times 10^{10}\,M^{-1}\,s^{-1}$) in dynamic quenching. This indicated that the fluorescence quenching process of BSA with the triazole compounds, as well as warfarin and ibuprofen, was primarily governed by a static quenching mechanism, rather than a dynamic one.[20]

The binding constant K_a and the number of binding sites n of synthesized compounds can be calculated by using the following double logarithm equation:

$$\log[(F_0 - F)/F] = \log K_a + n \log[Q]. \tag{2}$$

K_a and n are obtained from the intercept and slope of a linear plot of log (F_o – F)/F versus log [Q], respectively (Figure 3), and the values are summarized in Table 3.

Table 3. Binding constant and number of binding site of compounds **8a**–**8i**, warfarin, and ibuprofen with BSA at 298 K.

Entry	Compound	K_a (L mol^{-1})	**N**
1	**8a**	859	1.03
2	**8c**	288.1	0.89
3	**8d**	1175	1.23
4	**8h**	1051	0.94
5	**8i**	1820	0.96
6	Warfarin	641,000	1.07
7	Ibuprofen	56,870	1.26

The different substituents on the triazole ring were found to affect the binding ability of the 1,2,4-triazole-based derivatives with BSA. The presence of the methyl group at the *meta* position enhanced the affinity of the triazole derivative to BSA (entries 4 and 5), giving rise to higher K_a values. This might be due to an increase in molecular size, providing a larger hydrophobic area that can interact with the hydrophobic surface on the protein molecule.[21] However, when the methyl group was at the *para* position, a decrease in binding constant was observed (entry 4).

2.2.2. Thermodynamics of BSA binding

The interactions between the triazole compounds and BSA may involve hydrophobic, electrostatic, or van der Waals forces as well as hydrogen bonding. In order to elucidate the types of interaction occurring between triazole compounds and BSA, thermodynamic parameters such as free energy (ΔG), enthalpy (ΔH), and entropy (ΔS) were determined and these were used to demonstrate the type of binding taking place.

The four binding modes through which small molecules can bind to biological macromolecules are hydrogen bonds, hydrophobic forces, electrostatic forces, and van der Waals interactions. These interactions can be described by thermodynamic parameters of enthalpy and entropy energies. If $\Delta H < 0$ and $\Delta S < 0$, van der

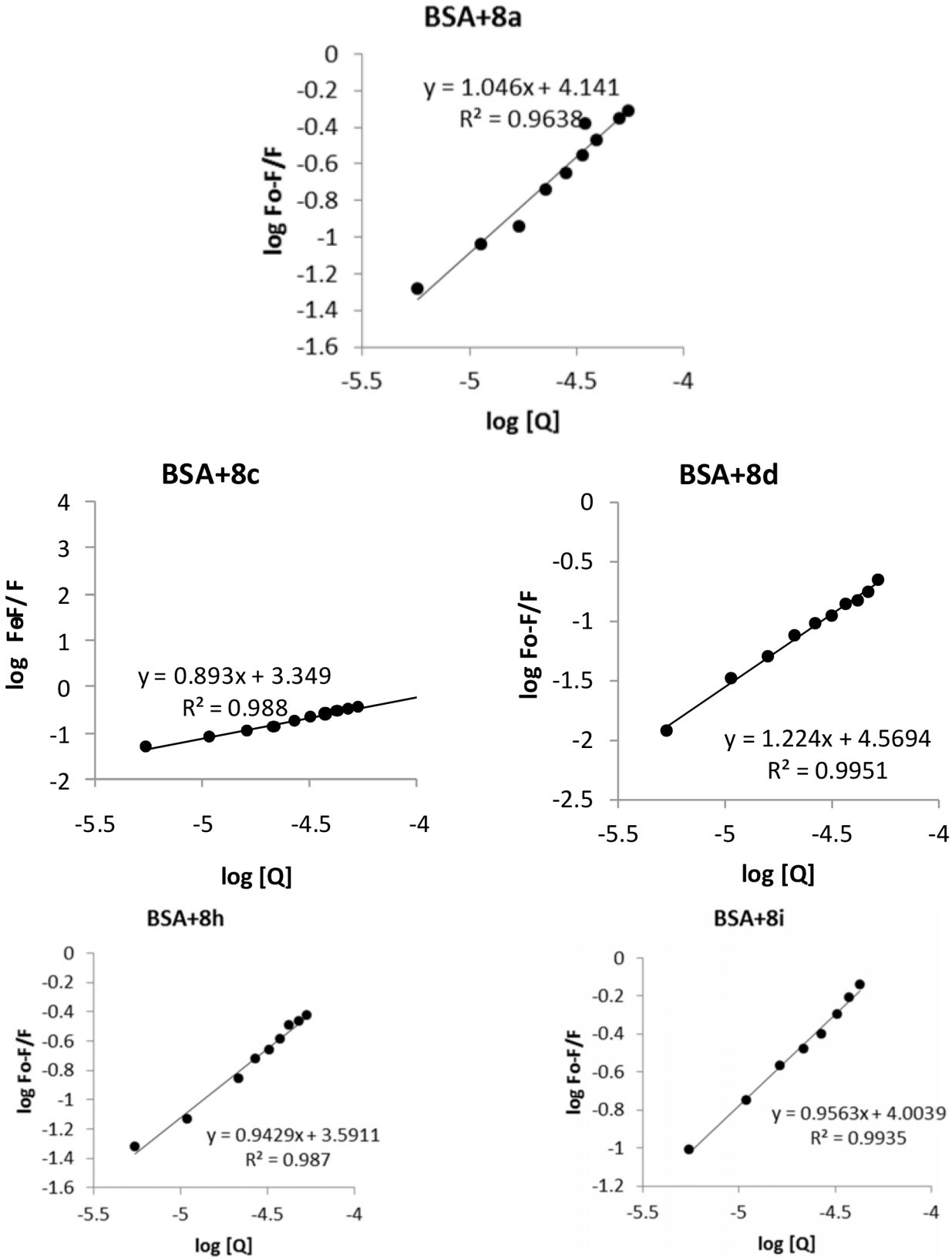


Figure 3. **a**–**e**) Linear double logarithm plot for BSA and **8a**, **8c**, **8d**, **8h**, and **8i** bindings.

Waals forces and hydrogen bonds play major roles in the binding reaction. If ΔH >0 and ΔS >0, hydrophobic interactions are dominant. Electrostatic forces are more prominent when ΔH <0 and ΔS >0.[22]

The thermodynamic parameters of selected triazole analogs binding to BSA were studied at three different temperatures. ΔG, ΔH, and ΔS values were estimated using Eqs. (3) and (4):

$$\Delta G = -RT \ln K, \tag{3}$$

where T is the experimental temperature, K_a is the binding constant at T, and R is the gas constant. The temperature-dependent thermodynamic parameters, ΔH and ΔS, can be analyzed using the van't Hoff formula:

$$\ln K_a = -(\Delta H/RT) + (\Delta S/R), \tag{4}$$

where K_a is the binding constant, R is the gas constant, and T is experimental temperature. To obtain values of ΔH and ΔS, the graph of ln K_a against 1/T was plotted (Figure 4) and the results are tabulated in Table 4. From the slope and intercept of the linear van't Hoff plot, values of ΔH and ΔS were obtained and the free energy change, ΔG, was also estimated using the following relationship:[1]

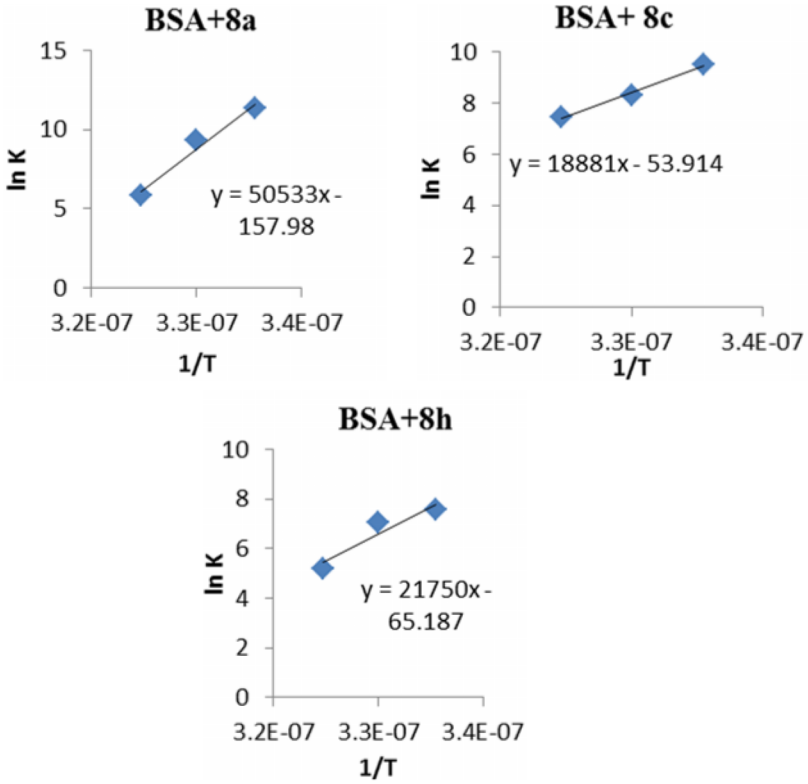


Figure 4. van't Hoff plots for BSA-(**8a**, **8c**, **8h**) systems.

$$\Delta G = \Delta H - T\Delta S. \tag{5}$$

From Figure 3, it can be observed that there is good linearity of the van't Hoff plots. The negative ΔH and negative ΔS indicated that the interactions with **8a**–**8i** are enthalpically driven but not entropically favorable. Moreover, the negative ΔG values at all temperatures for **8a**–**8i** proved that the interactions between the

Table 4. Binding constants and thermodynamic parameters obtained at three temperatures (298, 303, and 308 K).

System	Temp. (T) K	$1/T \times 10^{-3}$ K^{-1}	K_a L mol^{-1}	ln K_a	ΔG $kJmol^{-1}$	ΔH $kJmol^{-1}$	ΔS $Jmol^{-1}K^{-1}$
BSA-**8a**	298	3.356	8.350×10^4	11.33	–28.07	–420.13	–1313.45
	303	3.300	1.114×10^4	9.32	–23.48		
	308	3.247	3.364×10^2	8.12	–20.79		
BSA-**8c**	298	3.356	1.318×10^4	9.49	–23.51	–156.98	–448.24
	303	3.300	4.080×10^3	8.31	–20.93		
	308	3.247	1.687×10^3	7.43	–19.03		
BSA-**8i**	298	3.356	1.967×10^3	7.58	–18.78	–180.83	–541.96
	303	3.300	1.135×10^3	7.03	–17.71		
	308	3.247	1.826×10^2	5.21	–13.34		

synthesized compounds with BSA were spontaneous. From the thermodynamic parameters, the negative ΔH and ΔS values indicated that van der Waals forces and hydrogen bonding play major roles in the binding of compounds **8a**–**8i** to BSA and have a great contribution to the stability of the complex formed. The triazole derivatives contain electronegative atoms and therefore can form hydrogen bonds with the amino acid residues in BSA.

2.2.3. Site-selective binding of selected compounds on BSA in the presence of site markers

It is reported that the structure of BSA consists of three homologous domains named I, II, and III and each domain includes two subdomains called A and B. The major regions of ligand-binding sites on BSA are situated in hydrophobic cavities known as subdomains IIA (Sudlow's site 1) and IIIA (Sudlow's site II).

The binding locations are usually studied with site markers that have specific binding sites and that can help determine the drug-binding sites on BSA by performing competitive binding experiments. To determine the binding site of compounds **8a**–**8i** on BSA, warfarin and ibuprofen were selected as site markers for site I and site II, respectively. A varied amount of compounds **8a**–**8i** was added to a solution of site marker and BSA and the resulting mixture was excited at 295 nm.

The fluorescence quenching data of the **8a**–**8i** and BSA system in the presence of site markers were analyzed using Eq. (2) and the results are tabulated in Table 5. The binding constants significantly decreased in the presence of ibuprofen but to a lesser extent in the presence of warfarin. Therefore, this showed that ibuprofen competed with the triazole derivatives in the subdomain IIIA of BSA, demonstrating that the binding of the triazole derivatives with BSA primarily takes place at site II.

2.2.4. Synchronous study of the binding of 1,2,4-triazole analogs to BSA

Synchronous fluorescence spectroscopy was carried out to confirm the binding site of compounds **8a**–**8i** with BSA by monitoring the change in the microenvironment surrounding Trp and Tyr residues. The shift in the position of the fluorescence emission maximum corresponded to modification of the polarity around Trp and Tyr residues. A blue shift of λ_{max} indicated that the amino acid residues were situated in a more hydrophobic environment while a red shift of λ_{max} implied that the amino acid residues were in a polar environment. It is possible to study the change in the conformation of BSA induced by triazole-based synthesized compounds by measuring the fluorescence intensity of BSA before and after the addition of the compound. From the literature information, the synchronous fluorescence spectra obtained at $\Delta\lambda = 15$ nm and $\Delta\lambda = 60$ nm corresponded to

Table 5. Binding constant of selected triazole compounds with BSA in presence of site markers.

System	Site marker	K_a (L mol^{-1})	N
BSA-**8a**	Blank (without marker)	3.70×10^3	1.06
	Warfarin	2.70×10^3	0.95
	Ibuprofen	5.15×10^2	0.92
BSA-**8c**	Blank (without marker)	1.60×10^3	0.64
	Warfarin	1.24×10^3	1.05
	Ibuprofen	9.60×10^2	0.94
BSA-**8i**	Blank (without marker)	9.42×10^3	1.21
	Warfarin	3.10×10^3	0.86
	Ibuprofen	5.49×10^2	1.11

the Tyr and Trp residues, respectively.[23] The synchronous spectra for BSA-TDs are displayed in Figure 5 for $\Delta\lambda = 15$ nm and in Figure 6 for $\Delta\lambda = 60$ nm.

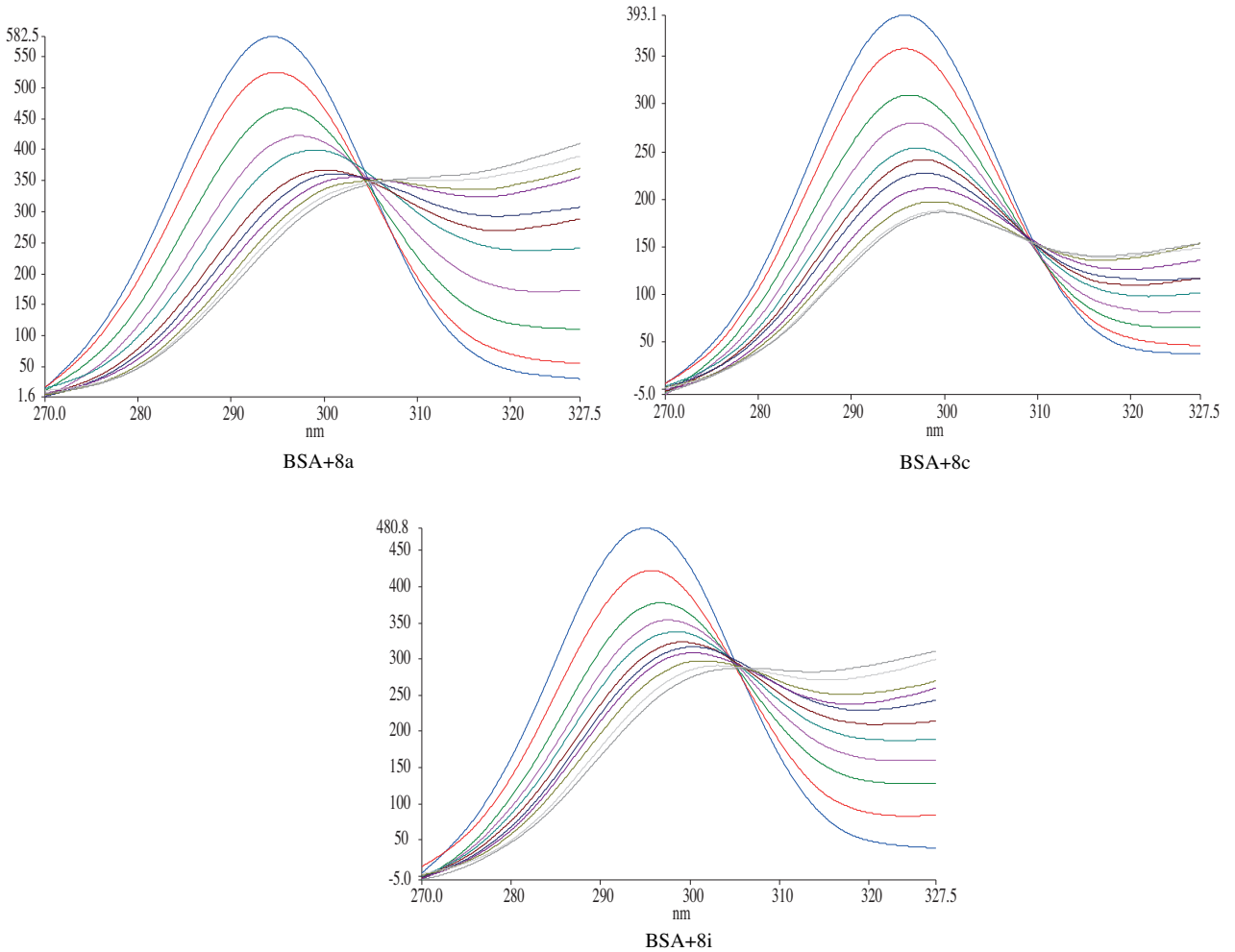


Figure 5. Synchronous fluorescence spectra of BSA in the presence of **8a**, **8c**, and **8i** at $\Delta\lambda = 15$ nm (red shift).

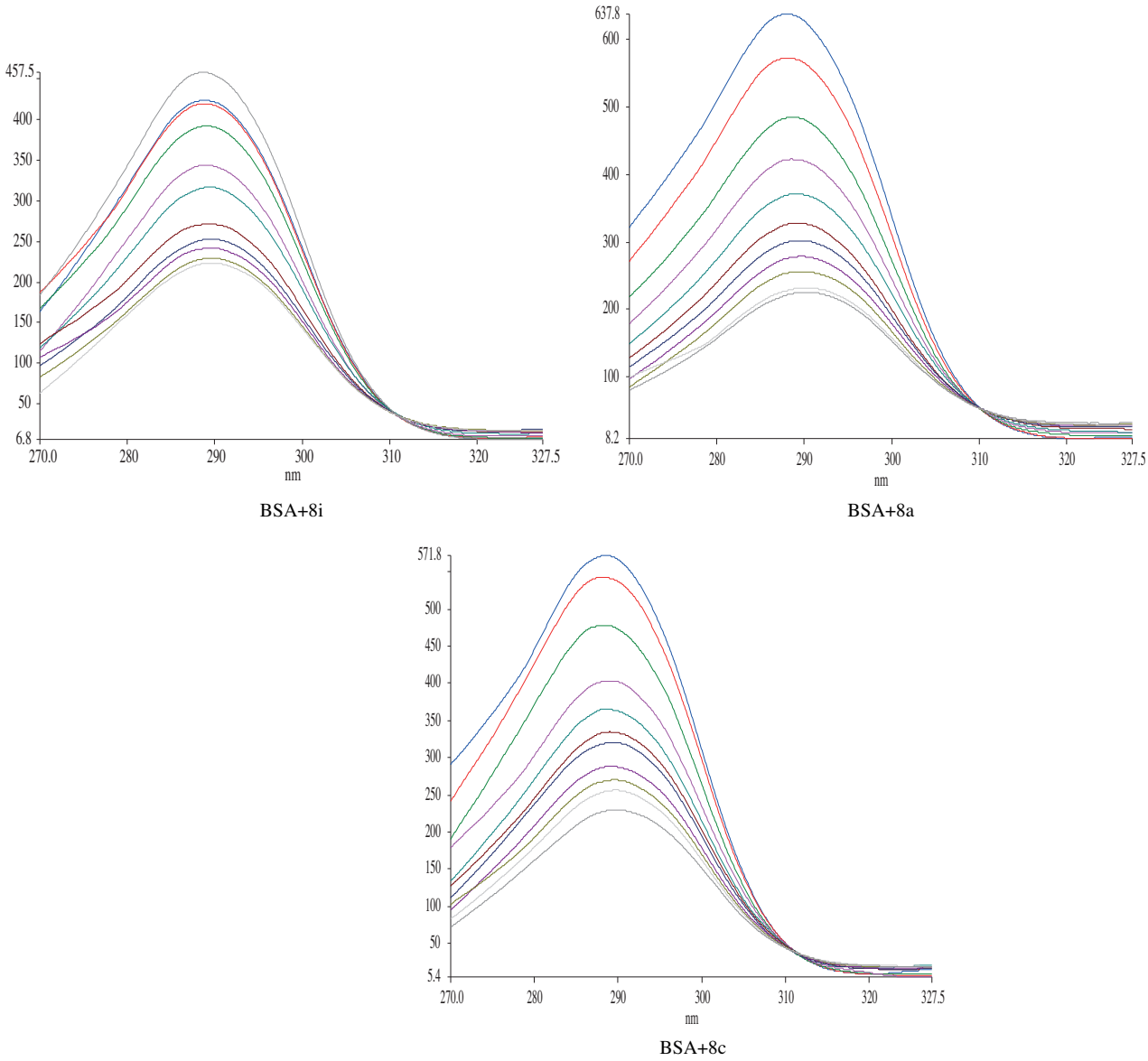


Figure 6. Synchronous fluorescence spectra of BSA in the presence of **8a**, **8c**, and **8i** at $\Delta\lambda = 60$ nm.

The results revealed that the fluorescence intensity of both Trp and Tyr decreased with a notable red shift at maximum emission $\Delta\lambda = 15$ nm in the presence of compounds **8a**–**8i**, whereas no significant shift was observed at $\Delta\lambda = 60$ nm for **8a**–**8i**. This observation confirmed that the triazole derivatives were bound to the tyrosine residue of BSA, in a similar manner to that of ibuprofen.

Infrared spectroscopy is considered to be a useful technique to investigate the secondary structure of proteins. In the FT-IR region, amide I, II, and III vibrations are related to the frequencies of the bands, especially that of amide I, as it is more sensitive to change of the secondary structure. It was reported that the amide I peak is situated in the region of 1600–1700 cm^{-1} (mainly C=O stretching) and the amide II peak at 1548 cm^{-1} (C-N stretch coupled with N-H bending mode).[1] Comparing the FT-IR spectra of BSA with and without triazole-derived compounds **8a**–**8i**, it was found that the peak of amide I, which corresponds to the

(C=O) stretching, shifted from 1651.72 cm^{-1} to 1652.05 cm^{-1}, indicating that the secondary structure of BSA was altered due to the interaction between compounds **8a**–**8i** and BSA. From FT-IR analysis, a change in the absorbance of BSA in the presence of compound **8a**–**8i** was observed (Figure 7). The spectrum of BSA deforms as the ratio of peak heights in the absence and in the presence of compounds **8a**–**8i** (diluted and concentrated) is different. This result indicates that the secondary structure of BSA has been altered due to the interaction of BSA with compounds **8a**–**8i**.

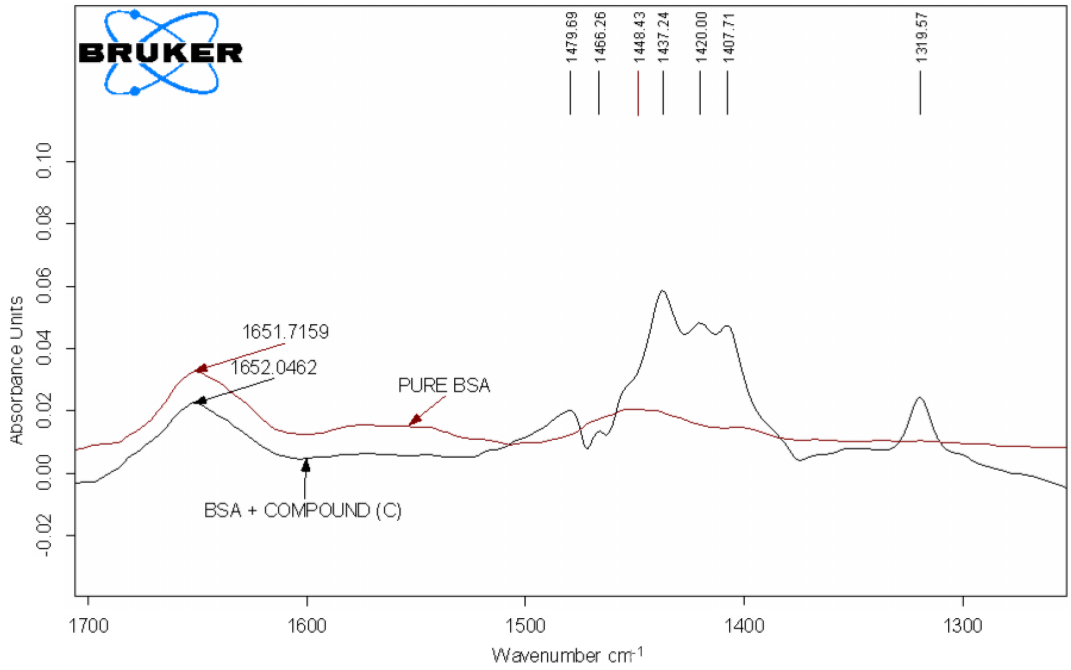


Figure 7. FT-IR difference spectra of free BSA and BSA + compound.

2.3. Conclusions

Results of the fluorescence studies showed that the triazole derivatives were able to interact with BSA, causing a static quenching of the fluorescence intensity of BSA in a similar way to that of the well-known drugs warfarin and ibuprofen. The presence and position of different substituents on the triazole ring was found to affect the binding affinity of the synthesized compounds for BSA. The thermodynamic parameters indicated that the binding between the triazole analogs and BSA was spontaneous and involved mostly van der Waals forces and hydrogen bonding. Synchronous fluorescence data and competitive binding of compounds **8a**–**8i** in the presence of warfarin and ibuprofen illustrated that compounds **8a**–**8i** were bound to the same binding site as that of ibuprofen, i.e. on tyrosine residues of BSA. This study showed that the 1,2,4-triazole-based compounds were able to bind to BSA and therefore can be effectively transported and eliminated in the body, which can be a useful guideline to open new avenues for the designing of the most suitable triazole derivatives for further drug design.

3. Experimental

3.1. General

All the chemicals were of analytical grade and were obtained from Sigma Aldrich and Alfa Aesar Ltd. and were provided by local chemical trading companies in Pakistan. Phosphate buffer (20 mM, pH 7.4), BSA,

calf-thymus DNA, Tris-buffered saline (0.05 M, pH 7.6), and ethidium bromide were purchased from Sigma Aldrich (St. Louis, MO, USA). IR spectra were recorded on a Bruker Alpha FT-IR spectrometer. Aluminum plates coated with silica gel were used to confirm the purity of synthesized compounds with an n-hexane : ethyl acetate mobile phase on TLC and visualized with a UV_{254} lamp. ^{1}H NMR (600 MHz, $CDCl_3$), ^{13}C NMR (150 MHz, $CDCl_3$), and EI-MS data were recorded using a Bruker spectrometer and JMS-HX-110 spectrometer, respectively.

3.2. Synthesis

3.2.1. Synthesis of ethyl 1-(4-nitrophenylsulfonyl)piperidin-4-carboxylate (3)

Ethyl 1-[(4-nitrophenyl)sulfonyl]piperidin-4-carboxylate (**3**) was synthesized by stirring 4-nitrobenzene sulfonyl chloride (**1**; 0.05 mol) with ethyl isonipecotate (**2**; 0.05 mol) at room temperature for 8 h in distilled water (30 mL). The pH of the reaction was maintained at 9–10 using 10% Na_2CO_3 solution. Completion of the reaction was confirmed by TLC and then chilled distilled water was added to precipitate the product, which was filtered, washed, and dried.

3.2.2. Synthesis of 1-(4-nitrophenylsulfonyl)piperidine-4-carbohydrazide (4)

Ethyl 1-[(4-chlorophenyl)sulfonyl]piperidin-4-carboxylate (**3**; 0.043 mol) was refluxed with hydrazine monohydrate (0.043 mol) using ethanol as a solvent for 5 h. TLC was used to monitor the reaction. At the completion of reaction, an excess of chilled distilled water was added with continuous shaking and the reaction mixture was kept at ice-cold temperature for 5 h. The obtained precipitate was filtered, washed, and dried at room temperature.

3.2.3. Synthesis of 5-(1-(4-nitrophenylsulfonyl)piperidin-4-yl)-4-phenyl-4H-1,2,4-triazole-3-thiol (5)

Compound **4** (0.030 mol) was refluxed with phenyl isothiocyanate (0.030 mol) in methanol for 5 h. The uncyclized precipitates obtained were filtered, washed, dried, and refluxed with 10% KOH for 6 h in distilled water. At the completion of the reaction as monitored by TLC, diluted HCl was added to the mixture drop by drop with continuous shaking until the pH was 5. After 30 min an off-white solid, compound **5**, was obtained, which was filtered, washed with distilled water, and dried at room temperature.

3.2.4. Synthesis of *N*-(substituted)-2-bromoacetamides (7a–8i)

Alkylaryl/aryl amines (**6a**–**6i**; 0.02 mol) were stirred with 2-bromoacetyl bromide (0.02 mol) in distilled water for 1 h. The pH was adjusted to 9–10 using 15% Na_2CO_3 solution. The formed precipitates were filtered, washed with distilled water, and dried. Those products that were not obtained in precipitate form were extracted using chloroform.

3.2.5. Synthesis of *N*-(substituted)-2-(5-(1-(4-nitrophenylsulfonyl)piperidin-4-yl)-4-phenyl-4*H*-1,2,4-triazol-3-ylthio)acetamide (8a–8i)

Compound **5** (0.0044 mol) was stirred with LiH in DMF (15 mL) for 30 min, and then an equimolar amount of N-(substituted)-2-bromoacetamides (**7a**–**7i**) was added. The whole mixture was stirred at room temperature for 2–4 h. The completion of the reaction was confirmed with TLC. Chilled distilled water was added after the completion of the reaction to obtain the targeted products, which were filtered, washed, and dried.

3.3. Spectral characterization of synthesized compounds

3.3.1. 2-(5-(1-(4-Nitrophenylsulfonyl)piperidin-4-yl)-4-phenyl-4*H*-1,2,4-triazol-3-ylthio)-*N*-phenylacetamide (8a)

Yellowish amorphous solid; yield: 79%; mp: 140–141 °C; molecular formula $C_{27}H_{26}N_6O_5S_2$; molecular mass: 578.66 g mol^{-1}; IR (KBr, υ_{max}, cm^{-1}): 3343 (N-H), 3109 (Ar C-H), 1654 (C=N), 1639 (C=O), 1584 (Ar C=C), 1538 (N=O), 1445 (S=O), 1150, 1146 (C-N), 619 (C-S); ^{1}H NMR ($CDCl_3$, 600 MHz, δ (ppm)): 10.27 (s, 1H, N-H), 7.57 (d, J = 7.8 Hz, 2H, H-2′′′′, H-6′′′′), 7.54 (d, J =8.8 Hz, 2H, H-2′′′, H-6′′′), 7.49 (d, J =8.9 Hz, 2H, H-3′′, H-5′′), 7.45 (d, J =7.7 Hz, 2H, H-3′′′, H-5′′′), 7.31 (t, J = 8.2 Hz, 1H, H-4′′′′), 7.21 (d, J = 7.7 Hz, 2H, H-3′′′′, H-5′′′′), 7.10 (t, J = 7.4 Hz, 1H, H-4′′′), 6.66 (d, J = 8.6 Hz, 2H, H-2′′, H-6′′), 4.11 (s, 2H, H-1′′′′′), 3.67–3.65 (m, 2H, H_{eq}-2′, H_{eq}-6′), 2.50–2.46 (m, 1H, H-4′), 2.27–2.23 (m, 2H, H_{ax}-2′, H_{ax}-6′), 2.04–2.00 (m, 2H, H_{eq}-3′, H_{eq}-5′), 1.97–1.83 (m, 2H, H_{ax}-3′, H_{ax}-5′); ^{13}C NMR (150 MHz, $CDCl_3$): δ 168.2 (C-2′′′′′), 166.6 (C-4′′), 158.0 (C-1′′), 150.5 (C-1′′′′′), 138.2 (C-2′′′′′), 132.4 (C-1′′′′), 130.7 (C-1′′′), 130.2 (C-3′′, C-5′′), 129.8 (C-2′′, C-6′′), 128.8 (C-2′′′′, C-6′′′′), 126.6 (C-2′′′, C-6′′′), 124.1 (C-3′′′, C-5′′′), 122.9 (C-4′′′), 119.7 (C-4′′′′), 113.9 (C-3′′′′, C-5′′′′), 45.5 (C-2′, C-6′), 36.1 (C-4′), 32.1 (C-1′′′′′), 29.4 (C-3′, C-5′); EI-MS (m/z): 578 [M]$^+$, 386 [$C_{18}H_{18}N_4O_4S$]$^+$, 295 [$C_{12}H_{13}N_3O_4S$]$^+$, 270 [$C_{11}H_{14}N_2O_4S$]$^+$, 186 [$C_6H_4NO_4S$]$^+$, 123 [$C_6H_5NO_2$]$^+$, 78 [C_6H_6]$^+$, 51 [C_4H_4]$^+$; Anal. Calcd. for $C_{27}H_{26}N_6O_5S_2$: C 56.04, H 4.53, N 14.52, O 13.82, S 11.08; found C 56.01, H 4.31, N 14.29, O 13.56, S 11.02.

3.3.2. *N*-(2-Ethylphenyl)-2-(5-(1-(4-nitrophenylsulfonyl)piperidin-4-yl)-4-phenyl-4*H*-1,2,4-triazol-3-ylthio)acetamide (8b)

Light yellowish amorphous solid; yield: 85%; mp: 137–138 °C; molecular formula: $C_{29}H_{30}N_6O_5S_2$; molecular mass: 606.72 g mol^{-1}; IR (KBr, υ_{max}, cm^{-1}): 3341 (N-H), 3134 (Ar C-H), 1652 (C=N), 1638 (C=O), 1596 (Ar C=C), 1546 (N=O), 1441 (S=O), 1125 (C-N), 633 (C-S); ^{1}H NMR ($CDCl_3$, 600 MHz, δ (ppm)): 9.66 (s, 1H, N-H), 7.66 (s, 1H, H-6′′′), 7.54 (d, J = 9.1 Hz, 2H, H-2′′′′, H-6′′′′), 7.49 (d, J =8.5 Hz, 2H, H-3′′, H-5′′), 7.39–7.38 (m, 1H, H-4′′′′), 7.20 (d, J = 7.7 Hz, 2H, H-3′′′′, H-5′′′′), 7.17 (d, J = 10.9 Hz, 1H, H-3′′′), 7.10 (t, J = 8.2 Hz, 1H, H-5′′′), 7.02 (t, J = 7.9 Hz, 1H, H-4′′′), 6.66 (d, J = 8.6 Hz, 2H, H-2′′, H-6′′), 4.11 (s, 2H, H-1′′′′′), 3.69–3.67 (m, 2H, H_{eq}-2′, H_{eq}-6′), 2.63 (q, J = 8 Hz, 2H, H-2′′′′′), 2.50–2.48 (m, 1H, H-4′), 2.33–2.29 (m, 2H, H_{ax}-2′, H_{ax}-6′), 1.98 (dq, J = 3.6, 13.8 Hz, 2H, H_{eq}-3′, H_{eq}-5′), 1.84–1.82 (m, 2H, H-3′, H-5′), 1.14 (t, J = 7.5 Hz, 3H, H-3′′′′′); ^{13}C NMR (150 MHz, $CDCl_3$): δ 168.8 (C-4′′′′′), 167.1 (C-4′′), 158.2 (C-1′′), 152.7 (C-1′′′′′), 150.5 (C-2′′′′′), 135.5 (C-1′′′′), 132.3 (C-1′′′), 130.7 (C-3′′′), 130.4 (C-3′′, C-5′′), 129.8 (C-2′′, C-6′′), 128.7 (C-6′′′), 127.5 (C-2′′′), 126.9 (C-2′′′′, C-6′′′′), 126.4 (C-5′′′), 125.3 (C-4′′′), 123.3 (C-4′′′′), 113.9 (C-3′′′′, C-5′′′′), 45.4 (C-2′, C-6′), 35.5 (C-4′), 31.9 (C-1′′′′′), 29.4 (C-3′, C-5′), 24.4 (C-2′′′′′), 14.2 (C-3′′′′′); EI-MS (m/z): 606 [M]$^+$, 386 [$C_{18}H_{18}N_4O_4S$]$^+$, 295 [$C_{12}H_{13}N_3O_4S$]$^+$, 270 [$C_{11}H_{14}N_2O_4S$]$^+$, 186 [$C_6H_4NO_4S$]$^+$, 123 [$C_6H_5NO_2$]$^+$, 106 [C_8H_{10}]$^+$, 51 [C_4H_4]$^+$; Anal. Calcd. for $C_{29}H_{30}N_6O_5S_2$: C 57.41, H 4.98, N 13.85, O 13.19, S 10.57; found C 57.41, H 4.66, N 13.67, O 13.06, S 10.39.

3.3.3. *N*-(4-Ethylphenyl)-2-(5-(1-(4-nitrophenylsulfonyl)piperidin-4-yl)-4-phenyl-4*H*-1,2,4-triazol-3-ylthio)acetamide (8c)

Yellowish amorphous solid; yield: 78%; mp: 111-112 °C; molecular formula: $C_{29}H_{30}N_6O_5S_2$; molecular mass: 606.72 g mol^{-1}; IR (KBr, υ_{max}, cm^{-1}): 3349 (N-H), 3138 (Ar C-H), 1657 (C=N), 1630 (C=O), 1598 (Ar

C=C), 1544 (N=O), 1449 (S=O), 1124 (C-N), 637 (C-S); ^{1}H NMR ($CDCl_3$, 600 MHz, δ (ppm)): 10.12 (s, 1H, N-H), 7.53 (d, J = 9.3 Hz, 2H, H-2‴′, H-6‴′), 7.52 (d, J =9.0 Hz, 2H, H-3″, H-5″), 7.50–7.48 (m, 1H, H-4‴′), 7.20 (d, J = 7.8 Hz, 2H, H-3‴′, H-5‴′), 7.19 (d, J = 8.3 Hz, 2H, H-2‴, H-6‴), 7.14–7.13 (m, 2H, H-3‴, H-5‴), 6.66 (d, J = 8.6 Hz, 2H, H-2″, H-6″), 4.12 (s, 2H, H-1⁗′), 3.72–3.70 (m, 2H, H_{eq}-2′, H_{eq}-6′), 2.60 (q, J = 4.3 Hz, 2H, H-2⁗′), 2.49–2.47 (m, 1H, H-4′), 2.26–2.22 (m, 2H, H_{ax}-2′, H_{ax}-6′), 1.99 (dq, J = 3.8, 9.0 Hz, 2H, H_{eq}-3′, H_{eq}-5′), 1.84–1.82 (m, 2H, H-3′, H-5′), 1.20 (t, J = 4.5 Hz, H-3⁗′); ^{13}C NMR (150 MHz, $CDCl_3$): δ 168.9 (C-4⁗′), 167.4 (C-4″), 158.0 (C-1″), 152.8 (C-1⁗′), 150.5 (C-2⁗′), 140.3 (C-1‴′), 135.8 (C-1‴), 132.1 (C-4‴), 130.7 (C-3″, C-5″), 129.8 (C-2″, C-6″), 128.2 (C-2‴′, C-6‴′), 126.9 (C-2‴, C-6‴), 124.2 (C-4‴′), 119.8 (C-3‴′, C-5‴′), 113.9 (C-3‴, C-5‴), 45.5 (C-2′, C-6′), 36.0 (C-4′), 32.1 (C-1⁗′), 29.4 (C-3′, C-5′), 28.3 (C-2⁗′), 15.7 (C-3⁗′); EI-MS (m/z): 606 $[M]^+$, 386 $[C_{18}H_{18}N_4O_4S]^+$, 295 $[C_{12}H_{13}N_3O_4S]^+$, 270 $[C_{11}H_{14}N_2O_4S]^+$, 186 $[C_6H_4NO_4S]^+$, 123 $[C_6H_5NO_2]^+$, 106 $[C_8H_{10}]^+$, 51 $[C_4H_4]^+$; Anal. Calcd. for $C_{29}H_{30}N_6O_5S_2$: C 57.41, H 4.98, N 13.85, O 13.19, S 10.57; found C 57.40, H 4.66, N 13.67, O 13.07, S 10.39.

3.3.4. *N*-(2-Ethyl-6-methylphenyl)-2-(5-(1-(4-nitrophenylsulfonyl)piperidin-4-yl)-4-phenyl-4*H*-1,2,4-triazol-3-ylthio)acetamide (8d)

Light green amorphous solid; yield: 81%; mp: 129–130 °C; molecular formula: $C_{30}H_{32}N_6O_5S_2$; molecular mass: 620.74 g mol^{-1}; IR (KBr, υ_{max}, cm^{-1}): 3332 (N-H), 3128 (Ar C-H), 1646 (C=N), 1644 (C=O), 1582 (Ar C=C), 1534 (N=O), 1443 (S=O), 1133 (C-N), 632 (C-S); ^{1}H NMR ($CDCl_3$, 600 MHz, δ (ppm)): 8.56 (s, 1H, N-H), 7.56 (d, J = 9.0 Hz, 2H, H-2‴′, H-6‴′), 7.50 (d, J =7.2 Hz, 2H, H-3″, H-5″), 7.21 (t, J = 8.0 Hz, 2H, H-3‴′, H-5‴′), 7.38–7.35 (m, 1H, H-4‴′), 7.16–7.13 (m, 2H, H-3‴, H-5‴), 7.05 (t, J = 6.8 Hz, 1H, H-4‴), 6.65 (d, J = 11.1 Hz, 2H, H-2″, H-6″), 4.09 (s, 2H, H-1⁗′), 3.71–3.66 (m, 2H, H_{eq}-2′, H_{eq}-6′), 2.40 (s, 3H, H-2⁗′), 2.33–2.29 (m, 2H, H_{ax}-2′, H_{ax}-6′), 2.25–2.24 (m, 1H, H-4′), 1.98–1.96 (m, 4H, H-3′, H-5′), 1.26 (q, J = 4.9 Hz, 2H, H-3⁗′), 1.08 (t, J = 7.5 Hz, 3H, H-4⁗′); ^{13}C NMR (150 MHz, $CDCl_3$): δ 169.2 (C-5⁗′), 167.8 (C-4″), 158.2 (C-1″), 155.8 (C-1⁗′), 152.1 (C-2⁗′), 135.3 (C-1‴′), 133.9 (C-1‴), 131.8 (C-2‴, C-6‴), 130.8 (C-3″, C-5″), 129.8 (C-2″, C-6″), 128.6 (C-2‴′, C-6‴′), 127.1 (C-4‴′), 126.4 (C-3‴, C-5‴), 125.0 (C-4‴), 116.4 (C-3‴′, C-5‴′), 45.3 (C-2′, C-6′), 36.1 (C-4′), 32.3 (C-1⁗′), 30.2 (C-3′, C-5′), 21.7 (C-2⁗′), 19.1 (C-3⁗′), 15.8 (C-4⁗′); EI-MS (m/z): 620 $[M]^+$, 386 $[C_{18}H_{18}N_4O_4S]^+$, 295 $[C_{12}H_{13}N_3O_4S]^+$, 270 $[C_{11}H_{14}N_2O_4S]^+$, 186 $[C_6H_4NO_4S]^+$, 123 $[C_6H_5NO_2]^+$, 120 $[C_9H_{12}]^+$, 66 $[C_5H_6]^+$; Anal. Calcd. for $C_{30}H_{32}N_6O_5S_2$: C 58.05, H 5.20, N 13.54, O 12.89, S 10.33; found C 58.01, H 5.09, N 13.32, O 12.78, S 10.24.

3.3.5. *N*-(2,3-Dimethylphenyl)-2-(5-(1-(4-nitrophenylsulfonyl)piperidin-4-yl)-4-phenyl-4*H*-1,2,4-triazol-3-ylthio)acetamide (8e)

Yellowish amorphous solid; yield: 78%; mp: 123–124 °C; molecular formula: $C_{29}H_{30}N_6O_5S_2$; molecular mass: 606.72 g mol^{-1}; IR (KBr, υ_{max}, cm^{-1}): 3340 (N-H), 3075 (Ar C-H), 1678 (C=N), 1665 (C=O), 1596 (Ar C=C), 1523 (N=O), 1435 (S=O), 1076 (C-N), 605 (C-S); ^{1}H NMR ($CDCl_3$, 600 MHz, δ (ppm)): 8.56 (s, 1H, N-H), 7.60 (d, J =8.04 Hz, 1H, H-6‴), 7.56 (d, J =8.8 Hz, 2H, H-2‴′, H-6‴′), 7.50 (d, J =8.7 Hz, 2H, H-3″, H-5″), 7.47 (t, J =10.44 Hz, 1H, H-4‴′), 7.19 (d, J =10.2 Hz, 1H, H-5‴), 7.07 (t, J =7.62 Hz, 2H, H-3‴′, H-5‴′), 6.98 (d, J =7.5 Hz, 1H, H-4‴), 6.64 (d, J =8.6 Hz, 2H, H-2″, H-6″), 3.94 (s, 2H, H-1⁗′), 3.71–3.69 (m, 2H, H_{eq}-2′, H_{eq}-6′), 2.29 (s, 3H, H-2⁗′), 2.27–2.22 (m, 2H, H_{ax}-2′,H_{ax}-6′), 2.18 (s, 3H, H-

3′′′′′), 2.11 (br.s, 1H, H-4′), 1.83–1.81 (m, 4H, H-3′, H-5′); ^{13}C NMR (150 MHz, $CDCl_3$): δ 168.9 (C-4′′′′′), 167.1 (C-4′′), 158.1 (C-1′′), 152.6 (C-1′′′′′), 150.5 (C-2′′′′′), 137.3 (C-1′′′′), 135.7 (C-1′′′), 132.8 (C-2′′′), 132.4 (C-3′′′), 130.8 (C-3′′, C-5′′), 129.8 (C-2′′, C-6′′), 127.6 (C-2′′′′, C-6′′′′), 126.9 (C-6′′′), 125.7 (C-5′′′), 124.2 (C-4′′′), 122.8 (C-4′′′′), 113.9 (C-3′′′′, C-5′′′′), 45.5 (C-2′, C-6′), 35.6 (C-4′), 32.1 (C-1′′′′′), 29.2 (C-3′, C-5′), 20.6 (C-2′′′′′), 13.8 (C-3′′′′′); EI-MS (m/z): 606 $[M]^+$, 386 $[C_{18}H_{18}N_4O_4S]^+$, 295 $[C_{12}H_{13}N_3O_4S]^+$, 270 $[C_{11}H_{14}N_2O_4S]^+$, 186 $[C_6H_4NO_4S]^+$, 123 $[C_6H_5NO_2]^+$, 106 $[C_8H_{10}]^+$, 51 $[C_4H_4]^+$; Anal. Calcd. for $C_{29}H_{30}N_6O_5S_2$: C 57.41, H 4.98, N 13.85, O 13.19, S 10.57; found C 57.23, H 4.78, N 13.66, O 13.11, S 10.36.

3.3.6. *N*-(2,5-Dimethylphenyl)-2-(5-(1-(4-nitrophenylsulfonyl)piperidin-4-yl)-4-phenyl-4*H*-1,2,4-triazol-3-ylthio)acetamide (8f)

Light brown amorphous solid; yield: 87%; mp: 122–123 °C; molecular formula: $C_{29}H_{30}N_6O_5S_2$; molecular mass: 606.72 g mol^{-1}; IR (KBr, υ_{max}, cm^{-1}): 3335 (N-H), 3077 (Ar C-H), 1679 (C=N), 1635 (C=O), 1598 (Ar C=C), 1525 (N=O), 1437 (S=O), 1089 (C-N), 609 (C-S); ^{1}H NMR ($CDCl_3$, 600 MHz, δ (ppm)): 9.62 (s, 1H, N-H), 7.75 (s, 1H, H-6′′′), 7.58 (d, J =6.8 Hz, 2H, H-2′′′′, H-6′′′′), 7.49 (d, J =8.6 Hz, 2H, H-3′′, H-5′′), 7.20 (d, J =7.8 Hz, 2H, H-3′′′′, H-5′′′′), 7.19 (d, J =7.6 Hz, 1H, H-3′′′), 7.06–7.04 (m, 1H, H-4′′′′), 6.86 (d, J =7.5, 10.2 Hz, 1H, H-4′′′), 6.65 (d, J =8.6 Hz, 2H, H-2′′, H-6′′), 4.11 (s, 2H, H-1′′′′′), 3.75–3.70 (m, 2H, H_{eq}-2′, H_{eq}-6′), 2.90–2.80 (m, 2H, H_{ax}-2′,H_{ax}-6′), 2.47–2.43 (m, 1H, H-4′), 2.30 (s, 3H, H-2′′′′′), 2.23 (s, 3H, H-3′′′′′), 1.99–1.98 (m, 2H, H_{eq}-3′, H_{eq}-5′), 1.83–1.81 (m, 2H, H_{ax}-3′, H_{ax}-5′); ^{13}C NMR (150 MHz, $CDCl_3$): δ 166.9 (C-4′′′′′), 166.9 (C-4′′), 158.1 (C-1′′), 152.6 (C-1′′′′′), 150.5 (C-2′′′′′), 136.2 (C-1′′′′), 135.9 (C-1′′′), 132.3 (C-2′′′), 130.7 (C-6′′′), 130.4 (C-3′′, C-5′′), 129.8 (C-2′′, C-6′′), 126.9 (C-2′′′′, C-6′′′′), 126.1 (C-3′′′), 125.6 (C-4′′′), 124.2 (C-5′′′), 122.5 (C-4′′′′), 113.0 (C-3′′′′, C-5′′′′), 45.4 (C-2′, C-6′), 36.7 (C-4′), 31.9 (C-1′′′′′), 29.3 (C-3′, C-5′), 21.1 (C-2′′′′′), 17.7 (C-3′′′′′); EI-MS (m/z): 606 $[M]^+$, 386 $[C_{18}H_{18}N_4O_4S]^+$, 295 $[C_{12}H_{13}N_3O_4S]^+$, 270 $[C_{11}H_{14}N_2O_4S]^+$, 186 $[C_6H_4NO_4S]^+$, 123 $[C_6H_5NO_2]^+$, 106 $[C_8H_{10}]^+$, 51 $[C_4H_4]^+$; Anal. Calcd. for $C_{29}H_{30}N_6O_5S_2$: C 57.41, H 4.98, N 13.85, O 13.19, S 10.57; found C 57.21, H 4.78, N 13.63, O 13.11, S 10.36.

3.3.7. *N*-(2,6-Dimethylphenyl)-2-(5-(1-(4-nitrophenylsulfonyl)piperidin-4-yl)-4-phenyl-4*H*-1,2,4-triazol-3-ylthio)acetamide (8g)

Light brown amorphous solid; yield: 77%; mp: 102–103 °C; molecular formula: $C_{29}H_{30}N_6O_5S_2$; molecular mass: 606.72 g mol^{-1}; IR (KBr, υ_{max}, cm^{-1}): 3421 (N-H), 3074 (Ar C-H), 1672 (C=N), 1637 (C=O), 1592 (Ar C=C), 1528 (N=O), 1431 (S=O), 1072 (C-N), 603 (C-S); ^{1}H NMR ($CDCl_3$, 600 MHz, δ (ppm)): 9.32 (s, 1H, N-H), 7.56 (d, J =7.6 Hz, 2H, H-2′′′′, H-6′′′′), 7.49 (d, J = 8.5 Hz, 2H, H-3′′, H-5′′), 7.20–7.19 (m, 3H, H-3′′′′, H-4′′′′, H-5′′′′), 7.09 (d, J =7.0 Hz, 2H, H-3′′′, H-5′′′), 7.06 (t, J =7.3 Hz, 1H, H-4′′′), 6.64 (d, J =8.5 Hz, 2H, H-2′′, H-6′′), 4.09 (s, 2H, H-1′′′′′), 3.68–3.66 (m, 2H, H_{eq}-2′, H_{eq}-6′), 2.32–2.25 (m, 1H, H-4′), 2.32–2.25 (m, 2H, H_{ax}-2′,H_{ax}-6′), 2.21 (s, 3H, H-2′′′′′), 2.20 (s, 3H, H-3′′′′′), 1.96 (dq, J = 3.7, 11.0 Hz, 2H, H_{eq}-3′, H_{eq}-5′), 1.84–1.82 (m, 2H, H_{ax}-3′, H_{ax}-5′); ^{13}C NMR (150 MHz, $CDCl_3$): δ 168.7 (C-4′′′′′), 167.2 (C-4′′), 158.1 (C-1′′), 152.8 (C-1′′′′′), 150.5 (C-2′′′′′), 135.3 (C-1′′′′), 133.9 (C-1′′′), 132.4 (C-2′′′, C-6′′′), 130.5 (C-3′′, C-5′′), 129.8 (C-2′′, C-6′′), 128.1 (C-2′′′′, C-6′′′′), 127.2 (C-4′′′′), 126.9 (C-3′′′, C-5′′′), 124.2 (C-4′′′), 113.9 (C-3′′′′, C-5′′′′), 45.4 (C-2′, C-6′), 34.9 (C-4′), 31.9 (C-1′′′′′), 29.3 (C-3′, C-5′), 18.2 (C-2′′′′, C-3′′′′′); EI-MS (m/z): 606 $[M]^+$, 386 $[C_{18}H_{18}N_4O_4S]^+$, 295 $[C_{12}H_{13}N_3O_4S]^+$, 270 $[C_{11}H_{14}N_2O_4S]^+$,

186 $[C_6H_4NO_4S]^+$, 123 $[C_6H_5NO_2]^+$, 120 $[C_9H_{12}]^+$, 66 $[C_5H_6]^+$; Anal. Calcd. for $C_{29}H_{30}N_6O_5S_2$: C 57.41, H 4.98, N 13.85, O 13.19, S 10.57; found C 57.21, H 4.78, N 13.66, O 13.10, S 10.36.

3.3.8. *N*-(3,4-Dimethylphenyl)-2-(5-(1-(4-nitrophenylsulfonyl)piperidin-4-yl)-4-phenyl-4*H*-1,2,4-triazol-3-ylthio)acetamide (8h)

Light green amorphous solid; yield: 82%; mp: 94–95 °C; molecular formula: $C_{29}H_{30}N_6O_5S_2$; molecular mass: 606.72 g mol^{-1}; IR (KBr, υ_{max}, cm^{-1}): 3422 (N-H), 3071 (Ar C-H), 1670 (C=N), 1634 (C=O), 1593 (Ar C=C), 1520 (N=O), 1439 (S=O), 1228, 1077 (C-N), 602 (C-S); ^{1}H NMR ($CDCl_3$, 600 MHz, δ (ppm)): 8.02 (s, 1H, N-H), 7.56 (d, J =7.2 Hz, 1H, H-6‴), 7.55 (d, J =8 Hz, 2H, H-2⁗, H-6⁗), 7.54 (d, J =8 Hz, 2H, H-3″, H-5″), 7.35 (s, 1H, H-2‴), 7.34–7.32 (m, 1H, H-4⁗), 7.20–7.19 (m, 2H, H-3⁗, H-5⁗), 7.06 (d, J =8 Hz, 1H, H-5‴), 6.66 (d, J = 6.8 Hz, 2H, H-2″, H-6″), 4.11 (s, 2H, H-1⁗), 3.73–3.71 (m, 2H, H_{eq}-2′, H_{eq}-6′), 2.47 (dt, J = 2.7, 11.8 Hz, 2H, H_{ax}-2′, H_{ax}-6′), 2.20 (s, 3H, H-2⁗), 2.13 (s, 3H, H-3⁗), 2.10 (m, 1H, H-4′), 1.84–1.82 (m, 4H, H-3′, H-5′); ^{13}C NMR (150 MHz, $CDCl_3$): δ 167.9 (C-4⁗), 166.4 (C-4″), 158.0 (C-1″), 152.8 (C-1⁗), 150.5 (C-2⁗), 137.1 (C-1‴), 135.9 (C-1‴), 132.5 (C-3‴), 132.4 (C-4‴), 130.7 (C-3″, C-5″), 129.8 (C-2″, C-6″), 126.9 (C-2⁗, C-6⁗), 121.1 (C-6‴), 120.2 (C-2‴), 119.5 (C-5‴), 117.3 (C-4⁗), 114.0 (C-3⁗, C-5⁗), 45.5 (C-2′, C-6′), 36.1 (C-4′), 32.2 (C-1⁗), 29.4 (C-3′, C-5′), 19.8 (C-2⁗), 19.1 (C-3⁗); EI-MS (m/z): 606 $[M]^+$, 386 $[C_{18}H_{18}N_4O_4S]^+$, 295 $[C_{12}H_{13}N_3O_4S]^+$, 270 $[C_{11}H_{14}N_2O_4S]^+$, 186 $[C_6H_4NO_4S]^+$, 177 $[C_{11}H_{15}NO]^+$, 123 $[C_6H_5NO_2]^+$, 106 $[C_8H_{10}]^+$, 66 $[C_5H_6]^+$, 51 $[C_4H_4]^+$; Anal. Calcd. for $C_{29}H_{30}N_6O_5S_2$: C 57.41, H 4.98, N 13.85, O 13.19, S 10.57; found C 57.22, H 4.78, N 13.64, O 13.11, S 10.36.

3.3.9. *N*-(3,5-Dimethylphenyl)-2-(5-(1-(4-nitrophenylsulfonyl)piperidin-4-yl)-4-phenyl-4*H*-1,2,4-triazol-3-ylthio)acetamide (8i)

Off-white amorphous solid; yield: 85%; mp: 127–128 °C; molecular formula $C_{29}H_{30}N_6O_5S_2$; molecular mass: 606.72 g mol^{-1}; IR (KBr, υ_{max}, cm^{-1}): 3323 (N-H), 3105 (Ar C-H), 1652 (C=N), 1638 (C=O), 1586 (Ar C=C), 1532 (N=O), 1442 (S=O), 1152, 1143 (C-N), 611 (C-S); ^{1}H NMR ($CDCl_3$, 600 MHz, δ (ppm)): 9.62 (s, 1H, N-H), 7.76 (s, 2H, H-2‴, H-6‴), 7.56 (d, J =7.4 Hz, 2H, H-2⁗, H-6⁗), 7.49 (d, J =8.6 Hz, 2H, H-3″, H-5″), 7.26 (d, J = 8.2 Hz, 2H, H-3⁗, H-5⁗), 7.05–7.04 (m, 1H, H-4⁗), 6.87 (s, 1H, H-4‴), 6.66 (d, J = 8.6 Hz, 2H, H-2″, H-6″), 4.11 (s, 2H, H-1⁗), 3.68–3.66 (m, 2H, H_{eq}-2′, H_{eq}-6′), 2.95–2.86 (m, 2H, H_{ax}-2′, H_{ax}-6′), 2.50–2.47 (m, 1H, H-4′), 2.30 (s, 3H, 2⁗), 2.24 (s, 3H, 3⁗), 2.01–2.00 (m, 2H, H_{eq}-3′, H_{eq}-5′), 1.96–1.81 (m, 2H, H_{ax}-3′, H_{ax}-5′); ^{13}C NMR (150 MHz, $CDCl_3$): δ 168.5 (C-4⁗), 166.9 (C-4″), 158.1 (C-1″), 152.6 (C-1⁗) , 150.5 (C-2⁗), 136.2 (C-1‴), 135.9 (C-1‴), 132.4 (C-3‴, C-5‴), 130.2 (C-3″, C-5″), 129.8 (C-2″, C-6″), 126.9 (C-2⁗, C-6⁗), 125.6 (C-2‴, C-6‴), 122.9 (C-4⁗), 113.9 (C-3⁗, C-5⁗), 45.4 (C-2′, C-6′), 35.6 (C-4′), 31.9 (C-1⁗), 29.3 (C-3′, C-5′), 21.1 (C-2⁗), 17.7 (C-3⁗); EI-MS (m/z): 606 $[M]^+$, 386 $[C_{18}H_{18}N_4O_4S]^+$, 295 $[C_{12}H_{13}N_3O_4S]^+$, 270 $[C_{11}H_{14}N_2O_4S]^+$, 186 $[C_6H_4NO_4S]^+$, 123 $[C_6H_5NO_2]^+$, 106 $[C_8H_{10}]^+$, 66 $[C_5H_6]^+$, 51 $[C_4H_4]^+$; Anal. Calcd. for $C_{29}H_{30}N_6O_5S_2$: C 57.41, H 4.98, N 13.85, O 13.19, S 10.57; found C 57.21, H 4.78, N 13.62, O 13.10, S 10.36.

3.4. Assays

3.4.1. BSA interactions using fluorescence measurements

For the determination of the BSA quenching constant, fluorometric titration of a solution of BSA (3 mL, 3 µM) in phosphate buffer (20 mM, pH 7.4) with compounds **8a**–**8i** (1 mg/mL in DMSO solvent) was carried out. The

solutions were excited at 295 nm and the intensities of the BSA solutions with and without compounds **8a**–**8i** were recorded at 336 nm at 298 K. For thermodynamic studies of BSA and synthesized derivatives, the above experiment was repeated at other two temperatures, 303 K and 308 K, respectively, for each of the synthesized compounds **8a**–**8i**. Thus, three sets of fluorescence spectra were obtained at three temperatures: 298, 303, and 308 K.

For site-selective binding studies, fluorometric titration of BSA solution (3 mL, 3 µM) with and without site markers (ibuprofen or warfarin) was carried out with the solutions of synthesized compounds (1 mg/mL in DMSO solvent). The solutions were scanned using an excitation wavelength of 295 nm at 298 K.

For the synchronous binding studies (at $\Delta\lambda$ 15 nm or 60 nm), BSA solution (3 mL, 3 µM) was titrated with each compound of the synthesized series of triazole derivatives or the site markers at an excitation wavelength of 295 nm.

3.4.2. FT-IR spectroscopy

The FT-IR analysis was carried out on a Bruker Alpha FT–IR spectrometer. The spectrum of phosphate buffer in D_2O was collected and subtracted from the spectrum of BSA to obtain the FT-IR spectrum of pure BSA. The change in the spectra of BSA before and after addition of compounds **8a**–**8i** was analyzed.

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References

1. Rehman, A. U.; Iqbal, J.; Abbasi, M. A,; Siddiqui, S. Z.; Khalid, H.; Laulloo, S. J.; Virk, N. A.; Rasool, S.; Shah, S. A. A. *Cogent Chem.* **2018**, *4*, 1-16.
2. Jiaxin, F. U.; Kaiwei, W.; Yushu, G. E.; Fenglei, J.; Xiaohong, S.; Yang, L.; Yi, L. *Sci. China Chem.* **2011**, *54*, 788-796.
3. Qingpeng, W.; Jingqing, Z.; Guri, D.; Kun, W.; Huizhen, Z.; Chenghe, Z. *Sci. China Chem.* **2012**, *55*, 2134-2153.
4. Mitsumori, T.; Bendikov, M.; Sedó, J.; Wudl, F. *Chem. Mater.* **2003**, *15*, 3759-3768.
5. Zbancioc, G. N.; Mangalagiu, I. I. *Tetrahedron* **2010**, *66*, 278-282.
6. Tozkoparan, B.; Kupeli, E.; Yesilada, E.; Ertan, M. *Bioorg. Med. Chem.* **2007**, *15*, 1808-1814.
7. Rabea, S. M.; El-Koussi, N. A.; Hassan, H. Y.; Aboul-Fadl, V. *Arch. Pharm.* **2006**, *339*, 32-40.
8. Abdel-Aal, M. T.; El-Sayed, W. A.; El-Kosy, S. M.; El-Ashry, S. H. *Arch. Pharm.* **2008**, *341*, 307-313.
9. Dumitrascu, F.; Caproiu, M. T.; Georgescu, F.; Draghici, B.; Popa, M. M.; Georgescu, E. *Synlett* **2010**, 2407-2410.
10. Jones, D. H.; Slack, R.; Squires, S.; Wooldridge, K. R. H. *J. Med. Chem.* **1965**, *8*, 676-680.
11. Ilango, K.; Valentina, P. *Pharm. Chem.* **2010**, *2*, 16-22.
12. Sughen, J. K.; Yoloye, T. *Pharm. Acta Helv.* **1978**, *58*, 64-68.
13. Shams, E. S. A.; Hazzaa, A. A. B. *Pharmazie* **1974**, *29*, 761-763.
14. Oruc, E. E.; Rollas, S.; Kandemirli, F.; Shvets, N.; Dimoglo, A. S. *Bioorg. Med. Chem. Lett.* **2004**, *12*, 5651-5659.
15. Wu, G.; Ouyang, L.; Liu, J.; Zeng, S.; Huang, W.; Han, B.; Wu, F.; He, G.; Xiang, M. *Mol. Diversity* **2013**, *17*, 271-283.
16. Duan, Y. Q.; Lei, H. G.; Min, S. G.; Duan, Z. Q. *Spectrosc. Spect. Anal.* **2009**, *29*, 2998-3002.
17. Jiang, H.; Chen, R.; Pu, H. *J. Lumin.* **2012**, *132*, 592-599.

18. Ghosh, S.; Dey, J. *J. Colloid Interface Sci.* **2015**, *458*, 284-292.
19. Zhang, H. M.; Fei, Z. H.; Tang, B. P.; Chen, J.; Tao, W. H.; Wang, Y. Q. *Mol. Biol. Rep.* **2012**, *39*, 4937-4947.
20. Valstar, A.; Almgren, M.; Brown, W.; Vasilescu, M. *Langmuir* **2000**, *16*, 922-927.
21. Prashant, M. K.; Madaiah, M.; Revanasiddappa, H. D. *Organic Chemistry* **2013**, *2013*, 1-12.
22. Teng, Y.; Liu, R.; Yan, S.; Pan, X.; Zhang, P.; Wang, M. *J. Fluoresc.* **2010**, *20*, 381-387.
23. Ghosh, S.; Jana, S.; Guchhait, N. *J. Phys. Chem.* **2012**, *116*, 1155-1163.