[FRTSSDS- June 2018]
DOI:10.5281/zenodo.1288393

ISSN 2348 – 8034
Impact Factor- 5.070

GLOBAL JOURNAL OF ENGINEERING SCIENCE AND RESEARCHES

IN VITRO BINDING ANALYSIS OF BENZIMIDAZOLE DERIVATIVES TO BSA: ACOUSTICAL, THERMODYNAMIC AND MOLECULAR MODELING STUDY

Pradip V. Tekade*, Ajay M. Pisudde, Om A. Mahodaya & Shrikant B. Thakare
Jankidevi Bajaj College of Science, Jamnalal Bajaj marg, Civil lines, Wardha (India) India

ABSTRACT

Current investigation is undertaken to reveal the binding affinity of benzimidazole derivatives to bovine serum albumin (BSA) by acoustical study at physiological pH in different solvents and its molecular modeling. Findings were interpreted by scatchard plot which showed an increase in association constants with increasing temperature and concentrations of the ligands. It is observed that, the binding supposed to be more in 1, 4-dioxane than DMSO and DMF. The binding study also involves determination of thermodynamic parameters. The values of Gibb's free energy (ΔG), enthalpy (ΔH) and entropy (ΔS) are calculated from van't Hoff equation. The negative ΔH and positive ΔS showed exothermic interaction between ligand and BSA. Similarly negative ΔG showed the spontaneity of the binding process. ΔG becomes more negative with increase in temperature, indicated feasibility of the reaction at high temperature. Molecular modeling confirmed the binding interaction having energy -167.08 kJ/mol.

Keywords- *Acoustical study, molecular modeling, Scatchard analysis, association constants, BSA, thermodynamic parameters.*

I. INTRODUCTION

2-(4-hydrophenyl)-1*H*-benzimidazole (4HPHBI) is an important heterocyclic compounds shows various biological properties especially, antimicrobial, antiviral, anticancer and antitumor[1-5]. Benzimidazole derivatives essentially show intraocular pressure lowering effect and hypotensive activity[6]. Serum albumins are the most abundant proteins in the circulatory system of wide variety of organisms, being the major macromolecules contributing to the osmotic blood pressure[7]. The structure of HSA explains numerous physiological phenomena and provides further insight in pharmacokinetics and its functional and physiological properties have been studied over several decades[8]. A variation in temperature is found to be a key factor in binding affinities of HSA[9], as evident from the drugs Ligustrazine[11], Ciprofloxacin[11], methotrexate[12] and cisplatin[13]. Various techniques are available to monitor the binding interactions of ligands to protein like NMR[14], isothermal titration calorimetry[15], U.V. visible absorbance[16], fluorescence[17], equilibrium and FT-IR and CD spectroscopy[18]. Molecular modeling also shows important aspects about protein-drug interaction[19-21]. It is difficult to obtain HSA for experimental purposes. HSA and BSA exhibit similar chemical properties due to high percentage of sequence identities. BSA in lieu of HSA is use in this study because of low cost and easy availability.

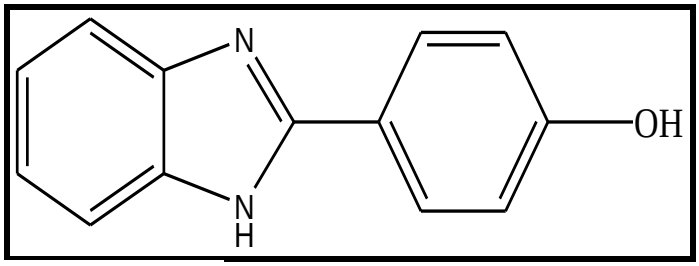


Figure 1: Structure of 2-(4-hydrophenyl)-1H-benzimidazole (4HPHBI)

In the view of above consideration, present study proposed to evaluate the effect of ligand concentration, temperature and polar/non polar solvent on binding interaction of 4HPHBI to BSA at physiological pH. The above

ISSN 2348 – 8034
Impact Factor- 5.070

study also involves the determination of thermodynamic parameters like free energy, enthalpy, entropy and molecular modeling.

II. MATERIALS AND METHOD

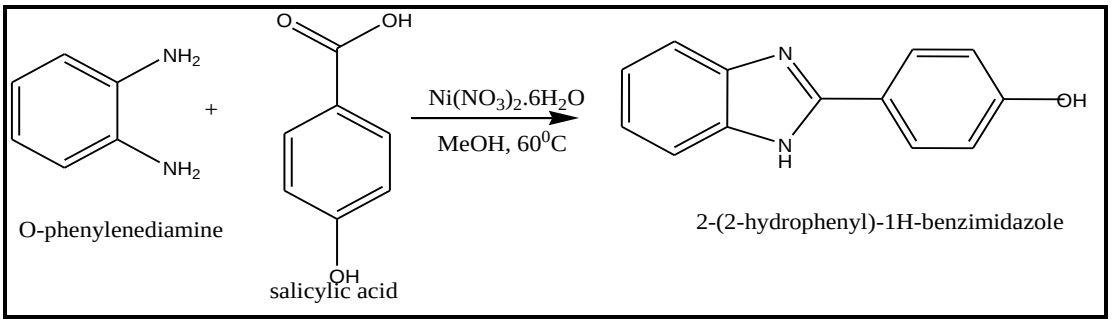
Multi-frequency ultrasonic interferometer (VI microsystem, Chennai, India), Hex 8.0, BSA (essential fatty acid free) purchased from Chemsworth Chemicals Ltd (India) and used without further purification and the ligand 4HPHBI. Basic buffer selected to maintain the physiological pH 7.4. For the synthesis, all the chemicals used are of A.R. grade of Merck India limited and purchased from commercial suppliers.

Optimization study

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

Preparation of 4HPHBI

2-(4-hydrophenyl)-1*H*-benzimidazole (4HPHBI) synthesized by known method[22]. The mixture of o-phenylenediamine (1 mmol) , salicylic acid (1mmol) and nickel nitrate hexahydrate [$(Ni(NO_3)_3.6H_2O)$] (5 mole %) was stirred in methanol (10mL) at 323 K temperature. The progress of the reaction was monitored by TLC. After completion of the reaction (2.5 minutes) the solvent was removed under reduced pressure. Cold water (15-25 mL) was added to the reaction mixture to get the product. The solid product was filtered and washed with cold water and air dried. The solid was recrystalized from ethanol .The obtained product was chromatographed on silica gel and eluted with CH_2Cl_2–MeOH (100:1) to obtain pure product. Melting point recorded using digital melting point apparatus Equiptronics (EQ 730). ^{1}H NMR spectra of the compound recorded in $CDCl_3$ on NMR instrument (500 MHz) using TMS as an internal standard from SAIF, CDRI Lucknow.



Scheme 1: Synthesis of ligand 2-(4-hydrophenyl)-1H-benzimidazole (4HPHBI)

Measurement of binding affinity

The binding interaction of 4HPHBI to BSA is expressed as binding constant or association constant, which is derived from the law of mass action. BSA (B) interacts with the 4HPHBI (L) to form the complex is given as,

$$B + L \rightleftharpoons BL$$

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

Binding strength of the ligand 4HPHBI to BSA is a measure of association constants.

Acoustical study

Ultrasonic is a versatile non-destructive and highly investigatory technique. Ultrasonic absorption in a medium provides important tools for evaluation of the structural, chemical and physical properties of medium[23]. Initially ultrasonic interferometer was set at 1MHz and different solutions of ligand 4HPHBI in solvents 1, 4-dioxane, DMSO, DMF have been prepared. The different concentrations were 1 mM, 1.5 mM, 2 mM, 2.5 mM, 3 mM, and 3.5 mM. 0.15µM BSA solution was prepared in basic buffer at physiological pH 7.4 and its ultrasonic velocity

ISSN 2348 – 8034
Impact Factor- 5.070

measured. Different concentrations of 4HPHBI mixed with BSA at 298 K and allowed to stand for 1 hr for maximum binding. Then, ultrasonic velocities of these complex solutions were recorded. Similar steps are performed at 303 K and 308 K and specific binding along with association constants have been determined using Scatchard plot.

Molecular modeling study

The Molecular modeling study of 4HPHBI to BSA was carried out on Hex 8.0 software. Hex 8.0 gives the value of efficient energy. PDB file of the crystal structure of BSA obtained from the RCSB data bank with ID 4F5S. Then 3D structure of 4HPHBI was developed. The structure of 4HPHBI has been drawn using Chem Draw and its 3D structure is developed. The obtained 3D structure arranged in a minimized energy form. The PDB files of ligands and BSA runs together on Hex 8.0, which gives the energy value of the newly formed complex showing its stability.

III. RESULT AND DISCUSSION

Initially, ultrasonic velocities of BSA solution in basic buffer at physiological pH were measured. The ultrasonic velocities of BSA are 1482.192, 1483.157 and 1485.220 m/s at temperatures is 298, 303 and 308K respectively. Then the ultrasonic velocities of 4HPHBI-BSA complexes were measured at different concentrations and temperatures 298, 303 and 308K. Ultrasonic velocities of 4HPHBI-BSA complexes measured at various concentrations of ligands and at different temperatures are mentioned in **Table 1**. The scatchard graph is plotted for specific binding versus percent ligand fraction and from this plot binding constants have been determined. The Scatchard analysis gives different association constants at different temperatures for 4HPHBI binding. The association constants in 1, 4-dioxane are 0.5011, 0.5017 and 0.5019 at temperature 298, 303 and 308K respectively. Similar analysis was carried out in DMSO and DMF and association constants have been calculated. The association constants in DMSO are 0.5004, 0.5011, 0.5015 and in DMF are 0.4999, 0.5012, 0.5016 at temperatures 298, 303 and 308 K respectively. The association constants increase with the increase in temperature. This increase in association constants supports the exothermic nature of reaction. This concluded the interaction of 4HPHBI to BSA by means of Vander Waal's interactions and hydrogen bonds. It is also observed that, with increased in concentration of the ligand, binding affinity increases; this probably enhances the pharmacological activity of the ligand. **Figures 2 to 4** shows the scatchard plots of BSA-4HPHBI binding in 1, 4-dioxane, DMSO and DMF respectively. The effect of temperature on BSA-4HPHBI binding is summarized in van't Hoff equation.

Table 1: Ultrasonic velocities of 4HPHBI -BSA complex solutions at different concentrations and temperatures

Temp. (^{0}K) Conc. (mM)	298 K			303 K			308 K		
	1, 4-dioxane	DMSO	DMF	1, 4-dioxane	DMSO	DMF	1, 4-dioxane	DMSO	DMF
1	1491.566	1482.696	1480.083	1498.696	1487.112	1488.091	1511.173	1495.212	1495.302
1.5	1490.277	1483.850	1481.189	1498.696	1489.190	1490.814	1509.850	1497.921	1497.965
2	1492.211	1484.499	1483.512	1501.959	1490.965	1490.989	1512.499	1499.566	1499.645
2.5	1493.503	1483.529	1483.836	1502.614	1490.612	1491.103	1508.529	1498.211	1503.523
3	1493.503	1485.870	1485.189	1502.614	1489.824	1490.867	1507.870	1499.860	1503.212
3.5	1404.191	1486.876	1484.091	1501.959	1494.590	1493.860	1513.827	1501.860	150.345
BSA	1490.921	1482.219	1480.192	1501.959	1486.57	1487.157	1505.894	1505.534	1493.220

ISSN 2348 – 8034
Impact Factor- 5.070

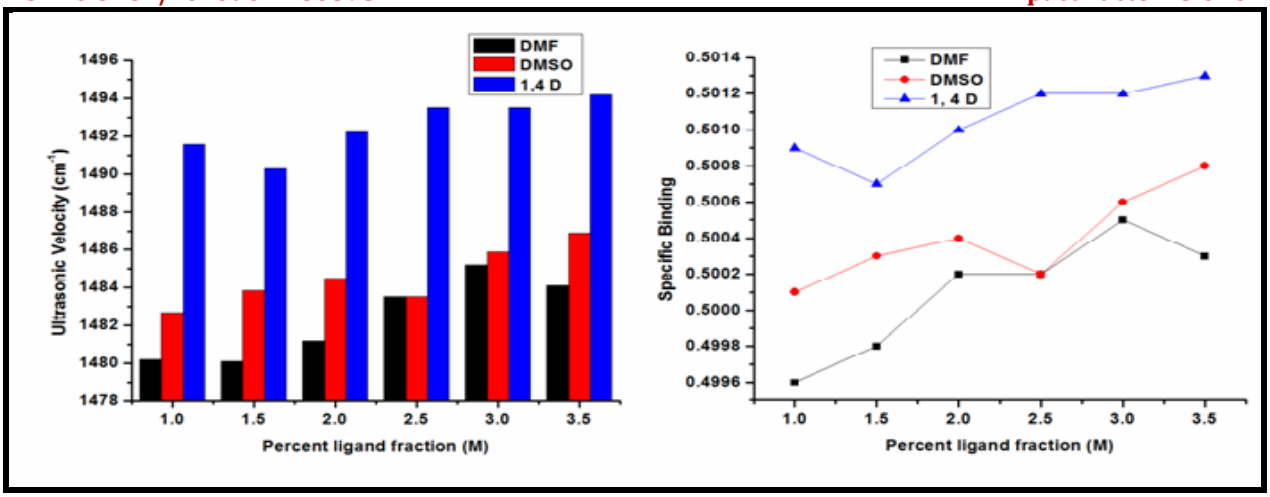


Figure 2: Scatchard plot of 4HPHBI-BSA complex in 1, 4 dioxane, DMSO, DMF at 298 K.

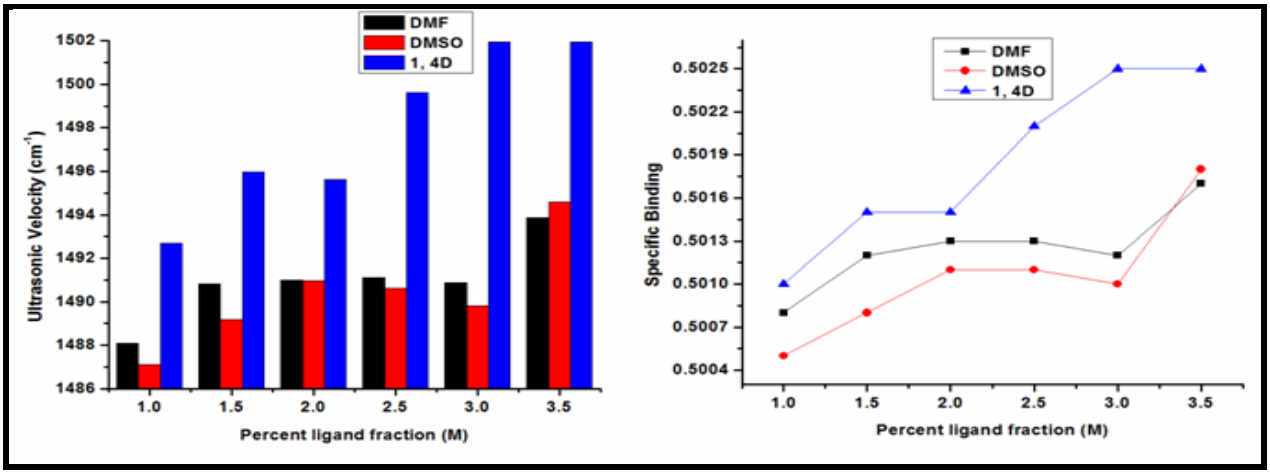


Figure 3: Scatchard plot of 4HPHBI-BSA complex in 1, 4 dioxane, DMSO, DMF at 303 K

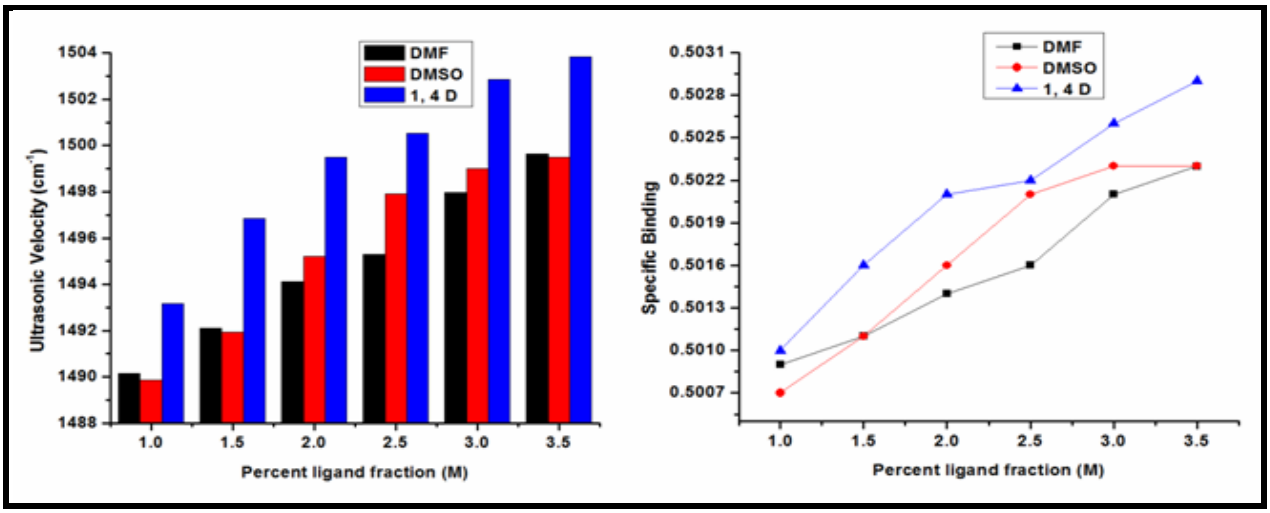


Figure 4: Scatchard plot of 4HPHBI-BSA complex in 1, 4 dioxane, DMSO, DMF at 308 K.

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Thermodynamic study:

In order to clarify the interaction of ligand 4HPHBI to BSA, the thermodynamic parameters (ΔG, ΔH and ΔS) have been calculated by using van't Hoff equation at the temperatures 298, 303 and 308 K. The values of ΔH and ΔS were calculated from the slope & intercept of the plot of lnk vs 1/T respectively.

$$\ln k = -\frac{\Delta H}{RT} + \frac{\Delta S}{R} \quad \ldots\ldots\ldots\ldots\ldots (1)$$

Graph plotted between **lnk vs 1/T** shows straight line with negative slope, as shown in figure 5.

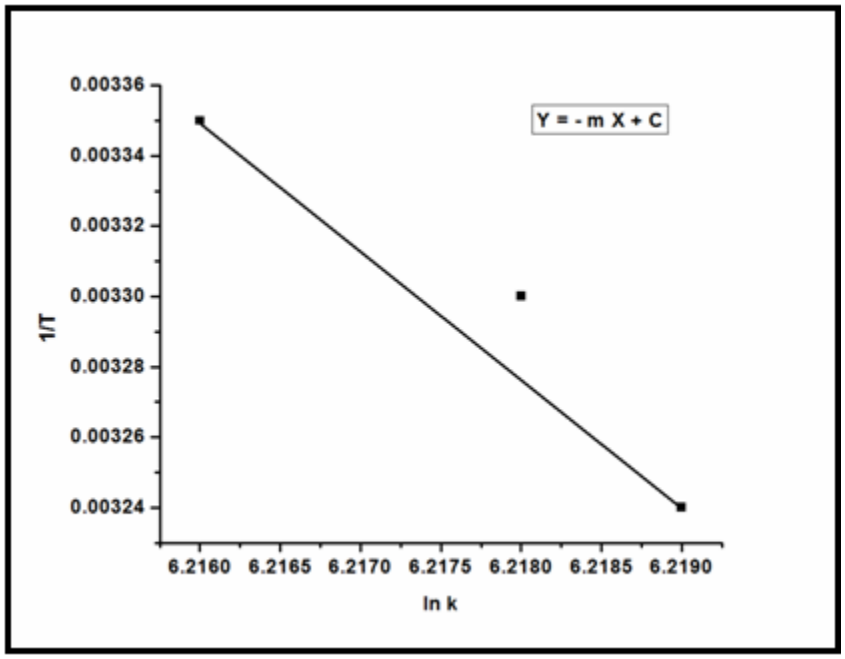

Figure 5: Graph of lnk vs 1/T in 1,4-dioxane for 4HPHBI-BSA complex

Table 2: Thermodynamic parameters at different temperature of 4HPHBI-BSA complex in 1, 4-dioxane

Sr. No.	Temp. (k)	ΔH J/mol	ΔG kJ/mol	ΔS J/mol
1	298	-226.72	-15.85	52.43
2	303		-16.11	
3	308		-16.37	

Negative value of ΔH and positive value of ΔS indicate that drug interaction with BSA are enthalpy and entropic driven. Positive value of entropy also shows that there is unfolding of BSA. For unfolding, process must be exothermic which is given by positive entropy and negative enthalpy (table 2). The specific electrostatic interaction is also characterized by the values of enthalpy and entropy. The negative value of ΔG supports the 4HPHBI-BSA complexation is feasible process at high temperature. Thus the overall stability of the complexes is indicated by Gibbs free energy. So, the hydrogen bonding, electrostatic and hydrophobic interactions are supposed to be possible factors contributing binding of the 4HPHB to BSA. The thermodynamic parameters in DMSO & DMF are not significant as that of 1, 4 dioxane.

ISSN 2348 – 8034
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Molecular modeling study

Molecular modeling is also an efficient method for measurement of interaction between protein and drug. Furthermore the binding interaction between BSA and 4HPHBI is studied by molecular modeling .The energy obtained from the study is the measure of binding of 4HPHBI to BSA. The energy obtained is -167.08, shows that the 4HPHBI efficiently binds with BSA. Diagrammatic representation of interaction between BSA and 4HPHBI is as shown below in figure 6.

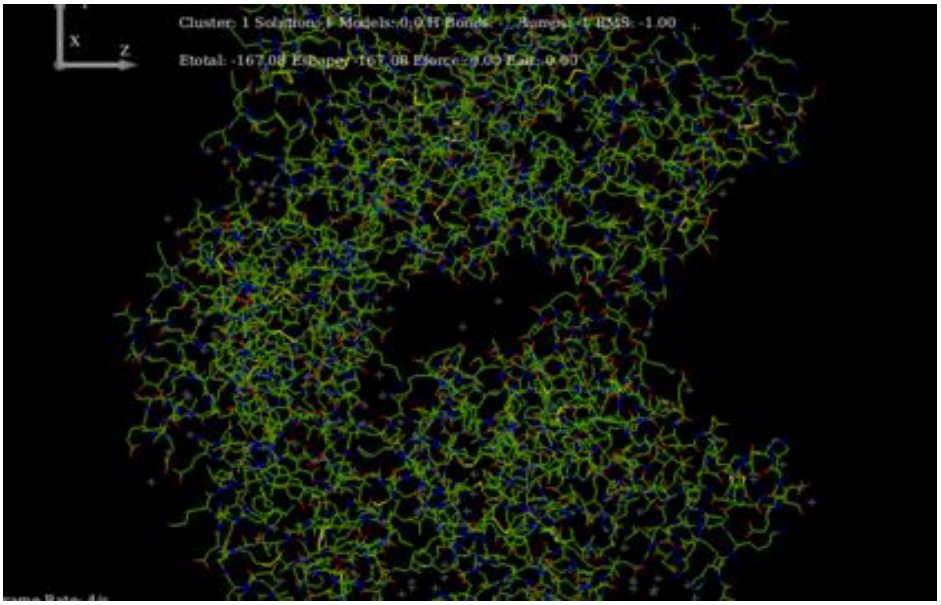

Figure 6: Molecular Modeling interaction between 4HPHBI and BSA.

IV. CONCLUSION

In the present study, the binding interaction of 2-(4-hydrophenyl)-1*H*-benzimidazole to BSA in 1, 4-dioxane, DMSO and DMF BSA has been reported by acoustical study at physiological pH and its molecular modeling study. The study also involves determination of thermodynamic parameters. Association constants for BSA-4HPHBI binding interaction increased with increase in temperatures, which clearly indicate exothermic nature of reaction. Negative value ΔG supports the spontaneity of the binding process. The scatchard analysis provided a non-linear curve on binding of ligand to the BSA, suggested the presence of at least two binding sites in BSA. The experimental results by acoustical study at different temperature clearly indicate that 4HPHBI interact to BSA by means of Vander Waal's interactions and hydrogen bonds in the hydrophobic packet of binding sites. It is also observed that binding affinity increases with increased in the concentrations and temperatures; this probably enhances the pharmacological activity of the 4HPHBI.

It is found that binding is more significant in 1, 4-dioxane than DMSO and DMF concluded from thermodynamic parameters. It may be due to aprotic and non polar nature of the 1, 4 dioxane. The thermodynamic parameters also indicated that the hydrogen bonding, electrostatic and hydrophobic interactions induce alterations in secondary structure of the BSA. Molecular docking is also used to confirm the binding of 4HPHBI with BSA. The binding energy of complex obtained is 167.08 $kJmol^{-1}$, this energy value indicates that the complex formed is stable concluding 4HPHBI is successfully bound to BSA.

[FRTSSDS- June 2018]
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ISSN 2348 – 8034
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