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In-Vitro Binding Interaction of 3-(Furan-2-Yl)-N-Phenylprop-2-Enamide With BSA: Equilibrium Dialysis and FT-IR Spectroscopic Study

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Abstract: This paper presented binding interaction of 3-(Furan-2-yl)-N-phenylprop-2-enamide (3FNPP2M) with bovine serum albumin (BSA) by equilibrium dialysis and FT-IR spectroscopy at physiological pH in various solvents. Findings were interpreted by scatchard plot in terms of association constants. The increased in association constants with increase in concentrations of the 3FNPP2M is observed. The binding interaction is found to be more in 1, 4-dioxane than DMSO and DMF.FT-IR study explained the binding interaction through shift in peak positions of amide I and II bands. FT-IR study confirmed the binding of 3FNPP2M with BSA in terms of changes in secondary structure of BSA. In this study, we have also reported the effect of foreign particles viz. arsenic and mercury on the binding interaction of 3FNPP2M with BSA. Reactivity and association of 3FNPP2M with BSA in presence of foreign moieties were calculated and compared.

Keywords: Association constant, BSA, Equilibrium dialysis, Foreign particles, FT-IR study, Scatchard plot.

I. Introduction

Pharmacologically important compounds which are widely used in different streams materials science, agro-chemistry, biomedical research and medicinal chemistry. N-heterocyclic compounds are also varying pharmacological importance. N-heterocyclic compounds has a prominent place in organocatalysis, organometallic and material chemistry shows various biological properties especially, antimicrobial, antiviral, anticancer and antitumor [1-4]. Plasma protein are the most abundant proteins in the circulatory system of wide variety of organisms. Association of drugs with these plasma protein is an important aspect to study. Variation in the temperature is found to be a key factor in binding affinities of plasma protein [5-6] as evident from various drugs viz. ligustrazine, ciprofloxacin [7], methotrexate [8] and cisplatin [9]. BSA is one of the important plasma protein which was used for the study of interaction with N-heterocyclic compounds. Various techniques are available to monitor the binding interactions of ligands to protein like NMR [10], isothermal titration calorimetry [11], U.V. visible absorbance [12], fluorescence [13], equilibrium & FT-IR [14], fluorescence and CD spectroscopy [15]. Equilibrium dialysis and FT-IR study also focused on the effect of foreign particles such as arsenic on protein-drug binding [16-17].

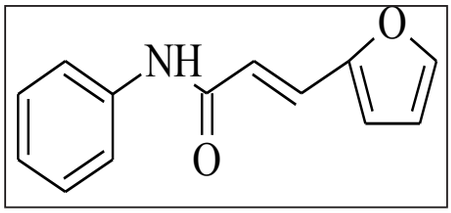


Fig. 1: Structure of 3-(Furan-2-yl)-N-Phenylprop-2-Enamide (3FNPP2M)

In the present study we evaluated the association of 3FNPP2M (Fig. 1) with BSA at different concentrations in different solvents by equilibrium dialysis. Along with this effect foreign particles and polar/non polar solvent on binding interaction of 3FNPP2M with BSA by equilibrium dialysis and FT-IR spectroscopic study at physiological pH.

II. Materials and Method

Dialysis membrane (molecular weight cut off 3500) used in the experiment purchased from Sigma Chemical Co. (USA). UV-VIS spectrophotometer (UV-1800, Shimadzu, Japan) and metabolic shaking incubator (REMI RS-24AC) used in the experiment. FT-IR measurements were taken at room temperature on a Bruker's FT-IR spectrophotometer (Alpha model, Germany) equipped with Zn-Se attenuated total reflection (ATR) accessory. BSA (essential fatty acid free) purchased from Chemsworth Chemicals Ltd (India) and used without further purification and the compound 3FNPP2M. Basic buffer was selected to maintain the physiological pH 7.4. For the synthesis, all the chemicals used were of A. R. grade of

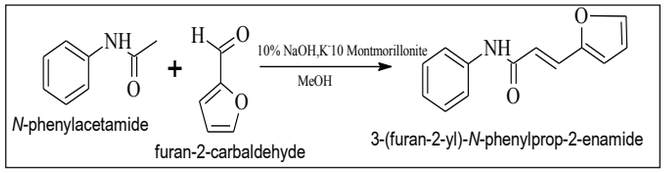
Merck India limited and purchased from commercial suppliers.

A. Optimization Study

3FNPP2M is insoluble in basic buffer at physiological pH. Hence mixture of buffer with non aqueous solvent such as 1, 4-dioxane, DMSO and DMF were used to dissolve 3FNPP2M. Different ratio of buffer: non-aqueous solvents were tried, but the complete solubility of 3FNPP2M was obtained at optimum ratio 30: 70:: non-aqueous solvent: buffer.

B. Preparation of 3FNPP2M

The synthesis of 3-(furan-2-yl)-N-phenylprp-2-enamide was reported by known method [18]. The mixture of acetanilide (0.05 mol), furan-2-carbaldehyde (0.05 mol), aqueous NaOH (10%, 1 mL), K-10 Montmorillonite and methanol (10 mL). The reaction mixture was then subjected to microwave irradiation for 56 seconds. After completion of the reaction (monitored by TLC) the resultant viscous mass was poured into ice water (10 mL) with vigorous stirring and left overnight for complete precipitation. The resultant solid product was filtered, washed with cold water, dried and recrystallized from ethanol.



Scheme 1: Synthesis of Ligand 3-(Furan-2-yl)-N-Phenylprop-2-Enamide (3FNPP2M)

C. Measurement of Binding Affinity

The binding interaction of 3FNPP2M with BSA is expressed as binding/association constants. The association constants are derived from the law of mass action. BSA (B) interacts with ligand 3FNPP2M (L) to form the BSA-3FNPP2M complex is given as:

$$B + L \rightleftharpoons BL$$

Hence, association constant $K_a = \frac{[BL]}{[BL]+[B]}$

Binding strength of the 3FNPP2M with BSA is a measure of association constants.

D. Equilibrium Dialysis

Various solutions of concentrations 1mM, 1.5 mM, 2 mM, 2.5 mM, 3 mM, 3.5 mM of ligand 3FNPP2M in 1, 4-dioxane, DMSO, DMF (30:70:: solvent: buffer) and 0.15 μM BSA solution were prepared. These solutions were mixed in 1:1 proportion and allowed to stand at room temperature for maximum binding interaction. 3.5 ml complex solution from each was poured into semi-permeable membrane and both the ends were sealed properly. These tubes were immersed into 100 ml conical flask containing 40 ml buffer. These conical flasks placed on a metabolic shaker for dialysis for 12 hrs and absorbance of bound fraction of 3FNPP2M with BSA was measured on UV spectrophotometer at 520 nm.

E. Effect of Foreign Particles

The binding interaction of 3FNPP2M to BSA was carried out in presence of foreign particles. 0.1M solutions of arsenic and mercury salts were prepared and mixed with 3FNPP2M-BSA complex solutions. These mixed ternary complex solutions were kept some time to study the effect of foreign particles on binding interaction.

F. FT-IR Study

Different concentrations of 3FNPP2M and the BSA as mentioned above mixed and allowed to stand at room temperature for maximum binding interaction. FT-IR measurements were carried out at room temperature on FT-IR spectrometer equipped with Zn-Se attenuated total reflection (ATR) method. Absorbance of BSA-3FNPP2M complexes were measured at room temperature.

III. Result and Discussion

A. Equilibrium Dialysis

Different absorbance values are obtained for BSA-3FNPP2M complexes in 1, 4-dioxane, DMSO and DMF, from the value of absorbance the values of specific binding are obtained. Fig. 2 shows the graph of absorbance versus concentrations of 3FNPP2M in 1, 4-dioxane, DMSO and DMF. Similarly, Fig. 3 shows the graph for specific binding against concentrations of 3FNPP2M in 1, 4-dioxane, DMSO and DMF. Fig. 3 is known as scatchard plot used to determine binding affinity of 3FNPP2M with BSA. The binding affinity of these complexes has been determined in terms of association constants. The association constants for BSA-3FNPP2M binding in 1, 4-dioxane, DMSO and DMF are 0.6191**,** 0.6765, 0.6278 respectively. It is seen that the association constant for BSA-3FNPP2M in 1, 4-dioxane is more than DMSO and DMF. Table I gives the values of absorbance and specific binding for the interaction of 3FNPP2M with BSA in 1, 4-dioxane, DMSO and DMF for different concentrations of 3FNPP2M.

TABLE I: ABSORBANCE AND SPECIFIC BINDING VALUES FOR BSA-3FNPP2M COMPLEX AT DIFFERENT CONCENTRATIONS OF 3FNPP2M IN 1, 4-DIOXANE, DMSO AND DMF

Sr. No.	*Concentrations of 3FNPP2M (mM)*	*BSA+ 3FNPP2M in 1,4 Dioxane*		*BSA+ 3FNPP2M in DMSO*		*BSA+ 3FNPP2M in DMF*	
		Absorbance	*Specific Binding*	*Absorbance*	*Specific Binding*	*Absorbance*	*Specific Binding*
1	1	0.286	0.5522	0.326	0.5842	0.312	0.5735
2	1.5	0.309	0.5711	0.419	0.6436	0.317	0.5774
3	2	0.378	0.6116	0.497	0.6817	0.339	0.5936
4	2.5	0.426	0.6474	0.602	0.7218	0.407	0.6369
5	3	0.433	0.6511	0.663	0.7407	0.439	0.6524
6	3.5	0.507	0.6860	0.772	0.7689	0.498	0.6821

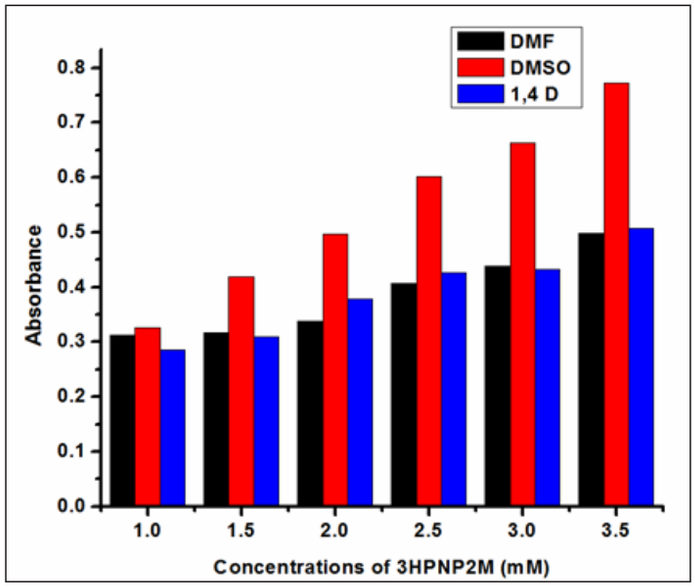


Fig. 2: Graph of Absorbance vs. Conc. of 3FNPP2M in 1, 4-Dioxane, DMSO and DMF

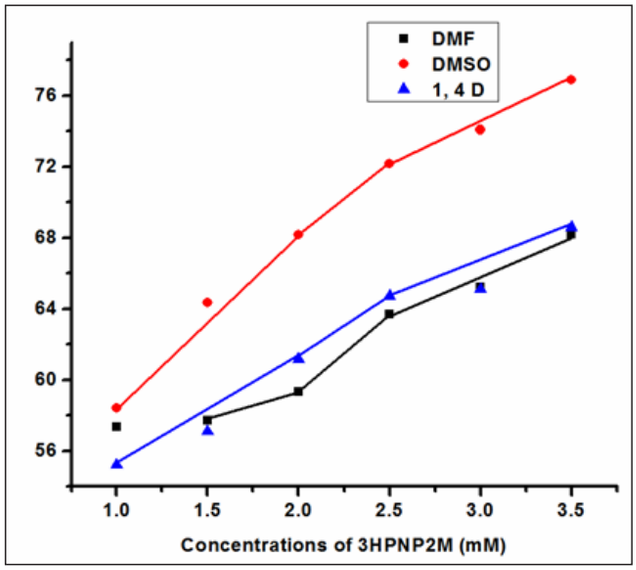


Fig. 3: Graph of Specific Binding vs. Conc. of 3FNPP2M in 1, 4-Dioxane, DMSO and DMF

B. Effect of Foreign Particles Arsenic and Mercury on 3FNPP2M-BSA Binding Interaction

Binding interaction of 3FNPP2M with BSA in presence of foreign particles viz. arsenic and mercury was studied. Different values of absorbance for 3FNPP2M-BSA in 1, 4-dioxane are obtained for different 3FNPP2M concentrations 1 mM, 1.5 mM, 2 mM, 2.5 mM, 3 mM and 3.5 mM respectively in presence of arsenic and mercury. Fig. 4 shows the graph of absorbance vs. concentrations of 3FNPP2M in 1, 4-dioxane in presence of arsenic and mercury. Fig. 5 shows the graph of specific binding against concentrations of 3FNPP2M in 1, 4-dioxane in presence of arsenic and mercury. The scatchard plot gives the association constants for the binding interaction of 3FNPP2M to BSA in presence of arsenic and mercury. The association constants in presence of arsenic and mercury are 0.4669 and 0.4810 respectively. Table II gives absorbance and specific binding values for BSA-3FNPP2M complex in presence of arsenic and mercury at different concentration of 3FNPP2M in 1, 4-dioxane.

Table II: Absorbance and Specific Binding Values for BSA+ 3FNPP2M Complex in Presence of Arsenic and Mercury at Different Concentrations of 3FNPP2M in 1, 4-Dioxane

Sr. No.	Concentrations of 3FNPP2M (mM)	Hg+BSA+3FNPP2M in 1,4-Dioxane		As+BSA+3FNPP2M in 1, 4-Dioxane	
		Absorbance	Specific Binding	Absorbance	Specific Binding
1	0.001	0.167	0.4406	0.161	0.4316
2	0.002	0.178	0.4564	0.174	0.4507
3	0.003	0.190	0.4726	0.189	0.4713
4	0.004	0.209	0.4964	0.203	0.4891
5	0.005	0.215	0.5035	0.197	0.4816
6	0.006	0.231	0.5214	0.214	0.5023

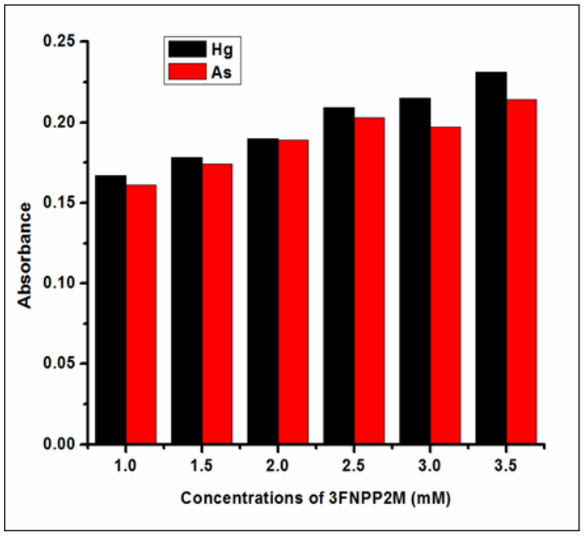


Fig. 4: Graph of Absorbance vs. Conc. of 3FNPP2M in 1, 4-Dioxane in Presence of Arsenic and Mercury

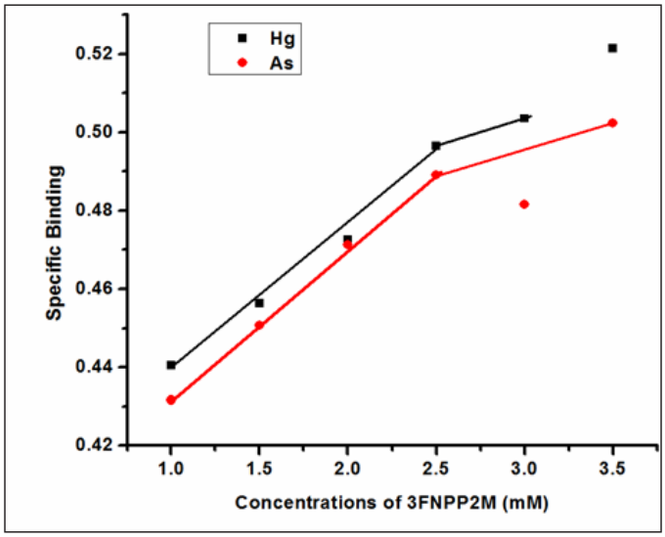


Fig. 5: Graph of Specific Binding vs. Conc. of 3FNPP2M in 1, 4-Dioxane in Presence of Arsenic and Mercury

C. FT-IR Study

The BSA-3FNPP2M binding interaction by FT-IR spectroscopy is analyzed by shifting of amide bands in BSA. The amide I band at 1635 cm^{-1} is due to C=O stretching and amide II band at 1543 cm^{-1} is due to C-N stretch coupled with N-H bending. A change in frequency of these bands is observed on binding of 3FNPP2M to BSA. The peak position of amide I is shifted to greater extent, however, a very small change in the amide II band has observed. As the concentration of drugs increases the frequency of the binding of amide I also increase (Fig. 6-8). It is concluded that amide I band is more sensitive to the changes of secondary structure of BSA than amide II. Fig. 6 shows the changes in the peak positions of amide bands of BSA-3FNPP2M complex in 1, 4-dioxane. Similarly, the shifting of amide bands in DMSO and DMF are shown in Fig. 7 and 8 respectively. In Fig. 6, 7 and 8**,** A shows spectrum of BSA and B, C, D, E, F, G represents spectra of BSA-3FNPP2M complexes for concentrations 1, 1.5, 2, 2.5, 3, 3.5 mM of 3FNPP2M respectively.

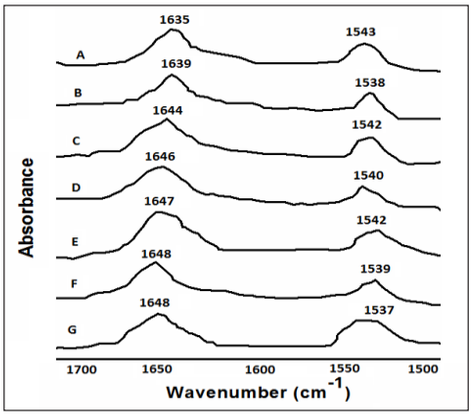


Fig. 6: Stacked FT-IR Spectra of BSA (A) and BSA-3FNPP2M (B-G) Complexes in 1, 4-Dioxane

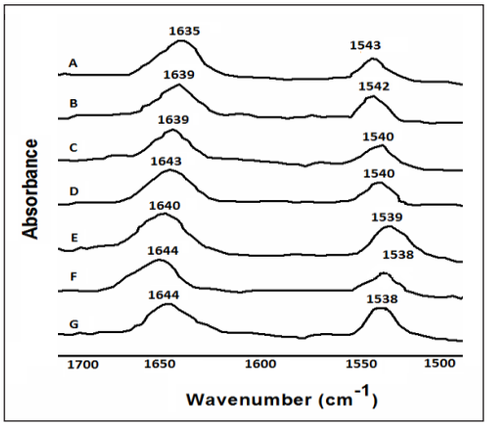


Fig. 7: Stacked FT-IR Spectra of BSA (A) and BSA-3FNPP2M (B-G) Complexes in DMSO

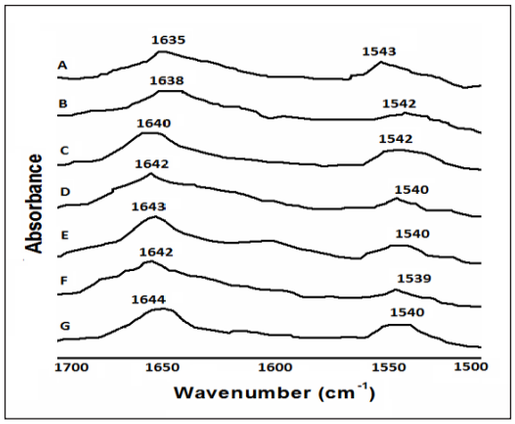


Fig. 8: Stacked FT-IR Spectra of BSA (A) and BSA-3FNPP2M (B-G) Complexes in DMF

IV. Conclusion

In this study, the binding interaction of 3-(Furan-2-yl)-N-phenylprop-2-enamide (3FNPP2M) with BSA in 1, 4-dioxane, DMSO and DMF BSA has been reported by equilibrium dialysis and FT-IR spectroscopy at physiological pH. Association constants for BSA-3FNPP2M binding interaction were calculated by using scatchard plot. The scatchard plot for the binding interaction of 3FNPP2M to BSA provided a non-linear curve, suggesting the presence of at least two binding sites in BSA. The binding affinity increased as the concentrations of the 3FNPP2M increases; this probably enhances the pharmacological activity of the 3FNPP2M. FT-IR spectroscopy shows that the binding interaction was mainly through amide I site by hydrophobic interaction, which changes the secondary structure of BSA. In this study, we have also reported the effect of foreign particles arsenic and mercury on the binding interaction of 3FNPP2M with BSA. It is seen that, in absence of foreign particle the association constants for 3FNPP2M-BSA are 68%, 63% and 62% in DMSO, DMF and 1, 4-dioxane, respectively. However, the associations constant in presence of foreign particles are decreased. Decrease in association constants in presence of arsenic and mercury are 47% and 48% in 1, 4-dioxane respectively. It is concluded that association and binding affinity of 3FNPP2M with BSA decreases in presence of foreign particles.

References

[1] G. Yadav, and S. Ganguly, "Structure activity relationship (SAR) study of benzimidazole scaffold for different biological activities: A mini-review," *European Journal of Medicinal Chemistry*, vol. 97, pp. 419-443, 2015.

[2] V. S. Padalkar, B. N. Borse, V. D. Gupta, K. R. Phatangare, V. S. Patil, P. G. Umape, and N. Sekar, "Synthesis and antimicrobial activity of novel 2-substituted benzimidazole, benzoxazole and benzothiazole derivatives," *Arabian Journal of Chemistry*, vol. 9, 2016.

[3] V. A. Sontakke, A. N. Kate, S. Ghosh, P. More, R. Gonnade, N. M. Kumbhar, V. S. Shinde, A. Kumbhar, and B. A. Chopade, "Synthesis, DNA interaction and anticancer activity of 2-anthryl substituted benzimidazole derivatives," *New Journal of Chemistry*, vol. 39, no. 6, 2015.

[4] A. A. M. El-Din, W. S. El-Serwy, A. S. A. El-All, N. M. A. El-Ebrashi, M. M. Abdalla, and A. A. El-Rashedy, "Synthesis, molecular modeling and bioevaluation of new benzimidazole derivatives as dual KSP (Kinesin Spindle Protein) and Aurora A Kinase inhibitors for antitumor activity," *JIPBS*, vol. 4, pp. 17-27, 2017.

[5] Q. Zhang, H. Yu, F. Z. Zhang, and Z. C.Shen, "Expression and purification of recombinant human serum albumin from selectively terminable transgenic rice," *Journal of Zhejiang University Sciences* B, vol.14, p. 867, 2013.

[6] X. M. He, and D. C. Carter, "Atomic structure and chemistry of human serum albumin," *Nature*, vol. 358, pp. 209-215, 1992.

[7] Y. J. Hu, Y. Zhang, Y. Ou-Yang, and Y. Liu, "Affinity and specificity of ciprofloxacin-bovine serum albumin interactions: Spectroscopic approach," *The Protein Journal*, vol. 29, no. 4, pp. 234-341, 2010.

[8] J. W. Paxton, "Protein binding of methotrexate in sera from normal human beings: Effect of drug concentration, pH, temperature, and storage," *Journal of Pharmacological Methods*, vol. 5, no. 3, pp. 203-213, 1981.

[9] G. Ferraro, A. Pica, I. R. Krauss, F. Pane, A. Amoresano, and A. Merlino, "Effect of temperature on the interaction of cisplatin with the model protein hen egg white lysozyme," *Journal of Biological Inorganic Chemistry*, vol. 21, no. 4, pp. 433-442, 2016.

[10] A. L. Skinner, and J. S. Laurence, "High-field solution NMR spectroscopy as a tool for assessing protein interactions with small molecule ligands," *Journal of Pharmaceutical Sciences*, vol. 97, no. 11, pp. 4670-4695, 2008.

[11] X. Li, G. Wang, D. Chen, and Y. Lu, "Binding of ascorbic acid and α-tocopherol to bovine serum albumin: A comparative study," *Molecular BioSystems*, vol. 10, no. 2, pp. 326-337, 2014.

[12] S. K. Chaturvedi, E. Ahmad, J. M. Khan, P. Alam, M. Ishtikhar, and R. H. Khan, "Elucidating the interaction of limonene with bovine serum albumin: A multi-technique approach," *Molecular BioSystems*, 11, no. 1, pp. 307-316, 2015.

[13] S. Baroni, M. Mattu, A. Vannini, R. Cipollone, S. Aime, P. Ascenzi, and M. Fasano, "Effect of ibuprofen and warfarin on the allosteric properties of haem–human serum albumin," *European Journal of Biochemistry*, vol. 268, no. 23, pp. 6214-6220, 2001.

[14] A. M. Pisudde, P. V. Tekade, S. D. Bajaj, and S. B. Thakare, "Effect of solvent on binding of diethyl 4-(4-hydroxyphenyl)-2, 6-dimethyl-1, 4-dihydro-pyridine-3, 5-dicarboxylate to BSA," *Heterocyclic Letters*, vol. 6, p. 679, 2016.

[15] D. Li, Y. Wang, J. Chen, and B. Ji, "Spectrochimica acta part A: Molecular and biomolecular spectroscopy," *Molecular and Biomolecular Spectroscopy*, vol. 79, no. 3, pp. 680-686, 2011.

[16] R. Uddin, N. Saffoon, and M. A. Alam, "Effect of arsenic on paracetamol binding to bovine serum albuminusing site specific probes," *International Current Pharmaceutical Journal*, vol. 1, no. 11, pp. 361-365, 2012.

[17] M. A. Alam, M. A. Awal, N. Subhan, and M. Mostofa, "In-vitro Relationship between protein-binding and free drug concentrations of a water-soluble selective beta-adrenoreceptor antagonist (atenolol) and its interaction with arsenic," *Journal of Health*, *Population And Nutrition*, vol. 27, no. 1, pp. 20-30, 2009.

[18] S. D. Bajaj, O. A. Mahodaya, and P. V. Tekade, "A green, microwave assisted and efficient protocol for synthesis of 2-(4-substituted phenyl)-1h-benzimidazole catalyzed by nickel nitrate and their molecular docking study," *Heterocyclic Letters*, vol. 6, no. 4, pp. 805-815, 2016.